UNIVERSITY OF SOUTHAMPTON

THE POTENTIAL OF ENTOMOGENOUS FUNGI TO CONTROL GLASSHOUSE PESTS AND BROWN PLANTHOPPER OF RICE

by

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ABSTRACT

FACULTY OF SCIENCE
BIOLOGY

Doctor of Philosophy

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Strains of Beauveria, Metarhizium anisopliae, Nomuraea rileyi, Paecilomyces, Syncephalastrum racemosum and Verticillium, have been examined for potential use as microbial insecticides, against the leafhopper, Hauptidia maroccana, Thrips tabaci and the planthopper, Nilaparvata lugens.

Methods have been developed for insect rearing and for maintaining insects at high humidity, utilizing whole plants, or leaf segments supported on agar.

In the laboratory, strains of M.anisopliae proved most pathogenic to test insects, but failed to provide reliable control of H.maroccana and N.lugens in the glasshouse. In contrast, a V.lecanii strain, only moderately pathogenic to T.tabaci in the laboratory, significantly reduced thrips populations on cucumbers under glass. In such glasshouses, humidity is generally highest at night, when temperature was maintained at 20°C.

V.lecanii conidia germinated more quickly than those of M.anisopliae at 20°C, and this correlated with the time required for cuticular penetration.

M.anisopliae readily produced conidia on semi-solid medium, but failed to produce economic yields of hyphal bodies in a variety of liquid media. V.lecanii produced hyphal bodies readily in submerged culture.

Further studies on fungal pathogens of T.tabaci and N.lugens are recommended, while the parasite, Anagrus atomus, might be developed to control H.maroccana.

INTRODUCTION

Currently, there is widespread concern over the reliance on insecticides to control insect pests. This anxiety arises from several sources; the occurrence of resistance to insecticides, environmental pollution, phytotoxicity and increased development costs, caused partly by the requirement of ever-more stringent safety tests. These factors have stimulated research into alternative methods of pest control, such as the use of predators, parasites and pathogens, to reduce insect numbers below the level at which they cause economic damage.

In the United Kingdom and in parts of Europe, the two major pests of cucumbers and tomatoes, the glasshouse red spider mite, Tetranychus urticae and the whitefly, Trialeurodes vaporariorum, are controlled biologically by the predatory mite Phytoseilius persimilis and the chalcid parasite Encarsia formosa, respectively. As a result, pesticide application to these crops has declined, and insects formerly controlled by sprays directed at the mite and whitefly, have increased to pest status. Two such insects are the onion thrips, Thrips tabaci, on cucumbers and the glasshouse leafhopper, Hauptidia maroccana, which occurs primarily on tomatoes. Both insects are susceptible to insecticides but these would also harm the predator and parasite, necessitating the abandonment of biological control and a return to the use of pesticides. Clearly, for thrips and leafhoppers, there is a need for specific control agents which can be integrated with the existing system of biological control.

The glasshouse provides an uniquely favourable environment in which to exploit fungi for pest control as two crucial parameters, temperature and humidity, can be manipulated. The fungus Verticillium lecanii is commercially available as Vertalec® for aphid control on chrysanthenmums, and as Mycotal® for control of whitefly on cucumbers, mainly

due to the efforts of Hall (1977). This success has prompted the present study to determine if entomogenous fungi might be used as specific control agents for the onion thrips and glasshouse leafhopper. The project was later extended to include the major rice pest, Nilaparvata lugens.

SECTION I

CONTROL OF INSECTS BY FUNGAL PATHOGENS - LITERATURE REVIEW

Fungi have long been recorded parasitising insects, but only in recent years have they been successfully manipulated to provide a reliable means of pest control. The biology, production and use of fungi as control agents, has been the subject of many reviews. Some early discussions were provided by Steinhaus (1949, 1954, 1956), Madelin (1963, 1968), Müller-Kogler (1965) and Roberts and Yendol (1971). More recently, Brady (1981) and Roberts and Humber (1981) provided a taxonomic viewpoint; Soper and Ward (1981) and Quinlan and Lisansky (1983) discussed production and formulation; Zacharuk (1981) gave a histopathological view, while Ferron (1978) and Hall and Papierok (1982), provided more general accounts. In addition, Burges (1981), contains many chapters on fungi and includes information on identification, Beauveria and Metarhizium, Verticillium lecanii, Hirsutella thompsonii, Nomuraea rileyi and the Entomophthorales. In view of the large number of reviews, a general account on the biology of entomogenous fungi will not be given here.

Entomogenous fungi occur in the classes Deuteromycetes, Zygomycetes, Oomycetes, Chytridiomycetes and Trichomycetes. In their recent review, Quinlan and Lisansky(1983) listed twelve fungi under development for use as mycoinsecticides, and included seven Deuteromycetes (Aschersonia spp., Beauveria bassiana, Culicinomyces clavosporus, Hirsutella thompsoni, Metarhizium anisopliae, Nomuraea rileyi, and Verticillium lecanii), four Zygomycetes (Conidiobolus obscurus, Entomophthora grylli, Erynia neoaphidis and Zoophthora radicans) and the Oomycete Lagenidium giganteum. Four Deuteromycetes are mass produced for pest control (B. bassiana, H. thompsoni, M. anisopliae and V. lecanii), while the most developed Zygomycete (C. obscurus) is at the pilot plant stage.

B. bassiana is commercially produced in the Soviet Union to control Colorado beetle (Ferron, 1978; Soper and Ward, 1981), while in the Peoples Republic of China it is routinely used on one million hectares, mainly against the European corn borer (Soper and Ward, 1981), but is also applied to rice for brown planthopper control (Pinnock, pers. comm.).

M. anisopliae is produced in Brazil for spittlebug control and relatively low doses (6×10^{11} - 1.2×10^{12} conidia ha^{-1}) gave a mean 65% mortality over an area of 4000 hectares (Ferron, 1981).

H. thompsoni, a virulent pathogen of eriophyid mites, has been developed for control of citrus rust mite (Mc Coy et al. 1971; Mc Coy and Selhime, 1974; Mc Coy, 1978, 1981). Mycar®, containing H. thompsonii, was first commercially produced in 1981, but proved unsatisfactory and was withdrawn to be released at a later date. Unlike most Deuteromycete fungi, Hirsutella only produces a single conidium per phialide and large spore numbers are difficult to produce. Thus, Mycar® contains primarily mycelia which prove difficult to stabilize.

In Europe, two strains of V. lecanii are produced as Vertalec® and Mycotal® for glasshouse control of chrysanthemum aphid and whitefly respectively, mainly due to the efforts of Hall (1981, 1982); Hall and Burges (1979).

Aschersonia spp are specifically active against whitefly and scales and are under development for control of glasshouse whitefly, both in W. Europe and the USSR. N. rileyi is pathogenic to Lepidoptera and has been isolated from the important pests Heliothis spp, Trichoplusia ni and Spodoptera littoralis (Getzin, 1961; Ignoffo, 1981). C. clavosporus has been isolated from mosquitoes in Australia and the USA (Russell et al., 1979) and together with the Deuteromycete Tolypocladium cylindrosporum, shows promise as a mosquito larvicide (Soares et al., 1979; Matawale, pers. comm.).

In contrast to the Deuteromycetes, no entomophthoran is produced commercially, and there are few records of succ-

essful insect control under field conditions. Wilding (1982) reviewed the data on field use and effectiveness of entomophthoran fungi, and in all cases, control was at best, insufficient. Furthermore, he concluded there was no published evidence for establishment of these fungi in insect populations by application of resting spores. Latgé (1982) calculated that effective aphid control by C. obscurus would require 1.2×10^{12} resting spores hectare $^{-1}$, which assuming optimum production, would require the yield of 1000-2000 l of fermenter medium, and clearly this process would be uneconomic. C. obscurus is perhaps the best studied of the entomophthoran fungi, but considerable work is required before use of this fungus for aphid control becomes a reality. Soper and Ward(1981) predicted a bright future for entomophthoran fungi, but this view appears overoptimistic, at least in the short term. However there there is a single record of the sucessful use of an entomophthoran fungus. Milner et al.(1982) reported the sucessful establishment of an introduced strain of Zoophthora radicans (originally isolated in Israel) on spotted alfalfa aphid. The aphid was first recorded in Australia in 1977 but the incidence of entomophthoran pathogens was low(Milner et al.,1980). Previous studies in the USA (Hall and Dunn, 1957) and in Israel (Kenneth and Olmert,1975) had indicated that some entomophthorans were capable of causing field epizootics. The most effective pathogen was Z.radicans, and this fungus persisted after its introduction and now causes epizootics of spotted alfalfa aphid and reduces the need for insecticide application.(Milner pers. comm.). This is generally regarded as the first transfer of an entomophthoran fungus from one country to another, that has resulted in successful pest control. Thus, it would seem that members of the Entomophthorales have a role to play in fungal control of insects, particularly where an insect has been introduced to a country where it does not normally occur.

The infection cycle in Deuteromycetes is simple : conidia germinate on host cuticle and penetrate to the haemolymph

where often hyphal bodies are formed. After a few days the insect dies, and if humidity is high, conidiophores erupt through the cuticle and sporulation occurs. Conidia are able to survive in soil (Milner and Lutton, 1976; Bell and Hamalle, 1970; Lingg and Donaldson, 1981). In addition, many species are saprophytic and can survive on other substrates in the absence of hosts.
By contrast, the life cycle of Zygomycetes is more complex: conidiospores are forcibly discharged and may produce primary, secondary, tertiary or quaternary conidia before penetrating cuticle. After a few days the insect dies and the fungus forms either conidia or resting spores. The factors stimulating resting spore formation are many and include humidity, temperature, photoperiod and developmental stage of the insect (Thaxter, 1888; Wilding, 1973; Papierok, 1978; Dedryver, 1980; Remaudière et al. 1981; Shimazu, 1979). Conidia are usually considered to be delicate and short-lived (Wilding, 1981) though some may persist in the soil for several months (Latteur, 1980), while resting spores germinate slowly and asynchronously (Latgé, 1982). Perhaps the major reason for the comparative success of the Deuteromycetes compared to the Zygomycetes is that the latter often prove difficult to culture in vitro. Indeed, some members of the genera Massospora and Neozygites remain obligate parasites and cannot be cultured outside the host, eg. N. parvispora (Samson et al., 1979a). By contrast, entomogenous Deuteromycetes conidiate freely on simple substrates eg. M. anisopliae on cereal grains (Aquino, 1975, 1977), while many form hyphal bodies in submerged culture eg. V. lecanii (Hall, 1977) and M. anisopliae (Adamek, 1963). Conidia have relatively thick walls and survive well at low temperatures (Clerk and Madelin, 1965; Daoust and Roberts, 1983) while there are reports of successful hyphal body stabilization (Blachère et al., 1973; Farques et al. 1979). The simple requirements of Deuteromycetes allow the addition of nutrients to spore sprays which permit growth and sporulation of the fungus and allow

prophylactic treatment (Hall and Turner unpub. obs.).

There have been a number of reports of fungi parasitizing thrips (Table 1.01), dating back to the nineteenth century. Krassilstschik (1886) recorded an Entomophthora and a Tarichium species on Thrips solanacearum while thrips infected with Entomophthora sphaerosperma were recorded by several workers. Interestingly, deep-ploughing of cereal stubbles encouraged B. bassiana to control overwintering thrips (Kurdjumov, 1913; Grivanov, 1939). Reyne (1921) was the first worker to record parasitism of thrips by Verticillium (as Cephalosporium) and further records are provided by Ekbom (1979) and Binns et al. (1982). Stradling (1968) described Thrips tabaci nymphs packed with black resting spores and later, Carl (1975), provided illustrations and described epizootics of the same fungus. Mac Leod et al. (1976) described the fungus as Entomophthora parvispora. However, the fungus was renamed Neozygites parvispora in the taxonomic revision of the Entomophthorales suggested by Remaudière and Hennebert (1980) and Remaudière and Keller (1980). Later, Samson et al. (1979) described a fungus causing epizootics in populations of T. tabaci in glasshouses, and described it as Entomophthora thripidum.

There are no records of fungi parasitizing Hauptidia maroccana, probably because the insect has been poorly studied. However, there are many records of fungi attacking related Hemiptera. Ben-Zeev and Kenneth (1981) reported Zoophthora radicans and Z. petchi infecting leaf-hoppers and frog hoppers. Devanesan et al. (1979)described infection of the leafhopper Nephotettix virescens by Fusarium equiseti,while Mathai et al.(1979) and Nayak and Srivastava (1979) recorded infection of rice leafhoppers by Syncephalastrum racemosum and B. bassiana respectively. In addition, there are many records of fungi parasitizing Nilaparvata lugens (Table 1.02). Kurvilla and Jacob (1979) reported 100% mortality of N. lugens within 3 days, after application of Fusarium oxysporum conidia in field conditions, while Balasubramanian (1979) recorded 60-70% mortality after application of V. lecanii conidia. Pinnock (pers. comm.)

Table 1.01

Fungal Pathogens of Thrips

Pathogen	Host	Reference
ENTOMOPHTHORACEAE		
Entomophthora sp.	Thrips solanacearum	Krassilstschik (1886) in Mac Leod et al. (1976)
Tarichium sp.	" "	"
Entomophthora sphaerosperma (Zoophthora radicans)	Thrips sp.	Thaxter (1886)
"	Thrips tabaci	Bourne and Shaw(1934) Bourne(1935) Bourne and Whitcomb(1935) Charles(1941)
Empusa(Entomophthora)grylli	Haplothrips ulmi Rhynchothrips hungaricus Acantothrips nudicornis Phloeothrips coriaceus Zygothrips minutus Taeniothrips atratus	Dyadechko (1964)
Entomophthora(Neozygites)parvispora	Thrips tabaci	
	T. fuscipennis	Carl (1975)
	T. major	Mac Leod et al.(1976)
	Taeniothrips atratus	Stradling (1968)
Entomophthora thripidum	Thrips tabaci	Ramakers (1976) Samson et al. (1979)
DEUTEROMYCETES		
Aspergillus sp.	Thrips fuscipennis T. validus T. tabaci T. physcipus Taeniothrips discolor Frankliniella intonsa Odontothrips loti O, phaleratus Anaphothrips obscurus Limothrips denticornis	Dyadechko (1964) " "
Beauveria bassiana	'Thrips'	Kurdjumov (1913)
	Haplothrips tritici	Grivanov (1939)
	Aptinothrips rufus Limothrips schmutzi Chirothrips manicatus Heliothrips haemorrhoidalis	Dyadechko (1964)

Table 1.01(cont)

Pathogen	Host	Reference
B. globulifera(bassiana)		Nowell(1916) Urich (1928) Callan(1943)
Cephalosporium(Verticillium)sp.	Heliothrips rubrocinctus	Reyne (1921)
Verticillium lecanii	Thrips tabaci	Ekbom (1979)
" "	Thrips tabaci	Binns et al. (1982)

Table 1.02

Fungal Parasites Recorded From *Nilaparvata lugens*

Pathogen	Country of Occurence	Reference
ENTOMOPHTHORACEAE		
Entomophthora coronata (*Conidobolus coronatus*)	Philippines	Gabriel (1968)
E. delphacis	Japan	Esaki and Hashimoto (1936) Sakai (1932), Shimazu(1976,1977) Aoki (1957)
E. fumosa	India	Samal et al.(1978)
E. nr. apiculata var *major*	Fiji	Hinckley (1963)
E. nr. coronata	Japan	Okado (1971)
E. sphaerosperma (*Zoophthora radicans*)	Japan	Shimazu (1979)
DEUTEROMYCETES		
Beauveria bassiana	India	Srivastava and Nayak (1978)
	China	Pinnock(pers.comm.)
	Japan	Aizawa (pers.comm.)
Fusarium oxysporum	India	Kuruvilla and Jacob (1979)
Hirsutella sp.	Philippines	Gabriel (1968,1970)
H. citriformis	Soloman Islands	Mac Quillan(1974)

Table 1.02 (cont)

Pathogen	Country of Occurence	Reference
Is aria farinosa (Paecilomyces farinosus)	Japan	Aoki (1957)
Metarhizium anisopliae	Philippines	Daoust and Roberts (1982)Roberts(pers. comm.)
Paecilomyces farinosus	India	Kuruvilla and Jacob (1980)
Verticillium lecanii	India	Balasubramanian (1979)Mancharan and Jayaraj(1980)

reported use of *B. bassiana* to control *N. lugens* in parts of China. In view of the large number of reports of fungi parasitising *N. lugens*, and the high humidity encountered in rice crops, the latter part of this study was devoted to *N. lugens*.

SECTION II

IMPORTANT FEATURES OF THE CUCUMBER AND RICE CROPS AND THEIR PESTS.

1. Cucumber

Throughout the United Kingdom cucumbers are grown under glass. There is a variety of cultivation methods, utilizing soil, straw bales, peat bags or 'Rockwool', as a growth medium and employing various training methods e.g. the umbrella and archway systems. Cucumbers are normally planted in December or January and removed in October, a 'break crop' of lettuce often being sown in the interval. In the late 1970's the area of cucumbers grown in England and Wales was 225 ha, thus comprising over 10% of the total glasshouse area. In continental Europe appreciable areas of cucumbers are grown, notably in the Netherlands where 720 ha are devoted to this crop.

2. Cucumber Pests and Their Control

Cucumbers are subjected to a variety of pests. The most important will be detailed here with emphasis on those species which were the subject of the present study, namely Tetranychus urticae, Thrips tabaci and Hauptidia maroccana.

a. Glasshouse red spider mite, Tetranychus urticae

This is a very serious pest. Adult mites hibernate in the glasshouse stucture and emerge in spring and feed on lower leaf surfaces, causing chlorotic speckling. They reproduce rapidly, the life cycle taking only 6½ days at 30°C. Many mite populations have developed resistance to acaricides, but fortunately this pest can be controlled by introducing the commercially produced predatory mite, Phytoseilius persimilis. In the classical use of P. persimilis, red spider mites are placed

on the cucumber plants (50 mites every fifth plant) before emergence of the overwintering mites. Predators are introduced one-two weeks later at the rate of five predators every fifth plant. At present some 70% of growers in England and Wales utilize the predator for controlling Tetranychus urticae on cucumber and this biological control method is also used throughout Europe.

b. Onion thrips, Thrips tabaci

Thrips tabaci is a polyphagous pest attacking cotton (Ghabn, 1948), tobacco (Federov, 1930), onions (Harris et al.1963) as well as cucumbers and chrysanthemums (Parr, 1968; Hussey et al. 1969; Gould, 1971). An excellent general account of thrips is given by Lewis (1973) and details of T. tabaci on cotton by Ghabn (1948). Binns et al.(1982) report the behaviour and distribution on glasshouse cucumbers. They found thrips most often on young apical foliage, both on upper and lower leaf surfaces and typical damage on cucumber is shown in Fig 2.01. Eggs are produced parthenogenetically. Second instar larvae fall to the ground and develop into prepupae which finally pupate under debris or in soil cracks. Adults and nymphs are illustrated in Fig. 2.02. T. tabaci is an increasing problem on cucumber due to the adoption of biological control (Section II e,g). They are more serious where crops are grown in a nutrient film or in 'Rockwool' (Bassett, 1981; Pickford, pers. comm.).
Thrips can be controlled by deltamethrin, diazinon, or malathion foliar sprays, or hexachlorohexane soil drenches (Morgan and Ledieu, 1979). However, these methods adversely affect the predatory mite,

Fig. 2.01 Damage to Cucumber Leaves Caused by Thrips tabaci.

2nd stage

×33

Adult

×44

Fig. 2.02 Stages in the Life Cycle of Thrips tabaci.

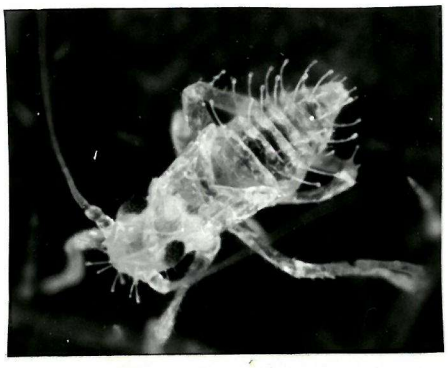
Phytoseilius persimilis and the whitefly parasite, Encarsia formosa, and cannot be used with biological control. Fortunately, alternative, selective methods are available. Polybutenes, together with the pyrethroid insecticide deltamethrin, are sprayed onto polythene sheets laid on the glasshouse floor. Larvae, falling to the floor for pupation, are trapped by the polybutenes and killed by the insecticide. Since the insecticide is confined to the polythene sheet it is selective. This method was developed by Pickford (pers.comm) and polybutenes containing deltamethrin are available commercially as Thripstick®. Ramakers (1981) claimed sucessful control of T. tabaci with the predatory mite Amblyseius mackenziei, but Bassett (1981) reports the failure of this method, which has not been generally accepted.

c. Glasshouse leafhopper, Hauptidia maroccana

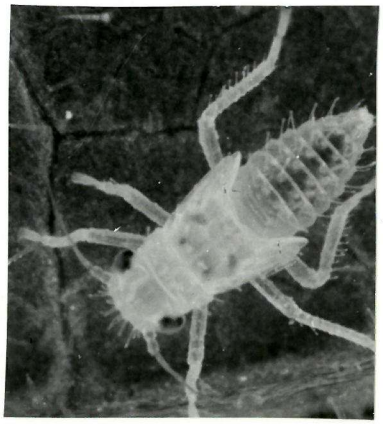
The first record of this jassid as a pest was damage on tomato, tabacco and other plants in 1918 (Anon, 1920). This insect was described as Zygina parvula and further damage was described by Rodman (1928). However, MacGill (1932) found all jassids causing damage on glasshouse plants to be Erythroneura pallidifrons, and considered earlier records to be misidentifications. Edwards (1924) described E. pallidifrons as Zygina pallidifrons, but recently it was described as Hauptidia maroccana (Dworakowska, 1970; Le Quesne and Payne, 1981). It was described briefly by Fox Wilson (1938) and in more detail by MacGill (1932). Eggs are laid singly in veins on the underside of leaves and there are five nymphal stages. The imagos are active and fly when disturbed. The various stages are illustrated in Fig. 2.03. At present H. maroccana is an occasional pest of cucumbers, occuring more frequently on tomato, but is of increasing importance. This increase is probably due to a reduction in pesticide application in glasshouses, occasioned by the increasing use of biological control (11, e,g).

1 st

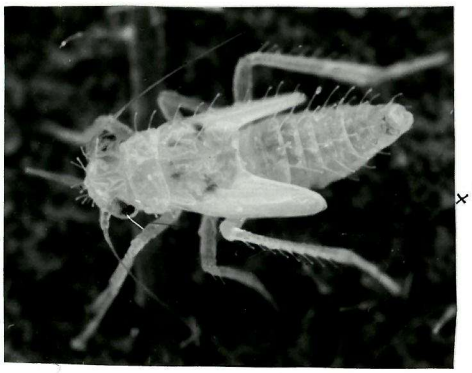
2 nd



3 rd



4 th



5 th

Adult

Fig. 2.03 Stages in the Life Cycle of H. maroccana

Insects extract sap from the underside of leaves and the first indication of their presence is chlorotic spotting of the upper leaf surface. When populations are high, spots coalesce to give more extensive chlorosis. Typical damage caused by H. maroccana on tomato is shown in Fig. 2.04.

Fig. 2.04 Damage to Tomato Caused by Hauptidia maroccana

d. Glasshouse whitefly, Trialeurodes vaporariorum

The whitefly is a serious pest causing damage directly by withdrawing sap and by producing honeydew which encourages the formation of sooty moulds. Eggs are laid on lower leaf surfaces. Newly hatched larvae are scale-like and have limited mobility for two days. They then become immobile. Many whitefly populations have become resistant to insecticides, so biological control is widely used by seasonal introductions of the chalcid parasite, Encarsia formosa. The recommended method is to introduce 12,500 parasites ha^{-1} after planting, until they become established on the crop. Occasionally, the host-parasite relationship becomes unbalanced and whitefly numbers increase. Populations can be reduced by spraying the plant tops with Pynosect 30(175ml, 50 lit H_2O-1) or by spraying the myco-insecticide Mycotal®.

e. Cotton aphid, Aphis gossypii

This aphid feeds on foliage, causing yellowing, or on developing fruit which are distorted or fail to develop. Control is by insecticides, the specific aphicide pirimcarb being particulary valuable.

f. Clover mite, Bryobia spp.

These mites sometimes infest cucumber early in the year and damage both foliage and fruit. They can be controlled with dicofol.

g. French-fly, Tyrophagus longior

This mite, common in straw and horse-manure, is a problem only where traditional cultivation methods are used. It attacks the apices of young plants causing leaf perforation and occasionally, shoot blindness. Control is by means of parathion.

h. Minor pests

These include fungus gnats (Sciara spp.) millipedes (Oxidus gracilis), root knot nematodes (Meloidogyne hapla and M. incognita), springtails (Collembola spp.), symphylids (Scutigerella immaculata), and woodlice (Armadillidium spp., Oniscus spp., Porcellio spp., Trichoniscus spp.). All these arthropods inhabit the soil and their incidence can be reduced by soil-sterilization prior to planting.

3. Cucumber Diseases and Their Control

Cucumbers are subject to many diseases including anthracnose, basal stem rot, black root rot, damping-off, grey mould, gummosis, powdery mildew, stem and fruit rot, root rot and Verticillium wilt. Chemicals must be applied to control disease, and fungicides are routinely sprayed against powdery mildew. The use of fungicides must be considered where it is hoped to exploit entomogenous fungi for pest control (V, 9)

4. Rice

a. General

Rice is perhaps the most important cereal crop in the world; in 1974 323,201 metric tons were produced from 136,791,000 hectares (Food and Agriculture Organisation, 1974).

Rice belongs to the tribe Oryzeae in the family Gramineae. Most cultivated varieties are in the diploid species Oryza sativa, though O. glaberrima is grown in parts of Africa.

An excellent account of rice, its production and associated pests and diseases is given by the Centre for Overseas Pests Research (1976) and only the most important features will be detailed here.

b. Agronomy

There are two main systems of rice cultivation, the upland or dry cultivation and the irrigated, or wet method. Upland rice is grown as a rain-fed crop under similar conditions to wheat, while irrigated rice is grown in flooded fields or 'paddies'. Irrigated rice is the most important and accounts for 90% of rice grown (Grist and Lever, 1969). There are several methods of producing irrigated rice. In Asia, pre-germinated seed is sown in nursery beds and then transplanted into 'paddies' when it is 30-40 days old. Transplantation is normally by hand, though mechanical methods are also available. Rice can also be sown directly in 'paddies' or into dry soil. In the latter method, fields are flooded immediately after sowing.
In areas where suitable growing temperatures occur for extended periods and water levels can be controlled, two or three rice crops may be grown in one season.

5. Rice Pests

a. General

Rice is subjected to many weeds, nematodes, crustacea, fungal, viral and bacterial pathogens, birds as well as many insects and mites. However, only insects will be detailed in table 2.01.

Table 2.01

Classification of the rice pests of Asia grouped as stem borers, leaf feeders or stem and root feeders. Adapted from Pest Control in Rice, PANS Manual No. 3. COPR.

Order	Family	Species	Notes
		STEM BORERS	
Lepidoptera	Pyralidae	Chilo auricilius	
"	"	C. polychrysus	
"	"	C. suppressalis	
"	"	Maliarpha separatella	
"	"	Tryporyza incertulas	
"	"	T. innotata	
"	Noctuidae	Sesamia inferens	
		LEAF FEEDERS	
Orthoptera	Acrididae	Oxya spp.	
"	"	Hieroglyphus banian	
"	"	H. nigroepletus	
Hemiptera	Eucelidae	Nephotettix cincticeps	Virus vector
"	"	N. nigropictus	Virus vector
"	"	N. virescens	Virus vector
"	Cicadellidae	Recilia dorsalis	Virus vector
"	Delphacidae	Unkanodes albifascia	Virus vector
"	"	Laodelphax striatellus	Virus vector
"	"	Sogatella furcifera	
"	"	Nilaparvata lugens	Virus vector very important
"	Aphidae	Rhopalosiphum padi	
"	Alydidae	Leptocorisa acuta	
"	"	L. costalis	
"	"	L. discoidalis	
"	"	L. oratorius	
"	"	L. lepida	
"	"	L. tagalica	
"	"	L. chinesis	
"	Coreidae	Cletus trigonus	
"	Pentatomidae	Scotinophara coarctata	
"	"	S. lurida	

Order	Family	Species	Notes
		LEAF FEEDERS	
Thysanoptera	Thripidae	Baliothrips biformis	
Lepidoptera	Limacodiae	Laroia bicolor	
"	Pyralidae	Cnaphalocrocis medinalis	
"	"	Nymphula depunctalis	
"	"	N. Vittalis	
"	Hisperiidae	Parnara gultata	
"	"	Pelopidas mathias	
"	"	Telicota augias	
"	Noctuidae	Spodoptera exempta	
"	"	S. exigua	
"	"	S. mauritia	
"	"	S. litura	
"	"	Naranga aenescens	
Diptera	Agromyzidae	Agromyza oryzae	
"	Ephydridae	Hydrellia griseola	
"	"	H. philippina	
"	"	H. sasakii	
Coleoptera	Chrysomelidae	Dicladispa armigera	
		STEM AND ROOT FEEDERS	
Orthoptera	Gryllotalpidae	Gryllotalpa africana	
Hemiptera	Aphidae	Rhopalosiphum rufiabdominalis	
"	Psedococcidae	Geococcus oryzae	
Diptera	Chloropidae	Chlorops oryzae	
"	Cecidomyiidae	Orselolia oryzae	
"	Muscidae	Atherigona spp.	

b. Nilaparvata lugens, the brown planthopper

The brown planthopper is widely distributed throughout Asia and parts of Australia (Dyck and Thomas, 1978). It was first recorded attacking rice in Korea in A.D. 18 (Okamoto, 1924) and in Japan in 697 (Suenaga and Nakatsuka, 1958). However, it is only in recent years that it has become a major pest (Mochida et al. 1977; Dyck and Thomas, 1978). The taxonomy and biology are described by Mochida and Okada (1979). Eggs are inserted, normally as groups, in the lower part of the plant, and hatch in 7-11 days. The five nymphal stages are completed in a further 10-15 days and each female can lay from 1-200 eggs. Insects remain at the base of the plant, unless populations are high, when they move upwards. In southern Japan five generations can occur on a single rice crop (Mochida, 1964a).

N. lugens damages plants by withdrawing sap and in some poorly understood way interferes with the plants translocatory system, with the result that areas of the plants are killed outright. N. lugens is also a virus vector, transmitting the agent of grassy stunt disease.

The rapid development, high fecundity, ability to transmit viruses and destructive mode of feeding combine to make N. lugens perhaps the most serious pest in Asia.

6. Control

Control often relies on chemicals, including the environmentally undesirable organochlorine compounds, such as dieldrin and DDT. Insecticides are often not available in developing countries and personnel are not adequately trained in their use. Furthermore, the protective clothing - deemed necessary for personnel applying toxic insecticides in the developed world - is often unavailable, or proves too cumbersome and hot, so is disregarded.

In addition, N. lugens rapidly acquires resistance to chemicals.

Clearly, there is a need for safe control agents for rice pests and, to date, very little attention has been given to the possibilities of using bacterial, fungal and viral pathogens for pest control on rice.

SECTION III

FUNGI - CLASSIFICATION AND MORPHOLOGY

MATERIALS AND METHODS

1. Scanning Electron Microscopy

Deuteromycete fungi were grown on membranes, while Entomophthorales, which grew poorly on this substrate were grown on Sabourauds dextrose agar. Membranes were prepared from dialysis tubing, previously boiled for 1 hr. to remove impurities, autoclaved in double - strength Sabourauds liquid medium (60g l^{-1}) and dried in a laminar flow cabinet. Sections ($1cm^2$) of membrane were positioned in the centre of a microscope slide (7.5 x3.8 cm) with double-sided Sellotape. The membranes were inoculated with conidia of the desired fungus, using a camel-hair brush and the slide inverted over distilled water, held in a glass staining block (4 x4cm). A seal was effected between the slide and block with grease(Edwards High Vacuum Co.). When, after 48-72 h ; considerable growth and sporulation had occurred, the membrane, or fungus on agar, was placed over 2% OsO_4 solution, for a 24 h . period of fixation. The specimens were then air dried in a fume cupboard for 3-4 days, mounted on stubs, gold-coated and viewed in a scanning electron microscope (T-20, Jeol, Japan) at 20 Kv.

2. The Deuteromycetes

The Deuteromycetes comprise those fungi which have no known sexual stage, and as such, are a heterogenous group. The most important genera encountered in this study are briefly described and the important diagnostic features illustrated.

a. The genus Beauveria

The fungi now described as Beauveria were formerly referred to as Botrytis by European authors, e.g. Balsamo (1836); Saccardo (1892) and as Sporotrichum by American workers (In De Hoog (1972)). Later, these entomogenous fungi were transferred to the genus Beauveria (Vuillemin, 1912; Petch, 1924). Some time later, MacLeod (1954) considered that Beauveria contained only two species. However, recently several new species have been described (Hoog, 1972; Hoog and Rao, 1975; Samson and Evans, 1982).

Hoog (1972) describes the typical features of the genus Beauveria. Colonies appear lanose, powdery or funiculose, rarely forming synnemata, white, yellowish or pinkish. Aerial hyphae are hyaline, loose or occasionally fasciculate. Conidiogenous cells arise from short, slightly swollen stalk cells in dense clusters, scattered, or in whorls; they consist of a globose to fusiform base and a geniculate, denticulate rachis. Condia are unicellular, hyaline, thin-walled, globose to ellipsoidal.
A perfect, or sexual stage, of B. bassiana was recorded, but not named by Schaerffenberg (1955). B. bassiana and B. brongniartii are the most important species and can be distinguished fairly readily. B. bassiana has globose or sub-globose conidia, while those of B. brongniartii are ellipsoidal. A less reliable taxonomic character is the amount of clustering of the conidiogenous cells. The structure of these fungi is shown in Figs. 3.01 and 3.02.

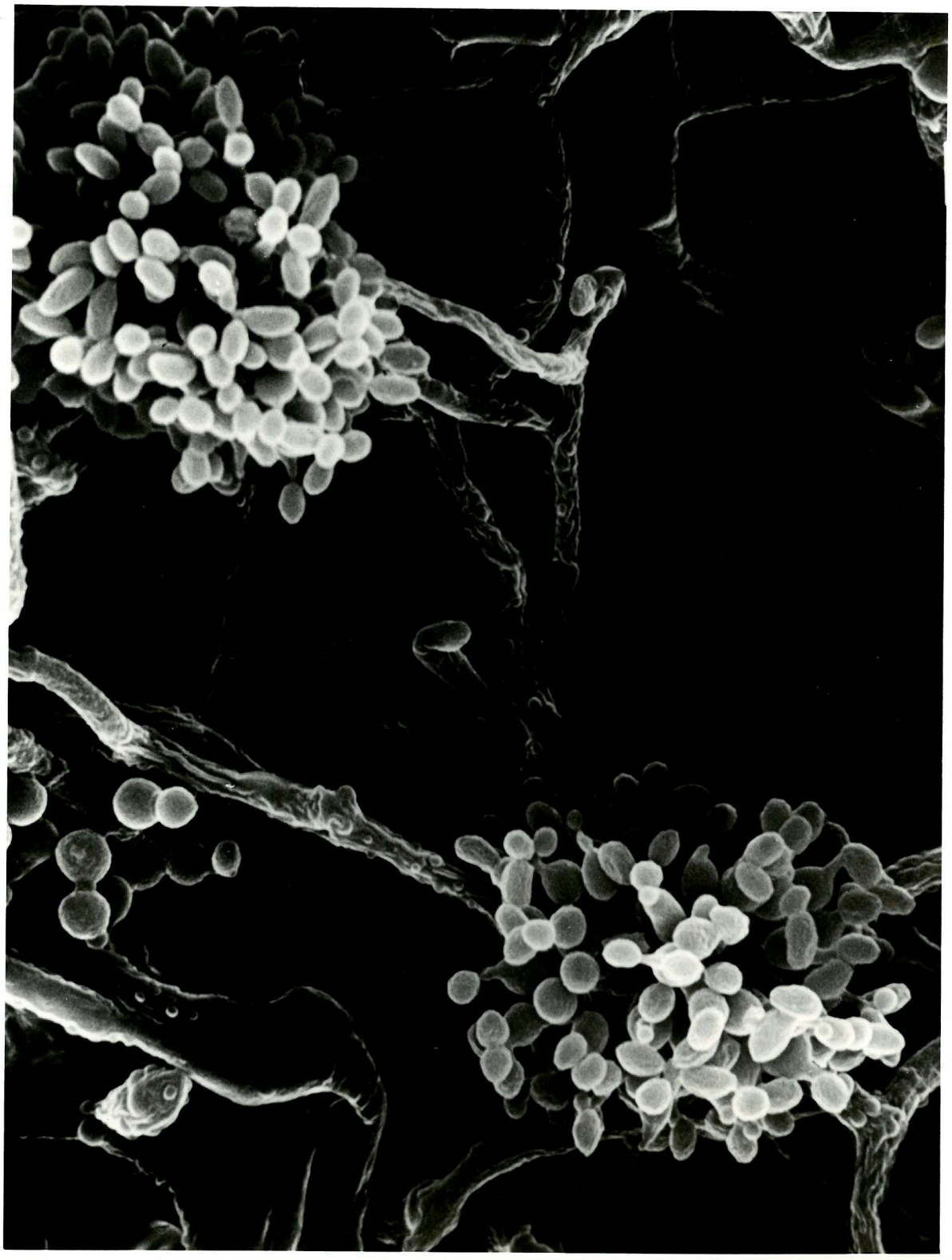


Fig. 3.01
Beauveria bassiana (63) showing clusters of globose to sub-globose conidiospores. x 2850.

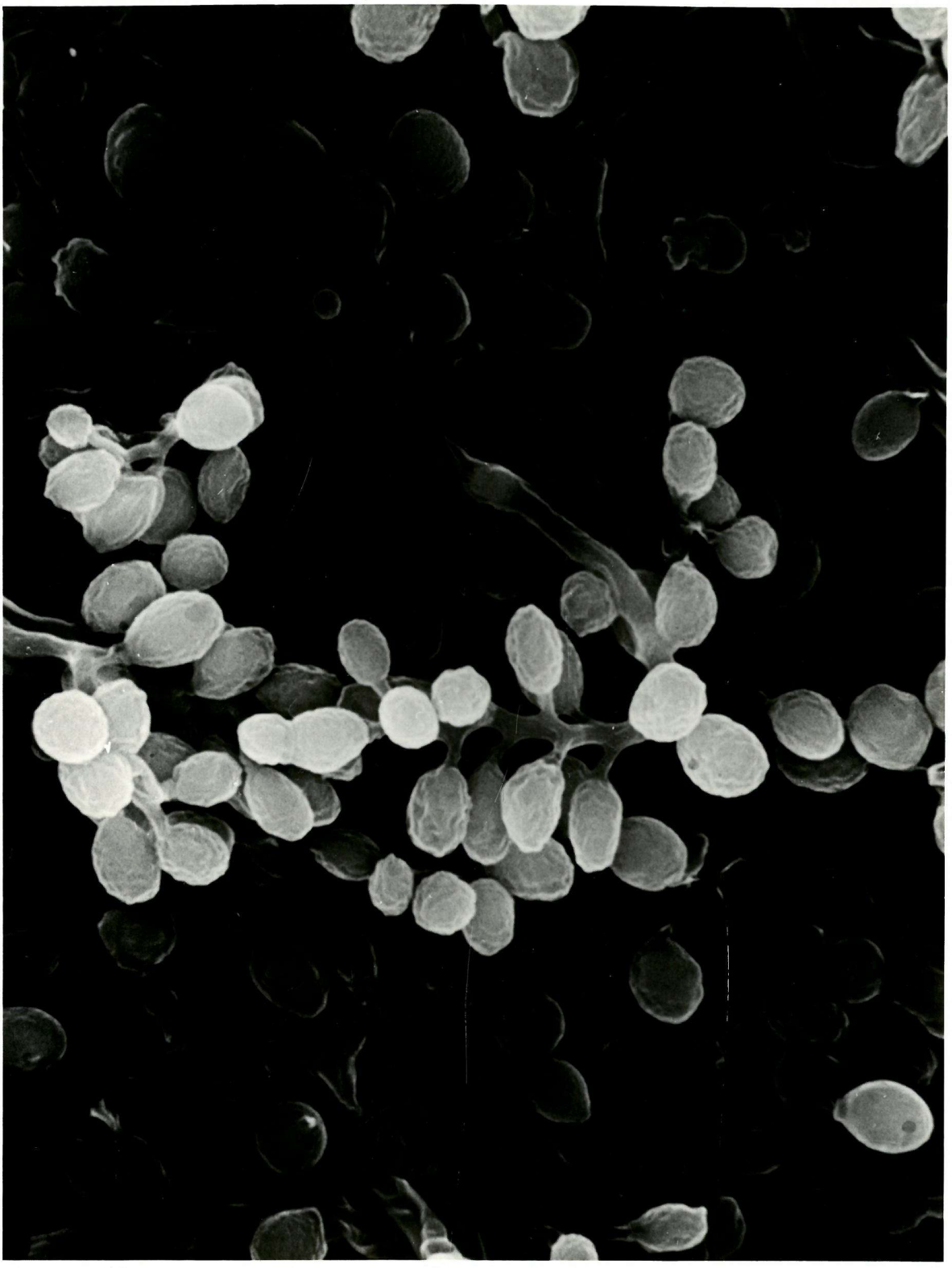


Fig. 3.02
Beauveria brongniartii (6), showing clusters of ellipsoidal conidiospores. x 4950.

b. The genus Metarhizium

The genus Metarhizium was first described by Sorokin (1883) though Metschnikoff (1879) recorded Entomophthora anisopliae four years earlier. Petch (1931) described Metarhizium album from Sri Lanka and later Metarhizium brunneum from the Philippines (Petch, 1934). However, these species are considered synonymous with Metarhizium anisopliae (Latch, 1965; Veen, 1968; Tulloch, 1976) and the genus Metarhizium includes only two species, M. anisopliae and M. flavoviride. These species differ in conidial morphology; cylindrical in M. anisopliae, elliptical in M. flavoviride. Brady (1979) describes M. anisopliae: "Colonies on potato dextrose agar have a white margin with clumps of conidiophores, which become coloured with the development of conidia, varying from buff to yellow-green or olivaceous or dark herbage green, occasionally pink. Spores appear in columns, often becoming confluent in the centre. Reverse are colourless or honey buff, sometimes pale luteous to citrine in the centre." The microscopic structure is illustrated in Figs. 3.03 and 3.04 and will not be detailed here. Conidial lengths of M. anisopliae vary (Johnston, 1915; Friederichs, 1920). The former author suggested M. anisopliae should be divided into formae major and minor. A recent review by Tulloch (1976) suggests the common short-spored form be designated var. anisopliae and reserves var. major for the long-spored type. This nomenclature is now accepted.

A perfect stage was recorded by Schaerffenberg (1959) as probably belonging to the Sphaeriales. However, this claim remains to be substantiated.

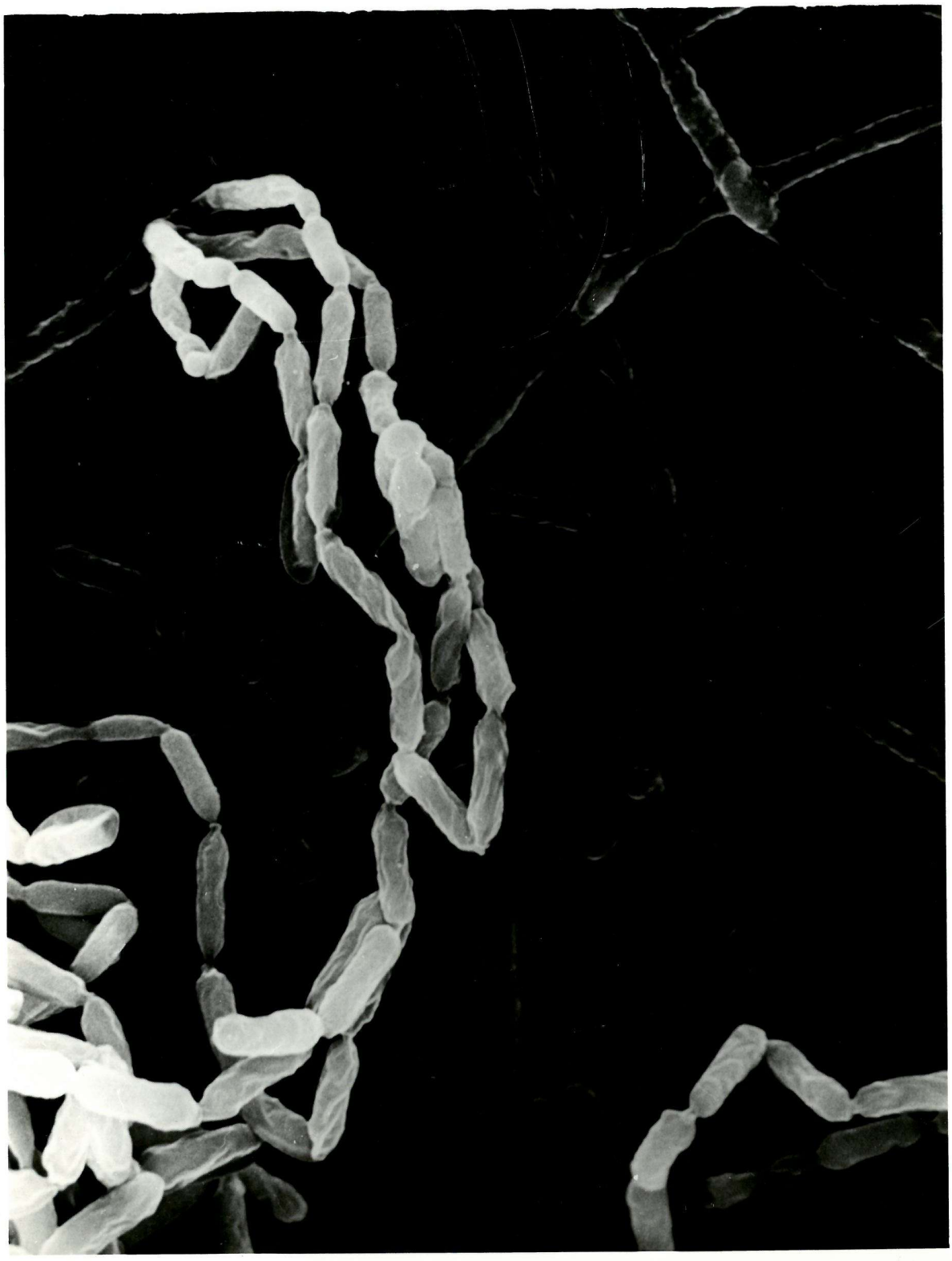


Fig. 3.03
Metarhizium anisopliae (ME2) showing chains of cylindrical conidospores. x 2850.

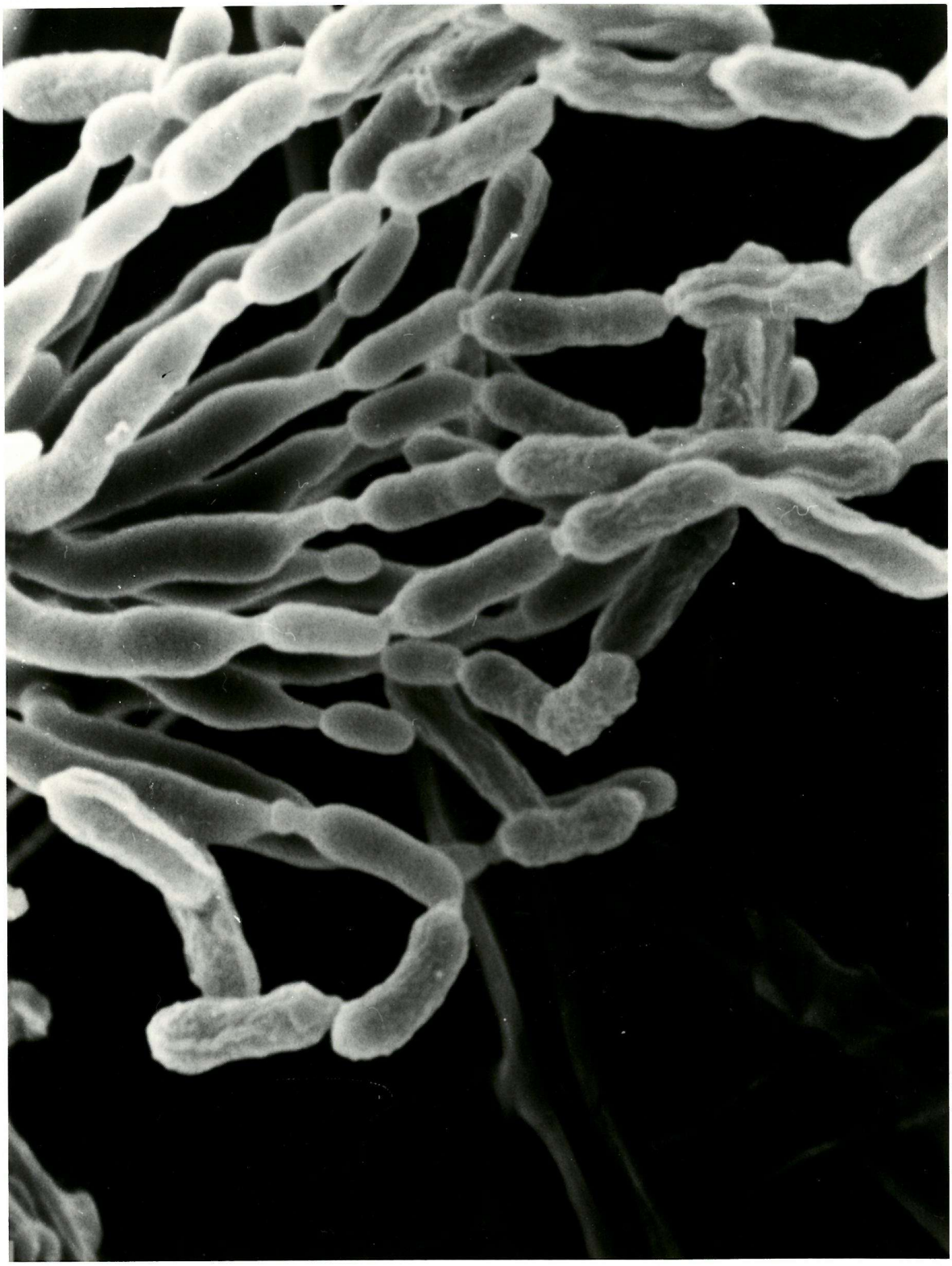


Fig. 3.04
Metarhizium anisopliae (ME2). x 7500.

c. The genus Paecilomyces

The genus Paecilomyces was created by Bainier (1907) who considered it was related to Penicillium. Brown and Smith (1957) monographed the genus and transferred some species of the genera Isaria and Spicaria to Paecilomyces. The genus Paecilomyces was broadened by Onions and Barron (1967) who included some monophialidic species, but Gams (1971) transferred most of these to the genus Acremonium.
The latest taxonomic revision was by Samson (1974) who monographed and re-defined the genus as "those species with verticillate conidiophores bearing divergent whorls of branches and phialides, which have a cylindrical or inflated basal portion and a long distinct neck and produce one celled hyaline conidia in basipetal chains". Samson (1974) proposed two sections; Paecilomyces and Isarioidea, the latter containing several entomogenous species. Several Paecilomyces genera have a known sexual stage. Stolk and Samson (1972) and Samson (1974) describe some members of the Ascomycete genera Byssochlamys, Talaromyces, and Thermoascus which have conidial states classified as Paecilomyces. More recently, Pacioni and Frizzi (1977) described P. farinosus as the conidial state of Cordyceps memorabilis. The morphology of P. fumosoroseus used in this study is shown in Fig. 3.05.

d. The genus Verticillium.

In 1839, Corda (In Gams, 1971) grouped all hyphomycetes with single-celled spore heads at the tip of simple phialides in the genus Cephalosporium. However, Gams (1971) has re-classified this group and placed many fungi, including the entomogenous Cephalosporium-like fungi, in a new section of Verticillium, sect. Prostrata. Members of the section characteristically have occasional mesotonous to acrotonous verticillate whorls of phialides and velvety-cotton, white or yellowish mycelium.
Within this section Prostrata, Gams included many fungi, formerly described as separate species, as Verticillium

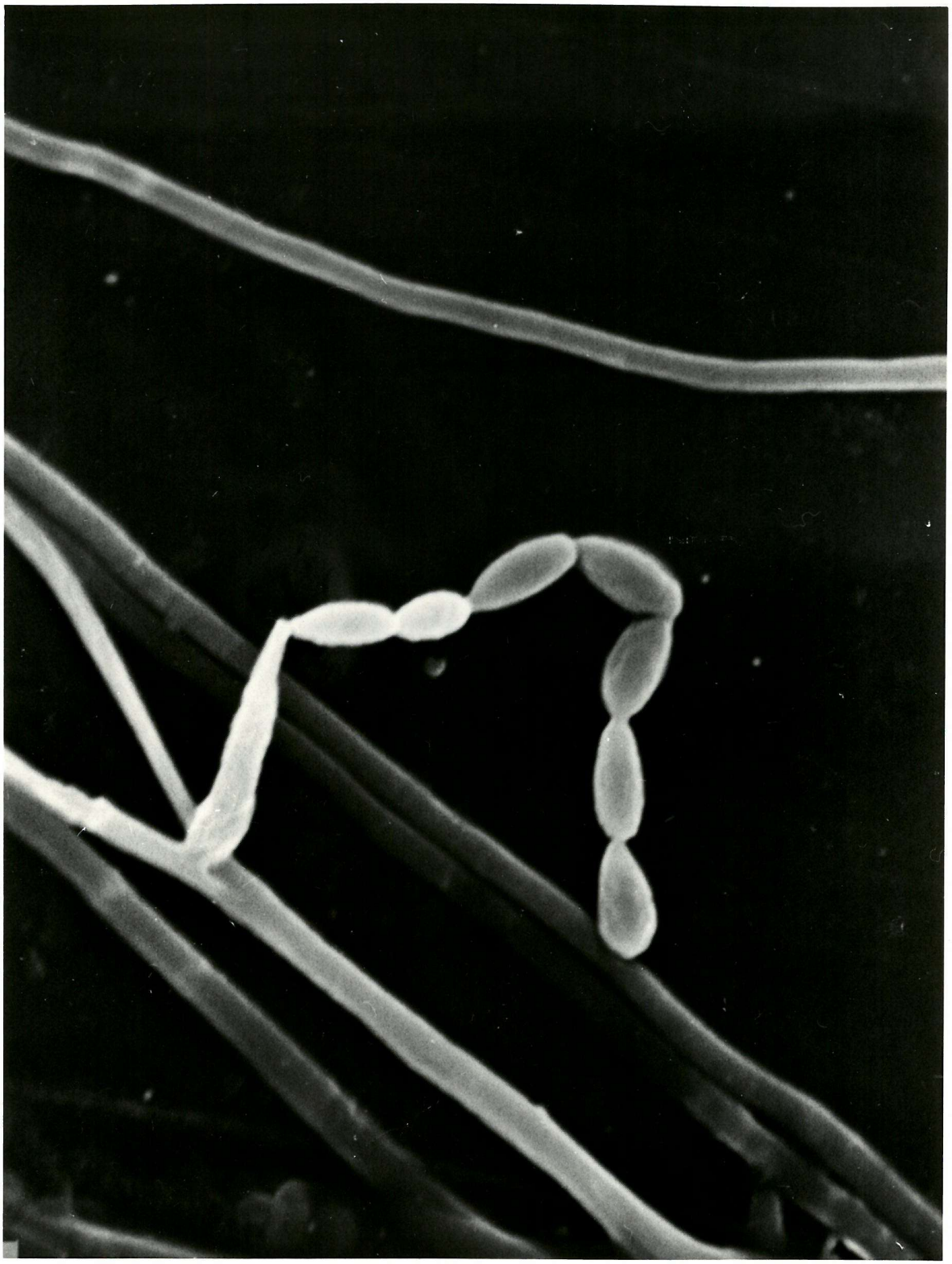


Fig. 3.05
Paecilomyces fumosoroseus (21) showing isolated conidiophore with chain of conidiospores.

lecanii. Balazy (1973) considered that Gams (1971) definition of V. lecanii was too wide and separated some species, eg. Cephalosporium eriophytis, from Gams' Verticillium lecanii complex, on the grounds of colony appearance and phialide length. Within Gams (1971) concept of V. lecanii, phialide length varies continuously, and Hall (1980) has found V. lecanii colony morphology to be extremely variable, particulary after continuous sub-culturing.

Considering the available data it appears that Gams (1971) concept of V. lecanii is valid, and all the strains used in this study conform to his description : "colonies on oatmeal and malt extract agars, white or pale yellow, cottony-velvety; underside colourless, but on malt extract agar light yellow to chrome yellow to ochre. Phialides awl-shaped, very variable in size, single or in small groups of verticillate whorls on hyphae of the aerial myceluim, occasionally with repeatedly branching conidiophores, which do not differ in structure from vegetative hyphae." The morphology of one strain of V. lecanii used in the present study (53-81) is shown in Fig. 3.06.

3. The order Entomophthorales.

The orders Entomophthorales, Mucorales, and Zoopagales belong to the class Zygomycetes and characteristically form zygospores, but lack flagellate spores. Members of the Entomophthorales are distinguished by the formation of forcibly discharged asexual spores, which are often termed conidia, but are probably sporangia which have evolved to function as single conidia (Benjamin, 1979). Most entomophthoralean fungi, some 150 spp., are parasites of insects and mites, while a few are parasitic on ferns, algae, tardigrades and nematodes.

Nowakowski (1883) proposed the family Entomophthoreae and divided it into 5 genera, Empusa, Lamia, Entomophthora, Tarichium and Completoria. A more comprehensive classification by Thaxter (1888) placed all insect pathogenic species in the genus Empusa.

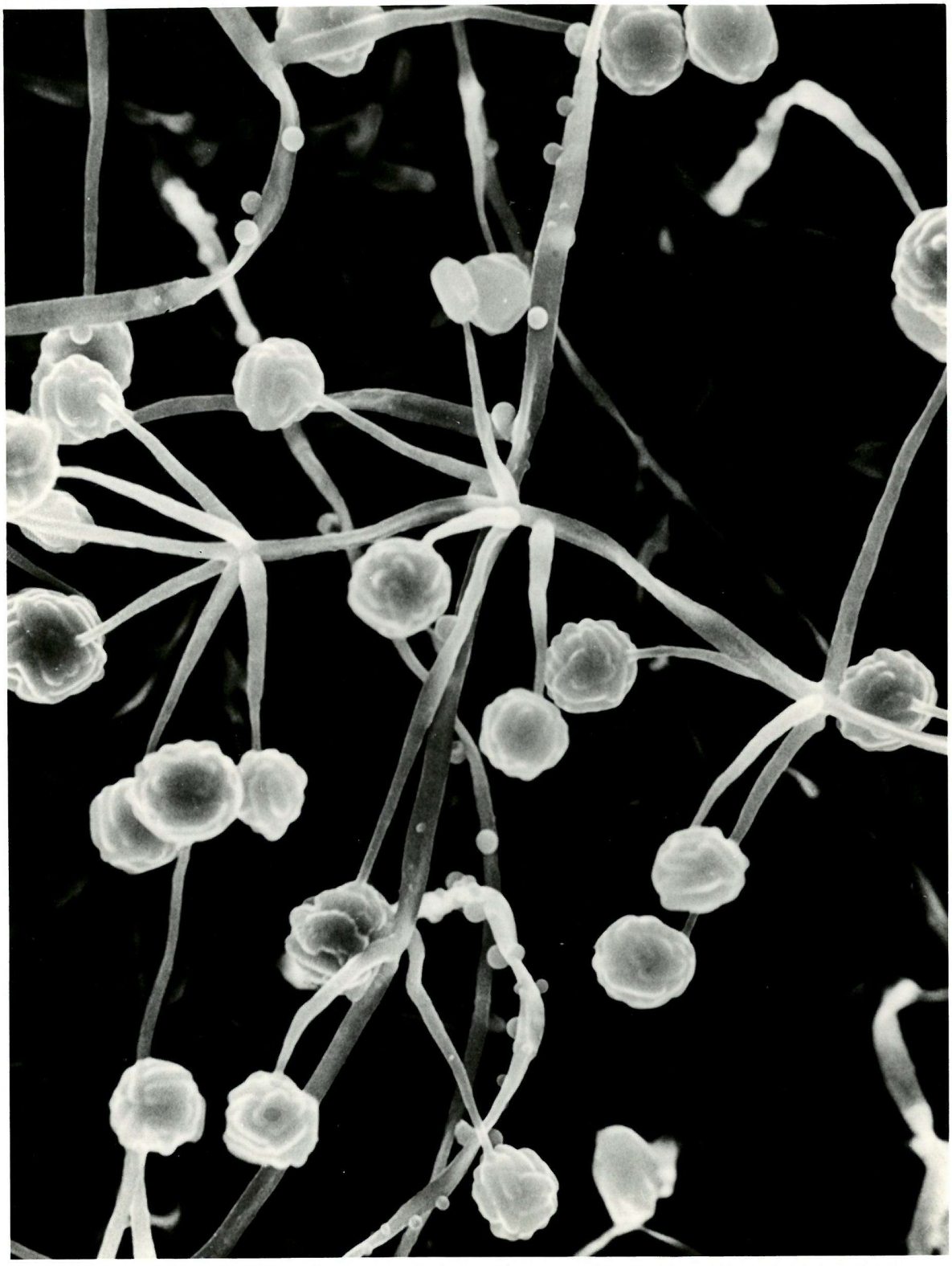


Fig. 3.06

Verticillium lecanii (53-81) showing whorls of conidiospores in slime heads. x 2100.

Later, Lakon (1915; 1919) re-erected the genus Entomophthora. This system remained largely unchanged until a new classification was developed by Batko (1964 a,b,c,d,e; 1966 a, b; 1974: Batko and Weiser, 196).
In this series of papers, Batko reviewed the genus Entomophthora and concluded that the variation was too great for all types to be included in one genus. Thus, he proposed several new genera. In recent years the classification of the Entomophthorales has been the subject of much debate. Remaudière and Hennebert (1980) and Remaudière and Keller (1980) published papers proposing a classification system, based on that of Batko, but differing in several important respects. In these papers they proposed six genera; Entomophthora, Conidiobolus, Neozygites, Massospora, Zoophthora, Erynia and Tarichium. The latter genus is regarded as a provisional form-genus, for types with unrecognized asexual stages, and as such, was not accepted on an equal basis with the other genera. However, the classification of Remaudière has been criticised by several workers. Humber (1981a) criticised the grouping because in some cases, it did nothing to reduce the biological heterogeneity of the reconized genera, while Humber (1981a, 1981b) and Ben-Ze'ev and Kenneth (1981, 1982) disagreed with many of the taxonomic criteria used. This controversy has triggered alternative classifications, the latest by Ben-Ze'ev and Kenneth (1982). These later classifications seek to establish phylogenetic relationships between members of the Entomophthorales and utilize such criteria as the number of nuclei contained in conidia. The classification of Ben-Ze'ev and Kenneth (1982) proposes the division of the Entomophthorales into the following genera; Conidiobolus Entomophaga, Entomophthora, Massospora, Triplosporium, Erynia, Erynia sensu. lato. Strongwellsea, Meristacrum, Ballocephala, Macrobiotophthora, Zygnemomyces, Tarichium and Entomophthora (nomina provisoria). It is likely that the latest classification will be accepted, and indeed from a chronological viewpoint, must be regarded as correct. However, it is likely that the classification of the Entomophthorales will continue to be the subject of much discussion.

In this study two species of Entomophthorales were encountered, Neozygites parvispora and Conidiobolus coronatus and the primary features of these fungi will be illustrated.

a. Neozygites parvispora.

1. Humber et al. (1981) and Ben-Ze'ev and Kenneth (1982) prefer the generic name Triplosporium. However the genus Neozygites is correct on nomenclatural priority Gustafsson (1965). N. parvispora, was originally described as Entomophthora parvispora, (Carl, 1975; Mac Leod et al., 1976) as a pathogen of Thrips spp. in Continental Europe. The characteristics of Neozygites are given by Ben-Ze'ev and Kenneth (1982) as: "nuclei entomophthoroid; conidia unitunicate, regularly tetranucleate; conidiophores unicellular, simple; conidial discharge active, by rounding-off". Thrips tabaci infected with N. parvispora is illustrated in Figs. 3.07 - 3.10.

b. Conidiobolus coronatus.

This species was first described by Costantin (1897) as Boudierella coronata, but later was referred to as Delacroixia coronata and Entomophthora coronata King (1976). It was renamed as Conidiobolus coronatus by Batko (1964 b) and independently by Srinivasan and Thirumalachar (1964). Ben-Ze'ev and Kenneth (1982) described the characteristic features of Conidiobolus as: "nuclei ancylistoid; conidia unitunicate, multinucleate; conidiophores unicellular, simple, rarely unicellular slightly branched; conidial discharge active by rounding-off". They retained the name C. coronatus but included it in the subgenus Delacroixia, characterised by the production of micro-conidia by multiplicative resporulation. King (1976) distingushed C. coronatus from other members of the genus by the presence of villose resting spores. The strain encountered in this study was isolated from Nilaparvata lugens and tentatively identified as C. coronatus. This identification was confirmed by Papierok (pers. comm.). The stucture is illustrated in Fig. 3.11.

Fig. 3.07
Neozygites parvispora conidiospores emerging through the cuticle of a Thrips tabaci adult. x 135.

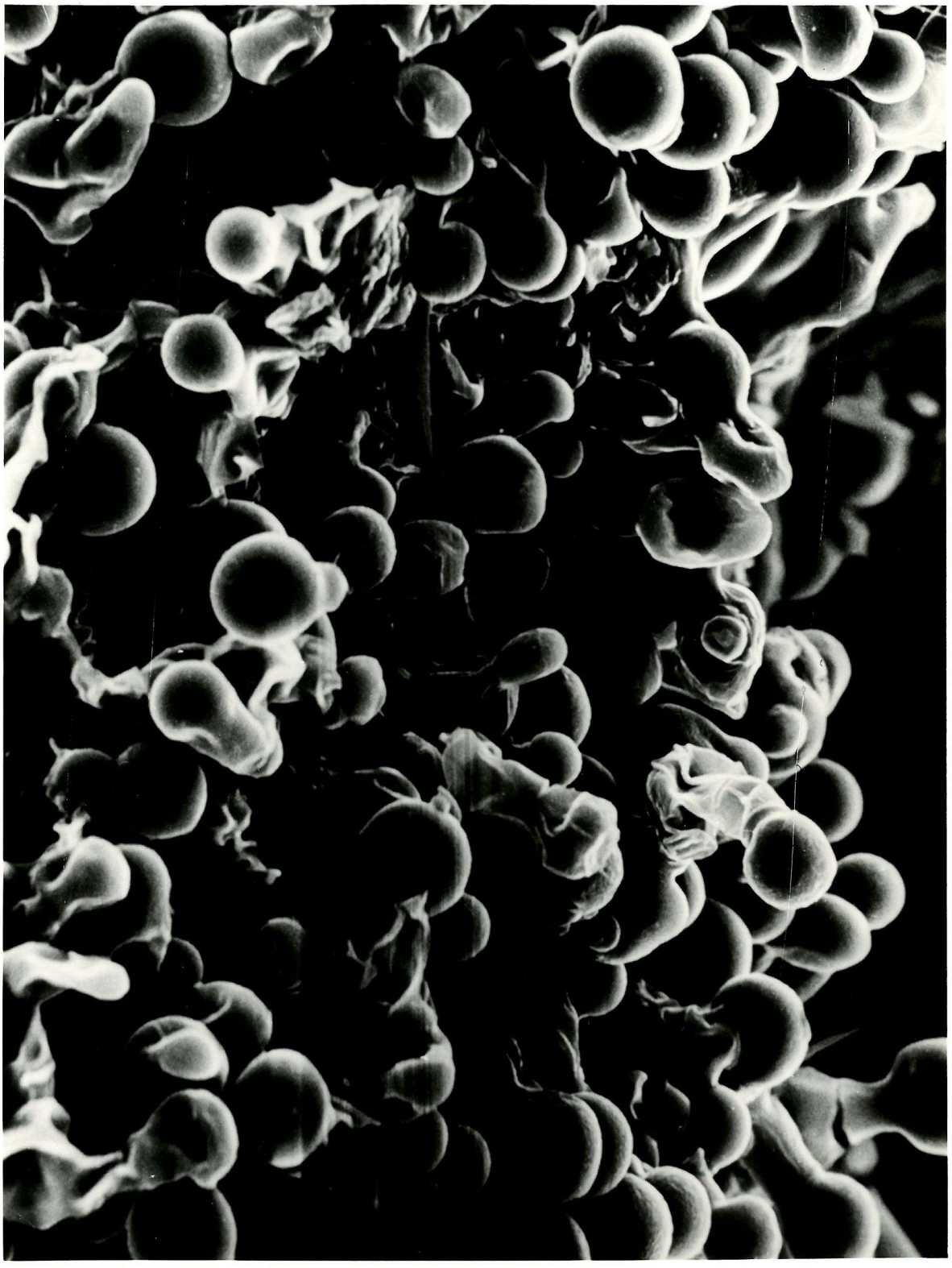


Fig. 3.08
Neozygites parvispora conidiospores.x 1350.

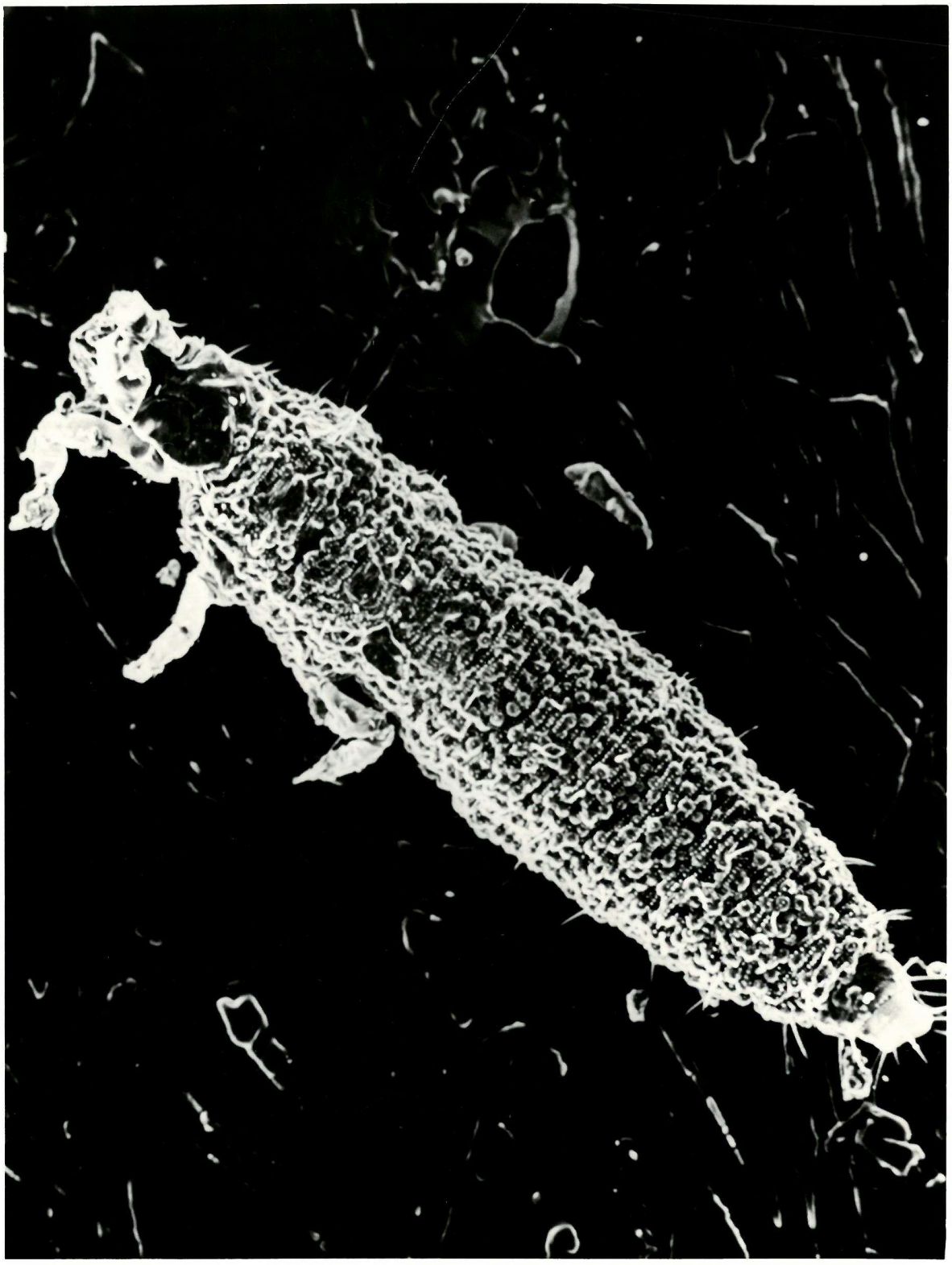


Fig. 3.09
Neozygites parvispora resting spores, contained inside a stage II Thrips tabaci larva. x 135.

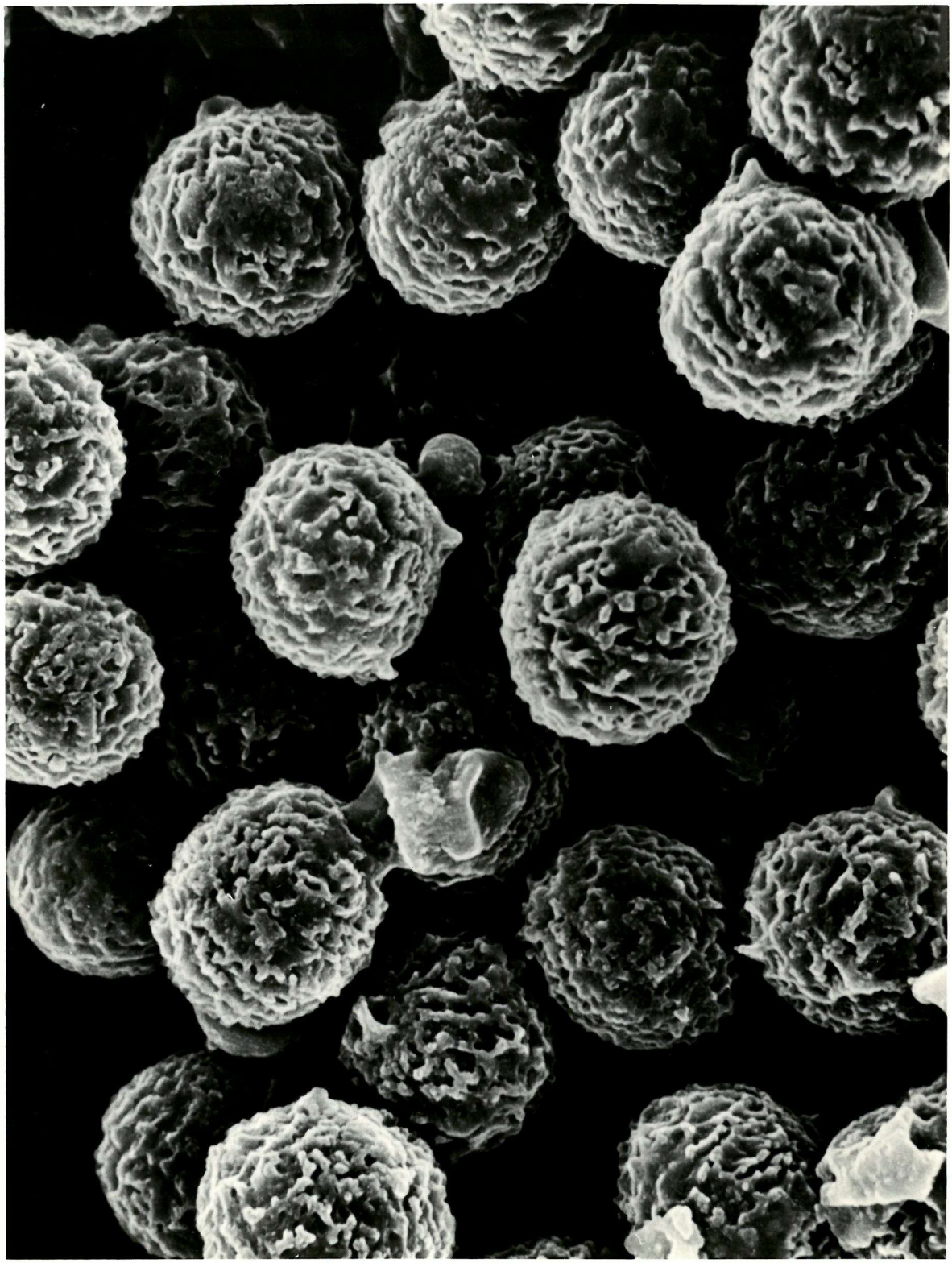


Fig. 3.10
Neozygites parvispora resting spores. x 2100.

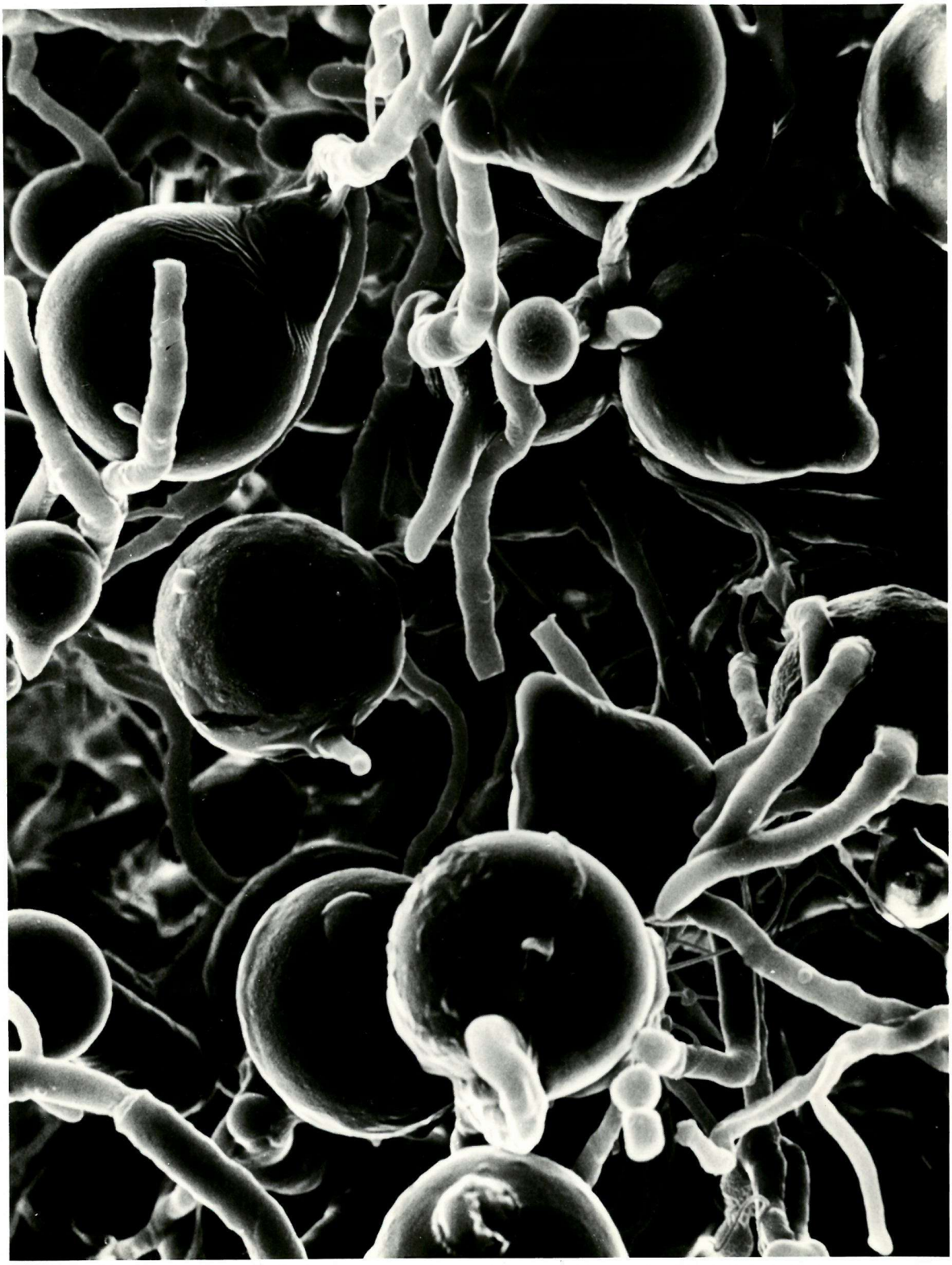


Fig. 3.11
Conidiobolus coronatus conidiospores growing on Sabouraud dextrose agar. x 1050.

SECTION IV

INSECTS- REARING, MAINTENANCE AND BIOLOGY

1. General

Insects were obtained from various sources (Table 4.01). Food plants bearing insects were positioned on galvanised iron trays (85 x 52 cm) and covered by perspex cages (80 x 53 x 48 cm). Each cage had a front opening (38 x 30 cm) closed with a 'Velcro' edged cotton flap, or with an aluminium plate, sealed with foam rubber insulation strip and secured to the cage at each corner. The plates had a central portion of 'Tigan' nylon mesh (21 cm^2). The rear of each cage had two holes (10 cm diam.), covered with fine cotton cloth, adpressed to which were two 4.5 cm diam plastic pipes, carrying an air supply, provided by an electric fan. This created a small positive pressure within the cages, which reduced humidity and helped to prevent unwanted insects from contaminating cultures.

Plants were grown in polyethylene pots (max diam 12.7 cm) placed in dishes (11.0 cm diam.) which were filled with water daily. At frequent intervals, dead plants were removed from cultures and cages were cleaned with detergent solution (Teepol).

Insects were captured with aspirators. A small model, made from a ball-point pen top and utilizing size 00 gelatin capsules (Farillon Ltd.) was used for small insects and mites, while a larger model fitted with 'Universal' bottles was used for brown planthoppers. When necessary, carbon dioxide was introduced into cages for one minute to anaesthetise insects. Aphids, anaesthetised, or immature insects, were handled individually with a camel-hair brush (size 00).

2. Production of Plants

All plants were grown in glasshouses.

a. Cucumber

Cucumber plants, cultivar Telegraph, were grown at 27°C until after cotylendon emergence, then at 21-27°C. During the winter months, supplementary lighting was provided by mercury vapour lamps (400 W. 16 h. daylength). Plants

Table 4.01

Source of the insects used in this study.

Insect	Source	Host
Hauptidia maroccana	G.C.R.I.	Cucumber
Thrips tabaci	G.C.R.I.	Cucumber
Tetranychus urticae	G.C.R.I.	French Beans
Phytoseilius persimilis	G.C.R.I.	French Beans
Aphis gossypii	G.C.R.I.	Cucumber
Macrosiphoniella sanborni	G.C.R.I.	Chrysanthemum
Nilaparvata lugens	Dr. E Harris C.O.P.R. Porton Down Salisbury WILTS.	Rice

G.C.R.I. - Glasshouse Crops Research Institute

were used for insect rearing when they had two or three true leaves (three - five weeks old).

b. Chrysanthemum

Chrysanthemum plants, cultivar Deep Tuneful, were grown from cuttings from a single source. Temperature was maintained as constant as possible and light levels increased where necessary with artificial light (mercury vapour lamps, 400 W 16 hour photoperiod).

c. Rice

Seedlings were grown in vermiculite in seed trays (32 x 22 x 5 cm) at a minimum of 21°C. After 8 days, when about 3 cm high, they were transplanted to 12.7 cm polyethylene pots (4 plants pot^{-1}) containing compost detailed in Table 4.02. Plants were irrigated frequently either with de-ionised water, or tap water acidified with concentrated sulphuric acid (0.11 ml $litre^{-1}$). The addition of acid was necessary to lower the pH, as higher pH's caused chlorosis This was probably because iron was unavailable, or not absorbed by roots, at high pH; foliar sprays of ferrous sulphate (0.5% w/v) reduced chlorosis and improved growth. From one week after potting, plants were watered with 200 ml of a solution containing 160 ppm N, 40 ppm P, 160 ppm K, 20 ppm Mg and 4 ppm Fe obtained by dilution of a stock solution (Table 4.03).After 6-10 weeks, pots were submerged in a nutrient solution containing 80 ppm N, 20 ppm P, 80 ppm K, 10 ppm Mg, 4 ppm Fe, contained in 18 cm diameter pots and the minimum temperature maintained at 27°C.

3. Insect Rearing

a. Hauptidia maroccana, the Glasshouse Leafhopper

Insects were reared on four cucumber plants per perspex cage, with approximately 200 adult leafhoppers being allowed to oviposit for 4 days. Insects were removed by vigorous shaking and plants transferred to a fresh cage, at 24-27°C and a 16 hour photoperiod, provided by fluorescent tubes (6,500 lux). Under these conditions, adult insects were produced in 28 days.

Table 4.02

Composition of Compost Used for Rice Cultivation

Ingredient	Rate ($bushel^{-1}$)
Irish sphagnum moss peat	0.037 m^3
Ammonium nitrate	13 g
Potassium nitrate	28 g
Superphosphate of lime (18.5% P_2O_5)	55 g
Dolomitic limestone	50 g
Frit WM 255	11 g
Ferrous sulphate	1 g
pH	approx. 4.8

Table 4.03

Composition of Rice Liquid Feed

Salt	Concentration ($g l^{-1}$)
Potassium dihydrogen orthophosphate	17.6
Potassium nitrate	28.7
Ammonium nitrate	35.6
Magnesium sulphate (Hydrated)	20.3
Ferrous sulphate (Hydrated)[a]	2.0

[a] made as separate solution

b. Thrips tabaci, the Onion Thrips

After trials with primula, cotton and cucumber plants, the latter were selected. Plants were stood on a 6 cm layer of peat and sand which provided suitable pupation sites. This substrate was kept slightly moist, as thrips pupae are prone to desiccation (Lewis, 1973). Plants were maintained at 25-29°C, in a 16 h photoperiod. Adult insects were harvested by shaking leaves over a white surface and aspirating the easily - visible thrips into gelatin capsules.

c. Tetranychus urticae, the Glasshouse Red Spider Mite

Mites were reared on french bean plants, cultivar Prince, in a small glasshouse, at 21°C and were collected in the same manner as thrips (IV, 3, b).

d. Phytoseilius persimilis, the Predatory Mite

Predators were reared on french bean plants infested with Tetranychus urticae produced as described earlier (IV, 3,c).

e. Aphis gossypii, the Cotton Aphid

This aphid was reared on cucumber at 24-7°C and a 16 h photoperiod. Aphids were encouraged to withdraw their stylets by gently touching their rear legs with a camel-hair brush, and transferred to 9 cm Petri dishes until required.

f. Macrosiphoniella sanborni, the Chrysanthemum Aphid

This aphid was reared on the susceptible chrysanthemum cultivar, Deep Tuneful, in similar conditions to A. gossypii (Wyatt, 1965; Hall, 1977).

g. Nilaparvata lugens, the Brown Planthopper

The susceptible rice cultivar TN1 was selected for rearing Nilaparvata lugens. Plants were trimmed to a height of 30 cm and the compost surface, together with the leaf bases, covered with aluminium foil to exclude the fungus Conidiobolus coronatus (III, 3, b; IV, 4, g, i). Plants, in perspex cages at 27-30°C and 16 hour photoperiod, were

exposed to about 100 adult planthoppers for 2 days to allow oviposition. Insects were removed by shaking plants and aspiration. When necessary, plants were replaced to provide a continuous food source.

4. Maintenance of Insects for Bioassay

a. Hauptidia maroccana

i) Early Methods

Insects were maintained on cucumber leaf discs supported on moist filter paper using the apparatus in Fig. 4.01. This method was unsatisfactory for the following reasons. Insects were kept in groups, which permitted disease transfer; observation was difficult; and most seriously, some insects became trapped in moisture and died. The modified Munger cells, used by Hall (1977) for aphid maintenance, were also unsuitable, as leafhoppers could not be seen easily without dismantling the apparatus, which resulted in many insects escaping.

ii) The Agar Method

Aliquots (2 ml) of agar (Technical Agar, No. 3, Oxoid Ltd. 15 g lit^{-1}) were placed in each compartment of a ten cm^2 plastic dish (Sterlin Ltd) and allowed to solidify in a laminar flow cabinet. Cucumber leaf discs (1.6 cm diam.), excised with a cork borer, were placed one compartment^{-1} on the solidified agar and the dish covered with 'Clingfilm' (Payne Scientific Co. Ltd). Dishes could be kept at 20°C for 2-3 days without leaf deterioration. Before use, a small hole was made in the 'Clingfilm' above each disc, insects placed singly on the leaf portions and the dish covered with 'Clingfilm'. A small hole was made above each disc with a mounted needle, which reduced condensation and allowed gas exchange. Occasionally, when dishes were incubated at 20°C, condensation occurred and made it difficult to see insects, so moisture was removed by placing dishes in a laminar flow cabinet for 20-30 min. A 25 - compartment dish used for maintaining insects is shown in Fig. 4.02.

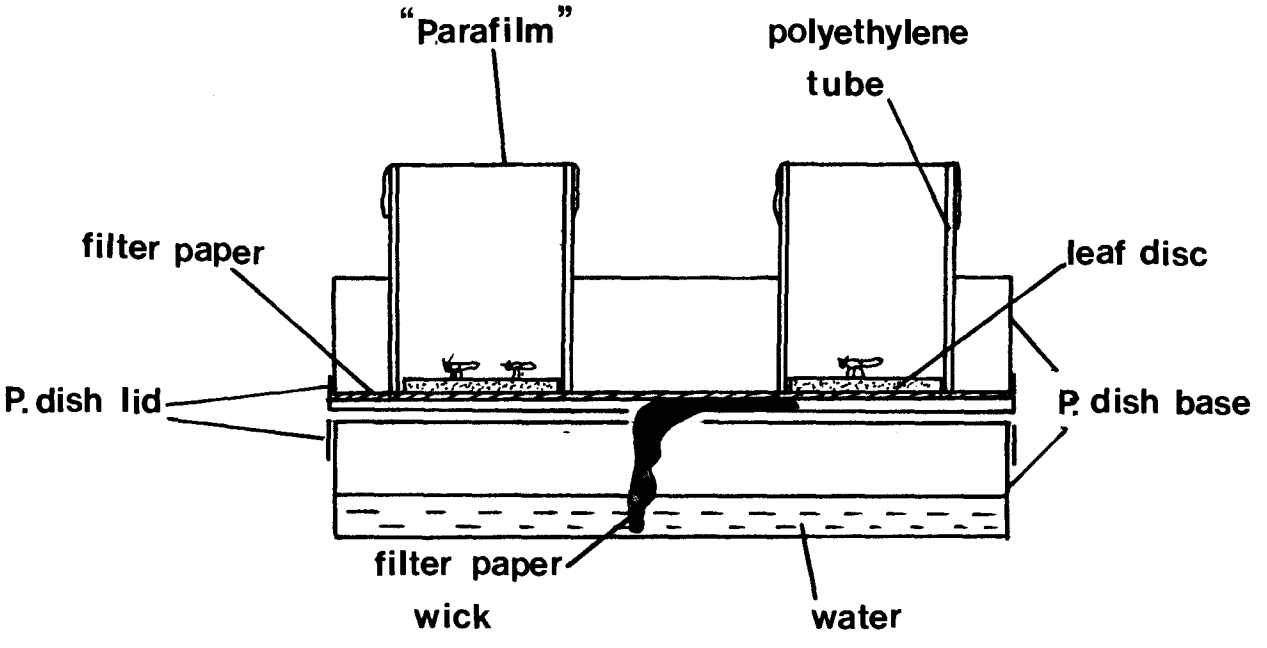


Fig. 4.01
Apparatus used in an attempt to maintain adult Hauptidia maroccana on leaf discs for bioassay.

Fig. 4.02
A square plastic dish containing agar and supporting cucumber leaf discs to provide a food source for insects, which were contained with 'Clingfilm'.x1

b. Thrips tabaci

i) Early Method

This method used polethylene Enzyme Linked Immunosorbent Assay (ELISA) trays to contain agar, leaf discs and insects. Molten agar (0.1 ml, 15 g l^{-1}) was placed in each cell and allowed to solidify, when cucumber leaf discs (0.6 cm diam) were placed, lower surface uppermost, on the agar surface. Thrips were placed individually on the discs and the plate covered with 'Clingfilm'. This method was discarded in favour of the method used for H. maroccana (IV, 4, a, ii) except holes were not made in the 'Clingfilm' as they enabled insects to escape.

c. Tetranychus urticae, Phytoseilius persimilis, Aphis gossypii and Macrosiphoniella sanborni.

The red spider and predatory mites and cucumber aphids were maintained on cucumber leaf discs on agar as described for T. tabaci (IV, 4, b, ii). For the predatory mite, at least three red spider mites were placed on each disc, two days before the predators, to provide a food source. The chrysanthemum aphid was maintained on chrysanthemum leaf discs, cultivar Deep Tuneful.

d. Nilaparvata lugens

i) Early Methods

Planthoppers were kept on rice leaf stems (1 cm high) supported in agar in Petri dishes (9 cm) or 25 - compartment, square plates (10 cm^2). This method proved unsatisfactory for adult insects as mortality levels were high and reached 11-40 percent in 8 days (Fig. 4.06). In contrast, nymphs survived well on rice stems, so this method was used to maintain immature insects both for assays and development studies.

ii) Whole Plant Method

Rice plants, grown individually in polyethylene pots (max. diam 3.6 cm) were about 20 cm high after 6-8 weeks. Insects were then placed on plants, trimmed to a height of 10 cm and stripped of dead leaves and contained in clear plastic propagators (Stewart Plastics Ltd.) (Fig. 4.03). Deaths

Fig. 4.03
Showing clear plastic propagators containing adult Nilaparvata lugens on rice plants.

using this method reached 50% in 6 days (Fig. 4.07), with about 20% covered with the fungus Conidiobolus coronatus. Some isolates of this fungus grow saprophytically in soil, while others are pathogens of various insects and mammals (King, 1976). Papierok (pers. comm.) showed the strain isolated from N. lugens was pathogenic to laboratory - reared aphids. Thus, it was assumed that C. coronatus was, at least in part, responsible for the observed mortality of N. lugens. To isolate planthoppers from C. coronatus, the compost of rearing plants was covered with aluminium foil (IV, 3, g) and the top 2 cm layer of compost of small plants, used for maintenance, replaced with clean peat/ sand. These precautions proved successful and mortality of untreated insects was much reduced (Fig. 4.07).

5. Biology

a. Hauptidia maroccana

i) Distribution on a Tomato Nursery

An infestation of H. maroccana on tomato, cultivar Sonatine, was studied on a commercial nursery. The population was 'mapped', over a 0·4 ha. block, by counting numbers of leafhoppers per leaflet, on every fifth plant on every fourth row.

ii) Occurrence Outside the Glasshouse

All plants immediately outside the glasshouse were examined for H. maroccana which could have been the source of the infestation.

iii) Life Cycle

Approximately 200 mature, adult leafhoppers were allowed to oviposit on three cucumber plants (21 days old) for 24hours. Leaf discs (1.6 cm diam)were excised and placed on agar in square plastic dishes. One dish was incubated at 20, 25 and 28^{o}C and a constant photoperiod of 16 hours. Insects were transferred to fresh leaf discs, immediately after hatching, and again after 10 days. The development stage of each insect was recorded daily.

b. Thrips tabaci

i) Life Cycle

Adult thrips were allowed to oviposit for 24 hours on cucumber plants, when excised leaf discs (1.6 cm diam) were maintained on agar at 20 or 25°C. Leaf discs were changed when necessary and development recorded daily.

c. Nilaparvata lugens

i) Life Cycle

About 100 adult females were allowed to oviposit on a rice plant for 24 hours, when they were removed and the plant incubated at 25°C. After emergence, nymphs were individually placed on one cm rice stem segments, positioned vertically in agar, in 10 cm^2 compartmentalised plastic dishes. The development of the planthoppers was recorded daily and rice stems replaced every five days.

RESULTS

6. Insect Maintenance

a. Hauptidia maroccana

Adult H. maroccana survived well on cucumber leaf discs supported on agar and mortality was below 10% after 12 days at 20 or 25°C (Fig. 4.04). The method had several advantages over other methods (IV, 4, a, i; Hall, 1977). Insects were readily observed, could be removed individually, handled without resort to anaesthesia and were maintained separately to prevent disease transmission.

b. Thrips tabaci

i) Early Method

Over half the adults died during 6 days incubation at 25°C (Fig. 4.05). Some 15% of the dead insects showed signs of Verticillium lecanii infection, though whether the fungus was growing parasitically or saprophytically was unknown. Subsequently, thrips were produced under drier conditions; the peat/sand under the cucumber plants was moistened only weekly. Another cause of death may have been insufficient food. The leaf discs (0.6 cm diam)

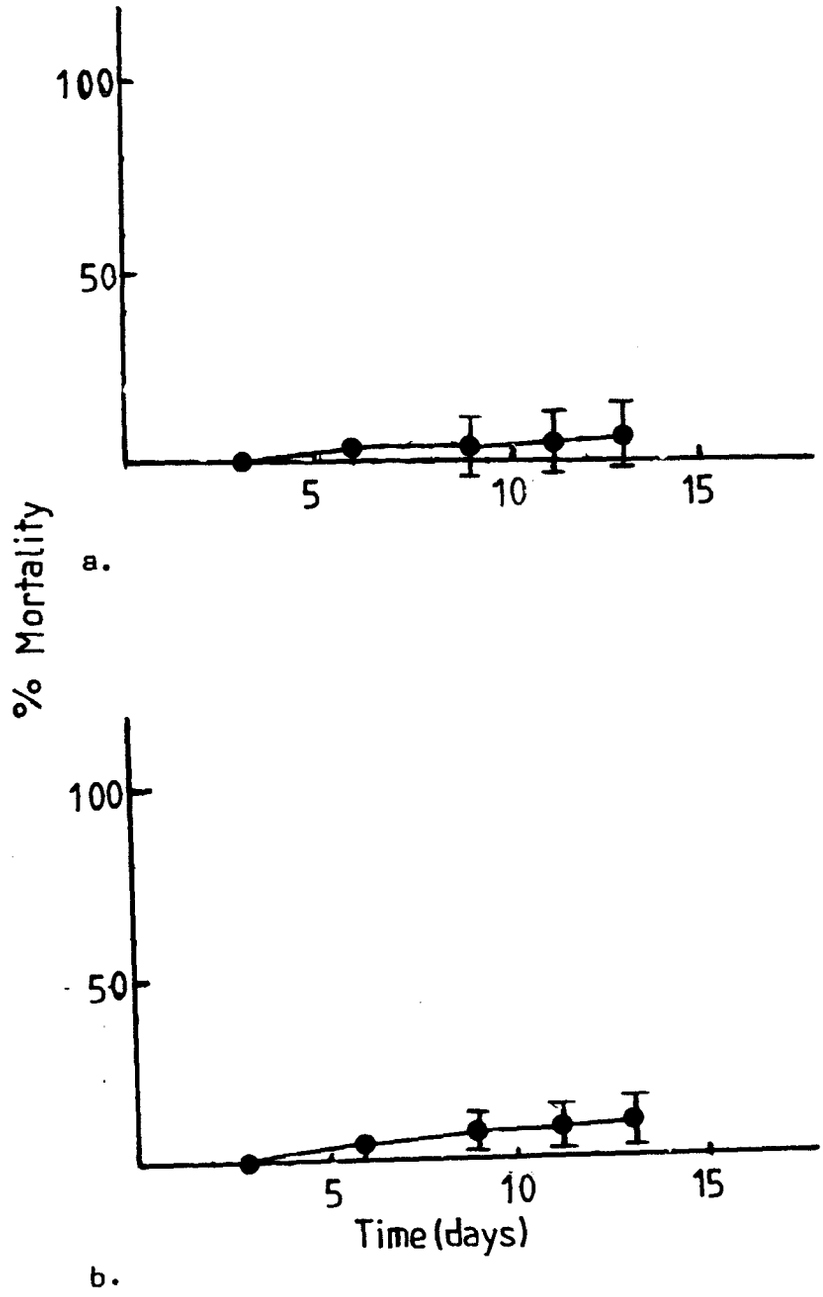


Fig. 4.04
Survival of adult Hauptidia maroccana on excised cucumber leaf discs, maintained on agar, at a. 20 and b. 25C. (Points are means of three replicates of 20 insects with 95% confidence limits (I)).

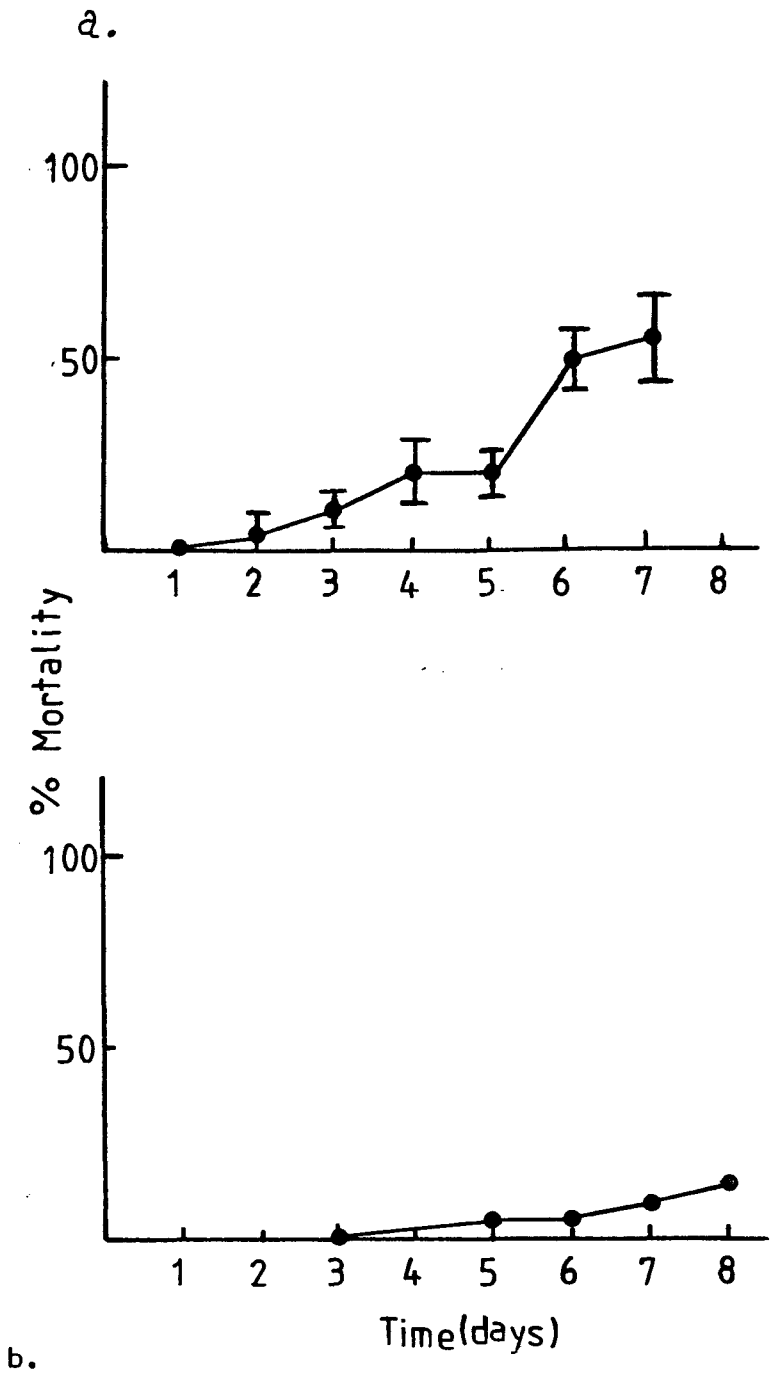


Fig. 4.05
Mortality of adult Thrips tabaci on cucumber leaf discs maintained on agar. a. 0.6 cm discs at 25°C; b. 1.6 cm discs at 20°C. (a. points are means of three replicates of 20 insects with 95% confidence limits, (I) b.unreplicated, 25 insects).

were small and after six days exposure to thrips, were in poor condition . This method was disgarded in favour of the agar method. (Fig. 4.02)

ii) The Agar Method

Survival of adult Thrips tabaci was satisfactory on 1.6 cm leaf discs and mortality never exceeded 10% after 7 days incubation at 20°C (Fig. 4.05). Occasionally, dead insects were found bearing V. lecanii, but the level was below 5%. Thrips were sometimes difficult to observe as they settled underneath leaf discs. However, by holding dishes over a strong light (60 W) thrips could be seen, and if alive, quickly moved in response to the light and heat. Despite careful sealing of the dish with 'Clingfilm', insects moved between leaf discs and thus cross contamination between thrips could not be eliminated.

c. Tetranychus urticae, and Macrosiphoniella sanborni

Red spider mites and chrysanthemum aphids survived well on leaf discs maintained on agar. Mortality rarely exceeded 5 % after 6 days incubation at 20°C.

d. Phytoseilius persimilis

Predatory mites survived well and 86% were alive after 5 days. However, by this time mites had consumed all the T. urticae provided as a food source, and survival over a longer period requires further study.

e. Nilaparvata lugens

Survial of adult planthoppers on excised rice stems (1 cm) supported in agar was poor, mortality reaching 40% in eight days (Fig. 4.06). In contrast, planthoppers contained on whole rice plants with propagators, survived well provided precautions were taken to eliminate the fungus Conidiobolus coronatus (IV, 3, g; 4, g) (Fig. 4.07).

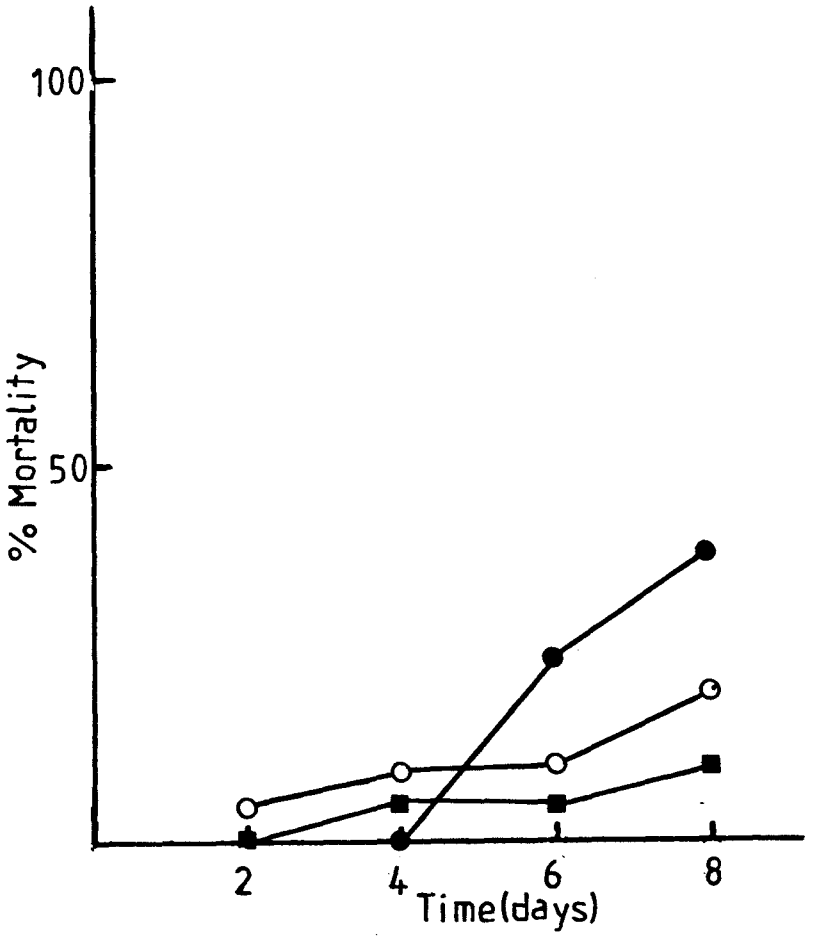


Fig. 4.06
Mortality of Nilaparvata lugens adult (brachypterous ♂ and ♀) on excised rice stems supported on agar in a square Petri dish (●), round Petri dish with (■) or without (○) filter paper to absorb moisture (25 insects treatment^{-1}).

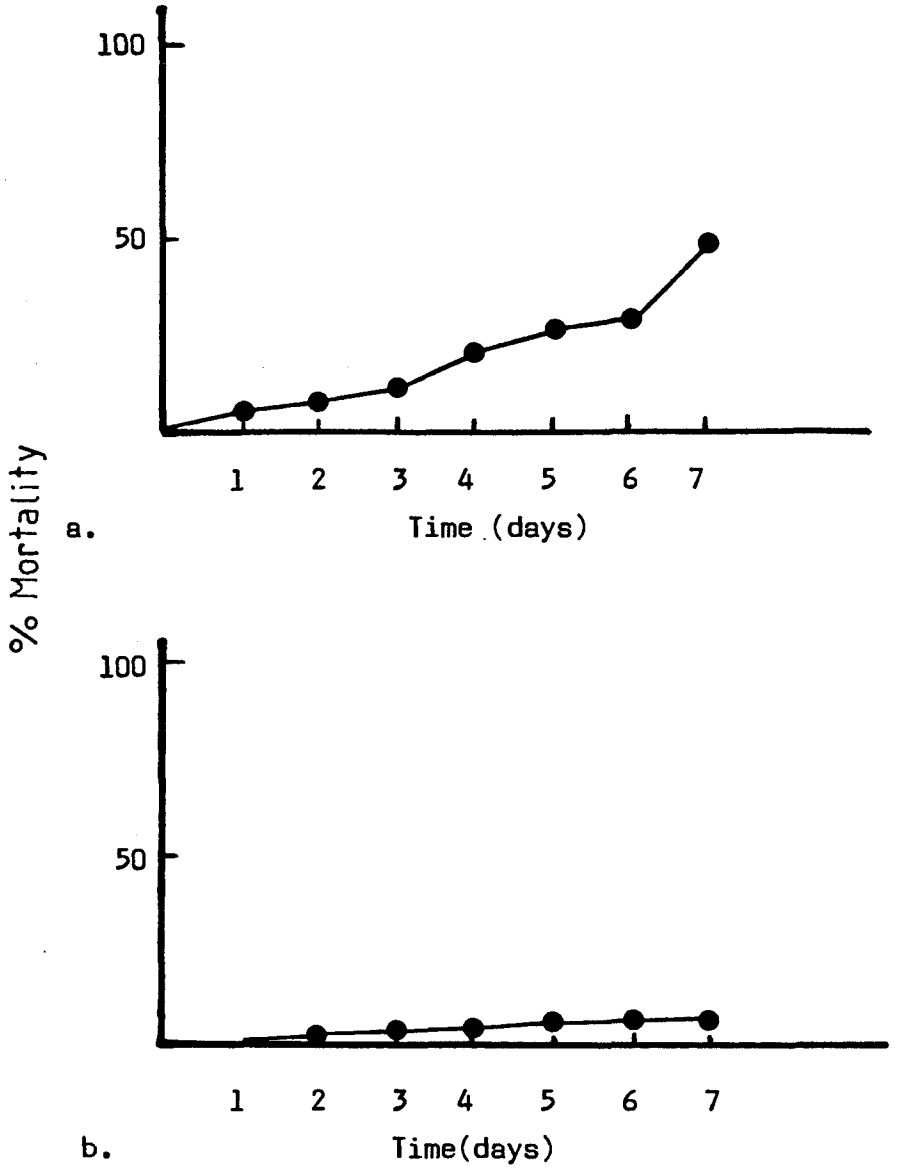


Fig. 4.07
Mortality of adult brachypterous Nilaparvata lugens, contained on rice plants at 25°C, a. before and b. after precautions were taken to eliminate Conidiobolus coronatus (Points show percentage mortality of 25 insects).

7. Biology

a. Hauptidia maroccana

i) Distribution on a Commercial Tomato Crop

The distribution of H. maroccana on a 0·4 ha block of tomatoes is illustrated in Fig. 4.08. Insects were most numerous in one area of the crop, which suggests that the outbreak spread from a central point. Thus, it is likely that leafhoppers were brought into the glasshouse on a limited batch of young seedlings, possibly as eggs, though the possibility that insects come in from outside cannot be dismissed.

ii) Occurrence on Weeds

H. maroccana was observed on Urtica dioica, Lamium purpureum and Chenopodium album and typical jassid 'feeding marks' were seen on two other weed species (Table 4.04). However, numbers were low, a total of 30, and only on nettles were nymphs observed. Mac Gill (1934) reported H. maroccana to feed on many plants and, in the laboratory, escaped insects fed on such diverse plants as coconut and early purple orchids.

iii) Life Cycle

The life cycle of H. maroccana was shorter at 25° than at 20°C (Fig. 4.09). At 28°C, only a few nymphs hatched and these all died before reaching the fourth instar. Thus, a constant temperature of 28°C is above the maximum for complete development of H. maroccana, though most insects can survive regular short periods of high temperature, as occur in glasshouses.

b. Thrips tabaci

The development period of T. tabaci, from egg to adult emergence, was shorter at 25°C (13-15 days; Fig. 4.10. a.) than at 20°C (18-21 days; Fig. 4.10. b.). Thus thrips can multiply rapidly and become a serious pest.

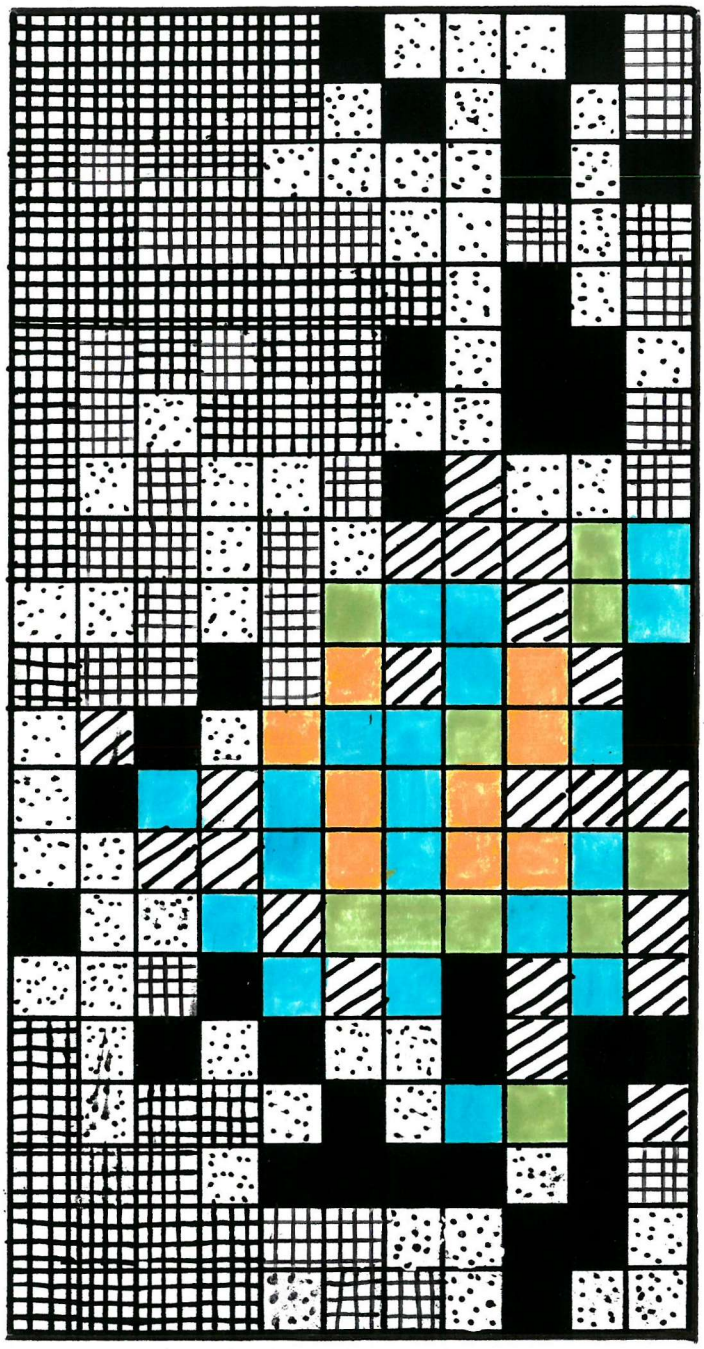


Fig. 4.08

Distribution of Hauptidia maroccana on a tomato nursery. Each square represents mean number of nymphs leaflet^{-1} on every fifth plant of every fourth row. 0-2, 2.1-4, 4.1-6, 6.1-8, 8.1-10, 10.1-12, > 12.

Table 4.04

Occurrence of Hauptidia maroccana on weeds outside an infested tomato glasshouse.

Species	Feeding Marks	Nymphs	Adults
Urtica dioica	+	+[a]	+
Lamium purpureum	+	-	+
Chenopodium album	+	-	+
Stellaria media	+	-	-
Veronica chamaedrys	+	-	-

+ Observed

- Not observed

[a] Indicates likely breeding on plant

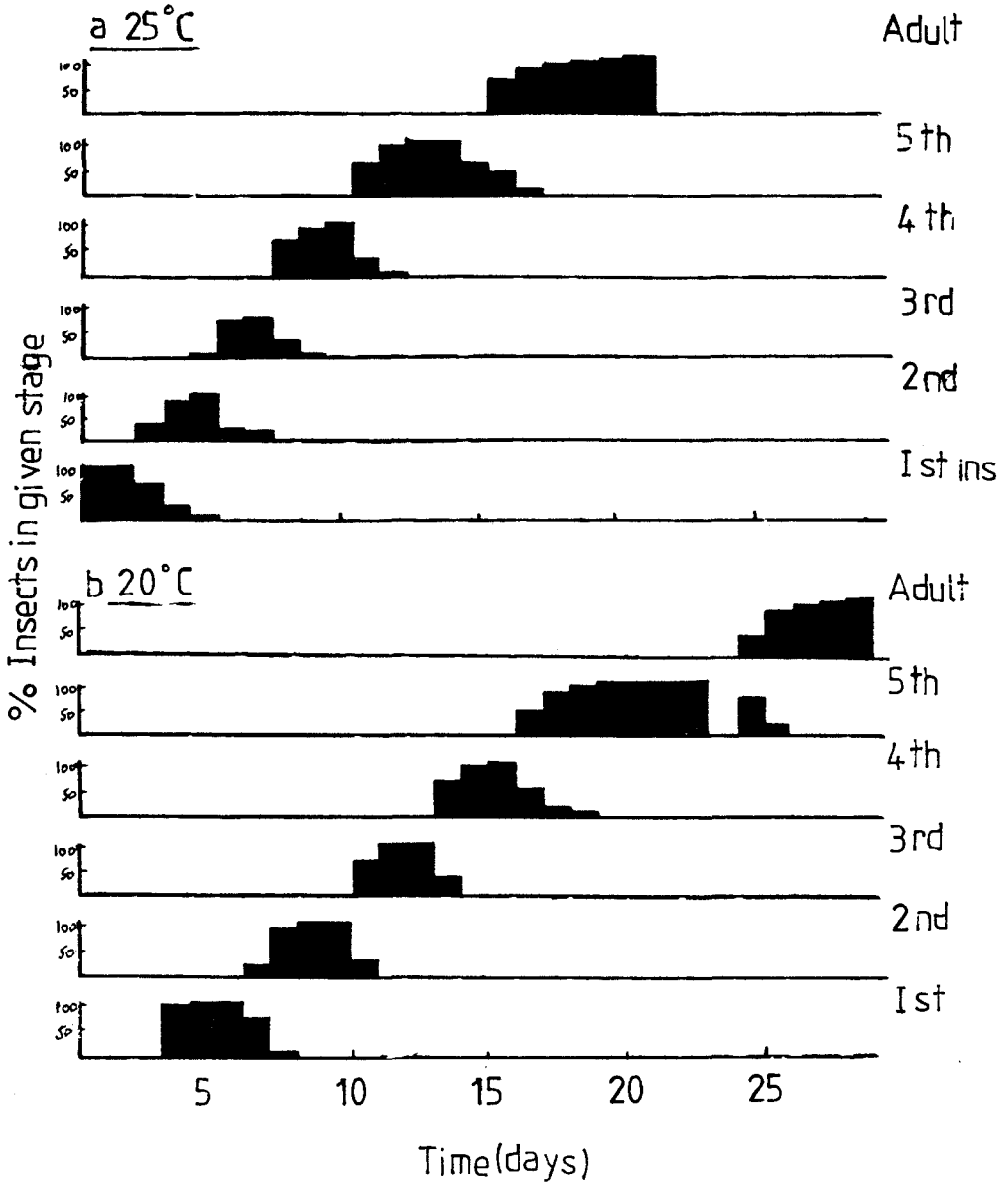


Fig. 4.09
Development of Hauptidia maroccana at a. 25 and b. 20°C. Eggs laid on cucumber plants and excised discs maintained on agar.

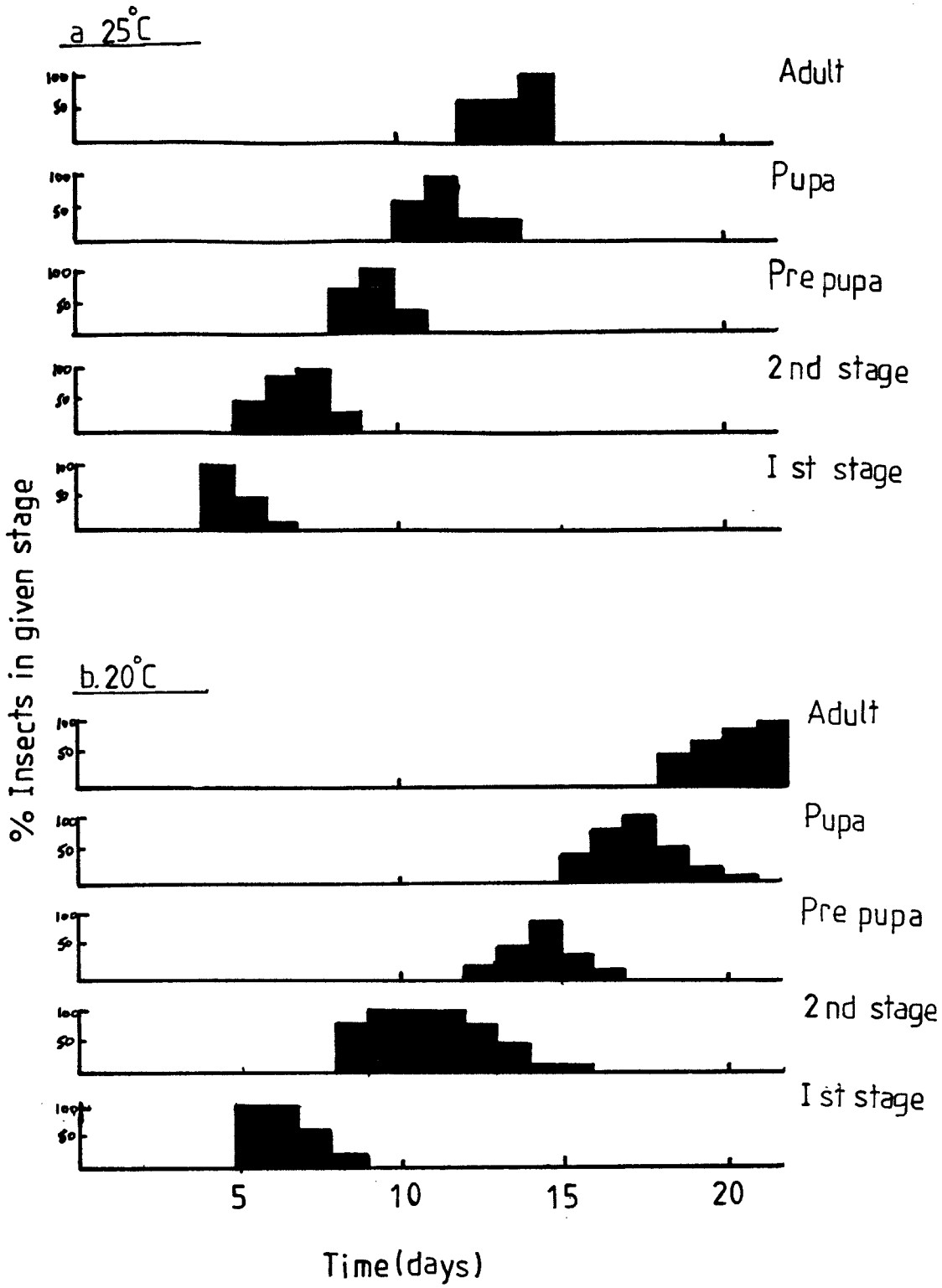


Fig. 4.10
Development of Thrips tabaci at a. 25 and b. 20°C. Eggs laid on cucumber plants and excised discs maintained on agar.

c. Nilaparvata lugens

At 25°C, adults were produced 26-29 days after oviposition (Fig. 4.11). First and second instars were short (one-two days) and stadia lengthened as maturity approached, the fifth instar lasting approximately six days.

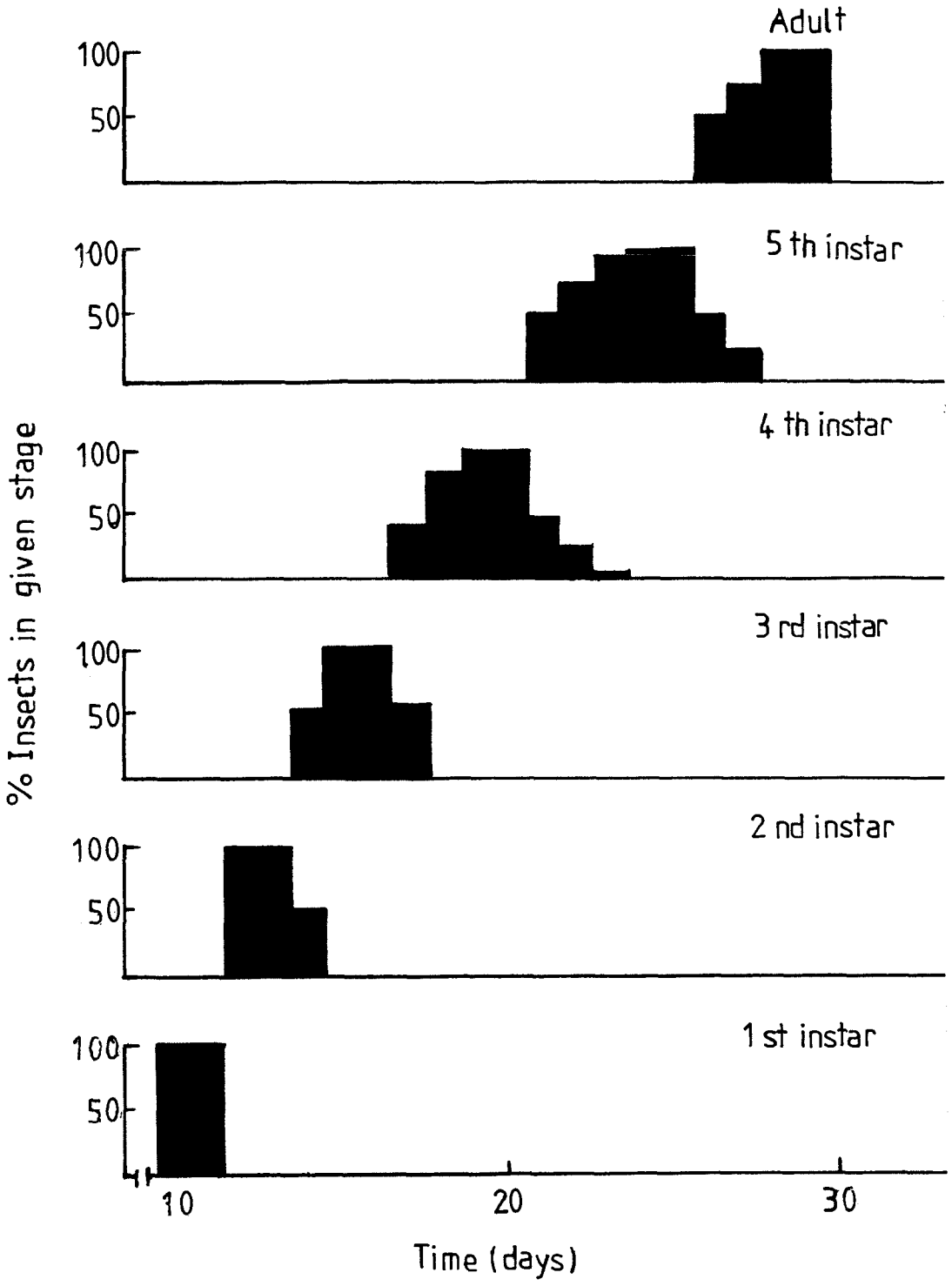


Fig. 4.11

Life cycle of N. lugens. Adults allowed to lay eggs for 24 hours on rice plant, removed and plant maintained at 25°C. On hatching insects transferred to rice stems supported on agar and observed daily.

DISCUSSION

The primary purpose of this section was to develop reliable methods of maintaining insects at the high humidities required to assess fungal pathogenicity to insects. With H. maroccana this proved relatively easy and adults survived well on agar supported leaf discs, both at 20° and 25°C. Mortality was slightly higher at 25° than 20°C, probably due to the more rapid deterioration of leaf discs at the higher temperature.

T. tabaci also survived well on large (1.6 cm diam) cucumber leaf discs when insects were produced in dry conditions, but could not be confined on single leaf discs, due to their ability to move through small apertures (Lewis, 1973). Occasionally, insects became trapped in condensation, but this was much reduced by incubation under fluorescent lights.

Adult N. lugens proved more difficult, and intact rice plants were used to maintain insects. The most serious disadvantage of this method was that insects were maintained in groups, so that cross-transmission of fungi between treated insects could not be eliminated. This is particularly important with Verticillium lecanii, which can grow and sporulate on aphids within 24 hours on conidial application (Hall, 1977).

Conidiobolus coronatus has been isolated from N. lugens on several occasions (Soper, pers. comm.) and might be considered as a control agent. However, C. coronatus has been isolated from mammals, including man, (In King, 1976), and although the strains are probably distinct, its use as a control agent is precluded. The method used to prevent contamination of insect cultures by C. coronatus was not entirely satisfactory, as honeydew falling on the aluminium foil encouraged development of fungi eg. Aspergillus sp. This was reduced by replacing plants frequently and by keeping cultures dry. Other methods to prevent C. coronatus contaminating cultures could be devised, utilizing antibiotics incorporated into the compost, though any effect on planthoppers would have to be ascertained.

Development studies were undertaken as data at constant temperatures are sparse, particularly for H. maroccana, and to provide information on ecdyses rates. Mac Gill (1932) reported the life cycle (egg laying to adult emergence) of H. maroccana (as Erythroneura pallidifrons) was completed in a mean of 35.5 days at 21°C. In the present study, development was complete in 33-37 days at 20° ± 1°C, but was more rapid at 25°C, taking 24-29 days. Mac Gill (1932) also observed that nymphs spent longer in the fourth and fifth instars, than in earlier stages and this was confirmed by the present study. Ghabn (1948) studied development of T. tabaci on cotton at uncontrolled temperature (mean 26, range 18-33°C). Under these conditions the life cycle was complete in a mean of 15 days, which agrees well with the 13-15 day period observed here, for complete development at 25°C. At a constant 25 ± 1°C, the life cycle of N. lugens was completed in 26-29 days. Suenaga (1963) and Mochida (1964a) reported more rapid development on rice seedlings, the life cycle taking a mean 24.7 days at 25°C. The slower development rate, observed here, may have been due to a sub-optimal food supply and future experiments might utilize whole plants as a food source, though observation would be more difficult.

Immature insects, developed rapidly at 25°C, a temperature which occurs commonly in glasshouse or rice 'paddies'. This rapid succession of moults can enable insects, contaminated by fungal spores, to escape infection. Thus, the rate of ecdysis and fungal spore germination must be considered when assessing a fungus for insect control. This theme will be expanded in later sections of this thesis.

SECTION V

FUNGI - BIOLOGY

MATERIALS AND METHODS

1. General

a. Media

Complex media were obtained from Oxoid Ltd., London.

Sabouraud dextrose agar

mycological peptone	10g
glucose	40g
agar	15g

In one litre of distilled water,
pH approximately 5.2

Sabouraud liquid medium

pancreatic digest of casein	5g
peptic digest of fresh meat	5g
glucose	20g

In one litre of distilled water,
pH approximately 5.7

Potato dextrose agar

potato extract	4g
glucose	20g
agar	15g

In one litre of distilled water,
pH approximately 5.6

Czapek - Dox agar

Potassium nitrate	2.0g
Potassium chloride	0.5g
Magnesium glycerophoshate	0.5g
Ferrous sulphate	0.01g
Potassium sulphate	0.35g
sucrose	30.0g
Oxoid agar No. 3	12.0g

In one litre of distilled water,
pH approximately 6.8

b. Surfactants

Triton X-100 (B.D.H. Chemicals Ltd., U.K.) was used as a wetting and dispersal agent of conidia, at a concentration of up to 0.1%. Germination of Metarhizium anisopliae (strain ME2) conidia on agar, was similar whether spores were placed directly on to the substrate or applied as a water suspension containing 1.0% Triton X-100.

c. Temperature Measurement

Temperature was preferentially recorded with a thermograph but otherwise spot thermometer readings were taken over the time course of the experiment.

d. Humidity Control

Humidities were maintained by saturated salt solutions (Table 5.01) held in sealed glass containers.

e. Total Spore Counts

Total counts of both conidia and hyphal bodies were made in an improved Neubauer haemocytometer. Before use, the haemocytometer was cleaned thoroughly in running water and dried using tissues. The cover slip was moistened by exhalation, applied to the counting chamber and agitated until 'Newtons Rings' became visible, thus indicating the correct chamber depth had been obtained. Both chambers were then carefully flooded with a suspension containing approximately 10^6 spores ml^{-1}. The number of spores in the central 1 mm^2 triple-lined square was determined, for each chamber. As variations in adhesion of the slide and coverslip can occur and thus give high variations between counts (Norris and Powell, 1961), the procedure was repeated four times and the spore concentration calculated as the mean of the four counts.

f. Assessment of Spore Viability

Viability of conidia and hyphal bodies was determined using the method of Hall (1977). Sterile Sabouraud dextrose agar (SDA) was spread in three areas on a glass microscope slide, and the slide placed in a Petri dish (9 cm diam.)

Table 5.01

Percentage relative humidities over saturated salt solutions at 20 and 25°C

Salt	Percent relative humidity 20°C	25°C
$K_2 Cr_2 O_7$	98[a]	98[ac]
$K_2 SO_4$	97[a]	97.5[a]
$Na_2 H PO_4 . 7H_2O$	95[a]	95[a]
KNO_3	93.5[a]	92.5[ac]
$Zn SO_4 . 7H_2O$	-	88.5[a]
Na Cl	76[ab]	-
$Na Br . 2H_2O$	59[a]58[bc]	-

a Winston and Bates (1960)

b Carson (1931)

c Solomon (1951)

lined with moist filter paper. Single drops of test suspension, containing approximately 10^6 spores ml^{-1}, were placed on the agar and the whole incubated at a defined temperature for 12-24 h. Viable spores produced a germ tube easily visible with phase-contrast microscopy. On each agar pool 100 spores were examined and the viability expressed as a percentage.

g. Production and Harvesting of Conidiospores

Conidiospores were cultured by spreading silica gel crystals, used to lyophylize conidia for storage (V, 9. c), on to SDA plates which were incubated at 23°C for 7 days, when fungi were re-cultured on fresh SDA plates. After a further 10 days incubation, spores were harvested by flooding plates with sterile distilled water, containing 0.01 - 0.1% Triton X-100, and agitating with a glass spreader. After removal of hyphal debris by filtration through cheese cloth or tissue, the spores were centrifuged and washed twice.

h. Culturing and Harvesting of Hyphal bodies

Hyphal bodies of M. anisopliae (ME2) were cultured by inoculating conidia into 250 ml conical flasks containing 50 ml of a medium comprising 40 g litre molasses (Tate and Lyle Ltd), 20 g yeast extract (Difco Ltd) and 0.5% Tween 80. Flasks were plugged with cotton wool and incubated on a reciprocal shaker (135 r.p.m., 26°C). Yields were optimal after 5 days (VII, 8, e) when hyphal bodies were harvested by filtration through cheesecloth or tissue, centrifuged, washed three times and resuspended in .025% Triton X-100.

2. Source of strains used in this study

Strains were obtained from many sources (Table 5.02)

3. Section of the most Suitable Medium for Routine Use

The rate of mycelial growth of M. anisopliae (ME2) and Verticillium lecanii (53-81) was determined on SDA plates of varying volume (5, 10, 15, 20, 25 ml) and on Czapeck Dox, Potato dextrose and Nutrient agars. Plugs (0.6 cm diam.) were removed from 72-hour old confluent cultures

Table 5.02

Details of Fungi Used in This Study

Species	Strain	Host	Isolated by
Beauveria bassiana	1	Thosea exigua	A. Gillespie
"	31	Leptinotarsa decemlineata	La Miniere
"	32	" "	"
"	43	Nilaparvata lugens	K. Aizawa
"	45	Nephotettix cincticeps	"
"	50	" "	"
"	53	" "	"
"	63	Mythimna unipuncta	La Miniere
Beauveria brongniartii	6	Melolontha melolontha	La Miniere
"	40	" "	S. Keller
"	65	" "	"
Conidiobolus coronatus	1	Nilaparyata lugens	A. Gillespie
Metarhizium anisopliae	Pemphigus	Pemphigus treherne	S. Foster
"	ME2	Leafhopper	D. Roberts
Metarhizium anisopliae	ME3	Otiorhynchus sulcatus	R. Hall
"	52	Melolontha melolontha	S. Keller
"	1140		
"	D r	Leafhopper	D. Roberts

Nomuraea rileyi	-	Antcarsia gemmatalis	C.M. Ignoffo
Paecilomyces fumosoroseus	21	—	La Miniere
Paecilomyces spp.	2	Melolontha melolontha	S. Keller
Synecephalastrum racemosum		Soil	IMI 77605
Verticillium fusiformis	1	—	B. Ekbom
Verticillium lecanii	1-72	Macrosiphoniella scriborni	R. Hall
"	7-73	Uromyces appendiculates	"
"	11-73	Contact Lens	IMI 176057
"	12-74	Pulvineria floccifera	IMI 79606
"	15-74	Scolytus scolytus	G. Barson
"	16-75	Erysiphe graminis	L. Leeming
"	19-79	Trialeurodes vaporariorum	R. Hall
"	25-79	Aphis gossypii	N. Hussey
"	27-79	Phorodon humuli	R. Hall
"	28-79	Hauptidia maroccana	A. Gillespie
"	33-79	Otiorhynchus sulcatus	R. Hall
"	41-81	Coffee scale	P. Kanagaratnan
"	53-81	Thrips tabaci	A. Gillespie
"	Tt	Thrips tabaci	A. Gillespie

and after inversion, placed in matching holes in the centre of agar plates. For each treatment, five replicate plates were inoculated, wrapped in aluminium foil to exclude light and incubated at 25 ± 1°C. At seven day intervals the diameters of resultant colonies were recorded; two diameters at right angles were measured with a ruler and the mean calculated. Sporulation was assessed visually on a subjective 1-4 scale; 4 indicating maximum sporulation.

4. Effect of Temperature on Germination and Mycelial Growth

Conidial germination was determined, at 20°C, or over a range of temperatures, on SDA, as described earlier for spore viability (V, 1. f). After various periods of incubation the proportion of 300 spores per treatment with a visible germ tube was determined, using phase contrast microscopy. The effect of temperature on mycelial growth was determined in a similar way to that described earlier (see V, 3), at temperatures from 13-32°C. Colony diameters were measured weekly for up to 4 weeks and rates of mycelial growth expressed as mean radial extension (mm day^{-1}).

5. Effect of Humidity on Germination and Sporulation

a. Spore Germination

The method adopted was modified from that used by Manners and Hossain (1963). Conidia of M. anisopliae (ME2) and V. lecanii (1-72), failed to germinate on glass slides, so were held on sections of dialysis tubing previously autoclaved in double-strength Sabouraud liquid medium (III, 1). Unwashed conidia were placed on membranes using a camel-hair brush (size 0), incubated over distilled water or saturated salt solutions, contained in glass staining blocks for 24 hours at 25 ± 0.5°C, and scored for germination using phase-contrast microscopy. A spore was considered to have germinated when it had produced a visible germ tube.

Prior to use, sealed humidity chambers were maintained at 25°C for 24 hours to allow humidities to stabalize and membranes were transferred rapidly to minimize humidity changes.

b. Sporulation

i. In vitro

Conidia were placed on SLM - treated membranes at 25°C and a nominal 100% relative humidity for 24 hours to allow spore germination. Membranes were then transferred to various humidities and observed daily for sporulation for four days.

ii. In vivo

Adult Hauptidia maroccana were treated with various fungi by immersion in conidial suspensions (10^7 spores ml^{-1}) then maintained on leaf discs supported on agar (VI, IV, 2, a, ii). Immediately after death, but before sporulation occurred on cadavers, insects were positioned on slides with double-sided Sellotape and placed over distilled water or saturated salt solutions. After four days the presence or absence of sporulation was recorded.

6. Effect of Light on Sporulation

a. Numbers of Conidia Produced in Constant Light or Dark

SDA plates were inoculated with 1 ml of a suspension containing 10^7 conidia ml^{-1} and spores distributed evenly with a bent glass rod. These plates were positioned 80 cm from a bank of four fluorescent tubes (5000 lux at plate level) or in darkness, under an inverted plastic tray, aerated by a small electric pump. Temperature was monitored with mercury thermometers, positioned with the bulbs inside un-inoculated SDA plates, and located with plates of both treatments. Hence any temperature differences at the agar surfaces that occurred between the two treatments, as a result of radiant heat, could be detected. Plates were incubated at 20 $\pm$ 1°C: after 12 days spore production was estimated by suspending a 12 cm diam. disc from each plate in 10 ml of water containing 0.05% Triton X-100 together with two glass balls (0.3 cm diam.) and mixed for three min. In order to check that all spores were removed, plugs were re-treated in fresh liquid for three min. Spore concentrations were estimated using an improved Neubauer haemocytometer.

b. The Effect of Fluorescent Light, Near Ultraviolet Light and Dark on Conidial Production

SDA plates were inoculated with conidia and incubated in darkness at 25°C for 24 hours to permit spore germination. This pre-incubation was essential as near ultraviolet light can impair conidial germination (Osman and Valadon, 1981). Plates were then positioned under fluorescent lights, in darkness, or under near ultraviolet lights at 20°C; after 12 days, sporulation was estimated as described earlier (V, 6, a).

7. Effect of Fungicides on Metarhizium anisopliae (ME2)

A range of fungicides (Table 5.03) was incorporated into molten Sabouraud dextrose agar, at 40-50°C, at 0.1, 1 and 10 times the recommended rate, poured into Petri plates (20 ml plate^{-1}) and pipetted in three discrete pools, on to clean glass slides. M. anisopliae conidia (ME2) were prepared as described earlier (V , 1, f) and single drops of a suspension containing 10^6 spores ml^{-1} placed on to each agar pool on five replicate slides. The slides were incubated in 9 cm Petri dishes lined with damp filter paper, in darkness at 25°C; after 24 hours, germination was determined by phase contrast microscopy or after staining with cotton blue in lactophenol. Conidia placed on SDA without added fungicides served as controls. Mycelial growth from 0.6 cm mycelial plugs inserted in SDA Petri plates, containing fungicides was measured. The plugs were obtained from a 72 hour old confluent culture of M. anisopliae (ME2). Five replicate plates for each pesticide concentration were incubated at 23°C; after 8 days colony diameters were recorded and compared with those attained on untreated SDA plates.

8. Growth of Metarhizium anisopliae on Rice After Application of Conidia With Skimmed Milk

Eight-week old rice plants, variety TN1, were reduced to 20 cm in height and dead leaves removed. Individual plants were then thoroughly sprayed with a suspension of M. anisopliae conidia (1.8 x 10^6 spores ml^{-1}) in 0.025% Triton X-100, or in various concentrations of skimmed

Table 5.03

Effect of fungicides on Conidial Germination and Mycelial Growth of Metarhizium anisopliae (ME2)

Fungicide	Recommended rate of use (grams active ingredient 100 litres H_2O^{-1})
benomyl	25
bupirimate	17.5
carbedazim	30
chlorothalonil	108
dinocap	6.25
etridiazole	25
imazalil	30
iprodione	50
pyrazophos	15
thiophanate - methyl	50
triforine	25
zineb	140

milk (Oxoid Ltd; 0.05, 0.5, 1.0, 2.5%). Immediately after spraying, plants were dried in a laminar flow cabinet; after 2 h, 0.2 g leaf samples were taken from each plant. Plants were then maintained in perspex cages at a nominal 100% relative humidity and 25°C; after 7 days, further leaf samples were obtained. Leaf samples were placed in 'Universal' bottles, containing 10 glass balls (approx. diam. 0.2 cm) and 10 ml of 0.025% Triton X-100 and agitated, using a vortex mixer, for 3 min. Spore concentrations were determined by spreading suspensions on SDA Petri plates, containing 80 $\mu g\ ml^{-1}$ streptomycin sulphate (Sigma Ltd.) and counting resultant M. anisopliae colonies after 4 days incubation at 25°C.

9. Storage

a. On Agar Slopes

SDA slopes (3 ml) in Bijou bottles, inoculated with conidia of various fungi, were incubated at 25 ± 1°C; after 10 days, conidial viability was assessed on agar ($\bar{V}$, 1, f). Eight slopes of each fungus were then stored in darkness at 20, 2 or -20°C. Two slopes of each treatment were sampled for viability 24 h after storage and at intervals over the next 2 years.

b. In 0.025% Triton X-100

Conidia and hyphal bodies of M. anisopliae (ME2) were produced and harvested in 0.025% Triton X-100 as described earlier ($\bar{V}$, 1, g: $\bar{V}$, 1, h). Aliquots (5 ml) of each suspension containing approximately 10^6 spores ml^{-1}, were placed in 'Universal' bottles, with the caps loosely fitted, and stored at 2, 10, 20, 25, or 30°C. Spore viability was assessed on SDA slides ($\bar{V}$, 1, f).

c. On Silica Gel

Storage of fungi on silica gel was first described by Perkins (1962) who suspended spores in skimmed milk before application to the crystals. In the present study, spores were applied in distilled water as described by Bell and Hamalle (1974). Soda-glass tubes (15 x 1.6 cm) containing approximately 10 g of silica gel (BDH Ltd., England) and

plugged with cotton wool, were dry-sterilized for five days, at approximately 130°C. When cool, about 10^8 conidia in 1 ml of 0.025% Triton X-100 were applied to the crystals, plugs replaced and the tubes sealed with Parafilm (American Can Co. USA). Tubes were agitated vigorously to disperse both conidia and the heat produced as the crystals absorbed water, then stored in darkness at 2°C. Viability, determined by placing crystals on SDA plates and incubating for 7 days at 23°C, was assessed 24 h after storage and at intervals for the next 2 years.

d. On Cadavers at Various Humidities

Cadavers of Hauptidia maroccana, bearing sporulating mycelium of M. anisopliae (ME2) or Beauveria bassiana (63) and obtained as described later (VI, 1, b.), were placed on Crystal polypot lids at 100, 97, 93, 76 or 58% relative humidity and 20 ± 1°C in the apparatus shown in Fig. 5. 01. After storage for 24 hours, and at intervals thereafter, spores from one insect per replicate were removed by vortex agitation in 0.025% Triton X-100 and conidial viability determined on SDA (V, 1, f). Three insects, one from each of three replicate jars at each humidity, were used at all sampling dates.

RESULTS

10. Growth on Solid Media

a. Type of Medium

i. Metarhizium anisopliae (ME2)

Growth of M. anisopliae (ME2) was maximal on Sabouraud dextrose (SDA) and Potato dextrose agars (PDA) (Fig. 5.02, a). Sporulation was assessed visually and was highest on SDA (Table 5.04)

ii. Verticillium lecanii (53-81)

Growth of V. lecanii (53-81) was similar on SDA, Czapek-Dox and Nutrient agars, but was significantly slower on PDA (Fig. 5.02, b). Sporulation was similar on all media tested.

Since all Deuteromycetes studied grew and sporulated well on SDA, it was selected for routine use.

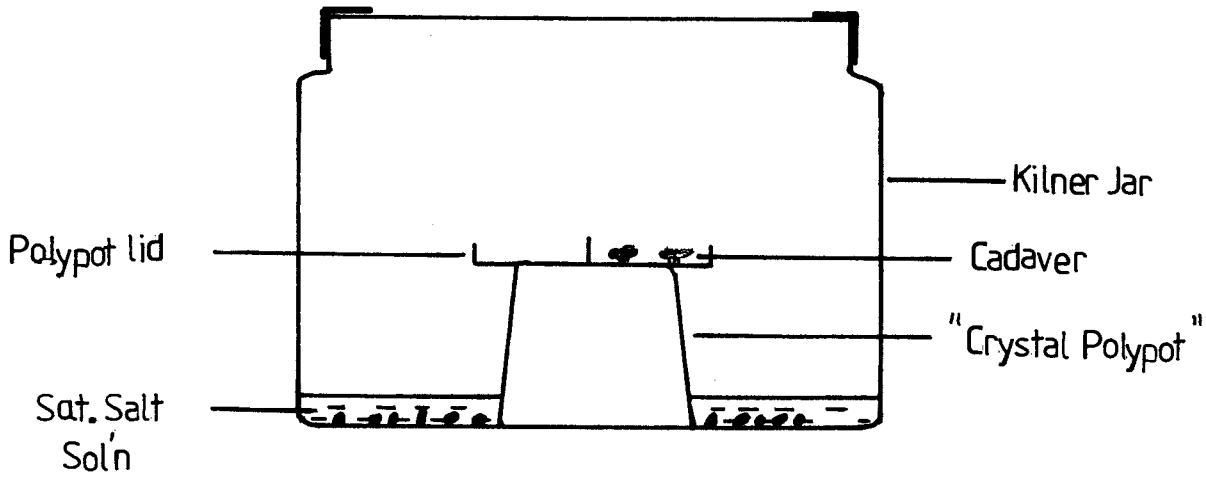


Fig. 5.01
Apparatus used for maintaining sporulating cadavers of Hauptidia maroccana at controlled humidities to assess conidial survival.

Table 5.04

Effect of Medium on Sporulation of *Metarhizium anisopliae* (ME2) (20 days at 25 ± 0.5°C)

Agar Medium	Degree of sporulation[a]
Sabouraud dextrose	+ + + +
Nutrient	+ + + +
Potato dextrose	+ +
Czapek - Dox	+

a + + + + indicates maximum sporulation

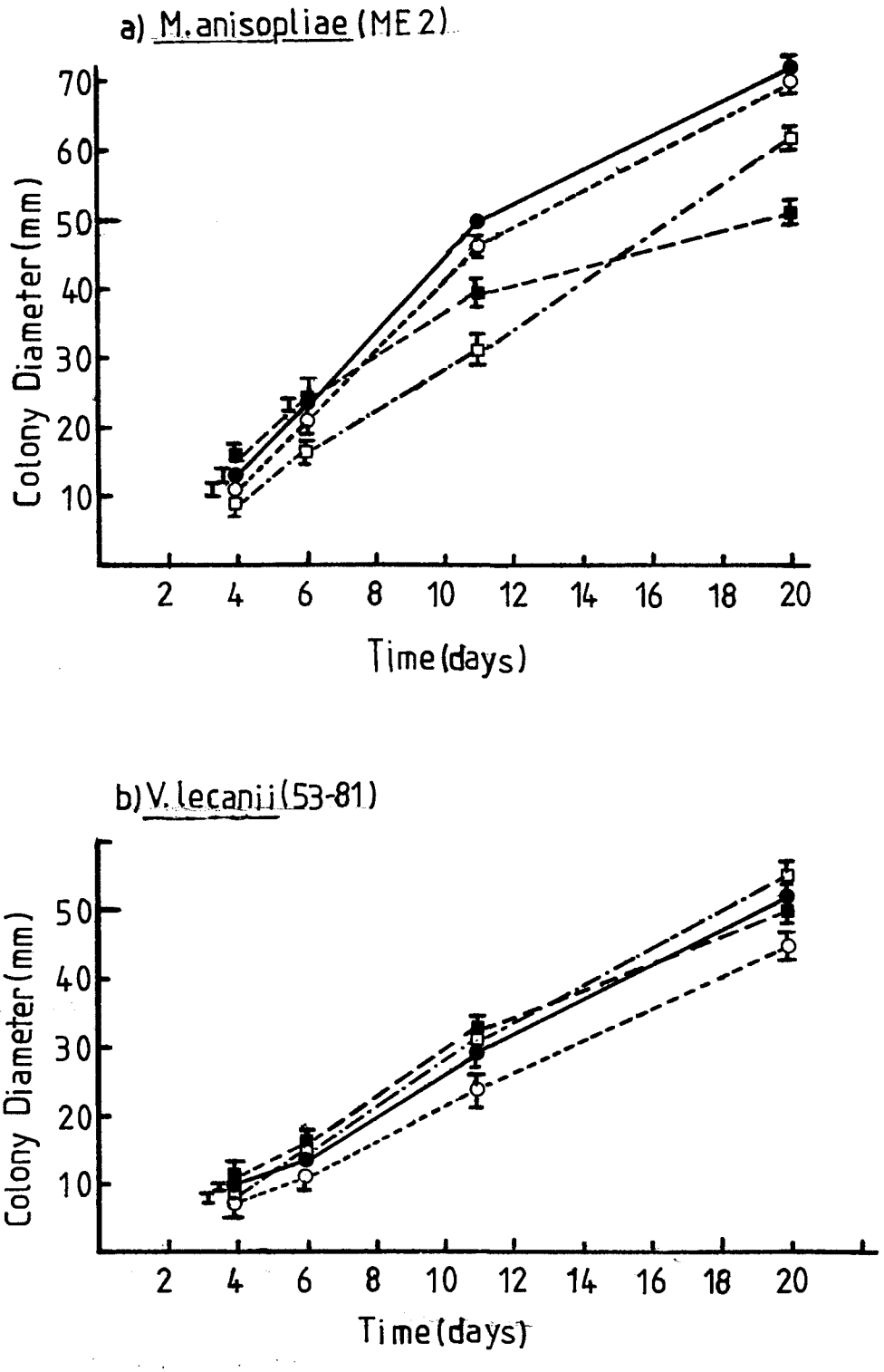


Fig. 5.02

Growth rates of a) Metarhizium anisopliae (ME2) and b) Verticillium (53-81) on Sabouraud dextrose (●) potato dextrose (○) Czapek-Dox (■) and nutrient (□) agar, at 25°C. (Points are means of 5 replicates with 95% confidence limits).

b. Thickness of Medium

Growth on SDA plates in volumes of medium above 10 ml was similar both for M. anisopliae (ME2) and V. lecanii (53-81), while on 5 ml of medium, growth was significantly reduced (Fig. 5.03). Chaudhuri (1923) reported growth to be affected by agar depth, but from this study the volume, provided it exceeded 10 ml, and hence the depth, was not critical. As a precaution, plate volumes were standardised at 20 ml when comparative growth measurements were made.

11. Effect of Temperature

a. Spore Germination

A detailed study of the effect of temperature on conidial germination on SDA was made with M. anisopliae (ME2) and V. lecanii (53-81). Other strains were examined only at 20°C, which is the night time temperature for cucumber production and generally coincides with the period of high glasshouse humidity.

i. Metarhizium anisopliae

Germination of M. anisopliae (ME2) conidia occurred rapidly from 23-30°C, virtually all conidia germinating in 12 hours (Fig. 5.04). Outside this range, and particulary at 20°C and below, germination was slower. Strains 52 and Map germinated more rapidly than 1140, ME2 and Ma Dr at 20°C, but still required 22 hours incubation to approach 100% germination (Fig. 5.05).

ii. Verticillium lecanii

Conidia of V. lecanii (53-81) germinated quickly at temperatures from 20-27°C and most rapidly at 23°C (Fig. 5.06). Germ tube formation proceeded more slowly at 30°C, and was considerably reduced at 10 and 13°C.

A variety of V. lecanii isolates was compared with M. anisopliae (ME2) (Fig. 5.07). Conidia of V. lecanii strains 1-72 and 7-73 germinated particulary rapidly at 20°C and all strains germinated more quickly than M. anisopliae (ME2).

iii. Beauveria

All six strains of B. bassiana and one strain of B. brongniartii

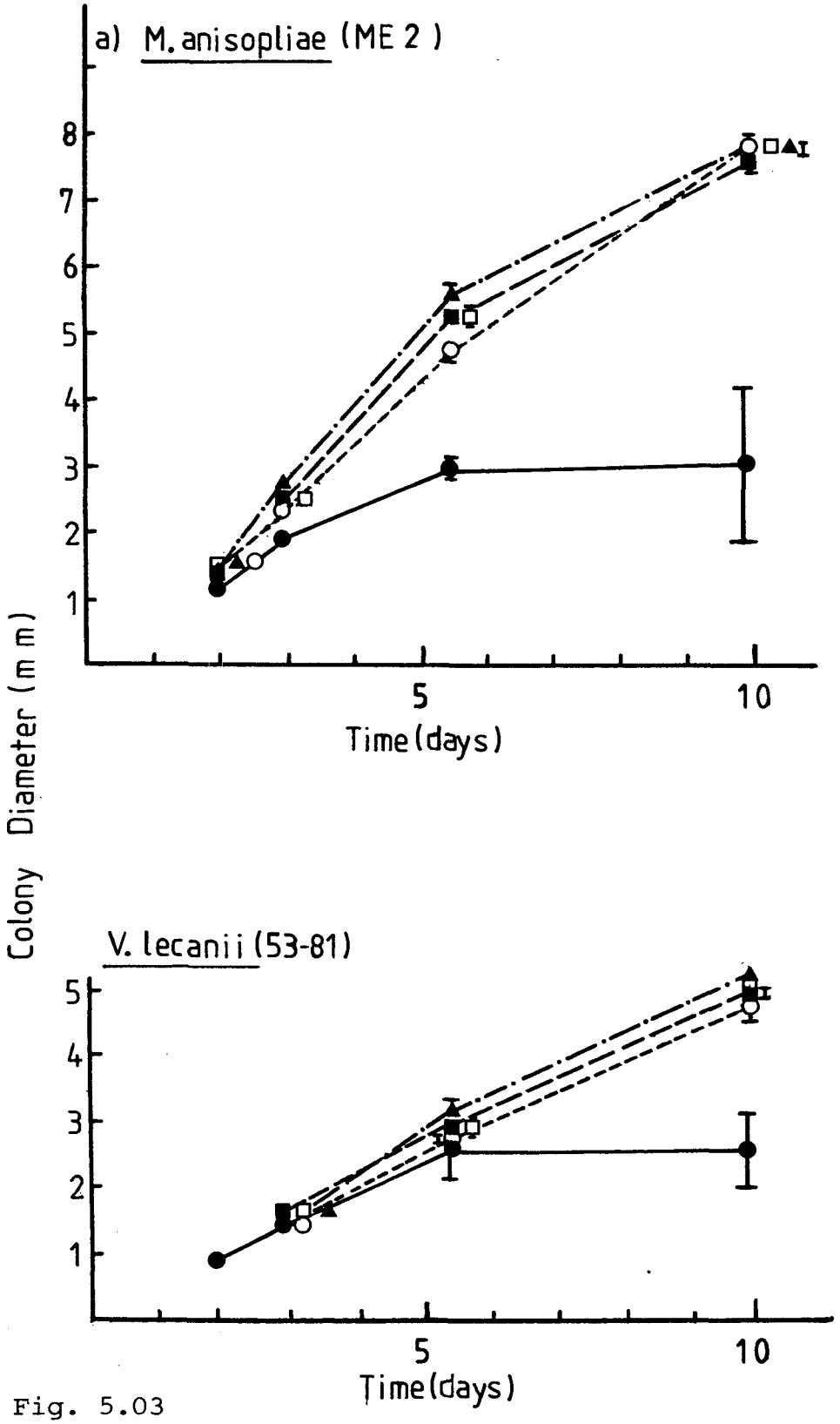


Fig. 5.03

Growth at 25°C of a) Metarhizium anisopliae and b) Verticillium lecanii on Sabouraud dextrose agar plates of varying volume; ● 5ml, ○ 10ml, ■ 15ml, □ 20ml, △ 25ml (Points are means of 5 replicates, with 95% confidence limits at 11 and 20 days)

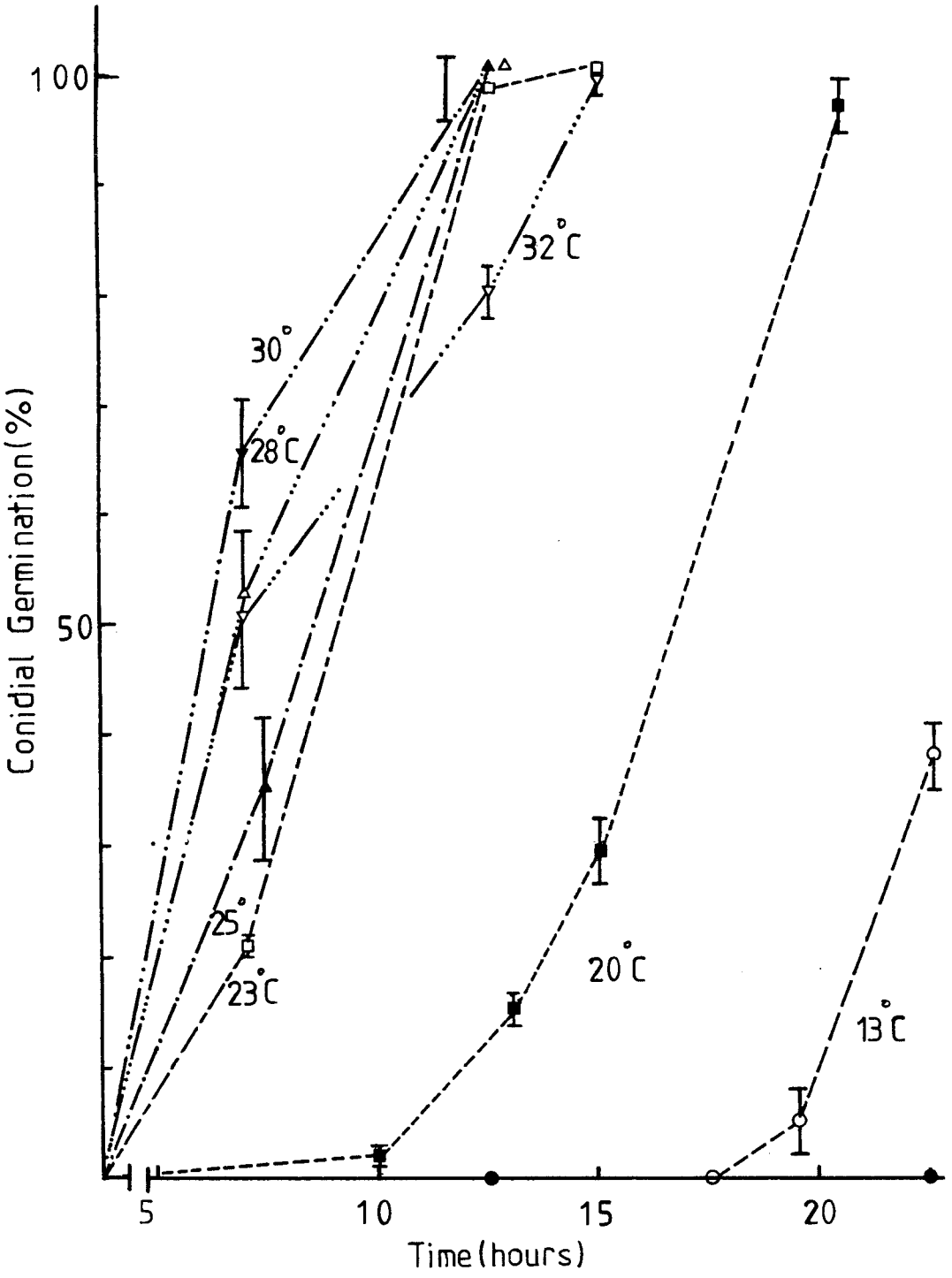


Fig. 5.04

Effect of temperature on conidial germination rates on SDA of Metarhizium anisopliae (ME2). (Points are means of three replicates with 95% confidence limits).

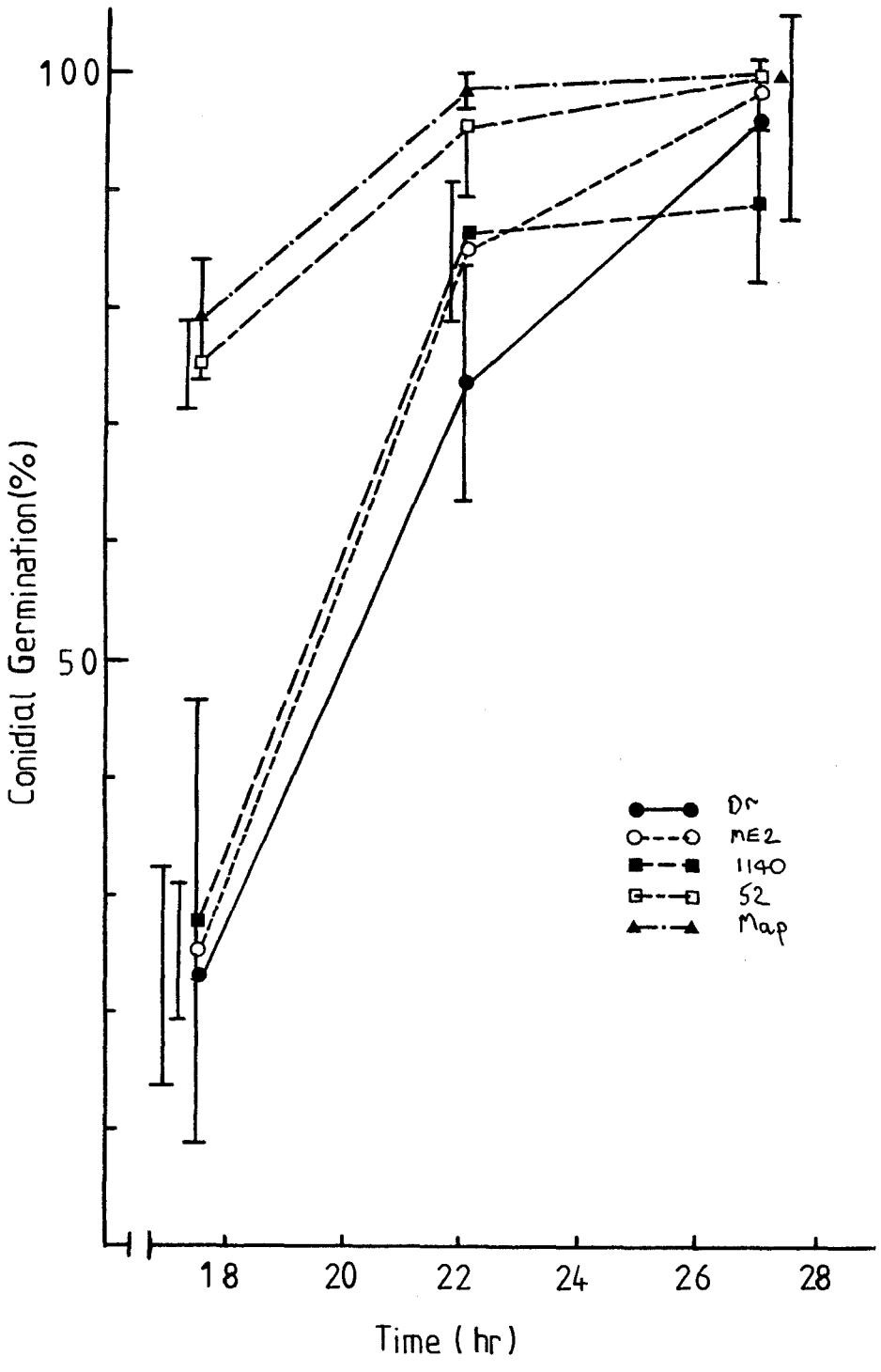


Fig. 5.05
Conidial germination rates of Metarhizium anisopliae strains at 20°C on Sabouraud dextrose agar. (Points are means of 3 replicates with 95% confidence limits).

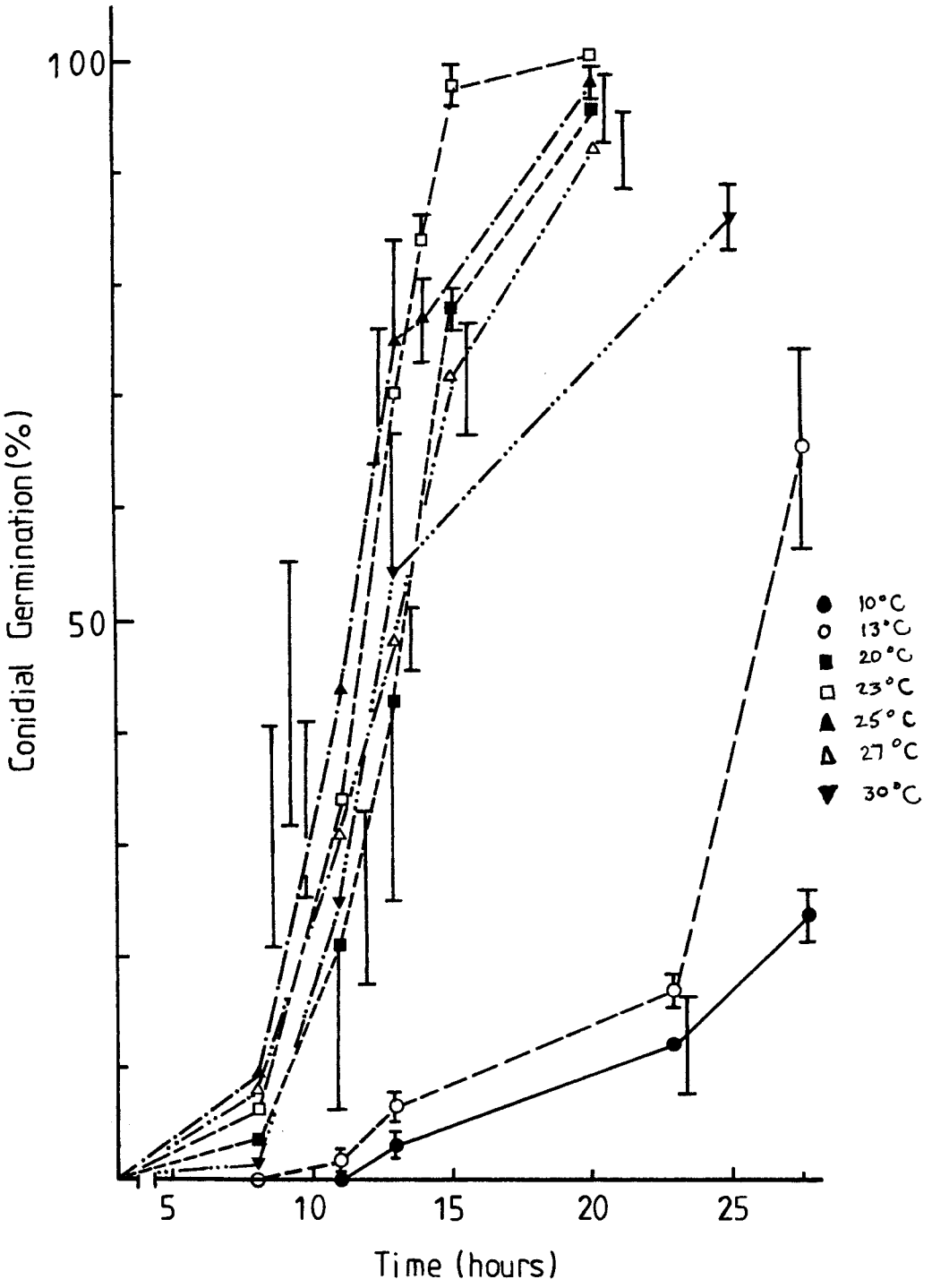


Fig. 5.06
Effect of temperature on conidial germination rates of Verticillium lecanii (53-81) (Points are means of 3 replicates with 95% confidence limits).

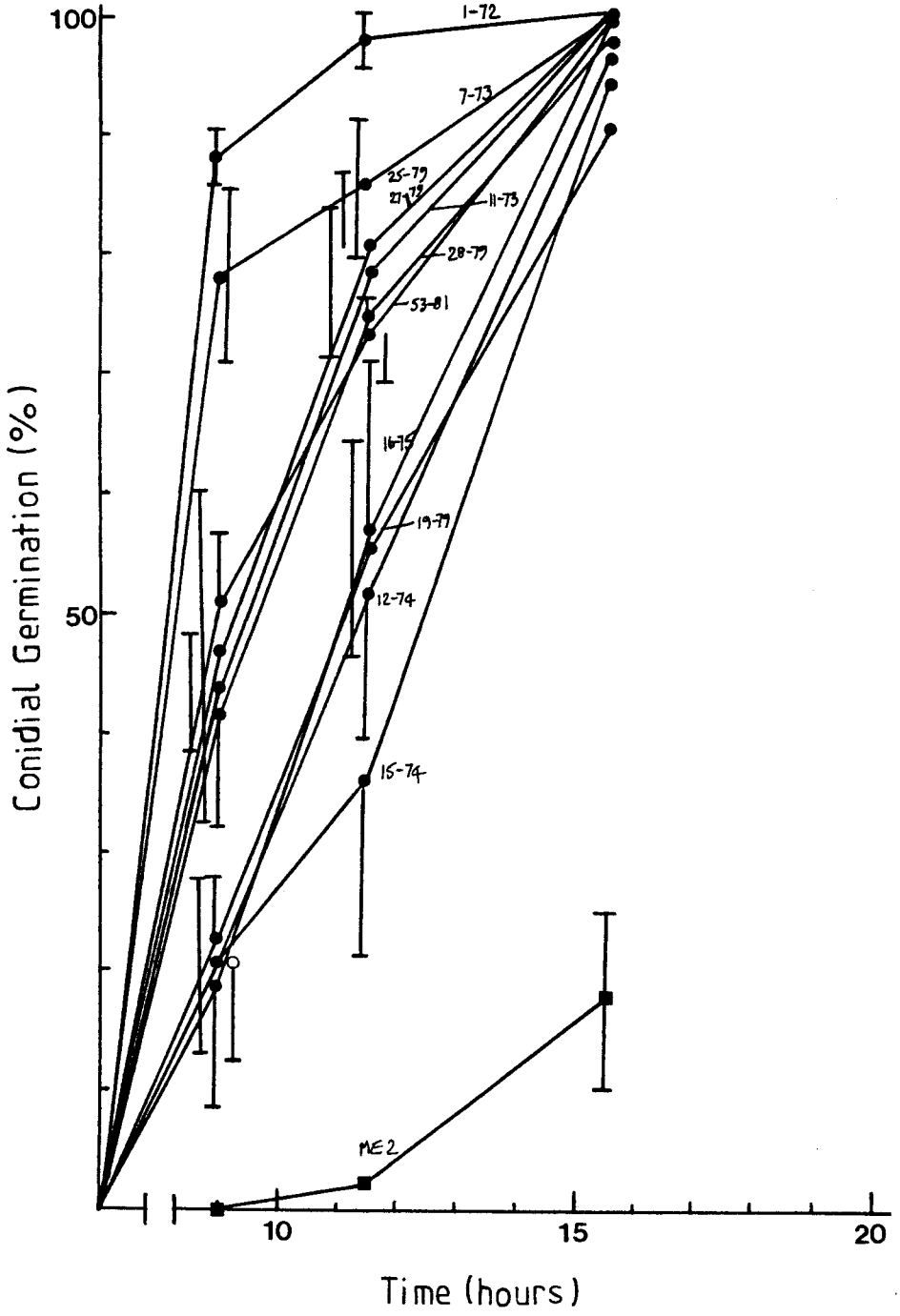


Fig. 5.07
Conidial germination rates of Verticillium lecanii strains at 20°C on Sabouraud dextrose agar, in comparison with Metarhizium anisopliae (ME2). (Points are means of 3 replicates with 95% confidence limits).

germinated slowly at 20°C and required at least 27 h for 90% conidial germination (Fig. 5.08). One strain of B. bassiana, isolated from a coleopterous larva in Sabah, germinated very slowly, requiring 27 hours incubation at 20°C to achieve 50% germination.

iv. Paecilomyces, Syncephalastrum and Verticillium

V. lecanii conidia germinated quickly while those of Paecilomyces took significantly longer. After incubation for 17½ hours, conidial germination of V. lecanii reached 72 and 85%, while only 41 and 50% of Paecilomyces conidia germinated in the same time (Fig. 5.09).

b. Mycelial Growth

i. Metarhizium anisopliae

The effect of temperature on mycelial growth rates of five strains of M. anisopliae is depicted in Fig. 5.10. All strains grew at temperatures from 13-30°C, though the optimal temperature varied. Strain Map grew best at 23-5°C while strains ME2, ME3, 1140 and 52 had optimal mycelial growth rates at 25-28°C. Growth of all strains at 32°C was either absent, or below 0.02 cm day^{-1}. Maximum growth rates in strain ME2 exceeded 0.4 cm day^{-1}, which was nearly twice that of the slowest growing strain, Map.

ii. Beauveria

Growth rates of 2 strains of B. bassiana and one strain of B. brongiartii are shown in Fig. 5.11. Growth of B. bassiana (63) was optimal at 23-25°C while strain 31 grew most quickly at 25-30°C. B. brongniartii had a lower temperature optimum and grew best at 23°C. Growth of all strains was inhibited at 32°C and B. bassiana (31), grew very slowly at 30°C. Maximum growth rates of all strains were low and ranged from .18 to .27 cm day^{-1}.

iii. Condiobolus coronatus, Paecilomyces fumosoroseus and Verticillium lecanii

Mycelial growth rates of C. coronatus, P. fumosoroseus (21) and V. lecanii (53-81) are shown in Fig. 5.12.

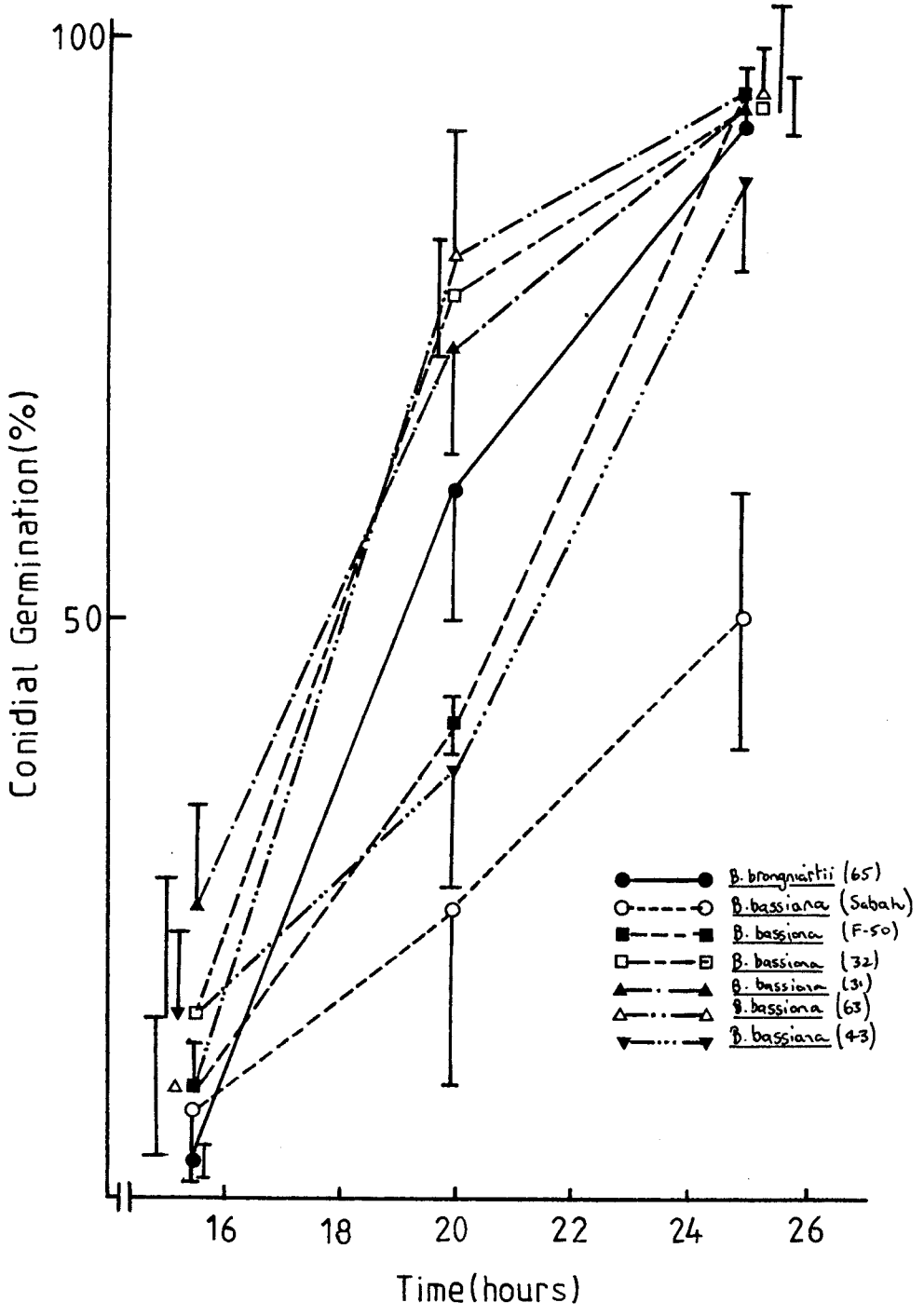


Fig. 5.08

Conidial germination rates of Beauveria strains at 20°C on Sabouraud dextrose agar. (Points are means of 3 replicates with 95% confidence limits).

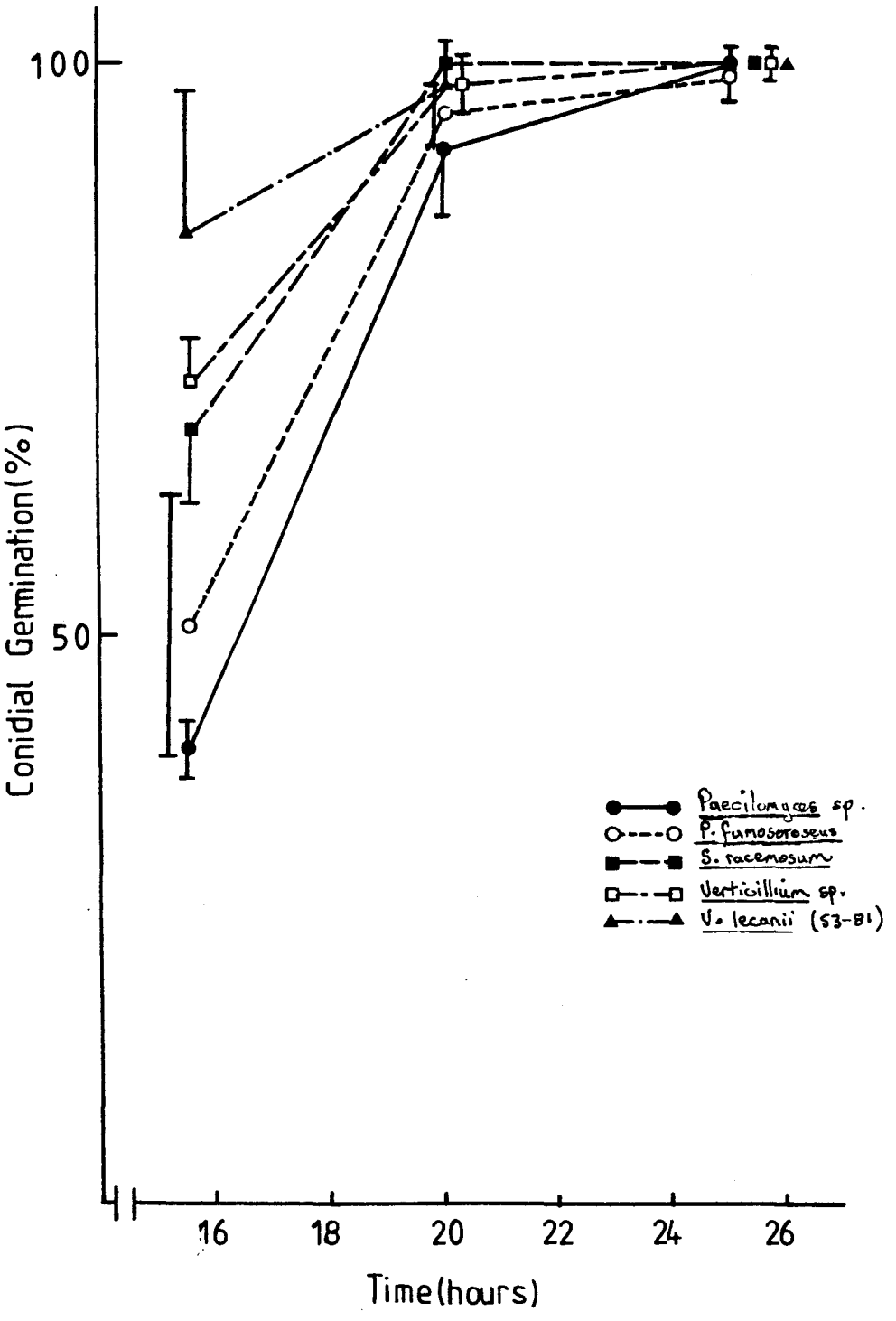


Fig. 5.09

Conidial germination rates of various entomogenous fungi at 20°C on Sabouraud dextrose agar. (Points are means of 3 replicates with 95% confidence limits).

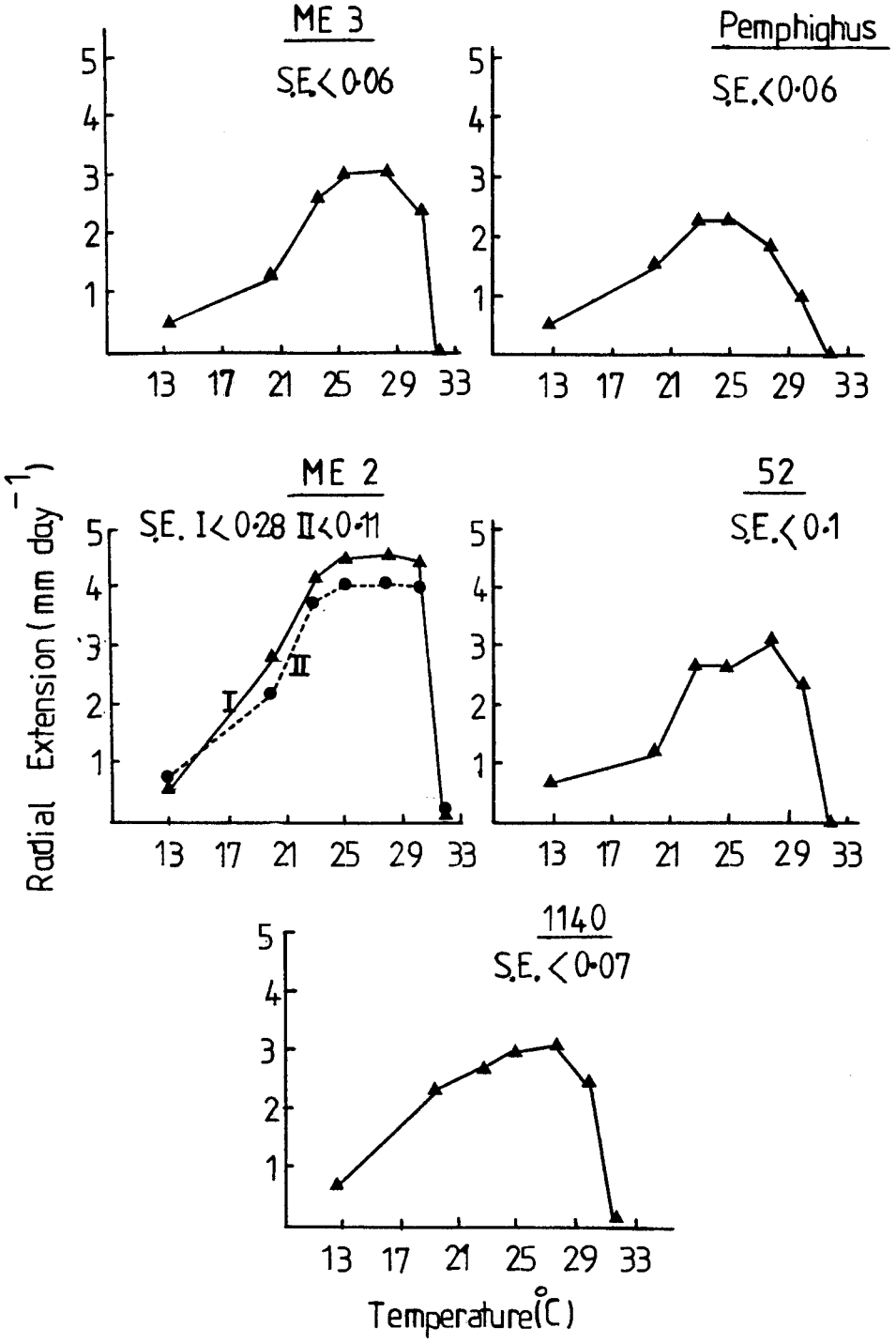


Fig. 5.10

Growth rates at various temperatures of Metarhizium anisopliae strains on Sabouraud dextrose agar. (Points are means of 5 replicates; I and II indicate two separate experiments).

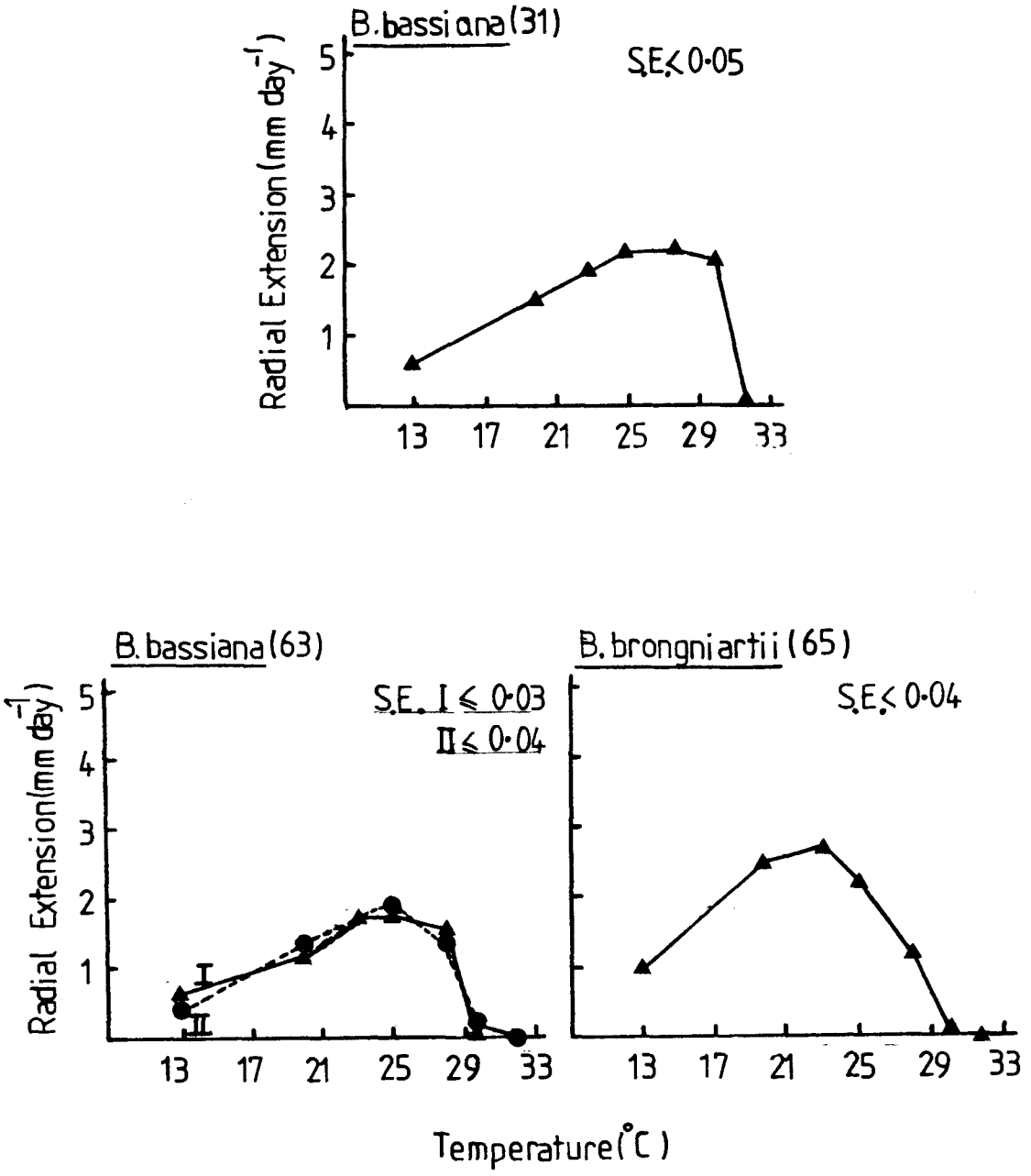


Fig. 5.11
Growth rates at various temperatures of Beauveria strains on Sabouraud dextrose agar. (Points are means of 5 replicate plates; I and II indicate two separate experiments).

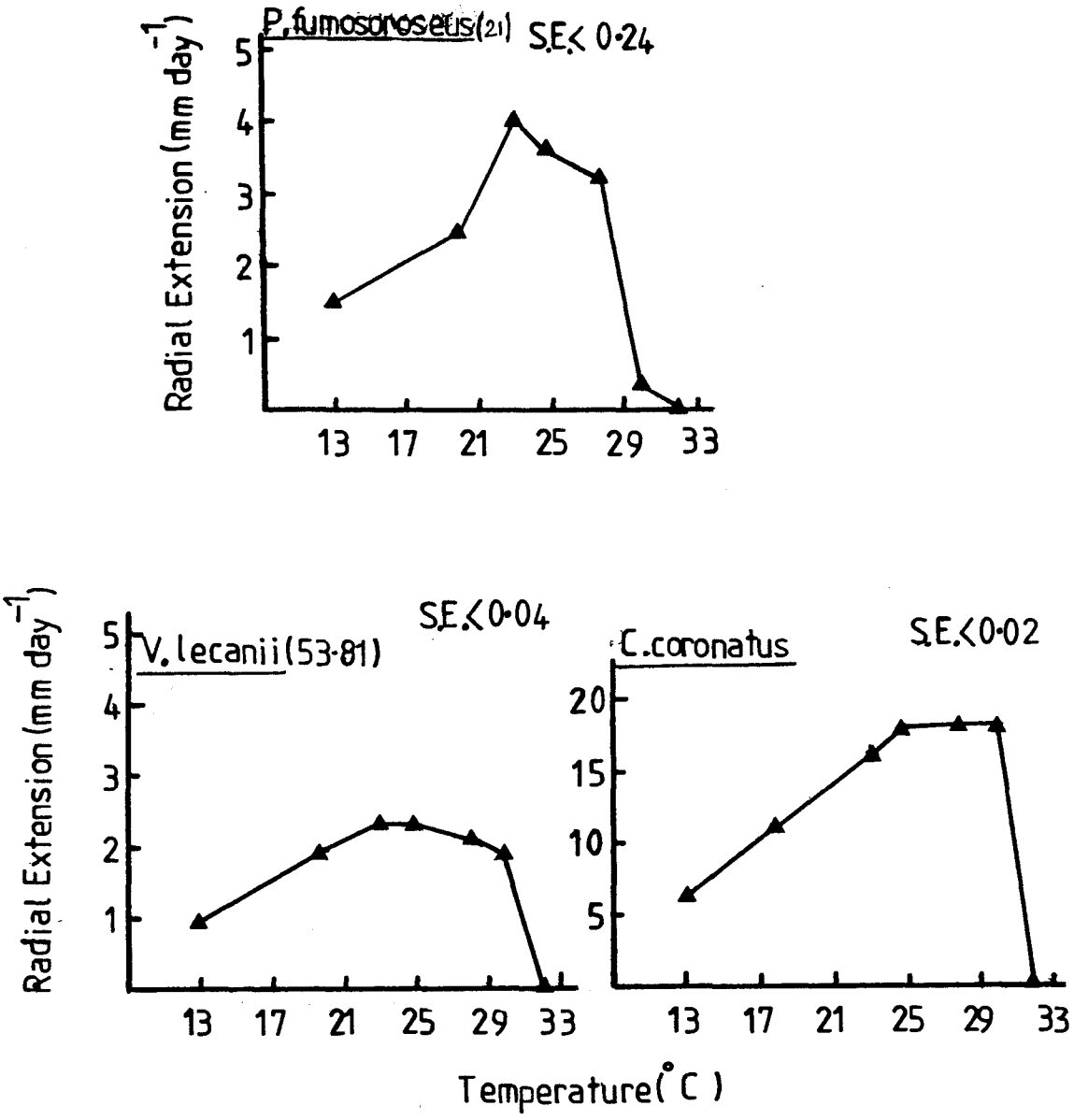


Fig. 5.12
Growth rates at various temperatures of a. Verticillium lecanii (53-81), b. Conidiobolus coronatus (1) and c. Paecilomyces fumosoroseus on Sabouraud dextrose agar. (Points are means based on 5 replicate plates).

C. coronatus grew optimally at 25-30°C but growth was absent at 32°C. Growth was extremely rapid, reaching 1.8 cm day^{-1}.
P. fumosoroseus (21) grew most rapidly at 23°C, slowly at 30°C and not at all at 32°C.
V. lecanii (53-81) grew best at 23-25°C and not at 32°C.

12. Effect of Humidity

a. Spore Germination

Conidia of both M. anisopliae (ME2) and V. lecanii (1-72) germinated at relative humidities from 95-100%, but not at 92.5 and 88.5% (Fig. 5.13). Germination of M. anisopliae conidia was significantly less in experiment II, but followed the same pattern. Evidence that potassium nitrate, used to maintain a humidity of 92.5%, was not toxic to fungi, was provided when a contaminating fungus was observed growing at this humidity.

b. Sporulation

Results obtained in vitro were similar to those from in vivo experiments. M. anisopliae (ME2), B. bassiana (63), P. fumosoroseus (21) and V. lecanii (53-81) sporulated at relative humidities from 95-100% but not below these levels (Table 5.05-06). Thus, levels of relative humidity required for conidial germination and sporulation are identical.

13. Effect of Light on Sporulation

a. Conidial Production in Constant Light or Dark

Light increased conidial production in all fungi examined (Fig. 5.14). However, the increase in sporulation was only significant ($p = 0.05$) for P. fumosoroseus (21) and V. lecanii (1-72). P. fumosoroseus produced 2.5 times more conidia in light than in darkness, cf. 4.7 times for V. lecanii (1-72).
Small-spored fungi (P. fumosoroseus, B. bassiana) produced significantly more conidia than those with large spores (M. anisopliae, V. lecanii (1-72)).

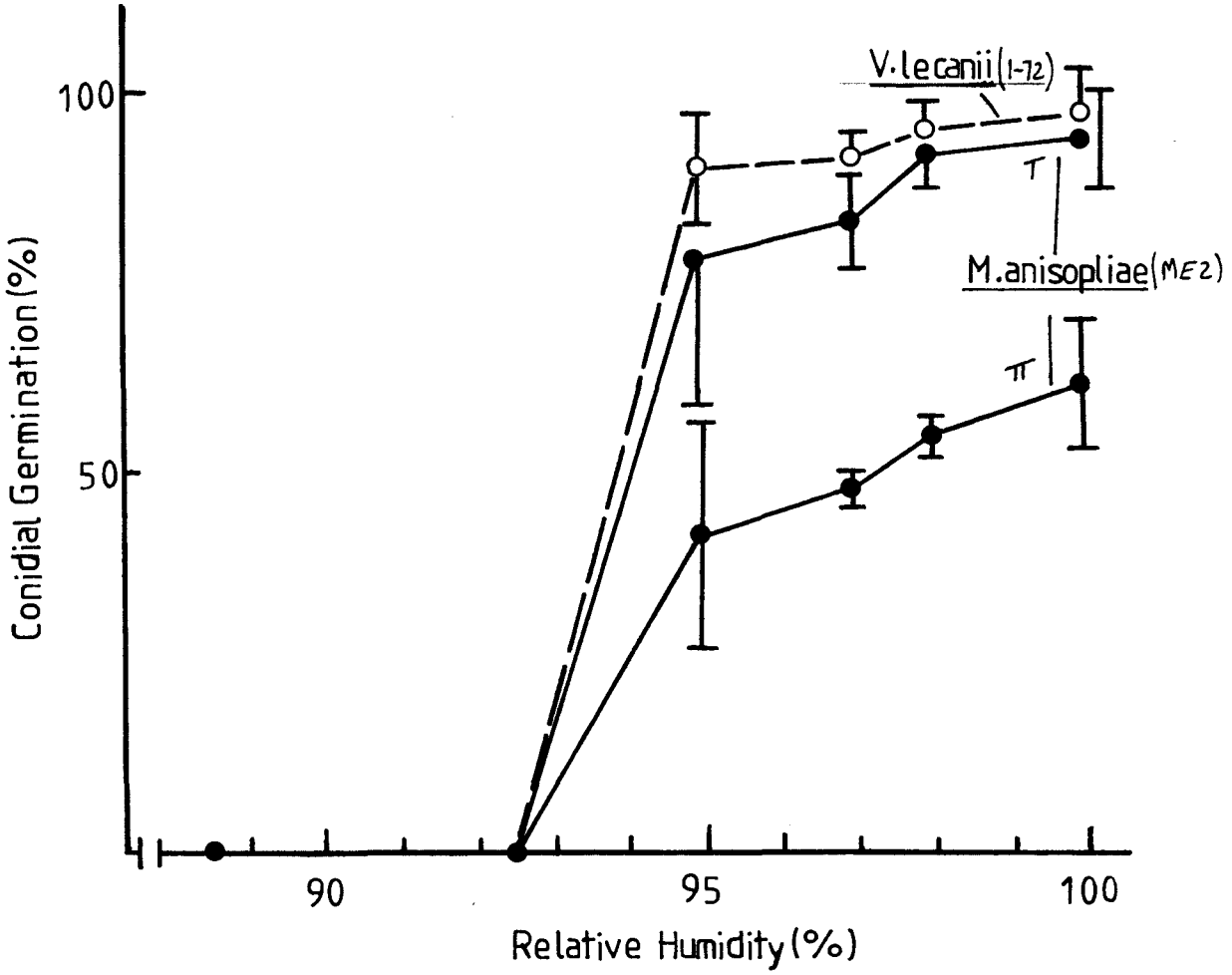


Fig. 5.13
The effect of relative humidity on conidial germination of Metarhizium anisopliae (ME2) and Verticillium lecanii (1-72) Points are means of three replicates with 95% confidence limits.

Table 5.05

The Effect of Relative Humidity on Sporulation of Fungi, *in vitro*.

Species and Strain	Percent relative humidity					
	88.5	92.5	95	97	98	100
Metarhizium anisopliae (ME2)	-	-	+	+	+	+
Beauveria bassiana (63)	-	-	+	+	+	+
Paecilomyces fumosoroseus (21)	-	-	+	+	+	+
Verticillium lecanii (53-81)	-	-	+	+	+	+

\+ sporulation

\- no sporulation

Results are based on three membranes treatment^{-1} maintained at each humidity for 96 h at 25°C

Table 5.06

The Effect of Relative Humidity on Sporulation of Fungi, in vivo.

Species and Strain	Percent relative humidity					
	88.5	92.5	95	97	98	100
Metarhizium anisopliae (ME2)	-	-	+	+	+	+
Beauveria bassiana (63)	N.A.[a]	-	+	+	+	+
Beauveria brongniartii (65)	-	-	+	+	+	+
Verticillium lecanii (53-81)	-	-	+	+	+	+

\+ Sporulation

\- No sporulation

Observations made on 10 cadavers per humidity maintained for 96 h at 25°C

a Not available

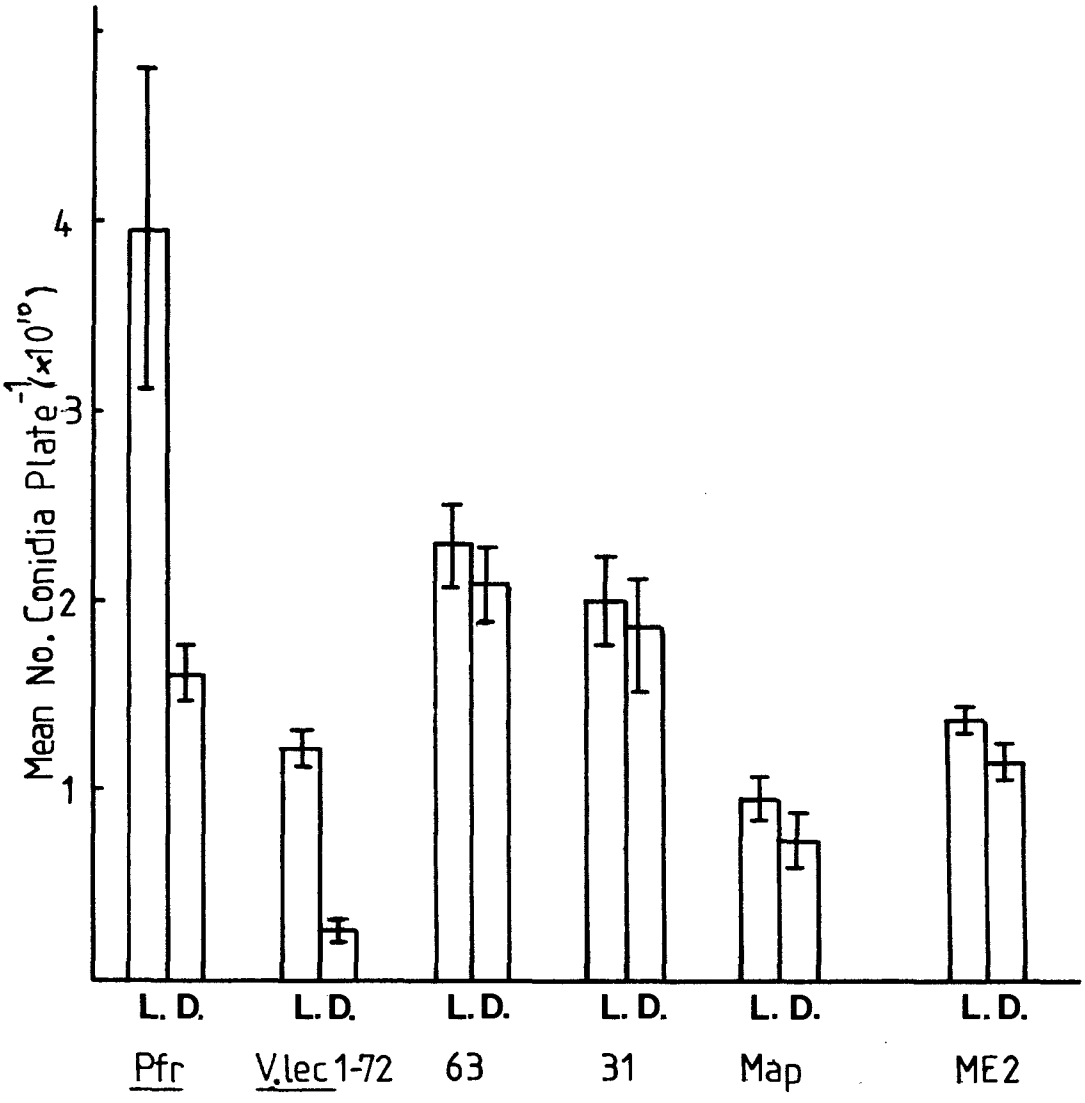


Fig. 5.14
Conidial production (9-cm Petri plate^{-1}) of Paecilomyces fumosoroseus (21), Verticillium lecanii (1-72), Metarhizium anisopliae (ME2, Map) and Beauveria bassiana (31, 63) on SDA at 20C, after 12 days incubation in fluorescent light (L) or darkness (D). (Histogram shows mean yields of 5 plates treatment^{-1} with 95% confidence limits).

b. The Effect of Light, Near Ultra Violet Light or Dark on Conidial Production

Sporulation of P. fumosoroseus (21) and V. lecanii (1-72, 53-81) in response to different light regimes varied (Fig. 5.15).

P. fumosoroseus produced most spores in fluorescent light (4.8×10^{10} conidia plate^{-1}). In darkness sporulation was significantly ($p = .001$) less (1.9×10^{10} conidia plate^{-1}) while under Near ultraviolet illumination (Nuv) the reduction was dramatic, only 2.8×10^{8} spores being produced.

V. lecanii (53-81) responded differently. Most spores were produced in Nuv light (1.75×10^{10} conidia plate^{-1}), slightly less in fluorescent light (6×10^{9}) and only 1.6×10^{8} in darkness. In contrast V. lecanii (1-72) produced most spores in fluorescent light (1.1×10^{10} conidia plate^{-1}) and conidial production was less under conditions of Nuv illumination (6.3×10^{9}) or darkness (2.4×10^{9}).

Fungal morphology was also affected by light. Dark- grown P. fumosoroseus plates were white and 'flat' while those in Nuv or fluorescent light were pink and had a raised, floccose appearance (Fig. 5.16 a). Light also increased pigmentation in V. lecanii; dark-grown plates were white while those in Nuv or fluorescent light had pronounced yellow pigmentation (Fig. 5.16 b). Osman and Valadon (1981) observed similary increased pigmentation in Verticillium agaricinum grown in Nuv and showed the increase was due to increased carotenoid production.

These results agree well with those of the previous experiment (V, 13, a) where fluorescent light stimulated conidial production of P. fumosoroseus (21) and V. lecanii (1-72) by 2.5 and 4.7 times respectively that obtained in darkness. In this experiment corresponding increases were 2.5 and 4.6.

Sporulation of the fungi examined was increased by fluorescent light, while the response to Nuv light varied, even between V. lecanii strains. Why this differential response occurred is not known.

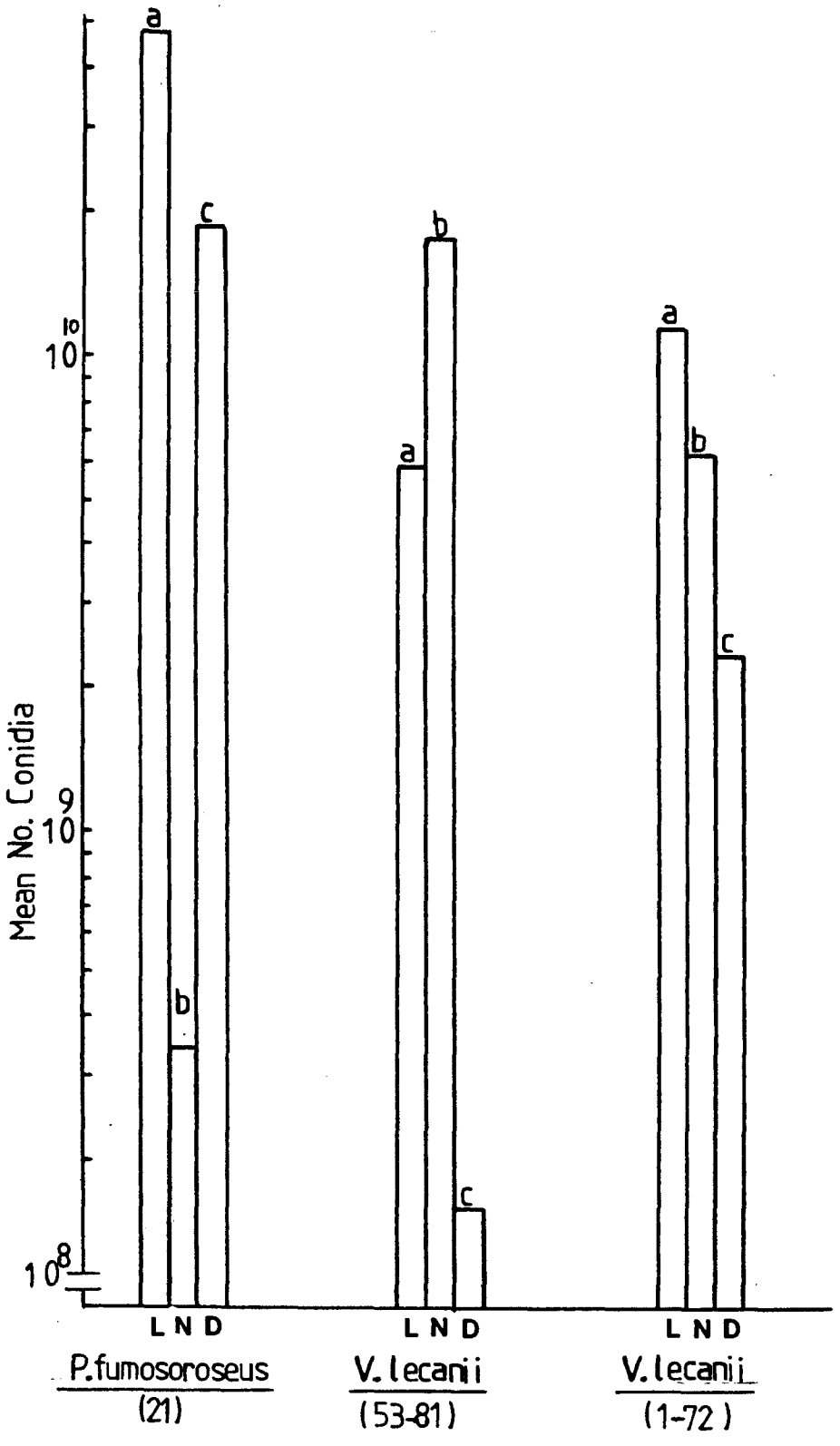
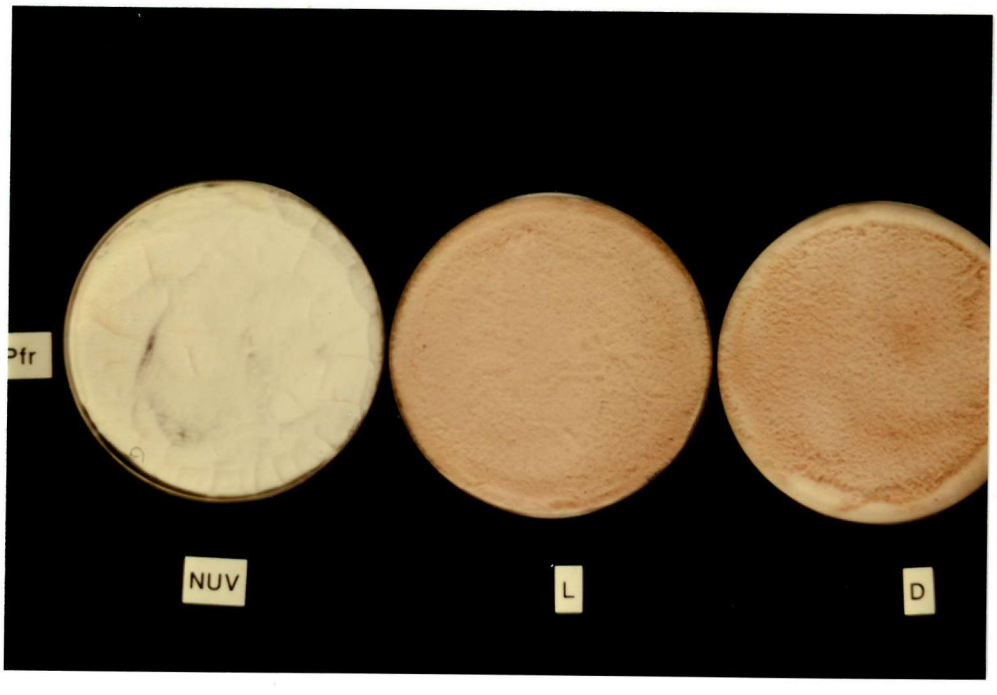


Fig. 5.15

Conidial production (9cm diam. Petri plate^{-1}) of Paecilomyces fumosoroseus (21) and Verticillium lecanii (53-81, 1-72) on SDA at 20°C, after 12 days incubation in fluorescent light (L), near ultraviolet (N) or darkness (D). Mean yields of 5 plates treatment^{-1}. Each fungus produced significantly different numbers of conidia in the various radiation regimes.

a



b

Fig. 5.16
Cultures of a) Verticillium lecanii (53-81, 1-72) and b) Paecilomyces fumosoroseus (21) grown in Near ultraviolet light (NUV) fluorescent light (L) and darkness (D).

14. The Effect of Fungicides on Metarhizium anisopliae

a. Conidial Germination

The effect of 12 fungicides, incorporated into SDA, on conidial germination of M. anisopliae (ME2) is shown in Fig. 5.17. Variation between replicates was low and standard errors were generally below one and ranged from 0.24-3.0. All fungicides, except benomyl and chlorthalonil, had little effect on germination at 0.1 times the recommended rate, but at ten times this rate, inhibited germination to varying degrees. Carbendazim, dinocap, etridiazole and bupirimate had no effect, while thiophanate - methyl, iprodione and pyrazophos reduced germination to about 80%. Imazalil (60%) and zineb (17% germination) were more strongly inhibitory and benomyl, triforine and chlorothalonil prevented spore germination. The least toxic compounds were bupirimate and dinocap, which reduced germination only slightly at ten times the recommended dose, while all other fungicides tested, completely inhibited germination at this rate.

b. Mycelial Growth

Mycelial growth of M. anisopliae (ME2) was generally more sensitive to fungicides than spore germination (Fig. 5.18). Variation between replicates was negligible (S.E. $<$ 1.0; range 0.2 - 0.97). Benomyl, carbendazim and imazalil completely inhibited mycelial growth at all concentrations, while only zineb and etridiazole had no effect at the lowest rate. At recommended rates all fungicides reduced mycelial growth and at the highest concentration inhibition was either complete or very strong.

c. Sporulation

Sporulation was assessed visually over the area of mycelial growth and no attempt was made to account for the loss in spore production caused by reduced rates of radial

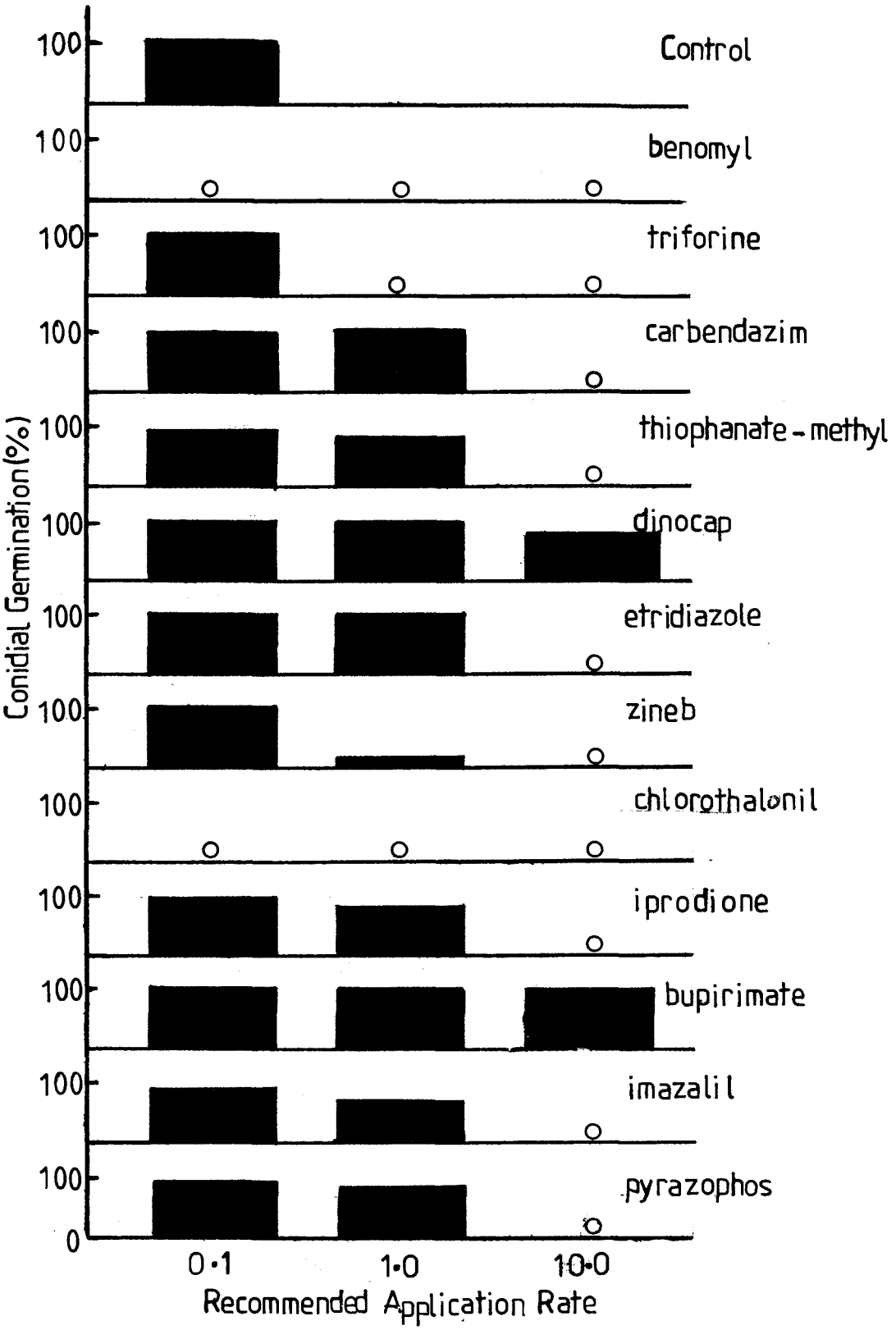


Fig. 5.17

The effect of fungicides incorporated into Sabouraud dextrose agar on conidial germination of Metarhizium anisopliae (ME2). (Means of 5 replicates treatment^{-1}).

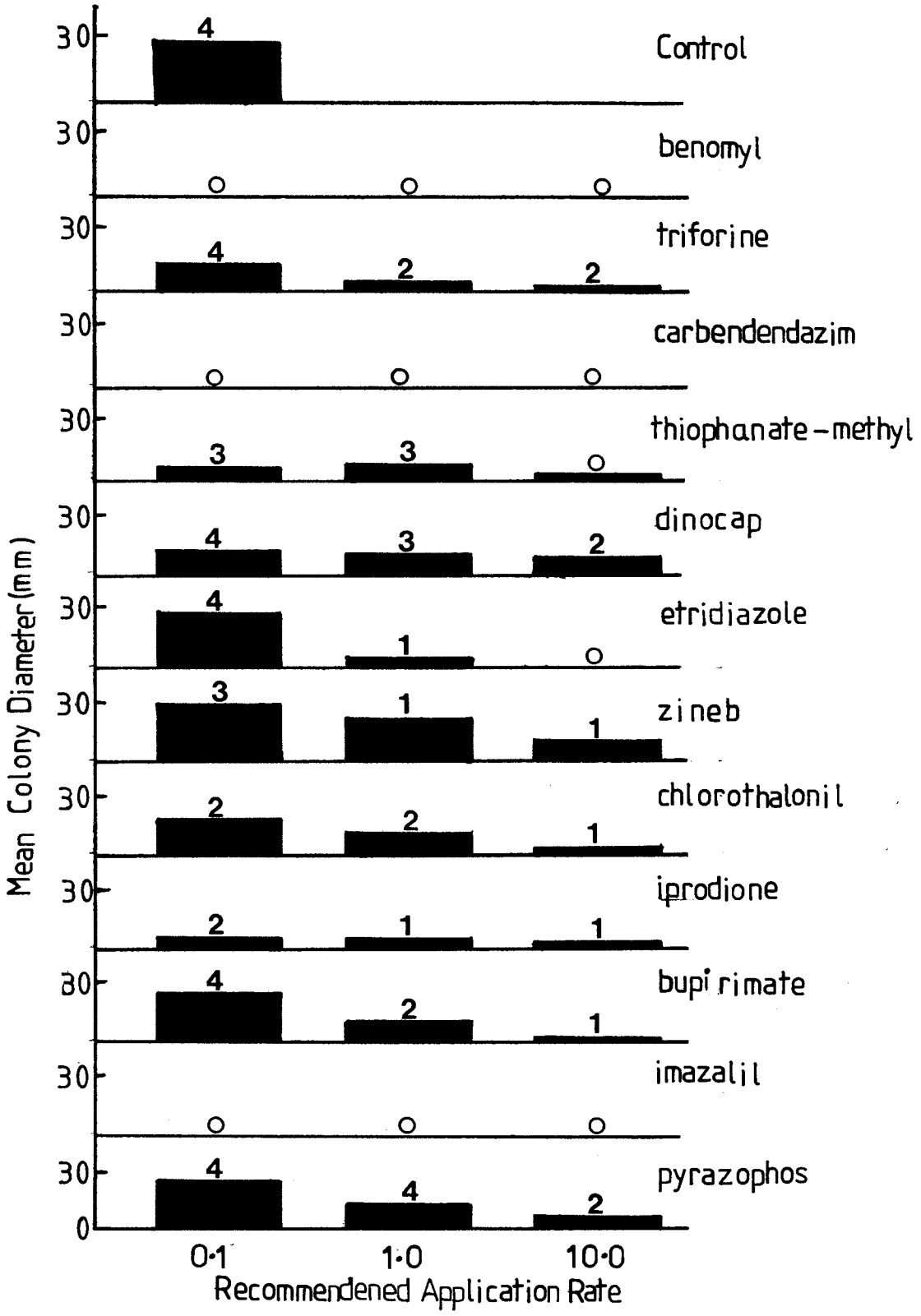


Fig. 5.18

The effect of fungicides incorporated into Sabouraud dextrose agar on mycelial growth of Metarhizium anisopliae (ME2). (Means of 5 replicates treatment^{-1}: suffix indicates degree of sporulation - 4 max).

growth. Degrees of sporulation are shown in Fig. 5.18. Sporulation was greatest where M. anisopliae was grown on SDA containing pyrazophos, dinocap or triforine. Other fungicides inhibited sporulation strongly, particulary at the highest incorporation rate (iprodione, chlorothalonil, zineb and etridiazole) while some reduced sporulation less (thiophanate-methyl and bupirimate). Obviously, where mycelial growth was absent, no sporulation occurred.

15. Growth and Sporulation of Metarhizium anisopliae on Rice Plants

Numbers of M. anisopliae (ME2) conidia deposited on rice plants were similar when spores were applied in 0.025% Triton X-100 or 0.5% skimmed milk (Table 5.07). A reduced milk concentration (0.05%) resulted in the deposition of less conidia, while higher rates (1.0, 2.5%) increased spore-leaf adhesion.

All tested concentrations of skimmed milk increased numbers of conidia on leaves, compared to those obtained with 0.025% Triton X-100, seven days after treatment. This increase was presumably due to germination and subsequent sporulation of applied conidia, which utilized substances in the skimmed milk as nutrients. Microscopic examination of leaves, treated with conidia, contained in 1.0 or 2.5% skimmed milk, showed characteristic long chains of M. anisopliae conidia growing from milk deposits, and thus confirmed that sporulation had occurred.

Application of conidia, together with 0.5% skimmed milk, increased conidial numbers on leaf surfaces by 12 times, compared to conidia applied without nutrients. This concentration of added nutrients (5 g l^{-1}) approaches the maximum which could be included in a final product, and while increased amounts of skimmed milk greatly increased sporulation, they are unlikely to be adopted commercially. Thus, it might be rewarding to search for alternative compounds more stimulatory to sporulation.

Formulations of fungi which allow growth and sporulation after application to leaf surfaces, are extremely desirable, as they afford a measure of persistence, provide inocula for insects missed by the initial spore spray and permit

Table 5.07

Production of conidia on rice plants after spraying Metarhizium anisopliae (ME2) spores together with skimmed milk

Percent skimmed milk	Conidia gram leaf^{-1} after 0 days	7 days[a]	Increase in conidial production compared to control
0[b]	1.75×10^5	2.5×10^5	—
0.05	6.0×10^4	7.5×10^5	x 3
0.5	1.65×10^5	3.0×10^6	x 12
1.0	3.4×10^7	1.85×10^7	x 74
2.5	3.4×10^5	1.23×10^8	x 492

a Estimated by plate counts

b 0.025% Triton x-100

One plant used for each treatment; assessments made using leaf tissue collected at random

prophylatic application. Three commercially produced myco-insecticides; Mycar®, containing Hirsutella thompsonii, Mycotal® and Vertalec® (V. lecanii), contain nutrients which permit growth and sporulation of fungus after application.

16. Storage

a. On SDA slopes

All fungi, except M. anisopliae (ME2), were viable 862 days after storage at 2 or -20°C (Table 5.08). Cultures maintained at 20°C were viable 51 days after storage, but not after 210 days. All fungi were stored in 'Bijoux' bottles with the cap loose, as cultures lose viability rapidly when in sealed containers, presumably due to oxygen lack or accumulation of volatile toxic metabolites (Hall, 1977). Cultures stored at 20°C became desiccated and this probably accounted for their rapid loss of viability. Generally, conidia survived longer at 2 than at -20°C, though the situation was reversed for B. bassiana (63) and P. fumosoroseus (21)

b. In .01% Triton x-100 solution

Conidia and hyphal bodies of M. anisopliae (ME2) were stored in 0.025% Triton x-100, at a range of temperatures. A surfactant was essential as M. anisopliae conidia were extremely hydrophobic and would not suspend in distilled water. Hall (1977) showed that concentrations of 0.2% Triton X-100 had little effect on conidial survival of V. lecanii (1-72)

Conidia and hyphal bodies died most rapidly at 2°C (Figs. 5.19-20) and only 23 and 59% respectively, were viable after 130 days. Survival of hyphal bodies was similar at 10, 20 and 25°C, and 97 days after storage, 84-91% of spores were viable. Hyphal bodies survived less well at 30°C and viability declined to 76% after 97 days. Conidia survived slighty better. After 97 days storage at 10, 20 or 25°C, 97-100% of conidia were viable, compared to 91% for those stored at 30%.

The rapid death of both conidia and hyphal bodies at 2°C was surprising. Usually fungal spores survive well at low temperatures. Daoust et al. (1982) reported that

Table 5.08

Survival of conidia stored as S.D.A. slopes at 20, 2 and $^{-}20^{o}C$, with time. Figures are the mean of two replicate samples.

Species and strain	Temp. C	Percentage viability of conidia ± S.E at selected times after storage (days).						
		0	2	8	51	210	336	862
Metarhizium	20		97±2	100±0	99±1	0		
anisopliae	2	99±0	92±1	98±0	93±2	90±2	27±22	0±0
ME2	-20		87±1	91±1	72±2	80±6	20±3	6±0
Metarhizium	20		99±2	95±3	98±1	0		
anisopliae	2	97±2	97±2	96±2	94±0	89±2	86±5	21±5
Pemphigus	-20		93±1	86±1	81±1	56±3	61±2	2±1
Beauveria	20		99±0	89±2	N.A.	0		
bassiana	2	100±0	100±0	72*	82±15	64±3	87±3	24±5
31	-20		92±5	98±0	N.A.	85±0	50±36	11±2
Beauveria	20		100±0	100±1	1	0		
bassiana	2	100±0	100±0	98±1	N. A.	86±1	17±15	4±2
63	-20		99±1	99±0		88±2	86±	86±1
Paecilomyces	20		95±0	96±3	97±0	0		
fumosoroseus	2	98.5±2	99±1	98±1	98±1	96±0	96±0	20±5
21	-20		95±1	92±2	96±1	90±1	61±2	18±16
Verticillium	20		96±3	97±1	74±5	0		
lecanii	2	98±0	99±1	100±0	87±6	75±2	65±10	60±3
1-72	-20		99±1	91±2	90±2	81±1	69±8	3±1
Verticillium	20		99±0	99±1	98±1			
lecanii	2	100±0	98±1	100±1	98±1	94±1	98±1	52±40
Zp.	-20		99±0	98±1	97±0	97±1	96±1	88±5

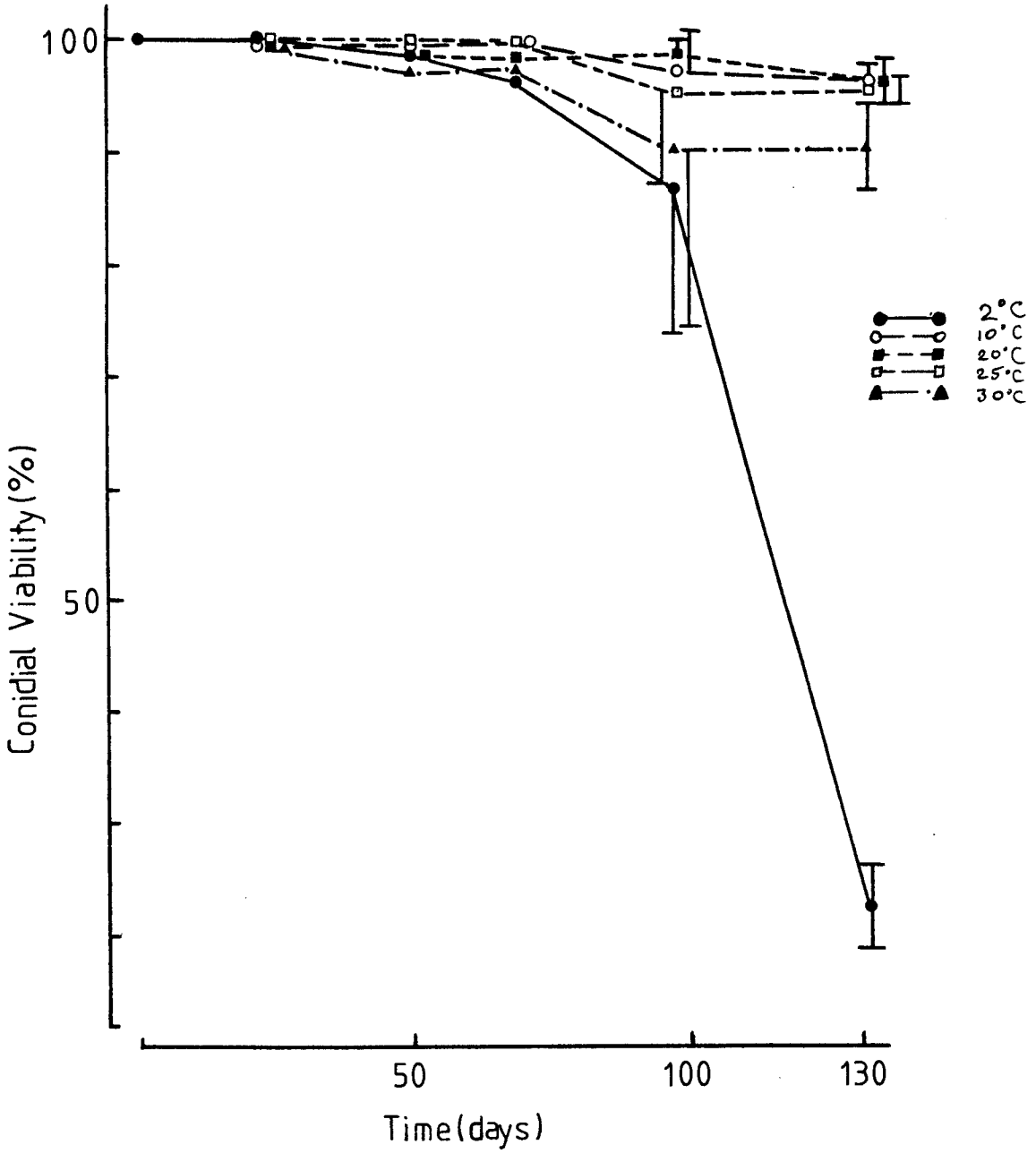


Fig. 5.19
Survival of conidia of Metarhizium anisopliae, as indicated by germination on Sabouraud dextrose agar, stored in 0.01% Triton X-100 at various temperatures. (Points are means of 3 replicates with 95% confidence limits).

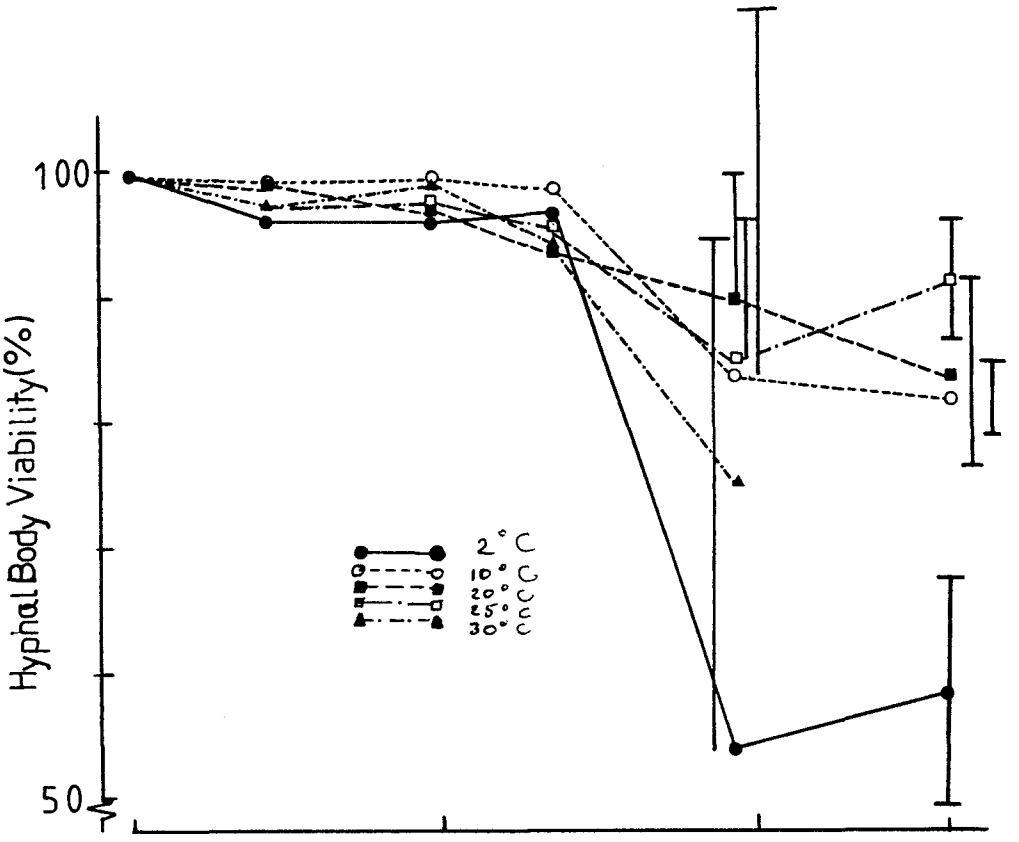


Fig. 5.20
Survival of hyphal bodies of Metarhizium anisopliae, as indicated by germination on Sabouraud dextrose agar, after storage in 0.01% Triton X-100 at various temperatures. (Points are means of 3 replicates with 95% confidence limits.)

M. anisopliae conidia, stored in water at 20°C lost viability within 4 months, while at 4°C, 42% were still viable. Further study is needed, but time did not permit additional experiments.

c. On silica gel

Generally, strains of Deuteromycete fungi survived well on silica gel (Table 5.08). In contrast Conidiobolus coronatus, a member of the Entomophthorales, could not withstand the drying process and died within 24 h. M. anisopliae isolates survived poorly; after 730 days storage strain ME2 was dead, while only 33% of tubes containing strains ME3 and Map contained viable conidia. B. brongniartii survival was adequate and the use of several tubes ensured survival for 584 or 730 days. Survival of B. bassiana, Paecilomyces and V. lecanii isolates was excellent and all tubes contained viable conidia after 730 days. Thus, survival on silica gel varied with different species, but can be recommended for some.

M. anisopliae survival might be prolonged at temperatures above 2°C as suggested by earlier experiments ($\bar{V}$, 15, a). Storage of strains on silica gel has several advantages. Spores are maintained in a dehydrated state, thus growth and possible variation is eliminated; strains do not have to be sub-cultured and sampling is rapidly accomplished.

d. Effect of Relative Humidity on Conidiospore Survival

i. Metarhizium anisopliae

Conidia of M. anisopliae (ME2) survived well at high relative humidities but poorly at humidities below 93% (Fig. 5.21). After 126 days storage at relative humidities of 93, 97 or 100%, approximately 90% of conidia were viable, while at 76 or 58% all conidia were dead.

ii. Beauveria bassiana

Conidia of B. bassiana (63) survived less well than those of M. anisopliae (ME2) (Fig. 5.21). Conidia survived longest at high relative humidities; after 126 days at 100, 97 or 93% r.h. , 61, 53 and 10% of conidia were viable, while at 76 or 58% r.h., all conidia were dead. Some evidence

Table 5.08

Survival of fungal isolates stored on silica gel at 2°C

Species and Strain	Viability on selected days after storage determined as growth(+) or no growth(-) onSDA						
	1	145	194	241	344	584	730
Beauveria bassiana							
1	++			++			
31	+++	+++			+++		+++
32	+++	+++					+++
43	++			++			
45	++			++			
50	++			++			
53	++			++			
63	+++	+++			+++		+++
Beauveria brongniartii							
6	+++	+++			+++		+--
40	++		++			+-	
65	++		++			+-	
Conidiobulus coronatus							
1	--					--	
Metarhizium anisopliae							
ME2	+++	+++			++-		---
ME3	+++	+++			+++		+--
Map	+++	+++			++-		+--
52	++		++			+-	
1140	++		++			++	
Paecilomyces fumosoroseus							
21	+++	+++			+++		+++
Paecilomyces sp.							
2	++		++			++	
Synecphalastrum racemosum							
	++		++			++	
Verticillium lecanii							
1-72	+++	+++			+++		+++
53-81	++			++			
Zp	+++	+++			+++		+++
T.t.	+++	+++			+++		++c

c. Crystal contaminated

Variable sampling dates as strains stored on three dates; 25/7/80; 22/12/80 and 30/11/81 .

Each + represents one replicate tube.

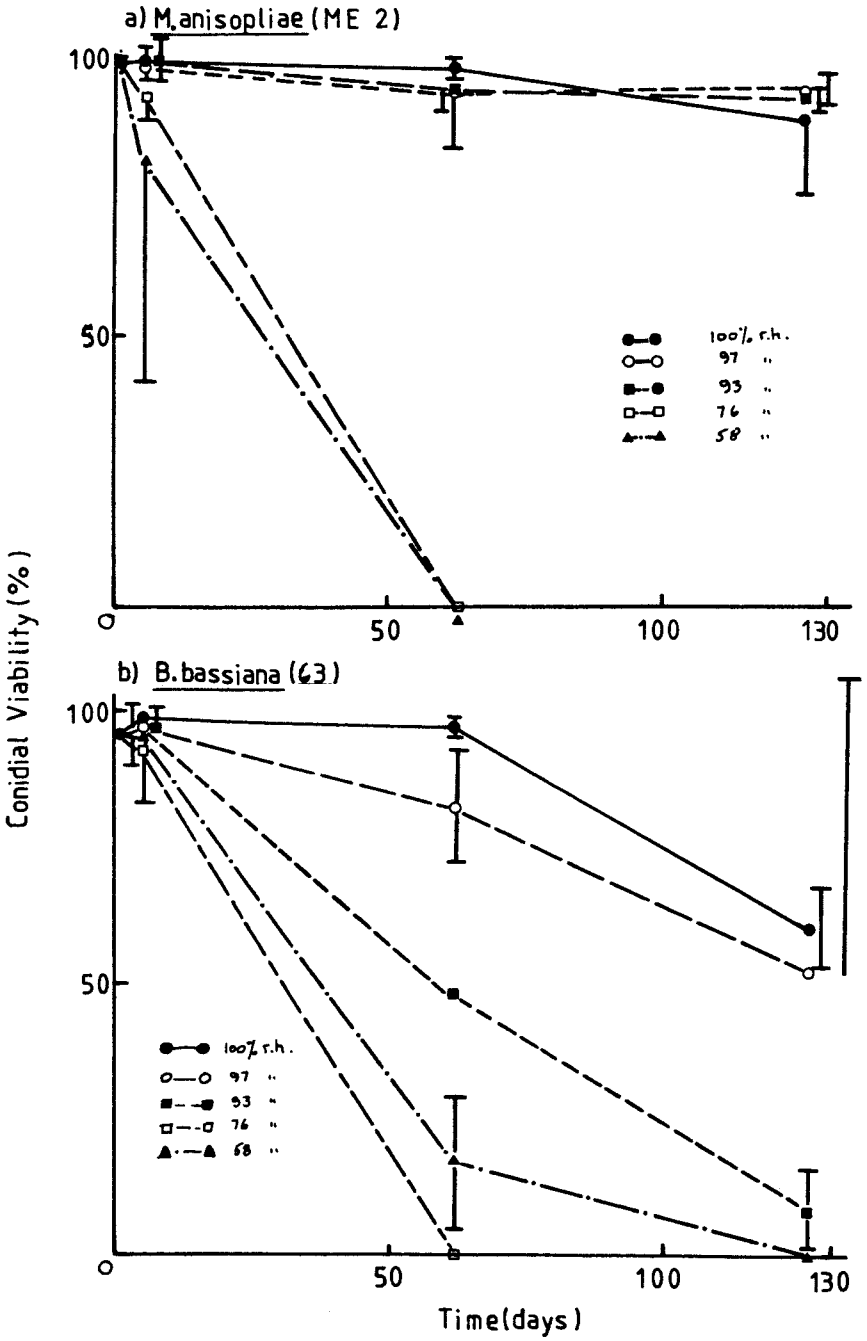


Fig. 5.21
Survival of conidia, estimated by germination on Sabouraud dextrose agar, of a) Metarhizium anisopliae (ME2) and b) Beauveria bassiana (63) on cadavers of Hauptidia maroccana maintained at various humidities at 20°C. (Points are means of 3 replicates with 95% confidence limits).

of longer survival at low humidities, as reported by Clerk and Madelin (1965) was obtained as conidia survived longer at 58% r.h. than at 76%.

DISCUSSION

The main aim of the experiments in this section was to obtain data relevant to the production and use of fungi as myco-insecticides. Thus, this discussion concentrates on practicalities and includes purely scientific aspects only to explain observed data.

Optimum temperatures for conidial germination and mycelial growth on agar, may not be directly applicable *in vivo*. Penetration of insect cuticle is partly dependent on enzymes (Brobyn and Wilding, 1977; Lambiase and Yendol, 1977; Grula *et al.*, 1978) and many entomogenous fungi produce proteases, chitinases and lipases *in vitro* (see Hall and Papierok, 1982). The optimum temperatures for conidial germination and mycelial growth may differ from those for enzyme production and/or activity. However, spore germination and limited mycelial growth must precede penetration and there is probably a close correlation between temperatures optimal for growth on agar, and those for infection. Storage rot disease caused by phytopathogenic fungi are usually most severe at temperatures favouring pathogen mycelial growth (Weimer and Harter, 1923; Lauritzen and Harter, 1925; Lauritzen, 1929; Wellman, 1932). Similar data for entomogenous fungi are lacking, though Doberski (1981 b) reported greater infection of *Scolytus scolytus* by *M. anisopliae* and *B. bassiana* as temperatures were increased from 2° to 20°C, a result which correlates well with data presented in this section. Further, in this study, mortality of *Hauptidia maroccana*, treated with *M. anisopliae* (ME2), was highest at 25°C and was significantly greater than at 13 or 20°C (VI, 4, α, ī×). Conidial germination rates are of great importance, particularly where fungi are considered for control of glasshouse pests, as in this environment, humidities sufficient for fungal growth generally only occur at night (VII, 5, α). Conidia of *Beauveria*, *Metarhizium* and *Paecilomyces* germinated slowly at 20°C (the night temperature for cucumber production) while those of *V. lecanii* produced germ tubes more rapidly.

Pekrul and Grula (1979) demonstrated cuticular penetration by B. bassiana after 16-18 hours at 25°C, immediately after conidial germination, or following limited mycelial growth. Hughes (unpub. obs.) showed similar direct penetration in V. lecanii and the present study demonstrated aphid infection with V. lecanii (1-72) occurred after 16 hours at 20°C (VI,a ,vii). In contrast, M. anisopliae penetrated insect cuticle only after appressorial formation (Zacharuk, 1973; Schabel, 1978), a process which may extend the time needed for infection. The present study demonstrated infection of H. maroccana and M. anisopliae (ME2) required 48 h at 20°C. Conidial germination rates are also important when control of frequently ecdysing insects is considered. Farques and Vey (1974) showed Colorado beetle larvae could avoid B. bassiana infection by moulting and shedding the inoculum, an effect likely to be most important with slow-germinating fungi. Mutants of M. anisopliae, hyper-virulent to mosquito larvae, all had conidial germination rates higher than those of wild type (Al-Aidross and Roberts, 1978; Al-Aidross and Seifert, 1980). Of the fungi tested in glasshouses, only V. lecanii reliably controlled target insects (VII, 6,a b,c) and also controlled aphids and whitefly (Hall and Burges, 1979; Hall, 1982) with the result that this fungus is used commercially. It is likely that the success of V. lecanii in the glasshouse environment is correlated with its rapid conidial germination.

M. anisopliae strains differed in their response to temperature, both in mycelial growth rate at a given temperature and in temperature optima. One strain, M. anisopliae (52), grew similarly at 23 and 25°C, but more rapidly at 28°C. This unusual profile has also been observed by Pinnock(pers. comm.) and is probably indicative of a change from a low to a high temperature metabolism. Growth profiles of two B. bassiana strains were also distinct. One, B. bassiana(63) grew optimally at 25°C and slowly at 30°C, while B. bassiana (31) grew optimally at 25-30°C. Variations in optimum and maximum growth temperatures might be expected to correlate with climatic conditions in the area of isolation: sub-tropical strains may have higher temperature optima than

those from temperate areas (Brown and Wood, 1953). However, no such correlation was found with V. lecanii strains (Hall, 1977; Hall and Turner, unpub. obs.). In the present study, the Map strain of M. anisopliae, isolated in the U.K., had a lower temperature optimum than the strain ME2 isolated in Brazil but others (ME3, 52, 1140) isolated in N. Europe, also had high optimal growth temperatures.
A relative humidity (r.h.) of 95% was necessary for conidial germination and sporulation in all fungi examined. Walstad et al. (1970) observed germination and sporulation of M. anisopliae at relative humidities of 92.5%, when conidia were positioned on agar and applied in distilled water - this addition of moisture may have increased humidities above the calculated levels. Ferron (1981) recorded germination at 92% though his report lacked experimental details. B. bassiana conidia on glass slides germinated at relative humidities from 94-100% (Hart and MacLeod, 1955). Conidia of M. anisopliae (ME2) germinated poorly on glass slides ($<$ 1%) which necessitated the use of dried membranes, previously autoclaved in SLM, to provide nutrients. Sporulation on cadavers was possibly influenced by residual moisture in the dead insects. However, sporulation on membranes in vitro was similar to that in vivo, so this possibility can be discounted.
The humidity encountered by conidia on insect cuticles may be above ambient due to microclimates created by transpiration from spiracles, intersegmental membranes, or stomata. Kramer (1980) reported infection of flies with Entomophthora muscae at a nominal 0% r.h. and Doberski (1981 b) observed infection of Scolytus scolytus with M. anisopliae and B. bassiana at relative humidities of 51%. In the present study, mortalities of Thrips tabaci, treated with V. lecanii, were higher on lower than upper leaf surfaces (VII, 6, α) probably due to a higher microclimate humidity on lower surfaces, caused by more numerous stomata, protection from solar radiation, or reduced air movement.
Stimulation of conidial production by light has often been reported in Deuteromycetes. Hedgecock (1906) observed

diurnal, daylight - induced, zonation in Cephalosporium and Hall (1977) demonstrated V. lecanii (1-72) produced more conidia in constant light than in dark. The present study confirms the result of Hall (1977) and also showed light stimulated conidial production in V. lecanii (53-81) and Paecilomyces fumosoroseus (21). Near ultraviolet light (NUV) also stimulates conidial production in many fungi including Aspergillus ornatus, Penicillum isariiforme, Trichoderma viride and Septoria spp. (Cooke and Jones, 1970; Lee and Jones, 1974; Tan, 1978). This light impaired conidial germination of Verticillium agaricinum (Osman and Valadon, 1981) and to avoid such effects in the present work, conidia were germinated in darkness, before placement in NUV for study of its effects on growth. Sporulation of V. lecanii strains responded differently to the various light regimes. Conidial production of strain 1-72 was highest in fluorescent light while 53-81 produced most conidia in NUV. Sporulation of P. fumosoroseus (21) was inhibited by NUV but stimulated by fluorescent light. Hall (1933) found light inhibited conidiation of Monilinia fructicola but stimulated that of M. fructigena and Leach (1962) found Ascochyta pisi isolates varied in their ability to form pycnidia in darkness. Thus, it is impossible to predict how a fungal species, or strain, will respond to irradiation. The mechanism by which light exerts its effect on sporulation is poorly understood. Flavin photoreceptors and cytochrome systems have been implicated for Alternaria solani and Neurospora crassa (Lukens, 1963; Munoz and Butler, 1975). In NUV - irradiated cultures of Ascochyta pisi a different receptor molecule was found and designated "P 310", which stimulated sporulation of several fungi responding to NUV (Trione and Leach, 1969). Presumably these receptor molecules initiate metabolic pathways that lead to conidiophore formation.

These results are of most importance when producing fungi for use as myco-insecticdes. Semi-solid fermentations of V. lecanii or P. fumosoroseus in light would result in increased conidia production and thus make the process more economic. In the field there would be sufficient light to stimulate conidial production on cadavers and

thus provide inoculum for disease spread.
When conidial production was estimated on SDA plates, small-spored strains (P. fumosoroseus, B. bassiana) produced more than those with larger spores (M. anisopliae, V. lecanii; 1-72). Thus, it is likely commercial production of numbers of conidia would be maximal with small-spored strains. Productivity must be considered when analysing bioassay data. Selection of the strain most pathogenic to the pest may not be the best choice, as control might be achieved more economically with a less virulent strain able to produce more conidia.
Osman and Valadon (1981) reported increased hyphal wall thickness of V. agaricinum grown in NUV. If similar increases occurred in V. lecanii conidia, they might be more resistant to desiccation and easier to store than non irradiated conidia.
The effect of fungicides on M. anisopliae was studied by incorporation of compounds into agar, a method employed by many workers (Ramaraje Urs et al., 1967; Olmert and Kenneth, 1974; Zimmermann, 1976; Wilding and Brobyn, 1980; Hall, 1981). Wilding (1972), working with V. lecanii (cited as Cephalosporium aphidicola), measured growth inhibition around fungicide - treated filter paper discs and observed that benomyl and triarimol prevented growth. However, previous treatment of cucumber plants with triarimol did not impair infection of aphids with V. lecanii. Hall (1981) observed that many pesticides, inhibitory to V. lecanii when incorporated in agar, only slightly reduced aphid mortality, particulary when applied before rather than after spore sprays. Irvine (unpub. obs.) and Yendol and Hamlin (1973) also observed that in vitro results did not always correlate with those in vivo. Hall (1981) found mycelial growth of V. lecanii was less affected by pesticides than spore germination, but for M. anisopliae the reverse was generally observed: iprodione, imazalil and carbendazim had little effect on spore germination, but strongly inhibited mycelial growth. Benomyl strongly inhibited Erynia neoaphidis (cited as Entomphthora aphidis), V. lecanii and B. bassiana (Wilding, 1972; Olmert and Kenneth, 1974; Wilding and Brobyn, 1980; Hall, 1981) and completely

prevented conidial germination and mycelial growth of M. anisopliae. Bupirimate and dinocap had little effect on germination of M. anisopliae, even at ten times the recommended application rate, and could probably be included with M. anisopliae in a programme of integrated control. Other fungicides require further testing in vivo before recommendations can be made. Several workers have shown pesticide use can be integrated with fungal control of insects. Kanagaratnan et al.(1982) reported that infection of glasshouse whitefly by V. lecanii was unaffected by dimethirimol, applied for mildew control and Clark et al. (1982) reported that integration of a fungicide with B. bassiana did not impair infection of Colorado beetle. In eastern Europe, B. bassiana is commonly used, in combination with reduced doses of insecticides, to control the same insect (Ferron, 1978).

All fungi in the present work, except M. anisopliae, survived for 862 days when stored at 2° or $-20^{\circ}C$. Cultures stored at $20^{\circ}C$ were dead after 210 days, desiccation probably being partly responsible. Above $0^{\circ}C$, survival of fungi generally decreases with increasing temperature (Anderson et al.,1948; Kawakami and Mikuni, 1965; Galanopoulus and Tribe, 1974). Conidia and hyphal bodies of M. anisopliae, suspended in 0.01% Triton X-100, survived poorly at $2^{\circ}C$,compared to 10°, 20°, 25°, or $30^{\circ}C$. This result contrasts with the data of Daoust et al. (1982) who observed optimal survival of M. anisopliae conidia, stored in water, at low temperatures. However, poor survival at low temperature has been reported previously. Berger (1970) observed that while cultures of the bacterium, Pseudomonas solanacearum, survived for 10 years in distilled water at room temperature, they died rapidly when refrigerated. Mc Ginnis et al. (1974) showed many fungal cultures survived for 4 years, when stored in water at room temperature and recommended this method for preservation of stock cultures.

Fungi were first preserved on silica gel by Perkins (1962). Bell and Hamalle (1974) reported that many entomogenous fungi survived for three years at $-20^{\circ}C$. In the present study, B. bassiana, Paecilomyces spp. and V. lecanii survived well and all replicate tubes contained viable

conidia after 584 or 730 days. In contrast, M. anisopliae and Beauveria brongniartii survived poorly and many tubes contained no viable spores after 584 days. Butterfield et al. (1974) reported virtually all fungi tested survived for 8 years, when stored in liquid nitrogen and this method should be used for long term storage of cultures, the use of silica gel being reserved to preserve cultures for routine laboratory use.

Most conidia of M. anisopliae, on insect cadavers, survived for 126 days at 93, 99 or 100% r.h. while at 76 or 58% all died within 62 days. These results agree with those of Clerk and Madelin (1965) who observed M. anisopliae conidia survived well at high and low humidities but poorly at intermediate ones.

The most lethal humidity for unwashed conida was 45%, while for washed (or wetted) conidia it increased to 65%. The latter humidity commonly occurs in glasshouses (VII,5,a) and thus conidia, which commenced germination during the night, might be even more vulnerable and lose viability more rapidly. Clerk and Madelin (1965) demonstrated that conidia of B. bassiana died more rapidly at 76, than at 34 or 0% r.h., but did not study survival at high moisture levels. In the present work conidia of B. bassiana (63) survived longest at relative humidities from 93-99%. Optimum r.h. for survival of B. bassiana conidia in soil at 25°C varied between strains. One strain survived best at 100% r.h. while the other retained similar viability at 33 or 100% (Lingg and Donaldson, 1981). Thus, B. bassiana conidia would also survive poorly in glasshouses.

Perhaps the most striking feature of the results discussed in this section is the variation encountered between strains of the same fungal species. Not surprisingly, strains also differ in pathogenicity to insects (e.g. Farques, 1976; Hall, 1977; Doberski, 1981 a; Daoust and Roberts, 1982; VI,4,abc) and strain variation must be taken into account when fungi are considered for use as myco-insecticides.

SECTION VI

FUNGUS/INSECT INTERACTIONS

MATERIALS AND METHODS

1. General

a. Preparation and Standardisation of Conidia and Hyphal Bodies

Conidial and hyphal body suspensions were produced and standardised as described previously ($\bar{V}$, 1, e,g,h) and where possible, used immediately. Where this was impracticable suspensions were stored in darkness at 2°C for a maximum of 2 weeks. Before use, the viability of conidia and hyphal bodies was ascertained by the agar slide technique ($\bar{V}$, 1, f).

b. Assay Procedures

Three methods were employed to assess the pathogenicity of fungal isolates against insects and mites.

i) Direct Immersion in Spore Suspensions

This technique was adapted from that described by Hall (1977). Conidial or hyphal body suspensions were prepared in water containing either 0.01 or 0.025% Triton X-100, and 20 ml poured into a filter paper-lined Buchner funnel (Whatman GFA, 4.7 cm diam; funnel, 4.7 cm diam). Where necessary, insects were anaesthetised by a 1 min exposure to carbon dioxide and immersed in the conidal or hyphal body suspension, using a camel hair brush (size 0). Suction was then applied to drain the Buchner funnel, by means of a filter pump, and the insects gently removed and placed on a food source of plants or leaf discs (VI, a, b, c, d).

ii) Contagion From Cadavers

This method was used to assess the ability of fungal strains to spread from diseased to healthy insects. Adult Hauptidia maroccana were infected with conidia and maintained on leaf discs (1.6 cm diam) at 25°C and a nominal 100% r.h., until sporulating mycelia covered the dead insects. Cadavers were then carefully positioned in the centres of

fresh, cucumber leaf discs (1.6 cm diam) which were supported on agar, contained in a square Petri dish. Adult H. maroccana were then anaesthetised and placed on the leaf discs, care being taken to ensure the healthy insects were positioned away from the cadaver. The Petri dishes were then sealed with Clingfilm and maintained at the desired temperature.

iii) Contagion from Verticillium lecanii Growing on Nutrients Applied to Cucumber Leaf Discs

A hand - held compression sprayer was used to thoroughly wet 3 to 4 - week old cucumber plants with suspensions of commercially produced Verticillium lecanii products ($2.5\ g\ l^{-1}$). The plants were then maintained at a nominal 100% r.h. and 25°C for 4 days, when areas of leaf where V. lecanii was growing on formulation ingredients were excised, using a cork borer (1.6 cm diam), and placed on agar contained in a square Petri dish. Insects or mites were then placed individually on the leaf discs, the dishes sealed with Clingfilm and incubated at 20°C.

2. Pathogenicity Testing and Bioassay

a. Hauptidia maroccana

i) Initial Screening Experiments

Batches of approximately twenty H. maroccana adults were anaesthetised and immersed in 0.01% Triton X-100, or a similar solution containing approximately 5×10^7 conidia ml^{-1}, of either Beauveria bassiana (strains 32 and 63), Metarhizium anisopliae (ME2), Paecilomyces fumosoroseus (21) or Verticillium lecanii (28-79, 1-72 and Tt). The treatments were replicated three times, except for V. lecanii (1-72), where only one batch of insects was treated. Following removal of the spore suspensions, insects were maintained on 1.6 cm diam. leaf discs at 23 ± 1°C. In a further experiment, groups of twenty insects were treated with 0.01% Triton X-100, or a similar solution containing approximately 5×10^7 conidia ml^{-1} of M. anisopliae (ME2, 52 and 1140), Paecilomyces sp. or Nomuraea

rileyi. Treatments were replicated twice, except for N. rileyi, where there were sufficient conidia for only one replicate. Treated insects were maintained on cucumber leaf discs (1.6 cm diam.) at 25°C.

ii) Multiple Dosage Assays

For each fungal strain, a range - finding assay was conducted to estimate the spore concentrations (in 0.025% aqueous Triton X-100), which when used to immerse adult H. maroccana, resulted in mortalities from 10 to 90% after 6 days incubation at 25°C and 100% r.h. With all strains examined, serial dilutions of spores, over a range of four logarithms, were necessary to achieve the desired mortalities. A series of assays was conducted with M. anisopliae (ME2), as initial screening experiments suggested this strain was most pathogenic to H. maroccana. Three groups of twenty anaesthetised H. maroccana adults were briefly immersed in each of four conidial suspensions which ranged from approximately 10^4 - 10^7 spores ml^{-1}. The assay was repeated six times over a period of 9 months, a different batch of insects being used for each assay. A further two non-replicated assays were performed where only twenty insects were treated with each spore suspension. In order to compare the pathogenicity of a number of fungal isolates against adult H. maroccana, replicated four dosage assays were conducted with each of the following strains; B. bassiana (31, 63); B. brongniartii (65); M. anisopliae (ME3, 5 , 1140, Map) and P. fumosoroseus (21). Generally, two assays were conducted for each strain.

iii) Comparison of the Pathogenicity of Metarhizium (ME2) Conidiospores and Hyphal Bodies.

In a non-replicated assay, conidial and hyphal body suspensions (in 0.025% aqueous Triton X-100) were used to immerse adult H. maroccana which were then maintained on leaf discs at 25°C. The assay was repeated with a fresh batch of insects.

iv) Comparison of the Pathogenicity of Metarhizium anisopliae (ME2) Conidia to Laboratory or Glasshouse Reared Hauptidia maroccana

During this study H. maroccana were generally reared in the laboratory. A small glasshouse (2.5 x 1m) was also kept planted with cucumbers to maintain a stock of H. maroccana. Laboratory and glasshouse-reared insects were immersed in a range of M. anisopliae (ME2) conidial suspensions, (in 0.025% aqueous Triton X-100) and maintained on leaf discs at 25°C. The experiment was repeated.

v) Transmission of Fungi From Diseased to Healthy Insects

Cadavers bearing sporulating mycelia of B. bassiana (63) M. anisopliae (ME2) or P. fumosoroseus (21), were placed on cucumber leaf discs, healthy H. maroccana positioned on the discs away from the dead insects, contained with Clingfilm and incubated at 25°C. Three replicates were used for each treatment.
The above procedure was repeated with cadavers bearing sporulating mycelia of B. bassiana (63) P. fumosoroseus and M. anisopliae (1140).

vi) Relationship Between Spore Concentration and Number of Spores Adhering to Insects

Bioassays were conducted with M. anisopliae (52) and P. fumosoroseus (21). After removal of conidial suspensions, ten insects were carefully removed from the filter paper and placed in Bijoux bottles (5 ml), containing 1, 2, or 3 ml of sterile water, with 0.025% Triton X-100 and approximately five glass balls (0.3 cm diam). The Bijoux were agitated for 1 min, using a vortex mixer, which macerated the insects. Suitable volumes of suspension were then plated on to Sabouraud dextrose agar, containing 80 µg ml streptomycin sulphate, to provide approximately 300 colonies plate^{-1} after 3 days incubation at 25°C.
To check the agitation did not impair conidial viability,

3 ml aliquots of a M. anisopliae (52) conidial suspension (4.0×10^4 conidia ml^{-1}) were placed in each of ten Bijoux. Half were subjected to vortexing while the remainder were shaken gently. Suitable aliquots were then plated onto SDA and colonies counted after 3 days incubation at 25°C.

vii) Period of High Humidity Required for Infection

Approximately one hundred adult insects were immersed in a conidial suspension of M. anisopliae(ME2), containing 5×10^7 spores ml^{-1}(0.025% Triton X-100), transferred to cucumber leaf discs and maintained at 20°C. Most insects were contained in square Petri dishes but approximately 20 insects were immediately placed on a leaf disc (8 cm diam) supported on agar in a Petri dish (9 cm diam) and contained with fine nylon mesh. After 24, 48 and 72 hours, groups of approximately twenty insects were anaesthetised with carbon dioxide and transferred to large cucumber leaf discs. Mortality was estimated at 24 h intervals for a period of 5 days.

The experiment was repeated : two hundred insects were infected and separate groups maintained at 20 or 25°C.

viii) Pathogenicity of Verticillium lecanii strains

Adult insects were immersed in 0.025% Triton X-100, or similar solutions containing 5×10^7 conidia ml^{-1} of M. anisopliae (ME2), or V. lecanii strains 1-72, 15-74, 16-75, 19-79, 27-79 or 53-81, and maintained on leaf discs at 20°C. Three groups of approximately twenty insects were used for each treatment.

ix) Effect of Temperature on Mortality

Adult insects were immersed in 0.025% Triton X-100, or similar solutions containing 5×10^7 conidia ml^{-1} of either B. bassiana (63), M. anisopliae (ME2) or P. fumosoroseus (21). With B. bassiana, four groups of insects were treated and two each maintained at 20 or 25°C, while with M. anisopliae 3 groups were maintained at the same temperature. P. fumosoroseus - treated insects were maintained at 13°C (2 replicates), 20, (3 rep) or 25°C (3 rep), while

one group of Triton - treated insects were maintained at each temperature.

b. Thrips tabaci

i) Initial Screens

Adult Thrips tabaci were briefly immersed in 0.01% Triton X-100 or similar solutions containing containing (approx. 5×10^7 conidia ml^{-1}) of either B. bassiana (31, 32, 63) M. anisopliae (ME2, Map), P. fumosoroseus (21) or V. lecanii (1-72, Tt or Zp). Insects were transferred to cucumber leaf discs (0.6 cm diam), supported on agar, and maintained at 23 ± 1°C. Three replicate groups of approximately twenty insects were used for each treatment.

In a second experiment, single groups of twenty adult insects were immersed in 10^7 conidia ml^{-1} (with 0.025% aqueous Triton X-100) of either B. bassiana (31, 32, 63), B. brongniartii (6, 65) M. anisopliae (ME2, 52, 1140, Map), P. fumosoroseus (21), V. lecanii (53-81) or Verticillium sp. (Keller) and maintained on cucumber leaf discs (1.6 cm diam) at 20°C.

ii) Comparison of Commercial Verticillium lecanii Products

Cucumber plants were sprayed with Mycotal®, Thriptal® or Vertalec® (Tate and Lyle Ltd; 2.5 g l^{-1}) and incubated for 4 days, when leaf discs (1.6 cm diam) were removed and placed on agar (VI, 1, b). Adult T. tabaci were anaesthetised with carbon dioxide (1 min exposure) and positioned on the leaf discs; for each treatment twenty insects were individually placed on leaf discs and maintained at 20°C. Unsprayed leaf discs were used to maintain insects as controls and the experiment repeated.

c. Tetranychus urticae - Red Spider Mite

i) Pathogenicity of Commercial Verticillium lecanii Products

The procedure was as described above (VI, 2, b, ii), except that it was not necessary to anaesthetise mites and they

were transferred directly from bean plants to the cucumber leaf discs.

d. Macrosiphoniella sanborni - Chrysanthemum Aphid

i) Period of High Humidity Required for Infection with Verticillium lecanii (1-72)

Approximately one hundred adult Macrosiphoniella sanborni were immersed in a conidial suspension of V. lecanii (1-72) containing 10^7 spores ml^{-1} (with 0.025% aqueous Triton X-100) and positioned on chrysanthemum leaf discs (cv Deep Tuneful), supported on agar in square Petri dishes. One group of twenty insects was contained on leaf discs by coating the sides of each cell with Fluon (Polytetrafluorethylene, BDH Co Ltd, England)-the sticky nature prevented aphids from walking on treated surfaces - while the remainder were contained with Clingfilm. After 16, 21.5, 40.5 and 66 hours, further groups of insects were transferred to excised leaf discs at ambient humidity. Occa sionally, aphids became trapped on the Fluon coating and these were disregarded in mortality assesments.

e. Phytoseiluis persimilis - Red Spider Mite Predator

i) Pathogenicity of Verticillium lecanii (53-81)

Groups of approximately twenty adult insects were immersed in .01% Triton X-100 or a similar solution containing 8 x 10^7 conidia ml^{-1} of V. lecanii (53-81). Insects were then placed on cucumber leaf discs (1.6 cm diam.), together with about four red spider mites to provide a food source. Insects were contained with Clingfilm at 20°C.

f. Nilaparvata lugens - Brown Planthopper

i) Initial Screening Experiments

Adult brachypterous Nilaparvata lugens were immersed in 0.025% Triton X-100 or similar solutions containing conidia (10^7 spores ml^{-1}) of B. bassiana (31, 32, 40, 43, 63), B. brongniartii (65), M. anisopliae (ME2, 52, 1140, Map), P. fumosoroseus (21), Paecilomyces sp., Syncephalastrum

racemosum, V. lecanii (53-81) or Verticillium sp. Approximately 25 planthoppers were used for each treatment and insects maintained on 1 cm high rice stem segments, positioned vertically in agar, at 25°C (IV, 4, d, i).
The experiment was repeated with treated insects being maintained on rice plants contained in propagators (IV, 4, d, ii).

ii) Replicated Experiment

Adult brachypterous N. lugens were immersed in 0.025% Triton X-100 or similar solutions containing 5×10^7 conidia ml^{-1} of B. bassiana (50), M. anisopliae (ME2, 52, 1140), or P. fumosoroseus (21). Three batches of approximately 20 insects were used for each treatment, except P. fumosoroseus where only two replicates were used, and treated insects were maintained on rice plants at 25°C.

iii) Bioassay

Adult brachypterous N. lugens were immersed in conidial suspensions (10^4, 10^5, 10^6, or 10^7 spores ml^{-1}, 0.025% Triton X-100) of M. anisopliae (ME2). Insects were maintained on rice plants at 25°C and the assay repeated three times with new batches of insects.

iv) Nymphs

Groups of about 20 N. lugens nymphs (3-5th instar) were immersed in .01% Triton X-100, or similar solutions containing 5×10^7 conidia ml^{-1} of B. bassiana (63), M. anisopliae (ME2, 52, 1140), P. fumosoroseus (21), or S. racemosum. Insects were maintained on rice stems at 25°C.

RESULTS

4. Pathogenicity Testing and Bioassay

a. Hauptidia maroccana

i) Initial Screening Experiments

Four days after conidial application, M. anisopliae and P. fumosoroseus treatments produced significantly greater

mortality (P = 0.05) than all other treatments (Fig. 6.01). By seven days after treatment, M. anisopliae (ME2), P. fumosoroseus (21) and B. bassiana (63) had killed nearly 100% of the treated insects and were significantly more pathogenic (P = 0.05) than B. bassiana (32), or V. lecanii Tt). V. lecanii strains 28-79 and 1-72 were of low pathogenicity and 7 day mortalities were not significantly different from control - treated insects.
In a second replicated experiment, M. anisopliae strains ME2, 52 and 1140 were significantly more pathogenic (P = 0.05) to H. maroccana adults, than Paecilomyces sp. or Nomuraea rileyi (Fig. 6.02).
Adult H. maroccana, killed by B. bassiana (63), M. anisopliae (ME2) or P. fumosoroseus (21) are shown in Figs. 6.03-05.

ii) Multiple Dosage Assays

Bioassays were read after 6 days and mortality data analysed by probit analysis, using the maximum-likelihood computer programme (Ross, 1970) in the program library of Rothamsted Experimental Station, Harpenden, England. An example of probit transformed data is provided in Fig. 6.06. The mean LC 50 of M. anisopliae (ME2) to adult H. maroccana was 5.5×10^5 conidia ml^{-1} after 6 days incubation at 25°C, as determined from a series of six replicated assays (Table 6.01). Slopes were low and ranged from 0.8 - 1.8. A slightly higher mean LC 50, 7.1×10^5 conidia ml^{-1}, was obtained from a series of six non-replicated assays (Table 6.02). With the replicated assays, LC 50 values ranged from 3.1×10^5 - 9.6×10^5 conidia ml^{-1} and fiducial limits for individual assays were close, differing by a factor of , at most, 3.3. In contrast, non-replicated assay LC 50 values, ranged from 2.3×10^5 - 11.1×10^5 conidia ml^{-1} and fiducial limits were wide, differing by factors of 3.5 - 6.9.
M. anisopliae strains ME3, 52, and 1140 had mean LC 50 values of 4.2×10^5, 7.6×10^5 and 2.4×10^5 conidia ml^{-1} respectively and thus had similar pathogenicity to M. anisopliae strain ME2 (Table 6.03). One strain, M. anisopliae

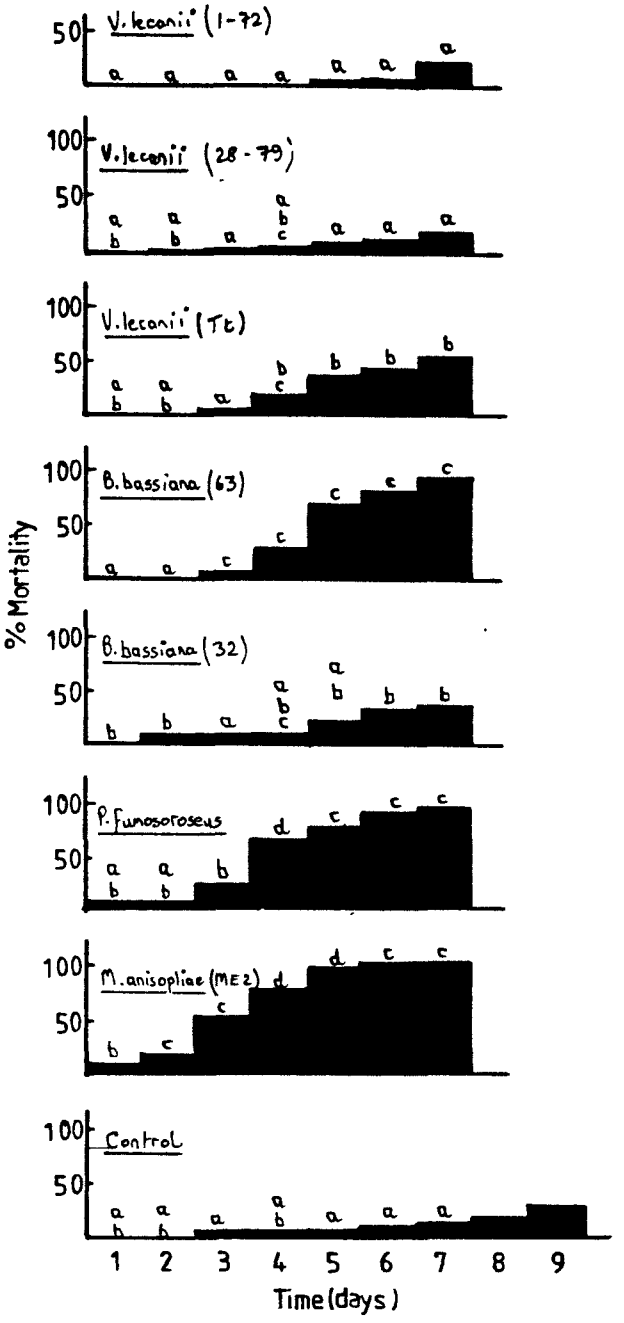


Fig. 6.01
Mortality of adult Hauptidia maroccana after immersion in 5 x 10^7 conidia ml^{-1} of various entomogenous fungi, and maintenance on cucumber leaf discs at 23 ± 1°C. (Histogram shows mean mortalities of three replicate batches of insects $treatment^{-1}$; for each sampling date, different letters indicate significant difference between fungi (P= 0.05).

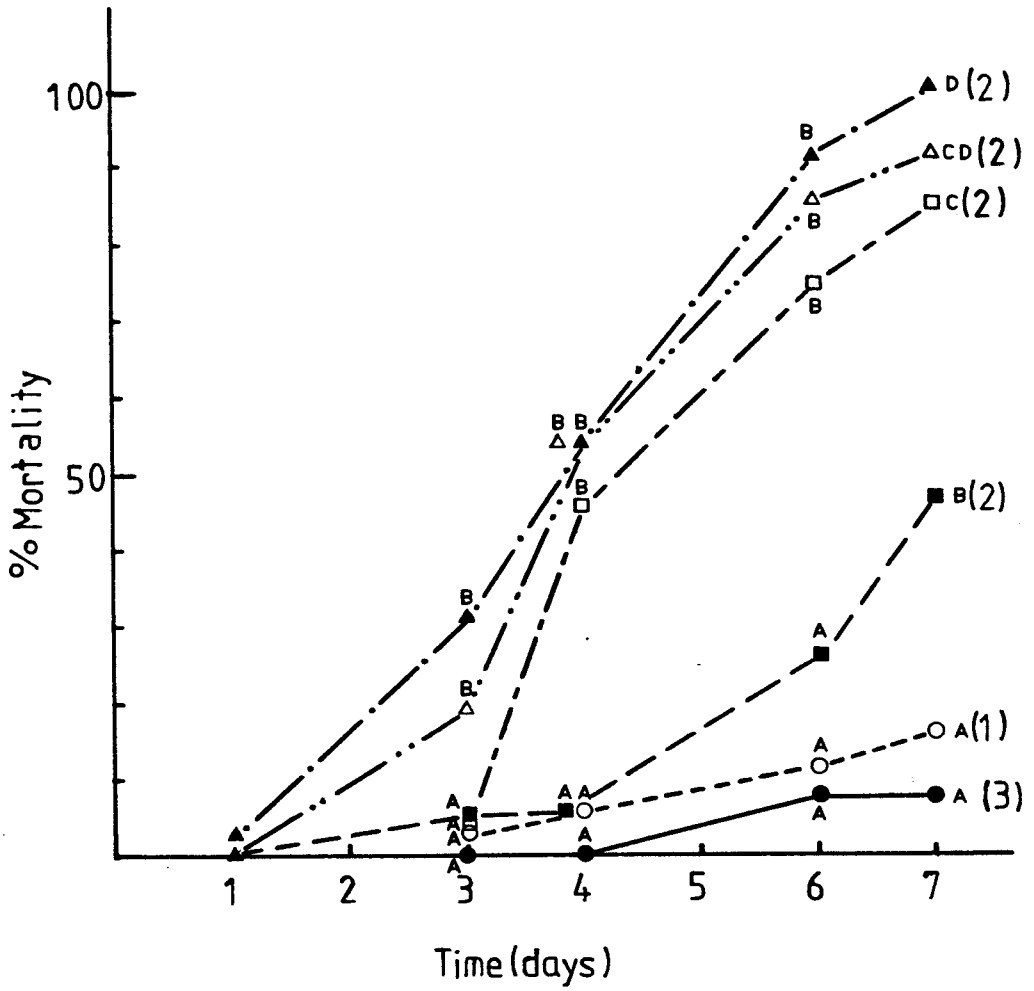


Fig. 6.02

Mortality of adult Hauptidia maroccana after immersion in 0.025% Triton X-100 (●—●) or surfactant containing 5 x 10^7 conidia ml^{-1} of Nomuraea rileyi (o---o), Paecilomyces sp. (■—■) or Metarhizium anisopliae strains ME2 (Δ··—Δ), 52 (□--□) and 1140 (▲—·▲) and maintenance on cucumber leaf discs at 25°C. Points are means; for each sampling date different letters indicate significant difference (p = 0.05). Numbers in parantheses indicate nos. of replicates.

Fig. 6.03
Adult Hauptidia maroccana showing sporulating mycelia of Beauveria bassiana (63). X 24.

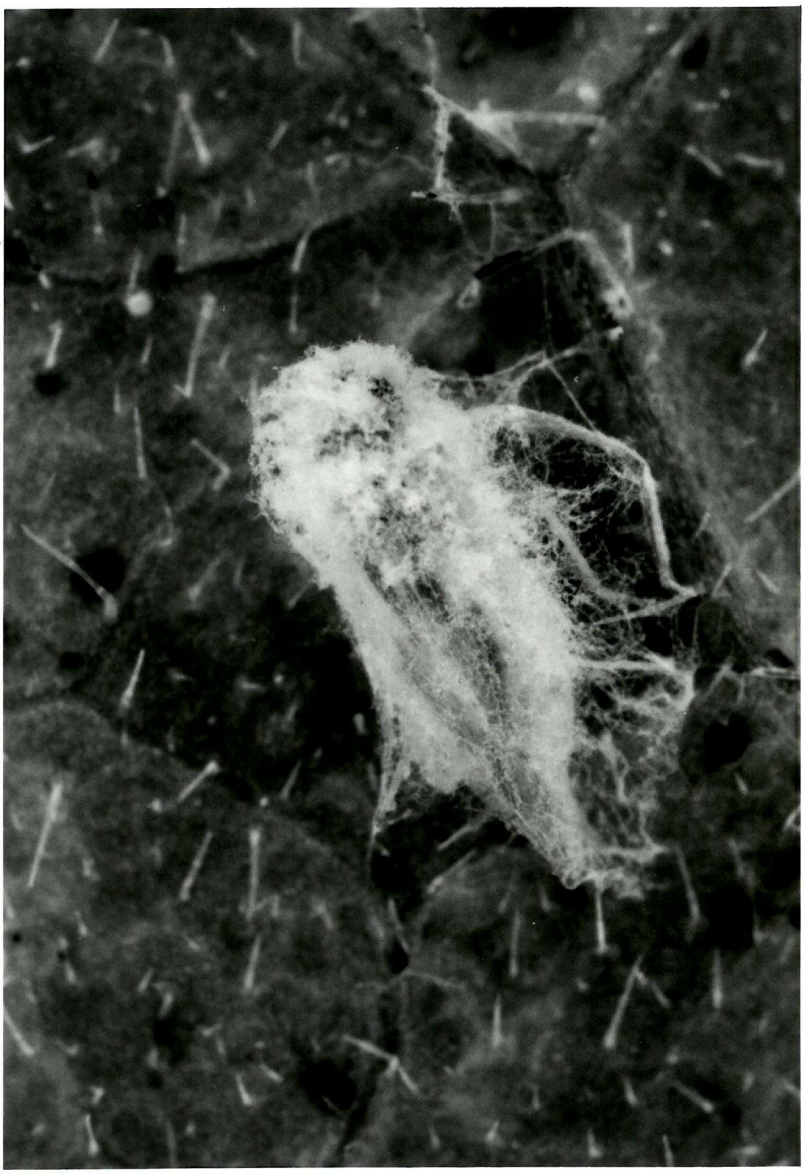


Fig. 6.04
Adult Hauptidia maroccana showing sporulating mycelia of Paecilomyces fumosoroseus (21). X 24.

Fig. 6.05
Adult Hauptidia maroccana showing sporulating mycelia of Metarhizium anisopliae (ME2). X 30

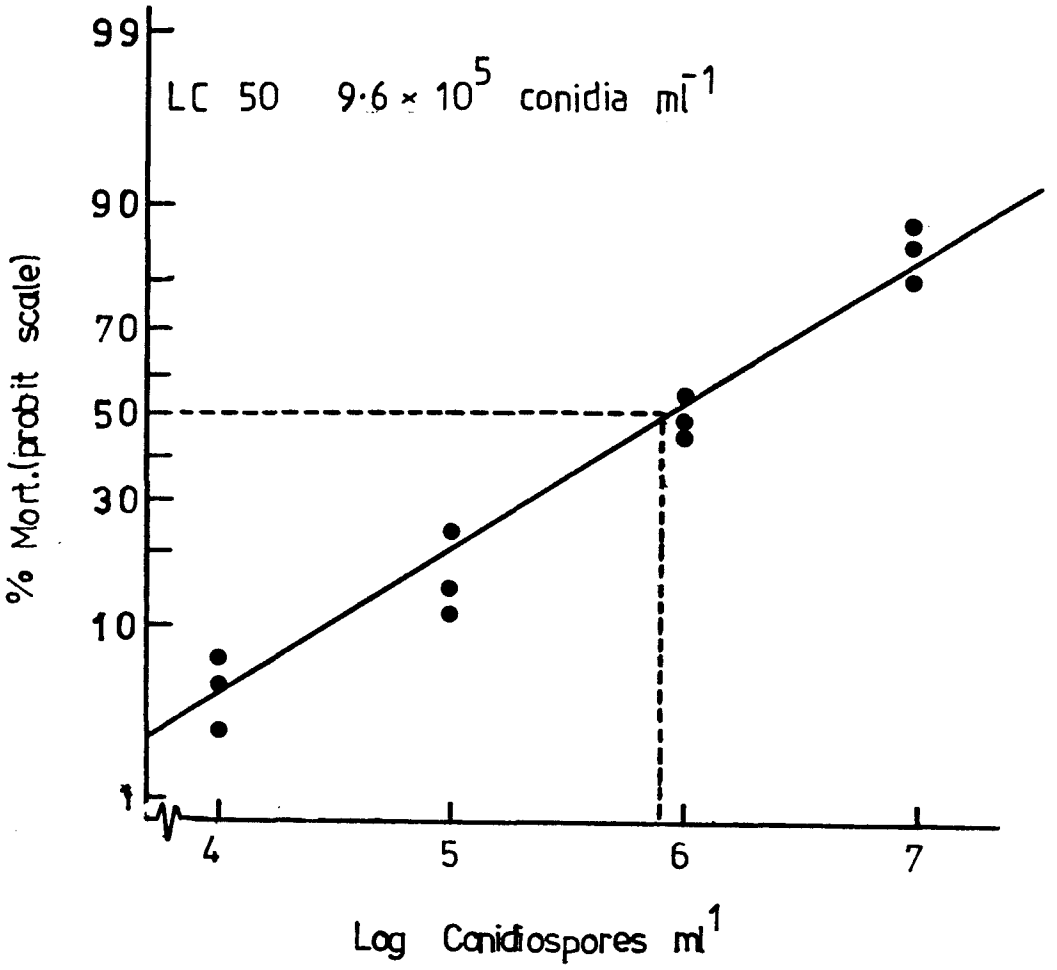


Fig. 6.06
Example of linear relationship between probit - transformed mortality of Hauptidia maroccana and log 10 concentration of Metarhizium anisopliae (ME2) conidiospores. Assay number 3, Table 6.01.

Table 6.01

Pathogenicity of Metarhizium anisopliae conidiospores (Strain ME2) to adult Hauptidia maroccana

I Replicated; 3 x 20 insects dose^{-1}. 4 doses.

Assay number	LC 50 with 95% fiducial limits ($\times 10^5$ spores ml^{-1})	Slope $\pm$ S.E.[a]	Chi^{2b}	d.f.[c]	p[b]
1	4.3 (2.6-6.5)	1.8±0.3	10.5	10	>0.05
2	4.0 (2.6-6.1)	1.2±0.1	10.4	10	>0.05
3	9.6 (6.2-15.1)	1.1±0.1	5.9	10	>0.05
4	3.1 (2.1-4.8)	1.2±0.2	13.5	10	>0.05
5*	7.2 (4.1-13.7)	0.8±0.1	6.8	9	>0.05
6	4.7 (2.5-7.3)	1.4±0.2	9.0	10	>0.05

Mean LC 50 5.5 x 10^5 conidia ml^{-1}

a Standard error
b heterogeneity about regression line
c degrees of freedom
* highest dose with only 2 replicates

Table 6.02

Pathogenicity of *Metarhizium anisopliae* Conidiospores (Strain ME2) to adult *Hauptidia maroccana*

II Unreplicated; 20 insects $dose^{-1}$. 4 doses

Assay number	LC 50 with 95% fiducial limits ($\times 10^5$ spores ml^{-1})	Slope $\pm$ S.E.[a]	Chi^{2b}	d.f.[c]	p[b]
1	11.1 (4.9-28.1)	1.0±0.2	1.7	2	>0.05
2	7.8 (3.4-19.1)	1.0±0.2	0.6	2	>0.05
3*	2.3 (0.8-5.5)	0.8±0.2	1.6	2	>0.05
4*	10.9 (4.9-23.6)	1.1±0.2	1.5	2	>0.05
5**	3.9 (2.1-7.3)	1.2±0.2	4.9	2	>0.05
6**	6.5 (3.5-12.2)	1.3±0.2	1.1	2	>0.05

Mean LC 50 7.5×10^5 conidia ml^{-1}

a Standard error
b heterogeneity about regression line
c degrees of freedom
* assays from Table 6.07
** assays from Table 6.06

Table 6.03

Pathogenicity of *Metarhizium anisopliae* conidiospores (Strains ME3, 52, 1140 and Map) to adult *Hauptidia maroccana*

Replicated assays; 3 x 20 insects dose. 4 doses.

Strain	Assay number	LC 50 with 95% fiducial limits ($\times 10^5$ spores ml^{-1})	Mean LC 50 ($\times 10^5$ spores ml)	Slope ± S.E.[a]	Chi^{2b}	d.f.[c]	p[b]
ME3	1	6.7(4.2-10.8)	4.2	100±0.1	10.9	10	>0.05
	2	1.7(1.1-2.8)		1.0±0.1	5.1	10	0.05
52	1	4.9(3.1-7.6)	7.6	1.0±0.1	19.0	10	>0.02
	2	10.3(5.7-19.4)		0.8±0.1	9.9	10	>0.05
1140	1	1.8(1.1-2.8)	2.4	1.1±0.1	9.1	10	>0.05
	2	3.0(2.1-4.6)		1.1±0.1	9.5	10	>0.05
Map *	1	140(71.5-322.0)		0.6±0.1	13.0	10	>0.05

a Standard error b heterogeneity about regression line c degrees of freedom

* assay read after 9 days

(Map) had an LC 50 values of 140×10^7 conidia ml^{-1} after 9 days incubation and was considerably less pathogenic than the other M. anisopliae strains tested.
B. bassiana strains 31 and 63 had mean LC 50 values of 16.4×10^5 and 46.5×10^5 conidia ml^{-1} respectively while B. brongniartii (65) had an LC 50 of 20.3×10^5 (Table 6.04).
P. fumosoroseus (21) had a mean LC 50 of 88.0×10^5 (Table 6.05).

iii) Comparison of the Pathogenicity of Metarhizium anisopliae (ME2) Conidiospores and Hyphal Bodies

Conidiospores and hyphal bodies of M. anisopliae (ME2) were of similar pathogenicity to H. maroccana adults (Table 6.06). Only two, non-replicated assays were conducted, due to a shortage of time and insects, but the data obtained strongly suggests similar pathogenicity of conidiospores and hyphal bodies.

iv) Pathogenicity of Metarhizium anisopliae (ME2) Conidiospores to Laboratory or Glasshouse Reared Hauptidia maroccana

Laboratory reared leafhoppers were slightly more susceptible to infection by M. anisopliae (ME2) than those reared in a glasshouse (Table 6.07). However, the differences were not significant with fiducial limits overlapping, and further assays would be required to determine if the difference observed was real or due to variation.

v) Transmission of Fungi From Diseased to Healthy Insects

Healthy insects acquired disease from cadavers (Fig. 6.07). In the first experiment the most pathogenic strain was M. anisopliae (ME2). However, it required 7 days to achieve 13% mortality and after a further 5 days some 26% of exposed insects were still apparently healthy, P. fumosoroseus spread poorly, and after 12 days, mortality of leaf-

Table 6.04

Pathogenicity of *Beauveria bassiana* (Strains 31 and 63) and *B. brongniartii* (65) conidiospores to adult *Hauptidia maroccana*

Replicated assays; 3 x 20 insects dose. 4 doses.

Strain	Assay number	LC 50 with 95% fiducial limits ($\times 10^5$ spores ml^{-1})	Mean LC 50 ($\times 10^5$ spores ml^{-1})	Slope ± S.E.[a]	Chi^{2b}	d.f.[c] [b]	p[b]
31	1	21.2(13.8-32.8)	16.4	1.1±0.1	6.2	10	>0.05
	2	11.6(7.1-18.7)		0.9±0.1	11.0	10	>0.05
63	1	88.7(55.6-151.0)	46.5	1.0±0.1	21.7	10	>0.01
	2	49.5(29.5-88.3)		1.0±0.1	12.6	10	>0.05
	3	27.4(15.4-45.8)		0.8±0.1	13.9	12	>0.05
65	1	20.3(13.3-30.9)		1.0 0,1	19.1	10	>0.01

a Standard error b heterogeneity about regression line c degrees of freedom

Table 6.05

Pathogenicity of Paecilomyces fumosoroseus (21) conidiospores to adult Hauptidia maroccana

Replicated assays; 3 x 20 insects dose^{-1}. 4 doses.

Assay number	LC 50 with 95% fiducial limits ($\times 10^5$ spores ml^{-1})	Slope $\pm$ S.E.[a]	Chi ^{2b}	d.f.[c]	p[b]
1	54.2 (26.8-121.3)	0.8±0.1	9.7	10	>0.05
2	131.3(60.2-392.0)	0.6±0.1	20.7	9	>0.01
3*	61.5 (31.7-146.6)	0.7±0.1	9.1	8	>0.05
4**	105.1(50.3-217.1)	1.0±0.2	1.2	2	>0.05

Mean LC 50 88.0 x 10^5 conidia ml^{-1}

a Standard error

b heterogeneity about regression line

c degrees of freedom

* Two highest doses replicated twice only

** Single replicates only

Table 6.06

Pathogenicity of Metarhizium anisopliae (Strain ME2) conidiospores and hyphal bodies to Hauptidia maroccana

Non replicated assays; 20 insects dose, 4 doses

Experiment number	Propagule type	LC 50 with 95% fiducial limits ($\times 10^5$ ml^{-1})	Slope ± S.E.[a]	Chi^{2b}	d.f.[c]	P[b]	potency ratio
1	Hyphal body	3.6(2.0-6.5)	1.2±0.2	1.9	2	>0.05	1.0
	Conidiospore	3.9(2.1-7.3)	1.2±0.2	4.9	2	>0.05	
2	Hyphal body	2.7(1.5-5.0)	1.4±0.2	2.0	2	>0.05	2.4
	Conidiospore	6.5(3.5-12.2)	1.3±0.2	1.1	2	>0.05	

a Standard error b heterogeneity about regression line c degrees of freedom

Table 6.07

Pathogenicity of *Metarhizium anisopliae* conidiospores strain (ME2) to adult *Hauptidia maroccana* reared for 2 years in the laboratory or under more natural conditions in a glasshouse

Non replicated assays; 20 insects dose, 4 doses

Experiment number	Source of insects	LC 50 with 95% fiducial limits ($\times 10^5$ spores ml^{-1})	Slope ± S.E.[a]	Chi^{2b}	d.f.[c b]	P[b]	Potency ratio
1	Laboratory	2.3(0.8-5.5)	0.8±0.2	1.6	2	>0.05	3.4
	Glasshouse	7.5(3.0-19.7)	0.8±0.2	2.8	2	>0.05	
2	Laboratory	10.9(4.9-23.6)	1.1±0.2	1.5	2	>0.05	1.4
	Glasshouse	15.3(7.6-34.5)	1.1±0.2	2.2	2	>0.05	

a Standard error b heterogeneity about regression line c degrees of freedom

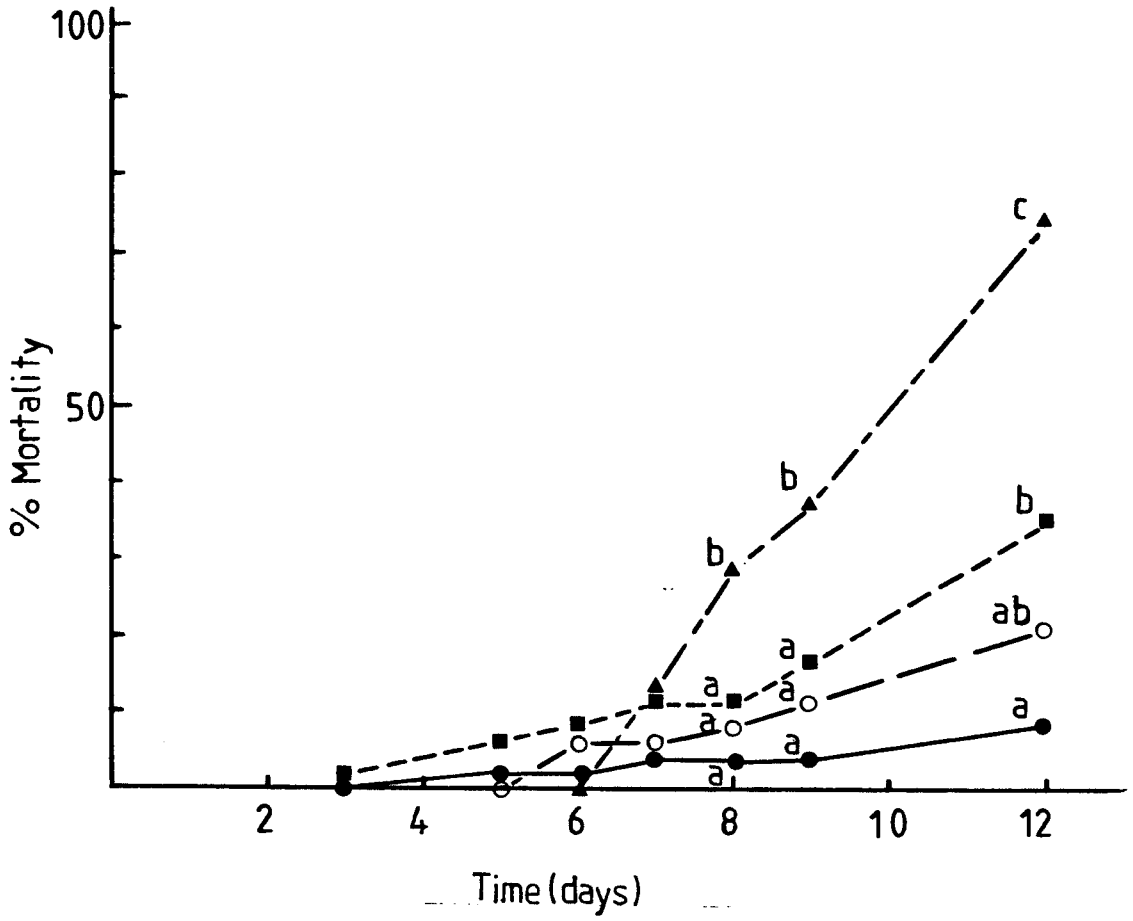


Fig. 6.07
Mortality of adult Hauptidia maroccana when maintained with cadavers on leaf discs at 25°C (●—●) or in similar fashion with cadavers bearing sporulating mycelia of Paecilomyces fumosoroseus (21) (o—o), Beauveria bassiana (63) (■--■) or Metarhizium anisopliae (ME2) (▲--▲). Points are mean mortalities of three replicates. For each sampling date different letters indicate significant difference (p = 0.05).

hoppers was not significantly greater than insects maintained without contact with fungi. Exposure of insects to B. bassiana (63) resulted in 35% mortality after 12 days.

In a second experiment M. anisopliae (1140) proved effective and from 5 days mortalities were significantly larger ($p = 0.05$) than other treatments. (Fig. 6.08). Again P. fumosoroseus (21) and B. bassiana (63) spread poorly and mortalities only reached 28 and 53% respectively after 12 days.

vi) Relationship Between Spore Concentration and Number of Conidia Adhering to Insects

Viability of M. anisopliae (52) conidia was unaffected by the agitation used to remove conidia from insects-non-agitated solutions contained a mean of 1.07×10^4 conidia ml^{-1}, while those agitated with glass balls contained 1.11×10^4 conidia. With M. anisopliae (52), approximately .008% of the spore numbers ml^{-1} (range .007-.0104%) adhered to adult H. maroccana, while a similar percentage was obtained for P. fumosoroseus (21); mean .0081, range .0049-.01 (Table 6.08).

Probit analysis of mortality data gave LD 5, 50 and 95 values as 1, 42 and 1581 conidia for M. anisopliae (52) and 2, 552 and 128000 for P. fumosoroseus (Table 6.09), illustrating the greater pathogenicity of M. anisopliae.

vii) Period of High Humidity Required For Infection

When adult insects were treated with M. anisopliae (ME2) conidia and maintained at 100% r.h. and 20°C for 24 hours, and then at ambient humidity for 4 or 6 days, mortality was below 10% (Fig. 6.09). When the time at 100% r.h. was increased to 48-49 , mortality reached 42% after 7 days (Expt 1) or 63% in 5 days (Expt 2) . A further increase in the high humidity period to 72-73 h resulted in about 60% mortality.

At 25°C, 24, 48, or 73 h exposures of treated insects to 100% r.h. resulted in approximately 90% mortality. However, in this experiment insects removed to ambient humidity straight after treatment also died; some 32% were dead after 5 days.

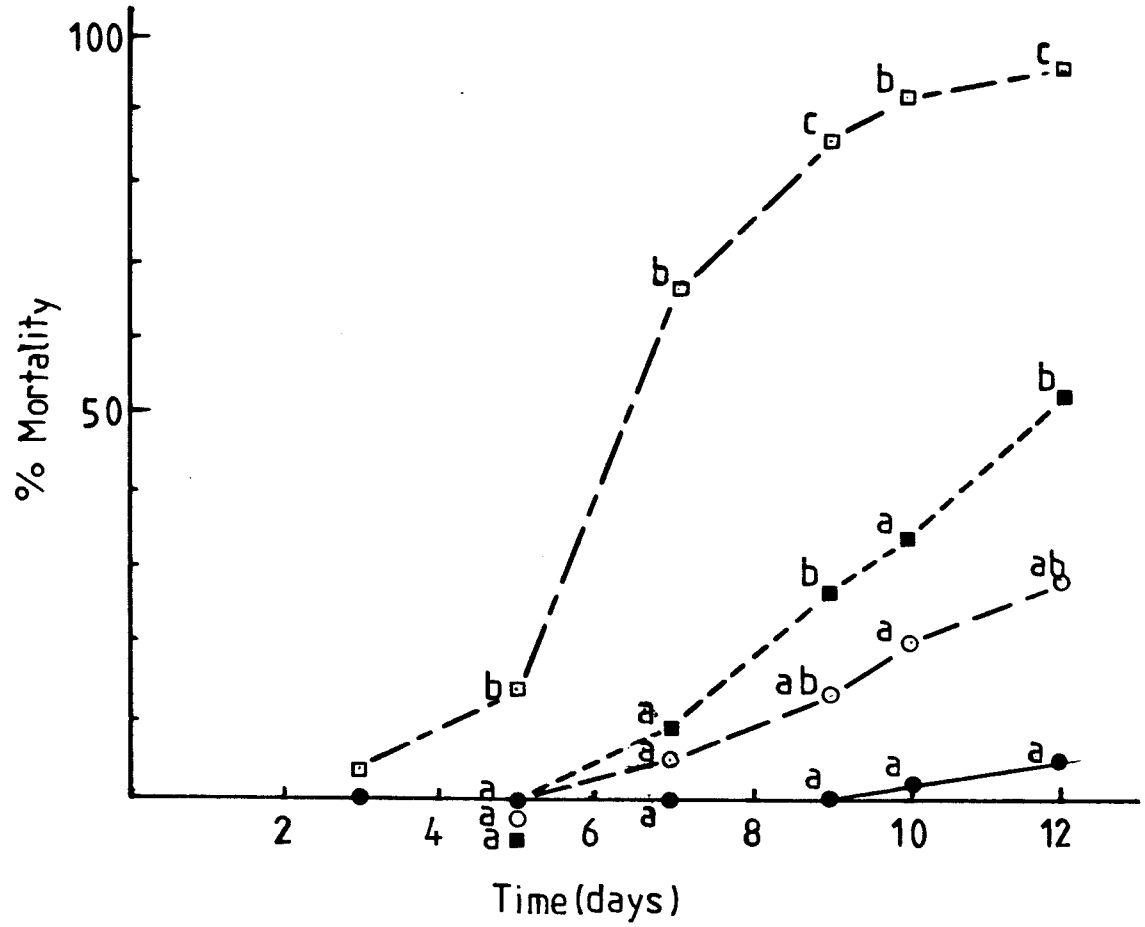


Fig. 6.08
Mortality of adult Hauptidia maroccana when maintained on leaf discs at 25°C (●—●) or in similar fashion with cadavers bearing sporulating mycelia of Paecilomyces fumosoroseus (21) (o—o), Beauveria bassiana (63) (■--■), or Metarhizium anisopliae (1140) (□--□). Points are mean mortalities of three replicates. For each sampling date different letters indicate significant difference (p = 0.05).

Table 6.08

The Relationship Between Spore Concentrations Used for Immersion of Adult Hauptidia maroccana and Numbers of Conidia Adhering to Insects

Metarhizium anisopliae (52)

Spore Concentration (ml^{-1})	Mean no. spores adhering to insects	Standard error	% number of spores ml^{-1} adhering to insects
8.56×10^3	0.6	0.3	0.0070
8.56×10^4	6.3	1.4	0.0074
8.56×10^5	89.0	7.7	0.0104
8.56×10^6	657.0	104.1	0.0077

Paecilomyces fumosoroseus (21)

Spore Concentration (ml^{-1})	Mean no. spores adhering to insects	Standard error	% number spores ml^{-1} adhering to insects
4.25×10^4	2.1	0.6	0.0049
4.25×10^5	37.0	5.2	0.0087
4.25×10^6	443.0	58.5	0.0104
4.25×10^7	3501.0	557.4	0.00823

Table 6.09

Pathogenicity of *Metarhizium anisopliae* (52) and *Paecilomyces fumosoroseus* (21) Conidia to Adult *Hauptidia maroccana*

Metarhizium anisopliae (52)

LD 50 with 95% fiducial limits	LD 5 with 95% fiducial limits	LD 95 with 95% fiducial limits	Slope ± S.E.[a]	Chi^{2b}	d.f.[c]	p[b]
41.7(26.6-64.9)	1.1(0.4-2.3)	1581(731.9-4903.8)	1.0±.1	16.3	10	0.05

Paecilomyces fumosoroseus (21)

551.8(271.6-1394.0)	2.4(0.4-7.1)	128000(20,110-463000)	0.7±.1	9.4	8	0.05

a Standard error

b heterogeneity about regression line

c degrees of freedom

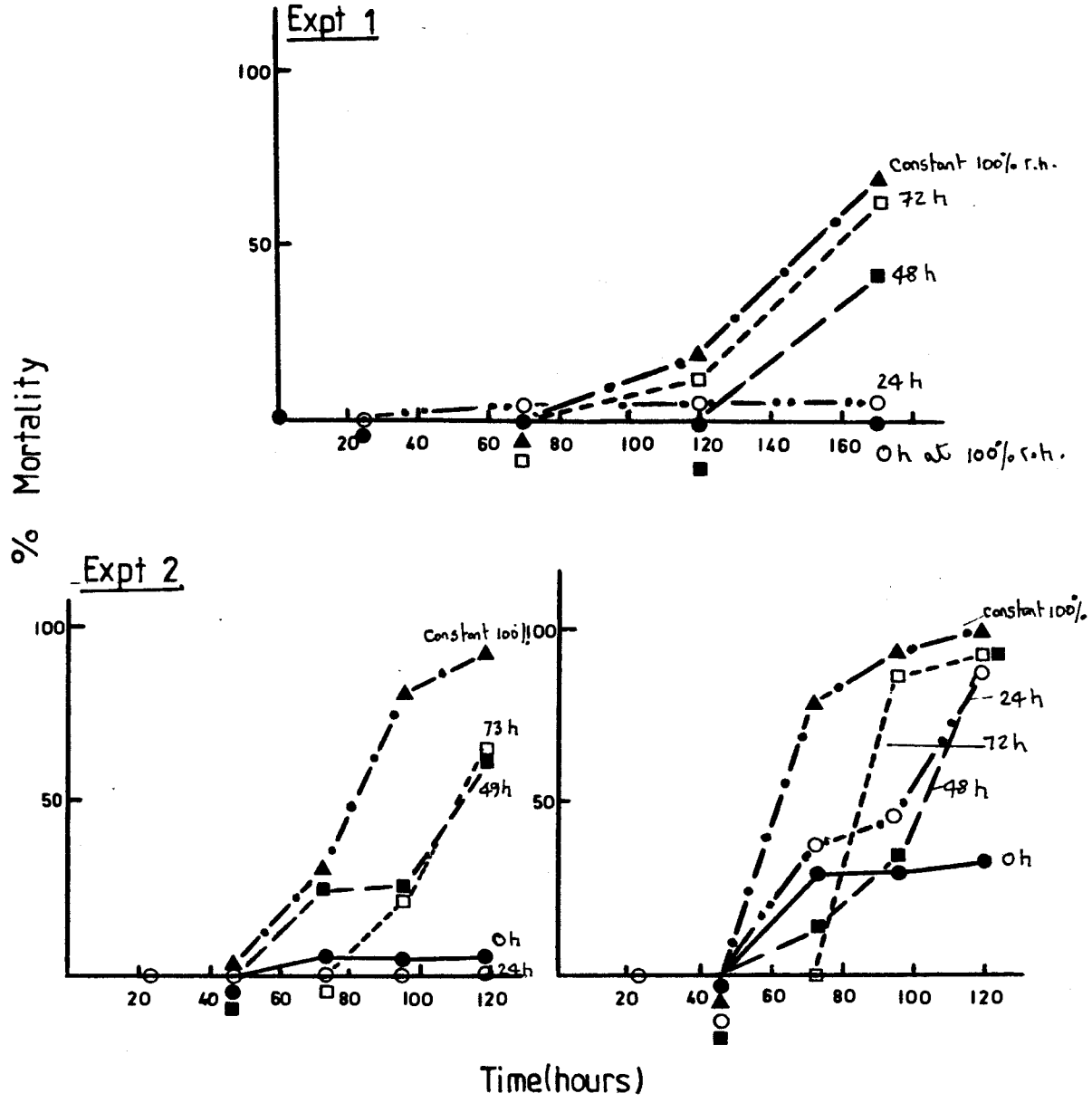


Fig. 6.09

Mortality of adult Hauptidia maroccana after immersion in 5 x 10^7 conidia ml^{-1} of Metarhizium anisopliae (ME2) and maintenance for varying time periods at 20 or 25°C and 100% relative humidity, before removed to ambient humidity. Points are percentage mortalities of 20 insects, $treatment^{-1}$.

viii) Pathogenicity of V. lecanii Strains

After 6, 7, 8 and 9 days incubation at 20°C, M. anisopliae (ME2) treatment had resulted in significantly greater ($p = 0.05$) mortality than all V. lecanii strains (Fig. 6.10). V. lecanii (27-79) treatment resulted in only 10% mortality after 9 days and was not significantly greater than control levels ($p = 0.05$). V. lecanii strains 15-74, 19-79 and 53-81 resulted in similar levels of mortality (83% after 9 days) and were more pathogenic than strain 1-72.

ix) Effect of Temperature on Mortality

Mortality of adult H. maroccana increased with temperature (Fig. 6.11). Incubation of B. bassiana (63) treated insects at 20 and 25°C, resulted in 62 and 72% mortality after 5 days. Corresponding figures for M. anisopliae were 78 and 100%. With P. fumosoroseus, the temperature effect was more marked and after 6 days at 25°C, 86% of insects were dead compared to only 28% at 20°C and 12% at 13°C.

b. Thrips tabaci

i) Initial Screens

All fungi tested, resulted in significant mortality after 4 days incubation at 23°C, with the most pathogenic strains being M. anisopliae (ME2), B. bassiana (31, 63) and P. fumosoroseus (21) (Fig. 6.12). However, control mortality reached 54% after 5 days and was thus unacceptably high. It is probable that high control mortality was due, at least in part, to a shortage of food source, as many cucumber leaf discs had deteriorated, becoming yellow and a few discs were dead.

In a second experiment where thrips were maintained on larger cucumber discs (1.6 cm diam.), at 20°C, control mortalities were much lower and reached only 15% after 7 days (Fig. 6.13). Again, all fungi tested caused appreciable mortality of Thrips tabaci - only, B. bassiana (32) had caused less than 50% mortality after 7 days. V. lecanii strains were less pathogenic than M. anisopliae, but due to their ability to kill insects in the glasshouse environment (Hall, 1977; Hall and Burges, 1979, Kanagaratnam et al. 1982; Hall, 1982) further study was concentrated on V. lecanii strains.

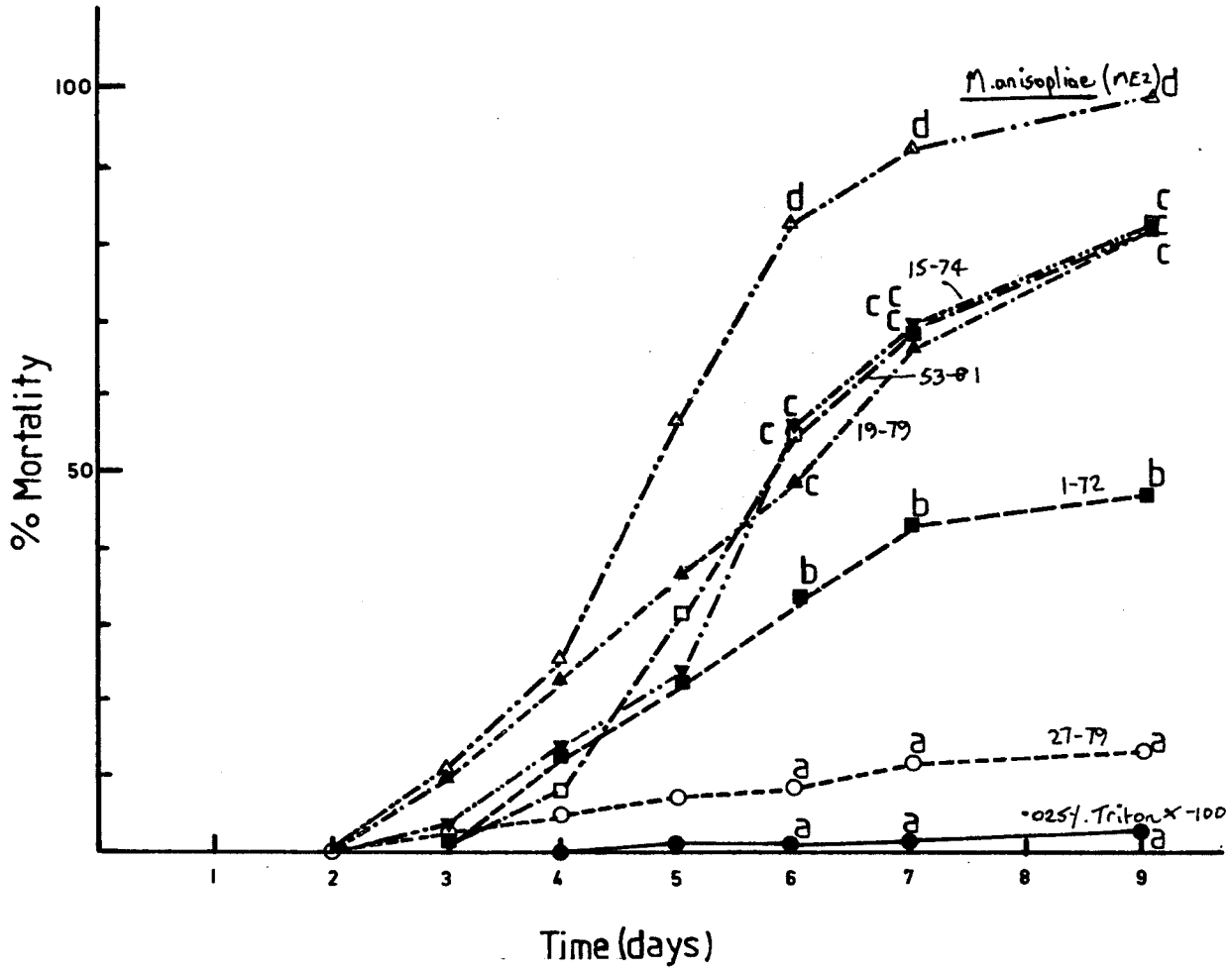


Fig. 6.10
Mortality of adult Hauptidia maroccana after immersion in 0.025% Triton X-100 or similar solutions containing 5×10^7 conidia ml^{-1} of Verticillium lecanii strains 27-79, 1-72, 19-79, 53-81, 15-74 or Metarhizium anisopliae (ME2) and maintenance at 20°C. Points are means of three replicates $treatment^{-1}$. For each sampling date, different letters indicate significant difference ($p = 0.05$).

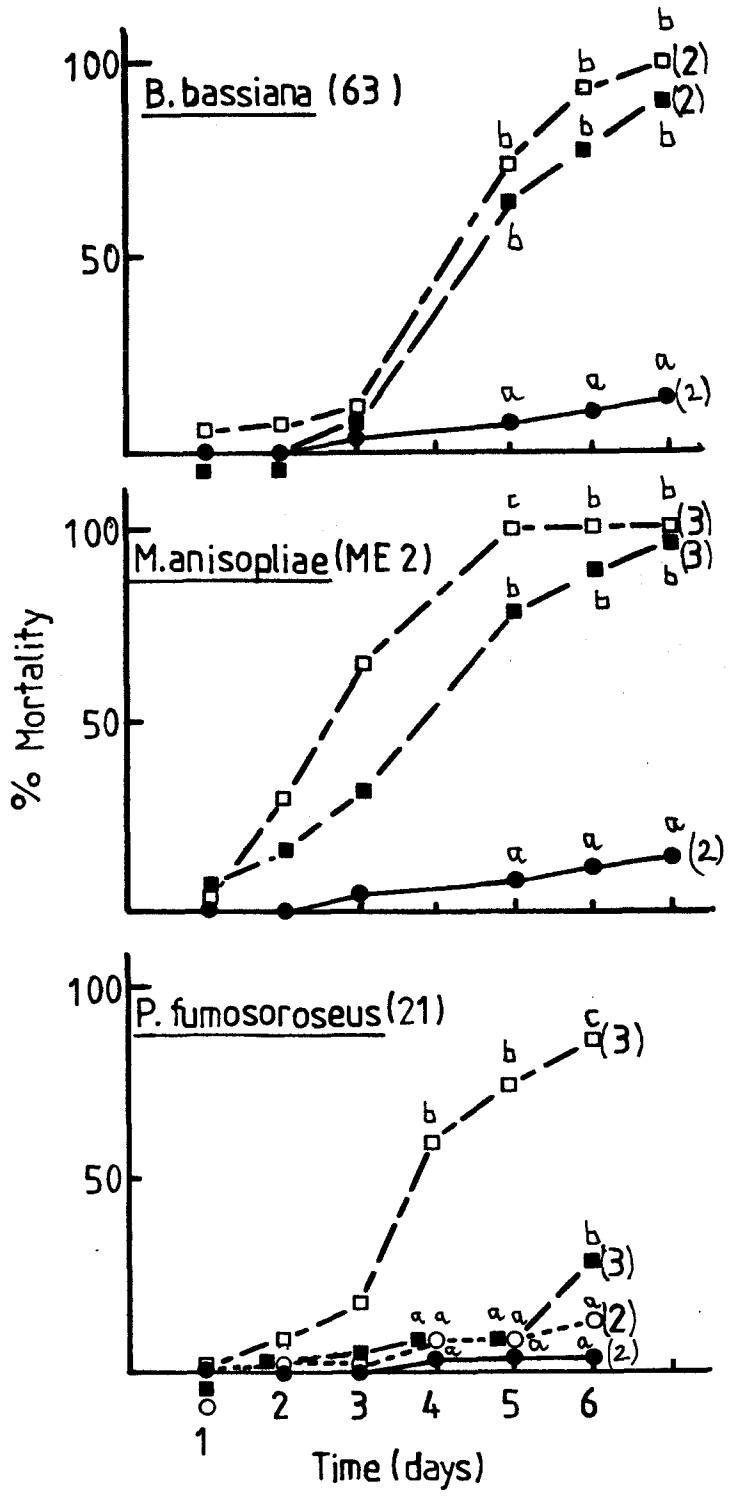


Fig. 6.11

Mortality of adult Hauptidia maroccana after immersion in 0.025% Triton X-100 (●—●) or surfactant containing 5×10^7 conidia ml^{-1} of Beauveria bassiana (63), Metarhizium anisopliae (ME2), or Paecilomyces fumosoroseus (21) and maintenance on leaf discs at 13 (o---o), 20 (■—■) or 25°C (□—□). Points are means. Numbers in parantheses indicate number of replicates of 20 insects. For each sampling date different letters indicate significant difference ($p = 0.05$)

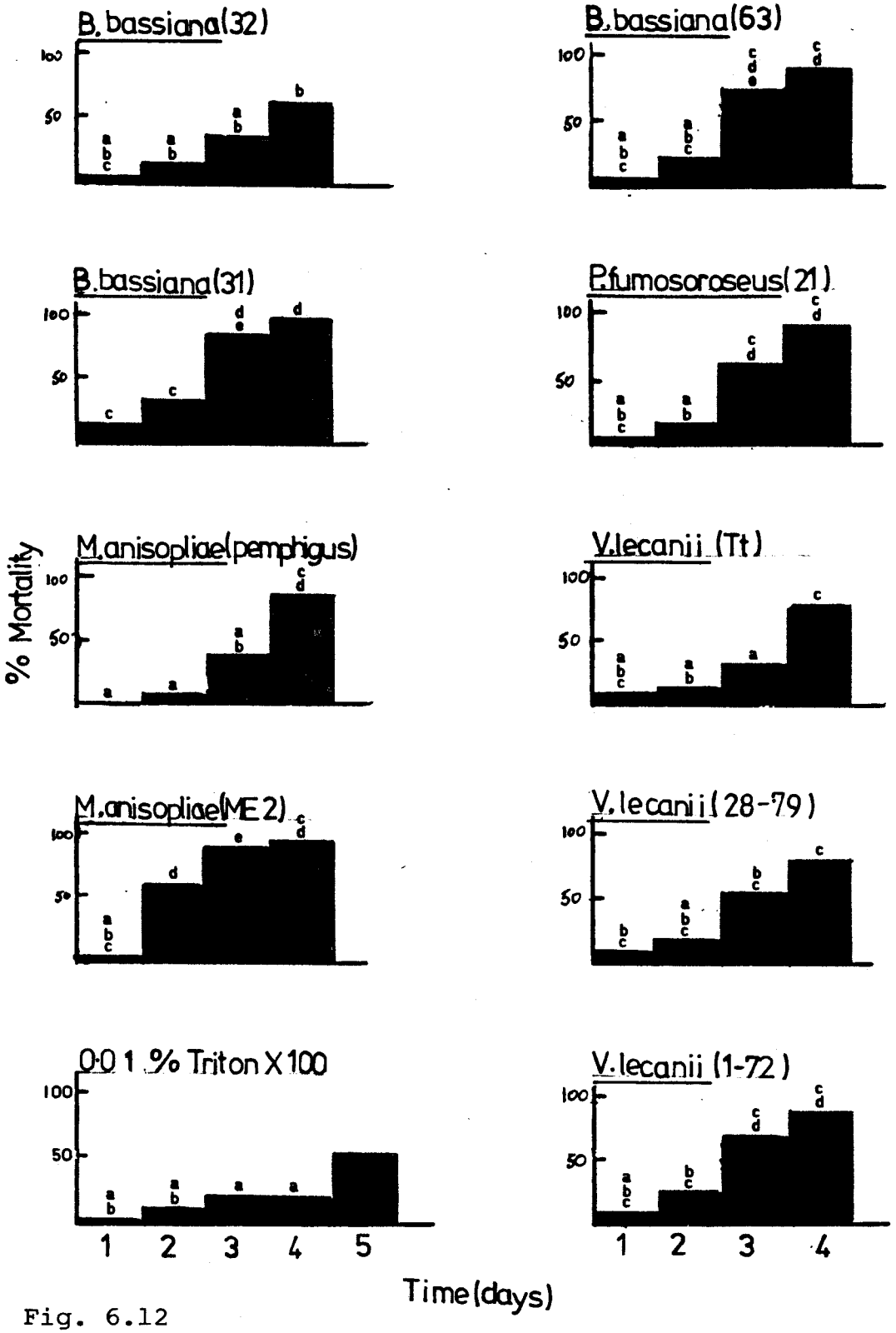


Fig. 6.12

Mortality of adult Thrips tabaci after treatment with various fungi (5×10^7 conidia ml^{-1}) and maintenance on leaf discs (diam. 0.6 cm) at 100% r.h. and 23°C. (Histogram shows mean mortalities of three replicates of 20 insects For each sampling date different letters denote significant difference between treatments at $P = 0.05$).

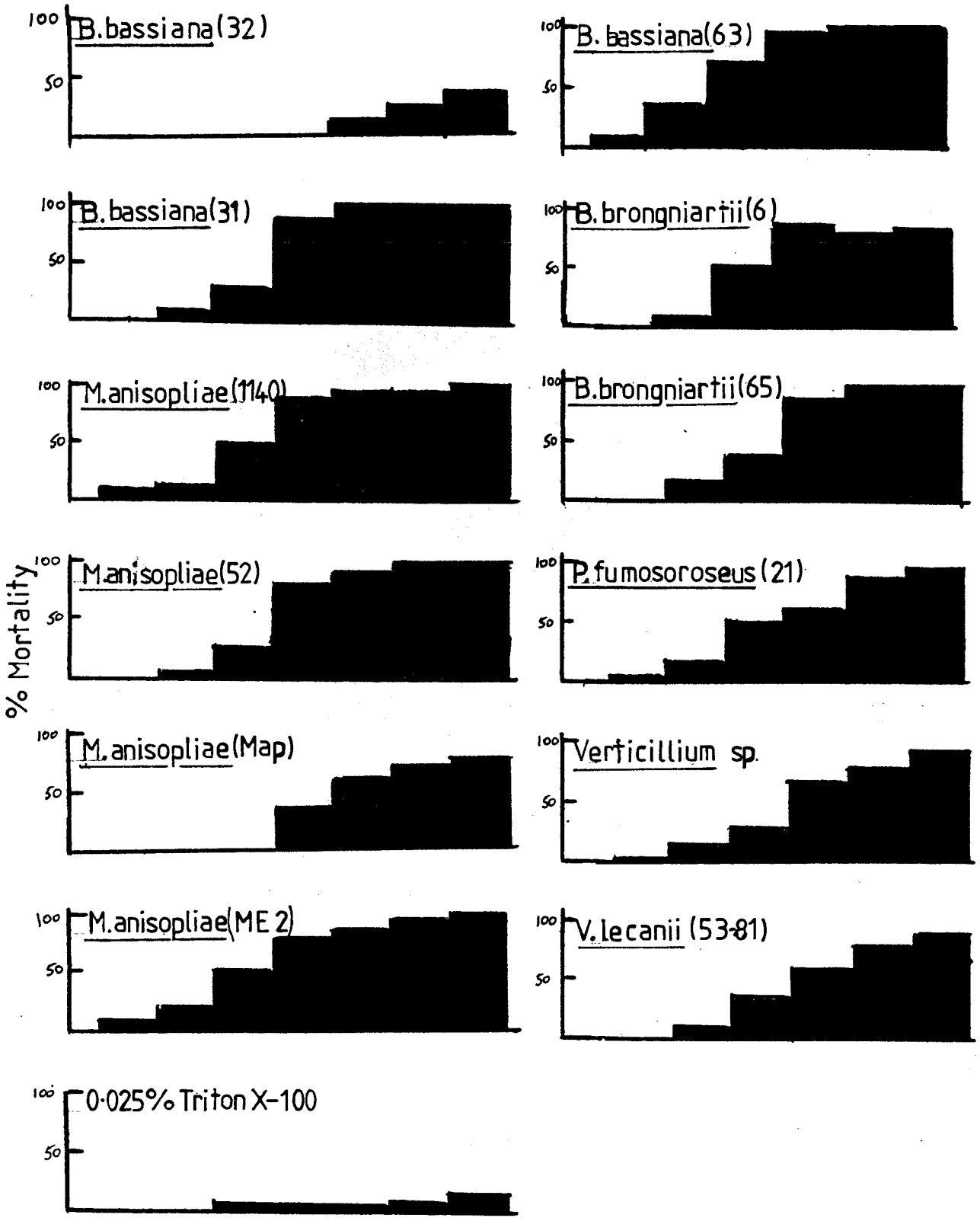


Fig. 6.13
Mortality of adult Thrips tabaci after immersion in 0.025% Triton X-100 or similar suspensions containing 10^7 conidia ml^{-1} of various fungi. Histogram shows % mortalities computed from approximately 20 insects $treatment^{-1}$.

ii) Comparison of Commercial Verticillium lecanii Products

Mycotal® was considerably more effective than Thriptal® (Fig . 6.14); mortalities after 4 days were Mycotal® 95% and 97% compared with Thriptal®, 13 and 44%. Sporulating V. lecanii mycelia was observed on dead insects and also growing from nutrient deposits on leaf surfaces (Fig. 6.15).

c. Tetranychus urticae - Red Spider Mite

i) Pathogenicity of Commercial Verticillium lecanii Products

Mycotal® was the most effective product, and 4 days after treatment had killed 88 and 95% of mites, compared to Thriptal®, 27 and 63% and Vertalec® 24% (Fig. 6.16). Possibly some of the mites became trapped in the mycelia growing on leaf surfaces and thus were killed. However, dead mites bore sporulating V. lecanii mycelia and no mites were observed to become entangled with the fungus for any length of time. Occasionally, mites were observed entangled, but all managed to free themselves within a few minutes. Mites infected with V. lecanii are shown in Fig. 6.17.

d. Macrosiphoniella saborni - Chrysanthemum Aphid

i) Period of High Humidity Required For Infection With Verticillium lecanii (1-72)

Aphids treated with V. lecanii (1-72) conidia and immediately placed at ambient humidity, remained healthy and after 90 hours, only 7% had died (Fig. 6.18). In con trast, insects maintained at a nominal 100% r.h. for 16, 21.5, 40.5 or 66 hours, suffered considerable mortality and 90 hours after treatment, mortalities were 56, 77, 100 and 92%, respectively. When treated aphids were maintained for 66 hours at a nominal 100% r.h. and then removed to ambient humidity, insects died rapidly and 6 hours after removal, 54% of such insects were dead. These insects had

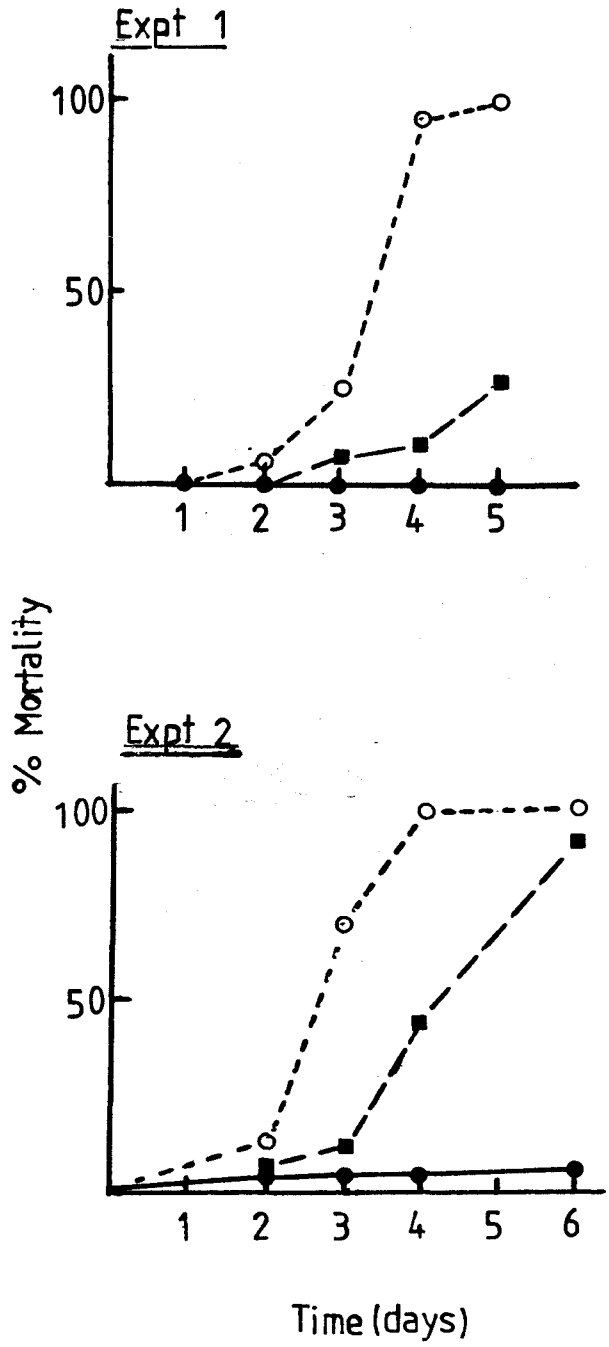


Fig. 6.14
Mortality of adult Thrips tabaci maintained on leaf discs (●—●) or on leaf discs previously sprayed with Mycotal® (o---o) or Thriptal® (■—■) and incubated at 100% r.h. and 25°C for 4 days. Twenty insects were used for each treatment.

Fig. 6.15

Thrips tabaci infected with Verticillium lecanii from Mycotal® growing on cucumber leaf discs.

×20

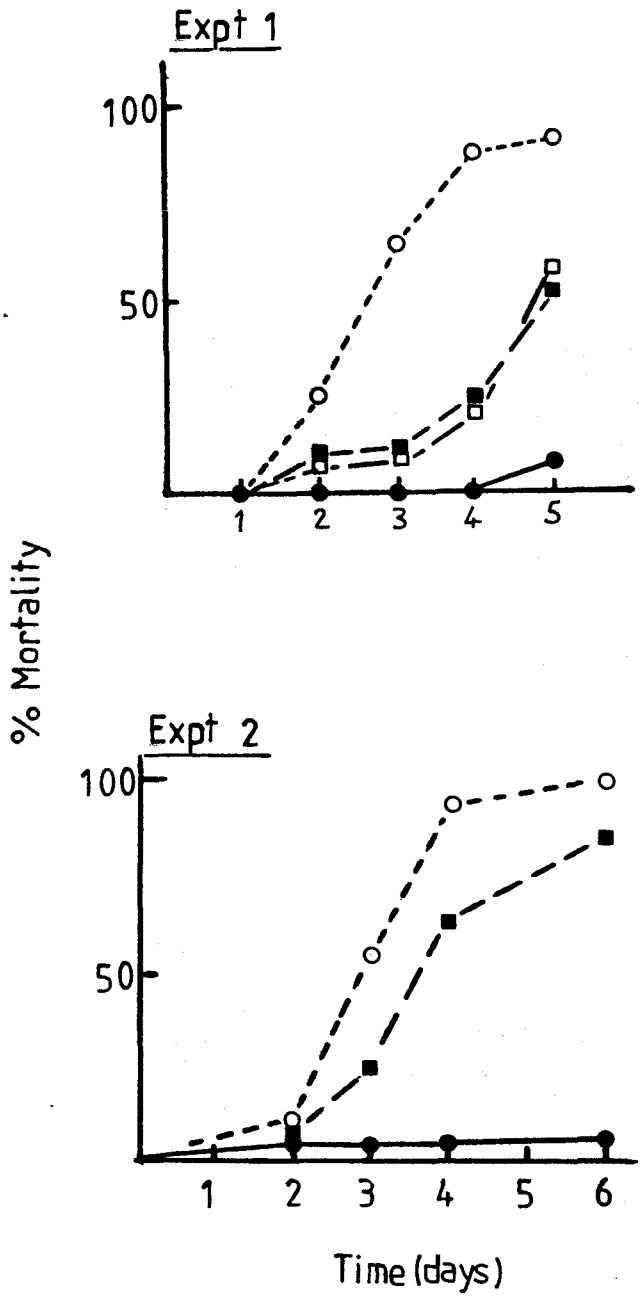


Fig. 6.16
Mortality of adult Tetranychus urticae maintained on leaf discs (●—●)at 20°C, or on leaf discs previously sprayed with Mycotal® (o--o), Thriptal®(■—■) or Vertalec® (□—□) and incubated at 100% r.h. and 25°C for 4 days. Twenty insects were used for each treatment.

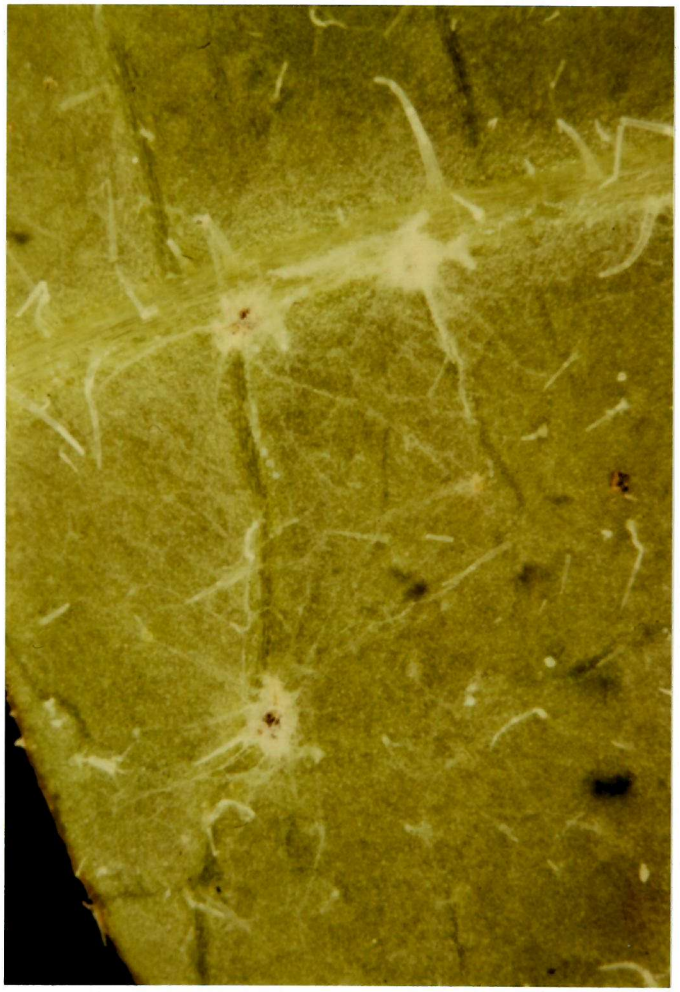


Fig. 6.17

Tetranychus urticae infected with *Verticillium lecanii* from Mycotal® growing on cucumber leaf discs

× 20

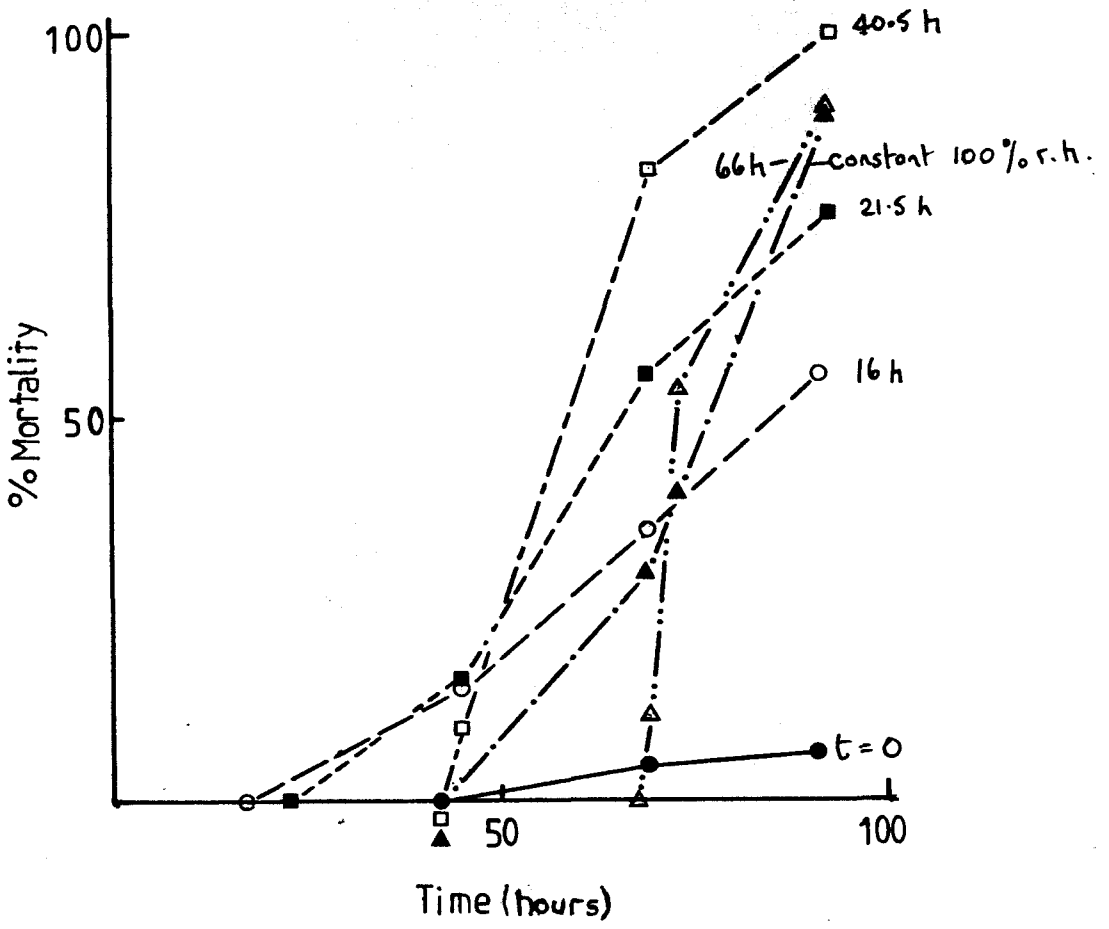


Fig. 6.18
Mortality of adult Macrosiphoniella sanborni after treatment with Verticillium lecanii (1-72) and maintenance at 100% r.h. for 0, 16, 21.5, 40.5 or 66 hours and then at ambient humidity at 20°C. Points are % mortalities calculated from 20 insects treatment.

a characteristic shrivelled appearance, and it is possible that death was due to dehydration as a result of fungal penetration. The experiment strongly suggests V. lecanii (1-72) is able to penetrate aphid cuticle and establish infection in as little as 16.5 hours.

e. Phytoseilius persimilis - Red Spider Mite Predator

Five days after treatment with V. lecanii (53-81), some 70% of predators were dead compared to 14% of those treated with only Triton X-100 (Fig. 6.19). Thus, the high dose of V. lecanii (53-81) conidia (8×10^7 spores ml^{-1}) used in this experiment, had an adverse affect on predators. However, a shortage of prey may have made predators more susceptible, as by 3 days after treatment all red spider mites had been consumed. Obviously, further work is required on this point.

f. Nilaparvata lugens - Brown Planthopper

i) Initial Screening Experiments

The first experiment used rice stem segments to maintain adult N. lugens after treatment with various fungi, and stems were in poor condition when the experiment was terminated after 6 days (Fig. 6.20). It is likely that insufficient food source contributed to the high control mortality; some 40% after 6 days. M. anisopliae strains ME2, 52 and 1140 and Paecilomyces sp. had killed all N. lugens after six days and were considerably more pathogenic than strains of B. bassiana, B. brongniartii, P. fumosoroseus, S. racemosum, V. lecanii and Verticillium sp, tested. B. bassiana (32, Sabah) S. racemosum and V. lecanii (53-81) were considered apathogenic and resulted in mortalities of only 31, 32, 25 and 35% after 6 days at 25°C; cf. control mortality, 40%.

When the experiment was repeated and insects maintained on rice plants, control mortalities were much reduced; 15% after 7 days (Fig. 6.21). The most pathogenic strains were M. anisopliae (ME2, 52, 1140) and Paecilomyces sp with Beauveria and Verticillium strains of low pathogeni-

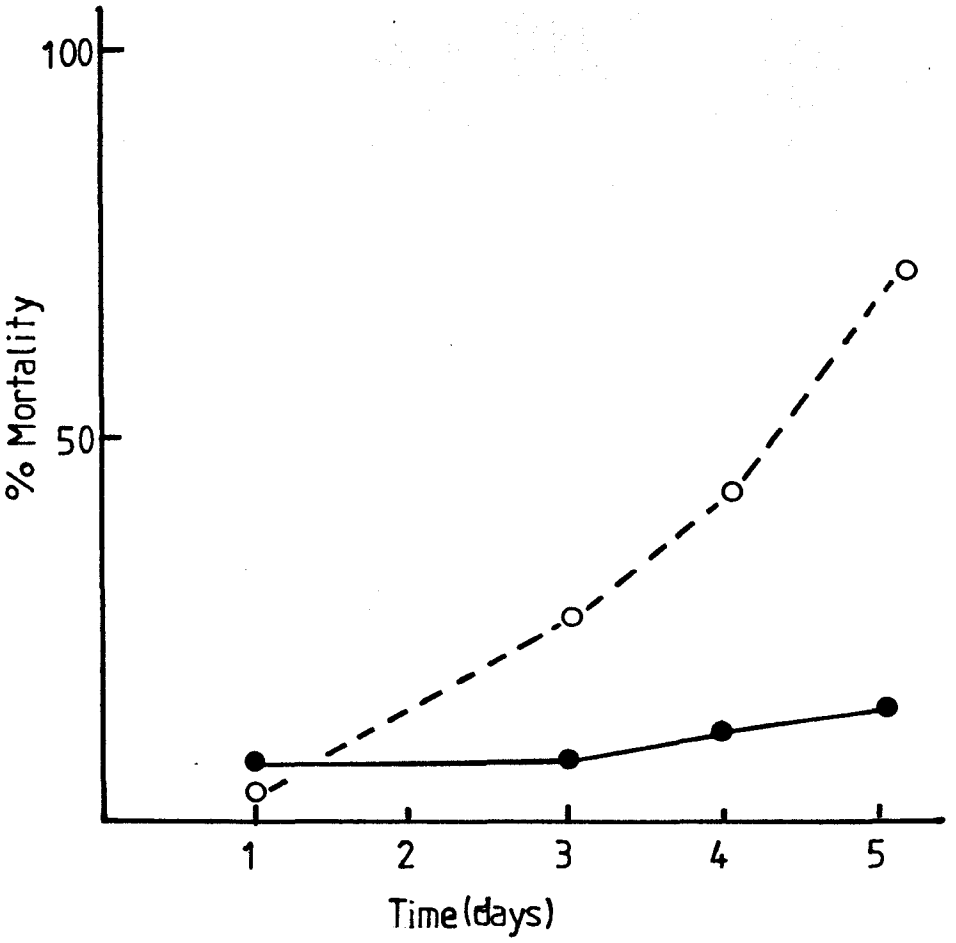


Fig. 6.19
Mortality of Phytoseilius persimilis adults after immersion in 0.01% Triton X-100 (●—●) or a similar solution containing 8 x 10^7 conidia ml^{-1} of Verticillium lecanii (53-81) (o--o). Points are percentage mortalities computed for approximately 25 insects treament.

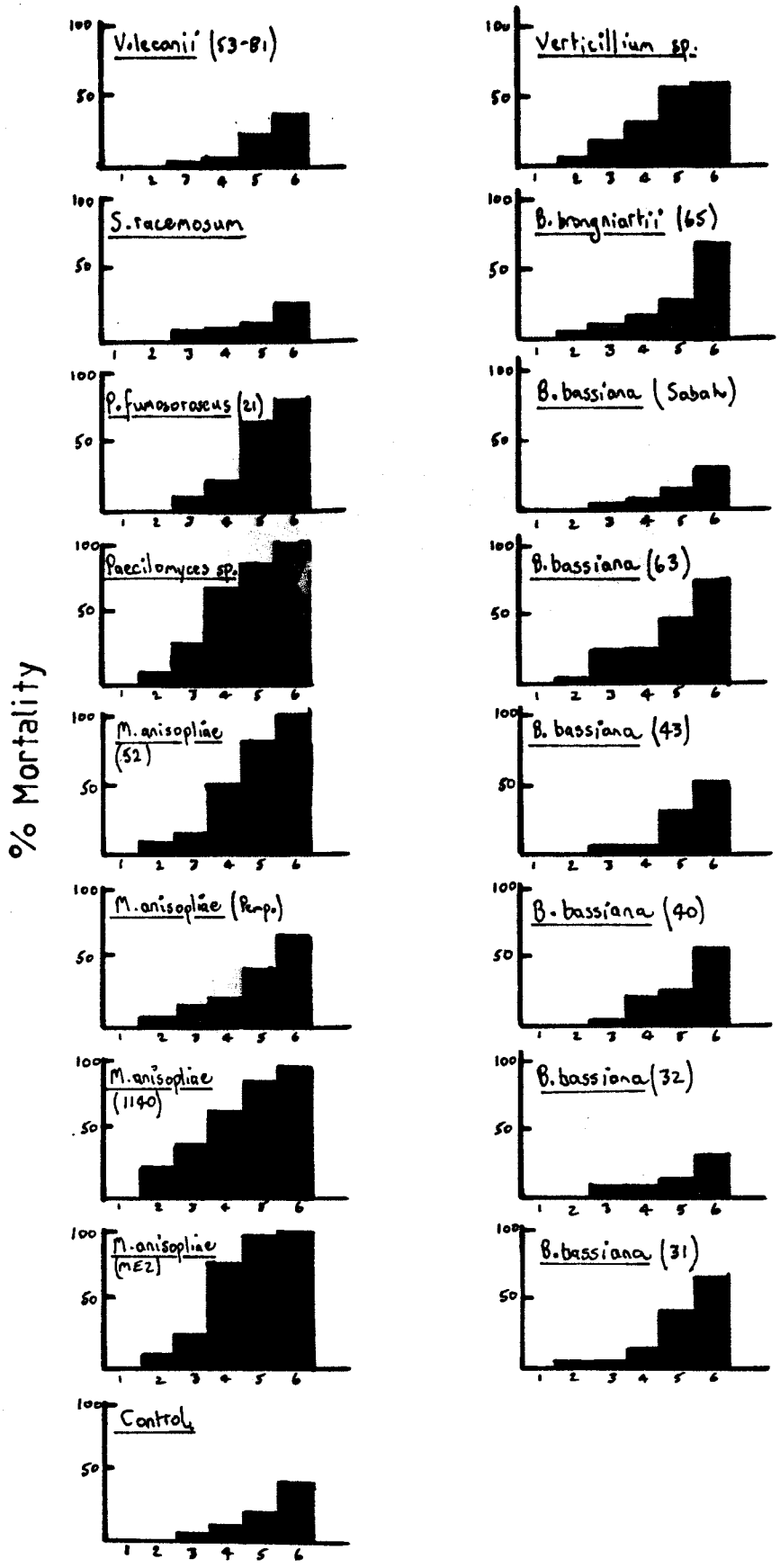


Fig. 6.20

Mortality of adult brachypterous Nilaparvata lugens after immersion in 0.025% Triton X-100, or similar solutions containing x 10^7 conidia ml^{-1} of various entomogenous fungi and maintenance on rice stem segments at 25°C. Histogram shows % mortalities computed from 20 insects $treatment^{-1}$.

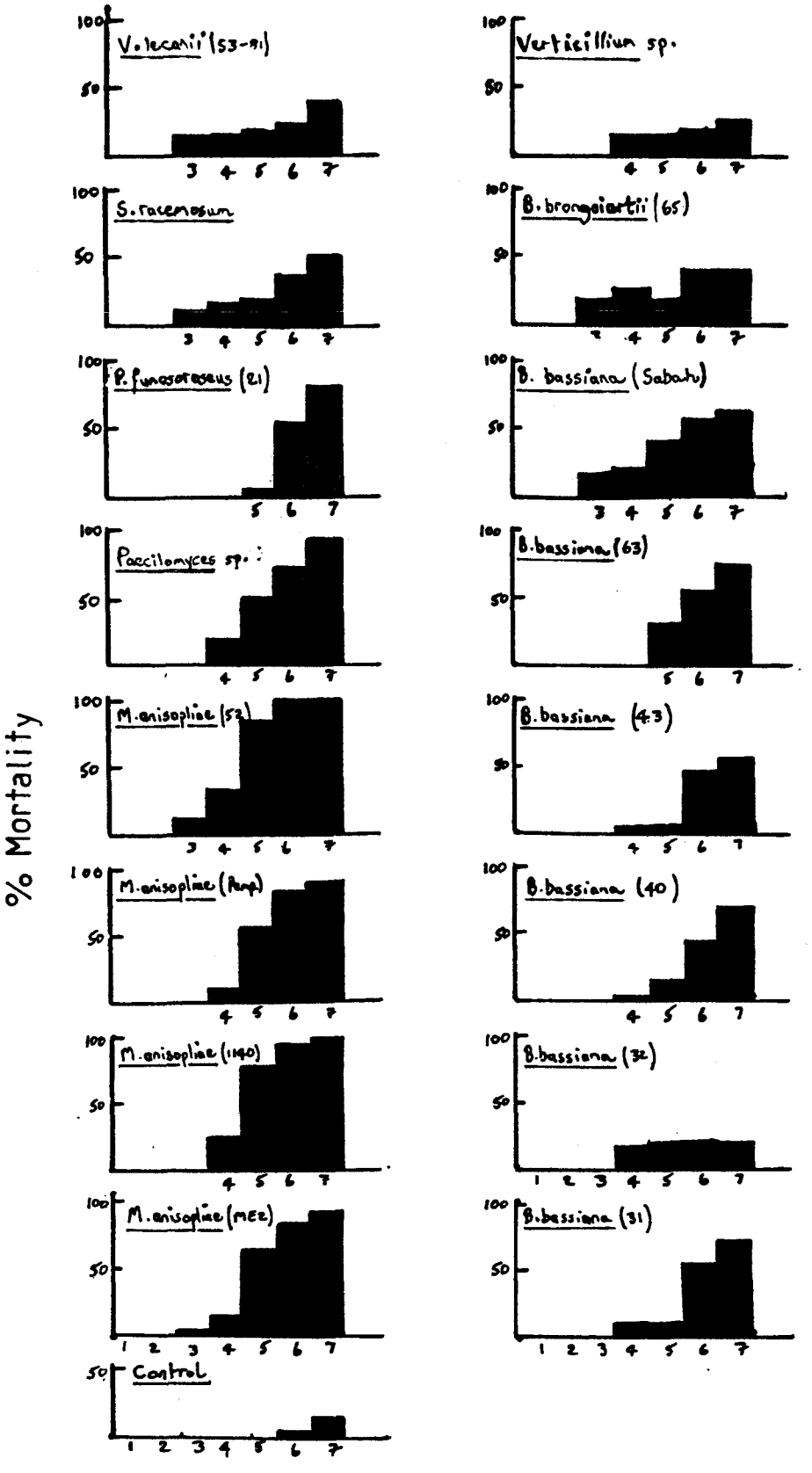


Fig. 6.21

Mortality of adult brachypterous Nilaparvata lugens after immersion in 0.025% Triton X-100 or similar solutions containing 10^7 conidia ml^{-1} of various entomogenous fungi and maintenance on rice plants at 25°C. Histogram show % mortalities computed from 20 insects $treatment^{-1}$.

city. This confirmed the results of the earlier experiment. Dead planthoppers infected with B. bassiana, M. anisopliae or Paecilomyces sp. are shown in Fig. 6.22.

ii) Replicated Experiment

M. anisopliae strains ME2, 52 and 1140 produced statistically identical mortalities ($p = 0.05$) and were more pathogenic than P. fumosoroseus (21) or B. bassiana (50) (Fig. 6.23).

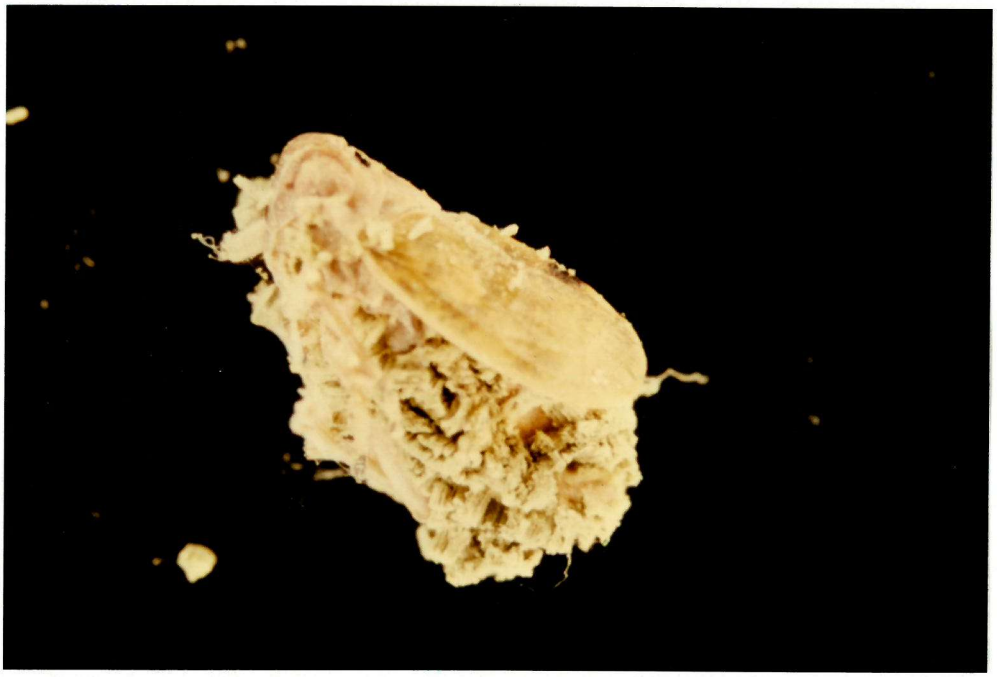
iii) Bioassays

The mean LC 50 value of M. aniospliae (ME2) was 18.6 x 10^5 conidia ml^{-1}, after 4 days incubation at 25°C (Table 6.10). Assays were read after 4 days, as planthoppers destructive mode of feeding damaged the rice plants, and control mortalities occasionally exceeded 5% after this time.

iv) Effect of fungi on nymphs

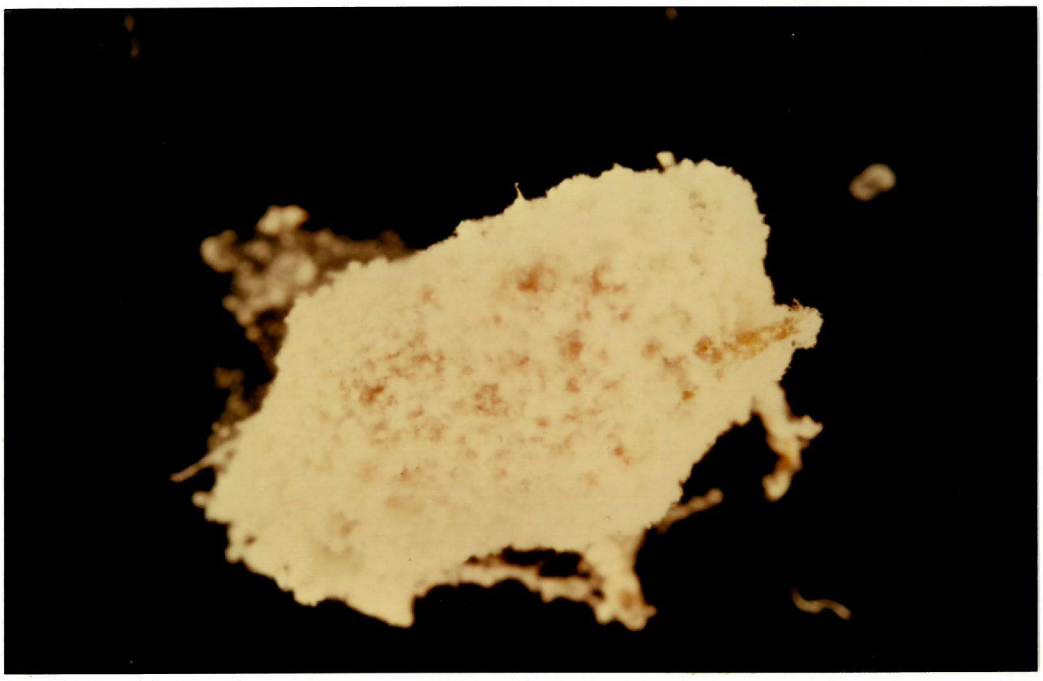
N. lugens nymphs proved susceptible to M. anisopliae (52, 1140) and immersion in 5 x 10^7 conidia ml^{-1} resulted in 94 and 85% mortality respectively after 7 days (Fig. 6.24) M. anisopliae (ME2) was less pathogenic and killed 71% in the same time. B. bassiana was only moderately pathogenic (57% mortality after 7 days) while S. racemosum killed only 16% in the same time.

a



b

Fig. 6.22
Adult Nilaparvata lugens infected with entomogenous fungi.
a. Beauveria bassiana, b. Metarhizium anisopliae



c

Fig. 6.22 (cont.)

c. Paecilomyces sp. ×13

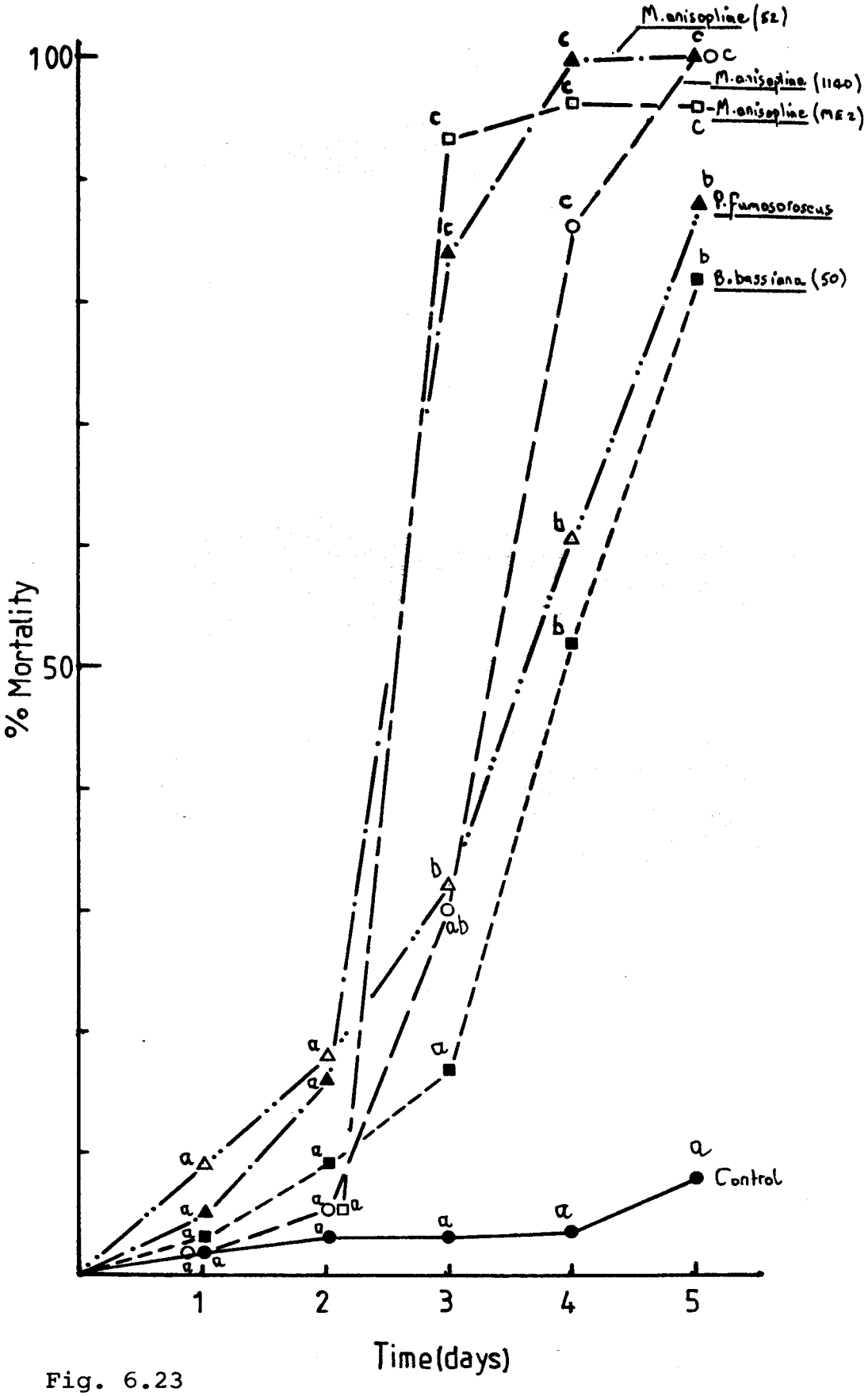


Fig. 6.23

Mortality of adult brachypterous Nilaparvata lugens after immersion in 0.025% Triton X-100 or similar solutions containing 5 x 10^7 conidia ml^{-1} of various entomogenous fungi, and maintenance on rice plants at 25°C. Points are means of 3 replicate groups of 20 insects treatment . For each sampling date different letters indicate significant difference (p = 0.05) between treatments.

Table 6.10

Pathogenicity of Metarhizium anisopliae (ME2) to adult Nilaparvata lugens

Non replicated assays; 20 insects dose^{-1}, 4 doses

Assay number	LC 50 with 95% fiducial limits (x 10^5 spores ml^{-1})	Slope ± S.E.[a]	Chi^{2b}	d.f.[c] [b]	p[b]
1	18.6(9.5-41.1)	1.5±0.6	2.0	2	>0.05
2	24.1(3.8-60.1)	1.4±0.5	1.2	2	>0.05
3	13.3(5.5-31.5)	0.9±0.2	1.9	2	>0.05

Mean LC 50 18.6 x 10^5 conidia ml^{-1}

a Standard error

b heterogeneity about regression line

c degrees of freedom

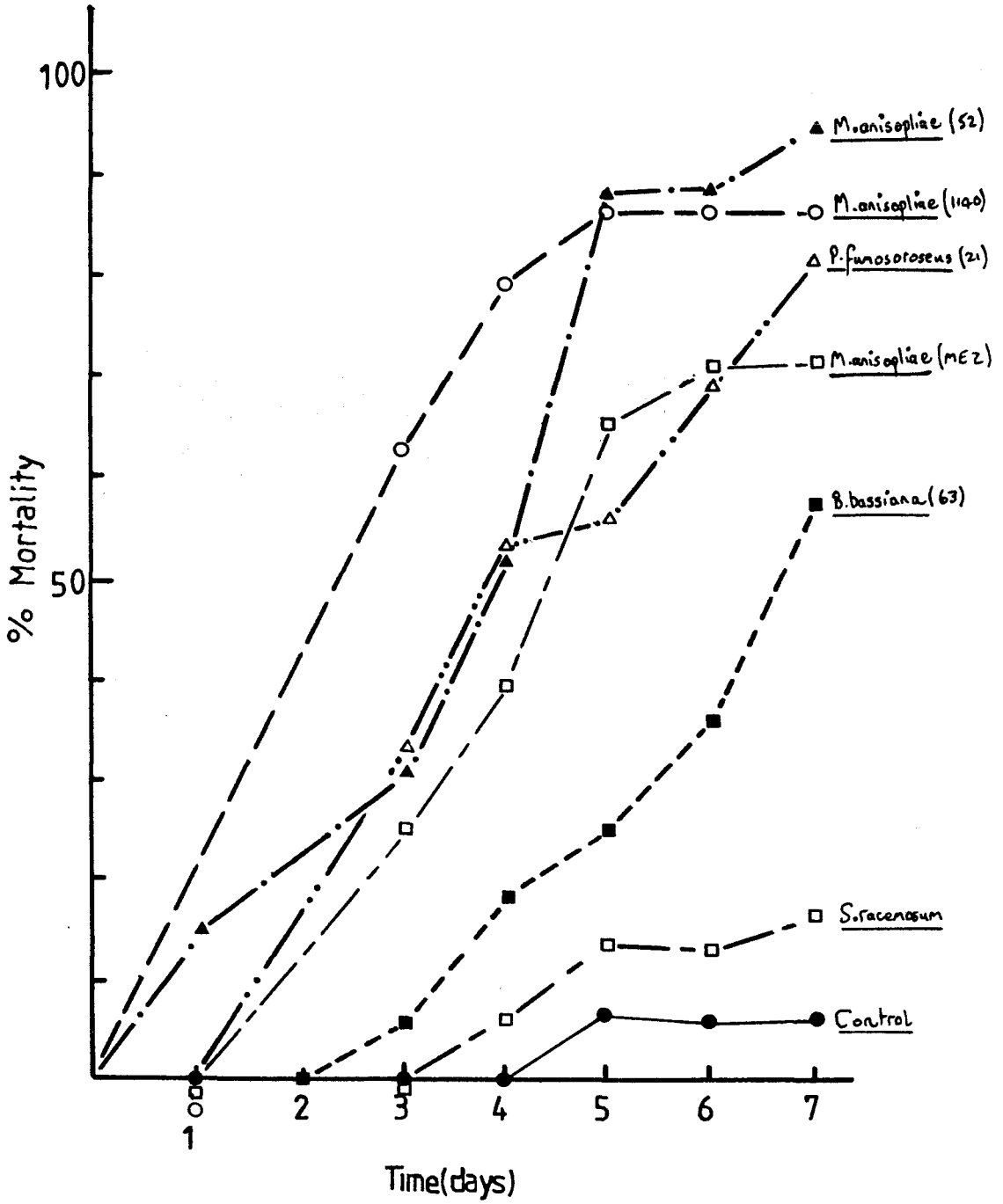


Fig. 6.24

Mortality of Nilaparvata lugens nymphs (3rd-5th instar) immersed in 0.01% Triton X-100 or similar solutions containing 5×10^{7} conidia ml^{-1} of various entomogenous fungi, and maintenance on rice stem segments at 25°C. Points indicate % mortalities computed from approximately 20 insects $treatment^{-1}$.

DISCUSSION

Preliminary screening experiments gave an indication of fungal pathogenicity under optimal conditions and allowed strains of low virulence to be discarded. In studies with H. maroccana and N. lugens, virulence was further assessed using multiple dosage assays, again conducted under conditions optimal for the fungus. However, the sucess of a fungus as a mycoinsecticide depends on many factors. Hall (1982) used the term infective potential, to describe fungal-induced mortality under optimum conditions and epizootic potential, to describe the ability of a fungus to spread from diseased to healthy insects. Hall (1982) treated adult aphids with conidia of V. lecanii strains and maintained treated insects on leaf discs, at a nominal 100% r.h. The mortality of adult aphids provided an estimate of infective potential, while progeny mortality was used to quantify epizootic potential. This system worked well for aphids and indicated the most effective strain for aphid control was V. lecanii (1-72) - a strain produced commercially for aphid control on glasshouse chrysanthemums. However, the system described by Hall (1982) is only of use with rapidly reproducing insects. In this study, a modified system was used to assess the potential of V. lecanii products. Thrips or mites were placed on leaf discs cut from plants previously sprayed with Vertalec®, Mycotal®, or Thriptal® and incubated at high humidity for 4 days. Fungus - induced mortality resulted from two factors; the pathogenicity of the fungal isolates and the ability of the isolate to spread. It is suggested that the above system would provide a useful quality control test for V. lecanii products, and may also be applicable to other insect/deuteromycete systems, by spraying plants with commercial products, or spore suspensions containing nutrients. Indeed, this study demonstrated growth and sporulation of M. anisopliae after application of spores and nutrients to rice plants (V, 15). Although the systems described above represent an improvement on direct treatment of insects with spores, they still

are conducted under constant high r.h., conditions which occur only rarely in the glasshouse or field. However, it would be very difficult to stimulate varying r.h. in the laboratory and such data can only be acquired in glasshouse or field experiments.

Screening experiments and multiple dosage bioassays demonstrated intraspecific variation in the pathogenicity of isolates of B. bassiana, M. anisopliae and V. lecanii. In addition, strains isolated from a particular insect were less pathogenic than those cultured from other hosts. For example, V. lecanii (28-79) isolated from H. maroccana, was less pathogenic to the host insect than strain Tt isolated from T. tabaci, and similarly, B. bassiana (43) isolated from N. lugens, was less virulent than strain 63. Probit analyses (Ross, 1970) of multiple dosage bioassays of M. anisopliae (ME2, and 1140) gave mean LC 50 values of approximately 6×10^5 and 2.4×10^5 spores ml^{-1} respectively, after 6 days incubation at 25°C and a nominal 100% r.h. Hall (1977) obtained a similar value in assays of V. lecanii (1-72) against M. sanborni, and his study led to commercialization of this strain as Vertalec®. Thus, it was considered that M. anisopliae (ME2, 1140) were sufficiently pathogenic to test further in glasshouse experiments.

Replication of doses within assays reduced variation slightly, but it is debatable whether this increased precision justified the increased number of insects and time. The slopes of probit-transformed dose/mortality data were low and ranged from 0.8-1.8. Hall (1976) obtained slopes from 1.36-3.02 for V. lecanii, while Burges and Thompson (1971), reviewing the slopes of bioassays for a range of pathogens, found fungal bioassay slopes to be similarly low. Estimation of spore numbers adhering to insects during immersion in spore suspensions, showed approximately 0.008% of the applied spore number ml^{-1}, adhered to H. maroccana. When these data were subject to probit analysis, LD 5 values were low; M. anisopliae (52) 1.1, P. fumosoroseus (21) 2.4, and thus it is likely that single spores can penetrate insect cuticle and establish infection.

This study demonstrated M. anisopliae (ME2 and 1140) were able to spread from cadavers and infect healthy insects. V. lecanii also has this ability (Hall, 1977, 1982). In contrast, B. bassiana (63) spread poorly and P. fumosoroseus failed to cause significantly greater mortality than insects maintained without cadavers. The ability of a fungus to spread is governed by the isolates pathogenicity, and also perhaps by the spore type. V. lecanii spores are contained in slime and it is possible this provides adhesion of spores to insect cuticle. By contrast, M. anisopliae is adapted for wind dispersal and has dry spores, which are readily dislodged from cadavers and picked up by insects. The poor spread of P. fumosoroseus (21) may be an artefact of the experimental technique. Insects killed by the fungus have a covering of mycelium which spreads around the cadaver and provides anchorage to the substrate. This mycelium was disturbed when insects were transferred to fresh leaf discs and so may have impaired spore transmission.

Pathogenicity of M. anisopliae (ME2) conidia and hyphal bodies was similar, a situation also found in the deuteromycetes, B. bassiana, Tolypocladium cylindrosporum and V. lecanii (Hall and Papierok, 1982). Hyphal bodies are more amenable to large - scale production as they can be produced in stirred tank reactors, whereas conidia are difficult to produce on a commercial scale (Quinlan and Lisansky, 1983).

At 20°C, V. lecanii was able to penetrate and infect aphids more rapidly than M. anisopliae (ME2) was able to establish infection in H. maroccana. This difference was probably due to V. lecanii germinating more rapidly than M. anisopliae at this temperature, and to a difference in the mode of penetration: V. lecanii penetrates aphids directly (Hughes, pers. comm.) while M. anisopliae penetrates only after formation of an appressorium (Zacharuk, 1981). N. lugens was most susceptible to strains of M. anisopliae. Roberts (pers. comm.) also reported N. lugens was killed by this fungus and reported laboratory cultures of this insect being treated with fungicide, to prevent M. anisopliae infection. In this study, strains of V. lecanii were of low pathogenicity, which contrasts with the reports of

Balasubramanian (1979) who described 60-70% mortality of N. lugens after treatment with V.lecanii.
The commercial V. lecanii product Mycotal® was pathogenic to both thrips and red spider mites,and if these results can be confirmed,it may be possible to control all the major cucumber pests with a single product.There are several reports of fungi parasitising mites.Oetting and Gardner (1982) demonstrated T. urticae was susceptible to H. thompsonii and Mc Coy(1978) showed the same fungus was pathogenic to citrus mites.Nemoto et al.(1979)observed spider mites infected with Entomophthora floridanum.
This section demonstrated isolates of fungi pathogenic to H.maroccana, T.tabaci, T.urticae and N.lugens and enabled selection of promising strains for further testing in glasshouse conditions.

SECTION VII

PRODUCTION OF FUNGI IN SUBMERGED CULTURE AND ON SEMI-SOLID MEDIUM

INTRODUCTION

Deuteromycete entomogenous fungi can be produced on semi-solid media, eg cereal grains contained in autoclavable polyethylene bags (' Aquino et al. 1975, 1977). However, large scale production would be facilitated by submerged culture, utilizing conventional fermenters. The experiments described here demonstrate production of Verticillium lecanii in submerged culture and attempts to obtain suitably high yields of Metarhizium anisopliae under similar conditions.

MATERIALS AND METHODS

1. General

   a. Media

Complex prepared media were obtained from Oxoid Ltd., England, with the following components litre $^{-1}$ of distilled water.

Dextrose Broth

'Lab-Lemco' Powder	3g
Tryptone	10g
Glucose	5g
Sodium chloride	5g

pH approximately 7.2

Czapek Dox Liquid Medium

Sodium nitrate	2g
Potassium chloride	0.5g
Magnesium glycerophosphate	0.5g
Ferrous sulphate	0.01g
Potassium sulphate	0.35g
Sucrose	30.0g

pH approximately 6.8

Glucose/ Yeast Extract Medium

Yeast Extract (Difco)	20g
Glucose	40g

pH approximately 6.5

Proflo Medium

Proflo [a]	10g
Bacto-Peptone (Difco)	2.0g
Glucose	15g
Yeast Extract (Difco)	2.0g
Magnesium sulphate	0.3g
Ferrous sulphate	0.02g
Zinc sulphate	0.02g
Calcium carbonate	1.0g

pH approximately 7.4

a. A partially defatted cooked cottonseed flour (Traders Oil Mill Co. U.S.A).

Adamek's (1963) Medium

Glucose	40g
Yeast Extract (Difco)	40g
Corn steep liquor	30g
Tween 80	0.4%

pH approximately 7.2

Basal Liquid Medium

Glucose	40g
Potassium nitrate	11.6g
Potassium dihydrogen orthophosphate	5.6g
di-Sodium hydrogen orthophosphate	3.38g
Ferrous sulphate	10mg
Cobalt chloride	1mg
Manganous chloride	1mg
Cupric sulphate	2.5mg
Zinc sulphate	10mg
Ammonium molybdate	1mg
Magnesium sulphate	0.5g
Calcium chloride [a]	0.1g

pH approximately 6.5

a. autoclaved separately

b. Antifoams

Polypropylene glycol (PPG) was used in all experiments at the rate of 1 drop $100ml^{-1}$ medium. Occasionally, when media containing the surfactant Tween 80 were used in fermenters, PPG inadequately prevented foaming and injections of sterile Antifoam A (Sigma Co. Ltd. England. 1ml 1.6 $litres^{-1}$) were required.

c. Preparation of Inoculum

Shake cultures were inoculated with conidial suspensions obtained by flooding 10-day old, SDA plates of M. anisopliae or V. lecanii with sterile distilled water, and filtering through sterile paper tissue. The hydrophobic nature of M. anisopliae conidia limited the spore concentration obtainable and suspensions used for inoculation were standardised at 10^5 conidia ml^{-1}. V. lecanii conidia wetted more readily and suspensions contained $3.24x10^8$ conidia ml^{-1}.

d. Small Scale Production Using Erlenmeyer Flasks

Aliquots of media (50 or 100ml), in conical flasks plugged with loose-fitting, non-absorbent cotton wool, were autoclaved (15lb sq in, 15 min), cooled, inoculated with media constituents requiring separate sterilization and 0.5ml aliquots of a water suspension containing defined numbers of conidia, then incubated on a rotary shaker (110 or 135 rpm) normally at 26 ± 1^oC.

Spore numbers were determined by removing 1ml aliquots of media with an adjustable micropipette (Labsystems O.Y., Finland), fitted with wide-bore tips (min diam 0.25cm) and counting suitable dilutions using a haemocytometer

Dry weight assessments were made by removing 10ml aliquots of media using glass pipettes with widened tips (min diam 0.3cm), vacuum filtration (GFA glass microfibre paper, 4.7cm diam. Whatman Co. Ltd.), washing resultant residues with distilled water and drying filter papers to constant weight at $70-80^oC$.

e. Medium Scale Production Using 1.6 l Fermenters

Fermenters comprised stainless steel impeller assemblies (Biolaffite S.A. France), 1.6 l capacity glass pods (Hampshire

Glass Co. Ltd. England), electric motors (125 watt, Parvalux Co. Ltd. England) and speed controllers (M.R. Supplies Co. Ltd., England. Fig. 7.01). Temperature was regulated by water circulated through a temperature controller (Churchill Co. Ltd. England.).
After placement of media into glass pods, the apparatus was assembled and ports for inoculation, sampling, air inlet and exhaust ports were fitted with autoclaved plastic tubes. An air filter (Gamma 12, grade 12-03 filter tube. Whatman Co. Ltd.) was fitted to the air inlet and all tubes plugged with non-absorbent cotton wool. All tubes, except the air exhaust, were then clamped and the apparatus autoclaved (15lb. sq. in. 20min.) and allowed to cool. Fermenters were inoculated with 50ml of either V. lecanii spores, previously grown in identical liquid medium to that contained in the fermenter (96h incubation, 25°C and 135 r.p.m.), or 0.01% Triton X-100 containing 10^7 M. anisopliae conidia ml^{-1}. Cultures were agitated (450 or 1500 rpm) and aerated with compressed air (50 or 100 l $hour^{-1}$). Spore numbers and fungal biomass were estimated as described earlier (VII, 1, a) using media expelled from the sampling port by pressure created by clamping the exhaust tube.

2. Production of Metarhizium anisopliae on Semi-solid Medium

Crushed barley grain (50g $flask^{-1}$) and 2-ml aliquots of corn oil (Keymarkets Co. Ltd. England.) were mixed in 250ml conical flasks and moistened by 35 ml of water. Necks were plugged with cotton wool, flasks autoclaved (15lb. sq. in. 20min) and while hot, shaken vigorously to break clumps of cereal grains. This process was not completely effective and a sterile glass rod was used to break up clumps of barley. Five flasks, inoculated with 2ml aliquots of a 0.025% TritonX-100 solution containing 10^8 M. anisopliae (ME2) conidia ml^{-1}, were incubated at 25 ± 1°C. Flasks were shaken vigorously after inoculation to distribute spores and briefly agitated on each of the next 3 days to maintain friability.

Samples of grain (1g) were removed with a sterile spatula, suspended in 0.025% TritonX-100 (10 or 20ml) and shaken for 3 min to remove conidia from the cereal grains. Resultant suspensions were suitably diluted and conidial numbers estimated using a haemocytometer. Further agitation failed to increase conidial numbers showing that 3 min shaking removed all spores from the grain. Further flasks were incubated at 20° (3 flasks), 25°(2) and 27°C (3).

3. Production of V. lecanii in Liquid Culture

a. Small Scale

Erlenmeyer flasks (250ml) containing 100ml glucose/yeast extract medium containing extra glucose (80g l^{-1} glucose, 20g l^{-1} yeast extract) were inoculated with 0.5 ml aliquots of V. lecanii (53-81) conidial suspensions, inoculated on a rotary shaker (110 rpm) at 25 ± 1°C and hyphal body production estimated after 43, 67 and 99 hours.

b. Medium Scale

Two 1.6 l fermenters, containing 1.5 l of sterile glucose/yeast extract medium (VII,1,a) were inoculated with V. lecanii, strain 53-81 or 19-79, at $5x10^7$ ml^{-1}, maintained at 25 ± 1°C with agitation (450 rpm) and aeration (50 lh^{-1}). Spore numbers and fungal biomass, were determined 23, 28, 47, 51.5, 62, 73.5 and 76 hours after inoculation.

4. Production of Metarhizium anisopliae in Submerged Culture in Erlenmeyer Flasks

a. Comparison of Spore Production Between Strains

i) Preliminary Experiment

Aliquots (100ml) of Sabouraud liquid medium (SLM) or SLM plus 0.5% Tween 80, were inoculated with M. anisopliae conidia, of strains ME2, ME3, 52 or 1140. Flasks were incubated on a rotary shaker (110 rpm) at 27 ± 1°C and qualitative growth assessments made after 48 and 72 hours.

ii) Quantitative Experiment

Media comprising molasses and yeast extract or molasses and peptone (40g l^{-1} molasses, 20g l^{-1} yeast extract

or Oxoid Bacteriological peptone) were inoculated with single loopfuls of M. anisopliae conidia of strains ME2, ME3, 52 or 1140. Flasks were incubated on a rotary shaker (110 rpm) at 26 ± 1°C and hyphal body production determined after 3 days. Spore yields in molasses/yeast extract medium, were computed from assessments made on three replicate flasks strain^{-1}, while those in peptone medium were made from single flasks.

b. Complex Prepared Media

Three flasks containing 100ml of either SLM, glucose/yeast extract, Czapek Dox or Proflo media were inoculated with M. anisopliae (ME2) conidia, incubated at 25 ± 1°C on a "wrist-action" shaker (Flask Shaker, Gallenkamp Co. Ltd. England. speed 2) and sampled after 24, 48 and 72 hours. Qualitative assessments of hyphal body production were made using phase contrast microscopy and expressed on a 1-5 scale (5 max). Hyphal body and dry weight production was estimated in glucose/yeast extract, Proflo, nutrient and dextrose broths, after 3 and 5 days respectively.

c. Adamek's Medium

Three flasks, containing 50ml of Adamek's medium, were inoculated with single loopfuls of conidia and incubated at 27 ± 1°C and 135 rpm. Spore production was determined after 24, 28, 48, 60 and 72 hours.

d. Effect of Carbon / Nitrogen Ratio

Erlenmeyer flasks, containing 50ml of basal medium with varying amounts of potassium nitrate to provide C/N ratios from 0 nitrogen to 2.5: 1, were inoculated with 0.5 ml aliquots of M. anisopliae (ME2) conidia. Three flasks of each C/N ratio were agitated on a rotary shaker at 26 ± 1°C. Hyphal body and dry weight yields were determined for each flask after 7 days, when pH was measured using pooled media from each group of three replicate flasks.

e. Effect of Nitrogen Source

i) Carbon Source Glucose

Modified basal media (50g l^{-1} glucose) were prepared

by the addition of separately autoclaved glucose, phosphate buffer, salt solutions and nitrogen sources (Table 7.01). and contained in Erlenmeyer flasks. Each flask, with 50ml of media containing 1.0g carbon and 0.1g N, was inoculated with M. anisopliae (ME2) conidia and incubated on a rotary shaker at 25 $\pm$ 1°C. Dry weights and hyphal body numbers were determined for all flasks (3 for each N source) after 7 days.

ii) Carbon Source Molasses

Since M. anisopliae (ME2) produced few hyphal bodies in modified basal liquid media ($<10^5 ml^{-1}$), the experiment was repeated using basal medium with glucose replaced by 80g lit^{-1} molasses. Hyphal body production was assessed after 3, 5 and 7 days, and dry weights at 7 days.

f. Effect of Carbon Source

Erlenmeyer flasks containing 50ml of 2% w.v. yeast extract (Oxoid), 60 mM phosphate buffer (KH_2PO_4 / Na_2HPO_4) and a minimum of 0.8 grams carbon, provided by either fructose, galactose, glucose, glycerol, lactose, maltose, mannitol, sorbitol or soluble starch, were inoculated with M. anisopliae conidia.

Quantities of sugars required were computed from the percentag of carbon in each sugar molecule calculated to provide a C : N ratio of 8 : 1. Similar flasks were prepared with molasses as carbon source, assuming that all the dry weight was sucrose. Three replicate flasks for each sugar were incubated at 26 $\pm$ 1°C on a rotary shaker and hyphal body production assessed after 3 and 5 days and pH and dry weight after 5 days.

g. Effect of Molasses and Yeast Extract Concentrations

Flasks containing variable amounts of molasses (United Molasses, 4, 6, or 8%) and yeast extract (Difco, 2, 3, or 4%) were inoculated with M. anisopliae (ME2) conidia and incubated on a rotary shaker (135 rpm) at 27 $\pm$ 1°C.

Table 7.01

Nitrogenous Compounds Added to Basal Medium to Determine Their Effect on Hyphal body and Dry Weight Production of *Metarhizium anisopliae*

Compound	Nitrogen (%)	Grams in 50ml of media
Ammonium sulphate	10.6	0.94
Potassium nitrate	13.9	0.72
Arginine	26.6	0.38
Cysteine (L)	11.6	0.87
Glycine	18.7	0.54
Proline	12.2	0.82
Urea	46.6	0.22
Cas amino acids	10.0	1.0
Casein hydrolysate	8.3	1.21
Corn Steep Liquor	8.0	1.25
Bach Peptone	14.3	0.62
Yeast Extract (Oxoid)	10.5	0.95
Yeast Extract (Difco)	9.5	1.05
Yeast Extract (Tate & Lyle)	5.7	1.76

h. Effect of Glucose Concentration

Glucose/yeast extract media containing 20g l^{-1} yeast extract and varying amounts of glucose (5, 10, 20, 40, 80, 160 g l^{-1}) were inoculated with M. anisopliae conidia. Three flasks of each sugar concentration were incubated at 26 $\pm$ 0.5°C and 110 rpm and hyphal body and dry weight production estimated after 3 and 5 days respectively.

i. Effect of Glucose and Tween 80 Concentration

Media containing 20g lit^{-1} yeast extract (Difco) and variable amounts of glucose (2, 4 or 8 g l^{-1}) and Tween 80 (0, 0.01, 0.05 or 0.1%) were inoculated with M. anisopliae (ME2). Hyphal body numbers in 3 replicate flasks $medium^{-1}$ were assessed after 2 and 4 days, and dry weight after 7 days incubation (26 $\pm$ 1°C, 135 rpm).
The experiment was repeated, using 80g l^{-1} glucose, 20g l^{-1} yeast extract and varying amounts of Tween 80 (as above) and hyphal body numbers determined after 4 days.

5. Production of M. anisopliae in 1.6 l Fermenters

a. Glucose/yeast Extract / Tween 80 Medium

A medium containing glucose (80g l^{-1}), yeast extract (Oxoid. 20g l^{-1}) and 0.1% Tween 80 was inoculated with M. anisopliae (ME2) conidia, aerated (100 l h^{-1}), stirred (1500 rpm) and maintained at 27°C. Hyphal body and dry weight assessments were made after 20.5, 26, 52.5, 68.5, 79 and 95 hours.
A similar experiment, conducted under identical conditions, utilizing an 8% glucose, 2% yeast extract and 0.5% Tween 80 medium was sampled after 49, 51, 57, 67, 74, 82, 92, 102 and 125 hours.

b. Molasses/Peptone Medium

A medium comprising 40g l^{-1} molasses and 20g l^{-1} Bacteriological Peptone (Oxoid) was inoculated with M. anisopliae (ME2) and sampled after 22, 44, 50, 58, 68, 74 and 93 hours.

RESULTS

6. Production of *Metarhizium anisopliae* Conidia on Semi-Solid Medium

M. anisopliae (ME2) grew and sporulated well on barley grain at 25°C and conidial production was maximal after 9 and 14 days when there was approximately 2×10^9 conidia gram dry barley grain^{-1} (Table 7.02).
Conidial production was always significantly higher ($p = 0.05$) at 25°C than at 20 or 27°C after 7, 9 and 13 days (Table 7.03). Some barley grains remained clumped together even after shaking and agitation with a glass rod. The addition of less water to the barley grain increases friability and therefore surface area (R.A. Hall unpub. obs.) and it is likely conidial production could be increased in this way.

7. Production of *Verticillium lecanii* in Submerged Culture

a. Small Scale - Erlenmeyer Flasks

V. lecanii (53-81) produced spores readily in glucose/yeast extract medium and numbers exceeded 10^9 ml^{-1}, 67 and 99 hours after inoculation (Table 7.04). Conidia germinated within 17 hours of inoculation and hyphal bodies were first noted after 22 hours. Hyphal body numbers increased rapidly and many secondary hyphal bodies were produced *i.e.* hyphal bodies produced further spores by budding in a way similar to yeast.

b. Medium Scale - 1.6-l Fermenters

V. lecanii strains 53-81 and 19-79 produced similar quantities of both hyphal bodies and biomass during the course of the fermentation (Fig. 7.01). Hyphal body numbers reached approximately 3×10^9 ml^{-1} after 62 hours, while biomass increased throughout the fermentation (26-28 mg ml^{-1} after 72h).

8. Production of *Metarhizium anisopliae* in Submerged Culture

a. Comparison of Spore Production Between Strains

After 2 or 3 days incubation in SLM, all strains had produced

Table 7.02

Number of Metarhizium anisopliae (ME2) Conidia Produced on Semi-solid Medium at 25°C

Time (days)	Mean no. conidia gram dry barley grain^{-1}	S.E.[a]
0	4.0×10^6	N.A.
4	1.36×10^8	8.24×10^6
6.5	9.11×10^8	6.75×10^7
9	1.89×10^9	6.97×10^7
14	2.38×10^9	1.05×10^8

a Standard error of the mean of 5 replicate flasks

N.A. Not available

Table 7.03

Number of Metarhizium anisopliae (ME2) Conidia Produced on Semi-solid Medium at 20°, 25°, or 27°C

Time (days)	Temperature (°C)	Mean no. conidia gram^{-1} dry barley grain[1]	L.S.D.[2]
7	20	2.62×10^{7a}	5.61×10^{7}
	25*	5.76×10^{8b}	
	27	4.27×10^{8c}	
9	20	5.49×10^{8a}	2.74×10^{8}
	25*	9.32×10^{8b}	
	27	5.97×10^{8a}	
13	20	3.63×10^{8a}	2.51×10^{8}
	25*	1.78×10^{9b}	
	27	1.17×10^{9c}	

1. Within each group of three means different letters denote significant difference (p = 0.05)
2. Least significant difference (p = 0.05) calculated on 2 (*) or three flasks temperature^{-1}

Table 7.04

Production of *Verticillium lecanii* (53-81) Hyphal Bodies in Submerged Culture (80g lit^{-1} glucose, 20g yeast extract, contained in Erlenmeyer flasks).

Time (hours)	Mean no. *V. lecanii* Hyphal bodies(ml^{-1})	S.E.
0	1.62×10^{6} a	N.A.
17	All conidia germinated	-
22	Hyphal bodies present	-
43	8.81×10^{8} b	3.15×10^{7}
67	1.24×10^{9} b	3.48×10^{7}
99	1.45×10^{9} b	5.45×10^{7}

a Conidial inoculum

b Hyphal bodies

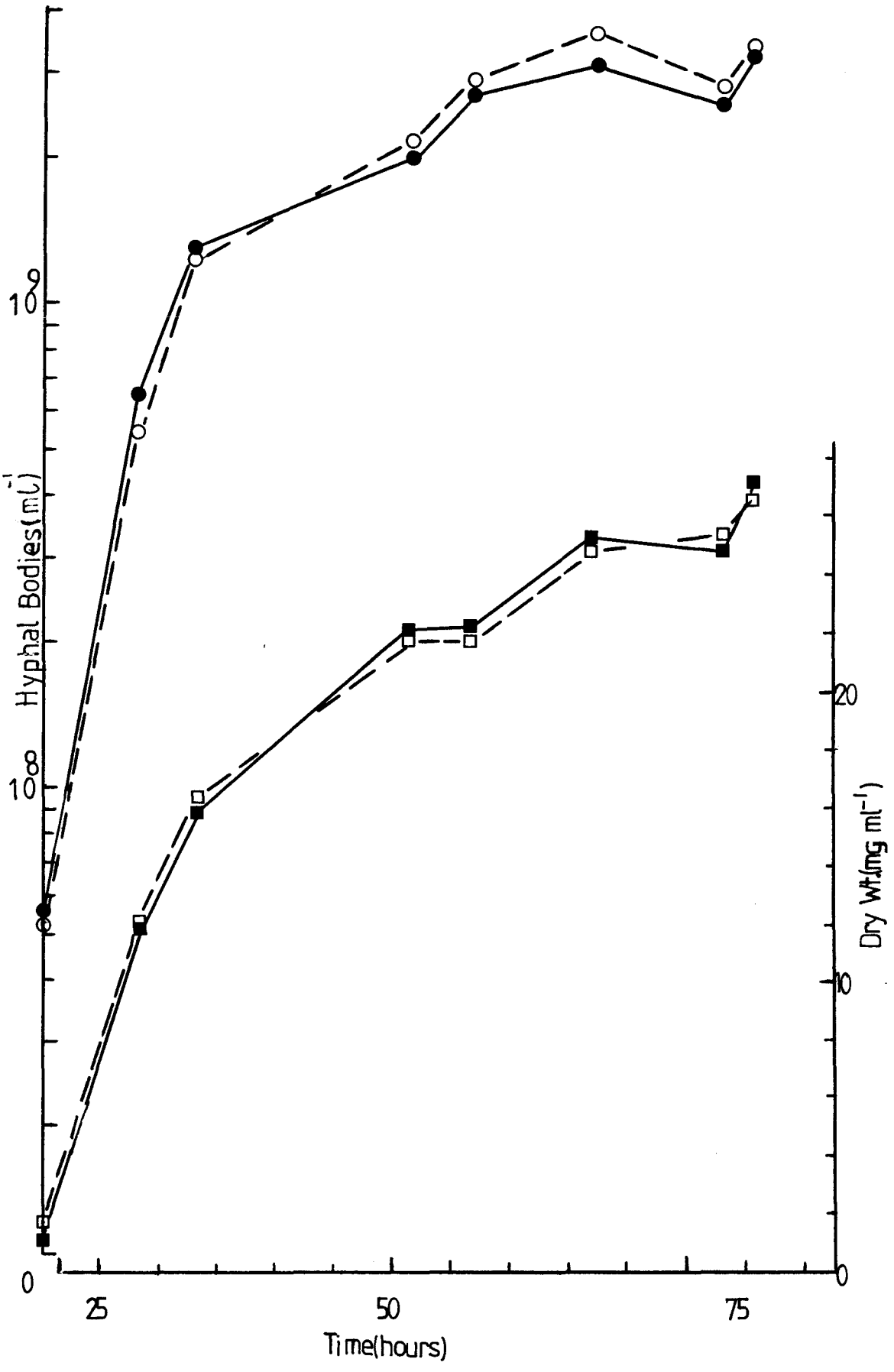


Fig. 7.0I

Hyphal body (● ○) and dry weight (■ □) production of V. lecanii strains 53-81 (● ■) and 19-79 (○ □) in submerged culture.

'balls' of dense mycelia (approx. diam. 0.5 cm) and hyphal bodies were absent. However, in SLM containing 0.5% Tween 80 growth was more homogenous, though still predominantly mycelial, and strain ME2 produced small numbers of spores.

M. anisopliae, strain ME2, produced many more spores than strains ME3, 52 and 1140, 3 days after conidial inoculation of molasses/yeast extract and molasses/peptone media (Table 7.05). No budding of spores was observed but they were formed singly on the side of mycelial elements.

Since all strains were of similar pathogenicity to test insects (VI) further studies were confined to strain ME2.

b. Complex, Prepared Media

Spore production was greatest in glucose/yeast extract or Proflo media when flasks were aerated on a wrist-action shaker (Table 7.06), while in rotary shaken cultures spore and biomass production was highest in glucose/yeast extract medium (Table 7.07).

c. Adamek's Medium

Spore production rapidly reached 1.5×10^8 ml^{-1} 48 hours after inoculation (Table 7.08). Generally spores were formed by a 'budding-like' process on the side of mycelial elements and secondary spore formation was only occasionally observed.

d. Effect of Carbon/Nitrogen Ratio

M. anisopliae grew poorly in basal liquid medium and maximal yields of spores (8.0×10^5 ml^{-1}) and biomass (4.28 mg ml^{-1}) occurred with a C : N ratio of 10 : 1 (Table 7.09).

e. Effect of Nitrogen Source

In basal media with single amino acids or inorganic salts as nitrogen source, growth was poor (dry weights $<$ 7mg ml^{-1}). In contrast, growth was considerable with organic, complex, nitrogen sources, and all dry weights exceeded 18 mg ml^{-1} (Table 7.10). Hyphal body numbers were too low for accurate assessments ($< 10^5 ml^{-1}$) regardless of nitrogen source.

Table 7.05

Hyphal Body Production of Metarhizium anisopliae Strains in Molasses/Yeast Extract or Molasses/Peptone Media

Strain	Mean no. hyphal bodies (ml^{-1})		
	Molasses/yeast extract	S.E.[a]	Molasses/peptone[b]
ME2	2.79×10^6	8.19×10^4	9.35×10^5
ME3	2.83×10^4	3.33×10^3	1.5×10^4
52	3.67×10^4	1.20×10^4	1.5×10^4
1140	2.65×10^5	4.19×10^4	1.35×10^5

a. Standard error of the mean of three replicate flasks

b. Unreplicated

Table 7.06

Production of Metarhizium anisopliae in Complex Prepared Media (Wrist action shaker 25 $\pm$ 1°C)

Medium	Hyphal bodies[a]			Mycelial growth[a]		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
SLM	-	+	++	b	++	+++
Glucose/yeast extract	-	+++	+++	b	++	+++
Czapek Dox	-	-	+	b	+	++
Proflo	-	+	++++	b	+++	+++

a. Estimated qualitatively on a 1-5 scale; 5 maximum.

b. Conidia germinated: germ tubes 10-20 µm long

Table 7.07

Production of Metarhizium anisopliae in Complex Prepared Media (Rotary Shaker, 110 rpm 25 ± 1°C)

Medium	Hyphal bodies[a] after 3 days	Mycelial growth[a] after 3 days	Dry weight[b] after 5 days (mg ml^{-1})
Glucose/yeast extract	+++++	++++	22.5
Proflo	++	++++	8.6[c]
Nutrient broth	+	++	1.3
Dextrose broth	+	++	5.5

a. Estimated on a 1-5 scale; 5 maximum

b. Mean of three replicates

c. Corrected for insoluble component of Proflo medium

Table 7.08

Hyphal Body Production of M. anisopliae in Adamek's Medium

Time (hours)	Mean no. hyphal bodies (ml^{-1})	S.E.[a]
24	-[b]	-
48	1.54×10^8	6.0×10^6
60	1.48×10^8	5.2×10^6
72	1.47×10^8	3.0×10^6

a. Standard error of the mean of 3 replicate flasks

b. Spores just starting to form on side of mycelial elements

Table 7.09

Effect of C/N Ratios on *Metarhizium anisopliae* Hyphal Body and Dry Weight Production in Basal Medium

C/N ratio	Hyphal body yield $\times 10^5$ (ml^{-1})	Dry Weight ($mg\ ml^{-1}$)	Final pH
2.5:1	1.77	2.78	6.17
5:1	2.97	4.26	6.24
10:1	8.00	4.28	6.24
20:1	4.67	4.50	6.40
50:1	2.37	4.35	5.90
100:1	2.60	3.96	5.72
200:1	1.37	2.64	6.32
No N-source	0	0.42	6.44

Table 7.10

Effect of N-Source on Dry Weight of Metarhizium anisopliae in Basal Medium with Glucose as C Source

Nitrogen source	Dry Weight (mg ml^{-1})	Final pH
Ammonium sulphate	2.6^{a}	4.6
Potassium nitrate	1.5^{a}	5.8
Arginine	4.3^{a}	4.8
Cysteine	N.A.	5
Glycine	3.7^{a}	5.8
Proline	4.0^{a}	5.7
Urea	2.2^{a}	6.2
Cas amino acids	18.1^{b}	4.1
Casein hydrolysate	20.6^{bcd}	3.7
Corn steep liquor	21.5^{cd}	4.5
Bacteriological peptone (Oxoid)	18.8^{bc}	4.8
Yeast extract(Oxoid)	23.5^{de}	4.2
Yeast extract(Difco)	21.8^{cd}	4.0
Yeast extract(Tate & Lyle)	26.4^{e}	3.1

Different letters indicate significant difference $p = 0.05$

Spore production was generally higher in media containing molasses (Table 7.11)and spore production was maximal in media containing nitrogen as peptone, Cas amino acids or yeast extract (Oxoid or Tate and Lyle). Biomass production, estimated by dry weight, was highest in media containing complex nitrogen sources.

f. Effect of Carbon Source

Spore production was greatest in media containing fructose, lactose, molasses, or starch as carbon source (Table 7.12). All sugars tested, except galactose, glucose and maltose, produced greater numbers of spores after 3 rather than 5 days. Starch and sorbitol produced maximal dry weights (26mg ml^{-1}), while lactose and galactose were utilized poorly (15 mg ml^{-1}), although lactose produced most spores. Values of pH ranged from 5.1-7.2 and were not correlated with biomass or spore production.

g. Effect of Molasses and Yeast Extract Concentration

Hyphal body yields were greatest in a medium comprising 40 g l^{-1} of both molasses and yeast extract (Table 7.13) and at 1.7×10^8 hyphal bodies ml^{-1} were similar to those obtained with Adamek's medium. A medium containing 80 g molasses and 40 g yeast extract l^{-1} produced slightly less spores ($p = 0.05$) but had the largest biomass (40.23 mg ml^{-1}). Values of pH ranged from 5.39-7.47 and did not correlate with spore or biomass production.

h. Effect of Glucose Concentration

Spore and dry weight production rose with increasing glucose concentration (Table 7.14). Spores were not detected at the lowest glucose concentration tested (0.5%) but there were 7.23×10^6 spores ml^{-1} in a medium containing 16% glucose.

i. Effect of Glucose and Tween 80 Concentration

Spore production was greatest in media rich in glucose (Table 7.15). Tween 80 reduced spore numbers when included in media containing 2 or 4% glucose, though the reductions were not significant ($p = 0.05$). In contrast, Tween 80 significantly ($p = 0.05$) increased spore production when

Table 7.11

Effect of N-Source with Molasses as C Source on Hyphal Body and Dry Weight Production of Metarhizium anisopliae in Liquid Medium

Nitrogen source	Hyphal bodies x 10^6 ml^{-1}			Dry weight (mg ml^{-1})	Final pH
	3 days	5 days	7 days		
Molasses Only	*0.16^{a}	0.15^{a}	1.03^{ab}	7.67^{a}	4.80
Ammonium sulphate	*0.04^{a}	0.70^{ab}	N.A.	12.45^{b}	4.48
Potassium nitrate	0.71^{a}	2.07^{abc}	N.A.	17.87^{cd}	4.62
Arginine	0.10^{a}	3.13*^{abcd}	6.6^{g}	14.27^{bc}	4.30
Glycine	0.013^{a}	2.87^{abc}	2.07^{abcd}	14.63^{bc}	4.85
Proline	*0.91^{a}	*2.25^{abc}	3.1^{bcde}	15.25^{bc}	4.30
Urea	0.4^{a}	1.23^{ab}	1.47^{abc}	7.67^{b}	5.17
Cas amino acids	4.09^{c}	4.00^{abcd}	4.3^{ef}	27.67^{f}	5.07
Casein hydrolsate	*0.02^{a}	*2.35^{abc}	2.65^{abcde}	11.40^{ab}	4.20
Corn Steep Liquor	*0.03^{a}	4.80^{bcd}	0.57^{a}	24.60^{ef}	5.32
Peptone (Bacteriological, Oxoid)	2.28^{b}	18.50^{e}	6.9^{g}	28.00^{f}	5.23
Yeast extract (Oxoid)	0.39^{a}	6.33^{cd}	6.37^{fg}	21.93^{de}	4.91
Bacto yeast extract (Difco)	0.64^{a}	2.20^{abc}	1.57^{abc}	27.17^{f}	5.42
Yeast extract (Tate & Lyle)	2.88^{b}	2.23^{abc}	3.43^{cde}	27.30^{f}	4.84

Within each column different letters indicate significant difference $p = 0.05$

N.A. Not available

* 2 replicates

Table 7.12

Effect of Carbon Source on Hyphal Body and Dry Weight Production of Metarhizium anisopliae (N-Source, yeast extract, C/N ratio 8:1, 135 rpm, 25 ± 1°C

C source	[x]Mean hyphal bodies (ml^{-1} x 10^6)		[x]Mean dry weight (mg ml^{-1})	[x]Mean pl
	3 days	5 days	5 days	5 days
Fructose	29.7^{d}	18.5^{b}	21.2^{b}	5.3^{cd}
Galactose	2.8^{a}	3.9^{*a}	14.7^{a}	6.1^{ef}
Glucose	4.2^{a}	5.1^{a}	20.3^{b}	5.1^{bc}
Glycerol	10.0^{b}	6.5^{a}	20.5^{b}	4.7^{a}
Lactose	34.6^{d}	26.3^{c}	11.8^{a}	7.2^{g}
Maltose	1.8^{a}	4.4^{a}	22.1^{b}	5.4^{d}
Mannitol	27.1^{cd}	6.4^{a}	21.6^{b}	5.9^{e}
Molasses	29.8^{d}	27.0^{c}	22.0^{b}	6.2^{f}
Sorbitol	18.4^{bc}	8.4^{a}	26.5^{c}	5.0^{b}
Starch	31.4^{d*}	17.1^{b}	28.1^{c}	6.2^{f}

x Means calculated on three replicate samples except *

* Two replicates

Within each column, different letters denote significant difference (p = 0.05)

Table 7.13

Hyphal Body and Dry Weight Production of *Metarhizium anisopliae* in Molasses/Yeast Extract Media

Molasses (g lit^{-1})	Yeast extract (g lit^{-1})	Hyphal body yield x 10^7 (ml^{-1})	Dry weight (mg ml^{-1})	pH
40	20	1.55^{a}	19.07	7.47
40	30	5.43^{b}	26.63	7.41
40	40	17.07^{e}	30.39	7.47
60	20	5.89^{b}	26.97	6.24
60	30	7.80^{c}	33.55	6.69
60	40	1.99^{a}	23.48	5.86
80	20	2.67^{a}	23.25	5.39
80	30	6.27^{bc}	28.65	5.67
80	40	14.63^{d}	40.23	6.19

Within each column different letters indicate means significantly different ($p = 0.05$)

Table 7.14

Hyphal Body and Dry Weight Production of *Metarhizium anisopliae* in Glucose/Yeast Extract Media

Glucose ($g\ lit^{-1}$)	Yeast extract ($g\ lit^{-1}$)	Hyphal body yield (ml^{-1})	Dry weight ($mg\ ml^{-1}$)
5	20	0^{a}	5.79^{a}
10	20	6.67×10^{3b}	8.71^{b}
20	20	1.17×10^{4b}	10.90^{bc}
40	20	1.32×10^{5b}	11.43^{c}
80	20	3.46×10^{6c}	14.78^{d}
160	20	7.23×10^{6d}	N.A.

Within each column different letters indicate means significantly different ($p = 0.05$)

Table 7.15

Effect of Glucose and Tween 80 Concentrations on Hyphal Body and Dry Weight Production of Metarhizium anisopliae

Glucose conc. (g lit^{-1})	Tween 80 conc. (%)	Hyphal body yield x 10^6 (ml^{-1})		Dry weight 7 days
		2 days	4 days	
20	0	7.7^{ab}	2.4^{a}	19.5^{a}
20	.01	1.9^{a}	1.2^{a}	19.4^{a}
20	.05	0.9^{a}	1.3^{a}	19.2^{a}
20	.1	2.4	0.5^{a}	22.5^{a}
40	0	24.1^{bc}	14.9^{ab}	27.8^{c}
40	.01	5.3^{ab}	7.7^{ab}	28.4^{c}
40	.05	5.5^{ab}	2.2^{a}	23.1^{ab}
40	.1	7.1^{ab}	3.7^{a}	30.6^{c}
80	0	31.5^{c}	24.3^{bc}	26.8^{bc}
80	.01	65.2^{d}	41.7^{cd}	28.2^{c}
80	.05	166.0^{e}	50.8^{d}	26.9^{bc}
80	.1	176.0^{e}	91.8^{e}	27.5^{c}

Within each column different letters indicate means significantly different ($p = 0.05$)

included in 8%-glucose media. An additional experiment confirmed that inclusion of Tween 80 in 8%-glucose medium increased spore production (Table 7.16).

9. Production of *Metarhizium anisopliae* in 1.6-l Fermenters

Spore and biomass production was similar in glucose/yeast extract media containing 0.1 or 0.5% Tween 80 (Table 7.17, 7.18). Conidia used for inocula germinated and produced germ tubes about 20 μ long after 20.5 hours incubation. After 26 hours, spores started to develop on the side of mycelial elements and later, spore production increased rapidly. In a medium containing 0.1% Tween 80, spore numbers exceeded 2 x 10^7 ml^{-1}, 52.5 hours after inoculation, while with 0.5% Tween 80 similar yields occurred after 74 hours. Spores then germinated to form sterile mycelia, which increased medium viscosity, clogged the fermenter, and reduced spore numbers. The various stages of growth are illustrated in Fig. 7.02. Dry weights increased throughout the fermentations and reached 20 mg ml^{-1} after 95 (0.1% Tween 80) or 125 hours.

In molasses/Peptone medium *M. anisopliae* developed similarly with spore numbers reaching 10^7 ml^{-1} after 50 hours, and then declining. Dry weight exceeded 18 mg ml^{-1} after 93 hours (Table 7.19).

Table 7.16

Effect of Tween 80 on Hyphal Body and Dry Weight Production of Metarhizium anisopliae

Tween 80 conc. (%)	Hyphal body yield x 10^6 (ml^{-1})	Dry Weight
0	5.2[a]	26.8[a]
0.01	10.5[ab]	26.9[a]
0.05	16.4[b]	27.5[a]
0.1	47.1[c]	28.2[a]

Within each column different letters indicate significant different (p = 0.05)

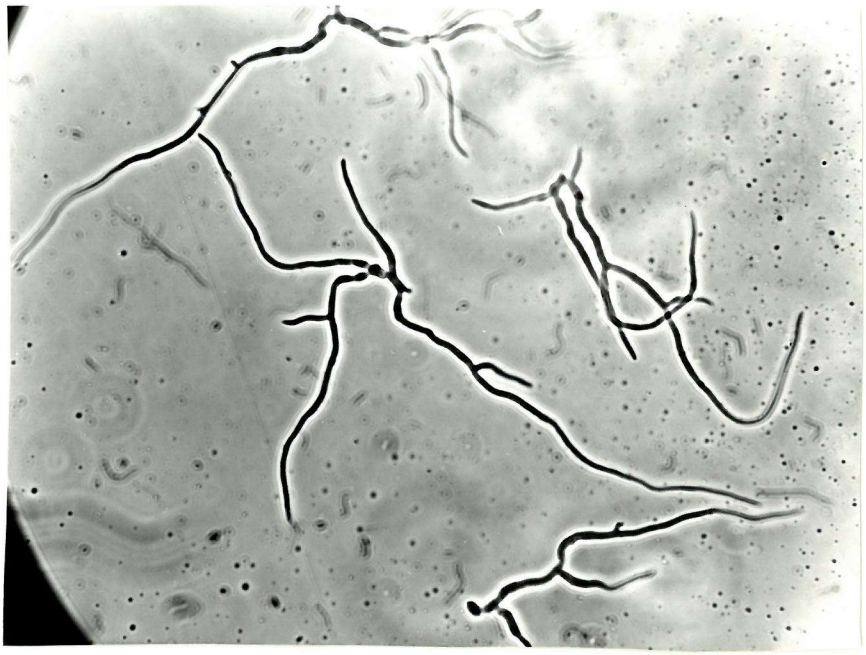
Table 7.17

Hyphal Body and Dry Weight Production of Metarhizium anisopliae in Glucose/Yeast Extract Medium in Fermenters. 0.1% Tween 80.

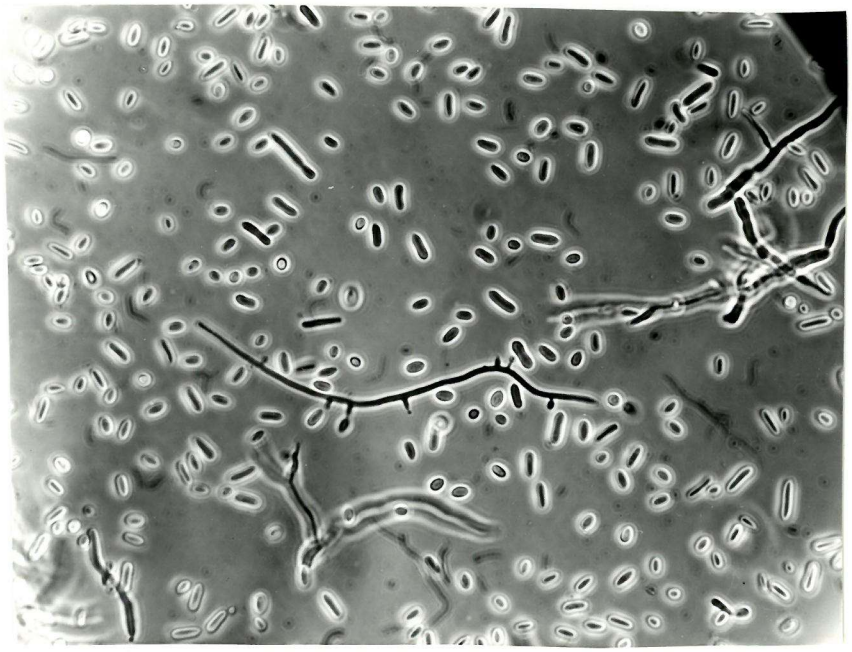
Time (hours)	Hyphal body yield (ml^{-1})	Dry weight (mg ml^{-1})
20.5	3 x 10^{4a}	0.45
26	3 x 10^{4b}	1.83
52.5	2.4 x 10^7	4.61
68.5	2.9 x 10^7	8.17
79	1.56 x 10^7	11.09
95	3.0 x 10^6	21.2

a Conidial inoculum, germ tubes c. 12μm. No hyphal bodies

b Hyphal body formation commencing



a. 20 hours

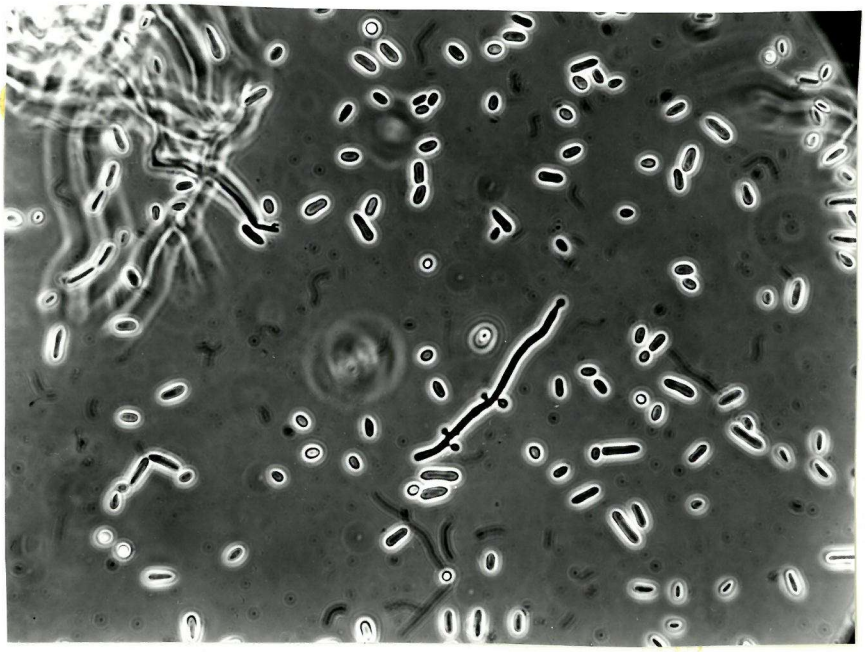


b. 49 hours

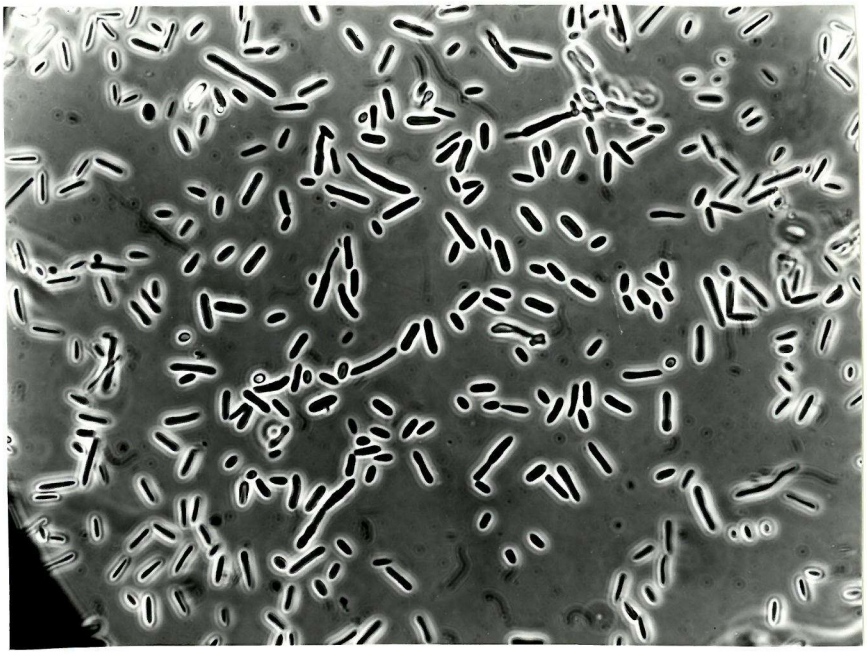
Fig. 7.02

Development of M. anisopliae in Submerged Culture. Medium 80g l^{-1} glucose, 20g l^{-1} yeast extract, 0.5% Tween 80.

× 850

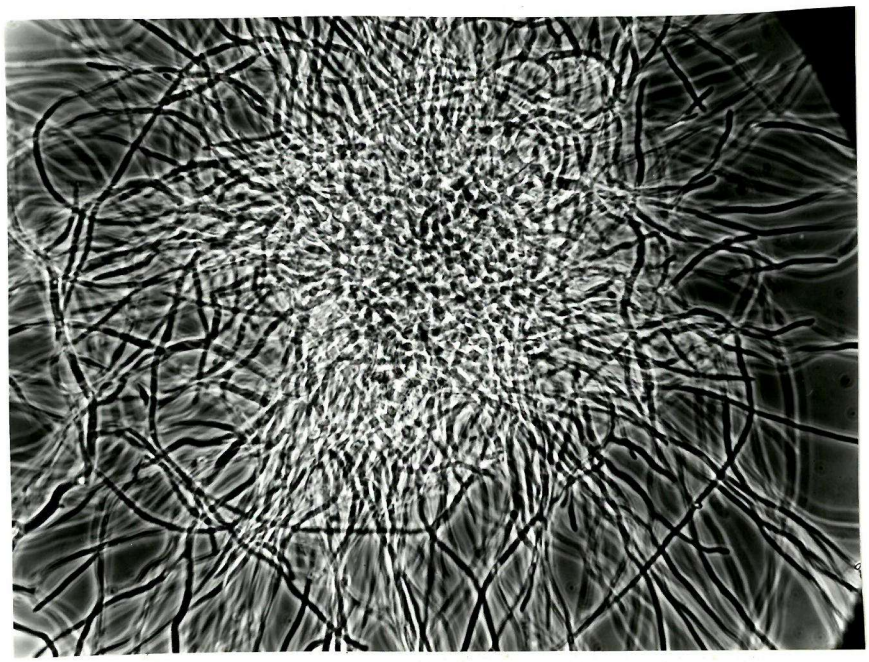


c. 49 hours



d. 92 hours

Fig. 7.02 (cont)



e. 125 hours

Fig. 7.02 (cont)

Table 7.18

Hyphal Body and Dry Weight Production of *Metarhizium anisopliae* in Glucose/Yeast Extract Medium in Fermenters. 0.5% Tween 80.

Time(hours)	Hyphal Bodies (ml^{-1})	Dry Weight (mg ml^{-1})
49	1.5×10^5	2.1
51	1.1×10^6	-
57	4.6×10^6	3.6
67	1.7×10^7	7.9
74	2.2×10^7	10.5
82	2.1×10^7	16.2
92	1.5×10^7	15.7
102	1.4×10^7	18.4
125	1.5×10^6	20.1

Table 7.19

Sporulation and Dry Weight of *Metarhizium anisopliae* in Molasses/Peptone Medium in Fermenters.

Time (hours)	Hyphal bodies (ml^{-1})	Dry weight (mg ml^{-1})
0	2.5×10^4	0.43
22	2.5×10^4	0.44
44	7.93×10^6	8.1
50	1.0×10^7	13.97
58	5.76×10^6	13.50
68	6.62×10^6	17.10
74	4.58×10^6	17.90
93	3.95×10^6	18.47

DISCUSSION

Many fungi exhibit dimorphism : an environmentally controlled, reversible change between yeast and mycelial morphologies (Romano, 1966). However, the determinants are poorly understood, with both nutritional and physical parameters being implicated. Ceratocystis ulmi grew mycelially in the presence of ammonium, arginine or asparagine, while proline induced a yeast morphology (Kulkarni and Nickerson, 1981). Phosphate, zinc or manganese levels were major morphological determinants in Ceratocystis minor, Candida albicans and Aspergillus parasiticus respectively (Biel et al.,1977; Yamaguchi, 1975; Detroy and Ciegler, 1971). Temperature may be the governing factor: Histoplasma capsulatum grew mycelially at 25°C, but developed a yeast-like form on increase to 37°C (Mahvi, 1965). Carbon dioxide, introduced into an anaerobic atmosphere, induced yeast-like growth of Mucor rouxii (Bartnicki-Garcia and Nickerson, 1962) and encouraged similar growth of Mycotypha africana and M. microspora (Schulz et al., 1974). Similar data on entomogenous fungi are scarce, though Hall and Latgé (1980) showed carbon dioxide promoted earlier hyphal body formation of V. lecanii. Several different spore types can be formed in liquid culture. Some fungi can be induced to conidiate; eg Aspergillus spp. (Anderson and Smith, 1977; Broderick and Greenshields, 1981); Paecilomyces varioti (Anderson et al., 1978); Neurospora crassa (Rossier et al., 1977) and Beauveria (Goral, 1973 a, b). Besides Beauveria, many other entomogenous fungi form propagules in liquid culture, and are variously termed conidia, blastospores or hyphal bodies. Adamek (1963) described conidial production of M.anisopliae in liquid culture. However, 'conidia' were not formed on typical phialides and though his description was technically correct - a conidium is an asexually produced spore (Ainsworth, 1961) use of this term should be reserved for spores formed on typical phialides. Samsinakova (1966) described hyphal body production of Beauveria bassiana in submerged culture and later (Samsinakova and Hrabetova, 1969) reported that hyphal bodies were formed by "breaking of mycelium, by budding or by splitting of mycelial hyphae". Goral (1975) provided

illustrations of B. bassiana in liquid culture forming hyphal bodies on the side of mycelia, in a way similar to that observed for M. anisopliae in this study. Nomuraea rileyi also forms hyphal bodies by budding from mycelial elements (Riba and Glandard, 1980). V. lecanii (1-72) reproduced in submerged culture by budding from mycelial elements, or directly from conidia , and Hall (1981) referred to the growth as a 'semi-yeast' morphology. In this study, V. lecanii (53-81) grew similarly, with occasionally three propagules being formed on a single spore, and this multiple budding is considered a true yeast morphology. Hall's (1981) description of a 'semi-yeast' morphology in V. lecanii is consistent with other reports of stages intermediate between yeast and mycelia. Lane and Garrison (1970) suggested dimorphism in Sporothrix schenckii, Histoplasma capsulatum, Blastomyces dermatitidis and Paracoccidioides brasiliensis occurred via a transitional form, having a combination of yeast and hyphal characters. Odds(1979) considered a similar form occurred in Candida albicans, while the arthrospores of Mucor rouxii, which were distinguished from yeast-like growth by Bartnicki-Garcia (1963), may also represent such a form. From the available evidence, it would seem that many reports of submerged spore production in entomogenous Deuteromycetes have described similar intermediate forms. I consider that the term blastospore -"a spore produced by budding, as in yeasts" (Ainsworth, 1961)-should not be used for spores of entomogenous Deuteromycetes produced in liquid culture. A better term is hyphalbody , originally reserved for the Entomophthoraceae (Thaxter, 1888; Ainsworth, 1961; Alexopoulos, 1962), but extended by Prasertphon and Tanada(1968) as follows: "Hyphal bodies are fragments of hyphae of various sizes and shapes, and are produced by budding and division within the host or in an artificial medium, ------".

Hyphal bodies, though often easier to produce than conidia, have several disadvantages. Hall(unpub. obs.) showed V. lecanii (1-72) hyphal bodies had thinner walls than conidia and this probably explains the shorter half life of hyphal

bodies (9 days at 4°C) compared to conidia (3.5-4 months, Quinlan and Lisansky, 1983). However, several workers have successfully stabilised hyphal bodies of Beauveria. Blachère et al. (1973) and Fargues et al. (1979) using different techniques, preserved viability of Beauveria brongniartii hyphal bodies for 8 months at 4 or 5°C, while Belova (1978) reported B. bassiana hyphal bodies maintained high viability and virulence for 1 year at similar temperatures. Hyphal bodies of B. brongniartii, B. bassiana and V. lecanii were pathogenic to Melolontha melolontha, Leptinotarsa decemlineata and Macrosiphoniella sanborni respectively (Keller, 1982; Fargues et al., 1979; Hall, 1977), while this study showed M. anisopliae hyphal bodies were pathogenic to Hauptidia maroccana. In contrast, hyphal bodies of Nomuraea rileyi were not pathogenic to Heliothis zea and Bombyx mori (Bell, 1975; Riba and Glandard, 1980).

Hyphal bodies are frequently found in the haemolymph of insects infected with fungi; eg V. lecanii (Hall, 1977), N. rileyi (Kish and Allen, 1978; Mohamed et al., 1978; Boucias and Pendland, 1982), M. anisopliae (Prasertphon and Tanada, 1968), Paecilomyces fumosoroseus and B. bassiana (Aoki et al. cited in Aoki and Yanase, 1970). Such elements circulate in the insect haemocoel and spread infection throughout the insect.

M. anisopliae readily produced conidia on barley grain and yields were similar to those of Aquino et al. (1975). In Brazil, Metaquino, a product containing M. anisopliae conidia, is produced on rice contained in polythylene bags and used for control of Mahanarva posticata and other froghoppers (Ferron, 1978). However, Brazilian use of large numbers of small containers poses problems of quality control, as all should be examined for purity and pathogenicity. Although numbers can be bulked for the latter observation, the obvious solution is to scale up semi-solid production, but this is difficult due to problems of air supply, contamination, regulation of fermentation and harvesting (Quinlan and Lisansky, 1983).

V. lecanii (53-81) was easily produced in Erlenmeyer flasks, and in 1.6-litre fermenters. Yields exceeded 3×10^9 hyphal bodies ml^{-1}, and Hall (unpub. obs.) has obtained yields of 7.6×10^9 ml^{-1} with increased nutrient

levels.

After inoculation of SLM with M. anisopliae conidia, balls of mycelia were formed, as also described by Adamek (1963). Similar mycelial balls were reported in Hirsutella (MacLeod, 1959) and N. rileyi (Riba and Glandard, 1980). In this study, addition of Tween 80 to media containing 20 g lit^{-1} yeast extract and 20 or 40 g lit^{-1} glucose prevented formation of mycelial balls of M. anisopliae but also reduced hyphal body formation (though not significantly), compared to similar media without surfactant. By constrast,Tween 80 (0.01-0.1%) prevented mycelial balls and also increased hyphal body formation when added to media containing 20 g lit^{-1} yeast extract and 80 g lit^{-1} glucose. Increased hyphal body formation by addition of Tween 80 was reported in M.anisopliae (Adamek, 1963) and in N.rileyi (Riba and Glandard, 1980).

Of four M. anisopliae strains studied, ME2 produced hyphal bodies most readily. Veen (1968) also reported similar strain variation.

Strain ME2 produced a maximum of about 10^8 hyphal bodies ml^{-1} both in Adamek's (1963) medium, and in molasses/yeast extract medium, but yields could not be increased above this level. M. anisopliae grew poorly in basal liquid media but yields were highest with a C:N ratio of 10:1, cf. Hall (1977) with V. lecanii. Inorganic or single amino acid nitrogen sources supported only limited growth, while on complex sources, eg yeast extract, growth was increased. Roberts (1966) found better growth of M. anisopliae with Neopeptone as nitrogen source compared with Na NO_3. Campbell et al. (1978) observed M. anisopliae produced maximal dry weights and hyphal bodies in media containing tryptophan, (5.1 g lit^{-1}), glutamic acid (3.5 g lit^{-1}) or histidine (3.5 g lit^{-1}). These nitrogen sources, not included in this study, should be investigated but would be too expensive to use commercially. Barnes et al. (1975) studied growth and sporulation of M. anisopliae on various peptone sources and found the best source for growth and hyphal body formation was yeast extract, while Bacteriological Peptone (Difco) was poor (9.6 g lit^{-1}). In this

study, Bacteriological Peptone (Oxoid) and molasses proved the best medium (dry weight 28 g l^{-1}, 1.85 x 10^7 hyphal bodies ml^{-1}) and confirms Barnes et al. (1975) observation that different peptone products can markedly affect growth. M. anisopliae varied in its ability to utilize different sugars, growing poorly on lactose and galactose. V. lecanii (1-72) produced maximal and minimal numbers of hyphal bodies in media containing galactose and lactose, respectively (Hall, 1977). By contrast, M.anisopliae produced hyphal bodies in the presence of lactose, though similar numbers were formed in media with carbon provided by starch, molasses, mannitol and fructose. The high yield obtained in media with molasses was encouraging, as molasses is a readily available, low-cost, carbon source. A combination of 40 g l^{-1} of both molasses and yeast extract resulted in a hyphal body yield of 1.7 x 10^8 ml^{-1} after 3 days, and was the highest yield obtained.

In glucose/yeast extract media, increased glucose levels resulted in greater hyphal body production and dry weights. High hexose levels have been frequently reported to increase yeast-like growth (Sypherd et al., 1978) and in this study some budding of M. anisopliae was observed in a 16% glucose medium.

Production of M. anisopliae in 1.6 l fermenters was disappointing and highest yields were 2.9 x 10^7 propagules ml^{-1}, a level not economic for commercial production. Further flask and fermenter studies are necessary, but time, and technical problems with the newly acquired fermenters, precluded this. The yields obtained in this study were some ten times less than those achieved by Hall (unpub. obs.) with V. lecanii (1-72), which has similar sized hyphal bodies. Thus M. anisopliae is probably better produced by semi-solid fermentation. Indeed, Soper and Ward (1981) considered this technique to be the method of choice and possibly the recent development of Samsinakova et al. (1981), utilizing nutrient filled polyethylene cushions to maximise surface area, could be utilized for M. anisopliae.

SECTION VIII

GLASSHOUSE EXPERIMENTS

MATERIALS AND METHODS

1. General

a. Spore Production

i. Conidia

Conidia were usually produced on Sabouraud dextrose agar, contained in stainless steel trays (31 x 26 x 5 cm or 25.5 x 20.5 x 5 cm), at 25°C. Each tray contained 500 or 700 ml of agar and was inoculated with five ml of a water suspension containing 0.025% Triton X-100 and approximately 5 x 10^7 conidia ml^{-1}. Conidia were distributed evenly using a bent glass rod and incubated for 8-10 days when spores were harvested and viabilities checked as described previously (V, 1, ef).

Metarhizium anisopliae conidia, used in the Nilaparvata lugens experiment, were produced on barley grain as described earlier (VII, 2).

ii. Hyphal bodies

Hyphal bodies of Verticillium lecanii were produced in a 1.6-l fermenter, as described previously (VII, 3, b). Spores were harvested and suspensions standardised as for conidia.

b. Commercial Products

Commercial products, produced by Tate and Lyle Ltd, were stored at 2°C and used as soon as possible after production, according to the manufacturers instructions. Dried powder was mixed to a paste with tap water, diluted and left at ambient temperature for five hours to permit spore re-hydration. Conidial numbers were determined by macerating 0.1 g samples of commercial products with 5ml sterile distilled water, in a Griffiths' tube and spreading resultant dilutions on SDA plates containing 100mg ml^{-1} streptomycin sulphate. Resultant colonies were counted after 4 days incubation at 25°C.

c. Additives

Additives to spore suspensions were dispersed in 250ml water before adding to the spray tank, which was then agitated vigorously.

d. Application

All treatments were applied with pneumatic sprayers of one litre capacity (The Master No. 5P/T, Philip B. Waldron Co., England) or 20 litre (CP3, Cooper Pegler Co. Ltd., England). Plants were sprayed until 'run off' and care was taken to provide coverage of the lower surfaces of leaves.

e. Temperature and Humidity Measurement

Temperature and humidity were monitored with thermohygrographs (Negretti and Zambra Ltd; Casella and Co. Ltd., England) positioned in each glasshouse, or compartment. Instruments on 30 cm high metal stands were positioned so the sensors were shaded by lower leaves. Instruments in glasshouses containing rice, were protected from direct sunlight by a paper towel. Thermohydrograph readings were checked at regular intervals against mercury thermometers and a whirling hygrometer.

f. Humidification

In one experiment, glasshouse moisture levels were increased with distilled water, dispersed by two humidifiers (Defensor 505, A.G. Switzerland).

g. Glasshouses

Experiments conducted at the Glasshouse Crops Research Institute utilized two glasshouses. One compartmentalised glasshouse consisted of five chambers (3.6 x 2.7 m; max. height 3.15 m) with concrete walls (1.05 m). Glass was fixed in cedarwood with putty which ensured the houses were relatively airtight. Heating was by thermostatically controlled steam pipes and temperature reduction at levels above the thermostat setting was achieved by hand-operated roof ventilators. The second glasshouse (12x12 m; max. height 4m) constructed of aluminium with automatic ventilators

was more typical of commercial glasshouses.

h. Plants

Cucumber plants were produced in 'whale-hide' pots (9cm diam) in the same way as plants used for rearing and planted directly in soil, or in peat bags (two plants bag^{-1}). When plants were 2m high, the terminal buds were removed to encourage development of lateral shoots to obtain an 'umbrella' system of training. Three lateral shoots on each plant were allowed to grow and these were stopped just before they reached soil level.

2. Experiments With the Glasshouse Leafhopper, Hauptidia maroccana

a. Preliminary Experiment

This experiment was conducted in two chambers of the compartmentalised glasshouse with the minimum temperature set at 20°C. Cucumber plants, variety Butchers' Disease Resistor (BDR), were soil-planted, twelve $compartment^{-1}$, when they had five - six leaves. One day after transplanting, plants were fumigated overnight with nicotine smoke, which killed any aphids or whitefly present. Following dispersal of the nicotine by ventilation adult H. maroccana were introduced (approx. 400 $house^{-1}$) and allowed to establish. First instar nymphs appeared 14 days later, indicating leafhoppers were successfully reproducing.

Conidial sprays (Table 8.01) were applied between 6 and 7 p.m. on August 15th, 5½ weeks after introduction of leaf hoppers, to populations ranging from 50-100 nymphs $leaf^{-1}$. One plant in each house was used for each treatment and sprayed plants were separated with untreated "spacer" plants. Relative humidity was increased in one house using two humidifiers.

Immediately sprayed foliage was dry, five sections (each 6 cm^2) were cut from each treated plant, maintained on moist filter paper at 25°C in the apparatus shown in Fig. 8.01 and mortality assessed after one, three and five days incubation.

Insect numbers were assessed one day before conidial application and again after three, five and 13 days, by counting numbers of leafhoppers on 10 leaves $plant^{-1}$.

Table 8.01

Treatments applied to cucumber for control of *Hauptidia maroccana*

Treatment[a]	Conidial concentration[b] (spores ml^{-1})	Viability[c] (%)
Untreated	-	-
.03% Triton X-100	-	-
B. bassiana (63)	2.32×10^7	98.7
P. fumosoroseus (21)	2.34×10^7	99.3
M. anisopliae (ME2)	2.46×10^7	99.7
V. lecanii (1-72)	2.34×10^7	99.7

a Conidia applied in distilled H_2O containing 0.03% Triton X-100

b Estimated by haemocytometer counts

c Estimated on SDA

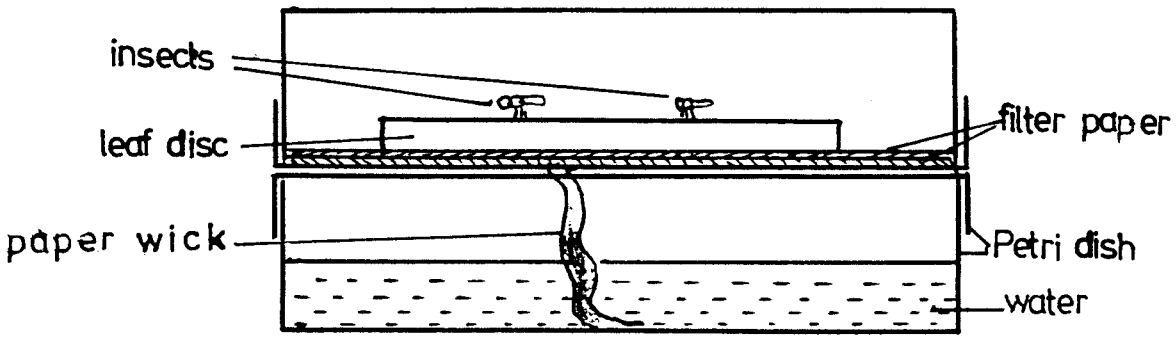


Fig. 8.01

Apparatus used to maintain excised cucumber leaf squares to assess mortality of Hauptidia maroccana under conditions optimal for fungal growth.

b. Experiment 2

This experiment utilized one chamber of the compartmentalized glasshouse. Cucumber plants (cv. Telegraph) were transplanted and fumigated as described earlier (VIII, 2, a). Approximately 100 adult H. maroccana were introduced on October 29th and a further 50 seven days later, when small numbers of Aphis gossypii were observed. These aphids were eradicated by 'spot' treatments of the specific aphicide pirimicarb (0.5 g l^{-1}). Prior to introduction of leafhoppers, mildew was controlled by bupirimate sprays on 13th (0.3 ml l^{-1}) and 17th (0.88 ml l^{-1}) of October.

Conidial sprays (Table 8.02) were applied to single plants between 11a.m. and 2p.m. on November 11th, to populations of 20-30 nymphs $leaf^{-1}$, with a one litre pneumatic sprayer. Leaf squares were removed after spraying and incubated in the laboratory (VIII, 2, a). Numbers of insects were counted on 10 leaves treated $plant^{-1}$, one day before conidial application and after six and 23 days.

c. Large Scale Experiment

This experiment utilized five chambers of the compartmentalized glasshouse, one being used for each treatment, which allowed the assessment of both adult and nymph populations. Cucumber plants, cultivar Farbiola, were transferred to the glasshouse on April 21st and fumigated with nicotine. Two days later, 120 adult H. maroccana were released in each house, followed by two further introductions of 60 insects on 29th April and 6th May. Nymphs were first observed on 7th May.

Mildew was controlled with bupirimate (0.88 ml l^{-1}), applied to upper leaf surfaces, at two-weekly intervals, three days before conidial application and 11 days after. Bupirimate, the least toxic mildewicide tested in laboratory experiments, had no effect on conidial germination of M. anisopliae (ME2) but reduced mycelial growth at the recommended rate of application, ($\bar{V}$, 14).

Conidial sprays (Table 8.03; c. 330 ml $plant^{-1}$) were applied with a 20 litre pneumatic sprayer, from 6-8p.m. on 25th May, to mean populations of 28.5 nymphs and 0.8 adults $leaf^{-1}$. When the foliage was dry, leaf squares were removed from eight leaves $treatment^{-1}$, maintained in the laboratory under

Table 8.02

Treatments applied to cucumber for control of Hauptidia maroccana

Treatment[a]	Conidial concentration (spores ml^{-1})	Viability[b]
Untreated	-	-
0.03% Triton X-100	-	-
B. bassiana (63)	5.17×10^7 [c]	99.2
P. fumosoroseus	4.97×10^7 [c]	99.7
M. anisopliae (ME2)	5.07×10^7 [c]	99.1
M. anisopliae (ME2) comm. form incl. barley grain	1.00×10^7 [d]	-

a All conidia applied in distilled H_2O containing 0.03% Triton X-100

b Estimated on SDA

c Estimated by haemocytometer counts

d Estimated by plate counts on SDA containing 100 mg ml^{-1} streptomycin sulphate

Table 8.03

Treatments applied to cucumber for control of *Hauptidia maroccana*

Treatment[a]	Conidial concentration (spores ml^{-1})	Viability[d] (percent)
0.05% Triton X-100	-	-
P. fumosoroseus	4.96×10^{7} [b]	98.4
M. anisopliae (1140)	3.8×10^{7} [b]	99.2
M. anisopliae (ME2)	4.9×10^{7} [b]	99.0
M. anisopliae (ME2) comm. form incl. barley grain	5.0×10^{6} [c]	-

a Conidia applied in distilled H_2O containing 0.05% Triton X-100

b Estimated by haemocytometer

c Estimated by plate counts on SDA

d Estimated on SDA

conditions optimal for fungal growth (VIII, 2, a) and leafhopper mortality assessed after one, three and six days. Numbers of insects on 20 labelled leaves treatment^{-1} were determined one day before spore application and after eight and 35 days, while populations on new lateral leaves that grew after spraying (10 treatment^{-1}), were estimated 35 and 46 days after treatment.

3. Experiment With Onion Thrips, Thrips tabaci

a. Preliminary Experiment

The experiment was conducted in September 1981 to assess the potential of a V. lecanii strain (53-81) isolated from Thrips tabaci in June. Established cucumber plants, cultivar Farbiola, soil-planted in two chambers of the compartmentalized glasshouse, were infested with adult T. tabaci in July. Populations developed slowly, but were sufficiently high by early September to damage the cucumber plants appreciably.

One group of plants was sprayed between 5-7p.m. on 8th September with a commercial formulation of V. lecanii (1.0 g l^{-1}; Tate and Lyle Ltd, 53-81) together with ingredients (total 1.5 g l^{-1}) which promoted fungal growth and sporulation on leaf surfaces (Hall and Turner, unpub. obs.). The concentration of viable V. lecanii propagules applied, estimated by plate counts, was 10^7 spores ml^{-1}. The other group of plants was similarly sprayed with nutrients and autoclaved V. lecanii formulation. Thrips populations were assessed by direct counts on 10 labelled lateral leaves chamber^{-1}, immediately prior to treatment and after 7 and 14 days.

b. Effect of Single Applications of the Commercial V. lecanii Products Mycotal® and Thriptal® on Thrips tabaci on Cucumber

This experiment utilized the aluminium-framed glasshouse. Cucumber plants, cultivar Farbiola, were planted in peat bags (Grobag, Fisons Ltd) on March 31st 1982 and watered through 'trickle' lines (three bag^{-1}) containing appropriate nutrients. After one day, 300 adult Thrips tabaci were evenly distributed throughout the glasshouse and a further

introduction of 200 insects made 17 days later. At approximately weekly intervals, leaves with high numbers of thrips larvae were removed, cut into pieces and distributed throughout the crop to obtain an even pest distribution.

Five days after planting, whitefly, Trialeurodes vaporariorum, were noticed on many cucumber plants. The most appropriate control method was introduction of the chalcid, Encarsia formosa, but this was precluded by the proximity of an experiment evaluating fungal control of whitefly, which needed to be free of parasites. Whitefly numbers were reduced by periodic manual squashing of adults and by spraying the tops of plants with bioresmethrin (11.7 ml l^{-1}) on May 14th.

Three replicate groups of eight plants, randomly distributed throughout the glasshouse, were sprayed with commercial V. lecanii products (Tate and Lyle Ltd, 2.5 g l^{-1}, at 500 ml $plant^{-1}$) between 6-7p.m. on May 25th. The treatments were Mycotal® (V. lecanii, 19-79), Thriptal® (V. lecanii, 53-81), and as control, autoclaved Thriptal®.

Thrips populations were assessed in two ways; (1) by direct counts performed on both upper and lower surfaces of 10 leaves per group of eight plants and (2) by trapping larval thrips on 'Boltac grease' (Pan Brittanica Ind. Ltd; Bassett, pers. comm.) applied to the base of 9 cm Petri dishes, positioned on alternate peat bags throughout the glasshouse. Direct counts were made one day before application and after 17 and 50 days, while Petri dishes were replaced every five days after spraying.

Powdery mildew was not observed during this experiment and no fungicides were applied.

c. Comparison of Mycotal® and V. lecanii (19-79 and 53-81) Hyphal bodies as Control Agents for T. tabaci on Cucumber

Cucumber plants, used for the previous experiment, were removed from the glasshouse and replaced with fresh cucumber plants, cultivar Farbiola, positioned in the used peat bags, and maintained as described earlier (VII, 3, b), on 15th July. Re-introduction of T. tabaci was unnecessary and insects soon became established on the cucumber plants.

Two groups of eight plants were sprayed with each of the treatments detailed in Table 8.04 (approx. 270 ml plant^{-1}) from 12-2p.m. on August 6th.
Thrips populations were assessed by trapping on 'Boltac grease' treated Petri dishes, replaced every seven days, and by direct counts 17 days after treatment (5 cm^2 areas on 10 replicate leaves).

4. Control of Nilaparvata lugens with Metarhizium anisopliae

This experiment utilized two chambers of the compartmentalized glasshouse, each with two beds (2.0 x 0.6 x 0.178 m), lined with 500 gauge polythylene sheet and supported by iron poles and wire. The beds were filled with rice compost 0.144 m deep (Table 4.02) and 65, three-four week old rice seedlings planted, 0.144 m apart, in each bed on June 24th. A minimum temperature was set at 25^{o}C, plants irrigated daily with acidified tap water and fed weekly with liquid nutrients (VI, 2, c). The area around the rice beds was kept continuously flooded to increase glasshouse humidity.
On August 10th, 50 adult N. lugens were evenly distributed on each bed of established rice plants and six days later, sprayed with water suspensions comprising M. anisopliae conidia (10^7 spores ml^{-1}), 1.5 g l^{-1} barley flour and 0.5 g l skimmed milk (Oxoid, Ltd). Two adjacent beds in one chamber, were sprayed with viable conidia (98.7%) while those in the other chamber were sprayed with autoclaved spores. Further similar treatments were made 14 days later. Insects on 20 plants bed^{-1} were counted before the first treatment and after 14 and 28 days.

Table 8.04

Treatments applied to cucumber for control of *Thrips tabaci*

Treatment	Spore concentration (ml^{-1})	Viability (%)
Autoclaved Mycotal® ($2.5\ g\ l^{-1}$)	-	0
Mycotal® ($2.5\ g\ l^{-1}$)	6.3×10^5	100.0
V. lecanii hyphal bodies (19-79)+$1.5\ g\ l^{-1}$ barley flour, $0.5\ g\ l^{-1}$ skimmed milk[a]	10^7	99.2
V. lecanii hyphal bodies (53-81)+$1.5\ g\ l^{-1}$ barley flour, $0.5\ g\ l^{-1}$ skimmed milk[a]	10^7	98.7

a. St. Ivel

RESULTS

5. Hauptidia maroccana

a. Preliminary Experiment

i. Chamber With Increased Humidity

Numbers of nymphs on untreated leaves declined, and 13 days after spraying other plants, there was a mean of only nine nymphs leaf^{-1} (Fig. 8.02 b). Only 1.8% of nymphs present were dead and nymphs probably moved from labelled leaves, or moulted into adults and dispersed. Conidial sprays of P. fumosoroseus, B. bassiana and M. anisopliae reduced nymph populations to similar levels, but the decline was attributed to fungal infection as approximately 85% of nymphs were dead, of which 88-98% bore sporulating mycelia. Leaves treated with V. lecanii had similar numbers of nymphs (mean no. live nymphs leaf^{-1} 24.2; % dead, 56.2) to those treated with 0.03% Triton X-100 (21.7 nymphs leaf^{-1}, 8.2% dead).

ii. Chamber With Normal Humidity

Thirteen days after treatment, mean numbers of nymphs on leaves left untreated, or sprayed with 0.03% Triton X-100, were 28.6 and 32.4 respectively (Fig. 8.02). Compared to these levels, populations were reduced on leaves sprayed with P. fumosoroseus, B. bassiana, M. anisopliae or V. lecanii which had 21.3, 12.6, 18.6 and 10.3, nymphs leaf^{-1} respectively, while corresponding percentages of nymphs dead were 48.8, 43.4, 31.6 and 77.8. Thus, mortality levels were generally lower than in the humidified house.

iii. Glasshouse Humidity Levels

In the humidified chamber relative humidity (r.h.) remained above 84% for 18 hours after conidial application and fell briefly to 76% before returning to 84-90% for the next 60 hours (Fig. 8.03 a). Thereafter, humidity levels were cyclical, approaching 90% at night but falling to about 75% during parts of the day, in response to an increase in temperature.

In the non humidified chamber, r.h. remained about 88% for 18 hours after spraying, then fell sharply to 75-80%

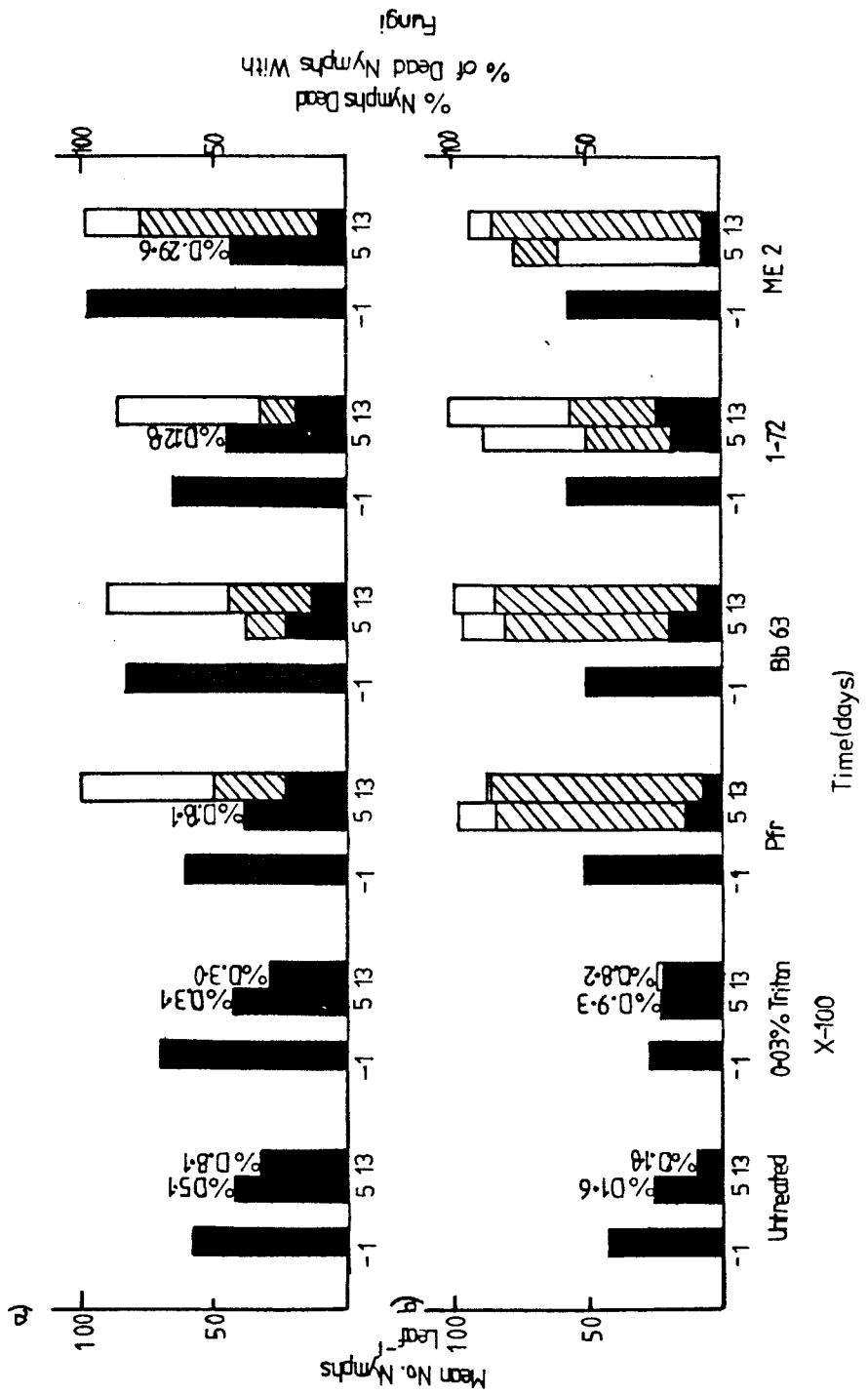


Fig. 8.02

Mean numbers of Hauptidia maroccana nymphs leaf^{-1} (■) before, and 3, 5 and 13 days after treatment of cucumber plants with Beauveria bassiana (63) Metarhizium anisopliae (ME2) Paecilomyces fumosoroseus (21) or Verticillium lecanii (1-72), ▨ % Nymphs dead, □ % dead nymphs with fungus: a, normal r.h.: b, increased r.h.

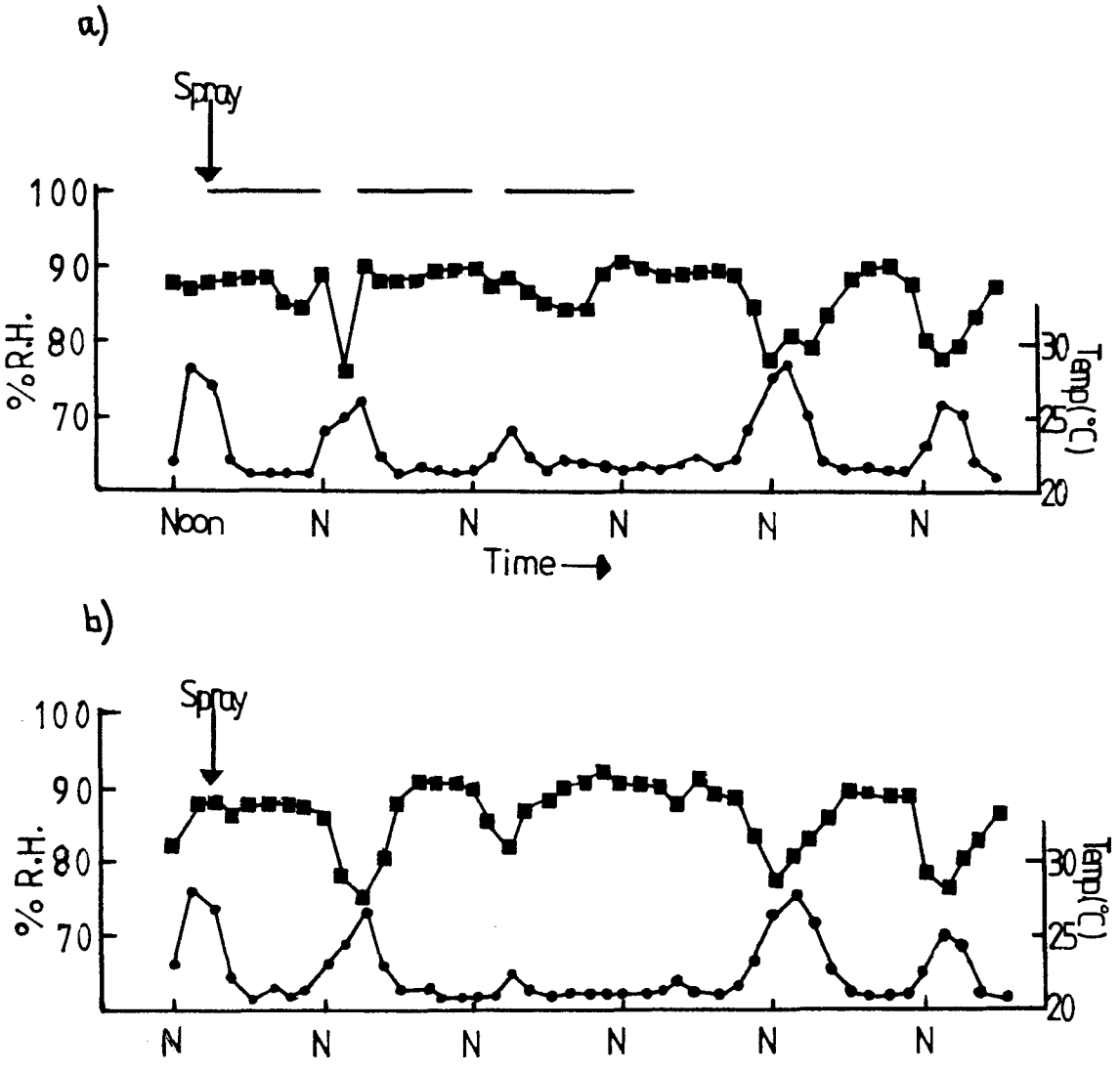


Fig. 8.03

Relative humidities and temperatures in glasshouse chambers after application of fungi to control Hauptidia maroccana : a. chamber with increased humidity; b. chamber with normal humidity; ——— Denotes period of humidification. Points are "spot readings" taken at 3 hour intervals from thermohygrographs. (● temp, ■ r.h.)

for 9 hours, before recovering to 90% (Fig. 8.03 b). For the next 60 hours r.h. was generally about 90% but fell briefly to about 80% . Humidity levels were then similar to those in the humidified house.

iv. Mortality of Hauptidia maroccana on leaves maintained at 25°C and a nominal 100% r.h.

One day after all treatments some 20% of insects were dead compared to only 2.8% of untreated insects (Fig. 8.04). Fungus requires more than 24 h to kill and insects probably drowned in the applied moisture.Three days after treatment, mortalities increased and all sprayed leaves had a significantly higher percentage of dead insects($p=0.05$) than unsprayed leaves.M.anisopliae treated insects were most vulnerable and 93.4 % were dead which was significantly greater($p = 0.05$) than all other treatments,except P. fumosoroseus.Five days after treatment,mortalities of fungi-treated insects were significantly greater($p = 0.05$) than untreated insects,though only M.anisopliae-induced mortality was significantly larger than that which occurred in insects treated with 0.03 % Triton X-100.

b. Experiment 2

i. Effect of Conidial Sprays on Populations of H.maroccana

Six days after treatment, populations of H. maroccana nymphs on plants sprayed with 0.03% Triton x-100, or left untreated, were similar to those before spraying (Fig. 8.05). In contrast, populations on leaves treated with fungi were reduced after the same time. However, 23 days after spore application, leaves of all six treatments had increased numbers of nymphs, compared to pre-treatment levels. Populations ranged from a mean of 78 $leaf^{-1}$ (untreated leaves) to 38 $leaf^{-1}$ on plants treated with M. anisopliae or B. bassiana . At no time after treatment were many dead nymphs observed (< 2%) and no insects showed evidence of fungal infection. Thus, the reduced populations on plants treated with fungi were probably not due to fungal infection and, in any case, the reductions were insufficient to achieve control.

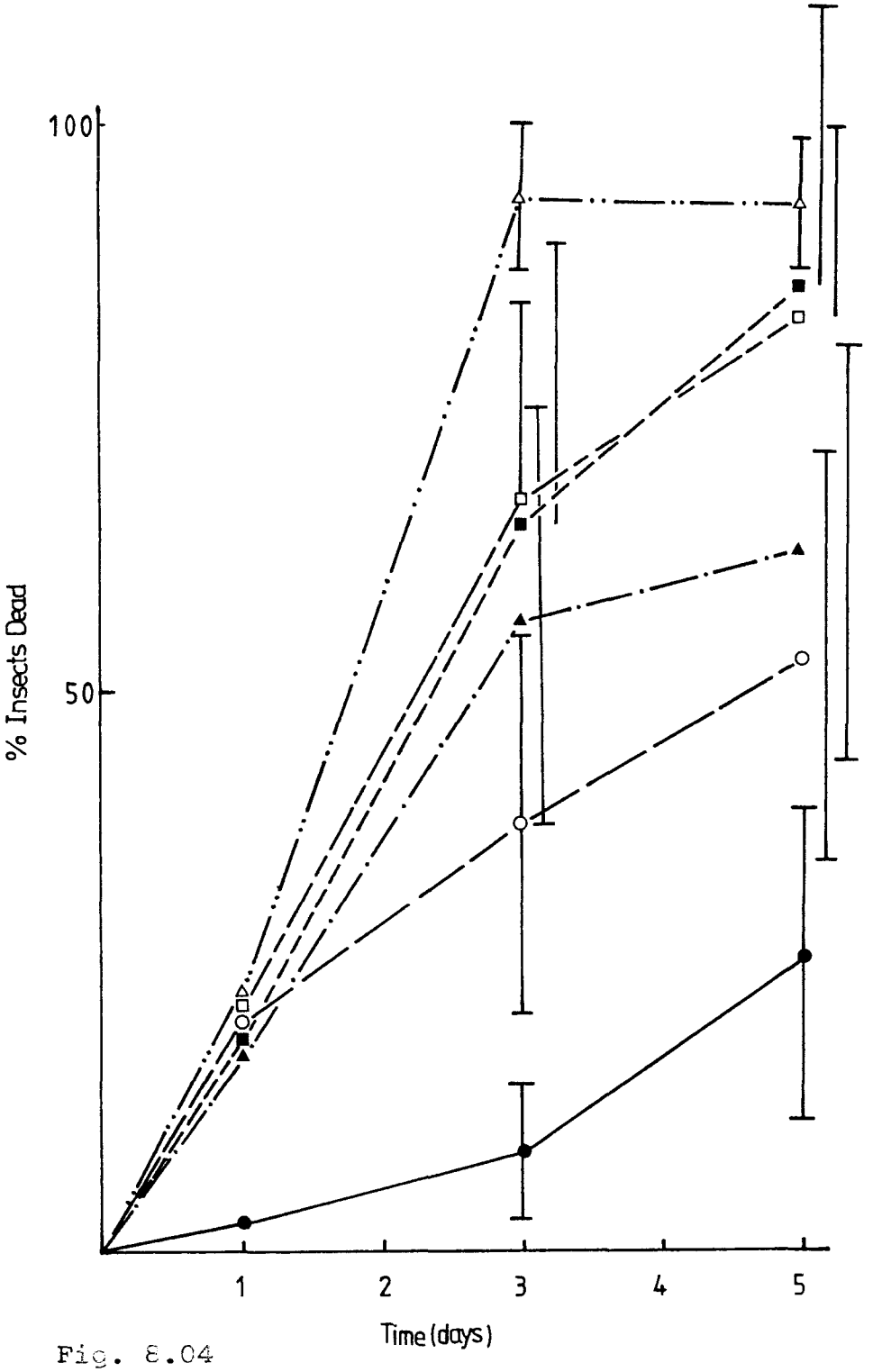


Fig. 8.04

Mortality of Hauptidia maroccana adults and nymphs on cucumber leaf squares removed after spraying and maintained at 25°C and 100% r.h. ● Untreated control, ○ 0.03% Triton X-100, ■ Paecilomyces fumosoroseus, □ Beauveria bassiana (63), Δ Verticillium lecanii (1-7), ▲ Metarhizium anisopliae (ME?). Points are means of 5 replicates with 95% confidence limits. (I).

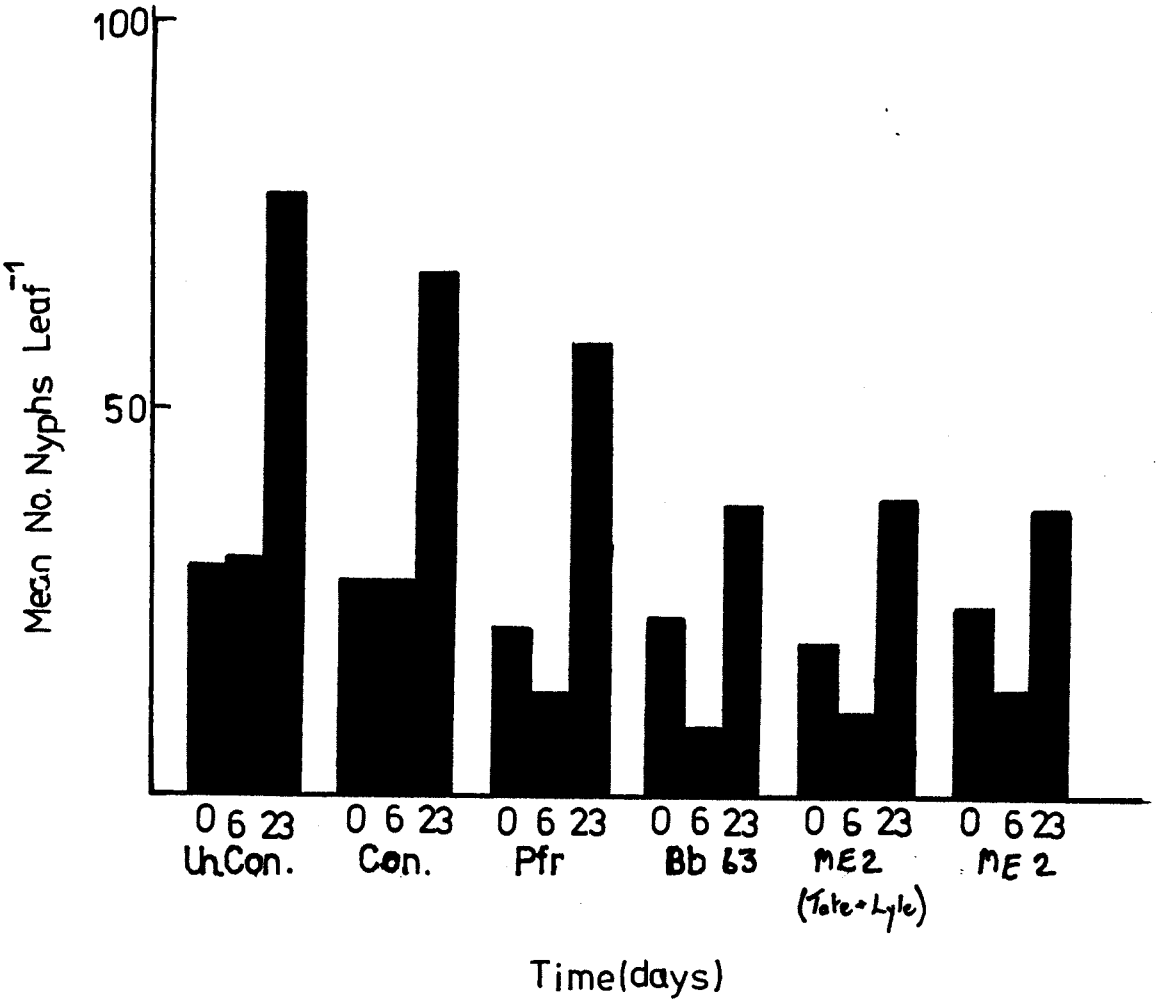


Fig. 8.05
Mean numbers of Hauptidia maroccana nymphs leaf before, and 6 and 23 days after treatment of cucumber plants with Beauveria bassiana (63), Paecilomyces fumosoroseus (21) or Metarhizium anisopliae (ME2) fresh spores or M. anisopliae (ME2) Tate and Lyle formulation.

Un. Con - Untreated Control
Con. 0.03% Triton X-100

ii. Glasshouse Humidity levels

During conidial application, r.h. ranged from 82-87% and then increased to approximately 90% for the next 15 hours (Fig. 8.06). Thereafter, r.h. fell and was between 68-72% from 27-39 hours after spore application, but increased to 84% and remained close to this level for the next 78 hours. During the next 8 days r.h. was generally above 80%, only twice falling below this level.

iii. Mortality of Hauptidia maroccana on Leaves Maintained at 25°C and a Nominal 100% r.h.

Treatment of insects with M. anisopliae, P. fumosoroseus, or B. bassiana resulted in significantly greater mortality ($p = 0.05$) after 5 and 7 days, when compared to controls. (Fig. 8.07) Between 90 and 100% of fungi-treated insects were dead after 7 days, compared to 24.9% of untreated insects and 15.9% of insects treated with 0.03% Triton x-100.

c. Large Scale Experiment

i. Effect of Conidial Sprays on Populations of Hauptidia maroccana

Eight days after conidial application, populations of H. maroccana nymphs on leaves treated with 0.05% Triton x-100, or P. fumosoroseus were greater than before treatment (Fig. 8.08). In contrast, conidial sprays of M. anisopliae (all three treatments) reduced numbers slightly. By 35 days after treatment, there were less nymphs (mean no. leaf^{-1}, 17-26) on plants treated with fungi, than those sprayed with surfactant alone (28 leaf^{-1}). Populations of adult H. maroccana increased throughout the experiment, irrespective of treatment, and 35 days after spore application, only P. fumosoroseus-treated plants had lower numbers than the control (0.05% Triton x-100).

The commercial formulation of M. anisopliae (ME2) grew slightly on lower leaf surfaces but microscope examination revealed few conidia.

The ineffectiveness of fungal treatments in killing insect on established foliage, was reflected in the high numbers of nymphs present on recently emerged (and therefore unsprayed)

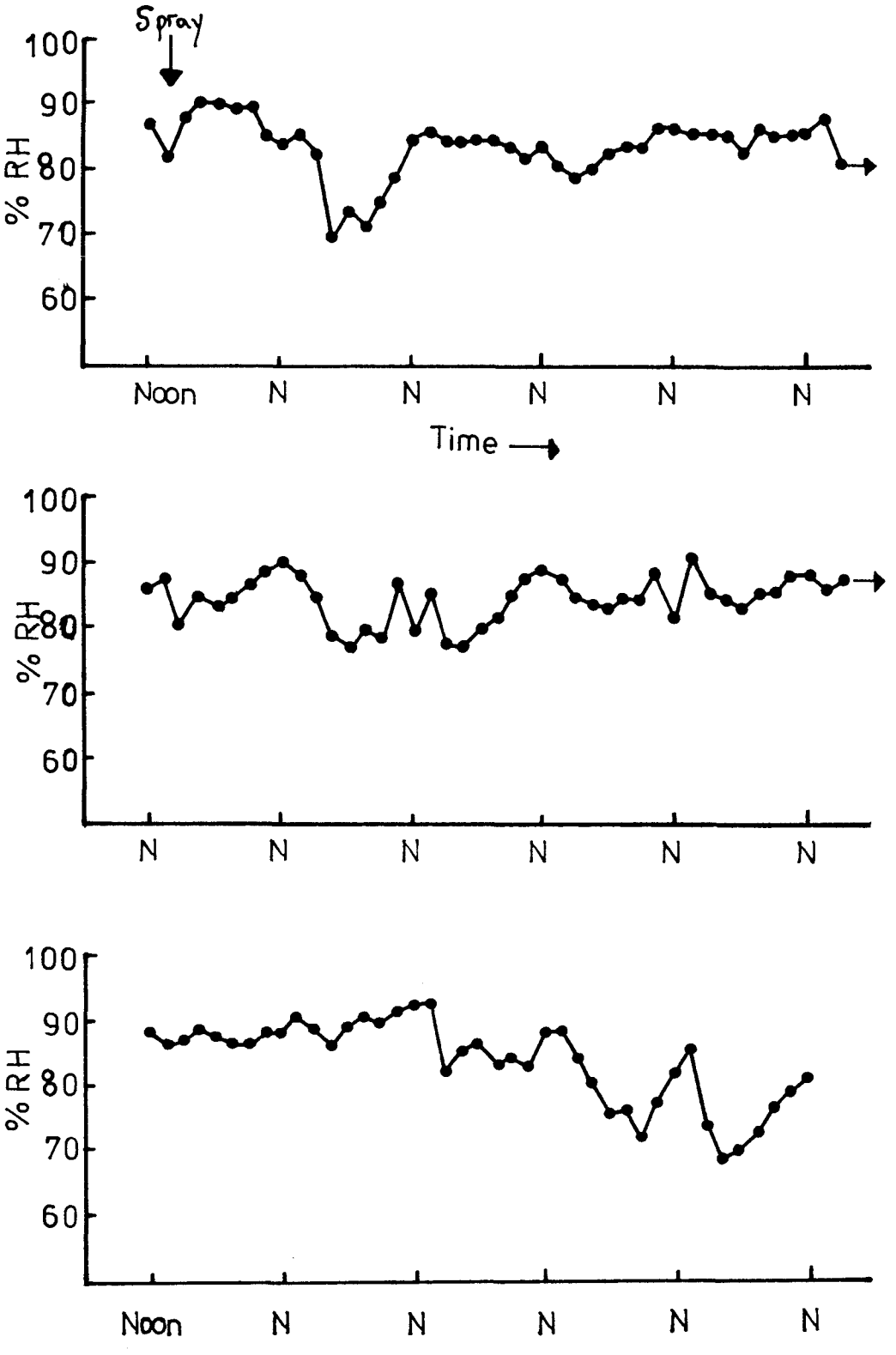


Fig. 8.06
Relative humidities in glasshouse chamber after treatment of cucumber with fungi to control Hauptidia maroccana. Points are "spot readings" taken at 3 hours intervals from thermohygrographs.

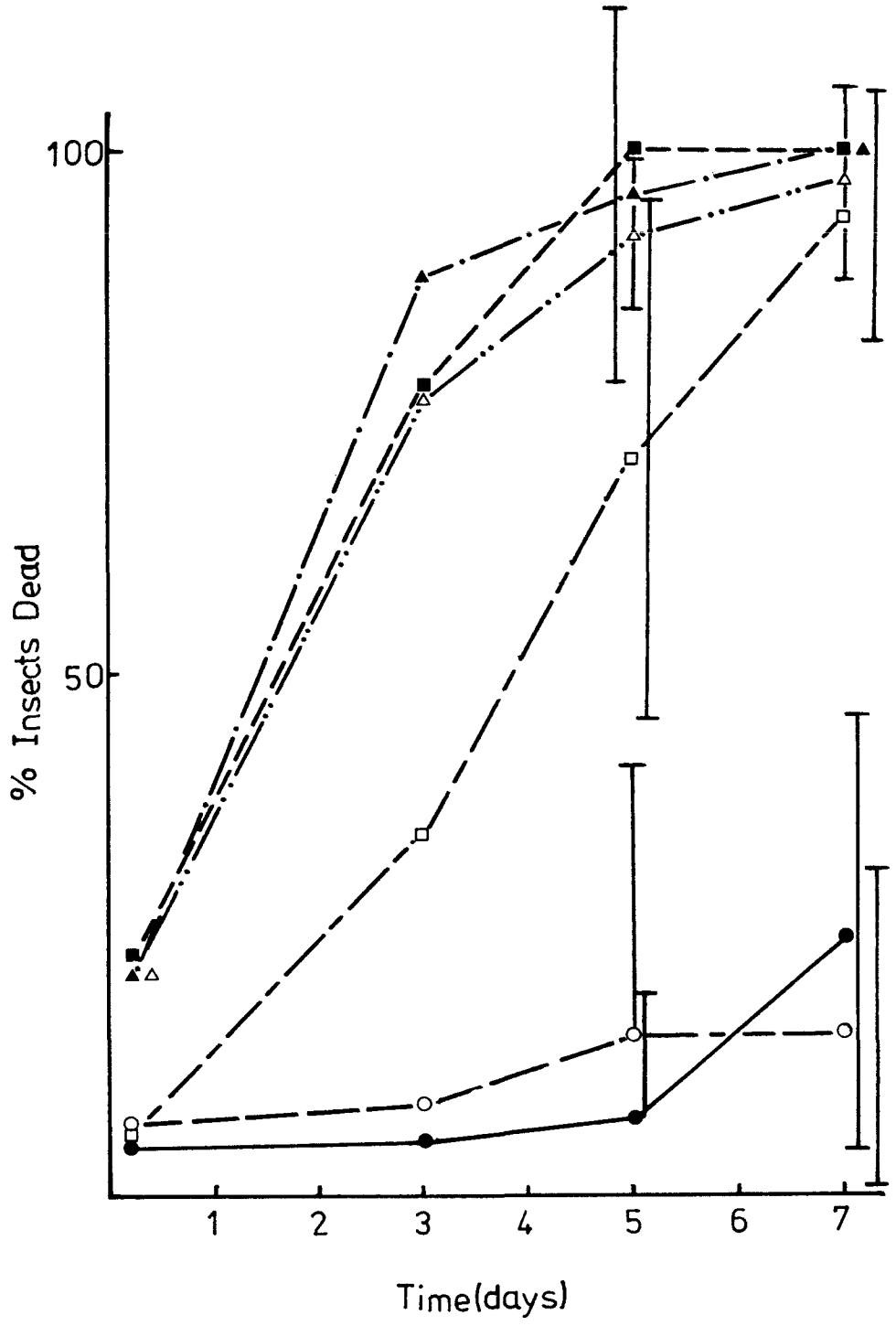


Fig. 8.07

Mortality of Hauptidia maroccana adults and nymphs on cucumber leaf squares removed after spraying and maintained at 25°C and 100% r.h. ● Untreated control, ○ 0.03% Triton X-100, ■ Paecilomyces fumosoroseus, □ Beauveria bassiana (63) ▲ Metarhizium anisopliae (ME2, Tate and Lyle formulation) △ M. anisopliae ME2 (fresh conidia). Points are means of 5 replicates with 95% confidence limits (I).

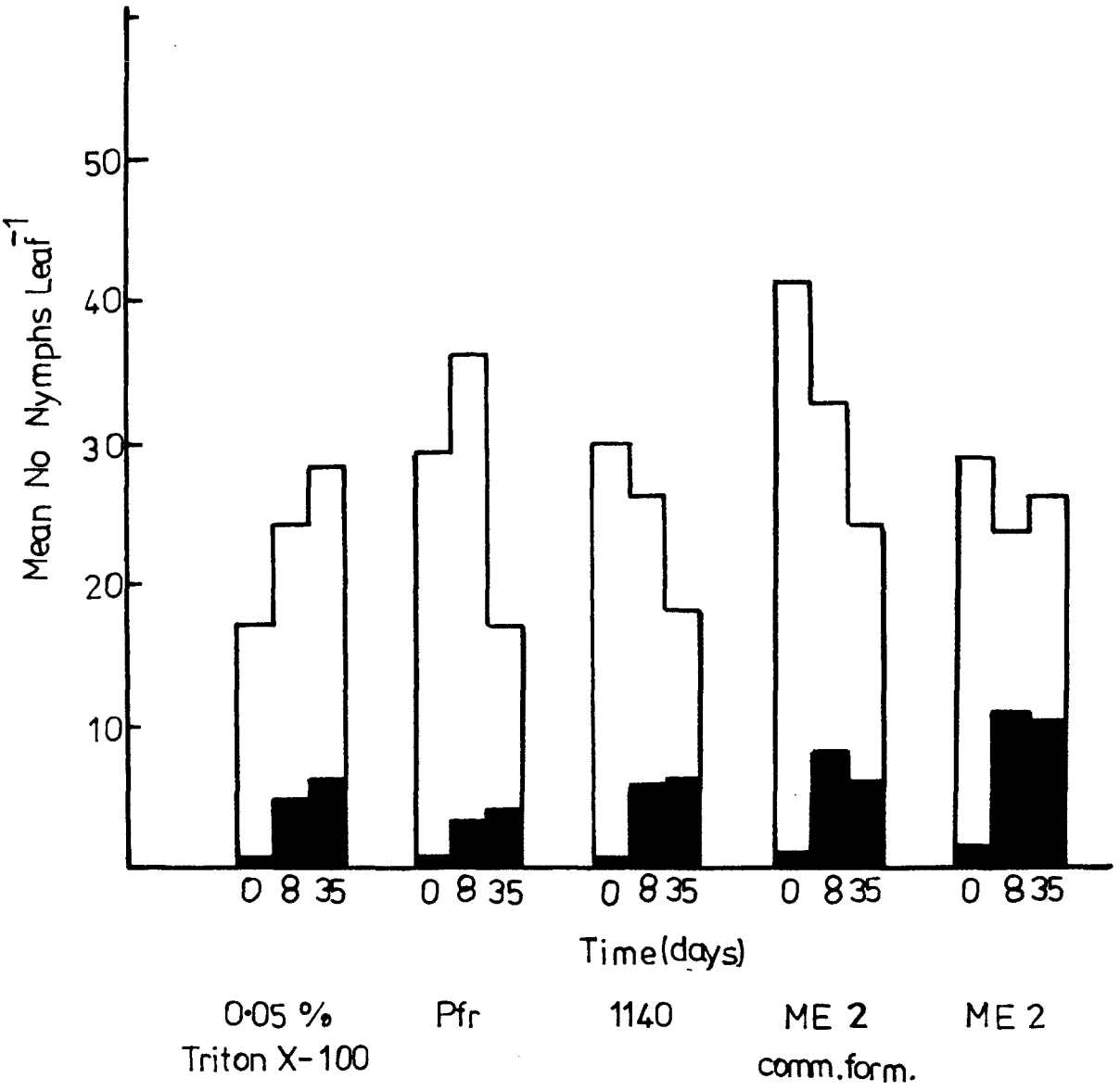


Fig. 8.08

Mean numbers of Hauptidia maroccana adults and nymphs leaf^{-1}, before and 8 and 35 days after application of Paecilomyces fumosoroseus (21) or Metarhizium anisopliae (ME2 fresh spores, ME2 comm. form. or strain 1140).

■ nymphs, □ adults.

leaves (Fig. 8.09). In all chambers, mean populations exceeded 27 nymphs leaf^{-1}, 35 and 46 days after treatment. Throughout the experiment, few dead insects were observed (> 3%) and only occasionally were these insects covered by fungi.

By 35 and 46 days after treatment, some unsprayed leaves of plants, treated with M. anisopliae or P. fumosoroseus, bore reddish-pink coloured H. maroccana eggs and developing larvae, which were shown to be parasited by the mymarid Anagrus atomus. The parasites' incidence was generally low, but 46 days after treatment, P. fumosoroseus sprayed plants had a mean of 31 parasitised eggs and larvae unsprayed leaf^{-1}.

ii. Glasshouse Relative Humidity

Immediately after spore application, r.h. was between 80 and 95%, but fell sharply 18 hours later to 60-70%, before increasing to 80-90% (Fig. 8.10). By 42 hours after conidial application, r.h. again fell to about 70%, and then remained above 80% for most of the next 4 days. Low r.h. occurred most frequently about mid-day, and at night r.h. was generally above 90%.

iii. Mortality of Hauptidia maroccana on leaves maintained at 25°C and a nominal 100% relative humidity

One day after treatment with M. anisopliae (1140), 42% of insects were dead which was significantly greater (p = 0.05%) than in all other treatments, (Fig. 8.11) except M. anisopliae (ME2 fresh conidia). Two days later, mortality of M. anisopliae (1140) treated insects had reached 80% and those treated with M. anisopliae (ME2) 51%, compared to P. fumosoroseus 12.9%, M. anisopliae (ME2 comm. form.) 28% and control treated (0.05% Triton X-100) 3%. After 6 days , 85-95% of M. anisopliae treated insects and 69% of those treated with P. fumosoroseus were dead, and these mortalities were significantly greater (p = 0.05) than control levels (15%).

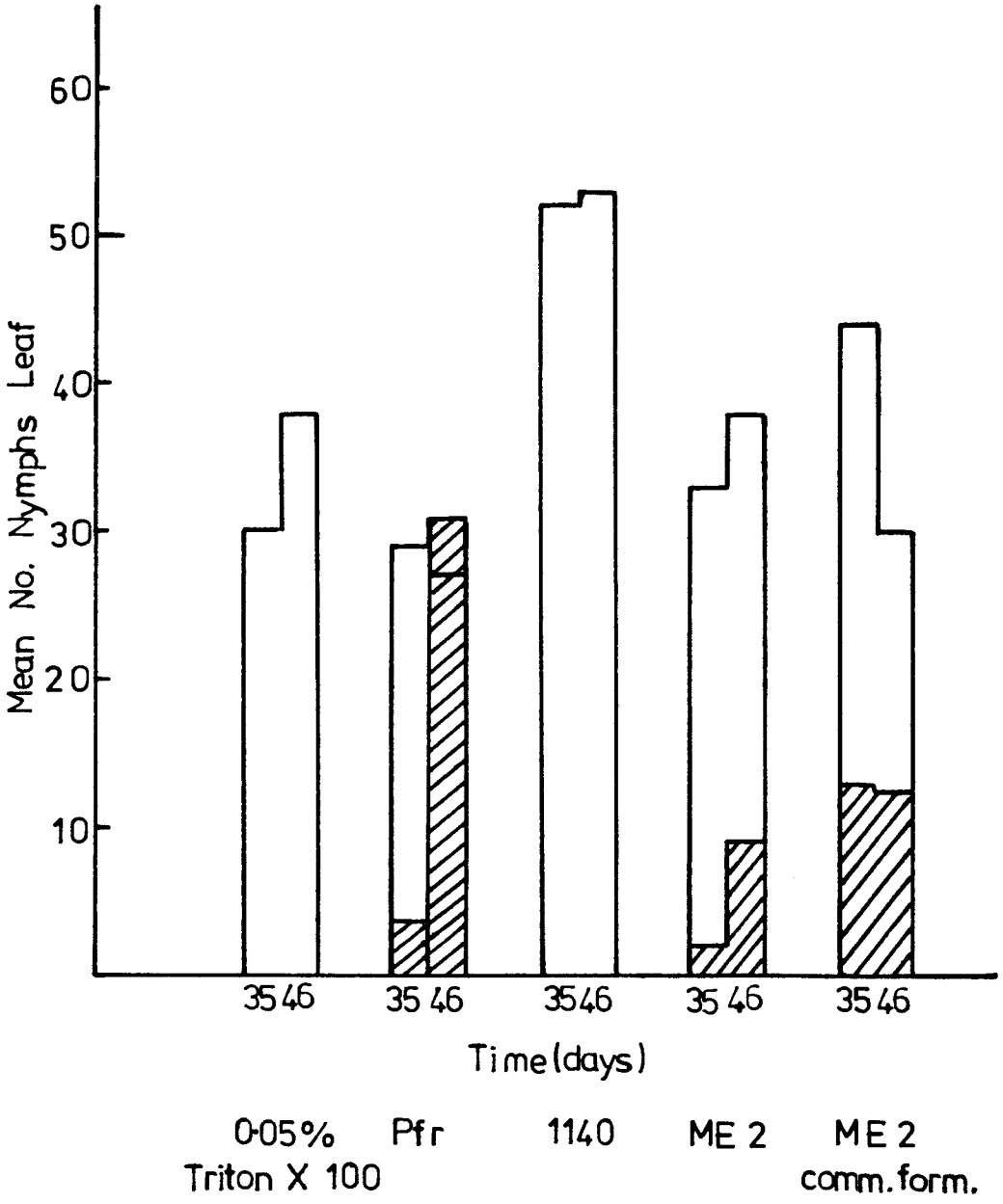


Fig. 8.09

Mean numbers of nymphs leaf^{-1} on new cucumber foliage 35 and 46 days after spraying established plants with Metarhizium anisopliae (ME2, fresh spores; or comm. form. Tate and Lyle) M. anisopliae (1140) or Paecilomyces fumosoroseus.

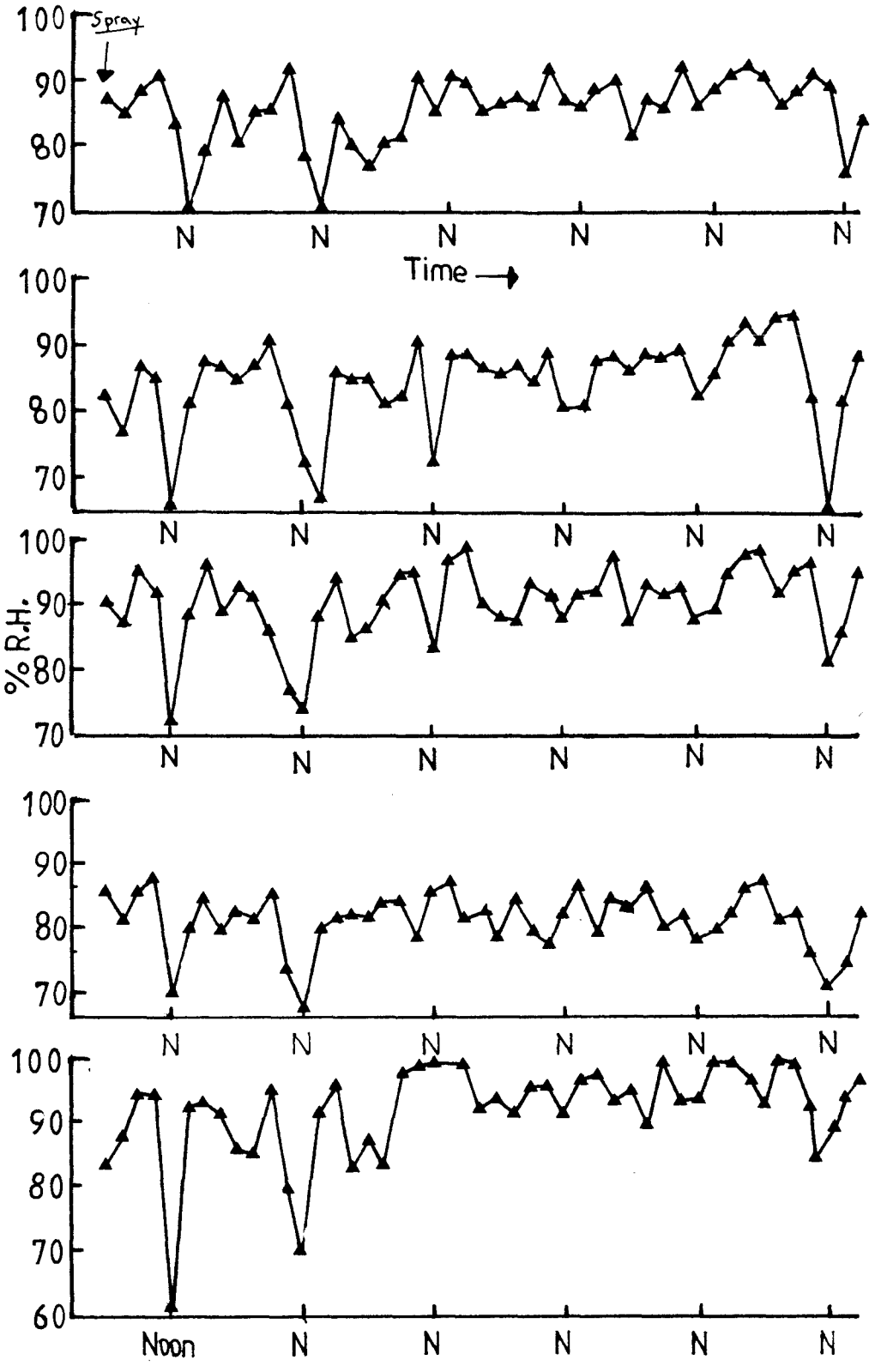


Fig. 8.10
Relative humidity levels in glasshouses after application of fungi to control Hauptidia maroccana. Points are spot readings taken at 3 hour intervals from thermohygrographs.

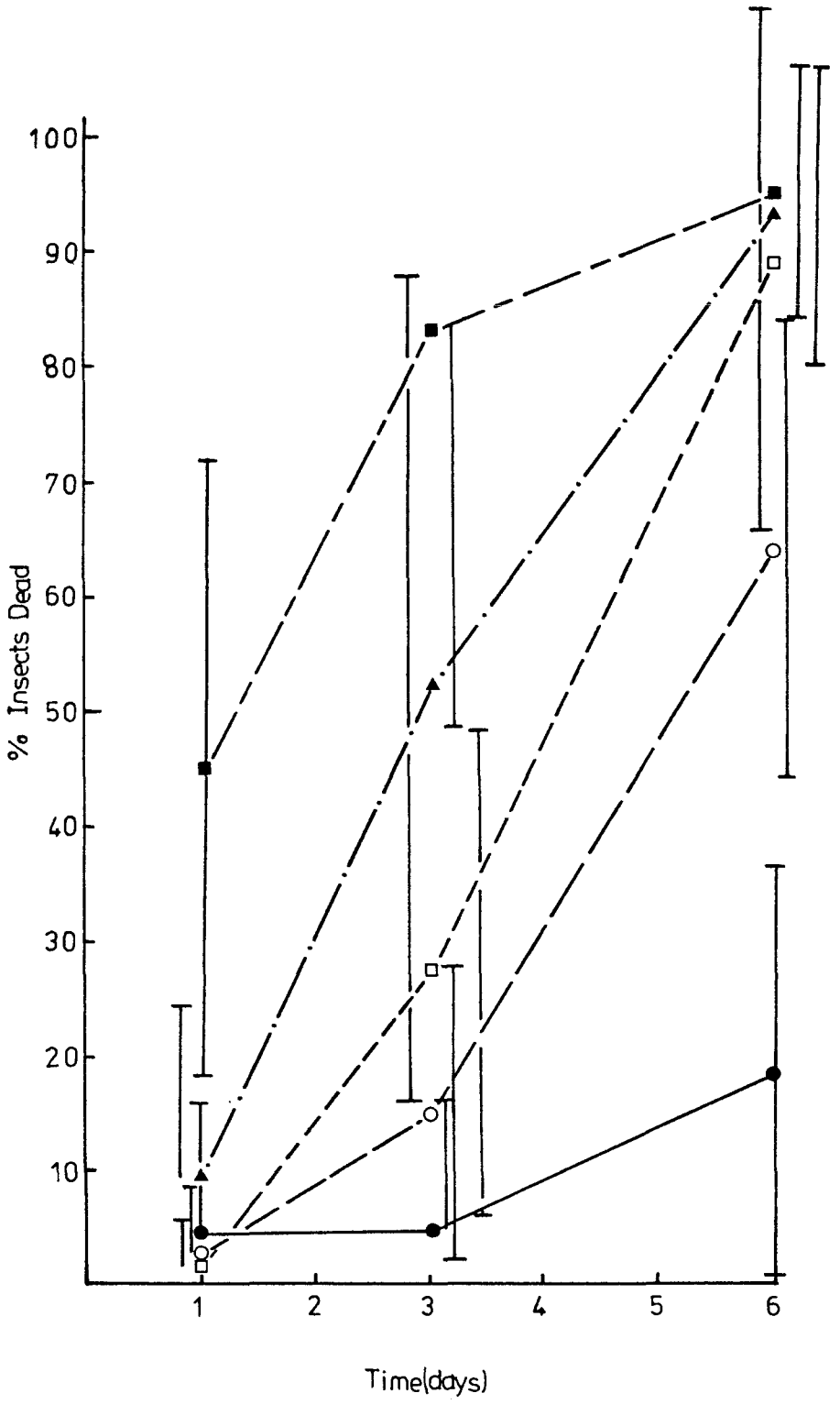


Fig. 8.11

Mortality of Hauptidia maroccana adults and nymphs on cucumber leaf squares removed after spraying and maintained at 25°C and 100% r.h. ● 0.05% Triton X-100, ○ Paecilomyces fumosoroseus, ■ Metarhizium anisopliae (1140), □ M. anisopliae (ME?, commercial formulation) ▲ M. anisopliae (ME2, fresh conidia). Points are mean of 5 replicates with 95% confidence limits (I).

6. Experiments With *Thrips tabaci*

a. Preliminary Experiment - The Effect of *V. lecanii* on Thrips Populations

i. Upper Leaf Surfaces

Populations of *T. tabaci* nymphs, treated with *V. lecanii*, increased during the 7 days after spore application, while populations treated with autoclaved *V. lecanii* control remained stable (Fig. 8.12). However, after 14 days, control treated nymphs had increased to a mean of 56 leaf^{-1}, while populations treated with *V. lecanii* declined to 10 leaf^{-1}. Populations of *T. tabaci* adults were greater on control leaves than on those sprayed with viable *V. lecanii* spores, both 7 and 14 days after treatment. On control leaves, less than 1% of thrips present were dead 14 days after treatment, compared to 66.7% of insects treated with viable *V. lecanii* spores(Table 8.05).

ii. Lower Leaf Surfaces

Numbers of both adult and immature thrips were reduced from pre-treatment levels, 7 days after application of viable *V. lecanii* spores (Fig. 8.12 b). In contrast, control treated nymphs increased from a mean of a 19 leaf^{-1} to 75 leaf^{-1} in the 7 days after spraying, while adult populations remained stable. After 14 days, *V. lecanii* had virtually eliminated thrips from treated foliage (mean 0.1 leaf^{-1}) and some 77% of insects were dead (Table 8.05), while control treated leaves still had a mean of 30 nymphs leaf^{-1}.

V. lecanii was first observed growing and sporulating on the surface of treated leaves 5 days after spore applicatio: Microscopic examination revealed mycelia emerging from spray deposits and spreading over the leaf, often growing between hairs and showed more extensive growth on lower, than on upper leaf surfaces.

iii. Glasshouse Relative Humidity

At the time of treatment, r.h. was above 90% and remained so for 6 hours, before falling to about 80% 6 hours later (Fig. 8.13). R.h. then increased to about 90%, at which

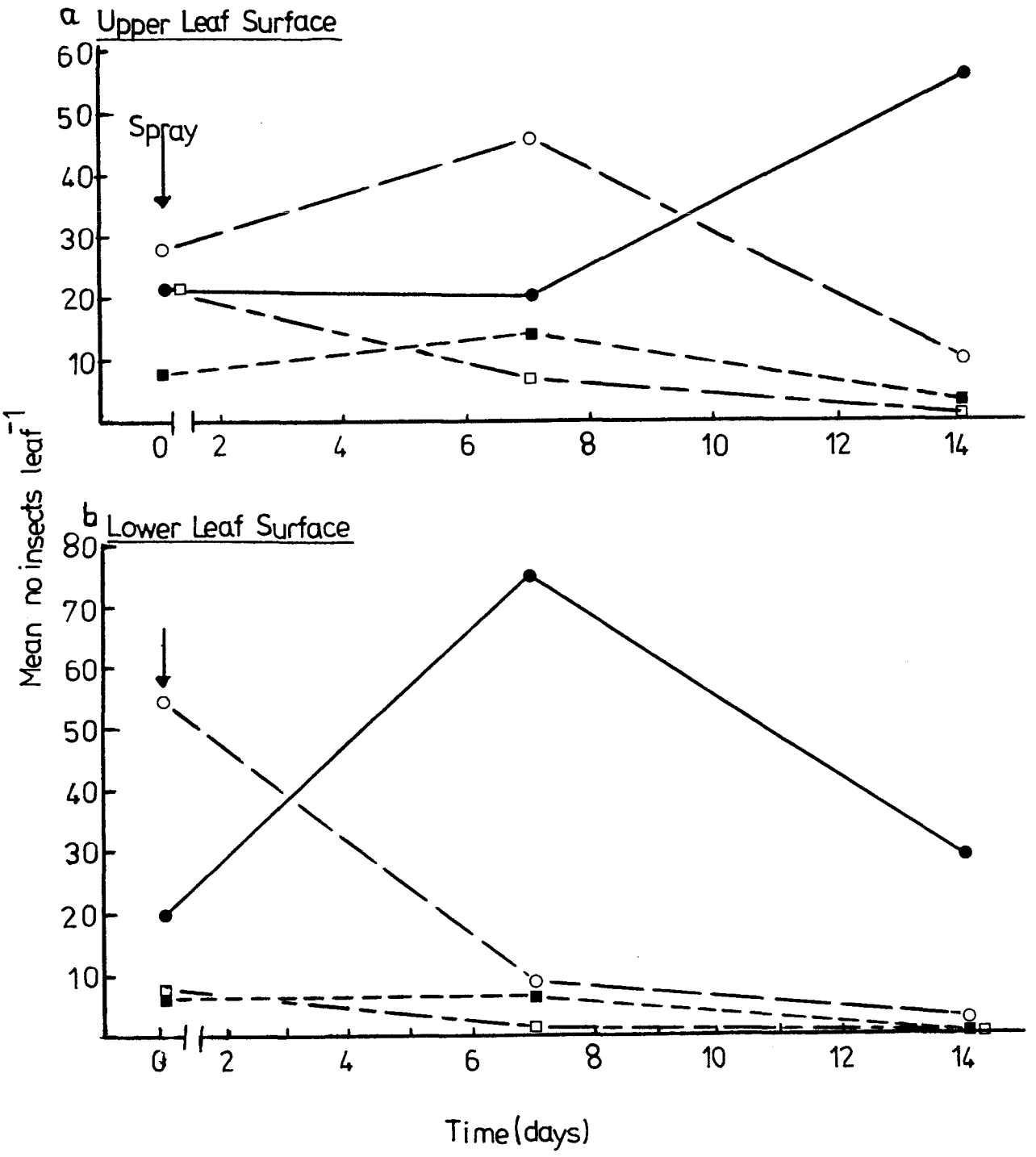


Fig. 8.12

Mean number of Thrips tabaci adults (■ □) and nymphs (● ○) leaf^{-1} before, and 7 and 14 days after treatment with Verticillium lecanii (53-81) and nutrients (open symbols) or nutrients alone (closed symbols).

Table 8.05

Percentage of thrips dead seven and 14 days after spraying with *V. lecanii* in preliminary experiment.

Treatment	Leaf surfaces	% thrips dead on selected days after treatment[a]	
		7	14
Control	Upper	0.8	1.0
	Lower	0.3	2.7
V. lecanii	Upper	6.1	66.7
	Lower	31.2	76.7

a Mean % adult and nymphs dead from observations on 10 leaves treatment^{-1}.

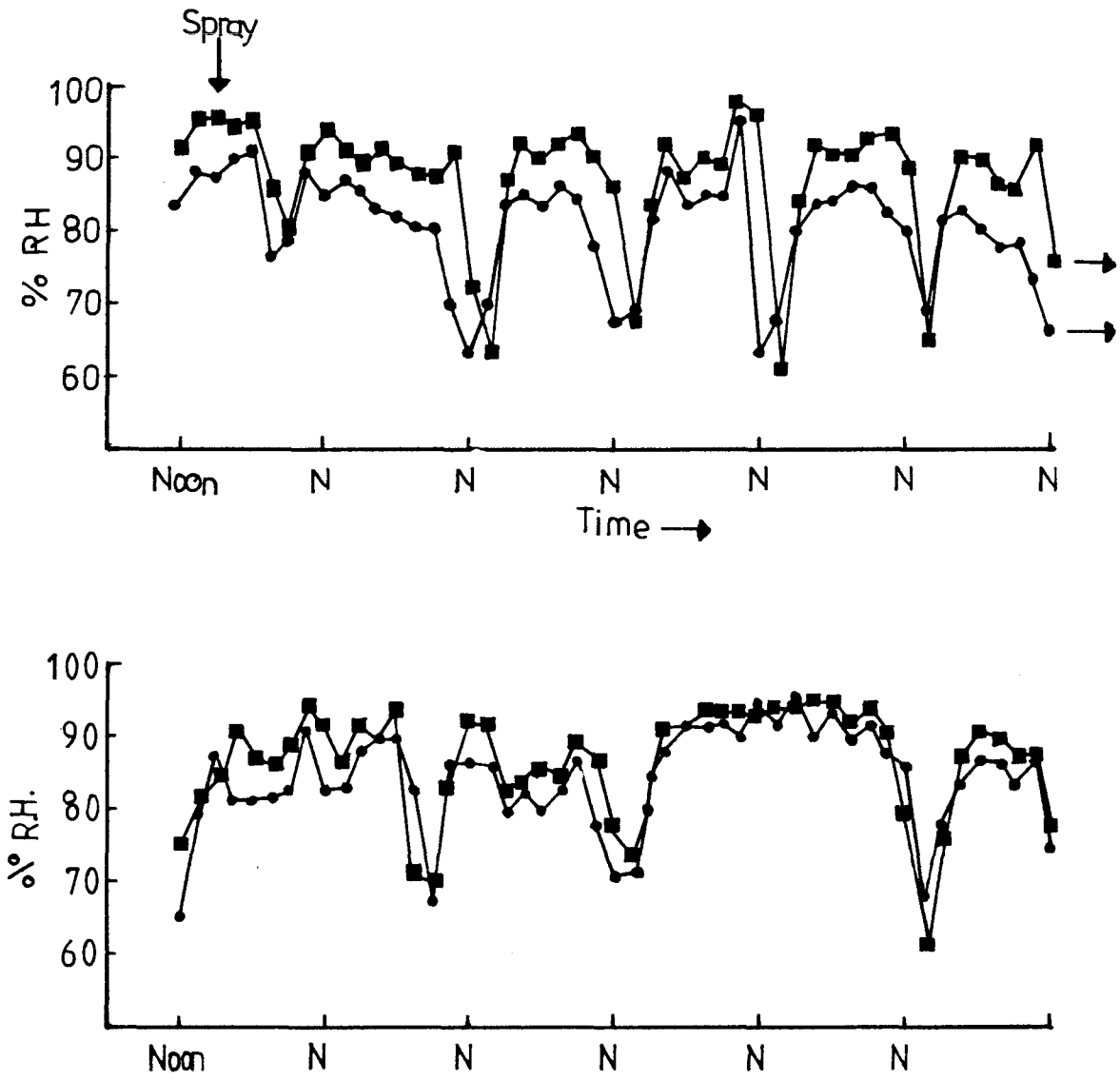


Fig. 8.13
Relative humidities in glasshouse chambers used to maintain cucumber plants after treatment with Verticillium lecanii (53-81) and nutrients (□), or nutrients alone (●). Points are "spot readings" taken at 3 hour intervals from thermohygrographs.

it remained for 24 hours, then fell sharply to about 65% Thereafter, humidity was generally cyclical, high at night, low during daytime, and minimal around noon.

b. Experiment 2 - Effect of Single Sprays of Mycotal® or Thriptal® on Thrips tabaci on Cucumber

i. Effect on Thrips tabaci Populations

Thrips populations, estimated from grease traps, increased on all plants during the 15 days after spraying, then declined sharply (Fig. 8.14). At 20, 25, 35, 40, and 45 days after treatment, significantly less ($p = 0.05$) thrips were present on plants sprayed with Mycotal® than on control plants treated with autoclaved Thriptal®. In contrast, at no time did Thriptal® treated plants have significantly less ($p = 0.05$) thrips than controls.

Direct counts of thrips, 17 days after treatment, showed both Mycotal® and Thriptal® treated plants had significantly ($p = 0.05$) lower numbers of live insects (a quarter to a half) per leaf than controls (Table 8.06). However, significantly more ($p = 0.05$) Mycotal® treated insects were dead (upper leaf surface, 26.3% dead, lower 35.8% dead) than those treated with Thriptal® (upper 9.6%, lower, 16.1%).

At 50 days after spore application, numbers of thrips on new foliage were infected with V. lecanii, demonstrating the ability of this fungus to spread (Table 8.07). Mycotal® was the most effective and killed some 39% of thrips on upper leaf surfaces and 35% on lower. Control plants had about 9% of thrips on new foliage covered in V. lecanii and the fungus probably spread from adjacent Mycotal® and Thriptal® treated plants.

ii. Glasshouse Relative Humidity

With the exception of a 33-hour period when r.h. was con-tinuously above 85%, r.h. was generally between 80-90% overnight but fell to 57-70% around midday (Fig. 8.15).

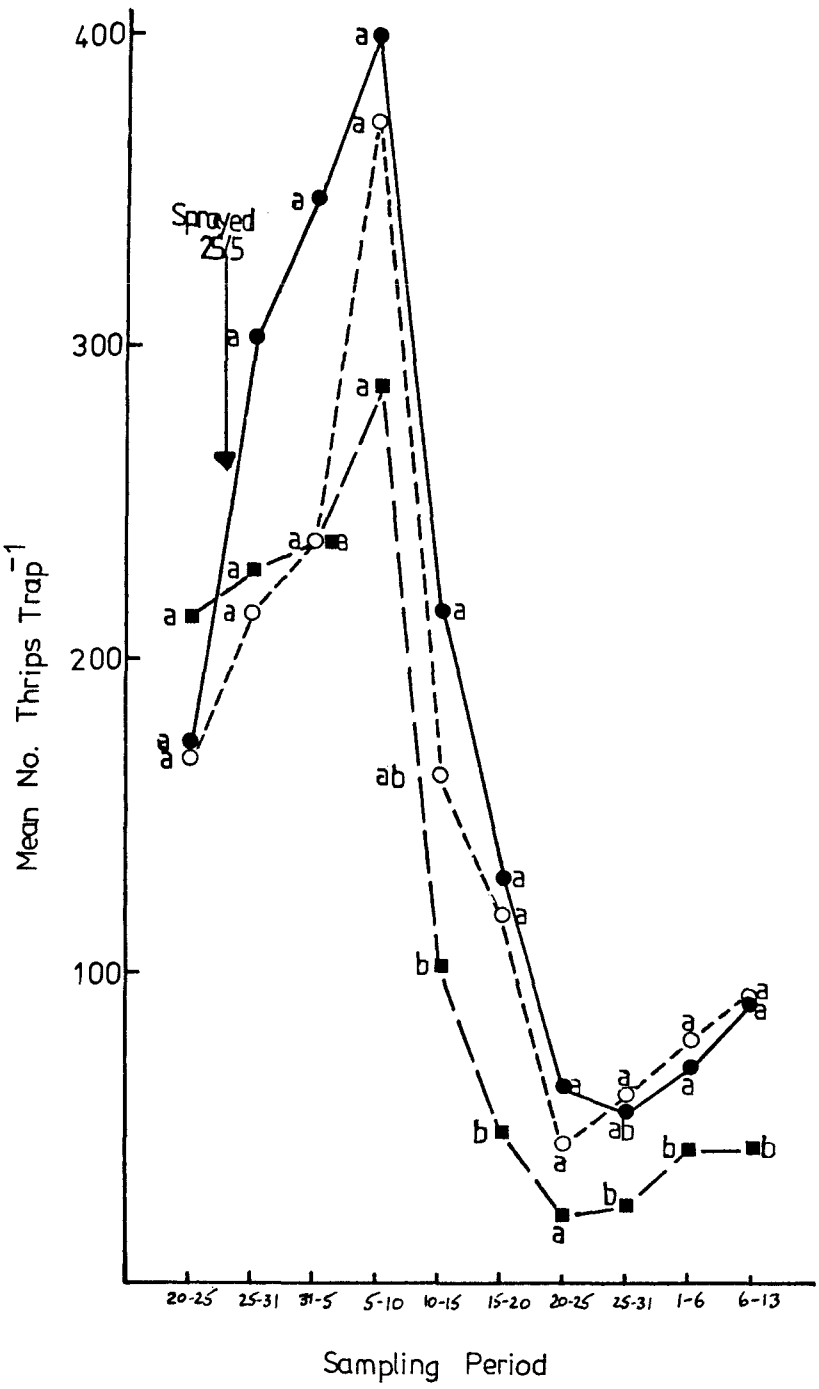


Fig. 8.14

Mean numbers of Thrips tabaci caught trap^{-1} after treatment of cucumbers with Mycotal® (■) Thriptal® (○) or autoclaved Mycotal® (●)(For each sampling date different letters denote significant difference (p = .05)).

Table 8.06

Numbers and percentages of thrips dead 17 days after spraying with V. lecanii as Mycotal® or Thriptal®

Leaf surface	Treatment	Mean number of thrips				
		Before spray	17 days after spray			
		Live	Live	Dead	+fungus	%dead
Upper	Control	28.6	98.1[a]	2.8[a]	2.2[a]	2.8[a]
	Mycotal®	17.9	51.2[b]	18.5[b]	18.2[b]	26.3[b]
	Thriptal®	19.9	48.0[b]	4.9[a]	4.5[a]	9.6[a]
Lower	Control	11.7	82.1[a]	1.0[a]	0.2[a]	1.2[a]
	Mycotal®	11.3	22.2[b]	11.0[b]	10.6[b]	35.8[b]
	Thriptal®	7.2	34.4[b]	6.6[c]	5.8[b]	16.1[c]

i Within each column of three figures; different letters denote significant differences (p = 0.05) determined by one way analysis of variance / Least significant difference techniques.

Table 8.07

Mortality of thrips on unsprayed foliage 50 days after spraying established cucumber plants with *V. lecanii* as Mycotal® or Thriptal®.

Leaf surface	Treatment	Mean number thrips		Mean
		Live	Dead	% dead
Upper	Control	8.2	0.9	8.9
	Mycotal®	3.5	2.4	38.6
	Thriptal®	6.9	0.7	6.8
Lower	Control	8.8	1.7	8.4
	Mycotal®	5.9	3.3	35.2
	Thriptal®	14.6	2.4	13.6

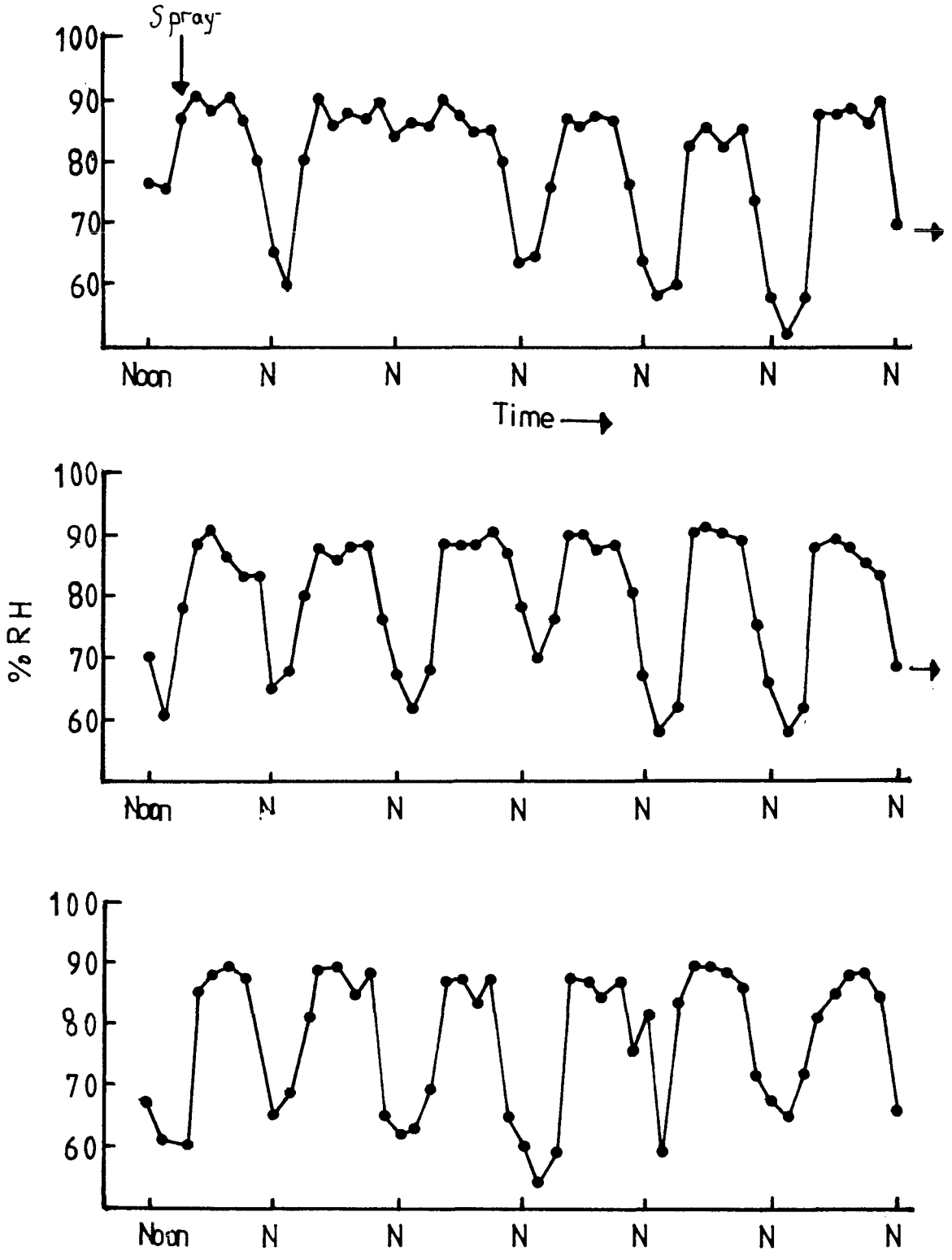


Fig. 8.15

Relative humidity levels in glasshouses after application of Verticillium lecanii as Mycotal® or Thriptal® for Thrips tabaci control. Points are "spot readings" taken at 3 hour intervals from thermohygrographs.

c. Experiment 3 - Effect of Single Sprays of *V. lecanii* Hyphal Bodies (strains 19-79 or 53-81), or Mycotal® on *Thrips tabaci*

i. Effect on *Thrips tabaci* Populations

Thrips populations, assessed by grease traps, on plants treated with autoclaved Mycotal® (control), Mycotal® or *V. lecanii* (19-79), increased after spraying, but then declined sharply (Fig. 8.16). Thrips populations on plants treated with *V. lecanii* (19-79 and 53-81), or non-autoclaved Mycotal® were similar to, but significantly lower ($p = 0.05$) than on control plants, during the sampling period 11-18 days after treatment and at all subsequqnt assessments. In contrast, populations treated with *V. lecanii* (53-81) declined immediately after spraying.

Direct counts of thrips 17 days after treatment showed thrips killed by *V. lecanii* and also by *Neozygites parvispora* (Table 8.08). Insects killed by *N. parvispora* were full of black resting spores and were easily distinguished from those killed by *V. lecanii* which bore white, cottony, mycelia. On upper leaf surfaces treated with viable *V.lecanii* spores, 66-76% of thrips were dead, of which 86-90% were infected with *V. lecanii*. Equivalent values for lower leaf surfaces were 87-95% and 98-100% respectively. On control treated leaves, mortality was lower (upper leaf surface 44% dead, lower 61%), only 47-60% being infected with *V. lecanii*, the remainder with *N. parvispora*.

ii. Glasshouse Relative Humidity

For almost two thirds of the period after treatment r.h. was between 80-90%, but fell around midday and was occasionally below 60% for short periods (Fig. 8.17).

7. Experiments With the Brown Planthopper, *Nilaparvata lugens*

a. Effect of Two Conidial Sprays of *Metarhizium anisopliae*

i. Effect on Planthopper Numbers

By 14 days after the first conidial application, adult planthoppers on both control and treated plants were

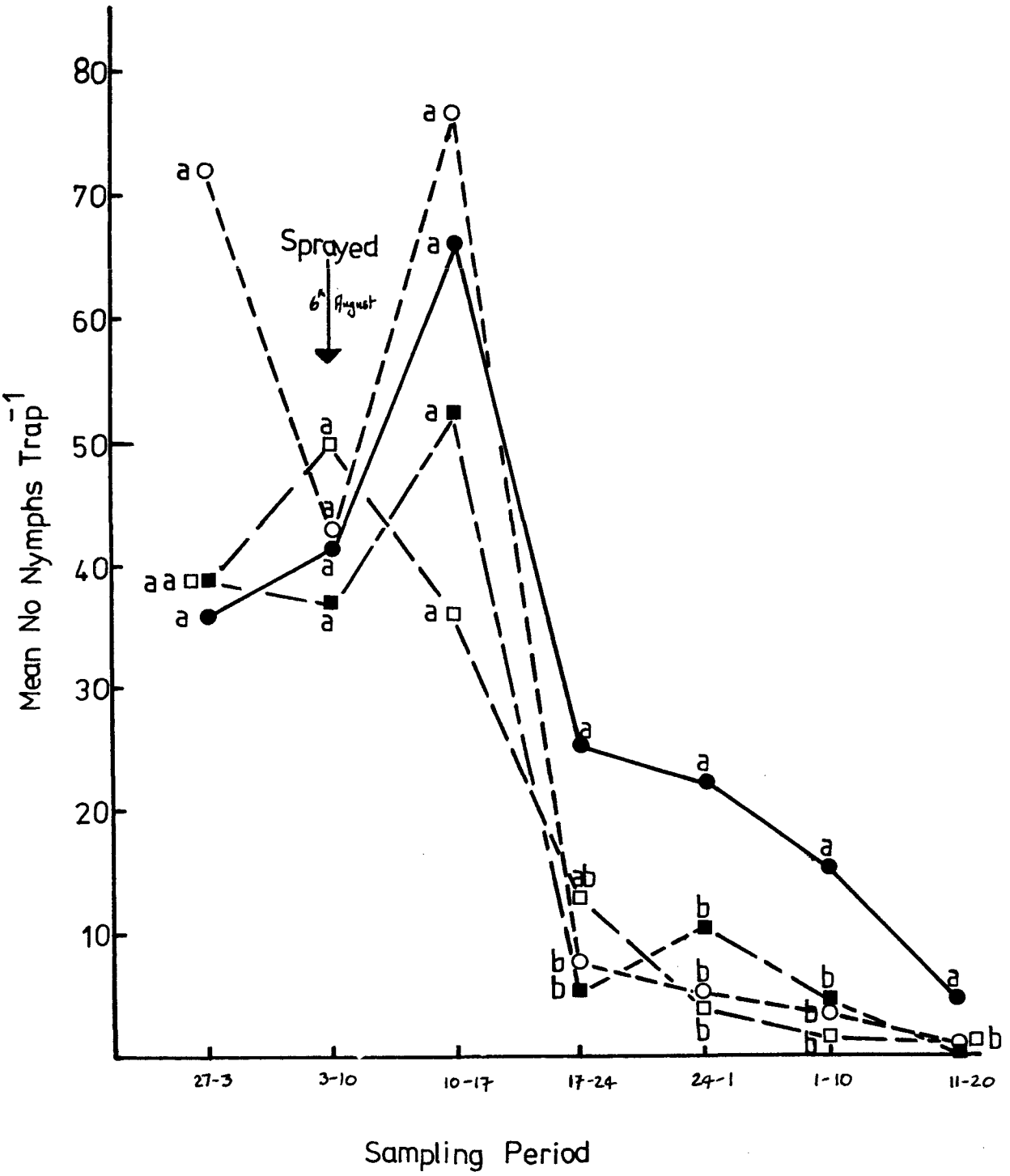


Fig. 8.16
Mean numbers of Thrips tabaci caught trap^{-1} after treatment of cucumbers with Mycotal^{R} (○), Verticillium lecanii hyphal bodies (19-79 (■) or 53-81 (□)) or autoclaved Mycotal^{R} (●). For each sampling period different letters denote significant difference (p = 0.05).

Table 8.08

Numbers of live and dead thrips, with mortality due to Verticillium lecanii and Neozygites parvispora, 17 days after spraying cucumbers with Verticillium lecanii

Leaf surface	Treatment	No. of Thrips			% infected by	
		Live	Dead	% Ø	V.lecanii	N.parvispora
Upper	Control	2.0	1.8	44.3	47.3	52.7
	Mycotal®	1.2	2.3	66.2	86.3	13.7
	V. lecanii (19-79)	1.2	3.3	76.0	89.9	10.1
	V. lecanii (53-81)	1.9	4.2	68.9	89.8	10.2
Lower	Control	1.5	2.6	61.4	60.3	39.7
	Mycotal®	0.45	3.1	87.5	98.4	1.6
	V. lecanii (19-79)	0.2	4.1	95.2	100	0
	V. lecanii	0.3	4.1	94.8	99	1.0

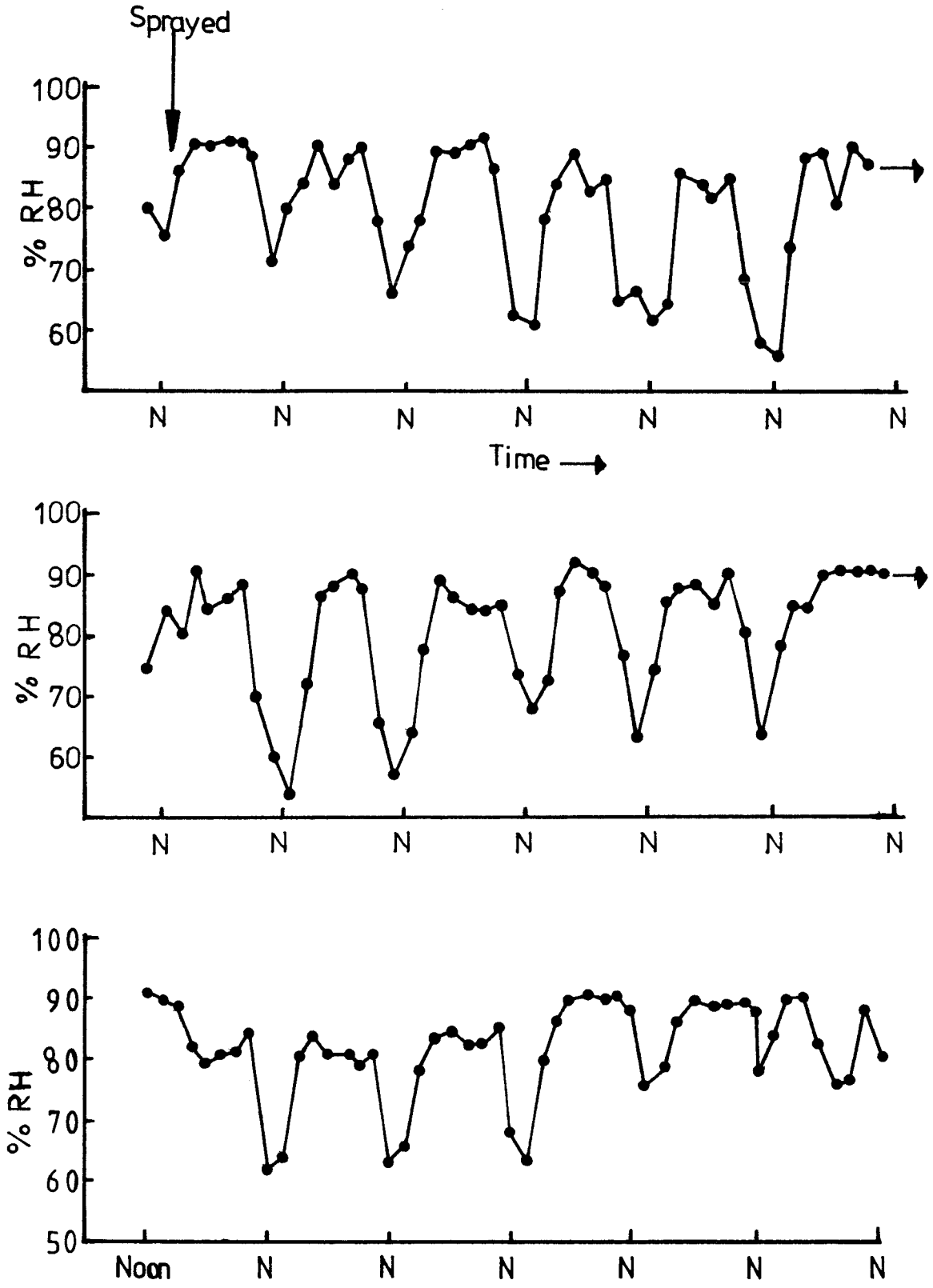


Fig. 8.17
Relative humidity levels in glasshouse after treatment of cucumber with Mycotal or Verticillium lecanii hyphal bodies. Points are 'spot readings' taken at 3 hour intervals from thermohygrographs.

reduced from pre-treatment levels (Fig. 8.18). In contrast, nymphs, which were absent before spraying, reached a mean of approximately 10 plant^{-1}. After a further 14 days, plants treated with viable conidia had more adults plants^{-1} (mean 10) than on control rice treated with autoclaved spores (mean 7.4). Coinciding with the increase in adults, nymph populations were reduced, the mean number on viable conidium treated plants being 3.4 and on control 5.4.
Dead planthoppers fell from rice plants onto compost and made mortality assessments on single plants impossible. Furthermore, dead insects were often eaten by sciarid larvae, present in the compost, which caused an underestimate of mortality. Numbers of M. anisopliae infected insects were low and 14 days after the first spore application, only about 1% of treated adults had succumbed and all nymphs were healthy. By day 28, some 6.6% of M. anisopliae-treated adults and 1% of nymphs were found infected with M. anisopliae, but none on control beds.
M. anisopliae did not grow on the plants, which indicated that either conditions were unfavourable, or insufficient conidia or nutrients adhered at spraying, to the vertical, hydrophobic leaves.
M. anisopliae was ineffective in controlling planthoppers and 5 weeks after the first spore application, most plants were dead, irrespective of treatment (Fig. 8.19).

ii. Glasshouse Relative Humidity

R.h. remained between 71-86% after treatment, with peaks generally occurring at night and troughs around midday (Fig. 8.20). Occasionally, humidity at night was reduced, compared to daytime levels, and this probably reflected the input of heat required to maintain the temperature at 25°C.

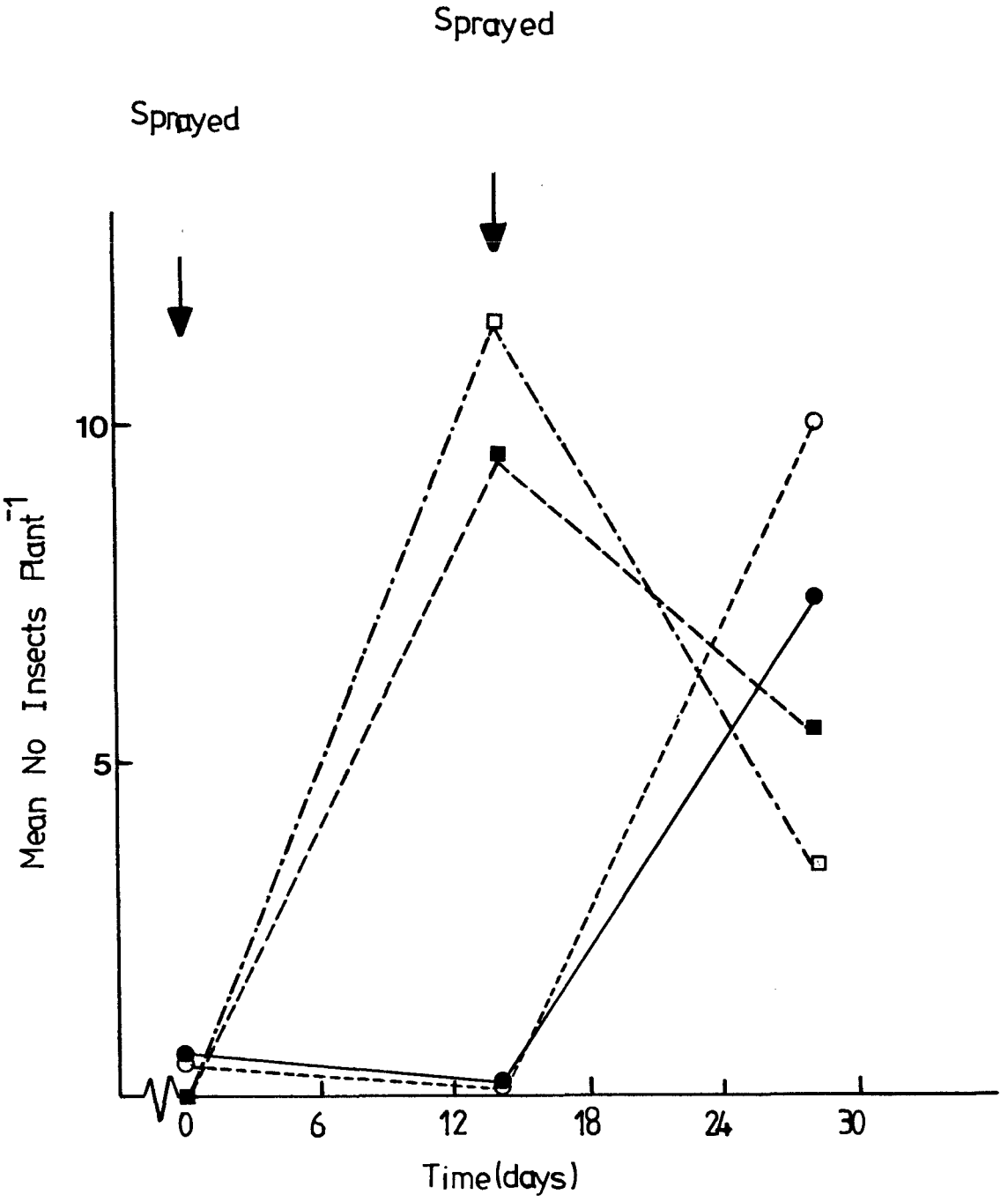


Fig. 8.18
Mean numbers of Niloparvata lugens adults (● ○) and nymphs (■ □) on rice plants before, and 14 and 28 days after application of Metarhizium anisopliae (ME2) and nutrients (open symbols) or nutrients only (closed symbols).

Fig. 8.19
Damage caused to rice plants by Nilaparvata lugens.

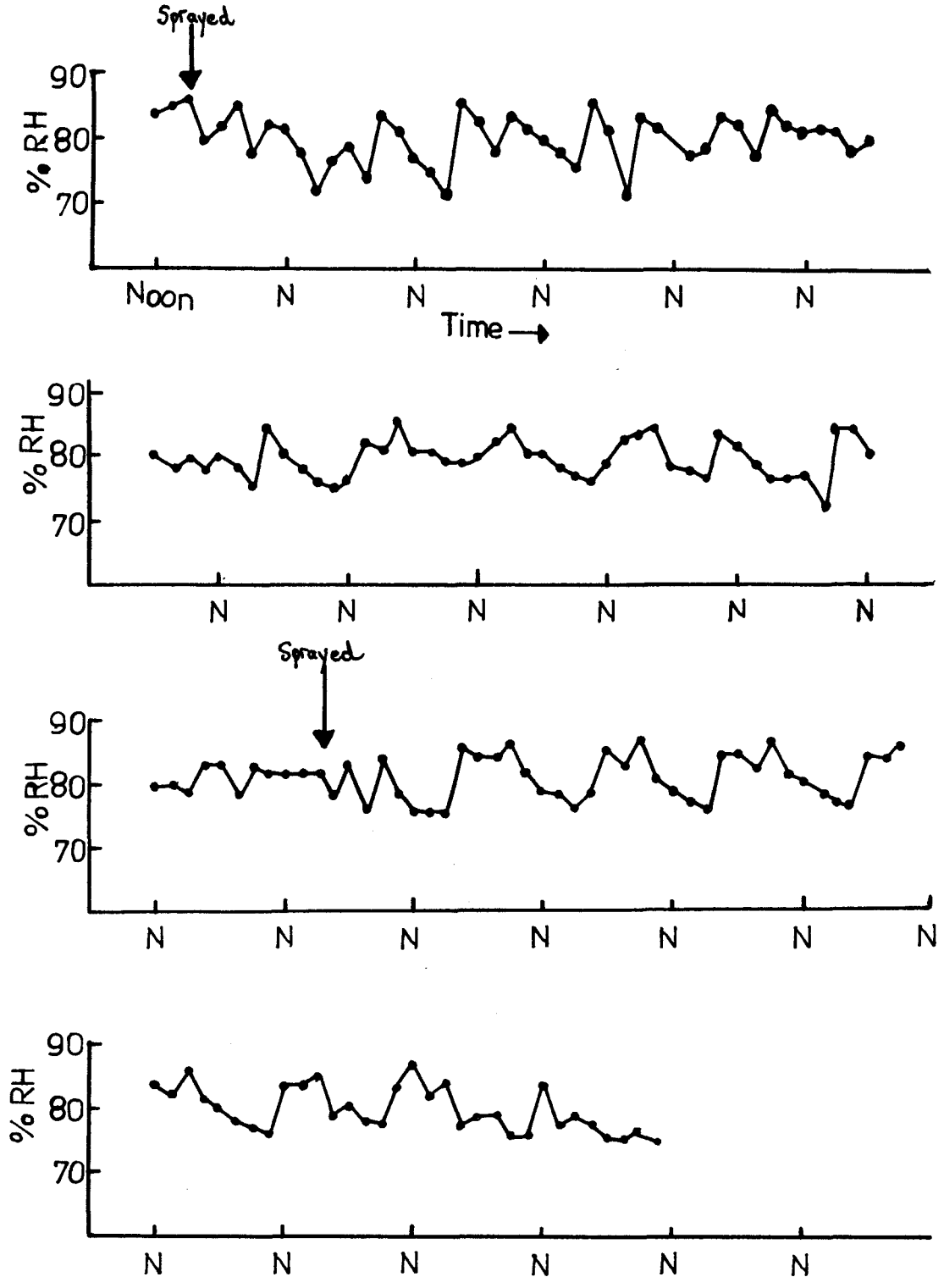


Fig. 8.20

Relative humidity in glasshouse after application of Metarhizium anisopliae to rice plants to control Nilaparvata lugens. Points are 'spot readings' taken every 3 hours from a thermohygrograph

DISCUSSION

The most critical factor affecting growth of fungi, and therefore, their use as myco-insecticides, is relative humidity (r.h.). At a given temperature, air can carry only a maximum amount of water vapour, and when it contains this amount, is described as saturated. The vapour pressure is then the saturation vapour pressure. When air is below saturation point, the r.h. is defined as the ratio of the actual vapour pressure to the saturation vapour pressure, at the same temperature, expressed as a percentage. For example air at 100°C can hold more than 100 times as much water vapour as air at 0°C, so in a closed system, r.h. falls with increased temperature.

If we first consider a glasshouse in a temperate region during summer, temperature generally falls at night and increases during day-time, coinciding with input of radiant heat from the sun. Thus, humidity is high at night and low during day-time. If weather is overcast, little radiant heat falls on the glasshouse and temperatures during night and day are similar. Ventilators do not open wide, consequently r.h. remains constantly high. Examples of both these situations are provided in Fig. 8.03.

During winter, glasshouse conditions may be markedly different. On a sunny day, radiant heat will increase both ambient and glasshouse temperatures and make use of artificial heat unnecessary. Thus, air inside the glasshouse will be humidified by transpiration from rapidly photosynthesising plants, the ventilators will not open wide while escaping air will be replaced by relatively warm air from outside. In these conditions r.h. may remain high during day-time. However, if the sky remains clear at night, ambient temperatures fall sharply and necessitate the use of artificial heat to maintain the minimum temperature. Air, escaping from the glasshouse, will be replaced by cold air from outside and when this air is heated, r.h. will fall. On an overcast day, less temperature fluctuations occur, and glasshouse r.h. remains more constant, with the level depending on the ambient temperature. Glasshouse humidities in winter are illustrated in Fig. 8.06. Another

factor influencing the glasshouse environment, particulary in winter, is condensation of water on cold glass, which lowers r.h.

Hygrographs are not the best instruments for measuring r.h., and are particulary innaccurate at high humidity. Electronic systems have recently been devised, but were unavailable for this study.

In the preliminary experiment with H. maroccana, all three fungi, M. anisopliae, B. bassiana and P. fumosoroseus killed many insects, particulary in the glasshouse chamber with increased humidity. In contrast, the last two experiments resulted in virtually no fungal infection. However, in all experiments, many insects were killed by fungi when leaf squares were transferred to optimal conditions (25°C, 100% r.h.). Thus, the lack of fungal infection was not due to apathogenic spores, inadequate coverage or adverse effect of applied mildewcides. Possibly temperatures, maintained with a minimum of 21°C, were too low for fungal infection? This possibility is unlikely, as all tested fungi grew well at 20°C on SDA, also many insects died following treatment with M. anisopliae and maintenance on leaf discs at this temperature (VI,a,x). The most likely cause of the lack of infection is inadequate glasshouse r.h. In the first experiment, the r.h. was around 90% for the most of the 4 days after treatment and fell only briefly to 75%. In the second experiment, 27 hours after treatment, the r.h. reached a minimum of 70% and remained below 80% for 15 hours, while in the third experiment, r.h. fluctuated rapidly and twice fell to 60-70% in the 2 days after treatment. The role of r.h. can be considered from two aspects. Was it sufficient to permit fungal growth and how did fungal spores survive sub-optimal levels? Microclimate humidities, encountered by conidia on insects feeding from lower leaf surfaces, are probably higher than those recorded by thermohygrographs. However, current apparatus does not permit 'spot' measurements, and the relationship (if any) between ambient and microclimate r.h. is unknown. Conidia of M. anisopliae, B. bassiana and P. fumosoroseus germinated slowly at 20°C and all probably require extended periods of high r.h. for infection (see V. Discussion). If conidia were unaffected by periods

of r.h. below that necessary for growth, they could germinate and infect insects during successive, night time moist periods and infection of H. maroccana might have been expected in experiments 1 and 3. In the second experiment, conducted in winter, r.h. was about 90% immediately after spraying and then fell and remained at about 85%, a level possibly insufficient for fungal growth. In the first experiment, spores must have survived the low period of humidity that occurred after spraying and the lower infection occurring in the non-humidified chamber, may be explained by greater spore mortality, due to the relatively longer period of unfavourable r.h. In experiments two and three, conidia probably died during the more pronounced low r.h. periods following spraying.

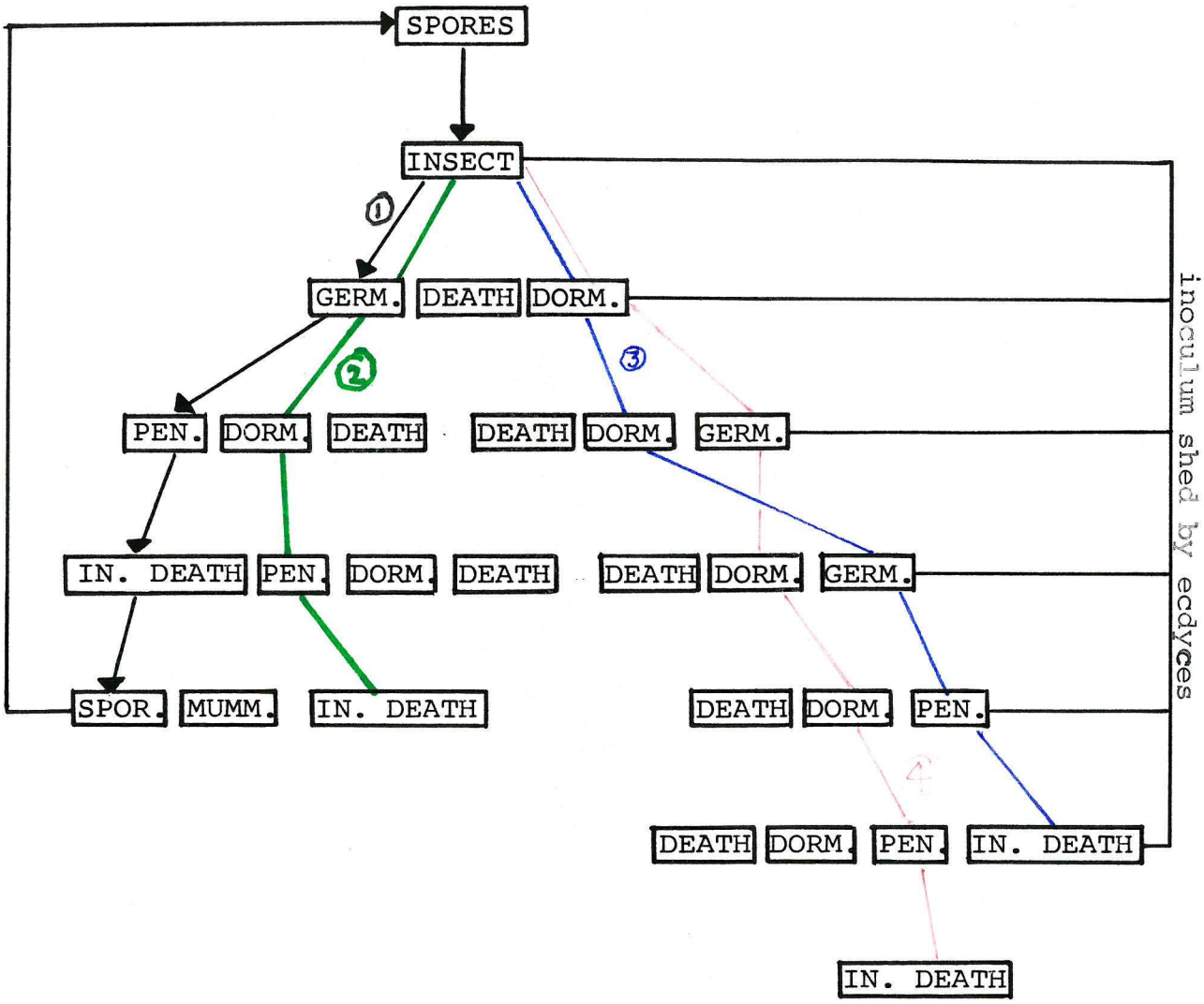
Low r.h. periods occurred after application of V. lecanii to cucumbers for thrips control but did not prevent fungal infection. It follows that V. lecanii must be less dependent than M. anisopliae on high humidity for germination, can germinate and penetrate rapidly or is unaffected by r.h.s unfavourable for growth. V. lecanii required the same r.h. for germination as M. anisopliae ($\geq$ 92.5) so the first possibility can be discounted. Hall (1977) showed that washed, ungerminated, V. lecanii (1-72) conidia survived well at 80-100% r.h. but poorly at 76% r.h.; results similar to those obtained for M. anisopliae (Clerk and Madelin, 1965). V. lecanii (1-72) conidia germinated rapidly on SDA at 20°C and about 50% of aphids treated with conidia, became infected after only 16 hours, at a nominal 100% r.h. By contrast, M. anisopliae treated leafhoppers acquired a similar level of infection only after 48 hours continuous high r.h. Similar experiments could not be conducted with thrips, owing to their small size and resultant containment problems. However, hyphal bodies of V. lecanii, used in the glasshouse experiments, also germinate quickly at 20°C, and it is likely that penetration and infection of thrips occurred rapidly. Thus, a proportion of thrips became infected with V. lecanii during the high r.h. period following spraying, and though some partly germinated V. lecanii spores doubtlessly died during the subsequent low r.h. period, a proportion probably

survived, and carried on growing when r.h. increased. After contacting an insect, spores can either remain dormant, germinate or die. This cycle is shown in Fig. 8.22, while Fig. 8.23 shows an alternative cycle under ideal conditions. Insect infection may occur without interruption (Fig. 8.22, Route 1), or more likely, proceed via other paths (eg Routes, 2-4). Obviously, the longer the process of penetration the greater the likelihood of insects avoiding infection. Thus, successful glasshouse pathogens, like V. lecanii, germinate and infect insects rapidly.

Despite the successes recorded with V. lecanii in glasshouses (Hall and Burges, 1979; Hall, 1982) there have also been failures. Kanagaratnan et al. (1982) reported a V. lecanii strain (1-72) from an aphid, killed whitefly hit by applied spores, but failed to spread, while Ekbom (1979a) reported only limited infection. Hall (1982) demonstrated dramatic whitefly control with a V. lecanii strain (19-79) from whitefly. The degree of success depends largely on using the 'correct' strain and this cannot be overemphasised.

Fungal control of glasshouse pests has concentrated on V. lecanii and reports concerning other pathogens are scarce. However, Aschersonia aleyrodis can be used in conjunction with the parasite Encarsia formosa to control whitefly (Ramakers et al. 1982) though Hall (unpub. obs.) demonstrated control was inferior to that provided by V. lecanii. Oetting and Gardner (1982) reported that Hirsutella thompsonii infected Tetranychus urticae in the laboratory (nominal 100% r.h.) but not in glasshouses. Dedryver and Rabasse (1982) demonstrated some mortality of lettuce aphids after application of Erynia neoaphidis mycelia: sporulation occurred on the glasshouse soil and resultant conidia infected a maximum 80% of aphids. By contrast, application of Conidiobolus obscurus resting spores resulted in low aphid mortality, probably because spores germinated both slowly and asynchronously.

In this study, Neozygites parvispora occurred naturally, and killed a maximum 53% of Thrips tabaci on untreated plants. However, attempts to cultivate this fungus in vitro were unsuccessful (Carl, 1975) and precludes easy use of N. parvispora as a control agent.



GERM. - germination
DORM. - dormancy
PEN. - penetration
SPOR. - sporulation
IN. DEATH - insect death
MUMM. - mummification

see p. 117

Fig. 8.22
Diagrammatic representation of a fungus/insect infection cycle under glasshouse conditions

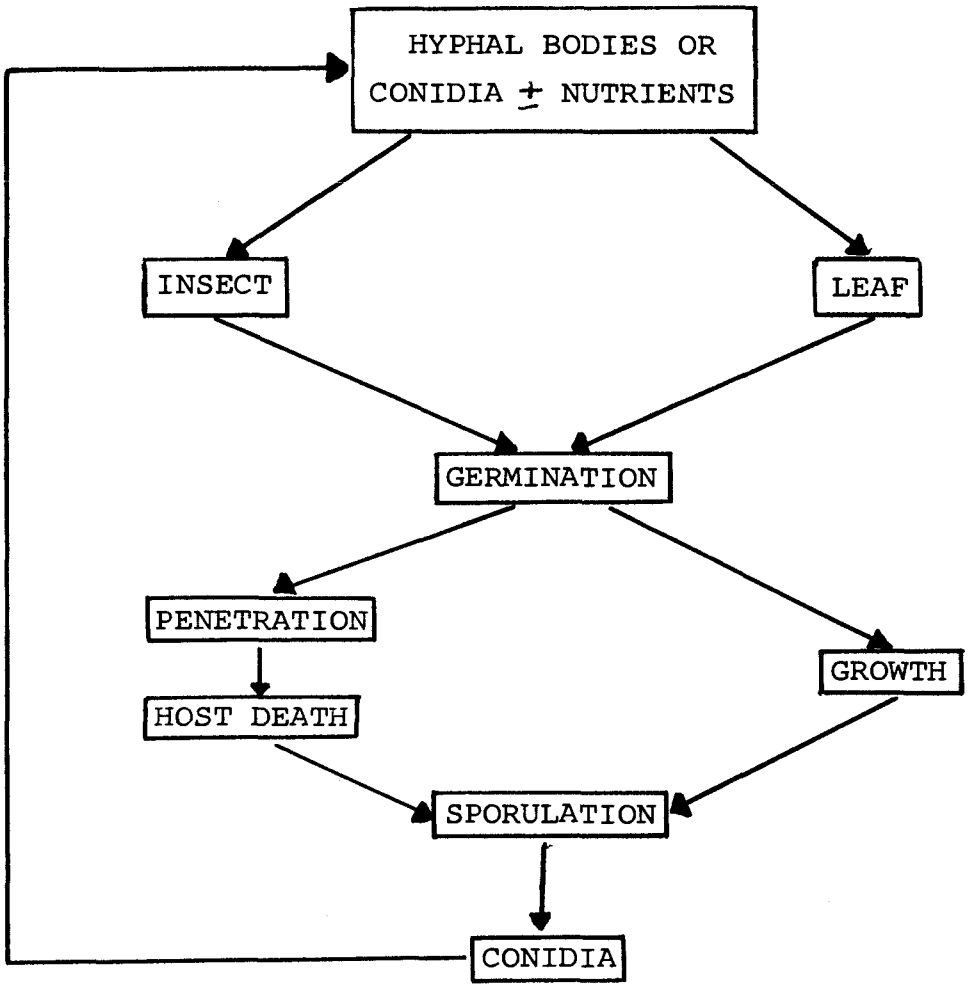


Fig. 8.23
Diagrammatic representation of a fungus/insect infection cycle under ideal conditions

In the experiment using M. anisopliae to control N. lugens, r.h. remained around only 80% after treatment, despite the frequent presence of free - standing water. This level of humidity was probably insufficient for fungal growth and resulted in low mortality. However, in rice paddies r.h. would probably be sufficient - ambient r.h. in the wet tropics is generally high - and attempts to use M. anisopliae for control of N. lugens should not be discounte Indeed, Roberts (pers. comm.) reported 80% mortality of field N. lugens populations after treatment with M. anisopliae, while Balasubramanian (1979) described 60-70% mortality with V. lecanii.

An anomaly exists between the similar performance of V. lecanii strains 53-81 and 19-79, when used as fresh spores, and the markedly different results obtained when used as formulated commercial products. Work by Hall (unpub. obs.) showed fresh spores of both strains are similarly pathogenic to whitefly and aphids, while this study demonstrates similar fermenter yields and pathogenicity to H. maroccana. Furthermore, both strains were isolated from glashouses in N. Humberside, and it seems reasonable to assume that the strains are identical. If this is correct, the most probable explanation is that the product Thriptal® did not contain spores of 53-81, but of another V. lecanii strain.

The hymenopteran parasite A. atomus was observed parasitising H. maroccana whenever large leafhopper populations occurred in glasshouses and was also reported by Mac Gill (1934). This suggests that controlled rearing and release of A. atomus in a manner similar to that used for Encarsia formosa, might provide a specific method of leafhopper control. Although this section provides no dramatic evidence that the use of fungi as mycoinsecticides may be extended to pests other than aphids and whitefly, it is considered further experiments on N. lugens and T. tabaci are worth-while, while studies on biological control of H. maroccana might be best concentrated on the parasite A. atomus.

SECTION IX

DISCUSSION

This study was initiated to extend the use of entomogenous fungi in the glasshouse environment. From this respect, the failure to control H.maroccana in glasshouse experiments, was disappointing. However, as a result of this study, a clearer idea of the essential requirements of a successful myco-insecticide has emerged. These can be listed as follows;

1) High pathogenicity to target insects.
2) Ability to spread and cause epizootics.
3) Rapid spore germination at temperatures occuring during periods of high humidity.
4) Ability to grow in culture and produce large numbers of conidia or hyphal bodies.
5) Ability to penetrate insect cuticle directly.

The latter attribute may be particularly important in the glasshouse environment where humidity fluctuates markedly.

V. lecanii (53-81) possessed all the necessary attributes and reduced thrips populations in the glasshouse. The effect might have been more dramatic, but for a naturally occuring epizootic of N. parvispora. The same fungus was also recorded reducing thrips populations on a commercial cucumber holding in N. Humberside, during 1981. However, N. parvispora cannot be cultured in vitro (Carl, 1975) which makes use as a microbial insecticide difficult. In contrast, V. lecanii (53-81) is easily cultured in semi-solid or liquid media.

Indeed, V. lecanii strains 53-81 and 19-79, provided identical control of thrips in glasshouses, and 19-79 is commercially available as Mycotal®, for whitefly control on cucumbers. Thus, it should be a simple matter to carry out further experiments to provide conclusive evidence of the potential of V. lecanii to control thrips. In addition, Mycotal® was shown to infect red spider mites in the laboratory and if these results are repeated in the glasshouse, we might have a situation where a single product would provide control of all three major cucumber pests. This is indeed

an exciting possibility, and such a situation would provide considerable savings to cucumber producers.
The glasshouse leafhopper, H. maroccana, is of increasing importance on tomatoes and suitable control methods are needed. This study attempted to find suitable fungal strains, but without success. However, tomato crops are grown at lower humidity than cucumbers and Mycotal®, though successful on cucumber, does not provide reliable whitefly control on tomatoes (Hall, pers. comm.). Therefore, it is suggested further studies are made of the parasite Anagrus atomus, to assess its potential for commercial production. During the course of this study, a natural infestation of H. maroccana was observed on a commercial tomato nursery. The pest was relatively localised and laboratory studies showed the insect required 4 weeks to complete its life cycle. Thus, it is suggested that Pynosect is applied at the first sign of infestation and, if necessary, re-introductions made of the predator P. persimilis and parasite E. formosa.

The failure of M. anisopliae to infect N. lugens on rice in the glasshouse should not be interpreted as meaning that this fungus has no potential as a microbial insecticide. In the glasshouse, it proved impossible to maintain a minimum temperature of 25°C, without use of heat, and this reduced the relative humidity. In a rice 'paddy', humidity would be continuously high and it is likely M. anisopliae would provide at least a measure of control. Indeed, Roberts (pers. comm.) reported 80% mortality of N. lugens after application of M. anisopliae conidia to a rice crop. Thus, it is considered further studies on microbial control of rice pests are worthwhile. Indeed, only by developing microbial insecticides for use on major crops, will microbial control gain widespread acceptance.

The use of fungi as mycoinsecticides, cannot be contemplated without careful consideration of a candidate organism's safety to non-target species and vertebrates. In this study, strains of M.anisopliae were highly pathogenic to thrips, leafhoppers and planthoppers and it is likely these strains are able to infect a broad range of insects. Veen(1968)

collated all the observations of this pathogen and found records from seven orders of insects. Thus,it is unlikely that applications of M.anisopliae conidia will provide specific control of a target insect,without affecting beneficial predators and parasites. However, rice is subject to such a range of insect pests (Table 2.01) that a specific control agent would be of limited use. In contrast to M. anisopliae, V.lecanii has a more limited host range and has found application in the complex, integrated pest control system, used in glasshouses in Western Europe.

Burges (1981) reviewed the data on safety of entomogenous fungi and reported only B.bassiana and Paecilomyces lilacinus (among the Deuteromycetes) and the entomophthoran, C.coronatus, as being recorded from man. Somewhat strangely, there are a number of records of entomogenous fungi from reptiles and Burges (1981) summarizes records of B.bassiana, M.anisopliae, Paecilomyces farinosus and P.lilacinus from crocodiles, lizards, terrapins and tortoises.

Of the fungi examined in this study, none grew above 32°C. This means they are unlikely to cause systemic mycoses in mammals, though the possibility remains that they may cause skin infections, as epidermal temperatures are generally lower than core temperatures. However,Burges(1981) contains reports of safety tests on strains of B.bassiana, M.anisopliae P.farinosus,N.rileyi, and H.thompsonii, and no lesions were caused in a range of laboratory animals.In addition,V.lecanii strains 1-72 and 19-79, have been cleared for use on chrysanthemums, or cucumbers and tomatoes, under the United Kingdom Pesticides Safety Precautions Scheme, after passing a range of safety tests (Tate and Lyle, unpub. obs.).Thus, the available data strongly suggests that entomogenous fungi, currently under consideration for use as mycoinsecticides, have no affect on the vast majority of vertebrates.This fact gives fungi an important advantage over the more frequently used chemical insecticides.

Another frequently quoted advantage of biological control is that resistance is unlikely to occur,and indeed no cases of resistance to the most commonly used microbial agent, Bacillus thuringiensis, have been recorded. However a note of caution must be sounded. Milner(1982), reported a lab-

oratory culture of the pea aphid, Acyrthosiphon pisum, contained two clones,one susceptible to the fungus Erynia neoaphidis,and the other completely resistant. However, studies with other isolates of the fungus,revealed two strains capable of infecting both aphid clones.Thus,at least for entomophthorans, it seems possible that insects could develop resistance to microbial insecticides and only time will reveal how serious a problem this will be.

The last few years have seen increased research into the whole area of biological control of pests, diseases and weeds.This has resulted in the commercialisation of several strains of microorganisms(Table 9.01). Most companies have concentrated on B.thuringiensis, but recently two myco-herbicides have been released.

The past year has seen the formation of at least two companies, Mycogen Inc. and Ecogen, devoted to applying the techniques of genetic engineering to the production of microorganisms for use in biological control. It is likely that initial studies will produce strains with wider host ranges,as at present mechanisms of pathogenicity are poorly understood.

The great increase in all aspects of microbial control,can only provide a better understanding of this aspect of biology and will undoubtedly result in the production of further microbial control agents. Thus,the future of invertebrate pathology is brighter now than ever before and by the end of the century, microbial pesticides will feature prominently in the product ranges of the major agrochemical companies.

Table 9.01

Commercial Products Containing Bacteria and Fungi for Microbial Control of Insects, Mites and Weeds

Company	Product	Target
Abbott Laboratories	B. thuringiensis	Lepidoptera
"	"	Mosquitoes and blackfly
"	H. thompsonii*	Citrus rust mite
"	Phytophthora citrovora	Milky vine weed
Biochem Laboratories	B. thuringiensis	Lepidoptera
"	"	Mosquitoes and blackfly
Pfizer Chemical Corporation	"	Lepidoptera
Reuter Laboratories	Bacillus popilliae	Japanese beetle
"	B.thuringiensis	Mosquitoes and blackfly
Sandoz	"	Lepidoptera
"	"	Mosquitoes and blackfly
"	"	Waxmoths
Tate and lyle	"	Lepidoptera
"	"	Mosquitoes and blackfly
"	V.lecanii	Aphids
"	"	Whitefly
Upjohn Laboratories	Colletotrichum gleosporoides	Northern joint vetch

* withdrawn 1982 to be released at a later date

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BIBLIOGRAPHY

ADÁMEK, L. 1963. Submerged cultivation of the fungus, Metarhizium anisopliae (Metsch.) Folia microbiol., (Praha), 10, 255-257.

AINSWORTH, G.C. 1958. Pathogenic yeasts. In "The chemistry and biology of yeasts". (A.H. Cook, ed.), pp.587-602. Academic Press, New York and London.

AINSWORTH, G.C. 1961. "Dictionary of the fungi", 5th ed., 547 pp. Commonwealth Mycological Institute, Kew, Surrey, England.

AIZAWA, K. 1980. Rice. In "Proc. of workshop on insect pest management with microbial agents: Recent achievements deficiencies and innovations". In. Path. Res. Center, Boyce Thompson Ins. Cornell Univ., Ithaca, N.Y. 14853, USA.

ALEXOPOULOS, C.J. 1962. "Introductory Mycology," 2nd ed., 611 pp. Wiley, New York.

AL-AIDROSS, K. and SEIFERT, A.M. 1980. Polysaccharide and protein degradation, germination and virulence against mosquitoes in the entomopathogenic fungus Metarhizium anisopliae. J. invert. Path., 36, 29-34.

AL-AIDROSS, K. and ROBERTS, D.W. 1978. Mutants of Metarhizium anisopliae with increased virulence toward mosquito larvae. Can. J. Gen. Cytol., 20, 211-219.

ANDERSON, A.L., HENRY, B.W., and MORGAN, T.L. 1948. The effect of temperature and relative humidity upon the viability of conidia of Piricularia oryzae. Phytopathology, 38, 574.

ANDERSON, J.G. and SMITH, J.E. 1971. The production of conidiophores and conidia by newly germinated conidia of Aspergillus niger (microcycle). J. gen. Microbiol., 69, 185-197.

ANDERSON, J.G., ARYEE, V. and SMITH, J.E. 1978. Microcycle Conidiation in Paecilomyces varioti. Fems Microbiol Lett., 3, 57-60.

AOKI, K. 1957. "Insect pathology". Gihodo, Tokyo

ANON, 1920. Bd. Agric. Fish., Misc. Pub., 23, 35.[In FOX WILSON, 1938].

AOKI, J. and YANASE, K. 1970. Studies on the nutrition and metabolism of pathogenic fungi of muscardine. (2) The influences of amino acid for the formation and multiplication of hyphal bodies of Spicaria fumoso-rosea (Wize) Vassil. and of Beauveria bassiana (Bals.) Vuill. J. Sericult. Sci. Japan, 39, 285-292.

AQUINO, de M.L., CAVALANTI, V.A., SENA, R.C. and QUEIROZ, G.F. 1975. Nova tecnologia de multiplicação do fungo Metarhizium anisopliae. Bol. Tecn. da CODE - CAP (Recife Pernambuco), 4, 1-31.

AQUINO, de L.M., VITAL, A.F., CAVALCANTI, V.A.L.B. and NASCIMENTO, M.G. 1977. Culture de Metarhizium anisopliae em sacos de polipropileno (Nota Prévia). Bol. Tecn. da CODE - CAP (Recife Pernambuco), 5, 7-11.

BAINIER, G. 1907. Mycothèque de l'école de Pharmacie. XI. Paecilomyces, genre nouveau de Mucédinées. Bull. trimest. Soc. mycol. Fr. 23, 26-27.

BAKER, R.T. 1981. Natural mortality of Currant Clearwing Synanthedon salmachus (Lepidoptera: Sesiidae), in New Zealand. N.Z.J. Zool., 8, 529-530.

BALASUBRAMANIAN, M. 1979. Pest management studies for rice brown planthopper in Tamil Nadu. In [Rev. appl. Ent. 1980, 68].

BALAZY, S. 1973. A review of entomopathogenic species of the genus Cephalosporium Corda (Mycota, Hyphomycetales). Bull. Soc. Amis. Sci. Lett., Poznan, D, 14, 101-137.

BALSAMO, 1836. Linnea 10, 611.

BARNES, G.L., BOETHEL, D.J., EIKENBARY, R.D., CRISWELL, J.T. and GENTRV, C.R. 1975. Growth and sporulation of Metarhizium anisopliae and Beauveria bassiana on media containing various peptone sources. J. invert. Path., 25, 301-305.

BARTNICKI-GARCIA, S. and NICKERSON, W.J. 1962. Induction of yeast like development in Mucor by carbon dioxide. J. bact., 84, 829-840.

BASSETT, P. 1981. Observations on biological and chemical control. Working Group on Integrated Control in glasshouses, International Organisation for Biological Control (OILB/SROP), Bull. SROP, 1981, p 4.

BATKO, A. 1964a. Notes on entomophthoraceous fungi in Poland, (Coll. Int. Pathol. Insectes, Paris. 1962). Entomophaga, Mém. Hors. Sér. 2, 129-131.

BATKO, A. 1964b. Remarks on the genus Entomophthora Fresenius 1856 non Nowakowski 1883 (Phycomycetes: Entomophthoraceae). Bull. Acad. Polon. Sci., Sér. Sci. Biol. 12, 319-321.

BATKO, A. 1964c. On the new genera: Zoophthora gen. nov., Triplosporium (Thaxter) gen. nov. and Entomophaga gen. nov. (Phycomycetes: Entomophthoraceae). Bull. Acad. Polon. Sci., Sér. Sci. Biol. 12, 323-326.

BATKO, A. 1964d. Remarks on the genus Lamia Nowakowski 1883 vs. Culicicola Nieuwland 1916 (Phycomycetes: Entomophthoraceae). Bull. Acad. Polon. Sci., Sér. Sci Biol. 12, 399-402.

BATKO, A. 1964e. Some new combinations in the fungus family Entomophthoraceae (Phycomycetes). Bull. Acad. Polon. Sci., Sér. Sci. Biol. 12, 403-406.

BATKO, A. 1966a. A new aphidicolous fungus from Poland, Zoophthora phalloides sp. nov. (Entomophthoraceae). Acta Mycol. 2, 17-13.

BATKO, A. 1966b. On the subgenera of the fungus genus Zoophthora Batko 1964 (Entomophthoraceae). Acta. Mycol. 2, 15-21.

BATKO, A. 1974. Filogeneza a structury taksonomiczne Entomophthoraceae. In "Ewolucja Biologiczna, Szkice Teoretyczne I Metodologiczne". (C. Nowinskiego ed.), pp. 251-272. Polska Akad. Nauk., Inst. Filozof. Sociol. Wroclaw.

BATKO, A. and WEISER, J. 1965. On the taxonomic position of the fungus discovered by Strong, Wells, and Apple: Strongwellsea castrans gen. et sp. nov. (Phycomycete: Entomophthoraceae). J. invert. Path. 1, 455-463.

BELL, J.V. 1975. Production and pathogenicity of the fungus Spicaria rileyi from solid and liquid media. J. invert. Path., 26, 129-130.

BELL, J.V. and HAMALLE, R.J.1970. Three fungi tested for control of the cowpea curculio, Chalcodermus aeneus . J. invert. Path., 15, 447-450.

BELL, J.V. and HAMALLE, R.J. 1974. Stability and pathogenicity of entomogenous fungi after prolonged storage on silica gel at -20°C. Can. J. Microbiol. 20, 639-642.

BELOVA, R.N. 1978. Development of the technology of Boverin production by the submersion method. In Proceedings of the First Joint US/USSR conference on the Production, Selection and Standardisation of Entomopathogenic Fungi of the US/USSR Joint Working Group on the Production of Substances by Microbiological Means. Ed. em. Ignoffo, Asra Information Resources, Natural Science Foundation, USA.

BENJAMIN, R.K. 1979. Zygomycetes and their spores. In "The Whole Fungus". (B. Kendrick, ed.), Vol. 2, pp.47-71. National Museum of Natural Sciences, Ottawa, Canada.

BEN-ZE EV, I. and KENNETH, R.G. 1981. Zoophthora radicans and Zoophthora petchi sp. nov. (Zygomycetes:Entomophorales), two species of the "sphaerosperma group" attacking leaf-hoppers and frog-hoppers (Hom.). Entomophaga, 26,131-142.

BEN -ZE EV, I. and KENNETH, R.G. 1982. Features - Criteria of Taxonomic Value in the Entomophthorales: 1. A Revision of the Batkoan Classification. Mycotaxon 14, 393-455.

BERGER, L.R. 1970. Proposed basis for the storage of viable bacterial clones in distilled water. In "Culture Collections of Microrganisms" (H. Iizuka and T. Hasegawa eds.), pp. 265-267. Univ. Park Press, Baltimore, Maryland.

BIEL, A.K., BRAND, J.M., MARKOVETZ, A.J. and BRIDGES, J.R. 1977. Dimorphism in Ceratocystis minor var. barrasii. Mycopathologia, 62, 179-182.

BINNS, E.S., HALL, R.A. and PICKFORD, R.J.J. 1982. Thrips tabaci Lind. (Thysanoptera - Thripidae) - Distribution and Behaviour on Glasshouse Cucumbers in Relation to Chemical and Integrated Control. Entomologists Monthly Magazine, 118 : 55-68.

BLACHÈRE, H., CALVEZ, J., FERRON, P., CORRIEU, G. and PERINGER, P. 1973. Etude de la formulation et de la conservation dune preparation entomopathogène à base de blastospores de Beauveria tenella (Delacr. Siemaszko). Ann. Zool. Écol. anum. 5, 69-79.

BOUCIAS, D.G. and PENDLAND, J.C. 1982. Ultrastructural studies on the fungus, Nomuraea rileyi, infecting the velvet bean caterpillar, Anticarsia gemmatalis. J. invert. Path., 39, 338-345.

BOURNE, A.I. 1935. Control of onion thrips. Mass. Expt. Stn. Bull. (Annual Rept., 1934), 315 : 42-44.

BOURNE, A.I. and SHAW, F.R. 1934. Notes on a fungus attacking onion thrips. J. Econ. Entomol., 27 : 860-861.

BOURNE, A.I. and WHITCOMB, W.D. 1935. Department of Entomology. Bull. Mass. Agric. Exp. Stn., 315 : 38-52.

BRADY, B.K.L. 1979. Metarhizium anisopliae. Descriptions of Pathogenic Fungi and Bacteria C. Mycol. Inst. No. 609.

BRADY, B.L. 1981. Fungi as parasites of insects and mites. Biocon. News and Inform., 2 ,281-296

BROBYN, P.J. and WILDING, N. 1977. Invasive and developmental processes of Entomophthora species infecting aphids. Trans. Br. mycol. Soc., 69, 349-366.

BRODERICK, A.J. and GREENSHIELDS, R.N. 1981. Sporulation of Aspergillus niger and Aspergillus ochraceus in Continuous submerged liquid culture. J. gen. Microbiol., 126, 193-202.

BROWN, A.H.S., and SMITH, G. 1957. The genus Paecilomyces Bainier and its perfect stage Byssochlamys Westling. Trans. Br. mycol. Soc., 40, 17-89.

BROWN, W. and WOOD, R.K.S. 1953. Ecological adaptations in fungi. Symp. Soc. Gen. Microbiol., 3, 326-339.

BURGES, H.D. 1981. "Microbial Control of Pests and Plant Diseases 1970-1980". 949 pp. Academic Press, New York and London.

BURGES, H.D. and THOMSON, E.M. 1971. Standardisation and assay of microbial insecticides. In " Microbial Control of Insects and Mites " (H.D. Burges and N.W. Hussey, Eds.), pp 591-622. Academic Press,New York and London

BUTTERFIELD, W., JONG, S.C., and ALEXANDER, M.T. 1974. Preservation of living fungi pathogenic for man and animals. Can. J. Microbiol., 20, 1665-1673.

CALLAN, E.M.C. 1943. Natural enemies of the cacao thrips. Bull. ent. Res., 34, 313-321.

CAMPBELL, R.K., PERRING, T.M., BARNES, G.L., EIKENBARY, R.D. and GENTRY, C.R. 1978. Growth and sporulation of Beauveria bassiana and Metarhizium anisopliae on media containing various amino acids. J. invert. Path., 31, 289-295.

CARILLI, A. and PACIONI, G. 1977. Growth and Sporulation of Cordyceps militaris (Linn. ex Fr.) Link in submerged culture Trans. Br. mycol. Soc. 68 : 237-243.

CARL, K.P. 1975. An Entomophthora sp. (Entomophthorales : Entomophthoraceae) pathogenic to Thrips spp. (Thysan.: Thripidae) and its potential as a biological control agent in glasshouses. Ento mophaga, 20, 381-388.

CARSON, F.T. 1931. Control of relative humidity in a small enclosed space. Paper Trade J., 93, 71-74.

CENTRE FOR OVERSEAS PEST RESEARCH, 1976. Pest Control in Rice. PANS Manual No. 3.

CHARLES, V.K. 1941. A preliminary check list of the entomogenous fungi of N.America. Tech. Bull. U.S. Dep. Agric., 21 ,707-785.

CHAUDHURI, H. 1923. Ann. Bot. 37, 519-539. (Cited in Cochrane, 1958).

CLARK, R.A., CASAGRANDE, R.A. and WALLACE, D.B. 1982. Influence of Pesticides on Beauveria bassiana, a pathogen of the Colorado potato beetle. Environ. Entomol., 11, 67-70.

CLERK, G.C. and MADELIN, M.F. 1965. The longevity of conidia of three insect-parasitizing hyphomycetes. Trans. Br. mycol. Soc. , 48, 193-209.

COCHRANE, V.W. 1958. Physiology of Fungi" John Wiley and Sons, Inc., New York. Chapman and Hall, Ltd., London.

COLE, G.T. and NOZAWA, Y. 1981. Dimorphism. In "Biology of conidial fungi, Vol. I". pp. 97-126. Academic Press, Inc., New York and London.

COOKE, B.M. and JONES, D.G. 1970. The effect of near-ultraviolet irradiation and agar medium on the sporulation of Septoria nodorum and S. tritici. Trans. Br. mycol. Soc., 54, 221-226.

COSTANTIN , J. 1897 . Sur une Entomophthorée nouvelle, Bull. Soc. Mycol. France , 13 , 38-43.

DAOUST, R.A. and ROBERTS, D.W. 1982. Virulence of natural and insect-passaged strains of Metarhizium anisopliae to mosquito larvae. J. invert. Path., 40, 107-117.

DAOUST, R.A. and ROBERTS, D.W. 1983. Studies on the prolonged storage of Metarhizium anisopliae conidia: Effect of growth substrate on survival and virulence against mosquitoes. J. invert. Path., 41, 161-170.

DAOUST, R.A., WARD, M.G. and ROBERTS, D.W. 1982. Effect of formulation on the virulence of Metarhizium anisopliae conidia against mosquito larvae. J. invert. Path., 40, 228-236.

DEDRYVER ,C.A. 1980. Premiers resultats concernant le rôle de trois espèces d'Entomophthora dans la limitation des populations de pucerons des céréales dans l'ouest de la France. Bull. Organ Int. Lutte Biol., Sect. Reg. Ouest Palearct., 4, 5-9.

DEDRYVER, C.A. and RABASSE, J.M. 1982. Attempt of biological control of the lettuce aphids in glasshouses with resting spores of Conidiobolus obscurus Hall and Dunn and mycelium of Erynia neoaphidis Remaud. and Henn. In "Invertebrate Pathology and Microbial Control" Ab. III rd int. Collogu. invert. Path. Microbial Cont., Brighton, 1982, p. 103.

DETROY, R.W. and CIEGLER, A. 1971. Induction of yeastlike development in Aspergillus parasiticus. J. gen. Microbiol. 65, 259-264.

DEVANESAN, S., JACOB, A., KURUVILLA, S. and MATHAI, S. 1979. Infection of Nephotettix virescens (Stil).(Cicadellidae : Hemiptera) by Fusarium equiseti (Corda) Sacc. Entomol., 4, 304-305.

DOBERSKI, J.W. 1981a. Comparative laboratory studies on three fungal pathogens of the elm bark beetle Scolytus scolytus: pathogenicity of Beauveria bassiana, Metarhizium anisopliae, and Paecilomyces farinosus to larvae and adults of S. scolytus. J. invert. Path., 37, 188-194.

DOBERSKI, J.W. 1981b. Comparative laboratory studies on three fungal pathogens of the elm bark beetle Scolytus scolytus : effect of temperature and humidity on infection by Beauveria bassiana, Metarhizium anisopliae, and Paecilomyces farinosus. J. invert. Path., 37, 195-200.

DWORAKOWSKA, I 1970. Three new genera of Erythroneurini (Auchenorrhyncha, Cicadellidae, Typhlocybinae). Bull. Acad. Pol. Sci., 18, 617-624.

DYADECHKO, N.P. 1964. "Thrips or fringe-winged insects (Thysanoptera) of the European part of the USSR". Urozhai Publishers, Kiev, USSR. Translated from Russian and Published by Amerind Pub. Co. Pot. Ltd., New Delhi, 1977. for the Agric. Res. Serv., USDA and the National Science Foundation, Washington D.C.

DYCK, V.A. and THOMAS, B. 1978. The brown planthopper problem. In "Brown planthopper : threat to rice production in Asia". pp. 3-17. International Rice Research Institute, Los Baños, Laguna, Philippines.

EDWARDS, J. 1924. On some new or little known British Cicadina. Entomologists Mon. Mag., 60, 52-58.

EKBOM, B.S. 1979a. Investigations on the potenial of a parasitic fungus (Verticillium lecanii) for biological control of the greenhouse whitefly (Trialeurodes vaporariorum). Swedish J. agric. Res., 9, 129-138.

EKBOM, B.S. 1979b. Biologisk bekämpning av växthus vita flygare (Trialeurodes vaporariorum). Kort historik och nagra framtidsvisioner. Entomol. Tidskr., 100, 1-10.

ESAKI, T. and HASHIMOTO,S. 1936. Report on the leafhoppers injurious to the rice plant and their natural enemies. Entomol Lab., Dep. Agric., Kyusha Imp. Univ. Publ., 4, 32 pp.

FARGUES, J. 1976. Specificité des champignons pathogènes imparfait (Hyphomycètes) pour les larves de Coléoptères (Scarabaeidae et Chrysomelidae). Entomophaga, 21, 313-323.

FARGUES, J. and VEY, A. 1974. Modalités d'infection des larves de Leptinotarsa decemlineata par Beauveria bassiana au cours de la mue. Entomophaga, 19, 311-323.

FARGUES, J., ROBERT, P.H. and VEY, A. 1979. Formulation des productions de musse de l'hyphomycète entomopathogène Beauveria en vue des applications phytosanitaires. Annls. Zool. - Écol. anim., 11, 247-257.

FEDEROV, S. M. 1930. Thrips tabaci as a pest of tobacco plant in the Crimea. Eos Madr., 6, 229-284.

FERRON, P. 1978. Biological control of insect pests by entomogenous fungi. Ann. Rev. Entomol., 23, 41-48.

FERRON, P. 1981. Pest control by the fungi Beauveria and Metarhizium. In "Microbial Control of Pests and Plant Diseases 1970-1980" (H.D. Burges, ed.), pp. 465-482. Academic Press, London and New York.

FOOD AND AGRICULTURE ORGANISATION, 1974. FAO Production Yearbook 1974, 28, 1, 2 FAO, Rome (1975)

FOX WILSON ,G. 1938. The glasshouse leaf-hopper Erythroneura pallidifrons Edw. J. R. hort. Soc., 63, 481-484.

FRIEDERICHS, K. 1920. Uber die pleophagie des insektenpilzes Metarrhizium anisopliae (Metch.) Sor., Zentbl. Bakt. Parasitkde, 50, 335-355.

FRIEDERICHS, K. 1930. Die Grundfragen und Gesetzmassigkeiten der Land - und Fortwirtschaftlichen Zoologie 463 pp. Berlin.

GABRIEL, B.P. 1968. Entomogenous microorganisms in the Philippines : new and past records. Philipp. Entomol., 1, 97-130.

GABRIEL, B.P. 1970. Additional records on the microbiota of Philippine insects. Philipp. Entomol., 1, 465-472.

GALANOPOULOS, N. and TRIBE, H.T. 1974. Conidial survival in Verticillium dahliae. Trans. Br. mycol. Soc., 63, 85-91.

GAMS, W. 1971. "Cephalosporium - artige Schimmelpilze (Hyphomycetes)". Gustav Fischer Vertag, Stuttgart.

GETZIN , L. W. 1961. Spicaria rileyi (Farlow) Charles, an entomogenous fungus of Trichoplusia ni Hubner). J. Insect Path., 3, 2-10.

GHABN, A.A. 1948. Contribution to the knowledge of the bilogy of Thrips tabaci Lind. in Egypt (Thysanoptera). Bull. Soc. Fouad Ier Ent., 32, 123-174.

GORAL, V.M. 1973b. The conditions of conidia formation of Beauveria bassiana (Bals.) Vuill. Proc. 5th int. Colloqu. Insect Path. Microbiol. Cont., Oxford, 1973, p 76 (abstract).

GORAL, V.M. 1973a. The influence of conditions of deep culture on the formation of Beauveria bassiana and tenella conidia, two entomo-pathogenic fungi. Zakhyst Roslyn. Kiev, 18, 66-72.

GORAL, V.M. 1975. Morphological characteristics of the life history of Beauveria bassiana (Bals.) Vuill., in submerged cultures. Mikologiya I Fitopatologya, 9, 98-103.

GOULD, H.J. 1971. Large-scale trials of an integrated coctrol programme for cucumber pests on commercial nurseries. Pl. Path., 20, 149-156.

GRIST, D.H. and LEVER, R.J.A.W. 1969. Pests of Rice.Longmans, Green and Co., Ltd.,London.

GRIVANOV, K.P. 1939. Deep-ploughing instead of burning the stubble for the control of wheat thrips (Haplothrips tritici Kurd.), Social Grain Fmg. 6, 182-188. [Rev. appl. Ent. (A) 28, 69-70].

GRULA, E.A., BURTON, R.L.,SMITH, R., MAPES, T.L.,CHEUNG, P.Y.K., PEKRUL, S., CHAMPLIN, F.R., GRULA, M. and ABEGAZ, B. 1978. Biochemical basis for entomopathogenicity of Beauveria bassiana. Proc. 1st Jt. US/USSR Conf. Prod. Select. Stand. Entomopathogenic Fungi, Proj V, Microbiol. Con. In. Pests, US/USSR Jt. Work. Gr. Prod. Sub. Microbiol. Means. (C.M. Ignoffo, Ed) Asra Information Resources, National Science Foundation, USA.

GUSTAFSSON, M. 1965. On species of the genus Entomophthora Fres. in Sweden. 1. Classification and Distribution. LantbrHogsk. Annlr., 31, 103-212.

HALL, I.M., and DUNN, P.H. 1957. Entomogenous fungi on the spotted alfalfa aphid. Hilgardia, 27, 159-181.

HALL, M.P. 1933. An analysis of the factors controlling the growth form of certain fungi, with especial reference to Sclerotinia (Monilia) fructigena. Ann. Bot. (London), 47, 543-578.

HALL, R.A. 1976. A bioassay of the pathogenicity of Verticillium lecanii conidiospores on the aphid Macrosiphoniella sanborni. J. invert. Path., 27, 41-48.

HALL, R.A. 1977. The potential of the fungus, Verticillium lecanii, as a control agent of glasshouse aphid pests. Ph.D. Thesis, Univ. Southampton, England.

HALL, R.A. 1980. Effect of repeated subculturing on agar and passaging through an insect host on pathogenicity, morphology, and growth rate of Verticillium lecanii. J. invert. Path., 36, 216-222.

HALL, R.A. 1981. Laboratory studies on the effects of fungicides, acaricides and insecticide on the entomopathogenic fungus, Verticillium lecanii. Ent. exp. appl., 29, 39-48.

HALL, R.A. 1982. Control of whitefly, Trialeurodes vaporariorum and cotton aphid, Aphis gossypii in glasshouses by two isolates of the fungus Verticillium lecanii. Ann. appl. Biol., 101, 1-11.

HALL, R.A. and BURGES, H.D. 1979. Control of glasshouse aphids with the fungus, Verticillium lecanii. Ann. appl. Biol. 93, 235-246.

HALL, R.A. and LATGÉ, J.P. 1980. Étude de quelques facteurs stimulant la formation in vitro des blastospores de Verticillium lecanii (Zimm.) Viégas. C.R. Acad. Sc. Paris, t. 291, Série D, 75-78.

HALL, R.A. and PAPIEROK, B. 1982. Fungi as biological control agents of arthropods of agricultural and medical importance. Parasitology, 84, 205-240.

HARRIS, H.M., DRAKE, C.J. and TATE, H.D. 1963. Observations on the onion thrips. Iowa St. J. Sci., 10, 155-172.

HART, M.P. and MAC LEOD, D.M. 1955. An apparatus for determining the effects of temperature and humidity on germination of fungous spores. Can. J. Bot., 33, 289-292.

HAWKER, L.E. 1957. The physiology of reproduction in fungi. Cambridge University Press, Cambridge.

HEDGECOCK, G.G. 1906. Zonation in artificial cultures of Cephalosporium and other fungi. Rep. Mo. Bot. Gard., 17, 115-117.

HINCKLEY, A.D. 1963. Ecology and control of rice planthoppers in Fiji. Bull. Entomol. Res., 54, 467-481.

HOOG, G.S., DE. 1972. The genera Beauveria, Isaria, Tritirachium and Acrodontium, gen. nov. Stud. Mycol., 1, 1-41.

HOOG, G.S., DE, and RAO, V. 1975. Some new Hyphomycetes. Persoonia, 8, 207-212.

HUMBER, R.A. 1981a. An alternative view of certain taxonomic criteria used in the Entomophthorales (Zygomycetes). Mycotaxon . 13, 191-240.

HUMBER, R.A. 1981b. Erynia (Zygomycetes : Entomophthorales): Validations and new species. Mycotaxon, 13, 471-480.

HUMBER, R.A., SOPER, R.S., MAC LEOD, D.M., TYRELL, D., KENNETH, R.G. and BEN-ZE'EV, I. 1981. Proposal to conserve the entomopathogenic fungal genus Triplosporium (Thaxter) Batko against Neozygites Witlaczil (Entomophthorales). Taxon, 30, 353-357.

HUSSEY, N.W., READ, W.H. and HESLING, J.J. 1969. "The Pests of Protected Cultivation". Edward Arnold(Publishers) Ltd., London.

IGNOFFO, C.M. 1981. The fungus,Nomuraea rileyi as a microbial insecticide. In" Microbial Control of Pests and Plant Diseases, 1970-1980.(ed. H.D. Burges), pp. 513-538. Academic Press,New York and London.

JOHNSTON, J.R. 1915. The Entomogenous Fungi of Porto Rico. Government of Porto Rice Board of Commisioners of Agriculture, Rio Pedras. Bul. 10.

KANAGARATNAM, P., HALL, R.A. and BURGES, H.D. 1982. Control of glasshouse whitefly, Trialeurodes vaporariorum, by an'aphid' strain of the fungus Verticillium lecanii. Ann. appl. Biol., 100, 213-219.

KAWAKAMI, K., and MIKUNI, Y. 1965. Effects of relative humidity and temperature on the viability of conidia of some muscardines. Sansi-Kenk u (Acta Sericologica), 56, 42-46.

KELLER, G. 1982. Effect of high temperatures and artificial sunlight on the viability of conidia of Metarhizium anisopliae. J. invert. Path., 40, 36-40.

KELLER, S. and WEUST, J. 1983. Observations sur trois espèces de Neozygites (Zygomycetes : Entomophthoraceae), Entomophaga, 28, 123-134.

KENNETH, R., and OLMERT, I. 1975. Entomopathogenic fungi and their insect hosts in Israel : additions. Israel J. Ent., 10, 105-112.

KING, D.S. 1976. Systematics of Conidiobolus (Entomophthorales) using numerical taxonomy.1. Biology and cluster analysis. Can. J. Bot., 54, 45-65..

KISH, L.P. and ALLEN, G.E. 1978. The biology and ecology of Nomuraea rileyi and a programme for predicting its incidence on Anticarsia gemmatalis in soybean. Fla. Agri. Exp. Stn., Tech. Bull. 795.

KRAMER, J.P. 1980. The house-fly mycosis caused by Entomopthora muscae : influence of relative humidity on infectivity and germination. J. New York ent. Soc., 88, 236-240.

KRASSILSTSCHIK, J.M. 1886. Insektenkrankheiten durch Pilze verursacht. Mem. der neurussischen Naturf. Ges., Odessa, 11, 74-171.

KULKARNI, R.K. and NICKERSON, K.W. 1981. Nutritional Control of dimorphism in Ceratocystis ulmi Exp. Mycol., 5, 148-154.

KURDJUMOV, N.V. 1913. The more important insects injurious to grain crops in Middle and South Russia. Stud. Poltava Agric. Exp. Stn. 17. Dept. Agric. Ent., 6, 119pp. [Rev. appl. Ent. (A), 2, 170-173].

KURUVILLA, S. and JACOB, A. 1979. Comparative susceptibility of nymphs and adults of Nilaparvata lugens Stål to Fusarium oxysporum Schlect and its use in microbial control. Agric. Res. J. Kerala, 17, 287-288.

KURUVILLA, S. and JACOB, A. 1980. Pathogenicity of the entomogenous fungus Paecilomyces farinosus(Dickson and Fries)to severral insect pests. Entomon., 5, 175-176.

LANE, J.W. and GARRISON, R.G. 1970. Electron microscopy of the yeast to mycelial phase conversion of Sporotrichum schenckii. Can. J. Microbiol., 16, 747-749.

LAKON, G. 1915. Zur Systematik der Entomophthoreen Gattung Tarichium. Z. Pflanzenkrankh. 25, 257-272.

LAKON, G. 1919. Die Insektenfeinde aus der Familie Entomophthoraceen. Z. Angew. Entomol. 5, 161-216.

LAMBIASE, J.T. and YENDOL, W.G. 1977. The fine structure of Entomophthora apiculata and its penetration of Trichoplusia ni. Can. J. Microbiol., 23, 452-464.

LATCH, G.C.M. 1965. Metar(r)hizium anisopliae (Metsch.) Sorok. strains in New Zealand and their possible use for controlling pasture inhabiting insects. N. Z. J. agri. Res. 8, 384-396.

LATGÉ, J.P. 1982. Production of Entomophthorales. Prog. and Abstr. 111rd int. Colloqu. invert. Path. and Microbial. Cont., Brighton, 1982, pp 164-169.

LATTEUR, G. 1980. The persistence of infectivity of conidia of Entomophthora obscura at different temperatures on the surface of an unsterilised soil. Acta Oecologica; Oecologia Applicata, 1, 29-34.

LAURITZEN, J.I. 1929. Rhizoctonia rot of turnips in storage. J. Agric. Res. (Washington D.C.), 38, 93-108.

LAURITZEN, J.I. and HARTER, L.L. 1925. The influence of temperature on the infection and decay of sweet potatoes by different species of Rhizopus. J. Agric Res. (Washington D.C.), 30, 793-810.

LEACH, C.M. 1962. The quantitative and qualitative relationship of ultraviolet and visible radiation to the induction of reproduction in Ascochyta pisi. Can. J. Bot., 40, 1577-1602.

LEE, N. and JONES, D.G. 1974. Rapid method for spore production in three Septoria species. Trans. Br. mycol. Soc., 62, 208-211.

LE QUESNE, W.J. and PAYNE, K.R. 1981. Cicadellidae (Typhlocybinae) with a check list of the Brtiish Auchenorhyncha (Hemiptera, Homoptera) In "Handbooks for the Identification of British Insects (ed. N.G. Fitton) II, Royal Entomological Society, London.

LEWIS, T. 1973. Thrips, Their Biology, Ecology and Economic Importance". Academic Press, New York and London.

LINGG, A.J. and DONALDSON, M.D. 1981. Biotic and abiotic factors affecting stability of Beauveria bassiana in soil. J. invert. Path., 38, 191-200.

LUKENS, R.J. 1963. Photo-inhibition of sporulation in Alternaria solani. Am. J. Bot., 50, 720-724.

MAC GILL, E.I. 1932. The biology of Erythroneura (Zygina) pallidifrons, Edwards. Bull. Entomol. Res., 23, 33-43.

MAC GILL,E.I. 1934. On the biology of Anagrus atomus (L.) Hal.:An egg parasite of the leaf-hopper Erythroneura pallidrofrons Edwards, Parasitology, 26, 57-63.

MAC LEOD, D.M. 1954. Investigations on the genera Beauveria Vuill. and Tritirachium Limber. Can. J. Bot. 32, 818-890.

MAC LEOD, D.M. 1959. Nutritional studies on the genus Hirsutella., Can. J. Bot., 37, 695-724 ; 819-832.

MAC LEOD, D.M., TYRRELL, D. and CARL, K.P. 1976. Entomophthora parvispora sp. nov., a pathogen of Thrips tabaci. Entomophaga, 21, 307-312.

MAC QUILLAN, M.J. 1974. Influence of crop husbandry on rice planthoppers (Hemiptera ; Delphacidae) in the Soloman Islands. Agro-Ecosystem 1, 339-358

MADELIN, M. F. 1963. Diseases caused by hyphomycetous fungi. In " Insect Pathology: An Advanced Treatise" (E.A. Steinhaus,ed.), Vol. 2,pp 233-271. Academic Press, New York and London.

MADELIN, M.F. 1968. Fungal parasites of invertebrates. 1. Entomogenous fungi. In " The Fungi :An Advanced Treatise" (G.C. Ainsworth and A.S. Sussman, eds.), Vol. 3, pp. 227-238. Academic Press, New York and London.

MAHVI, T.A. 1965. A comparative study of the yeast and mycelial phases of Histoplasma capsultum. I. Pathways of Carbohydrate dissimilation. J. infect. diseases, 115, 226-232.

MANCHARAN, N. and JAYARAJ, S. 1979. Recent trends in rice brown planthopper control. In "Proc. of a Collaquium on rice brown planthopper". Pub. Coimbatore, India; Sandoz (Indian) Limited.

MANNERS, J.G. and HOSSAIN, S.M.M. 1963. Effects of temperature and humidity on conidial germination in Erysiphe graminis. Trans. Br. mycol. Soc., 46, 225-234.

MATANMI, B.A. and LIBBY, J.L. 1975. Scanning eletron microscopy of Entomophthora virulenta and Conidiobolus coronatus (Entomophthorales : Entomophthoraceae) J. invert. Path., 22, 143-147.

MATHAI, S.; KURUVILLA, S. and JACOB, A. 1979. Syncephalastrum racemosum Cohnex Schroeter, an entomogenous fungus of rice leafhopper. Cicadella spectra. Entomon, 4, 215-216.

MC COY, C.W. 1978. Entomopathogens in arthropod pest control programs in citrus. In "Microbial Control of Insect Pests: Future Strategies in Pest Management Systems" (ed. G.E. Allen, C.M. Ignoffo and R.P. Jacques), pp. 211-224.

MC COY, C.W. and SELHIME, A.G. 1974. The fungus pathogen, Hirsutella thompsonii, and its potential for control of the citrus rust mite in Florida. Proc. Int. Citrus C ng., Murcia, Spain (1973), Vol. 2, pp. 521-527.

MC COY, C.W., SELHIME, A.G., KANAVEL, R.F. and HILL, A.J. 1971. Suppression of citrus mite populations with application of fragmented mycelia of Hirsutella thompsonii. J. invert. Path., 17, 270-276.

MC GINNIS, M.R., PADHYE, A.A. and AJELLO, L. 1974. Storage of stock cultures of filamentous fungi, yeasts, and some aerobic actinomycetes in sterile distilled water. Appl. Microbiol., 28, 218-222.

METSCHNIKOFF, E. 1879. Maladies des hannetons duble. Zapiski imperatorskogo obshcestua sel'skago Khozyaistra yuzhnoi rossii, 17-50.

MILNER, R.J. 1982. Occurence of pea aphids, Acyrthosiphon pisum, resitant to isolates of the fungal pathogen Erynia neoaphidis. Ent. exp. appl., 32, 23-27.

MILNER, R.J. and LUTTON, G.G. 1976. Metarhizium anisopliae: Survival of conidia in the soil. Proc. 1st Int. Colloqu. invert. Path and IX th Ann. Meeting Soc. invert. Path, Kingston, pp 428-430.

MILNER, R.J., TEAKLE, R.E., LUTTON, G.G., and DARE, F.M. 1980. Pathogens of the blue-green aphid Acyrthosiphon kondoi Shinji and other aphids in Australia. Aust. J. Bot., 28, 601-619.

MILNER, R.J., SOPER, R.S. and LUTTON, G.G. 1982. Field release of an Israeli strain of the fungus Zoophthora radicans (Brefeld) Batko for biological control of Therioaphis trifolii (Monell) f. maculata. J. Aust. ent. Soc., 21, 113-118.

MOCHIDA, O. 1964a.Ovi position in the brown planthopper, Nilaparvata lugens (Stal) (Hom., Auchenorrhyncha).I Ovi position and environmental factors with special reference to temperature and rice plant. Bull. Kyushu Agric. Exp. Stn., 10, 257-285.

MOCHIDA, O. 1964b. Ovisposition in the brown planthopper, Nilaparvata lugens (Stal) (Hum., Auchenorrhyncha). II The number of eggs in an egg group, especially in relation to the fecundity Jpn. J. Appl. Entomol.Zool. 8, 141-148.

MOCHIDA, O. and OKADA, T. 1979. Taxonomy and Biology of Nilaparvata lugens (Hom., Delphacidae). In "Brown Planthopper: Threat to Rice Production in Asia". pp. 21-43. Int. Rice Res. Inst., Philippines.

MOCHIDA, O., TATANG, S. and AYUK, W. 1977. Recent outbreaks of the brown planthopper in Southeast Asia (with special reference to Indonesia) In "The rice brown planthopper". pp. 170-191. FFTC (ASPAC), Taipei.

MOHAMED, A.A., SIKOROWSKI, P.P. and BELL, J.V. 1978. Histopathology of Nomuraea rileyi in larvae of Heliothis zea and in vitro enzymatic activity. J. invert. Path., 31, 345-352.

MOONEY, D.T. and SYPHERD, P.S. 1976. Volatile factor involved in the dimorphism of Mucor racemosus. J. bact., 126, 1266-1270.

MORGAN, W.M. and LEDIEU, M.S. 1979. Pest and Disease of Glasshouse Crops. In "The Pest and Disease Control Handbook" (N.E.A. Scopes ed.) British Crop Protection Council.

MORISON, G.D. 1947. Thysanoptera of the London area. London. Nat. (Suppl.), 59, 1-75.

MORISON, G.D. 1957. A review of British glasshouse Thysanoptera. Trans. R. Entomol. Soc. London, 109, 467-520.

MULLER-KOGLER, E. 1965. "Pilzkran Kheiten bei Insekten". Paul Parey in Berlin and Hamburg.

MUNOZ, V. and BUTLER, W.L. 1975. Photoreceptor pigment for blue light in Neurospora crassa. Plant Physiol. 55, 421-426.

NAYAK, P. and SRIVASTAVA, R.P. 1979. Occurence of Beauveria bassiana (Bals.) Vuilli. on certain rice pests Ind. J. Entomol., 41, 99-100.

NEMOTO, H., KOBAYASHI, M. and TAKIZAWA, Y. 1979. Scanning eletron microscopy of Entomophthora (Triplosporium) floridana (Zygomycetes: Entomophthorales) attacking the Sugi spider mite, Oligonychus hond oesis (Acarina : Tetranychidae), Appl. Entomol. Zool., 14, 376-382.

NORRIS, K.P., and POWELL, E.O. 1961. Improvements in deter mining total counts of bacteria. J.R. microsc. Soc., 80, 107-119.

NOWAKOWSKI, L. 1883. Entomophthoreae. Przycznek do znajomosci pasorzytnych grzybkow sprawiajacych pomor owadow. Pamietn. Wydz. Akad. Umiej. Krakow, 8, 153-183.

NOWELL, W. 1916. The fungus on cacoa thrips. Agric. News, Bridgetown, 15, 430.

ODDS, F.C. 1979. "Candida and Candidosis"University Park Press, Baltimore, Maryland.

OETTING, R.D., and GARDNER, W.A. 1982. Two-spotted spider mite susceptibility to the fungal pathogen Hirsutella thompsonii. In "Invertebrate Pathology and Microbial Control" Ab. III rd int. Colloqu. invert. Path. Microbial Cont., Brighton, 1982, p. 112.

OKADA, T. 1971. An entomophthoraceous fungus, Conidiobolus sp., separated from planthoppers, Laodelphax striatellus (Fallen) and Nilaparvata lugens (Stal) Proc. Assx. Plant Prot. Kyushu, 17, 107-110.

OKAMOTO, H. 1924. Jassidae and Falgoridae injurious to the rice plant in Korea. Rep. Agric. Exp. Stn. Chosen. 12: 1-37.

OLMERT, I., and KENNETH, R. 1974. Sensitivity of the entomopathogenic fungi, Beauveria bassiana, Verticillium lecanii and Verticillium spp. to fungicides and insecticides. Environ. Ent., 3, 33-38.

ONIONS, A.H.S., and BARRON, G.L. 1967. Monophialidic species of Paecilomyces. Mycol. Pap., 107, 1-25.

OSMAN, M. and VALADON, L.R.G. 1981. Effect of light(especially near-u.v.) on spore germination and ultrastruture of Verticillium agaricinum. Trans. Br. mycol. Soc., 77, 187-189.

PACIONI, G., and FRIZZI, G. 1977. Paecilomyces farinosus, the conidial state of Cordyceps memorabilis. Can. J. Bot. 56, 391-394.

PAPIEROK, B. 1978. Obtention in vivo des azygospores 'd' Entomophthora thaxteriana Petch, champignon pathogene de pucerons (Homopteres Aphididae). C.R. Acad. Sci. Paris, 286, 1053-1056.

PARR, W.J. 1968. Thysanoptera on cucumbers Rept. Glasshouse Crops Res. Inst., 1967, 80-81.

PARIS, S. and FERRON, P. 1979. Study of the virulence of some nutrients of Beauveria brongniartii (=Beauveria tenella). J. invert. Path. 34, 71-77.

PEKRUL, S., and GRULA, E.A. 1979. Mode of infection of the corn earworm (Heliothis zea) by Beauveria bassiana as revealed by scanning electron microscopy. J. invert. Path., 34, 238-247.

PERKINS, D.D. 1962. Preservation of Neurospora stock cultures with anhydrous silica gel. Can. J. Microbiol., 8, 591-594.

PETCH, T. 1924. Studies in entomogenous fungi. VI. Cephalosporium and associated fungi. Trans. Br. mycol. Soc. 10, 152-182.

PETCH, T. 1931. Notes on Entomogenous Fungi. Trans. Br. mycol. Soc., 16, 67-71.

PRASERTPHON, S. and TANADA, Y. 1968. The formation and circulation, in Galleria, of hyphal bodies of Entomophthoraceous fungi. J. invert. Path., 11, 260-280.

QUINLAN, R.J. and LISANSKY, S.G. 1983. Microbial Insecticides. In "Biotechnology" (H.J. Rehmand G. Reed, eds.), Vol. 3, pp. 234-254. Verlag Chemie, Weinheim.

RAMAKERS, P.M.J. 1976. Preliminary observations on an Entomophthora sp. on Thrips tabaci Lind. in Dutch glasshouses. Bull. Organ. Int. Lutte Biol., Sect Reg. Ouest Palearct., 4, 180-182.

RAMAKERS, P.M.J. 1981. Thrips Control Work in the Netherlands. Working Group on Integrated Control in Glasshouses, International Organization for Biological Control (OILB/SROP), Bull. SROP, 1981 p 7.

RAMAKERS, P.M.J., ROMBACH, M.C. and SAMSON, R.A. 1982. Application of the entomopathogenic fungus, Aschersonia aleyrodis in a integrated programme against the glasshouse whitefly, Trialeurodes vaporariorum. Prog. and Abstr. IIIrd int. Colloqu. invert. Path. and Microbial Cont., Brighton, 1982, p. 99.

RAMARAJE URS, N.V., GOVINDU, H.C. and SHIVASHANKARA SHASTRY, K.S. 1967. The effect of certain insecticides on the entomogenous fungi Beauveria bassiana and Metarhizium anisopliae. J. invert. Path., 9, 398-403.

REMAUDIÈRE, G. and HENNEBERT, G.L. 1980. Révision systématique de Entomophthora aphidis Hoffm. in Fres. Description de deux nouveaux pathogènes d'aphides. Mycotaxon. 11, 269-321.

REMAUDIÈRE, G. and KELLER, S. 1980. Revision systématique des genres d'Entomophthoraceae à potentialité entomopathogène. Mycotaxon. 11, 323-338.

REMAUDIÈRE, G., LATGE, J.P. and MICHEL, M.F. 1981. Ecologie comparée des Entomopaththoracees pathogènes de pucerons en France littorale et continentale, Entomophaga, 26, 157-178.

REYNE, A. 1921. De Cacaothrips (Heliothrips rubrocinctus Giard). Bull. Dep. Landb. Suriname, 44.

RIBA, G. and GLANDARD, A. 1980. Mise au point d'un milieu nutritif pour la culture profonde du champignon entomopathogène Nomuraea rileyi. Entomophaga, 25, 317-322.

ROBERTS, D.W. 1966. Toxins from the entomogenous fungus Metarhizium anisopliae. Production in submerged and surface cultures, and in inorganic and organic nitrogen media. J. invert. Path., 8, 212-221.

ROBERTS, D.W., and YENDOL, W.G. 1971. Use of fungi for microbial controlof insects. In "Microbial Control of Insects and Mites" (H.D. Burges and N.W. Hussey, eds.), pp. 125-149. Academic Press, New York and London.

ROBERTS, D.W., and HUMBER, R.A. 1981. Entomogenous Fungi. In "Biology of Conidial Fungi" Vol. 2., pp. 201-235. Academic Press, New York and London.

RODMAN, G.H. 1928. Notes on Erythroneura parvula Bott. (Homoptera). Entomologists Mon. Mag., 114, 201-203.

ROMANO, A.H. 1966. Dimorphism. In "The Fungi". (G.C. Ainsworth and A.S. Sussman, eds.), Vol 2, pp. 181-209. Academic Press, New York and London.

ROSS, G.J.S. 1970. The efficient use of function minimisation in non-linear maximum likelihood estimation. J.R. Statist. Soc. (Series C), 19, 205-221.

ROSSIER, C., THE-CAN TON-THAT, an TURIAN, G. 1977. Microcyclic microconidiation in Neurospora crassa.Exp. Mycology,1, 52-62.

RUSSELL, R.C., DEBENHAM, M.L. and LEE, D.J. 1979. A natural habitat of the insect pathogenic fungus, Culicinomyces, in the Sydney area. Proc. Li n. Soc. New South Wales, 103, 71-73.

SACCARDO, P.A. 1892. Sylloge Fungorum 4. Padova.

SAKAI, K. 1932. On the increase and decrease in a year of the enemies of rice leafhoppers near Oita, Kyushu. prel. report Oyo - Dobuts Zasshi, 4, 124-127.

SAMAL, P., MISRA, B.C., and NAYAK, P. 1978. Entomophthora fumosa Spears, on entomogenous fungus on rice brown planthoppers. Curr. Sci., 47, 241-242.

SAMSINAKOVA, A., KALALOVA, S., VLCEK, V. and KYBAL, J. 1981. Mass production of Beauveria bassiana for regulation of Leptinotarsa decemlineata populations. J. invert. Path., 38, 169-174.

SAMSINAKOVA, A. and HRABETOVA, E. 1969. Respiration of blastospores of the fungus Beauveria bassiana during submersed cultivation in the presence of certain sugars. J. invert. Path., 13, 382-385.

SAMSON, R.A 1974. Paecilomyces and some allied Hyphomycetes. Studies in Mycology. No. 6. Centraalbureau voor Schimmelcultures, Baarn. Netherlands.

SAMSON, R.A., RAMAKERS, P.M.J. and OSWALD, T. 1979a. Entomophthora thripidum, a new fungal pathogen of Thrips tabaci. Can. J. Bot., 57, 1317-1323.

SAMSON, R.A., STALPERS, J.A. and VERKERKE, W. 1979b. A simplified technique to prepare fungal specimens for scanning electron microscopy. Cytobios, 24, 7-12.

SAMSON, R.A. and EVANS, H.C. 1982. Two New Beauveria spp. from South America. J. invert. Path., 39, 93-97.

SCHABEL, H.G. 1978. Percutaneous infection of Hylobius pales by Metarhizium anisopliae. J. invert. Path., 31, 180-187.

SCHAERFFENBERG, . 1955. Zeitschrift fur Pflanzenpathologie und Pflanzenschutz. 62, 544-549.

SCHAERFFENBERG, B. 1959. In Brady, B.K.L. 1979.

SCHULZ, B.E., KRAEPELIN, G. and HINKELMANN, W. 1974. Factors affecting dimorphism in Mycotypha (Mucorales): a correlation with the fermentation/respiration equilibrium. J. gen. Microbiol., 82, 1-13.

SHIMAZU, M. 1976. Entomophthora delphacis isolated from the brown planthopper, Nilaparvata lugens (Stal) Jpn. J. Appl. Entomol. Zool., 20, 144-150.

SHIMAZU, M. 1977. Factors affecting conidial germination of Entomophthora delphacis Hori (Entomophthorales : Entomophthoraceae) Appl. Ent. Zool. 12, 260-264.

SHIMAZU, M. 1979. Resting spore formation of Entomophthora sphaerosperma Fresenius (Entomophthorales : Entomophthoraceae) in the brown planthopper, Nilaparvata lugens (Stal) (Hemiptera : Delphacidae) Appl. Ent. Zool. 14, 383-388.

SOARES, G.G., PINNOCK, D.E. and SAMSON, R.A. 1979. Tolypocladium, a new fungal pathogen of mosquito larvae with promise for use in microbial control. Calif. Mosq. Vec. Can Ass., 47, 51-54.

SOLOMON, M.E. 1951. Control of humidity with Potassium hydroxide, Sulphuric acid, or other solutions. Bull. ent. Res., 42, 543-554.

SOPER, R.S. and WARD, M.G. 1981. Production, formulation and application of fungi for insect control. In "Biological Control in Crop Production" (BARC Symposium No. 5; G.C. Papavizas, Ed.) Allenheld, Osman, Totowa.

SRINIVASAN, M.C. and THIRUMALACHAR, M.J. 1964. On the identity of Entomophthora coronata, Mycopathol. Mycol. Appl., 24, 294-296.

SRIVASTAVA, R.P. and NAYAK, P. 1978. A white muscardine disease on brown planthopper of rice. Curr. Sci., 47, 355-356.

STEINHAUS, E.A. 1949. "Principles of Insect Pathology" Mc Graw-Hill, New York, 757 pp.

STEINHAUS, E.A. 1954. The effects of disease on insect populations. Hilgardia, 23, 197-261.

STEINHAUS, E.A. 1956. Microbial control - the emergence of an idea. A brief history of insect pathology through the nineteenth century. Hilgardia, 26, 107-160.

SOROKIN, N. 1883. Plant parasites of Man and Animals as causes of infectious diseases 2, 268-291.

STOLK, A.C., and SAMSON, R.A. 1972. Studies on Talaromyces and related genera II. The genus Talaromyces. Stud. Mycol. 2, 1-65.

STRADLINS, D.J. 1968. Investigations on the natural enemies of the onion thrip (Thrips tabaci Lindman) A. Work undertaken at the Europeon Station during 1968., Interim Rept. Commonw. Inst. biol. Control, Dec 1968, 1-8.

SUENAGA, H. 1963. Analytical studies on the ecology of two species of planthoppers, the white back planthopper (Sogatella furcifera Horvath) and the brown planthopper (Nilaparvata lugens Stal) with special reference to their outbreaks. Bull. Kyushu Agric. Exp. Stn., 8, 1-152.

SUENAGA, H. and NAKATSUKA, K. 1958. Critical review of forecasting the occurences of planthoppers and leafhoppers on rice in Japan. Byogaity-Hassei Yosatu-Tokubetu - Hokoku, 1, 1-453.

SYPHERD, P.S., ORLOWSKI, M. and PETERS, J. 1979. Models of fungal dimorphism : Control of dimorphism in Mucor racemosus In "Microbiology - 1979" (D. Schlessinger, ed.),pp. 224-227. Am. Soc. Microbio., Washington D.C.

TAN, K.K. 1978. Light-induced fungal development. In "The Filamentous Fungi" (J.E. Smith and D.R. Berry, eds.), Vol. 3, pp. 334-357. Arnold, London.

THAXTER, R. 1888. The Entomophthoreae of the United States. Mem. Boston Soc. Nat. Hist. 4, 133-201.

TRIONE, E.J. and LEACH, C.M. 1969. Light - induced sporulation and sporogenic substances in fungi. Phytopathology, 59, 1077-1083.

TULLOCH, M. 1976. The Genus Metarhizium. Trans. Br. mycol. Soc., 66, 407-411.

URICH, F.W. 1928. San Thomé cacao industry. Trop. Agric. Trin., 5, 275-278.

VEEN, K.H. 1968. Recherches sur la maladie, due à Metarhizium anisopliae chez le criquet pèlerin. Meded. Landbouwhogeschool Wageningen, 68, 14-15.

VUILLEMIN, P. 1912. Beauveria, nouveau genre de Verticilliacees. Bull. Soc. bot. Fr. 29, 34-40.

WALSTAD, J.D., ANDERSON, R.F. and STAMBAUGH, W.J. 1970. Effects of environmental conditions on two species of muscardine fungi (Beauveria bassiana and Metarhizium anisopliae). J. invert. Path., 16, 221-226.

WEIMER, J.L. and HARTER, L.L. 1923. Temperature relations of eleven species of Rhizopus. J. Agric. Res. (Washington D.C.), 24, 1-40.

WEISER,J. 1968. Triplosporium tetranychi sp.n. (Phycomycetes, Entomophthoraceae), a fungus infecting the red mite Tetranychus althaeae HANST. Folia Parasitol., Praha, 15, 115-122.

WELLMAN, F.L. 1932. Rhizoctonia bottom rot and head rot of cabbage. J. Agric. Res. (Washington D.C.), 45, 461-469.

WILDING, N. 1972. The effect of systemic fungicides on the aphid pathogen Cephalosporium aphidicola. Pl. Path. 21, 137-139.

WILDING, N. 1982. Entomophthorales : field use and effectiveness. Prog. and Abstr. IIIrd int. Collogu. invert. Path. and Microbial Cont., Brighton, 1982, pp. 170-175.

WILDING, N. and BROBYN, P.J. 1980. Effects of fungicides on development of Entomophthora aphidis. Trans. Br. mycol. Soc., 75, 297-302.

WINSTON, P.W. and BATES, D.H. 1960. Saturated solutions for the control of humidity in biological research. Ecology, 41, 232-237.

WYATT, I.J. 1965. The distribution of Myzus persicae (Sulz.) on year - round chrysanthemums. 1. Summer season. Ann. appl. Biol., 56, 439-459.

YAMAGUCHI, H. 1975. Control of dimorphism in Candida albicans by Zinc : Effect on cell morphology and composition. J. gen. Microbiol. 86, 370-372.

YENDOL, W.E. and HAMLIN, R.A. 1973. Ecology of entomogenous viruses and fungi. Ann. New York Acad. Sci., 217, 18-30.

ZACHARUK, R.Y. 1973. Electron - microscope studies of the histopathology of fungal infections by Metarhizium anisopliae. Misc. Pub. ent. Soc. Am., 9, 112-119.

ZACHARUK, R.Y. 1981. Fungal Diseases of Terrestrial Insects. In "Pathogenesis of Invertebrate Microbial Diseases" (E.W. Davidson, ed.), pp. 367-402. Allenheld, Osman (Publ.) New York.

ZIMMERMANN, G. 1976. Uber die wirkung systemischer fungizide auf aphiden befallende Entomophthoraceen (Zygomycetes) in vitro. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz., 83, 261-269.

ADDENDA

MC COY, C.W. 1981. Pest control by the fungus, Hirsutella thompsonii. In "Microbial Control of Pests and Plant Diseases 1970 - 1980". (ed. H.D. Burges) ,pp. 499 — 512 .Academic Press,New York and London.

PETCH, T. 1934. Notes on entomogenous fungi. Trans. Br. mycol. Soc., 19 ,189.

WILDING, N. 1973. The survival of Entomophthora spp. in mummified aphids at different temperatures and humidities. J. invert. Path., 21 , 309- 311.

WILDING, N. 1981. Pest control by the Entomophthorales. In " Microbial Control of Pests and Plant Diseases 1970-1980". (ed. H.D. Burges), pp.539 - 554 .Academic Press, New York and London.