4 Biology, Ecology and Pest Management Potential of Entomophthorales

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Introduction

The Entomophthorales are an order of fungi placed in the subdivision Zygomycotina, class Zygomycetes. Currently six families in the Entomophthorales are recognized: Entomophthoraceae, Neozygitaceae, Completoriaceae, Ancylistaceae, Meristacraceae and Basidiobolaceae (Humber, 1989). The two most important families, from the standpoint of entomologists and invertebrate pathologists, are the Entomophthoraceae and the Neozygitaceae. Recent phylogenetic analysis of the Entomophthorales based on ribosomal DNA suggests the Entomophthorales are monophyletic and that *Basidiobolus* spp. may not belong in the Entomophthorales (Jensen *et al.*, 1998). The generic classification in the Entomophthorales is generally clear at the *sensu lato* level. However, more detailed classification into genera *sensu stricto* with or without subgenera is unclear and an area of debate (Remaudière and Keller, 1980; Humber, 1981, 1989; Ben-Ze'ev and Kenneth 1982a, b; Balazy, 1993). There are 200–300 known species in the Entomopthoraceae and 15 species in the Neozygitaceae (Keller, 1997). Undoubtedly, many more species in these two families remain to be described. Figures 4.1–4.24 show examples of a number of insect and mite species from different orders and families with typical external signs of infection by Entomophthorales.

Both hyphomycete and entomophthoralean species have evolved to exploit the ecological niches in which they exist; similarities and differences between the groups are given in Table 4.1. In general, Entomophthorales tend to have narrow host ranges, close associations with foliar insect or mite hosts and conspicuous epizootics (Evans, 1989). In comparison, hyphomycete fungi tend to have wide host ranges and epizootics usually occur only in insect populations in soil (Keller and Zimmerman, 1989).

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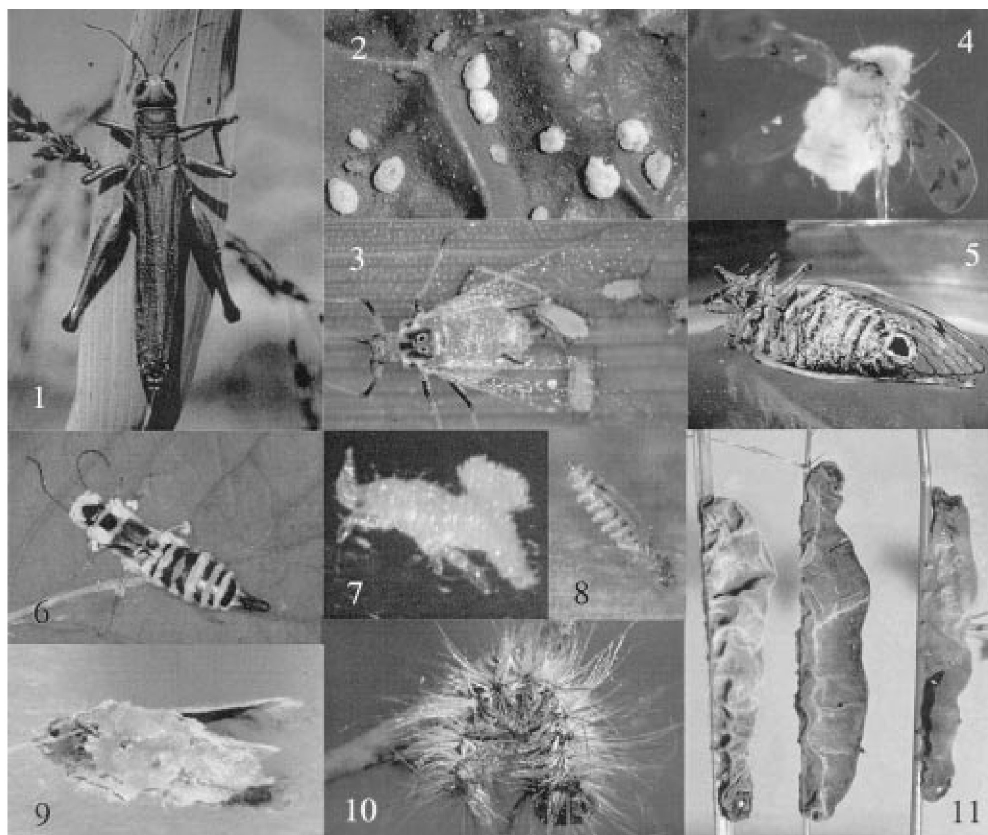

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Fig. 4.1. Differential grasshopper, *Melanoplus differentialis* (Orthoptera: Acrididae), infected with *Entomophaga grylli*. No external indication of infection is seen but the cadaver is filled with resting spores.
Fig. 4.2. Peach–potato aphids, *Myzus persicae* (Hemiptera: Aphididae), infected with *Erynia neoaphidis*.
Fig. 4.3. Alate grain aphid, *Sitobion avenae* (Hemiptera: Aphididae), infected by *E. neoaphidis*. The nymphs will become infected by conidia discharged from the infected adult.
Fig. 4.4. Bandedwinged whitefly, *Trialeurodes abutilonea* (Hemiptera: Aleyrodidae), infected with *Orthomyces aleyrodis*.
Fig. 4.5. Periodical cicada, *Magicicada trecassini* (Hemiptera: Cicadidae), infected with the resting spore stage of *Massospora cicadina*. The resting spores are spread in the environment through a hole in the abdomen of the still-living cicada.
Fig. 4.6. Earwig, *Forficula forficularia* (Dermaptera: Forficulidae), infected with *Zoophthora forficulae*.
Fig. 4.7. Onion thrips, *Thrips tabaci* (Thysanoptera: Thripidae), infected with *Entomophthora thripidum*. The conidiophores emerge in a bundle and discharge conidia from the still-living thrips. Photograph: Florian Freimoser and Anne Grundschober.
Fig. 4.8. Onion thrips infected with *Neozygites parvispora*. Photograph: Anne Grundschober.
Fig. 4.9. Adult diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae), infected with *Zoophthora radicans*. Both adults and larvae are susceptible to *Z. radicans*.
Fig. 4.10. Larva of gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), infected with *Entomophaga maimaiga*. Discharged conidia are seen on the hairs.
Fig. 4.11. Larvae of true army worm, *Pseudaletia unipuncta* (Lepidoptera: Noctuidae), infected with the conidial stage of *Erynia* (= *Furia*) *virescens*. The larvae die at the top of grass stems.

Table 4.1. General characteristics of the Entomophthorales and the Hyphomycetes.

Characteristic	Entomophthorales	Hyphomycetes
Conidia size and number per cadaver	>10 μm in length, relatively small numbers per cadaver	< 10 μm in length, relatively large numbers per cadaver
Conidia discharge	Active, with exceptions: *Massospora* spp., *Strongwellsea* spp. and some *Neozygites* spp.	Not active
Preformed mucus on conidia	Present	Not present with exceptions: *Verticillium* spp., *Hirsutella* spp., *Aschersonia* spp.
Rhizoids	Present in many species	Absent
Ability to modify host behaviour	Can modify behaviour, e.g, *Entomophora muscae*, *Entomophaga grylli*	Cannot modify behaviour with exceptions: *Sorosporella* spp.
Pre-death sporulation	Present in some species: *Entomophthora thripidum*, *Strongwellsea castrans* and *Massospora* spp.	Present in some species: *Verticillium lecanii*
Epizootics	Most common in foliar insects	Most common in soil insects
Host range	Generally narrow	Generally wide, with exceptions: *V. lecanii*, *Hirsutella thomsoni*
Toxin production	Not known	Observed in many species
Saprophytic	Not known, with exceptions: *Conidiobolus* spp.	Known in some species
Resting spores	Present in most species	Not present, with the exception of *Sorosporella* chlamydospores
Virulence	Few conidia required for infection	Many conidia required for infection, with exceptions: *V. lecanii*
Sporulation and germination rate	Fast	Slow
Higher-order conidium production	In all species	Only in *Aschersonia*

The most host-specific Hyphomycetes have life-history attributes similar to Entomophthorales and the Entomophthorales with widest host ranges have attributes similar to the Hyphomycetes (Table 4.1). In terms of exploitation in pest management some ecological attributes of the Entomophthorales are clearly very advantageous. Many species in the Entomophthorales play an important role in regulating host populations under certain conditions. They have caused spectacular epizootics in numerous insect populations and these epizootics commonly reduce the host population to near zero at a local scale: hence their great potential in pest management (Table 4.2).

Ecological, epizootiological and life-history attributes of each species–pest system must be understood to effectively exploit their potential, overcome their problems and design appropriate methods to integrate them into pest management strategies. In this chapter ecological case histories for a number of entomophthoralean species are described; the spatial and temporal distribution, persistence, dispersal and impact on host biology and behaviour are keys to their effective exploitation in specific environments. The use of these species in pest management is discussed with particular emphasis on future opportunities. Fungal species and insect hosts have been

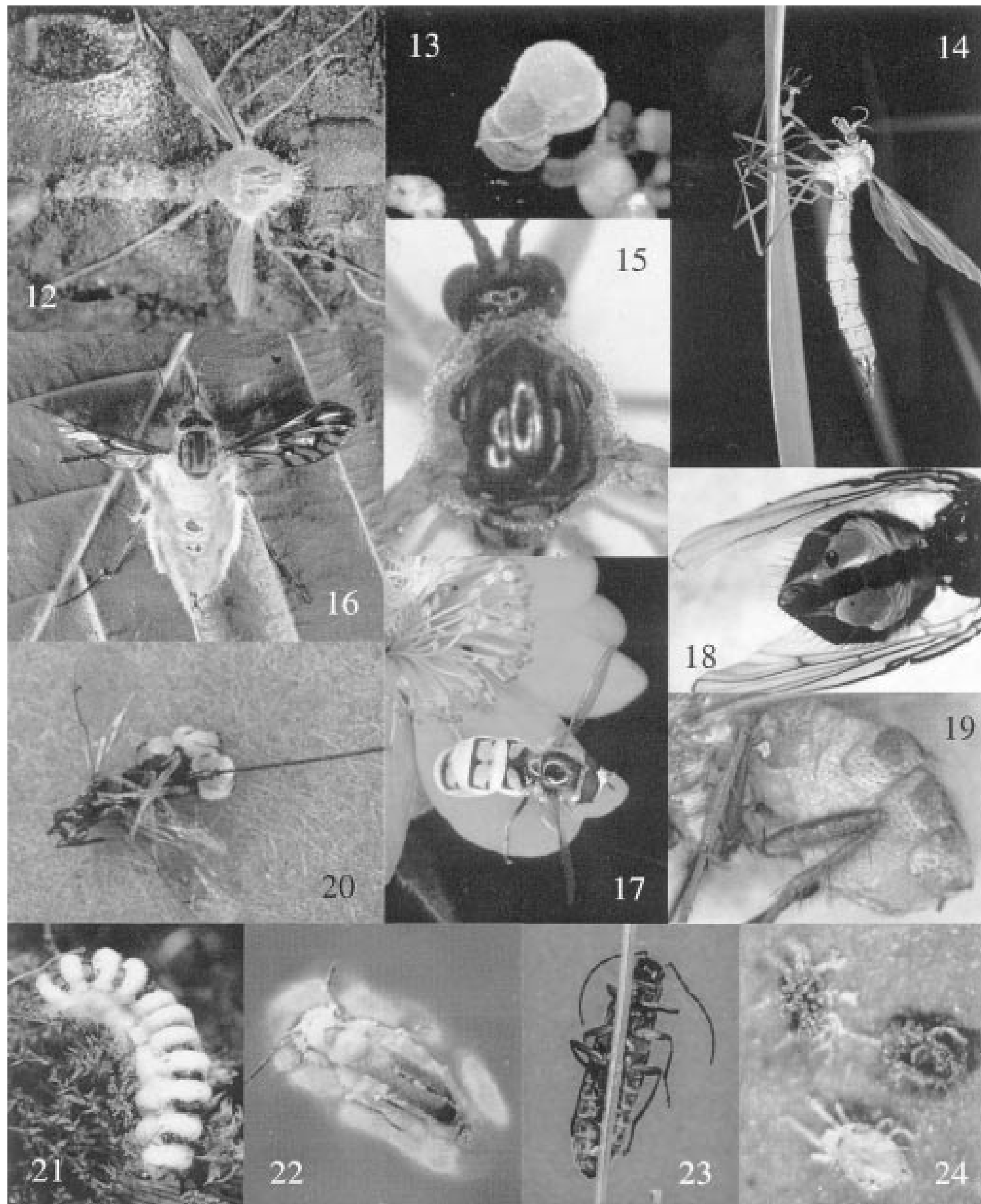

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Fig. 4.12. Nematoceous fly (Diptera: Chironomidae or similar), infected with *Erynia conica*. The cadaver is fixed by rhizoids to a piece of wood floating in the water. Discharged conidia are seen on the wood.

Fig. 4.13. Pupa of a snow-pool mosquito, *Aedes fitchii* (Diptera: Culicidae), infected with *Erynia aquatica*. Conidia are discharged from the floating pupa and infect newly emerging adults, which then die with the resting spore stage.

Fig. 4.14. Adult crane fly, *Tipula* sp. (Diptera: Tipulidae), grasping the vegetation with its legs. The cause of mortality is a fungus from the Entomophthorales (probably from the genera *Batkoa* or *Eryniopsis*). No fungal structures are seen before incubation in a moist chamber.

selected from complementary examples of pest management in agriculture, horticulture, forestry, livestock and rangelands.

Basic Biology

Entomophthoralean life cycles are often complex and usually involve at least two different types of spores: conidia and resting spores. The basic life cycle, as exemplified by *Entomophthora muscae*, is provided in Fig. 4.25. There are many elaborations and exceptions to the basic pattern, generally acting to increase the ability of a species to reach and infect its hosts and these are described in the following case histories.

Conidia are the spore forms responsible for infection during the season when hosts are active. Conidiophores emerge through membranous regions of the host, particularly through the intersegmental membranes, a hymenial layer of the fungus develops and copious numbers of primary conidia are actively discharged by hydrostatic pressure (Figs 4.26, 4.27, 4.30, 4.31). Tens of thousands of conidia can be produced from a single host, with numbers depending on cadaver biomass. While conidia are usually actively discharged from bodies of dead hosts, exceptions occur. For example, conidia are discharged from living thrips by *Entomophthora thripidum* (Fig. 4.7; Samson *et al.*, 1979) and conidia are not actively discharged from mealy-bug cadavers by some *Neozygites* species (B. Papierok, personal communication). Conidia of the cicada-infecting genus *Massospora* are produced within the abdomens of living adult hosts and, as adults fly, successive segments of the abdomen break off and conidia are dispersed (Fig. 4.5; Soper *et al.*, 1976). Species from the genus *Strongwellsea* produce conidia within the abdomens of living flies and an opening is formed in the ventral wall of the abdomen from which conidia are released as the living adult moves (Figs 4.18, 4.44–4.47).

Fig. 4.15. Adult *Ptychoptera contaminata* (Diptera: Ptychopteridae), infected with *Eryniopsis ptychopterae*. Conidiophores emerge on the sides of the thorax and on the abdomen.
Fig. 4.16. Snipe fly, *Rhagio mystaceus* (Diptera: Rhagionidae), infected with *Erynia* (= *Pandora*) *ithacensis*, attached by rhizoids to the underside of a beech-tree leaf.
Fig. 4.17. Hover fly (Diptera: Syrphidae), infected with a species from the *Entomophthora muscae* complex, presumably *E. syrphi*. Photograph: Holger Philipsen.
Fig. 4.18. Arctic fly, *Spilogone dorsata* (Diptera: Muscidae), infected with *Strongwellsea* sp. nov. Conidia are discharged from one or two abdominal holes in living individuals.
Fig. 4.19. Cabbage-root fly, *Delia radicum* (Diptera: Anthomyiidae), infected with *Strongwellsea castrans*. Bright orange resting spores fill the abdomen.
Fig. 4.20. Chalcid wasp, *Torymus druparum* (Hymenoptera: Torymidae), infected with *Entomophthora* sp.
Fig. 4.21. Larva of a beetle, *Lagria* sp. (Coleoptera: Tenebrionidae), infected with *Erynia* s. l. sp. Photograph: Neil Wilding.
Fig. 4.22. Adult cantharid beetle, *Rhagonycha fulva* (Coleoptera: Cantharidae), infected with *Erynia* (= *Pandora*) *lipai* and incubated in a moist chamber. Many discharged conidia are seen on the glass slide.
Fig. 4.23. Cantharid beetle, *R. fulva*, infected with *Tarichium rhagonycharum*. The specimen, filled with brown resting spores, is fixed by rhizoids to the vegetation.
Fig. 4.24. Cassava green mites, *Mononychellus tanajoa* (Acari: Tetranychidae), infected with *Neozygites* sp. The two cadavers at the top of the image have terminated sporulation. Photograph: Italo Delalibera.

Table 4.2. Examples of natural epizootics in insects and mites.

Host species	Main causative fungus	Reference
Acari: Tetranychidae		
Mononychellus tanajoa	*Neozygites floridana*	Delalibera *et al.*, 1992; Elliot *et al.*, 2000
Tetranychus urticae	*N. floridana*	Smitley *et al.*, 1986
Hemiptera: Aphididae		
Myzus persicae	*Erynia neoaphidis*	McLeod *et al.*, 1998
Aphis fabae	*E. neoaphidis*	Wilding and Perry, 1980
Microlophium carnosum	*E. neoaphidis*	Hemmati, 1999
Metopolophium dirhodum	*E. neoaphidis*	Hatting *et al.*, 1999; Yeo, 2000
Diuraphis noxia	*E. neoaphidis*	Wraight *et al.*, 1993
Sitobion avenae	*E. neoaphidis*	Schmitz *et al.*, 1993; Nielsen *et al.*, 2000a
Aphis gossypii	*Neozygites fresenii*	Silvie and Papierok, 1991; Steinkraus *et al.*, 1991, 1995
Aphis citricola	*N. fresenii*	Kuntz, 1925
A. fabae	*N. fresenii*	Dedryver, 1978
Therioaphis trifolii	*Zoophthora radicans*	Kenneth and Olmert, 1975
Rhopalosiphon padi	*Entomophthora planchoniana*	Nielsen *et al.*, 2000a
Elatobium abietinum	*N. fresenii*	Nielsen *et al.*, 2000b
Hemiptera: Lachnidae		
Schizolachnus piniradiatae	*Erynia canadensis*	Soper and MacLeod, 1981
Hemiptera: Pseudococcidae		
Planococcus citri	*Neozygites fumosa*	Speare, 1922
Hemiptera: Cicadellidae		
Amrasca biguttula	*Batkoa amrascae*	Villacarlos and Keller, 1997
Empoasca kraemeri	*Z. radicans*	Galaini-Wraight *et al.*, 1991
Empoasca fabae	*Z. radicans*	McGuire *et al.*, 1987c
Hemiptera: Cicadidae		
Massospora levispora	*Okanagana rimosa*	Soper *et al.*, 1976
Hemiptera: Aleyrodidae		
Trialeurodes abutilonea	*Orthomyces aleyrodis*	Steinkraus *et al.*, 1998b
Thysanoptera: Thripidae		
Frankliniella occidentalis	*Neozygites parvispora*	Montserrat *et al.*, 1998
Lepidoptera: Lymantriidae		
Euproctis chryssorhoea	*Entomophaga aulicae*	Speare and Colley, 1912
Lymantria dispar	*Entomophaga maimaiga*	Koyama, 1954; Hajek, 1997c
Orgyia vetusta	*E. aulicae*	Hajek *et al.*, 1996c
Lepidoptera: Noctuidae		
Pseudaletia unipuncta	*Furia virescens*	Steinkraus *et al.*, 1993c
Lepidoptera: Lasiocampidae		
Malacosoma disstria	*Furia crustosa*	MacLeod and Tyrrell, 1979
Lepidoptera: Geometridae		
Lambdina fiscellaria	*E. aulicae*	Otvos *et al.*, 1973
Lepidoptera: Tortricidae		
Choristoneura fumiferana	*Z. radicans*	Vandenberg and Soper, 1978

Table 4.2. continued

Host species	Main causative fungus	Reference
Choristoneura fumiferana	*E. aulicae*	Perry and Regnière, 1986
Lepidoptera: Plutellidae		
Plutella xylostella	*Z. radicans*	Ullyett and Schonken, 1940; Kanervo, 1946; Aruta *et al.*, 1974; Ooi, 1981; Yamamoto and Aoki, 1983; Riethmacher and Kranz, 1994
P. xylostella	*Erynia blunckii*	Tomiyama and Aoki, 1982
Diptera: Muscidae		
Musca domestica	*Entomophthora muscae* and *Entomophthora schizophorae*	Mullens *et al.*, 1987; Steinkraus *et al.*, 1993b; Watson and Petersen, 1993b
Ophyra aenescens	*E. muscae*	Watson and Petersen, 1993b
Diptera: Psilidae		
Chamaepsila rosae	*E. muscae*	Eilenberg and Philipsen, 1988
	Conidiobolus pseudapiculatus	Eilenberg, 1988
Diptera: Fanniidae		
Fannia spp.	*E. muscae*	Mullens *et al.*, 1987
Diptera: Anthomyiidae		
Delia antiqua	*E. muscae*	Carruthers *et al.*, 1985
Delia coarctata	*E. muscae*	Wilding and Lauckner, 1974
Delia floralis	*E. muscae* and *Strongwellsea castrans*	Klingen *et al.*, 2000
Delia radicum	*E. muscae* and *S. castrans*	Eilenberg and Michelsen, 1999; Eilenberg, 2000; Thomsen and Eilenberg, 2000
Orthoptera: Acrididae		
Camnula pellucida	*Entomophaga grylli*	Carruthers *et al.*, 1997
Dissosteira carolina	*E. grylli*	Carruthers *et al.*, 1997
Coleoptera: Curculionidae		
Hypera postica	*Zoophthora phytonomi*	Harcourt *et al.*, 1974

Conidia are relatively fragile and short-lived but can germinate quickly. They are generally sticky, being covered with preformed mucus, aiding in host attachment (e.g. Fig. 4.32). As a general pattern, if primary conidia land on non-host surfaces, they can produce higher-order conidia, e.g. a primary conidium may germinate to produce and actively discharge a secondary conidium and a secondary conidium may germinate to produce and actively discharge a tertiary conidium (e.g. Figs 4.35, 4.36). Although it seems possible that this process could continue until the protoplasm is depleted, the infectivity of successive generations of supernumerary conidia beyond secondary has not been investigated. As a basic pattern, supernumerary conidia are similar in shape to primaries but each generation of supernumerary conidia is slightly smaller in size. The production of successive generations of supernumerary conidia greatly increases the ability of the fungus to reach and infect hosts. With some genera, e.g. *Neozygites*, primary conidia are not infective, secondary conidia are always

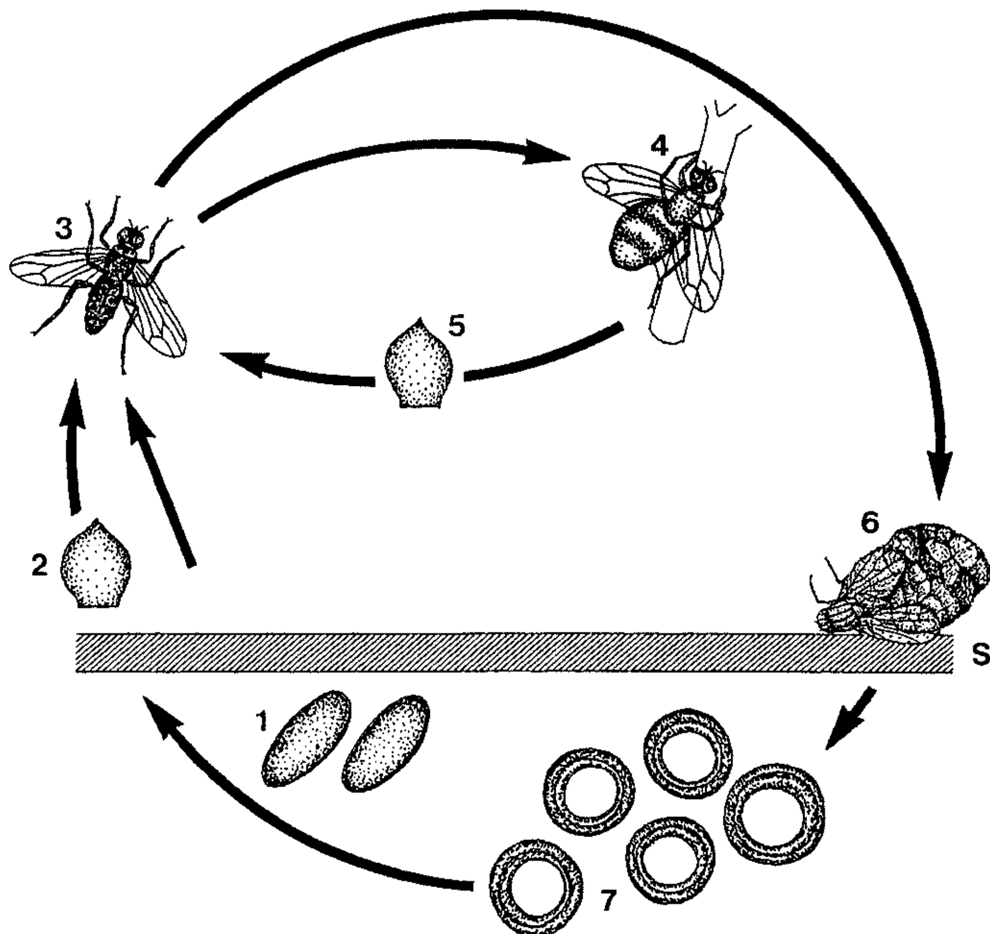

Fig. 4.25. Life cycle of *Entomophthora muscae* in the host, cabbage-root fly (*Delia radicum*, Diptera: Anthomyiidae).
1. Pupae of *D. radicum* overwinter in the soil. 2. During spring, infective conidia of *E. muscae* are produced and discharged from resting spores in the soil. 3. Adult *D. radicum* emerge from pupae during spring and become infected by conidia of *E. muscae*. 4. After the incubation period, *E. muscae* kills *D. radicum*. The dead fly is attached to the vegetation by rhizoids and legs. Conidiophores emerge from the abdomen and produce conidia.
5. Conidia are discharged from the cadaver and infect (eventually after replicative conidiation) other adult *D. radicum*. Several successive infection cycles can take place during the season in the host population. 6. After midsummer, some *E. muscae*-infected *D. radicum* develop resting spores instead of conidia. After the incubation period, flies die with resting spores and drop to the soil surface (S). The abdomen is filled with resting spores (azygospores). 7. Thick-walled resting spores survive in the soil surface layers during winter. In the next spring they will germinate and discharge primary conidia and the cycle is completed.

produced, and the infective secondaries may differ in shape and size from the primaries. In some genera, e.g. *Zoophthora*, higher-order conidia are not always actively discharged but rather produced on fine capilliconidiophores. The capilliconidium is borne on the top of the conidiophore some distance above the surface and remains attached until a host passes. The conidium is sticky and readily attaches to the host, breaking off from the conidiophore at a zone of weakness.

Little is known about host recognition, the first stage in the infection process. In general, conidia are not thought to require exogenous nutrients for growth. Typically, rounded appressoria are produced for host penetration (Fig. 4.37), although some studies report penetration without production of appressoria (Brobyn and Wilding, 1977; Lambiase and Yendol, 1977). Where fungal cells invade, the host cuticle is frequently delaminated and displaced or depressed, suggesting that mechanical pressure is used for cuticular penetration. In addition, histolysis of tissues beneath invading hyphae suggests the utilization of enzymes for penetration.

Once within hosts, some Entomophthorales initially grow as protoplasts, lacking sugar-rich cell walls. It is thought that this stage has evolved to escape detection by the host immune system (Beauvais *et al.*, 1989). Other species grow as unicellular hyphal bodies or coenocytic hyphae and do not have a protoplastic stage. Often, through most of the course of an infection, there are few overt symptoms, although host feeding may decrease as the infection progresses. Some infected insects display negative geotaxis directly prior to death; therefore, cadavers are located in elevated positions (e.g. *E. muscae* and *Entomophaga grylli*), allowing for more efficient dispersal of conidia after discharge. Cadavers of hosts killed by some species are fixed in place above the ground by rhizoids, which would also enhance chances for dispersal of conidia (Figs 4.28, 4.29). Host death is probably caused by physiological starvation of the host when the fungus has consumed all reserves. However, in one study of lepidopteran hosts infected with *Entomophaga aulicae*, a short-lived cell-lytic factor that was most active at or shortly after death is hypothesized as the cause of death (Milne *et al.*, 1994); the onset of cell-lytic activity was detected shortly before death, just as terminal behavioural changes occurred.

Many factors have been associated with the types of spores produced by cadavers but, in general, these factors act to synchronize fungal and host activity (e.g. Hajek, 1997b). The type of spore initiating an infection can play a part in subsequent fungal reproduction.

Resting spores are the most important way that Entomophthorales survive periods when hosts are not present or active. Resting spores of Entomophthorales are either formed by the union of two hyphae (zygospores) or by one hyphal cell rounding up at one end (azygospores). These spores are resistant, with a thick double wall. They are frequently dormant directly after production, often requiring a cold period of several months before germination is possible (Hajek, 1997a). Resting spores may germinate throughout the period of time that hosts are present in the field. Many resting spores do not germinate in the first year after production but survive for numerous years. This is thought to be how *Massospora* spp. infecting periodical cicadas persist during the extended intervals between insect outbreaks (e.g. 17 years). Once resting spores germinate they produce one to several forcibly ejected germ conidia or, in the case of *Neozygites* spp., capilliconidia, either of which is infective to new hosts. While knowledge of germ conidia is meagre, these spores are known to resemble primary conidia except that they can differ reproductively, e.g. by producing only conidia after infection, and thereby act to begin cycles of infection after a period of inactivity.

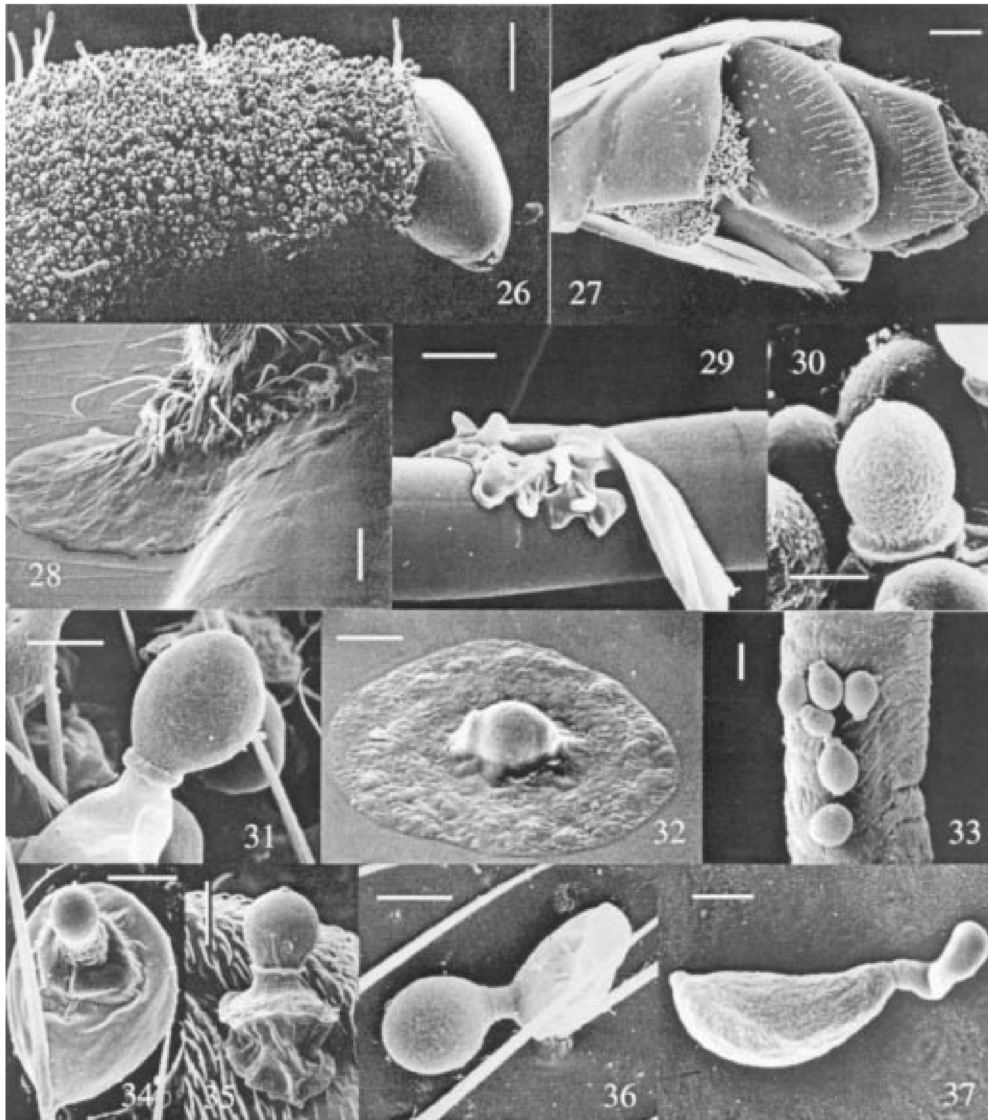

Fig. 4.26. Sciarid larva (Diptera: Sciaridae) infected with *Erynia* sp. Note cystidia above the conidiophore layer. Scale bar = 100 µm. SEM images, Figs 4.26–4.37: José Bresciani and Jørgen Eilenberg.
Fig. 4.27. Adult chalcid wasp, *Torymus druparum* (Hymenoptera: Torymidae), infected with *Entomophthora* sp. Conidiophores emerge only intersegmentally. Scale bar = 250 µm.
Fig. 4.28. Rhizoids and mucoid substances of *Entomophthora schizophorae* fix the mouthparts of an adult carrot fly, *Chamaepsila rosae* (Diptera: Psilidae), to the substrate. Scale bar = 10 µm.
Fig. 4.29. Rhizoid of *Zoophthora forficulae* attaching the earwig host, *Forficula forficularia* (Dermaptera: Forficulidae), to the vegetation. Scale bar = 10 µm.
Fig. 4.30. Primary conidium of *Entomophthora* sp. nov. being discharged from a cantharid beetle, *Cantharis livida* (Coleoptera: Cantharidae). Scale bar = 20 µm.
Fig. 4.31. Primary conidium of *Eryniopsis ptychopterae* formed at the end of the conidiophore. Scale bar = 20 µm.
Fig. 4.32. Discharged primary conidium of *Entomophthora schizophorae*. Note the material (wall laminae and mucus) surrounding the conidium, helping to attach the conidium to the insect cuticle. Scale bar = 10 µm.
Fig. 4.33. Discharged primary conidia of *Neozygites fresenii* attaching to aphid cuticle. Scale bar = 10 µm.
Fig. 4.34. Primary conidium of *E. schizophorae* attaching to the cuticle of a carrot fly and producing a secondary conidium. Scale bar = 20 µm.
Fig. 4.35. Fully formed secondary conidium of *Entomophthora muscae* formed by a primary conidium on the cuticle of a cabbage-root fly, *Delia radicum* (Diptera: Anthomyiidae). Scale bar = 20 µm.
Fig. 4.36. Primary conidium of *Enyniopsis ptychopterae* forming a secondary conidium on the cuticle of the host, *Ptychoptera contaminata* (Diptera: Ptychopteridae). Scale bar = 10 µm.
Fig. 4.37. A germinating capilliconidium (secondary conidium) of *N. fresenii.* Scale bar = 10 µm.

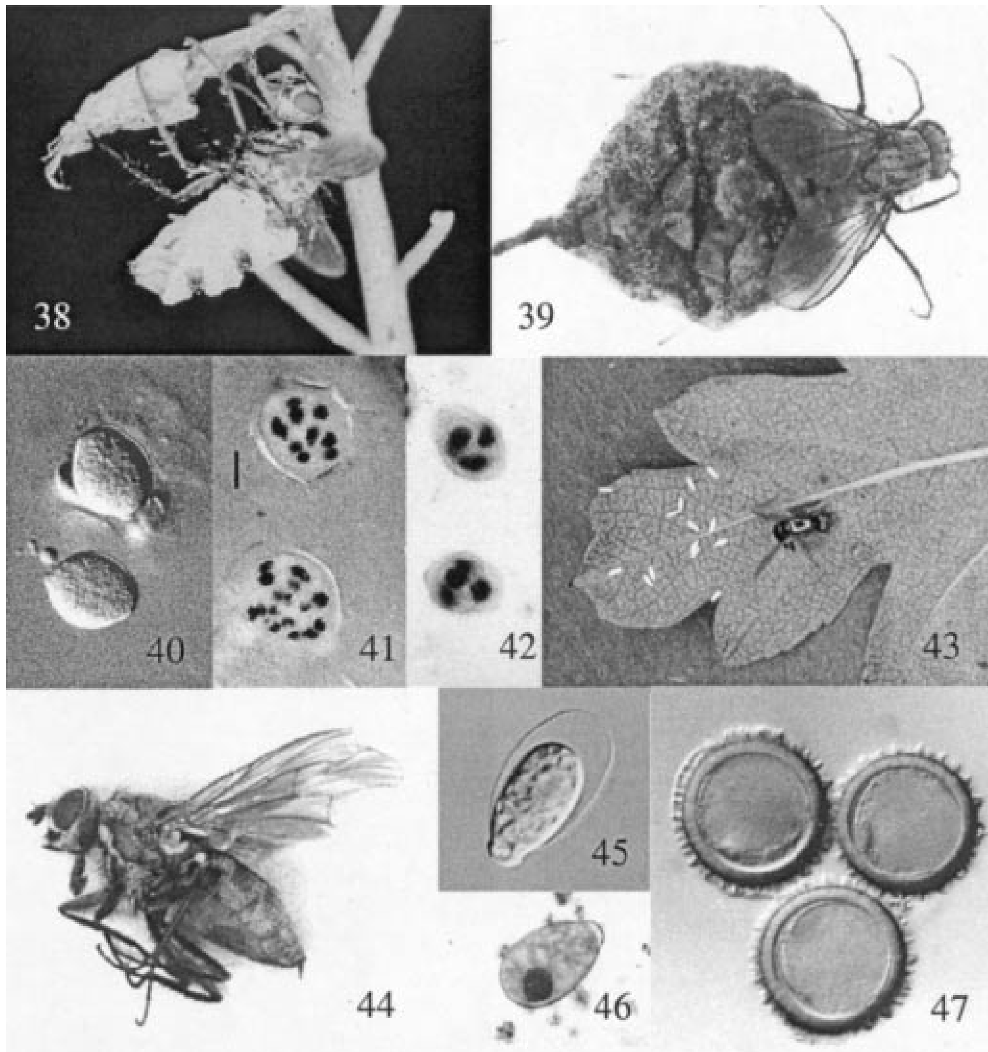

Fig. 4.38. Adult cabbage-root fly, *Delia radicum* (Diptera: Anthomyiidae), infected by *Entomophthora muscae*. The dead fly is attached to the vegetation and conidia are discharged.

Fig. 4.39. Cabbage-root fly, *D. radicum*, infected with *E. muscae*. The abdomen of the dead fly is filled with resting spores (azygospores).

Fig. 4.40. Primary conidia of *E. muscae*.

Fig. 4.41. Primary conidia of *E. muscae*. The nuclei are stained with aceto-orcein. Scale bar = 20 μm.

Fig. 4.42. Primary conidia of *Entomophthora schizophorae*. The nuclei are stained with aceto-orcein. Scale bar = 20 μm.

Fig. 4.43. Carrot fly, *Chamaepsila rosae* (Diptera: Psilidae), infected with *E. schizophorae*. The infected fly deposited eggs on leaves of *Crataegus* sp. 4 m above the soil surface before death due to infection.

Fig. 4.44. Cabbage-root fly, *D. radicum*, infected with *Strongwellsea castrans*. The primary conidia are discharged from living individuals though an abdominal hole.

Fig. 4.45. Primary conidium of *S. castrans*. Note detached spore wall layer. Scale bar = 20 μm.

Fig. 4.46. Primary conidium of *S. castrans*. The nucleus is stained with aceto-orcein. Scale bar = 20 μm.

Fig. 4.47. Resting spores of *S. castrans*. Note the spiny surface. Scale bar = 20 μm.

Ecological Case Histories

Entomophthora muscae, sensu lato *(Entomophthoraceae)*

Taxonomy and distribution

E. muscae was the first entomophthoralean fungus to be described (Cohn, 1855). In the last century, both the biology and potential of *E. muscae* for biological control of adult flies, especially the housefly, *Musca domestica*, have been studied extensively (reviewed by MacLeod *et al.*, 1976). The fungus is readily apparent as dead flies are attached to vegetation, walls, etc. by rhizoids emerging through the proboscis and by the legs of the dead flies (Fig. 4.38).

The genus *Entomophthora* is characterized by campanulate primary conidia (Figs 4.40, 4.41) and *E. muscae* is known from a range of dipteran hosts from different families of Cyclorrhapha, e.g. Muscidae, Anthomyiidae, Fanniidae. Keller (1984, 1987c) demonstrated that *E. muscae* was actually a complex of species and, at that time, *Entomophthora schizophorae* was described as a new species. *E. schizophorae* differs from *E. muscae* in only a few morphological features (e.g. fewer nuclei per conidium (Fig. 4.42)) but both have certain hosts in common. Recently *E. muscae sensu stricto* has been redescribed (Keller *et al.*, 1999). Both *E. muscae* and *E. schizophorae* are recorded in the field as infecting *M. domestica* and anthomyiid flies. While recent studies differentiate between the two species, earlier studies referring to *E. muscae* may indeed be studies of *E. schizophorae*. References referring solely to *E. schizophorae* are indicated in the text, although in most cases (life-cycle, growth *in vivo* and *in vitro*, use in biocontrol) the two species will be treated simultaneously as '*E. muscae*' *sensu lato*.

Host specificity

When considering both indoor and outdoor agricultural/livestock systems, observations indicate that *E. muscae* and *E. schizophorae* are not confined to one host species in specific situations, but may be transmitted to other dipteran species. First, during epizootiological studies of one dipteran host, other infected dipteran species were found at the same site and time (Mullens *et al.*, 1987; J. Eilenberg, unpublished). Secondly, transmission of the pathogen in the laboratory from one dipteran host to other dipteran hosts, including those in other families, has been successful in several cases (Kramer and Steinkraus, 1981; Mullens, 1989; Eilenberg *et al.*, 1990). It is therefore likely that several hosts can be involved in the epizootiology of the fungus, with transmission between different dipteran host species being common. Perhaps only some host species are involved in the entire life cycle; this could explain why resting spores are commonly found in only a limited number of host species. In addition to *E. muscae* and *E. schizophorae*, two similar species originally described as *E. muscae sensu lato* that attack higher Diptera have also been recognized: *Entomophthora syrphi* (Fig. 4.17) and *Entomophthora scatophagae*. They differ in morphological features and their host range in the field. The ability of these latter species to be transmitted between dipteran hosts has, however, not been examined thoroughly; *E. syrphi* was impossible to transmit to *M. domestica* in the laboratory and recent molecular data supports the hypothesis that *E. schizophorae* and *E. syrphi* are different from *E. muscae sensu stricto* (Jensen and Eilenberg, 2000).

The extent to which the natural host range of *E. muscae* and *E. schizophorae* goes beyond Diptera is still largely unknown. *E. muscae* has been recorded from Hymenoptera in the field and infection of Hymenoptera has been achieved in the laboratory. The hymenopteran species *Torymus druparum* occurs at the same time and at the same site as certain dipteran species and infections with fungal pathogens resembling *E. muscae* were found among *T. druparum*. In the laboratory it was possible to transmit *E. muscae* from *T. druparum* to *M. domestica* and *E. schizophorae* from *T. druparum* to *Chamaepsila rosae* (Eilenberg *et al.*, 1987; J. Eilenberg, unpublished). Cluster analysis of molecular data on *E. muscae* from *T. druparum* clustered among other *E. muscae* isolates from Diptera (Jensen and Eilenberg, 2000). The potential life cycle of *E. muscae* could, therefore, be even more complicated.

Epizootiology in annual cropping and livestock-related ecosystems

For some host–pathogen relationships the entire life cycle, including production of resting spores, has been observed in field populations: *Delia radicum*/*E. muscae* (Thomsen and Eilenberg, 2000), *Delia antiqua*/*E. muscae* (Carruthers *et al.*, 1985) and *Delia coarctata*/*E. muscae* (Wilding and Lauckner, 1974). For other host–pathogen relationships, resting spores occur rarely (e.g. *M. domestica*/*E. muscae* (Mullens *et al.*, 1987; Steinkraus *et al.*, 1993b)) or have never been seen in natural populations of the host, even during epizootics (e.g. *C. rosae*/*E. schizophorae* (Eilenberg and Philipsen, 1988)).

E. muscae and *E. schizophorae* have the ability to establish epizootics in populations of *M. domestica*, *Delia* spp. and *C. rosae*, with high prevalences of infection in the host population (*c.* 50–80%) developing within a few weeks (Table 4.2). Some epizootics show sharp peaks in infection and thus the epizootics occur rapidly, while other epizootics establish more slowly and are prolonged. Epizootics can be found both indoors and outdoors. Outdoor studies have shown that there is a tendency for epizootics during late summer and autumn to last longer than epizootics during spring and early summer.

Time to kill is strongly dependent on incubation temperature. At 21°C, *E. muscae* took 7 days to kill *D. antiqua* adults, and this time was extended to > 17 days at 10°C (Carruthers and Haynes, 1985). Similar patterns were observed for *C. rosae* infected with *E. schizophorae* (Eilenberg, 1987a) and *M. domestica* infected with *E. muscae* (Bellini *et al.*, 1992). Primary conidia are discharged from cadavers at a rate dependent on temperature. At 21°C, the peak discharge rate for *E. muscae* from *M. domestica* was 10–12 h post-mortem (Mullens and Rodriguez, 1985). For *C. rosae* infected with *E. schizophorae*, the peak discharge rate was approximately 8 h post-mortem at 20°C (Eilenberg, 1987a). The majority of primary conidia of *E. muscae* and *E. schizophorae* were discharged within 3.75 cm of the cadaver, although a few conidia were discharged as far as 8.75 cm (Six and Mullens, 1997). The total number of conidia discharged per infected individual was calculated to be 5.1×10^4 for *E. schizophorae* (Eilenberg, 1987a). The factors governing the total number of primary conidia per individual were cadaver weight and sex. Since females are larger than males, they produce more conidia (Mullens and Rodriguez, 1985). Secondary conidia of *E. muscae* are significantly more infective than primary conidia (Bellini *et al.*, 1992).

A number of studies have described significant behavioural aspects of the interaction between *E. muscae*/*E. schizophorae* and their hosts. During mating, males, of *M. domestica* are significantly more attracted to *E. muscae*-killed females compared with uninfected females (Møller, 1993). After contact, these males become infected and are

also capable of transmitting conidia to uninfected females during subsequent copulations (Watson and Petersen, 1993a). The fungus is favoured by these behavioural changes, which enhance the chances for dissemination of the fungus in the host population.

After infection, *E. muscae* induces further behavioural changes. *E. muscae*-infected *M. domestica* prefer higher temperatures than uninfected flies (the so-called 'behavioural fever'), with the result that the fungus may die and the fly survive (Watson *et al.*, 1993). This sort of self-cure or resistance based on behaviour is clearly disadvantageous for the fungus.

Host-plant recognition for ovipositing plant-feeding flies can be disturbed by fungus infection. Females of *C. rosae* infected with *E. schizophorae* did not recognize their host plants, even though they were physically capable of depositing eggs. This behaviour was documented in the field by Eilenberg (1987b) who found fertile eggs of *C. rosae* deposited on leaves in hedges at heights of several metres. The eggs were far away from the normal host plant, the carrot, with little chance of survival (Fig. 4.43). This behaviour will influence the effect of *E. schizophorae* on *C. rosae* populations, since infected females will not contribute to population growth.

Towards the end of an infection with *E. muscae*, *M. domestica* is severely affected. An infected fly passes through several phases governed by an endogenous clock, moving more and more sluggishly (Krasnoff *et al.*, 1995). The final result is that the flies attach themselves to the vegetation with their abdomen exposed and, after death, the fungus begins to discharge primary conidia.

These behavioural aspects are important for an understanding of host–pathogen interactions. The fungal-induced changes may eventually prove to be keys to success or failure in biological control programmes. Much has yet to be learnt, because all studies, except one, refer to *M. domestica*. No documentation of similar behavioural aspects in, for example, *Delia* spp. or comparative studies with other isolates of these fungi have been reported.

Neozygites fresenii *(Neozygitaceae)*

Taxonomy and distribution

The first member of this genus and family was described as *Empusa fresenii* from aphids by Nowakowski (1883). Then Witlaczil (1885) described an aphid infection as *Neozygites aphidis*, believing the pathogen to be a gregarine protozoan. Thaxter (1888), unaware of the description by Witlaczil, placed *E. fresenii* in a new subgenus, *Triplosporium*. Giard (1888) noted that the genera *Triplosporium* and *Neozygites* were synonymous. Remaudière and Keller (1980) replaced *Triplosporium* with *Neozygites*. Much of the literature prior to 1980 used the genus *Triplosporium*. Humber and Soper (1981) proposed conservation of the genus *Triplosporium* but this proposal failed, and 15 *Neozygites* species are currently recognized (Keller, 1997). More undescribed species of *Neozygites* from Collembola have recently been discovered (Dromph *et al.*, 1999).

N. fresenii has a worldwide distribution with reports of infected aphids from Africa (Silvie and Papierok, 1991), Australia (Milner and Holdom, 1986), Europe (Gustafsson, 1965a; Thoizon, 1970; Dedryver, 1978), India (Ramaseshiah, 1968), Israel (Bitton *et al.*, 1979), the South Pacific (Keller, 1997) and North America (Kuntz, 1925; Soper and MacLeod, 1963; Thaxter, 1888). This is considered the most common aphid pathogen of tropical regions (Remaudière, 1977). *Neozygites floridana* has

been reported from India (Ramaseshiah, 1971), West Africa (Yaninek *et al.*, 1996), Brazil (Delalibera *et al.*, 1992), Israel (Kenneth *et al.,* 1972), Poland (Mietkiewski *et al.*, 1993), and the USA (Brandenburg and Kennedy, 1981; Smitley *et al.*, 1986).

Host specificity

The members of the Neozygitaceae are specialized as pathogens of small arthropods, mainly mites (Acari), springtails (Collembola), thrips (Thysanoptera) and aphids (Hemiptera) (Keller, 1997). Because the hosts of the Neozygitaceae are small (Fig. 4.48), *Neozygites* spp. may be under-studied compared with species in the Entomophthoraceae, which infect larger arthropods, such as Lepidoptera, Diptera and Coleoptera.

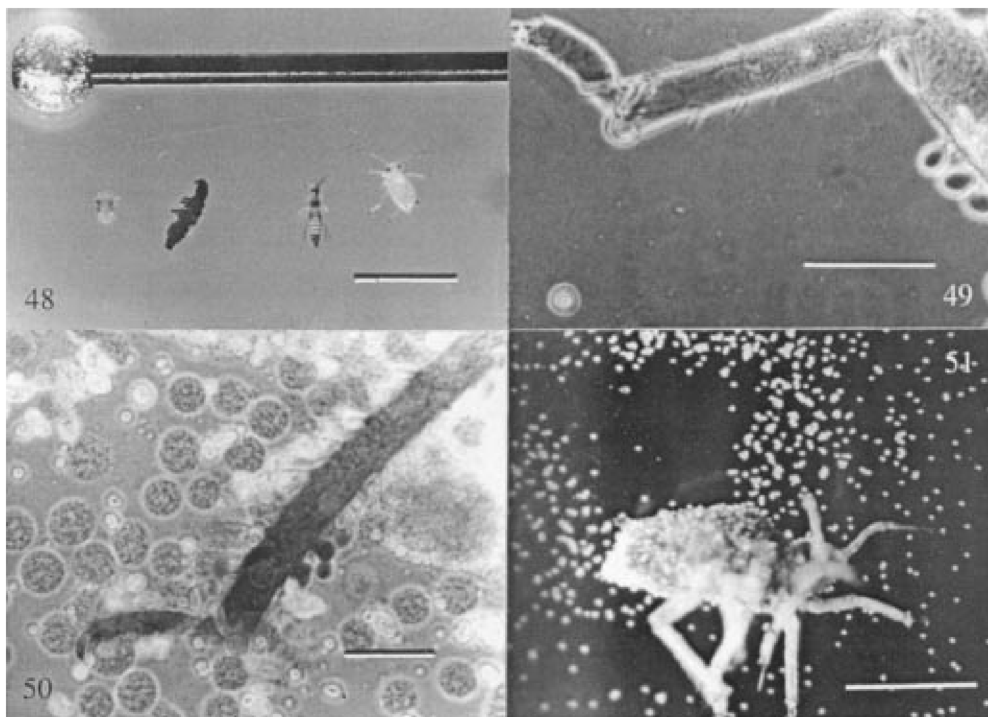

Fig. 4.48. Common hosts of Neozygitaceae next to an insect pin to show their small size. From left to right: spider mite, *Tetranychus urticae* (Acari: Tetranychidae); unidentified collembolan; unidentified thrips (Thysanoptera); cotton aphid, *Aphis gossypii* (Hemiptera: Aphididae). Scale bar = 2 mm.
Fig. 4.49. Leg of a cotton aphid showing infective capilliconidia (secondary spores) of *Neozygites fresenii* attached to the femur. The capilliconidia are the infective spore form and are tightly attached to the insect cuticle by the sticky mucoid hapteron on their apex. Scale bar = 100 μm.
Fig. 4.50. Cotton-aphid leg surrounded by protoplasts of *N. fresenii* liberated from the haemocoel of the host after squashing. Protoplasts are a wall-less vegetative form of the fungus and develop walls shortly before conidiophores are formed. Scale bar = 60 μm.
Fig. 4.51. *N. fresenii*-infected cotton aphid in conidial stage. Conidiophores have erupted through the host's integument, formed conidia and have explosively discharged conidia for several millimetres. Scale bar = 0.5 mm.

What limits the Neozygitaceae to small arthropods? Their common hosts, plant-feeding mites, aphids and thrips, are associated with plant structures. Ancestral *Neozygites* species may have made host shifts from mites to aphids or thrips, or vice versa, because of the close proximity of these various hosts. However, this does not explain why many other arthropods that occur in the same situations do not become infected. During *N. fresenii* epizootics in *Aphis gossypii* populations in Arkansas, many insects in a cotton field are contaminated with capilliconidia (the infective stage) of *N. fresenii* (D. Steinkraus, unpublished data), providing an opportunity for host shifts to occur. Yet hosts other than aphids have not been reported for *N. fresenii*. There also appears to be isolate specificity with respect to aphid host species (L.T. Villacarlos, unpublished).

Few experimental host-range studies have been conducted with *Neozygites* spp. At present it appears that *N. fresenii*, *Neozygites cinarae*, and *Neozygites microlophii* attack only aphids, while others, such as *Neozygites cucumeriformes* and *Neozygites parvispora*, are known only from Thysanoptera (Balazy, 1993) and *Neozygites sminthuri* only from Collembola (Keller and Steenberg, 1997). *N. floridana* and *Neozygites tetranychi* appear to be restricted to mites in the Tetranychidae. For more detailed discussion of host specificity of individual species in the Neozygitaceae, see Keller (1997).

Epizootiology in annual cropping ecosystems

The Neozygitaceae are the most important pathogens of Acari, Aphididae, Collembola and Thysanoptera in some geographical regions, particularly in tropical areas (Table 4.2). In addition to these specific examples, *Neozygites* spp. are recognized as important natural control agents of cassava green mite, *Mononychellus tanajoa,* in Brazil and West Africa (Fig. 4.24; Keller, 1997; Elliot *et al.*, 2000). Similar studies have shown the importance of *Neozygites* spp. in the natural control of mites on groundnuts (Boykin *et al.*, 1984), cotton (Carner and Canerday, 1968) and lima beans (Brandenburg and Kennedy, 1983). Their impact is also extremely fast; Klubertanz *et al.* (1991) found that a *Neozygites* sp. reduced two spotted spider mite, *Tetranychus urticae*, populations by up to 95% over a 6-day period on soybean.

There are a number of reasons why the Neozygitaceae are effective in causing epizootics in mites and aphids. First, their life cycles are rapid. The time from the initial aphid contact with a capilliconidium to the death of the host and fungal sporulation can be as short as 3 days (Steinkraus *et al.*, 1993a). Secondly, whereas some Entomophthorales attack only one host life stage, *Neozygites* spp. attack all stages, except eggs. Thirdly, a large number of primary conidia are produced per host, even though the hosts are small. The life cycle starts with the primary conidium, about 15 μm in diameter. Primary conidia germinate within 6–9 h to form capilliconidia, the infective stage. Almond-shaped capilliconidia are formed on the apex of capilliconidiophores and have a sticky mucoid hapteron. Capilliconidia are formed at the height of an aphid's femur. When aphids walk across a leaf, capilliconidia adhere tightly to the aphid (Fig. 4.49), rapidly germinate and penetrate the aphid's exoskeleton. Once within an aphid's haemolymph, the fungus initially reproduces vegetatively as protoplasts (Fig. 4.50) and, after 3 days, forms hyphal bodies. Three to four days after the aphid host is penetrated by the fungus, the aphid dies and about 3000 primary conidia of *N. fresenii* are explosively discharged per infected aphid (Fig. 4.51) (Steinkraus *et al.*, 1993a). Approximately 75% of these conidia enter the air, while 25% immediately hit the leaf on which the cadaver is located. Fourthly, during cotton aphid epizootics, the num-

ber of conidia of *N. fresenii* present in the air is immense. Conidia of *N. fresenii* are readily distinguished from other particulate matter in the air by their characteristic shapes, sizes and staining properties (Fig. 4.52) (Thaxter, 1888; Soper and MacLeod, 1963; Steinkraus *et al.*, 1991). Rotorod aerial spore traps (Fig. 4.53) collected up to 90,000 *N. fresenii* primary conidia per cubic metre of air during the night in a cotton field in Louisiana (Steinkraus *et al.*, 1999), similar to results reported previously from Arkansas (Steinkraus *et al.*, 1996b). When healthy sentinel *A. gossypii* were exposed overnight for 8 h to the air in a Louisiana cotton field during an epizootic, up to 50% of the aphids became infected (Steinkraus *et al.*, 1999).

The discharge of *Neozygites* conidia into the air appears to be strictly a mecha-

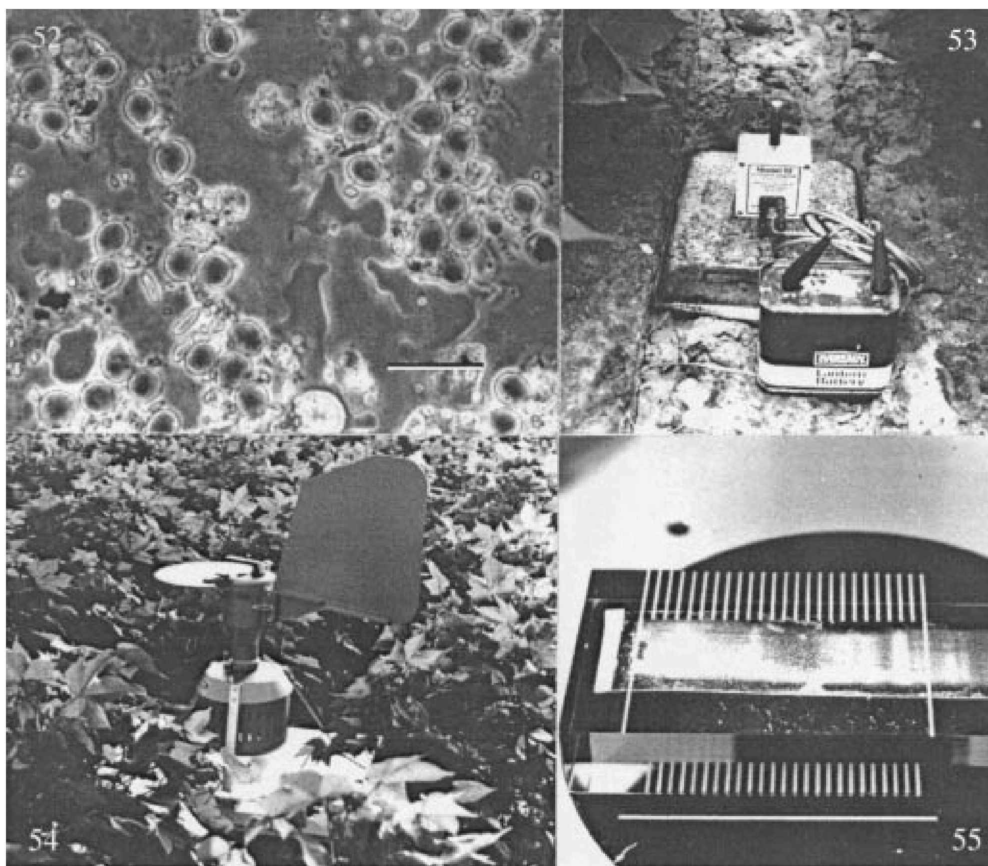

Fig. 4.52. Primary conidia of *Neozygites fresenii* trapped on the silicone grease-treated surface of a plastic rod from a rotorod aerial spore trap. The conidia were collected from the air in a cotton field in Louisiana during an epizootic in cotton aphids. Scale bar = 40 μm.
Fig. 4.53. Rotorod sampler in a cotton field. The battery-powered aerial spore sampler spins two plastic rods through the air, catching particles such as spores, dust and pollen.
Fig. 4.54. Burkard 7-day aerial spore trap in a cotton field. This trap sucks air through an orifice, aerial particles are impacted on a silicone grease-treated tape and can be quantified by hour for 7 days.
Fig. 4.55. A 24 h portion of the 7-day collection of aerial particles from a Burkard trap. Each 24 h section of the adhesive trap can be mounted on a slide, examined under a microscope and the spores present in the air quantified. Scale bar = 50 mm.

nism of dispersal, both short-range and long-range, and not directly involved in the infection process. Studies on the aerobiology of *N. fresenii* showed that most conidia in the air were primary conidia, with capilliconidia making up only 11.3% of the forms collected (Steinkraus *et al.*, 1996b). Most probably, capilliconidia found in the air were dislodged from the surfaces of leaves. Primary conidia of *N. fresenii* were damaged by relative humidities (RH) below 90%. Steinkraus and Slaymaker (1994) found that exposure of *N. fresenii* primary conidia to 75% RH for only 1 min significantly reduced subsequent capilliconidia formation to 29% versus 76% at 100% RH. The deleterious effect of low humidity on primary conidia is mitigated by the periodicity of discharge of *N. fresenii*. Counts of conidia caught in aerial spore traps (Figs 4.54, 4.55) indicated that active discharge of primary conidia occurred mainly between 0100 and 0300 h (Steinkraus *et al.*, 1996b, 1999). Capilliconidia are rapidly formed and are more resistant to lower humidities than primary conidia. Some capilliconidia remain infective for 2 weeks at 75% RH (Steinkraus and Slaymaker, 1994). Therefore, *N. fresenii* has the capability to kill its hosts shortly after nightfall, and then rapidly produce and discharge its primary conidia at night when the RH is high. Before daylight most of the low-humidity-sensitive primary conidia have germinated to form the more long-lived infective capilliconidia.

Some individual insects infected with *Neozygites* spp. form resting spores (zygospores) (Fig. 4.84). These spores are generally dark-walled and provide a long-lived spore form in the soil or on tree bark (Bitton *et al.*, 1979). In Israel resting spores of *N. fresenii* were synchronized to germinate in the spring concurrent with the build-up of *Aphis spiraecola* aphids on citrus trees (Bitton *et al.*, 1979). Thus, resting spores germinate under certain environmental conditions to produce capilliconidia that infect new hosts. Resting spores are not explosively discharged, and hosts infected with this stage of the pathogen generally become very fragile so that the host's cuticle is easily ruptured, dispersing resting spores on to plant surfaces and the soil.

The Neozygitaceae are unusual entomophthoraleans in that they appear to function best in hot weather. This is important because many crop pests, particularly mites and aphids, reproduce most rapidly during midsummer. For instance, *Erynia neoaphidis* has been found infecting *A. gossypii* on cotton in late autumn and *Myzus persicae* on winter spinach in Arkansas (Fig. 4.2), but has not been found during the summer in these areas (McLeod *et al.*, 1998). Thus, unlike *N. fresenii*, *E. neoaphidis* is of no importance in controlling the immense cotton aphid outbreaks that occur across the USA during June and July (Steinkraus *et al.*, 1995). The fact that *Neozygitaceae* are adapted to hot, humid, even tropical conditions has been reported by many researchers (Gustafsson, 1965a; Steinkraus *et al.*, 1991; Keller, 1997). In the Mississippi delta, epizootics of *N. fresenii* occur even in non-irrigated fields that have experienced no rain for up to a month before the epizootic. Epizootic development under these conditions is apparently supported by high night-time humidities.

Entomophaga maimaiga *(Entomophthoraceae) and other entomophthoralean fungi from forest Lepidoptera*

Several species of entomophthoralean fungi cause epizootics in outbreak species of forest-dwelling Lepidoptera in North America: *Entomophaga maimaiga* (Figs 4.56–4.60) from *Lymantria dispar*, *E. aulicae* and *Zoophthora radicans* from *Choristoneura fumiferana* and *Lambdina fiscellaria*, and *Erynia gastropatchae* (= *Furia crustosa*) from

Malacosoma disstria (Figs 4.61–4.63). *E. maimaiga* has been most studied and a recent review was published on this species (Hajek, 1999). In the following sections, comparisons are drawn among the four host–pathogen systems to investigate commonalities and differences in strategies developed by these different entomophthoralean species occupying similar ecological niches in forests.

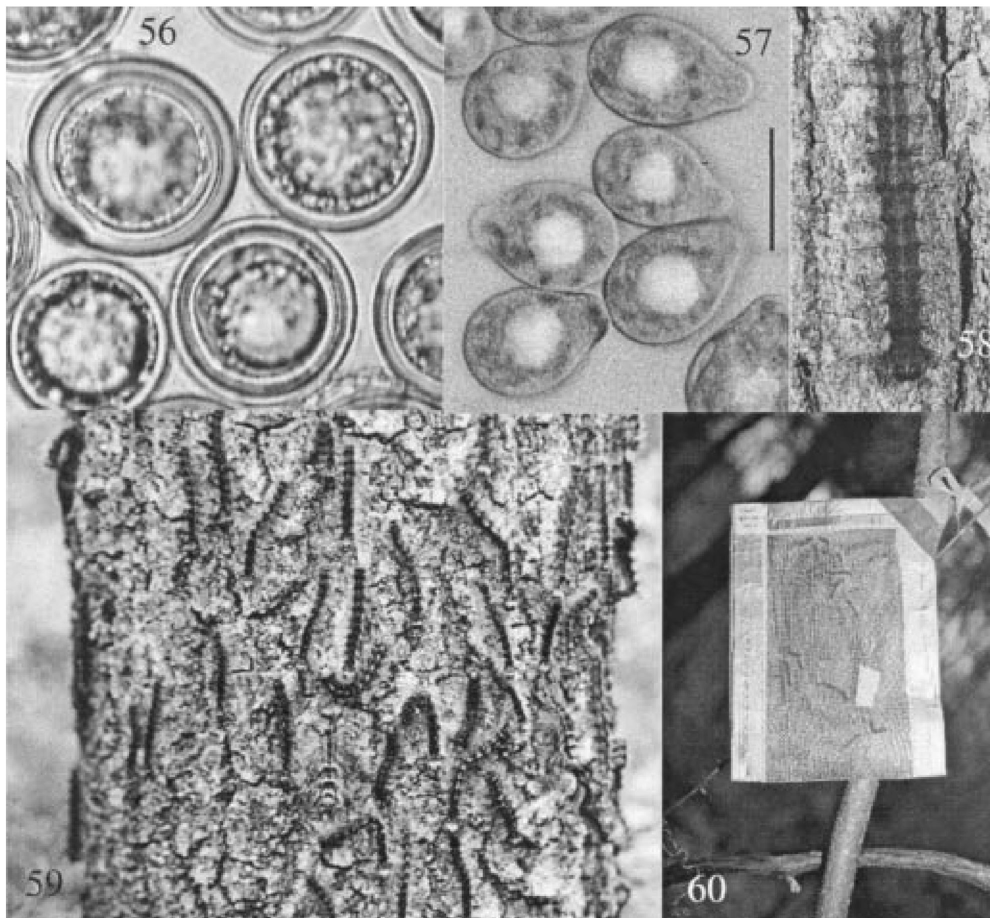

Fig. 4.56. Mature resting spores of *Entomophaga maimaiga.* Scale bar = 20 µm.
Fig. 4.57. Pear-shaped, primary conidia of *E. maimaiga.* The nuclei are stained with aceto-orcein. Scale bar = 20 µm.
Fig. 4.58. Cadaver of a late-instar larva of gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantridae), infected with *E. maimaiga*. Although there is no external indication of fungal infection, this cadaver is filled with resting spores (azygospores) and chacteristically is attached to tree bark by prolegs, with head downwards. Earlier instars killed by *E. maimaiga* externally produce conidia (see Fig. 4.10). Photograph: Donald Specker.
Fig. 4.59. An epizootic caused by *E. maimaiga* in late-instar gypsy moth. Photograph: Gary Bernon.
Fig. 4.60. Window-screening cages used to expose gypsy-moth larvae in the field to evaluate location and activity of *E. maimaiga* inoculum.

Taxonomy and distribution

E. maimaiga is thought to be native to northern Asia (Japan, northern China, Korea and far-eastern Russia) but has also been reported establishing epizootics in North America since 1989 (Andreadis and Weseloh, 1990; Hajek *et al.*, 1990b; Table 4.2). Comparisons at the molecular level have shown that *E. aulicae* is a species complex, with *E. maimaiga* included in one of four groups. Fungi in the other three groups currently retain the name *E. aulicae* (Walsh, 1996). While this species complex is worldwide in distribution, one of the *E. aulicae* groups (group III) is known only from one strain isolated in Europe (only one European isolate was available for comparison at the molecular level). Among the two remaining *E. aulicae* groups, many isolates belong to group I, which is associated with a diversity of lepidopteran families and is known from both North America and Japan. Group II has been isolated only from Noctuidea in North America.

Host specificity

The species *E. maimaiga* was initially differentiated from the *E. aulicae* species complex, in part, because this is the only member that is known to infect *L. dispar* (Soper *et al.*, 1988). Host specificity of this species has been extensively studied due to its potential application for control. In the laboratory, *E. maimaiga* infected 36% of 78 non-target lepidopteran species tested at a concentration of conidia yielding > 50% infection in *L. dispar*, although per cent infection was usually low (Hajek *et al.*, 1995a). The only non-targets consistently infected at high levels were species of Lymantriidae (the family including *L. dispar*). To evaluate host specificity in the field, 1511 lepidopteran larvae of 52 species belonging to seven lepidopteran families were collected and reared during *E. maimaiga* epizootics in sympatric *L. dispar* populations. Throughout the field season only two non-target individuals were infected: one lasiocampid and one noctuid (Hajek *et al.*, 1996a). Due to the discrepancy between host specificity in the laboratory (physiological host range) and in the field (ecological host range), it is hypothesized that, in addition to physiological interactions between host and pathogen, temporal and spatial factors are also very important in determining the host specificity of *E. maimaiga*.

There are two main conditions under which *E. maimaiga* is unable to successfully attack hosts. First, *E. maimaiga* can develop in some non-host lepidopterans if injected into the haemocoel, but it cannot penetrate the insect cuticle (Hajek *et al.*, 1995a). For other non-host lepidopterans, the fungus cannot survive even when injected directly into the haemocoel (Hajek *et al.*, 1995a). *E. maimaiga* and *E. aulicae* both occur in the host haemocoel as protoplasts lacking cell walls, which is thought to aid in escaping detection by hosts. However, prolonged elevated levels of prophenoloxidase have been detected in *L. dispar* larvae inoculated with *E. aulicae* (Bidochka and Hajek, 1998), suggesting that this species is detected by the immune response. Plasma membranes of protoplasts contain glycoproteins that differ between *E. maimaiga* and *E. aulicae*; it is hypothesized that these surface glycoproteins may lead to detection of protoplasts in non-permissive hosts (Bidochka and Hajek, 1996).

While *Z. radicans* has been isolated from many different insects, bioassays have demonstrated that at least some strains of this species may be quite host-specific, even to the family or species levels. *E. gastropatchae* has been isolated only from members of the genus *Malacosoma* and appears to be very host-specific (Figs 4.61–4.63). In

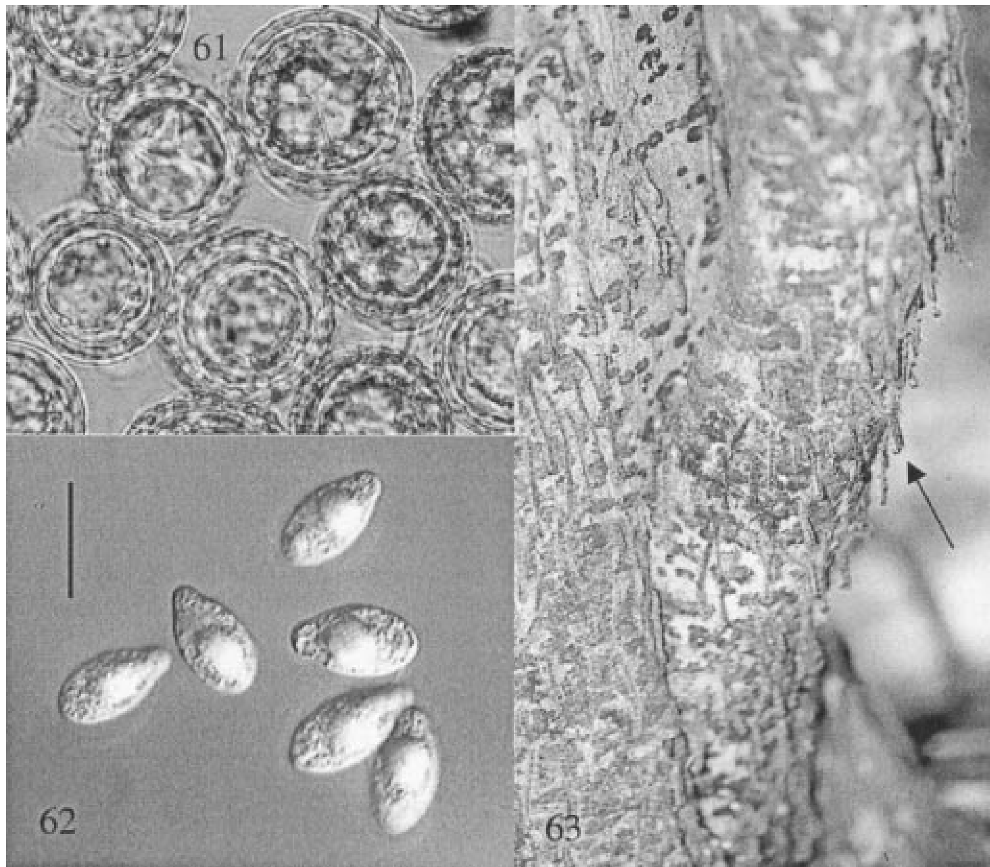

Fig. 4.61. Mature resting spores of *Erynia gastropachae* having undulate epispores. Scale bar = 20 μm.
Fig. 4.62. Primary conidia of *E. gastropachae.* Scale bar = 20 μm. Photograph: Melanie Filotas.
Fig. 4.63. Cadavers of late instars of *Malacosoma disstria* (Lepidoptera: Lasiocampidae) infected and killed by *E. gastropatchae.* Cadavers are filled with resting spores (azygospores) and hang head downwards (arrow).

contrast, *E. aulicae* group I has been isolated from many lepidopteran families, though, as with *Z. radicans*, this does not preclude specificity of individual strains (Walsh, 1996).

Epizootiology in forest ecosystems

Forest ecosystems differ substantially from agricultural or pasture environments, where much of the research on entomophthoralean fungi has been conducted. Many forests are natural ecosystems, and the soil and leaf litter often remain largely undisturbed. After host death, cadavers of entomophthoralean-killed larval Lepidoptera containing resting spores (azygospores in the case of *E. maimaiga*) are often found attached to tree trunks (Figs 4.58, 4.59, 4.63). *E. maimaiga*-infected cadavers eventually fall to the ground, where resting spores are rapidly released from them, especially when it rains

(Hajek *et al.*, 1998b). The majority of resting spores remain in the organic layer of the soil, with mean densities of 4751 *E. maimaiga* resting spores g^{-1} dry soil at the bases of trees after an epizootic (Hajek *et al.*, 1998a). The greatest concentrations of resting spores occur directly around the bases of trees (Hajek *et al.*, 1998a). Resting spores are retained near the soil surface, where, upon germination, germ conidia can be actively discharged. Although some resting spores of *E. maimaiga* germinate the year after production, many do not (Hajek and Humber, 1997). Bioassays conducted by caging larvae on soil have demonstrated that *E. maimaiga* resting spores can persist in undisturbed forest soil for at least 6 years (Weseloh and Andreadis, 1997).

Forests also differ from habitats with only herbaceous vegetation due to the depth of the plant canopy. Many forest Lepidoptera feed on foliage quite distant from the soil, but the principal reservoir of entomophthoralean resting spores is in the soil. Therefore, either the hosts must travel to the soil for primary infection to occur, or infective entomophthoralean conidia must travel to the hosts. Both germ conidia from germinating resting spores and primary conidia from the surfaces of cadavers are actively discharged. The extent to which germ conidia produced from soil-borne resting spores become airborne is not known. Studies of this phenomenon are clearly difficult to undertake because germ conidia are morphologically identical to primary conidia, although they can differ reproductively (Hajek, 1997a). Infections initiated by resting spores produce only conidia, while infections initiated by conidia from cadavers can produce either conidia or resting spores (Hajek, 1997b). For infections initiated by conidia discharged from cadavers, production of resting spores versus conidia has been associated with a diversity of factors: host age or moulting status, environmental conditions, including temperature, season, light and humidity, fungal dose, isolate or combination of isolates and attenuation (Hajek and Shimazu, 1996). Cadavers of *L. dispar* larvae producing *E. maimaiga* conidia are generally found in the foliage (Hajek *et al.*, 1998b). Therefore, when conidia are discharged from cadavers, there is a good chance that they will become airborne. Sampling the air-spora and exposing caged larvae (Fig. 4.60) within a forest demonstrated that *E. maimaiga* conidia were abundant in the air at particular times (Hajek *et al.*, 1999). Abundance of *E. maimaiga* conidia in the air was autocorrelated and positively associated with leaf wetness and wind and negatively associated with temperature. These results emphasized the episodic nature of conidial abundance and the association with weather events of prolonged duration. The movement of wind carrying entomophthoralean conidia would differ between agricultural settings with a low plant canopy and forests with a much deeper plant canopy. Studies of the dispersal of *E. maimaiga* have shown that rates of spread differ on local and regional scales. Presumably different mechanisms for dispersal of conidia occur within forest stands and above the forest canopy where airborne conidia are transported long distances (Dwyer *et al.*, 1998).

Some forest lepidopteran larvae have a great tendency to wander, especially as late instars. For each of the four species of fungi being discussed, infection may be enhanced when hosts travel to the ground. For *E. aulicae* and *Z. radicans*, infection was not noted until after *C. fumiferana* became fifth instars (Vandenberg and Soper, 1978; Perry and Regnière, 1986). Perry and Regnière (1986) deduced an association between increased infection in late instars and the peak of larval wandering at the fifth to sixth instar; in fact, an epizootic caused by *E. aulicae* occurred during a season of defoliation, which apparently enhanced infection due to increased wandering on the soil by larvae searching for food. Similarly, *E. gastropatchae* epizootics have only been noted in the field after *M. disstria* became late instars and wandered on the forest floor (M.J.

Filotas and R.S. Soper, personal communciations). Similarly, late instars of *L. dispar* wander in the leaf litter and rest there during daylight hours, while second and third instars primarily feed in the foliage. Few *E. maimaiga* infections have been found among early instars of *L. dispar*, with infection prevalence only increasing once late instars were present (Hajek, 1997c). Empirical data from all of these host–pathogen systems have fostered the hypothesis that resting spores predominantly initiate cycles of infection once late instars wander on the forest floor. However, early instars also have contact with the forest floor, either during neonate dispersal (e.g. *L. dispar* ballooning) or when early instars fall or are blown from trees. Studies of the activity of *E. maimaiga* resting spores demonstrate initiation of germination just prior to *L. dispar* egg hatch and continued germination throughout the period of larval activity (Hajek and Humber, 1997; A.E. Hajek, unpublished data). It is not known whether resting spores of *E. aulicae*, *E. gastropatchae* or *Z. radicans* germinate for such a prolonged period. Germination of *E. maimaiga* resting spores is positively associated with soil moisture (Hajek and Humber, 1997).

A simulation model of *E. maimaiga* infection cycles in *L. dispar* has demonstrated that infections initiated by conidia produced on killed hosts are primarily responsible for the exponential increase in infection characteristic of epizootics. Therefore, epizootics develop due to cycles of conidial infections initiated throughout the season when larvae contact the soil (predominantly as mobile stages, such as late instars) and become infected. Information about differential exposure to fungal conidia based on larval behaviour has been incorporated into a simulation model for *E. maimaiga* (Hajek *et al.*, 1993). The model estimated that four to nine cycles of infection could occur during the yearly field season of *c.* 2 months, with ambient moisture levels strongly influencing the number of infection cycles.

L. dispar larvae held at 20°C die from *E. maimaiga* infection approximately 4–6 days after conidial inoculation (Hajek *et al.*, 1993). *E. maimaiga* (Hajek, 1989) and *E. aulicae* (Tyrrell, 1990) infections did not cause overt changes among infected larvae until weight gain decreased 2 days or 1 day prior to death, respectively. Changes in the behaviour of *C. fumiferana* larvae began with sluggishness at *c.* 5 h before death to near-immobility 4 h later (Tyrrell, 1990). For *E. maimaiga*, after larval death there is a lag of *c.* 17 h before conidia are produced from cadavers (Hajek *et al.*, 1990a) or > 2 days before mature resting spores are produced (Hajek and Humber, 1997). Either type or both types of propagule can be produced from an individual cadaver. The primary determinant of the type of spore produced is larval instar, with conidia predominantly produced from *E. maimaiga*-killed early instars and resting spores produced within later instars (Hajek and Shimazu, 1996). Third to sixth instars of *L. dispar* infected with *E. maimaiga* were more abundant in the understorey vegetation than high in the tree canopy (Hajek and Webb, 1999). Cadavers of *E. maimaiga*-killed *L. dispar* larvae producing conidia are most frequently found on the undersides of twigs and leaf petioles of understorey vegetation, while cadavers containing resting spores are generally found attached to lower regions of tree trunks (Hajek *et al.*, 1998b). The extent to which the differential distribution of cadavers within trees is determined by the fungus is not known. However, healthy early instars of *L. dispar* generally occur in the tree canopy, so the general location of conidia-producing cadavers of early instars does not differ markedly from the location of healthy early instars. Alternatively, late instars of *L. dispar* travel from dark, sheltered locations (leaf litter or under bark flaps) to the tree canopy and back daily and therefore spend at least part of this time on tree trunks. The predominant distribution of late-instar cadavers on tree trunks (and not

in the leaf litter or tree canopy) suggests a fungal-induced behaviour causing larvae to be located on tree trunks at the time of death, rather than in lower sheltered locations or in the tree canopy.

Entomophaga grylli *(Entomophthoraceae)*

Taxonomy and distribution

E. grylli was first collected in Europe from a *Gryllus* sp. and named by Fresenius (1858) and since then has been variously known as *Entomophthora grylli*, *Empusa grylli* and *Conidiobolus grylli* (Carruthers *et al.*, 1997). Since the first collections in Europe, *E. grylli* has been collected worldwide; however, it is now believed that these collections are not the same species as the original (Carruthers *et al.*, 1997). As with other entomophthoralean species, *E. grylli* exists as a complex of pathotypes, but none of these has been raised to species status. *E. grylli sensu stricto* appears to have a mainly European distribution. Pathotypes 1 and 2 are from North America, pathotype 3 is from Australia and pathotype 4 is from Japan and new pathotypes are continually being recorded (Carruthers *et al.*, 1989; Humber, 1989). Pathotypes 1, 2, 3 and 4 are sometimes referred to as *E. macleodii, E. calopteni, E. praxibuli* and *E. asiatica,* respectively, and we await formal descriptions. All pathotypes have been recorded causing epizootics (some examples in Table 4.2).

Host specificity

All members of the *E. grylli* species complex are pathogens of grasshoppers and locusts and, as such, are not pathogenic to other organisms (MacLeod, 1963; Carruthers *et al.*, 1989). Each of the two pathotypes/species from North America has a very different host range. Pathotype 1 infects band-winged grasshoppers in the Oedipodinae (e.g. Streett and McGuire, 1990), whereas pathotype 2 is associated with species in the Melanoplinae. These acridid species occur in the same habitat, but laboratory and field studies indicate that there is little or no cross-infection of the two pathotypes between these hosts (Pickford and Riegert, 1964; Carruthers and Soper, 1987; Ramoska *et al.*, 1988). The biology of these two pathotypes is also very different. Both pathotypes produce resting spores, which produce infective germ conidia to initiate the infection cycle in their hosts. In pathotype 1, this culminates in infected insects either producing actively discharged conidia for further cycling, or harbouring resting spores for overwintering. In pathotype 2, actively discharged conidia never occur, and resting spores are produced in all infected hosts. This pathotype is therefore thought to have only one infection cycle from each resting spore (Carruthers *et al.*, 1997). This has made interpretation of field sampling in the past very difficult.

Different grasshopper species have variable levels of susceptibility to the four different pathotypes and this seems to be resistance conferred at the level of the cuticle rather than after penetration (Ramoska *et al.*, 1988). Of all the pathotypes tested, pathotype 3 has the widest host range among North American grasshoppers (Ramoska *et al.*, 1988). This has raised concerns related to its release in classical control; however, in the field, only eight out of 20 species of grasshopper were found infected, and they were all pest species (Carruthers and Onsager, 1993). Further research has helped to confirm the ecological/physiological host range of *E. grylli*, pathotype 3 (Carruthers *et al.*, 1997).

Epizootiology in rangeland ecosystems

Infection with *E. grylli* is commonly called 'summit disease' as infected and dying insects exhibit abnormal behaviour and climb to the top of vegetation, dying in a head-up position, grasping the plant stems (Fig. 4.64; Evans, 1989). Insect death occurs in the late afternoon and early evening, synchronizing sporulation and infection with optimal conditions of high humidity, cool temperatures and zero ultraviolet radiation during the night (Carruthers and Soper, 1987; Carruthers *et al.*, 1988, 1992). Given suitable abiotic conditions, *E. grylli* is able to sporulate within hours of host death (Fig. 4.65). If abiotic conditions are not favourable, the cadaver desiccates, but the fungus remains viable for extended periods of time, with the ability to rehydrate, sporulate and desiccate repeatedly (Sawyer *et al.*, 1997). Spore trap data from areas adjacent to epizootics revealed very few *E. grylli* conidia, although many smaller conidia from other fungal species were collected (Chatigny *et al.*, 1979; Carruthers *et al.*, 1997).

The fungus proliferates within infected insects as protoplasts, only forming hyphal bodies 1–2 days prior to host death (Funk *et al.*, 1990). One study of the development of *E. grylli* pathotype 2 within grasshoppers demonstrated that the fungus only affected fat body and neural tissue in early stages of infection, and muscle tissue was penetrated only after host death (Funk *et al.*, 1993). Speed of kill is temperature-related but death usually occurs within 7 days, more slowly when resting spores are produced (Ramoska *et al.*, 1988; Carruthers *et al.*, 1992).

At the time that hyphal bodies are produced, disease symptoms also become more obvious; the host becomes sluggish, stops feeding and begins abnormal posturing behaviour. Egg laying and feeding are both retarded prior to death (Carruthers *et al.*, 1997). Recent studies with pathotype 1 infecting *Campanula pellucida* have shown that host behaviour also has an impact on pathogen survival and development in the host. Acridids naturally bask in direct sunlight, raising their body temperatures by up to 10–15°C above air temperature. This can raise the temperature in the haemocoel above the thermal limit of *E. grylli* (Carruthers *et al.*, 1992). This is the natural behaviour of the insect and is not encouraged by infection. However, in some areas of the USA, during some seasons, significant fungus mortality could occur due to host basking, a behaviour which, therefore, may cure infected insects.

In pathotype 1, differentiation to produce resting spores is favoured in late-stage instars at warmer temperatures (Carruthers *et al.*, 1997). Infected insects harbouring resting spores (Fig. 4.66) fall to the ground, releasing the spores into the soil at a variable rate, depending on rain and wind (Fig. 4.67). Here, spores may lie dormant for two or more seasons. The proportion of resting spores germinating depends on abiotic conditions, and germination of resting spores at a given site can continue for several weeks (Carruthers *et al.*, 1997). For pathotype 1 (and 3), intra-season cycles of infection, with epizootic potential, are possible from cadavers producing actively discharged conidia; these conidia are extremely pathogenic (Carruthers *et al.*, 1991) but also much more susceptible to extreme conditions, such as low humidity, high temperature and ultraviolet radiation (Carruthers *et al.*, 1988; Firstencel *et al.*, 1990). The average time for 50% of conidia-producing cadavers to disappear was 2.8 days in field experiments in Arizona and was clearly influenced by rainfall. The older a cadaver became without sporulating, the less likely it was to sporulate successfully. However, regardless of cadaver age, highest rates of sporulation were predicted when there was high humidity at night with associated long periods of leaf wetness (Sawyer *et al.*, 1997).

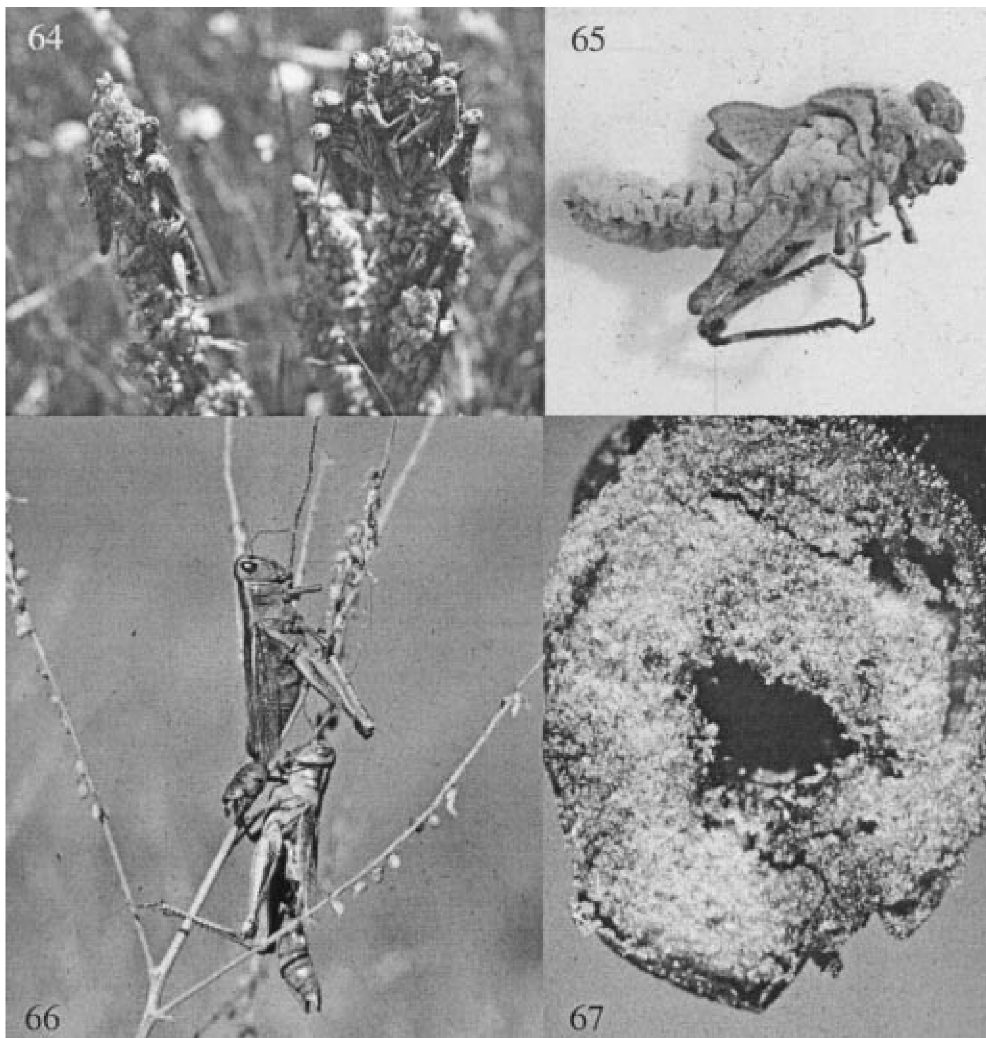

Fig. 4.64. Clear-winged grasshopper, *Campanula pellucida* (Orthoptera: Acrididae), infected with *Entomophaga grylli* pathotype 1. Infected cadavers sit in clusters at the top of the vegetation. Photographs Figs 4.64–4.67: Ray Carruthers.
Fig. 4.65. Dead *C. pellucida* with emerging conidiophores of *E. grylli* pathotype 1.
Fig. 4.66. Two-striped grasshopper, *Melanoplus bivittatus* (Orthoptera: Acrididae), infected with *E. grylli* pathotype 2. No conidiophores will emerge, but the bodies are filled with resting spores.
Fig. 4.67. Abdomen of *M. bivittatus* filled with resting spores of *E. grylli* pathotype 2.

Grasshoppers and locusts are highly mobile insects, and infection has little impact on their mobility during the early stages. Therefore, dispersing grasshoppers are likely to carry infection with them, at least over moderate distances. However, Carruthers *et al.* (1997) have suggested that long-range migration may, in part, have evolved as a way of escaping disease, particularly when the fungus can survive at a site for several seasons as resting spores. Carruthers *et al.* (1997) also noted that some species leave favoured feeding sites to lay eggs in more open areas, which may separate susceptible early instars from the overwintering sites of the fungus.

Erynia neoaphidis *(Entomophthoraceae)*

Taxonomy, distribution and host specificity

E. neoaphidis has a wide distribution, being recorded from Europe, Asia, Africa, North and South America, and Australasia (e.g. Wilding and Brady, 1984; Glare and Milner, 1991; Hatting *et al.*, 1999). Unusually, for such a common species, its genus remains a subject of debate, with different authors variously assigning it to *Erynia* (Keller, 1991), *Pandora* (Humber, 1989) and *Zoopthora* (Balazy, 1993), but in this text it will be considered as *Erynia. E. neoaphidis* has been recorded from > 70 species of aphids on annual and perennial crops, weeds and wild flowers (e.g. Wilding and Brady, 1984). Epizootics that contribute to the regulation of aphid populations are commonly recorded (Table 4.2) and, in some instances, the aphid population is reduced to near-zero on a local scale.

Virulence assays have tended to select highly pathogenic isolates under optimal conditions against a single life stage of a particular aphid species (e.g. Wilding, 1976). However, populations and biotypes of the same aphid species may differ significantly in their susceptibility; nymphs of the pea aphid *Acyrthosiphon pisum* are more susceptible than adults and alate adults are more susceptible than apterous adults (Milner, 1982, 1985a; Lizen *et al.*, 1985). Few studies have considered the relative susceptibility of a broader range of different aphid species or whether *E. neoaphidis* can move freely between different aphid species in the agroecosystem (Milner *et al.*, 1983). Glare and Milner (1991) suggest that those species that are most commonly found infected in the field are also those found most susceptible in laboratory assays. However, recent studies suggest this may not always be the case. Studies with an isolate of *E. neoaphidis* from the nettle aphid *Microlophium carnosum* showed that *A. pisum* was extremely susceptible, with an LC_{50} of 0.9 conidia mm^{-2}, while the cereal aphid *Sitobion avenae* was more resistant, with an LC_{50} of 34.9 conidia mm^{-2} (Hemmati, 1999). Yet epizootics of *E. neoaphidis* are common in both *A. pisum* and *S. avenae* in the UK, suggesting the possibility of pathotypes with specific host associations. Molecular techniques may be able to determine the genetic basis of some of these differences (Rohel *et al.*, 1997; Sierotzki *et al.*, 2000).

Epizootiology in annual cropping ecosystems

Infected aphids (Fig. 4.68) die at the end of the photophase, ensuring that sporulation occurs during the night, when conditions are humid, cool and free from ultraviolet radiation. The time of death is set by dawn, ensuring that aphids die at the end of the photophase (Milner *et al.*, 1984a). The position on the plant where infected aphids die appears to depend on the aphid species. For instance, in laboratory experiments, cadavers of *S. avenae* were found higher on wheat than uninfected individuals, but this was not the case for *A. pisum* cadavers (Roy, 1997). The effect in the field may be different. Cadavers are firmly attached to the foliage by discoid rhizoids and, at death, cystidia cause breaks in the cuticle around which the conidiophores emerge.

Conidiophores are branched and multinucleate, forming uninucleate lemon-shaped conidia, 18–35 × 10–15 μm in size (Fig. 4.69). They are actively discharged at an estimated velocity of between 5 and 19.5 m s^{-1} between 2 and 11 mm horizontally and 7 and 8 mm vertically away from the aphid cadaver, ensuring that they escape the boundary layer for dispersal. The maximum discharge distance and the total

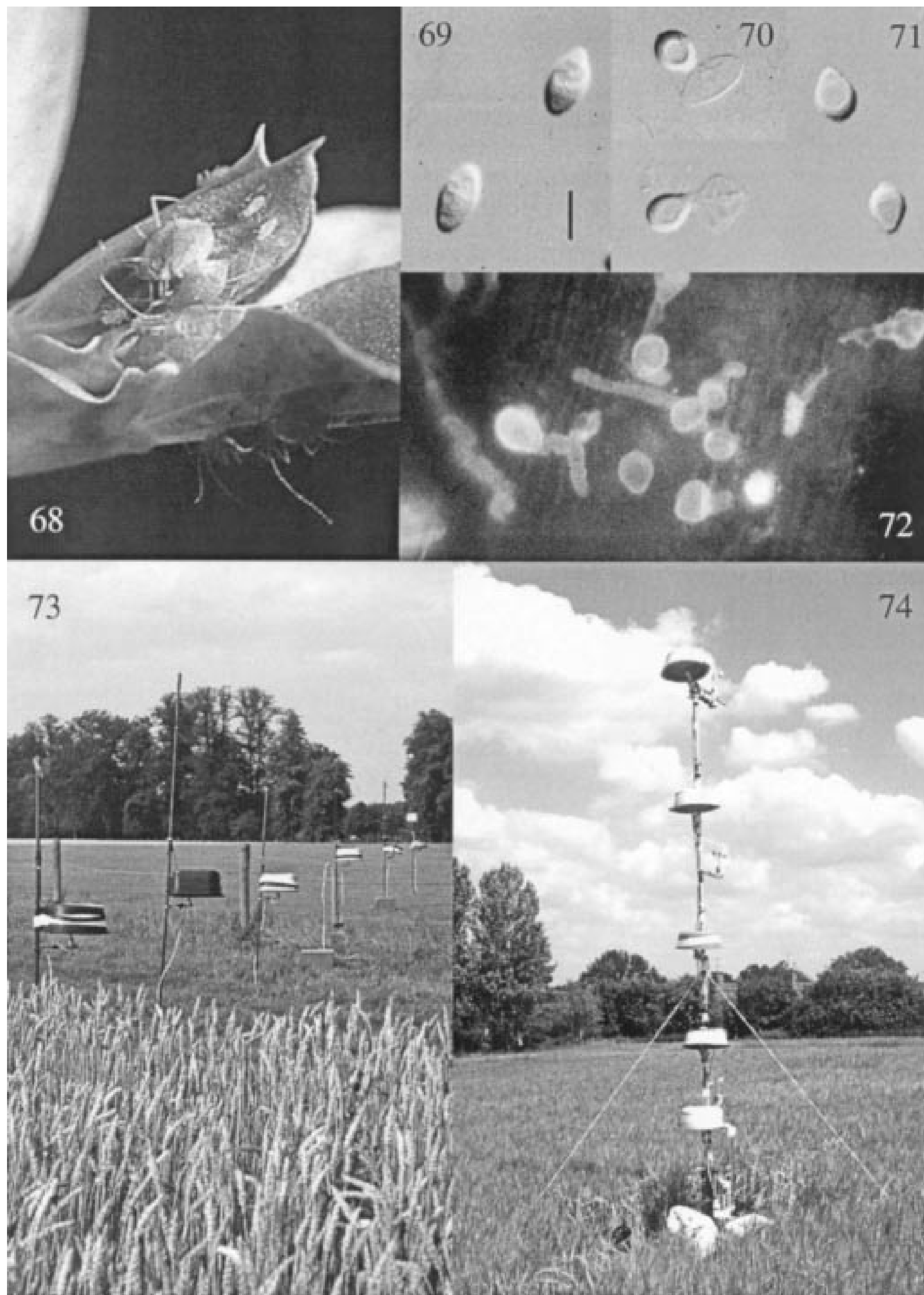

Fig. 4.68. *Erynia neoaphidis*-infected pea aphid, *Acyrthosiphum pisum* (Hemiptera: Aphididae). Conidiophores have erupted through the host's integument, have formed conidia and have explosively discharged conidia for several millimetres.
Fig. 4.69. Primary conidia of *E. neoaphidis.* Scale bar = 20 μm.
Fig. 4.70. Secondary conidia of *E. neoaphidis* being produced by primary conidia. Scale bar = 20 μm.

number of conidia produced are affected by temperature; greater numbers of conidia are discharged and they travel further at 18°C than at 10°C or 25°C (Hemmati *et al.*, 2001a). At 5°C, numbers of conidia produced are drastically reduced (Dromph *et al.*, 1997), as they are at 30°C (Glare and Milner, 1991). Infection of large aphid species results in the production of significantly more conidia than infection of small species (Glare and Milner, 1991); according to Hemmati (1999), *E. neoaphidis* produced 2.14 $\times 10^5$ conidia per cadaver of adult *A. pisum*, compared with 1.4 and 1.3×10^4 conidia per cadaver of the smaller aphids, *S. avenae* and *M. persicae*, respectively.

Sporulation begins within 2 h of aphid death and the majority of conidia are produced within 24 h at 20°C (Glare and Milner, 1991; Hemmati, 1999). Sporulation is significantly affected by humidity, being almost completely inhibited below 93% RH (Wilding, 1969). If humidities fall below 93% but then rise again, sporulation can recommence (Glare and Milner, 1991). Conidia produced within the first 4 h of sporulation germinated faster and were more infective to *A. pisum* aphids than conidia produced after 30 h (Morgan, 1994). Both sporulation and germination are inhibited by green leaf volatiles (Brown *et al.*, 1995; P.A. Shah, unpublished data).

Once discharged, many conidia enter the airstream with the potential to be passively dispersed. In the UK, conidia were sampled from the air using a Burkard aerial spore trap (Fig. 4.54) between June and August, when infected aphid populations were evident in the field (Wilding, 1970a). Greatest numbers of conidia and the establishment of epizootics were associated with periods of rainfall or just after periods of rainfall (Dean and Wilding, 1971, 1973). Most conidia were collected between 0400 and 0800 h in the morning when temperatures were between 10 and 16°C and RH was greater than 90%. The majority of conidia collected were primary conidia (Hemmati *et al.*, 2001b).

Using rotorod samplers at different heights above and distances away from a cereal field in the UK where an epizootic of *E. neoaphidis* was noted, the vertical and horizontal profiles of conidia have been measured (Figs 4.53, 4.73, 4.74). The majority of conidia were found at the height of the crop, but some were trapped up to 4 m above the crop, where they would certainly have been dispersed long distances from their origin, with the potential to infect new aphid populations. Conidia were trapped up to 20 m horizontally away from field sources (Hemmati, 1999).

Conidia are deposited on to foliage or hosts through a combination of settling and impaction. Rain will wash conidia out of the air. The velocity at which they settle (settling velocity) relates to the distance that they may travel before deposition and has been determined for *E. neoaphidis*. *In vitro*-produced conidia of *E. neoaphidis* are significantly larger than *in vivo*-produced conidia (Morgan, 1994). While primary conidia are lemon-shaped, secondary conidia are smaller and can either be of a similar shape or more rounded, depending on the temperature at which they were produced

Fig. 4.71. Secondary conidia of *E. neoaphidis.* Scale bar = 20 μm.

Fig. 4.72. *E. neoaphidis* conidia on aphid cuticle showing germ tubes and appressoria. Scale bar = 20 μm.

Fig. 4.73. *E. neoaphidis* conidia profiles are assessed horizontally away from a wheat crop in the UK. The rotorod conidium samplers are protected beneath rain covers and supported at the height of the crops on canes.

Fig. 4.74. *E. neoaphidis* conidia profiles are assessed vertically above a wheat crop in the UK. The rotorod conidium samplers are protected beneath rain covers and supported on a mast.

(Figs 4.70, 4.71; Morgan *et al.*, 1995). For this reason *in vitro* conidia have a faster settling velocity than *in vivo* conidia which, in turn, settle faster than secondary conidia. In epizootiological terms, this suggests that *in vitro*-augmented fungus may not disperse far initially from the release site and that secondary conidia may be responsible for longer distance dispersal than primary conidia (Hemmati, 1999).

When conidia are deposited, they adhere to host or non-host surfaces. On the host, both primary and secondary conidia can germinate and penetrate under favourable abiotic conditions (Fig. 4.72; Butt *et al.*, 1990). At 100% RH, the mean time for infection is 5.3 h at 10°C and 4.5 hours at 20°C (Glare and Milner, 1991). *E. neoaphidis* proliferates within the aphid as protoplasts, only developing hyphal bodies at or just before death (Butt *et al.*, 1981; Kobayashi *et al.*, 1984). The time to kill is dependent on temperature; in *A. pisum*, infection did not occur at 0°C or at 30°C and the time to kill varied from 5 to 16 days at 20 and 10°C, respectively (Wilding, 1970b). Infection has little impact on *A. pisum* behaviour until just prior to death, when infected aphids are unable to respond to alarm pheromone, though they can still produce it (Roy *et al.*, 1999). If dislodged, infected aphids are less able to return to the plant (Roy *et al.*, 1999). On non-host surfaces, secondary conidia are produced within a few hours (Fig. 4.70); 6 h were required for 30% and 80% of primary conidia to germinate to produce secondary conidia at 10°C and 18°C, respectively (Morgan, 1994). Optimal temperatures for germination are between 18 and 21°C (Glare and Milner, 1991). At suboptimal temperatures, increasing numbers of rounded secondary conidia are produced compared with lemon-shaped ones. Conidia produced at suboptimal temperatures and then placed at optimal temperatures still germinate more slowly than those produced at optimal temperatures (Morgan *et al.*, 1995). Germination to produce secondary conidia is also affected by pH and osmotic potential (Morgan, 1994).

E. neoaphidis resting spores have not been recorded in the field, and the mechanism for overwintering is largely unknown. The fungus may persist during winter as hyphal bodies in cadavers (Feng *et al.*, 1992) or it may reproduce at a slow rate in overwintering aphids (Byford and Reeve, 1969; Feng *et al.*, 1991; McLeod *et al.*, 1998). Nielsen *et al.* (1998) have suggested that *E. neoaphidis* may overwinter in the soil as thick-walled 'loriconidia', which are similar to resting spores. Although conidia can survive up to 8 months at –3°C and +4°C on soil and at least 32 days at +5°C on foliage in the laboratory, these times are significantly reduced under winter field conditions (Morgan, 1994; Schofield, 1998). This suggests that field temperatures in the UK during the winter are not consistently low enough to prevent germination and prolong the survival of conidia. In regions where the temperature is consistently lower during the winter, these times may be extended.

During the summer in the UK, conidia are likely to be inactivated by ultraviolet radiation. Brobyn *et al.* (1985) demonstrated that survival of conidia on bean foliage was related to position on the plant; conidia on the upper surfaces of leaves survived < 3 days, whereas those on the undersides of leaves survived for > 7 days. In laboratory studies, humidity was implicated in conidial survival (Brobyn *et al.*, 1987). The role of rain in persistence of conidia and cadavers on foliage and soil was examined recently. Simulated heavy rainfall for > 30 min removed significant numbers of conidia, particularly from the upper surfaces of leaves. Sixty minutes of heavy rainfall were necessary before significant numbers of cadavers were removed from foliage. Cadavers on the soil were completely destroyed by this amount of rain. Lighter rain is likely to have little impact on conidial and cadaver persistence, and high humidity associated

with rainfall is likely to prove more beneficial for sporulation, germination and transmission and might compensate for the negative effects of inoculum removal (Pell *et al.*, 1997b).

Zoophthora radicans *(Entomophthoraceae)*

Taxonomy and distribution

Z. radicans was originally described from *Pieris brassicae* (Brefeld, 1870) as *Empusa radicans* and has subsequently also been known as *Entomophthora sphaerosperma* and *Erynia radicans. Z. radicans* has a worldwide distribution and has been recorded from numerous insect orders, including Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera, Thysanoptera and Trichoptera (Glare and Milner, 1991). Epizootics have commonly been recorded (Table 4.2).

Host specificity

Although *Z. radicans* is recorded from numerous different insect groups, the literature suggests that individual isolates are better adapted to infecting taxonomically related insect hosts (Papierok *et al.*, 1984; Milner and Mahon, 1985; Goettel *et al.*, 1990). Several strains, for example, were unable to infect species from orders other than the order from which they were isolated (Papierok *et al.*, 1984; McGuire *et al.*, 1987a; Magalhaes *et al.*, 1988). Others were unable to infect insects from different families within the same order (Mietkiewski *et al.*, 1986). However, some studies have identified isolates with seemingly broader host ranges. A Malaysian isolate of *Z. radicans* (reference NW250) from the diamondback moth *Plutella xylostella* (Figs 4.75, 4.76) is unable to infect one species of hymenopteran parasitoid, *Cotesia plutellae*, but did infect another parasitoid, *Diadegma semiclausum* (Furlong and Pell, 1996; Fig. 4.83). *D. semiclausum* was 100 times less susceptible than the original host, *P. xylostella*, and was never found infected in the field during epizootics, suggesting a physiological but not ecological susceptibility; the parasitoid is less likely to receive a sufficiently high dose in the field. Poprawski *et al.* (1992) reported that an isolate of *Z. radicans* from *Diuraphis noxia* was also able to infect the aphid parasitoid *Aphelinus asychis*. Some isolates of *E. muscae* from Hymenoptera can also infect Diptera (Eilenberg *et al.*, 1987, J. Eilenberg, unpublished) and Mietkiewski *et al.* (1986) also found that a *Zoophthora* sp. from a hymenopteran was able to infect a small percentage of inoculated Lepidoptera. Susceptibility demonstrated in the laboratory may not relate to infection in the field. Susceptibility relates both to the physiology of an insect and to its behaviour, which may encourage or discourage the acquisition of conidia (Goettel, 1994; Roy *et al.*, 1998). It is likely that pathotypes of *Z. radicans* exist, as with other entomophthoralean species, and that each pathotype has a limited host range.

Epizootiology in annual cropping ecosystems

In *Z. radicans*-infected spotted alfalfa aphid, *Therioaphis trifolii* f. *maculata*, day length affects the time of death, although not as precisely as seen in aphids infected with *E. neoaphidis* (Milner *et al.*, 1984a); Glare and Milner (1991) suggest that some aphids killed by *Z. radicans* are filled with resting spores, the production of which does not

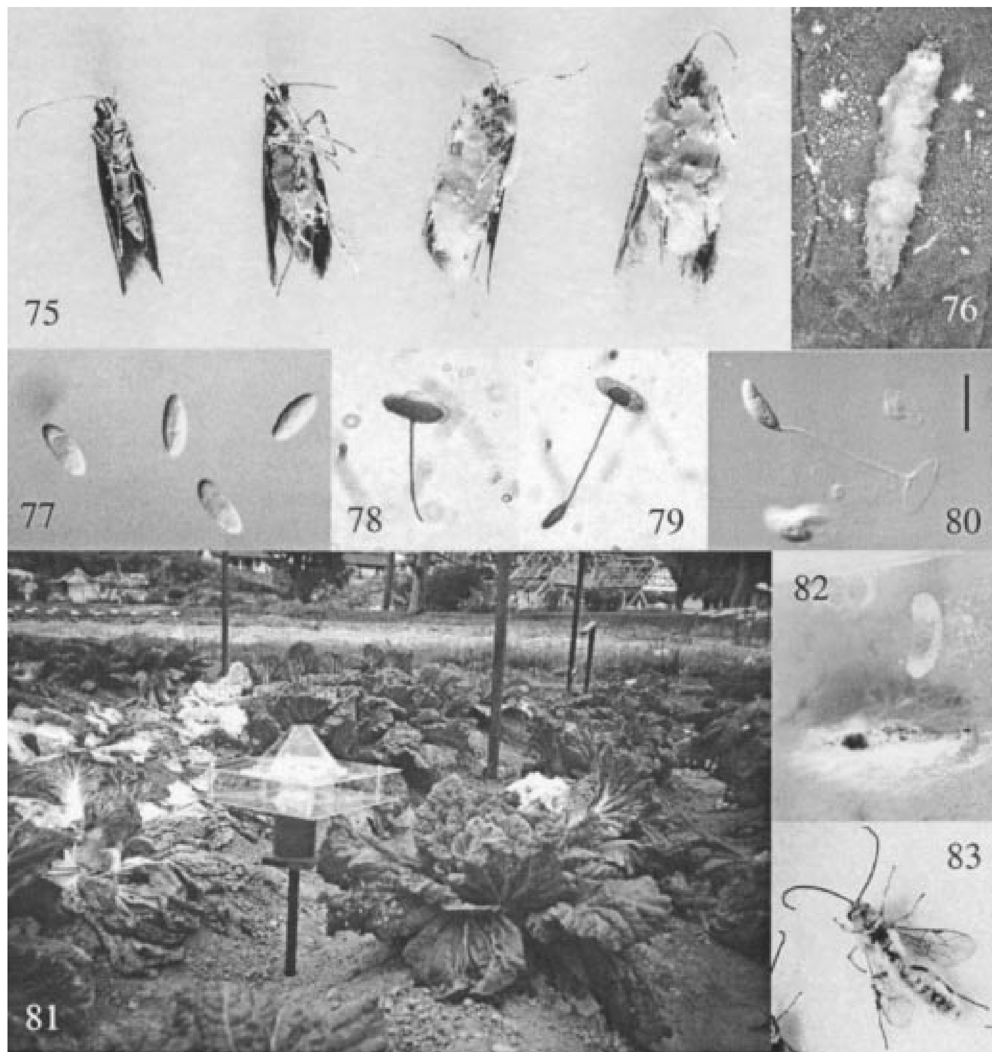

Fig. 4.75. *Zoophthora radicans*-infected adult diamondback moths, *Plutella xylostella* (Lepidoptera: Plutellidae), in the conidial stage. Conidiophores have erupted through the host's integument and formed conidia. The moth at the left of the image is uninfected.
Fig. 4.76. *Z. radicans*-infected larva of diamondback moth. The actively discharged conidia are seen as a halo around the cadaver.
Fig. 4.77. Primary conidia of *Z. radicans.* Scale bar = 20 μm.
Fig. 4.78. The formation of a capilliconidiophore from a primary conidium of *Z. radicans*. The conidium is stained in 10% cotton blue in lactophenol. Scale bar = 20 μm.
Fig. 4.79. The formation of a secondary conidum is initiated at the end of the capilliconidiophore of *Z. radicans*. The conidia is stained in 10% cotton blue in lactophenol. Scale bar = 20 μm.
Fig. 4.80. A fully formed capilliconidium of *Z. radicans* at the end of the capilliconidiophore. Scale bar = 20 μm.
Fig. 4.81. Autodissemination trap for *Z. radicans* in a vegetable crop, Cameron Highlands, Malaysia.

require a period of high humidity after host death. *Z. radicans* may produce actively discharged conidia or internal resting spores and, on occasion, both are produced from the same host.

Insects killed by *Z. radicans*, like those infected with *E. neoaphidis*, usually remain attached to the surface on which they have died by rhizoids or pseudorhizomorphs, which emerge from their ventral surface. A water-saturated or near-saturated environment is essential for active conidial discharge and subsequent germination and infection. Uninucleate, cigar-shaped primary conidia are discharged several millimetres from the cadaver with the potential to infect new hosts (Fig. 4.83).

Active discharge seems to follow a circadian rhythm, with more conidia being discharged in the dark than the light (Yamamoto and Aoki, 1983; Milner *et al.*, 1984a). In the spotted alfalfa aphid more conidia are produced at 25°C than at 15, 20 or 30°C and none are produced at 35°C (Milner and Lutton, 1983). Sawyer (1929) reported that sporulation was very low at 13°C and stopped entirely at 8°C. In contrast, Leite *et al.* (1996a) recorded reduced, but substantial, sporulation of *Z. radicans* on *Empoasca kraemeri* cadavers at 5 and 10°C compared with 15 and 20°C.

Discharged conidia are presumed to travel long distances in the air, although there have been no field measurements of aerial dispersal for *Z. radicans*. G.W. Riethmacher (personal communication) was able to collect conidia on microscope slides placed some distance from an epizootic occurring in *P. xylostella* on brassicas in the Philippines, suggesting that conidia were dispersed readily. In addition, the conidial settling velocities have been recorded for two isolates and these were less than those for species with larger conidia (Sawyer *et al.*, 1994; Hemmati, 1999).

Discharged conidia landing on appropriate hosts will infect them if the environment is saturated or near-saturated. Both primary and secondary conidia are thought to be infective (Wraight *et al.*, 1990; Pell *et al.*, 1993b) and the speed at which they germinate and infect is related to temperature (van Roermund *et al.*, 1984; Glare *et al.*, 1987). For isolates infective to the leafhopper *E. kraemeri*, conidia landing on a suitable host can develop either germ tubes with rounded or elongate appressoria for infection, long, slender capilliconidiophores bearing almond-shaped capilliconidia (Figs 4.78–4.80), or replicative conidiophores which actively discharge secondary conidia similar in shape to the primaries. Germination type is dependent on temperature; germ-tube production was greatest at 22°C (beginning within 0.7 h), capilliconidiophore production at 16.5°C (within 1.9 h) and replicative conidiophore production at 28–30°C (within 3.2 h) (Galaini-Wraight *et al.*, 1992).

The factors that affect the type of germination and subsequent development are clearly complex and differ depending on which type of conidia are formed and where they land on the host (Wraight *et al.*, 1990; Leite *et al.*, 1996b, c), what nutrients and pH are available (van Roermund *et al.*, 1984; Magalhaes *et al.*, 1990, 1991a, b) and the immune response of the host (Butt *et al.*, 1988). The type of secondary conidia produced can be dependent on whether the primary conidia were produced *in vivo* or *in vitro*; on 1% water agar capilliconidia were almost exclusively produced from *in*

Fig. 4.82. Dual infection and parasitization of diamondback moth larva: *Z. radicans* has emerged and is sporulating on the cadaver of the larva. In addition, the larva of the parasitoid, *Diadegma semiclausum* (Hymenoptera: Ichneumonidae), has also emerged and is about to pupate. Photograph: Mike Furlong.

Fig. 4.83. *Z. radicans*-infected parasitoid, *D. semiclausum* adult. Conidia are actively discharged from the dead host.

vitro-produced primaries, whereas greater numbers of actively discharged secondary conidia were produced from *in vivo*-produced primaries (J.K. Pell, unpublished).

After infection, *Z. radicans* proliferates throughout the host body as hyphae, with hyphal bodies forming near host death. No protoplast stages have been observed (Glare and Milner, 1991), although it is possible to produce them from walled stages *in vitro* (Glare *et al.*, 1989a). Time to kill varies between isolates and hosts and is dependent on temperature (Milner and Lutton, 1983; Leite *et al.*, 1996d). Fluctuating temperatures can extend the time to kill (Furlong *et al.*, 1995). *Z. radicans* isolates have been recorded as able to germinate and grow between 0 and 36°C, though most do not grow at or above 35°C (van Roermund *et al.*, 1984; Glare *et al.*, 1987; M.J. Furlong and J.K. Pell, unpublished data).

Infection may have an impact on host behaviour; food consumption by infected larvae of *P. xylostella* was not affected until the third day after infection (1 day prior to death). On the day of death, no food was consumed and, overall, infected larvae (inoculated as third instars) ate 44% less foliage than healthy larvae (Furlong *et al.*, 1997). The number of eggs laid by infected females (inoculated 3–6 h after eclosion) was also significantly reduced, even over the period of egg production prior to death (Furlong *et al.*, 1997). *Z. radicans* also inhibits the response to and production of sex pheromone in *P. xylostella*, thereby disrupting their mating behaviour (Reddy *et al.*, 1998). Changes in behaviour and reproductive rate contribute to pest management even before the host succumbs to disease.

Under certain circumstances, persistent resting spores (azygospores) are produced within the host in response to changing climatic factors, particularly low temperature and high humidity, high inoculum density, host age or inappropriate hosts (Ben-Ze'ev and Uziel, 1979; Shimazu, 1979; Perry *et al.*, 1982; McCabe *et al.*, 1984; McGuire *et al.*, 1987c; Glare *et al.*, 1989b). Resting spore production has not been observed in all isolates (Uziel *et al.*, 1982; Glare *et al.*, 1989b; Pell *et al.*, 1993), but the conditions for their production are not fully understood and may differ between isolates. Mixed infections with more than one isolate also encourage resting spore production, suggesting genetic recombination (Glare *et al.*, 1989b). Resting spore-filled cadavers are not attached by rhizoids and fall to the ground, where resting spores remain dormant during unfavourable climatic conditions. In the laboratory, they may remain dormant for 4 months at 4°C and 100% RH (Perry *et al.*, 1982). When resting spores germinate, in synchrony with host population increase (Perry and Fleming, 1989), they produce multiple, actively discharged, infective germ conidia.

Resting spores play a role in long-term persistence in the environment during unfavourable climatic conditions. The shorter-term survival of conidia between discharge and infection, necessary to ensure transmission, is also important in disease dynamics and can be affected by temperature, humidity, rain and ultraviolet radiation.

Conidia inoculated on to brassica foliage or soil in the field in Malaysia lost the ability to infect their host, *P. xylostella*, within 24 h (Furlong and Pell, 1997). This is similar to the time for conidial survival of a *Z. radicans* isolate from *T. trifolii* in Israel (Uziel and Shtienberg, 1993). In complementary laboratory studies at temperatures and day length simulating field conditions in Malaysia (12 : 12 h light : dark (L : D), 23 : 16°C), conidia survived for 16 days on both foliage and soil, suggesting that other factors reduced viability in the field (Furlong and Pell, 1997). Infectivity was lost faster on the upper than on the lower leaf surface and faster on dry than on moist soil, highlighting the importance of moisture in conidia survival (Furlong and Pell, 1997). Uziel and Kenneth (1991) also showed that *Z. radicans* conidia remained viable for extended

periods at high humidities. In a laboratory study, conidia viability, as measured using vital staining techniques, was lost most rapidly at RH < 95% and differed between isolates (Griggs *et al.*, 1999). One hour of simulated heavy rain on primary conidia on upper and lower leaves did not significantly reduce the ability of conidia to infect *P. xylostella* (Furlong and Pell, 1997). When this experiment was repeated with capilliconidia, the raindrops dislodged capilliconidia from their fine capilliconidiophores and washed them off the leaf within an hour. However, this is unlikely to have contributed to losses of inoculum observed in the field, particularly as persistence studies were repeated in the field (with and without rain) on several occasions with the same result (Furlong and Pell, 1997). Ultraviolet radiation is by far the most important factor in conidia mortality. Both primary conidia and capilliconidia are rapidly deactivated by ultraviolet radiation (within 3 min for primary conidia and 120 min for capilliconidia (Furlong and Pell, 1997). In simulated tropical sunlight, infectivity of primary conidia and immature capilliconidia fell to 10% within 8 h. Mature capilliconidia remained able to infect 40% of the larvae after 8 h exposure, corroborating the findings of Uziel and Shtienberg (1993) that mature capilliconidia were more resistant to ultraviolet radiation. The cadaver and the halo of conidia (high-density depositions) can provide some protection for conidia, and fungus within the host is also protected from ultraviolet radiation (M.J. Furlong, unpublished data).

Exploitation in Pest Management

The potential of Entomophthorales has largely been considered from the point of view of biological control approaches in isolation from other control measures. Although the potential to combine fungi with other strategies has always been accepted, this has only recently been practically addressed. Biological control is defined as:

> the use of natural enemies or competitor populations to suppress a pest population, making it less abundant and thus less damaging than it would otherwise be. It may be the result of purposeful actions by man or may result from the unassisted action of natural forces. Biological control may be employed either for suppression of crop or food pests, or for restoration of natural systems affected by adventive (nonnative) pests.
>
> (van Driesche and Bellows, 1996)

There are three types of biological control: introduction (or classical biological control), conservation and augmentation. Here, successful and unsuccessful examples where these three strategies have been applied are described, identifying opportunities and bottlenecks. Approaches that are moving towards integration of biological, chemical and cultural strategies (i.e. integrated pest management (IPM) in the true sense) are also discussed and future prospects evaluated.

Biological control: introduction of exotic Entomophthorales

When pest species are introduced to a new area, their natural enemies are often absent. In these cases, introductions of natural enemies may decrease populations of the new pest. Natural enemies for introduction can be collected from an exotic area, usually where the pest host is indigenous, and released in the area of introduction after

appropriate quarantine and evaluation (van Driesche and Bellows, 1996). This strategy is called classical biological control. Alternatively, exotic natural enemies may be introduced against a native pest, a strategy that has been called neoclassical biological control or use of a new association. As fungal isolates within a species can differ significantly in a number of characteristics, including host specificity (= pathotypes), the introduction of an exotic pathotype can be considered as an introduction of a 'new' natural enemy even if the fungal species is already present. Unfortunately, difficulties in identification and separation of species, or pathotypes within a species, can make it difficult to determine whether a pathotype is already present or not. In addition, many pest species have been transported worldwide over many centuries and it is difficult to determine whether pests have been introduced or are indigenous.

In any case, the goal of pathogen introductions is to release the natural enemy for establishment so that it will eventually create a self-maintaining system, which, in the long run, can be much less expensive than other control approaches. In some instances, exotic isolates have been introduced to test inoculative or inundative augmentation where the objective was to cause epizootics; such examples will be described later, because the methods used were appropriate for immediate control and not solely establishment. Several examples are described where exotic entomophthoralean species have been introduced for establishment against pests, whether native or introduced.

Entomophaga maimaiga *and* Lymantria dispar

In 1909, an entomophthoralean fungus found infecting *L. dispar* in Japan was brought to the northeastern USA, where *L. dispar* had been introduced. This fungus was introduced in 1910/11 by releasing infected insects but it was not considered to have become established at that time (Speare and Colley, 1912). Surprisingly, in 1989, an entomophthoralean fungus was found causing epizootics in *L. dispar* populations in seven northeastern states (Andreadis and Weseloh, 1990; Hajek *et al.*, 1990b). This fungus was identical to isolates from Japan (named *E. maimaiga*), but it was not at all certain whether the fungus found in 1989 had originated from the 1910/11 releases or from a hypothetical, more recent accidental introduction (Hajek *et al.*, 1995b). Since 1989, *E. maimaiga* has repeatedly caused epizootics, resulting in widespread maintenance of *L. dispar* populations at low densities. From 1989 to 1992, the spread of this fungus across much of the distribution of *L. dispar* in northeastern North America was documented (Elkinton *et al.*, 1991; Hajek *et al.*, 1996b). Some of the spread was due to human activity, including purposeful point introductions but possibly also accidental movement of soil containing resting spores. However, the seemingly simultaneous appearance of *E. maimaiga* over large areas where it had not previously been detected under appropriate conditions (i.e. host population present and adequate rainfall) suggests that much of the spread could be attributed to airborne movement of the conidia.

L. dispar was introduced to the Boston area of North America in 1868 or 1869 and has increased in distribution ever since. This early-spring, univoltine species has a great potential for outbreaks and yet, for periods of time, it can remain largely undetected in the forest. For several years after 1989, populations of *L. dispar* were dense enough to be noticeable in forests, and an outbreak was feared. At that time, *E. maimaiga* did not occur throughout the area infested with *L. dispar* and the fungus was not considered capable of spreading extensively on its own. Therefore, programmes were developed to introduce this fungus into areas where it did not occur, but a reli-

able method for *in vitro* production was not available. In a preliminary study, *E. maimaiga* was effectively redistributed by collecting resting spore-laden soil from the bases of trees where epizootics had occurred and releasing that soil around the bases of trees where control of *L. dispar* was required (Hajek and Roberts, 1991). In addition, weekly watering of the soil at the bases of the trees increased infection rates. To quantify the numbers of resting spores released, wet-sieving and density-gradient centrifugation were followed by spore counts (Hajek and Wheeler, 1994). Using this methodology, a redistribution programme was conducted in four states along the leading edge of *L. dispar* spread, where surveys had demonstrated that the fungus did not occur. *E. maimaiga* resting spores were released at 34 plots of 0.1 ha in 1991 and seven plots in 1992 (Hajek *et al.*, 1996b). During 1991, *E. maimaiga* infections were found in 28 of the 34 release plots with infection levels of > 40%. Once again, weekly watering of the soil at the bases of trees increased host infection. Infections were also found in four of the 15 control plots but with an average infection of only 0.5%. During 1992, an average of 72% infection was found in the 1992 release plots, and infection levels were high at all of the 1991 release sites.

Redistribution was also evaluated in central Michigan, using release of soil-borne resting spores and laboratory-infected larvae (Smitley *et al.*, 1995). In the second year after introduction, *E. maimaiga* caused 9–40% infection in release plots and infection was inversely correlated with defoliation. In the third year after fungal release, epizootics of *E. maimaiga* occurred at the two release sites. Throughout the study, there was no significant difference between the two methods for introduction. While quantification of resting spores in soil to be introduced is time-consuming, inoculation of larvae is much more so. Therefore, releases of *E. maimaiga* in other locations have predominantly focused on releasing resting spores.

Between 1990 and 1994, *E. maimaiga* was released at a total of 146 sites in eight states (A.E. Hajek, unpublished data). The principal method for redistribution has been movement of soil containing *E. maimaiga* resting spores. Releases of this fungus have continued along the leading edge of the ever-increasing distribution of gypsy moth. In areas newly colonized by gypsy moth, damage can be severe and the time for *E. maimaiga* to invade on its own cannot be predicted. Due to concerns about the many organisms that could be moved along with *E. maimaiga* resting spores when moving soil, redistribution now uses field-collected cadavers containing resting spores. This new method is not without drawbacks; it requires collecting cadavers from epizootics during the 3+-week period after larval mortality, when the majority of cadavers remain attached to tree-trunks (Hajek *et al.*, 1998b). After this time, many of the cadavers fall to the ground and decompose to release resting spores into the soil. Because the timing and location of epizootics are not predictable, cadaver collection requires a large effort during a relatively brief period to find and visit epizootic sites for cadaver collection. Cadaver collection is mostly done before the pest oviposits. Therefore, at-risk sites cannot be identified for immediate release. Methods have yet to be developed to ensure fungal survival during storage of resting spore-laden cadavers until such time that release sites have been identified. In addition, if the fungus is to be stored throughout winter, resting spores must receive conditions that satisfy their constitutive dormancy requirements. Studies are currently being conducted to identify these conditions. At present, although *E. maimaiga* occurs throughout a major part of the *L. dispar* distribution area, there is still a strong demand for inoculum along the leading edge of spread of this pest, where land managers and residents are unsure how long it will take for this fungus to become well established.

Zoophthora radicans and *Therioaphis trifolii* f. *maculata*

Spotted alfalfa aphid became an important pest of pasture legumes, particularly lucerne, when it was introduced into Australia in 1977. Although six species of fungi were found in aphids, they were at extremely low levels in spotted alfalfa aphid; *Z. radicans* was not recorded from this aphid (Milner *et al.*, 1980). In other countries *Z. radicans* was known to cause epizootics in spotted alfalfa aphid populations (Hall and Dunn, 1957; Kenneth and Olmert, 1975) and so was considered to have good potential for introduction. An isolate from Israel was chosen because of the similarities in climate between its area of origin and the site for release in Australia. The fungus was released either by the placement of sporulating *in vitro* cultures over lucerne plants infested with aphids or introducing laboratory-infected living and dead aphids at four sites (Milner *et al.*, 1982). Although infection levels were very low in the first few samples made at release positions, it was clear within 5 weeks that epizootics were in progress. Highest levels of infection were recorded close to the release points (3 m), extending horizontally as far as 15 m, with occasional infected aphids found throughout the field. No rain occurred during the epizootic but humidity remained high for prolonged periods each night. The fungus was able to survive as resting spores, enabling it to persist from year to year and subsequently spread on its own (Milner *et al.*, 1982). Although some isolates of *Z. radicans* can infect Hymenoptera, this fungus was never found infecting the spotted alfalfa aphid parasitoid *Trioxys complanatus* (Glare and Milner, 1991).

Zoophthora radicans and *Empoasca fabae* in the USA

The potato leafhopper, *Empoasca fabae,* is an important pest of lucerne, potatoes and other crops in the midwestern and northeastern USA and epizootics of *Z. radicans* were regularly observed in Wisconsin but rarely in states further south. In Illinois, *Z. radicans* had never been recorded from *E. fabae* despite intensive investigations between 1960 and 1985. Lack of this species in Illinois was considered to be due to the higher temperatures in this state. Isolates from Wisconsin were released against *E. fabae* in Illinois in 1984 using a method similar to that described by Milner *et al.* (1982). Initial infections were observed at the release sites but no subsequent horizontal transmission was recorded (McGuire *et al.*, 1987c). However, epizootics did occur widely the following year around the original release site and it was suggested that one of the isolates released might have become established and caused these epizootics. Observations in 1986 indicated that *Z. radicans* was again present in the same area and contributed to reductions in *E. fabae* populations (McGuire *et al.*, 1987b). No further research has been done to confirm the establishment of this pathogen and so the success of the introduction is not confirmed. The released isolate was also highly infective to *E. kraemeri*, but had limited virulence against aphids and did not infect Lepidoptera (McGuire *et al.*, 1987a).

Neozygites fresenii and *Aphis gossypii* in California

Classical biological control introductions of *N. fresenii* were made in cotton, in the San Joaquin Valley of California in 1994 and 1995 (Steinkraus and Rosenheim, 1995; Steinkraus *et al.*, 1998a). Cotton aphids in California lacked fungal pathogens, leaving a niche that could be filled by *N. fresenii*. Mid-August releases of isolates from

Arkansas, using dried infected aphids (Fig. 4.85), were moderately successful, resulting in the spread of the pathogen from the release areas. Fungus activity continued until September or October, despite environmental extremes and heavy predator activity (D.C. Steinkraus, unpublished data). Levels of infection were considered higher than those used to predict initiation of epizootics in the southeastern USA, but epizootics did not develop. Whether the pathogen will persist in California and cause long-term suppression of cotton aphid populations is unknown. Low RH in the San Joaquin Valley may limit the survival and spread of the fungus.

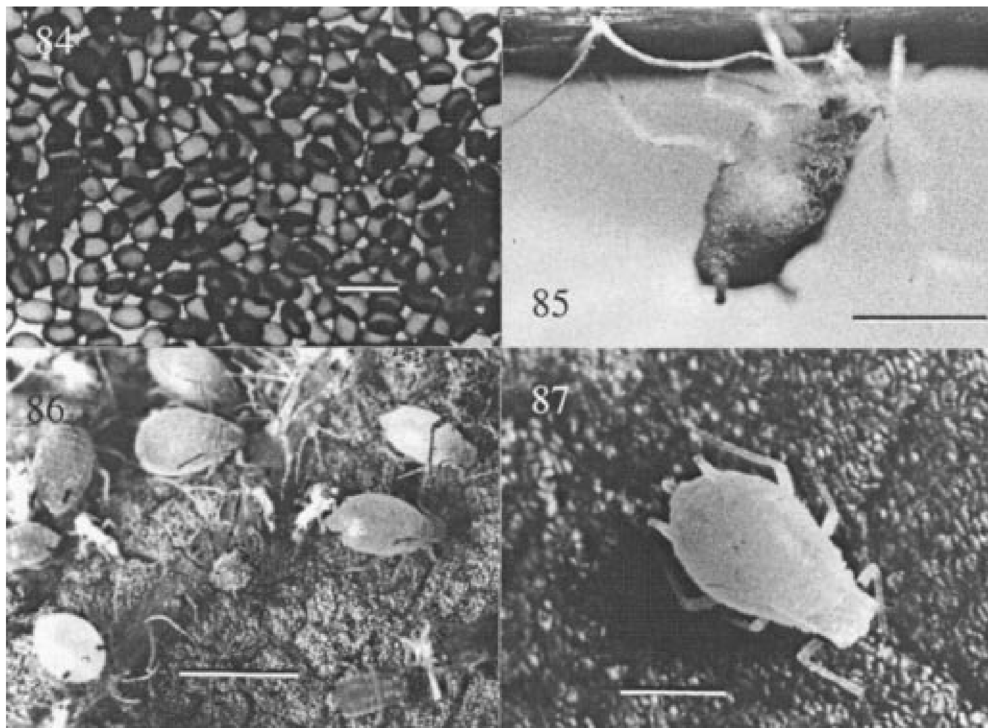

Fig. 4.84. Resting spores of *Neozygites fresenii* from *Aphis gossypii.* Scale bar = 50 μm.
Fig. 4.85. *N. fresenii*-infected cotton aphid. Infected aphids can be dried prior to formation of conidia, frozen and stored for years. Within an hour of being placed at room temperature in a humid chamber, conidia will form and be discharged from such aphid cadavers. Dried infected aphids are useful in laboratory and field experiments.
Fig. 4.86. Underside of a cotton leaf from a field in which an epizootic caused by *N. fresenii* is in progress. Several aphids killed by the fungus are visible, as well as several infected aphids, which are shiny and swollen and which will be killed by the fungus within hours. During sporulation each infected aphid cadaver will discharge conidia on to the leaf, into the air and on to surrounding aphids.
Fig. 4.87. Cotton aphid infected with *N. fresenii*. Three days after infection the aphid host becomes whitish, slightly swollen and sometimes shiny. When such an aphid is squashed on a slide and examined, thousands of protoplasts or hyphal bodies will be revealed. Scale bar = 0.5 mm.

Entomophaga grylli pathotype 3 and grasshoppers

E. grylli, pathotype 3, from Australia causes disease epizootics in Australia in areas similar to the rangelands of the western USA. In addition, although its general life cycle is very similar to that of pathotype 1, its host range is wider than that of either pathotype 1 or 2 from the USA. In fact, its host range is similar to the combined host range of the other two isolates (Ramoska *et al.*, 1988). Pathotype 3 sporulates more rapidly than pathotype 1, allowing it to produce conidia and infect hosts during a shorter dew period, although it is less able to undergo successive cycles of desiccation, rehydration and sporulation. Conidia of pathotype 3 are morphologically distinguishable from pathotype 1, so their spread in the field could be monitored easily. It was therefore considered to have great potential for introduction into the USA. An initial release of pathotype 3 was made in McKenzie County in North Dakota in 1989 (500 laboratory-infected fifth-instar nymphs were released in a 45-acre field site), but it was late in the season and, although low levels of sporulation occurred, little secondary transmission was observed. As the release was late in the season, resting spores were probably produced rather than infective conidia. Earlier-season releases (synchronized with early-instar grasshoppers and a high likelihood of high humidity) were made on five occasions in 1990 at the same site (500 infected insects released on each occasion). Sampling during the following 4 years indicated that pathotype 3 had become established with successive seasons of overwintering and reinfection (1991–1993). This was associated with significantly reduced populations of a number of pest grasshopper species in the release areas. This included nearly 100% infection of fifth-instar nymphs (i.e. prior to egg laying) of *C. pellucida* and *Melanophus sanguinipes* in 1991 (Carruthers and Onsager, 1993). DNA probes were used to evaluate persistence and spread (Bidochka *et al.*, 1996). In 1992, 23% infection was found with no infection > 1 km from the release sites. By 1993 and 1994, the Australian pathotype was rare or not found at all and researchers questioned the long-term survival of this pathotype in North America. Further releases over a wider area were not made due to concerns for the survival of indigenous non-pest grasshoppers (Lockwood, 1993a, b), although mycosis was only observed in eight out of 20 grasshopper species found at the release site and all eight species are pests of rangeland (Carruthers and Onsager, 1993; Carruthers *et al.*, 1997). The reverse situation was also tried, i.e. release of *E. grylli* pathotype 1 into Australia (Milner and Soper, 1983; Milner, 1985b). The fungus did cause initial infection, but did not establish outside the release cages.

Biological control: conservation of Entomophthorales

Biological control through conservation seeks to identify effective indigenous natural enemies and adopt management practices that conserve and promote them in the field. Fundamental is the assumption that the natural enemies exist locally and that they have the potential to suppress the pest. Management practices that favour entomophthoralean fungi may include provision of increased moisture and provision of overwintering sites or alternative hosts. Here we describe examples where conservation of Entomophthorales has been observed and/or attempted to encourage their activity.

Use of irrigation

Epizootics have been encouraged in several systems through irrigation to ensure high relative humidities for sporulation and infection. Wilding *et al.* (1986b) demonstrated that irrigation of field beans greatly increased the proportion of black bean aphids (*Aphis fabae*) infected with *E. neoaphidis* compared with plots that were not irrigated. *Erynia ithacensis* exists at enzootic levels in populations of the mushroom gnat, *Phoradonta flavipes*, in hothouses in China. Huang *et al.* (1992) found that they could increase the 14% background level of infection to 37% within 2 days and ultimately to 59% by spraying water on to wall corners, mushroom-bed racks and ceilings. Pickering *et al.* (1989) compared infection of *A. pisum* aphids in two areas of a pecan orchard in Georgia; sprinkle irrigation resulted in high levels of infection (74%) with *E. neoaphidis*, but no fungal mortality was noted in the area that was drip-irrigated. Similar results were also recorded by Hall and Dunn (1957) in irrigated lucerne.

Providing reservoirs/alternate hosts

In Switzerland, Keller and Suter (1980) recorded that economically unimportant aphid species developing in spring in meadows (lucerne) were important for the multiplication of entomophthoralean fungi. If aphid populations in these meadows were high in the spring, *E. neoaphidis* and *Conidiobolus obscurus* rapidly built up to sufficient levels to regulate aphid populations in adjacent fields of annual crops. When aphids were scarce in the spring, this did not happen. Feng *et al.* (1991) suggested that the earlier infections observed in *Metopolophium dirhodum*, an aphid feeding in exposed locations on cereal leaves, provided an increase in entomophthoralean inoculum throughout the field, with subsequent increases in infection of an aphid inhabiting sheltered locations (*D. noxia*) and a more xerophilic species (*S. avenae*). Similarly Powell *et al.* (1986b) found that entomophthoralean fungi were more common at the weedy edges of fields, and they presumed that this was because alternative aphid hosts were present and the weed canopy afforded a better environment for transmission than in the wheat fields. Other aphid-pathogenic species, such as *Zoophthora aphidis*, *Zoophthora phalloides* and *Entomophthora planchoniana*, are also known to overwinter in hosts in hedges and forest borders (Keller, 1987a, b, 1998; Nielsen *et al.*, 2001a)

The presence of hedgerows is vital to the persistence and spread of *E. schizophorae* and *E. muscae* in dipteran populations (Eilenberg, 1985, 1988, 1999). The prevalence of *E. schizophorae* in carrot fly, *C. rosae*, was always significantly greater in insects sampled from hedges than from carrot fields. Hedges are the preferred sites for flies to rest and therefore cadavers become attached there prior to sporulation, making hedges the sites where uninfected flies are more likely to receive inoculum (Eilenberg, 1987b). The same phenomenon, though less obvious, was also seen for the cabbage root fly, *D. radicum*, and infections of *E. muscae*; the prevalence of *E. muscae* was always greatest amongst flies caught in the hedges or flowers around the field (Eilenberg, 2000).

Biological control: augmentation of Entomophthorales to initiate early-season epizootics

When natural enemies are missing (in glasshouses, for example), late in arriving at new plantings or simply too scarce to provide control, their numbers may be increased

through releases or applications; this is termed augmentation. 'Inoculative releases' are those in which small numbers of the natural control agent are added early in the season so that they will reproduce and effect control for an extended period. 'Inundative release', or the mycoinsecticide approach, is used when pest control is required rapidly and exclusively by the natural enemy released and not by natural enemy progeny. There are several examples where natural levels of entomophthoralean fungi were supplemented to effect control in one of these ways. However, for Entomophthorales it is more usual that the intention is to inoculate the crop with the fungus early in the season so that the fungus can establish and multiply, retaining pest populations below the damage threshold. Whether an inoculative or inundative release, the intention is to effect control that season and to repeat applications in subsequent seasons. In many examples the trials are on a small scale and are not repeated, making the differentiation between the two approaches more difficult.

Usually, the isolate of fungus being released at a site was not collected from the same site. Due to the variability among entomophthoralean isolates within a species, it is then questionable whether the natural population is being 'augmented' or a novel isolate is being released for control. Regardless, these instances are included under the umbrella term of 'augmentation' when the objective is immediate or delayed pest control within a single crop season and not long-term control through self-perpetuating establishment (introduction). Generally, studies included in this section are those where an isolate being released is indigenous to the area or, if not indigenous, is not known to have characteristics significantly different from the native isolate, e.g. significant differences in specificity.

Entomophaga maimaiga *and* Lymantria dispar *in the USA*

Inoculative augmentation of the lepidopteran pathogen *E. maimaiga* was conducted to evaluate the potential of this method to cause earlier initiation of epizootics in areas where this fungus had already established (Hajek and Webb, 1999). A programme to investigate inoculative augmentation utilizing resting spores collected from the organic layer of soil directly at the bases of trees in locations where epizootics had occurred (Hajek *et al.*, 1998a) was established. Movement and release of resting spore-bearing soil required obtaining permissions from state and federal authorities, collection of soil from areas where quarantines of plant pathogens did not exist and screening collected soil to exclude larger organisms.

In spring, a relatively small number of resting spores were released around bases of trees in treatment sites (1×10^6 resting spores per site). Larvae were sampled throughout the field season, both from understorey vegetation and from the upper tree canopy. Larval survival was lower for treatment versus control plots on four of 12 sampling dates. While infection by *E. maimaiga* was always greater in treatment than in control plots, differences were not statistically significant due to variability among plots. At the end of the season, the only plots with severe defoliation were control plots where numbers of fifth-instar larvae were associated with defoliation levels. It was concluded that a treatment effect had been seen and this study was repeated in a second year to confirm results. However, during the second season, epizootics unexpectedly occurred throughout the region, causing a complete collapse in host populations and subsequently no defoliation. While the strategy of releasing resting spores of *E. maimaiga* shows promise, methods for *in vitro* production of resting spores are needed before such a technique could be considered operational. Efficient production meth-

ods would facilitate and improve such releases and make it possible to release standardized material and higher doses than previously possible.

Zoophthora radicans *and* Empoasca fabae *in the USA and Brazil*

In New York state, Wraight *et al.* (1986) applied a dry mycelial preparation of a *Z. radicans* isolate from central Brazil against populations of *E. fabae* on lucerne, on two occasions in September 1984. *Z. radicans* was not found infecting leafhoppers in surveys made in the area prior to release, and so technically this also constituted a classical introduction. However, the intention was to test the potential for inoculative/inundative augmentation on a pest population in an area free of any potentially contaminating fungi and so this study is included in this section. Dry particles of mycelium were broadcast late in the evening directly on to dew-covered foliage in 2 m × 2 m plots at a rate of 10 g m^{-2}. For the first release, temperatures were low for the first day and it was dry on the following 2 days, after which time, rain removed all the inoculum; fewer than 10% of nymphs became infected. On the second occasion, temperatures and humidities were higher, ensuring good sporulation; 80 and 64% of nymphs became infected in the two treatment plots, compared with 0% in the untreated plot. Wraight *et al.* (1986) suggested that, although the doses in the treatment were too high for large-scale application, the method showed promise as a way of initiating epizootics in leafhopper populations when climatic conditions were favourable. This was confirmed by subsequent trials (July 1986) in which applications of *Z. radicans* dry mycelium to small 'inoculation plots' in two fields of lucerne resulted in field-wide epizootics that decimated the leafhopper populations (Wraight and Roberts, 1987).

In 1990 and 1991 seven additional isolates of *Z. radicans* (all from Serbia) were released in test fields near Ithaca, New York, for experimental control of *E. fabae*. On each occasion, only three infected leafhoppers were found in extensive searches made immediately after release. Isolations were made from each of these six insects. Using random amplification of polymorphic DNA polymerase chain reaction (RAPD-PCR), it was possible to determine that five of these isolates were very similar to the released isolates and different from any other isolates included in molecular analysis, suggesting that the fungus had caused a low level of disease. The sixth isolate was similar to an isolate from aphids and was thought to represent a population endemic at the release site (Hodge *et al.*, 1995). Although infection by the released isolates could be confirmed, the prevalence was very low, and it was uncertain whether the isolates would eventually be able to establish epizootics.

Releases of *Z. radicans* against *Empoasca* sp. on bean plants (*Phaseolus*) in 1990 in São Paolo, Brazil (Leite, 1991), followed the same methodology for fungal mycelium production and release as used in central New York, USA, in 1984 (Wraight *et al.*, 1986). In this region, *Z. radicans* was already known to cause epizootics, but natural control was often achieved too late to prevent damage. The objective of this study was to initiate epizootics earlier in the crop cycle. An isolate of *Z. radicans* from Yugoslavia was selected for the test; it was considered to have exceptional biological control potential, because it was originally isolated from an epizootic in *Empoasca vitis* under drought conditions. Applications of 0.25–2.0 g of dried mycelium m^{-2} were made in 12 small subplots (2 m × 2 m) within a single 0.5 ha field. Over the range of concentrations applied, per cent infection was generally greater in plots receiving more fungus, although this was not always the case. On day 5 post-application, the average rate of infection in the treated plots was 25% compared with only 6% in untreated plots

located 5–36 m from the treatments. After 8 days, infection in the treated plots was still greater (34%) than in untreated plots (18%); however, the fungus spread rapidly during the following week and similar levels of infection were recorded in all plots (60–64%) after 15 days. The field-wide epizootic reduced leafhopper numbers to below the economic threshold (from 4.8 to 0.7 hoppers per trifoliate). In summary, infection foci were successfully established in the small plots, and the fungus spread rapidly through the entire field. A few cadavers of infected leafhoppers were found in part of the field at the time of the release. The proportion of infections caused by the resident versus introduced fungus was not determined, but researchers concluded that the epizootic was caused primarily by the released pathogen.

Zoophthora radicans *and* Plutella xylostella

Epizootics of *Z. radicans* in *P. xylostella* populations are common but unpredictable and often fail to prevent increase in pest densities above the economic threshold. In a similar experiment to that of Wraight *et al.* (1986), Pell and Wilding (1994) introduced a Taiwanese isolate of *Z. radicans* into caged populations of *P. xylostella* in the UK to test the potential for establishing early-season epizootics. *Z. radicans* had not previously been recorded from *P. xylostella* in the UK and so the problem of confusion from contamination by local isolates was removed. Between 36 and 68% of larvae were infected in treated cages compared with 0% in untreated cages, demonstrating the potential for augmentation of *Z. radicans*.

Entomophaga grylli *pathotype 1 and grasshoppers in the USA*

A number of 'probable' releases of *E. grylli* were made into North America in the early part of the last century. In many cases it is likely that the fungus was misidentified, and many of the releases were exotic material, including material from Natal, South Africa. Carruthers *et al.* (1997) question whether pathotypes 1 and 2 are actually native to North America, as is commonly accepted. However, regardless of whether the pathotype is native or exotic, augmentation was attempted in 1983 in New Mexico. Approximately 400 field-collected *C. pellucida* were injected with pathotype 1 (*in vitro*-produced protoplasts of an isolate originally collected from an epizootic in Arizona) and released in a 10 ha area near Underwood Flat, New Mexico. Intensive monitoring was carried out in the following years to determine infection levels in grasshopper populations. During the season of release, only 5% of the grasshoppers became infected, but, in the following season, infection levels reached 40%. No parallel control sites were available, but, as no infection was found prior to release, it was assumed that this level of infection was due to the release (Carruthers *et al.*, 1997). To make augmentative releases on a larger scale, methods for *in vitro* production of mycelial material possessing the capacity to sporulate are needed, especially as such methods would remove the constraint of having to inoculate by injection. To date this has not been possible, and so attempts to augment *E. grylli* pathotype 1 in rangeland sites were abandoned (Carruthers *et al.*, 1997).

Entomophthora muscae/Entomophthora schizophorae *and Diptera*

The use of *E. muscae* or *E. schizophorae* for biological control has been considered for a number of dipteran pests. So far, a number of augmentations for control of indoor

populations of *M. domestica* have been tried (Geden *et al.*, 1993; Kramer and Steinkraus, 1993; Shimazu and Kuramoto, 1994; Six and Mullens, 1996; Kuramoto and Shimazu, 1997). The releases, on dairy farms, at poultry facilities or in large cages, took place either by dispersing cadavers of fungus-killed flies or by releasing living fungus-infected *M. domestica*, or both. *E. muscae*/*E. schizophorae* was established in populations of *M. domestica* and, in certain cases, the fungus was more prevalent than in control sites. In no case, however, was it possible to document a decrease in *M. domestica* populations sufficient for control (Mullens *et al.*, 1987).

Releases using *E. muscae* or *E. schizophorae* are difficult to perform for various reasons. First, adult flies are difficult to monitor, precisely due to their behaviour, which is greatly influenced by external factors. This also hampers the possibilities of finding or establishing control plots close to treated plots and monitoring the prevalence of the fungus. For this reason, all release experiments have been done indoors on farms, e.g. poultry houses, with *M. domestica* as the target (Steinkraus *et al.*, 1993b; Six and Mullens, 1996; Kuramoto and Shimazu, 1997). Another problem is the lack of *in vitro* material for release.

Both *E. muscae* and *E. schizophorae* have been kept in culture *in vivo* in various hosts: *M. domestica*, *D. radicum*, *D. antiqua* and *C. rosae* (Kramer and Steinkraus, 1981; Carruthers and Haynes, 1985; Eilenberg, 1987a; Mullens, 1989). The disease was transmitted from sporulating cadavers to uninfected hosts and it was possible for the authors: (i) to keep cultures *in vivo* for a prolonged period of time; (ii) to study host–pathogen relationships, e.g. the effect of temperature on the mortality rate; and (iii) to determine the host range of the fungus. *In vivo* cultivation methods have been used for the production of large numbers of infected flies to be released alive or distributed as cadavers for biocontrol experiments in stables (Geden *et al.*, 1993; Steinkraus *et al.*, 1993b; Shimazu and Kuramoto, 1994).

Although early initiation of epizootics could be achieved artificially, it has proved difficult to control *M. domestica* indoors, even in a confined environment. This is due to a number of factors. Behavioural fever, for example, can influence the development of epizootics in animal houses (Kalsbeek *et al.*, 1999). The simultaneous presence of the two species *E. muscae* and *E. schizophorae* may have seriously biased several of the attempts, since the two species may have different biologies and epizootiology and therefore different requirements for the establishment of epizootics. Without doubt, there is a potential for the control of dipteran insects with *E. muscae* and *E. schizophorae*, but more cost-efficient and reliable *in vitro* systems for production require development.

Erynia neoaphidis *and* Neozygites fresenii *in aphids*

On some occasions, rates of epizootic development are enhanced by extended dew periods and rain and can follow the build-up of aphid populations very closely, retaining that population below the threshold at which spraying of synthetic chemical insecticides is necessary (Glare and Milner, 1991; J.K. Pell, personal observation). However, in many years, the development of epizootics is too late to prevent crop damage, particularly in high-value crops (e.g. salads), where the damage threshold is low. Inoculative augmentation has been attempted on several occasions to encourage the early impact of *E. neoaphidis* and *N. fresenii* in glasshouses and in the field. These trials have given variable results.

In the glasshouse Dedryver (1979) dispersed local isolates of *N. fresenii* in living

infected aphids on two occasions among *A. fabae*-infested bean plants. The glasshouse was maintained at a constant 18°C and was irrigated by misting, ensuring saturated or near-saturated conditions for 20 h day^{-1}. In untreated areas, aphid numbers reached a mean maximum of 1300 per plant after 19 days, whereas treated plants reached a mean maximum of 490 aphids per plant after 8 days. Between 80 and 90% infection in treated aphids was observed after 24–27 days. Latgé *et al.* (1982) and Silvie *et al.* (1990) applied fresh or dry mycelium of *E. neoaphidis* to aphid-infested plants as an aqueous spray in glasshouses. Although the fungus sporulated and was able to infect some aphids, population suppression through further horizontal transmission was not achieved. More recently, glasshouse trials of *E. neoaphidis* formulated as dried mycelium or in alginate beads and applied as a mycoinsecticide have shown some promise for control of *Macrosiphon euphorbiae* (Shah *et al.*, 2000).

Latteur and Godefroid (1983) introduced *in vitro*-produced mycelia of *E. neoaphidis* into populations of aphids on cereals at three field sites in France. Although the inoculum sporulated, there was no population suppression. In this study and in those of Latgé *et al.* (1982) and Silvie *et al.* (1990), tropical isolates of *E. neoaphidis* were used, although their intention was clearly to augment the existing fungal population, rather than to effect classical biological control. This is acceptable because there is no reason to believe that the exotic isolate represented a different pathotype of the fungus, although its temperature optimum was higher. In contrast, *E. grylli* pathotypes occurring on different continents have different biological and molecular characteristics.

Under field conditions, Wilding (1981) released local British isolates of *E. neoaphidis* and *N. fresenii* in fungus-killed aphid cadavers into *A. fabae* populations and was able to cause an early-season crash in aphid populations with both fungal species in 2 out of 4 years. The two species of fungi spread rapidly and some yield improvements were observed in 1 year. Both the successful years had below-average temperatures and above-average rainfall. In a further trial, plots were treated with *E. neoaphidis* in a similar way and, although 70% of aphids became infected, the fungus failed to multiply rapidly enough to protect the crop adequately. Irrigation increased the proportion of infected aphids, confirming that the fungus can be limited by dry conditions (Wilding *et al.*, 1986b). Similar effects were seen in cereals (Wilding *et al.*, 1990), with *E. neoaphidis* causing increased infection in treated plots but acting too slowly and unpredictably.

In a recent study by Poprawski and Wraight (1998) in Idaho, small pieces of sporulating *E. neoaphidis* mycelium were inserted directly into approximately 200 rolled wheat leaves with large colonies of Russian wheat aphid, *D. noxia*. After 5 days, inoculated tillers were sampled. The fungus had sporulated profusely and 18% of aphids were infected. The fungus spread rapidly to uninoculated tillers within subplots but was slow to spread to the rest of the field.

Bottlenecks in exploitation in biological control – mass production and formulation

Mass production is the most important bottleneck in the use of Entomophthorales as mycoinsecticides (inundative augmentation), when large quantities would be required, and, to a lesser extent, for smaller-scale use in inoculative augmentation and classical introduction. For all these strategies the quantity and, more importantly, the quality

of mass-produced materials must be reliable. Members of the Entomophthorales present a spectrum of nutritional requirements for growth *in vitro* (Latgé, 1981). Based on their nutritional requirements for growth, the Entomophthorales can be divided into four groups. *Conidiobolus* spp. grow quickly on standard media. *Batkoa, Erynia* and *Zoophthora* spp. are a little more difficult but can generally be grown on standard media with some supplements. *Entomophthora* and *Entomophaga* spp. need more complex media and *Strongwellsea* and *Neozygites* have proved difficult to grow in anything but tissue culture media (Keller, 1997).

Therefore, complexity regarding *in vitro* mass production can differ significantly among genera. This poses problems related to mass production. With respect to formulation and application, what are the options with respect to the propagule to be released: conidia, resting spores, hyphal bodies, dried mycelium? Cells of the Entomophthorales are relatively large, posing problems for conventional use in spray equipment; some sprayers have filters as fine as 10 μm, although most conventional hydraulic sprayers have openings of at least 150 μm. Conidia are also sticky, making them difficult to harvest from cultures and suspend uniformly in water. In addition, if, during formulation or application, the mucus surrounding conidia is lost, a vital adhesion mechanism could be lacking. As conidia are considered ephemeral, most work has considered only hyphal material and resting spores. To date, technology for economic mass production has not been developed to enable marketing any Entomophthorales for large-scale application. However, numerous studies have been conducted to develop methods for mass production; these have primarily targeted hyphal stages or resting spores.

Production of hyphal stages

The general hypothesis behind mass production of hyphal stages is that, after application in the field, these stages would rehydrate and produce conidia *in situ*. With this strategy as a goal, McCabe and Soper (1985) developed a mass production and drying method for *Z. radicans*, named the 'marcescence process'. The fungus was grown in 25 l fermentation vessels and then sprayed with 10% maltose and dried. Fungal mats were then milled, and hyphal fragments were subsequently released in the field against *E. fabae*. An unknown proportion of hyphal fragments stored in the freezer (–20°C) were viable for at least 1 year. Li *et al.* (1993), using an isolate of *Z. radicans* under development for *P. xylostella* control and a slightly modified marcescence process, found that dried mycelia could be stored at 4°C for up to 80 days, but did not survive freezing. These researchers suggested that the mucilage observed in scanning electron microscopy of mycelium was an important protectant during drying. Cold-temperature (4°C) drying of mycelia of this isolate was essential for storage; if the mycelia were dried under ambient conditions, they lost the ability to sporulate within 2 weeks (Pell *et al.*, 1998).

Nolan (1985) worked on developing media and conditions to optimize mass production of *E. aulicae* hyphal bodies that would be able to produce conidia. The formation and yield of protoplasts and hyphal bodies under varying conditions was investigated using several media including Grace's insect tissue culture media plus fetal calf serum. A protein-free defined medium including eight amino acids was developed that supported growth of protoplasts in both stationary and shaken cultures (Nolan, 1988). Subsequently, enhancement of hyphal body production through loose binding of early protoplast stages to a substrate was investigated. Hyphal bodies constituted

94% of fungal cells in the presence of substrates but only 42% in the absence of substrates. Using 14 l fermentation vessels, addition of a neutral or positively charged disc improved hyphal body production, especially by the fourth day. A disc with a net neutral charge (mylar) demonstrated greater yields than a positively charged disc (polypropylene) (Nolan, 1990). A negatively charged disc (Teflon) was found to inhibit the transition from protoplasts to hyphal bodies, with a recycling of protoplast developmental stages occurring instead (Nolan, 1991). Further studies in 14 l fermenters compared the effects of three different media (a basal medium consisting of 13 amino acids supplemented with either fetal calf serum, tryptic soy broth plus albumin or tryptic soy broth plus calcium caseinate) on amino acid uptake and production and glucose and oxygen utilization by *E. aulicae* (Nolan, 1993b). The medium supplemented with fetal calf serum (a major component of standard media for growing entomophthoralean cells in tissue culture) delayed protoplast growth in *E. aulicae*, possibly due to inhibition by free fatty acids, and supported the lowest hyphal body yield after 9 days. This basal medium supplemented with tryptic soy broth and calcium caseinate greatly increased yields to 3×10^8 hyphal bodies l^{-1}, while inducing some production of conidia during fermentation growth, demonstrating that conidia could be produced from these hyphal bodies. Hyphal bodies may be easily separated from the medium and they withstand applications as sprays. The medium supported growth of *E. aulicae* isolates from numerous geographical areas and is relatively inexpensive (Nolan, 1993a, 1998).

E. neoaphidis grows readily on complex solid media containing egg yolk and milk (Latgé *et al.*, 1978; Wilding and Brobyn, 1980). On a larger scale, mycelium has also been produced in 10 l batch fermenter culture in yeast extract, glucose and milk (Li *et al.*, 1993). More recently, a semi-defined medium containing oleic acid has been developed (Gray *et al.*, 1990). In this medium, oleic acid was required at low concentrations (0.02% v/v) but was toxic at high concentrations (0.2% v/v). The consequence of toxicity was that cultures in semi-defined liquid media only grew if the initial inoculum density was high. The presence of baffles in fermenters assisted growth and also resulted in greater wall growth (Nielsen *et al.*, 2000a). Gray and Markham (1997) developed a model to describe the growth kinetics of *E. neoaphidis* in liquid culture and, by applying predictions from the model, were able to grow *E. neoaphidis* in continuous culture for the first time. This model was able to account for some of the inconsistent results that were observed in simple batch fermenter culture. More work is required, particularly with respect to the C and N requirements during growth, if consistent, good-quality *E. neoaphidis* is to be produced on a large scale.

The most successful culture of *E. muscae* and *E. schizophorae in vitro* has been obtained using liquid media (Carruthers *et al.*, 1985; Eilenberg *et al.*, 1990, 1992; A.B. Jensen, unpublished) with growth as protoplasts (Latgé *et al.*, 1988). Conidia production from *in vitro* cultures has only been achieved on a very small scale, although these conidia are infective. Currently, material for experimentation and release is produced *in vivo* in various hosts: *M. domestica*, *D. radicum*, *D. antiqua* and *C. rosae* (Kramer and Steinkraus, 1981; Carruthers and Haynes, 1985; Eilenberg, 1987a; Mullens, 1989).

The *Neozygitaceae* are among the most advanced of the Entomophthorales in terms of host specialization and growth requirements. Numerous attempts to grow *Neozygites in vitro* have failed (Gustafsson, 1965b; Mietkiewski *et al.*, 1993) or reports are questionable (Kenneth *et al.*, 1972, in Keller, 1997); this led Keller (1997) to state that there is no growth in standard media. However, Butt and Humber (1989) successfully

cultured the vegetative cells of an unidentified *Neozygites* sp. from an infected *T. urticae*, in Grace's insect tissue culture medium and mammalian tissue culture medium 199. Delalibera (1996), Leite *et al.* (1996e) and others reported *in vitro* culture of *Neozygites* sp. isolated from mites, but growth was limited to the hyphal body stage. Grundschober *et al.* (1998) have recently successfully growth *N. parvispora in vitro*, which augurs well for future research with other *Neozygites* spp. They cultured two isolates of *N. parvispora* (from *Thrips tabaci*) using Grace's insect tissue culture medium supplemented with fetal bovine serum and pretreated lepidopteran haemolymph. Both the fetal bovine serum and the haemolymph were essential for sustained growth of hyphal bodies. These hyphal bodies were able to act as conidiophores and produce primary conidia. Interestingly, *E. thripidum*, another species from *T. tabaci*, which was also previously impossible to culture *in vitro*, grew in a similar medium but without the requirement for haemolymph (Freimoser *et al.*, 2000).

Successful *in vitro* cultures of *Neozygites* spp. have been reported only for species isolated from mites and thrips, all of which apparently do not form protoplasts. *N. fresenii* from aphids does form protoplasts prior to hyphal body formation, and this may be one factor influencing the lack of *in vitro* success with *Neozygites* spp. from aphids. At present, the prospects of producing *Neozygites* species *in vitro* for use as mycoinsecticides are uncertain. However, Grundschober *et al.* (1998) are determining the vital components in haemolymph necessary for the growth of hyphal bodies and may be able to apply this knowledge to other species. The production of mycelia of good quality in sufficient quantities for commercial applications requires further research in order to define the media requirements during production. For some entomophthoralean species, e.g. *E. aulicae* and *E. neoaphidis*, certain fatty acids, particularly oleic acid, promote growth and development (Nolan, 1988; Gray *et al.*, 1990). Often variation between fermenter batches of mycelium is high, even when conditions are as constant as possible, suggesting that vital components are missing (Pell *et al.*, 1998).

Dried insect cadavers containing entomophthoralean hyphal bodies are known to survive well at 4°C (Kenneth *et al.*, 1972; Wilding, 1973; Tyrrell, 1988; Pell and Wilding, 1992). Aphids infected with *N. fresenii* and dried in the hyphal body stage (Fig. 4.85) have been dried and frozen for years and the fungus still formed primary conidia within an hour of being rehydrated (Steinkraus *et al.*, 1993a). Entomophthoralean conidia produced *in vivo* are often more pathogenic than those produced *in vitro* (Papierok, 1982; Morgan, 1994). Through an understanding of the *in vivo* nutrient requirements of these fungi and their intrinsic ability to survive desiccation, it may be possible to improve the pathogenicity and storage potential of *in vitro*-produced fungus. For example, Nolan and Dunphy (1979) demonstrated that the incorporation of fungal sex hormones and insect growth hormones into *in vitro* cultures of *E. aulicae* (= *Entomophthora egressa*) encouraged the development of thick-walled hyphal bodies. The promotion of specific fungal structures more suited to the drying process by the addition of specific components to the culture medium could offer an opportunity to optimize production methods in the future.

Formulation of hyphal material

For application, mycelium produced in liquid culture must be formulated. Recent studies by Shah *et al.* (1998) have identified algination as a way of formulating *E. neoaphidis* mycelium for application. An optimal concentration of 1.5% sodium alginate in either

0.1 or 0.25 mol l^{-1} calcium chloride as a gelling agent was used to trap hyphae (220–620 μm × 7–19 μm fragments only) in a matrix. Granules containing 40% fungal biomass produced significantly more conidia than those containing 20% or 10% biomass and the algination process had no impact on infectivity of conidia produced compared with those from unformulated mycelia. Significantly more conidia were produced from granules supplemented with sucrose, starch or chitin (Shah *et al.*, 1999). Granules dried at 95% RH retained more activity than those dried at lower humidities suggesting rate of drying is critical. Storage for short periods was better at 10°C than 23 or 4°C, and a storage humidity of 65% was better than either 80 or 90% (Shah *et al.*, 1998). Although only small fragments could be formulated in this way, requiring filtration during harvesting, this still represents a promising formulation technique and a significant step forward for the practical application of *E. neoaphidis* and other Entomophthorales. Recent glasshouse trials using alginate granules and unformulated mycelia have given promising results (Shah *et al.*, 2000). In addition, hyphal bodies of *N. parvispora* produced *in vitro* could be entrapped in alginate from which they were capable of producing capilliconidia (Grundschober *et al.*, 1998). Commercial application methods for Entomophthorales are still required.

Production of resting spores

Some mass-production research programmes have focused on resting spores. However, only some species of Entomophthorales are known to produce resting spores *in vitro* (Table 4.3). Principal efforts have focused on aphid pathogens in the genus *Conidiobolus*, due to the ease with which members of this genus produce resting spores both in hosts and *in vitro*. Studies have been conducted to optimize production of resting spores using solid media. Soper *et al.* (1975) produced kilogram quantities of *Conidiobolus thromboides* (= *Entomophthora* sp. nr. *thaxteriana*) resting spores using media containing egg yolks, with yields approximating 2–3 g of spores per egg yolk. While the cost of labour and supplies precluded production on a commercial scale, resting spores could easily be produced for small-scale field trials. As part of this study, germinability of *in vitro*-produced resting spores was evaluated. The presence of selected chemicals, such as ethanol, *cis*-cinnamaldehyde, D-limonene, and *n*-nonanol, enhanced resting spore germination. Significant increases in germination were obtained after high-speed blending of resting spores or sonication. These studies were extended to investigate storage of the resting spores; storage of dry resting spores at 4°C for 1 year resulted in no change in germination.

A greater effort has been focused on *in vitro* production of resting spores by *Conidiobolus* spp. using liquid media. Initially, several species of Entomophthorales were used to test the effect of various sources of carbon (Latgé, 1975a), nitrogen (Latgé, 1975b) and lipids and fatty acids (Latgé and de Bievre, 1976) on fungal growth and sporulation. Fungal species or isolates within a species differed in their propensity to produce resting spores *in vitro*. This information helped with the development of semi-defined media for the production of resting spores by *C. thromboides* (= *Entomophthora virulenta*) (Latgé *et al.*, 1977, 1978) and *Conidiobolus obscurus* (= *Entomophthora obscura*) (Latgé, 1980). Darkness, 25°C and a pH of 6.5, with yeast extract as the nitrogen source and a 15% total nutrient concentration, maximized production of *C. thromboides* resting spores (Latgé *et al.*, 1978). For *C. obscurus*, which has more complex nutritional requirements than *C. thromboides*, culturing in aerated media containing 4% glucose and 1% yeast extract produced 0.5–1 × 10^6 resting spores ml^{-1}

Table 4.3. Species of Entomophthorales reported as producing resting spores *in vitro*.

Species	Host group of isolate	Reference
Basidiobolus ranarum	Aphididae	Latgé, 1975a
20 species of *Conidiobolus*	Various	King, 1977
Conidiobolus coronatus	Aphididae	Gustafsson, 1965b
Conidiobolus obscurus	Aphididae	Latgé and Perry, 1980
Conidiobolus pseudapiculatus	Tenthredinae	Keller, 1987c, 1991
Empusa sp.	Tortricidae	Sawyer, 1929
Entomophaga aulicae	Geometridae	Nolan *et al.*, 1976
Entomophaga conglomerata	?	Tyrrell, 1970
Entomophaga gigantea	Tipulidae	Keller, 1987c
Entomophaga limoniae	Limoniidae	Keller, 1987c
Entomophaga maimaiga	Lymnatriidae	Kogan and Hajek, 2000
Entomophaga tipulae	?	Tyrrell, 1970
Entomophthora culicis	Diptera	Gustafsson, 1965b
Entomophthora muscae[a]	Diptera	Schweizer, 1948
Entomophthora schizophorae	Diptera	Eilenberg *et al.*, 1990
Erynia aquatica	Nematocera	Keller, 1991
Erynia athaliae	Tenthredinidae	Keller, 1991
Erynia blunckii	Plutellidae	Keller, 1991
Erynia dipterigena	Diptera	Keller, 1991
Erynia neoaphidis	Aphididae	Uziel and Kenneth, 1986
Erynia virescens	Noctuidae	Keller, 1991
Furia crustosa	Lasiocampidae	MacLeod and Tyrrell, 1979
Pandora nouryi	Aphididae	Gustafsson, 1965b
Zoophthora elateridiphaga	Elateridae	Keller, 1991
Zoophthora phytonomi	Curculionidae	Ben Ze'ev and Jaques, 1990
Zoophthora radicans	?	Gustafsson, 1965c
Zoophthora viridis	Miridae	Keller, 1991

[a]The validity of these observations is in question.

within 6–8 days (Latgé, 1980). Chemically defined media have been developed for these two species (Perry and Latgé, 1980; Latgé and Sanglier, 1985).

Resting spores ($1–3 \times 10^6$ spores ml^{-1}) of *C. obscurus* were produced in 20 l fermenters using semi-defined media, but problems were encountered in larger fermenters (Remaudière, 1983). When batches in large fermenters (100–1000 l) were successful, yields matched those from small-scale laboratory studies. However, during many runs, fermenter batches failed due to contamination. Resting spore production is a relatively slow process, requiring 7–8 days, and, over this long time period, standard sterilization procedures for fungal fermentation were often not adequate. Once prespores are formed by the fourth day of fermentation, nutrients were no longer necessary and, to avoid contamination, prespores could be extracted from fermenters, washed in water and mixed with humid clay. After production, these spores require a period of 3 months at 4°C in a humid atmosphere before germination is possible. Therefore, the time necessary to evaluate the quality of fermentation products was judged prohibitive by industry (Latgé *et al.*, 1983). In addition, numerous scientists attempted to use *in vitro*-produced resting spores of *C. obscurus* for aphid control, and all of these attempts failed (Wilding *et al.*, 1986a). Reasons given for the failure included low germination of resting spores, asynchronous germination, and the fact that all field trials were

conducted in a single year during which aphid populations were low and weather conditions might have been detrimental for the development of epizootics.

Recently, *E. maimaiga* was found to produce resting spores in cell culture, and production of resting spores is being investigated *in vitro* (Kogan and Hajek, 2000). In Grace's insect tissue culture medium plus fetal calf serum, resting spore production requires 7–21 days, much longer than *C. obscurus*. *In vitro* resting spore production varies dramatically by fungal isolate, requires a large surface area : volume ratio in stationary tissue culture flasks, and can be inhibited by > 5% fetal calf serum or glycerol. At present, resting spores have only been produced in low volumes, with a maximum of 3×10^4 resting spores ml^{-1}, but studies are under way to investigate the potential for greater yields in larger volumes of less complex media. Studies of dormancy requirements and per cent germination of *in vitro*-produced resting spores are being conducted simultaneously (A.E. Hajek, unpublished data).

Integrated pest management – the way forward for exploitation of Entomophthorales

The potential of Entomophthorales in pest population regulation lies in the fact that they are common and effective natural antagonists in many insect and mite populations and can decimate those populations at some times of the year, surviving as dormant resting spores or in alternate hosts at times when host populations are absent or low. They have relatively narrow host ranges and a huge capacity for natural dispersal but are difficult to mass-produce.

There are trends in the ecological case histories described here, and in the details of how those species have been used. Classical biological control has been more successful in perennial crops compared with short-term annual cropping systems. In both the *E. maimaiga*/*L. dispar* system and the *Z. radicans*/lucerne aphid system, long-term control was assisted by the ability of the fungus to survive as resting spores and, in the case of the *Z. radicans*/lucerne aphid system, the careful selection of an isolate able to survive under the prevailing abiotic conditions. Undoubtedly, rapid disease spread was achieved through the active discharge and dispersal mechanisms of the two pathogens. In the forest system, long-term control was also enhanced by the leaf litter, which was hardly disturbed from year to year (aiding long-term persistence), and also through conservation (providing moisture in spring in areas where resting spores were released (Hajek and Roberts, 1991; Hajek *et al.*, 1996b)) and augmentation at the leading edge of spread. The presence of at least semi-permanent reservoir sites was also important for the *E. grylli*/grasshopper system.

Where pests are indigenous, existing in annual crops in fragmented agroecosystems, successful approaches learnt from strategies used in perennial crops may still be applied. In annual crops, conservation approaches have potential, particularly by the provision of permanent or semi-permanent refuges in the agroecosystem, i.e providing an environment similar to that in perennial crops. Inoculative augmentation has been attempted but even where infection levels were increased, spread was often unpredictable or too late to prevent crop damage. This is largely because, even though the pathogens have effective mechanisms of persistence and dispersal, the environment is transient and optimal conditions for disease transmission are unpredictable within it. Continued studies on the epizootiology of these fungi will contribute to our understanding of key parameters associated with epizootic establishment so that we can

manipulate conditions to make their occurrence more predictable and intense. Both Wilding (1982a) and Milner (1997) identified this problem and the possible solutions: use fungi as one component of an integrated management strategy or use them as inundative mycoinsecticides, in which case there is a severe bottleneck with respect to mass production and formulation for the Entomophthorales, as previously discussed. Mass production, formulation and application techniques present significant technological difficulties that will only be overcome through fundamental studies of fungal physiology. The mycoinsecticide strategy has greatest potential in glasshouse and high-value systems and even then should be integrated with other approaches. For annual, broad-acre, low-value crops, IPM holds the greatest potential.

IPM is:

> a pest management system that in the socioeconomic context of farming systems, the associated environment and the population dynamics of the pest species, utilizes all suitable techniques in as compatible a manner as possible and maintains the pest population levels below those causing economic injury.
>
> (Dent, 1995)

The spotted alfalfa aphid is no longer a significant pest in Australia for a number of reasons, only one of which was the classical introduction of *Z. radicans*, which undoubtedly contributed to its downfall (R.J. Milner, personal communication). Other control methods used were resistant cultivars, chemical insecticides and the build-up of natural enemies in unsprayed areas, i.e. using the principles of IPM. Today, the aphid is rare (R.J. Milner, personal communication).

IPM seeks to integrate multidisciplinary methodologies to develop pest management strategies that are practical, effective, economical and protective of both public health and the environment (Smith and Reynolds, 1966; Smith *et al.*, 1976). IPM systems will only be successful if they are designed based on an understanding of the key interactions between the pest, its natural enemies and the environment. The range of control measures available for use in IPM are pesticides, host-plant resistance, biological control, cultural control and interference methods (e.g. semiochemicals) (Dent, 1995). More information is required on the integration of Entomophthorales as biological control agents in IPM to exploit them predictably against a number of crop pests.

Fundamental to implementing IPM is the development of reliable pest monitoring methods that relate to an economic damage threshold. Assuming that the conventional use of an insecticide is the first method of control likely to be attempted, these thresholds allow the farmer to make informed decisions on whether to spray, i.e. only when pest damage has reached the predetermined 'action level' (van Emden, 1989; Dent, 1995). The likelihood of the pest population reaching the action threshold is related to other parameters, such as natural enemy abundance (including Entomophthorales), host-plant resistance, cultural practices and climatic variables, all of which are exploited in IPM. If pesticide applications can be reduced in this way, the farmer can make financial and environmental savings. This will also maintain the effectiveness of the currently available narrow-spectrum insecticides by reducing the selection pressure for resistance and so contributing to resistance management.

Compatibility between Entomophthorales and chemical pesticides?

Regardless of whether an entomophthoralean fungus is to be classically introduced, conserved or augmented in an environment as part of an IPM programme, it is essential

to understand how it might interact with the chemical insecticides and fungicides most commonly used for crop protection, in order to determine whether pesticide applications need to be temporally or spatially separated from the most susceptible life stage of the entomophthoralean natural enemy. Susceptibility of fungi to pesticides, particularly fungicides, can vary among fungal species, among isolates within a species and among chemicals (Poprawski and Majchrowicz, 1995; Hermann, 2000), and entomophthoralean fungi are generally more adversely affected by fungicides than hyphomycetes (Majchrowicz and Poprawski, 1993).

Numerous laboratory studies have examined the impact of fungicides on growth (Hall and Dunn, 1959; Soper *et al.*, 1974; Lagnaoui and Radcliffe, 1998), germination, sporulation, infectivity and persistence of Entomophthorales (e.g. Wilding and Brobyn, 1980). These laboratory studies are useful in indicating which chemicals are most likely to have an impact in the field, and need to be continually updated as new products come on to the market. Chemicals which have deleterious effects in the laboratory then need to be tested for field impact.

Fungicides applied to crops can interfere with entomophthoralean fungi, with resulting increases in pest populations. Nanne and Radcliffe (1971) found that potatoes sprayed with captafol, mancozeb and Bordeaux mixture had 1.7–2.6 times more aphids (*M. persicae*) late in the season compared with controls not receiving fungicide treatments. The proportion of aphids infected with entomophthoralean species was 4–5 times greater in the control plots than in the treated plots. Smith and Hardee (1996) found more cotton aphids (*A. gossypii*) and a reduced prevalence of *N. fresenii* in plots treated with carboxin compared with untreated control plots. During years of intensive fungicide spraying, increases in *M. persicae* numbers and potato leaf roll virus were reported by farmers in Minnesota (Lagnaoui and Radcliffe, 1998). In contrast, the numbers of *A. fabae* on field beans were not affected by weekly applications of maneb, benomyl, captafol, mancozeb and tridemorph, even though benomyl significantly decreased aphid mortality due to *E. neoaphidis* (Wilding, 1982b).

Pesticide resistance and the environmental and economic impacts of pesticides have been the greatest driving force behind the development of IPM. The generally accepted view of incompatibility between chemicals and biological control agents has particularly focused attention on understanding and evaluating the impact of natural enemies when assessing the need to apply a chemical (Kogan *et al.*, 1999). One criterion for such an assessment is the concept of 'inaction levels' of natural enemies, which was proposed by Sterling (1984) and has been applied to avoid negative impacts of chemicals on entomopathogenic fungi from the Hyphomycetes (Kogan *et al.*, 1977). Decisions on whether to apply an insecticide were made not only on the economic injury level of the pest, i.e. the 'action level/threshold', but also on the effective population density of the natural enemies or 'inaction level/threshold'. Mathematical models can also be used to predict expected populations of natural enemies and therefore the likelihood of a necessity to use an insecticide. Entomophthoralean fungi have also been used, or have the potential to be used, in combination with semiochemicals, insect natural enemies, host-plant resistance and other pathogens, which are all techniques moving towards true IPM and will be described below.

Use of 'inaction thresholds' and predictive models for IPM with Entomophthorales

NEOZYGITES FRESENII AND *APHIS GOSSYPII* IN THE USA. Since 1989 fungal epizootics

in cotton aphids have been documented in the mid-south USA (Steinkraus *et al.*, 1995). In 1991, the causal agent was identified as the fungus *N. fresenii* (Steinkraus *et al.*, 1991). Studies in the USA have shown that *N. fresenii* epizootics in cotton aphids occur yearly between June and August over wide areas of cotton production (Steinkraus *et al.*, 1995). Further research has indicated that epizootics can be predicted by careful diagnosis of aphid samples (Hollingsworth *et al.*, 1995). When prevalence reaches 15%, aphid declines caused by epizootics are usually imminent. This can be used to limit the use of chemical insecticides and thus save money and potentially assist in the conservation of both fungal and insect natural enemies of aphids.

Declines are more rapid in fields with large aphid populations. Field scouting for fungus-killed aphids is the simplest practical method for detection of fungus during early stages of epizootics (Figs 4.86, 4.87). However, it is not very accurate, even if a hand-lens or stereo-microscope is used. Aphids killed by pesticides may superficially resemble aphids killed by *N. fresenii*. With experience, it is possible to recognize recently killed aphids. Infected aphids are pale grey or tan in colour, somewhat crystalline in appearance, stand on their heads and are attached to the leaf by their mouth-parts. After aphids have been dead for several days, they are often overgrown by saprophytic fungi. These aphids are more noticeable because they are covered with brownish green, woolly fungi. Such aphids are often a good indicator of an ongoing epizootic. However, diagnosis of individual aphids with a phase microscope is preferable and much more accurate. Based on an understanding of the epizootiology of *N. fresenii*, it has been possible to avoid unnecessary applications of insecticides by monitoring *A. gossypii* populations, as discussed below.

In 1993, an extension-based service was established to determine the prevalence of the fungus in cotton aphids collected from fields in Arkansas (Steinkraus *et al.*, 1996a, 1998a; Steinkraus and Boys, 1997). In 1997, the service was expanded to include Louisiana and Mississippi and then expanded again in 1998 to include Alabama and Georgia. Prior to planting cotton each spring, extension agents and consultants are chosen in each state to participate in the programme. Participants are mailed aphid-sampling kits consisting of vials containing 70% ethanol, labels, instructions and pre-addressed express-mail envelopes. During the season, participants sample aphids whenever aphids are considered a problem. The method for determining fungus prevalence in a field is to collect aphids from five areas of a cotton field by rolling up infested leaves or cutting off leaf strips containing aphids and placing these in vials of 70% ethanol. The aphid samples are shipped by express mail to the diagnostic laboratory, where they are squashed and analysed microscopically.

In the laboratory, a random subsample of 50 aphids from each field is squashed in lactophenol–acid fuchsin stain on microscope slides. It is essential that the subsample be a random sample of aphids. No matter what the condition or size, each aphid must have a chance of being selected. With a phase microscope, each individual aphid is diagnosed for signs of the fungus at 200× magnification. Each aphid is carefully scanned for the presence of protoplasts, hyphal bodies, resting spores, conidiophores, conidia, secondary conidia or a combination of saprophytic fungi and *N. fresenii* structures. It takes about 1 h for an experienced operator to examine the aphids from one field.

The success of this service is based on the persistence of *N. fresenii* structures and the small, soft-bodied nature of *A. gossypii*. These attributes make it possible to identify both internal and external signs of infection in squashed aphids (Figs 4.88, 4.89). With training, each of the important structures of the pathogen is easily recognized.

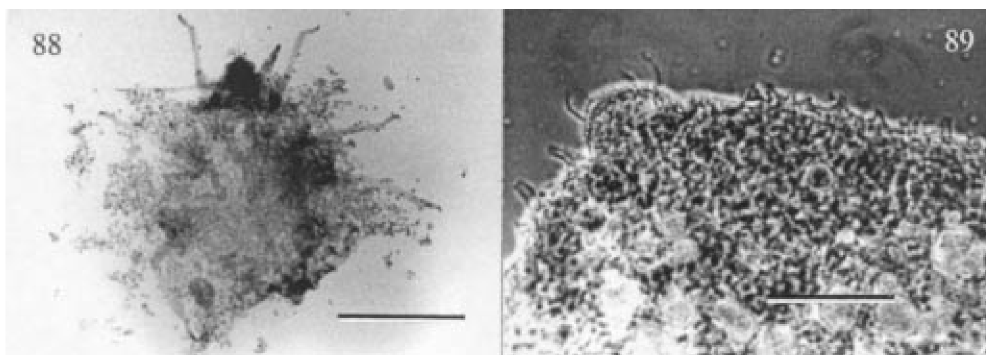

Fig. 4.88. *Neozygites fresenii*-infected cotton aphid squashed on a slide. This aphid contains the protoplast stage of the fungus. Scale bar = 0.5 mm.
Fig. 4.89. Abdominal region and cauda of an *N. fresenii*-infected cotton aphid immediately after sporulation. Remnants of conidiophores indicate that this aphid was killed by the fungus and had discharged conidia. Scale bar = 75 μm.

Capilliconidia, the infective stage, are extremely firmly attached to the legs and antennae of aphids (Figs 4.33, 4.49). Protoplasts and hyphal bodies are released from within infected squashed aphids (Fig. 4.50). Resting spores (Fig. 4.84) and conidiophores (Figs 4.51, 4.89) are also clearly visible. Therefore, when a random sample of aphids is diagnosed, an accurate estimate of the prevalence of *N. fresenii* in the aphid population can be determined.

Results are reported to senders within 24 h. If fungal prevalence is 15% or higher, there is a strong likelihood that the aphid population will decline within a week, due to the occurrence of an epizootic. If it is 50%, there is a strong likelihood that the aphid population will decline within a few days. The critical moment for scouting for the fungus is when a grower is considering an insecticide application for aphid control. If permanent plant damage is being caused by an aphid population and scouting reveals no fungus or very low levels of fungus, growers must use their best judgement in making crop management decisions. The presence or absence of the fungus in the field is an additional piece of information cotton crop consultants can use when making management decisions. This service has been considered useful by the majority of participants. When disease prevalence is 15% or more, growers frequently decide not to use an insecticide, thus saving money, preserving beneficial insects and reducing environmental contamination by pesticides (Steinkraus *et al.*, 1996a, 1998a; Steinkraus and Boys, 1997).

ZOOPHTHORA PHYTONOMI AND THE ALFALFA WEEVIL. Management practices to exploit the natural control potential of *Zoophthora phytonomi* against alfalfa weevil, *Hypera postica*, have been developed. In initial economic assessments, Brown and Nordin (1982) predicted that, in a lucerne field that has not received insecticide applications, the economic benefits from a naturally occurring epizootic were larger than the cost of insecticide use.

A model of this host/pathogen system was used to develop alfalfa weevil control recommendations that take advantage of the activity of *Z. phytonomi*. An early harvest date was recommended so that first cut coincided with the time of first incidence of

disease in weevils, resulting in earlier and more intense epizootics (Brown, 1987). First incidence of the fungus could be accurately predicted with the model. Harvesting the lucerne was thought to stimulate epizootics because larvae became concentrated in warm and humid wind-rows, optimizing chances for disease transmission (Brown and Nordin, 1986). Adequate weevil control resulted, with insecticide applications required only early in the season. While the early harvest date may reduce the profit from the first cutting, epizootics of *Z. phytonomi* could reduce larval weevil populations affecting the second cutting (Nordin, 1984). Benefits from epizootics were greater in wet years than dry years.

ENTOMOPHTHORA MUSCAE AND *DELIA ANTIQUA*, THE ONION FLY, IN THE USA. Six biological components were found to be important in the levels of *E. muscae*-induced control of the onion fly, *D. antiqua*. These included parameters related to the pathogen and primary host, the secondary host (the seed-corn maggot, *Delia platura*), the onion crop and adjacent crops and border plants (Carruthers and Soper, 1987; Carruthers *et al.*, 1985). A simulation model was developed and verified using laboratory and field data to describe the parameters affecting pest *D. antiqua* dynamics in the onion agroecosystem (Carruthers and Soper, 1987). Adding the alternate host significantly increased the prevalence of *E. muscae*; emergence of the seed-corn maggot earlier in the season than the onion maggot allowed *E. muscae* to reach higher levels earlier in the season. Close proximity of secondary hosts, in borders, etc., would aid in the natural control of onion maggot. The simulation model, in combination with laboratory and field experiments, also predicted deleterious impacts of pesticide applications on *E. muscae*. This information was used to advise onion growers in Michigan and led to a reduction in pesticide use while maintaining or increasing onion maggot control (Carruthers *et al.*, 1985; Carruthers and Soper, 1987).

Integrating Entomophthorales with behaviour-modifying chemicals (semiochemicals)

AUTODISSEMINATION. The hypothesis behind autodissemination is that pest behaviour can be manipulated using semiochemicals to encourage the spread of pathogens to susceptible conspecifics on the crop earlier in the season than they would normally spread. In this way disease epizootics would establish and decimate small early-season pest populations before the crop was damaged.

The strategy has been applied to the control of the diamondback moth, *P. xylostella*, using *Z. radicans*. Male moths are attracted into a specially designed fast-entry slow-exit trap in response to synthetic female sex pheromone. Whilst inside the trap, they become contaminated with infective conidia from a sporulating source of *Z. radicans*. On habituation to the pheromone, contaminated moths leave the trap and return to the crop, disseminating the pathogen among their own populations (= autodissemination). The benefits of this strategy over the inundative mycoinsecticide approach are threefold. Use of a specific sex pheromone targets the inoculum to the diamondback moth, as this is the only insect entering the trap. Only small quantities of fungal inoculum and pheromone are required, thereby limiting problems associated with mass production, formulation and storage. Whilst inside the trap, the fungus can be protected from the damaging effects of ultraviolet radiation, and an abiotic environment that favours sporulation and infection can be provided. If an epizootic develops, insecticide applications will be reduced.

Prototype traps have been designed and evaluated in the laboratory and the field to test this strategy, and this work is described here. Complementary laboratory studies have selected isolates, originating from the diamondback moth, with high virulence in the country where the strategy was to be tested (Pell *et al.*, 1993b) and low virulence against non-target organisms (Furlong and Pell, 1996). The prototype trap allows rapid entry of the insects to a central inoculation arena but delays departure, thereby ensuring heavy contamination of moths with infective conidia. The central arena of the trap contained the sporulating fungus and pheromone lure and was protected from rain and ultraviolet radiation by a pyramid of plastic placed above it. A water reservoir was connected by a wick to the central arena to ensure that the humidity remained high within the trap. Moths entered and left the central arena through baffles (Pell *et al.*, 1993a). The lure comprised a polythene vial impregnated with the synthetic pheromone, and fungus production could be adapted from the methods described by McCabe and Soper (1985), Li *et al.* (1993) and Pell *et al.* (1993b, 1998). The trap was placed either at the height of the crop or on the soil between rows of the crop during evaluation (Fig. 4.81).

In field evaluations made in the Cameron Highlands, Malaysia, moths responded to the synthetic sex pheromone and entered the trap at all times of the day and night. Moths entering the trap spent a mean of 88 s in the central inoculation arena. Parallel laboratory studies showed that when a sporulating source of *Z. radicans* was placed above and below the pheromone lure in the trap, the LT_{50} for a single visit by male moths to the inoculation arena was less than 60 s (Furlong *et al.*, 1995; Pell and Furlong, 1998). Therefore, the duration of the visits observed in the field would guarantee infection if appropriate quantities of inoculum were placed in the trap (Furlong *et al.*, 1995). Under field conditions, infected moths succumbed to *Z. radicans* within 4 days of inoculation. This was within the active lifetime of the healthy moths, which was vital as *Z. radicans* must develop within a living host and cannot grow and sporulate on an insect that has died before the fungus has fully completed its development (Furlong *et al.*, 1995).

The prototype trap was both complex in design and expensive to construct and therefore impractical, particularly for use by resource-poor farmers in Africa and Asia, the targeted users of the technology. Simpler traps have now been designed, based either on the standard Delta trap (Oecos, Kimpton, UK) or on materials such as soft-drink cans, which are readily available to farmers in these regions. A Delta trap with a Petri-dish insert (inoculation arena) and the pheromone lure (= Delta–Petri trap) was more effective at attracting moths (16.2 ± 4.2 moths entered in 15 min) than the prototype trap (7.9 ± 2.8 moths entered in 15 min) when tested in kale in Kenya (Pell and Furlong, 1998; J.K. Pell and M.J. Furlong, unpublished data). The duration of visits to the central inoculation arena of the Delta–Petri trap (79.9 ± 19.2 s) was less than to the same region of the prototype trap (122.2 ± 19.4 s) but was still within the time necessary to ensure infection of the moth (Pell and Furlong, 1998; J.K. Pell and M.J. Furlong, unpublished data). Simple traps also required modifications to compensate for the removal of the water reservoir in the prototype trap. To maintain high humidities within the inoculation chambers, the inner surfaces of the traps were coated with a layer of 1% tap-water agar. Simple traps incorporating a water reservoir and water agar surfaces are currently being tested in Australia (J.K. Pell and R. Vickers, unpublished data).

Once effective traps were designed that reliably contaminated insects with fatal doses of inoculum, it was essential to quantify dispersal and transmission of inoculum

from those insects to the remaining population. This has mainly been examined in laboratory studies. In a preliminary field trial, a fluorescent marker was placed in the inoculation arena. Moths became contaminated and were observed depositing the marker on foliage up to 5 m away from the trap, demonstrating that fungal dispersal would occur over at least that distance and almost certainly much further (Pell *et al.*, 1993a).

Dispersal of inoculum from contaminated moths to conspecifics can occur in two ways: passive transfer from contaminated males to other adults and larvae on the crop or, upon the death of the contaminated moth, transfer of fungal inoculum from the resulting cadaver to larvae and adults on the crop. In laboratory studies, although the dose of *Z. radicans* conidia acquired in the inoculation arena was sufficient to kill 90–100% of the moths, passive mechanical vectoring of *Z. radicans* to other adults and larvae was extremely limited; a mean of 15% of larvae on plants in contact with *Z. radicans*-contaminated moths became infected (Pell and Furlong, 1998). Larvae that die from infection are characteristically attached to the leaf surface by rhizoids and the sticky conidia were actively discharged, forming a halo around the dead insect. These cadavers caused disease in a significant proportion of the surrounding larval population (M.J. Furlong and J.K. Pell, unpublished data). In addition to ensuring the death of the male moths entering the trap, with the resulting potential to initiate epizootics, *Z. radicans* has detrimental impacts on its host prior to death (e.g. decreased feeding in infected larvae), which also contribute to a reduction in crop damage. If an epizootic is to establish, good levels of transmission are essential. Disease epizootiology requires further study in the field to better understand how to enhance the development of epizootics. In addition, extensive experiments to determine the number of traps and quantity of inoculum required to effect control are necessary. The number of traps required per hectare and the position and timing of their use require particular examination. Autodissemination also has potential in other systems, particularly with flies, and may overcome the problems associated with a highly mobile host and a fungus that is difficult to mass-produce. Using autodissemination will only require a small amount of inoculum (*in vivo* or *in vitro*) and would exploit the natural behaviour of flies. *M. domestica* adults are attracted to fungus-killed cadavers, thus encouraging their entry into inoculation devices, and adults are known to passively transfer disease in subsequent matings (Møller, 1993; Watson and Petersen, 1993a).

THE 'PUSH–PULL' STRATEGY. The 'push–pull' strategy or 'stimulo-deterrent diversionary strategy' (Miller and Cowles, 1990; Pickett *et al.*, 1991) aims to reduce pest colonization on the crop by reducing the attractiveness of that crop in a number of ways, including the use of antifeedants and semiochemicals, such as oviposition deterrents. At the same time that pests are 'pushed' away from the crop, they are 'pulled' into a highly attractive sacrificial or 'trap' crop nearby. The attractiveness of the sacrificial area is maximized by visual cues and semiochemicals, such as enhanced plant-derived attractants and insect-derived attractants, such as aggregation pheromones. Simultaneously, predators and parasitoids of the pest are also attracted into this area, or highly selective control agents could be used, such as entomopathogenic fungi. This strategy is currently being developed for protection of oil-seed rape crops in the UK and includes the use of the hyphomycete fungus *Metarhizium anisopliae* (Pickett *et al.*, 1995). However, there is no reason why entomophthoralean fungi could not be encouraged in the sacrificial area in a similar way, in conjunction with habitat management and the encouragement of insect natural enemies (see below).

Integrating Entomophthorales with insect natural enemies

Entomophthoralean fungi generally have limited host ranges and are therefore unlikely to directly infect insect natural enemies that are active in the same agroecosystem. This makes them suitable for integration with insect natural enemies for pest management. Apart from direct infection of insect natural enemies, other interactions can occur and have the potential for exploitation in IPM.

The presence of foraging predators and parasitoids in insect populations significantly increases transmission of entomophthoralean fungi (Furlong and Pell, 1996; Roy *et al.*, 1998). When starved, the predatory beetle *Coccinella septempunctata* fed on *E. neoaphidis*-infected *A. pisum* cadavers, although they rarely consumed entire cadavers (Pell *et al.*, 1997a; Roy *et al.*, 1998). Disease transmission from damaged and intact cadavers was not significantly different. However, the presence of the foraging predator on the plant significantly increased infection levels in aphids on that plant compared with plants where there was no predator foraging. *A. pisum* exhibits very strong escape responses in the presence of a predator, which is thought to encourage contact with inoculum and therefore increase transmission (Roy *et al.*, 1998). Complementary to the study of predator and *E. neoaphidis* interactions, Fuentes-Contreras *et al.* (1998) showed that the presence of foraging parasitoids, *Aphidius rhopalosiphi*, encouraged *E. neoaphidis* infection in *S. avenae* populations. In addition, the presence of the foraging parasitoid, *D. semiclausum*, significantly enhanced transmission of *Z. radicans* in *P. xylostella* larval populations (Furlong and Pell, 1996).

Pest populations are patchy in their distribution within the agroecosystem, especially early in the season, and so fungus dispersal between those populations is essential if fungi are to be effective control agents. Aerial dispersal clearly plays a role in passive movement of inoculum between aphid populations. More directed movement will occur through the movement of infected alate aphids, as with *Z. radicans* (Milner *et al.*, 1982). However, these are not the only dispersal mechanisms. For example, the aphid predator *C. septempunctata* can passively carry inoculum of *E. neoaphidis* between *A. pisum* populations while foraging and can thereby establish disease within those populations. This has been demonstrated in laboratory and field studies; in the laboratory, passive vectoring occurred even when the predator foraged on plants with only a single sporulating cadaver present (Pell *et al.*, 1997a; Roy *et al.*, 1998). These studies demonstrate that, with appropriate management, these interactions could be manipulated and encouraged to favour the fungus. This would be particularly appropriate in conjunction with conservation strategies and semiochemicals to manipulate the behaviour of the predators so as to favour dispersal of the pathogen (Roy and Pell, 2000). Parasitoids are not effective as vectors of *E. neoaphidis* to *S. avenae* or *Z. radicans* to *P. xylostella* larvae (Furlong and Pell, 1996; Fuentes-Contreras *et al.*, 1998). In both these cases the parasitoids were contaminated naturally during foraging. If they had been contaminated artificially with more inoculum, it might have been sufficient to facilitate vectoring. Poprawski and Wraight (1998) describe a release of the parasitoid *A. asychis* contaminated with *E. neoaphidis* for control of Russian wheat aphid, *D. noxia*. Aphids were parasitized by the wasp but did not become infected with the fungus. Although this trial was unsuccessful, there remains a potential that parasitoids could be useful in vectoring disease, given appropriate management systems.

Parasitoids and pathogens can interact antagonistically when they compete for resources within their host. For example, the parasitoid wasp *A. rhopalosiphi* continues to attack *E. neoaphidis*-infected cereal aphids until 1 day before fungus-induced

death (Brobyn *et al.*, 1988). Parasitoid development takes longer than fungus development, meaning that, at 20°C, parasitoid oviposition must occur at least 4 days before fungal infection if the parasitoid is to emerge (Powell *et al.*, 1986a). However, since the preferred weather conditions for parasitoids and fungi are contrasting (parasitoids are more active in warm dry conditions and entomophthoralean fungi are more active in cool humid conditions), Milner *et al.* (1984b) suggest that a combination of the two agents is still likely to afford the best control option. Similarly, in the *P. xylostella* natural enemy complex, *Z. radicans* has the potential to interact with two hymenopteran parasitoids, *C. plutellae* and *D. semiclausum*. As already discussed, *D. semiclausum* is susceptible to the fungus, though significantly less so than *P. xylostella*, and infected parasitoids do oviposit significantly fewer eggs than uninfected ones. Neither parasitoid can recognize infected larvae and therefore they oviposit into those larvae. Simultaneous infection and parasitism results in competition within the host. When parasitoid eggs are laid into infected larvae, the eggs are unable to develop successfully. However, if a parasitoid has completed most of its development in the larva prior to infection, it will emerge successfully (Furlong and Pell, 2000). On some occasions the fungus and the parasitoid can emerge from the same host (Fig. 4.82). All these experiments are laboratory-based, and so conclusions must be limited by the context in which the data were collected. In the field, the interactions may be spatially or temporally prohibited; parasitoids can be active under different climatic conditions from fungi, for example. In addition, with appropriate management systems in place, antagonistic interactions can be spatially or temporally avoided.

Habitat manipulation

Biological control using Entomophthorales has been most successful in perennial crop systems (e.g. forestry) or in systems where the pest spends significant periods of time in permanent habitats. This is in part due to the stable nature of the habitats, which act as reservoirs for the fungal natural enemy. In annual cropping systems, foliage is removed and the soil ploughed repeatedly, removing inoculum from the environment. Habitat manipulation could provide permanent or semi-permanent reservoirs in the agroecosystem, with the specific purpose of assisting persistence and early-season multiplication of entomophthoralean fungi. These reservoirs could be managed field margin strips adjacent to hedgerows (Roy and Pell, 2000). The potential of arthropod natural enemies to be conserved in the same areas would improve their chances of encouraging local transmission and vectoring to pest populations in adjacent crops (Roy and Pell, 2000). The success of environmental manipulation to encourage natural enemies relies on the characteristics of the pest, the natural enemy and the ecosystem (Fuxa, 1998). For example, the entomopathogen must be able to replicate extensively, must persist for long periods of time in the reservoir and/or have a sufficiently broad host range to exploit alternate hosts in the reservoir and must be able to disperse from the reservoir into pest hosts on crops. The potential of managed field margins as reservoirs for *E. neoaphidis* is currently under development in the UK (J.K. Pell, unpublished).

Integrating Entomophthorales with host-plant resistance

Plants differ in their nutritive value for herbivorous insects and have an impact on insect physiology and behaviour (Bartlet, 1995; Cole, 1996; Awmack *et al.*, 1997) and,

potentially, susceptibility to entomopathogenic fungi. Few studies have actually considered the role of the host plant on the susceptibility of insects to entomopathogenic fungi and most have focused on Hyphomycetes. In some studies fungal efficacy was synergized, and in others it was inhibited or unaffected (e.g. Hare and Andreadis, 1983; Ramoska and Todd, 1985; Inyang, 1997). In one study with *E. maimaiga*, time to kill was extended on plants less suitable for the host *L. dispar* (Hajek and St Leger, 1994). The effects of malnutrition or starvation, which might occur on unsuitable or resistant host plants, have also been examined. Starvation inhibited infection of the spotted alfalfa aphid *T. trifolii* f. *maculata* by *E. neoaphidis* (Milner and Soper, 1981) and *P. brassicae* by *Z. radicans* (Mietkiewski and van der Geest, 1986). Villacarlos *et al.* (1996) examined the effect of the host plant on *A. gossypii* susceptibility to *N. fresenii* and suggested that there is an impact of the host plant and that it differs with respect to the isolate. Recent work by Fuentes-Contreras *et al.* (1998) has shown that host-plant resistance can also have an impact on the interaction between the parasitoid *A. rhopalosiphi* and *E. neoaphidis* attacking *S. avenae*. In this study it extended the developmental time of the parasitoid but not of the fungus, thereby giving the fungus a competitive advantage. Integrated use of Entomophthorales and host-plant resistance will therefore require further research, particularly with the advent of new transgenic varieties of plants with resistance or partial resistance to pests (Schuler *et al.*, 1998).

Integrating Entomophthorales with other pathogens

The combined use of different pathogens with different biological attributes with respect to the biotic and abiotic environment could be extremely useful, not only in extending the spatial and temporal range over which a target pest can be controlled but also in targeting a number of pests on the same crop. Unfortunately, few studies have been made on interactions between any insect pathogens (Jaques and Morris, 1980). In the *E. maimaiga*/*L. dispar* system, a nuclear polyhedrosis virus (NPV) also causes epizootics and is well known to co-infect individual insects along with *E. maimaiga* (Hajek, 1997c). Co-infection studies suggest that these two important pathogens have minimal negative interactions; during simultaneous infections, *E. maimaiga* kills insects and produces conidia, but, for some treatments when the NPV infected before the *E. maimaiga*, the NPV produced fewer propagules (Malakar *et al.*, 1999).

Autodissemination of *Z. radicans* has the potential to be integrated with the use of other microbial pathogens against the diamondback moth. Complementary studies using *Beauveria bassiana* in the trap suggest that the greatest potential to effect control is given by using both pathogens together in the trap (Pell and Furlong, 1998).

Conclusion

Entomophthorales have great potential in pest management and have been used in a number of successful biological control programmes, particularly in long-term stable systems. They have also been successful in novel approaches under development for use in IPM. Future exploitation lies in their use as components in IPM, and will rely on an increasing understanding of their population genetics, physiology and epizootiology in a multitrophic environment.

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References

Andreadis, T.G., and Weseloh, R.M. (1990) Discovery of *Entomophaga maimaiga* in North American gypsy moth, *Lymantria dispar*. *Proceedings of the National Academy of Sciences, USA* 87, 2461–2465.

Aruta, M.C., Carillo, L.R. and Gonzalez, M.S. (1974) Determinacion para Chile de hongos entomopatogenos del genero *Entomophthora*. *Agro Sur* 2, 62–70.

Awmack, C.S., Harrington, R. and Leather, S.R. (1997) Host plant effects on the performance of the aphid *Aulocorthum solani* (Kalt.) (Homoptera: Aphididae) at ambient and elevated CO_2. *Global Change Biology* 3, 545–549.

Balazy, S. (1993) *Flora of Poland. Fungi (Mycota)* Vol. 24. *Entomophthorales*. Institute of Botany, Krakow, 356 pp.

Bartlet, E. (1995) Chemical cues to host-plant selection by insect pests of oilseed rape. *Agricultural Zoology Reviews* 7, 89–116.

Beauvais, A., Latgé, J.-P., Vey, A. and Prevost, M.-C. (1989) The role of surface components of the entomopathogenic fungus *Entomophaga aulicae* in the cellular immune response of *Galleria mellonella*. *Journal of General Microbiology* 135, 489–498.

Bellini, R., Mullens, B.A. and Jespersen, J.B. (1992) Infectivity of two members of the *Entomophthora muscae* complex (Zygomycetes: Entomophthorales) for *Musca domestica* (Dipt.: Muscidae). *Entomophaga* 37, 11–19.

Ben-Ze'ev, I. and Jaques, R.P. (1990) Entomopathogenic fungi in insects in alfalfa fields in south-western Ontario. *Proceedings of the Entomological Society of Ontario* 121, 71–78.

Ben-Ze'ev, I. and Kenneth, R.G. (1982a) Features-criteria of taxonomic value in the Entomophthorales: I. A revision of the Batkoan classification. *Mycotaxon* 14, 393–455.

Ben-Ze'ev, I. and Kenneth, R.G. (1982b) Features-criteria of taxonomic value in the Entomophthorales: II. A revision of the genus *Erynia* Nowakowski 1881 (= *Zoophthora* Batko (1964). *Mycotaxon* 14, 456–475.

Ben-Ze'ev, I. and Uziel, A. (1979) *Moniellia costalis* (Fitch), a new host for *Zoophthora radicans* (Brefeld) Batko and *Entomophthora planchoniana* Cornu (Zygomycetes: Entomophthoraceae) in Israel. *Phytoparasitica* 7, 159–167.

Bidochka, M.J. and Hajek, A.E. (1996) Protoplast plasma membrane glycoproteins in two species of entomopathogenic fungi. *Mycological Research* 100, 1094–1098.

Bidochka, M.J., and Hajek, A.E. (1998) A nonpermissive entomophthoralean fungal infection increases activation of insect prophenoloxidase. *Journal of Invertebrate Pathology* 72, 231–238.

Bidochka, M.J., Walsh, S.R.A., Ramos, M.E., St Leger, R.J., Silver, J.C. and Roberts, D.W. (1996) Fate of biological control introductions: monitoring an Australian fungal pathogen of grasshoppers in North America. *Proceedings of the National Academy of Sciences USA* 93, 918–921.

Bitton, S., Kenneth, R.G. and Ben-Ze'ev, I. (1979) Zygospore overwintering and sporulative

germination in *Triplosporium fresenii* (Entomophthoraceae) attacking *Aphis spiraecola* on citrus in Israel. *Journal of Invertebrate Pathology* 34, 295–302.

Boykin, L.S., Campbell, W.V. and Beute, M.K. (1984) Effects of pesticides on *Neozygites floridana* (Entomophthorales: Entomophthoraceae) and arthropod predators attacking the twospotted spider mite (Acari: Tetranychidae) in North Carolina peanut fields. *Journal of Economic Entomology* 77, 969–975.

Brandenburg, R.L. and Kennedy, G.G. (1981) Overwintering of the pathogen *Entomophthora floridana* and its host, the twospotted spider mite. *Journal of Economic Entomology* 74, 428–431.

Brandenburg, R.L. and Kennedy, G.G. (1983) Interactive effects of selected pesticides on the two-spotted spider mite and its fungal pathogen, *Neozygites floridana*. *Entomologia Experimentalis et Applicata* 34, 240–244.

Brefeld, O. (1870) Entwicklungsgeschichte der *Empusa muscae* und *Empusa radicans. Botanische Zeitschrift* 28, 161–166, 179–186.

Brobyn, P.J. and Wilding, N. (1977) Invasive and develomental processes of *Entomophthora* species infecting aphids. *Transactions of the British Mycological Society* 69, 349–366.

Brobyn, P.J., Wilding, N. and Clark, S.J. (1985) The persistence of infectivity of conidia of the aphid pathogen *Erynia neoaphidis* on leaves in the field. *Annals of Applied Biology* 107, 365–376.

Brobyn, P.J., Wilding, N. and Clark, S.J. (1987) Laboratory observations on the effect of humidity on the persistence of infectivity of conidia of the aphid pathogen *Erynia neoaphidis*. *Annals of Applied Biology* 110, 579–584.

Brobyn, P.J., Clark, S.J. and Wilding, N. (1988) The effect of fungus infection of *Metopolophium dirhodum* (Homoptera: Aphididae) on the oviposition behaviour of the aphid parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Aphidiidae). *Entomophaga* 33, 333–338.

Brown, G.C. (1987) A modelling approach to epizootiological dynamics of insect host–pathogen interactions and an implementation example. In: Shoemaker, C.A. and Brown, G.C. (eds) *Insect Pest Management Modelling*. John Wiley & Sons, New York.

Brown, G.C. and Nordin, G.L. (1982) An epizootic model of an insect–fungal pathogen system. *Bulletin of Mathematical Biology* 44, 731–740.

Brown, G.C. and Nordin, G.L. (1986) Evaluation of an early harvest approach for induction of *Erynia* epizootics in alfalfa weevil populations. *Journal of the Kansas Entomological Society* 59, 446–453.

Brown, G.C., Prochaska, G.L., Hildebrand, D.F., Nordin, G.L. and Jackson, D.M. (1995) Green leaf volatiles inhibit conidial germination of the entomopathogen *Pandora neoaphidis* (Entomophthorales: Entomophthoraceae). *Environmental Entomology* 24, 1637–1643.

Butt, T.M. and Humber, R.A. (1989) An immunofluorescence study of mitosis in a mite-pathogen, *Neozygites* sp. (Zygomycetes: Entomophthorales). *Protoplasma* 151, 115–123.

Butt, T.M., Beckett, A. and Wilding, N. (1981) Protoplasts in the *in vivo* lifecycle of *Erynia neoaphidis*. *Journal of General Microbiology* 127, 417–421.

Butt, T.M., Wraight, S.P., Galaini-Wraight, S., Humber, R.A., Roberts, D.W. and Soper, R. S. (1988) Humoral encapsulation of the fungus *Erynia radicans* (Entomophthorales) by the potato leafhopper *Empoasca fabae* (Hemiptera: Cicadellidae). *Journal of Invertebrate Pathology* 52, 49–56.

Butt, T.M., Beckett, A. and Wilding, N. (1990) A histological study of the invasive and developmental process of the aphid pathogen *Erynia neoaphidis* (Zygomycetes: Entomophthorales) in the pea aphid *Acyrthosiphon pisum*. *Canadian Journal of Botany* 68, 2153–2163.

Byford, W.J. and Reeve, G.L. (1969) *Entomophthora* species attacking aphids in England, 1962–1966. *Transactions of the British Mycological Society* 52, 342–346.

Carner, G.R. and Canerday, T.D. (1968) Field and laboratory investigations with *Entomophthora fresenii*, a pathogen of *Tetranychus* spp. *Journal of Economic Entomology* 61, 956–959.

Carruthers, R. I. and Haynes, D.L. (1985) Laboratory transmission *in vivo* of *Entomophthora*

muscae (Entomophthorales: Entomophthoraceae) in the onion fly, *Delia antiqua* (Diptera: Anthomyiidae). *Journal of Invertebrate Pathology* 45, 282–287.

Carruthers, R.I. and Onsager, J.A. (1993) Perspective on the use of exotic natural enemies for biological control of pest grasshoppers. *Environmental Entomology* 22, 885–903.

Carruthers, R.I. and Soper, R.S. (1987) Fungal diseases. In: Fuxa, J.R. and Tanada, Y. (eds) *Epizootiology of Insect Diseases.* Wiley Intersciences, New York, pp. 357–416.

Carruthers, R.I., Haynes, D.L. and MacLeod, D.M. (1985) *Entomophthora muscae* (Entomophthorales: Entomophthoraceae) in the onion fly, *Delia antiqua* (Diptera: Anthomyiidae). *Journal of Invertebrate Pathology* 45, 81–93.

Carruthers, R.I., Feng, Z., Ramos, M.E. and Soper, R.S. (1988) The effect of solar radiation on the survival of *Entomophaga grylli* conidia. *Journal of Invertebrate Pathology* 52, 154–162.

Carruthers, R.I., Humber, R.A. and Ramos, M.E. (1989) *Biological Control of Rangeland Grasshoppers Using a Fungal Pathogen from Australia: a Proposed Field Release of an Exotic Fungal Pathogen.* USDA-APHIS Report, 23 pp.

Carruthers, R.I., Sawyer, A.L. and Hural, K. (1991) Use of fungal pathogens as biological control agents of insect pests. In: Rice, B.J. (ed.) *Symposia Proceedings of the National Academy of Sciences*. National Academy of Sciences Press, Washington, DC, pp. 336–372.

Carruthers, R.I., Larkin, T.S., Firstencel, H. and Feng, Z. (1992) Influence of thermal ecology on the mycosis of a rangeland grasshopper. *Ecology* 73, 190–204.

Carruthers, R.I., Ramos, M.E., Larkin, T.S., Hostetter, D.L. and Soper, R.S. (1997) The *Entomophaga grylli* (Fresenius) Batko species complex: its biology, ecology and use for biological control of pest grasshoppers. *Memoirs of the Entomological Society of Canada* 171, 329–353.

Chatigny, M.A., Dimmick, R.L. and Harrington, J.B. (1979) Principles of deposition of microbiological particles. In: Edmonds, R.L. (ed.) *Aerobiology – the Ecological Systems Approach*. Dowden, Hutchinson and Ross, Stroudsberg, Pennsylvania, pp. 111–150.

Cohn, F. (1855) *Empusa muscae* und die Krankheit der Stubenfliegen. *Hedwigia* 1, 57–61.

Cole, R.A. (1996) Comparison of feeding behaviour and development of two brassica pests, *Brevicoryne brassicae* and *Myzus persicae* on wild and cultivated *Brassica* species and an analysis of the glucosinolate and amino acid profiles. *Breeding Resistance in Insects and Mites. IOBC Bulletin* 19, 56–61.

Dean, G.J. and Wilding, N. (1971) *Entomophthora* infecting the cereal aphids, *Metopolophium dirhodum* and *Sitobion avenae. Journal of Invertebrate Pathology* 18, 169–176.

Dean, G.J. and Wilding, N. (1973) Infection of cereal aphids by the fungus *Entomophthora*. *Annals of Applied Biology* 74, 133–138.

Dedryver, C.A. (1978) Facteurs de limitation des populations d'*Aphis fabae* dans l'ouest de la France. III. Répartition et incidence des différentes espèces d'*Entomophthora* dans les populations. *Entomophaga* 23, 137–151.

Dedryver, C.A. (1979) Déclenchement en serre d'une épizootie à *Entomophthora fresenii* Nowak. sur *Aphis fabae* Scop. Par introduction d'inoculum et régulation de l'humidité relative. *Entomophaga* 24, 443–453.

Delalibera, I. Jr (1996) Producao '*in vitro*' de *Neozygites* sp. (Zygomycetes: Entomophthorales) patogeno de *Mononychellus tanajoa*. *Proceedings Simposio Controle Biologico, Foz do Iguacu, Brasil*, p. 171.

Delalibera, I. Jr, Sosa Gomez, D.R., de Moraes, G.J. de Alencar, J.A. and Farias Araujo, W. (1992) Infection of *Mononychellus tanajoa* (Acari: Tetranychidae) by the fungus *Neozygites* sp. (Entomophthorales) in northeastern Brazil. *Florida Entomologist* 75, 145–147.

Dent, D. (1995) *Integrated Pest Management.* Chapman & Hall, London, 356 pp.

van Driesche, R.G. and Bellows, T.S. (1996) *Biological Control.* Chapman & Hall, New York, 539 pp.

Dromph, K.M., Pell, J.K. and Eilenberg, J. (1996) Infection of *Sitobion avenae* with *Erynia neoaphidis*. In: *Proceedings of the III International Colloquium on* Bacillus thuringiensis *and the XXIX Annual Meeting of the Society for Invertebrate Pathology, Cordoba, Spain, September*, p. 22 (abstract).

Dromph, K., Pell, J.K. and Eilenberg, J. (1997) Sporulation of *Erynia neoaphidis* from alate *Sitobion avenae*. In: *Insect Pathogens and Insect Parasitic Nematodes. IOBC Bulletin* 21, 91–94.

Dromph, K.M., Eilenberg, J. and Esbjerg, P. (2000) Natural occurrence of entomophthoralean fungi on collembolans. *IOBC/WPRS Bulletin* 23, 147–149.

Dwyer, G., Elkinton, J.S. and Hajek, A.E. (1998) Spatial scale and the spread of a fungal pathogen of gypsy moth. *American Naturalist* 152, 485–494.

Eilenberg, J. (1985) Relationship between the carrot fly (*Psila rosae* F.) and its fungal pathogens from Entomophthorales, particularly *Entomophthora muscae* (C.) Fres. PhD thesis, Department of Zoology, Royal Veterinary and Agricultural University, Copenhagen, Denmark, 109 pp.

Eilenberg, J. (1987a) The culture of *Entomophthora muscae* (C.) Fres. in the carrot fly (*Psila rosae* F.) and the effect of temperature on the pathology of the fungus. *Entomophaga* 32, 425–435.

Eilenberg, J. (1987b) Abnormal egg-laying behaviour of female carrot flies (*Psila rosae*) induced by the fungus *Entomophthora muscae*. *Entomologia Experimentalis et Applicata* 52, 17–24.

Eilenberg, J. (1988) Occurrence of fungi from Entomophthorales in a population of carrot flies (*Psila rosae* F.). Results 1985 and 1986. *IOBC/WPRS Bulletin* 11, 53–59.

Eilenberg, J. (2000) Entomophthorales on Diptera. In: Papp, L. and Darvas, B. (eds) *Manual of Palearctic Diptera*, Vol. 1, *General and Applied Dipterology*. Science Herald, Budapest, pp. 519–533.

Eilenberg, J. and Michelsen, V. (1999) Natural host range and prevalence of the genus *Strongwellsea* (Zygomycota: Entomophthorales) in Denmark. *Journal of Invertebrate Pathology* 73, 189–198.

Eilenberg, J. and Philipsen, H. (1988) The occurrence of *Entomophthorales* on the carrot fly (Psila rosae F.) in the field during two successive seasons. *Entomophaga* 33, 135–144.

Eilenberg, J., Bresciani, J. and Martin, J. (1987) *Entomophthora* species with *E. muscae* like primary spores on two new insect orders, Coleoptera and Hymenoptera. *Nordic Journal of Botany* 7, 577–584.

Eilenberg, J., Bresciani, J. and Latgé, J.P. (1990) Primary spore and resting spore formation *in vitro* of *Entomophthora schizophorae* and *E. muscae*, both members of the *E. muscae*-complex. *Cryptogamic Botany* 1, 365–371.

Eilenberg, J., Wilding, N. and Bresciani, J. (1992) Isolation *in vitro* of *Strongwellsea castrans* (Fungi: Entomophthorales) a pathogen of adult cabbage root flies, *Delia radicum* (Dipt.: Anthomyiidae). *Entomophaga* 37, 65–77.

Elkinton, J.S., Hajek, A.E., Boettner, G.H. and Simons, E.E. (1991) Distribution and apparent spread of *Entomophaga maimaiga* (Zygomycetes: Entomophthorales) in gypsy moth (Lepidoptera: Lymantriidae) populations in North America. *Environmental Entomology* 20, 1601–1605.

Elliot, S.L., de Moraes, G.J., Delalibera I. Jr, da Silva, C.A.D., Tamai, M.A. and Mumford, J.D. (2000) Potential of the mite-pathogenic fungus *Neozygites floridana* (Entomophthorales: Neozygitaceae) for control of the cassava green mite *Mononychellus tanajoa* (Acari: Tetranychidae). *Bulletin of Entomological Research* 90, 191–200.

van Emden, H.F. (1989) *New Studies in Biology: Pest Control.* Edward Arnold, London, 117 pp.

Evans, H.C. (1989) Mycopathogens of insects of epigeal and aerial habitats. In: Wilding, N., Collins, N.M., Hammond, P.M. and Webber, J.F. (eds) *Insect–Fungus Interactions.* Academic Press, London, pp. 205–238.

Feng, M.-G., Johnson, J.B. and Halbert, S.E. (1991) Natural control of cereal aphids (Homoptera: Aphididae) by entomopathogenic fungi (Zygomycetes: Entomophthorales) and parasitoids (Hymenoptera: Braconidae and Encyrtidae) on irrigated spring wheat in southwestern Idaho. *Environmental Entomology* 20, 1699–1710.

Feng, M.-G., Nowierski, R.M., Klein, R.E., Scharen, A.L. and Sands, D.C. (1992) Spherical hyphal bodies of *Pandora neoaphidis* (Remaudière and Hennebert) Humber (Zygomycetes: Entomophthorales) on *Acyrthosiphon pisum* Harris (Homoptera: Aphididae): a potential overwintering form. *Pan-Pacific Entomologist* 68, 100–104.

Firstencel, H., Butt, T.M. and Carruthers, R.I. (1990) A fluorescence microscopy method for determining the viability of entomophthoralean fungal spores. *Journal of Invertebrate Pathology* 55, 258–264.

Freimoser, F.M., Grundchober, A., Aebi, M. and Tuor, U. (2000) *In vitro* cultivation of the entomopathogenic fungus *Entomophthora thripidum*: isolation, growth requirements and sporulation. *Mycologia* 92, 208–215.

Fresenius G. (1858) Ueber die Pilzgattung *Entomophthora*. *Abhandlungender Senckenbergischen Naturforschenden Gesellschaft* 2, 201–210.

Fuentes-Contreras, E., Pell, J.K. and Niemeyer, H.M. (1998) Influence of plant resistance at the third trophic level: interactions between parasitoids and entomopathogenic fungi of cereal aphids. *Oecologia* 117, 426–432.

Funk, C.J., Ramoska, W.A. and Bechtel, D.B. (1990) Light and electron microscopic studies of protoplast development during *Entomophaga grylli* pathotype 2 infections in *Melanoplus differentialis*. *Journal of Invertebrate Pathology* 55, 207–214.

Funk, C.J., Ramoska, W.A. and Bechtel, D.B. (1993) Histopathology of *Entomophaga grylli* pathotype 2 infections in *Melanoplus differentialis*. *Journal of Invertebrate Pathology* 61, 196–202.

Furlong, M.J. and Pell, J.K. (1996) Interactions between the fungal entomopathogen *Zoophthora radicans* Brefeld (Entomophthorales) and two hymenopteran parasitoids attacking the diamondback moth, *Plutella xylostella* L. *Journal of Invertebrate Pathology* 68, 15–21.

Furlong, M.J. and Pell, J.K. (1997) The influence of environmental factors on the persistence of *Zoophthora radicans* conidia. *Journal of Invertebrate Pathology* 69, 223–233.

Furlong, M.J. and Pell, J.K. (2000) Conflicts between a fungal entomopathogen, *Zoophthora radicans*, and two larval parasitoids of the diamondback moth. *Journal of Invertebrate Pathology* 76, 85–94.

Furlong, M.J., Pell, J.K., Ong, P.C. and Syed, A.R. (1995) Field and laboratory evaluation of a sex pheromone trap for the autodissemination of the fungal entomopathogen *Zoophthora radicans* (Entomophthorales) by the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). *Bulletin of Entomological Research* 85, 331–337.

Furlong, M.J., Pell, J.K. and Reddy, G.V.P. (1997) Premortality effects of *Zoophthora radicans* (Zygomycetes: Entomophthorales) infection on larval feeding rate and adult fecundity in *Plutella xylostella* (Lepidoptera: Yponomeutidae). *Journal of Invertebrate Pathology* 70, 214–220.

Fuxa, J. (1998) Environmental manipulation for microbial control of insects. In: Barbosa, P. (ed.) *Conservation Biological Control*. Academic Press, London, pp. 255–268.

Galaini-Wraight, S., Wraight, S.P., Carruthers, R.I., Magalhaes, B.P. and Roberts, D.W. (1991) Description of a *Zoophthora radicans* (Zygomycetes: Entomophthoraceae) epizootic in a population of *Empoasca kraemeri* (Hemiptera: Cicadellidae) on beans in central Brazil. *Journal of Invertebrate Pathology* 58, 311–326.

Galaini-Wraight, S., Wraight, S.P., Carruthers, R.I. and Roberts, D.W. (1992) Temperature-dependent germination and host penetration of the entomophthoralean fungus *Zoophthora radicans* on the leafhopper *Empoasca kraemeri*. *Mycological Research* 96, 38–48.

Geden, C.J., Steinkraus, D.C. and Rutz, D.A. (1993) Evaluation of two methods for release of *Entomophthora muscae* (Entomophthorales: Entomophthoraceae) to infect house flies (Diptera: Muscidae) on dairy farms. *Environmental Entomology* 20, 1201–1208.

Giard, A. (1888) Note sur deux types remarquables d'Entomophthorées, *Empusa fresenii* Now. et *Basidiobolus ranarum* Eid. suivie de la description de quelques espèces nouvelles. *Compte Rendu des Seances de la Societe de Biologie Paris* 40, 783–787.

Glare, T.R. and Milner, R.J. (1991) Ecology of entomopathogenic fungi. In: Arora, D.K., Ajello, L. and Mukerji, K.G. (eds) *Handbook of Applied Mycology*, Vol. 2, *Humans, Animals, and Insects.* Marcel Dekker, New York, pp. 547–612.

Glare, T.R., Milner, R.J., Chilvers, G.A., Mahon, R.J. and Brown, W.V. (1987) Taxonomic implications of intraspecific variation amongst isolates of the aphid-pathogenic fungi

Zoophthora radicans Brefeld and *Z. phalloides* Batko (Zygomycetes: Entomophthorales). *Australian Journal of Botany* 35, 49–67.

Glare, T.R., Milner, R.J. and Chilvers, G.A. (1989a) Production, stabilisation and regeneration of protoplasts of *Zoophthora radicans* using a commercial enzyme. *Mycological Research* 92, 477–485.

Glare, T.R., Milner, R.J. and Chilvers, G.A. (1989b) Factors affecting the production of resting spores by *Zoophthora radicans* in the spotted alfalfa aphid *Therioaphis trifolii* f. *maculata. Canadian Journal of Botany* 67, 848–855.

Goettel, M.S. (1994) Host range and specificity in relation to safety of exotic fungi. In: *Proceedings of the VI International Colloquium on Invertebrate Pathology and Microbial Control. XXVII Annual Meeting of the Society for Invertebrate Pathology, Montpellier,* pp. 325–329.

Goettel, M.S., Poprawski, T.J., Vandenberg, J.D., Li, Z. and Roberts, D.W. (1990) Safety to nontarget invertebrates of fungal biocontrol agents. In: Laird, M., Lacey, L.A. and Davidson, E.W. (eds) *Safety of Microbial Insecticides.* CRC Press, Boca Raton, Florida, pp. 209–231.

Gray, S.N. and Markham, P. (1997) A model to explain the growth kinetics of the aphid-pathogenic fungus *Erynia neoaphidis* in liquid culture. *Mycological Research* 101, 1475–1483.

Gray, S.N., Robinson, P., Wilding, N. and Markham, P. (1990) Effect of oleic acid on vegetative growth of the aphid-pathogenic fungus *Erynia neoaphidis*. *FEMS Microbiology Letters* 68, 131–136.

Griggs, M.H., Vandenberg, J.D. and Sawyer, A.J. (1999) Effect of relative humidity on viability of primary conidia of *Zoophthora radicans. Journal of Invertebrate Pathology* 73, 315–320.

Grundschober, A., Tuor, U. and Aebi, M. (1998) *In vitro* cultivation and sporulation of *Neozygites parvispora* (Zygomycetes: Entomophthorales). *Systematic and Applied Microbiology* 21, 461–469.

Gustafsson, M. (1965a) On species in the genus *Entomophthora* Fres. in Sweden. I. Classification and distribution. *Lantbrukshögskolans Annaler* 31, 103–212.

Gustafsson, M. (1965b) On species of the genus *Entomophthora* Fres. in Sweden. II. Cultivation and physiology. *Lantbrukshögskolans Annaler* 31, 405–457.

Gustafsson, M. (1965c) On species of the genus *Entomophthora* Fres. in Sweden. III. Possibility of usage in biological control. *Lantbrukshögskolans Annaler* 35, 235–274.

Hajek, A.E. (1989) Food consumption of *Lymantria dispar* (Lepidoptera: Lymantriidae) larvae infected with *Entomophaga maimaiga* (Zygomycetes: Entomophthorales). *Environmental Entomology* 18, 723–727.

Hajek, A.E. (1997a) Ecology of terrestrial fungal entomopathogens. *Advances in Microbial Ecology* 15, 193–249.

Hajek, A.E. (1997b) *Entomophaga maimaiga* reproductive output is determined by the spore type initiating an infection. *Mycological Research* 101, 971–974.

Hajek, A.E. (1997c) Fungal and viral epizootics in gypsy moth (Lepidoptera: Lymantriidae) populations in central New York. *Biological Control* 10, 58–68.

Hajek, A.E. (1999) Pathology and epizootiology of the Lepidoptera-specific mycopathogen *Entomophaga maimaiga. Microbiology and Molecular Biology Reviews* 63, 814–835.

Hajek, A.E. and Humber, R.A. (1997) Formation and germination of *Entomophaga maimaiga* azygospores. *Canadian Journal of Botany* 75, 1739–1747.

Hajek, A.E. and Roberts, D.W. (1991) Pathogen reservoirs as a biological control resource: introduction of *Entomophaga maimaiga* to North American gypsy moth, *Lymantria dispar*, populations. *Biological Control* 1, 29–34.

Hajek, A.E. and St Leger, R.J. (1994) Interactions between fungal pathogens and insect hosts. *Annual Review of Entomology* 39, 293–322.

Hajek, A.E. and Shimazu, M. (1996) Types of spores produced by *Entomophaga maimaiga* infecting the gypsy moth *Lymantria dispar*. *Canadian Journal of Botany* 74, 708–715.

Hajek, A.E. and Webb, R.E. (1999) Inoculative augmentation of the fungal entomopathogen *Entomophaga maimaiga* as a homeowner tactic to control gypsy moth (Lepidoptera: Lymantriidae). *Biological Control* 14, 11–18.

Hajek, A.E. and Wheeler, M.M. (1994) Application of techniques for quantification of soil-borne entomophthoralean resting spores. *Journal of Invertebrate Pathology* 64, 71–73.

Hajek, A.E., Carruthers, R.I. and Soper, R.S. (1990a) Temperature and moisture relations of sporulation and germination by *Entomophaga maimaiga* (Zygomycetes: Entomophthoraceae), a fungal pathogen of *Lymantria dispar* (Lepidoptera: Lymantriidae). *Environmental Entomology* 19, 85–90.

Hajek, A.E., Humber, R.A., Elkinton, J.S., May, B., Walsh, S.R.A. and Silver, J.C. (1990b) Allozyme and RFLP analyses confirm *Entomophaga maimaiga* responsible for 1989 epizootics in North American gypsy moth populations. *Proceedings of the National Academy of Sciences USA* 87, 6979–6982.

Hajek, A.E., Larkin, T.S., Carruthers, R.I. and Soper, R.S. (1993) Modeling the dynamics of *Entomophaga maimaiga* (Zygomycetes: Entomophthorales) epizootics in gypsy moth (Lepidoptera: Lymantriidae) populations. *Environmental Entomology* 22, 1172–1187.

Hajek, A.E., Butler, L. and Wheeler, M.W. (1995a) Laboratory bioassays testing the host range of the gypsy moth fungal pathogen *Entomophaga maimaiga*. *Biological Control* 5, 530–544.

Hajek, A.E., Humber, R.A. and Elkinton, J.S. (1995b) Mysterious origin of *Entomophaga maimaiga* in North America. *American Entomologist* 41, 31–42.

Hajek, A.E., Butler, L., Walsh, S.R.A., Silver, J.C., Hain, F.P., Hastings, F.L., O'Dell, T.M. and Smitley, D.R. (1996a) Host range of the gypsy moth (Lepidoptera: Lymantriidae) pathogen *Entomophaga maimaiga* (Zygomycetes: Entomophthorales) in the field versus laboratory. *Environmental Entomology* 25, 709–721.

Hajek, A.E., Elkinton, J.S. and Witcosky, J.J. (1996b) Introduction and spread of the fungal pathogen *Entomophaga maimaiga* along the leading edge of gypsy moth spread. *Environmental Entomology* 25, 1235–1247.

Hajek, A.E., Walsh, S.R.A., Silver, J.C. and Strong, D.R. (1996c) A disjunct Californian strain of *Entomophaga aulicae* infecting *Orygia vetusta. Journal of Invertebrate Pathology* 68, 260–268.

Hajek, A.E., Bauer, L., Wheeler, M.M. and McManus, M.L. (1998a) Distribution of resting spores of the *Lymantria dispar* pathogen *Entomophaga maimaiga* in soil and on bark. *BioControl* 43, 189–200.

Hajek, A.E., Tatman, K.M., Wanner, P.H. and Wheeler, M.M. (1998b) Location and persistence of gypsy moth, *Lymantria dispar*, cadavers containing *Entomophaga maimaiga* azygospores. *Mycologia* 90, 754–760.

Hajek, A.E., Olsen, C.H., Elkinton, J.S. (1999) Dynamics of airborne conidia of the gypsy moth (Lepidoptera: Lymantriidae) fungal pathogen *Entomophaga maimaiga* (Zygomycetes: Entomophthorales). *Biological Control* 16, 111–117.

Hall, I.M. and Dunn, P.H. (1957) Entomogenous fungi on the spotted alfalfa aphid. *Hilgardia* 27, 159–181.

Hall, I.M. and Dunn, P.H. (1959) The effect of certain insecticides and fungicides on fungi pathogenic to the spotted alfalfa aphid. *Journal of Economic Entomology* 52, 28–29.

Harcourt, D.G., Guppy, J.C., MacLeod, D.M. and Tyrrell, D. (1974) The fungus *Entomophthora phytonomi* pathogenic to the alfalfa weevil, *Hypera postica. Canadian Entomologist* 106, 1295–1300.

Hare, J.D. and Andreadis, T.G. (1983) Variation in the susceptibility of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) when reared on different host plants to the fungal pathogen *Beauveria bassiana* in the field and laboratory. *Environmental Entomology* 12, 1892–1897.

Hatting, J.L., Humber, R.A., Poprawski, T.J. and Miller, R.M. (1999) A survey of fungal pathogens of aphids from South Africa with special reference to cereal aphids. *Biological Control* 16, 1–12.

Hemmati, F. (1999) Aerial dispersal of the entomopathogenic fungus *Erynia neoaphidis*. PhD thesis, University of Reading, UK, 186 pp.

Hemmati, F., Pell, J.K., McCartney, H.A., Clark, S.J. and Deadman, M.L. (2001a) Active discharge in the aphid pathogenic fungus *Erynia neoaphidis*. *Mycological Research* (in press).

Hemmati, F., Pell, J.K., McCartney, H.A. and Deadman, M.L. (2001b) Airborne concentrations of conidia of *Erynia neoaphidis* above cereal fields. *Mycological Research* (in press).

Hermann, D. (2000) *In vitro* effects of ten fungicides on the aphid pathogen *Erynia neoaphidis*. MSc thesis, Nottingham Trent University, UK.

Hodge, K.T., Sawyer, A.J. and Humber, R.A. (1995) RAPD-PCR for identification of *Zoophthora radicans* isolates in biological control of the potato leafhopper. *Journal of Invertebrate Pathology* 65, 1–9.

Hollingsworth, R.G., Steinkraus, D.C. and McNew, R.W. (1995) Sampling to predict fungal epizootics on cotton aphids (Homoptera: Aphididae). *Environmental Entomology* 24, 1414–1421.

Huang, Y., Zhen, B. and Li, Z. (1992) Natural and induced epizootics of *Erynia ithacensis* in mushroom hothouse populations of yellow-legged fungus gnats. *Journal of Invertebrate Pathology* 60, 254–258.

Humber, R.A. (1981) An alternative view of certain taxonomic criteria used in the Entomophthorales (Zygomycetes). *Mycotaxon* 13, 191–240.

Humber, R.A. (1989) Synopsis of a revised classification for the Entomophthorales (Zygomycotina). *Mycotaxon* 34, 441–460.

Humber, R.A. and Soper, R.S. (1981) Proposal to conserve the entomopathogenic fungal genus *Triplosporium* (Thaxter) Batko against *Neozygites* Witlaczil (Entomophthorales). *Taxon* 30, 353–357.

Inyang, E.N. (1997) Effect of the host plant on susceptibility of crucifer pests to the insect pathogenic fungus *Metarhizium anisopliae*. PhD thesis, University of London.

Jaques, R.P. and Morris, O.N. (1980) Compatibility of pathogens with other methods of pest control and with different crops. In: Burges, H.D. (ed.) *Microbial Control of Pests and Plant Diseases 1970–1980.* Academic Press, London.

Jensen, A.B. and Eilenberg, J. (2000) Genetic variation within the insect-pathogenic fungus genus *Entomophthora*, focusing on the *E. muscae* complex, using PCR-RFLP of the ITS II and the LSU rDNA. *Mycological Research* 105, 307–312.

Jensen, A.B., Gargas, A., Eilenberg, J. and Rosendahl, S. (1998) Relationships of the insect-pathogenic Order Entomophthorales (Zygomycota, Fungi) based on phyllogenetic analyses of the nuclear small subunit ribosomal DNA sequences (SSU rDNA). *Fungal Genetics and Biology* 24, 325–334.

Kalsbeek, V., Mullens, B.A. and Jespersen, J.B. (1999) Field studies of *Entomophthora* induced behavioural fever in *Musca domestica* in Denmark. In: *Proceedings of the XXXIII Annual Meeting of the Society for Invertebrate Pathology, Irvine, California, August*, p. 50.

Kanervo, V. (1946) Sporadic observations concerning diseases in certain species of insects 3. Diseases attacking *Plutella maculipennis* Curt. *Suomen Hyonteistieteellinen Aikakauskija* 12, 143–153.

Kanervo, V. (1949) On the epidemiology of the diamondback moth (*Plutella maculipennis* Curt.). *Suomen Hyonteistieteellinen Aikakauskija* 14, 99–105.

Keller, S. (1984) *Entomophthora muscae* als Artenkomplex. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 57, 131–132.

Keller, S. (1987a) Die Bedeutung ökologischer ausgleichsflächen für den Pflanzenschutz. *Mitteilungen der Schweizerischen Landwirtschaft* 35, 56–65.

Keller, S. (1987b) Observations on the overwintering of *Entomophthora planchoniana. Journal of Invertebrate Pathology* 50, 333–335.

Keller, S. (1987c) Arthropod-pathogenic Entomophthorales of Switzerland. I. *Conidiobolus, Entomophaga* and *Entomophthora. Sydowia* 40, 122–167.

Keller, S. (1991) Arthropod-pathogenic Entomophthorales of Switzerland. II. *Erynia, Eryniopsis, Neozigites, Zoophthora* and *Tarichium. Sydowia* 43, 39–122.

Keller, S. (1997) The genus *Neozygites* (Zygomycetes, Entomophthorales) with special reference to species found in tropical regions. *Sydowia* 49, 118–146.

Keller, S. (1998) The role of Entomophthorales in a sustainable agriculture. In: *Insect Pathogens and Insect Parasitic Nematodes. IOBC Bulletin* 21, 13–16.

Keller, S. and Steenberg, T. (1997) *Neozygites sminthuri* sp. nov. (Zygomycetes: Entomophthorales) a pathogen of the springtail *Sminthurus viridis* L. (Collembola: Sminthuridae). *Sydowia* 49, 21–24.

Keller, S. and Suter, H. (1980) Epizootiologische Untersuchungen über das Entomophthora-Auftreten bei feldbaulich wichtigen Blattlausarten. *Acta Oecologica, Oecologica Applicata* 1, 63–81.

Keller, S. and Zimmerman, G. (1989) Mycopathogens of soil insects. In: Wilding, N., Collins, N.M., Hammond, P.M. and Webber, J.F. (eds) *Insect–Fungus Interactions.* Academic Press, London, pp. 240–270.

Keller, S., Kalsbeek, V. and Eilenberg, J. (1999) Redescription of *Entomophthora muscae* (Cohn) Fresenius. *Sydowia* 51, 197–209.

Kenneth, R. and Olmert, I. (1975) Entomopathogenic fungi and their insect hosts in Israel: additions. *Israel Journal of Entomology* 10, 105–112.

Kenneth, R., Wallis, G., Gerson, U. and Plaut, H.N. (1972) Observations and experiments on *Triplosporium floridanum* (Entomophthorales) attacking spider mites in Israel. *Journal of Invertebrate Pathology* 19, 366–369.

King, D.S. (1977) Systematics of *Conidiobolus* (Entomophthorales) using numerical taxonomy. III. Descriptions of recognised species. *Canadian Journal of Botany* 55, 718–729.

Klingen, I., Meadow, R. and Eilenberg, J. (2000) Prevalence of fungal infections in adult *Delia radicum* and *Delia floralis* trapped on the edge of a cabbage field. *Entomologia Experimentalis et Applicata* 97, 265–274.

Klubertanz, T.H., Pedigo, L.P. and Carlson, R.E. (1991) Impact of fungal epizootics on the biology and management of the twospotted spider mite (Acari: Tetranychidae) in soybean. *Environmental Entomology* 20, 731–735.

Kobayashi, Y., Mogami, K. and Aoki, J. (1984) Ultrastructural studies on the hyphal growth of *Erynia neoaphidis* in the green peach aphid *Myzus persicae*. *Transactions of the Mycological Society of Japan* 25, 425–434.

Kogan, M., Turnipseed, S.G., Shepard, M., Oliveira, E.B. and Borgo, A. (1977) Pilot insect pest management program for soybean in southern Brazil. *Journal of Economic Entomology* 70, 659–663.

Kogan, M., Gerling, D. and Maddox, J.V. (1999) Enhancement of biological control in annual agricultural environments. In: Bellows, T.S. and Fisher, T.W. (eds) *Handbook of Biological Control Principles and Applications of Biological Control.* Academic Press, London, pp. 789–817.

Kogan, P.H. and Hajek, A.E. (2000) Formation of azygospores by the insect pathogenic fungus *Entomophaga maimaiga* in cell culture. *Journal of Invertebrate Pathology* 75, 193–201.

Koyama, R. (1954) Two epizootic diseases of the gypsy moth. *Shirin-Boeki* 27, 296–298.

Kramer, J.P. and Steinkraus, D.C. (1981) Culture of *Entomophthora muscae in vivo* and its infectivity for six species of muscoid flies. *Mycopathologia* 76, 139–143.

Kramer, J.P. and Steinkraus, D.C. (1993) Experimental introduction of the mycosis caused by *Entomophthora muscae* in a population of house flies (*Musca domestica*) in a poultry house. *Journal of the New York Entomological Society* 95, 114–117.

Krasnoff, S.B., Watson, D.W., Gibson, D.M. and Kwan, E.C. (1995) Behavioral effects of the entomopathogenic fungus, *Entomophthora muscae*, on its host *Musca domestica*: postural changes in dying hosts and gated pattern of mortality. *Journal of Insect Physiology* 41, 895–903.

Kuntz, W.A. (1925) Aphid diseases. In: *Florida Agricultural Experimental Station Report,* Gainesville, Florida, pp. 46R–49R.

Kuramoto, H. and Shimazu, M. (1997) Control of house fly populations by *Entomophthora muscae* (Zygomycotina: Entomophthorales) in a poultry house. *Applied Entomology and Zoology* 32, 325–331.

Lagnaoui, A. and Radcliffe, E.B. (1998) Potato fungicides interfere with entomopathogenic fungi impacting population dynamics of green peach aphid. *American Journal of Potato Research* 75, 19–25.

Lambiase, J.T. and Yendol, W.G. (1977) The fine structure of *Entomophthora apiculata* and its penetration of *Trichoplusia ni. Canadian Journal of Microbiology* 23, 452–464.

Latgé, J.-P. (1975a) Croissance et sporulation de 6 espèces d'Entomophthorales. I. Influence de la nutrition carbonée. *Entomophaga* 20, 201–207.

Latgé, J.-P. (1975b) Croissance et sporulation de 6 espèces d'Entomophthorales. II. Influence de diverses sources d'azote. *Mycopathologia* 57, 53–57.

Latgé, J.-P. (1980) Sporulation de *Entomophthora obscura* Hall & Dunn en culture liquide. *Canadian Journal of Microbiology* 26, 1038–1048.

Latgé, J.-P. (1981) Comparaison des exigences nutritionelles des Entomophthorales. *Annales de Microbiologie (Institut Pasteur)* 132B, 299–306.

Latgé, J.-P. and de Bievre, C. (1976) Influence des lipides et acides gras du jaune d'oeuf sur la croissance et la sporulation des Entomophthorales. *Annales de Microbiologie (Institut Pasteur)* 127A, 261–274.

Latgé, J.-P. and Perry, D.F. (1980) The utilization of an *Entomophthora obscura* resting spore preparation in biological control experiments against cereal aphids. *Bulletin SROP* 3, 19–25.

Latgé, J.-P. and Sanglier, J.J. (1985) Optimisation de la croissance et de la sporulation de *Conidiobolus obscurus* en mileu défini. *Canadian Journal of Botany* 63, 68–85.

Latgé, J.-P. Soper, R.S. and Madore, C.D. (1977) Media suitable for industrial production of *Entomophthora virulenta* zygospores. *Biotechnology and Bioengineering* 19, 1269–1284.

Latgé, J.-P., Remaudière, G., Soper, R.S., Madore, C.D. and Diaquin, M. (1978) Growth and sporulation of *Entomophthora virulenta* on semidefined media in liquid culture. *Journal of Invertebrate Pathology* 31, 225–233.

Latgé, J.-P., Silvie, P., Papierok, B., Remaudière, G., Dedryver, C.A. and Rabasse, J.M. (1983) Advantages and disadvantages of *Conidiobolus obscurus* and of *Erynia neoaphidis* in the biological control of aphids. In: Cavalloro, R. (ed.) *Aphid Antagonists.* A.A. Balkema, Rotterdam, the Netherlands, pp. 20–32.

Latgé, J.-P., Eilenberg, J., Beauvais, A. and Prevost, M.C. (1988) Morphology of *Entomophthora muscae* protoplasts grown *in vitro. Protoplasma* 146, 166–173.

Latteur, G. and Godefroid, J. (1983). Trial of field treatments against cereal aphids with mycelium of *Erynia neoaphidis* (Entomophthorales) produced *in vitro*. In: Cavalloro, R. (ed.) *Aphid Antagonists*, A.A. Balkema, Rotterdam, the Netherlands, pp. 2–10.

Leite, L.G. (1991) Estudo de alguns fatores que afetam a epizootia de *Zoophthora radicans* e utilização do fungo para o controle de *Empoasca* sp. MS thesis, Univ. São Paolo, Piracicaba, Brazil.

Leite, L.G., Alves, S.B., Wraight, S.P., Galaini-Wraight, S., Roberts, D.W. and Magalhães, B.P. (1996a) Conidiogênese de *Zoophthora radicans* (Brefeld) Batko sobre *Empoasca kraemeri* (Ross and Moore) à diferentes temperaturas. *Arq. Instituto Biológico, São Paulo* 63, 47–53.

Leite, L.G., Alves, S.B., Wraight, S.P., Galaini-Wraight, S. and Roberts, D.W. (1996b) Habilidade de infecçã de isolados de *Zoophthora radicans* sobre *Empoasca kraemeri. Scientia-Agricola* 53, 152–158.

Leite, L.G., Alves, S.B., Wraight, S.P., Galaini-Wraight, S. and Roberts, D.W. (1996c) Processos de germinação de isolados do fungo *Zoophthora radicans* sobre *Empoasca kraemeri. Scientia-Agricola* 53, 179–183.

Leite, L.G., Alves, S.B., Wraight, S.P., Galaini-Wraight, S. and Roberts, D.W. (1996d) Comportamento de isolados do fungo *Zoophthora radicans* sobre *Empoasca kraemeri. Ecosistema* 21, 16–22.

Leite, L.G., Smith, L., Moraes, G.J. and Roberts, D.W. (1996e) Production of hyphal bodies of the fungus *Neozygites* nr. *floridana 'in vitro'.* In: *Proc. Simposio Controle Biologico, Foz do Iguacu, Brazil*, p. 448.

Li, Z., Butt, T.M., Beckett, A. and Wilding, N. (1993) The structure of dry mycelia of the entomophthoralean fungi *Zoophthora radicans* and *Erynia neoaphidis* following different preparatory treatments. *Mycological Research* 97, 1315–1323.

Lizen, E., Latteur, G. and Oger, R. (1985) Sensibilité à infection par l'Entomophthorale *Erynia neoaphidis* Remaud. et Henn. du puceron *Acyrthosiphon pisum* (Harris) selon sa forme, son stade et son âge. *Parasitica* 41, 163–170.

Lockwood, J.A. (1993a) Environmental issues involved in biological control of rangeland grasshoppers with exotic agents. *Environmental Entomology* 22, 503–518.

Lockwood, J.A. (1993b) Benefits and costs of controlling rangeland grasshoppers with exotic organisms: search for a null hypothesis and regulatory compromise. *Environmental Entomology* 22, 904–914.

McCabe, D. and Soper, R.S. (1985) Preparation of an entomopathogenic fungal insect control agent. US patent document no. 4530834.

McCabe, D.E., Humber, R.A. and Soper, R.S. (1984) Observation and interpretation of nuclear reductions during maturation and germination of entomophthoralean resting spores. *Mycologia* 76, 1104–1107.

McGuire, M.R., Maddox, J.V. and Ambrust, E.J. (1987a) Host range studies of an *Erynia radicans* strain (Zygomycetes: Entomophthorales) isolated from *Empoasca fabae* (Homoptera Cicadellidae). *Journal of Invertebrate Pathology* 50, 75–77.

McGuire, M.R., Morris, M.J., Armbrust, E.J. and Maddox, J.V. (1987b) An epizootic caused by *Erynia radicans* (Zygomycetes: Entomophthoraceae) isolated from *Empoasca fabae* (Homoptera: Cicadellidae). *Journal of Invertebrate Pathology* 50, 78–80.

McGuire, M.R., Maddox, J.V. and Ambrust, E.J. (1987c) Effect of temperature on distribution and success of introduction of an *Empoasca fabae* (Homoptera: Cicadellidae) isolate of *Erynia radicans* (Zygomycetes: Entomophthoraceae). *Journal of Invertebrate Pathology* 50, 291–301.

MacLeod, D.M. (1963) *Entomophthorales infections.* In: Steinhaus, E.A. (ed.) *Insect Pathology: an Advanced Treatise,* Vol. 2. Academic Press, New York, pp. 189–232.

MacLeod, D.M. and Tyrrell, D. (1979) *Entomophthora crustosa* n. sp. as a pathogen of the forest tent caterpillar, *Malacosoma disstria* (Lepidoptera, Lasiocampidae). *Canadian Entomologist* 111, 1137–1144.

MacLeod, D.M., Müller-Kögler, E. and Wilding, N. (1976) *Entomophthora* species with *E. muscae*–like conidia. *Mycologia* 68, 1–29.

McLeod, P.J., Steinkraus, D.C., Correll, J.C. and Morelock, T.E. (1998) Prevalence of *Erynia neoaphidis* (Entomophthorales: Entomophthoraceae) infections of green peach aphid (Homoptera: Aphididae) on spinach in the Arkansas River Valley. *Environmental Entomologist* 27, 796–800.

Magalhaes, B.P., Lord, J.C., Wraight, S.P., Daoust, R.A. and Roberts, D.W. (1988) Pathogenicity of *Beauveria bassiana* and *Zoophthora radicans* to the coccinellid predators *Coleomegilla maculata* and *Eriopis connexa. Journal of Invertebrate Pathology* 52, 471–473.

Magalhaes, B.P., Butt, T.M., Humber, R.A., Shields, E.J. and Roberts, D.W. (1990) Formation of appressoria *in vitro* by the entomopathogenic fungus *Zoophthora radicans* (Zygomycetes: Entomophthorales). *Journal of Invertebrate Pathology* 55, 284–288.

Magalhaes, B.P., Humber, R.A., Shields, E.J. and Roberts, D.W. (1991a) Effects of environment and nutrition on conidium germination and appressorium formation by *Zoophthora radicans* (Zygomycetes: Entomophthorales): a pathogen of the potato leafhopper (Homoptera: Cicadellidae). *Environmental Entomology* 20, 1460–1468.

Magalhaes, B.P., Wayne, R., Humber, R.A., Shields, E.J. and Roberts, D.W. (1991b) Calcium-regulated appressorium formation of the entomopathogenic fungus *Zoophthora radicans. Protoplasma* 160, 77–88.

Majchrowicz, I. and Poprawski, T.J. (1993) Effects *in vitro* of nine fungicides on growth of entomopathogenic fungi. *Biocontrol Science and Technology* 3, 321–336.

Malakar, R., Elkinton, J.S., Hajek, A.E. and Burand, J.P. (1999) Within-host interactions of *Lymantria dispar* (Lepidoptera: Lymantriidae) nucleopolyhedrosis virus and *Entomophaga maimaiga* (Zygomycetes: Entomophthorales). *Journal of Invertebrate Pathology* 73, 91–100.

Mietkiewski, R.T. and van der Geest, L.P.S. (1986) Effect of starvation on mortality of *Pieris brassicae* L. larvae after infection with *Erynia radicans* (Brefeld). In: Samson, R.A., Vlak, J.M. and Peters, R. (eds) *Fundamental and Applied Aspects of Invertebrate Pathology: Proceedings of the IV International Colloquium on Invertebrate Pathology and XIXth Annual Meeting of the Society for Invertebrate Pathology. Society for Invertebrate Pathology*, Veldhoven, p. 234.

Mietkiewski, R.T., van der Geest, L.P.S. and Balazy, S. (1986) Preliminary notes on the pathogenicity of some *Erynia* strains (Mycophyta: Entomophthoraceae) towards larvae of *Pieris brassicae*. *Journal of Applied Entomology* 102, 499–504.

Mietkiewski, R., Balazy, S. and van der Geest, L.P.S. (1993) Observations on a mycosis of spider mites (Acari: Tetranychidae) caused by *Neozygites floridana* in Poland. *Journal of Invertebrate Pathology* 61, 317–319.

Miller, J.R. and Cowles, R.S. (1990) Stimulo-deterrent diversion: a concept and its possible application to onion maggot control. *Journal of Chemical Ecology* 16, 3197–3212.

Milne, R., Wright, T., Welton, M., Budau, C., Gringorten, L. and Tyrrell, D. (1994) Identification and partial purification of a cell-lytic factor from *Entomophaga aulicae*. *Journal of Invertebrate Pathology* 64, 253–259.

Milner, R.J. (1982) On the occurrence of pea aphids, *Acyrthosiphon pisum*, resistant to isolates of the fungal pathogen *Erynia neoaphidis*. *Entomologia Experimentalis et Applicata* 32, 23–27.

Milner, R.J. (1985a) Distribution in time and space of resistance to the pathogenic fungus *Erynia neoaphidis* in the pea aphid *Acyrthosiphon pisum*. *Entomologia Experimentalis et Applicata* 37, 235–240.

Milner, R.J. (1985b) Field tests of a strain of *Entomophaga grylli* from the USA for biocontrol of the Australian wingless grasshopper, *Phaulacridium vittatum*. In: Chapman, R.F. (ed.) *Proceedings of the 4th Australasian Conference on Grassland Invertebrate Ecology, Lincoln College, Canterbury.* Caxton Press, Canterbury, pp. 255–261.

Milner, R.J. (1997) Prospects for biopesticides for aphid control. *Entomophaga* 42, 227–239.

Milner, R.J. and Holdom, D.G. (1986) First record of *Neozygites fresenii* (Nowakowski) Batko, a fungal pathogen of aphids, in Australia. *Journal of the Australian Entomological Society* 25, 85–86.

Milner, R.J. and Lutton, G.G. (1983) Effect of temperature on *Zoophthora radicans* (Brefeld) Batko: an introduced microbial control agent of the spotted alfalfa aphid, *Therioaphis trifolii* (Monell) f. *maculata. Journal of the Australian Entomological Society* 22, 167–173.

Milner, R.J. and Mahon, R.J. (1985) Strain variation in *Zoophthora radicans*, a pathogen on a variety of insect hosts in Australia. *Journal of the Australian Entomological Society* 24, 195–198.

Milner, R.J. and Soper, R.S. (1981) Bioassay of *Entomophthora* against the spotted alfalfa aphid, *Therioaphis trifolii* f. *maculata. Journal of Invertebrate Pathology* 37, 168–173.

Milner, R.J. and Soper, R.S. (1983) The potential of *Entomophaga grylli* for biological control of wingless grasshoppers. In: *Proceedings of the Australian Entomological Society 14th Annual Meeting and Conference, Armidale*, p. 18.

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. *Australian Journal of Botany* 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. Journal of the Australian Entomological Society* 21, 113–118.

Milner, R.J., Mahon, R.J. and Brown, W.V. (1983) A taxonomic study of *Erynia neoaphidis* Remaudière and Hennebert (Zygomycetes: Entomophthoraceae) and description of the new species, *Erynia kondoiensis. Australian Journal of Botany* 31, 173–188.

Milner, R.J., Holdom, D.G. and Glare, T.R. (1984a) Diurnal patterns of mortality in aphids infected by entomophthoralean fungi. *Entomologia Experimentalis et Applicata* 36, 37–42.

Milner, R.J., Lutton, G.G., Bourne, J., Bailey, P. and Swincer, D. (1984b) A laboratory study of the interaction between aphids, fungal pathogens and parasites. In: *Proceedings of the 4th Australian Applied Entomological Research Conference, Adelaide, Australia,* pp. 375–381.

Møller, A.P. (1993) A fungus infecting domestic flies manipulates sexual behaviour of its hosts. *Behavioral Ecology and Sociobiology* 33, 403–407.

Montserrat, M., Castañé, C. and Santamaria, S. (1998) *Neozygites parvispora* (Zygomycotina: Entomophthorales) causing an epizootic in *Frankliniella occidentalis* (Thysanoptera: Thripidae) on cucumber in Spain. *Journal of Invertebrate Pathology* 71, 165–168.

Morgan, L.W. (1994) Survival, germination responses and infectivity of conidia of *Erynia neoaphidis* (Zygomycetes: Entomophthorales). PhD thesis, University of Cardiff, 192 pp.

Morgan, L.W., Boddy, L., Clark, S.J. and Wilding, N. (1995) Influence of temperature on germination of primary and secondary conidia of *Erynia neoaphidis* (Zygomycetes: Entomophthorales). *Journal of Invertebrate Pathology* 65, 132–138.

Mullens, B.A. (1989) Cross-transmission of *Entomophthora muscae* (Zygomycetes: Entomophthoraceae) among naturally infected muscoid fly (Diptera: Muscidae) hosts. *Journal of Invertebrate Pathology* 53, 272–275.

Mullens, B.A. and Rodriguez, J.L. (1985) Dynamics of *Entomophthora muscae* (Entomophthorales: Entomophthoraceae) conidial discharge from *Musca domestica* (Diptera: Muscidae) cadavers. *Environmental Entomology* 14, 317–322.

Mullens, B.A., Rodriguez, J.L. and Meyer, J.A. (1987) An epizoological study of *Entomophthora muscae* in muscoid fly populations on Southern California poultry facilities, with emphasis on *Musca domestica*. *Hilgardia* 55, 1–41.

Nanne, H.W. and Radcliffe, E.B. (1971) Green peach aphid populations on potatoes enhanced by fungicides. *Journal of Economic Entomology* 64, 1569–1570.

Nielsen, C., Hajek, A.E., Humber, R.A. and Eilenberg, J. (1998) Soil – a natural source of entomophthoralean fungi infecting aphids. In: *Insect Pathogens and Insect Parasitic Nematodes, IOBC Bulletin* 21, 45–48.

Nielsen, C., Eilenberg, J., Harding, S., Oddsdottir, E. and Haldórsson, G. (2000) Entomophthoralean fungi infecting the green spruce aphid (*Elatobium abietinum*) in the North-western part of Europe. *IOBC/WPRS Bulletin* 23, 131–134.

Nielsen, C., Eilenberg, J. and Dromph, K.M. (2001) *Entomophthorales on Cereal Aphids: Characterisation, Growth, Virulence, Epizootiology and Potential for Microbial Control.* Ministry of the Environment, Copenhagen, 58 pp.

Nolan, R.A. (1985) Protoplasts from Entomophthorales. In: Peberdy, J.F. and Ferenczy, L. (eds) *Fungal Protoplasts: Applications in Biochemistry and Genetics*. Marcel Dekker, New York, pp. 87–112.

Nolan, R.A. (1988) A simplified, defined medium for growth of *Entomophaga aulicae* protoplasts. *Canadian Journal of Microbiology* 34, 45–51.

Nolan, R.A. (1990) Enhanced hyphal body production by *Entomophaga aulicae* protoplasts in the presence of a neutral and a positively charged surface under mass fermentation conditions. *Canadian Journal of Botany* 68, 2708–2713.

Nolan, R.A. (1991) Influence of a negatively charged surface (Teflon disk) on *Entomophaga aulicae* protoplast morphogenesis under mass fermentation conditions. *Canadian Journal of Botany* 69, 2578–2581.

Nolan, R.A. (1993a) An inexpensive medium for mass fermentation production of *Entomophaga aulicae* hyphal bodies competent to form conidia. *Canadian Journal of Microbiology* 39, 588–593.

Nolan, R.A. (1993b) Physiological studies with the fungus *Entomophaga aulicae* during morphogenesis in three different media under fermentation conditions. *Canadian Journal of Microbiology* 39, 701–708.

Nolan, R.A. (1998) Growth media for entomophthoralean hyphal bodies. US Patent no. 5,728,572.

Nolan, R.A. and Dunphy, G.B. (1979) Effects of hormones on *Entomophthora egressa* morphogenesis. *Journal of Invertebrate Pathology* 33, 242–248.

Nolan, R.A., Dunphy, G.B. and MacLeod, D.M. (1976) *In vitro* germination of *Entomophthora egressa* resting spores. *Canadian Journal of Botany* 54, 1131–1134.

Nordin, G.L. (1984) Enhancement strategies for entomogenous fungi in integrated pest management. In: *Proceedings of the China National Academy of Science/United States National Academy of Science Joint Symposium on Biological Control of Insects, Beijing, China.* Science Press, Beijing, pp. 122–141.

Nowakowski, L. (1883) Entomophthoreae. Przyczynek do znajmosci pasorzytnych grzybków sprawiajacych pomór owadów. *Pamietn. Akad. Umiejetn. Krakowie, Wydz. Mat.-Przyr.* 8, 153–183.

Ooi, P.A.C. (1981) Microbial control of the diamondback moth in Cameron Highlands, Malaysia. *Malaysian Applied Biology* 10, 49–56.

Otvos, I.S., MacLeod, D.M. and Tyrrell, D. (1973) Two species of *Entomophthora* pathogenic to the eastern hemlock looper (Lepidoptera: Geometridae) in Newfoundland. *Canadian Entomologist* 105, 1435–1441.

Papierok, B. (1982) Entomophthorales: virulence and bioassay design. In: *Proceedings of the 3rd International Colloquium on Invertebrate Pathology, Brighton, UK,* pp. 176–181.

Papierok, B., Valadáo, B., Tôrres, L. and Arnault, M. (1984) Contribution to the study of the specificity of the entomopathogenic fungus *Zoophthora radicans* (Zygomycetes: Entomophthorales). *Entomophaga* 29, 109–119.

Pell, J.K. and Wilding, N. (1992) The survival of *Zoophthora radicans* Brefeld (Zygomycetes: Entomophthorales) isolates as hyphal bodies in mummified larvae of *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). *Entomophaga* 37, 649–654.

Pell, J.K. and Wilding, N. (1994) Preliminary caged field trial using the fungal pathogen, *Zoophthora radicans* Brefeld (Zygomycetes: Entomophthorales) against the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) in the UK. *Biocontrol Science and Technology* 4, 71–75.

Pell, J.K., Macaulay, E.D.M. and Wilding, N. (1993a) A pheromone trap for dispersal of the pathogen, *Zoophthora radicans* Brefeld (Zygomycetes: Entomophthorales) amongst populations of the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). *Biocontrol Science and Technology* 3, 315–320.

Pell, J.K., Wilding, N., Player, A.L. and Clark, S.J. (1993b) Selection of an isolate of *Zoophthora radicans* (Zygomycetes: Entomophthorales) for biocontrol of the diamondback moth *Plutella xylostella* (Lepidoptera: Yponomeutidae). *Journal of Invertebrate Pathology* 61, 75–80.

Pell, J.K., Furlong, M.J., Reddy, G.V.P., Poppy, G.M. and Roy, H.E. (1996) Dispersal of entomopathogenic fungi by manipulation of insect behaviour. In: *Proceedings of the XXIX Annual Meeting of the Society for Invertebrate Pathology and III International Colloquium on* Bacillus thuringiensis, *Cordoba, Spain, September*, p. 61 (abstract).

Pell, J.K., Pluke, R., Clark, S.J., Kenward, M.G. and Alderson, P.G. (1997a) Interactions between two aphid natural enemies, the entomopathogenic fungus, *Erynia neoaphidis* and the predatory beetle, *Coccinella septempunctata. Journal of Invertebrate Pathology* 69, 261–268.

Pell, J.K., Tydeman, C. and McCartney, H.A. (1997b) Impact of rainfall on the persistence and transmission of *Erynia neoaphidis*. *Insect Pathogens and Insect Parasitic Nematodes*, *IOBC Bulletin* 21, 49.

Pell, J.K. Barker, A.D.P., Clark, S.J., Wilding, N. and Alderson, P.G. (1998) Use of a novel sporulation monitor to quantify the effects of formulation and storage on conidia pro-

duction by dried mycelia of the entomopathogenic fungus *Zoophthora radicans*. *Biocontrol Science and Technology* 8, 13–21.

Perry, D.F. and Fleming, R.A. (1989) The timing of *Erynia radicans* resting spore germination in relation to mycosis of *Choristoneura fumiferana. Canadian Journal of Botany* 67, 1657–1663.

Perry, D.F. and Latgé, J.-P. (1980) Chemically defined media for growth and sporulation of *Entomophthora virulenta. Journal of Invertebrate Pathology* 35, 43–48.

Perry, D.F. and Regnière, J. (1986) The role of fungal pathogens in spruce budworm population dynamics: frequency and temporal relationships. In: Samson, R.A., Vlak, J.M. and Peters, D. (eds) *Fundamental and Applied Aspects of Invertebrate Pathology: 4th International Colloquium on Invertebrate Pathology, Wageningen, the Netherlands,* pp. 167–170.

Perry, D.F., Tyrrell, D. and Delyzer, A.J. (1982) The mode of germination of *Zoophthora radicans* zygospores. *Mycologia* 74, 549–554.

Pickering, J., Dutcher, J.D. and Ekbom, B.S. (1989) An epizootic caused by *Erynia neoaphidis* and *E. radicans* (Zygomycetes: Entomophthoraceae) on *Acyrthosiphon pisum* (Homoptera: Aphidae) on legumes under overhead irrigation. *Journal of Applied Entomology* 107, 331–333.

Pickett, J.A., Wadhams, L.J. and Woodcock, C.M. (1991) New approaches to the development of semiochemicals for insect control. In: *Proceedings of the Conference on Insect Chemical Ecology, Tábor.* Academia, Prague, and SPB Academic Publishers, The Hague, pp. 333–345.

Pickett, J.A., Butt, T.M., Doughty, K.J., Wallsgrove, R.M. and Williams, I.H. (1995) Minimising pesticide input in oilseed rape by exploiting natural regulatory processes. In: *Proceedings of the Ninth International Rapeseed Congress: Rapeseed Today and Tomorrow, Cambridge, UK,* pp. 565–571.

Pickford, R. and Riegert, P.W. (1964) The fungus disease caused by *Entomophthora grylli* Fres., and its effects on grasshopper populations in Saskatchewan in 1963. *The Canadian Entomologist* 96, 1158–1166.

Poprawski, T.J. and Majchrowicz, I. (1995) Effects of herbicides on *in vitro* vegetative growth and sporulation of entomopathogenic fungi. *Crop Protection* 14, 81–87.

Poprawski, T.J. and Wraight, S.P. (1998) Fungal pathogens of Russian wheat aphid (Homoptera: Aphididae). In: Quisenberry, S.S. and Peairs, F.B. (eds) *Response Model for an Introduced Pest – Russian Wheat Aphid.* Entomological Society of America, Lanham, Maryland, pp. 209–233.

Poprawski, T.J., Mercadier, G. and Wraight, S.P. (1992) Interactions between *Diuraphis noxia, Zoophthora radicans* and *Aphelinus asychis* – preliminary results of laboratory studies. In: Morrison, W.P. (compiler) *Proceedings of the Fifth Russian Wheat Aphid Workshop, 26–28th January, 1992, Fort Worth, Texas.* Great Plains Agricultural Council Publication 142, pp. 180–188.

Powell, W., Wilding, N., Brobyn, P.J. and Clark, S.J. (1986a) Interference between parasitoids (Hymenoptera: Aphidiidae) and fungi (Entomophthorales) attacking cereal aphids. *Entomophaga* 31, 293–302.

Powell, W., Dean, G.J. and Wilding, N. (1986b) The influence of weeds on aphid-specific natural enemies in winter wheat. *Crop Protection* 5, 182–189.

Ramaseshiah, G. (1968) *Entomophthora fresenii*, parasitic on aphids in India. *Journal of Invertebrate Pathology* 10, 439–441.

Ramaseshiah, G. (1971) Occurrence of an *Entomophthora* on tetranychid mites in India. *Journal of Invertebrate Pathology* 18, 421–424.

Ramoska, W.A. and Todd, T. (1985) Variation in efficacy and viability of *Beauveria bassiana* in the chinch bug (Hemiptera: Lygaeidae) as a result of feeding activity on selected host plants. *Environmental Entomology* 14, 146–148.

Ramoska, W.A., Hajek, A.E., Ramos, M.E. and Soper, R.S. (1988) Infection of grasshoppers by members of the *Entomophaga grylli* species complex. *Journal of Invertebrate Pathology* 52, 309–313.

Reddy, G.V.P., Furlong, M.J., Poppy, G.M. and Pell, J.K. (1998) *Zoophthora radicans* infection inhibits the response to and the production of sex pheromone in the diamondback moth. *Journal of Invertebrate Pathology* 72, 167–169.

Remaudière, G. (1977) Sur quelques Aphidoidea de la Polynésie Française. *Bulletin de Société Entmologique France* 82, 151–155.

Remaudière, G. (1983) Lutte biologique contre les pucerons des céréales au moyen des champignons Entomophthorales. In: Cavalloro, R. and Piavaux, A. (eds) *Comm. Eur. Comm. Prog. Integr. Biol. Control: Progress Report 1979–1981,* pp. 227–246.

Remaudière, G. and Keller, S. (1980) Révision systématique des genres d'Entomophthoraceae potentialité entomopathogène. *Mycotaxon* 11, 323–338.

Riethmacher, G.W. and Kranz, J. (1994) Development of disease incidence of Entomophthoraceae in field populations of *Plutella xylostella* in the Philippines. *Zeitschrift für Pflanzenkrakheiten-und-Pflanzenschutz* 101, 357–367.

van Roermund, H.J.W., Perry, D.F. and Tyrrell, D. (1984) Influence of temperature, light, nutrients and pH in determination of the mode of conidial germination in *Zoophthora radicans. Transactions of the British Mycological Society* 82, 31–38.

Rohel, E., Couteaudier, Y., Papierok, B., Cavelier, N. and Dedryver, C.A. (1997) Ribosomal internal transcribed spacer size variation correlated with RAPD-PCR pattern polymorphisms in the entomopathogenic fungus *Erynia neoaphidis* and some closely related species. *Mycological Research* 101, 573–579.

Roy, H.E. (1997) Interactions between aphid predators and the entomopathogenic fungus *Erynia neoaphidis*. PhD thesis, University of Nottingham, 206 pp.

Roy, H.E. and Pell, J.K. (2000) Interactions between entomopathogenic fungi and other natural enemies: implications for biological control. *Biocontrol Science and Technology* 10, 737–752.

Roy, H.E., Pell, J.K., Clark, S.J. and Alderson, P.G. (1998) Implications of predator foraging on aphid pathogen dynamics. *Journal of Invertebrate Pathology* 71, 236–247.

Roy, H.E., Pell, J.K. and Alderson, P.G. (1999) Effects of fungal infection on the alarm response of pea aphids. *Journal of Invertebrate Pathology* 74, 69–75.

Samson, R.A., Ramakers, P.M.J. and Oswald, T. (1979) *Entomophthora thripidum*, a new fungal pathogen of *Thrips tabaci*. *Canadian Journal of Botany* 57, 1317–1323.

Sawyer, A.J., Griggs, M.H. and Wayne, R. (1994) Dimensions, density and settling velocity of entomophthoralean conidia: implications for aerial dissemination of spores. *Journal of Invertebrate Pathology* 63, 43–55.

Sawyer, A.J., Ramos, M.E., Poprawski, T.J., Soper, R.S. and Carruthers, R.I. (1997) Seasonal patterns of cadaver persistence and sporulation by the fungal pathogen *Entomophaga grylli* (Fresenius) Batko (Entomophthoraceae) infecting *Camnula pellucida* (Scudder) (Orthoptera: Acrididae). *Memoirs of the Entomological Society of Canada* 171, 355–374.

Sawyer, W.H. Jr (1929) Observations on some entomogenous members of the Entomophthoraceae in artificial culture. *American Journal of Botany* 16, 87–121.

Schmitz, V., Dedryver, C.A. and Pierre, J.S. (1993) Influence of an *Erynia neoaphidis* infection on the relative rate of increase of the cereal aphid *Sitobion avenae*. *Journal of Invertebrate Pathology* 61, 62–68.

Schofield, G. (1998) Low temperature biology of the aphid natural enemies *Erynia neoaphidis* and *Diaeretiella rapae*. PhD thesis, University of Birmingham, 144 pp.

Schuler, T.H., Poppy, G.M., Kerry, B.R. and Denholm, I. (1998) Insect-resistant transgenic plants. *Trends in Biotechnology* 16, 168–175.

Schweizer, G. (1948) Uber die Kultur von *Empusa muscae* Cohn und anderen Entomophthoraceen auf kalt Sterilisierten nahrboden. *Planta* 35, 132–176.

Shah, P.A., Aebi, M. and Tuor, U. (1998) Method to immobilize the aphid-pathogenic fungus *Erynia neoaphidis* in an alginate matrix for biocontrol. *Applied and Environmental Microbiology* 64, 4260–4263.

Shah, P.A., Aebi, M. and Tuor, U. (1999) Production factors involved in the formulation of *Erynia neoaphidis* as alginate granules. *Biocontrol Science and Technology* 9, 19–28.

Shah, P.A., Aebi, M. and Tuor, U. (2000) Infection of *Macrosiphum euphorbiae* with mycelial preparations of *Erynia neoaphidis* in a greenhouse trial. *Mycological Research* 104, 645–652.

Shimazu, M. (1979) Resting spore formation of *Entomophthora sphaerosperma* Fresenius (Entomophthorales: Entomophthoraceae) in the brown plant hopper *Niliparvata lugens* (Stal.) (Hemiptera: Delphacidae). *Applied Entomology and Zoology* 14, 383–388.

Shimazu, M. and Kuramoto, H. (1994) Introduction of *Entomophthora muscae* (Zygomycotina: Entomophthorales) to caged house fly populations. *Applied Entomology and Zoology* 29, 563–569.

Sierotzki, H., Camestral, F., Shah, P.A., Tuor, U. and Aebi, M. (2000) Biological characteristics of selected *Erynia neoaphidis* isolates. *Mycological Research* 104, 213–219.

Silvie, P. and Papierok, B. (1991) Les ennemis naturels d'insectes du cotonnier au Tchad: premières données sur les champignons de l'ordre des Entomophthorales. *Coton Fibres Tropicale* 46, 293–308.

Silvie, P., Dedryver, C.A. and Tanguy, S. (1990) Application expérimentale de mycélium d'*Erynia neoaphidis* (Zygomycetes: Entomophthorales) dans des populations de pucerons sur laitues en serre maraîchère: étude du suivi de l'inoculum par caractérisation enzymatique. *Entomophaga* 35, 375–384.

Six, D.L. and Mullens, B.A. (1996) Seasonal prevalence of *Entomophthora muscae* and introduction of *Entomophthora schizophorae* (Zygomycotina: Entomophthorales) in *Musca domestica* (Diptera: Muscidae) populations of Southern California dairies. *Biological Control* 6, 315–323.

Six, D.L. and Mullens, B.A. (1997) Distance of conidial discharge of *Entomophthora muscae* and *Entomophthora schizophorae. Journal of Invertebrate Pathology* 67, 253–258.

Smith, M.T. and Hardee, D.D. (1996) Influence of fungicides on development of an entomopathogenic fungus (Zygomycetes: Neozygitaceae) in the cotton aphid (Hemiptera: Aphididae). *Environmental Entomology* 25, 677–687.

Smith, R.F. and Reynolds, H.T. (1966) Principles, definitions and scope of integrated pest control. In: *Proceedings of FAO (United Nations Food and Agriculture Organisation) Symposium on Integrated Pest Control*, Vol. 1, FAO, Rome, pp. 11–17.

Smith, R.F., Apple, J.L. and Bottrell, D.G. (1976) The origins of integrated pest management concepts for agricultural crops. In: Apple, J.L. and Smith, R.F. (eds) *Integrated Pest Management*. Plenum Press, New York, pp. 1–16.

Smitley, D.R., Kennedy, G.G. and Brooks, W.M. (1986) Role of the entomogenous fungus, *Neozygites floridana*, in population declines of the twospotted spider mite, *Tetranychus urticae*, on field corn. *Entomologia Experimentalis et Applicata* 41, 255–264.

Smitley, D.R., Bauer, L.S., Hajek, A.E., Sapio, F.J. and Humber, R.A. (1995) Introduction and establishment of *Entomophaga maimaiga*, a fungal pathogen of gypsy moth (Lepidoptera: Lymantriidae) in Michigan. *Environmental Entomology* 24, 1685–1695.

Soper, R.S. and MacLeod, D.M. (1963) Spore morphology of *Entomophthora fresenii* Nowakowski. *Journal of Insect Pathology* 5, 478–482.

Soper, R.S., and MacLeod, D.M. (1981) *Descriptive Epizootiology of an Aphid Mycosis.* USDA, Technical Bulletin 1632, 17 pp.

Soper, R.S., Holbrook, F.R. and Gordon, C.C. (1974) Comparative pesticide effects on *Entomophthora* and the phytopathogen *Alternaria solanii. Environmental Entomology* 3, 560–562.

Soper, R.S., Holbrook, F.R., Majchrowicz, I. and Gordon, C.C. (1975) Production of *Entomophthora* resting spores for biological control of aphids. *University of Maine (Orono) Life Sciences and Agriculture Experimental Station Technical Bulletin* 76, 1–15.

Soper, R.S., Smith, L.F.R. and Delyzer, A.J. (1976) Epizootiology of *Massospora levispora* in an isolated population of *Okanagana rimosa. Annals of the Entomological Society of America* 69, 275–283.

Soper, R.S., Shimazu, M., Humber, R.A., Ramos, M.E. and Hajek, A.E. (1988) Isolation and characterization of *Entomophaga maimaiga* sp. nov., a fungal pathogen of gypsy moth, *Lymantria dispar*, from Japan. *Journal of Invertebrate Pathology* 51, 229–241.

Speare, A. T. (1922) *Natural Control of the Citrus Mealybug in Florida. USDA Bulletin* 1117.

Speare, A.T. and Colley, R.H. (1912) *The Artificial Use of the Brown-tail Fungus in Massachusetts with Practical Suggestions for Private Experiments, with a Brief Note on a Fungous Disease of Gypsy Caterpillar.* Wright & Potter, Boston, Massachusetts.

Steinkraus, D.C. and Boys, G.O. (1997) Update on prediction of epizootics with extension-based sampling service. In: *Proceedings Beltwide Cotton Conferences, January 6–10, 1997, New Orleans.* National Cotton Council of America, Memphis, Tennessee, pp. 1047–1048.

Steinkraus, D.C. and Rosenheim, J. (1995) Biological factors influencing the epizootiology of cotton aphid fungus. In: *Proceedings Beltwide Cotton Conference, 1995, January 4–7, San Antonio, Texas.* National Cotton Council of America, Memphis, Tennessee, pp. 887–889.

Steinkraus, D.C. and Slaymaker, P.H. (1994) Effect of temperature and humidity on formation, germination, and infectivity of conidia of *Neozygites fresenii* (Zygomycetes: Neozygitaceae) from *Aphis gossypii* (Hemiptera: Aphididae). *Journal of Invertebrate Pathology* 64, 130–137.

Steinkraus, D.C., Kring, T.J. and Tugwell, N.P. (1991) *Neozygites fresenii* in *Aphis gossypii* on cotton. *Southwestern Entomologist* 16, 118–122.

Steinkraus, D.C., Boys, G.O. and Slaymaker, P.H. (1993a) Culture, storage, and incubation period of *Neozygites fresenii* (Entomophthorales: Neozygitaceae), a pathogen of the cotton aphid. *Southwestern Entomologist* 18, 197–202.

Steinkraus, D.C., Geden, J.G. and Rutz, D.A. (1993b) Prevalence of *Entomophthora muscae* (Cohn) Fresenius (Zygomycetes: Entomophthoraceae) in house flies (Diptera: Muscidae) on dairy farms in New York and induction of epizootics. *Biological Control* 3, 93–100.

Steinkraus, D.C., Mueller, A.J. and Humber, R.A. (1993c) *Furia virescens* (Thaxter) Humber (Zygomycetes: Entomophthoraceae) infections in the armyworm, *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae) in Arkansas with notes on other natural enemies. *Journal of Entomological Science* 28, 376–386.

Steinkraus, D.C., Hollingsworth, R.G. and Slaymaker, P.H. (1995) Prevalence of *Neozygites fresenii* (Entomophthorales: Neozygitaceae) on cotton aphids (Homoptera: Aphididae) in Arkansas cotton. *Environmental Entomology* 24, 465–474.

Steinkraus, D.C., Boys, G.O., Hollingsworth, R.G., Bacheler, J.S., Durant, J.A., Freeman, B.L., Gaylor, M.J., Harris, F.A., Knutson, A., Lentz, G.L., Leonard, B.R., Luttrell, R., Parker, D., Powell, J.D., Ruberson, J.R. and Sorenson, C. (1996a) Multistate sampling for *Neozygites fresenii* in cotton. In: *Proceedings 1996 Beltwide Cotton Research Conferences, Nashville, Tennessee.* National Cotton Council of America, Memphis, Tennessee, pp. 735–738.

Steinkraus, D.C., Hollingsworth, R.G. and Boys, G.O. (1996b) Aerial spores of *Neozygites fresenii* (Entomophthorales: Neozygitaceae): density, periodicity, and potential role in cotton aphid (Hemiptera: Aphididae) epizootics. *Environmental Entomology* 25, 48–57.

Steinkraus, D.C., Boys, G.O., Bagwell, R.D., Johnson, D.R., Lorenz, G.M., Meyers, H., Layton, M.B. and O'Leary, P.F. (1998a) Expansion of extension-based aphid fungus sampling service to Louisiana and Mississippi. In: *1998 Beltwide Cotton Conferences, San Diego, California*. National Cotton Council of America, Memphis, Tennessee, pp. 1239–1242.

Steinkraus, D.C., Oliver, J.B. Humber, R.A. and Gaylor, M.J. (1998b) Mycosis of bandedwinged whitefly (*Trialeurodes abutilonea*) (Homoptera: Aleyrodidae) caused by *Orthomyces aleyrodis* gen. & sp. nov. (Entomophthorales: Entomophthoraceae). *Journal of Invertebrate Pathology* 72, 1–8.

Steinkraus, D.C., Howard, M.N., Hollingsworth, R.G. and Boys, G.O. (1999) Infection of sentinel cotton aphids (Homoptera: Aphididae) by aerial conidia of *Neozygites fresenii* (Entomophthorales: Neozygitaceae). *Biological Control* 14, 181–185.

Sterling, W. (1984) *Action and Inaction Levels in Pest Management*. Texas Agricultural Experimental Station Bulletin 1480, College Station, Texas.

Streett, D.A. and McGuire, M.R. (1990) Pathogenic diseases of grasshoppers. In: Chapman, R.F. and Joern, A. (eds) *Biology of Grasshoppers.* Wiley Interscience, New York, pp. 483–516.

Thaxter, R. (1888) The Entomophthoreae of the United States. *Memoirs of the Boston Society of Natural History* 4, 133–201.

Thoizon, G. (1970) Spécificité du parasitisme des aphides par les Entomophthorales. *Annales de Société de Entomologie Francais (N.S.)* 6, 517–562.

Thomsen, L. and Eilenberg, J. (2000) *Entomophthora muscae* resting spore formation and germination *in vivo* in *Delia radicum. Journal of Invertebrate Pathology* 76, 127–130.

Tomiyama, H. and Aoki, J. (1982) Infection of *Erynia blunckii* (Lak. Ex. Zimm.) Rem. et Henn. (Entomophthorales: Entomophthoraceae) in the diamondback moth *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). *Applied Entomology and Zoology* 17, 375–384.

Tyrrell, D. (1970) Production of resting spores of some *Entomophthora* species on artificial media. *Bi-Monthly Research Notes, Department of Fisheries and Forestry, Ottawa, Canada* 26, 12–13.

Tyrrell, D. (1988) Survival of *Entomophaga aulicae* in dried insect larvae. *Journal of Invertebrate Pathology* 52, 187–188.

Tyrrell, D. (1990) Pathogenesis of *Entomophaga aulicae*. I. Disease symptoms and effect of infection on weight gain of infected *Choristoneura fumiferana* and *Malacosoma disstria* larvae. *Journal of Invertebrate Pathology* 56, 150–156.

Ullyett, G.C. and Schonken, D.B. (1940) *A Fungus Disease of* Plutella maculipennis *Curt. in South Africa with Notes on the Use of Entomogenous Fungi in Insect Control.* Union of South Africa, Department of Agriculture and Forestry Science Bulletin No. 218, Entomology Series No. 5.

Uziel, A. and Kenneth, R.G. (1986) *In vitro* resting spore production in *Erynia neoaphidis*. In: Samson, R.A., Vlak, J.M. and Peters, D. (eds) *Fundamental and Applied Aspects of Insect Pathology. Fourth International Colloquium on Invertebrae Pathology, Veldhoven,* p. 230.

Uziel, A. and Kenneth, R.G. (1991) Survival of primary conidia and capilliconidia at different humidities in *Erynia* (subgen. *Zoophthora*) spp. and in *Neozygites fresenii* (Zygomycetes: Entomophthorales), with special emphasis on *Erynia radicans*. *Journal of Invertebrate Pathology* 58, 118–126.

Uziel, A. and Shtienberg, D. (1993) Effect of meteorological variates on persistence of primary conidia and capilliconidia of *Erynia radicans* (Zygomycetes: Entomophthorales) under natural conditions. *Annals of Applied Biology* 122, 441–450.

Uziel, A., Kenneth, R.G. and Ben-Ze'ev, I. (1982) Spore types and other structures formed in adult housefly and medfly incubated with strains of *Erynia radicans*. In: *Third International Colloquium of Invertebrate Pathology, Brighton, England*, p. 191 (abstract).

Vandenberg, J.D. and Soper, R.S. (1978) Prevalence of Entomophthorales mycoses in populations of spruce budworm, *Choristoneura fumiferana. Environmental Entomology* 7, 847–853.

Villacarlos, L.T. and Keller, S. (1997) *Batkoa amrascae* Keller & Villacarlos, a new species of Entomophthorales (Zygomycetes) infecting the cotton leafhopper, *Amrasca biguttula* (Ishida) (Homoptera: Cicadellidae) in the Philippines. *Philippine Entomologist* 11, 81–86.

Villacarlos, L.T., Pell, J.K. and Steinkraus, D.S. (1996) Pathogenicity of *Neozygites fresenii* isolates from the Philippines and the United States. In: *Proceedings of the III International Colloquium on* Bacillus thuringiensis *and the XXIX Annual Meeting of the Society for Invertebrate Pathology, Cordoba, Spain, September*, p. 88 (abstract).

Walsh, S.R.A. (1996) Development of molecular markers for the detection and differentiation of *Entomophaga* strains pathogenic for insects. PhD dissertation, University of Toronto, Canada.

Watson, D.W. and Petersen, J.J. (1993a) Sexual activity of male *Musca domestica* (Diptera: Muscidae) infected with *Entomophthora muscae* (Entomophthoraceae: Entomophthorales). *Biological Control* 3, 22–26.

Watson, D.W. and Petersen, J.J. (1993b) Seasonal activity of *Entomophthora muscae* (Zygomycetes: Entomophthorales) in *Musca domestica* L. (Diptera: Muscidae) with reference to temperature and relative humidity. *Biological Control* 3, 182–190.

Watson, D.W., Mullens, B.A. and Petersen, J.J. (1993) Behavioral fever response of *Musca domestica* (Diptera: Muscidae) to infection of *Entomophthora muscae* (Zygomycetes: Entomophthorales). *Journal of Invertebrate Pathology* 61, 10–16.

Weseloh, R.M., and Andreadis, T.G. (1997) Persistence of resting spores of *Entomophaga*

maimaiga, a fungal pathogen of the gypsy moth, *Lymantria dispar*. *Journal of Invertebrate Pathology* 69, 195–196.

Wilding, N. (1969) Effect of humidity on the sporulation of *Entomophthora aphidis* and *E. thaxteriana*. *Transactions of the British Mycological Society* 53, 126–130.

Wilding, N. (1970a) *Entomophthora* conidia in the air-spora. *Journal of General Microbiology* 62, 149–157.

Wilding, N. (1970b) The effect of temperature on the infectivity and incubation periods of the fungi *Entomophthora aphidis* and *E. thaxteriana* for the pea aphid *Acyrthosiphon pisum*. In: *Proceedings of the 4th International Colloquium of Insect Pathology, Maryland, USA,* pp. 84–88.

Wilding, N. (1973) The survival of *Entomophthora* spp. in mummified aphids at different temperatures and humidities. *Journal of Invertebrate Pathology* 21, 309–311.

Wilding, N. (1976) Determination of the infectivity of *Entomophthora* spp. In: *Proceedings of the 1st International Colloquium of Invertebrate Pathology, Kingston, Ontario, Canada*, pp. 296–300.

Wilding, N. (1981) The effect of introducing aphid-pathogenic Entomophthoraceae into field populations of *Aphis fabae. Annals of Applied Biology* 99, 11–23.

Wilding, N. (1982a) Entomophthorales: field use and effectiveness. *Proceedings of the 3rd International Colloquium on Invertebrate Pathology, Brighton, UK*, pp. 170–175.

Wilding, N. (1982b) The effect of fungicides on development of *Entomophthora aphidis. Transactions of the British Mycological Society* 75, 297–302.

Wilding, N. and Brady, B.L. (1984) *Descriptions of Pathogenic Fungi and Bacteria,* Set 82, Nos 812, 814, 815, 817, 820. CMI, Kew, UK.

Wilding, N. and Brobyn, P.J. (1980) Effects of fungicides on the development of *Entomophthora aphidis*. *Transactions of the British Mycological Society* 75, 279–302.

Wilding, N. and Lauckner, F.B. (1974) *Entomophthora* infecting wheat bulb fly at Rothamsted, Hertfordshire, 1967–1971. *Annals of Applied Biology* 76, 161–170.

Wilding, N. and Perry, J.N. (1980) Studies on *Entomophthora* in populations of *Aphis fabae* on field beans. *Annals of Applied Biology* 94, 367–378.

Wilding, N., Latteur, G. and Dedryver, C.A. (1986a) Evaluation of *Entomophthorales* for aphid control: laboratory and field data. In: Samson, R.A., Vlak, J.M. and Peters, D. (eds) *Fundamental and Applied Aspects of Invertebrate Pathology: 4th International Colloquium on Invertebrate Pathology* Wageningen, the Netherlands, pp. 159–162.

Wilding, N., Mardell, S.K. and Brobyn, P.J. (1986b) Introducing *Erynia neoaphidis* into a field population of *Aphis fabae*: form of the inoculum and effect of irrigation. *Annals of Applied Biology* 108, 373–385.

Wilding, N., Mardell, S.K., Brobyn, P.J., Wratten, S.D. and Lomas, J. (1990) The effect of introducing the aphid-pathogenic fungus *Erynia neoaphidis* into populations of cereal aphids. *Annals of Applied Biology* 117, 683–691.

Witlaczil, E. (1885) *Neozygites aphidis*, eine neue Gregarine. *Archiven für Mikrobiologie und Anatomie* 24, 599–603.

Wraight, S.P. and Roberts, D.W. (1987) Insect control efforts with fungi. *Developments in Industrial Microbiology* 28, 77–87.

Wraight, S.P., Galaini-Wraight, S., Carruthers, R.I. and Roberts, D.W. (1986) Field transmission of *Erynia radicans* to *Empoasca* leafhoppers in alfalfa following application of a dry mycelial preparation. In: Samson, R.A., Vlak, J.M. and Peters, D. (eds) *Fundamental and Applied Aspects of Invertebrate Pathology: 4th International Colloquium of Invertebrate Pathology, Veldhoven*, p. 233.

Wraight, S.P., Butt, T.M., Galaini-Wraight, S., Allee, L.L., Soper, R.S. and Roberts, D.W. (1990) Germination and infection processes of the entomophthoralean fungus *Erynia radicans* on the potato leafhopper *Empoasca fabae. Journal of Invertebrate Pathology* 56, 157–174.

Yamamoto, M. and Aoki, J. (1983) Periodicity of conidial discharge of *Erynia radicans. Transactions of the Mycological Society of Japan* 24, 487–496.

Yaninek, J.S., Saizonou, S., Onzo, A., Zannou, I. and Gnanvossou, D. (1996) Seasonal and habitat variability in the fungal pathogens, *Neozygites* cf. *floridana* and *Hirsutella thompsonii*, associated with cassava mites in Benin, West Africa. *Biocontrol Science and Technology* 6, 23–33.

Yeo, H. (2000) Biorational selection of mycoinsecticides for aphid management. PhD thesis, University of Nottingham, 295pp.