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Use of Hyphomycetous Fungi for Managing Insect Pests

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Introduction

The development of resistance to chemical insecticides and concerns over the deleterious effects of chemicals on environmental and human safety have provided a strong impetus for the development of microbial control agents for use in integrated control of insect pests. A diverse assemblage of microorganisms are currently under consideration as control agents of insects, including viruses, bacteria, protozoa and fungi. Of the fungi, considerable effort has focused on the development and utilization of entomopathogenic Hyphomycetes (see next section). There are numerous examples of the efficacious suppression of pest insects with this group of microorganisms demonstrating their considerable potential as biological control agents (BCAs). However, their application has not always provided consistent suppression of insect pests. The factors responsible for the initiation and development of epizootics in insect populations are extremely complex, involving interactions among the pathogen(s), insect host, environment and time. An understanding of this dynamic interaction is important, and elucidation of the factors that limit disease initiation and development may allow us to overcome constraints and thereby achieve efficacious control of insect pests.

Reviews dealing with entomopathogenic Hyphomycetes and their development as microbial control agents include those of Ferron (1985), McCoy *et al.* (1988), Evans (1989), Ferron *et al.* (1991), Glare and Milner (1991), Roberts and Hajek (1992), Tanada and Kaya (1993), Hajek and St Leger (1994), Boucias and Pendland (1998) and Wraight and Carruthers (1999). For methods and techniques used to study Hyphomycetes, readers are referred to Goettel and Inglis (1997), Butt and Goettel (2000), Goettel *et al.* (2000a) and Lacey and Kaya (2000). In this chapter we provide

a broad overview of the major factors influencing the efficacy of hyphomycetous fungi and provide examples of successful use of entomopathogenic Hyphomycetes in pest control, emphasizing some of the strategies that can be used to optimize their impact on pest populations.

Entomopathogenic Hyphomycetes

Fungi are a phylogenetically diverse group of microorganisms that are all heterotrophic (absorptive nutrition) eukaryotes, unicellular (i.e. yeasts) or hyphal (i.e. filamentous), and reproduce by sexual and/or asexual spores. The true fungi (kingdom: *Mycota*) are divided into four divisions: the *Chytridiomycota*, *Zygomycota*, *Ascomycota* and *Basidiomycota* (Hawksworth *et al.*, 1995). Although a variety of criteria are used, the most common character used to assign fungi to natural groups (i.e. based on genetic relationships) relies on sexual fruiting structures. However, a large group of fungi, including many entomopathogenic taxa, have lost the ability to produce or rarely produce sexual spores. This group of fungi was traditionally placed in the formed division, *Deuteromycota*, within the artificial class, Hyphomycetes. The members of this class are characterized by mycelial forms that bear asexual spores, termed 'conidia', borne on specialized conidiogenous cells. Conidiogenous cells are often formed on simple or branched hyphae, termed 'conidiophores', or from aggregations of conidiophores, referred to as synnemata (a group of erect and sometimes fused conidiophores bearing conidia at the apex only or on both apex and sides) or sporodochia (a mass of short conidiophores produced in a cushion-like structure).

Members of the Hyphomycetes were traditionally distinguished from fungi that produced conidia in conidiomata (i.e. *Coelomycetes*). These fungi produced conidia in discrete, often flask-shaped, conidiomata, termed 'pycnidia', or in saucer-shaped conidiomata, termed 'acervuli'. Most mycologists no longer accept *Deuteromycota* and its subclasses as formal taxonomic assemblages, and these fungi are typically handled together as 'mitosporic fungi'. Although few taxa of entomopathogenic mitosporic fungi have been connected with a sexual state, most exhibit ascomycetous affinities, including septate hyphae possessing relatively simple septal pores and lamellate hyphal walls with a thin electron-dense outer layer and a relatively thick electron-transparent inner layer. Furthermore, many of these fungi have been correlated with members of the *Ascomycota* on the basis of DNA homology (i.e. 18S ribosomal DNA. When a mitosporic fungus has been connected with a sexual state, it is referred to as the 'anamorph' or 'anamorphic state'. The sexual or perfect state alone is termed the 'teleomorphic state', whereas the whole fungus and all its 'morphs' (i.e. the sexual and asexual states together) are referred to as the 'holomorph'.

The nomenclature of fungi is governed by the International Code (see also selected papers of the IXth International Congress of Mycology of the International Union of Microbiological Societies in 1999). The Code permits the different states of fungi to be given separate names. However, if a teleomorph is present, the name automatically refers to that morph even if the anamorph is also evident (i.e. the correct name of a holomorph is that of its teleomorph). For example, the entomopathogenic taxon, *Paecilomyces farinosus* is the name given to the anamorphic state of the teleomorph, *Cordyceps memorabilis* (Domsch *et al.*, 1980). Since this fungus has been correlated with a teleomorphic state, according to the rules of nomenclature it must be named *C. memorabilis*. For fungi not correlated with a teleomorphic state, the use of the gen-

eral term 'mitosporic fungi' has been advocated (Hawksworth *et al.*, 1995). However, the use of 'mitosporic fungi', is not without controversy, and numerous authors have argued that the anamorphic and teleomorphic phases of a fungus are determined not simply by the type of cellular processes (e.g. mitosis) that precede sporulation, but also by morphological features. Largely due to tradition, we have retained the use 'entomopathogenic Hyphomycetes' in this chapter. For a more comprehensive review on the nomenclature of ascomycetes the reader is referred to Seifert (2000).

We have already indicated that the asexual spores produced by mitosporic fungi are termed 'conidia' but some taxa can also produce asexual resting spores, termed 'chlamydospores'. Traditionally, the classification of Hyphomycetes to genera was primarily based on spore characteristics and on the degree of aggregation of conidiophores into more complex conidiomatal structures (e.g. synnematal or sporodochial conidiomata). Beginning with Hughes (1953), the study of conidium formation on conidiogenous cells (i.e. conidiogenesis) provided an additional tool for classifying the Hyphomycetes. The most common entomopathogenic genera of hyphomycetous fungi include *Aspergillus*, *Beauveria*, *Culicinomyces*, *Hirsutella*, *Metarhizium*, *Nomuraea*, *Paecilomyces*, *Tolyposcladium* and *Verticillium*. Each of these taxa is defined by its characteristic conidiogenesis (e.g. Barron, 1968; Samson *et al.*, 1988; Humber, 1997). However, placement of hyphomycetous fungi within formed genera based on conidiogenesis does not necessarily reflect phylogenetic groupings, and the application of molecular methods is shedding new light on generic and species concepts within this group of entomopathogenic fungi. Many of the above taxa of entomopathogenic Hyphomycetes are believed to possess a *Cordyceps* teleomorphic state (Ascomycota: Hypocreales).

A few taxa of entomopathogenic fungi rarely or never produce exogenous conidia. Two examples of such fungi are *Aschersonia* and *Sorosporella*. Conidia of *Aschersonia* are formed from conidiogenous cells within flask-shaped structures (i.e. pycnidia embedded in light to brightly coloured, hemispherical or cushion-shaped stromata, somewhat shrunken, opening by wide pores or ruptures) and are traditionally handled within the 'artificial' class, *Coelomycetes*; *Aschersonia* has been correlated with a *Hypocrella* teleomorph (Ascomycota: Hypocreales). *Sorosporella* is another mitosporic taxon that is a pathogen of insects (e.g. acridids). While *Sorosporella* readily produces brick-red chlamydospores in insect cadavers, it does not usually produce conidia. However, germinating chlamydospores can produce mycelium from which conidia are formed. The conidial state of this fungus is *Syngliocladium* (Petch, 1942).

Pathogenesis and Epizootiology

Fungi are unique among the insect pathogens in that they infect their hosts primarily through the external cuticle, although a few taxa (e.g. *Culicinomyces*) are able to invade through the alimentary canal. Conidia of most taxa of entomopathogenic Hyphomycetes strongly adhere to insect cuticles, and their attachment to cuticles is thought to involve non-specific adhesion mechanisms mediated by the hydrophobicity of the conidial cell wall (Boucias *et al.*, 1988, 1991). Once a propagule comes in contact with an appropriate insect cuticle, it may germinate and it may produce penetration structures (e.g. germ-tube swelling, appressorium or extracellular sheath) from which penetration hyphae are formed. Even if germination does occur, the fungus may not be able to penetrate the cuticle, due to a number of factors, such as an

inappropriate environment (e.g. conditions of moisture) and/or the presence of inhibitory factors, such as fatty acids or melanin, within the cuticle. To penetrate the cuticle, the entomopathogenic Hyphomycetes utilize a combination of enzymatic and mechanical mechanisms. The production of exoproteases are important in penetration by *Metarhizium anisopliae* and other fungi, but other enzymes, such as endoproteases, esterases, lipases, chitinases and chitobiases, are involved as well (St Leger, 1993; Boucias and Pendland, 1998; Butt *et al.*, 1998a).

Once the fungus reaches the haemocoel, it grows as hyphal bodies, which are single- or multicelled structures that lack a formal cell wall but do contain a thin, fibrillar layer on the plasma membrane (some forms are often referred to as 'blastospores'). Before the fungus can proliferate in the haemocoel, it must often overcome the insect's defence response, and the production of toxins by the fungus can debilitate the efficacy of the defence response mounted by the insect (see Chapter 12). The insect may respond to fungal infection using humoral (e.g. phenoloxidase, lectins and/or host defence proteins and peptides) and/or cellular (e.g. phagocytosis and/or encapsulation) mechanisms (Bidochka *et al.*, 1997; Boucias and Pendland, 1998). However, hyphal bodies of some species (e.g. *Nomuraea rileyi*) do not appear to be phagocytosed by haemocytes, either because they lack specific surface residues and therefore are not recognized by humoral lectins (i.e. opsonization) or because they mimic surface epitopes on insect haemocytes (Boucias and Pendland, 1998). Insect death may result from a combination of actions, including depletion of nutrients, physical obstruction or invasion of organs and toxicosis. For example, *Beauveria bassiana* produces a number of toxic compounds, including beauvericin, bassianolide and oosporein (see Chapter 12). Perhaps the best-studied toxins produced by entomopathogenic Hyphomycetes are the destruxins produced by *M. anisopliae*. Several of these are known to induce tetanic paralysis (Dumas *et al.*, 1996), while others can be immunosuppressive (Cerenius *et al.*, 1990).

Following death, the fungus often grows saprotrophically within the host, and metabolites produced by the various taxa (e.g. oosporein produced by *B. bassiana* and *Beauveria brongniartii*) may be involved in the competitive exclusion of competing microorganisms from the cadaver. Soon after host death, and under favourable conditions, hyphae emerge from the cadaver; they produce conidiogenous cells, sporulation occurs on the host surface and the conidia are liberated. Conidial dispersal is passive, relying principally on wind but other factors, such as rain, can play a role in dissemination. As mentioned previously, many hyphomycetous fungi (e.g. *Beauveria*, *Metarhizium* and *Paecilomyces*) produce conidia possessing hydrophobic properties due to cysteine-rich proteins called hydrophobins within the rodlet layer of the cell wall. In contrast, *Verticillium lecanii* (syn. *Cephalosporidium lecanii*) possesses hydrophilic conidia. The hydrophobicity of the conidial cell wall influences the biology of these fungi and is an important factor in deployment against insect pests.

Epizootics of hyphomycetous fungi can occur in field populations of insects, and they can be important in the natural regulation of insect pests. An epizootic is the result of a complex interaction among the host, the pathogen and the environment over time (i.e. the disease tetrad), and relatively little is known about the factors responsible for the initiation and continuance of epizootics for most fungal species. A threshold of inoculum is required to induce disease, and many pest managers have simply focused on the deposition of large quantities of virulent propagules on to an insect host. Often this approach has resulted in inadequate suppression of insect pests, since the inoculum threshold is not static and it is influenced by all aspects of the disease

tetrad (e.g. host susceptibility and environment). Much research is needed before we shall be able to adequately predict the conditions under which hyphomycetous fungal epizootics are initiated and developed. Such an understanding is necessary if we are to use these pathogens successfully in integrated pest management (IPM) programmes.

Perhaps the best-studied entomopathogenic hyphomycetous fungus from an epizootiological perspective is *N. rileyi* (Carruthers and Soper, 1987). This fungus often causes natural epizootics in populations of noctuids, such as soybean loopers (*Pseudoplusia includens*) and velvet-bean caterpillars (*Anticarsia gemmatalis*) in soybean. Numerous factors, such as the timing of the initial infection of the host population, the developmental lag associated with the disease incubation period, which is related to the host instar, the rate of spread of the pathogen (i.e. transmission) and various environmental parameters, are all known to control the occurrence of epizootics (Ignoffo *et al.*, 1977a; Kish and Allen, 1978; Ignoffo, 1981). Primary inoculum (i.e. the inoculum that initiates disease within a population) is thought to originate from ovipositing moths (Kish, 1975) or by overwintering inoculum within the field (Ignoffo *et al.*, 1977a). Secondary inoculum (i.e. inoculum produced from diseased individuals within a population) in field environments depends on the movement of infected host larvae and on the dispersal of airborne conidia from cadavers (Kish and Allen, 1978). Initially, infected insects occur in limited loci, but, given appropriate conditions, disease can spread very rapidly throughout a field (Fuxa, 1984). Infected hosts generally exhibit a more highly aggregated spatial pattern, and this probably contributes to higher densities of secondary inoculum in the vicinities of initial loci. Kish and Allen (1978) developed one of the first quantitative epizootiological models, based on a series of stimulus–response experiments, and model predictions were found to accurately predict field mortality in ~50% of the field situations examined. Due to the complexity of the factors controlling epizootic development, the use of quantitative models can provide critical information on the factors influencing disease. Such information can subsequently be used to facilitate the efficacious suppression of insect pests using inundative deployment strategies.

Factors Influencing Efficacy

The pathogen

Pathogenicity is the qualitative ability of a pathogen to cause disease and it is determined by a variety of factors, including the physiology of the host (e.g. defence mechanisms), the physiology of the fungus (e.g. pathogenicity factors, such as the production of enzymes and toxins) and the environment. Fungi, as a group, have one of the widest host ranges among the pathogens of arthropods. However, host spectra vary widely, depending on fungal species. For instance, *Aschersonia aleyrodinis* only infects whiteflies, and *N. rileyi* almost exclusively infects lepidopterans within the Noctuidae. In contrast, species such as *B. bassiana* and *M. anisopliae* have much wider host ranges, spanning numerous orders within the Arthropoda. It is now recognized that *B. bassiana* and *M. anisopliae* contain a diverse assemblage of genotypes and probably comprise ‘species complexes’. Therefore, it is not surprising that within these taxa (grouped on morphological characters) individual isolates or pathotypes can exhibit a substantially restricted host range. Physiological host range is usually determined within controlled

environment settings, and it is a very flexible characteristic where a variety of factors will determine the outcome (e.g. dose or application method). While the ability of a pathogen to incite disease is essential, a complex array of additional factors determines its suitability as a prospective BCA.

An important consideration in selecting a strain is its virulence, which is the quantitative amount of disease that a pathogen can incite in a group of insects. In a field setting, propagule densities must be sufficiently high to ensure a good probability that an insect will come in contact with an adequate number of propagules to exceed the inoculum threshold. Since a highly virulent pathogen will require fewer propagules to incite disease, selection of virulent genotypes has obvious consequences for efficacious microbial control of insects. Unfortunately, much of the research quantifying the virulence of entomopathogenic Hyphomycetes isolates (usually expressed as a median lethal dose or LD₅₀) has been conducted under conditions that are not necessarily indicative of field environments (e.g. constant and moderate temperatures). However, a few studies have attempted to mimic conditions that may limit the efficacy of entomopathogens in field environments (e.g. environmental constraints) in an attempt to select genotypes able to incite disease under such conditions.

The ability of an entomopathogenic Hyphomycetes species to persist in an environment is another important attribute of a successful BCA. For propagules that exhibit good persistence, there will be a higher probability of an insect coming in contact with sufficient propagules to cause disease. As mentioned previously, inoculum thresholds are dynamic and the prolongation of propagule survival until conditions are conducive for disease development will result in more efficacious control. For example, during sunny periods grasshoppers can optimize their body temperatures by basking. Despite the deposition of large numbers of virulent conidia on to grasshoppers, disease progression is prevented or reversed under such conditions. However, during cool overcast periods, when grasshoppers are incapable of elevating their body temperature, Inglis *et al.* (1997a) hypothesized that the inoculum threshold may drop sufficiently to permit disease initiation. By comparing disease development in grasshoppers inoculated with *B. bassiana* and placed in cages that were either exposed to full sunlight or protected from ultraviolet-B (UVB) radiation, they observed substantially more disease in the UVB-protected cage environments, and the onset of disease occurred during a relatively cool overcast period. There was no difference in the ability of grasshoppers to elevate their body temperatures between cage environments, but conidial persistence was enhanced in the UVB-protected environment. Their observations supported the hypothesis that prolonging conidial persistence until conditions are conducive for disease to develop – in this case, the grasshoppers were unable to elevate their body temperatures during an overcast period – can substantially enhance the efficacy of entomopathogenic Hyphomycetes against insect pests. Further research on the interaction among persistence, inoculum thresholds and efficacy is warranted.

A number of entomopathogenic Hyphomycetes frequently cause natural epizootics, and the ability of a pathogen to cycle and disperse is an important factor in the development of these epizootics. As indicated previously, transmission of *N. rileyi* inoculum is an important factor in the development of an epizootic. Kish and Allen (1978) summarized the salient factors influencing dispersal of *N. rileyi* in velvet-bean caterpillar populations in soybean, which are: (i) conidiogenesis on cadavers required periods of high humidity ($\geq 70\%$), but conidia will form on cadavers independently of fluctuations in relative humidity as long as the cadavers do not rapidly

desiccate; (ii) dry windy conditions promote conidial dispersal; (iii) dry windy conditions retard conidial germination and infection, but promote infection if they are followed by humid conditions, providing no excess of free water exists; (iv) rain and vegetative wetting promote conidiogenesis on cadavers; (v) conidia are washed to the ground by rain or heavy vegetative wetting by dew; (vi) an excess of free water during the height of an epizootic has little net effect on the course of the epizootic, but an excess of free water in the early stages of an epizootic (< 10% infection) may retard the spread of infection, if it follows conidiogenesis but precedes conidial dispersal; and (vii) an alternation of wet and dry conditions is necessary for spread of infection, and short periods of vegetative wetting and high humidity with longer periods of dry conditions with light winds favour the increase and spread of infections.

The insect host

A complex array of physiological and morphological factors influence the susceptibility of insect pests to entomopathogenic fungi. Examples include population density, behaviour, age, nutrition, genetics and exposure to injuries caused by mechanical, chemical or non-microbial agents (e.g. predators and parasites). A detailed review of the influence of host factors on disease development is beyond the scope of this chapter, and here we focus on important factors influencing the efficacy of entomopathogenic Hyphomycetes in managing insect pests.

One of the most important paradigms in microbial control is that 'stressed' animals are more susceptible to entomopathogens than non-stressed animals (e.g. Steinhaus, 1958a; Vago, 1963). While a variety of factors are thought to stress insects and predispose them to entomopathogens (e.g. crowding, nutrition, exposures to chemical stressors, environment), the physiological mechanisms (i.e. depressed immune response) of stress and the influence of many environmental parameters on the physiological predisposition of insects to entomopathogens are relatively poorly understood. Insect nutrition is a very important factor regulating the susceptibility of insects to entomopathogens, and it is an often overlooked factor in disease development. Inadequate nutrition often leads to increased susceptibility to entomopathogens, and the utilization of resistant plant genotypes to induce nutritional stress can substantially enhance the efficacy of entomopathogens. Conversely, diet can also decrease the susceptibility of insect pests to entomopathogenic Hyphomycetes. For example, Ekesi *et al.* (2000) found that thrips (*Megalurothrips sjostedti*) were less susceptible to *M. anisopliae* on certain cow-pea cultivars because of plant-derived fungistatic compounds. The concentration of secondary metabolites in plants is said to be higher in young leaves than in older leaves, but older leaves contain fewer nutrients (i.e. nitrogen and water) (Fenny 1992).

Declining nutrient and water content in the mature foliage of perennial plants was reported to reduce the growth rates of lepidopteran larvae compared with those of closely related species feeding on younger leaves or on the foliage of herbaceous plants (Krischik and Denno, 1983). It has also been suggested that high protein concentrations in an insect's diet can counterbalance the toxic effect of secondary metabolites, such as alkaloids (Costa and Gaugler, 1989). It is now recognized that insects may sequester antifungal compounds from their diets as a defence against entomopathogens, and increasing attention is now focusing on the impact of insect

nutrition on entomopathogen success. The inclusion of physiological stressors in the formulation of entomopathogenic Hyphomycetes is another obvious strategy that can be applied to enhance the efficacy of entomopathogens, and this strategy will also be discussed later in the chapter.

Another host factor that has been demonstrated to play an important role in the success of entomopathogens is the development stage of an insect. Not all stages in an insect's life cycle are equally susceptible to infection by entomopathogenic Hyphomycetes. In some situations, immature insects are more susceptible to infection than mature insects. For example, young larvae of the European corn-borer (*Ostrinia nubilalis*) are more susceptible to *B. bassiana* than older larvae (Feng *et al.*, 1985). In contrast, adult western flower thrips (*Frankliniella occidentalis*) were more susceptible to *V. lecanii* than larvae (Vestergaard *et al.*, 1995). Most host factors, such as insect developmental rates, cannot be considered independent of environment (e.g. temperature). High temperatures accelerate insect development and will reduce the time between molts, which can subsequently reduce the prevalence of infection due to loss of inoculum on exuviae.

Insect density is of particular importance in the epizootiology of disease. As the density of insects increases, there is a higher probability of an insect coming into contact with a pathogen (i.e. with infected individuals or with the pathogen directly). This is particularly true of viral epizootics, but limited research has focused on epizootics caused by entomopathogenic Hyphomycetes. Crowding has also been shown to stress individuals within a population, and subsequently predispose them to infection by non-fungal entomopathogens (Steinhaus, 1958b). However, the impact of crowding on the predisposition of insects to infection by entomopathogenic Hyphomycetes has not received much attention.

The behaviour of insects can influence epizootic development, and can affect the dispersal of an entomopathogen. For example, insects infected with entomophthoralean fungi often climb to the tops of plants just prior to death (i.e. summit disease syndrome), where they die firmly clasping the plant (e.g. grasshoppers infected with *Entomophaga grylli*). Such adaptations help ensure that spores contact potential hosts within and beneath the plant canopy, although such behaviour has not been reported for insects infected with hyphomycetous fungi. Another behavioural trait that can influence transmission of a pathogen is grooming. Conidia of *M. anisopliae* are spread among individual termites by grooming (Kramm *et al.*, 1982). While grooming can contribute to disease severity, the sanitation behaviour exhibited by many social insects can also limit the spread of an entomopathogen. For example, the termite *Reticulitermes flavipes* is very resistant to entomopathogenic Hyphomycetes (e.g. *B. bassiana*) not because of any endogenous defence mechanisms, but as a result of complex social behaviours, including the removal of infected individuals from the colony (Boucias *et al.*, 1996).

The environment

A variety of environmental factors have been shown to have dramatic effects on the efficacy of entomopathogens against insect pests. Salient parameters influencing the success of entomopathogenic Hyphomycetes against insects are solar radiation, temperature, water availability, precipitation and wind. Although we most often focus on a particular variable, environmental parameters interact with each other in their impact

on entomopathogens and, where possible, these factors should be addressed interactively.

Solar radiation

One of the most important parameters affecting propagule persistence in epigeal habitats is deactivation by solar radiation. Conidia, hyphal bodies and hyphae of all taxa of hyphomycetous fungi are highly susceptible to damage by solar radiation, and in particular the UVB portion of the solar spectrum (285–315 nm). However, significant differences in susceptibility to irradiation among taxa and strains within species have been observed. For example, Fargues *et al.* (1996) observed that conidia of *M. anisopliae* var. *acridum* (syn. *Metarhizium flavoviride*) were generally the most resistant to artificial sunlight (295–1100 nm at a UVB irradiance of 0.3 W m^{-2}) followed by conidia of *B. bassiana*, *M. anisopliae* and *Paecilomyces fumosoroseus*. While the mechanisms of resistance to solar radiation within the entomopathogenic fungi have not been extensively studied, resistance to UV damage should be considered in selecting genotypes of fungi for use in IPM programmes.

The microhabitat in which fungi are deployed is another important factor influencing their persistence. The survival of conidia deposited on substrates exposed to direct solar radiation is substantially reduced relative to propagules in protected locations, such as within plant canopies (Inglis *et al.*, 1993). Since many insect pests feed or exist for periods of time within the plant canopy, the influence of microclimate should not be overlooked. Another strategy is to apply conidia on the abaxial surface of leaves (e.g. Sopp *et al.*, 1990) to enhance persistence, but this can be a difficult task (Wraight and Carruthers, 1999). The degree to which the targeting of abaxial leaf surfaces will be efficacious will also depend on the behaviour of the target insect pest. Some insects naturally feed on the undersides of leaves (e.g. whiteflies), but it may be necessary to stimulate other pest insects to move to the abaxial surface of leaves. For example, Amiri *et al.* (1999) demonstrated that crude preparations of destruxins from *M. anisopliae* applied to the abaxial leaf surface caused diamondback moth (*Plutella xylostella*) and mustard beetle (*Phaedon cochleariae*) larvae to move to the adaxial leaf surface.

Even within shaded areas, propagules will eventually be killed due to indirect irradiance (Smits *et al.*, 1996a, b), but conidial survival may be sufficiently prolonged to enhance the efficacy of control. While the UVB component of the solar spectrum is detrimental to fungal propagules, irradiation at higher wavelengths may be beneficial by stimulating photoreactivation counteracting the harmful effects of UV radiation. Although we are not aware of a photoreactivation phenomenon specifically within insect-pathogenic fungi, it has been demonstrated in other fungi (Leach, 1971) and consequently should not be overlooked.

The rapid inactivation of infectious propagules by solar radiation is considered by many to be a major impediment to the successful commercialization of entomopathogens against insect pests of field crops, and considerable effort has focused on the protection of entomopathogens. The most popular approach has involved the incorporation of solar blockers and/or UVB-absorbing chemicals (i.e. sunscreens) in formulations of propagules. A diverse array of sunscreens have been tested with entomopathogenic viruses and *Bacillus thuringiensis*, and to a lesser extent with fungi. A summary of sunscreens used to protect microorganisms is provided by Bernhard *et al.* (1998) and Burges and Jones (1998). While a number of sunscreens have

demonstrated excellent protection of fungal propagules in controlled environment studies, reports of enhanced persistence or efficacy in field settings are less common. For example, several oil-soluble sunscreens significantly increased the survival of *B. bassiana* and *M. anisopliae* var. *acridum* conidia exposed to artificial radiation (Moore *et al.*, 1993; Inglis *et al.*, 1995a), but they did not enhance survival in field settings (Inglis *et al.*, 1995a; Shah *et al.*, 1998). In contrast, a solar blocker (clay) and a UV-absorbing optical brightener (Tinopal) did increase the field persistence of *B. bassiana* conidia on grass leaves exposed to sunlight (Inglis *et al.*, 1995a). It has long been recognized that pigmented propagules (e.g. melanin) of the dematiaceous Hyphomycetes are substantially more resistant to solar inactivation than non-melanized fungi. Formulation of *B. thuringiensis* in melanin provided excellent photoprotection (Liu *et al.*, 1993), and the formulation of melanin and other polyphenolics (e.g. lignin) may increase the persistence of entomopathogenic Hyphomycetes.

While research to date has focused on survival of propagules on plant tissues and/or on inanimate objects, very little is known about the persistence of propagules on insects. Many researchers consider this to be unimportant because the amount of time a fungus spends on the outside of the insect is limited (i.e. germination and penetration occur within 24 h). However, some evidence indicates that conidia on insects are as susceptible to solar deactivation as they are on inanimate objects or leaf surfaces (Inglis *et al.*, 1997a), and that prolonging the survival of fungal propagules on the surface of insects until conditions are favourable for disease development may enhance efficacy in field environments (e.g. Inglis *et al.*, 1997a). The adverse effects of solar radiation on entomopathogens are often influenced by other variables as well, and often these act as confounding effects. For example, both the direct (e.g. germicidal) and indirect (e.g. host behaviour) effects of solar radiation influence the efficacy of *B. bassiana* against grasshoppers (Inglis *et al.*, 1996a, 1997a). Research that determines whether prolonging the survival of fungal propagules will subsequently translate to increased field efficacy and the impact of other variables on this relationship is warranted.

Temperature

Temperature is one of the principal factors influencing entomopathogen efficacy. It is well documented that ambient temperature influences the rate of infection and time to death of insects treated with entomopathogenic hyphomycetous fungi. Given that a window of opportunity often exists for the pathogen to infect its host (e.g. conditions of humidity or solar radiation may be suitable for short periods of time), the influence of temperature on the infection process is very important. For example, the optimum temperature for *M. anisopliae* infecting adult thrips is $\sim 23^{\circ}\text{C}$ (Vestergaard *et al.*, 1995), and a decrease in temperature of $3\text{--}5^{\circ}\text{C}$ increases the time to death by ~ 1 day. A delay in death can be critical to a pest manager, especially in the management of pests with explosive population growth rates and/or pests of high-value crops (e.g. within greenhouses). Ideally, the thermal characteristics of isolates should be matched to the microhabitats in which they will be deployed for optimal performance.

The optimum temperature for most entomopathogenic Hyphomycetes is between 20 and 25°C , but infection and disease can occur at temperatures ranging between 15 and 30°C . Above 30°C , the vegetative growth of most taxa is inhibited and growth usually ceases at $\sim 37^{\circ}\text{C}$. The inability of an entomopathogenic fungus to grow at mammalian body temperature is an important consideration in registration (see Chapter

13). Similarly to variation in solar deactivation, considerable variability exists among genotypes in their thermal characteristics. For instance, Fargues *et al.* (1997b) found that four isolates of *M. anisopliae* var. *acridum* equally induced 98–100% mortality in the desert locust at 25 and 30°C, and none at 40°C, but there were significant differences among the isolates at 35°C, with mortalities ranging from 40 to 100%. A number of researchers have investigated the possibility of selecting genotypes possessing specific thermal characteristics (e.g. higher optimal and cardinal temperatures) from specific geographical locations (e.g. if an isolate comes from a warm country it may tolerate high temperatures, or conversely if it comes from cold climates it will perform better at low temperatures). Some studies have reported no or weak correlations between the geographical origin and thermal characteristic (McCammom and Rath, 1994; Fargues *et al.*, 1997a; Ouedraogo *et al.*, 1997). Others have observed a stronger relationship between temperature characteristics *in vitro* and place of origin. For example, Vidal *et al.* (1997a) measured the effects of temperature on the growth rates of various isolates of *P. fumosoroseus* originating from various insect hosts (primarily *Bemisia tabaci*, and some species of Lepidoptera) from the southern USA, Europe, Pakistan, Nepal and India. Similarly to other studies, they observed considerable variability among isolates in their ability to grow at various temperatures. They also reported that most isolates originating from Europe exhibited growth at 8–30°C with optimal growth rates at 20°C, 20–25°C or 25°C. The temperature range for the isolates from the southern USA (both humid and dry subtropical climates) and from west Asia (humid tropical climate) was broader (8–35°C) with optimal growth at 25°C, 25–28°C or 28°C. Indian isolates possessed the greatest tolerance to high temperatures (32°C and 35°C). The thermal biology of insects in field settings is extremely complex, and it still remains to be determined whether isolates possessing slightly higher optimal growth rates *in vitro* will translate into increased efficacy in field conditions.

Within field settings, daily temperatures can fluctuate substantially. For example, during May–July, when control of grasshoppers is usually implemented in the northern great plains of North America, night-time temperatures of less than 5°C and day-time temperatures of 25–35°C are common. Fluctuations in temperature have been shown to significantly influence the *in vitro* growth of a number of hyphomycetous fungi. However, the influences of fluctuating temperatures *in vivo* are less common. Inglis *et al.* (1999) measured the effects of oscillating temperature on the competitive infection and colonization of grasshoppers (*Melanoplus sanguinipes*) by *B. bassiana* and *M. anisopliae* var. *acridum* applied alone and in combination. Nymphal mortality and proliferation of the fungi in the haemocoel were measured in four environments with the same mean daily temperature (25°C) but differing in the degree to which they oscillated daily (constant 25°C, 20 to 30°C, 15 to 35°C or 10 to 40°C). In general, as the amplitude of temperature increased, growth rates for both fungi decreased. A similar trend was observed for nymphal mortality.

While the relationship between fungal proliferation in the haemocoel and disease development is poorly understood, Inglis *et al.* (1999) showed that both *B. bassiana* and *M. anisopliae* var. *acridum* proliferated in the haemocoel of inoculated grasshoppers, and that the size of fungal populations within haemocoels was significantly correlated with mortality. Although the same temperature regime was repeated on a daily basis, in nature temperature oscillations are variable and the influence of irregular oscillations on entomopathogens may further influence efficacy. The identification of temperature thresholds and the effect of cumulative exposures to high and low temperatures on efficacy of entomopathogenic Hyphomycetes, and the application of mathematical

models will facilitate our understanding of the effects of temperature on insect–fungal interactions. This will lead to the identification and implementation of strategies to overcome constraints of temperature on efficacy in entomopathogens.

The thermal constraints on entomopathogenic Hyphomycetes are not only the result of ambient temperatures but may also be exacerbated by host behaviour (i.e. thermoregulation). For example, many insects elevate their body temperatures higher than ambient by either directly or indirectly intercepting solar radiation (e.g. basking) (Heinrich, 1993, 1996). The influences of thermoregulation on disease development are less well documented, but basking behaviour in a number of insects, including houseflies (Watson *et al.*, 1993) and various acridids (e.g. Inglis *et al.*, 1996a), has been shown to reduce the severity of disease. Many acridids possess an optimal temperature of ~40°C, and optimization of body temperature via basking has been shown to adversely affect *B. bassiana* and to a lesser extent *M. anisopliae* var. *acridum* (Inglis *et al.*, 1996a, 1997b, 1999). The cardinal temperature for *B. bassiana* conidial germination and hyphal development is ~35°C, and the prevalence of mycosis decreased by ~45% and 90% in nymphs allowed to optimize their body temperature by basking for only 1 and 2 h day⁻¹, respectively (Inglis *et al.*, 1996a). While most insects possess lower temperature optima than acridids, these insects may exhibit a ‘behavioural fever’ response to infection. Behavioural fever is defined here as the elevation of body temperatures higher than normal levels using behavioural means. Most research on behavioural fever has utilized thermal gradients in which the body temperature of infected and non-infected insects is compared. For example, Inglis *et al.* (1996a) observed that a significantly higher prevalence of *B. bassiana*-infected nymphs selected hotter positions than non-infected nymphs on a heat gradient.

Field observations of behavioural fever are less common. Applying a grab-and-stab method at various times after application of *M. anisopliae* var. *acridum* against the Senegalese grasshopper (*Oedaleus senegalensis*), Blanford *et al.* (1998) observed that the body temperatures of grasshoppers sprayed with the fungus were 3°C higher on average than those of control grasshoppers, but that the fungus still provided good suppression, indicating that the behavioural fever response to infection did not confer resistance to disease in the treated population. However, behavioural fever in acridids infected with *M. anisopliae* var. *acridum* appears to increase the time to death and may also raise the inoculum threshold required for successful disease initiation and progression (Blanford *et al.*, 1998). The degree to which other insects exhibit a behavioural fever response and the implications of fever on the successful use of entomopathogens to manage insect pests in laboratory and field conditions is currently uncertain.

In insects possessing optimal temperatures that inhibit disease or that exhibit a behavioural fever response to infection, a number of strategies to overcome this constraint have been proposed. As mentioned previously, the selection of genotypes with the ability to grow and incite disease at higher temperatures is one possibility. Another strategy that is currently being pursued involves the use of behavioural modifiers and/or physiological stressors (e.g. Goettel *et al.*, 2000b). For example, sublethal doses of some insecticides have been observed to alter the behaviour of insects (e.g. following application insects become cryptic). Recently, renewed interest has focused on the use of insecticides (e.g. imidacloprid and diflubenzuron) in combination with entomopathogens. How these compounds interact with the pathogen and the insect host has not been well documented. Two other strategies that might be used to overcome the effects of high temperature include the use of novel targeting strategies (e.g. insect

stages at times of the year when behavioural optimization of body temperature is unlikely) and the development of predictive models that will facilitate the deployment of entomopathogens under suitable conditions (i.e. windows of opportunity).

Relative humidity

Relative humidity can influence fungal efficacy in several ways. Humidity, in combination with temperature, influences evaporation of spray droplets, which can result in the loss of small particles and thereby adversely affect targeting. Furthermore, moisture can also have very significant effects on the persistence of fungal inocula. For the most part, fungal conidia usually exhibit greatest stability under cool and dry conditions (Roberts and Campbell, 1977; Daoust and Roberts, 1983a; Hedgcock *et al.*, 1995; Hong *et al.*, 1997). In contrast, conidia of other fungi (e.g. *M. anisopliae*) survive better at moderate temperatures when relative humidity is high (e.g. Daoust and Roberts, 1983b). Not only is water essential for propagule germination, but it also regulates conidiogenesis on cadavers that have died from mycosis. In all instances, conidiogenesis on insect surfaces requires high moisture, and the production of conidia may influence horizontal transmission of the pathogen. For example, Fargues and Luz (2000) observed that a relative humidity of at least 97% was required for conidial production of *B. bassiana* on mummified cadavers of *Rhodnius prolixus* (vectors of *Trypanosoma*, the causal agent of Chagas' disease), and suggested that the occurrence of high humidity is the most crucial climatic constraint limiting the recycling ability of *B. bassiana* against *R. prolixus*. While high ambient humidity is a prerequisite for external conidiogenesis, evidence indicates that some taxa (e.g. *B. bassiana*) can form conidia within the haemocoel of cadavers under conditions of low humidity (Fernandes *et al.*, 1989).

The prerequisite for water during germination has led to the general belief that moist conditions are essential for effective use of fungi in microbial control against insects. This may hold true for some fungal taxa against some insect pests, but it is unlikely to hold true for all fungi in all circumstances. A number of studies indicate that dry conditions during or immediately following application of fungal propagules are less detrimental than previously thought. For example, ambient humidity does not influence infectivity of insects with *B. bassiana* and *M. anisopliae* var. *acridum* (e.g. Ramoska, 1984; Marcandier and Khachatourians, 1987; Fargues *et al.*, 1997b; Ferron, 1997). The ability of these fungi to germinate and infect the host under conditions of low ambient humidity is attributed to sufficient moisture within microhabitats. For example, a boundary layer surrounds plant vegetation, and a microclimate of high humidity probably also surrounds the integument of insects (e.g. due to water loss). Cuticular folds in insect exoskeletons may also be sites of high moisture. Although few studies have attempted to enhance infectivity of insects under conditions of low humidity, Bateman *et al.* (1993) reported that infection of desert locusts was enhanced for *M. anisopliae* var. *acridum* conidia applied in oil. On the basis of this report, many researchers now conclude that oil enhances infectivity at low humidities. However, Fargues *et al.* (1997b) found that *M. anisopliae* var. *acridum* conidia in oil did not enhance infectivity of desert locusts relative to an aqueous formulation. Evidence now indicates that there are complex interactions between inoculation method, carrier and humidity and this must be taken into consideration. Reports of spreading of propagules applied in oil over the surface of the integument (e.g. Inglis *et al.*, 1996b) and the influence that this has on infectivity (e.g. impact of microclimate) should be

addressed along with the impact of spatial distributions of propagules on the integument. Invert oils (water in oil emulsion) have been shown to enhance germination and penetration of plants by phytopathogenic fungi under low-moisture conditions, and the use of invert oils to enhance the infectivity of insects may prove to be beneficial.

Rainfall

In addition to increasing humidity, rainfall can serve to dislodge and disperse conidia from substrates as well as aid in the dispersion of propagules. The effects of precipitation and/or dew on the foliar persistence of entomopathogenic viruses and *B. thuringiensis* have been relatively extensively studied. In contrast, little is known about the persistence of entomopathogenic fungal propagules on insects and on foliage. Conidia of hyphomycetous fungi strongly adhere to insect cuticles (e.g. Boucias *et al.*, 1988, 1991). Anecdotal evidence, primarily based on poor recovery of conidia from insects following vigorous washing, has led to the opinion that rain does not 'wash off' significant numbers of conidia from the integument of insects (Burgess, 1998). However, recent evidence indicates that rain does cause significant removal of *B. bassiana* and *M. anisopliae* conidia from foliage of monocotyledonous and dicotyledonous plants (Inglis *et al.*, 1995b, 2000; Inyang *et al.*, 2000) and the integument of Colorado potato beetle larvae (*Leptinotarsa decemlineata*) (Inglis *et al.*, 2000). Rain-tower experiments indicated that *M. anisopliae* conidia are readily dispersed by rain splash, but very little inoculum was found in the soil outside sprayed plots in a subsequent field experiment, raising questions as to the importance of rain in the redistribution of inoculum (Inyang *et al.*, 2000). While rain has been shown to remove fungal conidia from leaves and insect cuticles, the influence of a number of confounding variables, such as canopy density and architecture and insect behaviour, on conidial persistence remains to be determined.

Stickers are frequently incorporated into viral and *B. thuringiensis* formulations to increase the retention of spray deposits on foliage, and a diverse array of commercial stickers have been used to enhance persistence of these entomopathogens (Bernhard *et al.*, 1998). Very limited research has focused on the utilization of sticker formulations to enhance the persistence of fungal propagules. The formulation of *B. bassiana* conidia in vegetable and mineral oils only marginally improved the retention of conidia on potato leaves and not on Colorado potato beetle larvae (Inglis *et al.*, 2000). Additional research on the influence of 'stickers' on persistence of entomopathogens applied in oil-based formulations is necessary, but caution must be exercised in choosing stickers. Stickers may prevent the transfer of conidia from sprayed leaf surfaces to insects, but those that remain liquid and/or can be subsequently transferred to the passing insect may prove useful.

Soil factors

Many hyphomycetous fungal entomopathogens are considered to be soil-borne microorganisms and have demonstrated considerable potential against soil pests (Keller and Zimmermann, 1989). However, soil is an extremely complex milieu, and a number of factors, including soil type (i.e. texture, cation exchange capacity, organic matter content, pH, etc.), moisture (i.e. water availability) and the presence of a viable soil microflora, can influence the persistence and/or efficacy of entomopathogenic hyphomycetous fungi. Furthermore, a variety of strategies (e.g. formulation and appli-

cation methods, such as tillage) have been tested in an attempt to enhance the success of entomopathogenic Hyphomycetes in soil.

ABIOTIC FACTORS. Many entomopathogenic fungi are able to withstand high and variable temperatures, as well as conditions of high moisture and drought stress, in soils (Roberts and Campbell, 1977), and a number of studies have demonstrated that conidia applied directly on soil surfaces or incorporated into soil following application exhibit considerable persistence in temperate climates (Muller-Kogler and Zimmermann, 1986; Gaugler *et al.*, 1989; Storey *et al.*, 1989; Inglis *et al.*, 1997c). For example, *M. anisopliae* was found to persist for at least 7 years in pasture soils at levels equivalent to the applied dose (Rath *et al.*, 1997).

The influence of various soil factors on the occurrence or persistence of entomopathogenic Hyphomycetes has been addressed in a number of survey studies. In these studies, researchers have attempted to correlate the abundance of fungi with various soil parameters, but, due to the complexity of these factors, it is often not possible to draw conclusions about the effects of factors on either survival or efficacy. While the results of controlled experiments are less numerous than survey data, several studies have addressed the influence of various soil factors on persistence and efficacy of entomopathogenic Hyphomycetes. In a variety of experimental settings, soil moisture has been shown to adversely influence the persistence of *B. bassiana*, *B. brongniartii*, *M. anisopliae* and *V. lecanii* and others in soil. In most circumstances, limited vertical movement of conidia occurs in soils. However, in other situations, physical loss of inoculum may occur (Storey and Gardner, 1987, 1988). For example, Storey and Gardner (1988) observed that the vertical movement of *B. bassiana* conidia was correlated with the water infiltration value of soils, with most loss occurring in sandy relative to finer-textured soils. However, even in the sandy soil (87% sand), greater than 85% of the conidia applied to the soil remained at the soil surface. Soil texture and organic matter appear to be the most important factors determining vertical movement of fungal propagules in water, and sandy-textured and soils low in organic matter tend to retain fewer propagules than clay-textured and organic soils (Ignoffo *et al.*, 1977b; Storey and Gardner, 1988; Keller and Zimmermann, 1989; Fornallaz, 1992). The mechanisms responsible for the high retention of conidia in such soils are unknown, but may relate to their high cation exchange capacity and/or to their reduced pore sizes. Furthermore, the vertical movement of the propagules is not only dependent on the type of soil, but also on properties of the propagules themselves. For example, conidia of *B. brongniartii* are relatively large (~8 µm in length) and are less prone to be vertically displaced relative to smaller propagules.

The influence of other parameters, such as pH and ionic conductivity, are less well understood. A number of studies have demonstrated no or minimal effects of soil pH on the distribution of entomopathogenic Hyphomycetes (e.g. Rath *et al.*, 1995). In contrast, Groden and Lockwood (1991) observed that fungistasis against *B. bassiana* was correlated with pH but not with other soil characteristics, including texture and organic matter content. In a later study, Groden and Dunn (1996) found that soil pH and nitrogen fertilizers had an impact on the germination of *B. bassiana* conidia, but not on the infection of the Colorado potato beetle. Fungistatic effects on soil-borne phytopathogens are often expressed most strongly in neutral or slightly acidic soils (Lockwood, 1977), and the release of plant-derived phenolic compounds can inhibit entomopathogenic fungi in soils (Lopez-Llorca and Olivares-Bernabeu, 1997).

A variety of strategies have been applied in an attempt to increase the persistence

of entomopathogenic Hyphomycetes in soil. Incorporation of conidia into soils is thought to increase their survival by protecting propagules from solar radiation and buffering them from extremes of temperature and moisture (Gaugler *et al.*, 1989), and number of application strategies have been developed to facilitate efficient application (e.g. injection with a seed drill). However, conditions of temperature in the top few centimetres of soil may range over 40°C between daylight and night hours, and temperatures in excess of 50°C can occur (Carruthers and Soper, 1987). Therefore, incorporation of propagules into soil to enhance persistence will depend not only on climatic factors (e.g. ambient temperatures and vegetation), but also on other factors, such as shading or the depth to which propagules are incorporated. In addition to injections, the encapsulation of propagules is another formulation strategy that may facilitate penetration and persistence in soil, but this has not been extensively tested with entomopathogenic Hyphomycetes.

A number of formulations have been shown to influence the persistence of propagules in soil. The application of conidia in oil or oil-emulsion sprays on to the surfaces of soil have generally not substantially enhanced their persistence relative to aqueous preparations of entomopathogenic fungal conidia (e.g. Inglis *et al.*, 1997c). In contrast, other formulations have been shown to increase the survival of propagules in soil. For example, coating *B. bassiana* conidia in clay increased their persistence in various soils and with various water activities under controlled environment conditions (Studdert *et al.*, 1990), but it did not affect mortality against beet army worms (*Spodoptera exigua*) (Studdert and Kaya, 1990a). Considerable interest has focused on the use of granular formulations of entomopathogenic Hyphomycetes. A variety of different materials have been used to prepare granules (e.g. alginate), and conidia or mycelia are incorporated into the granule matrix. The ability of the fungus to grow and sporulate in and/or on the granule is usually desirable, and a number of materials that facilitate growth and sporulation have been tested. Increased persistence and efficacy of fungi applied in granules in soils have been observed (e.g. Storey *et al.*, 1989; Krueger *et al.*, 1992), and propagules applied in granule formulations have provided satisfactory field activity against a number of economically important soil pests. Several of these products have been commercialized (Shah and Goettel, 1999; see also next section – ‘Mycoinsecticides in IPM’).

The most extensively studied parameters affecting the efficacy of entomopathogenic Hyphomycetes are soil texture and moisture (e.g. water availability). However, other factors, such as temperature, pH and the organic matter content of soil, may also have an impact on the efficacy of hyphomycetous fungi against insect pests. Soil moisture has been shown to have a significant impact on the efficacy. For example, Krueger *et al.* (1991) demonstrated that a higher number of chinch-bugs (*Blissus leucopterus leucopterus*) died from infection by *B. bassiana* under conditions of low relative to high soil moisture. Similarly, Studdert and Kaya (1990a) observed that emergence of beet army-worm adults was substantially reduced in drier (≤ 37 bars) than wetter soils (≥ 15 bars) inoculated with *B. bassiana* conidia, and a substantially higher density of conidia was required to reduce adult emergence in the wetter soils. Management practices, such as the degree of tillage, can also have an impact on entomopathogen efficacy. Grivanov (1940) reported that deep ploughing of cereal stubble increased the efficacy of *B. bassiana* against overwintering thrips, but the reason for the increased efficacy is uncertain. Much more research is needed on how agro-management practices can be altered to increase the efficacy of entomopathogenic Hyphomycetes against soil-dwelling insects.

BIOTIC FACTORS. Although many entomopathogenic Hyphomycetes are cosmopolitan in soil, very little is known about the saprotrophic ability of most taxa. Much circumstantial evidence suggests that many entomopathogenic Hyphomycetes (e.g. *B. bassiana* and *M. anisopliae*) are relatively weak competitors in soil, and it is common to observe relatively restricted vegetative growth emanating from insect cadavers that have died from mycosis in soil (e.g. Gottwald and Tedders, 1984). Supporting this supposition are observations of reduced vegetative growth in soils containing high to moderate levels of organic matter (Studdert and Kaya, 1990b) and in non-sterilized versus sterilized soils (e.g. Pereira *et al.*, 1993).

Studies attempting to elucidate the influence of the soil microflora on the persistence and efficacy of entomopathogenic Hyphomycetes are based on either anecdotal evidence or experimentation in which the microflora is eliminated (e.g. by sterilization) or modified (e.g. by organic amendments). Experimentation is often plagued by the extreme complexity of soils and by a variety of potentially confounding variables. For example, a number of researchers have compared persistence and/or efficacy of entomopathogenic Hyphomycetes in sterilized relative to unsterilized soils. The survival of *B. bassiana* conidia in non-sterile soil amended with carbon and/or nitrogen sources was found to be greatly decreased (Lingg and Donaldson, 1981). In contrast, populations of *B. bassiana* showed dramatic increases in sterile soil treated in the same manner, and Lingg and Donaldson (1981) postulated that the observed fungistasis in the non-sterile soils may have been due to the prevalence of the soil fungus *Penicillium urticae*, which produced a water-soluble inhibitor of *B. bassiana in vitro*. In another study, Inglis *et al.* (1998) observed that grasshoppers ovipositing into soils containing conidia of *B. bassiana* were highly susceptible to the fungus. While there was no effect of soil texture on mortality, on the number of eggs laid, on positioning of egg pods in the soil profile or on populations of *B. bassiana* recovered from female abdomens, a higher prevalence of mortality was observed for females ovipositing into sterilized than non-sterilized sandy-loam and clay-loam soils. All sterilization methods cause changes to the physical and/or chemical properties of soil, and extreme care must be taken in the interpretation of these types of studies.

Interactions among environmental variables

As indicated previously, the epizootics involve a dynamic interaction among the pathogen, host and environment in time. While the environment component of the disease tetrad is crucial to the success of a BCA, it is often overlooked. Furthermore, the interaction is extremely complex, and environmental parameters are rarely, if ever, static in time. Very few studies have attempted to empirically elucidate the importance of fluctuating climatic factors on the efficacy of entomopathogenic Hyphomycetes. A recent example by Fargues and Luz (2000) investigated the influence of fluctuating moisture and temperature regimes on sporulation of *B. bassiana* on *R. prolixus* cadavers. They found that high humidity was the most crucial parameter, but the intensity of conidial production was enhanced when diurnal temperatures were high (28 and 35°C). These types of mechanistic studies, while complex, are of paramount importance, and the integration of empirical studies with epizootiological modelling will enhance our understanding of epizootic initiation and development and facilitate the efficacious use of entomopathogenic Hyphomycetes.

Mycoinsecticides in IPM

There are three primary application strategies utilized against insects: (i) classical; (ii) inoculative (i.e. augmentative); and (iii) inundative approaches. In epigeal environments, entomopathogenic Hyphomycetes have primarily been applied utilizing an inundative application or microbial pesticide strategy. In soil environments, both inundative and inoculative strategies have been employed.

Entomopathogenic Hyphomycetes applied against insect pests using the inundative deployment strategy are frequently developed and assessed based on a chemical pesticide model and, in most instances, entomopathogens fit this model poorly. Here, we define a chemical pesticide model as the application of a specific quantity of an active ingredient to an insect population with the goal of inciting catastrophic and rapid mortality, thereby quelling an outbreak. Entomopathogenic Hyphomycetes are often relatively slow-acting or their effects may be sublethal. Furthermore, their efficacy is often assessed independent of environmental conditions. It is clear that new paradigms must be formulated and implemented within a biologically based framework if entomopathogens are to further contribute to the integrated management of insect pests.

Under optimal conditions, the death of an insect usually takes between 3 and 5 days from the time of application. In field environments, death can take substantially longer. This is usually due to conditions of suboptimal environment (see 'Factors Influencing Efficacy – The Environment'), which can prolong disease initiation and progress. For example, Sahelian grasshoppers treated with *M. anisopliae* var. *acridum* died more slowly when allowed to thermoregulate than those that were incubated in the shade (Langewald *et al.*, 1999). Disease progression by *B. bassiana* was arrested in Mormon crickets incubated at 37°C, but resumed when the crickets were transferred to 25°C, suggesting that mycosis could be typically delayed in the field by high temperatures and thermoregulation (J.H. Turnbow, personal communication).

Despite the delayed onset of mortality in field environments, the feeding behaviour of the insect pest can be affected during the period between infection and death. Although chemical pesticide models rarely take crop consumption into consideration in assessing efficacy, reduced feeding following the application of entomopathogenic Hyphomycetes can have a significant impact on crop protection. For instance, two examples in which reduced feeding was observed following infection are the Colorado potato beetle infected with *B. bassiana* and grasshoppers infected with *M. anisopliae* var. *acridum*. Fargues *et al.* (1994) observed that, 2 days following application of *B. bassiana*, substantial reductions (-57%) in consumption of foliage were observed in Colorado potato beetle larvae. Using faecal production as an indicator of food consumption, Thomas *et al.* (1997) also showed considerable reductions in feeding by grasshoppers (*Zonocerus variegatus*) infected with *M. anisopliae* var. *acridum* prior to death (< 7 days). A similar effect of *M. anisopliae* var. *acridum* on the acridid *Rhammatocerus schistocercoides* was observed in Brazil (Faria *et al.*, 1999).

An aspect of entomopathogen efficacy that is almost always neglected is the sublethal effects of infection. Sublethal infections occur when an insect does not die but various aspects of its biology are affected none the less. Reductions in feeding and fecundity and slowed developmental rates have been well documented for several viral and protozoan entomopathogens. In contrast, relatively little research has focused on the sublethal effects of entomopathogenic Hyphomycetes on insect pests. Fargues *et al.* (1991) demonstrated that the reproductive potential of Colorado potato beetle

females surviving infection by *B. bassiana* was reduced at 22°C but not at 25°C. Adults of the hymenopteran parasitoid of Russian wheat aphid (*Aphelinus asychis*) treated with *P. fumosoroseus* and incubated at high humidity were significantly less active (e.g. percentage of time walking, walking speed and distance covered) than untreated insects (Lacey *et al.*, 1997). Development times were prolonged and the predation efficacy of the coccinellid predator, *Serangium parcesetosum*, was similarly reduced in individuals sprayed with *B. bassiana* (Poprawski *et al.*, 1998). Authurs and Thomas (1999) observed that brown locusts (*Locusta pardalina*) infected by *M. anisopliae* var. *acridum* were more susceptible to predation. Similar effects have been shown for Sahelian grasshoppers (Thomas *et al.*, 1998). These recent reports demonstrate that sublethal effects occur in insect pests infected with entomopathogenic Hyphomycetes, and this should be considered in the evaluation of entomopathogenic fungi against insects.

Examples of hyphomycetous fungi used to manage insect pests

Entomopathogenic Hyphomycetes have been employed against a variety of different insect pests in a variety of different agroecosystems and they have demonstrated varying degrees of success. It is not possible to present a comprehensive review of the literature in this chapter, and we attempt to present examples in which efficacious management of insect pests has been achieved and/or novel approaches have been employed.

Glasshouses

Perhaps the greatest potential for managing pests with entomopathogenic Hyphomycetes is in glasshouses, where environment can be manipulated in favour of the pathogen. Several taxa have demonstrated excellent suppression of insect pests in greenhouses, including *Aschersonia* spp., *B. bassiana*, *M. anisopliae*, *P. fumosoroseus* and *V. lecanii*. Considerable research has focused on the development of *V. lecanii* against a variety of insect pests of glasshouse crops, including chrysanthemums (Hall and Burges, 1979; Hall, 1981). Aphids are economically important pests of chrysanthemums in glasshouses throughout the world, and the application of *V. lecanii* has demonstrated good efficacy against a variety of aphid species (Rombach and Gillespie, 1988). In addition, *V. lecanii* has shown considerable potential for managing whiteflies and thrips in greenhouse crops. Infection and penetration of aphids by *V. lecanii* can occur under conditions of reduced humidity (Hsiao *et al.*, 1992), but sporulation and transmission require high (~100%) humidity (Milner and Lutton, 1986).

Chrysanthemums are a short-day plant, and the normal procedure of covering plants with polyethylene sheeting from mid-afternoon until morning the following day during summer months creates a suitable environment (i.e. high humidity) for disease to occur. A single application of *V. lecanii* conidia just before plants were covered with the polyethylene was observed to provide satisfactory control of the important aphid pest, *Myzus persicae*, but less so of two minor aphid pests (Hall and Burges, 1979). It is also possible to easily and safely create high-humidity environments by fogging water over greenhouse crops at night. Helyer *et al.* (1992) observed that four consecutive nights of high humidity per week or a cycle of two nights of high humidity and two nights of ambient humidity resulted in excellent control of aphids, thrips and whiteflies by *V. lecanii* with no adverse impacts on the crop. The efficacy of *V.*

lecanii for controlling pests other than aphids, thrips and whiteflies (e.g. mites, nematodes and rusts) has also been demonstrated in a number of glasshouse crops (Verhaar *et al.*, 1996). The ability of *V. lecanii* to infect other fungi (i.e. mycoparasites) appears to be unique among the entomopathogenic Hyphomycetes (Askary *et al.*, 1998).

P. fumosoroseus (PRF-97®) and *B. bassiana* (Botaniguard®) are two other fungi that have recently been registered against an array of greenhouse pests, including aphids, thrips, whiteflies and spider mites (Shah and Goettel, 1999). See section on whiteflies below for examples of the use of fungi for control of whiteflies in field crops.

Field crops

COLORADO POTATO BEETLE. The Colorado potato beetle is an economically important pest of potatoes and other solanaceous crops throughout the world. The development of resistance to insecticides has prompted considerable research into alternative methods, including the application of *B. bassiana*, for managing this pest. Some studies have shown no or limited efficacy, while others have observed significant suppression of Colorado potato beetle (Campbell *et al.*, 1985; Hajek *et al.*, 1987; Anderson *et al.*, 1988; Jaques and Laing, 1988; Drummond and Groden, 1996; Poprawski *et al.*, 1997; Lacey *et al.*, 1999a). *B. bassiana* (e.g. Boverin) was used extensively (> 70 types of crops) in the former Soviet Union and satellite countries against a variety of insect pests. Against Colorado potato beetle, Boverin was applied alone or in combination with sublethal doses of dichlorodiphenyltrichloroethane (DDT) (Ferron, 1978). Several insecticides have been tested in combination with *B. bassiana* against Colorado potato beetle in North America, and some results have demonstrated an additive effect (e.g. Anderson *et al.*, 1989).

A number of studies have observed that foliar applications of *B. bassiana* to potatoes in North America provided satisfactory suppression of Colorado potato beetle populations. Foliar application of *B. bassiana* was used effectively in conjunction with the bacterium *B. thuringiensis*, and densities of beetles in the fungus–bacterium-treated plots declined yearly relative to other treatments (Drummond and Groden, 1996). Poprawski *et al.* (1997) observed that applications of *B. bassiana* conidia at 3–4-day intervals early in the season effectively reduced densities of older larvae and provided substantial foliar protection; larval densities were 10, 21 and 41 larvae per plant for the *B. bassiana*, insecticide (i.e. esfenvalerate, piperonyl butoxide, oxamyl and carbofuran) and control treatments, respectively. Lacey *et al.* (1999a) also observed significant effects of *B. bassiana* against Colorado potato beetle. Five weekly applications of conidia were made, but poor control of beetles was observed prior to row closure, after which point fair to good control was observed. Yields of tubers were rated as fair in plots treated with *B. bassiana*, but lower numbers of overwintering adult Colorado potato beetle were observed in these plots relative to the chemical control. Furthermore, the fungus enabled good survival in predatory Heteroptera and other non-target insects relative to the insecticide treatment. Reasons for the differential success among experiments in managing Colorado potato beetle with *B. bassiana* are currently unknown. While a number of reasons have been suggested for the poor efficacy of *B. bassiana* in some field trials, relatively limited research has specifically focused on the elucidation of constraints on disease development. The requirement for adequate canopy development (e.g. Lacey *et al.*, 1999a) suggests that microclimate is an important factor for efficacy. A commonality in most successful trials is that multiple applications of

B. bassiana have been utilized, which may ensure the presence of adequate inoculum until conditions are adequate for disease development.

In addition to foliar applications of *B. bassiana* against larvae and adults, the amendment of soils with conidia against overwintering adults has also been tested. Overwintering adults are often killed by indigenous *B. bassiana* in soil (e.g. Weber and Ferro, 1993), and applications of the fungus on to foliage or directly on to the soil surface have significantly reduced populations of emerging adults (Watt and LeBrun, 1984; Cantwell *et al.*, 1986; Anderson *et al.*, 1988).

EUROPEAN CORN-BORER. *B. bassiana* applied to maize foliage can effectively control European corn-borers (Hsiu *et al.*, 1973; Lewis and Bing, 1991; Lewis *et al.*, 1996). Recently, it was realized that *B. bassiana* forms an endophytic relationship with maize which has exciting possibilities for the management of insect pests. Initially, Bing and Lewis (1991) observed that most of the maize plants treated at a whorl stage of development with liquid or granular formulations of *B. bassiana* conidia became internally colonized by the fungus. Subsequent studies confirmed that *B. bassiana* entered maize tissues and, once in the plant, the fungus remained there throughout the growing season and provided season-long suppression of European corn-borer larvae (Bing and Lewis, 1991, 1992a, b, 1993; Lewis and Bing, 1991).

The timing of *B. bassiana* application was shown to influence the efficacy of endophytic *B. bassiana*. For example, maize plants inoculated with *B. bassiana* early in the season (e.g. at the whorl and late-whorl stage of development) had significantly more larval tunnelling than plants infested later in the season (i.e. at the pre-tassel stage) relative to their respective control treatments (Bing and Lewis, 1992a). This was attributed to more extensive colonization of plants by *B. bassiana* at later stages of development. Within maize plants, *B. bassiana* is commonly isolated within the pith of the plant during plant growth and senescence (Bing and Lewis, 1992b).

The adhesion, subsequent growth and penetration of maize leaves are very similar morphologically to the germination and penetration of insect cuticles by *B. bassiana* (Wagner and Lewis, 2000). On leaves, conidia germinate regardless of topographical signals, and hyphae from germinating conidia penetrate the cuticle directly; microscopic observations suggest both an enzymatic and a mechanical mechanism of penetration. Once in the plant, hyphae grow within the leaf apoplast and, in some cases, within the xylem elements. The ability to grow within xylem tissues may explain the systemic nature of the fungus within maize plants, which is important for its efficacy against European corn-borers. *B. bassiana* occurs naturally as an endophyte of maize in several tillage systems, and endophytism has been shown to be compatible with a number of chemical and microbial insecticides (e.g. Lewis *et al.*, 1996).

In the USA, substantial attention is now focusing on the use of *B. thuringiensis*-transgenic maize to control European corn-borers, and Lewis *et al.* (2000) observed no difference in rates of endophytism by *B. bassiana* between *B. thuringiensis*-transgenic and near-isogenic lines of maize. Studies to determine the impact of *B. thuringiensis*-transgenic maize and endophytic *B. bassiana* are currently in progress (L.C. Lewis, personal communication). The impact of endophytic *B. bassiana* on other insect pests of maize and whether *B. bassiana* exists as an endophyte in other plants are currently uncertain. Preliminary results from West Africa indicate that local strains of *B. bassiana* can exist endophytically within maize and provide some action against the corn-borer *Sesamia calamistis* (Cherry *et al.*, 1999).

LEPIDOPTEROUS (NOCTUIDAE) PESTS OF SOYBEAN. As indicated previously, *N. rileyi* frequently causes natural epizootics in noctuid populations. Unfortunately, naturally occurring epizootics often occur too late in the growing season to prevent economic crop losses (Ignoffo *et al.*, 1976; Kish and Allen, 1978; Boucias *et al.*, 1984; Fuxa, 1984), and a number of researchers have attempted to induce epizootics earlier in the growing season in an attempt to reduce losses. Most attempts to induce epizootics have involved the application of conidia as sprays or dusts, which have often resulted in high levels of disease (Getzin, 1961; Ignoffo *et al.*, 1976, 1978; Mohamed *et al.*, 1978; Ignoffo, 1981). However, the prolonged lag period between infection and disease development in older larvae allows them to continue feeding, which can result in unacceptable levels of damage (Carruthers and Soper, 1987). In an attempt to enhance crop protection, Ignoffo *et al.* (1976) demonstrated that early-season applications against young larvae could provide acceptable suppression of populations if the environmental conditions were adequate to allow epizootic development. Other researchers have attempted to increase early-season inoculum densities by distributing cadavers throughout the field (Sprenkel and Brooks, 1975), manipulating conditions of moisture through the application of irrigation (Kish and Allen, 1978) and manipulation of cultural practices, such as using early planting dates (Sprenkel *et al.*, 1979). Research has continued to focus on foliar applications of *N. rileyi*, but some evidence indicates that incorporation of conidia into soil may be an efficacious method of targeting some noctuids (Devi, 1995).

WHITEFLIES. The whiteflies *Bemisia argentifolii* and *B. tabaci* are two of the most important agricultural pests, affecting numerous crops worldwide. Damage occurs through direct feeding, vectoring of viruses, honeydew secretion and associated sooty-mould development. *P. fumosoroseus* is one of the most common fungal pathogens found attacking whiteflies and is responsible for epizootics that can significantly affect both greenhouse and field populations of the pests (Lacey *et al.*, 1996). However, natural epizootics are sporadic and cannot be depended upon for management of these pests.

P. fumosoroseus causes rapid infection and death of all whitefly stages; under optimal conditions, hyphae are present within the haemocoel within 24 h of inoculation, death occurs between 24 and 48 h, hyphae emerge and conidiogenesis occurs on the surface of the cadaver within 72 h (Osborne *et al.*, 1990). Optimal growth rates are between 20 and 30°C, with optima related to the microclimate of the fungal isolate's biotope (Vidal *et al.*, 1997a). Highly virulent isolates of *P. fumosoroseus* with considerable control potential against whiteflies are widespread and numerous (Lacey *et al.*, 1996; Vidal *et al.*, 1997b; Wraight *et al.*, 1998). Wraight *et al.* (2000) demonstrated that infection can take place at ambient relative humidities as low as 25%. Hyphal bodies are more virulent than conidia (Lacey *et al.*, 1999b) and can be rapidly produced in liquid culture, remaining viable and virulent following drying (Jackson *et al.*, 1997). *P. fumosoroseus* demonstrated limited lethal and sub-lethal effects on *S. parcesetosum*, an important coccinellid predator of whiteflies, suggesting that the integration of these two control agents in IPM may be possible (Poprawski *et al.*, 1998).

Inundative application of *P. fumosoroseus* conidia or hyphal bodies has been used successfully to manage whiteflies in field crops. In small-scale field trials using portable air-assist sprayers, multiple applications of *P. fumosoroseus* at 4–7-day intervals provided > 90% mortality of late-instar whiteflies on cucumber, cantaloupe melons and zucchini

squash (Wraight *et al.*, 2000). Although effects on nymphs were highly significant, the effects on adult whiteflies were minimal. Commercial products based on this fungus are now available for whitefly control (Shah and Goettel, 1999; Wraight *et al.*, 2000).

Although only occasionally found as a naturally occurring pathogen of whiteflies (Lacey *et al.*, 1996), *B. bassiana* has demonstrated comparable efficacy to *P. fumosoroseus* against whiteflies, and several isolates have been commercially developed for their control (Shah and Goettel, 1999; Wraight *et al.*, 2000). Mycotech Corporation, Butte, Montana, has developed an emulsifiable oil formulation, which can be readily targeted and applied with low-volume air-assist sprayers or moderate- to high-volume hydraulic sprayers (Wraight and Carruthers, 1999). Because whiteflies primarily inhabit the undersides of leaves, a special effort has to be made to adequately target this area. This can be accomplished by spraying upward from below the canopy level, using nozzles mounted on swivels on vertical tubes. For crops with low canopies (e.g. cucurbits), high-pressure hydraulic sprayers, fitted with drop nozzles carried at or slightly above canopy levels are effective. Application of a wettable powder formulation of *B. bassiana* (Mycotrol) at 560 g ha⁻¹ at 2–4-weekly applications in cucumbers and five to seven applications in cantaloupe melons consistently provided 65–75% control of first-generation whitefly larvae (Wraight *et al.*, 2000).

Pasture and grassland

COMMON COCKCHAFFER. Adults of the common or European cockchafer (*Melolontha melolontha*) feed on forest and fruit-tree leaves, in particular oaks, beech, maple, sweet chestnut, walnut and plum. Larvae are polyphagous, and they attack the roots of various crops including fruit or forest trees, cereals, red beet, potato and grasses. A host-specific strain of *B. brongniartii* has been tested in large-scale field tests in grassland regions in Austria, Italy and Switzerland over the last two decades. A number of formulations have been tested (Keller *et al.*, 1997; Strasser, 1999). In an attempt to use egg-laying females as vectors of the fungus, hyphal bodies were applied to woodland borders in 1985 and 1988 and, at two-thirds of the 15 sites, 50–80% reductions in cockchafer populations were observed (Keller *et al.*, 1997). From these trials, the authors concluded that spraying the adults with hyphal bodies to control cockchafer larvae has both advantages and disadvantages. The great advantage is that only a small area must be treated because the egg-laying females disseminate the fungus to an area 50 times larger than the treated one. However, the quantity of hyphal bodies needed can only be produced in large fermenters on a commercial scale, and they cannot be stored for more than 4 weeks without loss of efficacy (Keller *et al.*, 1997).

Cockchafer flight depends strongly on weather conditions and the spraying of the adults can only be done during a short period (i.e. when females reach the swarming sites). Therefore, efficacious application often necessitates the use of helicopters, which is less cost-effective. To overcome the disadvantages of applying hyphal bodies, an alternative method of applying *B. brongniartii* in and/or on barley kernels was developed (Aregger, 1992; Zelger, 1993; Strasser, 1999). Based on the results of field experiments by Fornallaz (1992), colonized barley was applied with a slit seeder at various times of the year to pastures, grasslands, sports fields, orchards, forests, tree nurseries and vineyards. The incorporation of inoculum into soil to a depth of 5–10 cm was found to increase efficacy by placing the fungus in close contact with the pest. A threshold of inoculum in soil is required to ensure epidemic levels in the pastures ($> 2 \times 10^4$

spores g^{-1} dry weight of soil (Ferron, 1979), and the results of field trials conducted between 1995 and 1999 with the barley inoculum indicated that population sizes of *B. brongniartii* increased continuously through five applications conducted in autumn 1994 and spring and autumn of 1995 until 1997 (Strasser, 1999). With the exception of the site with sandy-textured soil, fungal populations within soils persisted at above threshold levels. The application of *B. brongniartii* using barley kernels provided sufficient suppression of cockchafer populations after only 2 years of application ($> 20\%$ prevalence of mycosis), and populations were reduced from > 70 larvae m^{-2} to less than 22 larvae m^{-2} after 5 years. Since the spring of 1998, no relevant damage by *M. melolontha* has been reported at treated sites (Strasser, 1999).

RED-HEADED PASTURE COCKCHAFER. The subterranean scarab, *Adoryphorus couloni*, is an important root-feeding pest of pasture and field crops, and the application of *M. anisopliae* to pasture soils has shown considerable potential for the control of cockchafers in Australia. Conidia can be applied directly to soil or the fungus can be applied on/in grains by direct drilling at the time of pasture renovation. Application of *M. anisopliae* to pasture soil in the winter caused substantial mortality of larvae and pupae by 27 weeks, and decline in populations continued in subsequent years (Rath *et al.*, 1995). Reductions in larval numbers led to greater retention of pasture grasses, reduced weed invasion and an increase in pasture productivity. Throughout the 4-year duration of the study, *M. anisopliae* remained at levels equal to or above the applied concentrations. While autumn applications may not be sufficient to control pest populations initially, the excellent persistence of *M. anisopliae* in soil and effects on subsequent populations demonstrate the potential for long-term management of cockchafers in pastures. In some instances, it is not necessary to re-inoculate soil for a 5–10-year period. A commercial product of *M. anisopliae* (i.e. BioGreen®) is currently being marketed for use against red-headed pasture cockchafers in Australia (Shah and Goettel, 1999).

GRASSHOPPERS AND LOCUSTS. Acridids have a tremendous impact on agricultural production throughout the world. They are capable of decimating cultivated crops, pastures and rangeland, and historically have been indirectly responsible for death by starvation or death by diseases associated with starvation of untold thousands of people annually. *M. anisopliae* var. *acridum* is being developed as a BCA of acridids in various regions of the world, including Africa (e.g. Lomer *et al.*, 1997a), Australia (e.g. Milner, 1997) and Brazil (e.g. Magalhaes *et al.*, 2000). In a large research programme conducted in Africa, conidia are produced on rice (Jenkins *et al.*, 1998) and they are dried and stored for various periods of time at low (>18 months) and/or ambient (~ 12 months) temperatures (Lomer *et al.*, 1997a). Conidia are formulated in oils (primarily paraffinic oils used alone or as blends with botanical oils) and applied at ultra-low volumes, using hand-held Micro-Ulva applicators.

Trials conducted at a variety of locations in Africa have demonstrated satisfactory suppression of field populations of grasshoppers (e.g. *Z. variegatus*, *Hieroglyphus daganensis* and *O. senegalensis*) by *M. anisopliae* var. *acridum* in a number of instances (Lomer *et al.*, 1997a); in some cases, reductions in field populations of $\geq 70\%$ have been observed (e.g. Kooyman *et al.*, 1997; Lomer *et al.*, 1997a,b). Field testing of the fungus against locusts has been problematic, primarily because of the high mobility of locust adults and nymphs. Efficacy has been demonstrated in captured locusts that were maintained in cages following the application of conidia (Lomer *et al.*, 1997a).

However, efficacy in cages does not necessarily correlate with reductions in field populations of acridids, due to the substantially different microclimatic conditions between the two environments (Inglis *et al.*, 1997a), and extreme care must be taken in extrapolating efficacy from cage assessments. The efficacy of *M. anisopliae* var. *acridum* is probably enhanced by secondary spore pick-up; there are observations that acridids coming in contact with vegetation infested with conidia pick up inoculum and often become infected and die by mycosis (Bateman *et al.*, 1998; Thomas *et al.*, 1998). Furthermore, the fungus can persist in fragments of infected grasshopper cadavers and survive adverse environmental conditions (e.g. the Sahelian dry season), which potentially serves as a source of secondary inoculum (i.e. horizontal transmission), enhancing the efficacy of *M. anisopliae* var. *acridum* against acridids (Thomas *et al.*, 1996). However, the biotic and abiotic factors regulating horizontal transmission in field settings are still poorly understood.

Tree crops

The coconut-palm rhinoceros beetle, *Oryctes rhinoceros*, is a major pest of Asian- and Pacific-grown coconut- and oil-palms. The adults feed on palm fronds, boring into the axils and destroying plant tissues, and the immature stages are found in soil and litter (e.g. Bedford, 1980). Larvae are naturally infected by *M. anisopliae*, and the fungus is considered an important natural mortality factor (Carruthers and Soper, 1987). A control programme, involving the release of *M. anisopliae* and a baculovirus (*Rhabdionvirus oryctes*), was initiated in Tonga to manage rhinoceros beetles. The virus was found to induce epizootics, but a low prevalence of infections caused by *M. anisopliae* was observed 15 months after release (Young, 1974). Two years after release, *M. anisopliae* was barely detectible in natural breeding populations. The beetles are highly susceptible to *M. anisopliae*, and reasons for the poor efficacy in the field are speculative. Anecdotal evidence suggests that limited dispersal and longevity of inoculum are the most likely reasons for the poor efficacy observed (Latch and Falloon, 1976; Carruthers and Soper, 1987). More recent research using *M. anisopliae* has focused on the inundative application of conidia against the beetles, and the use of the fungus in IPM programmes with the baculovirus (Young, 1986).

Forests

While a number of entomopathogenic Hyphomycetes have demonstrated considerable potential as BCAs against a diverse array of insect pests of forest trees in controlled environments or small-scale field tests, there are relatively few examples of large-scale applications of entomopathogenic Hyphomycetes against forest pests. However, a substantial effort in China has gone into the widespread application of entomopathogenic Hyphomycetes against forest insect pests. For example, the annual production of *B. bassiana* conidia in China in the late 1980s was ~10,000 t which allowed the treatment of 0.8–1.3 million ha (Feng *et al.*, 1994). A major forest pest in China is the pine caterpillar (*Dendrolimus* spp.), and large-scale applications of *B. bassiana* in oil or oil-emulsion formulations have provided excellent suppression of caterpillars; more than 300,000 ha of forest were sprayed by aeroplane over a 5-year period and mortalities ranging from 43 to 93% were recorded during this period (Pan and Zheng, 1988). In some instances, repeated applications of *B. bassiana* in a given year resulted in better control (Lü and Zhao, 1988). The cost of managing pine caterpillars with *B. bassiana*

was low (\leq US\$3 ha⁻¹) regardless of the application method, and has been shown to be a cost-effective management practice (Feng *et al.*, 1994).

Urban pests

TERMITES. Termites cause considerable damage to wood structures throughout the world. A number of entomopathogenic Hyphomycetes, including *M. anisopliae* and *B. bassiana*, have demonstrated considerable potential for controlling termites. These entomopathogens are commonly isolated from termite colonies (e.g. Milner *et al.*, 1998a). In Australia, the application of *M. anisopliae* conidia to mound- and tree-nesting termites has been shown to incite substantial mortality, but indirect treatment using feeding sites and baits was not successful (Milner and Staples, 1996; Milner *et al.*, 1998b). In Brazil, a high prevalence of termite mortality (~100%) was observed in 19 of 20 nests treated with *M. anisopliae*, and the remaining nest exhibited 70% mortality (Alves *et al.*, 1995).

The efficacy of entomopathogenic Hyphomycetes against termites is facilitated by the horizontal transmission of conidia between individuals (Kramm *et al.*, 1982; Rosengaus and Traniello, 1997), and one of the major factors limiting the efficacy involves the behavioural response of healthy termites to conidia and diseased termites. The frequency of grooming increases during and after exposure to *M. anisopliae* conidia, and this increases the survival of termites (Rosengaus *et al.*, 1998a). Termites in direct contact with high concentrations of conidia can also exhibit a striking vibratory display, which appears to convey information to unexposed nest-mates, which are stimulated to stay clear of the infested individual (Rosengaus *et al.*, 1999).

Microorganisms associated with soil, the termite gut and gallery systems can be inhibitory to the fungus (Boucias *et al.*, 1996; Rosengaus *et al.*, 1998b; Ramakrishnan *et al.*, 1999). While the repellence of viable conidia to termites may represent an obstacle in the development of effective bait formulations, this characteristic has been used to provide a barrier treatment in soil (Milner and Staples, 1996). Evidence now indicates that it may be possible to overcome the repellency of conidia, and Rath and Tidbury (1996) observed that *M. anisopliae* conidia formulated in attapulgitic clay and surfactant were attractive to termites. Almeida *et al.* (1998) reported that the formulation of *B. bassiana* conidia in a bait with insecticides provided good suppression of termites in sugar cane in Brazil. Since infection of termites primarily occurs through the external integument, the efficacy of bait formulations will be dependent on the degree to which individuals become surface-contaminated during handling and ingestion of the bait. Therefore, development of bait formulations should focus on baits that promote tactile contact by termites, thereby enhancing transfer of conidia to the integument surface.

As indicated earlier, sublethal doses of chemicals can adversely affect the behaviour of termites. Most of the work on behavioural modifiers has focused on the insecticide imidacloprid. Imidacloprid is a systemic, chloronicotinyl insecticide that causes a blockage in a type of neuronal pathway (nicotinerpic), which leads to the accumulation of acetylcholine, resulting in insect paralysis and, at high enough doses, death (Kidd and James, 1991). A number of researchers have observed that combining entomopathogenic Hyphomycetes with sublethal doses of imidacloprid substantially increases efficacy of fungi against termites (Boucias *et al.*, 1996; Almeida *et al.*, 1998; Ramakrishnan *et al.*, 1999). This is attributed to the interference with normal tunnelling and termite grooming and associated trophallaxis activities, factors that play a

major role in conferring resistance to entomopathogenic Hyphomycetes. At present, a commercial formulation of *M. anisopliae* (i.e. BioBlast®) has demonstrated substantial control of termites on pine boards in the USA, but was more effective when used with a chemical barrier (Quarles, 1999).

COCKROACHES. The Bio-Path® Cockroach Control Chamber is the first microbial agent registered for control of cockroaches in the USA. Designed like a traditional bait station, cockroaches entering the station become infested with conidia of *M. anisopliae*. In the Bio-Path system, *M. anisopliae* is present on an agar medium, and the cockroaches entering the bait station come in contact with conidia. Horizontal transmission is important for the efficacy of entomopathogenic Hyphomycetes against cockroaches (Kaakeh *et al.*, 1996). Once cockroaches have left the station they may infest other individuals with inoculum from the trap, or they become infected and eventually die, and conidia subsequently sporulating on the cadaver serve as a source of secondary inoculum (Fehrenbach, 1993; Andis, 1994; Milner, 1994). The fungus has been marketed as being safe for use in hotels, restaurants and museums. Additional research has focused on the incorporation of entomopathogenic Hyphomycetes into bait formulations in which cockroaches become surface-infested during ingestion of the bait substrate (e.g. Mohan *et al.*, 1999). Dead infected cockroach nymphs are not cannibalized, suggesting an avoidance behaviour by healthy nymphs (Kaakeh *et al.*, 1996). The incorporation of imidacloprid into baits has been shown to increase the efficacy of *M. anisopliae* against cockroaches (Kaakeh *et al.*, 1997), but the mechanism of action is uncertain at present.

Compatibility with agrochemicals

Entomopathogenic Hyphomycetes will not supplant the need for chemical pesticides in all commercial production systems. For example, insecticides may be needed to suppress a rapidly expanding pest population or to control pests not targeted by fungi. Fungicides will often be required to control plant diseases, but many fungicides possess broad spectra of activity and they can adversely affect the efficacy of entomopathogenic fungi. Herbicides and plant growth regulators are also used extensively in most agroecosystems, and their compatibility with fungi is often uncertain. While it is not possible to draw specific conclusions on the compatibility of all agrochemicals with entomopathogenic Hyphomycetes, it is crucial that the pest manager be cognizant of the adverse impacts that these chemicals can have on entomopathogen efficacy. Furthermore, it is often necessary to obtain information on the compatibility of specific fungal strains with the agrochemicals used in a particular agroecosystem. This will allow growers to select appropriate compounds and schedule fungal or chemical treatments accordingly to minimize any deleterious effects on pathogen efficacy (i.e. compartmentalization).

Considerable research has focused on the influence of various agrochemicals on the germination and growth of entomopathogenic fungi *in vitro*. Here we attempt to summarize the salient findings of this research. All classes of agrochemicals are potentially inhibitory to entomopathogenic Hyphomycetes, including herbicides, insecticides and fungicides. There is a tremendous array of agrochemicals currently in use throughout the world, and their inhibitory properties will vary, both between and within chemical classes. In some instances, carriers and/or adjuvants associated with the active

ingredient are responsible for the fungistatic properties of a chemical pesticide, and the compositions of the formulations also vary tremendously.

The inhibitory effects of agrochemicals on the germination and growth of entomopathogenic Hyphomycetes often vary among taxa and strains (e.g. Vanninen and Hokkanen, 1988; Anderson *et al.*, 1989; Majchrowicz and Poprawski, 1993; Li and Holdom, 1994). Therefore, it may be possible to select genotypes which are naturally less susceptible. Another approach is to 'genetically engineer' resistance to pesticides. In this strategy, a gene conferring resistance to a specific pesticide is introduced into a susceptible entomopathogen. For example, *M. anisopliae* was transformed with a gene conferring resistance to the fungicide benomyl (Benlate®) (Goettel *et al.*, 1990a), and *P. fumosoroseus* was transformed with a gene conferring resistance to the herbicide gluphosinate (Cantone and Vandenberg, 1999). However, pesticides that are inhibitory in the laboratory do not always exhibit the same action in field environments. This can be a function of pesticide concentration or due to compartmentalization (i.e. the entomopathogen may never come in contact with the pesticide). For example, Keller *et al.* (1993) observed that one-third of the fungicides tested *in vitro* completely inhibited mycelial growth of *B. brongniartii* and the others either prevented or reduced conidial germination. Despite the highly inhibitory nature of the fungicides *in vitro*, the growth of the fungus on barley kernels on soils sprayed with fungicides was substantially less inhibited, and the authors concluded that most of the fungicides tested would not have an adverse effect on *B. brongniartii* applied to soils. In another study, Jaros-Su *et al.* (1999) demonstrated that the timing of fungicide application was not detrimental to *B. bassiana* applied against the Colorado potato beetle if applications were made asynchronously. These examples emphasize the potential inaccuracy of extrapolating field inhibition from *in vitro* results. There are several examples where fungicides that are highly inhibitory *in vitro* are successfully used in conjunction with entomopathogenic fungi to manage insect pests. For instance, in chrysanthemum and cucumber production, *V. lecanii* and benomyl are commonly used together (Gardner *et al.*, 1984; van der Schaaf *et al.*, 1991).

While the potential inhibitory effects of pesticides on entomopathogenic Hyphomycetes cannot be ignored, there are numerous examples where the application of chemical pesticides have enhanced the efficacy of entomopathogens against insect pests. As indicated previously, sublethal doses of chemical insecticides can act as physiological stressors and/or behavioural modifiers and thereby predispose insects to disease. There are now a number of examples where the application of entomopathogenic Hyphomycetes with sublethal doses of insecticides has substantially enhanced the efficacy of pathogens. An early example of this phenomenon was reported by Easwaramoorthy *et al.* (1978), where two insecticides that inhibited *V. lecanii* *in vitro* enhanced the efficacy of the fungus when they were applied in combination against the coffee green scale. Anderson *et al.* (1989) detected higher insect mortality when *B. bassiana* and sublethal concentrations of insecticides were applied to control Colorado potato beetle, attributing the higher rates to synergism between the two agents. A recent example of enhanced efficacy for combination treatments is for *B. bassiana* and *M. anisopliae* applied with imidacloprid against the sugar-cane rootstock borer weevil (*Diaprepes abbreviatus*) (Quintela and McCoy, 1998). As indicated previously, imidacloprid is a neural toxin that can substantially affect the behaviour of various insects, such as termites (Boucias *et al.*, 1996). The enhanced efficacy of entomopathogenic Hyphomycetes applied in combination with imidacloprid against the sugar-cane rootstock borer weevils was attributed to reduced mechanical removal of

conidia from the surfaces of larval cuticles; imidacloprid severely impaired larval movement in soil (Quintela and McCoy, 1998). However, in contrast, only additive effects were obtained when *B. bassiana* was applied with diflubenzuron against acridids in Mali (Delgado *et al.*, 1999). Diflubenzuron, a member of a larger group of highly selective insecticides known as benzoylphenylureas, works by inhibiting chitin synthesis, and the cuticle of insects treated with diflubenzuron provides less resistance to penetration by hyphae of entomopathogenic Hyphomycetes (Hassan and Charnley, 1989). This would suggest that increased efficacy of an entomopathogenic fungus would occur due to enhanced penetration, but, if this occurred, it did not translate into increased mortality in the trial against field populations of acridids in Mali.

The beneficial effects of insecticides may also potentially expand the pest host range of fungal agents. For example, *P. fumosoroseus* was not effective against greenhouse infestations of the aphids *Aphis gossypii* and *Macrosiphoniella sanborni*, but when applied with azadirachtin (Margosan-O®) efficacy was enhanced and good control of these aphids was attained, thereby increasing the cost-effectiveness of the chemical control strategy (Lindquist, 1993). If greater efficiency can be obtained, fewer applications will be required to achieve the desired degree of pest control. Many researchers are now taking a mechanistic approach to such research, which will facilitate the efficacious use of entomopathogen combinations.

As modern agriculture moves to adopt more 'environmentally friendly' practices, there is increasing interest in the use of 'biorational' pesticides, such as insect growth regulators, insecticidal soaps and horticultural oils; these materials have minimal effects on beneficial insects, low mammalian toxicity and reduced residual activity. Some of these products are effective against insect pests, and they have excellent potential for inclusion in IPM strategies (Allen *et al.*, 1993). However, there are few documented studies on interactions between biorational pesticides and entomopathogenic fungi. As the mode of action of most biorational pesticides relies on disruption of an insect-specific process, they should be relatively innocuous to fungi. While the specific mechanisms of activity differ among the products, they all affect the insect cuticle – its formation, deposition, structure or integrity – to varying degrees. Insect growth regulators and botanicals, such as neem (azadirachtin) directly interfere with insect development and cuticle formation or the moulting process (Staal, 1987; Rembold, 1989). If deposition, hardening and tanning of the new cuticle are affected, its ability to serve as an effective barrier to fungal infection is compromised and the chances of a lethal mycosis developing may be enhanced (Zimmermann, 1994). Synergism between *Metarhizium* spp. and benzoylphenylurea insecticides (e.g. diflubenzuron and teflubenzuron), which interfere with chitin synthesis, have been demonstrated for a number of insects (Hassan and Charnley, 1983; Joshi *et al.*, 1992). Alternatively, if the mode of action of the biorational pesticide can induce morphogenetic damage, preventing moulting and maturation, then successful fungal invasion is more likely to occur. Insecticidal soaps affect the waxy layers of the insect cuticle. These layers prevent desiccation and are important in the insect defence system; their disruption could predispose insects to fungal infection. Horticultural oils kill insects by asphyxiation, and insecticidal oils have been used in the formulation of several fungal species, enhancing efficacy and application against the target host insect (see Chapters 10 and 11).

Compatibility with other biological control agents

The contribution of predators, parasitoids and pathogens is an important aspect of IPM. Consequently, the use of entomopathogenic Hyphomycetes in any IPM programme must take into consideration their possible deleterious effects on other components, especially other biological agents. On the other hand, many Hyphomycetes may act additively or synergistically with other biological agents, especially other entomopathogens.

Predators and parasitoids

Safety to non-target organisms and more specifically to predators and parasites is of primary concern when registering a microbial control agent (see Chapter 13). While many entomopathogenic Hyphomycetes have wide physiological host ranges, their pathogenicity and virulence vary by species and strain and by insect host (Goettel *et al.*, 1990b; see also Chapter 13). Entomopathogenic Hyphomycetes have been shown to be compatible with a number of beneficial insects (Bethke and Parrella, 1989; Goettel *et al.*, 1990b; James and Lighthart, 1994). Although laboratory tests are often a prerequisite in first establishing the susceptibility of non-targets to candidate fungal strains, there are often poor correlations between these and field test data (see Chapter 13). In laboratory tests, higher doses of inoculum are generally used against stressed hosts, which are held under conditions favouring infection and disease development. Under field conditions, the likelihood of high infection rates occurring in non-targets is greatly reduced. Even if some agents are incompatible with certain predators or parasites, consideration must be given to the usually devastating effects to most predators and parasites when chemical pesticides are used instead.

Entomopathogens

Surprisingly little attention has focused on the possibility of co-applying entomopathogens, such as viruses, protozoa and some bacteria, with Hyphomycetes fungi to enhance efficacy. The high infectivity and often debilitating effects on insects and the substantially different modes of action relative to fungi, which may reduce interspecies antagonism, would indicate that there is great potential in this approach. There are only a few reports where entomopathogenic Hyphomycetes have been combined with either viruses or protozoa (e.g. Lecuona and Alves, 1988). A number of researchers have tested combinations of bacteria and entomopathogenic Hyphomycetes. In some instances, increased efficacy was observed. For example, Glare (1994) observed that the co-application of *M. anisopliae* and *Serratia entomophila* provided synergistic mortality of early- but not later-instar grass grub (*Costelytra zealandica*) larvae. Lewis *et al.* (1996) observed that the application of *B. thuringiensis* to whorl-stage and pollen-shedding maize enhanced the suppression of European corn-borers by endophytic *B. bassiana*. In other instances, there was no apparent advantage of the co-treatment (e.g. Lewis and Bing, 1991). Understanding how the two pathogens interact during pathogenesis may facilitate efficacious use of pathogen combinations.

Nematodes are increasingly being considered for control of a range of insects (e.g. Tomalek, 1994; Ehlers, 1998). The work of Barbercheck and Kaya (1990, 1991) indicated that co-infections by nematodes and *B. bassiana* could speed up the lethal infection rate and cause higher mortality in a treated pest population. Antagonistic

interactions between *B. bassiana* and the nematodes in dual-infected hosts, however, could adversely affect pathogen or progeny development. This could affect the spread of the fungus or the nematode within the pest population, but, if inundative releases of both organisms were contemplated in a control strategy, this would not necessarily prevent their combined use.

Very few studies have reported the combined application of entomopathogenic Hyphomycetes with the aim of increasing efficacy. As indicated previously, the detrimental effects of high temperatures, exacerbated by the ability of acridids to elevate their body temperatures, is now recognized as an important constraint on the use of *B. bassiana* against grasshoppers. Substantial fluctuations in diurnal temperatures occur in the northern plains of North America, and Inglis *et al.* (1997b, 1999) tested the hypothesis that the co-application of *B. bassiana* and *M. anisopliae* var. *acridum* could be used to increase the temperature range over which the individual fungi alone would incite mortality in acridids. In controlled environment studies, they observed that the final mortality was greater for the combination treatment than for *M. anisopliae* var. *acridum* alone in a simulated hot-temperature environment, and equal to *B. bassiana* alone in a simulated cool-temperature environment. While these were relatively simple simulations, they demonstrate the potential value of utilizing fungal 'cocktails' to overcome the constraints of temperature on entomopathogenic hyphomycetous fungi against acridids.

Some innovative strategies

Development of IPM often requires innovative strategies. Hyphomycetous fungi offer unique opportunities and strategies for use in IPM programmes. For example, recent studies by Butt *et al.* (1998b) showed that honey-bees could be used to disseminate fungal inoculum and the improved targeting resulted in better control of pollen beetles (*Meligethes aeneus*) in oil-seed rape (= canola) than conventional spraying methods. This method has also been shown to control seed weevil (*Ceutorhynchus assimilis*) and has the potential to control most floral pests, including thrips (T.M. Butt, unpublished observations). Many entomopathogenic fungi fail to kill bees, partly because of the elevated temperatures in the beehive (Butt *et al.*, 1994). The 'push-pull' strategy is another novel pest control method, which, briefly, entails insect pests being driven out of the main crop with feeding deterrents and drawn into a trap crop, where they could be controlled by inundation with benign pathogens, such as *M. anisopliae*. To encourage pests into the trap crop, lures such as favoured plant varieties (i.e. those more attractive than the crop) and chemical attractants may be used.

Semiochemicals

When evaluating *V. lecanii* for control of *A. gossypii*, Hockland *et al.* (1986) observed an increased infection rate among aphids in plots sprayed with the aphid alarm pheromone, (E)- β -farnesene. In response to the pheromone, the aphids became agitated and increased their movement over the treated leaf surface, and in so doing, acquired more infective conidia, resulting in higher rates of infection. Could a similar approach be considered for other insect pests? Teerling *et al.* (1993) identified an alarm pheromone in droplets of anal fluid produced by western flower thrips. Both nymphs and adults responded to the pheromone by walking away from the source of

application. The thrips are highly thigmotactic and often abundant inside flowers, where they are difficult to reach with contact insecticides, including fungi. If the alarm pheromone could stimulate insects to vacate these refugia and increase their exposure to conidia deposited on leaves or petals, better control might be achieved.

Host-plant resistance

As discussed earlier, the host plant can influence the susceptibility of insects to fungal infection. In addition to considerations of resistance to insect pests and phytopathogens, it may also be beneficial to consider the efficacy of entomopathogens in selecting plant germ-plasm. Insects tend to be more stressed on resistant plants, and perhaps more prone to infection. Furthermore, if the developmental rate is affected, then the intermoult period is extended, providing a longer period of susceptibility to infection. Differences in insect susceptibility have been observed, depending on the type of host plant on which insects have been reared (Hare and Andreadis, 1983; Ramoska and Todd, 1985). While traits conferring resistance to insects have been bred into many agricultural crops, most plant breeding programmes, particularly in ornamentals, have focused on the development of resistance to phytopathogens. Although this in itself may not directly affect insect pests, the use of disease-resistant cultivars will decrease or eliminate the application of fungicides, etc., thereby reducing harmful effects on entomopathogenic fungi used against insect pests. Furthermore, the development of resistance to plant viruses transmitted by insect vectors (e.g. thrips, whitefly and aphids) would reduce the need to maintain plants virtually insect-free, allowing for greater flexibility in the type of control tactics that can be incorporated into an insect pest control programme.

Trap crops

Trap crops are crops that are grown to attract insect pests and thereby protect primary crops from pest attack. Once the insects are attracted to the trap crop, they are usually managed there, thus decreasing or eliminating the need to target the entire field. While trap crops that are preferred hosts for the insect pest are commonly used, a number of other strategies may be used, such as the 'push-pull method'. Limited work has focused on the use of entomopathogens to manage insect pests in trap crops, but this may represent an excellent strategy for deploying entomopathogenic Hyphomycetes. Since mortality is partly dependent upon the application rate of the fungus and host density, this strategy may significantly improve the effectiveness of fungal BCAs on pest populations. It may also be possible to modify environmental conditions within the trap crops to favour infection and disease development within pest insects. For example, Amiri *et al.* (1999) showed that forcing adults and larvae of *P. cochleariae* to the underside of Chinese cabbage leaves with a feeding deterrent increased their susceptibility to *M. anisopliae*. The enhanced efficacy may have resulted from higher humidity, which can be important in the infection process and on sporulation on cadavers, and increased conidial persistence, because they are less exposed to harmful UV radiation and are less likely to be dislodged by rain (Inglis *et al.*, 1993; Inyang *et al.*, 2000).

Conclusions

Fungi will not be cure-alls for pest problems on all crops and in all agricultural settings, and it is unlikely that they will ever totally supplant the management of insect pests with chemical insecticides. Nevertheless, they represent a valuable management resource to be utilized within an IPM framework, and will contribute significantly to reductions in chemical pesticide use (Lacey and Goettel, 1995). Research to identify constraints on efficacy and the utilization of biologically based strategies to overcome these constraints will continue to increase the consistency of insect suppression with entomopathogenic Hyphomycetes. Furthermore, the integration of this group of fungi with other management practices will require detailed compatibility studies and the development of effective guidelines for their use. For example, in a typical agroecosystem, a range of pest species, including phytopathogens and insects, are encountered and have to be combated simultaneously. Thus the role of mycoinsecticides in pest control must be viewed within the context of a total crop management programme. Such integration will be more complex than the use of traditional chemical control tactics, and will rely upon efficient monitoring to optimize the levels of control obtained from each application. Recent developments portend well for the future use of entomopathogenic Hyphomycetes in pest management programmes.

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