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The fungal past, present, and future: Germination, ramification, and reproduction

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The history of observation and research on fungal pathogens of invertebrates dates back thousands of years. In the era before microscopes, fungi were visible to the naked eye and observation of them helped give birth to invertebrate pathology as a modern field of study. Early observations of disease in useful insects, the honey bee and the silkworm, included documentation of mycoses. Both general and particular historical aspects of fungal entomopathogens and their use as microbial control agents have been thoroughly reviewed by others (Alves, 1998; Boucias and Pendland, 1998; Hajek and St. Leger, 1994; McCoy et al., 1988; Roberts and St. Leger, 2004; Steinhaus, 1949, 1975; Tanada and Kaya, 1993). In this review, we will attempt a contemporary review of key advances in our knowledge of the many aspects of fungal pathogen–insect host interactions and fungal evolution and systematics. We focus primarily on terrestrial fungi, in particular the Deuteromycota and Entomophthorales, and we include works on entomogenous Oomycetes, which were traditionally classified as fungi but are now recognized as phylogenetically distinct (Hawksworth et al., 1995). Fungi as microbial control agents are covered within a separate review by Lord, but here we review aspects of the research done on *Metarhizium anisopliae* var *acridum* for locust control. A comprehensive international program facilitated a wide array of studies that serve to illustrate well the marriage of basic and applied research needed to develop a fungal pathogen for use as a microbial control agent. We conclude with our own outlook on where research is headed and what needs to be addressed in the future. Because of space limitations, we have used selected case studies to illustrate the rich past, exciting present, and promising future of research on fungi. The papers we cite are merely representative of a large body of information resulting from the work of researchers from many different laboratories.

1. Fungal pathogen–insect host interactions

1.1. Infection processes and pathogenicity

Fungi differ from other insect pathogen groups in their ability to invade a host by penetrating its cuticle. Upon landing on a potential host, a fungal propagule initiates a series of steps that could lead to a compatible (infection) or a noncompatible (resistance) reaction. Alternatively, a propagule landing on an insect may elicit no reaction because of an absence of recognition between the fungus and the insect. In a compatible reaction, fungal recognition and attachment proceed to germination on the host cuticle, followed by penetration into the cuticle and colonization of the insect hemocoel. Infection eventually culminates in the rupture of the host cuticle for external fungal growth prior to spore formation and dispersal. Except in fungal species that are dispersed by flight or movement of infected hosts, e.g., *Massospora* spp. and *Strongwellsea castrans* (Batko and Weiser, 1965; Humber, 1976; Soper, 1974), death of the infected host usually occurs during colonization of the hemocoel, wherein the host suffers depletion of nutrients, or starvation, as was shown in *Culex pipiens quinquefasciatus* larvae infected

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with the oomycete *Lagenidium giganteum* (Domnas et al., 1974). Review articles on the general infection process have been written (Hajek and St. Leger, 1994), along with more focused treatments on specific aspects (Boucias and Pendland, 1991; Charnley and St. Leger, 1991; Kerwin and Washino, 1986; Roberts and Aist, 1984; St. Leger, 1991; Vey et al., 2001).

The infection process involves complex insect host–fungal pathogen interactions that vary among fungi and even among different strains of a species for a given host. The initial step in infection, attachment, may be passive and nonspecific as has been shown by Boucias et al. (1988) for entomopathogenic deuteromycetes *Beauveria bassiana*, *M. anisopliae*, and *Nomuraea rileyi* on host and nonhost insects. In these fungi, attachment is due to the hydrophobic interaction between the insect cuticle and the well-organized fascicles of rodlets in the conidia. These rodlets are formed from the self-assembly of hydrophobic proteins or hydrophobins in fungal aerial structures (Kershaw and Talbot, 1998). These hydrophobins were found in *B. bassiana* aerial conidia but not in blastospores (Bidochka et al., 1995). A mucilaginous coat also permits passive attachment in Entomophthorales and some deuteromycetes (Boucias and Pendland, 1991; Brey et al., 1986). In contrast, attachment was shown to be selective in two *M. anisopliae* strains that were specific to their scarabaeid hosts (Fargues, 1976; Vey et al., 1982).

Among aquatic entomogenous fungi, attachment followed by encystment of zoospores also varies from general to specific. In *L. giganteum*, initial contact between zoospores and mosquito larvae is due to negative geotaxis favoring encounters with mosquito larvae near the water surface. The initial number of encysted zoospores varies among different mosquito species and does not reflect host susceptibility (Golkar et al., 1993). In contrast, selective attachment followed by encystment was shown by Zebold et al. (1979) for the chytridiomycetes *Coelomomyces* spp., which have an obligatory alternating life cycle between a mosquito host and a copepod host (Whisler et al., 1975). They observed bands of encysting zoospores along intersegmental areas and along the head capsule of susceptible mosquitoes and found none on resistant mosquito species. Attachment of *Coelomomyces* spp. on mosquito larvae appears to be dependent on chemical and physical cues on the host cuticle (Kerwin, 1983).

In terrestrial fungi germination proceeds with the formation of a penetrant germ tube (Boucias and Pendland, 1991) or a germ tube and appressorium (Madelin et al., 1967; Zacharuk, 1970a), which forms a thin penetration peg that breaches the insect cuticle via mechanical (turgor pressure) and/or enzymatic means (e.g., proteases) (Zacharuk, 1970b). An exocellular mucilage, proposed to enhance binding to the host cuticle, is also secreted by several entomogenous fungi during the formation of infective structures (see review by Boucias and Pendland, 1991). In *M. anisopliae*, appressorium formation, hydrophobins, and the expression of cuticle-degrading proteases are triggered by low nutrient levels (St. Leger et al., 1992), demonstrating that the fungus senses environmental conditions or host cues at the initiation of infection.

The production of cuticle-degrading enzymes, proteases, chitinases, and lipases, has long been recognized as an important determinant of the infection process in various fungi, facilitating penetration as well as providing nourishment for further development (Charnley, 1984; Dean and Domnas, 1983; Samsináková et al., 1971). A study by Smith et al. (1981) using *Heliothis zea* "larval ghosts" demonstrated that a sequence of proteinase followed by chitinase enzymatic digests was needed to dissolve the insect cuticle. Among the proteases found in entomopathogenic fungi, the chymoelastase Pr1 has been well characterized and its role in cuticle invasion has been established (see review by St. Leger, 1994). Ultrastructural studies of *M. anisopliae* penetration sites on *Manduca sexta* larvae have shown high levels of Pr1 coincident with hydrolysis of cuticular proteins (Goettel et al., 1989; St. Leger et al., 1989). Pr1 inhibition studies also showed delayed mortality in *Ma. sexta* larvae, resulting from delayed penetration of the cuticle (St. Leger et al., 1988). Furthermore, construction of a *M. anisopliae* strain with multiple copies of the gene encoding Pr1 and overexpressing the protease resulted in 25% reduction of time to death in *Ma. sexta* compared to those infected by the wild-type strain (St. Leger et al., 1996). For reviews on the role of enzymes and proteases in pathogenicity of entomogenous fungi see Charnley and St. Leger (1991) and St. Leger (1994).

Inside the insect hemocoel the fungus switches from filamentous hyphal growth to yeast-like hyphal bodies or protoplasts that circulate in the hemolymph and proliferate via budding (Boucias and Pendland, 1982; Prasertphon and Tanada, 1968; Zacharuk, 1971). Later the fungus switches back to a filamentous phase and invades internal tissues and organs (Mohamed et al., 1978; Prasertphon and Tanada, 1968; Zacharuk, 1971). In vitro studies by Freimoser et al. (2003) showed that proliferation of *Entomophthora thripidum* as protoplasts was followed by differentiation into hyphal growth and formation of mycelia during depletion of nitrogen in the culture medium, suggesting that morphogenesis was induced under low-nutrient conditions. The fungus later erupts through the cuticle and an external mycelium covers all or parts of the host. Formation of infective spores ensues under appropriate environmental conditions (Boucias and Pendland, 1982; McCauley et al., 1968). Under suboptimal conditions, some fungi form resting structures inside the cadaver as in the case of *N. rileyi* under conditions of

low relative humidity and temperature (Pendland, 1982).

Fungi also produce secondary metabolites, derivatives from various intermediates in primary metabolism, some of which have insecticidal activities (see review by Vey et al., 2001). For entomopathogens producing these toxins, infection has been shown to result in more rapid host death (McCauley et al., 1968) compared to strains that do not produce these metabolites (Kershaw et al., 1999; Samuels et al., 1988). The insecticidal properties of destruxins, cyclic depsipeptide toxins from *Metarhizium* spp., first described by Kodaira (1961) and shown to be produced in wax moth and silkworm larvae by Roberts (1966) and Suzuki et al. (1971), have been tested against various insects (see review by Roberts, 1981). Currently, over 28 different destruxins have been described, mostly from *Metarhizium* spp., with varying levels of activities against different insects (Vey et al., 2001). The level of destruxin has been correlated with virulence (Al-Aïdroos and Roberts, 1978) and host specificity (Amiri-Besheli et al., 2000). Studies on the activities of destruxins have also shown modulation of the host cellular immune system, including prevention of nodule formation (Huxham et al., 1989; Vey et al., 2001) and inhibition of phagocytosis (Vilcinskas et al., 1977) in infected insects. Other representative toxins produced by entomopathogenic fungi include oosporein, beauvericin, and bassianolide from *Beauveria* spp. (Eyal et al., 1994; Gupta et al., 1994; Suzuki et al., 1977), efrapeptins (=tolypin) from *Tolypocladium* spp. (Krasnoff et al., 1991; Weiser and Matha, 1988), and hirsutellin from *Hirsutella thompsonii* (Mazet and Vey, 1995).

1.2. Sporulation and dispersal

Studies on the outward growth of vegetative structures and subsequent differentiation to reproductive structures are few compared to studies on the initial stages of infection. While the understanding of the early stages of infection has provided clear implications for developing insect control strategies, knowledge of the conditions for spore development can enable efficient mass production technologies. One of the few studies on fungal external growth from host hemocoel was conducted by Brobyn and Wilding (1977) on *Entomophthora* spp. developing in aphids. They observed coordinated outward growth of conidiophores, either converged in defined groups or ungrouped, rupturing the cuticle to emerge evenly over the host surface. Conidial discharge followed, leaving the host cadaver collapsed and with very little tissue. Boucias and Pendland (1982) also observed the formation of a confluent mycelial mat produced by emerging *N. rileyi* conidiophores over the entire surface of infected *Anticarsia gemmatalis* larvae. Conidiation following external growth appears to be regulated by blue light as has been shown by Sanchez-Murillo et al. (2004) in *Paecilomyces fumosoroseus*. This concurs with the study by Glare (1987) demonstrating maximal conidial production of *N. rileyi* on infected hosts following exposure to light and with studies on other fungi that identified blue light as one of the environmental cues for sporulation (Linden et al., 1997; Turian, 1974).

In contrast to the scarcity of studies on the morphogenesis of conidiation, studies on environmental factors affecting fungal sporulation on infected cadavers have been more numerous. Most of these studies examined the role of relative humidity and temperature as key environmental factors in spore production and dissemination of terrestrial fungi, triggering potential epizootics in a host population. Early studies by MacLeod et al. (1966) and Wilding (1969) pointed to the importance of these factors in sporulation and disease cycling.

Propagules of terrestrial fungi may be discharged actively, as in the case of Entomophthorales ejecting conidia or capilliconidia from cadavers (Eilenberg et al., 1986), or passively, via air currents and rain splash (Fernandez-Garcia and Fitt, 1993; Garcia and Ignoffo, 1977) and movement of infected insects (Humber, 1976). As for sporulation events, environmental factors are critical in determining the level of airborne inocula (Harper et al., 1984; Steinkraus et al., 1996). Hajek (1997) has reviewed spore dispersal and the ecology of terrestrial fungi. Reviews by Benz (1987) and Carruthers and Soper (1987) cover environmental factors in disease epizootics and fungal disease epizootics.

1.3. Host response/insect immunity

The insect host is not a passive player in the infection process and neither is death imminent once infection has been initiated. Insects employ both cellular and humoral defenses to combat microbial infection. A fungus encounters activated host defense mechanisms from the time it attaches and attempts to penetrate the cuticle. In the host integument are phenolic compounds and cuticular oxidases that provide defense by melanization around a penetrating tube. A study by Golkar et al. (1993) showed that intense and diffuse melanization in *Anopheles gambiae* larvae, provoked by encystment of *L. giganteum* zoospores, led to encapsulation of fungal germ tubes and resulted in reduced mortality among infected larvae. In contrast, a weak and localized melanization in *Aedes aegypti* larvae resulted in near 100% mortality even though a lower number of encysted zoospores were observed compared to *A. gambiae*.

Fungi that successfully penetrate the host hemocoel encounter hemocytes engaged in encapsulation, nodule formation, and phagocytic activities (see reviews by Gillespie et al., 1997; Vey and Gotz, 1986). Activation of the host's innate defense system follows upon its detection of the invading fungus via changes in the properties

of the cuticle basement membrane (Gunnarson, 1988) and substances associated with the fungal cell wall (Butt et al., 1996; Unestam and Soderhall, 1977). In most cases, entomogenous fungi are able to overcome host defenses by continuing to grow even after having been phagocytized and by suppressing the spreading ability of granulocytes, which prevents nodule formation (Hung et al., 1993). In addition to the capability to suppress the host cellular defense, *B. bassiana* hyphal bodies can elude the defensive response in *Spodoptera exigua* larvae (Hung and Boucias, 1992). The absence of a well-defined cell wall, with its potential elicitors of detection, allows hyphal bodies to evade detection and phagocytosis even in the presence of immunocompetent hemocytes (Pendland and Boucias, 1993).

Genetic studies on *Drosophila melanogaster* have shown that infections with *B. bassiana* or *M. anisopliae* elicit activation of the Toll signaling pathway. This pathway, one of two distinct signaling cascades in the fly's innate immune response to microbial infection, controls the expression of antifungal peptide genes (i.e., *drosomycin* and *metchnikowin*) (de Gregorio et al., 2002; Lemaitre et al., 1997). In addition to hemocytic and humoral responses, behavioral changes in infected insects, like behavioral fever exhibited by infected grasshoppers (Carruthers et al., 1992) and houseflies (Watson et al., 1993), can affect disease development and, consequently, host resistance. Except for obligate pathogens whose development is synchronized with the host life cycle, most virulent strains of fungi evade detection and overcome innate defenses, killing the host in a relatively short period of time (Pendland and Boucias, 1993).

1.4. Host range and specificity

Fungal infections of insects in most orders and in all life stages have been observed, although infections of immature holometabolous insects are more commonly documented (McCoy et al., 1988; Tanada and Kaya, 1993). Among different fungal species, or even among strains of a given species, host range can vary significantly. For obligate pathogens with complex life cycles that have apparently coevolved with their hosts, specificity is often restricted to a narrow range. Examples of this specificity include the entomophthorans *Massospora* spp., limited to one genus of cicadas (Soper, 1974), and *S. castrans*, limited to anthomyiid flies (Eilenberg and Michelsen, 1999). In contrast, host range among deuteromycetes, particularly *B. bassiana*, includes numerous genera of many insect orders (McCoy et al., 1988). It should be noted, however, that some host range descriptions rely on laboratory bioassay studies (physiological host range) and do not necessarily reflect true host range in nature. Insect host and fungal pathogen biology and ecology could limit the chance of encounter between potential hosts and pathogen. Furthermore, typical bioassay conditions that include a high dose and optimal relative humidity and temperature do not reflect variable field conditions.

Host range is determined by specificity, defined as the reciprocal adaptations and affinities between a pathogenic organism and the entirety of its host species (Fargues and Remaudiere, 1977). Factors that determine whether an insect–fungus interaction leads to successful infection are the same factors that mediate specificity, from attachment (Zebold et al., 1979), to germination on host cuticle (Altre et al., 1999; Boucias and Pendland, 1984; Smith and Grula, 1981; Wraight et al., 1990), to successful evasion of host defenses (Golkar et al., 1993; Hung and Boucias, 1992; Pendland and Boucias, 1993). In addition, specificity depends on the natural environmental context under which a pathogen and potential hosts coexist; true biological host range is determined by spatial and temporal factors (Carruthers et al., 1997; Hajek et al., 1996a) as well as physiological interactions.

2. Systematics and evolution

Entomogenous fungi have been reported from the phyla Ascomycota, Basidiomycota, Chytridiomycota, Zygomycota and Deuteromycota and have been traditionally classified based on their life cycles and the morphological features of their different life stages. Biological features such as ecology and host range have limited value given fungal diversity and environmental adaptability. Life cycle studies and key morphological features have provided stable characters for describing biological species and for taxonomic studies of fungi with a sexual stage. For example, two closely related species of *Coelomomyces*, *C. dodgei* and *C. punctatus*, have sporangia with similar sizes, shapes, and other features, and they occur sympatrically with overlapping mosquito host range (Couch and Bland, 1983). Hybridization studies, however, resulted in only partially viable zygotes (Federici, 1982), and the discovery of gated gametangial dehiscence further provided a mechanism of temporal reproductive isolation (Federici, 1983), supporting the classification of two distinct species.

Species recognition or delineation is not as precise in asexual fungi. The Deuteromycota is a temporary classification for fungi with no known sexual, or teleomorph, stage. The lack of adequate and informative morphological features in this group has resulted in recognized species complexes, which are particularly problematic in the case of the economically important species like *B. bassiana*, *M. anisopliae*, and *Lecanicillium* (= *Verticillium*) *lecanii* (Humber, 1997; Mugnai et al., 1989). These taxonomic ambiguities, unfortunately, have not been resolved, and identification of entomogenous deuteromycetes still relies mainly on morphological characters.

However, the availability of molecular systematic markers has led to initial studies clarifying taxonomic grouping among these fungi. For key literature on identification and classical taxonomy of different entomopathogenic fungi see Humber (1997).

Resolution of the taxonomy and systematics of entomogenous deuteromycetes requires finding their teleomorph or correlation of molecular data on the asexual form with the suspect teleomorph (i.e., *Cordyceps* spp.). For species within recognized complexes, an exhaustive sampling of representative strains and analysis using several appropriate molecular markers should clarify taxonomic status and help guide discovery of the true teleomorph. A study by Rehner and Buckley (2005) on *Beauveria* phylogeny using nuclear ribosomal internal transcribed spacer and elongation factor 1-α revealed that *B. bassiana* consists of two unrelated phyletic lineages. One clade of cosmopolitan distribution includes the Asian teleomorph *Cordyceps staphylinidaecola*. This result corroborates data obtained by Liu et al. (2001, 2002) connecting *Beauveria* to the *Cordyceps* teleomorph. Studies by Liu et al. (2001, 2002) also connected other *Beauveria* spp., as well as *Hirsutella* sp., *Metarhizium* sp., and *Paecilomyces* spp. to *Cordyceps* teleomorphs.

Molecular systematics requires different markers at different levels of study, with each marker having a limited useful range in fungal systematics (Bruns et al., 1991). These markers, together with sequencing data, if necessary, allow inference of intraspecific to phylogenic relations among fungi. While most of the molecular studies conducted so far on entomopathogenic fungi are on intra- or interspecific diversity of a handful of strains and have limited taxonomic value, they have provided valuable information addressing questions on fungal biology and biological control. Markers for intraspecific diversity, including random amplified polymorphic DNA, restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms, and microsatellites, have been used to develop probes for tracking pathogens released in the field (Bidochka et al., 1996; Castrillo et al., 2003; Enkerli et al., 2001), to examine origin and relationships among isolates (Hajek et al., 1990; Hodge et al., 1995), and to follow epizootics (Hajek et al., 1996b). For example, the fungus *Entomophaga maimaiga* was discovered in populations of the gypsy moth, *Lymantria dispar*, in the northeastern part of the United States in 1989 (Andreadis and Weseloh, 1990). The fungus had previously been reported only from gypsy moth populations in Japan. Using allozyme and RFLP markers, Hajek et al. (1990) identified the strain responsible for epizootics in the northeast as originating from Japan. It probably spread from introductions into North America conducted in 1910–1911 and has produced epizootics under suitable environmental conditions.

Markers for studying genotypic diversity applied to an adequate number of field samples can also reveal a pathogen's genetic population structure and indicate the role of various evolutionary forces in shaping it. Population studies of entomogenous fungi have often focused on natural selection, specifically, host selection pressure versus local adaptation (Bidochka et al., 2001; Enkerli et al., 2001; Maurer et al., 1997a). The varying results of these studies are probably due to differences in sampling schemes and local conditions. While natural selection could limit diversity in a given population, genetic changes mediated by mutation, recombination, and gene flow could effect the opposite result. The roles of other evolutionary forces, mating systems, migration, mutation, and genetic drift, await further study on both local and global scales.

Most of the available population or genetic diversity studies have focused on deuteromycetes and have assumed a clonal mode of reproduction. Studies exploring sources of genetic variation among these asexual fungi reported the presence of double-stranded RNA (Castrillo et al., 2004; Leal et al., 1994; Sugimoto et al., 2003), transposons (Maurer et al., 1997b), and chromosomal polymorphism (Viaud et al., 1996). Genetic change via the parasexual cycle has also been investigated by examining vegetative compatibility groups and the potential for asexual recombination (Cantone and Vandenberg, 1998; Castrillo et al., 2004; Paccola-Meirelles and Azevedo, 1991). A recent study on *B. bassiana* populations from agricultural fields in Denmark detected the presence of mating types MAT1 and MAT2 in one phylogenetic group, suggesting the potential for sexual reproduction. The skewed ratio of mating types observed, however, suggests some level of clonal structure and that a mixed reproduction strategy may be in effect (S. Rehner, personal communication). The asexual method appears to be the common mode of reproduction and may be advantageous for these fungi in rapid dissemination of host-selected genotypes for colonizing insect populations during epizootics.

Fungal repositories play a critical role in facilitating research to resolve systematic questions. Key repositories include the USDA-ARS Collection of Entomopathogenic Fungal Cultures (www.ppru.cornell.edu/mycology/), the CAB International collection (formerly International Mycological Institute) (www.cabi-bioscience.org), and the American Type Culture Collection (www.atcc.org). These and others provide researchers with fungal isolates from various insect hosts collected from various locations throughout the world for molecular systematic studies from intraspecific to phylogenetic levels. While most isolate collections are not appropriate for population studies, they do provide essential representative host and geographic samples. Furthermore, these collections serve as banks for valuable germplasm; the isolates represent potential microbial control agents,

sources of useful genes and gene products, and largely untapped reservoirs for medicinal products.

3. Microbial control agents: the LUBILOSA Program example

The impetus to study entomopathogenic fungi has been driven primarily by the potential of these organisms as control agents against insect pests. The history of microbial control, including fungi and other pathogens, has been reviewed in a separate article by Lord. However, we address here the LUBILOSA (Lutte Biologique Contre les Locustes et Sauteriaux, or Biological Control of Locusts and Grasshoppers, www.lubilosa.org) program because it provides an excellent example of the need to understand fungal biology, insect host biology, and their interactions to develop and deliver an effective pest management option. The program was initiated in 1989 based on ideas by Prior and Greathead (1989) to address the problem of locust and grasshopper outbreaks in the Sahel and northwest Africa. Microbial control using entomogenous fungi was seen as a promising alternative to chemical insecticides, which resulted in environmental problems and which posed health hazards to humans and livestock (Prior et al., 1992). The project entailed basic studies including exploration for (Shah et al., 1997) and evaluation of pathogens attacking locusts and grasshoppers (Bateman et al., 1996; Prior et al., 1995), characterization of potential control agents (Bridge et al., 1997), and studies on the interaction of target insects with the selected pathogen, *M. anisopliae* var *acridum* (= *M. flavoviride*) (Blanford and Thomas, 2001; Moore et al., 1992). Along with these basic studies were the development of new, or adaptation of available, technologies to mass produce (Jenkins and Goettel, 1997), formulate, and apply massive quantities of the fungus over large areas and under the arid conditions under which the pests abound (Bateman, 1997; Kooyman and Godonou, 1997; Moore and Caudwell, 1997). Studies to determine the effects of spatial and temporal variables on insect growth in predicting the optimal time for field application of the mycoinsecticide have also been conducted (Lomer et al., 2001).

From these studies, a mycoinsecticide, Green Muscle®, was developed based on *M. anisopliae* var. *acridum* (strain IMI 330189) in oil formulations for ultralow-volume applications. Laboratory and cage studies demonstrated the efficacy of the selected strain against all acridids and a host range effectively limited to the superfamily Acridoidea (Lomer et al., 2001). By formulating dried conidia in oil, either in a low-tech formulation for hand-held or vehicle-mounted sprayers or a high-tech formulation for aerial applications, field efficacy was improved probably via increased adhesion of conidia to insect cuticle and by overcoming the need for high humidity during the germination step of the infection process. Field efficacy of the mycoinsecticide against various species of locusts and grasshoppers has been demonstrated in studies in Africa (Lomer et al., 2001). For example, field trials in West Africa against the rice grasshopper, *Hieroglyphus daganensis*, showed that the product did better than the chemical standard, fenithrothion, except in speed of kill. Moreover, while grasshopper numbers in fenithrothion-treated plots recovered (reinvasion) after 10 days, host numbers in Green Muscle-treated plots continued to decline, requiring only a single application of the product for season-long control (Langewald et al., 1999). Ecological studies also showed that the fungus sporulated in cadavers in the field, resulting in horizontal transmission to new hosts and fungal survival and vertical transmission between seasons (Arthurs and Thomas, 1999). Efficacy, however, may be affected by the ability of locusts and grasshoppers to thermoregulate and maintain body temperatures that are detrimental to fungal growth and development of infection (e.g., Blanford et al., 1998).

4. Future directions: where are we going?

We have attempted in this review to provide an assessment of our current knowledge of entomopathogenic fungi by highlighting some of the accomplishments of the past few decades. These accomplishments also guide our insight on research trends and directions for the future. The major thrust in research will continue to be the exploration and development of fungi as biological control agents, aided by newly emerging ideas on fungal biology from genomic studies. While economic, environmental, and political trends determine which insects are of primary importance (e.g., the common focus on invasive species and key agricultural pests) and thus which pathogens will receive research funding, new findings in more fundamental fields will help direct additional research. Along with critical studies on specific pathogen–insect host interactions in the context of their ecology, we see the following areas of importance.

4.1. Comparative fungal genomics

Genome sequencing projects provide information that can help elucidate various aspects of fungal biology, including gene regulation and recombination, metabolic processes, environmental sensing, growth and reproduction, and pathogenesis. Comparative genome studies of other fungi (noninsect pathogens) have already revealed that numerous genes similar to those required for pathogenesis are present in saprophytic fungi, suggesting that the pathogenic lifestyle involves modification of processes required for basic survival. Having a genomic sequence of one or more entomopathogenic fungi would

lead to advances in our understanding of their life strategies and pathogenesis and aid in development of fungi as biological control agents. It is likely we will have such sequences within just a few years. It is fortunate that several insect genomes are also under study. The genetics of insect defense systems, especially in response to fungal infection, are critical in understanding host–pathogen interactions.

4.2. Fungal ecology and natural history

As revealed by genomic studies, fungi have the genetic repertoire to modify their lifestyles to adapt to variable environments. Information on the natural history of these organisms in contexts outside of their insect hosts will aid in the selection of strains and development of strategies for utilization. It is thus important that exploration and collection studies continue and that microbial control efforts not be limited to currently available mycoinsecticides and their inundative releases.

4.3. Genetic stability and genetic recombination

Genetic studies of entomopathogenic fungi have revealed molecular mechanisms of genetic variation; thus, the common assumption that most of the asexual fungi are genetically stable has been refuted. Genetic changes via horizontal transfer of cytoplasmic elements or recombination (sexual or asexual) require further study. The findings from such studies will guide field application decisions for both wild-type and recombinant strains which may be released inundatively as microbial control agents.

4.4. Taxonomic resolution of deuteromycetes

Most fungal strains under consideration or development as insect microbial control agents are Deuteromycota. Genomic studies at any level, from interspecific to population, require clear definitions of the taxonomic status of the species of interest. Molecular systematic studies utilizing numerous strains (particularly including strains from Asia, where most of the teleomorphs have been discovered) and adequate and appropriate markers need to be pursued.

5. Conclusion

The 20th century brought phenomenal advances in our knowledge of fungal biology, cultivation, and use. A worldwide community of researchers works on many fronts to grasp the dynamics of fungal populations, to reveal their organismal and cellular mechanisms, and to decipher their genetic code. We are striving to deploy fungi to help manage pests and to exploit fungal genes and their products for new uses. We are gaining a much deeper understanding of the interactions of fungi with other agents of pest management and the trophic cascades in which they are involved. New technologies allow us to track, with increasing accuracy, the fate of fungi released into the environment. This encouraging state of affairs points to a future that is daunting but bright. The current assemblage of invertebrate fungal pathologists is relatively small and the struggle for adequate research funding is never-ending. However, in coming years, we are confident that an ever-wider array of techniques will be available to biologists and that our continued creativity will enable us to take full advantage of them.

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