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INTERACTIONS BETWEEN FUNGAL PATHOGENS AND INSECT HOSTS

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INTRODUCTION

Fungal diseases in insects are common and widespread and often decimate insect populations in spectacular epizootics. Virtually all insect orders are susceptible to fungal diseases. Fungi infect insects by breaching the host cuticle; they are the principal pathogens among sucking insects because these hosts cannot ingest other pathogens that infect through the gut wall. Fungi are also particularly important for control of Coleoptera, because viral and bacterial diseases are rare among beetles. Entomopathogenic fungi are associated with insects living in diverse habitats, including fresh water, soil, soil surfaces, and aerial locations.

Currently, widely publicized environmental concerns and health risks associated with use of synthetic chemical insecticides have stimulated efforts to develop biological-control agents as alternatives or supplements to these chemicals. Consequently, much recent interest in mycoinsecticides has led to the marketing of several of them. Nevertheless, of the 700 species of entomopathogenic fungi currently known, only 10 species have been, or are presently being, developed for control (85, 89, 101, 130, 136), and the full potential of entomopathogenic fungi has not been approached. To develop fungi for control purposes, we need to understand the requirements for the high levels of disease transmission in the field that are characteristic of epizootics. In-depth epizootiological studies of several systems have been undertaken, in some cases resulting in simulation models used for experimentation with these complex systems as well as for development of control

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strategies. On a more basic level, too little is known about the genetic and molecular basis of fungal pathogenicity to insects, and this has precluded the genetic engineering of fungi to improve control potential. However, here again studies have gained impetus in recent years with the application of rigorous microbiological and enzymatic techniques focusing on the biological significance of well-characterized enzymes and toxins in insect-pathogen interactions.

In recent years, several authors have comprehensively reviewed research on fungal entomopathogens (47, 52a, 101, 144, 162), while more specific reviews have focused on fungal infection processes (12, 28, 29, 92), host barriers to infection (9, 137), behavior of infected hosts (41), epizootiology and modeling (26, 114), control using entomopathogenic fungi (136, 174), and fungal pathogens of specific groups of hosts, e.g. soil-dwelling insects (87), aphids (80), leafhoppers (152), and grasshoppers (4). This article does not attempt to provide an additional encyclopedic summary of the literature. Rather, we concentrate on recent examples of studies that illustrate the general trends in interactions between insect hosts and fungal pathogens leading to the development of epizootics.

STAGES OF DISEASE DEVELOPMENT

The Fungal Infection Process

THE CUTICLE AS AN INFECTION BARRIER Fungal germlings are continually moving through different environments during cuticular penetration. They respond to these changes by invoking adaptive biochemical processes and cellular differentiation to form a series of specific morphological structures. For example, germ tubes of *Metarhizium anisopliae* develop appressoria (located at the cuticle surface), infection pegs (in the epicuticle), penetrant hyphae and penetrant plates (in the procuticle), and yeast-like hyphal bodies (blastospores) for dispersal through the hemocoel (Figure 1). These morphological transitions suggest that germlings are constantly sensing their environment and adjusting in order to colonize insect tissue and counteract potential host responses. Infection structures probably evolved as a mechanism by which the pathogen overcomes host barriers. For example, appressoria represent an adaptation for concentrating physical energy and lytic enzymes over a very small area to make the entry process more efficient.

Cuticular barriers have yet to be completely characterized for a single insect species, but available data suggest that most, if not all, barriers can be part of a typical resistance response acting in sequence or simultaneously (137).

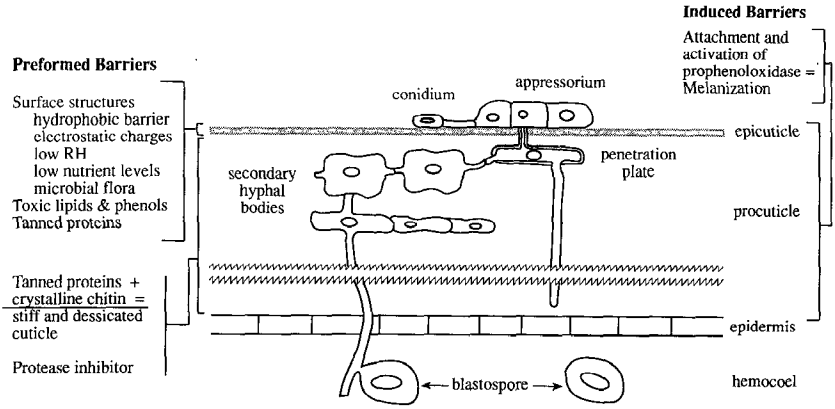

Figure 1 Schematic representation of infection structures of *Metarhizium anisopliae* and cuticular resistance barriers in cross-section of insect cuticle.

Pathogenesis is initiated by adhesion of a conidium (spore) to the insect cuticle. Secretion of an adhesive mucus as the conidium swells during pregermination development supplements the initial hydrophobic interactions between the conidium and the cuticle surface (12). An infection can be aborted on the epicuticle if a factor essential for a phase of adhesion, microbial development, or pathogenesis is absent (137). Specifically, infection may be prevented by low humidity (fungi require water for germination and extension growth), an inability to utilize available nutrients on the cuticle surface, or absence of factors necessary for recognition of a susceptible host or penetrable infection site.

Recognition of a susceptible host can include both chemical and topographical signals. The effects of surface topography on appressorium formation were studied using *Manduca sexta* cuticles and plastic replicas of the cuticular surface. Appressoria were only produced after extensive growth over the microfolds of the cuticle surface of early (1 day) fifth-instar larvae. These microfolds interfere with adhesion so that the fungus does not receive appropriate induction signals from the surface. In contrast, germination on the comparatively flat surface of 5-day fifth-instar larvae allows appressorium formation close to the conidium (141).

For some systems, the failure of fungi to invade insect cuticle has been attributed to the presence of inhibitory compounds (phenols, quinones, and lipids) on the cuticle surface (e.g. 88, 150). However, only circumstantial evidence indicates that any of these compounds are involved in disease resistance (137). Penetration of the epicuticle is either by infection pegs

produced from the underside of appressoria (Figure 1) or by direct entry of germ tubes (117, 175). The epicuticle is multilayered, and each layer has its own properties. The outer epicuticle appears in most insects to be mechanically fragile and may be penetrated by a weak force. Its resistance to enzymic degradation and its impermeability (99) suggest that, until physically disrupted, it could prevent passage of cuticle-degrading fungal enzymes. The inner epicuticle is thought to consist of polymerized lipoprotein stabilized by quinones (33). This composition implies toughness, but enzymes produced by entomopathogens may surmount this obstacle (29).

Once the epicuticle is breached, progress by the penetration peg through the cuticle may be more or less direct via penetrant hyphae, or penetrant structures may extend laterally, producing penetrant plates. These lateral expansions can cause fractures that favor penetration (13) and may facilitate dispersal of the pathogen's cuticle-degrading enzymes (56). The procuticle acts as a physical barrier by its impermeability to pathogen secretions, its resistance to degradation by many pathogen enzymes, and its mechanical resistance to penetration. The degree of resistance depends on the combined effects of the cuticle's thickness (32), the tensile strength imparted to the cuticle by the system of chitin lamellae (75), and the degree of cuticle hardening by sclerotization. Insects with heavily sclerotized body segments are usually invaded via arthrodial membranes or spiracles (28).

Cuticular melanization, induced by physical damage or β-1,3 glucans on the fungal cell wall (151), is common (e.g. 18, 113). Melanization often occurs too late, or in insufficient magnitude, to stop strong or fast-growing pathogens (137), but assessing the role of melanic responses in disease resistance is difficult because of insufficient knowledge of the quantities of melanin required to influence infection and the ways in which melanic reactions might hinder fungal growth.

METABOLITE PRODUCTION BY FUNGI Pathogenic fungi possess an intriguing array of mechanisms that permit them to break down and assimilate host materials while overcoming host-resistance mechanisms. For the most part, the fungal metabolites assist the pathogen with (*a*) physical aspects of ingress, e.g. cuticle-degrading enzymes that actively destroy or modify the structural integrity of the host; (*b*) inhibition of selective processes or enzymes of the host; and (*c*) interference with the regulatory systems of the host. Such damage, associated with disease symptoms, may be produced both by the pathogen's enzymes and by its low-molecular-weight metabolites (toxins).

Undoubtedly, many pathogen enzymes are important determinants of virulence because they enable the pathogen to coexist with the changing metabolic processes associated with the host's diseased state. As yet, we

have little definitive information as to which role(s) a particular enzyme or toxin is assuming. The gross physiological disturbances associated with infection processes make it difficult to distinguish between the initial site of the function perturbed and secondary or spurious events. Recombinant DNA techniques for isolation of specific genes are, however, advancing our knowledge. For example, a recently cloned protease (Pr1) from *M. anisopliae* (140) solubilizes cuticle proteins to assist penetration and provide nutrients for further growth (56, 139, 143). With the development of satisfactory transformation technology in *M. anisopliae* (55), we can now isolate numerous presumptive virulence genes and use disruptive mutagenesis to define factors that control pathogenicity.

The destructive effects of pathogen proteases on cuticles can be attributed, at least in part, to the structural importance and enzymatic accessibility of protein polymers in the cuticle. However, chitin fibers also contribute to cuticle structure as a mechanical barrier to penetration and as a stabilizer of the cuticular protein matrix, as evidenced by the synergistic effect of dual applications of Dimilin, an inhibitor of chitin synthesis, and *M. anisopliae* (75). Ultrastructural observations confirmed that treatment of cuticle with Dimilin massively enhanced fungal penetration (75) and increased susceptibility to Pr1, compared with control cuticle (RJ St. Leger, AK Charnley & RM Cooper, unpublished data).

Manipulation of pathogen enzymes, particularly Pr1, has enhanced investigators' understanding of cuticle structure and of how cuticles are naturally degraded. Further characterization of the regulation of enzyme systems will likely enable manipulation of enzyme levels through chemical and biotechnological procedures for the purpose of insect control (93).

Once fungi invade the hemocoel, the host may be killed by some combination of mechanical damage produced by fungal growth, nutrient exhaustion, and toxicosis (52). The relative importance of these mechanisms varies with the specific fungal isolate or host. Many entomopathogenic fungi produce toxins, but although some toxins are fully described chemically, the rarity of toxicological studies leaves their role in pathogenesis unclear (52, 135). As an exception, the cyclic depsipeptide destruxins are produced by isolates of *M. anisopliae* in amounts that correlate with toxicosis and differential virulence of isolates against some insects (145, 146). Destruxins affect various organnellar targets (e.g. mitochondria, endoplasmic reticula, and nuclear membranes), paralyzing cells and causing disfunction of the midgut, Malpighian tubules, hemocytes, and muscle tissue (145, 166, 167). Other toxins include the cyclic depsipeptides beauvericin (*Verticillium lecanii*) and bassianolide (*Beauveria bassiana*), which may function principally as endocellular ionophores, leucinostatins, and efrapeptins (linear peptides from *Paecilomyces* and *Tolypocladium* spp., respectively) with

antimicrobial activity (94), and cytochalasins (*M. anisopliae*), which may paralyze host cells (135).

Period of Lethal Infection

HOST DEFENSES AFTER INFECTION After penetration, fungi proliferate within the body of the host, frequently as a life stage that does not naturally survive outside of hosts. The outcome of an infection depends on the pathogen's genetic potential to grow rapidly, to penetrate host-induced barriers, and to resist toxic chemicals. These qualities must be related to the initial strength of the host's constitutive defenses and the speed or magnitude of its inducible responses. Insect immunity is a remarkably underexplored research area. Before we know what properties of a fungal pathogen need to be genetically improved for insect control, we must understand those factors responsible for an insect's susceptibility or resistance to a pathogen.

Little is known of how an insect recognizes a fungus as nonself so that its immune responses are initiated. The suggested role of phenoloxidase activation in recognition (151) is ambiguous because recognition may occur in the absence of melanization (132). Recruitment factors have been described that stimulate phagocytosis, deplete plasmatocytes, mobilize servile hemocytes, and promote nodule formation (34). An opsonin (a galactose-binding lectin) induced in the hemolymph mediates cellular recognition of *Nomuraea rileyi* blastospores (120). Cell walls of *N. rileyi* hyphal bodies, the stage successfully living within the host hemocoel, contain few β-1,3 glucans, while mycelial fragments with high levels of β-1,3 glucans are recognized and encapsulated (9). In contrast, the insect phagocytes can nonspecifically recognize *N. rileyi* conidia (12). The charge and wettability of a fungal surface apparently regulates hemocyte adhesion in several species (96, 176).

The main reaction in the cellular antifungal defense mechanism is encapsulation of the fungus, which is rapidly melanized (59). During encapsulation, granulocytes are attracted to the fungus and may engulf it (phagocytosis), then plasmatocytes are recruited and form a pseudotissue in concentric layers, thus differentiating a granuloma (nodule) in which the fungus may be lysed (59). Encapsulation only provides protection for hosts against weakly virulent pathogens. In the case of hypervirulent strains of a fungal species, hosts are either unable to form typical granulomas or fungi overcome the encapsulation and continue to grow (82). For example, the successful development of *B. bassiana* within hosts is based on simply overcoming the host hemocyte response (77); numbers of granulocytes are dramatically reduced 3 days after fungal challenge (81). Hung et al (82) propose that the cellular-defense response is the initial target of metabolites

produced by *B. bassiana*. These metabolites block the recruitment of hemocytes required for nodule formation, although the initial recognition and phagocytosis responses remain functional. *M. anisopliae* produces immunodepressive substances, such as the toxin destruxin E, that inhibit the formation of nodules by paralyzing hemocytes (27, 83). Other fungal species employ differing methods to avert cellular defense reactions. In *Entomophaga aulicae,* colonization of the hemocoel is carried out by protoplasts, which lack cell walls and therefore do not attract hemocytes (35). In *N. rileyi,* a mucus sheath masks germ tubes from host lectins (119).

Although cellular reactions against fungi are well documented, the question of humoral immunity to fungal mechanisms in insects has not been convincingly treated (63). Most published accounts of humoral reactions to fungi describe a complex of inducible, fungitoxic protease inhibitors (e.g. 11, 95). Comparisons between strains of *Bombyx mori* revealed a general polymorphism in which individual protease inhibitors were controlled by codominant alleles (36, 37). Variation between species, and, more importantly, intraspecific variation, implies potential for selection of increased resistance to disease in this beneficial insect.

Antifungal factors besides protease inhibitors can occur in the hemolymph of infected insects and prevent lethal infections. Recently, Iijima et al (84a) characterized a small (67–amino acid) antifungal protein produced in *Sarcophaga peregrina*. Interestingly, sarcotoxin (an antibacterial member of the cecropin family) has no activity against fungi alone but greatly facilitates the action of the antifungal protein.

On the level of the whole organism, specific behaviors of some insect hosts can defend against potentially lethal fungal infection. The grasshopper *Camnula pellucida* basks in the sun, which raises internal body temperatures above the optimum for development of *Entomophaga grylli,* and grasshoppers thereby recover from infection (23). Heightened body temperatures also cure *Musca domestica* of *Entomophthora muscae* infections, although heightened temperatures are only beneficial for a specific window of time during disease incubation (170).

TRITROPHIC-LEVEL INTERACTIONS Development of fungal pathogens within hosts can be influenced not only by immune reactions of hosts, but also indirectly by host food. For example, leafcutter bees, *Megachile rotundata,* reared on natural provisions were generally less susceptible to *Ascosphaera aggregata* as compared with bees reared on artificial provisions (57).

The majority of studies of tritrophic-level interactions have compared the impact of host-plant species on development of fungal pathogens within herbivorous insects. The few in vitro studies have demonstrated that the addition of glycoalkaloids or a diversity of plant extracts to media can

inhibit growth of *B. bassiana* (30, 127) and *N. rileyi* (51a). Adult chinch bugs, *Blissus leucopterus leucopterus,* inoculated with *B. bassiana,* demonstrated higher mortality when fed wheat, barley, or artificial diet compared with corn or sorghum (129). In addition, few cadavers of chinch bugs that had eaten corn or sorghum produced conidia, demonstrating an additional inhibitory effect of these foods, presumably resulting from fungistatic secondary plant chemicals. When the glycoalkaloid α-tomatine was added to artificial diet, development of *N. rileyi* in *Helicoverpa zea* was partially prevented at LC_{50} and was inhibited at LC_{90} (51a).

Further studies have confirmed that disease development is linked to the effects of plant nutritional quality on host growth rate. Thus, while similar mortality levels and disease-incubation times were observed when polyphagous larvae of *Lymantria dispar* infected with *Entomophaga maimaiga* were reared on foliage of five tree species (AE Hajek, unpublished data), the duration from infection to host death was increased for slow-growing larvae eating the poorly utilized *Acer rubrum. E. maimaiga* protoplasts were grown in tissue-culture media plus hemolymph from larvae reared on the five tree species. Protoplast growth rates were equivalent, suggesting that host-plant factors inhibitory to fungal growth were absent in the larval hemolymph, where protoplasts normally proliferate.

Time-mortality relationships between *N. rileyi* and another polyphagous host species, *Spodoptera littoralis,* did not differ for third-instar larvae reared on four readily utilized plant species (42). Comparisons of susceptibility of *Leptinotarsa decemlineata* to *B. bassiana* across species of *Solanum* with different levels of glycoalkaloids resulted in similar levels of larval mortality (31). In contrast, Hare & Andreadis (73) found decreased susceptibility to *B. bassiana* in *L. decemlineata* eating *Solanum* species that were nutritionally optimal. In part, these differences may have resulted from the methodology, because in the latter study insects were infected *per os,* an unnatural mode of infection (1). Empirical field data from *Helicoverpa armigera* on three different crops demonstrated that cadavers of more *N. rileyi*–killed larvae sporulated when larvae had eaten plants optimal for growth (58). The confusion regarding whether larval growth rate is positively (e.g. 58; AE Hajek, unpublished data) or negatively (e.g. 73) associated with successful pathogenesis may reflect the basic differences among fungal entomopathogens in their dependence on hosts. For example, many species of Entomophthorales are almost obligate pathogens and cannot be grown in vitro or are difficult to culture, while many Hyphomycetes are easily grown in vitro and can readily live as saprophytes in nature.

The endophytic occurrence of *B. bassiana* in corn plants provides a very different example of interactions among three trophic levels. When corn

plants were colonized endophytically by *B. bassiana*, *Ostrinia nubilalis* populations were suppressed throughout the season (6, 7).

BEHAVIORAL CHANGES OF LETHALLY INFECTED HOSTS In many systems, a reduction in feeding is one of the first overt changes in an infected host (e.g. 64, 110). This response provides an often-overlooked benefit of fungal pathogens infecting pest insects. Additional behavioral changes have been documented in some systems during the period of lethal infection. Female carrot flies, *Psila rosae*, infected with *E. muscae* do not lay their eggs near food plants, as healthy females do, thereby reducing the chances for egg survival (38). Infected ants change their normal routines to avoid contact with conspecifics (144), e.g. ground-dwelling ants frequently climb shrubs to die.

Aphids, ants, grasshoppers, planthoppers, and flies infected with diverse fungi are all known to move to elevated locations just before dying (144). Although the physiological basis for this response is not understood, *Choristoneura fumiferana* larvae infected with *E. aulicae* produce a small, labile peptide around the time of death. Synthesis of the peptide correlates with abnormal predeath behavioral symptoms that may be associated with these changes (105). Many hosts are known to die in positions with wings or elytra spread away from the body or with part of the body detached from the substrate to expose sporulating areas of the cadaver. Host mortality in elevated locations or in exposed positions would clearly enhance spore dispersal. Although these aberrant, predeath behaviors are not well understood, selective pressures on the fungus may have favored evolution of host-pathogen interactions that ultimately promote spore dispersal.

Disease Transmission

Dispersal of infective propagules to a new host represents a most perilous part of the fungal life cycle. Processes of spore production and discharge, spore dispersal, and spore survival and germination frequently depend on environmental conditions. The enormous numbers of spores produced per cadaver, e.g. 2.6×10^6 *E. maimaiga* conidia/fifth-instar *L. dispar* at 15°C (148), partially compensate for the high probability that the vast majority of spores produced will not survive and infect a new host.

Processes influencing spore production have not been well studied at the biochemical or physiological level for many host-pathogen systems. Host death is necessary before spores are produced in many, but not all, systems. At least eight entomophthoralean species cause host mortality in diurnal patterns, with host death frequently occurring over a specific span of photophase (light) hours (108, 111, 164). Diurnal timing of mortality and

the time required from death to initiation of sporulation indicate that many species produce spores during scotophase (dark) hours when humidity is higher.

Fungal pathogens in the Zygomycetes and Oomycetes frequently produce two spore types, a short-lived, actively dispersing spore and a long-lived, environmentally resistant spore. In vitro studies have determined that the presence of sterols promotes the production of resistant oospores in *Lagenidium giganteum* (90). Among the Entomophthorales, factors influencing resting spore production are not well understood; in vivo production of resting spores of *Zoophthora radicans* in *Therioaphis trifolii* f. *maculata* is promoted by lower temperatures and higher relative humidity (RH) and varies with fungal isolate, fungal strain attenuation, and dose (54). Interestingly, coinfection of aphids with different isolates of *Z. radicans* yields increased resting spore production, suggesting that their production may be at least partially genetically determined by a cytoplasmic factor.

For many fungal entomopathogens, production of aerially dispersing spores is associated with RH. The entomophthoralean species *Zoophthora phalloides* (53) and *E. maimaiga* (66) produce and discharge primary conidia in abundance only at a constant RH of >95%. In *E. aulicae,* RH is not limiting for much of the interval after death while the fungus develops within cadavers, changing from protoplasts to hyphal bodies. However, high RH is definitely necessary for some time prior to spore discharge (D Tyrrell, personal communication). In contrast, laboratory studies have demonstrated that *E. muscae* is better adapted to drier conditions with abundant conidial discharge at 50% RH and higher (112). *Erynia* sp. that infects *Hypera postica* discharges progressively fewer conidia, over a shorter duration, in response to decreasing moisture levels (104). In some instances, cadavers can discharge spores, then undergo a dry period when production abates, after which cadavers rehydrate and discharge spores again (71, 104; AJ Sawyer, personal communication).

After spores are produced and discharged, they must survive until contacting a new host. Frequent causes of spore mortality include high temperatures, desiccation, and solar radiation (e.g. 20, 171, 177). In the soil environment, decreased spore survival can also be caused by fungistatic effects varying by site and year that may be caused by Actinomycetes or other saprophytic microorganisms (61, 87, 121). Airborne, asexual spores (conidia) of entomophthoralean species, in particular, have frequently been considered very short-lived and inappropriate for biological-control use. However, studies now demonstrate that *Pandora neoaphidis* conidia can survive prolonged periods of 40–50% RH and 90% RH, while infectivity at 70% RH declines rapidly (14). Furthermore, capilliconidia of three entomophthoralean species can survive extended exposure to 50% RH (165).

Such tolerance of fairly dry environments would allow for greater spore survival during dispersal.

Fungal pathogens spread in a variety of ways over varying distances. For example, on a localized scale, infected insects can move prior to death (98), spores of Oomycetes actively move in water (162), or spores can be spread by rain splash (45). Movement of soil-inhabiting fungal entomopathogens is probably limited, although spores can be washed through the soil, or fungal mycelia originating in cadavers can locally spread through soil (87, 160). Spores of soil-inhabiting fungi that infect insects living above-ground can leave the soil environment by attaching to surfaces of growing plants (84). Although other animal species can vector entomopathogens (e.g. 62), potential spread by these means has never been documented.

Fungal pathogens of aerial and epigeal insect stages are frequently anemochorous, and active spore liberation can aid spore dispersal. In still air or light winds, conidia of *E. muscae* should remain in close proximity to the spore source (19). However, for the few systems studied, spores of entomopathogenic fungi are occasionally very abundant in air samples, suggesting that aerial dispersal is not uncommon. *Entomophthora gammae* conidia are characteristically abundant in the air between 2000 and 0600 hs during periods with RH near 100% (74). Abundant *E. maimaiga* conidia found in aerial samples have been associated with infection of *L. dispar* larvae caged adjacent to a spore sampler (AE Hajek & JS Elkinton, unpublished data), demonstrating that airborne conidia are alive and infective. Long-distance spread by *E. maimaiga* from 1989–1992 was documented by sampling larvae and cadavers of *L. dispar* (40; AE Hajek & JS Elkinton, unpublished data). During 1992 alone, *E. maimaiga* simultaneously spread into many areas of northern Virginia, where it had never before occurred, suggesting movement of *E. maimaiga* conidia on weather systems.

Recent studies suggest that, in at least one system, even if fungal pathogens arrive in the correct habitats, potential hosts may be able to detect them. Larval scarabs were repelled by mycelia of *M. anisopliae* in soil, and although conidia were not deterrents (168), extracts of mycelia deterred both adults and larvae from feeding. However, adult scarabs preferentially laid eggs in the presence of mycelia, possibly because mycelial respiration mimics growing plant roots (168).

GENETIC DIVERSITY IN HOST-PATHOGEN SYSTEMS

Many species of fungal entomopathogens are composed of a genetically diverse group of strains, but these strains cannot usually be differentiated by morphological criteria. Until 1980, little information other than variation

in pathogenicity was known about the genetics of entomopathogenic fungi. The advent of improved biochemical and molecular techniques has rapidly expanded our knowledge of intraspecific variability in fungi.

Antigens raised against fungal proteins can differentiate fungal species (e.g. 65) but frequently not strains within a species. Allozymes provided the first unambiguous markers available in sufficient numbers to enable reliable genetic studies of entomopathogenic fungal species (10, 134, 154). Several enzyme systems are polymorphic and sufficiently well resolved for reliable genetic studies of *M. anisopliae* (142), *B. bassiana* (138), and the *E. aulicae* species complex (67, 68). Random amplification of polymorphic DNA (RAPD) (5) and restriction fragment length polymorphisms (RFLPs) of genomic DNA (67, 68, 169) have provided additional markers for population analysis. Other molecular approaches applied recently include the analysis of rRNA sequences to distinguish between *Tolypocladium* and the genus *Beauveria* (128) and contour-clamped homogeneous electric field (CHEF) fractionation of *M. anisopliae* chromosomes to distinguish different isolates (149).

RFLP probes and isozymes have been used in concert to evaluate strains of the *E. aulicae* species complex causing epizootics across the northeastern United States in gypsy moth (*L. dispar*) populations (67). This fungus had never been reported from North American gypsy moth before, although there was an attempt to introduce it for biological control in 1910–1911 (155). Results from these biochemical techniques were in agreement that this northeastern pathogen was identical to *E. maimaiga,* a member of the *E. aulicae* species complex from Japan, and differed from indigenous strains of this complex. These same techniques have been further used to demonstrate that different members within the *E. aulicae* species complex can coexist sympatrically in different lepidopteran hosts (68). Only lack of a greater diversity of isolates within this complex and associated biological information prevents clarification of species-level relationships within this group.

The use of the newly available biochemical and molecular markers has facilitated other studies on species structure and geographical spread. For example, in recent allozyme studies (138, 142), the level of genetic distance observed between cluster groups of *B. bassiana* and *M. anisopliae* indicated that each represents a species aggregate, components of which display overlapping genetic variability, with some isolates currently assigned to other rarer species in the same genus. Except for isolates of *M. anisopliae* var. *majus,* most *Metarhizium* isolates are homozygous at each locus in a manner consistent with haploidy. In spite of the maintenance of high diversity in *B. bassiana* and *M. anisopliae,* the majority of isolates are contained in a few geographically widespread genotypic classes. The persistence of these

genotypes over time and space suggests that, in many situations, these fungi have a clonal population structure. Other aspects of the allozyme data (the magnitude of genetic distances between populations, gene diversity, and the pattern of distribution of genotypic classes) indicate the early and effective operation of heterokaryon incompatibility. The most common multilocus genotypes of *B. bassiana* have achieved worldwide distribution, suggesting a means of long-distance dispersal. *M. anisopliae* resembles *B. bassiana* in the localized distribution of many genotypic classes. Many of these localized classes of *M. anisopliae* contain only strains isolated from Coleoptera, and they presumably evolved through pathogenic specialization to these hosts. Geographic isolation and/or the very limited dispersal of *M. anisopliae* spores may also be important in the evolution of different genotypes.

Intraspecific variability of fungi has often been characterized by differences in pathogenicity. However, host insect populations are not genetically static in their responses to fungal pathogenicity. Among aphid populations, clones resistant to certain isolates of *P. neoaphidis* are distributed in specific areas of Australia, coexisting among susceptible clones at low levels (106, 107). The evolution of this variability is somewhat puzzling because it is believed that only a small population of these aphids was initially introduced to Australia and these aphids reproduce only asexually. Presumably, genetic variation in host susceptibility could have arisen through one or more mutations or extranuclear inheritance mechanisms. Coexistence of clones is partially maintained by differential temperature optima and dispersal capabilities (79, 107). In North America, variation in susceptibility to one strain of *P. neoaphidis* has been found among 60 clones of pea aphid, *Acyrthosiphon pisum,* originating from within the same field (K Hural, personal communication). Likewise, one of two clones of *A. pisum* was resistant to a strain of *Conidiobolus obscurus* (116).

Sibling groups of leafcutter bees from the same original population differ in susceptibility to chalkbrood, *A. aggregata* (157). Matings between resistant lines resulted in loss of resistance, suggesting a polygenic basis for the resistance, which is easily disrupted by out-crossing (158). These first documented cases of variability in susceptibility among insect hosts to fungal pathogens demonstrate the potential occurrence of coevolution between hosts and fungal pathogens.

EPIZOOTIOLOGY OF FUNGAL DISEASES

An epizootic is defined as an unusually large number of cases of a disease in a host population (162). The subjectiveness of this definition allows it to encompass the diversity of host-pathogen systems, e.g. some diseases

are always uncommon even when most abundant. However, among systems that have been comprehensively studied, diseased hosts are usually very abundant during epizootics. The past 10–15 years have seen an impressive growth in the number of publications on the epizootiology of fungal diseases. Analyses of some host-pathogen systems have used up to three years of empirical data (3, 72, 123, 153, 163). This type of long-term study is essential for drawing generalizations regarding interactions in nature. However, for those systems characterized by uncommon hosts or with infrequent occurrences of epizootics, long-term data are extremely difficult to collect.

The methodologies and knowledge gained from studies of plant-disease epidemiology and vertebrate epidemiology, as well as basic population dynamics, can provide a foundation for investigations of the epizootiology of fungal diseases. Many studies of fungal epizootics quantify only disease prevalence and host density, although overall pathogen density is an integral part of the system and needs to be quantified. Methods for optimizing accuracy in calculating percent parasitism (e.g. 60) could have clear application to calculations of percent infection. In addition, impact of fungal pathogens is seldom evaluated as a component of overall host ecology, such as in life-table analysis.

Accuracy of sampling designs used to quantify disease prevalence has never been determined. Sampling designs should particularly be developed around potential changes in the behavior of infected insects. Fortunately, cadavers of cereal aphids in spring wheat have the same clumped distribution as uninfected insects so the same sampling design can be used for healthy insects, fungal-infected insects, and resulting cadavers (43). Quantification of field populations of fungal pathogens can be fairly simple if this involves counting only cadavers. However, in many systems, the pathogen resides not only in cadavers but also in reservoirs, frequently the soil. Quantification of fungi in soil requires very different techniques compared with evaluation of disease prevalence and host density. Fungal reservoirs in soil can be quantified using insects as bait (178), growing the fungus on selective substrates (87), or by directly counting spores (e.g. 102), but all of these procedures can be very time consuming.

Epizootiological studies are usually initiated with detailed accounts describing the natural history of the disease, phenology of both pathogen and host, impact of the pathogen on host populations, and association of epizootics with weather conditions, with frequent emphasis on moisture in the form of RH, condensation, or rainfall. Questions raised regarding key aspects of host-pathogen interactions are subsequently approached with laboratory studies. For example, laboratory studies of the phenology of *Z. radicans* resting spore germination at constant temperatures suggest that the

timing of *C. fumiferana* infection is, at least in part, regulated by the timing of resting spore germination (122).

Critical interpretation of empirical data acquired from epizootiological studies requires careful choice of appropriate statistics for analysis. Regression analyses of epizootics of the aquatic fungus *Coelomomyces punctatus,* which has a complex life cycle including a copepod intermediate host and an anopheline primary host, determined that the single factor most closely associated with prevalence of infection was copepod abundance (3). Multiple regression analyses determined that the fungal inoculum and number of hours of condensation were the variables most closely associated with *Z. radicans* epizootics in *Empoasca fabae* populations (51). Infection of the blackmargined aphid, *Monellia caryella,* by three entomophthoralean species was correlated with minimum temperature, and during periods above 8°C, infection was positively correlated with the total hours of leaf wetness during the previous 5, 6, and 7 days (39). Patterns of infection of *L. dispar* larvae caged on the forest floor suggested that *E. maimaiga* resting spores germinated 1–2 days after precipitation (172).

In fact, many examples in the literature link epizootics with levels of ambient moisture for many host-pathogen systems. However, one of the few studies evaluating spatial dynamics of epizootics determined that weather factors did not limit the prevalence of *Zoophthora canadensis* in the woolly pine needle aphid *Schizolachnus piniradiatae* (153). Instead, fungal inoculum and host density were associated with infection level, although the association of host density was weak unless the spatial distribution of the hosts was also considered. Over years with equivalent host densities, decreased aggregation of hosts was associated with increased disease prevalence because aphid colonies contained fewer individuals but were more closely spaced. Another study evaluating disease dispersion demonstrated that larvae of three species of Lepidoptera infected with *N. rileyi* were more aggregated than uninfected larvae (49). This disease was first found in a few loci but subsequently spread through a soybean field, even while disease prevalence was low. These data agree with empirical observations that fungal diseases are distributed as foci of infection before subsequent spread of the disease (87, 171). Unfortunately, studies of the spatial distribution of disease are rare, although this information is imperative to understanding density relations as well as fungal spread.

Generalized patterns for the long-term temporal dynamics of fungal diseases have been hypothesized (156) and substantiated in the *S. piniradiatae–Z. canadensis* system (153). Low-density host populations have low percentages of infection, resulting in host increase (preepizootic phase). Host populations reach high densities and, after a lag, disease epizootics

cause population decline (epizootic phase). Even when host populations are reduced, infection levels remain high owing to abundant fungi in the environment (postepizootic phase). Therefore, these fungal pathogens are acting with delayed density dependence, as has been found empirically in the *E. muscae–Delia antiqua* system (22).

The ecology of most host-pathogen systems is not understood well enough to predict the influences of the biotic community on development of epizootics. Vegetation type should influence disease prevalence in systems with tritrophic-level interactions (as described above). Presence of alternate hosts in the community may allow increases in inoculum, resulting in increased infection in primary hosts. For example, sympatric aphids are differentially infected by *P. neoaphidis* in California and, while *P. neoaphidis* kills few *Acyrthosiphon kondoi,* pathogen density is increased, resulting in greater infection of the more susceptible *A. pisum* (125). Interactions among pathogens and other natural enemies could potentially impact host and pathogen populations. However, when *Diatraea saccharalis* parasitized for 1–6 days were inoculated with *M. anisopliae,* the fungus was not harmful to three species of parasitoids (48). In contrast, infection of cereal aphids by fungal pathogens soon after parasitization prevented development of aphidiids, although fungi were not found invading parasitoid tissues (126). Conversely, fungal infection of hosts in more advanced stages of parasitization impaired fungal development. As our understanding of primary interactions within host-pathogen systems increases, appreciation of community-level influences may aid in understanding and predicting development of epizootics.

Modeling

With current levels of expertise, model development is the ultimate step toward understanding and predicting fungal epizootics. Theoretical models have been developed to answer basic ecological questions about simplified, generalized host-pathogen systems and have served as a basis for modeling entomopathogenic disease systems (e.g. 2). These basic models have been adapted to include the reservoirs for pathogens frequently found in fungal-insect systems (76) or reproduction of infected insects prior to death (147). A generalized model can be used to investigate system stability, high pathogenicity, short lifespan of pathogen propagules, and high host reproductive rate, all of which affect localized stability in host-entomopathogen systems. In contrast, model results suggest that pathogen transmissibility and pathogen production do not influence local stability (15).

For specific host-fungus systems, regression models or differential equations decribing treatment-response relationships have been developed using experimental data (e.g. 21, 66, 147). However, in nature, multitudes of

parameters influencing host-pathogen interactions vary in intensity over space and time. The effects of a range of combinations of these parameters are not known and cannot be determined experimentally and, in many cases, analytical analysis is intractable. Therefore, treatment-response data have been used to develop complex simulation models for experimental manipulation of host-pathogen systems. The breadth of information must be large to develop complex simulation models that can accurately mimic disease dynamics. Complex simulation models have been developed for diseases of several agricultural pests (16, 17, 19, 44), grasshoppers on pastures (24), and forest defoliators (69, 124).

Simulation models have been useful in integrating quantitative experimental data as well as in guiding empirical research. More importantly, model results have been validated using field data, and simulation experiments have also been used to predict the outcomes of changes in specific factors. Both model results and field experiments were used to investigate application of *B. bassiana* before, during, or after *O. nubilalis* larvae were introduced to plants (44). The model accurately predicted timing of larval mortality when *B. bassiana* was applied at the same time or before *O. nubilalis*. However, when *O. nubilalis* larvae were placed on corn plants before *B. bassiana*, the model underestimated time to death. Model results therefore suggested that this application sequence produced delayed interactions in the field. In an examination of practical pest-control questions, a complex model simulating interactions between an *Erynia* sp. and *Hypera postica* was used to investigate retention of windrows after alfalfa harvest in an effort to aggregate *H. postica* adults in a moist environment, thereby enhancing conditions for epizootic development (16). In addition, thresholds for early insecticide applications were evaluated. After field validation, investigators proposed new recommendations for control of *H. postica* using cultural practices to enhance *Erynia* sp. infection levels.

A simulation model has also been used to test the timing of *L. dispar* infection by resting spores of *E. maimaiga* (69). Experiments to study this phenomenon are impossible in the forest environment. Behavior of *L. dispar* larvae varies by instar, which can alter larval exposure to the reservoir of resting spores in the soil. Following the incorporation of coefficients for instar-specific exposure of larvae to germinating resting spores, the model results demonstrated that resting spore infection of late instars was important in the development of epizootics. This model was also used to evaluate the ability of *E. maimaiga* to cause abundant infection under weather conditions throughout the northeastern US. The rapid spread and establishment by *E. maimaiga* throughout this area between 1989 and 1992 have validated model results.

A model of the *E. grylli–C. pellucida* system demonstrated that, while

both initial host and pathogen densities influenced resulting infection levels, the impact of host density was by far more dramatic (24). These results are reasonable because pathogens can rapidly increase in abundance during a field season if conditions are favorable, while host population densities are relatively slow at changing. The extent of density dependence of natural enemies has historically been a major concern in biological control, and the basic model used for most fungal pathogen systems assumes density dependence (2, 16). Unfortunately, experimental studies testing host density relationships of fungal diseases are sorely lacking.

Host-density thresholds for epizootic development have been documented in the *H. postica–Erynia* sp. system (17). The concept of a theshold host density above which epizootics can develop as defined by modelers varies. Recent analyses demonstrate that host-density thresholds are not static but are sensitive to initial prevalence of disease and spatial and temporal dynamics of hosts (115). In agreement, field studies of the *Z. radicans–Empoasca kraemeri* system suggest a threshold pathogen density (cadavers of fungal-killed leafhoppers) before epizootics develop (51). Largely unexplored is the suggestion that an environmental threshold must be reached before epizootics can develop (161). For example, epizootics of *Z. radicans* in *E. kraemeri* do not develop when condensation occurs for <9 h per night (51).

CONTROL POTENTIAL

The impact of fungal epizootics on host populations can be very dramatic, and many attempts have been made to harness this potential for pest-control purposes. Control strategies that have been successful include permanent introduction and establishment, augmentative releases, and environmental manipulation or conservation (50). For all strategies, experience has highlighted the unique nature of interactions among fungi and hosts in specific systems. Novel approaches to control are being developed to manipulate these species-specific interactions and enhance disease prevalence.

The majority of control programs utilizing fungi rely on augmentative releases, frequently using inundative application technology similar to that used for standard chemical pesticides. Whether inundative or inoculative, these releases are generally considered augmentative because of the ubiquitous nature of pathogens. Most fungal releases differ fundamentally from applications of chemical pesticides. After fungi are released, pathogen densities should increase through disease transmission as the pathogen repeatedly cycles through the host population. Different fungal species are released using varying doses, differing fungal life stages, and differing

application strategies because of system-specific expectations based on secondary transmission levels necessary for pest control. In one example, *Beauveria brongniartii* was applied to soil-inhabiting grubs of *Melolontha melolontha* using two different techniques, and the speeds at which epizootics developed were compared (86). Barley kernels colonized by *B. brongniartii* and drilled into soil resulted in control of *M. melolontha* in orchards that was superior to control from chemical pesticides. The alternative strategy of spraying swarming adults so that egg-laying females transported *B. brongniartii* to breeding sites resulted in slower, possibly more host-density dependent, development of epizootics (86). To control mosquitoes in rice fields, two types of spores are applied simultaneously; application of both sexual and asexual spores of *L. giganteum* resulted in both immediate and long-term mosquito control under varying environmental conditions (91).

The majority of control efforts have emphasized inundative augmentation, the use of so-called mycoinsecticides. Inundative releases have resulted in some control successes, notably among greenhouse pests, rice pests, spittlebugs, and pine caterpillars (133, see 100a, 136, 174), and currently, several entomopathogenic fungi are commercially available for inundative release (136). Selection of an optimal strain is crucial to successful control, e.g. a strain of *M. anisopliae* tolerant to low Tasmanian temperatures has been used successfully to control *Adoryphorus couloni,* a pasture scarab (131). In addition, cost-efficient fungal mass production and effective formulations are critical elements in development of mycoinsecticides (100a).

Novel techniques to enhance fungal infection are under evaluation in pest systems that are generally difficult to control using other methods. *Plutella xylostella* males are attracted to pheromone traps where they are dosed with *Z. radicans,* which they subsequently carry to insecticide-resistant larvae in the field (118). *B. bassiana* and *Bacillus thuringiensis* were applied simultaneously to corn plants for control of *O. nubilalis* (97). Although activity of these two agents was not synergistic, *B. bassiana* applied to first-generation larvae persisted in plants and caused infections in the second generation. Baits containing fungal pathogens are being developed for control of cryptic scarab grubs in pastures (87; DE Pinnock, personal communication). Fungus-containing baits have also been investigated for control of fire ants, *Solenopsis invicta* (159), and leaf-cutting ants (8) in soil; direct applications of fungal pathogens to these social insects have proven ineffective.

Permanent introduction and establishment of entomopathogenic fungi has been successfully used for 19 different host-pathogen systems at 26 locations (136). Recent classical biological-control efforts have used fungal strains originating in areas climatically similar to release sites (109; RI Carruthers,

personal communication). However, recent releases have been troubled by public concern over potential negative side-effects (100), and scientists are presently trying to objectively clarify concerns (25) and establish clearer guidelines to direct future releases (103).

Entomophthoralean resting spores have not previously been used extensively for control purposes because of their prolonged dormancy and asynchronous germination (46). However, a small-scale comparison of methods for introduction and establishment of the gypsy moth pathogen *E. maimaiga* in northeastern North America revealed superior infection levels when resting spores were introduced and moistened on a weekly basis (70). Based on these results, resting spores were released during 1991–1992 at 39 sites where this fungus had not previously been detected. *E. maimaiga* became established at the majority of sites and, where water was applied weekly to spores, increased infection levels resulted (AE Hajek & JS Elkinton, unpublished data). To address public concerns, host-specificity trials demonstrated that *E. maimaiga* is quite specific to the Lymantriidae and generally causes low levels of infection only in some species of related families (AE Hajek & L Butler, unpublished data).

The environmental sensitivity of some fungal stages has led to development in a few systems of conservation strategies for control. Many fungal stages are sensitive to desiccation, and as a consequence, techniques have frequently been aimed at increasing moisture levels. As mentioned previously, altered timing of alfalfa harvest has been suggested to maximize *H. postica* infection by an *Erynia* sp. in moist environments within windrows (16). Field studies of *Aphis fabae* populations demonstrated that irrigation was associated with increased infection by *P. neoaphidis* and *C. obscurus,* although *Neozygites fresenii* and *Entomophthora planchoniana* infection levels did not increase (173). In mushroom hothouses in China, *Erynia ithacensis* infection levels in gnat populations were increased by spraying water daily in areas where cadavers were abundant (78). Many fungi are also sensitive to pesticides, especially fungicides, and pesticide applications have been altered in conjunction with the use of fungi for insect control (26). Potential for overcoming pesticide sensitivity in entomopathogenic fungi has been demonstrated with an isolate of *M. anisopliae* transformed experimentally to benomyl resistance (55).

An underexplored research area is the detection, isolation, characterization, and commercial development of the toxins of entomopathogenic fungi for insect control (135). These advances may be aided by recombinant DNA techniques; however, the fact that toxins from *Metarhizium* spp. (destruxins) are the products of complex, multigenic pathways will increase the difficulty of isolating and manipulating specific toxin genes.

CONCLUSIONS

Naturally occurring epizootics of fungal diseases of insects are ever-present reminders of the potential of these pathogens for insect control. However, epizootics are complex phenomena; a profusion of interacting processes in specific states are necessary for development of epizootics. In past years, attempts to use fungal entomopathogens for inundative releases, similar to use of synthetic chemical insecticides, have frequently been unsuccessful, and we now realize that to harness epizootics, we must understand which interactions are critical determinants of pathogenicity and epizootic development. Research is progressing simultaneously along many avenues, and the technology needed to make further progress in these fields of research is now available. Biochemical and molecular investigations of host-pathogen interactions are defining those attributes yielding increased pathogenicity and are aimed at manipulation of specific fungal processes. Investigations at the organismal level encompass studies of the development and activity of various stages of host and pathogen, frequently in association with variations in environmental conditions. Ultimately, data from some host-pathogen systems have been assembled to create models used for answering questions about conditions necessary for epizootic development on the population and community levels. These collective studies advance our knowledge of fungal diseases in general, and this knowledge will also be useful in further developing our ability to use fungi to control insects.

The species of entomopathogenic fungi that have significant impacts on host populations are many and diverse. Unfortunately, few fungal-insect systems have been studied in great detail. Studies conducted to date, however, provide frameworks for further investigation and generate hypotheses to be tested in other host-pathogen systems. Obviously, with the great diversity of fungi and the many routes by which they have achieved their success as pathogens, not everything learned about a few model systems will apply throughout other systems. Still, we are developing a knowledge base from which to compare systems and discover commonalities in pathogenicity and epizootiology.

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Literature Cited

1. Allee LL, Goettel MS, Gol'berg A, Whitney HS, Roberts DW. 1990. Infection by *Beauveria bassiana* of *Leptinotarsa decemlineata* larvae as a consequence of fecal contamination of the integument following *per os* inoculation. *Mycopathologia* 111:17–24
2. Anderson RM, May RM. 1981. The population dynamics of microparasites and their invertebrate hosts. *Trans. R. Philos. Soc. Ser. B* 291:451–524
3. Apperson CS, Federici BA, Tarver FR, Stewart W. 1992. Biotic and abiotic parameters associated with an epizootic of *Coelomomyces punctatus* in a larval population of the mosquito *Anopheles quadrimaculatus*. *J. Invertebr. Pathol.* 60:219–28

3a. Arora DK, Ajello L, Mukerji KG, eds. 1991. *Handbook of Applied Mycology*, Vol. 2, *Humans, Animals, and Insects*. New York: Marcel Dekker

4. Bidochka MJ, Khachatourians GG. 1991. Microbial and protozoan pathogens of grasshoppers and locusts as potential biocontrol agents. *Biocontr. Sci. Technol.* 1:243–59
5. Bidochka MJ, McDonald MA, St. Leger RJ, Roberts DW. 1993. Differentiation of species and strains of entomopathogenic fungi by random amplification of polymorphic DNA (RAPD). *Curr. Genet.* In press
6. Bing LA, Lewis LC. 1991. Suppression of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) by endophytic *Beauveria bassiana* (Balsamo) Vuillemin. *Environ. Entomol.* 20:1207–11
7. Bing LA, Lewis LC. 1992. Temporal relationships between *Zea mays*, *Ostrinia nubilalis* (Lep.: Pyralidae) and endophytic *Beauveria bassiana*. *Entomophaga* 37:525–36
8. Blowers M, Jackson CW, Knapp JJ. 1992. Effect of composition of alginate granules on their potential as carriers of microbial control agents against the leaf-cutting ant *Atta sexdens*. In *Biology and Evolution of Social Insects*, ed. J Billen, pp. 145–51. Leuven/ Belgium: Leuven Univ. Press
9. Boucias D, Latgé J-P. 1988. Fungal elicitors of invertebrate cell defense systems. In *Fungal Antigens*, ed. E Drouhet, GT Cole, L de Repentigny, J-P Latgé, B Dupont, pp. 121–37. New York: Plenum
10. Boucias DG, McCoy CM, Joclyn OJ. 1982. Isozyme differentiation among 17 geographical isolates of *Hirsutella thompsonii*. *J. Invertebr. Pathol.* 39: 329–37
11. Boucias DG, Pendland JC. 1987. Detection of protease inhibitors in the haemolymph of resistant *Anticarsia gemmatalis* inhibitory to the entomopathogenic fungus, *Nomuraea rileyi*. *Experientia* 43:336–39
12. Boucias DG, Pendland JC. 1991. Attachment of mycopathogens to cuticle: the initial event of mycosis in arthropod hosts. See Ref. 29a, pp. 101–28
13. Brey PT, Latgé J-P, Prevost MC. 1986. Integumental penetration of the pea aphid, *Acyrthosiphon pisum*, by *Conidiobolus obscurus*. *J. Invertebr. Pathol.* 48:34–41
14. Brobyn PJ, Wilding N, Clark SJ. 1987. Laboratory observations on the effect of humidity on the persistence of infectivity of conidia of the aphid pathogen *Erynia neoaphidis*. *Ann. Appl. Biol.* 110:579–84
15. Brown GC. 1984. Stability in an insect-pathogen model incorporating age-dependent immunity and seasonal host reproduction. *Bull. Math. Biol.* 46:139–53
16. Brown GC. 1987. Modeling. See Ref. 50a, pp. 43–68
17. Brown GC, Nordin GL. 1982. An epizootic model of an insect-fungal pathogen system. *Bull. Math. Biol.* 44:731–39
18. Butt TM, Wraight SP, Galaini-Wraight S, Humber RA, Roberts DW, Soper RS. 1988. Humoral encapsulation of the fungus *Erynia radicans* (Entomophthorales) by the potato leafhopper *Empoasca fabae* (Homoptera: Cicadellidae). *J. Invertebr. Pathol.* 52:49–56
19. Carruthers RI. 1981. *The biology and ecology of* Entomophthora muscae *in the onion agroecosystem*. PhD thesis, Michigan State Univ., East Lansing, Mich.
20. Carruthers RI, Feng Z, Ramos ME, Soper RS. 1988. The effect of solar radiation on the survival of *Entomophaga grylli* (Entomophthorales: Entomophthoraceae) conidia. *J. Invertebr. Pathol.* 52:154–62
21. Carruthers RI, Feng Z, Robson DS, Roberts DW. 1985. In vivo temperature-dependent development of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) mycosis of the European corn borer, *Ostrinia nubilalis* (Lepidoptera:

Pyralidae). *J. Invertebr. Pathol.* 46: 305–11
22. Carruthers RI, Haynes DL, MacLeod DM. 1985. *Entomophthora muscae* (Entomophthorales: Entomophthoraceae) mycosis in the onion fly, *Delia antiqua* (Diptera: Anthomyiidae). *J. Invertebr. Pathol.* 45:81–93
23. Carruthers RI, Larkin TS, Firstencel H, Feng Z. 1992. Influence of thermal ecology on the mycosis of a rangeland grasshopper. *Ecology* 73:190–204
24. Carruthers RI, Larkin TS, Soper RS. 1988. Simulation of insect disease dynamics: an application of SERB to a rangeland ecosystem. *Simulation* 51: 101–9
25. Carruthers RI, Onsager JA. 1993. A perspective on the use of exotic natural enemies for biological control of pest grasshoppers. *Environ. Entomol.* In press
26. Carruthers RI, Soper RS. 1987. Fungal diseases. See Ref. 50a, pp. 357–416
27. Cerenius L, Thornquist PO, Vey A, Johansson MW, Soderhäll, K. 1990. The effect of the fungal toxin destruxin E on isolated crayfish haemocytes. *J. Insect Physiol.* 36:785–89
28. Charnley AK. 1989. Mechanisms of fungal pathogenesis in insects. In *The Biotechnology of Fungi for Improving Plant Growth*, ed. JM Whipps, RD Lumsden, pp. 85–125. London: Cambridge Univ. Press
29. Charnley AK, St. Leger RJ. 1991. The role of cuticle-degrading enzymes in fungal pathogenesis in insects. See Ref. 29a, pp. 267–86
29a. Cole GT, Hoch HC, eds. 1991. *The Fungal Spore and Disease Initiation in Plants and Animals*. New York: Plenum
30. Costa SD, Gaugler R. 1989. Sensitivity of *Beauveria bassiana* to solanine and tomatine: plant defensive chemicals inhibit an insect pathogen. *J. Chem. Ecol.* 15:697–706
31. Costa SD, Gaugler R. 1989. Influence of *Solanum* host plants on Colorado potato beetle (Coleoptera: Chrysomelidae) susceptibility to the entomopathogen *Beauveria bassiana*. *Environ. Entomol.* 18:531–36
32. David WAL. 1967. The physiology of the insect integument in relation to the invasion of pathogens. In *Insects and Physiology*, ed. JWL Beament, JE Treherne, pp. 17–35. London: Oliver & Boyd
33. Dennell R. 1946. A study of an insect cuticle: the larval cuticle of *Sarcophaga faculata* Pand. (Diptera). *Proc. R. Soc. London Ser. B* 133:348–73
34. Dunn PE. 1986. Biochemical aspects of insect immunology. *Annu. Rev. Entomol.* 31:321–39
35. Dunphy GB, Nolan RA. 1982. Cellular immune response of spruce budworm larvae to *Entomophthora egressa* protoplasts and other test particles. *J. Invertebr. Pathol.* 39:81–92
36. Eguchi M. 1982. Inhibition of the fungal protease by haemolymph protease inhibitors of the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). *Appl. Entomol. Zool.* 17:589–90
37. Eguchi M, Ueda K, Yamashita M. 1984. Genetic variants of protease inhibitors against fungal protease and alpha-chymotrypsin from haemolymph of the silkworm, *Bombyx mori*. *Biochem. Genet.* 22:1093–1102
38. Eilenberg J. 1987. Abnormal egg-laying behaviour of female carrot flies (*Psila rosae*) induced by the fungus *Entomophthora muscae*. *Entomol. Exp. Appl.* 43:61–65
39. Ekbom BS, Pickering J. 1990. Pathogenic fungal dynamics in a fall population of the blackmargined aphid (*Monellia caryella*). *Entomol. Exp. Appl.* 57:29–37
40. Elkinton JS, Hajek AE, Boettner GH, Simons EE. 1991. Distribution and apparent spread of *Entomophaga maimaiga* (Zygomycetes: Entomophthorales) in gypsy moth (Lepidoptera: Lymantriidae) populations in North America. *Environ. Entomol.* 20: 1601–5
41. Evans HC. 1989. Mycopathogens of insects of epigeal and aerial habitats. See Ref. 172a, pp. 205–38
42. Fargues J, Mániania NK. 1992. Variabilité de la sensibilité de *Spodoptera littoralis* [Lep.: Noctuidae] a l'Hyphomycete entomopathogène *Nomuraea rileyi*. *Entomophaga* 37: 545–54
43. Feng M-G, Nowierski RM. 1992. Spatial patterns and sampling plans for cereal aphids [Hom.: Aphididae] killed by entomophthoralean fungi and hymenopterous parasitoids in spring wheat. *Entomophaga* 37:265–75
44. Feng Z, Carruthers RI, Larkin TS, Roberts DW. 1988. A phenology model and field evaluation of *Beauveria bassiana* (Bals.) Vuillemin (Deuteromycotina: Hyphomycetes) mycosis of the European corn borer, *Ostrinia nubilalis* (Hbn.) (Lepidoptera: Pyralidae). *Can. Entomol.* 120:133–44

45. Fernandez-Garcia E, Fitt BDL. 1993. Dispersal of the entomopathogen *Hirsutella cryptosclerotium* by simulated rain. *J. Invertebr. Pathol.* 61:39–43
46. Ferron P. 1978. Biological control of insect pests by entomogenous fungi. *Annu. Rev. Entomol.* 23:409–42
47. Ferron P, Fargues J, Riba G. 1991. Fungi as microbial insecticides against pests. See Ref. 3a, pp. 665–706
48. Folegatti MEG, Alves SB. 1987. Interaction between the fungus *Metarhizium anisopliae* (Metsch.) Sorok., and the main parasitoids of the sugar cane borer, *Diatraea saccharalis* (Fabr.). *An. Soc. Entomol. Brasil* 16:351–62
49. Fuxa JR. 1984. Dispersion and spread of the entomopathogenic fungus *Nomuraea rileyi* (Moniliales: Moniliaceae) in a soybean field. *Environ. Entomol.* 13:252–58
50. Fuxa JR. 1987. Ecological considerations for the use of entomopathogens in IPM. *Annu. Rev. Entomol.* 32:225–51
50a. Fuxa JR, Tanada Y, eds. 1987. *Epizootiology of Insect Diseases*. New York: Wiley
51. Galaini-Wraight S, Wraight SP, Carruthers RI, Magalhaes BP, Roberts DW. 1991. Description of a *Zoophthora radicans* (Zygomycetes: Entomophthoraceae) epizootic in a population of *Empoasca kraemeri* (Homoptera: Cicadellidae) on beans in central Brazil. *J. Invertebr. Pathol.* 58:311–26
51a. Gallardo F, Boethel DJ, Fuxa JR, Richter A. 1990. Susceptibility of *Heliothis zea* (Boddie) larvae to *Nomuraea rileyi* (Farlow) Samson: effects of α-tomatine at the third trophic level. *J. Chem. Ecol.* 16:1751–59
52. Gillespie AT, Claydon N. 1989. The use of entomogenous fungi for pest control and the role of toxins in pathogenesis. *Pest. Sci.* 27:203–15
52a. Glare TR, Milner RJ. 1991. Ecology of entomopathogenic fungi. See Ref. 3a, pp. 547–612
53. Glare TR, Milner RJ, Chilvers GA. 1986. The effect of environmental factors on the production, discharge, and germination of primary conidia of *Zoophthora phalloides* Batko. *J. Invertebr. Pathol.* 48:275–83
54. Glare TR, Milner RJ, Chilvers GA. 1989. Factors affecting the production of resting spores by *Zoophthora radicans* in the spotted alfalfa aphid, *Therioaphis trifolii* f. *maculata*. *Can. J. Bot.* 67:848–55
55. Goettel MS, St. Leger RJ, Bhairi S, Jung MK, Oakley BR, Staples RC. 1989. Transformation of the entomopathogenic fungus *Metarhizium anisopliae* using the *Aspergillus nidulans benA3* gene. *Curr. Genet.* 17:129–32
56. Goettel MS, St. Leger RJ, Rizzo NW, Staples RC, Roberts DW. 1989. Ultrastructural localization of a cuticle-degrading protease produced by the entomopathogenic fungus *Metarhizium anisopliae* during penetration of host (*Manduca sexta*) cuticle. *J. Gen. Microbiol.* 135:2233–39
57. Goettel MS, Vandenberg JD, Duke GM, Schaalje GB. 1993. Susceptibility to chalkbrood of alfalfa leafcutter bees, *Megachile rotundata*, reared on natural and artificial provisions. *J. Invertebr. Pathol.* 61:58–61
58. Gopalakrishnan C, Narayanan K. 1989. Epizootiology of *Nomuraea rileyi* (Farlow) Samson in field populations of *Helicoverpa* (= *Heliothis*) *armigera* (Hubner) in relation to three host plants. *J. Biol. Control* 3:50–52
59. Götz, P. 1986. Encapsulation in arthropods. In *Immunity in Invertebrates*, ed. M. Brehélin, pp. 153–70. Berlin: Springer-Verlag
60. Gould JR, Elkinton JS, Van Driesche RG. 1992. Suitability of approaches for measuring parasitoid impact on *Lymantria dispar* (Lepidoptera: Lymantriidae) populations. *Environ. Entomol.* 21:1035–45
61. Groden E, Lockwood JL. 1991. Effects of soil fungistasis on *Beauveria bassiana* and its relationship to disease incidence in the Colorado potato beetle, *Leptinotarsa decemlineata*, in Michigan and Rhode Island soils. *J. Invertebr. Pathol.* 57:7–16
62. Gugnani HC, Okafor JI. 1980. Mycotic flora of the intestine and other internal organs of certain reptiles and amphibians with special reference to characterization of *Basidiobolus* isolates. *Mykosen* 23:260–68
63. Gupta AP. 1986. *Hemocytic and Humoral Immunity in Arthropods*. New York: Wiley
64. Hajek AE. 1989. Food consumption by *Lymantria dispar* (Lepidoptera: Lymantriidae) larvae infected with *Entomophaga maimaiga* (Zygomycetes: Entomophthorales). *Environ. Entomol.* 18:723–27
65. Hajek AE, Butt TM, Strelow LI, Gray SM. 1991. Detection of *Entomophaga maimaiga* (Zygomycetes: Entomophthorales) using enzyme-linked im-

munosorbent assay (ELISA). *J. Invertebr. Pathol.* 58:1–9
66. Hajek AE, Carruthers RI, Soper RS. 1990. Temperature and moisture relations of sporulation and germination by *Entomophaga maimaiga* (Zygomycetes: Entomophthoraceae), a fungal pathogen of *Lymantria dispar* (Lepidoptera: Lymantriidae). *Environ. Entomol.* 19:85–90
67. Hajek AE, Humber RA, Elkinton JS, May B, Walsh SRA, Silver JC. 1990. Allozyme and RFLP analyses confirm *Entomophaga maimaiga* responsible for 1989 epizootics in North American gypsy moth populations. *Proc. Natl. Acad. Sci. USA* 87:6979–82
68. Hajek AE, Humber RA, Walsh SRA, Silver JC. 1991. Sympatric occurrence of two *Entomophaga aulicae* (Zygomycetes: Entomophthorales) complex species attacking forest Lepidoptera. *J. Invertebr. Pathol.* 58:373–80
69. Hajek AE, Larkin TS, Carruthers RI, Soper RS. 1993. Simulating the dynamics of *Entomophaga maimaiga* (Zygomycetes: Entomophthorales) epizootics in gypsy moth populations. *Environ. Entomol.* In press
70. Hajek AE, Roberts DW. 1991. Pathogen reservoirs as a biological control resource: introduction of *Entomophaga maimaiga* to North American gypsy moth, *Lymantria dispar,* populations. *Biol. Contr.* 1:29–34
71. Hajek AE, Soper RS. 1992. Temporal dynamics of *Entomophaga maimaiga* after death of gypsy moth (Lepidoptera: Lymantriidae) larval hosts. *Environ. Entomol.* 21:129–35
72. Harcourt DG, Guppy JC, Tyrrell D. 1990. Phenology of the fungal pathogen *Zoophthora phytonomi* in southern Ontario populations of the alfalfa weevil (Coleoptera: Curculionidae). *Environ. Entomol.* 19:612–17
73. Hare JD, Andreadis TG. 1983. Variation in susceptibility of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) when reared on different host plants to the fungal pathogen, *Beauveria bassiana,* in the field and laboratory. *Environ. Entomol.* 12:1892–97
74. Harper JD, Herbert DA, Moore RE. 1984. Trapping patterns of *Entomophthora gammae* (Weiser) (Entomophthorales: Entomophthoraceae) conidia in a soybean field infested with the soybean looper, *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae). *Environ. Entomol.* 13: 1186–90
75. Hassan AEM, Charnley AK. 1989. Ultrastructural study of the penetration by *Metarhizium anisopliae* through Dimilin-affected cuticle of *Manduca sexta*. *J. Invertebr. Pathol.* 54:117–24
76. Hochberg ME. 1989. The potential role of pathogens in biological control. *Nature* 337:262–65
77. Hou RF, Chang J. 1985. Cellular defense response to *Beauveria bassiana* in the silkworm, *Bombyx mori*. *Appl. Entomol. Zool.* 20:118–25
78. Huang Y, Zhen B, Li Z. 1992. Natural and induced epizootics of *Erynia ithacensis* in mushroom hothouse populations of yellow-legged fungus gnats. *J. Invertebr. Pathol.* 60:254–58
79. Hughes RD, Bryce MA. 1984. Biological characterization of two biotypes of pea aphid, one susceptible and the other resistant to fungal pathogens, coexisting on lucerne in Australia. *Entomol. Exp. Appl.* 36:225–29
80. Humber RA. 1991. Fungal pathogens of aphids. In *Proceedings on Aphid-Plant Interactions: Populations to Molecules,* ed. DC Peters, JA Webster, CS Chlouber, pp. 45–56. Stillwater: Okla. State Univ.
81. Hung S, Boucias DG. 1992. Influence of *Beauveria bassiana* on the cellular defense response of the beet armyworm, *Spodoptera exigua*. *J. Invertebr. Pathol.* 60:152–58
82. Hung S-Y, Boucias DG, Vey A. 1993. Effect of *Beauveria bassiana* and *Candida albicans* on the cellular defense response of *Spodoptera exigua*. *J. Invertebr. Pathol.* 61:179–87
83. Huxham IM, Lackie AM, McCorkindale NJ. 1989. Inhibitory effects of cyclodepsipeptides, destruxins, from the fungus *Metarhizium anisopliae* on cellular immunity in insects. *J. Insect Physiol.* 35:97–105
84. Ignoffo CM, Garcia C, Hostetter DL, Pinnell RE. 1977. Laboratory studies of the entomopathogenic fungus *Nomuraea rileyi:* soil-borne contamination of soybean seedlings and dispersal of diseased larvae of *Trichoplusia ni*. *J. Invertebr. Pathol.* 29:147–52
84a. Iijima R, Kurata S, Natori S. 1993. Purification, characterization, and cDNA cloning of an antifungal protein from the hemolymph of *Sarcophaga peregrina* (flesh fly) larvae. *J. Biol. Chem.* 268:12055–61
85. Keller S. 1992. Control of *Melolontha melolontha* with *Beauveria brongniartii:* comparison of two methods. *Annu. Meeting Soc. Invertebr. Pathol., 25th,* p. 175 (Abstr.)

86. Keller S. 1992. The *Beauveria-Melolontha* project: experiences with regard to locust and grasshopper control. In *Biological Control of Locusts and Grasshoppers*, ed. CJ Lomer, C Prior, pp. 279–86. Wallingford, UK: CAB International
87. Keller S, Zimmermann G. 1989. Mycopathogens of soil insects. See Ref. 172a, pp. 205–38
88. Kerwin JL. 1984. Fatty acid regulation of the germination of *Erynia variabilis* conidia on adults and puparia of the lesser housefly, *Fannia canicularis*. *Can. J. Microbiol.* 30:158–61
89. Kerwin JL. 1992. EPA registers *Lagenidium giganteum* for mosquito control. *Soc. Invertebr. Pathol. Newsl.* 24:8–9
90. Kerwin JL, Washino RK. 1986. Regulation of oosporogenesis by *Lagenidium giganteum:* promotion of sexual reproduction by unsaturated fatty acids and sterol availability. *Can. J. Microbiol.* 32:294–300
91. Kerwin JL, Washino RK. 1986. Ground and aerial application of the sexual and asexual stages of *Lagenidium giganteum* (Oomycetes: Lagenidiales) for mosquito control. *J. Am. Mosq. Control Assoc.* 2:182–89
92. Khachatourians GC. 1991. Physiology and genetics of entomopathogenic fungi. See Ref. 3a, pp. 613–64
93. Kramer KJ, Hopkins TL, Shaefer J. 1988. Insect cuticle structure and metabolism. In *Biotechnology for Crop Protection, American Chemical Society Symposium Series 379*, ed. P Hedin, J Menn, R Hollingworth, pp. 160–85. Washington, DC: Am. Chem. Soc.
94. Krasnoff SB, Gupta S, St. Leger RJ, Renwick JAA, Roberts DW. 1991. Myco- and entomotoxigenic properties of the efrapeptins: toxins of the fungus *Tolypocladium niveum*. *J. Invertebr. Pathol.* 58:180–88
95. Kucera M, Weiser J. 1985. Different course of proteolytic inhibitory activity and proteolytic activity in *Galleria mellonella* larvae infected by *Nosema algerae* and *Vairimorpha heterosporum*. *J. Invertebr. Pathol.* 45:41–46
96. Lackie AM. 1983. Effect of substratum wettability and charge on adhesion in vitro and encapsulation in vivo by insect haemocytes. *J. Cell. Sci.* 63:181–90
97. Lewis LC, Bing LA. 1991. *Bacillus thuringiensis* Berliner and *Beauveria bassiana* (Balsamo) Vuillemin for European corn borer control: program for immediate and season-long suppression. *Can. Entomol.* 123:387–93
98. Lloyd M, White J, Stanton N. 1982. Dispersal of fungus-infected periodical cicadas to new habitat. *Environ. Entomol.* 11:852–58
99. Locke M. 1984. The structure of insect cuticle. See Ref. 134a, pp. 38–53
100. Lockwood JA. 1992. Neoclassical biological control: a double-edged sword. *Soc. Invertebr. Pathol. Newsl.* 24:6–8
100a. McCoy CW. 1990. Entomogenous fungi as microbial pesticides. In *New Directions in Biological Control: Alternatives for Suppressing Agricultural Pests and Diseases*, ed. RR Baker, PE Dunn, pp. 115–38. New York: Liss
101. McCoy CW, Samson RA, Boucias DG. 1988. Entomogenous fungi. In *CRC Handbook of Natural Pesticides*, Vol. 5, *Microbial Insecticides*, pp. 151–236. Boca Raton: CRC
102. McDonald RM, Spokes JR. 1981. *Conidiobolus obscurus* in arable soil: a method for extracting and counting azygospores. *Soil Biol. Biochem.* 13: 551–53
103. Maddox JV, McManus ML, Jeffords MR, Webb RE. 1990. *Exotic insect pathogens as classical biological control agents with an emphasis on regulatory considerations*. Presented at Annu. Meet. Entomol. Soc. Am., New Orleans
104. Millstein JA, Brown GC, Nordin GL. 1983. Microclimatic moisture and conidial production in *Erynia* sp. (Entomophthorales: Entomophthoraceae): in vivo production rate and duration under constant and fluctuating moisture regimes. *Environ. Entomol.* 12:1344–49
105. Milne R, Wright T, Welton M, Budau C, Gringorten L, Tyrrell D. 1992. Identification and partial purification of a cell lytic factor from *Entomophaga aulicae*. *Annu. Meeting Soc. Invertebr. Pathol., 25th*, p. 230 (Abstr.)
106. Milner RJ. 1982. On the occurrence of pea aphids, *Acyrthosiphon pisum*, resistant to isolates of the fungal pathogen *Erynia neoaphidis*. *Entomol. Exp. Appl.* 32:23–27
107. Milner RJ. 1985. Distribution in time and space of resistance to the pathogenic fungus *Erynia neoaphidis* in the pea aphid *Acyrthosiphon pisum*. *Entomol. Exp. Appl.* 37:235–40
108. Milner RJ, Holdom DG, Glare TR. 1984. Diurnal patterns of mortality in aphids infected by entomophthoran fungi. *Entomol. Exp. Appl.* 36:37–42
109. Milner RJ, Soper RS, Lutton GG.

1982. Field release of an Israeli strain of the fungus *Zoophthora radicans* (Brefeld) Batko for biological control of *Therioaphis trifolii* (Monell) f. *maculata*. *J. Aust. Entomol. Soc.* 21: 113–18

110. Moore D, Reed M, Le Patourel G, Abraham YJ, Prior C. 1992. Reduction in feeding by the desert locust, *Schistocerca gregaria*, after infection with *Metarhizium flavoviride*. *J. Invertebr. Pathol.* 60:304–7
111. Mullens BA. 1985. Host age, sex, and pathogen exposure level as factors in the susceptibility of *Musca domestica* to *Entomophthora muscae*. *Entomol. Exp. Appl.* 37:33–39
112. Mullens BA, Rodriguez JL. 1985. Dynamics of *Entomophthora muscae* (Entomophthorales: Entomophthoraceae) conidial discharge from *Musca domestica* (Diptera: Muscidae) cadavers. *Environ. Entomol.* 14:317–22
113. Murrin F, Nolan RA. 1987. Ultrastructure of the infection of spruce budworm larvae by the fungus *Entomophaga aulicae*. *Can. J. Bot.* 65:1694–1706
114. Onstad DW, Carruthers RI. 1990. Epizootiological models of insect diseases. *Annu. Rev. Entomol.* 35:399–419
115. Onstad DW, Maddox JV, Cox DJ, Kornkven EA. 1990. Spatial and temporal dynamics of animals and the host-density threshold in epizootiology. *J. Invertebr. Pathol.* 55:76–84
116. Papierok B, Wilding N. 1979. Mise en evidence d'une différence de sensibilité entre 2 clones du puceron du pois *Acyrthosiphon pisum* Harr. (Homoptères: Aphididae), exposé à 2 souches du champignon Phycomycète *Entomophthora obscura* Hall & Dunn. *C. R. Acad. Sci. Ser. D.* 288:93–95
117. Pekrul S, Grula EA. 1979. Mode of infection of the corn earworm (*Heliothis zea*) by *Beauveria bassiana* as revealed by scanning electron microscopy. *J. Invertebr. Pathol.* 34:238–47
118. Pell JK, Macaulay EDM, Wilding N. 1993. A pheromone trap for dispersal of the pathogen *Zoophthora radicans* Brefeld (Zygomycetes: Entomophthorales) amongst populations of the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). *Biocontrol Sci. Technol.* In press
119. Pendland JC, Boucias DG. 1992. Ultrastructural localization of carbohydrate in cell walls of the entomogenous hyphomycete *Nomuraea rileyi*. *Can. J. Microbiol.* 38:377–86
120. Pendland JC, Heath MA, Boucias DG. 1988. Function of a galactose-binding lectin from *Spodoptera exigua* larval haemolymph: opsonisation of blastospores from entomogenous Hyphomycetes. *J. Insect Physiol.* 34: 533–40
121. Pereira RM, Stimac JL, Alves SB. 1993. Soil antagonism affecting the dose-response of workers of the red imported fire ant, *Solenopsis invicta*, to *Beauveria bassiana* conidia. *J. Invertebr. Pathol.* 61:156–61
122. Perry DF, Fleming RA. 1989. The timing of *Erynia radicans* resting spore germination in relation to mycosis of *Choristoneura fumiferana*. *Can. J. Bot.* 67:1657–63
123. Perry DF, Régnière, J. 1986. The role of fungal pathogens in spruce budworm population dynamics: frequency and temporal relationships. In *Fundamental and Applied Aspects of Invertebrate Pathology, 4th Int. Colloq. Invertebrate Pathology*, ed. RA Samson, JM Vlak, D Peters, pp. 167–70. Wageningen, The Netherlands: Grafisch bedrijf Ponsen & Looijen
124. Perry DF, Whitfield GH. 1985. The interrelationships between microbial entomopathogens and insect hosts: a system study approach with particular reference to the Entomophthorales and the eastern spruce budworm. In *Animal-Microbial Interactions*, ed. JM Anderson, ADM Rayner, D Walton, pp. 307–31. London: Cambridge Univ. Press
125. Pickering J, Gutierrez AP. 1991. Differential impact of the pathogen *Pandora neoaphidis* (R. & H.) Humber (Zygomycetes: Entomophthorales) on the species composition of *Acyrthosiphon* aphids in alfalfa. *Can. Entomol.* 123:315–20
126. Powell W, Wilding N, Brobyn PJ, Clark SJ. 1986. Interference between parasitoids [Hym.: Aphidiidae] and fungi [Entomophthorales] attacking cereal aphids. *Entomophaga* 31:293–302
127. Raghavaiah G, Jayaramaiah M. 1987. Antifungal activity of selected plant extracts against the white muscardine fungus. *Curr. Res.* 17:62–64
128. Rakotonirainy MS, Dutertre M, Brygou Y, Riba G. 1991. rRNA sequence comparison of *Beauveria bassiana*, *Tolypocladium cylindrosporum* and *Tolypocladium extinguens*. *J. Invertebr. Pathol.* 57:17–22
129. Ramoska WA, Todd T. 1985. Variation in efficacy and viability of *Beauveria bassiana* in the chinch bug (Hemiptera: Lygaeidae) as a result of

feeding activity on selected host plants. *Environ. Entomol.* 14:146–48
130. Rath AC. 1992. The long-term control of the scarab, *Adoryphorus couloni*, with *Metarhizium anisopliae*. *Annu. Meeting Soc. Invertebr. Pathol., 25th*, p. 180. (Abstr.)
131. Rath AC, Koen TB, Worladge D, Anderson GC. 1990. Control of the subterranean pasture pest *Adoryphorus couloni* (Coleoptera: Scarabaeidae) with *Metarhizium anisopliae* isolate DAT F-001. *Proc. 5th Int. Colloq. Invertebr. Pathol.* p. 35 (Abstr.)
132. Ratner S, Vinson SB. 1983. Phagocytosis and encapsulation: cellular immune responses in Arthropoda. *Am. Zool.* 23:185–94
133. Ravensberg WJ, Malais M, Van der Schaaf DA. 1990. *Verticillium lecanii* as a microbial insecticide against glasshouse whitefly. In *Brighton Crop Protection Conf. Pests and Diseases*, pp. 265–68. Surrey, UK: Br. Crop Prot. Council
134. Riba G, Bouviar-Fourcade I, Caudal A. 1986. Isoenzymes polymorphism in *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) entomogenous fungi. *Mycopathologia* 96:161–69
134a. Roberts DW, Aist JR, eds. 1984. *Conference Report on Infection Processes of Fungi*. New York: Rockefeller Foundation
135. Roberts DW, Gupta S, St. Leger RJ. 1992. Metabolite production by entomopathogenic fungi. *Pesqui. Agropecu. Bras.* 27:325–47
136. Roberts DW, Hajek AE. 1992. Entomopathogenic fungi as bioinsecticides. In *Frontiers in Industrial Mycology*, ed. GF Leatham, pp. 144–59. New York: Chapman & Hall
137. St. Leger RJ. 1991. Integument as a barrier to microbial infections. In *The Physiology of Insect Epidermis*, ed. A Retnakaran, K Binnington, pp. 286–308. Australia: CSIRO
138. St. Leger RJ, Allee LL, May B, Staples RC, Roberts DW. 1992. World-wide distribution of genetic variation among isolates of *Beauveria* spp. *Mycol. Res.* 96:1007–15
139. St. Leger RJ, Charnley AK, Cooper RM. 1987. Characterization of cuticle-degrading proteases produced by the entomopathogen *Metarhizium anisopliae*. *Arch. Biochem. Biophys.* 253: 221–32
140. St. Leger RJ, Frank DC, Roberts DW, Staples RC. 1992. Molecular cloning and regulatory analysis of the cuticle-degrading protease structural gene for the entomopathogenic fungus *Metarhizium anisopliae*. *Eur. J. Biochem.* 204:991–1001
141. St. Leger RJ, Goettel M, Roberts DW, Staples RC. 1991. Pre-penetration events during infection of host cuticle by *Metarhizium anisopliae*. *J. Invertebr. Pathol.* 58:168–79
142. St. Leger RJ, May B, Allee LL, Frank DC, Roberts DW. 1992. Genetic differences in allozymes and in formation of infection structures among isolates of the entomopathogenic fungus, *Metarhizium anisopliae*. *J. Invertebr. Pathol.* 60:89–101
143. St. Leger RJ, Staples RC, Roberts DW. 1991. Changes in translatable mRNA species associated with nutrient deprivation and protease synthesis in *Metarhizium anisopliae*. *J. Gen. Microbiol.* 137:807–15
144. Samson RA, Evans HC, Latgé J-P. 1988. *Atlas of Entomopathogenic Fungi*. Berlin: Springer-Verlag
145. Samuels RI, Charnley AK, Reynolds SE. 1988. The role of destruxins in the pathogenicity of three strains of *Metarhizium anisopliae* for the tobacco hornworm *Manduca sexta*. *Mycopathologia* 104:51–58
146. Samuels RI, Reynolds SE, Charnley AK. 1988. Calcium channel activation of insect muscle by destruxins, insecticidal compounds produced by the entomopathogenic fungus, *Metarhizium anisopliae*. *Comp. Biochem. Physiol.* 90C:403–12
147. Schmitz V, Dedryver C-A, Pierre J-S. 1993. Influence of an *Erynia neoaphidis* infection on the relative rate of increase of the cereal aphid *Sitobion avenae*. *J. Invertebr. Pathol.* 61:62–68
148. Shimazu MS, Soper RS. 1986. Pathogenicity and sporulation of *Entomophaga maimaiga* Humber, Shimazu, Soper and Hajek (Entomophthorales: Entomophthoraceae) on larvae of the gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae). *Appl. Entomol. Zool.* 21:589–96
149. Shimizu S, Arai Y, Matsumoto T. 1992. Electrophoretic karyotype of *Metarhizium anisopliae*. *J. Invertebr. Pathol.* 60:185–87
150. Smith RJ, Grula EA. 1981. Nutritional requirements for conidial germination and hyphal growth of *Beauveria bassiana*. *J. Invertebr. Pathol.* 37:222–30
151. Soderhäll K. 1982. Fungal cell wall beta-1,3-glucans induce clotting and phenoloxidase attachment to foreign surfaces of crayfish hemocyte lysate. *Dev. Comp. Immunol.* 5:565–73

152. Soper RS. 1985. Pathogens of leafhoppers and planthoppers. In *The Leafhoppers and Planthoppers*, ed. LR Nault, JG Rodriguez, pp. 469–88. New York: Wiley
153. Soper RA, MacLeod DM. 1981. Descriptive epizootiology of an aphid mycosis. *USDA Tech. Bull. 1632*. 17 pp.
154. Soper RS, May B, Martinell B. 1983. *Entomophaga grylli* enzyme polymorphism as a technique for pathotype identification. *Environ. Entomol.* 12: 720–23
155. Speare AT, Colley RH. 1912. *The Artificial Use of the Brown-Tail Fungus in Massachusetts, with Practical Suggestions for Private Experiment, and a Brief Note on a Fungous Disease of Gypsy Caterpillar*. Boston: Wright & Potter
156. Steinhaus EA. 1949. *Principles of Insect Pathology*. New York: McGraw-Hill
157. Stephen WP, Fichter BL. 1990. Chalkbrood (*Ascosphaera aggregata*) resistance in the leafcutting bee (*Megachile rotundata*). I. Challenge of selected lines. *Apidologie* 21:209–19
158. Stephen WP, Fichter BL. 1990. Chalkbrood (*Ascosphaera aggregata*) resistance in the leafcutting bee (*Megachile rotundata*). II. Random matings of resistant lines to wild type. *Apidologie* 21:221–31
159. Stimac JL, Pereira RM, Alves SB, Wood LA. 1990. Field evaluation of a Brazilian strain of *Beauveria bassiana* for control of the red imported fire ant, *Solenopsis invicta*, in Florida. *Proc. 5th Int. Colloq. Invertebr. Pathol.* p. 337 (Abstr.)
160. Studdert JP, Kaya HK. 1990. Water potential, temperature, and soil type on the formation of *Beauveria bassiana* soil colonies. *J. Invertebr. Pathol.* 56:380–86
161. Tanada Y. 1964. Epizootiology of insect diseases. In *Biological Control of Insect Pests and Weeds*, ed. P DeBach, EI Schlinger, pp. 548–78. London: Chapman & Hall
162. Tanada Y, Kaya HH. 1993. *Insect Pathology*. San Diego: Academic
163. Thorvilson HG, Pedigo LP. 1984. Epidemiology of *Nomuraea rileyi* (Fungi: Deuteromycotina) in *Plathypena scabra* (Lepidoptera: Noctuidae) populations from Iowa soybeans. *Environ. Entomol.* 13:1491–97
164. Tyrrell D. 1987. Induction of periodic mortality in insect larvae infected with *Entomophaga aulicae*. *Annu. Meeting Soc. Invertebr. Pathol., Gainesville, Fla.* p. 53 (Abstr.)
165. Uziel A, Kenneth RG. 1991. Survival of primary conidia and capilliconidia at different humidities in *Erynia* (subgen. *Zoophthora*) spp. and in *Neozygites fresenii* (Zygomycotina: Entomophthorales), with special emphasis on *Erynia radicans*. *J. Invertebr. Pathol.* 58:118–26
166. Vey A, Quiot JM. 1989. Effect cytotoxique in vitro et chez l'insecte hote des destruxines, toxines cyclodepsipeptidiques produites par le champignon entomopathogène *Metarhizium anisopliae*. *Can. J. Microbiol.* 35: 1000–8
167. Vey A, Quiot JM, Vago C, Fargues J. 1985. Effet immunodepresseur de toxines fongiques: inhibition de la réaction d'encapsulement multicellulaire par les destruxines. *C. R. Acad. Sci. (Paris) Ser. 3* 300:647–51
168. Villani MG. 1992. Behavioural response of scarabs to inundative release of entomopathogens in turfgrass mesocosms: implications for consistent results in the field. *Annu. Meeting Soc. Invertebr. Pathol., 25th*, p. 178 (Abstr.)
169. Walsh SRA, Tyrrell D, Humber RA, Silver JC. 1990. DNA restriction fragment length polymorphisms in the rDNA repeat unit of *Entomophaga*. *Exp. Mycol.* 14:381–92
170. Watson DW, Mullens BA, Petersen JJ. 1993. Behavioral fever response of *Musca domestica* (Diptera: Muscidae) to infection by *Entomophthora muscae*. *J. Invertebr. Pathol.* 61:10–16
171. Weiser J. 1982. Persistence of fungal insecticides: influence of environmental factors and present and future applications. In *Microbial and Viral Pesticides*, ed. E Kurstak, pp. 531–57. New York: Dekker
172. Weseloh RM, Andreadis TG. 1992. Mechanisms of transmission of the gypsy moth (Lepidoptera: Lymantriidae) fungus, *Entomophaga maimaiga* (Entomophthorales: Entomophthoraceae) and effects of site conditions on its prevalence. *Environ. Entomol.* 21:901–6
172a. Wilding N, Collins NM, Hammond PM, Webber JF, eds. 1989. *Insect-Fungus Interactions*. London: Academic
173. Wilding N, Mardell SK, Brobyn PJ. 1986. Introducing *Erynia neoaphidis* into a field population of *Aphis fabae*: form of the inoculum and effect of irrigation. *Ann. Appl. Biol.* 108:373–85

174. Wraight SP, Roberts DW. 1987. Insect control efforts with fungi. *Dev. Ind. Microbiol.* 28:77–87
175. Zacharuk RY. 1981. Fungal diseases of terrestrial insects. In *Pathogenesis of Invertebrate Microbial Diseases*, ed. EW Davidson, pp. 367–402. New Jersey: Allanheld, Osmun
176. Zahedi M, Denham DA, Ham PJ. 1992. Encapsulation and melanization responses of *Armigeres subalbatus* against inoculated Sephadex beads. *J. Invertebr. Pathol.* 59:258–63
177. Zimmermann G. 1982. Effect of high temperatures and artificial sunlight on the viability of conidia of *Metarhizium anisopliae*. *J. Invertebr. Pathol.* 40:36–40
178. Zimmermann G. 1986. The "Galleria bait method" for detection of entomopathogenic fungi in soil. *Z. Angew. Entomol.* 102:213–15

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