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Ecological factors in the inundative use of fungal entomopathogens

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Abstract Fungal entomopathogens have been developed in numerous countries as biocontrol agents with more than 100 mycoinsecticide products commercially available in 2006. The chief, perhaps sole, use of these mycoinsecticides has been as inundative agents, within a chemical paradigm. Large numbers of propagules are applied in an attempt to overwhelm by brute force many of the factors that keep a pathogen in nonepizootic equilibrium with its host. This review attempts to summarize what we know about the abiotic and biotic factors that affect the efficacy of these mycoinsecticides in both foliar and soil applications. Sunlight, humidity, temperature, and phylloplane-associated factors can affect both immediate efficacy and persistence on plants. Likewise, soil texture-moisture interactions, temperature, and a host of biotic factors can affect mycoinsecticides in the soil. Despite much research, our understanding of these ecological aspects is imperfect, especially in a holistic, dynamic sense.

Keywords *Metarhizium* · *Beauveria* · Persistence · Efficacy · UV · Humidity · Temperature · Phylloplane · Soil

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Introduction

The advent of chemical insecticides in the mid twentieth century created the concept that insect pests could be all but eliminated from threatened crops. A succession of compounds has appeared since then. Initially, many were quite toxic and environmentally damaging. In recent years, however, new materials have appeared, which address human and environmental safety concerns caused by the earlier materials. In parallel, we have realized the inadvisability of using chemicals as stand-alone, catastrophic mortality factors, and integrated pest management schemes have evolved to employ a variety of cultural, chemical, and biological tools to manage (not eradicate) pest invasion to a point below an economic threshold. Biological tools, including microbial agents, have received increasing attention as alternatives to chemicals within this context. Nevertheless, the chemical paradigm, in which a material is used to efficiently, simply, and quickly eradicate a pest problem, still persists. Microbials are too often merely substituted for chemical pesticides.

Among entomopathogens, fungi have attracted a lot of attention as biologically based pesticides. Per Faria and Wraight (2007), 129 mycoinsecticide products (fungus-based formulations targeting insects) were commercially available worldwide in 2006. Fungi used in these products are primarily ascomycetes including: *Beauveria bassiana* (Bals.) Vuill.; *B. brongniartti* (Sacc.) Petch; *Metarhizium anisopliae* (Metsch.) Sorokin*., sensuo latum, M. acridum* (formerly *M. anisopliae* var. *acridum*) (Driver and Milner) J.F. Bischof., Rehner and Humber stat. nov.; *Isaria fumosorosea* Wize (formerly *Paecilomyces fumosoroseus*); *Lecanicillium longisporum* and *muscarium* (Petch) R. Zare and W. Gams (formerly *Verticillium lecanii*); and *Hirsutella thompsoni* F.E. Fisher. In addition, the Oomycete *Lagenidium giganteum* Couch has been commercialized in the US. Inundative use of *Nomuraea rileyi* (Farl.) Samson and *Aschersonia aleyrodis* Webber has been studied and the latter fungus was briefly commercialized in Europe. Despite the number of products, mycoinsecticides have not captured a significant market share of the biopesticide market, especially in the US and EU. A principle reason is that, compared to chemical pesticides, these mycoinsecticides lack consistent, speedy efficacy in combating insect pest problems, and are more complicated to use, despite their obvious safety. The chemical paradigm too often pervades the use of biopesticides.

Fungal entomopathogens, as well as other microbial agents, can be used in several ways (Fuxa 1987; Eilenberg et al. 2001). Classical biological control involves introducing a novel fungus for permanent establishment and long term pest control. This subject is discussed by Hajek and Delalibera (2009). Inoculative biological control has the expectation that the agent will multiply, spread, and provide extended control of an insect pest, but only for a finite period. These approaches require several key characteristics, notably the ability for reproduction and horizontal transmission to create epizootic spread. Alternatively, the crop environment can be manipulated to enhance resident microbial agent populations (conservation biocontrol), as discussed by Pell et al. (2009).

The fourth approach is to inundate a crop with a microbial agent in much the same manner as a chemical pesticide. Insect control is achieved only by the organisms that were applied; there is little or no epizootic spread. Several reviews on the use of fungal entomopathogens in field crops are those of Feng et al. (1994), Wraight and Carruthers (1999), Inglis et al. (2001), Shah and Pell (2003), and Goettel et al. (2005). My review will focus on the ecological aspects affecting the efficacy of these fungi when they are used in an inundative approach in foliar and soil arenas. Understanding these factors, which affect both efficacy and persistence, will allow intelligent manipulation of insect, pathogen, crop, and especially their environment, to achieve satisfactory management of an insect pest population within the context of inundative use.

Why inundate a crop with an entomopathogenic fungus? There are regulatory and economic, as well as technical, reasons. Pesticide regulations, which include microbial agents, require generation of human and environmental safety, and in some countries, replicated verification of efficacy, a process that can require approximately US$1–1.5 M, in addition to normal development costs. If money is to be invested in commercializing a microbial pest control agent, there must be return on investment, which in turn means repeat sales. Inoculation of a crop with a self-replicating organism (classical biocontrol) defeats this purpose.

There are technical reasons for employing inundative use. Unlike the Entomophthorales, the Hypocreales, particularly *Beauveria* spp. and *Metarhizium* spp., do not commonly cause natural, large-scale epizootics among insects in annual crops, nor have many classical or inoculative biological control introductions been successful, with the possible exception of *Lecanicillium* spp. (Hajek et al. 2005). Most cropping systems and their insect pests are transient in nature, being present for only one growing season, sometimes for only a few weeks. In addition, widespread adoption of crop rotation on large areas of monoculture creates a very temporally unstable environment for any microbial agent. Annual disruption in habitat not only removes the insect hosts, but in many cases directly destroys the microbial agent. Inundation with a microbial agent, sometimes repeatedly, is therefore necessary. Inundation attempts to overcome many of the factors that keep a pathogen in nonepizootic equilibrium with its host, by overwhelming the habitat with sheer numbers of infectious propagules. Inundative use also fits, for better or worse, into the familiar chemical paradigm—farmers simply apply the fungus as they

would a chemical pesticide with the expectation of rapid, extreme efficacy.

The entomopathogenic Hypocreales have, in particular, received considerable commercial attention over the past 30–35 years because they lend themselves to in vitro mass production of sufficient quantities of infective propagules (aerial conidia, submerged conidia, or blastospores) for use in an inundative approach. In most or all cases, the propagules need to be formulated with additives to provide shelf life, inert diluents, spreaders, stickers, and emulsifiers (Jaronski 1997; Burges 1998). The propagule types and ecological considerations in formulating entomopathogenic fungi are discussed by Jackson et al. (2009). For the sake of simplicity, I will use "spore" as a generic description of the different propagules in this review, unless a specific propagule type is indicated in a cited example.

Inundative use against foliar pests

A mycopesticide can be employed inundatively using a variety of delivery methods: ground or aerial ultralow volume (ULV) sprays, medium to high-volume broadcast or directed sprays, as dusts, as granules, or distributed via autodissemination devices. Application technology has been thoroughly discussed by a number of authors (Bateman et al. 2007; Chapple et al. 2007; Mierzejewski et al. 2007; Vega et al. 2007).

Winning the numbers game

Inundative use of fungal entomopathogens, as well as other pathogens, is a "numbers game," in which one applies sufficient numbers of spores to overwhelm an insect population. Unlike other insect pathogens, these fungi are percutaneously infectious agents. They act by contact. An insect can acquire spores directly from impingement of a spray or indirectly from contact with a fungus-contaminated surface. Behavior of the insect and the nature of the plant canopy determines which of these two routes is more important. Immature whiteflies, being sessile, need the fungus "to come to them." Large insects positioned prominently in a habitat, such as gregarious locusts or migrating Mormon crickets (*Anabrus simplex* Haldeman), also present a direct target for spray applications. For insects that actively move about their habitat, e.g., thrips and Heteroptera, acquisition of spores from the environment can be more important. Some insects, e.g., nongregarious locusts and grasshoppers, present a mixed picture, in which both direct and indirect acquisition of propagules are important (Lobo Lima et al. 1992; Johnson et al. 1992). In other cases, the situation is more complex. An example is larval *Trichoplusia ni* (Hübner) on cabbage versus beans. With cabbage, applications of a *B. bassiana* resulted in nearly equal mortalities among insects exposed to direct spray contact or exposed to spray residue, whereas on beans, direct spray contact provided significant insect mortality, but mortality due to residual contact was ineffective (Behle 2006). Nevertheless, efficacy is based on the number of propagules that end up contacting the host cuticle.

Typically, inundative use of fungal entomopathogens in a field or glasshouse crop involves application of at least 10^{13}–10^{14} propagules ha^{-1} (Wraight and Carruthers 1999), although standard rates of the commercial *M. acridum* against African locusts and grasshoppers are 2–2.5 $\times$ 10^{12} conidia ha^{-1} (van der Valk 2007), with successful control being achieved under certain circumstances with 1–1.25 $\times$ 10^{12} ha^{-1} in Australia (D. Hunter personal communication). Broadcast application of 1 $\times$ 10^{13} conidia ha^{-1} translates to 1 $\times$ 10^{5} conidia cm^{-2} on a planar surface, or, theoretically, 2.5 $\times$ 10^{4} conidia cm^{-2} on a crop with a typical leaf area index of 4 (Scurlock et al. 2001).

Why so many spores? Target insects have to acquire a sufficient number of conidia for infection to occur. Tens to thousands of conidia are needed per insect for a median effective dose (LC_{50} or LD_{50}). For example, in laboratory bioassays using larval *Plutella xylostella* (L.), LC_{50}s of 11–6,500 conidia cm^{-2} of sprayed surface were observed among 41 isolates of *B. bassiana* (Wraight et al. 2009).

Additionally, the dose-mortality response with fungal entomopathogens typically has a low regression slope value. The implication of this phenomenon is that very large increments in the number of spores are needed for commensurate increase in efficacy. We can gain some insights into this phenomenon from published bioassay and field efficacy data. Data from field trials of *M. acridum* IMI330189 (Green Muscle™) and FI985 (Green Guard™) against various acridids indicate that a consistently efficacious (>80% insect

mortality) field rate is 2.5×10^{12} conidia ha^{-1} (Hunter et al. 2001; van der Valk 2007), which translates to 2.5×10^4 conidia cm^{-2} planar surface. A locust with a cross-sectional target area of a approximately 6 cm^2 could be expected to acquire approximately 1.5×10^5 conidia from a direct spray at the above rate; a small grasshopper, approximately 5×10^4 conidia. In direct, topical bioassays of *M. acridum* FI985 and the wingless grasshopper, *Phaulacridium vittatum* (Sjöstedt), the log dose-response regression slope was 2.08 (Milner 1997). An eightfold increase in dose was needed to go from the LD_{50} (1,212 conidia per insect) to the estimated LD_{95} (9,240 conidia per insect). For the Migratory Locust, *Locusta migratoria* L., the slope was 1.30, requiring a 19-fold increase from the LD_{50} (4363 conidia) to the estimated LD_{95} (7.94×10^4 conidia). Using a spray application onto various larval Lepidoptera and substrate, Wraight et al. (2009) reported one *B. bassiana* isolate had a regression slope of 0.97. The result of this low slope was that a 57-fold increase was needed to go from the LC_{50} to the LC_{95} (2.5×10^6 conidia cm^{-2}). This last concentration corresponds to 2.5×10^{14} conidia ha^{-1} on a planar surface, higher when leaf area index is included.

There have been very few field studies where propagules per unit area of leaf surface have actually been measured as a basis for understanding efficacious rates. Notable among these are Poprawski et al. (1997), Wraight et al. (2000), and Wraight and Ramos (2002). Wraight et al. (2000) applied *B. bassiana* GHA against *Bemisia tabaci* (Gennadius) in various crops, monitoring spray coverage with plastic coverslips on which conidial deposition rates could be determined. Their application of 5×10^{13} conidia ha^{-1} with an air-assisted electrostatic sprayer achieved $1.7–2.8 \times 10^5$ conidia cm^{-2} on the lower surfaces of cucumber (*Cucumis sativus* L.) or melon (*Cucumis melo* L.) leaves. A parallel application of 1×10^{14} conidia ha^{-1} yielded $3.9–4.8 \times 10^5$ conidia cm^{-2}. They subsequently observed a 69% reduction in large nymphs after one application of 1×10^{14} conidia ha^{-1}, and 90% reduction after two sprays of the higher rate and four sprays at the lower rate, at 4-day intervals. To place these data in context, Wraight et al. (1998) observed an LC_{50} of approximately 2.5×10^4 cm^{-2} for this *B. bassiana* isolate in laboratory bioassays. Based on their mean log-dose regression slope for this fungus (1.09), a theoretical LC_{95} would be on the order of 8×10^5 conidia cm^{-2} of sprayed surface. Wraight and Ramos (2002) also monitored spray coverage using plastic coverslips when applying commercial *B. bassiana* formulations at 1.25, 2.5 or 5×10^{13} conidia ha^{-1} for the control of Colorado potato beetle (*Leptinotarsa decemlineata* Say). When the fungus was applied using upward pointing spray nozzles placed below the canopy, they observed mean conidial deposition rates on upper and lower leaf surfaces of $7.31–11.4 \times 10^4$ and $2.6–6.5 \times 10^4$ cm^{-2}, respectively. These rates of fungus yielded 10–65% beetle reduction depending on frequency of application. An LC_{95} of 2.3×10^4 conidia cm^{-2} *B. bassiana* GHA for second instar larvae was based on an LC_{50} of 1,460 conidia cm^{-2} and a regression slope of 1.37 (Furlong and Groden 2003). In summary, much of the published data indicates that considerable numbers of spores have to be applied for good efficacy, and that large additional increments of fungus are needed to achieve increasing levels of efficacy, when control relies upon infections from only the applications. There is an economic context to these inundative rates. An internet survey in 2009 of prices of the commercial *B. bassiana* product in the US (Jaronski unpublished data) yielded an average sales price of US$25 per 1×10^{13} conidia (plus shipping). Efficacious rates, such as discussed earlier, imply a user cost of US$25–250 ha^{-1} per spray using this product, clearly restricting use to very high margin crops. The Green Muscle *M. acridum,* with its greater infectivity and virulence for Acrididae, cost US$9–18 ha^{-1} in 2007 at the $2.5–5.0 \times 10^{12}$ conidia ha^{-1} rate (Jaronski unpublished data).

How can we change the mathematics of application rates? One way is to concentrate the conidia into a narrower, targeted zone. An example is application of *B. bassiana* against larval whitefly species in cucurbits. The target insect resides on the underside of the plant's leaves, which are generally a layer of umbrella-like structures. Figure 1 illustrates a directed spray application using conventional spray equipment that directs most of the spray into the cucurbit canopy. Similarly, by using a backpack sprayer with hydraulic drop nozzles pointing upwards, Wraight and Ramos (2002) were able to increase the conidial deposition on leaf undersides 6- to 30-fold. In cotton, fungus sprays can be applied to the undersides of the leaves by use of a horizontal bar preceding the hydraulic spray boom. This bar bends the cotton

Fig. 1 Directed spray application of mycoinsecticide using conventional spray equipment that directs most of the spray into the cucurbit canopy. (Top) Spray designed to treat two rows of cucurbits. (Bottom) Close-up of drop tube and nozzle arrangement. Note height of nozzles in relation to plant canopy and rearward direction of spray

plants and exposes the leaf undersides to the spray (Jaronski, unpublished data).

A second method is to concentrate a broadcast application of spores into a directed band over the row of crop ("band over row"). For instance, sugarbeet root maggot (*Tetanops myopaeformis* Röder) adults oviposit into the upper soil surface within 3 cm of the emerging seedling. One approach in controlling this insect has been to apply conidial suspensions of *M. anisopliae* in a 12.5-cm band-over-row application just before oviposition begins (Jaronski et al. 2007). If applied in a broadcast spray, 5×10^{13} conidia ha^{-1} would result in a level of 4.9×10^{5} conidia cm^{-2} of soil surface. With the banded application soil, levels become 2.4×10^{6} cm^{-2}, a fivefold increase at the same rate per hectare, and confined to the actual oviposition site. Similarly, spores can be placed in the path of insects, such as on fiber bands wrapped around tree trunks to control the Asian longhorned beetle (*Anoplophora glabripennis* Britton and Sun) (DuBois et al. 2004; Shanley et al. 2009), affording considerable economies.

A different approach is to bring the insect to the fungus, using a bait or attractant formulation. For example, linoleic and linolenic acid-rich vegetable oils can be used to draw grasshoppers to fungus-treated strips spaced every 20–30 m rather than applying the fungus broadcast). This approach has been validated on rangeland using chemical insecticide (Lockwood et al. 2001) and is being pursued with fungi. Also, pheromones can be used to draw target insects into trap stations where the insects become dusted with conidia. This approach, thoroughly reviewed by Vega et al. (2007), is being used operationally against Japanese beetle (*Popillia japonica* L.) in the Azores. Alternatively, another insect can be used to carry fungal conidia specifically to the target insect's habitat. An example is use of bumblebees (*Bombus* spp.) and honey bees (*Apis mellifera* L.) to vector *B. bassiana* conidia to greenhouse crops and canola (*Brassica rapae* L.) (Al-Mazra'Awi et al. 2006a, b) to control thrips and *Lygus lineolaris* Palisot de Beauvois. Baverstock et al. (2009) discuss using insect behavior to enhance fungal entomopathogen efficacy. Lastly, efficacy could be increased by combination with other microbials such as *Bacillus thuringiensis* Berliner (Wraight and Ramos 2005) or chemical pesticides.

Environmental factors affecting the fungi in foliar use

Once spores are applied to foliage, their levels decline, the rate of decline affected by a number of factors: sunlight, rain, temperature, humidity, leaf surface chemistry, and phylloplane microbiota. The fungal spores, once on the insect cuticle, usually invade the body of their hosts within 24 h. During the initial infection process—spore activation, germination, initial cuticular penetration—the fungi continue to be susceptible to many of the same environmental factors. Once inside the host's body, the fungi continue to be affected by temperature, and, indirectly, humidity, via its effects on overall insect health, and become exposed to new, humoral factors, which themselves can be affected by food plant suitability, partitioning of insect resources among reproduction, movement and immunity, temperature, etc. During the past few years new discoveries (e.g., Lemaitre and Hoffmann 2007; Müller et al. 2008) indicate insect humoral immunity may be more important than previously thought, even with fungal entomopathogens. These diverse factors can combine to limit the efficacy of mycoinsecticides applied at economically acceptable rates. At the same time, certain of these aspects can be manipulated, at least theoretically, to enhance efficacy.

Sunlight

It is generally recognized that sunlight, particularly the UV-A and UV-B components, is a major mortality factor of fungal propagules on the phylloplane and is largely responsible for short persistence of mycoinsecticides in the epigeal habitat. There have been an number of laboratory studies using artificial UV sources (e.g., Ignoffo et al. 1977b; Hunt et al. 1994; Inglis et al. 1993, 1995a; Alves et al. 1998; Lee et al. 2002). Outdoor studies have been far fewer (Gardner et al. 1977; Inglis et al. 1995a, 1997a, b; Smits et al. 1996; Braga et al. 2001b; and Behle 2006). In general, the half-life of fungal conidia under natural, outdoor sunlight, in terms of percent viability or viable numbers per unit area, is 3–4 h (Roberts and Campbell 1977; Braga et al. 2001a), although Inglis et al. (1997a) observed a half-life of approximately one day in a North American short-grass prairie, and Sabbahi et al. (2008) observed viable conidia on sprayed strawberry foliage for up to six days. As measured by insect efficacy during field trials, however, persistence may be as long as 8–14 days, at least in the case of *M. acridum* under African subtropical, semi-arid conditions (summarized by van der Valk 2007). The UV-A component (320–400 nm) represents about 95% of total solar UV and is associated with conidial death and delayed germination (Braga et al. 2001a), but the UV-B component (280–320 nm) is considered more damaging (e.g., Moore et al. 1993) and has been the general focus of most studies. Both components have to be considered because they have different modes of action, while more realistic, outdoor studies need to be conducted.

In addition to outright conidial mortality, several authors have noted delayed germination as well (Braga et al. 2001a). This latter aspect has bearing on overall efficacy because it gives advantage to rapidly molting insects such as aphids, and earlier instars of many Lepidoptera and Coleoptera. Some sort of recovery from UV damage may be possible (Braga et al. 2001a), but this aspect is not well understood and needs further inquiry.

The effect of sunlight on persistence can be affected by location of the spores (abaxial vs. adaxial leaf surfaces) (Jaronski, unpublished data), formulation (Alves et al. 1998; Edgington et al. 2000; Cohen and Joseph 2009; Thompson et al. 2006), and fungal species or strain (Ignoffo and Garcia 1992; Fargues et al. 1996; Fernandes et al. 2007). With regards to the last aspect, conidia of *I. fumosorosea* were the most susceptible, while *M. acridum* were the most resistant to UV irradiation followed by *B. bassiana* and *M. anisopliae* (Fargues et al. 1996). Significant differences also existed among isolates within each species (Fargues et al. 1996; Fernandes et al. 2007). There may also be an interaction between temperature and sensitivity to UV radiation (Smits et al. 1996). A caveat about some of the published studies on this subject is that photodegradation of conidia on glass is faster than on leaf or agar surface (Inglis et al. 1997a) so that some data have to be interpreted with caution.

Survival on the lower surface of leaves, especially when there is considerable lateral shading by adjacent canopy, can be considerable (Fig. 2). During persistence studies of *B. bassiana* GHA (as Mycotrol^{TM} 22WP) in southern California, viabilities of conidia applied to the lower and upper surfaces of melon (*Cucumis melo* L.) leaves were followed on a daily basis using germination tests of spores washed off leaf surfaces (Jaronski unpublished data). Conidial viability on leaf undersides decreased approximately 9–11% day^{-1}. On upper leaf surfaces viabilities dropped by 47% day^{-1}. As the melon canopy grew and expanded, the rate of conidial death on adaxial surfaces decreased to 1.2–1.6% day^{-1} although neither the amount of daily solar radiation nor air temperatures varied. Adjacent canopy increasingly protected conidia on lower surfaces. The host plant itself may also have a role in photoinactivation of conidia. In parallel tests, conidial viability on adaxial

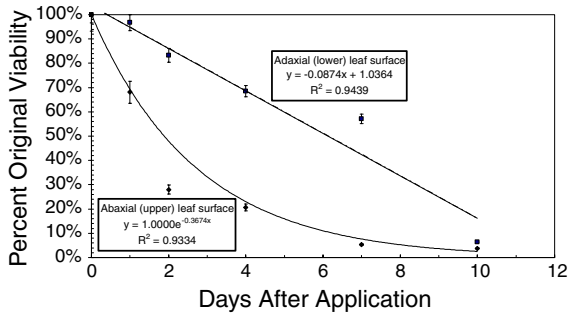

Fig. 2 Conidial residual life of *Beauveria bassiana* GHA as Mycotrol^{TM} 22WP on abaxial and adaxial leaf surfaces of *Cucumis melo* at Brawley CA, USA late May 1995. Each point is the mean of three replicate leaves. Error bars represent SD

cotton leaf surfaces decreased 24.3% vs. 4.2% day^{-1} for melon leaves (Jaronski unpublished data).

A number of UV protectants have been evaluated and a few with practical potential identified (Jackson et al. 2009). Inglis et al. (1995a) identified a number of water soluble and oil-soluble UV protectants in laboratory tests. When the candidates were tested outdoors, however, the degree of protection was greatly reduced for all protectants and was inconsistent between two replicate trials. Nevertheless, there was a quantitative indication of the potential of photoprotection under realistic outdoor conditions—25–37%. More recently, Reddy et al. (2008) identified 1–10 g Tinopal UNPA-GX^{TM} L^{-1} of carrier as providing significant UV protection of a *B. bassiana*. The LT_{50}, in terms of hours of exposure to natural sunlight, was increased by 26%. While these results seem encouraging, one has to retain a sense of practicality. Use of 1 g Tinopal L^{-1}, as was tested by Reddy et al. (2008), in an aqueous spray applied at 187 L ha^{-1}, which is a low but common application rate for insect control on US vegetables, would cost US$99 when the optical brightener is obtained as 85% technical grade material from the manufacturer (2009 prices). Such an additional cost is rarely practical. The above rates of photoprotectant in an ultra low volume (ULV) oil formulation, which is typically applied at 1–2 L ha^{-1} could be more feasible. But such low rates, although common in some instances, for example locust control in Africa and Australasia, are rare in the United States and the EU.

A critical question remains: how much must persistence be improved for a significant increase in field efficacy? Twofold? Fourfold? More? In many intended uses of a commercial mycoinsecticide, farmers apply fungus to their crops repeatedly over the crop cycle. This is certainly the case with *B. tabaci* in the southwestern US, where season-long (6–8 weeks) weekly applications of a mycoinsecticide, for example, *B. bassiana*, can be necessary. One implication of the interaction of repeated mycoinsecticide sprays with a constant loss of viable conidia is a fluctuating, "sawtooth" variation in the overall levels of fungus in the crop (Fig. 3). (The simple models presented here ignore other sources of conidial losses, such as physical loss from canopy due to rain or wind, or dilution of conidial concentrations on leaf surfaces due to canopy growth.) A more-or-less constant (9%) daily loss occurs in the

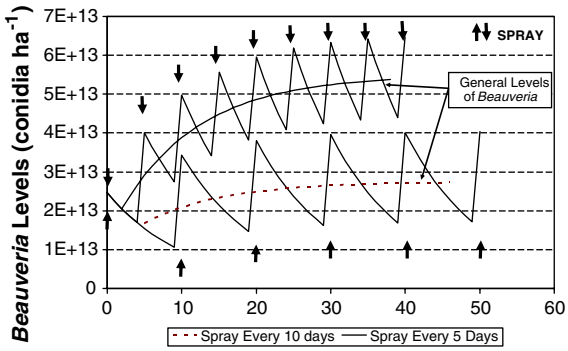

Fig. 3 Effect of a 9% day^{-1} loss of *Beauveria bassiana* GHA due to sunlight at two spray schedules of 1 Kg Mycotrol^{TM} 22WP (2.5 × 10^{13} conidia) ha^{-1}. Applications of *B. bassiana* (arrows) are either every five or ten days. At each spray the fungus titers increase but then decrease due to solar radiation. Degradation rate is based on observations of Mycotrol 22WP persistence on *Cucumis melo* adaxial leaf surfaces at Brawley CA, USA late May–June 1995 and 1996. Trends assume no expansion of plant canopy

levels of viable conidia as affected by application intervals of five vs. ten days for six weeks during the melon-growing season (Fig. 4). More frequent replenishment of fungal conidial levels may serve to overcome loss in conidial viability over time. With a 10-day schedule, the "average" conidial titers plateau at a low level, which may not be sufficient for control, while more frequent applications rapidly increase overall titers. In 1995 Wraight et al. (unpublished) observed considerably reduced efficacy from a 10-day application schedule of Mycotrol versus a 5-day schedule, although continuing oviposition by adult whiteflies probably was also a factor.

The persistence studies of *B. bassiana* GHA in cucurbits, presented earlier, may provide some insight into the degree of photoprotection necessary. The data were used to model fluctuations in conidial densities on plant leaf surfaces. Trends in conidial levels resulting from a change in conidial loss, from 24 to 12% day^{-1} ("50% protection" from UV), with reapplication of fungus every seven days (as recommended by the company for whiteflies in cucurbits) are depicted in Fig. 4. With 24% daily loss in conidial viability, weekly applications of fungus are needed to maintain conidial levels in the crop (ignoring new growth and canopy expansion). The initial level of 2.5 × 10^{13} conidia ha^{-1} quickly drops by more than one-half and then fluctuates around an

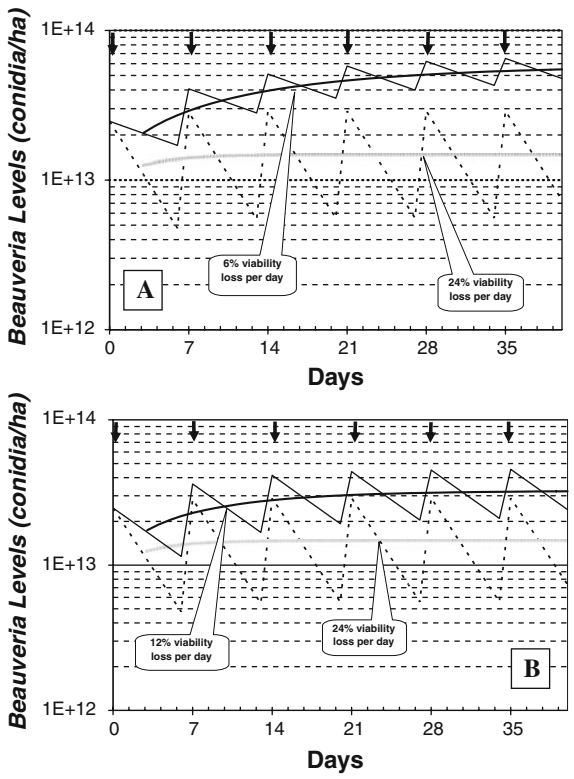

Fig. 4 Effect of reducing *Beauveria* GHA photodegradation rate from 24% day^{-1} to 6% (**A**) or 12% day^{-1} (**B**) on conidial levels in a crop (e.g., melons for whitefly control) subject to weekly sprays of 2.5×10^{13} conidia ha^{-1}. Applications of *Beauveria* (arrows) are weekly. At each spray the fungus titers increase but then subsequently decrease due to solar radiation at a daily rate of 24, 12 or 6%, accordingly. Trends assume no expansion of plant canopy. Legend: spaced dash 24% viability loss day^{-1}, dash 6% or 12% viability loss day^{-1}

equilibrium level of $1.4–1.5 \times 10^{13}$ conidia ha^{-1} with the repeated weekly sprays. If a UV protectant decreases the daily loss in viability by one-half, to 12%, the weekly applications may result in greatly decreased fluctuations and a gradual accumulation of fungus to levels slightly greater than the original application (Fig. 4B). If, instead, 75% protection was achieved, a reduction in the rate of viability loss to 6% day^{-1} (Fig. 4A), the trends in conidial numbers might increase through the growing season to about twice the original level. This model is, of course, a gross simplification and ignores a number of factors, including conidial landing on non-target plant surfaces, physical loss of conidia from leaf surfaces, canopy expansion, insect movement to or oviposition on new foliage flush, and critical concentrations of conidia and rate response of efficacy (slope of the rate-efficacy regression, discussed in a previous section). But the model is a starting place. Obviously, carefully designed field experiments with effective photoprotectants are a critical need to resolve this question.

Rainfall

Rain events following application of fungal propagules can be catastrophic for efficacy. Relatively few controlled studies have been conducted regarding this aspect, notably those of Inglis et al. (1995b, 2000) and Inyang et al. (1998). In their earlier work, Inglis and his coworkers observed that *B. bassiana* conidia suffered rates of removal of 25–47% from alfalfa (*Medicago sativa* L.) and 51–56% from wheat (*Triticum* spp.) leaflets with as little as 30 min of simulated rain (either 27 or 113 mm h^{-1}). The conidia were applied as aqueous suspensions without wetting agents. Later on, they examined the rainfastness of a series of commercial and experimental formulations (Inglis et al. 2000). Only conidia in a nonemulsifiable oil carrier resisted simulated rainfall of 77 mm h^{-1}. Several emulsifiable suspensions (ES) and a wettable powder formulation washed off leaves as readily as conidia applied in water only (Fig. 5). What is instructive is that the total volume of spray had an effect on rainfastness for the commercial Mycotrol ES formulation—the 0.8% spray had greater rainfastness than 0.125% spray. The inference is that the volume of oil confers a degree of rainfastness. Inyang et al. (2000) observed that 39–76% of *M. anisopliae* conidia applied in three different formulations were lost from oilseed rape leaves after 1 h of simulated rainfall (rate of simulated rain not given). The least removal of conidia occurred with a safflower oil-Shellsol T^{TM} carrier, once again indicating that oil-based formulations may be more rainfast. Similarly, Wraight and Ramos (2002) observed better efficacy of an ES formulation of *B. bassiana* than a wettable powder formulation against Colorado potato beetle in potatoes when their field trial was beset by frequent rainfall during the fungus application phase. Nevertheless, significant rain or overhead irrigation, after a spray application of a mycopesticide, may be a major detriment to efficacy. At the same time in certain situations, rainfall can act in the dispersal of conidia especially

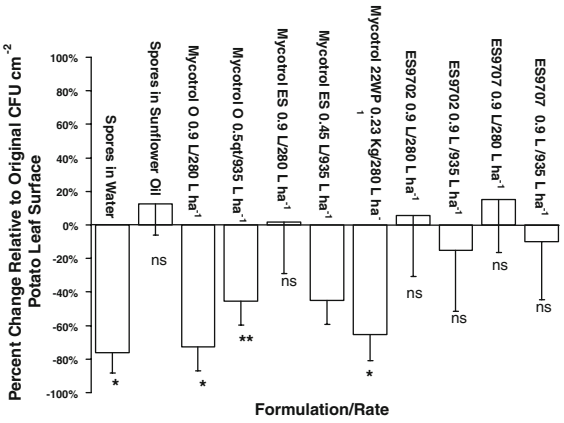

Fig. 5 Rainfastness of commercial and experimental formulations of *Beauveria bassiana* on potato leaves after a simulated rainfall of 77 mm h^{-1} for 30 min. The rate, 280 L ha^{-1}, represents typical application rate onto vegetables in US; 935 L ha^{-1} was the manufacturer's recommended spray volume for whiteflies in cucurbits and cotton. Symbols: * significantly different from 0% change, at $P = .05$; ns not significant. Error bars represent SE. (Data adapted from Inglis et al. 2000)

to the soil beneath the plant canopy (Bruck and Lewis 2002) and thus enhance efficacy. There also seem to be differences among plants in terms of persistence of spores on foliage, differences that may be mediated by leaf cuticle chemistry. There were significant differences in retention of conidia on lettuce (*Lactuca sativa* L.) and celery (*Apium graveolens* L.) following significant natural rainfall during a field trial (Kouassi et al. 2003). Rain reduced the numbers of CFU on celery by 92% but only 10% on lettuce. This latter aspect is an area that needs further research.

Temperature

Ambient temperatures can affect fungal entomopathogen field efficacy. For example, efficacy of *B. bassiana* GHA against *Lygus hesperus* Knight was greatly reduced in small plot field tests in July but not June of the same year, even though the insect is quite susceptible to this fungus (Noma and Strickler 1999). While optimal germination and growth rates of fungal entomopathogens range between 23°C and 28°C, growth, in general, rapidly slows above 30°C, and ceases for most isolates at 34–37°C. Similarly, conidial germination is adversely affected by temperatures above 30°C. In the Noma and Strickler (1999) study, temperatures within the plant canopy were observed to be in excess of 30°C during the July trials; the upper thermal limit for this strain is 32–34°C. In subsequent laboratory experiments, Noma and Strickler (2000) demonstrated that infections were greatly reduced at 35°C, compared to 25°C. Temperatures below 16°C increasingly slow germination and growth rates for most of the fungal entomopathogens, and thus affect efficacy in terms of a longer survival of the target population (Inglis et al. 1999; Ihara et al. 2008). This can have important bearing on mycopesticide field efficacy in northern climes and also on temperate rangeland where night time insect body temperatures can be <10°C for more than 6 h day^{-1}. Night time ground temperatures even reached 5–6°C, in South and North Dakota during the Summer of 2003 during a grasshopper field trial (Jaronski unpublished data). The temperature data shown in Fig. 6, using thermal surrogates (Lactin and Johnson 1998), represent the maximum temperatures that could be achieved during a 24 h cycle by grasshoppers on the ground and in the plant canopy. Not only are mid-day body temperatures in excess of the upper thermal limit for *B. bassiana* GHA, due to normal basking as well as 'behavioral fever' thermoregulation (see below), but night time temperatures are cold enough to greatly slow fungal growth within the insects. The result is that there can be only a few hours each day during which temperatures are permissive for fungal growth. For example, the temperature observations as represented in the top graph in Fig. 6, and made during successive days during the same field trial. They were used to construct a heat budget for *B. bassiana* GHA (Fig. 7). As can be seen in Fig. 7, only 6–7 h each day were permissive for growth (based on upper and lower cutoff temperatures for 50% of fastest fungal growth) on sunny days and 11–16 h on partly cloudy days. The end result is that considerable time can elapse before infected insects succumb to infection, time for the insects to damage a crop. An example is *B. bassiana* GHA used against grasshoppers. Johnson and Goettel (1993) and Inglis et al. (1997b) observed that even though a considerable proportion of the targeted grasshopper population was infected, few died in the field within the observation period.

There are considerable differences in temperature tolerances among the fungal entomopathogens, even among isolates of the same species (e.g., Fargues et al. 1996; Bugeme et al. 2009), so that a candidate

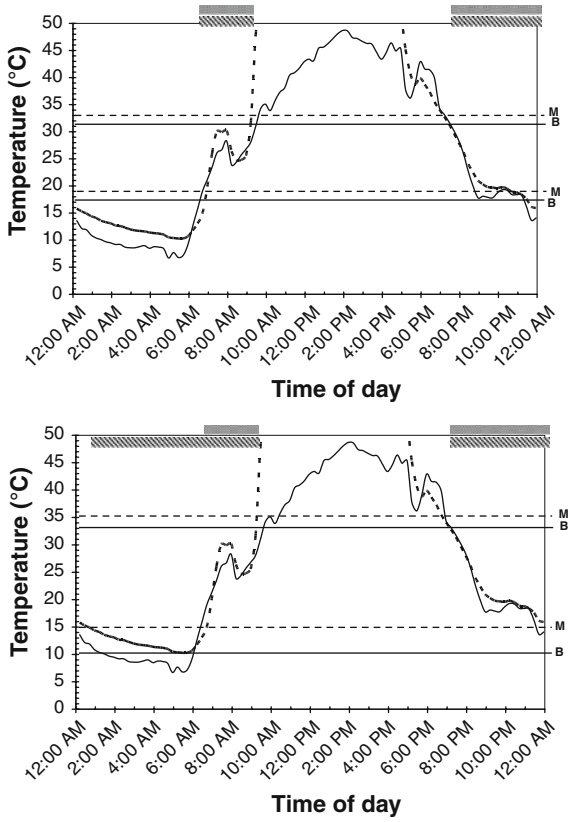

Fig. 6 Maximum potential grasshopper body temperatures based on heat absorbance of thermal surrogates July 10, 2003, on mixed grass prairie, Edgemont, South Dakota USA, and parallel effects on entomopathogenic fungus growth. Legends: dash temperatures recorded in surrogate in plant canopy 10 cm above ground; spaced dash temperatures of a thermal surrogate placed on ground simulating a basking grasshopper; horizontal lines associated with "B" and "M" are upper and lower temperature thresholds for 50% (top graph) or 20% (bottom graph) of maximum growth rate of *Beauveria bassiana* GHA and *Metarhizium acridum* IMI33189 respectively; patterned horizontal bars represent duration of permissive temperatures for fungus growth, ▒ *Metarhizium acridum* IMI330189; ▨ *Beauveria bassiana* GHA

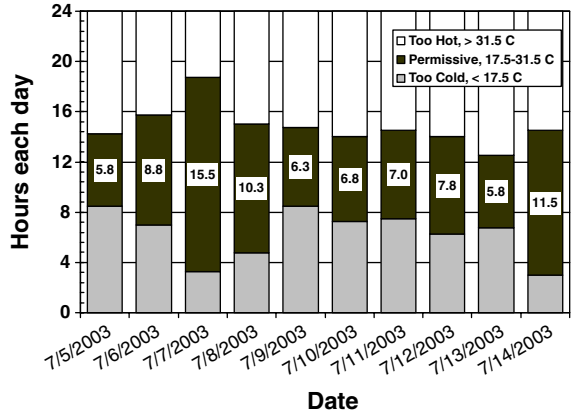

Fig. 7 Heat budget for *Beauveria bassiana* GHA based on temperature observations of thermal surrogate placed in grass canopy, in July 2003 at Edgemont, South Dakota, USA, and on the upper and lower temperature limits for 50% fastest growth rate for this strain

fungus may be identified for better heat tolerance to suit intended use, either by itself or to complement a second fungus with the opposite temperature tolerance. This latter approach was tried by Inglis et al. (1999). There are isolates with some degree of cold tolerance (Rath et al. 1995; Li and Feng 2009) including ones that grow at 8°C (De Croos and Bidochka 1999) and this attribute may make them superior to others for the control of insects in colder situations. Temperature tolerance should be one of the criteria for candidate selection if proposed uses so justify the concern. The downside is that ability of a microorganism to grow at 36–37°C raises concerns about pathogenicity for homeothermic vertebrates. Whether these differences are reflected in differences in field efficacy remains to be determined.

There is an additional aspect. Almost all thermal tolerance work has been done using constant temperatures, typically examining radial growth of colonies on agar media at a range of temperatures, e.g., Fargues et al. (1997). Only a few researchers have examined fluctuating temperatures (Inglis et al. 1999; Fargues and Luz 2000; Devi et al. 2005). In nature, temperatures fluctuate to a considerable extent in some habitats. Furthermore, certain insects (grasshoppers, houseflies, cockroaches) demonstrate active thermoregulation whereby they maintain their body temperatures several degrees above ambient by absorbing heat directly from the sun as well as from warm substrate (Carruthers et al. 1992). This thermoregulatory behavior can be pronounced upon infection with a pathogen, a phenomenon termed "behavioral fever" (Watson et al. 1993; Inglis et al. 1996; Kalsbeek et al. 2001). Thus insects, for example grasshoppers, can be infected following inundative application of a fungal pathogen, but do not die unless they are prevented from thermoregulating (Inglis et al. 1997a, b; Ouedraogo et al. 2004). An assumption in this phenomenon is that fungal growth resumes when temperatures become permissive. This is not always the case. Many isolates of

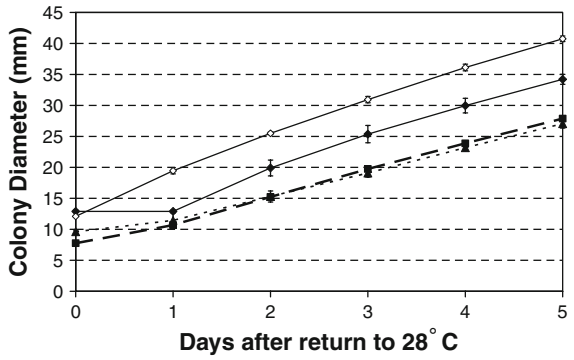

Fig. 8 Effect of a transient, 6 h exposure to 41°C on the subsequent radial growth at 28°C of *Beauveria bassiana* Strain GHA, *Metarhizium anisopliae* Strain F52, and *M. acridum* IMI330189. The mean colony diameter of *B. bassiana* in the 41°C treatment was significantly smaller one day after return of cultures to 27°C than for counterpart cultures grown at constant 27°C (T test statistic 23.66, 4 df, $P < .001$). Subsequent rate of growth (slope) was not significantly different from the 27°C treatment. Neither *M. anisopliae* F52 nor *M. acridum* IMI330189 displayed a significant lag and their rates of growth were not significantly different in the two treatments (data for the two *Metarhizium* at 27°C not shown). [diamond] *B. bassiana* 27°C; [small filled diamond] *B. bassiana* transient 41°C; [large filled triangle] *M. anisopliae* F52 transient 41°C; [filled square] *M. acridum* IMI330189 transient 41°C. Error bars represent 95% Confidence Limits

B. bassiana and *M. anisopliae* demonstrate a delayed resumption of normal growth after exposure to short periods of temperatures above their normal threshold (Jaronski, Keyser and Roberts, unpublished data). Figure 8 represents the effect of a 6-h exposure to 41°C on subsequent in vitro radial growth of a *B. bassiana* and two *M. anisopliae* isolates. This exposure time and temperature would be encountered by fungi infecting the Mormon cricket (Turnbow 1998). The commercial *B. bassiana* GHA displays a 1-day delay before normal growth rate is resumed. This delay becomes more pronounced with higher temperature and increased exposure time. Neither *M. anisopliae* F52 nor *M. acridum* isolates IMI330189 (Green Muscle™) and FI985 (Green Guard™) demonstrate a growth delay after 6 h at 41°C. F52 shows delays in resuming growth only after 9 h exposure to 41°C or 3 h at 44°C. In contrast, IMI330189 and FI985 require more than 6 h at 44°C or 18 h at 41°C before they show delayed resumption of growth. Thus, fluctuating temperatures can have more than a simple subtractive effect on efficacy and at least partially explain underestimates in time to onset of Mormon cricket or grasshopper field mortality predicted by simple heat budgets (Jaronski and Foster unpublished data).

Another aspect is indirect effect of temperature, especially high temperatures, on efficacy, as mediated through the insect's defense system. Larval *Galleria mellonella* L. exposed to 38°C for 30 min, then injected with *B. bassiana* blastospores may have a longer survival time than non-heat shocked larvae (Wojda et al. 2009). Heat-shocked larvae had elevated humoral anti-yeast and lysozyme activity and galiomycin expression in response to subsequent infection. While the purpose of inundative application of a mycopesticide is to overcome such defenses, the latter may still be manifested through slowed efficacy.

Humidity

While there is a requirement for high humidity for spore germination in vitro (e.g., Lazzarini et al. 2006), insects can become infected at much lower humidity. It is generally thought that infection is independent of ambient relative humidity (Ferron 1977; Marcandier and Khachatourians 1987; James et al. 1998; Lord 2005). But this is not true in all cases, viz., Luz and Fargues (1999), who observed a humidity threshold of >96% for efficacy of *B. bassiana* against *Rhodnius prolixus* Stål. Similarly, Yasuda et al. (1997) observed reduced efficacy of against *Cylas formicarius* Fabricius at <43% relative humidity. There are other examples in the literature, e.g., Altre and Vandenberg (2001), Lazzarini et al. (2006). The fungi *H. thompsonii* and *Lecanicillium* spp. may represent an extreme example of high humidity requirement for efficacy. Key to efficacy of *H. thompsonii* is very high humidity for at least 24 h (McCoy 1981). The current recommendations for commercial *Lecanicillium* spp. are application with subsequent relative humidity of at least 80–95% at the leaf surface, for 10–12 h per day for several days (Koppert 2009a, b). Thus the dependence of infection on humidity depends upon the insect, and its ecology, especially in relation to the phylloplane and its microclimate. Oil based formulations seem to overcome this problem (Ibrahim et al. 1999).

Phylloplane microhabitat vs. macrohabitat as it affects environmental variables

In considering environmental effects on a mycoinsecticide, one must differentiate the ambient environment, within canopy habitat, and, especially, leaf-surface microhabitat, especially for small target insects such as whitefly nymphs, aphids, thrips, and mites. Ambient temperature and humidity measurements, taken above the crop canopy can have little relationship to conditions within the canopy. For example, Shipp et al. (2003) observed that ambient humidity had little effect on *B. bassiana* activity against aphids, thrips and whiteflies on cucumber leaves under greenhouse conditions.

Each leaf on a plant and even different parts of a leaf have their own equilibrium temperature with the environment, based on sensible and latent heat losses vs. net heat gain from irradiation, and thus have a unique microclimate. During the day, upper leaf surfaces can be 10°C greater than ambient, while lower surfaces can be 1–2°C below ambient (Burrage 1971). Plant geometry affects leaf temperatures. Sunlight penetrates plant canopy in a reduced intensity and changed spectrum as determined by leaf angle and leaf area distribution. With crops having vertical leaves, the angle of the sun is most important with greatest penetration being at mid-day. The sunlight in turn affects leaf temperature. For example, leaves of 24-cm rye grass (*Lolium* spp.) can vary by as much as 6–7°C from air temperature at mid-day. On plants with horizontally held leaves, e.g., beans (*Phaseolus vulgaris* L.), the upper leaf surfaces were 2.5°C higher than the air while the lower surfaces were 3°C lower than ambient (Willmer 1986). In addition, temperatures can vary by as much as 2–3°C across a leaf surface (Burrage 1971). Above 33°C, leaf evapotranspiration can keep the leaf cooler than the surrounding air, but this is affected by leaf canopy and the leaf's position therein. Ferro et al. (1979) recorded abaxial apple (*Malus domestica* Borkh.) leaf temperatures 12°C lower than air temperature when the air was 38°C. Similarly, Chu et al. (1994) observed that leaves of cotton could be 5–7°C cooler than ambient under hot desert conditions, which may explain the efficacy of *B. bassiana* GHA against whitefly nymphs in Arizona cotton when air temperatures were in excess of 48°C (Jaronski et al. 1997).

The critical factor in humidity microclimate is the leaf boundary layer (LBL), which can be defined as the transition zone above the leaf surface in which wind speed increases with distance from the surface. The LBL can be 1–10 mm thick (Bonan 2008; Willmer 1986) although other sources cite a thickness of 2–3 cm in greenhouse tomato (*Solanum lycopersicum* L.) leaves (Boulard et al. 2002). Vesala (1998) examined the complexity of the factors affecting the thickness of the boundary layer. He divided the leaf boundary layer into two regions, an upper "adhering air layer" and a "lower superstomatal air layer." The former is affected by the size and shape of the leaf, presence of other leaves and wind velocity. The latter is affected by number of stomata per cuticle area, pore radius, leaf radius, wind velocity, and stomatal resistance. The boundary layer of air above a leaf surface is affected by leaf topology, radiation temperature, and air movement. More simply put, the humidity at the leaf surface is affected by evapotranspiration rate and wind velocity which combine to control the rate at which water vapor is transferred through the boundary layer. For more details about the physics of LBL, see Schuepp (1993).

The LBL can cause relative humidity immediately adjacent to the leaf surface to be higher than the ambient humidity. In cabbage (*Brassica oleracea* Linne) leaves, the ambient relative humidity (RH) of 70% increased to 90% 1 cm above both upper and lower leaf surfaces, and increased from 56% RH to 70% within 5 mm of waterlily (*Nymphaea* spp.) leaf (Willmer 1986). Within the immediate proximity of leaf stomata, RH could be 95–99% at 1 mm above leaf surface. Ramsay et al. (1938) observed an RH of 40% at 1 mm above the leaf surface of dock (*Rumex* spp.) vs. 10% ambient RH, and 95% vs. 50% ambient with a tulip (*Tulipa* sp.) leaf. A study by Boulard et al. (2002) is the most detailed and potentially relevant to the use of entomopathogenic fungi. They observed a 20–30% increase above ambient RH at 5 mm above tomato leaf surface in the morning, 7–10% at the end of the day. As the wind speed in the immediate vicinity of the leaf surface exceeds 0.36 km h^{-1}, however, turbulence disrupts the LBL and relative humidity approaches ambient (Gates 1968). This aspect creates a very complex and dynamic situation on the leaf surface especially in outdoor crops, but even with glasshouse plants. Large insects such as adult beetles, grasshoppers and late

instar Lepidoptera, are probably less influenced by the LBL because their size places much of their bodies above it. Nevertheless infection via the tarsi and ventral surfaces of large insects may still be under the influence of the LBL.

There seems to be no comparable information about boundary layers above the insect cuticle. One can infer that there is a boundary layer and sufficiently high humidity to allow conidial germination and penetration into the cuticle from bioassays where the ambient RH during incubation was less than required for in vitro conidial germination, e.g., Ferron (1977), Marcandier and Khachatourians (1987), and Ramoska (1984). Charnley (1989) mentions that infection is often through the cuticle of the mouthparts, intersegmental folds, and spiracles, regions where the humidity may be higher than on other parts of the cuticle. However independence from humidity is not universal. A number of authors report a direct relationship between ambient humidity and infection rate.

Influence of phylloplane chemistry

Plant cuticle comprises a mesh of insoluble polymers, cutin and cutan, infused with a mixture of lipids, mostly long-chain (C20–C40) fatty acids and derivatives. Above this matrix is a layer of epicuticular waxes either crystalline or smooth in appearance. See Beattie (2002) and Andrews and Buck (2002) for more information. Plant cuticular compounds have the potential of affecting spore persistence on the phylloplane, and the susceptibility of insects to infection. The plant can either affect spore acquisition by insects or spore persistence. Persistence can be affected either by simple physical removal from the leaf surface (without rain) or toxicity from chemicals lethal to the spore. Inyang et al. (1998) observed that twice as many mustard beetles became infected when exposed to treated Chinese cabbage leaves than oil seed rape, with turnip leaves being intermediate. According to Poprawski et al. (2000) whitefly nymphs reared on tomatoes were significantly less susceptible to infection by *B. bassiana* and *I. fumosorosea* than whiteflies reared on cucumber. Lygus bug mortalities from *B. bassiana* were significantly different between celery and lettuce (Kouassi et al. 2003). Ugine et al. (2007) described a strong difference—sevenfold—in thrips infection rates from *B. bassiana* on beans and impatiens due to a much greater acquisition of conidia from bean leaves. There are, however, other insect-fungal entomopathogen systems where the plant had no effect, e.g., Colorado potato beetle-*Beauveria* (Costa and Gaugler 1989), *Spodoptera-Nomuraea* (Fargues and Maniania 1992). Such plant-associated inconsistency has important implications for adoption of these fungi for microbial pest control. Insect control on some crops may be far more amenable than others. There is very little information regarding effects of plant hybrid or variety, which effects may be important due to the many varieties of any crop plant in common use.

Leaf topography seems to affect the numbers of spores acquired by insects from treated surfaces (Inyang et al. 1998). Host plant and leaf age had significant influence on conidial attachment to beetle abdomens, less so to thoraces. These effects were paralleled by insect mortality from fungus infection. Notably, insect mortality from mycosis decreased drastically with increasing delay between leaf treatment and addition of insects (73–77% at three days to 0–10% at nine days), with little difference between Chinese cabbage (*Brassica rapa* L.) and oilseed rape (*B. napus* L.). The authors postulated that this effect was due to leaf expansion and thinning of the conidial levels on the leaf surface, but a leaf-surface-associated mortality of conidia could also have been at work. Similarly, Ugine et al. (2007) reported that the LD_{50} of *B. bassiana* GHA in western flower thrips (*Frankliniella occidentalis* L.) was almost sevenfold greater on impatiens (*Impatiens walleriana* Hook.f.) than on beans. This differential effect was paralleled by a different extent to which thrips acquired conidia from leaf surfaces.

A thorough study of the effect of plant cuticular compounds on fungal entomopathogens was reported by Inyang et al. (1999). Leachates of turnip (*Brassica rapa* var. *rapa* L.), Chinese cabbage, and oilseed rape had both stimulatory and inhibitory effects on conidial germination. There were differences among leachates from the three plants and among different solvents used. In vivo germination on leaf cuticle was stimulated by dewaxing the leaf surfaces. There was also significantly higher conidial germination on young (77%) versus old (40%) turnip leaves, but this was not the case with the other two plants. Water treatment, such as might occur during rain or heavy dew periods, resulted in higher germination on

Chinese cabbage and old turnip leaves, but not rape. Fungal virulence for larval mustard beetle (*Phaedon cochleariae* Fabricius) was enhanced by both leaf extracts and cuticle leachates. The authors hypothesized that this enhancement was a result of accelerated germination and higher effective dose. The in vitro fungistasis from cuticular leachates did not occur on the insect cuticle, highlighting the potential pitfalls of in vitro studies. From a practical aspect, any effect of plant cuticular compounds becomes significant if spore germination on insect cuticle and penetration are affected. Ostensibly, spores on the plant cuticle surface remain dormant until picked up by an insect. Fungistasis on the leaf cuticle is beneficial. If fungistasis is absent or lost, as happened when leaves were dewaxed, spores can germinate on the plant cuticle (Inyang et al. 1999), and are potentially lost from the effective dose of fungus presented to the insects. Implications for consistent control by fungal entomopathogens on a range of plants are considerable. This type of research needs to be expanded to other crop plants. For further exploration of this topic see Muller and Riederer (2005), Cory and Hoover (2006), and Cory and Ericsson (2009).

Tritrophic effects on efficacy may also be exerted via plant effects on the host insect. Nutrition has bearing on overall health of an insect, and suboptimal nutrition may mediate effects of fungal entomopathogens. For example, Thungrabeab et al. (2006) reported that two species of thrips reared on cotton or *Saintpaulia* spp. (Gesneriaceae) were much less susceptible to *B. bassiana* than those reared on bean, leek (*Allium ampeloprasum* var. *porrum* L.), cucumber, or daisy (*Bellis perennis* L.). Similarly, *B. tabaci* reared on cucumber, tomato, melon, green pepper (*Capsicum annuum* L.), potato, eggplant (*Solanum melongena* L.), marrow (*Cucurbita* spp.), cabbage, bean, or cotton displayed different susceptibilities to *B. bassiana* with significant differences in median survival times (Santiago-Alvarez et al. 2006). Nutritional differences were suggested as a causal factor. Hare and Andreadis (1983) observed that host plant affected susceptibility of Colorado potato beetle larvae to *B. bassiana*. The plant most suitable for insect growth produced larvae with the least susceptibility to the fungus. Furthermore, potato plants grown in the glasshouse were less suitable for the insect than field-grown plants, resulting in greater susceptibility to *B. bassiana*. This subject is treated in more detail by Cory and Ericcson (2009).

There is evidence that phytopathogens may have an effect on susceptibility of an insect to a fungus (Rostas and Hilker 2003). Treatment of mustard beetle larvae with *M. anisopliae* resulted in 100% mortality when insects were on leaves infected with *Alternaria brassicae* Berk. but only 50% mortality when they fed on uninfected leaves. The beetles were feeding on symptom-free plant parts and displayed slowed development, indicating either suboptimal nutrition or the effects of chemical changes in the plant accompanying Systemic Acquired Resistance (SAR). The influence of multitrophic interactions on fungus efficacy is a largely unexplored area but one with considerable potential bearing on field efficacy, especially enhancing entomopathogen efficacy via SAR.

Influence of pesticide residues on the phylloplane

There is a considerable body of literature on the effects of pesticides on fungal entomopathogens, most recently summarized by Klingen and Haukeland (2006). Most of this data is based on in vitro laboratory assays in which germination and vegetative growth is observed on agar media incorporating an insecticide or fungicide, or tests where spores are incubated with operational concentrations of pesticides for varying periods of time, then plated on germination media (e.g., Clark et al. 1982; Mietkiewski and Gorski 1995; Todorova et al. 1998). While these approaches readily identify innocuous agrochemicals, they can yield "false positives"—chemicals that have an adverse effect in vitro but not in vivo. The critical arena for chemical-spore interaction is on the leaf surface where the spore is dormant under most conditions until it contacts the insect cuticle. Some agrochemicals are rapidly absorbed after application by the leaf. For example, the strobilurin fungicides are toxic in vitro to fungal entomopathogens as well as to a wide range of fungi (da Silva and Neves 2005). The strobilurin derivative fluoxastrobin, however, is absorbed into the plant leaf within 15 min of application (Arysta LifeScience 2009) rendering contact between already present, or concurrently applied fungal spores to a very short exposure. Spores applied after the fungicide should have no contact with the residues. A more realistic

strategy is to test the effects of spore deposits on plant surfaces that have been treated before, after, or concurrently with the chemical under study, an approach adopted by Mycotech for their pesticide compatibility recommendations (Laverlam International 2005). The spores are incubated for a set period of time, then washed off, rinsed by centrifugation and plated on agar media for germination.

The insect cuticle as it affects efficacy

While epicuticular substances clearly stimulate spore germination (or otherwise cuticular route of infection would not be possible), some insects possess fungistatic compounds. For example, Smith and Grula (1982) showed that cuticular extracts from larval *Helicoverpa zea* Boddie inhibited *B. bassiana* conidial germination. A more striking example may be *Nezaria viridula* L. SosaGomez et al. (1997) observed conidial germination of *M. anisopliae* on *N. viridula* cuticle was much lower than on other insect cuticle substrates, parallel to reduced infectivity for that insect. Only 5–20% of the conidia on *N. viridula* cuticle produced germ tubes, attributed to presence of the aldehyde, (E)-2-decenal. In addition, the cuticular topography affected conidial binding. Similarly, a pentane extract of *Melolontha melolontha* L. or *Ostrinia nubilalis* Hubner cuticle inhibited conidial germination and hyphal growth of a *B. bassiana* non-pathogenic to each insect (Lecuona et al. 1997), while in each case, a pathogenic isolate was not inhibited. In exploring the basis of differential susceptibility of *G. mellonella*, *Dendrolimus pini* L., and *Calliphora vicina* Robineau-Desvoidy to an entomophthoralean fungus, *Conidiobolus coronatus* (Costantin) Batko, Golebiowski et al. (2008) observed that reduced susceptibility to infection was associated with presence of C14, C16, and C20 fatty acids in *C. vicina*, but direct causation was not proven. In such studies caution should be taken in extrapolating in vitro germination tests with cuticular extracts to in vivo situations. The in vitro situation may not parallel in vivo conditions. The immediate environment for conidial attachment and germination, and the molecular concentration of stimulants or inhibitors cannot be easily duplicated. For example, free fatty acid toxicity to *B. bassiana* conidia was dependent on nutritional conditions (Smith and Grula 1982). Nevertheless, current evidence does indicate that the insect cuticle surface can mediate successful infection and thus the efficacy of inundatively used fungi against some insects. The existence of highly pathogenic isolates for most insects, however, implies that fungi can be found for which these barriers are unimportant.

Interaction with biotic components of the foliar environment

Phylloplane microflora

The phylloplane is replete with a great variety of microorganisms. Biofilms are almost ubiquitous on the phylloplane and are often 20 μm in depth and up to 1 mm in length (Morris et al. 1997). Aerial conidia of species such as *B. bassiana* and *M. anisopliae*, being dormant until they contact insect cuticle, are probably unaffected, at least as inferred from various on-leaf persistence studies where UV effects are absent. The ability of *H. thompsoni* to germinate and grow vegetatively on the leaf surface, then conidiate (McCoy 1981; McCoy and Couch 1982), infers that, at least in the citrus phylloplane microhabitat, the microflora is innocuous. Relatively few studies regarding any interaction between fungal entomopathogen conidia and microorganisms have been reported and all were done in vitro, creating another area ripe for investigation.

Non-target invertebrates and vertebrates

One of the characteristics of most of the fungal entomopathogens is their specificity to the Arthropoda. Published studies (see reviews by Zimmermann 2007a, b, 2008), as well as publicly released registration data, have demonstrated general safety for healthy vertebrates. There is relatively little data about the effect of vertebrates on the inundative release of fungal entomopathogens, but birds do attack locusts infected with *M. acridum* and can have a significant impact on treated populations (Mullie 2009). Nontarget invertebrates, for their part, have the potential of vectoring a fungal entomopathogen. An example are the Collembola, which seem refractive to infection by fungal entomopathogens and able to vector several fungal entomopathogen species to larval *Tenebrio mollitor* larvae, at least in a laboratory setting (Dromph 2003). Many predatory and

parasitic insects seem to be ecologically protected from serious impact by inundatively applied fungal entomopathogens (Jaronski et al. 1998) and they have the potential to vector the fungal spores (Roy et al. 2001; Baverstock et al. 2009), as well as to complement the fungi in reducing an insect population. For instance, simultaneous use of predators, parasitoids, and mycopesticides can provide additive effects under greenhouse conditions (Labbe et al. 2009). There was a considerably faster elimination of aphids on leaf disks over which *Lecanicillium longisporum*-treated *Orius laevigatus* Fieber had walked (Down et al. 2009). However this potential dispersal of conidia was not universal. In the same study *F. occidentalis* were only slightly more affected than controls under the same conditions, and *B. tabaci* not at all. Conidia of *I. fumosorosea* were vectored by *Hippodamia convergens* Guérin-Méneville to healthy aphids and caused a variable proportion of the aphids to become infected (Pell and Vandenberg 2002), with greatest vector efficiency after the beetles fed among sporulated aphid cadavers. The authors pointed out this phenomenon might facilitate the spread of mycopesticide application within and between fields and therefore improve the efficacy. Predators might also act as vectors moving fungal inoculum into cryptic feeding sites. However, this potential dispersal of fungal conidia is not universal. See the recent review by Furlong and Pell (2005) for more details about fungal entomopathogen-natural enemy interactions.

Other insect pathogens

There are a few laboratory studies examining interaction of a fungal entomopathogen with another insect pathogen, or two fungal pathogens within the same host. The presence of another pathogen may make the target insects more susceptible to a fungal entomopathogen. For example, Brinkman and Gardner (2000) observed that fire ants (*Solenopsis invicta* L.) from microsporidian-infected colonies were 4.5 times more susceptible to *B. bassiana* than ants from healthy colonies, based on the LD_{50} ratio. Similarly, at low doses, joint infections of *Paranosema* (*Nosema*) *locustae* Canning and *B. bassiana* had a faster onset of mortality than nymphs with single infections and at high doses were synergistic (Tounou et al. 2008). Infections of *M. acridum* and a *B. bassiana* complemented each other under oscillating high and low temperatures (Inglis et al. 1999). But this mutually beneficial situation may not always be the case. A notable exception is a study by Thomas et al. (2003), in which the in vivo interactions of virulent and avirulent fungal entomopathogens in locusts were variable and were affected by the order of infection and by environmental conditions, particularly temperature. The significance of inter-pathogen interactions would depend upon the prevalence of the endemic pathogen in the target population, and would probably be manifested by degree of efficacy from the applied fungus.

Inundative use against soil-dwelling pests

Extensive discussions of abiotic and biotic factors affecting persistence and efficacy of fungal entomopathogens in the soil were published recently (Klingen and Haukeland 2006; Jaronski 2007). Therefore, the present review will only mention highlights.

Use of mycoinsecticides in the soil presents a different situation than foliar applications. All dose transfer to the insect is indirect—the insect pest must come into contact with the fungus spores. The key with all tactics is to create an infectious "minefield" of fungal spores to intercept the insects as they migrate through the soil and around the plant roots (Jaronski et al. 2005). In moving through the minefield the insects must physically contact and acquire sufficient numbers of spores for infection.

One approach is to apply conidia in aqueous suspension or as a dust into the soil or into the plant crown. Soil drenches do not carry conidia very far into all but the coarsest textured soils or potting mixes, limiting this approach (Ignoffo et al. 1977a; Storey and Gardner 1988; Storey et al. 1989). But soil drenches are still useful if spores can be applied to intercept insects dropping into the soil for pupation (e.g., *Curculio caryae* Horn, the pecan weevil; and *Rhagoletis indifferens* Curran, the cherry fruit fly), or neonates hatching from eggs laid on or just in the soil surface (e.g., *T. myopaeformis,* or *Delia* spp.).

The "numbers game" in the soil arena

Use of fungal entomopathogens in the soil arena is also a numbers game. There are several estimates of

the LC_{50} and LC_{95} of fungi in terms of colony forming units or spores, per cm^{-3} or g of soil (e.g., Ferron 1981; McDowell et al. 1990; Bruck 2005; Ekesi et al. 2002; Bruck et al. 2005). The exact value, however, depends on the specific soil characteristics (sterile vs. nonsterile, organic content, texture, etc.); size and behavior of the insect; and specific environmental conditions (moisture, temperature). Data from numerous lab assays and field trials indicate an efficacious level is approximately 10^5–10^6 colony forming units (CFU) cm^{-3} or g^{-1} soil with better isolates. In broadcast application of spores with incorporation to a depth of 10 cm, the volume of the arena is 1×10^9 cm^3 ha^{-1}, requiring 10^{14}–10^{15} spores ha^{-1} at the previously mentioned levels. Where the target insect tends to be restricted to a specific location in the soil, e.g., neonate sugarbeet root maggot (Jaronski et al. 2005), requirements can be reduced by concentrating spore application to that specific arena. For example, a 10-cm-wide, banded application of spores in water, centered on the bases of seedling sugar beets, with a target soil penetration of 1 cm (the oviposition zone for the sugarbeet root maggot adult fly) reduces the arena volume to 1.64×10^7 cm^3 ha^{-1} for, potentially, a 100-fold reduction in spores needed per hectare for a given spore concentration in that zone. The distribution of spores in soil, however, is extremely heterogeneous, even with a thorough soil drench. The soil consists of a complex network of soil pores ranging from 5 to 500 μm wide, depending upon the soil texture and upon compaction. On a larger scale, cracks in dry soil will conduct spore suspensions into the larger spaces, leaving large zones devoid of spores. Also, spores do not readily move subsequent to deposition by the aqueous carrier except in the sandiest soils (Ignoffo et al. 1977a; Storey and Gardner 1988).

The fungal entomopathogen often has to persist until the target insects arrive in the specific arena. This persistence may have to range from a few days to a number of weeks or months. For example, efficacious titers of fungi applied at planting for control of corn rootworm must persist for about a month before the eggs of the insect hatch. Sugarbeet root maggots hatch 4–8 weeks after typical planting so that a fungus applied at planting must persist at efficacious levels for at least that long. Fungal persistence in the soil is very variable, from a few weeks (Storey et al. 1989) to more than 40 weeks (Kabaluk et al. 2007).

Application of fungi on granules change the numbers game. Presuming the granule is covered with conidia, a soil insect has merely to brush against it to acquire a large dose of spores. Furthermore, nutritive granules allow for the fungus to germinate, grow and resporulate increasing the titer of spores. A critical concentration of granules is still needed for acceptable efficacy. In replicated bioassays, using third-instar sugarbeet root maggot larvae in a clay soil and at optimal moisture and temperature for the fungus, four or more granules (corn grit granules, 0.5–1 mm diameter, coated with *M. anisopliae* conidia and having a titer of 1,400 granules g^{-1}) per cm^3 of soil were needed for >90% efficacy in laboratory bioassays (Jaronski et al. 2005). If such granules are applied broadcast and incorporated into the top 10 cm of soil, one would need 2,858 kg ha^{-1} to achieve four granules per cm^3 soil. Application amounts decrease to 351 kg granules ha^{-1} if granules are applied in a 15-cm band over the row (with 61 cm row spacing) and incorporated to a depth of 5 cm. The critical concentration of granules could be achieved at 1.9 kg ha^{-1} if the granules are applied in-furrow (essentially a band 1 cm wide, 1 cm thick). In furrow application may not properly intercept the target insects, however. The nature of the granule can also change the numbers game. For example, granular formulations of the newly discovered *M. anisopliae* microsclerotia (Jackson and Jaronski 2009; Jaronski and Jackson 2008, 2009) have a laboratory LC_{90} of 0.5 grains cm^{-3} soil against sugarbeet root maggot, possibly because of attraction (Jaronski and Jackson 2009). Thus, the above amounts needed per hectare would theoretically decrease by a factor of eight.

Effect of soil abiotic and biotic factors

Abiotic factors

Primary abiotic factors affecting the efficacy of fungal entomopathogens are soil texture (pore size distribution), temperature, and moisture. Other physical factors in soil—pH, cation exchange capacity, and inorganic salts—do not seem to have any important impact on fungal entomopathogen infectivity or persistence.

Soil texture and moisture interact in a complex relationship that also involves the size and movement behavior of the insect. Soil texture, particularly the size distribution of pore spaces, affects the infectivity of spores by evidently mediating physical contact. For example, the LC_{50} of *B. bassiana* IL116 for second-instar *Diabrotica undecimpunctata howardii* Barber (southern corn rootworm) ranged from 9.0×10^4 to 2.25×10^6 CFU g^{-1} soil in ten different soils all held at 25% field capacity (Jaronski 2007). There was no correlation with soil type and the differences were not due to differences in conidial viabilities. Similarly, Kabaluk et al. (2007) observed that efficacy of *M. anisopliae* for wireworms (Elateridae) differed significantly among sand, clay, and organic soils at the same moisture level. The moisture content of a soil interacts with soil texture to further complicate effects on efficacy. Infection and mortality of third instar sugarbeet root maggot larvae by *M. anisopliae* F52 were significantly affected by soil type and moisture in five soils and three moisture levels (Table 1) even though levels of fungus (CFU g^{-1} soil) were not different (Jaronski et al. 2005). To complicate matters, different fungal isolates may respond differently to different soil types and moisture levels (Jaronski 2007).

Just as in the foliar arena, temperature in the soil influences efficacy. Soil temperatures, at levels more than 5 cm below the surface, tend to be cool, especially in the mesic, frigid, and cryic regions, which encompass much of North America, the northern half of Europe and Asia, and the southern portion of South America. For example, in Tennessee (Lat. 34–36°N), soil temperatures at 10 cm depth generally are above 15°C only between Julian Day 110 and 270, while in Oregon and southern Michigan (Lat. 41–44°N) that period is Julian Day 150–280 (data drawn from Zheng et al. 1993). In tropical and subtropical regions, soil temperatures are higher and usually within the optimal range for most fungal entomopathogens. Low soil temperatures often prolong the duration before mortality from mycosis is achieved. For example, in evaluating a *M. anisopliae* for the control of the pasture scarab beetle, *Adoryphorous couloni* Burmeister, in Tasmania, Rath et al. (1995), observed that LT_{50} values increased from 36 to 189 days when the treated insects were incubated at 5 vs. 15°C. In a Canadian study, wireworms exposed to *M. anisopliae* and incubated at 12°C escaped infection for over 60 days whereas those incubated at 18°C suffered considerable mortality from mycosis (Kabaluk and Ericsson 2007). The wireworms also needed at least an initial 48 h exposure at 18°C for fatal infection to occur, presumably to allow conidia to germinate and the fungi to penetrate the wireworm cuticle. Soil temperatures in cooler latitudes can thus be a major factor in timely efficacy and extensive selection of appropriate isolates are warranted. Most of the fungal entomopathogens studied, e.g., Fargues et al. (1997), display slowed germination and growth at temperatures below 15°C although there are isolates with better tolerance to cooler temperatures (Bidochka et al. 2001). Temperature tolerances must be contrasted with not only regional and seasonal soil

Table 1 Mortality of third instar sugarbeet root maggot (SBRM) after two weeks of exposure to *Metarhizium anisopliae* F52 (2.5×10^6 conidia g^{-1} dry soil), in six soils at three moisture levels (10, 15, 30% field saturation for each soil type)

Soil	Snd:Cl:Slt	FS (%)	SBRM mortality	
			Mean (%)	SD (%)
Clay	11:56:33	10	11	4c
		15	44	10b
		30	84	12a
Clay	16:51:33	10	22	4c
		15	9	10c
		30	98	4a
Loam	35:19:46	10	56	21b
		15	100	0a
		30	100	0a
Clay loam	39:31:30	10	5	4c
		15	100	0a
		30	98	4b
Sandy clay loam	56:21:23	10	47	18b
		15	80	9a
		30	58	10b
Sandy loam	75:13:12	10	100	0a
		15	100	0a
		30	98	4a

Snd:Slt:Cl is the sand:silt:clay ratio for each soil. Data are means and SD of three replicate assays each with three replicates per treatments. In all cases the numbers of colony forming units g^{-1} soil were not significantly different from each other at start and end of each assay. Mean mortalities followed by different letters are significantly different (Tukey's HSD test, $P = 0.05$)

temperatures but also temperatures in the specific soil arena and time of use.

Agricultural inputs (fertilizer, pH modifiers, pesticides) and practices can have major impacts on soil microbial and macrobial populations (Stewart 1991). However, there have been very few in situ studies with fungal entomopathogens. Most studies have focused on correlation between fungal entomopathogen titers and agricultural practices. There have been very few manipulative in situ studies where agricultural inputs are controlled variables. See Jaronski (2007) and Klingen and Haukeland (2006) for more information on this topic.

Agrichemicals, especially fungicides, can have direct bearing on fungal persistence. Most studies have been concerned with in vitro fungal-pesticide interactions. Using an agar incorporation approach is useful in identifying harmless pesticides but may imply false adverse effects from a particular chemical. One must remember that for the most part, conidia in soil remain ungerminated; germination on an insect's cuticle may be isolated from the effects of a soil pesticide; and, once inside an insect, a fungus may well be insulated from adverse effects of a pesticide in the soil. There is a lack of realistic, in situ studies that examine potential interference of agrochemicals in fungal entomopathogen efficacy. Often the best, most realistic approach is within the context of a field trial or at least outdoor, in-field microcosm. For instance, it was demonstrated that of 13 fungicides toxic in vitro, none had adverse impact on *M. anisopliae* in commercial potting media under realistic conditions, even when applied twice during the observation period (Bruck 2009a).

Biotic factors

Biotic factors, primarily soil microbiota, are important particularly with regards to persistence of fungi. A typical soil can contain 10^8–10^9 bacteria, "several metres of fungi", 10^5 soil protozoa, 10–20 nematodes, and 0–100 arthropods per gram (Tugel et al. 2000). In general, most natural soils exhibit a fungistasis for fungal entomopathogen conidia (e.g., Pereira et al. 1993) as well as other fungi (Stotzky 1972). This fungistasis is removed by soil sterilization, after which fungal titers can increase by several orders of magnitude. In one of the few manipulative experiments, the fungistatic effect of a natural and a synthetic soil was destroyed by autoclaving and restored by inoculation with bacteria, actinomycetes or fungi (Ho and Ko 1986). Interestingly, soil fungistasis does not operate on fungal entomopathogens when they are on nutritive granules or *M. anisopliae* microsclerotia (e.g., Jaronski and Jackson 2008, 2009). This ostensible anomaly indicates that fungistasis may be due more to nutritional factors than antibiosis. The typical subsequent trend in fungal titers is a decline in CFU, the rapidity of which depends on a number of as yet incompletely understood factors. What is known about the effect of microflora underscores the complexity of relationships in time and space (Jaronski 2007). Jaronski et al. (2007) examined the in vitro interactions between 30 sugarbeet rhizoplane bacteria and each of three isolates of *B. bassiana* and *M. anisopliae*. There were qualitative differences among the fungal species and isolates in their response to the various bacteria. A general trend appeared to be greater inhibition of conidial germination by Gram negative (G−) than Gram positive (G+) species. Hyphal growth of the fungi was generally not inhibited by any of the bacteria. More G+ bacteria were inhibited by *M. ansiopliae* than by *B. bassiana*, and fewer G− bacteria were inhibited by either fungus. It should be added that their in vitro observations may not be necessarily reflected in vivo. Certainly, the availability of nutrients in vivo can be much lower and more tightly restricted to minute foci within the soil. At the same time, soil fungistasis does not seem to be completely effective because soil insects do become infected in nature.

When fungi are applied as a seed coat, the key requirement is that the fungi colonize the growing root system and subsequently sporulate. Using green fluorescent protein (gfp)-labeled *M. anisopliae,* Hu and St Leger (2002), observed rhizosphere colonization by *M. anisopliae* in field plots. Bruck (2005) indirectly observed a higher titer of *M anisopliae* in *Picea abies* (L.) H. Karst rhizosphere than in the bulk potting medium, although the observations are complicated by use of fungus on rice grain spent substrate and peat- or bark-based media rather than soil. Using gfp transformants, Jaronski et al. (2007) observed that several isolates of *M. anisopliae* and *B. bassiana* could only colonize the rhizoplane of young sugarbeet seedlings in vitro in an agar-based system. In gnotobiotic media—sterile clay soil, sterile potting

mix, vermiculite + 10% Hoagland's Solution—rhizoplane colonization was not observed, regardless of whether conidia were applied to the seed coat or added to the medium itself and seeds thereafter added. Root colonization was also not observed with chard (*Beta vulgaris* var. *cicla*), bean, or maize (*Zea mays* L.) seedlings. A prerequisite for colonization is conidial germination in the rhizosphere or on the rhizoplane. Jaronski et al. (2007) determined that conidial germination was almost nonexistent in root exudate of two-leaf sugar beets, but reached about 50% after 24 h in exudate from four-leaf sugar beets, cabbage and chard. In contrast, germination was >95% in oat, rye, or bean root exudate, as well as in 1% neopeptone, or in Sabouraud dextrose broth. The subject of fungal entomopathogens in the rhizosphere is treated further by Bruck (2009b).

Among the Protistan microfauna, soil amoebae have the potential to reduce fungal levels by direct mycophagy (Bryant et al. 1982). Several species of amoeba have the potential for direct ingestion of conidia but the fate of such conidia is not known. Members of the Vampyrellidae are known to perforate spores of plant pathogenic fungi (Anderson and Patrick 1985). They may also have the potential of attacking fungal entomopathogen conidia and mycelium. Soil mesofauna, such as Collembola, orbatid and prostigmatid mites, can also potentially affect fungal titer in the soil by feeding. Collembola have been proposed as biocontrol agents of plant pathogenic fungi (Curl 1988; Lartey et al. 1994). These insects seem to be somewhat refractory to infection by at least several isolates of fungal entomopathogens, and conidia were attractive to three species of Collembola (Dromph and Vestergaard 2002). Collembolan grazing can suppress *Rhizoctonia solani* Kühn and *R. cerealis* Hoeven, both in laboratory and field situations (Shiraishi et al. 2003 cited in Friberg et al. 2005). Effects on entomopathogenic titers in soil are not known. Much less is known about the impact of soil mites. I refer the interested reader to Friberg et al. (2005) for an introduction to the subject.

Summary and closing thoughts

Inundative use of fungal entomopathogens seeks to overcome by sheer numbers many of their disadvantages as classical biocontrol agents and the many environmental factors that mitigate their impact on a target insect population under natural conditions. Inundation can work to create transient epizootics to manage an insect population. To do so consistently, practicably, and within economic constraints is the challenge. We have learned a lot about how the key environmental variables of temperature, moisture and ultraviolet light in the foliar arena, and temperature, moisture and soil characteristics in the soil arena can affect the success or failure of these fungi. The bulk of studies have focused on one or a few variables at a time, for example as spore mortality factors, very often in the laboratory, not in real situations. That is a start. These variables interact, however, producing complex, dynamic effects on the spores. The existing body of knowledge is only a beginning to our understanding.

Development of models incorporating multiple variables is critically needed to better understand the many factors that operate together to affect efficacy of a fungal entomopathogen. Efforts have been made in this regard: Pinnock and Brand (1980) and Brand and Pinnock (1980) in a general sense; Galaini (1984) regarding Colorado potato beetle in potatoes; Yang et al. (1997) with the citrus rust mite; Feng et al. (1985) concerning European corn borer; Knudsen and Schotzko (1999) regarding modeling *B. bassiana* epizootics in Russian wheat aphid; Boulard et al. (2002) and Vidal et al. (2003) with regards to whiteflies in glasshouse tomatoes; Klass et al. (2007a, b) regarding climate suitability for locust control by *M. acridum*; and Polar et al. (2008) with ticks on livestock. Hesketh et al. (2009) further address models of fungi in natural populations of insects. The models should not be an end to themselves, but rather serve as tools to refine our understanding of environmental variables in a holistic perspective, comprehend how and when the fungi can work in an inundative use, and inspire methods to enhance efficacy. Further, the lessons from the models must be taken into operational situations.

We need to reconsider the best use of inundative release. These organisms are not chemicals. The chemical paradigm often involves use of insecticides, by themselves, once an outbreak has occurred and reached, or passed, the economic injury level. This "fire extinguishing" philosophy is not appropriate for fungal entomopathogens. The fungi work "too slowly", and repeated applications at short intervals

must often be made. The best use of mycoinsecticides is pest outbreak prevention with applications targeting the first immigrants into the crop to prevent pest population establishment and increase to the economic injury level. This has become a guiding philosophy of at least one biopesticide company in advising growers how to use its fungus (Bioworks Inc 2008). A prime example of using fungal entomopathogens in a proactive, preventative, rather than reactive, strategy is deployment of *M. acridum* against African locust (Lecoq 2004; Showler 1997).

Lastly, we need to integrate these organisms with other tools, such as predators and parasites, and cultural practices, to create sustainable, biologically based systems, where possible, and not use the fungi by themselves. For example, predators and parasites can act efficiently on the survivors during mycopesticide use. In the glasshouse environment, combined use of a mycopesticide, parasites and predators has been a viable option (Labbe et al. 2009). The fungi can also be combined with other microbial pest control agents, such as *B. thuringiensis tenebrionis* to overcome the disadvantages of each, such as for Colorado potato beetle (Wraight and Ramos 2005). Only through intelligent use of fungal entomopathogens will they become significant tools for farmers.

References

Al-Mazra'Awi MS, Shipp L, Broadbent B, Kevan P (2006a) Biological control of *Lygus lineolaris* (Hemiptera: Miridae) and *Frankliniella occidentalis* (Thysanoptera: Thripidae) by *Bombus impatiens* (Hymenoptera: Apidae) vectored *Beauveria bassiana* in greenhouse sweet pepper. Biol Control 37(1):89–97

Al-Mazra'Awi MS, Shipp L, Broadbent B, Kevan P (2006b) Dissemination of *Beauveria bassiana* by honey bees (Hymenoptera: Apidae) for control of tarnished plant bug (Hemiptera: Miridae) on canola. Environ Entomol 35(6): 1569–1577

Altre JA, Vandenberg JD (2001) Factors influencing the infectivity of isolates of *Paecilomyces fumosoroseus* against diamondback moth, *Plutella xylostella*. J Invertebr Pathol 78(1):31–36

Alves RT, Bateman RP, Prior C, Leather SR (1998) Effects of simulated solar radiation on conidial germination of *Metarhizium anisopliae* in different formulations. Crop Prot 17(8):675–679

Anderson TR, Patrick ZA (1985) Mycophagous amoeboid organisms from soil that perforate spores of *Thielaviopsis basicola* and *Cochliobolus sativus*. Phytopathology 68: 1618–1626

Andrews JH, Buck JW (2002) Adhesion of yeasts to leaf surfaces. In: Lindow SE, Hect-Porter EI, Elliott VJ (eds) Phyllosphere microbiology. American Phytopathological Society, St Paul MN, pp 53–68

Arysta LifeScience (2009) Evito product information. Available via http://www.arystalifescience.com/default.asp?V_DOC_ID=1758 Cited 22 Sep 2009

Bateman RP, Matthews GA, Hall FA (2007) Ground-based application equipment. In: Lacey LA, Kaya HK (eds) Field manual of techniques in invertebrate pathology, 2nd edn. Springer, New York, pp 73–98

Baverstock J, Roy HE, Pell JK (2009) Entomopathogenic fungi and insect behavior: from unsuspecting hosts to targeted vectors. BioControl. doi 10.1007/s10526-009-9238-5 (this SI)

Beattie GA (2002) Leaf surface waxes and the process of leaf colonization by microorganisms. In: Lindow SE, Hect-Porter EI, Elliott VJ (eds) Phyllosphere microbiology. American Phytopathological Society, St Paul MN, pp 3–26

Behle RW (2006) Importance of direct spray and spray residue contact for infection of *Trichoplusia* in larvae by field applications of *Beauveria bassiana*. J Econ Entomol 99(4):1120–1128

Bidochka MJ, Kamp AM, Lavender TM, Dekoning J, deCroos JNA (2001) Habitat association in two genetic groups of the insect-pathogenic fungus *Metarhizium anisopliae*: uncovering cryptic species? Appl Environ Microbiol 67(3):1335–1342

Bioworks Inc (2008) Aphid management. BotaniGard ES Technical Bulletin B-Aphids/2-09. Available via http://www.bioworksbiocontrol.com/botanigard/pdf/BotaniGard%20AphidMgtTechBulletin.pdf. Cited 5 Sep 2009

Bonan GB (2008) Ecological climatology concepts and application, 2nd edn. Cambridge University Press, Cambridge

Boulard T, Mermier M, Fargues J, Smits N, Rougier M, Roy JC (2002) Tomato leaf boundary layer climate: implications for microbiological whitefly control in greenhouses. Agr Forest Metereol 110:159–176

Braga GUL, Flint SD, Miller CD, Anderson AJ, Roberts DW (2001a) Both solar UVA and UVB radiation impair conidial culturability and delay germination in the entomopathogenic fungus *Metarhizium anisopliae*. Photochem Photobiol 74(5):734–739

Braga GUL, Flint SD, Miller CD, Anderson AJ, Roberts DW (2001b) Variability in response to UV-B among species and strains of *Metarhizium* isolated from sites at latitudes from 61°N to 54°S. J Invertebr Pathol 78:98–108

Brand RJ, Pinnock DE (1980) Application of biostatistical modeling to forecasting the results of microbial control trials. In: Burges HD (ed) Microbial control of pests and plant diseases 1970–1980. Academic Press, London, pp 667–693

Brinkman MA, Gardner WA (2000) Enhanced activity of *Beauveria bassiana* to red imported fire ant workers (Hymenoptera: Formicidae) infected with *Thelohania solenopsae*. J Agric Urban Entomol 17(4):191–195

Bruck DJ (2005) Ecology of *Metarhizium anisopliae* in soilless potting media and the rhizosphere: implications for pest management. Biol Control 32:155–162

Bruck DJ (2009a) Impact of fungicides on *Metarhizium anisopliae* in the rhizosphere, bulk soil and *in vitro*. Biocontrol 54:597–606

Bruck D (2009b) Fungal entomopathogens in the rhizosphere. BioControl. doi 10.1007/s10526-009-9236-7 (this SI)

Bruck DJ, Lewis LC (2002) Rainfall and crop residue effects on soil dispersion and *Beauveria bassiana* spread to corn. Appl Soil Ecol 20(3):183–190

Bruck DJ, Snelling JE, Dreves AJ, Jaronski SJ (2005) Laboratory bioassays of entomopathogenic fungi for the control of *Delia radicum* (L.) larvae. J Invertebr Pathol 89:179–183

Bryant RJ, Woods LE, Coleman DC, Fairbanks BC, McClellan JF, Cole CV (1982) Interactions of bacterial and amebal populations in soil microcosms with fluctuating moisture content. Appl Environ Microbiol 43(4):747–752

Bugeme DM, Knapp M, Boga HI, Wanjoya AK, Maniania NK (2009) Influence of temperature on virulence of fungal isolates of *Metarhizium anisopliae* and *Beauveria bassiana* to the two-spotted spider mite *Tetranychus urticae*. Mycopathologia 167(4):221–227

Burges HD (1998) Formulation of mycoinsecticides. In: Burges HD (ed) Formulation of microbial biopesticides. Kluwer Academic Publishers, Dordrecht, pp 7–30

Burrage SW (1971) The micro-climate at the leaf surface. In: Preece TF, Dickinson CH (eds) Ecology of leaf surface micro-organisms. Academic Press, New York, pp 91–101

Carruthers RI, Larkin TS, Firstencel H (1992) Influence of thermal ecology on the mycosis of a rangeland grasshopper. Ecology 73(1):190–204

Chapple AC, Downer RA, Bateman RP (2007) Theory and practice of microbial insecticide application. In: Lacey LA, Kaya HK (eds) Field manual of techniques in invertebrate pathology, 2nd edn. Springer, Dordrecht, pp 9–34

Charnley AK (1989) Mechanisms of fungal pathogenesis in insects. In: Whipps JM, Lumsden RD (eds) Biotechnology of fungi for improving plant growth. Cambridge Univ Press, Cambridge, pp 85–125

Chu CC, Akey DH, Henneberry TJ (1994) Response of cotton plants to aldicarb soil application. J Agr Assoc China 167:75–90

Clark RA, Casagrande RA, Wallace DB (1982) Influence of pesticides on *Beauveria bassiana*, a pathogen of the Colorado potato beetle. Environ Entomol 11(1):67–70

Cohen E, Joseph T (2009) Photostabilization of *Beauveria bassiana* conidia using anionic dyes. Appl Clay Sci 42(3–4):569–574

Cory J, Ericsson JD (2009) Fungal entomopathogens in a tritrophic context. BioControl. doi 10.1007/s10526-009-9247-4 (this SI)

Cory JS, Hoover K (2006) Plant-mediated effects in insect-pathogen interactions Trends. Ecol Evol 21(5):278–286

Costa SD, Gaugler R (1989) Influence of *Solanum* host plants on Colorado potato beetle (Coleoptera: Chrysomelidae) susceptibility to the entomopathogen, *Beauveria bassiana*. Environ Entomol 18:531–536

Curl EA (1988) The role of soil macrofauna in plant disease suppression. CRC Crit Rev Pl Sci 7:175–198

da Silva RZ, Neves PMOJ (2005) Techniques and parameters used in compatibility tests between *Beauveria bassiana* (Bals) Vuill and *in vitro* phytosanitary products. Pest Manage Sci 61(7):667–674

De Croos JNA, Bidochka MJ (1999) Effects of low temperature on growth parameters in the entomopathogenic fungus *Metarhizium anisopliae*. Can J Microbiol 45(12):1055–1061

Devi KU, Sridevi V, Mohan CM, Padmavathi J (2005) Effect of high temperature and water stress on *in vitro* germination and growth in isolates of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuillemin. J Invertebr Pathol 88(3):181–189

Down RE, Cuthbertson AGS, Mathers JJ, Walters KFA (2009) Dissemination of the entomopathogenic fungi, *Lecanicillium longisporum* and *L. muscarium*, by the predatory bug, *Orius laevigatus*, to provide concurrent control of Myzus persicae, *Frankliniella occidentalis* and *Bemisia tabaci*. Biol Control 50(2):172–178

Dromph KM (2003) Collembolans as vectors of entomopathogenic fungi. Pedobiologia 47(3):245–256

Dromph KM, Vestergaard S (2002) Pathogenicity and attractiveness of entomopathogenic hyphomycete fungi to collembolans. Appl Soil Ecol 21(3):197–210

Dubois T, Li Z, Jiafu H, Hajek AE (2004) Efficacy of fiber bands impregnated with *Beauveria brongniartii* cultures against the Asian longhorned beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae). Biol Control 31(3):320–328

Edgington S, Segura H, Del a Rosa W, Williams T (2000) Photoprotection of *Beauveria bassiana*: testing simple formulations for control of the coffee berry borer. Intl J Pest Mgt 46(3):169–176

Eilenberg J, Hajek A, Lomer C (2001) Suggestions for unifying the terminology in biological control. BioControl 46(4):387–400

Ekesi S, Maniana NK, Lux SA (2002) Mortality in three African tephritid fruit fly puparia and adults caused by the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*. Biocont Sci Technol 12(1):7–17

Fargues J, Luz C (2000) Effects of fluctuating moisture and temperature regimes on the infection potential of *Beauveria bassiana* for *Rhodnius prolixus*. J Invertebr Pathol 75(3):202–211

Fargues J, Maniania NK (1992) Variabilité de la sensibilité de *Spodoptera littoralis* (Lep; Noctuidae) à l'hyphomycète entomopathogène *Nomuraea riley*. Entomophaga 37:545–554

Fargues J, Goettel MS, Smits N, Ouedraogo A, Vidal C, Lacey LA, Lomer CJ, Rougier M (1996) Variability in susceptibility to simulated sunlight of conidia among isolates of entomopathogenic hyphomycetes. Mycopathologia 135(3):171–181

Fargues J, Goettel MS, Smits N, Ouedraogo A, Rougier M (1997) Effect of temperature on vegetative growth of *Beauveria bassiana* isolates from different origins. Mycologia 89(3):383–392

Faria MR, Wraight SP (2007) Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Biol Control 43:237–256

Feng Z, Carruthers RL, Roberts DW, Robson DS (1985) Age-specific dose-mortality effects of *Beauveria bassiana* on the European corn borer, *Ostrinia nubilalis*. J Invertebr Pathol 46:259–264

Feng MG, Poprawski TJ, Khachatourians GG (1994) Production, formulation, and application of the entomopathogenic

fungus *Beauveria bassiana* for insect control: current status. Biocont Sci Technol 4:3–34
Fernandes ÉKK, Rangel DEN, Moraes ÁML, Bittencourt VREP, Roberts DW (2007) Variability in tolerance to UV-B radiation among *Beauveria* spp. isolates. J Invertebr Pathol 96(3):237–243
Ferro DN, Chapman RB, Penman DR (1979) Observations on insect microclimate and insect pest management. Environ Entomol 8:1000–1003
Ferron P (1977) Influence of relative humidity on the development of fungal infection caused by *Beauveria bassiana* (Fungi Imperfecti, Moniliales) in imagines of *Acanthoscelides obtectus* (Col.: Bruchidae). Entomophaga 22(4):393–396
Ferron P (1981) Pest control by the fungi *Beauveria* and *Metarhizium*. In: Burges HD (ed) Microbial control of pests and plant diseases. Academic Press, London, pp 465–481
Friberg H, Lagerlöf J, Rämert B (2005) Influence of soil fauna on fungal plant pathogens in agricultural and horticultural systems. Biocont Sci Technol 15(7):641–658
Furlong MJ, Groden E (2003) Starvation induced stress and the susceptibility of the Colorado potato beetle, *Leptinotarsa decemlineata*, to infection by *Beauveria bassiana*. J Invertebr Pathol 83(2):127–138
Furlong MJ, Pell JK (2005) Interactions between entomopathogenic fungi and arthropod natural enemies. In: Vega FE, Blackwell M (eds) Insect-fungal associations: ecology and evolution. Oxford University Press, New York, pp 51–73
Fuxa JR (1987) Ecological considerations for the use of entomopathogens in IPM. Ann Rev Entomol 32:225–251
Galaini, S (1984) The efficacy of foliar applications of *Beauveria bassiana* conidia against *Leptinotarsa decemlineata.* MS thesis, Cornell University, Ithaca
Gardner WA, Sutton RM, Noblet R (1977) Persistence of *Beauveria bassiana*, *Nomuraea rileyi* and *Nosema necatrix* on soybean foliage. Environ Entomol 6:616–618
Gates DM (1968) Transpiration and leaf temperature. Rev Plant Physiol 19:211–238
Goettel MS, Eilenberg J, Glare T (2005) Entomopathogenic fungi and their role in regulation of insect populations. In: Gilbert LI, Iatrou K, Gill S (eds) Comprehensive molecular insect science. Elsevier, Amsterdam, pp 361–406
Golebiowski M, Malinski E, Bogus MI, Kumirska J, Stepnowski P (2008) The cuticular fatty acids of *Calliphora vicina, Dendrolimus pini* and *Galleria mellonella* larvae and their role in resistance to fungal infection. Insect Biochem Mol Biol 38(6):619–627
Hajek A, Delalibera I (2009) Fungal pathogens as classical biological control agents against arthropods. BioControl. doi 10.1007/s10526-009-9253-6 (this SI)
Hajek AE, McManus ML, Delalibera I (2005) Catalogue of introductions of pathogens and nematodes for the classical biological control of insects and mites. US Dept Agric For Serv, For Health Technol Enterprise Team 2005-05
Hare JD, Andreadis TG (1983) Variation in the susceptibility *of Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) when reared on different host plants to the fungal pathogen *Beauveria bassiana* in the field and in the laboratory. Environ Entomol 12:1892–1897
Hesketh H, Roy H, Eilenberg J, Pell J, Hails R (2009) Challenges in modelling complexity of fungal entomopathogens in semi-natural populations of insects. BioControl. doi 10.1007/s10526-009-9249-2 (this SI)
Ho WC, Ko WH (1986) Microbiostasis by nutrient deficiency shown in natural and synthetic soils. J Gen Microbiol 132(10):2807–2815
Hu G, St Leger RJ (2002) Field studies of a recombinant mycoinsecticide (*Metarhizium anisopliae*) reveal that it is rhizosphere competent. Appl Environ Microbiol 68: 6383–6387
Hunt TR, Moore D, Higgins PM, Prior C (1994) Effect of sunscreens, irradiance and resting periods on the germination of *Metarhizium flavoviride conidia*. Entomophaga 39(3/4):313–322
Hunter DM, Milner RJ, Spurgin PA (2001) Aerial treatment of the Australian plague locust, *Chortoicetes terminifera* (Orthoptera: Acrididae) with *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes). Bull Entomol Res 91:93–99
Ibrahim L, Butt TM, Beckett A, Clark SJ (1999) The germination of oil-formulated conidia of the insect pathogen, *Metarhizium anisopliae*. Mycol Res 103(7):901–907
Ignoffo C, Garcia C (1992) Influence of conidial color on inactivation of several entomogenous fungi (Hyphomycetes) by simulated sunlight. Environ Entomol 21(4): 913–917
Ignoffo CM, Garcia C, Hostetter DL, Pinnell RE (1977a) Vertical movement of *Nomuraea rileyi* through sand and loam soil. J Econ Entomol 70:163–164
Ignoffo CM, Hostetter DL, Sikorowski PP, Sutter G, Brooks WM (1977b) Inactivation of representative species of entomopathogenic viruses, a bacterium, fungus and protozoan by an ultraviolet light source. Environ Entomol 6:411–415
Ihara F, Toyama M, Mishiro K, Yaginuma K (2008) Laboratory studies on the infection of stink bugs with *Metarhizium anisopliae* strain FRM515. Appl Entomol Zool 43(4):503–509
Inglis GD, Goettel MS, Johnson DL (1993) Persistence of the entomopathogenic fungus, *Beauveria bassiana* on phylloplanes of crested wheatgrass and alfalfa. Biol Control 3:258–270
Inglis GD, Goettel MS, Johnson DL (1995a) Influence of ultraviolet light protectants on persistence of the entomopathogenic fungus, *Beauveria bassiana*. Biol Control 5:581–590
Inglis GD, Johnson DL, Goettel MS (1995b) Effects of simulated rain on the persistence of *Beauveria bassiana* on leaves of alfalfa and wheat. Biocont Sci Technol 5(3):365–369
Inglis GD, Johnson DL, Goettel MS (1996) Effects of temperature and thermoregulation on mycosis by *Beauveria bassiana* in grasshoppers. Biol Control 7(2):131–139
Inglis GD, Johnson DL, Goettel MS (1997a) Effects of temperature and sunlight on mycosis (*Beauveria bassiana*) of grasshoppers under field conditions. Environ Entomol 26:400–409
Inglis GD, Johnson DL, Goettel MS (1997b) Field and laboratory evaluation of two conidial batches of *Beauveria bassiana* (Balsamo) Vuillemin against grasshoppers. Can Entomol 129:171–186

Inglis GD, Duke GM, Kawchuk LM, Goettel MS (1999) Influence of oscillating temperatures on the competitive infection and colonization of the migratory grasshopper by *Beauveria bassiana* and *Metarhizium flavoviride*. Biol Control 14(1):111–120

Inglis GD, Ivie TJ, Duke GM, Goettel MS (2000) Influence of rain and conidial formulation on persistence of *Beauveria bassiana* on potato leaves and Colorado potato beetle larvae. Biol Control 18:55–64

Inglis GD, Goettel MS, Butt TM, Strasser H (2001) Use of hyphomycetous fungi for managing insect pests. In: Butt TM, Jackson C, Magan N (eds) Fungi as biocontrol agents: progress problems and potential. CABI Publishing, Wallingford, pp 23–70

Inyang EN, Butt TM, Beckett A, Archer S (1998) The effect of plant growth and topography on the acquisition of conidia of the insect pathogen *Metarhizium anisopliae* by larvae of *Phaedon cochleariae*. Mycol Res 102(11):1365–1374

Inyang EN, Butt TM, Beckett A, Archer S (1999) The effect of crucifer epicuticular waxes and leaf extracts on the germination and virulence of *Metarhizium anisopliae* conidia. Mycol Res 103(4):419–426

Inyang EN, McCartney HA, Oyejola B, Ibrahim L, Pye BJ, Archer SA, Butt TM (2000) Effect of formulation, application and rain on the persistence of the entomogenous fungus *Metarhizium anisopliae* on oilseed rape. Mycol Res 104(6):653–661

Jackson MA, Jaronski ST (2009) Production of microsclerotia of the fungal entomopathogen *Metarhizium anisopliae* and their use as a biocontrol agent for soil-inhabiting insects. Mycol Res 113(8):842–850

Jackson MA, Dunlap CA, Jaronski ST (2009) Ecological considerations in producing and formulating fungal entomopathogens for use in insect biocontrol. BioControl. doi 10.1007/s10526-009-9240-y (this SI)

James RR, Croft BA, Shaffer BT, Lighthart B (1998) Impact of temperature and humidity on host-pathogen interactions between *Beauveria bassiana* and a coccinellid. Environ Ent 27(6):1506–1513

Jaronski ST (1997) New paradigms in formulating mycoinsecticides. In: Goss GR, Hopkinson MJ, Collins HM (eds) Pesticide formulations and applications systems: 17th Volume, ASTM STP 1328. American Society for Testing and Materials, Philadelphia, pp 99–112

Jaronski ST (2007) Soil ecology of the entomopathogenic ascomycetes: a critical examination of what we (think) we know. In: Maniana K, Ekesi S (eds) Use of entomopathogenic fungi in biological pest management. Research SignPosts, Trivandrum India, pp 91–144

Jaronski ST, Jackson MA (2008) Efficacy of *Metarhizium anisopliae* microsclerotial granules. Biocont Sci Technol 18(8):849–863

Jaronski ST, Jackson MA (2009) Further Progress with *Metarhizium* microsclerotial production. IOBC/WPRS Bull 45:275–278

Jaronski ST, Rosinska J, Brown C, Osterlind R, Staten R, Craft R, Antilla L (1997) Impact of *Beauveria bassiana* Mycotech strain GHA, buprofezin, and pyriproxyfen on whitefly predators in Arizona cotton. In: Silverleaf whitefly 1997 supplement to the 5-Year National research and action plan: progress review, technology transfer and new research and action plan (1997–2001). p 49

Jaronski ST, Lord J, Rosinka J, Bradley C, Hoelmer K, Simmons G, Osterlind R, Brown C, Staten R, Antilla L (1998) Effect of *Beauveria bassiana*-based mycoinsecticide on beneficial insects under field conditions. In: The 1998 Brighton conference pests and diseases, British crop protection council Brighton, vol 2. pp 651–657

Jaronski ST, Grace JA, Schlothauer R (2005) *Metarhizium anisopliae* for biocontrol of sugarbeet root maggot: constraints and challenges. Proc 33rd Bienn Mtg Amer Soc Sugar Beet Technol, pp 185–187

Jaronski ST, Fuller-Schaeffer C, Jung K, Boetel M, Majumdar A (2007) Challenges in using *Metarhizium anisopliae* for biocontrol of sugarbeet root maggot, *Tetanops myopaeformis*. Bull IOBC/WPRS 30(7):119–124

Johnson DL, Goettel MS (1993) Reduction of grasshopper populations following field application of the fungus *Beauveria bassiana*. Biocont Sci Technol 3:165–175

Johnson DL, Goettel MS, Bradley C, van der Paaw H, Maiga B (1992) Field trials with the entomopathogenic fungus *Beauveria bassiana* against grasshoppers in Mali, West Africa, July 1990. In: Lomer CJ, Prior C (eds) Biological control of locusts and grasshoppers. CAB International, Wallingford, pp 296–310

Kabaluk JT, Ericsson JD (2007) Environmental and behavioral constraints on the infection of wireworms by *Metarhizium anisopliae*. Environ Entomol 36(6):1415–1420

Kabaluk T, Goettel M, Ericsson J, Erlandson M, Vernon B, Jaronski S, Mackenzie K, Cosgrove L (2007) Promise versus performance: Working toward the use of *Metarhizium anisopliae* as a biological control for wireworms. Bull IOBC/WPRS 30(7):69–76

Kalsbeek V, Mullens BA, Jespersen JB (2001) Field studies of Entomophthora (Zygomycetes: Entomophthorales)—induced behavioral fever in *Musca domestica* (Diptera: Muscidae) in Denmark. Biol Control 21(3):264–273

Klass JI, Blanford S, Thomas MB (2007a) Development of a model for evaluating the effects of environmental temperature and thermal behaviour on biological control of locusts and grasshoppers using pathogens. Agric For Entomol 9:189–199

Klass JI, Blanford S, Thomas MB (2007b) Use of a geographic information system to explore spatial variation in pathogen virulence and the implications for biological control of locusts and grasshoppers. Agric For Entomol 9:201–208

Klingen I, Haukeland S (2006) The soil as a reservoir for natural enemies of pest insects and mites with emphasis on fungi and nematodes. In: Eilenberg J, Hokkanen HMT (eds) An ecological and sociological approach to biological control. Springer, Dordrecht, pp 145–211

Knudsen GR, Schotzko DJ (1999) Spatial simulation of epizootics caused by *Beauveria bassiana* in Russian wheat aphid populations. Biol Control 16(3):318–326

Koppert (2009a). Mycotal product information. Available via http://www.koppert.com/pests/thrips/products-against-thrips/detail/mycotal-1/. Cited 26 Aug 2009

Koppert (2009b) Vertelac production information. Available via http://www.koppert.com/pests/aphid/products-against-aphids/detail/vertalec-2/. Cited 26 Aug 2009

Kouassi M, Coderre D, Todorova SI (2003) Effect of plant type on the persistence of *Beauveria bassiana*. Biocont Sci Technol 13(4):415–427

Labbe RM, Gillespie DR, Cloutier C, Brodeur J (2009) Compatibility of an entomopathogenic fungus with a predator and parasitoid in the biological control of greenhouse whitefly. Biocont Sci Technol 19(4):429–446

Lactin DJ, Johnson DL (1998) Environmental, physical, and behavioral determinants of body temperature in grasshopper nymphs (Orthoptera: Acrididae). Can Entomol 130(5):551–577

Lartey RT, Curl EA, Peterson CM (1994) Interactions of mycophagous Collembola and biological control fungi in the suppression of *Rhizoctonia solani*. Soil Biol Biochem 26(1):81–88

Laverlam International (2005) BotaniGard ES compatibility with fungicides. Tech Update Bull 98F1015A, Available via http://www.laverlamintl.com/pdf/BotaniCompiFungENG.pdf. Cited 1 Sep 2009

Lazzarini GMJ, Rocha LFN, Luz C (2006) Impact of moisture on *in vitro* germination of *Metarhizium anisopliae* and *Beauveria bassiana* and their activity on *Triatoma infestans*. Mycol Res 110(4):485–492

Lecoq M (2004) Vers une solution durable au problème du criquet pèlerin? Secheresse 15(3):217–224

Lecuona R, Clement JL, Riba G, Joulie C, Juarez P (1997) Spore germination and hyphal growth of *Beauveria* sp on insect lipids. J Econ Entomol 90(1):119–123

Lee A, Minho A, Yoon C-S, Yun T, Kim H-S, Yoo J-K (2002) Selection of a highly virulent *Verticillium lecanii* strain against *Trialeurodes vaporariorum* at various temperatures. J Microbiol Biotechnol 12(1):145–148

Lemaitre B, Hoffmann J (2007) The host defense of *Drosophila melanogaster*. Ann Rev Immunol 25:697–743

Li J, Feng M-G (2009) Intraspecific tolerance of *Metarhizium anisopliae* conidia to the upper thermal limits of summer with a description of a quantitative assay system. Mycol Res 113(1):93–99

Lobo Lima ML, Brito JM, Henry JE (1992) Biological control of grasshoppers in the Cape Verde Islands. In: Lomer CJ, Prior E (eds) Biological control of locusts and grasshoppers. CAB International, Wallingford, pp 287–295

Lockwood JA, Narisu, Schell SP, Lockwood DR (2001) Canola oil as a kairomonal attractant of rangeland grasshoppers: An economical liquid bait for insecticide formulation. Intl J Pest Mgt 47:185–194

Lord JC (2005) Low humidity, moderate temperature, and desiccant dust favor efficacy of *Beauveria bassiana* (Hyphomycetes: Moniliales) for the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bruchidae). Biol Control 34(2):180–186

Luz C, Fargues J (1999) Dependence of the entomopathogenic fungus, *Beauveria bassiana*, on high humidity for infection of *Rhodnius prolixus*. Mycopathologia 146(1):33–41

Marcandier S, Khachatourians GG (1987) Susceptibility of the migratory grasshopper, *Melanoplus sanguinipes* (Fab.) (Orthoptera: Acrididae), to *Beauveria bassiana* (Bals.) Vuillemin (Hyphomycete): influence of relative humidity. Can Entomol 119:901–907

McCoy CW (1981) Pest control by the fungus *Hirsutella thompsonii*. In: Burges HD (ed) microbial control of pests and plant diseases 1970–1980. Academic Press, New York, pp 499–512

McCoy CW, Couch TL (1982) Microbial control of the citrus rust mite with the mycoacaricide, Mycar. Fla Entomol 65(1):116–126

McDowell JM, Funderburk JE, Boucias DG, Gilreath ME, Lynch RE (1990) Biological activity of *Beauveria bassiana* against lesser cornstalk borer, *Elasmopalpus lignosellus* (Lepidoptera; Pyralidiae) on both soil and leaf substrates. Environ Entomol 19(1):182–190

Mierzejewski K, Reardon RC, Thistle H, Dubois NR (2007) Conventional application equipment: aerial application. In: Lacey LA, Kaya HK (eds) Field manual of techniques in invertebrate pathology, 2nd ed. edn. Springer, Dordrecht, pp 99–126

Mietkiewski R, Gorski R (1995) Growth of selected entomopathogenic fungi species and isolates on media containing insecticides. Acta Mycol 30(1):27–33

Milner RJ (1997) *Metarhizium flavoviride* (FI985) as a promising mycoinsecticide for Australian acridids. Mem Ent Soc Can 171:287–300

Moore D, Bridge PD, Higgins PM, Bateman RP, Prior C (1993) Ultra-violet radiation damage to *Metarhizium flavoviride* conidia and the protection given by vegetable and mineral oils and chemical sunscreens. Ann Appl Environ Biol 122:605–616

Morris CE, Monier JM, Jacques MA (1997) Method for observing microbial biofilms directly on leaf surfaces and recovering them for isolation of culturable microorganisms. Appl Environ Microbiol 63:1570–1576

Muller C, Riederer M (2005) Plant surface properties in chemical ecology. J Chem Ecol 31(11):2621–2651

Müller U, Vogel P, Alber G, Schaub G (2008) The innate immune system of mammals and insects. Contrib Microbiol 15:21–44

Mullie W (2009) Does bird predation enhance the impact of Green Muscle for grasshopper control—experiences from cleared woodland in Central Senegal. Metaleptea special conference issue 10th international congress orthopterology. Available via http://140.247.119.138/OrthSoc/pdf/metaleptea_special_2009.pdf. cited 5 Sep 2009

Noma T, Strickler K (1999) Factors affecting *Beauveria bassiana* for control of lygus bug (Hemiptera: Miridae) in alfalfa seed fields. J Agricult Urban Ent 16(4):215–233

Noma T, Strickler K (2000) Effects of *Beauveria bassiana* on *Lygus hesperus* (Hemiptera: Miridae) feeding and oviposition. Environ Ent 29(2):394–402

Ouedraogo RM, Goettel MS, Brodeur J (2004) Behavioral thermoregulation in the migratory locust: a therapy to overcome fungal infection. Oecologia 138(2):312–319

Pell JD, Vandenberg JD (2002) Interactions Among the Aphid *Diuraphis noxia*, the Entomopathogenic Fungus *Paecilomyces fumosoroseus* and the Coccinellid *Hippodamia convergens*. Biocont Sci Technol 12(2):217–224

Pell JD, Hannam J, Steinkraus D (2009) Conservation biological control using fungal entomopathogens. BioControl. doi 10.1007/s10526-009-9245-6 (this SI)

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 61:156–161

Pinnock DE, Brand RJ (1980) A quantitative approach to the ecology of the use of pathogens for insect control. In: Burges HD (ed) Microbial control of pests and plant diseases 1970–1980. Academic Press, London, pp 655–665

Polar P, Moore D, Kairo MTK, Ramsubhag A (2008) Topically applied myco-acaricides for the control of cattle ticks: overcoming the challenges. Exp Appl Acarol 46:119–148

Poprawski TJ, Carruthers RI, Speese J III, Vacek DC, Wendel LE (1997) Early-season applications of the fungus *Beauveria bassiana* and introduction of the hemipteran predator *Perillus bioculatus* for control of Colorado potato beetle. Biol Control 10(1):48–57

Poprawski TJ, Greenberg SM, Ciomperlik MA (2000) Effect of host plant on *Beauveria bassiana*- and *Paecilomyces fumosoroseus*-induced mortality of *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). Eviron Entomol 29(5):1048–1053

Ramoska WA (1984) The influence of relative humidity on *Beauveria bassiana* infectivity and replication in the Chinch bug, *Blissus leucopterus*. J Invertebr Pathol 43:389–394

Ramsay JA, Butler CG, Sang JH (1938) The humidity gradient at the surface of a transpiring leaf. J Exp Biol 15:255–265

Rath AC, Anderson GC, Worledge D, Koen TB (1995) The effect of low temperatures on the virulence of *Metarhizium anisopliae* (DAT F-001) to the subterranean scarab, *Adoryphorus couloni*. J Invertebr Pathol 65(2):186–192

Reddy NP, Khan PAA, Devi KU, Victor JS, Sharma HC (2008) Assessment of the suitability of Tinopal as an enhancing adjuvant in formulations of the insect pathogenic fungus *Beauveria bassiana* (Bals.) Vuillemin. Pest Manag Sci 64:909–915

Roberts DW, Campbell AS (1977) Stability of entomopathogenic fungi. Misc Publ Ent Soc Amer 10(3):19–76

Rostas M, Hilker M (2003) Indirect interactions between a phytopathogenic and an entomopathogenic fungus. Naturwissenschaften 90:63–67

Roy HE, Pell JK, Alderson PG (2001) Targeted dispersal of the aphid pathogenic fungus *Erynia neoaphidis* by the predator *Coccinella septempunctata*. Biocont Sci Technol 11(1):101–112

Sabbahi R, Merzouki A, Guertin C (2008) Efficacy of *Beauveria bassiana* (Bals.) Vuill. against the tarnished plant bug, *Lygus lineolaris* L., in strawberries. J Appl Entomol 132(2):124–134

Santiago-Alvarez C, Maranhao EA, Maranhao E, Quesada-Moraga E (2006) Host plant influences pathogenicity of *Beauveria bassiana* to *Bemisia tabaci* and its sporulation on cadavers. BioControl 51(4):519–532

Schuepp PH (1993) Tansley review no. 59, Leaf boundary layers. New Phytol 125:477–507

Scurlock JMO, Asner GP, Gower ST (2001) Worldwide historical estimates and bibliography of leaf area index, 1932–2000. ORNL Technical Memorandum TM-2001/268. Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

Shah PA, Pell JK (2003) Entomopathogenic fungi as biological control agents. Appl Microbiol Biotechnol 61(5/6):413–423

Shanley RP, Keena M, Wheeler MM, Leland J, Hajek AE (2009) Evaluating the virulence and longevity of nonwoven fiber bands impregnated with *Metarhizium anisopliae* against the Asian longhorned beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae). Biol Control 50:94–102

Shipp JL, Zhang Y, Hunt DWA, Ferguson G (2003) Influence of humidity and greenhouse microclimate on the efficacy of *Beauveria bassiana* (Balsamo) for control of greenhouse arthropod pests. Environ Entomol 32(5):1154–1163

Shiraishi H, Enami Y, Okano S (2003) *Folsomia hidakana* (Collembola) prevents damping-off disease in cabbage and Chinese cabbage by *Rhizoctonia solani*. Pedobiologia 47:33–38

Showler AT (1997) Proaction: strategic framework for today's reality. In: Krall S, Peveling R, Ba Diallo D (eds) New Strategies for Locust Control. Birkhauser, Basel, pp 461–465

Smith RJ, Grula EA (1982) Toxic components on the larval surface of the corn earworm (*Heliothis zea*) and their effects on the germination and growth of *Beauveria bassiana*. J Invertebr Pathol 39(1):15–22

Smits N, Fargues J, Rougier M, Goujet R, Itier B (1996) Effects of temperature and solar radiation interactions on the survival of quiescent conidia of the entomopathogenic hyphomycete *Paecilomyces fumosoroseus* (Wize) Brown and Smith. Mycopathologia 135(3):163–170

SosaGomez DR, Boucias DG, Nation JL (1997) Attachment of *Metarhizium anisopliae* to the southern green stink bug *Nezara viridula* cuticle and fungistatic effect of cuticular lipids and aldehydes. J Invertebr Pathol 69(1):31–39

Stewart WDP (1991) The importance of sustainable agriculture on biodiversity among invertebrates and microorganisms. In: Hawksworth DL (ed) The biodiversity of microorganisms and invertebrates, its role in sustainable agriculture. CAB International, Wallingford, pp 122–130

Storey GK, Gardner WA (1988) Movement of an aqueous spray of *Beauveria bassiana* into the profile of four Georgia soils. Environ Entomol 17:135–139

Storey GK, Gardner WA, Tollner EW (1989) Penetration and persistence of commercially formulated *Beauveria bassiana* conidia in soil of two tillage systems. Environ Entomol 18:835–839

Stotzky G (1972) Activity, ecology, and population dynamics of microorganisms in soil. CRC Crit Rev Microbiol 2(1):59–137

Thomas MB, Watson EL, Valverde-Garcia P (2003) Mixed infections and insect-pathogen interactions. Ecol Lett 6(3):183–188

Thompson SR, Brandeberg R, Arends JJ (2006) Impact of moisture and UV degradation on *Beauveria bassiana* (Balsamo) Vuillemin conidial viability in turfgrass. Biol Control 39:401–407

Thungrabeab M, Blaeser P, Sengonca C (2006) Effect of temperature and host plant on the efficacy of different entomopathogenic fungi from Thailand against *Frankliniella occidentalis* (Pergande) and *Thrips tabaci* Lindeman (Thysanoptera : Thripidae) in the laboratory. J Plant Dis Protect 113(4):181–187

Todorova S, Coderre D, Duchesne R-M, Cote J-C (1998) Compatibility of *Beauveria bassiana* with selected fungicides and herbicides. Biol Control 27(2):427–433

Tounou AK, Kooyman C, Douro-Kplndou OK, Poehling HM (2008) Interaction between *Paranosema locustae* and *Metarhizium anisopliae* var. *acridum*, two pathogens of the desert locust, *Schistocerca gregaria* under laboratory conditions. J Invertebr Pathol 97(3):203–210

Tugel A, Lewandowski A, Happe-von Arb D (eds) (2000) Soil Biology Primer. Soil and Water Conservation Society, Ankeny

Turnbow JH (1998) Temperature-sensitive *Beauveria bassiana* mycosis in the Mormon cricket, *Anabrus simplex*. MS Thesis. Montana State University, Bozeman

Ugine TA, Wraight SP, Sanderson JP (2007) A tritrophic effect of host plant on susceptibility of western flower thrips to the entomopathogenic fungus *Beauveria bassiana*. J Invertebr Pathol 96(2):162–172

Van der Valk H (2007) Review of the efficacy of *Metarhizium anisopliae* var. *acridum* against the desert locust plant production and protection division, locusts and other migratory pest group N. AGP/DL/TS/34. 81 pp. Available via http://www.fao.org/ag/locusts/common/ecg/1295/en/TS34e.pdf. Cited 10 Aug 2009

Vega FE, Dowd PF, Lacey LA, Pell JK, Jackson DM, Klein MG (2007) Dissemination of beneficial microbial agents by insects. In: Lacey LA, Kaya HK (eds) Field manual of techniques in invertebrate pathology, 2nd edn. Springer, New York, pp 127–146

Vesala T (1998) On the concept of leaf boundary layer resistance for forced convection. J Theor Biol 194:91–100

Vidal C, Fargues J, Rougier M, Smits N (2003) Effect of air humidity on the infection potential of hyphomycetous fungi as mycoinsecticides for *Trialeurodes vaporariorum*. Biocont Sci Technol 13(2):183–198

Watson DW, Mullens BA, Petersen JJ (1993) Behavioral fever response of *Musca domestica* (Diptera: Muscidae) to infection by *Entomophthora muscae* (Zygomycetes: Entomophthorales). J Invertebr Pathol 61(1):10–16

Willmer P (1986) Microclimate effects on insects at the plant surface. In: Juniper B, Southwood R (eds) Insects and the Plant Surface. E Arnold, New York, pp 65–80

Wojda I, Kowalski P, Jakubowicz T (2009) Humoral immune response of *Galleria mellonella* larvae after infection by *Beauveria bassiana* under optimal and heat-shock conditions. J Insect Physiol 55(6):525–531

Wraight SP, Carruthers RI (1999) Production, delivery and use of mycoinsecticides for control of insect pests of field crops. In: Hall FR, Menn JJ (eds) Methods in biotechnology, vol 5, Biopesticides: use and delivery. Humana Press, Totowa, New Jersey, pp 233–269

Wraight SP, Ramos ME (2002) Application parameters affecting field efficacy of *Beauveria bassiana* foliar treatments against Colorado potato beetle *Leptinotarsa decemlineata*. Biol Control 23:164–178

Wraight SP, Ramos ME (2005) Synergistic interaction between *Beauveria bassiana* and *Bacillus thuringiensis tenebrionis*-based biopesticides applied against field populations of Colorado potato beetle larvae. J Invertebr Pathol 90(3):139–150

Wraight SP, Carruthers RI, Bradley CA, Jaronski ST, Lacey LA, Wood P, Galaini-Wraight S (1998) Pathogenicity of the entomopathogenic fungi *Paecilomyces* spp. and *Beauveria bassiana* against the Silverleaf whitefly, *Bemisia argentifolii*. J Invertebr Pathol 71:217–226

Wraight SP, Carruthers RI, Jaronski ST, Bradley CA, Garza CJ, Galaini-Wright S (2000) Evaluations of the entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* for the microbial control of the silverleaf whitefly, *Bemisia argentifolii*. Biol Control 17:203–217

Wraight SP, Ramos ME, Avery PB, Jaronski ST, Vandenberg JD (2009) Comparative virulence of *Beauveria bassiana* isolates against lepidopteran pests of vegetable crops. J Invertebr Pathol (in press)

Yang Y, Allen JC, Knapp JL, Stansly PA (1997) An age-structured population model of citrus rust mite: a fruit-mite-fungal pathogen system. Ecol Modeling 104(1):71–85

Yasuda K, Tokays K, Uehara K (1997) Effects of temperature, humidity and conidial density on infection by *Beauveria bassiana* of adult sweet potato weevil, *Cylas formicarius* (Fabricius) (Coleoptera: Curculionidae). Jap J Appl Entomol Zool 41(1):55–58

Zheng D, Hunt ER Jr, Running SW (1993) A daily soil temperature model based on air temperature and precipitation for continental applications. Clim Res 2:183–191

Zimmermann G (2007a) Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocont Sci Technol 17(5–6):553–596

Zimmermann G (2007b) Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. Biocont Sci Technol 17(9–10):879–920

Zimmermann G (2008) The entomopathogenic fungi *Isaria farinosa* (formerly *Paecilomyces farinosus*) and the *Isaria fumosorosea* species complex (formerly *Paecilomyces fumosoroseus*): biology, ecology and use in biological control. Biocont Sci Technol 18(9):865–901

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