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REVIEW

The production and uses of *Beauveria bassiana* as a microbial insecticide

Gabriel Moura Mascarin[1] · **Stefan T. Jaronski**[2]

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Abstract Among invertebrate fungal pathogens, *Beauveria bassiana* has assumed a key role in management of numerous arthropod agricultural, veterinary and forestry pests. *Beauveria* is typically deployed in one or more inundative applications of large numbers of aerial conidia in dry or liquid formulations, in a chemical paradigm. Mass production is mainly practiced by solid-state fermentation to yield hydrophobic aerial conidia, which remain the principal active ingredient of mycoinsecticides. More robust and cost-effective fermentation and formulation downstream platforms are imperative for its overall commercialization by industry. Hence, where economics allow, submerged liquid fermentation provides alternative method to produce effective and stable propagules that can be easily formulated as dry stable preparations. Formulation also continues to be a bottleneck in the development of stable and effective commercial *Beauveria*-mycoinsecticides in many countries, although good commercial formulations do exist. Future research on improving fermentation and formulation technologies coupled with the selection of multi-stress tolerant and virulent strains is needed to catalyze the widespread acceptance and usefulness of this fungus as a cost-effective mycoinsecticide. The role of *Beauveria* as one tool among many in integrated pest management, rather than a stand-alone management approach, needs to be better developed across the range of crop systems. Here, we provide an overview of mass-production and formulation strategies, updated list of registered commercial products, major biocontrol programs and ecological aspects affecting the use of *Beauveria* as a mycoinsecticide.

Keywords Mycoinsecticides · Fermentation · Pests · Formulation · Blastospores · Conidia · White muscardine · Biocontrol

Introduction

Microbial biopesticides are undergoing a great momentum worldwide stimulated by societal, governmental and market-driven demands for chemical-free residue on foods, decreased reliance on chemical pesticides, increased growth of organic agriculture and expansion and consolidation of integrated pest management (IPM) programs (Ravensberg 2011, 2015). Interest in developing microbial biopesticides has recently emerged even among large chemical agricultural companies for expansion of their portfolios for sustainable management of pests and diseases in vegetable crops. At least 586 insect species are resistant to $\geq$1 of 325 chemical insecticides and to five insecticidal traits in genetically modified organisms (GMO) (Sparks and Nauen 2014). The recent concerns about adverse effects of neonicotinoids on bee populations (van der Sluijs et al. 2013) has further increased the need and desire to develop biological methods to manage insect pests. Discovery of new synthetic pesticides has become increasingly more difficult and costly. Companies have had to screen at least 150,000 chemicals to find one new, commercially acceptable, synthetic pesticide, which requires an investment of >$250 million and takes at least 10 years to be launched into the

✉ Gabriel Moura Mascarin
gmmascar@gmail.com; gabriel.mascarin@embrapa.br

[1] EMBRAPA Rice and Beans, Rod. GO-462, km 12, Zona Rural, St. Antônio de Goiás, GO 75375-000, Brazil

[2] United States Department of Agriculture, Agriculture Research Service, Pest Management Research Unit, Northern Plains Agricultural Research Laboratory, 1500 N. Central Avenue, Sidney, MT 59270, USA

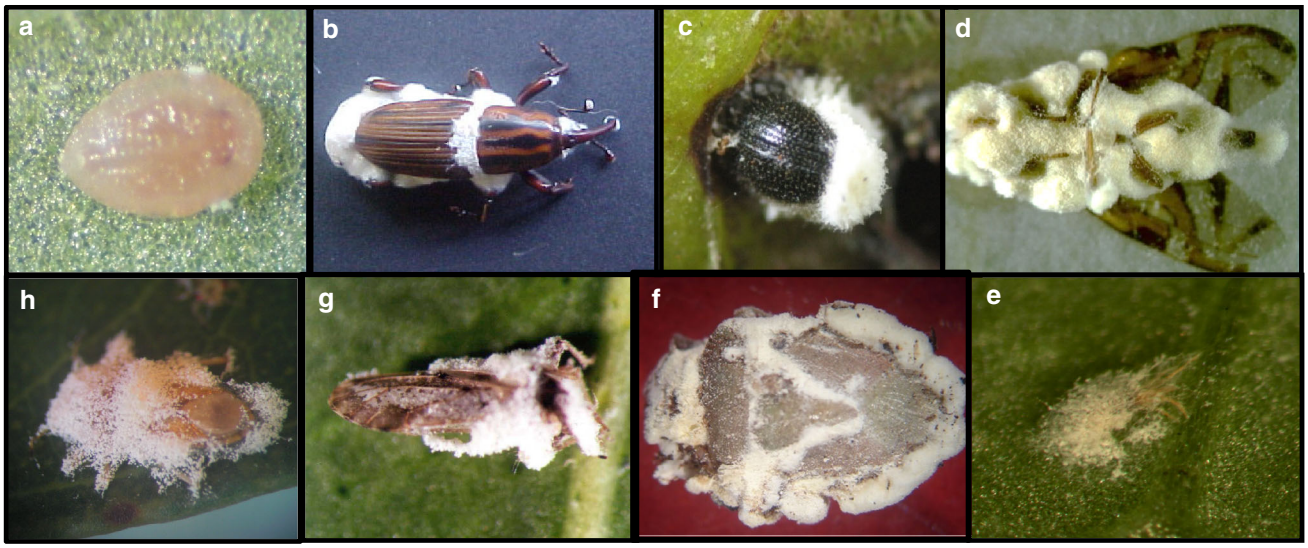

Fig. 1 Broad range of infected arthropods by *Beauveria bassiana* s.l.: **a** Nymph of the silverleaf whitefly *Bemisia tabaci* biotype B (Credits: Gabriel M. Mascarin); **b** Banana weevil *Metamasius hemipterus* (Credits: Rogério B. Lopes); **c** Coffee berry borer *Hyphotenemus hampei* (Credits: Gabriel M. Mascarin); **d** Fruitfly *Anastrepha fraterculus* (Credits: Gabriel M. Mascarin); **e** Spider mite *Tetranychus urticae* (Credits: Gabriel M. Mascarin); **f** Soybean stinkbug *Nezara viridula* (Credits: Gabriel M. Mascarin); **g** Citrus psyllid *Diaphorina citri* (Credits: Luiz F. L. Padulla); **h** Eucalyptus bronze bug *Thaumastocoris peregrinus* (Credits: Gabriel M. Mascarin)

market (McDougall 2016). On the other hand, overall investment in a microbial biopesticide is in the order of $1–2 million and it only takes about 3–5 years to get to market (Glare et al. 2012; Marrone 2014). The plant protection industry has been witnessing a steady growth of the global biopesticide market since the 1980s (15.6 % compound annual growth rate [CAGR]) and was reported to capture about 5.8 % of the global plant protection market by 2014 ($3.3 billion US Dollars) (Marrone 2014). Optimistic forecasts expect that this biocontrol sector will reach $6.60 billion by 2020 at a CAGR of 18.8 % from 2015 to 2020 (Markets and Markets 2015). Among the microbial biopesticides, fungi represented almost 19.4 % of the total biopesticides sold in 2010 (CPL Business Consultants 2010).

The fungi have evolved fascinating parasitic lifestyles that can be exploited in biological control of pests and diseases, and are the commonest cause of microbial disease in invertebrates as well as the most diverse group with nearly 1000 species that play a key role as natural mortality factors of insects and arachnids (ticks and mites) (Humber 2008; Boomsma et al. 2014). Unlike viruses, protozoans, and bacteria, which require specific routes of infection (i.e., through ingestion), the majority of entomopathogenic fungi infect arthropods by direct penetration of the host cuticle and thus function mainly as contact pathogens. Fungal entomopathogens have long been sought in all categories of biological control—classical, augmentative, and conservation (Lord 2005; Lacey et al. 2015)—and have proven to be pivotal in IPM strategies because they can cause frequent epizootics in a density-dependent fashion (Hajek and St. Leger 1994).

Most species of entomopathogenic fungi belong to one of two divisions, the Entomophthoromycota and the Ascomycota, with *Beauveria* being in the latter, within the Cordycipitaceae of the Hypocreales. For much of its history, until the advent of molecular taxonomic techniques, *Beauveria* was placed within the Deuteromycetes, because a sexual stage was not known. Recent DNA-sequence based analyses have since allied *Beauveria* with the Ascomycete genus *Cordyceps* and resulted in its reassignment (Rehner and Buckley 2005).

Despite the exceptional epizootic ability observed for biotrophic entomophthoralean fungi, fungal biopesticides (mycoinsecticides and mycoacaricides) are predominantly based on the anamorphic phases of the hypocrealean—*Beauveria* spp., *Metarhizium* spp., *Isaria* spp. (formerly in the genus *Paecilomyces*), and *Lecanicillium* spp. (formerly *Verticillium*) (Faria and Wraight 2007). These Ascomycetes have been readily developed because of a saprophytic lifestyle, paralleling their entomopathogenicity, allowing for cost effective mass production unlike the Entomophthorales, which are predominantly host specific biotrophs. The cosmopolitan, naturally soil-inhabiting *Beauveria bassiana* Balls. (Vuill.) sensu lato is a facultative necrotrophic pathogen of a broad host arthropod range spanning almost all orders of insects and extending to ticks and mites (Rehner et al. 2011) (Fig. 1) (The term *Beauveria* will be used hereafter to refer to the morphological species when no other information is known).

Since the last reviews on *Beauveria* by Feng et al. (1994) and Zimmermann (2007), a vast literature related to this entomopathogen has been produced that allows us to

take a snapshot of the progresses and recent discoveries on its life histories, biocontrol potential and commercial development. Faria and Wraight (2007) determined almost 40 % of the total mycoinsecticides were based on *Beauveria*, although presently many products are no longer available in the biopesticide market. Here, we cover the current knowledge of mass production, formulation and applications of *Beauveria* in different regions of the world and we put efforts on updating the list of commercial *B. bassiana* products worldwide.

Brief history of *Beauveria*, the "white muscardine" disease

In 1835, the entomologist Agostino Bassi discovered the causal agent of pebrine disease that turned legions of Italy's silkworms into white mummies (Lord 2005). The characteristic appearance of cadavers—covered with a white powdery layer—gave rise to the descriptor, white muscardine disease. The fungus was subsequently named after Bassi by Vuillemin. One of the first and most prominent early attempts in extensively using *Beauveria* took place in the US Midwest for control of chinch bugs, *Blissus leucopterus*, in the mid-1800s (Lord 2005). This fungus is a well-known pathogen of a broad host range arthropods capable of infecting >700 species of hosts, including various species in Acari and Insecta (Inglis et al. 2001; Zimmermann 2007) (Fig. 1). It is one of the most intensively studied fungal entomopathogens from which more than thousands of isolates have been collected from different parts of the world (Rehner et al. 2011). Of 6451 scientific articles, reviews and patents retrieved in a recent literature survey from 1945 to 2015 using Web of Science, we have found *Beauveria* appearing in 37.7 % publications (followed by *Metarhizium* spp. with 34.9 %), which confirms its importance as a biocontrol, industrial, and pharmaceutical microbial agent.

The increasing taxonomic complexity of *Beauveria*

Until recently, the genus *Beauveria* was thought to contain only the very common and ubiquitous species *bassiana*, a less common *B. brongniartii*, and a rare *B. album* with all placed within the Deuteromycota because their sexual stages were unknown (deHoog 1972). Additional species—*amorpha, caledonica, velata,* and *vermiconia*—were subsequently created, but the species *bassiana* remained intact (deHoog and Rao 1975; Samson and Evans 1982; Bissett and Widden 1986; Rehner et al. 2006). The advent of DNA-sequence-based diagnostics has resulted in the creation of a number of new species, splitting *Beauveria* into two species, the original *bassiana* and the new, phylogenetically distinct but morphologically similar, *pseudobassiana*, (Rehner and Buckley 2005; Rehner et al. 2011). In addition, both *B. bassiana* and *B. brongniartii* have been associated with the teleomorphic genus *Cordyceps* (Rehner and Buckley 2005; Sung et al. 2007). Both *C. bassiana* and *C. brongniartii* are very rarely observed and the role of the teleomorphic life phase is really unknown. Additional cryptic species within *Beauveria* are suspected (Rehner et al. 2006; Ghikas et al. 2010). This increasing taxonomic complexity within the genus *Beauveria* has made the true taxonomic status of many commercial and experimental *Beauveria* strains uncertain, and many broader past phenotypic characterizations in the literature unclear.

Pathogenesis by *Beauveria*

The basic pathogenic life cycle of *Beauveria* is briefly outlined in Fig. 2. Infection begins via attachment of conidia to the host cuticle through physical forces followed by germination and penetration of cuticular layers with the aid of hydrolytic enzymes (e.g., proteases, lipases, chitinases), mechanical pressure and other factors (Ortiz-Urquiza and Keyhani 2013). When the growing hyphae reach the nutrient-rich hemolymph, the fungus is capable of budding into single-celled, yeast-like blastospores (or hyphal bodies) that are specialized structures to rapidly proliferate and exploit nutrients, colonize internal tissues, and evade the host immune system (Humber 2008). A variety of toxic metabolites (antimicrobial peptides) are produced during colonization. They are involved in host immune suppression, accompanied by destruction of host internal tissues and nutrient depletion, and hence leading to host death (Ortiz-Urquiza et al. 2010; Gibson et al. 2014). Studies have demonstrated that pathogenesis and virulence of *Beauveria* isolates appear to be linked with the production of in vivo toxicogenic metabolites, cuticle-degrading and anti-oxidant enzymes, and active vegetative development within a host leading to physiological starvation of the host (Quesada-Moraga and Vey 2003; Zimmermann 2007; Ortiz-Urquiza et al. 2010, 2015). However, insect-toxic secreted peptides of *Beauveria* may not always be a requirement for virulence (Quesada-Moraga et al. 2006). *Beauveria* is considered an environmentally safe biocontrol agent that poses zero or minimal threat to human health and is generally harmless to non-target organisms (Zimmermann 2007).

This dimorphic cycle in *Beauveria* has been considered a virulence trait, since yeast-like blastospores have evolved to evade host immune defenses and to exploit host nutrients

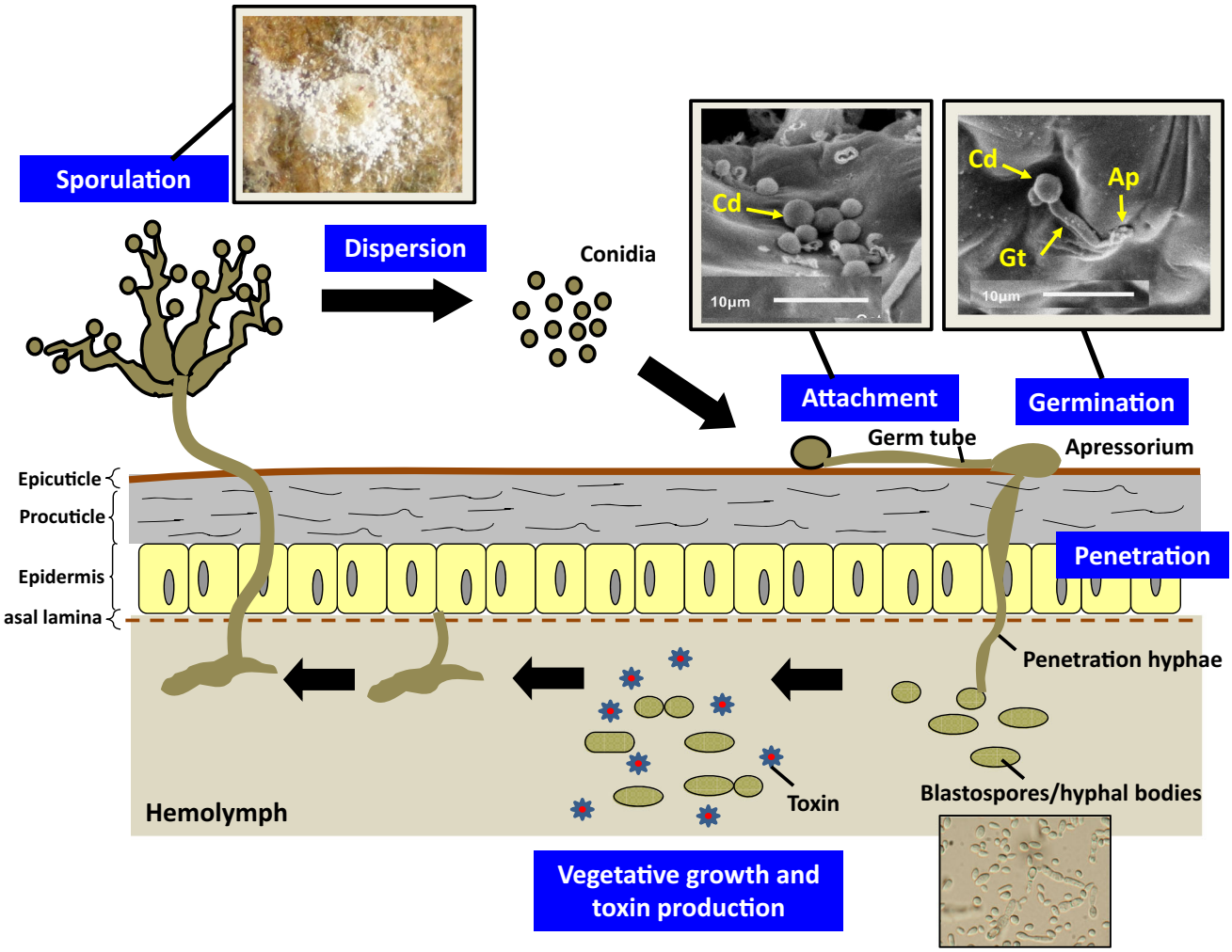

Fig. 2 Overview of the basic infection cycle depicted by *Beauveria bassiana* in invertebrates. Typically, asexual spores (i.e., conidia) are dispersed by wind, rain splash, or even by arthropod vectors that assist the fungus to establish infections in susceptible hosts. Firstly, conidia (or in some cases blastospores) attach to insect cuticle by electrostatic and chemical forces and upon rehydration and chemical stimuli they germinate and form a germ tub that may form a specialized structure namely appressorium (i.e., an enlarged cell expansion bearing key hydrolytic cuticle-degrading enzymes) or penetration peg, which enable the growing hyphae to breach the host integument. From the appressorium or penetration peg, the fungus makes its way in by penetrating all cuticle layers using a battery of hydrolytic enzymes (e.g., proteases, chitinases, lipases), mechanical pressure and other factors (e.g., oxalate) until reaching a nutrient-rich environment, the hemolymph. In the hemolymph, the fungus undergoes a morphogenetic differentiation switching from filamentous growth to single-celled, yeast-like hyphal bodies or blastospores that strategically exploit nutrients, colonize internal tissues and evade the host immune system. During this stage of infection the fungus can also secrete toxic metabolites that aids in host immune suppression and therefore support successfully colonization of the host. These events culminate ultimately in host death. Due to its semelparous life-history and upon host death, conidiophores emerges from the host's dead body (mummified cadavers) after a few days and produce newly infective conidia (sporulation) for dissemination and to continue the pathogen's life cycle. The arrows indicate the direction of fungal growth (adapted from Humber 2008; Ortiz-Urquiza and Keyhani 2013; Valero-Jiménez et al. 2014)

in a rapid fashion (Pendland et al. 1993; Holder et al. 2007). In contrast to aerial conidia, the lack of hydrophobin constituents or hydrophobic rodlet layers has been suggested that electrostatic charges found on blastospore surface may play an important role in the host-pathogen interaction (Holder et al. 2007). Blastospores of *Beauveria* have shown to be similarly virulent or even superior compared with aerial conidia or submerged conidia towards numerous arthropod pests (Hegedus et al. 1992; Holder et al. 2007; Mascarin et al. 2015a).

Once the host dies, the fungus emerges from the dead cadaver and produces aerial conidia on the surface when environmental conditions, especially humidity, are permissive. Conidia can be disseminated by wind, rain splash and other abiotic and biotic factors. The genes and biochemical mechanisms underlying the infection cycle and

virulence of *Beauveria* have been addressed in excellent reviews by Ortiz-Urquiza and Keyhani (2013), Ortiz-Urquiza et al. (2015), and Valero-Jiménez et al. (2014). Reviews of the complex landscape of multi-trophic interactions that exist and modulate fates in fungal entomopathogens, with emphasis on *Beauveria* and its arthropod hosts from a community perspective are illustrated by Fig. 3. Readers can also consult the review by Hesketh et al. (2010) for more details.

Viruses in fungal species are more common than previously thought, and particularly for *B. bassiana*, numerous mycoviruses of double-stranded RNA from different families have been identified. In a survey of Canadian entomopathogenic fungi, Melzer and Bidochka (1998) observed 2 of 12 *Beauveria* isolates contained dsRNA viruses. The ecological role of these mycoviruses in fungal entomopathogens are still obscure and results from available literature are contradictory in terms of the deleterious (side-) effects that these small DNA or RNA sequences have on biological traits of *B. bassiana*. Data for a strain of *Metarhizium anisopliae* s.l. generated by Melzer and Bidochka (1998) imply that spore production and virulence may be affected by the presence of a dsRNA virus. Although this subject is out of the scope of this paper, we invite interested readers to consult Herrero et al. (2012) for a review of this subject.

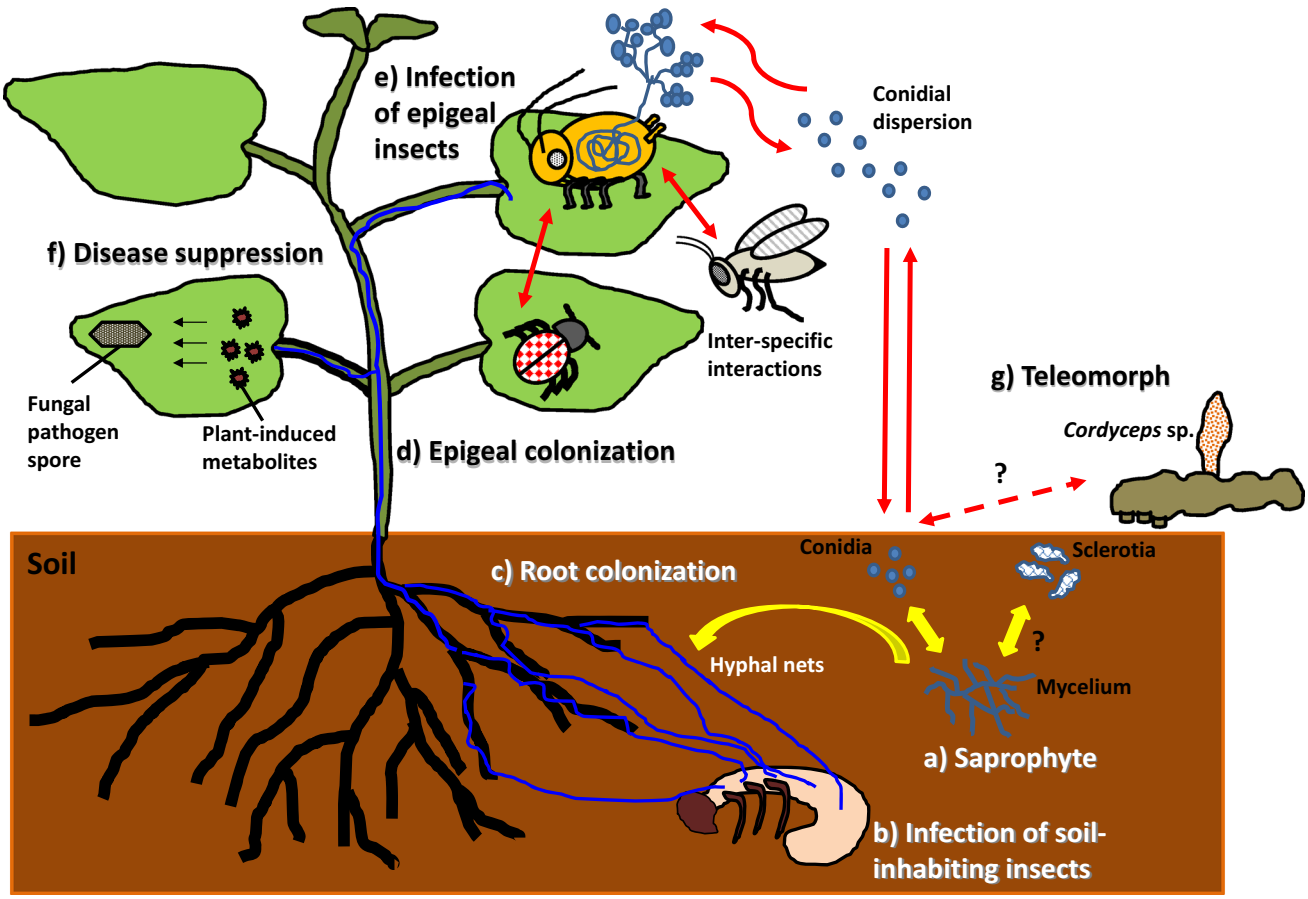

Fig. 3 Conceptual summary of the basic lifestyles in *Beauveria bassiana* and its putative multi-trophic interactions with plants, arthropods, soil, and other microbes within a landscape community scenario: **a** Saprophyte life history is taken place in the soil with conidia shifting to mycelium, whereas the ability of this fungus to form sclerotium remains unknown, as this propagule has been observed neither under in vitro nor under natural conditions; **b** *Beauveria* also infects soil-inhabiting insects and **c** it may transfer nitrogen from the insect to the plant through establishment of root endophyte colonization; **d** If it has endophyte capability, *Beauveria* can colonize bottom-up the aerial plant tissues, including stems, leaves and seeds; **e** Epigeal susceptible insects may get contaminated and then eventually infected by spores of the fungus from dead, sporulated cadavers, air-borne spores or perhaps from endophytic colonization; other organisms such as predators and parasitoids may interact with the fungus by vectoring its spores; **f** Disease suppression in planta may take place by endophytic colonization that triggers systemic resistance defences or by direct antagonism (antibiosis or nutrient competition); **g** Teleomorph of *Beauveria* has been reported to be related to *Cordyceps* sp. and appears to be only found in Asia, where it is commonly used in Chinese medicine; however it remains unclear how the asexual (anamorph) stage shifts to the sexual reproductive stage in nature (Adapted from Behie et al. 2012; Meyling and Eilenberg 2007)

Environmental limitations

Efficacy is intrinsically mediated by environmental abiotic factors, most noticeably humidity, temperature, rainfall, and solar radiation (UV-A and B) (McCoy et al. 2002; Jaronski 2010; Fernandes et al. 2015); and biotic factors, such as host age, susceptibility, behavior. Humidity is considered a critical environmental factor in both laboratory and field efficacy of *Beauveria*. Although there are minor intraspecific differences, *Beauveria* has a general upper temperature limit for growth of 34–36 °C; higher temperatures may greatly reduce efficacy, e.g., Noma and Strickler (1999); Ugine (2011). The phylloplane microclimate of insects, esp. small insects in intimate contact with the leaf surface such as *Bemisia tabaci* nymphs or thrips, can be very different from the general ambient temperature and humidity and thus the fungus can have efficacy beyond that predicted from the latter (Jaronski 2010). Plant architecture, chemical and physical properties of surfaces where fungi are applied constitute another array of challenges that remarkably influence directly or indirectly the host-pathogen interactions and thus the ultimate outcome of biocontrol. For example, western flower thrips were six times more susceptible to *Beauveria* when exposed on *Phaseolus vulgaris* leaf disks than on *Impatiens walleriana* (garden impatiens) leaf disks (Ugine et al. 2005). The thrips evidently acquired significantly more conidia from treated bean leaf surfaces than the impatiens surfaces. Plant volatiles as well as plant surface chemistry can affect survival and biocontrol effectiveness of fungal entomopathogens as reviewed by Cory and Ericsson (2010).

Although these stresses can pose a limitation to the efficacy of *Beauveria*-based mycoinsecticides under field conditions, strategies have been devised to overcome or ameliorate fungal performance under such stresses. Oil-based formulations can greatly increase infectivity of conidia (Bateman et al. 1993). Such formulations can also result in considerable rainfastness of conidia (Inglis et al. 2000). More tolerant and virulent phenotypes (isolates) can be selected through screening and conidial vigor optimized via nutritional and physical manipulation during fungal growth (Jackson 1997; Rangel et al. 2015). Genetic engineering has also been proposed as an approach to create more environmentally robust strains (St. Leger and Wang 2010).

Mass production

Mass production of *Beauveria* as well as the other fungal entomopathogens is required by the typical inundative use strategy, requiring a cost-effective production and stabilization process that delivers a large number of viable, infective propagules (Jackson et al. 2010). For additional information on mass production of fungal entomopathogens, readers can refer to reviews by Feng et al. (1994), Jackson et al. (2010), Jaronski and Jackson (2012) and Jaronski (2013). Downstream pipelines involving production and post-harvesting processes to achieve *Beauveria* mycopesticides are illustrated in Figs. 4 and 5.

Solid substrate fermentation

Aerial conidia are the primary infective propagule of *Beauveria* and other hypocrealean entomopathogens. This hydrophobic asexual spore is relatively easy and inexpensive to mass-produce. Typically, aerial conidia are commonly produced with solid-substrate fermentation (SSF) using sterilized, moistened cereal grains (Jaronski 2013). Solid-substrate fermentation can be laborious and time-consuming, but is very well suited for low technology artisanal production.

To produce aerial conidia, a one- or two-stage technique for mass production can be used. In single-stage production, the substrate is directly inoculated with conidia from solid culture. Two-stage production involves production of an inoculum, typically blastospores, using submerged liquid fermentation, which inoculum is then used for the solid substrate phase.

A pilot factory in the former Soviet Union was able to produce 22 tons of *B. bassiana* as Boverin annually (6×10^9 conidia g^{-1}) (Ferron 1981). Solid-substrate fermentation is still the main production system of aerial conidia in Brazil (Li et al. 2010). In large-scale production facilities, some companies adopted the tray method (Alves and Pereira 1989) or the mushroom spawn bag to produce conidia of *Beauveria*, with yields greatly dependent on the substrate, oxygen level, initial moisture and isolate. Earlier tray method trials in the 1980s provided yields up to 6.2×10^9 conidia/g of steamed rice (Alves and Pereira 1989), which continues to be the major starch-rich substrate used in SSF for conidial production of *Metarhizium* spp. and *Beauveria* spp. in Brazil. The solid substrate fermentation process can be significantly scaled up and automated, however. The U.S. commercial strain GHA is routinely produced using an automated solid-substrate fermentation system that yields approximately 2.2×10^{13} conidia kg^{-1} for 114 kg of high purity conidial powder (1.4×10^{17} conidia) from a batch size of 6.35 metric tons of a sterilized cereal grain in a cycle of 10 days (Jaronski, unpublished observations).

Filamentous fungi, such as *Beauveria* and *Metarhizium* spp., have also been produced on fabric strands (viz., non-woven fiber cloth) embedded with a suitable artificial culture medium. The fungus grows relatively quick on this

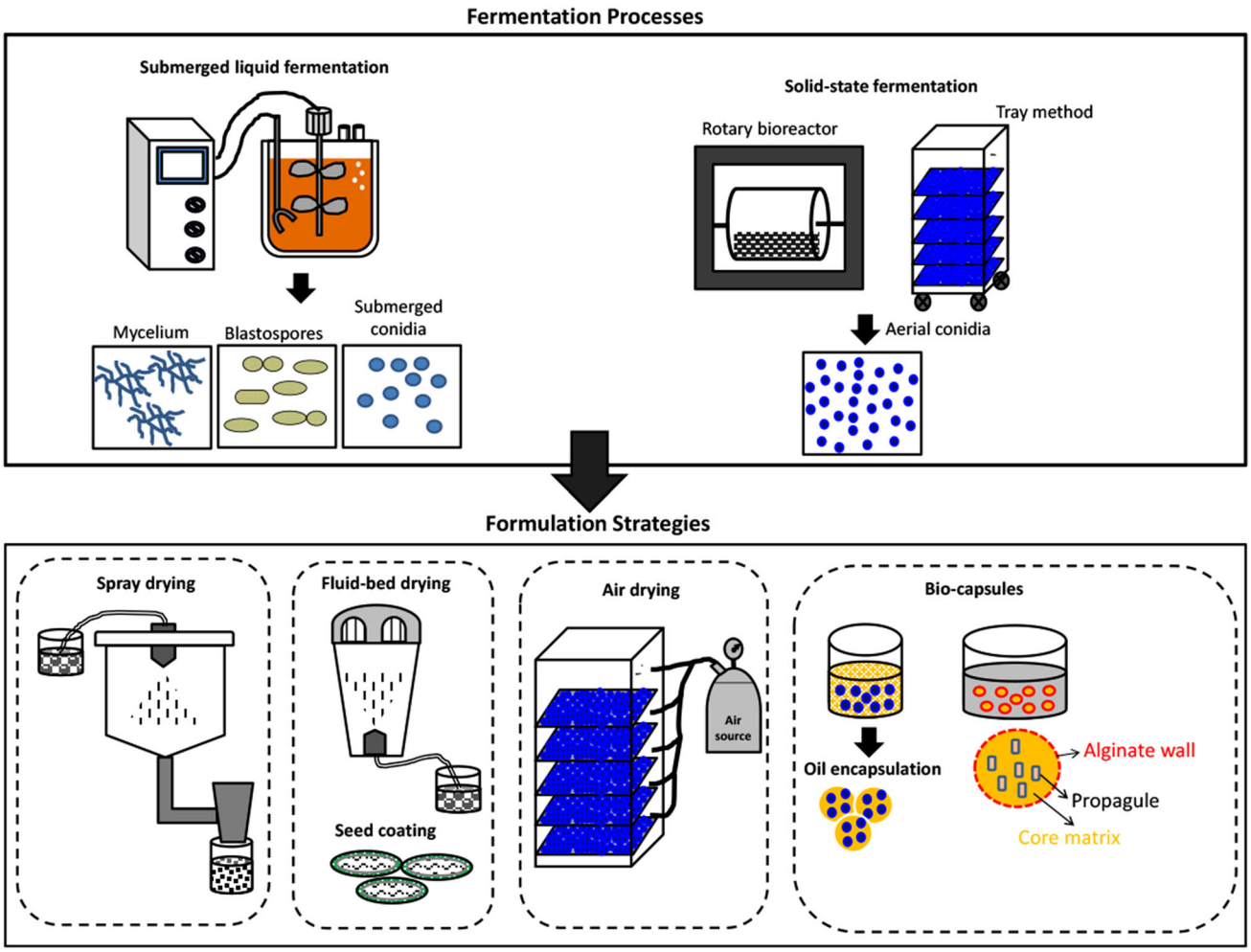

Fig. 4 Overview of fermentation and formulation strategies used or in development for *Beauveria bassiana*-based mycoinsecticides. Different industrial fermentation processes can be employed to obtain propagules for use as the active ingredient of commercial mycoinsecticides. Subsequently, post-harvesting strategies including formulation and drying processes are elaborated to give the final shape to the product and can be accomplished by a variety of methods already developed by food and pharmaceutical industry, such as spray drying, fluid-bed drying, air drying, seed coating, and encapsulation (i.e., coacervation, emulsion, cross-linked polymer coatings), just to name a few

nutrient-rich matrix and ultimately produces a large number of conidia per square meter of cloth surface (Jenkins and Lomer 1994; Dubois et al. 2004). This production system is performed vertically or horizontally and can support the application of 'fungal bands' for management of wood-boring beetles and gypsy moth.

Typically, the solid substrate cultures are dried before conidia are harvested. In artisanal production, the intact dry substrate is frequently sold directly to the user, who washes the conidia from the substrate and sprays them on the crop. On a larger scale, the solid substrate is air-dried and conidia removed by mechanical classification. Such procedure is essential if the conidia are to be incorporated into an oil-based formulation. In some systems, conidia are washed off using an aqueous liquid carrier with surfactant, which enhances conidial dislodgment from the solid matrix. This conidial suspension is then mixed with inerts or additives that are part of the formulation, centrifuged, and dried, parallel to what is done for industrial baking yeast (Mascarin, unpublished observations). The drying step after harvesting is critical for acceptable shelf life and efficiency depends on the drying speed, temperature, relative humidity during drying, and initial and final moisture contents.

Submerged liquid fermentation

Most entomopathogenic hypocrealean fungi display dimorphic growth in liquid media, which growth resembles the pattern observed with growth in the insect hemocoel. *Beauveria bassiana* can form submerged hydrophilic propagules, termed blastospores, in liquid fermentation, in

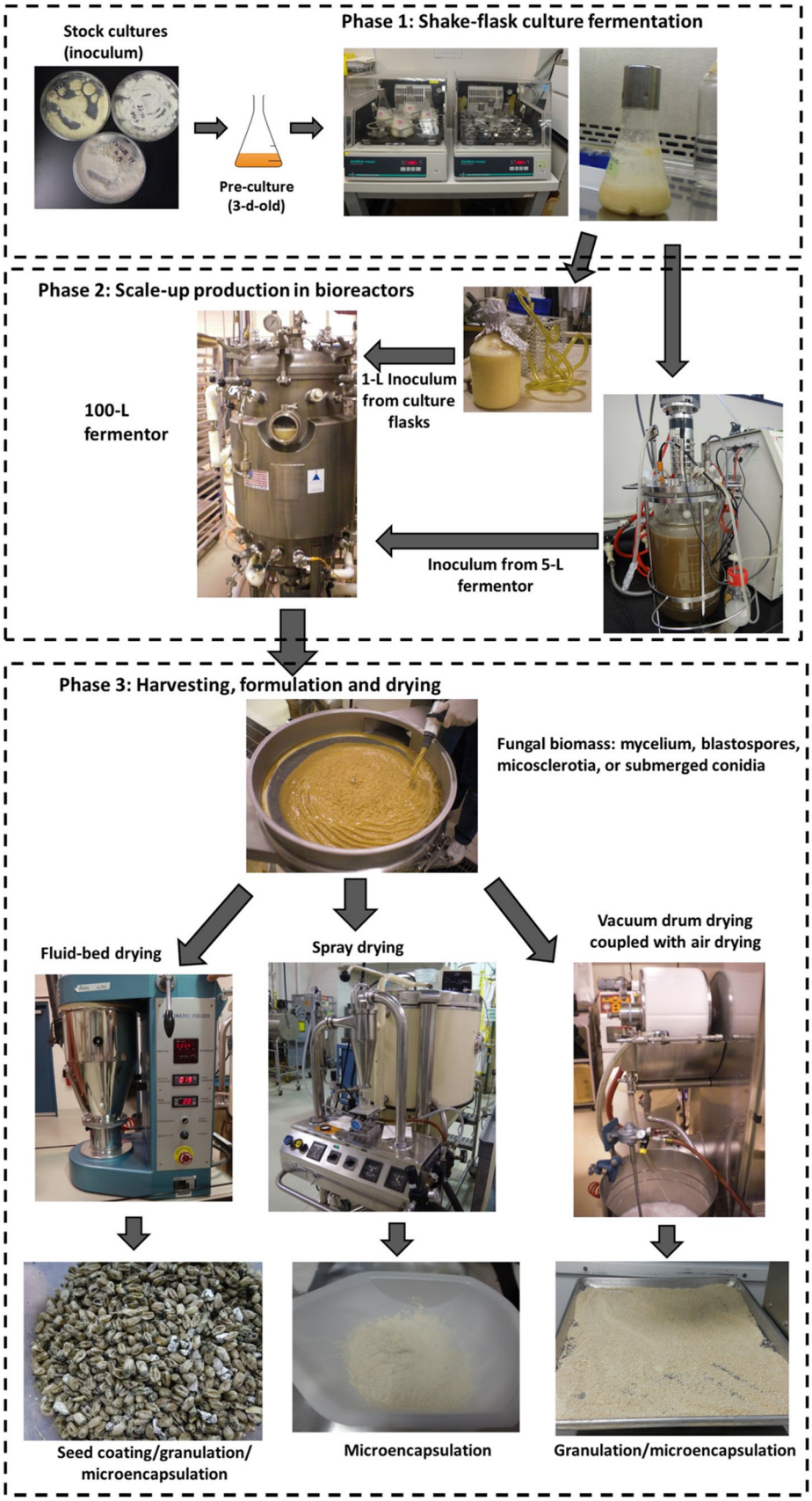

Fig. 5 Schematic diagram of downstream processing for liquid culture fermentation of different types of fungal biomass (mycelium, blastospores, microsclerotia, or submerged conidia) including mass production, harvesting, formulation and drying (Adapted from Mascarin et al. 2016)

addition to hyphae (or mycelium), and submerged conidia through microcycle conidiation. Although hyphal bodies and blastospores are commonly used as synonyms, some studies have pointed out that the former can be mono-nucleate or multi-nucleate vegetative cells produced in vivo within the hemolymph, while the latter is exclusively mono-nucleate single cells that are produced in vitro using artificial nutrient-rich broth (Holder and Keyhani 2005). Blastospores are divergent from aerial conidia and submerged conidia based on their morphological, physical, biochemical, and pathological properties (Hegedus et al. 1992; Holder et al. 2007). Pronounced differences in physicochemical surface characteristics of cell wall were described for aerial conidia, in vitro blastospores and submerged conidia of *Beauveria* (Holder et al. 2007).

In contrast to *Metarhizium* spp. that are capable of producing compact, melanized hyphal aggregates, termed microsclerotia, which function as overwintering structures (Jackson and Jaronski 2009), no such propagule has been observed by any *Beauveria* species up to date during liquid culture fermentation.

Previous works that attempted to mass-produce *Beauveria* blastospores using liquid culture methods produced yields of $<1 \times 10^9$ blastospores mL^{-1} with fermentation times >5 days, poor desiccation tolerance, and poor storage stability (Samsinakova 1966; Humphreys et al. 1989; Rombach 1989; Vidal et al. 1998; Vega et al. 2003; Pham et al. 2009; Chong-Rodriguez et al. 2011; Lohse et al. 2014). Low aeration rates coupled with inadequate nutrition were responsible for these poor results. More recently, researchers have identified critical nutritional and environmental conditions required for the rapid production of high concentrations of blastospores of *Beauveria* and related species using high aeration rates and high osmotic pressure under appropriate nutritional conditions (Mascarin et al. 2015b). Increasing aeration rates to better oxygenate by altering volume to surface ratio and/or speed of agitation have substantially improved blastospore yields in 2–3 days of fermentation. The result was blastospore concentration as high as 2×10^9 mL^{-1} with >60 % of these cells viable after desiccation through spray drying or air drying (Mascarin et al. 2015b, 2016; Jackson and Mascarin 2016). Further improvements in blastospore yield were achieved by increasing the osmotic pressure of liquid cultures using ionic or nonionic osmolytes, including carbohydrates and salts, resulting in concentrations up to 3×10^9 blastospores mL^{-1} with no negative impact on desiccation tolerance of these cells when subjected to air or spray drying process (Mascarin et al. 2015b, 2016; Jackson and Mascarin 2016).

Insect hemolymph has a hyperosmotic environment (0.7–1.2 MPa); blastospores appear to be fit to cope with such abiotic stress. In contrast with low yields of aerial conidia produced under osmotic stress conditions on solid culture media (Rangel et al. 2015), enhanced blastospore production seems to be induced by increased osmotic pressure in the liquid medium (Mascarin et al. 2015b). Blastospores of *Beauveria* may have the ability to accumulate endogenous osmoprotectant compounds, such as glycerol, in order to maintain an osmotic balance, and this process has been linked to the high osmolarity glycerol (HOG) pathway, in which osmosensing proteins are responsible to mediate adaptation to the insect hemocoel (Xiao et al. 2012). Blastospores produced in liquid media amended with high concentrations of glucose (≥10 %) have shown to retain good desiccation tolerance followed up by greater infectivity towards whitefly nymphs in comparison to cells produced in lower osmotic stress liquid media amended with 4 % glucose (Mascarin et al. 2015b). These hyperosmotic-produced blastospores also exhibited a shift from normal oblong shape to smaller spherical cells.

Submerged liquid fermentation allows good control of the nutritional and environmental conditions required for the production of blastospores of *Beauveria*. The use of stirred bioreactors produces a homogenous nutritional environment where temperature, dissolved oxygen, and pH can be monitored and controlled thus improving blastospore yields and reducing contamination (see Fig. 5). Manipulation of the medium composition and physical characteristics, such as aeration rate, temperature, osmotic pressure and pH, make this process easy to assess proper nutritional and environmental conditions for growing rapidly large amounts of active, stable blastospores of *Beauveria*. Cost-effective media components are a key element for the success of liquid fermentation. Blastospores may be better fitted for use against small insects living within the high humidity boundary layer of the phylloplane (whiteflies, aphids and psyllids), because these spores germinate and infect the host faster than aerial conidia, reducing exposure to adverse environmental effects. Conversely, aerial conidia may be more suitable for large insects, such as grasshoppers and caterpillars, living in a drier ambient environment and where oil-based formulations are desired. The decision of which is the better propagule for the active ingredient in a biological product will depend on numerous factors, including the cost of the production medium and formulation, production yields, virulence, persistence in the field, tolerance to environmental stresses, host target, delivery system, market size, and consistent performance under field conditions.

Blastospores produced under proper nutritional and physical conditions during submerged liquid fermentation were amenable to air drying and spray drying retaining excellent shelf life at both refrigerated and non-refrigerated conditions. Spray drying with skim milk powder amended with ascorbic acid has shown promising to extend shelf life

of blastospores at 28 °C (Jackson and Mascarin 2016; Mascarin et al. 2016). Fluid-bed drying and its variations comprise drying processes that can be applied to seed coating or to make sprayable or granule products containing blastospores or conidia of *Beauveria* (see Figs. 4, 5).

Beauveria formulations

Formulation technology is of paramount importance for commercial mycopesticide development and offers a broad array of choices and processes. Formulation is important to provide commercial "robustness" by facilitating transport, handling, and application; extending room temperature shelf life; enhancing insecticidal activity for more consistent efficacies; improving persistence after application; affording protection against abiotic environmental stresses, without altering the physicochemical properties of fungal propagules (Jaronski 1997; Burges 1998; Brar et al. 2006). Formulation strategies can also broaden the target host-spectrum of a mycoinsecticide, to new markets and alleviate the burden of registering different strains for specific arthropod hosts (Ravensberg 2011). Mycoinsecticide formulations in industry are usually proprietary, which situation limits our knowledge about the compositions or components that integrate into successful, and unsuccessful, products.

On the simplest level, such as in South American artisanal production, intact dry sporulated culture is provided to the user, who washes and filters the conidia from the substrate using a diluted wetting agent and sprays them onto the target crop (Jaronski, unpublished observations).

More sophisticated, commercial *Beauveria* products can be divided into dry and liquid formulations, which usually contain compatible adjuvants, fillers and other necessary additives to form a stable preparation. Formulation type will be driven by the fungal propagule (conidium or blastospore), delivery strategy and arthropod target behavior and habitat. Need for inexpensive and natural (biodegradable) formulation components represents a challenge to the product final cost and environmental concerns. Drying processes encompassing spray drying, air drying, rotary vacuum drum drying, fluid-bed drying are some of the large-scale techniques suitable for stabilizing *Beauveria* propagules for formulation, enabling a critical low water content for satisfactory shelf life (see Figs. 4 and 5). Some formulation components, such as exogenous nutrients, osmoprotectants, and oils can be added during growth or during drying to enhance desiccation tolerance and extend shelf life. A basic understanding on how fungal propagules interact with their host target or respond to their target environment constitutes an important guide for formulation development. Toxicity of the formulation ingredients such as long-chain alkyl based surfactants for different fungal propagules should be also carefully addressed, because blastospores and other vegetative fungal cells can be more sensitive than aerial conidia (Jaronski 1997; Jackson et al. 2010). Investments in formulation strategies are generally linked with post-harvesting processes involving drying for dry products or oil- or water-based preparations for liquid products. New formulation strategies using organic or synthetic biopolymers along with microencapsulation techniques open new opportunities to develop stable and effective formulated *Beauveria* mycoinsecticides.

Oil formulations have been demonstrated to improve infection at low humidity (Prior et al. 1988; Bateman et al. 1993), thermal stress tolerance (Hedgecock et al. 1995; Hong et al. 1997), survival under solar radiation (Moore et al. 1993; Alves et al. 1998), and efficiency of application to large areas with ultralow volume (ULV) application (Burges 1998). Dry aerial conidia, which are hydrophobic, are readily incorporated into neat oil (for ULV applications) or emulsifiable oil formulations for subsequent dilution with water for spraying (Burges 1998). Despite the advantages outlined for oil-based formulations of *Beauveria* and other fungal entomopathogens by some researchers, contrary reports document similar efficacy of oil and aqueous preparations of conidia, equivalent persistence on phylloplanes after conventional or ultra-low-volume (ULV) applications (Inglis et al. 1993; Behle et al. 2009). The apparent short persistence of oil-based formulations may be attributed to absorption of oil by plant tissues, subsequently leading to loss of any photoprotection. Burges (1998) and Brar et al. (2006) compiled a list of numerous carriers/additives used in formulation strategies for microbial biocontrol agents.

Solar radiation, particularly the UV-A and -B spectra, is a major factor in the short post-application persistence of *Beauveria* as well as other fungi (summarized in Zimmermann 2007). Most research on protecting spores from solar radiation in oil formulations has focused on oil soluble sunscreens, which may protect spores in laboratory bioassays on glass (Moore et al. 1993; Hunt et al. 1994), but may fail to protect spores on natural hydrophobic surfaces or improve efficacy in field trials (Inglis et al. 1995; Burges 1998). Reflective minerals, such as titanium dioxide or silicon dioxide, some clays and anionic food dye-clay complexes have been proposed as UV-blockers for conidia of *Beauveria* (Burges 1998; Foster 2000; Cohen and Joseph 2009).

While many positive results have been reported, many of these additives are either not cost-effective, e.g., oxybenzophenone sunblocks, or are toxic, e.g., Congo Red. Cross-linked lignin coatings of *Beauveria* conidia have afforded remarkable solar protection when exposed to simulated sunlight, yet these formulations presented lower virulence than unformulated conidia (Leland and Behle 2005).

Improved persistence of lignin coating formulations may outweigh the negative effects on efficacy. Behle et al. (2009) reported that soyscreen oils containing feruloylated soy glycerides (i.e., soybean oil+ferulic acid) protect *Beauveria* conidia from UV degradation, because these molecules naturally absorb damaging UV wavelengths, but field efficacy against *Trichoplusia ni* in cabbage was unsatisfactory.

Packaging system can affect the formulation, particularly its shelf life. Generally, low water content (<5 % gravimetric moisture content), low temperature, and reduced oxygen levels are critically involved in prolonged shelf life of fungal propagules. Active packaging approaches can involve incorporation of oxygen and moisture scavengers to prolong fungal survival during long-term storage (Jin et al. 1993; Faria et al. 2012; Mascarin et al. 2016). Challenges in formulation still comprise a major bottleneck in the expansion of commercial mycoinsecticides. Nevertheless, in recent years formulated products have appeared in the marketplace mainly based on wettable powders, oil dispersions or emulsifiable suspensions of aerial conidia (Table 1).

An interesting strategy consists of incorporating certain virulence-related extracellular enzymes, which are produced in the supernatant of liquid fermentation cultures, into the formulated product. Kim and Je (2010) efficiently precipitated and immobilized a thermostable chitinase enzyme derived from the supernatant of *Beauveria* cultures by using 0.5 % (w/v) attapulgite (Attagel®, BASF) as the mineral precipitant. Bioassays with this chitinase-based formulation not only retained activity at 50 °C but also exhibited excellent insecticidal activity against cotton aphids when mixed with 0.01 % (v/v) polyoxyethylene-(3)-isotridecyl ether (TDE-3) as a spreading agent. Attapulgite and related minerals presenting colloidal and sorptive properties are suitable thickening agents for use in liquid flowable formulations, because they can effectively stabilize and suspend particulate matters, such as microbial cells.

Formulation of *Beauveria* for seed coatings or foliar spray applications using liquid-produced submerged conidiospores was designed aiming at the successful establishment of endophytic colonization coupled with the suppression of target insects (Lohse et al. 2015). Nevertheless, these same authors found that the spray application appeared the most effective means of promoting endophytic colonization in oil-seed rape plant tissues, comparatively to soil application, as in the latter *Beauveria* growth was hampered by competition of natural soil microbiota.

Fungal bands incorporating *Beauveria* consist of a formulation targeting adult beetles or gypsy moth larvae that climb up and down the trees and have a high likelihood of contaminating themselves when walking across or under the fungal bands. The bands may be treated with conidia in an oil-based formulation or actually used as a culture substrate by impregnation with media, inoculated with fungus and incubated until it sporulates. Once produced the fungal bands can be attached around branches or tree trunks with or without allelochemical lures. This is a simple, low-cost delivery approach for fungal dissemination within the target insect population. The 'fungal band' application technology was originally developed in Japan with *B. brongniartii* to combat native cerambycids attacking orchards (Higuchi et al. 1997), and has been investigated for the integrated management of invasive destructive wood-boring beetles in North America (Ugine et al. 2013).

A novel formulation based on electrostatically charged micro-powder composed of kaolin, carnauba wax and *Beauveria* conidia has been recently designed to control grain storage arthropods (Storm et al. 2011). The principle behind this innovative formulation (Entostat® Bb38, see Table 1) resides primarily in the use of the hydrophobicity of the spores and of the electrostatic attraction of the carrier particles resulting in an efficient carrier system that is able to deliver spores to target insect cuticle surfaces more efficiently than oil-based formulations.

Development of blastospore formulations is not as advanced as for conidia and demands research to identify compatible adjuvants that are non-toxic to this cell type. Although blastospores are by nature hydrophilic, oil-based formulations that were unlikely to work for this propagule form were successfully conceived for blastospores of *I. fumosorosea* using corn oil, sodium alginate and isotridecyl alcohol ethoxylated-3EO (TDE-3). Increased thermotolerance and greater efficacy toward whitefly nymphs under greenhouse conditions were achieved (Kim et al. 2013), but shelf life of this formulation is uncertain. Microencapsulation by coacervation is the oldest true encapsulation method that can be widely applied to design liquid concentrated emulsions or solid microcapsules of fungal propagules using natural bio-based polymers. This technology offers a diverse array of options to convey innovative and stable formulations of *Beauveria* propagules (Lohse et al. 2015). It is noteworthy to point out that variations in formulation outcomes may arise from genetic variability among isolates of *Beauveria* species and each case should be dealt with care. Pre-existing techniques in food, pharmaceutical or biotechnology industry provide a myriad of methods that can be adjusted to mycoinsecticide production systems and thus facilitate cost-effective scale-up fermentation-formulation strategies.

Potential for resistance

Although it is a feature unlikely to happen with fungal biopesticides, insect resistance to *Beauveria* as well as other fungal entomopathogens is inherent to the arms race between host and pathogens with >300 million year co-

Table 1 Registered commercial products containing propagules of *Beauveria bassiana* for use in biological control against various arthropod pests

Trade name	Strain	Formulation[a]	Type of propagule	Target pest	Concentration and recommended dosage[b]	Manufacturer	Registration
Ballvéria	IBCB-66	WP	Conidia	*Bemisia tabaci* biotype B	1×10^9 CFU/g (application rate: 0.15–0.25 $\times$ 10^{12} conidia/ha)	Ballagro Agro-Tecnologia Ltda.	Brazil
Beauveria JCO	IBCB-66	WP	Conidia	*Cosmopolites sordidus*, *Dalbulus maidis*, *Tetranychus urticae*, *Bemisia tabaci* biotype B	0.6×10^9 CFU/g (application rate: 0.75–8.0 $\times$ 10^{12} conidia/ha)	JCO Indústria e Comércio de Fertilizantes Ltda.	Brazil
Bouveriz WP Biocontrol	IBCB-66	WP	Conidia	*Cosmopolites sordidus*, *Dalbulus maidis*, *Tetranychus urticae*, *Bemisia tabaci* biotype B	8×10^9 CFU/g (application rate: 0.75–8.0 $\times$ 10^{12} conidia/ha)	Biocontrol Sistema de Controle Biológico Ltda.	Brazil
Bovebio	IBCB-66	WP	Conidia	*Cosmopolites sordidus*, *Dalbulus maidis*, *Tetranychus urticae*, *Bemisia tabaci* biotype B	1.48×10^9 CFU/g (application rate: 0.74–8.14 $\times$ 10^{12} conidia/ha)	Biofungi Ind. e Com. De Def. Biológicos e Inoculantes Ltda.	Brazil
Bovemax EC	CG 716	EC	Conidia	*Hypothenemus hampei*, *Diaphorina citri*, *Hedypathes betulinus*	1.5×10^9 conidia/mL (application rate: 2.25×10^{11} conidia/plant or 2.25–3.0 $\times$ 10^{12} conidia/ha)	Novozymes BioAg Produtos para Agricultura Ltd.	Brazil
Boveril WP PL63	ESALQ-PL63	WP	Conidia	*Hypothenemus hampei*, *Tetranychus urticae*, *Gonipterus scutellatus*	1.48×10^9 CFU/g (application rate: 0.05–2.0 $\times$ 10^{12} conidia/ha)	Koppert do Brasil Sistemas Biológicos Ltda.	Brazil
Granada	CB-66	WP	Conidia	*Cosmopolites sordidus*, *Dalbulus maidis*, *Tetranychus urticae*, *Bemisia tabaci* biotype B	1.0×10^9 CFU/g (application rate: 1.11–11.84 $\times$ 10^{12} conidia/ha)	Laboratório de Biocontrole Farroupilha Ltda	Brazil
BotaniGard	GHA	CS, WP, ES	Conidia	Aphids, foliage-feeding Lepidoptera, leaf-feeding beetles, leafhoppers, plant hoppers, mealybugs, grasshopper, mormon cricket, locust, mole cricket; plant bugs, psyllids, scarab beetles, stem-boring lepidoptera, thrips, weevils, whiteflies, European corn borer.	4.4×10^{10} conidia/g or 2.16×10^{10} conidia/mL (application rate: 4.0–8.0 $\times$ 10^{12} conidia/ha)	Laverlam International	United States, Spain, Greece, Italy, Canada, Mexico, Approved in Germany, Switzerland, Korea, Austria
Mycotrol	GHA	WP	Conidia	Similar to BotaniGard	2×10^{10} conidia/g (application rate: 4.0–8.0 $\times$ 10^{12} conidia/ha)	Emerald Bioagriculture Corp.	Mexico (ES only), United States

Table 1 continued

Trade name	Strain	Formulation[a]	Type of propagule	Target pest	Concentration and recommended dosage[b]	Manufacturer	Registration
Mycotrol-O	GHA	ES	Conidia	Similar to BotaniGard	2×10^{10} conidia/mL (application rate: 4.0–8.0 $\times 10^{12}$ conidia/ha)	Emerald Bioagriculture Corp.	United States
Naturalis-L	ATCC 74040	CS	Conidia	Ant, aphid, armyworm, bollworm, budworm, chinch bug, citrus blackfly, Colorado potato beetle, corn borer, cutworm, Elateridae, European chafer, European crane fly larvae, fleahopper, fungus gnat, grasshopper, green June beetle, Japanese beetle, leaf-feeding caterpillar, leafhopper, looper, Lygus bug, mealybug, millipede, mite, mole cricket, Northern masked chafer, pear psylla, psyllid, root weevil, shore fly, sod webworm, sowbug, spittlebug, tarnished plant bug, Tetranychid mite, thrip, tomato fruitworm, weevil, whitefly	2.3–6.9 $\times 10^7$ conidia/mL (application rate: 2.3–6.9 $\times 10^{10}$ conidia/ha)	Troy Biosciences Inc.	Korea, Mexico, United States, United Kingdom, Austria, Italy, Switzerland, Spain, Greece
balEnce	HF23	ES	Conidia	Flies and beetles in poultry and livestock facilities	5.6×10^9 CFU/mL	Terragena	USA
CornGard	Not specified	WG	Conidia	Lepidoptera (Crambidae)	Not specified	Mycotech Corp.	USA
Beaublast	K4B3	CS	Blastospores	Aphid, psylla, Thysanoptera, whitefly	Not specified	Biotelliga Ltd.	New Zealand
Beaugenic	K4B1	WP	Conidia	Aphid, thrips, whitefly	Not specified	Biotelliga Ltd.	New Zealand
Ostrinil	147	G	Conidia	*Paysandisia archon* and *Ostrinia nubilalis*	5.8×10^8 conidia/g (25 kg/ha corn; 4–10 g/palm plant)	Arysta Lifescience S.A.S.	France
Seremoni	Not specified	CS	Conidia	Greenhouse whitefly: *Trialeurodes vaporariorum*; two spotted spider mite: *Tetranychus urticae*	1.0×10^6 CFU/g	Dongbu HiTek Co., Ltd.	Korea
Racer	NCIM 1216 ATCC 26851	WP	Conidia	Rice leaf folder, *Helicoverpa armigera*, *Spodoptera litura*, loopers, leaf eating caterpillars, mealy bugs, coffee berry borers, fruit borers, cotton boll worm, root grubs, surface living larvae and nymphs.	1.0×10^8 CFU/g	Agrilife Biosolutions for Soils & Crops	India
Daman	IPL/BB/M1/01	WP	Conidia	Root borers, caterpillars, sucking pests, locusts, Colorado potato beetles	2×10^8 CFU/g	ILP	India
Nagestra	IPL/BB/M1/01	Liquid	Conidia	Root borers, caterpillars, sucking pests, locusts, Colorado potato beetles	2×10^9 CFU/mL	ILP	India

Table 1 continued

Trade name	Strain	Formulation[a]	Type of propagule	Target pest	Concentration and recommended dosage[b]	Manufacturer	Registration
Bb-Protec	R444	WP	Conidia	Whiteflies, herbivorous mites, wire worms,	$\geq 2 \times 10^9$ spores/g (application rate 1 g/L water)	Andermatt Biocontrol	Switzerland
Boverosil	Not specified	WP	Conidia	Coleoptera (Curculionidae) and stored pests	Not specified	Not specified	Czech Republic
Boverol	Not specified	WP	Conidia	Coleoptera (Chrysomelidae)	Not specified	Fytovita	Czech Republic
Bea-Sin	Not specified	WP or Liquid	Conidia	Whitefly (*Bemisia tabaci*)	5.33×10^9 conidia/g (480 g/200 L) or 1.2×10^9 conidia/mL (2 L/ha)	Agrobionsa	Mexico
Bio-Fung	Not specified	Not specified	Conidia	Orthoptera	Not specified	Centro de Sanidad Vegetal de Guanajuato (CESAVEG)	Mexico
Bassianil	Not specified	WP	Conidia	*Anthonomus grandis*, *Hypothenemus hampei*, *Metamasius* sp., *Ancognatha* sp., *Collaria* sp., *Phyllophaga* sp., *Ryncophorus* sp., *Empoasca* sp., *Corytucha* sp., *Thrips* sp., *Bemisia tabaci, Trialeurodes vaporariorum*	1×10^8 conidia/g (application rate: 1–2 g/L of water)	Biotropic S.A.	Mexico
PHC BEA TRON	Abn Bb102	WP	Conidia	*Bemisia tabaci, Phyllophaga* spp., *Hypothenemus hampei*, *Acigona loftini*, *Aeneolamoa* spp.	5×10^9 conidia/g (application rate 240 g/ha)	PHC Health Care de Mexico	Mexico
Becan	Not specified	Not specified	Conidia	Coleotera, Diptera, Hemiptera, Lepidoptera, Orthoptera	Not specified	Newbiotechnic S.A.	Spain
Trichobass-P	Not specified	WP	Conidia	Coleoptera (Curculionidae, Scarabaeidae), Hemiptera (Aleyrodidae), Acari (Tetranychidae)	Not specified	Trichodex S.A.	Spain
Trichobass-L	Not specified	OD	Conidia		Not specified		Spain
Beauvedieca	Not specified	Not specified	Conidia	Coleoptera (Curculionidae)	Not specified	Liga Agricola Industrial de La Can˜a de Azucar (LAICA)	Costa Rica, Panama
Nativo 2 SC	Not specified	CS	Conidia	Coleoptera (Curculionidae)	Not specified	Bayer Cropscience S.A.	Costa Rica

Table 1 continued

Trade name	Strain	Formulation[a]	Type of propagule	Target pest	Concentration and recommended dosage[b]	Manufacturer	Registration
Ago Biocontrol Bassiana 50	Not specified	Not specified	Conidia	Coleoptera, Diptera, Hemiptera, Lepidoptera	Not specified	Ago Biocontrol	Colombia
Agronova	Not specified	Not specified	Conidia	Coleoptera (Curculionidae, Scarabaeidae), Lepidoptera(Noctuidae, Nymphalidae, Sphingidae)	Not specified	Live Systems Technology S.A.	Colombia
Cebiopest	Not specified	Not specified	Not specified	Not specified	Not specified	Fundacion Centro de Biotecnologia Mariano Ospina Perez	Colombia
Conidia	Not specified	WG	Conidia	Coleoptera (Curculionidae)	Not specified	Hoechst Schering AgrEvo	Colombia
Brocaril 50 WP	Not specified	WP	Conidia	Coleoptera (Curculionidae)	2.0×10^9 conidia/g (application rate: 1.0–2.0×10^{11} conidia/ha)	Laverlam S.A.	Colombia
Brocavec	Not specified	Not specified	Conidia	Not specified	Not specified	Empresa Colombiana de Productos Veterniarios Vecol S.A.	Colombia
MicosPlag[c]	CENICAFE Bb9205	WP	Conidia	Plant parasitic nematodes and *Hypothenemus hampei*	5.0×10^4 CFU/g (application rate: 100–300 g/ha)	Orius Biotecnología	Colombia
3B	Not specified	TK	Conidia + hyphae	*Hypothenemus hampei*	3.7×10^7 conidia/g (application rate: 639 g/ha)	PROCAFE HQ	El Salvador
Bazam	Not specified	WP	Conidia	Coleoptera (Chrysomelidae, Curculionidae), Hemiptera (Aleyrodidae, Aphididae), Lepidoptera (Noctuidae, Plutellidae), Acari (Tetranychidae)	Not specified	Escuela Agrícola Panamericana	Honduras
Bauveril	Not specified	WP	Conidia	Coleoptera (Curculionidae, Scarabaeidae), Lepidoptera (Castniidae)	Not specified	Laverlam S.A.	Colombia, Dominican Republic

Table 1 continued

Trade name	Strain	Formulation[a]	Type of propagule	Target pest	Concentration and recommended dosage[b]	Manufacturer	Registration
Bb Plus	Not specified	WP	Conidia	Hemiptera (Aphididae), Acari (Tetranychidae)	Not specified	Biological Control Products SA (Pty) Ltd.	South Africa
BroadBrand	PPRI 5339	EC	Conidia (Minimum 4×10^9 viable spores/mL)	*Plutella xylostella*, *Thaumatotibia leucotret*, *Aonidiella aurantii*, *Tetranychus urticae*, *Phthorimaea operculella,* stinkbug, thrips, whiteflies	4.0×10^9 conidia/mL (application rate: $2.0–4.0 \times 10^{12}$ conidia/ha)	BASF South Africa (Pty) Ltd.	South Africa
Bb Weevil	Not specified	CP	Conidia	Coleoptera (Curculionidae)	Not specified	Biological Control Products SA (Pty) Ltd.	South Africa
Eco-Bb	R444	WP	Conidia	Red spider mites, whitefly and various other agricultural insect pests	2.0×10^9 conidia/g (300–600 g/ha)	Plant Health Products (Pty) Ltd.	South Africa
Beauvitech	J25	WP	Conidia	Whiteflies, thrips, aphids	1.0×10^{10} conidia/g (250 g/ha)	Dudutech Ltd.	Kenya
Proecol	Not specified	Not specified	Conidia	Lepidoptera (Noctuidae)	Not specified	Probioagro S.A.	Venezuela
BioExpert	Not specified	Not specified	Conidia	Hemiptera (Aleyrodidae), Thysanoptera (Thripidae)	Not specified	Live Systems Technology S.A.	Colombia
Baubassil	Not specified	Not specified	Conidia	Coleoptera, Hemiptera, Lepidoptera	Not specified	Productos Biológicos Perkins Ltda	Colombia
Bazam	Not specified	WP	Conidia	Coleoptera (Chrysomelidae, Curculionidae), Hemiptera (Aleyrodidae, Aphididae), Lepidoptera (Noctuidae, Plutellidae) + Acari (Tetranychidae)	Not specified	Produtos Ecológicos, Guatemala; Escuela Agrícola Panamericana, Honduras	Honduras, El Salvador, Guatemala, Jamaica, Nicaragua
Mirabiol	Not specified	TK	Conidia + hyphae	Coleoptera (Curculionidae)	1.13×10^9 conidia/g (4.84×10^{11} conidia/ha)	Union de Cooperativas Agropecuarias (UCA Miraflor)	Nicaragua

Table 1 continued

Trade name	Strain	Formulation[a]	Type of propagule	Target pest	Concentration and recommended dosage[b]	Manufacturer	Registration
Bb Vinchuca	Bb10	ES	Conidia	Hemiptera (Reduviidae)	1.0–2.0 × 10^{10} conidia/g (application rate: 5.0 × 10^{8} conidia/m^2)	Laboratorios Biagro S.A.	Argentina
Bb Moscas	Not specified	OD, RB	Conidia	Diptera (Muscidae)	Not specified	Laboratorios Biagro S.A.	Argentina
BiolisaMadara	Not specified	Fiber band	Hyphae and conidia	Coleptera (Cerambycidae)		Nitto Denko	Japan
In2Care Mosquito Trap	GHA	Trap	Conidia + pyriproxyfen	Mosquitoes (Culicidae)	Not specified	Univar Environmental Sciences	USA
Entostat Bb38	IMI389521	Wax based electrostatic micro powders	Conidia	Grain storage insects	Not specified	Exosect Ltd.	UK
Biostop F	Not specified	Not specified		Cabbage moth, Colorado potato beetle, rosa moth, codling moth, cabbage aphids, tabacco thrips, beet flea leaf aphid, gypsy moth	>10^{8} CFU/mL	Invivo Ltd. Moscow	Russia
Boverin	Not specified	WP	Blastospores	Hemiptera (Aleyrodidae), Thysanoptera (Thripidae), Acari (Tetranychidae)	Not specified	Biodron	Russia

[a] *WP* Wettable powder, *WG* wettable dispersible granule, *EC* emulsifiable concentrate, *ES* liquid emulsifiable suspension, *CS* concentrated suspension, *G* granule, *OD* oil dispersion, *RB* ready for use (bait), *CP* contact powder, *TK* technical concentrate (see also formulation details in Faria and Wraight 2007)

[b] Frequency of applications may vary among product formulations and manufacturer's recommendations according to the target pest or crop system

[c] MicosPlag® is a unique mixture of *Metarhizium anisopliae*, *Beauveria bassiana* and *Purpureocillium lilacinum* used as a biological nematicide and insecticide in Colombia

evolutionary history underscoring remarkably behavioral, biochemical and genetic interactions (Pedrini et al. 2015; Sigwart et al. 2015). Insects have pathogen recognition systems and immune defenses. Infection by *Beauveria* and other fungi caused differential expression of numerous gene systems (ChengXiang et al. 2014). Several laboratory studies have identified innate immune responses to *Beauveria* and other fungi (Wojda et al. 2005; Kangassalo et al. 2015). An increase in these defense systems could result in evolution of resistance. Nevertheless, no reports of cases of true host (genetic basis) resistance to *Beauveria* under field conditions have yet been documented.

Using *Beauveria*

Current commercial products

Due to the growing demand for chemical-free crops and tightening regulations on pesticide residues, especially in developed countries, especially from Europe and North America, federal regulatory agencies are encouraging the approval of more biopesticides annually as compared to conventional chemicals. With the growing acceptance of microbial biopesticides as an efficient crop protection alternative with eco-friendly footprint, several leading agrochemical companies are increasingly leaning towards biopesticides sector.

Beauveria, along with *Metarhizium,* are the major fungal insect pathogens exploited for biocontrol, mainly because of their ubiquitous, cosmopolitan distribution and easy mass production using artificial media. Together, both species comprise nearly 70 % of all commercial mycoinsecticides, according to the last comprehensive review (Faria and Wraight 2007). Not surprisingly, almost 90 % of mycoinsecticides sold in the world are composed of solid-substrate-produced aerial conidia. In contrast, vegetative propagules encompassing blastospores, mycelium and compact hyphal aggregates (microsclerotia), represent only a small portion (<10 %) of these products. Growing interest in exploring these vegetative propagules, however, has arisen from the short incubation time and laborsaving procedure involved in liquid culture fermentation processes.

For example, Koppert in Brazil produces *Beauveria* (Boveril® WP, strain ESALQ-PL63) using solid-state fermentation on moistened rice with a yield reaching up to 10 tons of pure dry conidia for spray application on nearly 50,000 ha, comprising mainly coffee and eucalyptus plantations. This product has been recommended for the control of spider mites (*Tetranychus urticae*), whiteflies (*B. tabaci*), eucalyptus snout beetle (*Gonipterus scutellatus*), coffee berry borer (*Hypothenemus hampei*). Whiteflies on soybean crops in Brazil comprise a huge market for *Beauveria,* estimated to be >30 million hectares. In the U.S., the European Union, Japan, and Mexico, *Beauveria* strain GHA, as BotaniGard® and Mycotrol® products, are widely used against thrips, Hemiptera including whitefly, aphids, psyllids, mealybugs, plantbugs, leafhoppers, a wide range of Curculionidae and Chrysomelidae in the Coleoptera, and Orthoptera, such as grasshoppers (Faria and Wraight 2001, 2007; Lacey et al. 2008). More interestingly, a mixture of *M. anisopliae*, *Purpureocillium lilacinum* and *B. bassiana* with the commercial name of MicosPlag® is sold in Colombia for the control of plant parasitic nematodes as well as insect pests, including the coffee berry borer (see details in Table 1). Additionally, Indian biopesticide market appears to have several *B. bassiana* mycoinsecticides, which can be found on websites (e.g., http://dir.indiamart.com/search.mp?ss=beauveria), but they showed critical missing data (i.e., recommended dosage, concentration, manufacturer and target pests) and thus were not listed in Table 1.

Most *Beauveria*-based products are wettable powder (WP) followed by concentrated suspension (CS) and emulsifiable suspension (ES) formulations (Table 1). A few products of *Beauveria* have been formulated in auto-dissemination devices for specific targets such as Tephritidae (Ekesi et al. 2007), flies (Bb Moscas®, Argentina) and beetles (BiolisaMadar®, Japan). The integrated approach laid on the combination of *Beauveria* and other natural-derived botanical insecticides is a reality and has given successful results in numerous cases (Islam and Omar 2012). Some companies, e.g. LAM International (Butte, MT, USA), are adding specific botanical compounds to their *Beauveria*-based products, such as azadirachtin (neem) as a strategy to achieve synergism for ultimate improvement in effectiveness of pest control under field conditions.

How is *Beauveria* being used?

Historically, *Beauveria*, as well as the other entomopathogenic Ascomycetes, has been used in an inundative manner, in a chemical paradigm, as it were. Typically, very large quantities of propagules (5×10^{12}–5×10^{13} ha^{-1}), usually aerial conidia, are applied to the target insect and its habitat. In a survey of the literature we find that, in most cases, evaluative research has sought to achieve a high degree of control, much like the chemical insecticides being used in the target agroecosystems. This tendency has often been continued into operational use, leading to sometimes disappointing results, in which insect control is inferior to that of the chemical standards.

Early efforts to remedy this deficiency involved combinations of mycoinsecticides with sublethal doses of

chemical insecticides, previously selected on basis of biological compatibility with the fungal agent, to increase mortality in some insect pests (Furlong and Groden 2001; Farenhorst et al. 2010). A side benefit, however, of such approaches lies in the fact that the use of lower chemical concentrations pose minimum environmental pollution.

While laboratory and field testing of various *Beauveria* isolates for the control of silverleaf whitefly, *B. tabaci* biotype B, in the US and Brazil identified good candidates (Wraight et al. 2000; Faria and Wraight 2001; Vicentini et al. 2001; Mascarin et al. 2013), traditional application approaches were not very successful for operational control. Whitefly nymphs are sessile on abaxial surfaces of leaves, which makes delivery of conidia to them difficult with conventional sprays. To overcome this obstacle, successful whitefly population reduction was achieved with *Beauveria* being sprayed multiple times at intervals of 4–5 days using an electrostatic spray device that effectively delivered conidia to nymphs in cucurbit crops in the US (Wraight et al. 2000). Similarly, Wraight and Ramos (2002) were able to increase the conidial delivery of conidia 6- to 30-fold to leaf undersides using a backpack sprayer with hydraulic drop nozzles pointing upwards. Another approach for cucurbit crops is to use nozzles on drop tubes positioned almost in the canopy and faced rearwards, pictured in Jaronski (2010). Other alternative delivery mechanisms of *B. bassiana* have been devised for augmentative or inoculative applications that employ relatively small amount of fungal inoculum by using a variety of application methods, namely auto-inoculative devices baited with pheromones or kairomones, e.g., Lopes et al. (2014); placement of conidia in the path of migrating insects (Shanley et al. 2009), and autodissemination (Dowd and Vega 2003; Storm et al. 2011; Jenkins et al. 2013); use of attractants (summarized by Vega et al. 2007), vector technology using honeybees and bumble bees (Shipp et al. 2006), seed coatings for soil application, and endophytes for plant colonization. These less conventional delivery mechanisms promote efficient use of microbial control agents with reduced amounts of inoculum for particular conditions, in which inundative strategy is not suitable or not economically feasible.

Joint action involving *B. bassiana* with other entomopathogens can also render synergistic efficacy in integrated biologically based management of some target insects, especially when both agents have distinct mode of action as for instance the insect pathogenic bacterium *Bacillus thuringiensis* (Wraight and Ramos 2005). Integrated use of botanical insecticides, such as neem-based products, and *B. bassiana* is also feasible and may result in enhanced pest control (Islam et al. 2010).

The wide natural genetic variability within *Beauveria* has enabled screening for more virulent strains, a frequent goal in this field (Mascarin et al. 2013; Quesada-Moraga et al. 2006; Valero-Jiménez et al. 2014), and strains more tolerant to heat, cold and ultraviolet (UV) radiation stresses (Fernandes et al. 2007, 2008). These efforts are directed toward making a better *Beauveria,* with improved persistence and more consistent efficacy under field conditions.

Effective approaches for improving virulence and other fitness traits in *Beauveria* through the manipulation of nutritional and physical environment during fungal growth have been documented. Exposure of mycelial growth to certain stressful abiotic conditions, including pH, hyperosmolarity, UV radiation, photoperiod regime, and nutrient starvation have rendered infectious conidia to some extent with improved virulence and stress resistance, but accompanied usually by greatly lower yields (Rangel et al. 2015). Different carbon sources greatly influenced the biocontrol fitness of *Beauveria* conidia. Crespo et al. (2002) found out that alkane-grown conidia of *Beauveria* exhibited greater virulence to the bean weevil, *Acanthoscelides obtectus*, than when cultivated only in a glucose-amended medium. More intriguingly and unexpectedly, blastospores of *Beauveria* cultivated in low water activity liquid media imposed by high levels of glucose not only displayed morphological changes but also enhanced virulence against whitefly nymphs (*B. tabaci*) (Mascarin et al. 2015b). Earlier studies have shown that lowering the water activity in growth media amended with ionic or nonionic osmolytes for filamentous entomopathogenic fungi have resulted in accumulation of endogenous reserves composed mainly of sugar alcohols (e.g., erythritol, mannitol, glycerol) and threhalose in the spores that have been associated to faster speed of germination and greater virulence even under low relative humidities (Hallsworth and Magan 1994; Magan 2001).

There has been a growing effort to create superior *Beauveria* strains by direct genetic manipulation or by genetic recombination using protoplast fusion with improved virulence in terms of faster speed of kill and reduced lethal doses (Couteaudier et al. 1996; St. Leger and Wang 2010). One goal is to overcome the perceived slower speed of kill (St. Leger and Wang 2010). Efforts to enhance *Beauveria* have even extended to expression of the *Bacillus thuringiensis* Vip3Aa insecticidal protein in a genetically transformed strain (XiaoFang et al. 2011), or pathogenicity-associated proteinase and esterase genes from *M. anisopliae* (Rodríguez and Góngora 2005) for enhanced virulence. The novel RNA-guided mutagenesis based on the CRISPR-Cas9 technology or similar may accelerate and facilitate research in this area and open new avenues to exploiting *Beauveria.* With the recent publication on the complete genome of *B. bassiana* (Xiao et al. 2012), researchers now have the opportunity to progress into the complex mechanisms involved in different life-histories of

this fungus and manipulate its genes for various industrial purposes, including biocontrol. However, despite the unlimited potential progress in genetically engineered fungal entomopathogens to overcome obstacles not solved by non-engineering approaches, strict regulatory issues and lack of studies probing their safety to non-target hosts in outdoor environments, as well as public reluctance, may hamper development of genetically modified commercial mycoinsecticides.

All these efforts stem from the opinion that *Beauveria* does not "work well enough." What is forgotten all too often is that *Beauveria*, as well as other microbial agents, is just one tool among several in integrated pest management, which seeks to not to eliminate a pest population but rather to manage it to keep it below economic threshold. Thus, the fungus does not have to possess the efficacy of a chemical pesticide.

A new role for *Beauveria*?—as an endophyte

New insights on the life histories of the entomopathogenic Ascomycetes, particularly *Beauveria*, have led to their recognition as endophytes (Fig. 3). This ecological role was first observed by Wagner and Lewis (2000), who found *B. bassiana* growing in maize stems, causing considerable mortality in larvae of the European corn borer, *Ostrinia nubilalis*. In subsequent years there has been an increasing number of reports of asymptomatic endophytic colonization of plants by *Beauveria*, either occurring naturally or as a result of deliberate inoculation (reviewed by Vega et al. 2008). Endophytic *B. bassiana s.l.* has subsequently been reported in cocoa (Posada and Vega 2006), opium poppy (Quesada-Moraga et al. 2009), date palm (Gómez-Vidal et al. 2009), coffee (Posada et al. 2007), tomato (Ownley et al. 2008), grapes (Jaber 2015), banana (Akello et al. 2009), bean (Akutse et al. 2014), sorghum (Tefera and Vidal 2009), pine (Reay et al. 2010), cotton (Gurulingappa et al. 2010), and rape (Lohse et al. 2015). Deliberate endophytism by *Beauveria* has been accomplished by root dips into spore suspensions, treating seeds, even spraying plants (Landa et al. 2013). *Beauveria* evidently can be transmitted vertically via seeds from endophytically colonized opium poppy plants, which evidently also affords protection against the stem gall wasp, *Iraella luteipes* (Quesada-Moraga et al. 2014). In broad bean, it has been attributed to provide a protective presence against leaf miner as manifested by significant reduction in larval survival and damage (Akutse et al. 2014). Ostensible endophytic colonization of cotton by *Beauveria* was associated with significantly decreased aphid reproduction (Gurulingappa et al. 2010). Similarly, *Beauveria* endophytism in cotton affected *Helicoverpa zea* development and survivorship (Lopez and Sword 2015), and *Cosmopolites sordidus* larval survival and damage in banana (Akello et al. 2008). Dual systemic protection, against plant pathogens as well as insect pests, promoted by this endophytic association has been proposed (Ownley et al. 2008; Quesada-Moraga et al. 2009).

The mechanisms underlying the insecticidal and microbicidal properties of endophytic *Beauveria* remain obscure. In many of the reported cases, plant protection was not accompanied by overt insect death from mycosis. *Beauveria* produces a number of known metabolites—oosporein, several bassianolides, beauvericin, tenellin, bassianin, oxalic acid—and undoubtedly still others as yet unidentified. Vidal and Jaber (2015) suggested that an observed reduced survival of insects exposed to plants endophytically colonized by fungal entomopathogens, including *Beauveria*, may be related to production of one or more of these fungal metabolites that could affect the insects directly or cause a reduced fitness via systemic defense mechanisms elicited in the host plant, or a combination of these factors. Endophytic colonization of plant tissues by *Beauveria* and *Lecanicillium* spp. may trigger an induced systemic defense in host plants, which, in turn, affects the biological fitness of harmful herbivores (Goettel et al. 2008; Vidal and Jaber 2015). Evidence has been accumulating about the induction of herbivore defenses in plant by microorganisms (Karban and Johnson 2011), so it is possible that this phenomenon exists with endophytic *Beauveria*. *Metarhizium anisopliae* has been shown to elicit phytohormonal effects in soybeans (Khan et al. 2012) and the same may be possible with *Beauveria*. Strategies to deliver endophytic *Beauveria* to host plants seem more cost-effective in comparison to the inundative biocontrol strategy, mainly because the fungal endophyte will be protected from environmental abiotic stresses once inside the plant, promoting longer persistence, and requiring less inoculum to initiate host colonization. Furthermore, appropriate formulation of *Beauveria* endophytes has been devised to facilitate high endophytic establishment in oilseed rape plants via aerial or ground application of viable fungal propagules produced by submerged liquid fermentation (Lohse et al. 2015). In summary, there is a strong evidence of the multiple roles of biocontrol and plant enhancer performed by *Beauveria*. Successful implementation of *Beauveria* as a protective endophyte in commercial vegetable crops, however, will depend on plant variety, nutritional status of the plant, soil physical–chemical-microbial characteristics, fungal isolate, delivery system of fungal application, fungal inoculum density, and weather conditions. Inconsistent endophytic colonization of plant crops has been the major problem in full development of *Beauveria* endophytes.

Exemplar model IPM systems incorporating *Beauveria*

Fungal biopesticides, including *Beauveria*, are very suitable for incorporating into IPM programs, as they play a key role in mitigation resistance evolution to synthetic pesticides and reduction of chemical reliance. For an extensive overview on the main microbial control programs for arthropod pests in agroecosystems and other semi-natural landscapes, readers can refer to the book of Lacey and Kaya (2007). Here we present a few examples in which *Beauveria* is used within the context of IPM.

Coffee berry borer (*Hypothenemus hampei*): *Beauveria* is the most common natural fungal pathogen of coffee berry borer (CBB) worldwide. Previous reports document natural infection rates ranging from <1 to 91 % in different coffee country producers (Aristizábal et al. 2016). Efficacy of *Beauveria* against CBB under field conditions relies on numerous factors, including weather conditions, strain, concentration, virulence, application efficiency, infestation level. Applications of *Beauveria* are performed with sprayable formulations via two ways: (1) conidia are sprayed onto CBB female founders when they migrate from refuges or parchment coffee areas during the peak flight activity; (2) the fungus is applied to fallen infested berries on the ground with resultant decrease in CBB infestation up to 75 % in Colombia (Aristizábal et al. 2016). Typically, conidia are the primary infective propagule used for spray applications against CBB at concentrations ranging from 1×10^{11} to 1×10^{12} conidia ha^{-1}. In Brazil, up to two sequential applications of *Beauveria,* at label rate of 1×10^{12} conidia ha^{-1} in 800 L water during the CBB flight activity peak, when relative humidity is >60 %, have resulted in low infestation levels (Mascarin GM, unpublished data). Ideally, initial infestation (damaged berries) by CBB should be <2 % for sustained suppression by *Beauveria*. Once the insect bores into the berry, the application of *Beauveria* has much lower efficacy because the insect and its progeny are protected. Use of organosilicone surfactants in inundative sprays increases fungus delivery to the insect and efficacy (Jaronski, unpublished data). Biological control combining *Beauveria* and other natural enemies, including parasitoid wasps and entomopathogenic nematodes, has recently gained strength. Within an IPM context, other control techniques including sanitization of the fallen berries and use of mass capturing with methanol-ethanol traps, along with *Beauveria* use will aid CBB suppression consequently mitigating crop losses (Aristizábal et al. 2012). An integrated approach for CBB management in Hawaii is being intensively implemented with biological control agents along with cultural practices (Jaronski, unpublished data), and a summary of practices being pursued in Hawaii has been incorporated into a YouTube video (Kona Coffee Farmers Association 2012).

Potato Colorado beetle (*Leptinotarsa decemlineata*): Organic agriculture offers an exceptional opportunity of deploying microbial biocontrol agents for pest control. Recently, *Beauveria* was shown to be an important fungal pathogen of the Colorado Potato Beetle, *Leptinotarsa decemlineata*, in organic potato. Along with other natural enemies it can strongly mitigate damage by this insect pest (Crowder et al. 2010). Other studies also reveal the combined or complimentary biological control exerted by *Beauveria* along with other natural enemies including predators, parasitoid wasps as well as microbial insect pathogens. *Beauveria* can be combined with other microbial pest control agents, such as *B. thuringiensis* var. *tenebrionis,* to overcome the disadvantages of each, such as for Colorado potato beetle (Wraight and Ramos, 2005).

Glasshouse crops: The high value, limited expanse, intensive production practices, need for human safety and a contained environment have encouraged glasshouse growers to pursue biobased IPM systems. In the glasshouse environment, combined use of a mycopesticide, parasites and predators has been a viable option (Labbe et al. 2009). Efforts to do so have very recently been extensively summarized by Gonzalez et al. (2016). Furthermore, efforts to integrate microbials with pollinator-vectored technology have been experimented with *Beauveria* to several insect pests on crops, with numerous successful cases in Canada. A system was developed to deliver *Beauveria* conidia by bumble bees to target western flower thrips (*Frankliniella occidentalis*), greenhouse whitefly (*Trialeurodes vaporariorum*), and green peach aphid (*Myzus persicae*), major pests of greenhouse crops (Shipp et al. 2006). Greenhouse cage trials have shown that bee-vectored *B. bassiana* can cause substantial mortality of *Lygus*, whiteflies, thrips and aphids (up to 80 % mortality) when tested on greenhouse tomato and sweet pepper. The bees deliver the fungal spores directly to the flowers and leaves where the pests are found.

Future prospects

The continuous expansion of microbial control with regard to the entomopathogenic hypocrealean fungi, such as *Beauveria*, is indispensable for promoting sustainable solutions to safeguard food and nutrition security. Genetic selection or genetic recombination for virulent and multi-stress abiotic tolerant strains coupled with reliable and cost-effective mass production and formulation strategies will contribute to generating resilient, safe and effective *Beauveria* mycoinsecticides for use in different biocontrol approaches. Nevertheless, *Beauveria* and the other

microbial biocontrol agents are not silver bullets and really should be implemented within the context of integrated management programs for pest control. Intelligent and effective delivery systems will also support the usefulness of *Beauveria*. Since biopesticides are still mirrored in the legislation system designed for chemical pesticides, albeit with less stringent requirements, more favorable regulatory rules are needed to deal with microbial biopesticides in order to reduce time and costs for registration and, hence, promote wider commercial availability especially in the European Union (Ehlers 2011). Due to the growing maturity of a substantial biopesticide industry, especially with the recent involvement of large ag-chemical companies, a solid foundation is being created for progress in biological control strategies using *Beauveria* and other microbial biocontrol agents. New findings on the versatile ecological roles displayed by *Beauveria* with multiple effects add function and value to end users and will facilitate its eventual commercialization as a mycopesticide or as a plant growth promoter (biostimulant). Finally, we believe there is a need for improving education and extension of growers and extensionists about the availability and utility of *Beauveria* in order to foster its widespread adoption into comprehensive pest management strategies.

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References

Akello J, Dubois T, Coyne D, Kyamanywa S (2008) Endophytic *Beauveria bassiana* in banana (*Musa* spp.) reduces banana weevil (*Cosmopolites sordidus*) fitness and damage. Crop Prot 27(11):1437–1441

Akello J, Dubois T, Coyne D, Hillnhutter C (2009) *Beauveria bassiana* as an endophyte in tissue-cultured banana plants: a novel way to combat the banana weevil *Cosmopolites sordidus*. Acta Hortic 828:129–138

Akutse KS, Fiaboe KKM, Van Den Berg J, Ekesi S, Maniania NK (2014) Effects of endophyte colonization of *Vicia faba* (fabaceae) plants on the life-history of leafminer parasitoids *Phaedrotoma scabriventris* (Hymenoptera: Braconidae) and *Diglyphus isaea* (Hymenoptera: Eulophidae). PLoS ONE 9(10):e109965

Alves SB, Pereira RM (1989) Production of *Metarhizium anisopliae* (Metsch.) Sorok. and *Beauveria bassiana* (Bals.) Vuill. in plastic trays. Ecossistema 14:188–192

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 17:675–679

Aristizábal LF, Lara O, Arthurs SP (2012) Implementing an integrated pest management program for coffee berry borer in a specialty coffee plantation in Colombia. J Integr Pest Manag 3(1):G1–G5

Aristizábal LF, Bustillo AE, Arthurs SP (2016) Integrated pest management of coffee berry borer: strategies from Latin America that could be useful for coffee farmers in Hawaii. Insects 7:E6

Bateman RP, Carey M, Moore D, Prior C (1993) The enhanced infectivity of *Metarhizium flavoviride* in oil formulations at low humidities. Ann Appl Biol 122:145–152

Behie SW, Zelisko PM, Bidochka MJ (2012) Endophytic insect parasitic fungi translocate nitrogen directly from insects to plants. Science 336:1576–1577

Behle RW, Compton DL, Laszlo JA, Shapiro-Ilan DI (2009) Evaluation of soyscreen in an oil-based formulation for UV protection of *Beauveria bassiana* conidia. J Econ Entomol 102(5):1759–1766

Bissett J, Widden P (1986) A new species of *Beauveria* from Scottish moorland soil. Can J Bot 66:361–362

Boomsma JJ, Jensen AB, Meyling NV, Eilenberg J (2014) Evolutionary interaction networks of insect pathogenic fungi. Ann Rev Entomol 59:467–485

Brar SK, Verma M, Tyagi RD, Valéro JR (2006) Recent advances in downstream processing and formulations of *Bacillus thuringiensis* based biopesticides. Process Biochem 41:323–342

Burges HD (1998) Formulation of microbial biopesticides: beneficial organisms, nematodes and seed treatments. Kluwer Academic, Dordrecht, p 412

ChengXiang H, GuangXing Q, Tao G, Kun G, ZhongHua P, HeYing Q, XiJie G (2014) Differentially expressed genes in the silkworm (*Bombyx mori*) against infection by *Beauveria bassiana*. Acta Entomol Sin 57(1):13–24

Chong-Rodriguez MJ, Maldonado-Blanco MG, Hernandez-Escareno JJ, Galan-Wong LJ, Sandoval-Coronado CF (2011) Study of *Beauveria bassiana* growth, blastospore yield, desiccation-tolerance, viability and toxic activity using different liquid media. Afr J Biotech 10:5736–5742

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

Cory JS, Ericsson JD (2010) Fungal entomopathogens in a trithrophic context. Biocontrol 55:75–88

Couteaudier Y, Viaud M, Riba G (1996) Genetic nature, stability, and improved virulence of hybrids from protoplast fusion in *Beauveria*. Microb Ecol 32(1):1–10

CPL Business Consultants (2010) The 2010 worldwide biopesticides market summary, vol 1. CPL Scientific, Wallingford, p 39

Crespo R, Juarez MP, Dal Bello GM, Padin S, Fernandez GC, Pedrini N (2002) Increased mortality of *Acanthoscelides obtectus* by alkane-grown *Beauveria bassiana*. Biocontrol 47(6):685–696

Crowder DW, Northfield TD, Strand MR, Snyder WE (2010) Organic agriculture promotes evenness and natural pest control. Nat Lett 466:109–113

deHoog GS (1972) The genera *Beauveria*, *Isaria*, *Tritrachium* and *Acrodontium* n. gen.nov. Stud Mycol 1:1–41

deHoog GS, Rao V (1975) Some new hyphomycetes. Persoonia 8:207–212

Dowd PF, Vega FE (2003) Autodissemination of *Beauveria bassiana* by sap beetles (Coleoptera: Nitidulidae) to overwintering sites. Biocontrol Sci Technol 13:65–75

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

Ehlers R (2011) Regulation of biological control agents. Springer, Dordrecht **416**

Ekesi S, Dimbi S, Maniania NK (2007) The role of entomopathogenic fungi in the integrated management of tephritid fruit flies

(Diptera: Tephritidae) with emphasis on species occurring in Africa. In: Ekesi S, Maniania NK (eds) Use of entomopathogenic fungi in biological pest management. Research SignPost, Kerala, pp 239–274

Farenhorst M, Knols BGJ, Thomas MB, Howard AFV, Takken W, Rowland M, N'Guessan R (2010) Synergy in efficacy of fungal entomopathogens and permethrin against West African insecticide-resistant *Anopheles gambiae* mosquitoes. PLoS ONE 5(8):e12081

Faria M, Wraight SP (2001) Biological control of *Bemisia tabaci* with fungi. Crop Prot 20:767–778

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

Faria M, Hotchkiss JH, Wraight SP (2012) Application of modified atmosphere packaging (gas flushing and active packaging) for extending the shelf life of *Beauveria bassiana* conidia at high temperatures. Biol Control 61:78–88

Feng MG, Poprawski TJ, Khachatourians GG (1994) Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: current status. Biocontrol Sci Techn 4:3–34

Fernandes EKK, Rangel DEN, Moraes AML, Bittencourt VREP, Roberts DW (2007) Variability in tolerance to UV-B radiation among *Beauveria* spp. isolates. J Invertebr Pathol 96:237–243

Fernandes EKK, Rangel DEN, Moraes AML, Bittencourt VREP, Roberts DW (2008) Cold activity of *Beauveria* and *Metarhizium*, and thermotolerance of *Beauveria*. J Invertebr Pathol 98:69–78

Fernandes EKK, Rangel DEN, Braga GUL, Roberts DW (2015) Tolerance of entomopathogenic fungi to ultraviolet radiation: a review on screening of strains and their formulation. Curr Genet 61(3):427–440

Ferron P (1981) Pest control by the fungi *Beauveria* and *Metarhizium*. pp. 465–483. In: Burges HD (ed) Microbial control of pest and plant diseases 1970–1980. Academic Press, London, New York, p 949

Foster JP (2000) A process for coating biological pesticides and compositions therefrom, U. S. Patent 6,113,950, 19 pp

Furlong MJ, Groden E (2001) Evaluation of synergistic interactions between the Colorado potato beetle (Coleoptera: Chrysomelidae) pathogen *Beauveria bassiana* and the insecticides, imidacloprid, and cyromazine. J Econ Entomol 94(2):344–356

Ghikas DV, Kouvelis VN, Typas MA (2010) Phylogenetic and biogeographic implications inferred by mitochondrial intergenic region analyses and ITS1-5.8S-ITS2 of the entomopathogenic fungi *Beauveria bassiana* and *B. brongniartii*. BMC Microbiol 10:174

Gibson DM, Donzelli BGG, Krasnoff SB, Keyhani NO (2014) Discovering the secondary metabolite potential encoded within entomopathogenic fungi. Nat Prod Rep 31:1287–1305

Glare TR, Caradus J, Gelernter W, Jackson T, Keyhani N, Kohl J, Marrone P, Morin L, Stewart A (2012) Have biopesticides come of age? Trends Biotechnol 30:250–258

Goettel MS, Koike M, Kim JJ, Aiuchi D, Shinya R, Brodeur J (2008) Potential of *Lecanicillium* spp. for management of insects, nematodes and plant diseases. J Invertebr Pathol 98:256–261

Gómez-Vidal S, Salinas J, Tena M, Lopez-Llorca LV (2009) Proteomic analysis of date palm (*Phoenix dactylifera* L.) responses to endophytic colonization by entomopathogenic fungi. Electrophoresis 30(17):2996–3005

Gonzalez F, Tkaczuk C, Dinu MM, Fiedler Z, Vidal S, Zchori-Fein E, Messelink GJ (2016) New opportunities for the integration of microorganisms into biological pest control systems in greenhouse crops. J Pest Sci 89(2):295–311

Gurulingappa P, Sword GA, Murdoch G, McGee PA (2010) Colonization of crop plants by fungal entomopathogens and their effects on two insect pests when in planta. Biol Control 55(1):34–41

Hajek AE, St. Leger RJ (1994) Interactions between fungal pathogens and insect hosts. Annu Rev Entomol 39:293–322

Hallsworth JE, Magan N (1994) Effect of carbohydrate type and concentration on polyhydroxy alcohol and trehalose content of conidia of three entomopathogenic fungi. Microbiology 140:2705–2713

Hedgecock SD, Moore D, Higgins PM, Prior C (1995) Influence of moisture content on temperature tolerance and storage of *Metarhizium flavoviridae* in an oil formulation. Biocontrol Sci Technol 5:371–377

Hegedus DD, Bidochka MJ, Miranpuri GS, Khachatourians GG (1992) A comparison of the virulence, stability and cell-wall-surface characteristics of three spore types produced by the entomopathogenic fungus *Beauveria bassiana*. Appl Microbiol Biotechnol 36:785–789

Herrero N, Duenas E, Quesada-Moraga E, Zabalgogeazcoa I (2012) Prevalence and diversity of viruses in the entomopathogenic fungus *Beauveria bassiana*. Appl Environ Microbiol 78:8523–8530

Hesketh H, Roy HE, Eilenberg J, Pell JK, Halls RS (2010) Challenges in modelling complexity of fungal entomopathogens in semi-natural populations of insects. Biocontrol 55:55–73

Higuchi T, Takeshi S, Shuji S, Mizobata T, Kawata Y, Nagai J (1997) Development of biorational pest control formulation against longicorn beetles using a fungus, *Beauveria brongniartii* (Sacc.) Petch. J Ferment Bioeng 84:236–243

Holder DJ, Keyhani NO (2005) Adhesion of the entomopathogenic fungus *Beauveria* (*Cordyceps*) *bassiana* to substrata. Appl Environ Microbiol 71:5260–5266

Holder DJ, Kirkland BH, Lewis MW, Keyhani NO (2007) Surface characteristics of the entomopathogenic Fungus *Beauveria* (*Cordyceps*) *bassiana*. Microbiology 53:3448–3457

Hong TD, Ellis RH, Moore D (1997) Development of a model to predict the effect of temperature and moisture on fungal spore longevity. Ann Bot 79:121–128

Humber RA (2008) Evolution of entomopathogenicity in fungi. J Invertebr Pathol 98:262–266

Humphreys AM, Matewele P, Trinci APJ (1989) Effects of water activity on morphology, growth and blastospore production of *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces farinosus* in batch and fed-batch culture. Mycol Res 92:257–264

Hunt TR, Moore D, Higgins PM, Prior C (1994) Effect of sunscreens, irradiance and resting periods on the germination of *Metarhizium flavoviridae* conidia. Entomophaga 39:313–322

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 (1995) Influence of ultraviolet light protectants on persistence of the entomopathogenic fungus, *Beauveria bassiana*. Biol Control 5:581–590

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 23–69

Islam MT, Omar DB (2012) Combined effect of *Beauveria bassiana* with neem on virulence of insect in case of two application approaches. J Anim Plant Sci 22(1):77–82

Islam MT, Castle SJ, Ren S (2010) Compatibility of the insect pathogenic fungus *Beauveria bassiana* with neem against

sweetpotato whitefly, *Bemisia tabaci*, on eggplant. Entomol Exp Appl 134(1):28–34
Jaber LR (2015) Grapevine leaf tissue colonization by the fungal entomopathogen *Beauveria bassiana* s.l. and its effect against downy mildew. Biocontrol 60:103–112
Jackson MA (1997) Optimizing nutritional conditions for the liquid culture production of effective fungal biological control agents. J Ind Microbiol Biotechnol 19:180–187
Jackson MA, Jaronski ST (2009) Production of microsclerotia of the fungal entomopathogen *Metarhizium anisopliae* and their potential for use as a biocontrol agent for soil-inhabiting insects. Mycol Res 113:842–850
Jackson MA, Mascarin GM (2016) Stable fungal blastospores and methods for their production, stabilization and use. Patent US20160075992
Jackson MA, Dunlap CA, Jaronski S (2010) Ecological considerations in producing and formulating fungal entomopathogens for use in insect biocontrol. Biocontrol 55:129–145
Jaronski ST (1997) New paradigms in formulating mycoinsecticides. In: Goss GR, Hopkinson MJ, Collins HM (eds) Pesticide formulations and application systems, 17th volume, ASTM STP 1328. American Society for Testing and Materials, Philadelphia, pp 99–114
Jaronski ST (2010) Ecological factors in the inundative use of fungal entomopathogens. Biocontrol 55:159–185
Jaronski ST (2013) Mass production on entomopathogenic fungi: state of the art. In: Morales-Ramos JA, Rojas MG, Shapiro-Ilan DI (eds) Mass production of beneficial organisms. Elsevier Inc., Amsterdam, pp 357–415
Jaronski ST, Jackson MA (2012) Mass production of entomopathogenic hypocreales. In: Lacey LA (ed) Manual of techniques in invertebrate pathology. Academic Press, San Diego, pp 257–286
Jenkins NE, Lomer CJ (1994) Development of a new procedure for the mass production of conidia of *Metarhizium flavoviride*. Bull OILB/SROP 17(3):181–184
Jenkins NE, Barbarin AM, Rajotte EG, Thomas MB (2013) Compositions and methods for bed bug control using entomopathogenic fungi. Patent WO2013116454
Jin X, Grigas KE, Johnson CA, Perry P, Miller DW (1993) Method for storing fungal conidia. U.S. Patent 5,989,898
Kangassalo K, Valtonen TM, Roff D, Pölkki M, Dubovskiy IM, Sorvari J, Rantala MJ (2015) Intra- and trans-generational effects of larval diet on susceptibility to an entomopathogenic fungus, *Beauveria bassiana*, in the greater wax moth, *Galleria mellonella*. J Evol Biol 28(8):1453–1464
Karban R, Johnson MTJ (2011) The ecology and evolution of induced resistance against herbivores. Funct Ecol 25(2):339–347
Khan AL, Hamayun M, Khan SA, Kang SM, Shinwari ZK, Kamran M, Ur Rehman S, Kim JG, Lee IJ (2012) Pure culture of *Metarhizium anisopliae* LHL07 reprograms soybean to higher growth and mitigates salt stress. World J Microbiol Biotechnol 28(4):1483–1494
Kim JS, Je YH (2010) A novel biopesticide production: attagel-mediated precipitation of chitinase from *Beauveria bassiana* SFB-205 supernatant for thermotolerance. Appl Microbiol Biotechnol 87(5):1639–1648
Kim JS, Je YH, Skinner M, Parker BL (2013) An oil-based formulation of *Isaria fumosorosea* blastospores for management of greenhouse whitefly *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). Pest Manag Sci 69(5):576–581
Kona Coffee farmers Association (2012) Controlling the coffee berry borer. https://www.youtube.com/watch?v=yQbqIrA9BiA&spfreload=10. Accessed 11 July 2016
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. Biocontrol Sci Technol 19(4):429–446
Lacey LA, Kaya H (2007) Field Manual of Techniques in Invertebrate Pathology. In: Application and evaluation of pathogens for control of insects and other invertebrate pests, 2nd edn. Springer, Dordrecht, p 870
Lacey LA, Wright SP, Kirk AA (2008) Entomopathogenic fungi for control of *Bemisia tabaci* biotype B: foreign exploration, research and implementation. In: Gould J, Hoelmer K, Goolsby J (eds) Classical biological control of *Bemisia tabaci* in the United States—a review of interagency research and implementation. Springer, Dordrecht, pp 33–69
Lacey LA, Grzywacz D, Shapiro-Ilan DI, Frutos R, Brownbridge M, Goettel MS (2015) Insect pathogens as biological control agents: back to the future. J Invertebr Pathol 132:1–41
Landa BB, López-Díaz C, Jiménez-Fernández D, Montes-Borrego M, Muñoz-Ledesma FJ, Ortiz-Urquiza A, Quesada-Moraga E (2013) In-planta detection and monitorization of endophytic colonization by a *Beauveria bassiana* strain using a new-developed nested and quantitative PCR-based assay and confocal laser scanning microscopy. J Invertebr Pathol 114(2):128–138
Leland JE, Behle RW (2005) Coating *Beauveria bassiana* with lignin for protection from solar radiation and effects on pathogenicity to *Lygus lineolaris* (Heteroptera: Miridae). Biocontrol Sci Technol 15(3):309–320
Li ZZ, Alves SB, Roberts DW, Fan MZ, Delalibera I, Tang J, Lopes RB, Faria M, Rangel DEN (2010) Biological control of insects in Brazil and China: history, current programs and reasons for their successes using entomopathogenic fungi. Biocontrol Sci Technol 20:117–136
Lohse R, Jakobs-Schonwandt D, Patel AV (2014) Screening of liquid media and fermentation of an endophytic *Beauveria bassiana* strain in a bioreactor. AMB Express 4:47
Lohse R, Jakobs-Schönwandt D, Vidal S, Patel AV (2015) Evaluation of new fermentation and formulation strategies for a high endophytic establishment of *Beauveria bassiana* in oilseed rape plants. Biol Control 88:26–36
Lopes RB, Laumann RA, Moore D, Oliveira MWM, Faria M (2014) Combination of the fungus *Beauveria bassiana* and pheromone in attract and kill strategy against the banana weevil *Cosmopolites sordidus*. Entomol Exp Appl 151(1):75–85
Lopez DC, Sword GA (2015) The endophytic fungal entomopathogens *Beauveria bassiana* and *Purpureocillium lilacinum* enhance the growth of cultivated cotton (*Gossypium hirsutum*) and negatively affect survival of the cotton bollworm (*Helicoverpa zea*). Biol Control 89:53–60
Lord JC (2005) From Metchnikoff to Monsanto and beyond: the path of microbial control. J Invertebr Pathol 89:19–29
Magan N (2001) Physiological approaches to improving the ecological fitness of fungal biocontrol agents. In: Butt TM, Jackson CW, Magan N (eds) Fungi as biocontrol agents. CAB Publishing, Oxon, pp 239–325
Markets and Markets (2015) Biopesticides market by type (bioinsecticides, biofungicides, bioherbicides, and bionematicides), origin (beneficial insects, microbials, and biochemical), mode of application, formulation, crop type and region—global forecast to 2020. http://www.marketsandmarkets.com/Market-Reports/biopesticides-267.html. Accessed 06 June 2016
Marrone PG (2014) The market and potential for biopesticides. In: Gross AD, Coats JR, Duke SO, Seiber JN (eds) Biopesticides: state of the art and future opportunities, vol 1172. ACS Symposium Series, Washington, pp 245–258
Mascarin GM, Kobori NN, Quintela ED, Delalibera I Jr (2013) The virulence of entomopathogenic fungi against *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae) and their conidial production using solid substrate fermentation. Biol Control 66:209–218

Mascarin GM, Jackson MA, Kobori NN, Behle RW, Delalibera I Jr (2015a) Liquid culture fermentation for rapid production of desiccation tolerant blastospores of *Beauveria bassiana* and *Isaria fumosorosea* strains. J Invertebr Pathol 127:11–20

Mascarin GM, Jackson MA, Kobori NN, Behle RW, Dunlap CA, Delalibera I Jr (2015b) Glucose concentration alters dissolved oxygen levels in liquid cultures of *Beauveria bassiana* and affects the formation and efficacy of blastospores. Appl Microbiol Biotechnol 99(16):6653–6665

Mascarin GM, Jackson MA, Behle RW, Kobori NN, Delalibera I Jr (2016) Improved shelf life of dried *Beauveria bassiana* blastospores using convective drying and active packaging processes. Appl Microbiol Biotech. doi:10.1007/s00253-016-7597-2

McCoy C, Quintela ED, Faria M (2002) Environmental persistence of entomopathogenic fungi. In: Baur ME, Fuxa JR (eds) Factors affecting the survival of entomopathogens. Louisiana State University Agricultural Center, Southern Cooperative Series, Bulletin

McDougall P (2016) The cost of new agrochemical product discovery, development and registration in 1995, 2000 and 2005–8 and 2010 to 2014. R&D Expenditure in 2014 and Expectations for 2019. Final Report for Crop Life America and the European Crop Protection Association. http://www.croplifeamerica.org/wp-content/uploads/2016/04/Phillips-McDougall-Final-Report_4.6.16.pdf. Accessed 06 June 2016

Melzer MJ, Bidochka MJ (1998) Diversity of double-stranded RNA viruses within populations of entomopathogenic fungi and potential implications for fungal growth and virulence. Mycologia 90:586–594

Meyling NV, Eilenberg N (2007) Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: potential for conservation biological control. Biol Control 43:145–155

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

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

Ortiz-Urquiza A, Keyhani NO (2013) Action on the surface: entomopathogenic fungi versus the insect cuticle. Insects 4:357–374

Ortiz-Urquiza A, Riveiro-Miranda L, Santiago-Álvarez C, Quesada-Moraga E (2010) Insect-toxic secreted proteins and virulence of the entomopathogenic fungus *Beauveria bassiana*. J Invertebr Pathol 105(3):270–278

Ortiz-Urquiza A, Luo Z, Keyhani NO (2015) Improving mycoinsecticides for insect biological control. Appl Microbiol Biotech 99(3):1057–1068

Ownley BH, Griffin MR, Klingeman WE, Gwinn KD, Moulton JK, Pereira RM (2008) *Beauveria bassiana*: endophytic colonization and plant disease control. J Invertebr Pathol 98:267–270

Pedrini N, Ortiz-Urquiza A, Huarte-Bonnet C, Fan Y, Juárez MP, Keyhani NO (2015) Tenebrionid secretions and a fungal benzoquinone oxidoreductase form competing components of an arms race between a host and pathogen. Proc Natl Acad Sci 112(28):E3651–E3660

Pendland JC, Hung SY, Boucias DG (1993) Evasion of host defense by in vivo-produced protoplast-like cells of the insect mycopathogen *Beauveria bassiana*. J Bacteriol 175:5962–5969

Pham TA, Kim JJ, Kim K (2009) Production of blastospore of entomopathogenic *Beauveria bassiana* in a submerged batch culture. Mycobiology 37:218–224

Posada F, Vega FE (2006) Establishment of the fungal entomopathogens *Beauveria bassiana* (Ascomycota:Hypocreales) as an endophyte in cocoa seedlings (*Theobroma cacao*). Mycologia 97:208–213

Posada F, Aime MC, Peterson SW, Rehner SA, Vega FE (2007) Inoculation of coffee plants with the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales). Mycol Res 111(6):748–757

Prior CP, Jollands G, Le P (1988) Infectivity of oil and water formulations of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) to the cocoa weevil pest *Pantorhytes plutus* (Coleoptera: Curculionidae). J Invertebr Pathol 52:66–72

Quesada-Moraga E, Vey A (2003) Intra-specific variation in virulence and in vitro production of macromolecular toxins active against locust by *Beauveria bassiana* and effects of in vivo and in vitro passage on these factors. Biocontrol Sci Technol 13:323–340

Quesada-Moraga E, Maranhão EA, Valverde-Garcia P, Santiago-Álvarez C (2006) Selection of *Beauveria bassiana* isolates for control of the whiteflies *Bemisia tabaci* and *Trialeurodes vaporarium* on the basis of their virulence, thermal requirement and toxicogenic activity. Biol Control 36:274–287

Quesada-Moraga E, Munoz-Ledesma FJ, SantiagoAlvarez C (2009) Systemic protection of *Papaver somniferum* L. against *Iraella luteipes* (Hymenoptera: Cynipidae) by an endophytic strain of *Beauveria bassiana* (Ascomycota: Hypocreales). Environ Entomol 38:723–730

Quesada-Moraga E, López-Diaz C, Landa BB (2014) The hidden habit of the entomopathogenic fungus *Beauveria bassiana*: first demonstration of vertical plant transmission. PLoS ONE 9(2):e89278

Rangel DEN, Braga GUL, Fernandes EKK, Keyser CA, Hallsworth JE, Roberts DW (2015) Stress tolerance and virulence of insect-pathogenic fungi are determined by environmental conditions during conidial formation. Curr Genet 61(3):383–404

Ravensberg WJ (2011) A roadmap to the successful development and commercialization of microbial pest control products for control of arthropods. Springer, Dordrecht

Ravensberg WJ (2015) Crop protection in 2010: towards a natural, efficient, safe and sustainable approach. International Symposium on Biopesticides, Swansea University, UK, 7–9 Sept 2015. http://www.ibma-global.org/upload/documents/201509wrpresentationinswansea.pdf. Accessed 11 Feb 2016

Reay SD, Brownbridge M, Gicquel B, Cummings NJ, Nelson TL (2010) Isolation and characterization of endophytic *Beauveria* spp. (Ascomycota: Hypocreales) from *Pinus radiata* in New Zealand forests. Biol Control 54(1):52–60

Rehner SA, Buckley EP (2005) A Beauveria phylogeny inferred from ITS and EF1-a sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97:84–98

Rehner SA, Posada F, Buckley EP, Infante F, Castillo A, Vega FE (2006) Phylogenetic origins of African and Neotropical *Beauveria bassiana* s.l. pathogens of the coffee berry borer, *Hypothenemus hampei*. J Invertebr Pathol 93:11–23

Rehner SA, Minnis AM, Sung G-H, Luangsa-ard JJ, Devotto L, Humber RA (2011) Phylogeny and systematics of the anamorphic, entomopathogenic genus *Beauveria*. Mycologia 103:1055–1073

Rodríguez CML, Góngora BCE (2005) Transformation of *Beauveria bassiana* Bb9205 with *pr1A*, *pr1 J*, and *ste1* genes of *Metarhizium anisopliae* and evaluation of the pathogenicity on the coffee berry borer. Rev Colomb Entomol 31(1):51–58

Rombach MC (1989) Production of *Beauveria bassiana* (Deutoromycotina, Hyphomycetes) sympoduloconidia in submerged culture. Entomophaga 34:45–52

Samsinakova A (1966) Growth and sporulation of submerged cultures of the fungus *Beauveria bassiana* in various media. J Invertebr Pathol 8:395–400

Samson RA, Evans HC (1982) Two new *Beauveria* spp. from South America. J Invertebr Pathol 39:93–97

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

Shipp L, Kapongo JP, Kevan P, Sutton J, Broadbent B (2006) Bumblebees: an effective delivery system for microbial control agents for arthropod pest and disease management. IOBC WPRS Bull 29:47–51

Sigwart M, Graillot B, Lopez CB, Besse S, Bardin M, Nicot PC, Lopez-Ferber M (2015) Resistance to bio-insecticides or how to enhance their sustainability: a review. Front Plant Sci 6:381

Sparks TC, Nauen R (2014) IRAC: mode of action classification and insecticide resistance management. Pestic Biochem Physiol 121:122–128

St. Leger RJ, Wang C (2010) Genetic engineering of fungal biocontrol agents to achieve greater efficacy against insect pests. Appl Microbiol Biotechnol 85:901–907

Storm CG, Wakefield ME, Chambers J (2011) Control of arthropod infestation using particles comprising an entomopathogen and wax. Patent WO2011157983 A1

Sung G-H, Hywel-Jones NL, Sung J-M, Luangsa-ard JJ, Shrestha B, Spatafora JW (2007) Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. Stud Mycol 57:5–59

Tefera T, Vidal S (2009) Effect of inoculation method and plant growth medium on endophytic colonization of sorghum by the entomopathogenic fungus *Beauveria bassiana*. Biocontrol 54(5):663–669

Ugine T (2011) The effect of temperature and exposure to *Beauveria bassiana* on tarnished plant bug *Lygus lineolaris* (Heteroptera: Miridae) population dynamics, and the broader implications of treating insects with entomopathogenic fungi over a range of temperatures. Biol Control 59(3):373–383

Ugine TA, Wraight SP, Sanderson JP, Enkegaard A (2005) Differential susceptibility of western flower thrips (*Frankliniella occidentalis*) to *Beauveria bassiana*, as a function of host plant species. Bull OILB/SROP 28(1):271–274

Ugine TA, Jenkins NE, Gardescu S, Hajek AE (2013) Comparing fungal band formulations for Asian longhorned beetle biological control. J Invertebr Pathol 113:240–246

Valero-Jiménez CA, Debets AJM, van Kan JAL, Schoustra SE, Takken W, Zwaan BJ, Koenraadt CJM (2014) Natural variation in virulence of the entomopathogenic fungus *Beauveria bassiana* against malaria mosquitoes. Malar J 13:479

van der Sluijs Jeroen P, Simon-Delso N, Goulson D, Maxim L, Bonmatin JM, Belzunces LP (2013) Neonicotinoids, bee disorders and the sustainability of pollinator services. Curr Opin Environ Sustain 5(3–4):293–305

Vega FE, Jackson MA, Mercadier M, Poprawski TJ (2003) The impact of nutrition on spore yields for various fungal entomopathogens in liquid culture. World J Microbiol Biotechnol 19:363–368

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

Vega FE, Posada F, Aime MC, Pava-Ripoll M, Infante F, Rehner AS (2008) Entomopathogenic fungal endophytes. Biol Control 46:72–82

Vicentini S, Faria M, De Oliveira RVM (2001) Screening of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) Isolates against nymphs of *Bemisia tabaci* (Genn.) biotype B (Hemiptera: Aleyrodidae) with description of a bioassay method. Neotrop Entomol 30:97–103

Vidal S, Jaber LR (2015) Entomopathogenic fungi as endophytes: plant-endophyte-herbivore interaction and prospects for use in biological control. Curr Sci 109(1):46–54

Vidal C, Fargues J, Lacey LA, Jackson MA (1998) Effect of various liquid culture media on morphology, growth, propagule production, and pathogenic activity to *Bemisia argentifolii* of the entomopathogenic Hyphomycete, *Paecilomyces fumosoroseus*. Mycopathologia 143:33–46

Wagner BL, Lewis LC (2000) Colonization of corn, *Zea mays*, by the entomopathogenic fungus *Beauveria bassiana*. Appl Environ Microbiol 66:3468–3473

Wojda I, Kowalski P, Jakubwicz T (2005) Humoral immune response of *Galleria mellonella* larvae after infection by *Beauveria bassiana* under optimal and heat-shock conditions. J Insect Physiol 55:525–531

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) Synergictic interaction between *Beauveria bassiana*- and *Bacillus thuringiensis tenebrionis*-based biopesticides applied against field populations of Colorado potato beetle larvae. J Invertebr Pathol 90:139–150

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

Xiao G, Ying SH, Zheng P, Wang ZL, Zhang S, Xie XQ, Shang Y, St. Leger RJ (2012) Genome perspectives on the evolution of fungal entomopathogenicity in *Beauveria bassiana*. Sci Rep 2:483

XiaoFang R, ZhenBang L, Hong Z, Bo H, ZengZhi L (2011) Generation of transgenic *Beauveria bassiana* strain with *Vip3Aa* gene and its enhanced virulence on *Dendrolimus punctatus*. J Anhui Agric Univ 38(5):736–741

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