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Review

Molecular interactions between entomopathogenic fungi (Hypocreales) and their insect host: Perspectives from stressful cuticle and hemolymph battlefields and the potential of dual RNA sequencing for future studies

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ABSTRACT

Entomopathogenic fungi of the order Hypocreales infect their insect hosts mainly by penetrating through the cuticle and colonize them by proliferating throughout the body cavity. In order to ensure a successful infection, fungi first produce a variety of degrading enzymes that help to breach the insect cuticle, and then secrete toxic secondary metabolites that facilitate fungal invasion of the hemolymph. In response, insect hosts activate their innate immune system by triggering both cellular and humoral immune reactions. As fungi are exposed to stress in both cuticle and hemolymph, several mechanisms are activated not only to deal with this situation but also to mimic host epitopes and evade the insect's immune response. In this review, several components involved in the molecular interaction between insects and fungal pathogens are described including chemical, metabolomics, and dual transcriptomics approaches; with emphasis in the involvement of cuticle surface components in (pre-) infection processes, and fungal secondary metabolite (non-ribosomally synthesized peptides and polyketides) analysis. Some of the mechanisms involved in such interaction are also discussed.

1. Introduction

Insect infectious diseases have been known since the pioneering studies of Agostino Bassi, who in the 19th century postulated the more general idea that microorganisms are able to cause diseases in animals. Although Bassi isolated the fungal pathogenic agent of the white muscardine, currently known as *Beauveria bassiana*, from silkworm cadavers, knowledge about its teleomorph *Cordyceps* spp. infecting insects is believed to come from ancient China *circa* 2700 BC. It had been reported as "Chinese plant worm" by the entomologist de Reaumur, who mentioned that some specimens were sent from China to France in the early 18th century (Steinhaus, 1956). Moreover, current molecular techniques evidence a several-million-year coevolutionary history between entomopathogenic fungi and their invertebrate hosts. Insects represent a good model for studies of interactions with fungal pathogens due to their relatively short generation times; thus, experimental coevolution insights between both organisms can be obtained after only a few generations or experimental transfers, providing insight into the mechanisms involved in such interactions (Joop and Vilcinskas, 2016).

Entomopathogenic fungi can be considered as contact bioinsecticides, because they mostly invade the host by penetrating through the insect cuticle. During the first steps of infection, fungal conidia attach to the surface by nonspecific hydrophobic and electrostatic mechanisms. Several strains also secrete mucous substances that help conidia adsorption into and start dissolution of the surrounding cuticle (Boucias and Pedland, 1991). Then, spore germination can occur if the cuticular microclimate is adequate. In this regard, both germination stimulators and inhibitors have been detected on the host surface (Pedrini and Juárez, 2008). To penetrate the cuticle, entomopathogenic fungi form specialized structures named appressoria. These swollen cells form in turn the penetration hyphae (or penetration pegs) that utilize a combination of enzymatic and mechanical (pressing) mechanisms to pass through the different cuticular layers and to reach the hemolymph (Ferron, 1985; Boucias and Pedland, 1998).

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Once inside the insect cavity, the fungus switches to a different, yeast-like cell type, called hyphal bodies. These cells are often referred to as blastospores when they are produced artificially in culture media. Hyphal bodies are single- or multi-celled structures delimited by septa that are able to multiply quickly by division or budding. Depending on the fungal isolate, these cells can invade the entire insect by a tissue-specific sequential process and finally produce insect mummification; they can also secrete toxic compounds known as secondary metabolites (Boucias and Pedland, 1998). These substances can either facilitate the fungal invasion (Ferron, 1985; Roberts et al., 1992) or act as immunosuppressive compounds conferring resistance against host defense (Trienens and Rohlfs, 2012). As response to the fungal infection, the insects trigger their innate immune system. There are two types of insect immune reactions: 1) the cellular response involving phagocytosis, hemocyte aggregation, and pathogen encapsulation; and 2) the humoral response that includes the induction of a variety of antimicrobial peptides, lectins, and the prophenoloxidase cascade.

Although excellent review articles about entomopathogenic fungal virulence factors and insect response to fungal infection have been recently published (Valero-Jiménez et al., 2016; Butt et al., 2016; Wang and Wang, 2017), the aim of this contribution is to focus on some aspects not fully covered to date, such as the fungal response to the stressful cuticle embedded with antimicrobial compounds secreted by insects, the bottleneck exhibited by current biochemistry techniques to unambiguously assign a pathogenic role to fungal secondary metabolites during infection, as well as to highlight the applications of dual transcriptomic approaches to help understand the coevolutionary arms race between entomopathogenic fungi and their insect hosts.

2. Insect cuticle degradation by entomopathogenic fungi

2.1. Compositional compounds

2.1.1. Hydrocarbons

The most external surface of the insect cuticle, the epicuticle, is formed by a thin lipid layer making it hydrophobic, which facilitates the attachment of the also hydrophobic conidia (Fig. 1). In most insect species, a major component of the epicuticle is a mix of straight-chain and methyl-branched, saturated and unsaturated hydrocarbons. The composition of this hydrocarbon blend was shown to be a key factor in the success of conidia attachment and penetration: insects with saturated straight and branched chains are more susceptible to entomopathogenic fungi than insects with predominantly unsaturated (alkenes and/or alkadienes) chains (Pedrini et al., 2007). This might represent a first attempt of insects to stop fungal infection, i.e. the possibility of covering its surface with hydrocarbons of different chemical nature that provide a potential advantage to defend against the entry of fungi. However, entomopathogenic fungi have evolved to produce a variety of hydroxylating enzymes (Pedrini et al., 2010; Lin et al., 2011; Zhang et al., 2012; Huarte-Bonnet et al., 2017a) that can successful assimilate hydrocarbons and thus help degrade the lipid cuticular layer (for a review on this topic, see Pedrini et al., 2007, 2013; Keyhani 2018).

2.1.2. Protein and chitin

The next cuticle layer that fungi must go through is the procuticle, which contains the major bulk cuticle components, protein and chitin (Fig. 1). From mid-1980s to mid-1990s, St. Leger et al. characterized a variety of hydrolytic enzymes implicated in procuticle degradation, i.e., proteases (St. Leger et al., 1987a,b, 1988a,b, 1994a), peptidases (St. Leger et al., 1993, 1994b), and chitinases (St. Leger et al., 1986, 1996a,b). This information has subsequently facilitated obtaining fungal strains that overexpress a subtilisin-like protease (Pr1) (St. Leger et al., 1996c), a chitinase (Fang et al., 2005), and a fusion protein with both activities (Fang et al., 2009), all of which improved virulence against insect hosts. In fact, Pr1 expression and activity is currently used as a virulence marker in *Metarhizium* species (Wang et al., 2002; Small and Bidochka, 2005; Rosas-García et al., 2014).

2.2. Secreted compounds that are deposited on the cuticle

2.2.1. Antifungal aldehydes and ketones

Some insects have exocrine glands, mostly in their thorax, abdomen, and/or legs, which produce and secrete defensive compounds that repel and irritate predators. Although most of them are volatiles, these substances embed into the cuticle after they are released and also confer protection against invasive microorganisms. Some insects that are resistant to infection by entomopathogenic fungi share a characteristic: at least one compound containing a carbonyl group is present in their secretions; e.g., the family Pentatomidae produce saturated and α,β-unsaturated aldehydes (Aldrich, 1988) and many species belonging to the family Tenebrionidae secrete aromatic ketones (quinones) (Eisner, 1966). Sosa-Gómez et al. (1997) reported that the aldehyde (E)-2-decenal, a primary component of the scent gland of the stink bug *Nezara viridula*, was selectively fungistatic to entomopathogenic fungi; whereas da Silva et al. (2015) demonstrated that a mix of (E)-2-hexenal, (E)-2-octenal, and (E)-2-decenal released by the rice stalk stink bug is responsible for the fungal growth inhibition detected in *Metarhizium anisopliae*. On the other hand, both methyl- and ethyl-benzoquinone were the only compounds of the volatile blend secreted by the red flour beetle *Tribolium castaneum* able to inhibit both germination and growth of *B. bassiana* (Pedrini et al., 2015). Moreover, these authors moved forward and detected a fungal counterpart responsible for quinone degradation. By overexpression of a fungal 1,4-benzoquinone reductase, *B. bassiana* became more virulent against the red flour beetle *T. castaneum*, but not against other non-quinone secreting beetles (Pedrini et al., 2015). This was the first evidence that genetic manipulation can specifically intercede in the ongoing coevolutive arms race between an entomopathogenic fungus and its insect host. This snapshot showed that the red flour beetle is currently winning the race, but the fungus seems to be working on a solution to reverse this situation (Leal, 2015). Moreover, an experimental coevolution assay that includes a transcriptomic analysis of *B. bassiana* cyclically exposed to *T. castaneum* benzoquinones suggests that many oxidative stress resistance fungal genes are induced by this toxic compound (Rafaluk et al., 2017). Further episodes from this coevolutionary story are expected.

3. Insect cavity invasion by entomopathogenic fungi

3.1. Fungal secondary metabolite production

Secondary metabolites are defined as organic compounds that, although not directly involved in the normal growth and development of an organism, are crucial for its survivability and interaction with other organisms (Pichersky and Gang, 2000). Filamentous fungi produce them mainly for use as antimicrobial, and insect pathogenic fungi (Fig. 1) also use them to facilitate fungal invasion of the insect cavity and/or act as an immunosuppressant (Rohlfs and Churchill, 2011; Trienens and Rohlfs, 2012). *B. bassiana* synthesize beauvericin and bassianolide (cyclooligomer non-ribosomal peptides), a variety of beauverolides (cyclic peptides), oosporein (dibenzoquinone), bassiatin (diketomorpholine), and tenellin (2-pyridone); whereas *Metarhizium* spp. produce mainly

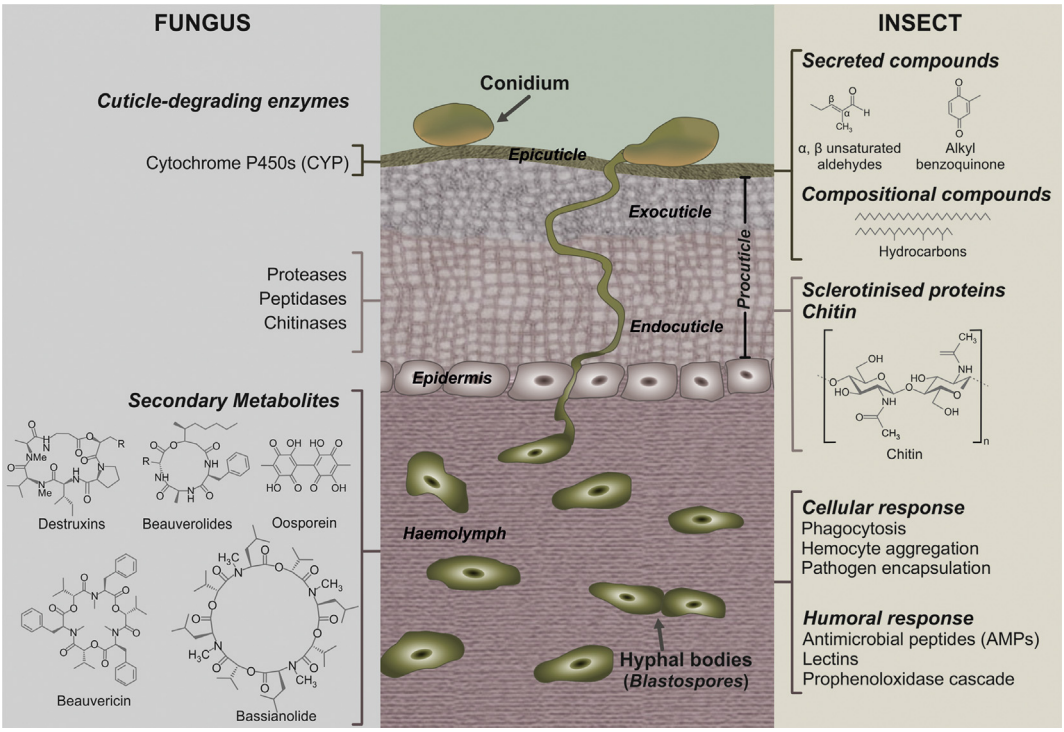

Fig. 1. Scheme of the infection process of an entomopathogenic fungus in an insect host. Some examples of fungal cuticle-degrading enzymes and fungal toxic secondary metabolites secreted in hemolymph are shown, as well as the composition of insect surface barrier and immune response against fungal invasion.

destruxins, a family of cyclic hexadepsipeptides composed of an α-hydroxy acid and five amino acid residues (Gibson et al., 2014) (Fig. 1). Although the importance of these toxic compounds has been frequently linked with virulence, their roles remain poorly understood. By using a metabolomics approach in combination with *ex vivo* insect tissue culturing, de Bekker et al. (2013) showed that entomopathogenic fungi exhibit tissue specific strategies for secondary metabolites production: *B. bassiana* secretes 80 % of the beauverolides produced in live tissues, whereas *Metarhizium brunneum* secretes destruxins mostly when in contact with dead tissues. This result suggests that *Beauveria* uses beauverolides to attack living tissues and thus these might be important in fungal virulence, and *Metarhizium* produces destruxins mainly with an antimicrobial role to protect the insect cadaver from competitive microbial degradation. These results are in concordance with a previous report by Skrobek et al. (2008), who reported destruxin peaking at the time of insect death.

The study by de Bekker et al. (2013) also characterized several novel beauverolides and destruxins that have not been described before, probably because previous attempts to identify them were done on whole insect rather than on insect tissues. Xu et al. (2016) also reported new destruxins produced on artificial medium but not on insects. Secondary metabolites are known to synthesize by gene clusters, including non-ribosomal peptides synthetases (NRPS), polyketides synthetases (PKS), and hybrid NRPS-PKS genes (Süssmuth et al., 2011). Most of the secondary metabolite core genes are silent during laboratory cultivation in artificial media, and require specific situations that induce their expression, such as are provided by a more natural environment, i.e., either during insect tissue culturing (de Bekker et al., 2013) or during whole insect infection (Lobo et al., 2015). Taken together, these data begin to complete the information gap about gene clusters and secondary metabolites produced, because several of the known clustered genes have no secondary metabolite assigned and *vice versa* (Gibson et al., 2014; Liu et al., 2017). For example, the toxic protein bassiacridin was isolated *B. bassiana* by chromatographic methods (Quesada-Moraga and Vey, 2004), and other insect-toxic secreted proteins were partially purified from *B. bassiana* and characterized as virulence factors against locusts (Quesada-Moraga and Vey, 2003; Ortiz-Urquiza et al., 2010); however, the gene(s) responsible of their synthesis remains unknown. A handful of the biosynthetic pathways have already been chemically and genetically characterized, e.g. for destruxins (Pedras et al., 2002; Wang et al., 2012), tenellin (Eley et al., 2007), beauvericin (Xu et al., 2008), bassianolide (Xu et al., 2009), and oosporein (Feng et al., 2015). The core genes involved in biosynthesis of beauverolides, the same as bassiatin, are still unknown. An insight into the core gene involved in synthesis of secondary metabolites in *Metarhizium* spp. reveals that 12 gene clusters are conserved in seven species of *Metarhizium*; a higher number of clusters are shared by at least two species, and from 4 to 11 gene clusters are species-specific (Hu et al., 2014). Generalist species of *Metarhizium* contain more secondary metabolites gene clusters than the specialists, and both conserved and divergent evolutions may have occurred in secondary metabolite genes during fungal speciation (Xu et al., 2016). These results in *Metarhizium* spp. add more complexity to the intricate correlation between gene clusters, secondary metabolites production, and host specification. In this respect, metabolomics data by mass spectrometry detection revealed that the seven *Metarhizium* species studied can be grouped based on their association to fungal host specificity (Xu et al., 2016).

Regarding the role of secondary metabolites during pathogenesis, the information available is not fully conclusive. NRPS and PKS gene disruption approaches indicate that beauvericin plays an important but dispensable role in virulence, and bassianolide is a highly significant virulence factor against insect hosts (Xu et al., 2008, 2009). On the contrary, tenellin does not seem to contribute to *B. bassiana* virulence (Eley et al., 2007). More detailed

information is available for oosporein, which was reported to act by evading insect immunity and thus facilitating fungal multiplication inside hosts (Feng et al., 2015). Destruxins have broad insecticidal effects, as was previously reviewed by Pedras et al. (2002); however, some differences in virulence through host species have been assigned to differences in the insect physiology of the target sites for destruxin activity and/or its detoxification (Wang et al., 2012).

Although liquid chromatography techniques coupled to mass spectrometry are useful to detect secondary metabolites from mycelial cakes, free-cell cultures, and pooled insects, their sensitiveness does not permit the detection of the few molecules that are expected to be produced by the fungus when it is growing inside the hemocoel of individual insects. In this regard, Lobo et al. (2015) set up a method based in qPCR to detect the expression of genes involved in the biosynthesis of these metabolites when the fungus grows inside its insect host. This technique allows not only obtaining an absolute quantification of NRPS and PKS transcripts, but also evaluates the response of the insect immune system genes during infection. The dynamics of the infection can be easy to follow; as shown in Fig. 2, *B. bassiana* induces toxin genes during the first days of infection, perhaps to be used as virulence factors, and then in moribund insects and/or cadavers to protect them from competitive microorganisms. However, during the middle-stage infection (and in conjunction with toxin decay), insects attempt to respond by inducing defensin, an antimicrobial peptide (Lobo et al., 2015). This dual qPCR approach represented a first but limited contribution to decipher the dynamics of entomopathogenic fungal infection at the molecular level. However, current dual RNA-seq techniques will contribute to a better and extensive comprehension of this phenomenon, as is detailed below.

4. Insect response to fungal infection and fungal strategies to evade the insect's immune system

Insects respond to microbial invasion by activating two types of innate immune reactions. The cellular response involves phagocytosis, hemocyte aggregation, and pathogen encapsulation. The humoral response includes the induction of several antimicrobial peptides (AMPs), lectins, and the prophenoloxidase cascade (Pal and Wu, 2009) (Fig. 3). There are some differences in the recognition of microbial cells inside body cavity; specifically for

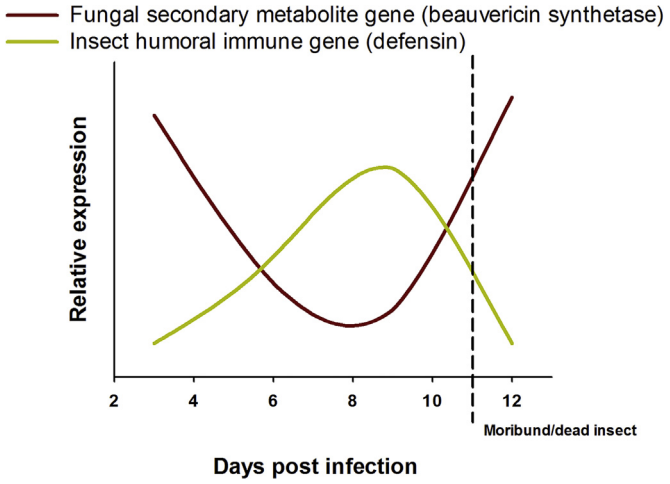

Fig. 2. Time-course expression of fungal genes encoding toxic non-ribosomal peptides (beauvericin synthetase) and insect genes encoding antimicrobial peptides (defensin) during infection of *Triatoma infestans* with *Beauveria bassiana*. Data were obtained from Lobo et al. (2015).

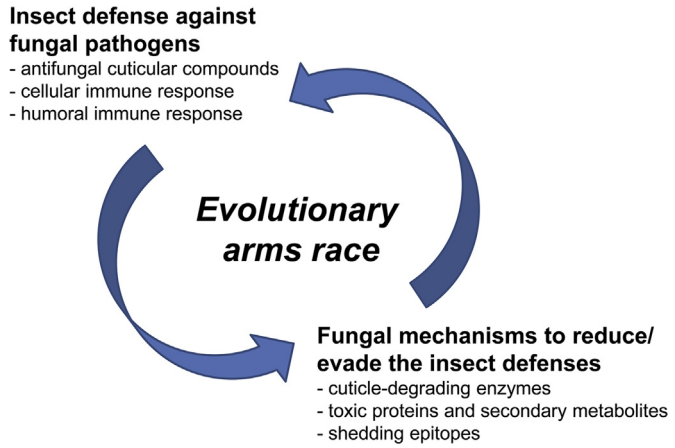

Fig. 3. Scheme showing some of the aspects participating in the interaction between insect pathogenic fungi and their hosts from a coevolutionary perspective.

entomopathogenic fungi, blastospores and hyphal bodies are weaker inductors of the immune system than conidia and hyphae, probably due to the different structures and lesser quantities of surface carbohydrates (epitope profile) exhibited by the former (Pendland et al., 1993). However, entomopathogenic fungi evolved more specific mechanisms to avoid the immune response of the insect host (Fig. 3), including shedding/changing these epitopes to escape from hemocyte encapsulation, as well as down-regulating protease activities. As stated before, proteases are used for cuticle degradation, and once inside an insect they can also degrade host defense molecules and thus help in fungal invasion (Vilcinskas, 2010). However, high protease activity can activate the prophenoloxidase cascade, resulting in high melanin production, which is toxic to both hosts and fungi (Butt et al., 2016; Wang and Wang, 2017). Thus, an accurate equilibrium must be managed by the fungus to ensure the weakness of insect immune system to allow colonization of the body cavity, but take care to neither be too injurious to the host facilitating competitive proliferation of other opportunistic microbes nor produce early host death before generating enough critical fungal biomass to continue the infective cycle.

The up-regulation of AMPs is regulated mainly by the Toll signal transduction pathway; the resulting peptides are then secreted into the hemocoel to prevent microbial proliferation (Pal and Wu, 2009). Fungi counterattack by secreting secondary metabolites that (as described above) have antibiotic and insecticide activities, although most of them also act as immunosuppressors by down-regulating the AMPs cascade, such as destruxins in *Metarhizium* spp. (Wang et al., 2012), myriocin in *Isaria sinclairii* (de Melo et al., 2013), and oosporein in *B. bassiana* (Feng et al., 2015).

5. Stress management by entomopathogenic fungi during infection

Lovett and St. Leger (2015) stated that stress is the rule rather than the exception for entomopathogenic fungi, as fungal cells are exposed to several stressful conditions in each of the infection stages. During conidia attachment to the cuticle, fungi have to cope with harmful environmental conditions such as solar radiation and fluctuating humidity and temperature (reviewed by Rangel et al., 2015a,b), the same as biotic conditions as antagonistic microbes and antifungal compounds released by insects (Toledo et al., 2011; Singh et al., 2016). Thus, entomopathogenic fungi trigger several mechanisms as response to high temperatures (Fernandes et al.,

2010; Rangel et al., 2010; Barreto et al., 2016), ultraviolet radiation (Braga et al., 2001a,b,c; Rangel et al., 2004, 2005; Fernandes et al., 2007, 2015), visible radiation (Rangel et al., 2011), oxidative stress during cuticular hydrocarbon degradation (Huarte-Bonnet et al., 2015, 2018), chemical insecticides used in combination with fungal pathogens in integrated pest management (Forlani et al., 2014), and secreted antifungal compounds (Rafaluk et al., 2017). During hemolymph invasion, hyphal bodies are exposed to the oxidative stress triggered by insect immune response (as detailed above) and thus they response by inducing several transcription factors that activate the expression of genes related with oxidative stress (Chu et al., 2016), secondary metabolites synthesis (Lobo et al., 2015), heat-shock proteins (Liao et al., 2014), among many others (for review see Ortiz-Urquiza and Keyhani, 2015; Wang et al., 2016).

6. Unusual battles: alternative modes of action of entomopathogenic fungi

There are reports about alternative routes of infection of fungal pathogen of terrestrial arthropods interacting with aquatic insects, i.e., living some portion of their life cycle in the water. In this sense, *M. anisopliae* kill the aquatic larvae of mosquitoes without adhering to the host cuticle. Lacey et al. (1988) proposed that the larvae movement on the surface for air intake (breathing) affects the water tension and thus facilitates the entry of floating conidia through the respiratory tract and/or the feeding channel. This "alternative" infective cycle leads to larval mortality between 6 and 24 h after ingestion. Some authors suggest that mortality is due to hemolymph colonization by the fungus (Riba et al., 1986; Bukhari et al., 2010), although the action of fungal toxins released was also proposed to promote insect death (Crisan, 1971; Lacey et al., 1988). Butt et al. (2013) shed light on the mechanism by which this fungus, adapted to terrestrial hosts, kills aquatic larvae of *Aedes aegypti*. Although ingested conidia fail to germinate and are expelled in faecal pellets, insect mortality appears to be linked to autolysis triggered by caspase activity. The pathway is regulated by Hsp70 and inhibited, in infected larvae, by protease inhibitors. Thus, the "traditional" host–pathogen response does not occur, since the species have not evolved to interact. The fungus retains pre-formed pathogenic determinants which mediate host mortality but, unlike true aquatic fungal pathogens, does not recognize and colonize its host.

7. Pathogen or host transcriptomics?: Dual RNA-seq promises successful research on entomopathogenic fungi–insect interaction

The development and improvement of transcriptomics techniques during the last 15 y, from microarrays to RNA-seq, has provided a panoramic view of gene expression during different stages of development in entomopathogenic fungi. Wang and St. Leger (2005) studied >1700 arrayed-genes in *Metarhizium acridum* grown on cuticle from different hosts and reported that a third of them are over-expressed by cuticle extract from host (locust) compared with non-host (beetles) species. Aside from genes implicated in pathogenicity, these authors also found that host extracts up-regulate genes for cell division and accumulation of cell mass, whereas fungi grown in the presence of beetle extracts are enriched in genes for detoxification and redox processes, similar to those reported in *B. bassiana* exposed to cuticular secretions of beetles (Rafaluk et al., 2017). Similar approaches, i.e., fungal expressed sequence tag (EST) analysis after addition of insect extracts to the culture media were assayed in both *Metarhizium* and *Beauveria* species, resulting in specific induction of genes involved in metabolism, pathogenicity, detoxification, and signal transduction (Freimoser et al., 2005; Cho et al., 2006a,b; Mantilla et al., 2012). In addition, transcriptomics has contributed information on the insect immune response to fungal challenges. Xiong et al. (2015) characterized the immunotranscriptome of non-host lepidopteran pest species infected with *B. bassiana*, and found that immune-related genes were activated in fat body but suppressed in hemocytes; conversely, bacterial infection triggered expression of the same genes in both tissues. An RNA-seq approach was employed on strains of silkworm that are either susceptible and resistant to *B. bassiana* infection; a suite of genes encoding for cuticular proteins, serine proteinase inhibitors, antimicrobial peptides, and toxins detoxification may be related to such resistance (Xing et al., 2017).

All this information can offer, however, only half the story. The development of next generation sequencing (NGS) technologies have allowed first a quickly increasing number of complete genomes sequenced from both the host and pathogen; and then, due to improvement in both depth and sensitivity of high-throughput RNA sequencing, have enabled a novel field of study on the host–pathogen interaction from a molecular perspective: dual RNA-seq (Westermann et al., 2012). This technique allows researchers a) to simultaneously capture all classes of coding and noncoding transcripts in both organisms, b) to map these transcripts against both reference genomes, and finally c) to have a more accurate insight of this interaction. Westermann et al. (2017) recently reviewed all the applications of dual RNA-seq in the last five years, since the concept of dual RNA-seq was introduced, but only focused on bacterial pathogens. The first report about dual RNA-seq applied to the entomopathogenic fungi–insect host system was by Chu et al. (2016), who studied the interaction between *B. bassiana* and the diamond-back moth. The authors showed that fungus-infected insects expressed fewer genes than non-infected insects at 24 h after infection, almost the same number of genes at 36 h, and twice at 48 h after treatment. On the other hand, the fungus increased the number of genes exclusively expressed after 2 d of infection, tripling this number compared with 24 h and 36 h after infection. The KEGG pathways revealed that at 24 h, *B. bassiana* highly expressed genes involved in both antioxidant activity (ten genes) and peroxidase activity (seven genes), which indicates the importance of oxidative stress during fungal infection (Chu et al., 2016). Recently, Dong et al. (in press) used dual RNA-seq to show that alternatively splicing mediates fungal adaptation to host niches. The authors demonstrated that *B. bassiana* expressed two splicing variants of an autophagy-related gene during growth into hemolymph, but only one of them is crucial to mitigate the oxidative stress scenario and thus facilitate fungal development inside the insect, body cavity invasion, and ultimately, therefore, the virulence.

8. Conclusion and perspectives

Coevolution between a host and pathogen was early addressed by Van Valen (1973), who proposed the Red Queen Hypothesis to capture the idea that both organisms, competing for survival, must do their best just to maintain the *status quo* in the coevolutionary race. Its implementation for coevolution studies between entomopathogenic fungi and their insect host was also applied to explain the interaction between fungal-derived proteinases and insect proteinase inhibitors (Vilcinskas, 2010), and between fungi and either insect cuticular hydrocarbons (Pedrini et al., 2013) or their volatile secretions (Pedrini et al., 2015). Moreover, it was also useful to explain the several million-year-scale coevolutive race between both components, which lead to understand for example the specialization or generalization of *Metarhizium* species. Wang

et al. (2016) stated that the specialist *M. acridum* retained existing proteins useful to interact with locust in a rapid evolution strategy, whereas generalist *Metarhizium* spp. opted for protein family expansion, extensive gene duplication, and horizontal gene transfer, among other mechanisms, for fungal interaction with larger insect orders. Recently, Joop and Vilcinskas (2016) used the Red Queen dynamics to review the interactions between *B. bassiana* virulence factors and the immune defenses of either susceptible (greater wax moth) or resistant (red flour beetle) hosts, describing opposing dynamics of fungal infection in response to the different scenarios raised by both insects. Data available to date begin to close the existing information gap; however, dual RNA-seq technology promises to revolutionize this field, allowing unveil in the next few years the molecular mechanisms of the interaction between any fungus–insect system.

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References

Aldrich, J.R., 1988. Chemical ecology of the Heteroptera. Annu. Rev. Entomol. 33, 211–238.
Barreto, L.P., Luz, C., Mascarin, G.M., Roberts, D.W., Arruda, W., Fernandes, É.K.K., 2016. Effect of heat stress and oil formulation on conidial germination of *Metarhizium anisopliae* s. s. on tick cuticle and artificial medium. J. Invertebr. Pathol. 138, 94–103.
Boucias, D.G., Pedland, J.C., 1991. Attachment of mycopathogens to cuticle. The initial events of mycoses in arthropod hosts. In: Cole, G.T., Hoch, H.C. (Eds.), The Fungal Spore and Disease Initiation in Plant and Animals. Springer, Boston, pp. 101–127.
Boucias, D.G., Pedland, J.C., 1998. Principles of Insect Pathology. Kluwer Academic Publishers, Boston.
Braga, G.U.L., Flint, S.D., Messias, C.L., Anderson, A.J., Roberts, D.W., 2001a. Effects of UVB irradiance on conidia and germinants of the entomopathogenic Hyphomycete *Metarhizium anisopliae*: a study of reciprocity and recovery. Photochem. Photobiol. 73, 140–146.
Braga, G.U.L., Flint, S.D., Miller, C.D., Anderson, A.J., Roberts, D.W., 2001b. Both solar UVA and UVB radiation impair conidial culturability and delay germination in the entomopathogenic fungus *Metarhizium anisopliae*. Photochem. Photobiol. 74, 734–739.
Braga, G.U.L., Flint, S.D., Miller, C.D., Anderson, A.J., Roberts, D.W., 2001c. Variability in response to UV-B among species and strains of *Metarhizium* isolated from sites at latitudes from 61 degrees N to 54 degrees S. J. Invertebr. Pathol. 78, 98–108.
Bukhari, T., Middelman, A., Koenraadt, C.J.M., Takken, W., Knols, B.G.J., 2010. Factors affecting fungus-induced larval mortality in *Anopheles gambiae* and *Anopheles stephensi*. Malar. J. 9, 22.
Butt, T.M., Greenfield, B.P.J., Greig, C., Maffeis, T.G.G., Taylor, J.W.D., Piasecka, J., Dudley, E., Abdulla, A., Dubovskiy, I.M., Garrido-Jurado, I., Quesada-Moraga, E., Penny, M.W., Eastwood, D.C., 2013. *Metarhizium anisopliae* pathogenesis of mosquito larvae: a verdict of accidental death. PLoS One 8 (12), e81686.
Butt, T.M., Coates, C.J., Dubovskiy, I.M., Ratcliffe, N.A., 2016. Entomopathogenic fungi: new insights into host–pathogen interactions. Adv. Genet. 94, 307–364.
Cho, E.M., Boucias, D., Keyhani, N.O., 2006a. EST analysis of cDNA libraries from the entomopathogenic fungus *Beauveria* (Cordyceps) *bassiana*. II. Fungal cells sporulating on chitin and producing oosporein. Microbiology 152, 2855–2864.
Cho, E.M., Liu, L., Farmerie, W., Keyhani, N.O., 2006b. EST analysis of cDNA libraries from the entomopathogenic fungus *Beauveria* (Cordyceps) *bassiana*. I. Evidence for stage-specific gene expression in aerial conidia, *in vitro* blastospores and submerged conidia. Microbiology 152, 2843–2854.
Chu, Z.J., Wang, Y.J., Ying, S.H., Wang, X.W., Feng, M.G., 2016. Genome-wide host-pathogen interaction unveiled by transcriptomic response of diamondback moth to fungal infection. PLoS One 11 (4), e0152908.
Crisan, E., 1971. Mechanism responsible for release of toxin by *Metarhizium* spores in mosquito larvae. J. Invertebr. Pathol. 17, 260–264.
da Silva, R.A., Quintela, E.D., Mascarin, G.M., Pedrini, N., Lião, L.M., Ferri, P.H., 2015. Unveiling chemical defense in the rice stalk stink bug against the entomopathogenic fungus *Metarhizium anisopliae*. J. Invertebr. Pathol. 127, 93–100.
de Bekker, C., Smith, P.B., Patterson, A.D., Hughes, D.P., 2013. Metabolomics reveals the heterogeneous secretome of two entomopathogenic fungi to *ex vivo* cultured insect tissues. PLoS One 8 (8), e70609.
de Melo, N.R., Abdrahman, A., Greig, C., Mukherjee, K., Thornton, C., Ratcliffe, N.A., Vilcinskas, A., Butt, T.M., 2013. Myriocin significantly increases the mortality of a non-mammalian model host during *Candida* pathogenesis. PLoS One 8, e78905.
Dong, W.X., Ding, J.L., Gao, Y., Peng, Y.J., Feng, M.G., Ying, S.H., 2017. Transcriptomic insights into the alternative splicing-mediated adaptation of the entomopathogenic fungus *Beauveria bassiana* to host niches: autophagy-related gene 8 as an example. Environ. Microbiol. https://doi.org/10.1111/1462-2920.13862 (in press).
Eisner, T., 1966. Beetle's spray discourages predators. Nat. Hist. 75, 42–47.
Eley, K.L., Halo, L.M., Song, Z., Powles, H., Cox, R.J., Bailey, A.M., Lazarus, C.M., Simpson, T.J., 2007. Biosynthesis of the 2-pyridone tenellin in the insect pathogenic fungus *Beauveria bassiana*. ChemBioChem 8, 289–297.
Fang, W., Leng, B., Xiao, Y., Jin, K., Ma, J., Fan, Y., Feng, J., Yang, X., Zhang, Y., Pei, Y., 2005. Cloning of *Beauveria bassiana* chitinase gene Bbchit1 and its application to improve fungal strain virulence. Appl. Environ. Microbiol. 71, 363–370.
Fang, W., Feng, J., Fan, Y., Zhang, Y., Bidochka, M.J., St. Leger, R.J., Pei, Y., 2009. Expressing a fusion protein with protease and chitinase activities increases the virulence of the insect pathogen *Beauveria bassiana*. J. Invertebr. Pathol. 102, 155–159.
Feng, P., Shang, Y., Cen, K., Wang, C., 2015. Fungal biosynthesis of the bibenzoquinone oosporein to evade insect immunity. Proc. Natl. Acad. Sci. U. S. A. 112 (36), 11365–11370.
Fernandes, É.K.K., Rangel, D.E.N., Moraes, Á.M.L., Bittencourt, V.R.E.P., Roberts, D.W., 2007. Variability in tolerance to UV-B radiation among *Beauveria* spp. isolates. J. Invertebr. Pathol. 96, 237–243.
Fernandes, É.K.K., Keyser, C.A., Chong, J.P., Rangel, D.E.N., Miller, M.P., Roberts, D.W., 2010. Characterization of *Metarhizium* species and varieties based on molecular analysis, heat tolerance and cold activity. J. Appl. Microbiol. 108, 115–128.
Fernandes, É.K.K., Rangel, D.E.N., Braga, G.U.L., Roberts, D.W., 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., 1985. Fungal control. In: Kerkut, G.A., Gilbert, L.I. (Eds.), Comprehensive Insect Physiology, Biochemistry and Pharmacology. Academic Press, New York, pp. 313–346.
Forlani, L., Juárez, M.P., Lavarías, S., Pedrini, N., 2014. Toxicological and biochemical response of the entomopathogenic fungus *Beauveria bassiana* after exposure to deltamethrin. Pest Manag. Sci. 70, 751–756.
Freimoser, F.M., Hu, G., St. Leger, R.J., 2005. Variation in gene expression patterns as the insect pathogen *Metarhizium anisopliae* adapts to different host cuticles or nutrient deprivation *in vitro*. Microbiology 151, 361–371.
Gibson, D.M., Donzelli, B.G.G., Krasnoff, S.B., Keyhani, N.O., 2014. Discovering the secondary metabolite potential encoded within entomopathogenic fungi. Nat. Prod. Rep. 31, 1287–1305.
Hu, X., Xiao, G.H., Zheng, P., Shang, Y.F., Su, Y., Zhang, X.Y., Liu, X., Zhan, S., St. Leger, R.J., Wang, C., 2014. Trajectory and genomic determinants of fungal-pathogen speciation and host adaptation. Proc. Natl. Acad. Sci. U. S. A. 111 (47), 16796–16801.
Huarte-Bonnet, C., Juárez, M.P., Pedrini, N., 2015. Oxidative stress in entomopathogenic fungi grown on insect-like hydrocarbons. Curr. Genet. 61 (3), 289–297.
Huarte-Bonnet, C., Kumar, S., Saparrat, M.C.N., Girotti, J.R., Santana, M., Hallsworth, J.E., Pedrini, N., 2017a. Insights into hydrocarbon assimilation by eurotialean and hypocrealean fungi: roles for CYP52 and CYP53 clans of cytochrome P450 genes. Appl. Biochem. Biotechnol. https://doi.org/10.1007/s12010-017-2608-z.
Huarte-Bonnet, C., Paixão, F.R.S., Ponce, J.C., Santana, M., Prieto, E.D., Pedrini, N., 2018. Alkane-grown *Beauveria bassiana* produce mycelial pellets displaying peroxisome proliferation, oxidative stress, and cell surface alterations. Fungal Biol. 122, 457–464.
Joop, G., Vilcinskas, A., 2016. Coevolution of parasitic fungi and insect hosts. Zoology 119, 350–358.
Keyhani, N.O., 2018. Lipid biology in fungal stress and virulence: entomopathogenic fungi. Fungal Biol. 122, 420–429.
Lacey, C.M., Lacey, L.A., Roberts, D.W., 1988. Route of invasion and histopathology of *Metarhizium anisopliae* in *Culex quinquefasciatus*. J. Invertebr. Pathol. 52, 108–118.
Leal, W.S., 2015. Snapshot of insect–fungus arms race. Proc. Natl. Acad. Sci. U. S. A. 112 (28), 8519–8520.
Liao, X., Lu, H.L., Fang, W., St. Leger, R.J., 2014. Overexpression of a *Metarhizium robertsii* HSP25 gene increases thermotolerance and survival in soil. Appl. Microbiol. Biotechnol. 98 (2), 777–783.
Lin, L.C., Fang, W.G., Liao, X.G., Wang, F.Q., Wei, D.Z., St. Leger, R.J., 2011. The MrCYP52 cytochrome P450 monoxygenase gene of *Metarhizium robertsii* is important for utilizing insect epicuticular hydrocarbons. PLoS One 6 (12), e28984.
Liu, H., Xie, L., Wang, J., Guo, Q., Yang, S., Liang, P., Wang, C., Lin, M., Xu, Y., Zhang, L., 2017. The stress-responsive and host-oriented role of nonribosomal peptide synthetases in an entomopathogenic fungus, *Beauveria bassiana*. J. Microbiol. Biotechnol. 27 (3), 439–449.

Lobo, L.S., Luz, W.C., Fernandes, E.K.K., Juárez, M.P., Pedrini, N., 2015. Assessing gene expression during pathogenesis: use of qRT-PCR to follow toxin production from the entomopathogenic fungus *Beauveria bassiana* during infection and immune response of the insect host *Triatoma infestans*. J. Invertebr. Pathol. 128, 14–21.

Lovett, B., St. Leger, R.J., 2015. Stress is the rule rather than the exception for *Metarhizium*. Curr. Genet. 61 (3), 253–261.

Mantilla, J.G., Galeano, N.F., Gaitan, A.L., Cristancho, M.A., Keyhani, N.O., Gongora, C.E., 2012. Transcriptome analysis of the entomopathogenic fungus *Beauveria bassiana* grown on cuticular extracts of the coffee berry borer (*Hypothenemus hampei*). Microbiology 158, 1826–1842.

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, 270–278.

Ortiz-Urquiza, A., Keyhani, N.O., 2015. Stress response signaling and virulence: insights from entomopathogenic fungi. Curr. Genet. 61 (3), 239–249.

Pal, S., Wu, L.P., 2009. Lessons from the fly: pattern recognition in *Drosophila melanogaster*. In: Kishore, U. (Ed.), Target Pattern Recognition in Innate Immunity. Springer, New York, pp. 162–174.

Pedras, M.S.C., Zaharia, L.I., Ward, D.E., 2002. The destruxins: synthesis, biosynthesis, biotransformation, and biological activity. Phytochemistry 59 (6), 579–596.

Pedrini, N., Crespo, R., Juárez, M.P., 2007. Biochemistry of insect epicuticle degradation by entomopathogenic fungi. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 146, 124–137.

Pedrini, N., Juárez, M.P., 2008. Entomopathogenic fungi and their host cuticle. In: Capinera, J. (Ed.), Encyclopedia of Entomology, second ed. Springer-Verlag, Heidelberg, pp. 1333–1336.

Pedrini, N., Zhang, S., Juárez, M.P., Keyhani, N.O., 2010. Molecular characterization and expression analysis of a suite of cytochrome P450 enzymes implicated in insect hydrocarbon degradation in the entomopathogenic fungus *Beauveria bassiana*. Microbiology 156, 2549–2557.

Pedrini, N., Ortiz-Urquiza, A., Huarte-Bonnet, C., Zhang, S., Keyhani, N.O., 2013. Targeting of insect epicuticular lipids by the entomopathogenic fungus *Beauveria bassiana*: hydrocarbon oxidation within the context of a host-pathogen interaction. Front. Microbiol. 4, 24.

Pedrini, N., Ortiz-Urquiza, A., Huarte-Bonnet, C., Fan, Y., Juárez, M.P., Keyhani, N.O., 2015. Tenebrionid secretions and a fungal benzoquinone oxidoreductase form competing components of an arms race between a host and pathogen. Proc. Natl. Acad. Sci. U. S. A. 112 (28), 3651–3660.

Pendland, J.C., Hung, S.Y., Boucias, D.G., 1993. Evasion of host defense by *in vivo*-produced protoplast-like cells of the insect mycopathogen *Beauveria bassiana*. J. Bacteriol. 175, 5962–5969.

Pichersky, E., Gang, D.R., 2000. Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. Trends Plant Sci. 5, 439–445.

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

Quesada-Moraga, E., Vey, A., 2004. Bassiacridin, a protein toxic for locust secreted by the entomopathogenic fungus *Beauveria bassiana*. Mycol. Res. 108, 441–452.

Rafaluk, C., Yang, W., Mitschke, A., Rosenstiel, P., Schulenburg, H., Joop, G., 2017. Highly potent host external immunity acts as a strong selective force enhancing rapid parasite virulence evolution. Environ. Microbiol. 19 (5), 2090–2100.

Rangel, D.E.N., Braga, G.U.L., Flint, S.D., Anderson, A.J., Roberts, D.W., 2004. Variations in UV-B tolerance and germination speed of *Metarhizium anisopliae* conidia produced on insects and artificial substrates. J. Invertebr. Pathol. 87, 77–83.

Rangel, D.E.N., Braga, G.U.L., Anderson, A.J., Roberts, D.W., 2005. Influence of growth environment on tolerance to UV-B radiation, germination speed, and morphology of *Metarhizium anisopliae* var. *acridum* conidia. J. Invertebr. Pathol. 90, 55–58.

Rangel, D.E.N., Fernandes, É.K.K., Dettenmaier, S.J., Roberts, D.W., 2010. Thermotolerance of germlings and mycelium of the insect-pathogenic fungus *Metarhizium* spp. and mycelial recovery after heat stress. J. Basic Microbiol. 50, 344–350.

Rangel, D.E.N., Fernandes, É.K.K., Braga, G.U.L., Roberts, D.W., 2011. Visible light during mycelial growth and conidiation of *Metarhizium robertsii* produces conidia with increased stress tolerance. FEMS Microbiol. Lett. 315 (2), 81–86.

Rangel, D.E.N., Alder-Rangel, A., Dadachova, E., Finlay, R.D., Kupiec, M., Dijksterhuis, J., Braga, G.U.L., Corrochano, L.M., Hallsworth, J.E., 2015a. Fungal stress biology: a preface to the fungal stress responses special edition. Curr. Genet. 61 (3), 231–238.

Rangel, D.E.N., Braga, G.U.L., Fernandes, E.K.K., Keyser, C.A., Hallsworth, J.E., Roberts, D.W., 2015b. Stress tolerance and virulence of insect-pathogenic fungi are determined by environmental conditions during conidial formation. Curr. Genet. 61 (3), 383–404.

Riba, G., Keita, A., Soares, G.G., Ferron, P., 1986. Comparative studies of *Metarhizium anisopliae* and *Tolypocladium cylindrosporum* as pathogens of mosquito larvae. J. Am. Mosq. Contr. Assoc. 2, 469–473.

Roberts, D.W., Gupta, S., St. Leger, R.J., 1992. Metabolite production by entomopathogenic fungi. Pesqui. Agropecuária Bras. 27, 325–347.

Rohlfs, M., Churchill, A.C.L., 2011. Fungal secondary metabolites as modulators of interactions with insects and other arthropods. Fungal Genet. Biol. 48, 23–34.

Rosas-García, N.M., Avalos-de-León, O., Villegas-Mendoza, J.M., Mireles-Martínez, M., Barboza-Corona, J.E., Castañeda-Ramírez, J.C., 2014. Correlation between Pr1 and Pr2 gene content and virulence in *Metarhizium anisopliae* strains. J. Microbiol. Biotechnol. 24 (11), 1495–1502.

Singh, D., Son, S.Y., Lee, C.H., 2016. Perplexing metabolomes in fungal-insect trophic interactions: a *terra incognita* of mycobiocontrol mechanisms. Front. Microbiol. 7, 1678.

Skrobek, A., Shah, F.A., Butt, T.M., 2008. Destruxin production by the entomogenous fungus *Metarhizium anisopliae* in insects and factors influencing their degradation. BioControl 53, 361–373.

Small, C.L., Bidochka, M.J., 2005. Up-regulation of Pr1, a subtilisin-like protease, during conidiation in the insect pathogen *Metarhizium anisopliae*. Mycol. Res. 109 (3), 307–313.

Sosa-Gómez, D.R., Boucias, D.G., Nation, J.L., 1997. Attachment of *Metarhizium anisopliae* to the southern green stink bug *Nezara viridula* cuticle and fungistatic effect of cuticular lipids and aldehydes. J. Invertebr. Pathol. 69, 31–39.

St. Leger, R.J., Cooper, R.M., Charnley, A.K., 1986. Cuticle-degrading enzymes of entomopathogenic fungi: regulation of production of chitinolytic enzymes. J. Gen. Microbiol. 132, 1509–1517.

St. Leger, R.J., Charnley, A.K., Cooper, R.M., 1987a. Characterisation of cuticle-degrading proteases produced by the entomopathogen *Metarhizium anisopliae*. Arch. Biochem. Biophys. 253, 221–232.

St. Leger, R.J., Cooper, R.M., Charnley, A.K., 1987b. Production of cuticle-degrading enzymes by the entomopathogen *Metarhirzium anisopliae* during infection of cuticles from *Calliphora vomitoria* and *Manduca sexta*. J. Gen. Microbiol. 133, 1371–1382.

St. Leger, R.J., Durrands, P.K., Cooper, R.M., Charnley, A.K., 1988a. Regulation of production of proteolytic enzymes by the entomopathogenic fungus *Metarhizium anisopliae*. Arch. Microbiol. 150, 413–416.

St. Leger, R.J., Durrands, P.K., Charnley, A.K., Cooper, R.M., 1988b. Role of extracellular chymoelastase on the virulence of *Metarhizium anisopliae* for *Manduca sexta*. J. Invertebr. Pathol. 52, 285–293.

St. Leger, R.J., Staples, R.C., Roberts, D.W., 1993. Entomopathogenic isolates of *Metarhizium anisopliae*, *Beauveria bassiana*, and *Aspergillus flavus* produce multiple extracellular chitinase isozymes. J. Invertebr. Pathol. 61, 81–84.

St. Leger, R.J., Bidochka, M.J., Roberts, D.W., 1994a. Isoforms of the cuticle-degrading Pr1 proteinase and production of a metalloproteinase by *Metarhizium anisopliae*. Arch. Biochem. Biophys. 313, 1–7.

St. Leger, R.J., Bidochka, M.J., Roberts, D.W., 1994b. Characterization of a novel carboxypeptidase produced by the entomopathogenic fungus *Metarhizium anisopliae*. Arch. Biochem. Biophys. 314, 392–398.

St. Leger, R.J., Joshi, L., Bidochka, M.J., Rizzo, N.W., Roberts, D.W., 1996a. Biochemical characterization and ultrastructural localization of two extracellular trypsins produced by *Metarhizium anisopliae* in infected insect cuticles. Appl. Environ. Microbiol. 62, 1257–1264.

St. Leger, R.J., Joshi, L., Bidochka, M.J., Rizzo, N.W., Roberts, D.W., 1996b. Characterization and ultrastructural localization of chitinases from *Metarhizium anisopliae, M. flavoviride*, and *Beauveria bassiana* during fungal invasion of host (*Manduca sexta*) cuticle. Appl. Environ. Microbiol. 62, 907–912.

St. Leger, R.J., Joshi, L., Bidochka, M.J., Roberts, D.W., 1996c. Construction of an improved mycoinsecticide overexpressing a toxic protease. Proc. Natl. Acad. Sci. U. S. A. 93, 16349–16354.

Steinhaus, E., 1956. Microbial control—the emergence of an idea. A brief history of insect pathology through the nineteenth century. Hilgardia 26 (2), 107–160.

Süssmuth, R., Muller, J., von Dohren, H., Molnar, I., 2011. Fungal cyclooligomer depsipeptides: from classical biochemistry to combinatorial biosynthesis. Nat. Prod. Rep. 28, 99–124.

Toledo, A.V., Alippi, A.M., de Remes Lenicov, A.M.M., 2011. Growth inhibition of *Beauveria bassiana* by bacteria isolated from the cuticular surface of the corn leafhopper, *Dalbulus maidis* and the planthopper, *Delphacodes kuscheli*, two important vectors of maize pathogens. J. Insect Sci. 11 (1), 29.

Trienens, M., Rohlfs, M., 2012. Insect-fungus interference competition - the potential role of global secondary metabolite regulation, pathway-specific mycotoxin expression and formation of oxylipins. Fungal Ecol. 5, 191–199.

Valero-Jiménez, C.A., Wiegers, H., Zwaan, B.J., Koenraadt, C.J.M., van Kan, J.A.L., 2016. Genes involved in virulence of the entomopathogenic fungus *Beauveria bassiana*. J. Invertebr. Pathol. 133, 41–49.

Van Valen, L., 1973. A new evolutionary law. Evol. Theor. 1, 1–30.

Vilcinskas, A., 2010. Coevolution between pathogen-derived proteinases and proteinase inhibitors of host insects. Virulence 1 (3), 206–214.

Wang, B., Kang, Q., Lua, Y., Bai, L., Wang, C., 2012. Unveiling the biosynthetic puzzle of destruxins in *Metarhizium* species. Proc. Natl. Acad. Sci. U. S. A. 109 (4), 1287–1292.

Wang, J.B., St. Leger, R.J., Wang, C., 2016. Advances in genomics of entomopathogenic fungi. Adv. Genet. 94, 67–105.

Wang, C., Typas, M.A., Butt, T.M., 2002. Detection and characterisation of pr1 virulent gene deficiencies in the insect pathogenic fungus *Metarhizium anisopliae*. FEMS Microbiol. Lett. 213 (2), 251–255.

Wang, C., St. Leger, R.J., 2005. Developmental and transcriptional responses to host and nonhost cuticles by the specific locust pathogen *Metarhizium anisopliae* var. *acridum*. Eukaryot. Cell 4, 937–947.

Wang, C., Wang, S., 2017. Insect pathogenic fungi: genomics, molecular interactions, and genetic improvements. Annu. Rev. Entomol. 62, 73–90.

Westermann, A.J., Gorski, S.A., Vogel, J., 2012. Dual RNA-seq of pathogen and host. Nat. Rev. Microbiol. 10, 618–630.

Westermann, A.J., Barquist, L., Vogel, J., 2017. Resolving host-pathogen interactions by dual RNA-seq. PLoS Pathog. 13 (2), e1006033.

Xing, D., Yang, Q., Jiang, L., Li, Q., Xiao, Y., Ye, M., Xia, Q., 2017. RNA-seq analyses for two silkworm strains reveals insight into their susceptibility and resistance to *Beauveria bassiana* infection. Int. J. Mol. Sci. 18, 234.

Xiong, G.-H., Xing, L.-S., Lin, Z., Saha, T.T., Wang, C., Jiang, H., Zou, Z., 2015. High throughput profiling of the cotton bollworm *Helicoverpa armigera* immunotranscriptome during the fungal and bacterial infections. BMC Genom. 16, 321.

Xu, Y., Orozco, R., Wijeratne, E.M.K., Gunatilaka, A.A.L., Stock, S.P., Molnar, I., 2008. Biosynthesis of the cyclooligomer depsipeptide beauvericin, a virulence factor of the entomopathogenic fungus *Beauveria bassiana*. Chem. Biol. 15, 898–907.

Xu, Y., Orozco, R., Wijeratne, E.M.K., Espinosa-Artiles, P., Gunatilaka, A.A.L., Stock, S.P., Molnar, I., 2009. Biosynthesis of the cyclooligomer depsipeptide bassianolide, an insecticidal virulence factor of *Beauveria bassiana*. Fungal Genet. Biol. 46, 353–364.

Xu, Y., Luo, F., Li, B., Shang, Y., Wang, C., 2016. Metabolic conservation and diversification of *Metarhizium* species correlate with fungal host-specificity. Front. Microbiol. 7, 2020.

Zhang, S., Widemann, E., Bernard, G., Lesot, A., Pinot, F., Pedrini, N., Keyhani, N.O., 2012. CYP52X1, representing new cytochrome P450 subfamily, displays fatty acid hydroxylase activity and contributes to virulence and growth on insect cuticular substrates in entomopathogenic fungus *Beauveria bassiana*. J. Biol. Chem. 287, 13477–13486.