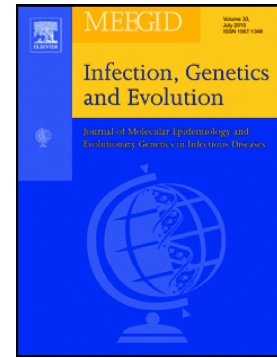# Accepted Manuscript

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Tracing the coevolution between *Triatoma infestans* and its fungal pathogen *Beauveria bassiana*

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Abstract

The chemical control of *Triatoma infestans*, the major Chagas disease vector in southern South America, has been threatened in the last years by the emergence of pyrethroid-resistant bug populations. As an alternative approach, the efficacy of the entomopathogenic fungus *Beauveria bassiana* to control *T. infestans* populations (regardless their pyrethroid susceptibility) has been demonstrated. Growing research efforts on the interaction between *T. infestans* and *B. bassiana* by molecular, ecological, biochemical and behavioral traits has allowed framing such interaction as an evolutionary arms race. This review will focus on the relationships established in this particular host-pathogen system, compiling available data on the relevance of fungal pathogenesis, insect behavior, population dynamics and human intervention to favor fungal dissemination in bug populations. The current snapshot shows the fungus ahead in the evolutionary arms race and predicts a promissory landscape for the biological control of Chagas disease vectors.

Keywords: arms race, biological control, Chagas disease vectors, entomopathogenic fungi, insect behavior.

1. Introduction

Coevolutionary processes involve reciprocal, adaptive genetic changes in two or more interacting species populations. In the particular case of host-pathogen interaction, both antagonistic organisms share a strong connection and apply selective pressure on each other (Joop and Vilcinskas, 2016; Woolhouse et al., 2002). This selective pressure acts as a major driving force for natural selection between the involved organisms and drives them to continuously adapt; the host changing their defense mechanisms, and their counterpart pathogens developing means to overcome the new defenses (Clay and Kover, 1996; Brockhurst et al., 2014).

In host-pathogen interaction, most of the metabolites that are used in the crosstalk between pathogens and host cells are chemically similar or identical. At the metabolic level, chemical signalling events enabling pathogens to sense anatomical location and the local physiology of the host, and microbial metabolic pathways dedicated to circumvent host immune mechanisms, together with some metabolites considered as central points of competition between the host and pathogens have been recently reviewed in bacteria (Olive and Sassetti, 2016). The outcome of this interaction is determined by the nature of the damage resulting from the host-pathogen relationship. Immune responses arise from the detection of danger signals produced by pathogens. Thus, host damage is often a requirement for the induction of a pathogen-specific immune response (Casadevall and Pirofski, 2000).

The interaction between insects and their microbial pathogens has been a subject of study for the past 50 years, and served as a model system for studying the coevolutionary processes involved in invertebrate-pathogen interaction (recently reviewed by Lovett and

St. Leger, 2017; Pedrini, 2017; Wang and Wang, 2017). Among all insect pathogens including bacteria, virus, nematodes and entomopathogenic fungi (Lacey et al., 2015), the most common microbial pathogens of insects are fungi (Lovett and St. Leger, 2017). Over the past 400 million years, both insects and fungi have coevolved a wide array of interactions; specifically, entomopathogenicity is a trait that has evolved in all major fungal phyla (Araujo and Hughes, 2016; Blackwell and Vega, 2005). Most of the available information about insect-fungi interaction is based on two model species, *Beauveria bassiana* and *Metarhizium anisopliae* (Ascomycota: Hypocreales); their experimental tractability has allowed explore a broad range of ecological, evolutive and behavioral traits and more recently molecular mechanisms involved in such interactions (Lovett and St. Leger, 2017). Both species are the most frequently used entomopathogenic fungi as biological control agents, having a wide host range that includes insects and other arthropods (Lacey et al., 2015). A few of these underlying mechanisms that take place in arthropod-fungal interactions and might define the competing aspects of an evolutionary arms race have been described in the insect models *Galleria mellonella* and *Tribolium castaneum* (Joop and Vilcinskas, 2016; Pedrini et al., 2015; Pedrini, 2017; Vilcinskas, 2010).

Chagas disease (American trypanosomiasis) is the major vector borne infectious disease caused by a parasite in Latin America. Its etiological agent *Tripanosoma cruzi* is transmitted and spread by triatomine insects through an infective bite. Triatominae subfamily (Hemiptera: Reduviidae) comprises 149 species grouped into 18 genera and five tribes (Justi and Galvão, 2017; Schofield and Galvão, 2009). Although all triatomine species are potential vectors of *T. cruzi*, their likely sanitary risk is directly linked to their ability to adapt and survive in contact with human habitats. Thus, from an epidemiologic

view and based on domiciliation success, *Triatoma infestans* and *Rhodnius prolixus* are considered the most relevant vectors of Chagas disease (WHO, 2000). The Southern Cone Initiative and similar initiatives for Central America and Andean countries resulted in efficient chemical control of *T. infestans* and *R. prolixus* domiciliated populations (Schofield and Dias, 1999; Hashimoto and Schofield, 2012). However, the detection of growing numbers of *T. infestans* residual populations in the Gran Chaco geographic area (shared by Argentina, Bolivia and Paraguay) showed failures in chemical control due to high levels of insecticide resistance (Mougabure-Cueto and Picollo, 2015; Picollo et al., 2005).

In parallel, during the last 10 years, there was a continuously growing research on the ability of *B. bassiana* to attack and kill *T. infestans* populations, and thus this fungus was proposed by our laboratory as an efficient and safe biological tool against this vector, regardless bug susceptibility to chemical insecticides (Forlani et al., 2011; 2015; Pedrini et al., 2009). *T. infestans* and *B. bassiana* are a good example of insect-entomopathogenic fungi interacting system, coevolutive traits between them are currently a subject of undergoing research. This review intend to compile available recent information about the interactions and the relationships established in this particular system, in an attempt to unravel coevolutive traits that both participants might have acquired during such interaction, and also to highlight the relevance of human intervention taking advantage of insect behavior to favor fungal dissemination in domestic and peridomestic bug populations.

2. An overview on the interaction between triatomines and entomopathogenic fungi

The first studies on the potential of *B. bassiana* as biological control tool against triatomines were done by Romaña et al. (1987). From then, several biological and biochemical traits on the interaction between triatomine insects and their fungal pathogens have been described, including the very initial mechanism of cuticle penetration (Napolitano and Juárez, 1997; Juárez et al., 2000, Pedrini et al., 2007), the effects of molting and starvation on the insect susceptibility to the fungal pathogen (Luz et al., 2003a), the importance of conidial autodissemination on the overall insect mortality (Forlani et al., 2011), and the effect of fungal infection in female fecundity and oviposition (Forlani et al., 2015) and in immune response (Flores-Villegas et el., 2016; Lobo et al., 2015). Also, a good number of fungal isolates with potential to be used in the biological control of triatomines have been reported (Garcia et al., 2016; Juárez et al., 2000; Luz et al., 1998; Lecuona et al., 2001).

Interestingly, fungal pathogens have been isolated from naturally infected triatomines; the first reported was an *Evlachovaea*-like fungi in Brazil (Luz et al., 2003b) and then the Argentinian isolates of *B. bassiana* and *Paecilomyces lilacinus* (Ascomycota: Hypocreales) in Córdoba and Santiago del Estero provinces, respectively (Marti et al., 2005; 2006). More recently, a native strain of *B. bassiana* was isolated from a *T. infestans* cadaver in Salta province (R. M. Cardozo, personal communication).

The landmark that triggered a second research wave on *T. infestans* and its pathogen *B. bassiana* was the detection of several pyrethroid-resistant foci of *T. infestans* in the Gran Chaco geographical region (Mougabure-Cueto and Picollo, 2015). Several laboratory and

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field studies showed the efficacy of this entomopathogenic fungus against both susceptible and resistant *T. infestans* populations (Forlani et al., 2015; Juárez et al., 2008; Pedrini et al., 2009). Furthermore, its use has been approved and successfully applied in rural houses infested with resistant bugs in Salta province, Argentina (A. Gentile and R.M. Cardozo, personal communication).

Another interesting point to be addressed is the susceptibility of *T. cruzi*-infected triatomines to fungal pathogens. Garcia et al. (2016) reported higher survival rates of *T. cruzi*-infected *R. prolixus* compared to non-parasitized bugs after treatment with *B. bassiana*. The authors suggested that the parasite might trigger the host's immune system and thus help acquire better defenses against the attack of other pathogen, e.g., an entomopathogenic fungus. This apparent reduction in the virulence of a secondary infective agent should be further explored; in particular, under similar environmental conditions to those bugs are exposed at their natural habitats; there are no reports on this regard neither on *T. infestans* nor other triatomines.

3. *Triatoma infestans* **as a host for** *Beauveria bassiana*

The insect cuticle is the first barrier against contact insecticides, and also is the point of entry for entomopathogenic fungi such as *B. bassiana*. In triatomines, the epicuticle — the outermost protective surface— is covered by a thin layer of mostly saturated lipids with very long chains (between 20 to more than 40 carbons), the predominant components are hydrocarbons, wax esters, fatty alcohols and either free or esterified fatty acids (Juárez and Calderón-Fernández, 2007). Among other functions, its physico-chemical properties help

delay the entry of chemical pesticides (Juárez, 1994). The role of the cuticle in the

pyrethroid resistance mechanism has been revealed by the first time in *T. infestans*

populations from the Gran Chaco region (Pedrini et al., 2009). Pyrethroid-resistant insects

have a thicker cuticle, together with a higher content of epicuticular hydrocarbons

compared to susceptible insects; these biochemical data correlated well with reduced

penetration of the insecticide and thus a decrease in the effective dose (Juárez et al., 2010;

Pedrini et al., 2009). Cuticle thickness is most likely related with the overexpression of

selected cuticle protein genes from the integument of pyrethroid-resistant insects

(Calderón-Fernández et al., 2017).

The epicuticle is also the first barrier to be beaten by the fungus prior initiation of

the infection process within the insect body. Entomopathogenic fungi have the ability to

grow using its host cuticle hydrocarbons as the sole carbon source; furthermore, this

mechanism is inducible and was shown to help reduce the time to kill (Napolitano and

Juárez, 1997; Pedrini et al., 2009). Interestingly, *B. bassiana* efficiently degrades the

epicuticle of *T. infestans* regardless its chemical insecticide resistance level (Pedrini et al.,

2009). Thus, these bioinsecticide properties make the fungus ideal to counter insecticide

resistance.

4. *Beauveria bassiana* as a pathogen of *Triatoma infestans*

Entomopathogenic fungi are the predominant natural pathogens in arthropod

populations, therefore these microbes have a formidable potential as pest control agents

(Lacey et al., 2015). *B. bassiana* is the entomopathogenic fungi most frequently tested

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against triatomine, and its potential against *T. infestans* has already being exploited (Forlani et al., 2015; Pedrini et al., 2009). In order to improve the horizontal transmission and residual activity parameters of a fungal formulation, the success relies on the fungal strain, the propagule stability and the formulation optimization (de Faria and Wraight, 2007; Forlani et al., 2011).

Several aspects of the biochemical processes involved in the interaction between *B. bassiana* and *T. infestans* cuticle were already examined (Crespo et al, 2000; Napolitano and Juarez 1997; Pedrini et al. 2007; 2009; 2010). The attachment and adhesion of the conidia to the epicuticle layer is in general dependent on the chemical composition of the host cuticle and the possibilities of the microbe to breach this structure (Pedrini et al., 2007). The utilization of *T. infestans* epicuticular hydrocarbons by entomopathogenic fungi was first demonstrated by Napolitano and Juárez (1997). Hydrocarbons were shown to be the preferred epicuticle lipid component for fungal growth. These components are fully metabolized to CO_2, used for energy production and utilized in the biosynthesis of cellular components. Furthermore, the hydrocarbons extracted from insects were shown to be better substrates than synthetic hydrocarbons of similar structure (Crespo et al., 2000; Napolitano and Juárez, 1997). However, growth on alkanes causes major changes in fungal metabolism, e.g., altering the profile of fatty acids and other lipids compared with fungi grown in rich media (Crespo et al., 2000, Juárez et al., 2000; 2004), and also display an oxidative stress scenario (Huarte-Bonnet et al., 2015). A set of fungal hydrocarbon-assimilating enzymes allow the degradation of host cuticle lipids (Alconada and Juárez, 2006; Pedrini et al., 2006; 2010), and help growth solely on very long chain hydrocarbons (Pedrini et al., 2010) boosting its ability to breach *T. infestans* cuticle (Pedrini et al., 2009).

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The degradation pathway starts with alkane's activation by terminal oxidation to the corresponding alcohol derivative, which is oxidized by alcohol and aldehyde dehydrogenases. Biochemical studies showed that the resulting activated fatty acids might be incorporated in membranes, stored as triacylglycerides, or enter the β-oxidation pathway in peroxisomes (Juárez et al. 2004; Pedrini et al. 2007). The first oxidation round of the alkane substrate is catalyzed by microsomal cytochrome P450 (CYP) enzymes (Pedrini et al., 2007; 2010). Microbial genes of the CYP52 subfamily have been reported to participate in alkane degradation (Ohkuma et al., 1995; Seghezzi et al., 1992). Up-regulation of these genes was shown in *B. bassiana* during growth in several synthetic alkanes, as well as in *T. infestans* hydrocarbon extracts (Huarte-Bonnet et al., 2017a,b; Pedrini et al., 2010).

Once the cuticle is breached, the fungus moves into the insect's hemocoel and develops cells that can elude the host's immune system (Pendland et al., 1993). During this invasive step, *B. bassiana* infects and progressively degrades different tissues until the host's death; emerging then through the cuticle (Valero-Jiménez et al., 2016). At this point, secretion of compounds that show antibiotic properties can help prevent competition for nutrients with other microorganisms (Pedrini, 2017). The potential role of these compounds also in fungal virulence against *T. infestans* is discussed below.

5. Biological and behavioral traits from *Triatoma infestans* may aid its natural enemy?

Blood-sucking arthropods have undergone an evolutionary selection process leading to key morphological, physiological and behavioral adaptations. Among Triatomines, some behavioral traits such as shelter recognition, predation risk, circadian rhythms, chemical

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communication and microclimatic preferences are shared (Guerenstein and Lazzari, 2009; Sant´Anna et al., 2017). Among a variety of chemical communication traits, adult *T. infestans* secrete alarm pheromones in the presence of predators (Manrique et al. 2006). Volatile and contact chemical signals mediate sex communication and mating success (Cocchiararo-Bastias et al., 2011, Manrique and Lorenzo, 2012). Also, aggregation behavior is mediated by thigmotaxis, by volatile cues from their faeces, and by contact chemical signals from their cuticle surface – mostly specific fatty acid components facilitating arresting behavior in selected shelters (Lorenzo Figueiras et al., 2009). This typical aggregation behavior help maintain triatomines in reduced size shelters during daylight and thus remaining in close contact to each other, favoring fungal horizontal transmission (also known as autodissemination process). Thus, it was reported that conidia autodissemination contributes significantly to the overall insect mortality and is dependent of the bug size (Pedrini et al., 2009). Also, the fungal horizontal transmission is associated to bug density and is very active for several days, since an infected bug was able to transmit fungal conidia by horizontal transmission to a healthy bug at least until one week after being in contact with a conidia powder formulation (Forlani et al., 2011).

Another behavioral trait worth commenting is the characteristic camouflage phenomenon exhibited by triatomines. In order to hide themselves, *T. infestans* have been reported to cover their bodies with soil dust, helping to hide themselves from predators in a typical camouflage adaptation reported in other members of the family Reduviidae, and likewise probably in thermoregulation (Brandt and Mahsberg, 2002; Zeledón et al., 1973). Remarkably, this behavior is also observed when the dust is a powder formulation of *B.*

bassiana conidia (Pedrini et al., 2009). Thus, the initial inoculum with a fungal formulation might be favored by triatomine behavior.

In summary, some behavioral traits might aid in some way the pathogen *B. bassiana* to infect an initial host with a high dose of conidia that can then be actively spreaded by autodissemination during daylight bug aggregation (Forlani et al., 2011, 2015; Pedrini et al., 2009). In the arms race to survive, *T. infestans* has a series of traits that naturally put them behind its pathogen, and as we detailed below, it might be even more noticeable due to human intervention.

6. Molecular basis of infective/defensive mechanisms in the *Beauveria bassiana-Triatoma infestans* system

During the invasive process, insect pathogenic fungi produce and secrete an array of toxic and immunosuppressive compounds (secondary metabolites) that help pathogen invasion and overcome the host defenses (Ferron, 1985; Trienens and Rohlfs, 2012). *B. bassiana* produces the cyclooligomer nonribosomal peptides beauvericin and bassianolide, the diketomorpholine bassiatin, the cyclic peptides beauverolides, the dibenzoquinone oosporein, and the 2-pyridone tenellin (Gibson et al., 2014; Molnar et al., 2010). They have a different degree of influence in the invasion/infection process depending on the host (Eley et al., 2007; Feng et al., 2015; Xu et al., 2008; 2009). In the specific pair *B. bassiana* infecting *T. infestans*, it was possible to determine the expression levels of the fungal synthetase genes encoding for tenellin (*BbtenS*), beauvericin (*BbbeaS*) and bassianolide (*BbbslS*) in fungus-infected insects (Lobo et al., 2015). The expression profiles in different

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stages of the infective process suggested that these three genes display differential roles. Both *BbtenS* and *BbbeaS* were found to be highly expressed in conidia-treated insects at day 3 and day 12 post-treatment, in coincidence with the mortality peaks previously observed in *T. infestans* infected with *B. bassiana* (Luz et al., 1998), suggesting a role as virulence factors. The high expression levels of *BbtenS* and *BbbeaS* detected at day 12 post-treatment also agrees with the high levels of secondary metabolites found in moribund/dead insects probably helpful to protect cadavers from competitive microorganisms (Pedrini, 2017).

T. infestans responds to *B. bassiana* infection by activating its humoral immune system; in a dual qPCR approach, Lobo et al. (2015) measured three genes involved in such response, including prophenoloxidase, hemolectin and defensin in the same samples containing RNA from both insect and fungus. During the middle-stage infection, and in conjunction with toxin decay, insects attempt to respond by inducing the three genes mentioned (Lobo et al., 2015). This result suggests that insect host immune responses are critical in attempts to limit or to stop the fungal infections. Although a correlation was found between the peak of expression of immunity related genes and the time course of fungal infection, the immune response is modulated by the infective fungal dose, and host responses may not be uniform and rather highly dependent on the inoculum. In conclusion, once the cuticle is breached and the fungus reaches the hemolymph, the immune response is overcome; the fungal "invader" proliferates throughout the host and death is mostly certain. Again, the major barrier to fungal infection seems to be the insect cuticle, and as the fungus evolved to degrade it and to invade the body cavity, it is seemingly ahead in the arms race.

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This dual qPCR approach represented a first but limited contribution to decipher the dynamics of the molecular interaction between triatomines and entomopathogenic fungi. However, current dual RNA-seq techniques will contribute to a better and extensive comprehension of this phenomenon, since this analysis allows mapping simultaneously the expression pattern of all genes, including their allelic variants, in both organisms during the infection process (Pedrini, 2017).

7. A case of success: Control of *Triatoma infestans* populations with "attraction-infection" traps based on *Beauveria bassiana* conidia

Taking advantage of some of the biological and behavioral features of both organisms described before, a box containing attractants for triatomines and a powder formulation containing *B. bassiana* conidia and diatomaceous earth was designed and used to control *T. infestans* populations in the Argentina-Bolivia border (Juárez et al., 2008). The infective box variants included both long distance attractants (CO_2) (Pedrini et al., 2009) and contact aggregation pheromones, i.e., cuticular lipids extracted from *T. infestans* (Forlani et al., 2015; Lorenzo Figueiras et al., 2009). Thus, insects may well be attracted to the trap by CO_2, a well-known attractant of most blood-sucking arthropods including *T. infestans* (Guerenstein and Lazzari, 2009), and once inside the box eventually induced to maximize their permanence in close contact with contact aggregation pheromones (Forlani et al., 2015) and the powder formulation. After loading with a high dose of conidia favored by the camouflage behavior, insect might leave the box and return to their nests, an ideal place for an efficient horizontal transmission. The results showed 50-60% bug mortality in

rural village houses infested with pyrethroid-resistant insects after one or two monthly

interventions using 6 boxes/room (Pedrini et al., 2009; Forlani et al., 2015). Furthermore, a

stage-specific matrix model was developed to describe the effects of *B. bassiana* infection

on *T. infestans* population dynamics and demonstrated that the number of boxes per house

is the main driver of the reduction of the total domestic bug population (Forlani et al.,

2015). Thus, this low cost, low tech, ecologically friendly methodology using a

pheromone-containing infective box is a promising tool against indoor populations of

triatomines; repeated fungal applications could eventually halt infection transmission as is

predicted by available mathematical models (Pedrini et al., 2009).

8. Conclusions and remarks

As a general rule, pathogenic fungi have developed many strategies to overcome the

insect host defenses; a key phase is the adhesion and penetration of the cuticle since once

inside the insect, the fungal pathogen has almost "won the survival battle". An opposite

situation has been observed in Coleoptera, both the red flour beetle and the rice stalk stink

bug secrete respectively benzoquinones and α,β-unsaturated aldehydes. These repellent

components wrapping the insect cuticle prevent the fungus to attach, colonize and grow

(Pedrini et al., 2015; da Silva et al., 2015). Once inside the body cavity, the fungus

produces a plethora of secondary metabolites that facilitate the fungal invasion and/or

interfere with the insect immune response, although some differences in their effects have

been assigned through both host and fungal species and thus their role as virulence factor is

still discussed (Lovett and St. Leger, 2017; Wang et al., 2012). The insects´ immune

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response is triggered after penetration, but the entomopathogenic fungi have evolved specific mechanisms to avoid the host response, including shedding epitopes to escape from hemocyte encapsulation (Pendland et al., 1993), which often leads to a successful infectious cycle.

For the particular system of *T. infestans* and *B. bassiana*, all the previous statements are true. Once *B. bassiana* has attached to the insects´ cuticle the degradation process begins. The cuticular hydrocarbon profile of *T. infestans* (Juárez and Calderón-Fernández, 2007) offers no resistance to fungal penetration (Pedrini et al., 2007), and the humoral immune defensive response of *T. infestans* shows a delayed activation to this aggression making the insect not capable to prevent fungal infection (Lobo et al., 2015). *B. bassiana* is "running forward" since it is able to overcome whatever barrier the insect interposes.

As *B. bassiana* is used worldwide as an integral part of biological control strategies, among them vector control programs, it is put in contact with a wider spectrum of insect populations that it would naturally be, or at least in a shorter time span. So the question rises, Are we helping *B. bassiana* "win" by using it as a biotechnological tool? Human intervention in the contact dynamics of these two organisms could be affecting the way they establish their interactions. An example of this is the use of biopesticide box traps, which helps *B. bassiana* spread faster and more efficiently (Forlani et al., 2015; Pedrini et al., 2009). Is the evolutive and adaptive rate of *B. bassiana* faster and more efficient than *T. infestans* or is this particular arms race biased because of human intervention? In the coevolutive adaptations, the human intervention should be considered a major driving force of the evolutive path this system undergoes. The further the research goes and the more

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information becomes available, the more questions seem to emerge regarding the human impact on the coevolutive link between *B. bassiana* and *T. infestans*.

Many cues at the molecular level remain unknown; some of them are currently being addressed in order to get a better understanding on what events take place when the fungus reaches the hemolymph, and which molecular pathways became involved in the response to the infection. The host initial response appears to be insufficient to overcome the amount of fungal resources after colonization has been established. A suite of immune-related genes was recently shown to be expressed in the integument of *T. infestans* (Calderón-Fernández et al., 2017), suggesting an earlier start of the immune system response, i.e., at the cuticular epidermal layer. There is yet a lot further to be explored regarding the coevolutive relationship between *T. infestans* and *B. bassiana*. In this snapshot of the current situation, attack and defense by the fungal pathogen and the host are being identified and assessed. The knowledge and comprehension of this information may aid to develop better biopesticides, design more efficient vector management strategies and therefore better vector-borne disease prevention.

Competing interests

The authors declare no conflict of interest.

Acknowledgments

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Table 1. Effectiveness of several isolates of *Beauveria bassiana* against *Triatoma infestans* and *Rhodnius prolixus*, under different laboratory conditions.

Insect	Stage	Fungal exposure	Fungal dose	Humidity	Mortality percentage	References
Triatoma infestans	Nymphs	Immersion	$1\times10^7\text{-}10^8$ conidia/ml	$\geq 90\%$	90-100	Luz et al., 1998; Lecuona et al., 2001
	Nymphs	Immersion	$1\times10^7\text{-}10^9$ conidia/ml	50%	50-100	Luz et al., 1998; Pedrini et al., 2009
	Nymphs	Immersion	1×10^8 conidia/ml	35%	54-68	Lecuona et al., 2001
	Adults	Immersion	1×10^8 conidia/ml	90%	90-100	Lecuona et al., 2001
	Nymphs	Contact with treated filter paper	$3\times10^6\text{-}10^7$ conidia/cm^2	50%	40-94	Luz et al., 1999
	Nymphs	Contact with powder formulation	3×10^8 conidia/cm^2	50%	82-100	Forlani et al., 2011
	Adults	Contact with powder formulation	3×10^8 conidia/cm^2	50%	88	Forlani et al., 2011
Rhodnius prolixus	Nymphs	Immersion	1×10^7 conidia/ml	> 90%	50-98	Cazorla-Perfetti and Morales-Moreno, 2016
	Nymphs	Contact with treated filter paper	~ 1×10^6 conidia/cm^2	80%	100	Garcia et al., 2016
	Nymphs	Sprayed onto insect	3×10^5 conidia/cm^2	> 90%	50-100	Romaña and Fargues, 1992
	Adults	Sprayed onto insect	3×10^5 conidia/cm^2	> 90%	100	Romaña and Fargues, 1992

This is not an inclusive list, it only provides representative examples.

Highlights

- The interaction between triatomines and their fungal pathogens is reviewed

- Some mechanisms involved in fungal attack and insect defense are identified

- Such interaction can be framed within an evolutionary arms race

- The fungus seems to be ahead since it overcome whatever barrier its host interposes

- *B. bassiana* is an effective tool to control pyrethroid-resistant *T. infestans*