DOI: http://dx.doi.org/10.1590/1678-992X-2017-0043

SCIENTIA AGRICOLA

ISSN 1678-992X

Susceptibility of *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae), a *Eucalyptus* pest, to entomopathogenic fungi

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Edited by: Alberto Soares Corrêa

Received February 03, 2017
Accepted February 11, 2018

ABSTRACT: *Thaumastocoris peregrinus* Carpintero and Dellape (Hemiptera: Thaumastocoridae) is a sap-sucking insect that has become a major pest of eucalypts. The entomopathogenic fungi *Beauveria bassiana* (Bals.-Criv.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin have the potential to control insect pests. This study evaluated the susceptibility of *T. peregrinus* to two commercial products based on conidia of *B. bassiana* and *M. anisopliae*. The fungi were sprayed onto adults of *T. peregrinus* at a concentration of 1×10^8 conidia mL^{-1} to evaluate their pathogenicity and conidial production on the insect cadavers. *Beauveria bassiana* caused 100 % mortality, while *M. anisopliae* caused more than 80 % mortality of *T. peregrinus* adults 11 days after fungi application. The fungi colonized the head and thorax regions and caused high mortality rates through conidial production. Pathogenicity of entomopathogenic fungi *B. bassiana* and *M. anisopliae* to *T. peregrinus* show potential to use these fungi in integrated pest management.
Keywords: *Beauveria bassiana*, *Metarhizium anisopliae*, biological control, bronze bug

Introduction

The bronze bug *Thaumastocoris peregrinus* Carpintero and Dellape (Hemiptera: Thaumastocoridae), native to Australia, is as a major pest to species of genus *Eucalyptus* (Laudonia and Sasso, 2012; Garcia et al., 2013; Souza et al., 2016). This pest has been introduced to more than 10 countries in Europe, Africa, South America and Oceania (Saavedra et al., 2015).

The short life cycle and high reproductive potential of females of *T. peregrinus* allow rapid population growth of this pest in the field (Soliman et al., 2012; Nadel et al., 2015). The damages caused for bronze bug reduces the photosynthetic capacity and causes death to trees that are severely infested (Jacobs and Neser, 2005; Nadel et al., 2010).

There are no effective control methods for *T. peregrinus*; therefore, the search for natural biological agents is essential. Biological control is the main approach to reduce damage by exotic insect pests in *Eucalyptus* (Wingfield et al., 2013). The egg parasitoid *Cleruchoides noackae* Lin and Huber (Hymenoptera: Mymaridae) is the only available biological control agent used against *T. peregrinus* (Barbosa et al., 2017).

Entomopathogenic fungus associated with the bronze bug was reported in Brazil. *Zoophthora radicans* (Entomophthorales: Entomophthoraceae) seems to be virulent against *T. peregrinus* and low densities of this insect were associated with high fungal infection levels (Mascarin et al., 2012). However, studies are needed to confirm virulence and potential for control of *T. peregrinus* with entomopathogenic fungi.

Beauveria bassiana (Bals.-Criv.) Vuill. (Ascomycota: Hypocreales: Cordycipitacea) and *Metarhizium anisopliae* (Metsch) Sorokin (Hypocreales: Clavicipitaceae) can rapidly spread in the field (Meyling et al., 2009; Costa et al., 2015) and regulate *Eucalyptus* pest populations (Sun et al., 2008; Echeverri-Molina and Santolamazza-Carbone, 2010). Thus, this study evaluated the susceptibility of *T. peregrinus* to two commercial products based on conidia of *B. bassiana* and *M. anisopliae*.

Materials and Methods

Insect collection

Thaumatocoris peregrinus was reared in Botucatu (22°53′09″ S; 48°26′42″ W; 804 m), São Paulo State, Brazil, at 24 ± 2 °C, 60 ± 10 % RH and 12L: 12D photoperiod, from insects collected in the field. Adults of the bronze bug were reared in bouquets of eucalyptus branches secured with a piece of foam in 500 mL Erlenmeyer flasks filled with water to prevent the insects from submerging.

Commercial products

The fungus *M. anisopliae* was obtained from the commercial products Toyobo - 4 × 10^9 conidia mL^{-1} (MTO) and Usina Paulista - 1.9 × 10^9 conidia mL^{-1} (MUS) and *B. bassiana* from Koppert- 5 × 10^8 conidia mL^{-1} - strain ESALQ-PL63 (BIT) and Usina Paulista - 4.8 × 10^9 conidia mL^{-1} (BUS). These mycoinsecticides are formulated as soluble powders. The conidia count per mL was performed in a Neubauer chamber (to confirm commercial product concentration) and then diluted in distilled water to standardize the concentration of all mycoinsecticides at 1 × 10^8 conidia mL^{-1}. Tween (20 0.02 %) adjuvant was added. The control used only distilled water and Tween 20 0.02 %. The products were stored in a freezer for 40 (BIT), 30 (BUS and MUS) and 21 (MTO) days. The product manufacturers guarantee minimum fungi viability of 90 %.

Bioassays

Eight adults of *T. peregrinus* were placed on Petri dishes (8.5 cm diameter) with diluted agricultural gel (hydroplan- EB/HyC, SNF S.A Floger) (1 g in 400 mL of distilled water) near a leaf of *Eucalyptus urophylla* S.T. Blake (Myrtaceae) with an area of 16.5 cm^2 per plot with five replications (40 insects per treatment). The mycoinsecticides (2 mL of suspension) (treatments: *M. anisopliae* (Toyobo – MTO and Usina – MUS); *B. bassaina* (Boveril - BIT and Usina - BUS) and control (distilled water and Tween 20 (0.02 %)) were sprayed on Petri dishes with insects with a Potter spray tower and transferred to a room with temperature regulated at 25 ± 3 °C, 60 ± 10 % RH with a photoperiod of 12h12 L:D. *T. peregrinus* adult mortality was evaluated at one, two, three, five, seven, nine, and eleven days after treatment application (DAA) and dead insects were transferred to plastic pots (100 mL) with a damp cotton ball and stored without light (mortality data confirmation). In addition, dead adults of *T. peregrinus* were stored under refrigeration in Karnovsky gel (2.5 glutaraldehyde, paraformaldehyde 2.0 %, phosphate buffer 0.05 M, pH 7.2) and analyzed with a DSM 940 A scanning electron microscope (SEM) (Carl Zeiss, Jena, Germany) of the Federal University of São Paulo, 21 days after mycoinsecticide application. Fungal development sites in the insect in tegument were identified from the photomicrographs.

Quantification of sporulation on *T. peregrinus* cadavers

Conidial production was evaluated in four *T. peregrinus* cadavers after 21 days of mycoinseticide application, with five replications per treatment (20 insects per treatment). Four insects were washed with 10 mL distilled water and Tween 20 0.02 %. The conidia in this solution were counted in a Neubauer chamber. Potential of conidial production was obtained as recommended for *Hypothenemus hampei* Ferrari (Coleoptera: Scolytidae) (Neves and Hirose, 2005) by dividing the production of different isolates by the production of isolate with lower production.

Statistical analyses

The mortality data were submitted to the analysis of variance with the F test and the means compared by the Tukey test ($p \leq 0.05$) with the SAS (Statistical Analysis System, version 9.0). Data were submitted to the survival analysis with the Kaplan-Meier estimator (Log-rank method) using Origin Pro (OriginLab Corporation, version 9.1). Bronze bugs that survived to the end of the experiment (11 DAA) were treated as censored data.

Results

The mycoinsecticides were pathogenic to adults of *T. peregrinus* at the dose of 1 × 10^8 conidia mL^{-1}. *Beauveria bassiana* and *M. anisopliae* penetrated and presented mycelial growth in the insect body, mainly in the head and thorax structures of *T. peregrinus*. *Beauveria bassiana* (BIT) mycelial growth was observed in the mouthparts and on intersegmental membranes of prothoracic legs in the thigh and trochanter of *T. peregrinus* (Figures 1A-E). The mycelial growth of *B. bassiana* (BUS) was more evident on the legs (Figure 1G). Mycelia of *M. anisopliae* (MUS) grew on the head, especially in the mouthparts (Figure 1F) with the hyphae (MTO) visible on the thorax (Figure 1H).

Mycoinsecticides with the *B. bassiana* fungus caused 100 % mortality of *T. peregrinus* (BUS: 8.00 ± 0.0 and BIT: 8.00 ± 0.0) and commercial products with *M. anisopliae* caused 83 and 88% mortality, respectively (Figure 2) (MTO: 6.66 ± 0.5 and MUS: 7.00 ± 0.5) ($F = 59.3$, $p < 0.05$), of *T. peregrinus* adults 11 days after fungi application.

The conidiogenesis on *T. peregrinus* from all fungi (Table 1) confirms the sporulation detected with SEM images (Figure 1). The BUS showed the highest conidial production on insect cadavers.

Discussion

The entomopathogenic action of *B. bassiana* and *M. anisopliae* on *T. peregrinus* is important because these fungi have a wide distribution and host range, easy production, with low risk to humans, animals and the

Table 1 – Number of conidia per cadaver of *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae) after 11 days of application (mean ± SE) and potential of conidial production after 21 days of application of *Beauveria bassiana* (Usina Paulista - BUS; Koppert - BIT) and *Metarhizium anisopliae* (Toyobo – MTO; Usina Paulista - MUS) formulated as commercial products at the concentration of 1 × 10^8 conidia mL^{-1} (25 ± 3 °C and a photoperiod of 12 h) (n = 20).

Treatment	Conidia/cadaver	[1]Potential of conidial production
BUS	144.9 ± 9.5	1.3
BIT	117.4 ± 8.5	1.0
MTO	141.4 ± 16.4	1.2
MUS	112.6 ± 16.0	-

[1]Potential of conidial production = conidia produced per product × divided by the conidia produced by MUS.

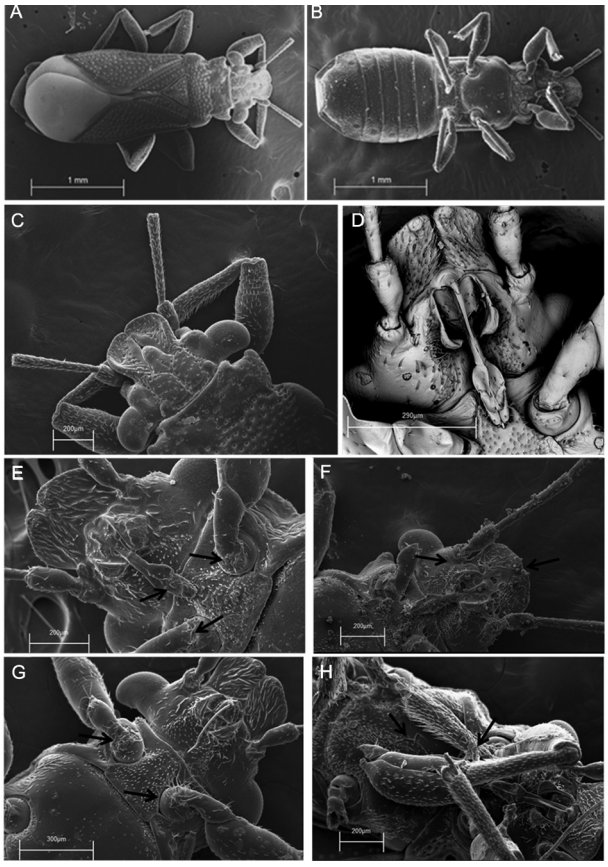

Figure 1 – Dorsal (A) and ventral view of the body (B), dorsal view (C) and ventral (D) of the head of *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae) without the application of mycoinsecticides. Ventral view of the head of this insect with application of *Beauveria bassiana* (Koppert - BIT) (E) and (Usina Paulista -BUS) (G) and *Metarhizium anisopliae* (Toyobo - MTO) (F) and (Usina Paulista - MUS) (H) at the concentration 1×10^8 conidia mL^{-1}. Black arrows indicate conidia attached to the host cuticle.

environment, and they penetrate the external cuticle of arthropods (Hussain et al., 2014). The use of fungi can complement the biological control of *T. peregrinus* with *Cleruchoides noackae* (Hymenoptera: Mymaridae), *Hemerobius bolivari* (Neuroptera: Hemerobiidae), and *Chrysoperla externa* (Neuroptera: Chrysopidae) as the main natural enemies reported for this pest (Nadel and Noack, 2012; Souza et al., 2012; Garcia et al., 2013). However, entomopathogenic fungi can cause adverse effects to the biological life history parameters of natural enemies (Agboton et al., 2013; Wu et al., 2015). Thus, the application of entomopathogenic fungi should be carefully adjusted to complement the biological systems of pest control (Furlong, 2004; Oreste et al., 2016). Temporal separation of the fungus application and parasitoid release could reduce antagonism and enhance pest control (Chow et al., 2016). This would reduce the possible detrimental effects of fungi on parasitoid development.

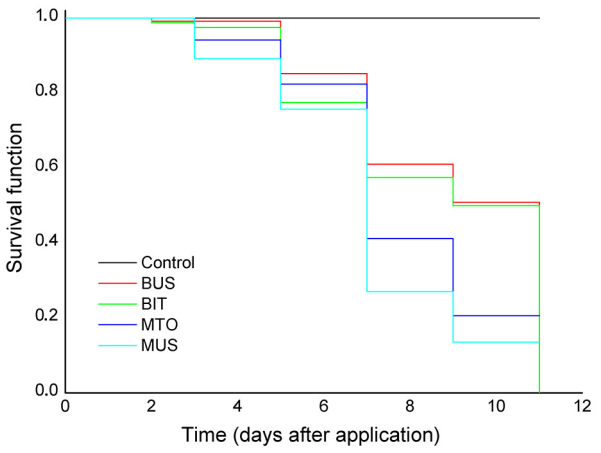

Figure 2 – Survival curves of *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae) up to 11 days after application of *Beauveria bassiana* (Usina Paulista - BUS; Koppert - BIT) and *Metarhizium anisopliae* (Toyobo – MTO; Usina Paulista - MUS) formulated as commercial products at the concentration of 1×10^8 conidia mL^{-1} and control using the Kaplan-Meier method and compared using the log-rank test ($X^2 = 191.2$; $p = 0.001$).

Mycelial growth of *B. bassiana* in *T. peregrinus* is more evident on the legs, similar to reports for fungus *Hirsutella thompsonii* on mite *Varroa destructor* (Acari: Varroidae) (Peng et al., 2002). The basal portion of the legs provides a favorable microclimate (e.g., higher humidity for germination and growth) and the lower sclerotized tissue of the intersegmental membrane of the insect favors fungi development. Besides, high hair density facilitates conidia attachment. In our study, colonization by *B. bassiana* began in the labium and spread to other parts of the bodies of *Myzus persicae* Sulzer (Hemiptera: Aphididae) and *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) (Amnuaykanjanasin et al., 2013).

Colonization of *T. peregrinus* mouthparts by *M. anisopliae* confirms reports of this fungus in the buccal cavity of blowfly *Lucilia cuprina* Wiedemann (Diptera: Calliphoridae) (Leemon and Jonsson, 2012). Fungal attachment in highly susceptible host locations is essential for successful pathogenesis (Amnuaykanjanasin et al., 2013). Conidia densities of *B. bassiana* and *M. anisopliae* were greater on legs, wings, and thorax of *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), *Bactericera cockerelli* Sulc. (Hemiptera: Triozidae) and *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) (Rios-Velasco et al., 2014).

Conidial production by fungi in *T. peregrinus* cadavers shows the dissemination capacity of these biological agents in the field (Ramos et al., 2004). High sporulation rate and epizootic potential are important characteristics for such control agents (Charley, 1997) allowing greater permanence (Alves and Lecuona, 1998) and residual effects of isolates in the field.

Susceptibility of *T. peregrinus* to the mycoinsecticides *B. bassiana* and *M. anisopliae* is similar to that reported for other Hemiptera pests, such as *Nilaparvata lugens* Stål (Hemiptera: Delphacidae) (Li et al., 2014), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) (Orduño-Cruz et al., 2015), *Nezara viridula* Linnaeus (Hemiptera: Pentatomidae) (Raafat et al., 2015), *Aeneolamia* spp. (Hemiptera: Cercopidae) (Hernández-Domínguez et al., 2016) and *Glycaspis brimblecombei* (Dal Pogetto et al., 2011a; 20011b).

Damage by *T. peregrinus* in eucalyptus plantations and the need for products that comply with forest certification requirements (Zanuncio et al., 2016; Lemes et al., 2017) show the importance of entomopathogenic fungi for the management of this pest. These studies are significant because *B. bassiana* and *M. anisopliae* caused 79 % mortality of adults of *N. lugens* 10 days after inoculation (Li et al., 2014) and 50 and 60 % mortality, respectively, to *D. citri* (Lezama-Gutiérrez et al., 2012). Other pathogenic fungi for these insects include *Hirsutella citriformis* Kuwayama (Ascomycota: Hypocreales: Ophiocordyceps) with 100 % mortality for *D. citri* (Orduño-Cruz et al., 2015). The microbial action of entomopathogenic fungi is slow and requires relatively long periods to induce insect mortality compared to chemicals (Lomer et al., 2001); however, infected insects have low feeding activity (Avery et al., 2009; Pelizza et al., 2013) and, therefore, damage caused by *T. peregrinus* may be reduced after application of entomopathogenic fungi. The aggregative behavior of *T. peregrinus* (Jacobs and Neser, 2005; Noack and Rose, 2007; Noack, 2009) may facilitate epizootic conditions for these microbial agents, similar to reports for *Zoophthora radicans* (Entomophthorales: Entomophthoraceae) (Mascarin et al., 2012).

Pathogenicity shows that *B. bassiana* and *M. anisopliae* have potential for the biological control of *T. penegrinus* and therefore should be considered in the integrated management of this pest. However, field studies are still needed. This is the first report on pathogenicity of these fungi to *T. peregrinus*.

Acknowledgements

We extend our thanks to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Programa Cooperativo sobre Proteção Florestal of the Instituto de Pesquisas e Estudos Florestais (PROTEF/IPEF) for the financial support. Dr. Phillip Villani revised and corrected the English language used in this manuscript.

Authors' Contributions

Conceptualization: Soliman, E.P., Wilcken, C.F. Data acquisition: Soliman, E.P., Wilcken, C.F., Firmino, A.C., Dal Pogetto, H.F.A. Data analysis: Soliman, E.P., Castro, B.M.C., Wilcken, C.F., Firmino, A.C., Barbosa, L.R., Zanuncio, J.C. Design of Methodology: Soliman, E.P., Wilcken, C.F., Firmino, A.C., Dal Pogetto, H.F.A. Writing and editing: Soliman, E.P., Castro, B.M.C., Wilcken, C.F., Dal Pogetto, H.F.A., Barbosa, L.R., Zanuncio, J.C.

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