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Selection of entomopathogenic fungi to control stink bugs and cotton boll weevil[1]

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ABSTRACT

Entomopathogenic fungi stand out in the biological control of several agriculturally important insects. Six isolates of *Metarhizium anisopliae*, *Cordyceps javanica*, *Beauveria* sp. and *B. bassiana* were screened to control *Anthonomus grandis*, *Euschistus heros*, *Oebalus poecilus*, *O. ypsilongriseus* and *Thyanta perditor*, important insect pests of soybean, cotton and rice. The bioassays were conducted in a completely randomized design, with four replications (10 insects/replication). Significant differences for virulence were observed between the tested fungal species and isolates. For *A. grandis*, the most virulent isolate was *M. anisopliae* BRM 2335, followed by *Beauveria* BRM 14527 and BRM 67744 [82.5 to 97.5 % of mortality; average lethal time (LT_{50}) of 5.9 to 7.8 days]. *M. anisopliae* BRM 2335 was also highly virulent to the four stink bug species (75 to 97.5 % of mortality; LT_{50} of 5.2 to 9.7 days). For the stink bugs, *Beauveria* sp. BRM 67744 was infectious to *O. poecilus* (75 % of mortality), but failed to control *E. heros* (16.9 % of mortality). *C. javanica* BRM 27666 and BRM 14526 showed average virulence to the stink bugs and *A. grandis* (17.5 to 57.3 % of mortality; LT_{50} of 6.0 to 9.7 days). *M. anisopliae* was consistently more virulent to the stink bugs than the other fungi. Therefore, *M. anisopliae* BRM 2335 was selected for further studies under screenhouse and field conditions to control *A. grandis* and other stink bug species, especially *E. heros*.

KEYWORDS: *Metarhizium anisopliae*, *Cordyceps javanica*, *Beauveria bassiana*, epizootics.

RESUMO

Seleção de fungos entomopatogênicos para controle de percevejos e bicudo-do-algodoeiro

Os fungos entomopatogênicos destacam-se no controle biológico de diversos insetos de importância agrícola. Seis isolados de *Metarhizium anisopliae*, *Cordyceps javanica*, *Beauveria* sp. e *B. bassiana* foram selecionados para o controle de *Anthonomus grandis*, *Euschistus heros*, *Oebalus poecilus*, *O. ypsilongriseus* e *Thyanta perditor*, importantes insetos-pragas da soja, algodão e arroz. Os bioensaios foram conduzidos em delineamento inteiramente casualizado, com quatro repetições (10 insetos/repetição). Foram observadas diferenças significativas, em termos de virulência, entre as espécies fúngicas e isolados testados. Para *A. grandis*, *M. anisopliae* BRM 2335 foi o isolado mais virulento, seguido por *Beauveria* BRM 14527 e BRM 67744 [82,5 a 97,5 % de mortalidade; tempo letal médio (TL_{50}) de 5,9 a 7,8 dias]. *M. anisopliae* BRM 2335 também foi altamente virulento para as quatro espécies de percevejo (75 a 97,5 % de mortalidade; TL_{50} de 5,2 a 9,7 dias). Para os percevejos, *Beauveria* sp. BRM 67744 foi infeccioso para *O. poecilus* (75 % de mortalidade), mas falhou no controle de *E. heros* (16,9 % de mortalidade). *C. javanica* BRM 27666 e BRM 14526 apresentaram virulência mediana para os percevejos e *A. grandis* (17,5 a 57,3 % de mortalidade; TL_{50} de 6,0 a 9,7 dias). *M. anisopliae* foi consistentemente mais virulento aos percevejos do que os outros fungos. Portanto, *M. anisopliae* BRM 2335 foi selecionado para estudos posteriores em casa telada e campo para o controle de *A. grandis* e outras espécies de percevejo, principalmente *E. heros*.

PALAVRAS-CHAVE: *Metarhizium anisopliae*, *Cordyceps javanica*, *Beauveria bassiana*, epizootias.

INTRODUCTION

Entomopathogenic fungi, with about 90 genera and more than 700 species, represent the largest share of organisms used in the microbial control of insects worldwide (Khachatourians & Qazi 2008). Unlike viruses, bacteria and protozoa that infect their host through the digestive tract, entomopathogenic fungi infect their host primarily by directly penetrating the insect cuticle (St. Leger & Wang 2010). Consequently,

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they are pathogens of many insect species and can infect all developmental stages: eggs, larvae, pupae, nymphs and adults.

Brazil has the largest microbial control program worldwide using *Metarhizium anisopliae* (Metsch.) Sorok. and *Beauveria bassiana* (Bals.) Vuill. for insect control (Mascarin et al. 2019). These fungi have a wide spectrum of activity and, therefore, they can infect a wide variety of arthropod species and be an alternative to chemical pesticides (Khan et al. 2012, Castro et al. 2016, Ríos-Moreno et al. 2016, Van Lenteren et al. 2018).

Stink bugs (Hemiptera: Pentatomidae) are important insect pests and can cause serious damage to vegetables, nuts and commodity crops such as soybean [*Glycine max* (L.) Merr.], corn (*Zea mays* L.) and cotton (*Gossypium* spp.). The stink bugs *Euschistus heros*, *Oebalus poecilus*, *O. ypsilongriseus* and *Thyanta perditor* are pod and seed feeders. Their feeding can cause direct consequences on yield and/or other parameters related to grain quality during pod development and seed filling. They are generally hard to control, and their populations can surpass the damage threshold (Sosa-Gómez et al. 2020).

Cotton boll weevil, *Anthonomus grandis* (Coleoptera: Curculionidae), is a devastating insect pest affecting cotton in the Americas (Cohen et al. 2023). It usually feeds upon and lays eggs inside flower buds, where hatched larvae feed and pupate, making it difficult to control, causing abscission or reduction in fiber quality (Grigolli et al. 2017, Arruda et al. 2021, Paim et al. 2021). Insecticide sprays are the main control tool used by producers to reduce the insect population. When no control strategy is adopted, outbreaks of boll weevils from the beginning of flowering until the cut-out (end of square production) can cause significant losses, even reaching 100 % (Abrapa 2018, Oliveira et al. 2022). Until just over 10 years ago, such losses ranged between 51 and 74 million dollars in Brazilian crops (Oliveira et al. 2013). The implementation of area-wide pest control programs in the Brazilian Savanna (Cerrado) has allowed more effective control and, currently, boll weevil infestations fluctuate between 5 and 9 % (Belot et al. 2016).

The fungal strains selected for this study have already shown promise to control other insect pests or have important characteristics for insect control. *Metarhizium anisopliae* BRM 2335 was isolated from the rice stalk stink bug *Tibraca limbativentris* Stal, 1860 (Heteroptera: Pentatomidae) in the 1980s in epizootic occurrence (Martins et al. 1986). Since then, several laboratory, screenhouse and field studies confirmed the high virulence of this strain to the rice stalk stink bug and several other arthropods, including cattle tick, *Rhipicephalus microplus* (Martins & Lima 1994, Martins et al. 1997 and 2004, Quintela et al. 2013, Silva et al. 2015).

The two strains of *Cordyceps javanica* BRM 14526 and BRM 27666 were isolated from insects at epizootic conditions and showed a high virulence to the whitefly *Bemisia tabaci* (Mascarin et al. 2018, Santos et al. 2018, Boaventura et al. 2021). The strain BRM 27666 of *C. javanica* was registered in August 2022 as a wettable powder named Lalguard Java for whitefly control after seven years of collaborative research between the Embrapa and Lallemand Plant Care Brazil (Patos de Minas, MG, Brazil) (Brasil 2022).

Beauveria sp. BRM 67744 was isolated from the southern green stink bug *Nezara viridula* (Heteroptera: Pentatomidae) in epizootic occurrence in a screenhouse. *Beauveria bassiana* BRM 14527 was isolated from the caterpillar *Rupela albinella* (Lepidoptera: Crambidae) and is considered promising in the control of *B. tabaci* (Mascarin et al. 2013). *Beauveria bassiana* IBCB 66 is registered by several companies to control different insect pests and is the most used strain for whitefly control in Brazil (Brasil 2022).

According to the high virulence of the isolates aforementioned for arthropod pests, and since virulence of entomopathogenic fungi varies according to fungal species and strain, this study aimed to select entomopathogenic fungi virulent to the stink bugs *E. heros*, *O. poecilus*, *O. ypsilongriseus* and *T. perditor*, as well as to the cotton boll weevil *A. grandis.*

MATERIAL AND METHODS

The studies were conducted at the Empresa Brasileira de Pesquisa Agropecuária (Embrapa Arroz e Feijão), in Santo Antônio de Goiás, Goiás state, central Brazil (16º30'20"S; 49º16'55"W), between May 10, 2019, and January 29, 2021.

Cotton boll weevil *A. grandis* was reared in a screenhouse on reproductive-stage cotton (*Gossypium hirsutum* L.) and on artificial diet according to Monnerat et al. (2000). Neotropical

brown stink bug *E. heros* nymphs were reared on green bean pods (*Phaseolus vulgaris* L.), okra fruits (*Abelmoschus esculentus* L.), soybean seeds (*Glycine max* L.) and peanut (*Arachis hypogaea* L.) under laboratory conditions, according to Silva et al. (2008). *O. poecilus*, *O. ypsilongriseus* and *T. perditor* were reared in a screenhouse on reproductive-stage rice (*Oryza sativa* L.).

The isolates were obtained from the collection of the Embrapa Arroz e Feijão (Table 1). *M. anisopliae* BRM 2335 was identified through the sequence analysis of the elongation factor 1-alpha gene, following the protocol described in Bischoff et al. (2009). BRM 27666 and BRM 14526 were identified as *C. javanica*, based on the tree concatenated with the regions ITS (579pb), LSU (884pb), RPB1 (779pb), RPB2 (1124pb) and TEF (1018pb) (Bayesian inference), according to Mongkolsamrit et al. (2018). The *B. bassiana* BRM 14527 identity was confirmed by molecular analysis, using the nucleotide sequence of the divergent domain (d1/d2) at the distal end of the 26S rRNA gene (Lo Cascio & Ligozzi 2011). The *B. bassiana* IBCB 66 was identified by molecular analysis with the ITS4 region by J. E. M. Almeida, in 2023.

Conidia were grown on potato-dextrose-agar (PDA) for 10-15 days and immediately suspended in 10 mL of sterile aqueous solution of 0.01 % (v/v) Tween 80 into 50 mL plastic centrifuge tubes. The suspension was vigorously agitated on a vortex mixer for 1 min and filtered through two layers of 30 μm pore-sized nylon cheesecloth. The filtered suspension (10 mL) was vortexed again for 1 min before application, and conidial concentrations were enumerated by a hemocytometer (Brightline Improved Neubauer, New Optik®, Brazil) at 400× magnification. The conidial germination for all isolates exceeded 90 % on PDA after 16 h at 26 ºC. Only conidia with germ tubes greater than conidial diameter were considered germinated.

Six experiments were conducted to determine the fungi virulence to the insects. The tested insect species, fungal species (strain and concentrations), location of insect rearing and the provided food are described in Table 2.

For all the experiments, the insects were anesthetized with carbon dioxide gas (CO_2) for 15 sec before fungal inoculation. One mL of fungal suspension was applied to ten insects kept in Petri dishes (60 mm), in a Potter Tower calibrated at 20 psi of working pressure. The control was treated with a sterile aqueous solution of 0.01 % (v/v) Tween 80.

The experiments were conducted in a completely randomized design, with four replicates, each consisting of ten insects, totaling forty per treatment. The experiments were maintained at room temperature. The temperature and relative humidity in the laboratory were monitored at 1 h intervals by two dataloggers (Hobo® U12-012, Onset Computer Corp. Ltd., Massachusetts). Small variations were observed for the datalogger measurements, with an average of 28 ± 3 ºC and 57 ± 15 % of relative humidity.

Groups of ten insects were fed with five cotton flower buds (*A. grandis*), two green bean pods (*E. heros*) in gerbox-type boxes (110 × 110 × 35 mm), and two rice panicles (*O. poecilus*, *O. ypsilongriseus* and *T. perditor*) in plastic cages (150 × 100 mm). All the foods were previously surface sterilized with sterile aqueous solution of 5 % (v/v) sodium hypochlorite (2 to 2.5 % of NaOCl) for 15 min and rinsed twice with distilled water.

Dead and live insects were evaluated daily between the third and fourteenth day after the treatment. To confirm the mortality by the fungi, the cadavers were transferred to Petri dishes (60 mm) with a wet cotton and maintained at room temperature. Insects were considered infected by the fungi when mycelial or conidial growth was observed on the insect cadaver.

Table 1. Fungal species and isolates, original insect host, place of origin and collection year in Brazil.

Fungal	Isolates	Original host	Geographic origin (city/state) and collection year
Cordyceps javanica	BRM 27666	*Bemisia tabaci* (Aleyrodidae)	Porangatu, Goiás, 2013
	BRM 14526	*Rupela albinella* (Crambidae)	Arari, Maranhão, 2012
Metarhizium anisopliae	BRM 2335	*Tibraca limbativentris* (Pentatomidae)	Santo Antônio de Goiás, Goiás, 1985
Beauveria bassiana	IBCB 66	*Hypothenemus hampei* (Curculionidae)	São José do Rio Pardo, São Paulo, ≈1984
	BRM 14527	*Rupela albinella* (Crambidae)	Arari, Maranhão, 2012
Beauveria sp.	BRM 67744	*Nezara viridula* (Pentatomidae)	Santo Antônio de Goiás, Goiás, 2020

Table 2. Tested insect species, fungal species (strain and concentrations) and location of insect rearing, and food provided during the bioassay development.

Insect species	Fungal species and strain	Concentration (conidia mL^{-1})	Insect origin	Food source
Anthonomus grandis	*Cordyceps javanica* BRM 27666, BRM 14526 *Metarhizium anisopliae* BRM 2335 *Beauveria bassiana* IBCB 66	1×10^8	Laboratory	Cotton squares
A. grandis	*C. javanica* BRM 27666 *M. anisopliae* BRM 2335 *B. bassiana* BRM 14527 *Beauveria* sp. BRM 67744	1×10^8	Field and screenhouse	Cotton squares
Euschistus heros	*C. javanica* BRM 27666 *M. anisopliae* BRM 2335 *B. bassiana* BRM 14527 *Beauveria* sp. BRM 67744	5×10^7	Laboratory	Bean pods
Oebalus poecilus	*C. javanica* BRM 27666 *M. anisopliae* BRM 2335 *B. bassiana* BRM 14527 *Beauveria* sp. BRM 67744	1×10^8	Screenhouse	Rice panicles
O. ypsilongriseus	*C. javanica* BRM 27666 *M. anisopliae* BRM 2335	5×10^7; 5×10^8	Screenhouse	Rice panicles
Thyanta perditor	*C. javanica* BRM 27666 *M. anisopliae* BRM 2335	2×10^7; 2×10^8	Screenhouse	Rice panicles

The virulence of all fungal isolates was expressed and compared in terms of percent of mortality, confirmed mortality (% of insect cadavers with fungal sporulation) and average lethal time (LT_{50}) for the different insect species.

For all the experiments, overall and confirmed mortality curves were adjusted according to non-linear models and compared using the Wilcoxon-Mann-Whitney (*A. grandis*, *E. heros*, *O. poecilus* and *T. perditor*) and the chi-square (*O. ypsilongriseus*) tests ($p < 0.05$). To estimate the LT_{50}, non-linear models (log-logistic, logistic, Gompertz or Weibull) were fitted, and values were compared by the overlap of their 95 % confidence intervals (95 % CI) using the Package 'drc' (Gottschalk & Dunn 2005). The LT_{50} values were not estimated for treatments where mortality did not reach 50 %. The R statistical software, version 4.2.2, was used for all the analyses (R Core Team 2023).

RESULTS AND DISCUSSION

In the first experiment, *M. anisopliae* BRM 2335 caused a significantly higher mortality of adults (97.5 % total and 92.5 % confirmed mortality) and a shorter average lethal time (LT_{50} = 5.9 days) than the other isolates (Figures 1A, 1B and 2A; Tables 3 and 4). No differences in total mortality were observed between *C. javanica* BRM 27666 and BRM 14526, as well as *B. bassiana* IBCB 66. The total mortalities were significantly different from the control for all the fungal isolates, except for *C. javanica* BRM 14526 (Figures 1A, 1B and 4A; Tables 3 and 4). Confirmed mortality was significantly higher for all the isolates than for the control, except for *B. bassiana* IBCB 66. No growth of *B. bassiana* IBCB 66 was observed on adult cadavers (Figure 1B; Table 3).

In the second experiment, *M. anisopliae* BRM 2335 and *B. bassiana* BRM 14527 were significantly more virulent to *A. grandis* adults than the other isolates, causing mortalities above 73.0 %. However, no significant differences among the isolates were observed for LT_{50} (Figures 1C, 1D and 3 B; Tables 3 and 4). No differences in confirmed mortality were observed for *M. anisopliae* BRM 2335, *B. bassiana* BRM 14527 and *Beauveria* sp. BRM 67744. The mortality of adults by *C. javanica* BRM 27666 was very low and similar to the control (Figures 1C, 1D and 4A; Table 3).

Both *M. anisopliae* BRM 2335 and *B. bassiana* BRM 14527 at 5×10^7 conidia mL^{-1} caused significantly higher total mortalities of second instar *E. heros*, when compared to *C. javanica* BRM 27666 and *Beauveria* sp. BRM 67744. The mortalities

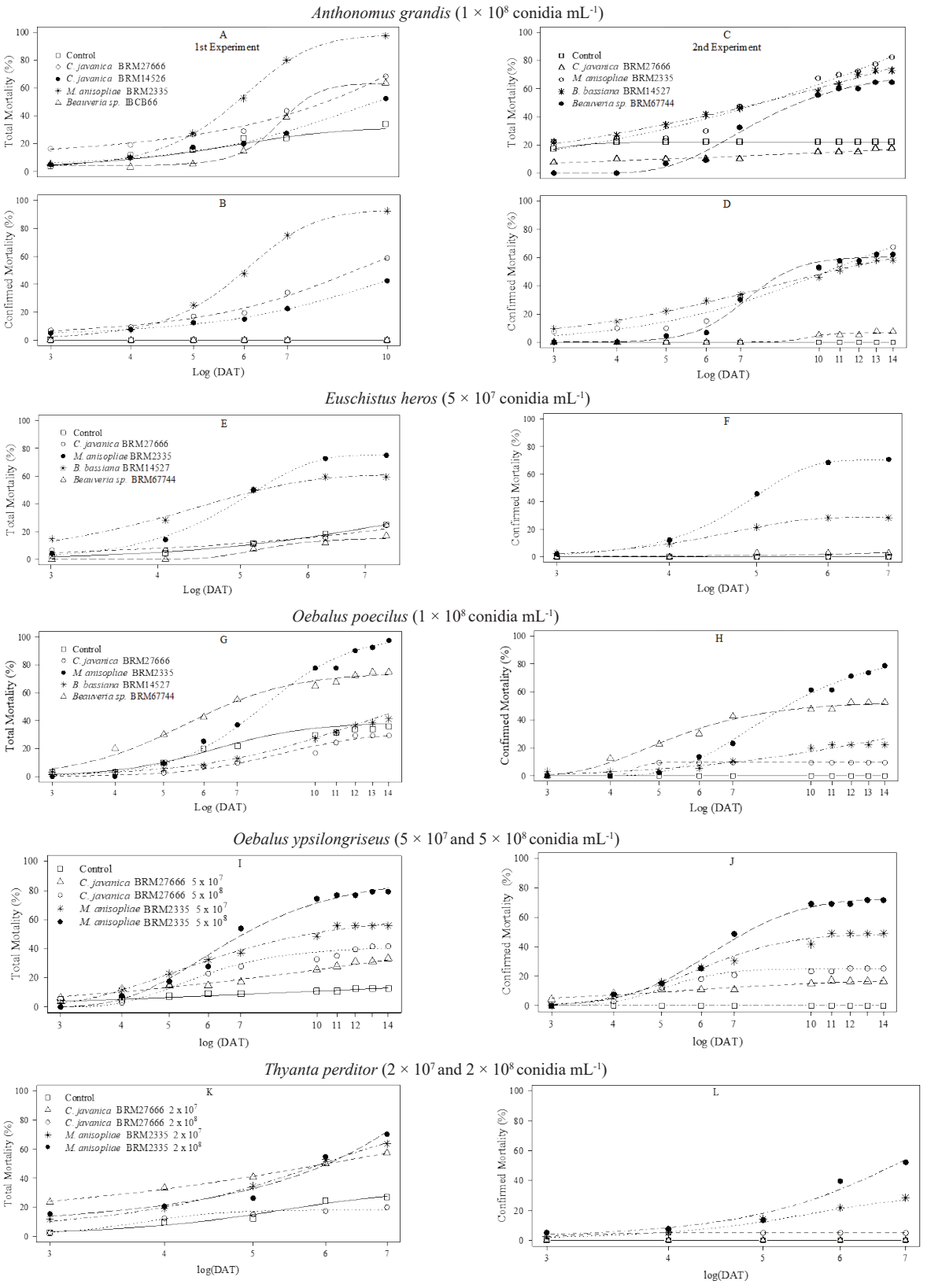

Figure 1. Cumulative and confirmed mortality at different days post-inoculation (DAT) of *Anthonomus grandis*, *Euschistus heros*, *Oebalus poecilus*, *Oebalus ypsilongriseus* and *Thyanta perditor* treated with concentrations of *Metarhizium anisopliae*, *Cordyceps javanica* and *Beauveria* spp. Curves were adjusted according to non-linear log-logistic (A and G), Weibull (C, E, F, H, I, K and L), Gompertz (J) and logistic (B and D) models.

Photos: Larissa Moreira de Sousa

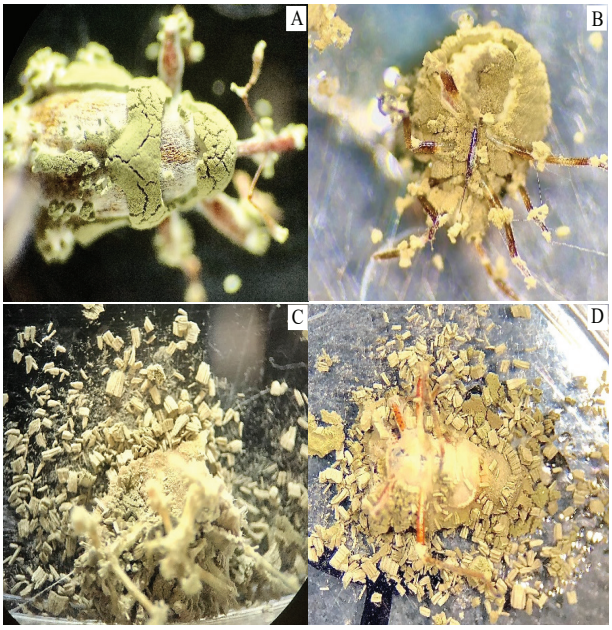

Figure 2. Adults of *Anthonomus grandis* (A), *Oebalus* spp. (C), *Thyanta perditor* (D) and nymphs of *Euschistus heros* (B) infected with *Metarhizium anisopliae* BRM 2335, with dense sporulation on the cadavers and around it.

Photos: José Francisco Arruda e Silva (A); Larissa Moreira de Sousa (B, C and D)

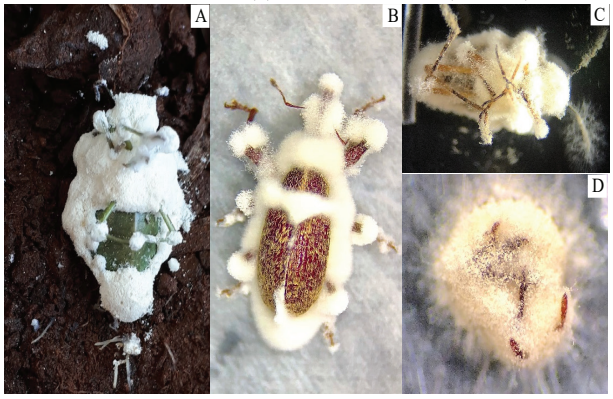

Figure 3. *Beauveria* sp. BRM 67744 isolated from the southern green stink bug *Nezara viridula* (Heteroptera: Pentatomidae) in epizootic occurrence in a screenhouse (A). Adults of *Anthonomus grandis* (B), *Oebalus poecilus* (C) and nymphs of *Euschistus heros* (D) infected with *Beauveria* spp.

Photos: Larissa Moreira de Sousa

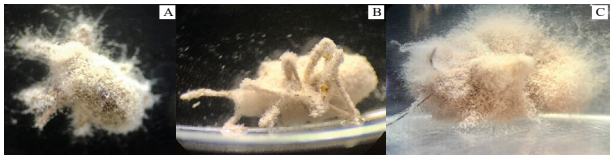

Figure 4. Adults of *Anthonomus grandis* (A), *Oebalus* spp. (B) and *Thyanta perditor* (C) infected with *Cordyceps javanica*.

were similar to the control for *Beauveria* sp. BRM 67744 and *C. javanica* BRM 27666 (Figures 1E, 1F and 3D; Tables 3 and 4). A higher confirmed mortality was observed for *M. anisopliae* (70.6 %) and differed from the other isolates (Figure 2B). The LT_{50} were 5.2 and 5.1 days for BRM 2335 and BRM 14527, respectively (Table 4).

M. anisopliae BRM 2335 and *Beauveria* sp. BRM 67744 at 1×10^8 conidia mL^{-1} were significantly more virulent to *O. poecilus* adults than the other isolates, causing mortalities of 97.5 and 75 % (78.6 and 52.5 % confirmed mortality), respectively (Figures 1G, 1H, 2C and 3C; Table 3). The LT_{50} was lower for BRM 67744 (6.6 days) than for BRM 2335 (7.9 days) (Table 4). The total and confirmed mortalities of adult *O. poecilus* by *B. bassiana* BRM 14527 and *C. javanica* BRM 27666 were very low and similar to the control (Figures 1G, 1H and 4B; Table 3).

No differences in mortality of adult *O. ypsilongriseus* by *M. anisopliae* BRM 2335 at the two tested concentrations (5×10^8 and 5×10^7 conidia mL^{-1}) were observed, and mortalities ranged from 55.7 to 79.2 % (48.9 to 71.4 % confirmed mortality) (Figures 1I, 1J and 2C; Table 3). However, the estimated LT_{50} values were lower for BRM 2335 at 5×10^8 conidia mL^{-1} (7.1 days), when compared to 5×10^7 conidia mL^{-1} (9.7 days) (Table 4). The mortality of *O. ypsilongriseus* by *M. anisopliae* BRM 2335 at 5×10^7 conidia mL^{-1} (55.7 % total and 48.9 % confirmed mortality) was similar to *C. javanica* BRM 27666 at 5×10^8 conidia mL^{-1} (41.5 % total and 25.2 % confirmed mortality). No differences for confirmed mortality were observed for *C. javanica* BRM 27666 at 5×10^7 conidia mL^{-1} and control (Figures 1I and 1J; Tables 3 and 4).

The mortalities of adult *T. perditor* by *M. anisopliae* BRM 2335 at 2×10^7 and 2×10^8 conidia mL^{-1} were similar to *C. javanica* BRM 27666 at 2×10^7 conidia mL^{-1} (Figures 1K, 1L and 2D; Tables 3 and 4). Unexpectedly, a higher mortality was observed for *C. javanica* BRM 27666 at 2×10^7, when compared to 2×10^8 conidia mL^{-1} (Figure 4 C). At 2×10^8 conidia mL^{-1}, *C. javanica* BRM 27666 was significantly less virulent than *M. anisopliae* BRM

Table 3. P values (p ≤ value) of the comparisons of mortality curves for *Anthonomus grandis*, *Euschistus heros*, *Oebalus poecilus*, *Oebalus ypsilongriseus* and *Thyanta perditor* after treatment with *Metarhizium anisopliae* (Ma), *Cordyceps javanica* (Cj) or *Beauveria* spp. isolates at different concentrations. The Wilcoxon-Mann-Whitney and chi-square (*Oebalus ypsilongriseus* only) rank sum tests were used for P values calculation. Curves were considered significant at p ≤ 0.05.

Anthonomus grandis (1st experiment)				
Treatment	BRM 27666	BRM 14526	BRM 2335	IBCB 66
Total mortality				
Control	0.0486	0.3741	< 0.001	< 0.001
BRM 27666	-	0.1649	0.0159	0.3176
BRM 14526	-	-	< 0.001	0.9535
BRM 2335	-	-	-	< 0.001
Confirmed mortality				
Control	< 0.001	< 0.001	< 0.001	1.000
BRM 27666	-	0.3829	0.0200	< 0.001
BRM 14526	-	-	0.0020	< 0.001
BRM 2335	-	-	-	< 0.001

Anthonomus grandis (2nd experiment)				
Treatment	BRM 27666	BRM 2335	BRM 14527	BRM 67744
Total mortality				
Control	0.3566	< 0.001	< 0.001	0.0045
BRM 27666	-	< 0.001	< 0.001	0.0011
BRM 2335	-	-	0.6985	0.0366
BRM 14527	-	-	-	0.0275
Confirmed mortality				
Control	0.8875	0.0047	0.0012	0.0015
BRM 27666	-	0.0062	0.0016	0.0021
BRM 2335	-	-	0.5777	0.2681
BRM 14527	-	-	-	0.1045

Euschistus heros				
Treatment	BRM 27666	BRM 2335	BRM 14527	BRM 67744
Total mortality				
Control	0.8231	< 0.001	< 0.001	0.5657
BRM 27666	-	< 0.001	< 0.001	0.5169
BRM 2335	-	-	0.2301	< 0.001
BRM 14527	-	-	-	< 0.001
Confirmed mortality				
Control	1.000	< 0.001	0.0162	0.8625
BRM 27666	-	< 0.001	0.0162	0.8625
BRM 2335	-	-	0.0015	< 0.001
BRM 14527	-	-	-	0.0256

Oebalus poecilus				
Treatment	BRM 27666	BRM 2335	BRM 14527	BRM 67744
Total mortality				
Control	0.2401	< 0.001	0.4624	0.0019
BRM 27666	-	< 0.001	0.4543	< 0.001
BRM 2335	-	-	< 0.001	0.0925
BRM 14527	-	-	-	< 0.001
Confirmed mortality				
Control	0.3428	< 0.001	0.1023	< 0.001
BRM 27666	-	< 0.001	0.3994	< 0.001
BRM 2335	-	-	< 0.001	0.0925
BRM 14527	-	-	-	0.0051

Oebalus ypsilongriseus				
Treatment	Cj 5×10^7	Cj 5×10^8	Ma 5×10^7	Ma 5×10^8
Total mortality				
Control	0.1949	0.0276	< 0.001	< 0.001
Cj 5×10^7	-	0.3247	0.0372	< 0.001
Cj 5×10^8	-	-	0.2269	0.0055
Ma 5×10^7	-	-	-	0.0979
Confirmed mortality				
Control	0.1532	0.0185	< 0.001	< 0.001
Cj 5×10^7	-	0.3482	0.0067	< 0.001
Cj 5×10^8	-	-	0.0701	< 0.001
Ma 5×10^7	-	-	-	0.0621

Thyanta perditor				
Treatment	Cj 2×10^7	Cj 2×10^8	Ma 2×10^7	Ma 2×10^8
Total mortality				
Control	0.0092	0.6468	0.0259	0.0213
Cj 2×10^7	-	0.0063	0.3711	0.3771
Cj 2×10^8	-	-	0.0132	0.0104
Ma 2×10^7	-	-	-	0.8065
Confirmed mortality				
Control	1.0000	0.6171	0.0719	0.0022
Cj 2×10^7	-	0.6171	0.0719	0.0022
Cj 2×10^8	-	-	0.1785	0.0087
Ma 2×10^7	-	-	-	0.1976

2335. No differences in confirmed mortality were observed for *C. javanica* BRM 27666 and control. There were no differences for all the fungal isolates and concentrations for LT_{50} (6 days) (Table 4).

This study found significant differences in virulence between the tested fungal species and isolates. For the cotton boll weevil *A. grandis*, the most virulent isolate was *M. anisopliae* BRM 2335, followed by *Beauveria* BRM 14527 and BRM 67744. Other studies also confirmed the potential of *M. anisopliae* and *B. bassiana* for microbial control of cotton boll weevil in laboratory and field experiments (Camargo et al. 1985, Coutinho & Cavalcanti 1988, Giometti et al. 2010, Nussenbaum & Lecuona 2012). For stink bugs, *M. anisopliae* was consistently more virulent than the other fungi, *Beauveria* and *Cordyceps*.

Several researchers showed that variation in the susceptibility of a host to fungal infection depends on the species and genetic variability between isolates (Hajek & St. Leger 1994, St. Leger 1995, Castrillo et al. 2005, Islam et al. 2021). Insects have evolved several mechanisms (cellular and humoral defenses) to keep the pathogens at bay. These complex processes involve the production of antimicrobial proteins, lipids and metabolites in the epicuticle

Table 4. Estimates of parameters of non-linear models and average lethal times (LT_{50}) of *Anthonomus grandis*, *Euschistus heros*, *Oebalus poecilus*, *Oebalus ypsilongriseus* and *Thyanta perditor* treated with *Cordyceps javanica*, *Metarhizium anisopliae* or *Beauveria* spp. isolates at different concentrations (conidia mL^{-1}).

Fungal isolate	Model	Model parameters[a]				LT_{50} (d) (CI95 %)[c]
		b	c	d	e	
Anthonomus grandis (1st experiment)						
Cordyceps javanica BRM 27666	Log-logistic	-0.29	-0.00	1.37	9.99	8.1 (6.7-9.4)
C. javanica BRM 14526		-0.49	-0.00	0.69	7.68	9.7 (9.1-10.3)
Metarhizium anisopliae BRM 2335		-1.21	0.01	0.98	5.84	5.9 (5.6-6.1)
Beauveria bassiana IBCB 66		-2.01	0.04	0.63	6.79	7.4 (7.1-7.7)
Anthonomus grandis (2nd experiment)						
M. anisopliae BRM 2335	Weibull	-0.40	-[b]	5.50	69.01	7.8 (5.4-10.2)
B. bassiana BRM 14527		-0.41	-	3.23	35.46	7.7 (6.5-8.9)
Beauveria sp. BRM 67744		-3.32	-	0.72	6.82	9.2 (8.6-9.7)
Euschistus heros						
M. anisopliae BRM 2335	Weibull	6.29	-	0.75	4.98	5.2 (4.9-5.4)
B. bassiana BRM 14527		3.71	-	0.61	4.39	5.1 (4.7-5.5)
Oebalus poecilus						
M. anisopliae BRM 2335	Log-logistic	-4.97	-	1.02	2.07	7.9 (7.8-7.9)
Beauveria sp. BRM 67744		-4.15	-	0.75	1.71	6.6 (6.5-6.6)
Oebalus ypsilongriseus						
M. anisopliae BRM 2335 5×10^7	Weibull	-2.26	-	0.63	5.06	9.7 (9.1-10.3)
M. anisopliae BRM 2335 5×10^8		-2.78	-	0.89	5.81	7.1 (6.7-7.5)
Thyanta perditor						
C. javanica BRM 27666 2×10^7	Weibull	-0.47	-	0.76	4.62	6.0 (5.6-6.4)
M. anisopliae BRM 2335 2×10^7		-0.81	-	0.82	5.41	6.0 (4.8-7.1)
M. anisopliae BRM 2335 2×10^8		-0.46	-	3.33	9.77	6.0 (5.0-7.1)

CI = 95 % confidence intervals. [a] Model parameters: b: slope factor around the "e" parameter; c: lowest asymptote of the curve; d: upper asymptote of the curve; e: inflection point of the curve. [b] The parameter is not part of the model. [c] LT_{50}: values were not estimated for treatments where mortality did not reach 50 %.

that provides defense by melanization around a penetrating tube; secretions for behavioral adaptions, including induced fever, grooming and removal of cuticle when moving from one growth stage to another, what effectively results in cleansing of the insect's outer surface; increased body temperature to adapt to changes in biochemical and environmental conditions; and recruitment of antibiotic or other defense compound producing (symbiotic) bacteria (Castrillo et al. 2005, Ortiz-Urquiza & Keyhani 2013, Qu & Wang 2018). In general, the fungi capacity to cause infection is mainly related to the biochemical processes involved in germ tube formation and host colonization, thickness and chemical composition of the host cuticle, body exudates or defensive secretions, maturation of the host immune system, host species, body mass and age of the insects (St. Leger et al. 1991, Silva et al. 2015, Sosa-Gómez & Alves 2000, Rosengaus et al. 2000).

In the case of some fungal infections, several species of stink bugs deploy biochemical barriers (aldehyde production) that are quite efficient (Sosa-Gómez et al. 1997, Sosa-Gómez & Moscardi 1998, Pike 2014, Silva et al. 2015). These chemicals affect spore adhesion and germination, vegetative growth and sporulation (Borges et al. 1993, Sosa-Gómez et al. 1997, Lopes et al. 2015, Silva et al. 2015, Pedrini 2018). Studies conducted with *M. anisopliae* BRM 2335 (tested in our studies) by Silva et al. (2015) demonstrated that the differential susceptibility of the rice stalk stink bug *T. limbativentris* to fungus infection was age-specific (early instar nymphs were more susceptible than late instars and adults) and partly mediated by fungistatic properties of aldehydes (E)-2-hexenal, (E)-2-octenal and (E)-2-decenal, which were produced by scent glands of both nymphs and adults. Other studies conducted with *N. viridula*, an important stink bug of soybean, showed similar results related to fungistatic compounds (Borges et al. 1993, Sosa-Gómez et al. 1997).

Despite these morphological and biochemical barriers of stink bugs to fungal infections, *M. anisopliae* BRM 2335 was highly virulent to the four stink bugs species (mortalities ranging from 75 to

97.5%). In addition, the percentage of cadavers with fungal sporulation (i.e., confirmed mortality) was very similar to the total insect mortalities. Besides the high virulence, this isolate showed a high sporulation rate in all the insect cadavers. The sporulating growth covered the cadaver and destroyed all the insect's tissues (Figures 2A-D).

Some other studies also demonstrated the virulence of *Metarhizium* to stink bugs. The most virulent strain of *Metarhizium* sp. ARSEF 4556 caused over 90 % of mortality for *E. heros* and the green-belly stink bug *Dichelops furcatus* (F.) (Resquín-Romero et al. 2020). Similar results were found for different *B. bassiana* isolates, with mortalities of *E. heros* adults above 95 % (Zambiazzi et al. 2012, Nora et al. 2021). According to Sosa-Gómez et al. (1997), the exposure of soybean stink bugs to high levels of conidia from various *B. bassiana* and *M. anisopliae* strains was required to elicit a lethal mycosis under laboratory conditions. *Piezodorus guildinii* was shown to be more susceptible than *N. viridula* to either *B. bassiana* or *M. anisopliae*. In field cages, *M. anisopliae* achieved infection levels of 48 and 41 % at day 30 for *P. guildinii* and *N. viridula*, respectively, whereas the infection level in *E. heros* reached 33 % (Sosa-Gómez & Moscardi 1998). Both *M. anisopliae* and *B. bassiana* were also virulent to *O. poecilus*, *O. pugnax* and *O. mexicana* under laboratory and field conditions (Alves et al. 1986, Luz et al. 1999, Patel et al. 2006, Santos et al. 2006, Cortez-Madrigal et al. 2022).

Natural epizootics by fungi in field conditions have not been reported in the soybean stink bug complex in Brazil (Sosa-Gómez & Moscardi 1998). Moscardi et al. (1988) observed isolated cases of mycosis (< 0.5 %) caused by *B. bassiana* and *M. anisopliae*. However, the *Beauveria* sp. BRM 67744 used in our studies was isolated from the southern green stink bug *N. viridula* (Heteroptera: Pentatomidae) in epizootic occurrence in a rearing colony in a screenhouse at the Embrapa Arroz e Feijão (Figure 3A). This isolate decimated the *Nezara* population. For an epizootic wave to be initiated, once it reaches the host, the pathogen's level of virulence is of utmost importance (Shapiro-Ilan et al. 2012). Therefore, we expected the BRM 67744 to show a high virulence to the tested stink bugs, what was not the case. This isolate was infectious to *O. poecilus* (75 % of mortality), but failed in controlling *E. heros* (16.9 % of mortality, similarly to the control).

This study also showed that *C. javanica* is host-specific and virulent to some groups of insects. *C. javanica* is the most prevalent fungi attacking whiteflies worldwide (Lacey et al. 1993, 1996 and 2008, Humber 2000, Faria & Wraight 2001, Quintela et al. 2016, Boaventura et al. 2021), but showed a median virulence to stink bugs and cotton boll weevil.

According to our results, *M. anisopliae* BRM 2335 was consistently more virulent than the other fungi, *Beauveria* and *Cordyceps*. This fungus was then selected for further studies with the cotton boll weevil *A. grandis* and the other species of stink bugs, mainly *E. heros*. The main pest in cotton production in Brazil is *A. grandis*, and due to its capacity to damage flower buds, an average number of 20 insecticide sprays are carried out per season (reaching almost 40 sprays per season in some areas) (Miranda 2006, Miranda & Rodrigues 2015, Belot et al. 2016). Among the control strategies for managing stink bugs, spraying with chemical insecticides has been the most used (Sosa-Gómez et al. 2020). However, controlling them only with insecticides has not been efficient, since insecticides are basically limited to three chemical groups: organophosphate, pyrethroid and neonicotinoid. This has led to their reduced susceptibility to insecticides and resulted in control failures (Sosa-Gómez et al. 2009).

Entomopathogenic fungi can be an important alternative to be used alone or in combination with chemicals for the management of these important pests. Our research group already tested *M. anisopliae* alone and in mixtures with sublethal concentrations of chemical insecticides for *A. grandis* and *E. heros* control (Vieira et al. 2022). The idea is to rapidly kill with chemicals, avoiding damage to the crops by the insects, and maintain the control for a long period with the fungus, since it starts to kill after 4-5 days.

CONCLUSIONS

1. For *Anthonomus grandis*, the most virulent isolate was *Metarhizium anisopliae* BRM 2335, followed by *Beauveria* BRM 14527 and BRM 67744;
2. *Metarhizium anisopliae* was consistently more virulent to the stink bugs *Euschistus heros*, *Oebalus poecilus*, *Oebalus ypsilongriseus* and *Thyanta perditor* than the other tested fungi, *Beauveria* and *Cordyceps*.

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