Horizontal transmission of the entomopathogenic fungus *Beauveria bassiana* (Unioeste 76 strain) among adults of *Euschistus heros* (Fabricius, 1798) (Hemiptera: Pentatomidae)

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ABSTRACT

The horizontal transmission of the entomopathogenic fungus *Beauveria bassiana* Unioeste 76 strain among adults of *Euschistus heros* was evaluated in the laboratory and greenhouse using sporulated cadavers and live insects contaminated as a source of inoculate. Adult insects in the laboratory bioassay either received treatments with dry conidia or were sprayed with an oily dispersion formulation, and the treatments were comprised of 10, 30, or 50% of contaminated insects. For the greenhouse bioassay, nylon cages were prepared, with soybean plants grown in pots. A total of 20 healthy brown stink bugs were released along with one or three sporulated cadavers of brown stink bugs, attached to the plant stem. In the bioassay with live insects, 20 stink bugs were released, and the treatments were comprised of 10, 30, or 50% contaminated insects (2:18, 6:14, and 10:10, fungus-treated insects : healthy insects). In all experiments, the control group was comprised only of healthy insects. Dead stink bugs were daily disinfested and transferred to a wet chamber for confirmation of death by fungus. Confirmed mortality was observed, which shows the occurrence of horizontal transmission. Mortality was higher when more contaminated insects were used in the population, in most treatments. The occurrence of transmission in the laboratory and the greenhouse emphasizes the potential of the Unioeste 76 strain as mortality agent and as an agent for maintaining the inoculum potential for the brown stink bug.

Keywords: auto-dissemination; biological control; microbial control; entomopathogen; soybeans.

INTRODUCTION

Soybean, *Glycine max* (L.) Merrill, is an oilseed with high protein content used in human diet and in the diet of animals, especially in the production of animal feed, bran, and soy oil (Gazzoni; Dall'agnol, 2018). Brazil is currently the largest world producer of soybeans, with a production of approximately 120 million tons (CONAB, 2022).

The brown stink bug, *Euschistus heros* (Fabricius, 1798) (Hemiptera: Pentatomidae), is currently considered the most important soybean pest in Brazil, due to the damages it causes and because it is the species with the highest frequency of occurrence and with the highest abundance in the country (Zerbino; Panizzi, 2019; Sosa-Gómez et al., 2020).

Chemical control is the most frequently used method to reduce the brown stink bug population (Bueno et al., 2013; Tuelher et al., 2018). However, the occurrence of populations resistant to chemical insecticides has become increasingly more frequent, and chemical control has become increasingly ineffective (Sosa-Gómez et al., 2020). Entomopathogenic fungi are known to be an alternative for the control of the brown stink bug. Recent studies proved the virulence of some *Beauveria bassiana* (Bals.-Criv.) Vuill. (Ascomycota: Hypocreales) strains with capacity for mass production in the laboratory (Dall Nora et al., 2021; Silva-Santana et al., 2022). In addition, there are several fungus-based products in Brazil for the control of the brown stink bug, although they are made from the same isolates (AGROFIT, 2022).

Fungi may also act by being transmitted inside a population, through the contact of a healthy insect with another insect, that is infected, or with sporulated cadavers (Hesketh et al., 2009). The capacity for horizontal transmission is a factor of great importance for its use as an agent for population control since infected individuals might be good vectors of the fungus in spreading the infection to healthy individuals and leading to the outbreak of epizootics in the field (Hajek; St. Leger, 1994; Furlong; Pell, 2001).

Even though the occurrence of horizontal transmission of *B. bassiana* has been proven between insects and mites (Cárcamo et al., 2015; Forlani et al., 2015; Conceschi et al., 2016; Mannino et al., 2018; Hassemer et al., 2020; Nascimento et al., 2020), there are no reports of its occurrence among pentanomids. Therefore, the present study aimed to evaluate the horizontal transmission of *B. bassiana* Unioeste 76 in adults of *E. heros*.

MATERIAL AND METHODS

The experiments were conducted in two stages: in the laboratory and the greenhouse. All insects used in the experiment were reared in the laboratory, maintained in plastic containers, and fed with a natural diet, adapted from Oliveira et al. (2016).

Horizontal transmission was evaluated based on two different sources of conidia:
- Transmission of the fungus from infected cadavers (fungus-carrying cadavers) to healthy individuals;
- Transmission of the fungus from live infected stink bugs (fungus-treated insects) to healthy individuals with either dry conidia or oil dispersion formulation.

The *B. bassiana* Unioeste 76 strain was used, previously selected as the most virulent against the brown stink bug (Silva-Santana et al., 2022). The fungus was multiplied in pre-cooked parboiled rice and plastic bags (Leite et al., 2003). Conidia were separated using a nylon sieve, and, after drying in a silica gel desiccator for seven days at 20 ± 1°C, they were separated using a metal 32-mesh sieve.

Preparing the fungus-treated insects

An oil dispersion formulation was prepared (a mixture of fungal conidia, emulsifier, and vegetable oil) (Faria; Wraight, 2007; Alves et al., 2015). The formulation was diluted in distilled water (final concentration of 10^9 conidia/mL). *E. heros* adults within 96 hours after emergence were dorsally identified in the pronotum with nontoxic yellow paint. The insects were submerged for 10 seconds in 4 mL of the formulation diluted in water and transferred to plastic containers. After drying, they were used in the laboratory and greenhouse experiments. Another group of identified insects was comprised of adult stink bugs and transferred to Petri dishes containing 0.5-g dry conidia for every 20 insects and slightly agitated for 60 seconds. The treated insects were called fungus-treated insects.

Preparing fungus-carrying cadavers

A suspension was prepared with dry conidia from the *B. bassiana* Unioeste 76 strain, in a 0.01% Tween 80 solution in distilled water (1×10^9 conidia/mL). *Euschistus heros* adults were immersed in the suspension for 15 seconds, transferred to plastic containers, and maintained in an acclimatized room (26 ± 2°C, relative humidity 60 ± 10%, and 14 hours of light). Dead insects were daily removed and submerged in 70% alcohol, 2% sodium hypochlorite, and distilled water, and transferred to a moistened filter paper in Petri dishes. The dishes were placed in a plastic container with polyurethane foam saturated with distilled water (wet chamber) and incubated for seven days to stimulate conidiogenesis (26 ± 2°C, relative humidity 60 ± 10%, and 14 hours of light). Cadavers were selected according to the uniformity of conidiogenesis. They were called fungus-carrying cadavers, and destined for the experiment in the greenhouse.

Transmission experiments in the laboratory

Fungus-treated insects (contaminated with the formulated fungus or with dry conidia) and healthy insects were transferred to plastic containers (8-cm height × 10-cm diameter) with a screened cap, at 2:18, 6:14, and 10:10 (fungus-treated insects : healthy insects ratio) ratios. In the control group, only 20 healthy insects were used. The insects were maintained in an acclimatized room (25 ± 1°C; 12 hours of photophase; relative humidity 60 ± 10%). Four replicates were prepared for each treatment and control group.

The experiment was daily evaluated for 10 days. The dead insects were removed, disinfected, and incubated in a wet chamber as previously described. The experiment was replicated twice.

Experiments in greenhouse

Plastic pots (4.9 L) were prepared with organic substrate and soil, in which soybean plants of the Brasmax Lança variety were grown and daily irrigated. When the plants reached the R1 phase, they were individualized in cages with nylon screens and metal structure (80-cm height × 30-cm diameter) and maintained in a greenhouse with controlled temperature. The pots were placed on a wooden structured and protected with plastic plates with water to prevent predator ants from attacking the stink bugs.

An Onset Hobo UX100-003 data logger was installed inside the greenhouse to monitor temperature and humidity continuously during the experimentation period.

Bioassay in a greenhouse with fungus-treated insects

The insects released into the previously prepared cages were already identified and treated with the fungus in the oil dispersion formulation at 2:18, 6:14, and 10:10 (fungus-treated insects : healthy insects) ratios. In the control group, only healthy insects were released. For each treatment and the control, five replicates were prepared.

Bioassay in a greenhouse with fungus-carrying cadavers

Another group of cages with soybeans plants was used with the fungus-carrying cadavers attached to the stems of soybeans using safety pins at random heights, at 1:20 and 3:20 (fungus-carrying cadavers : healthy insects) ratios per cage. Afterward, 20 laboratory-reared brown stink bugs healthy adults (96 hours after emergence) were released. In the control group, only healthy insects were released. Seven replicates were prepared for each treatment and the control.

In the two experiments, evaluations were daily performed for 10 days. The dead insects were removed, disinfected, and incubated in a wet chamber as previously described.

Evaluations and statistical analysis.

Total mortality data and confirmed mortality by fungus were corrected according to the Schneider-Orelli formula (Püntener, 1981), and, after that, they were analyzed regarding normality (Shapiro-Wilk's test, $p < 0.05$) and homogeneity of variance (Levene's test, $p < 0.05$). Means were compared using the HSD Tukey's test ($p < 0.05$).

RESULTS

Transmission experiments in the laboratory

In the cages with live insects contaminated by dry conidia, mortality varied between 73.5 and 100% and was higher in the presence of 10 fungus-treated insects ($F = 46.22$; $p < 0.05$), equivalent to 50% of the contaminated population (Table 1). Confirmed mortality ranged from 77.8 to 98.7% and was higher in the presence of 10 fungus-treated insects in the population ($F = 144.73$; $p < 0.05$) (Table 1). Total mortality of insects contaminated with the formulation varied between 10.7 and 53.4% and confirmed mortality ranged from 7.6 to 50.7%, with a significant difference among the three treatments ($F = 114.7$; $p < 0.05$). Confirmed mortality was higher when more fungus-treated insects were used in both laboratory bioassays (Table 1).

Comparing confirmed mortality from two different sources of inoculum, it was noticeable that transmission was higher when fungus-treated insects were infected with dry conidia than with the oil dispersion formulation, with significant difference in all treatments (Table 1).

Table 1. Mortality of adults of *Euschistus heros* in the presence of live insects treated with *Beauveria bassiana* Unioeste 76 strain, in laboratory.

Fungus-treated insects : healthy insects ratio	Mortality (%)	
	Dry conidia	Formulation (OD)
	Total mortality	
2:18	73.5 ± 10.1 Ab	10.7 ± 4.8 Bc
6:14	88.4 ± 2.7 Aab	36.8 ± 4.1 Bb
10:10	100.0 ± 0.0 Aa	53.4 ± 3.5 Ba
	Confirmed mortality	
2:18	77.8 ± 8.3 Ab	7.6 ± 1.4 Bc
6:14	87.2 ± 3.2 Aab	32.9 ± 3.1 Bb
10:10	98.7 ± 1.2 Aa	50.72 ± 2.7 Ba

OD: oily dispersion; means (± standard error of mean) followed by the same uppercase letter in the rows and lowercase letters in the columns do not differ from each other (Tukey's test, $p < 0.05$).
Source: Elaborated by the authors.

Bioassay in a greenhouse with fungus-treated insects

Total mortality of *E. heros* was observed in the treatments with two, six, or ten fungus-treated insects among the total of 20 insects, ranging from 36.2 to 53.9%, with no significant difference between treatments (Table 2).

Table 2. Mean mortality of *Euschistus heros* adults in the presence of live insects treated with *Beauveria bassiana* Unioeste 76 strain, on soybean plants, in a greenhouse.

Fungus-treated insects : healthy insects ratio	Mortality (%)	
	Total	Confirmed
2:18	36.2 ± 3.74 Aa	20.0 ± 1.58 Bb
6:14	57.1 ± 8.89 Aa	41.0 ± 3.32 Aa
10:10	53.9 ± 3.67 Aa	49.0 ± 3.67 Aa

Means (± standard error mean) followed by the same uppercase letter in the rows and lowercase letters in the columns do not differ from each other (Tukey's test, $p < 0.05$).
Source: Elaborated by the authors.

Comparing confirmed mortality among treatments, the treatment with two fungus-treated insects in the population obtained 20% of insects killed by the fungus (10% fungus-treated and 10% healthy). In the treatment with six fungus-treated insects, 41% of the insects were killed by the fungus (29% fungus-treated and 12% healthy). Finally, with 10 fungus-treated insects in the population, confirmed mortality was 49% (31% fungus-treated and 18% healthy). In the treatments with six and ten fungus-treated insects in the population, confirmed mortality was significantly higher than in the treatment with only two fungus-treated insects ($F = 4.91$; $p < 0.05$).

Temperatures between 12.1 and 32.1°C were observed, with daily mean temperature of 21.7°C and daily mean relative air humidity of 83.4%, ranging from 75.8 to 98.4% during the evaluation period.

Bioassay in a greenhouse with fungus-carrying cadavers

Mortality was 26.7% in the presence of one fungus-carrying cadaver, and the treatment with three cadavers had 29.7% mortality. There was no significant difference between the treatments (Table 3). In the presence of three fungus-carrying cadavers in the environment and 20 healthy insects, confirmed mortality was 31.1%, significantly higher than the treatment with one cadaver, which transmitted the fungus to an average of 19.4% of healthy insects. During the experimental period, the mean daily temperature was 24.4°C, with minimum and maximum temperatures of 19.4 and 34.6°C, respectively, and air relative humidity ranged between 74.8 and 90.6%, with a mean daily value of 83.8%.

Table 3. Mean mortality of *Euschistus heros* adults in the presence of fungus-carrying cadavers with *Beauveria bassiana* Unioeste 76 strain, on soybean plants, in a greenhouse.

Fungus-treated insects : healthy insects ratio	Mortality (%)	
	Total	**Confirmed**
1:20	26.7 ± 6.87 a	19.4 ± 3.49 b
3:20	29.7 ± 3.25 a	31.1 ± 4.19 a

Means (± standard error mean) followed by the same lowercase letters in the columns do not differ from each other (Tukey's test, $p < 0.05$).
Source: Elaborated by the authors.

DISCUSSION

Preliminary bioassays in the laboratory had higher values of confirmed mortality compared to those obtained in the greenhouse. This difference is likely because there is more control of environmental variables in the laboratory and insects were confined in containers with smaller space than the cages with plants, which promoted more encounters, that are directly proportional to the horizontal transmission of entomopathogenic fungi (Baverstock et al., 2010). Gregarious behavior and population density are factors that might have benefitted transmission (Forlani et al., 2015; Conceschi et al., 2016; Mannino et al., 2018).

It was also noticeable that mortality (both total and confirmed) in the treatment with formulated fungus was significantly lower than that obtained with dry conidia. The formulation might have influenced the time of conidial germination, growth, infection, and conidiogenesis. This contrasts with what was observed with dry conidia, which is the "naturally" found form of the fungus. Chergui et al. (2020) also found that different forms of application/contact or even sources of inoculum might have affected horizontal transmission.

The results also proved the occurrence of horizontal transmission of *B. bassiana* Unioeste 76 strain from contaminated *E. heros*, both sporulated cadavers and live individuals in the greenhouse. As horizontal transmission is an important aspect in the applicability of a biological fungus-based insecticide, its occurrence contributes to and emphasizes the potential of the Unioeste 76 strain of *B. bassiana* as a control agent for this species.

A previous study proved there was no conidiogenesis of *B. bassiana* on dead insects incubated above 34°C, while relative air humidity between 75 and 90% was considered suitable for conidia formation (Sosa-Gómez; Alves, 2000). Temperature and relative air humidity are therefore key factors in infecting insects. During the experimental period, the variation in the environmental conditions of the greenhouse might have interfered with the results. However, even under unstable conditions, the horizontal transmission of the fungus between *E. heros* adults emphasizes the susceptibility of the brown stink bug to the Unioeste 76 strain and this strain capacity to infect and colonize the host.

Despite relatively low values of confirmed mortality in the greenhouse, the occurrence of horizontal transmission might help the fungal environment dissemination. Also, it might cause an early outbreak of the epizootic (Membang et al., 2021) through the contamination of a healthy insect in contact with either a sporulated cadaver or an infected individual. It is worth noticing that transmission might occasionally be favored by air or water currents, which must be taken into consideration when using insect attract-and-infect devices using fungi (Vega et al., 1995). Moreover, individuals sprayed with fungi might be potentially transmitters of the pathogen to other individuals in the same area that had not been sprayed with enough conidia. Additionally, they might migrate to nearby areas, thus favoring fungal dissemination by area. These phenomena need to be assessed in future studies under field conditions.

CONCLUSIONS

There was a horizontal transmission of the fungus *B. bassiana* Unioeste 76 strain from contaminated insects, both live and dead *E. heros* adults. The transmission rate, in most treatments, was higher when there was a greater quantity of inoculum source in the population, with mortality being higher with longer exposure times to the fungus.

Arq. Inst. Biol., São Paulo, v.91, 1-8, e00262022, 2024

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