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# Entomopathogenic fungal activity against pupae and adult *Haematobia irritans* (Diptera: Muscidae)

Dinalva Alves Mochi, Antonio Carlos Monteiro *, Ana Carolina Ribeiro Machado, Luciana Yoshida

*Departamento de Produção Vegetal, Faculdade de Ciências Agrárias e Veterinárias (FCAV), Universidade Estadual Paulista (Unesp), Via de Acesso Prof. Paulo Donato Castellane, s/no, CEP 14884-900, Jaboticabal, SP, Brazil*

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ABSTRACT

The horn fly *Haematobia irritans* is one of the most important ectoparasites associated with grazing bovines. This study investigated the pathogenic activity of *Metarhizium anisopliae* (E9, IBCB425 and IBCB159), *Beauveria bassiana* (JAB06, JAB07 and AM09), *Isaria fumosorosea* (=*Paecilomyces fumosoroseus*) (IBCB133 and CB75) and *Isaria farinosa* (=*Paecilomyces farinosus*) (CG189 and CG195) fungi isolates on pupae and adult *H. irritans*. Groups of 20 pupae and 30 adult flies were respectively bathed and sprayed with fungal isolate suspensions containing $10^6$, $10^7$ and $10^8$ conidia $ml^{-1}$ in bioassays conducted in laboratories. In both assays the adult flies were fed bovine blood for 15 days, and death rates were assessed daily. The E9 and IBCB425 *M. anisopliae* isolates caused pupae death at concentrations of $10^7$ and $10^8$ conidia $ml^{-1}$, and the JAB07 and AM09 *B. bassiana* isolates caused higher pupae mortality at a concentration of $10^8$ conidia $ml^{-1}$. *I. farinosa* isolates were the most effective considering pupae mortality, with the CG195 inducing more deaths (56.6%) in the $10^8$ conidia $ml^{-1}$ concentration suspension. Adult flies were more susceptible to the fungi's pathogenic action, since the E9 isolate of *M. anisopliae* and all of the *B. bassiana* induced death in 100% of the flies at the $10^8$ conidia $ml^{-1}$ concentration suspension. The *I. fumosorosea* and *I. farinosa* isolates, on the other hand, were less effective in controlling adult flies. In both stages, but mostly the adult phase, pathogenicity was great at higher conidial concentrations.



## 1. Introduction

Of all external bovine parasites, the horn fly *Haematobia irritans* (Diptera: Muscidae), stands out for the damage and concern it has caused worldwide regarding cattle production. In the U.S.A., *H. irritans* is responsible for millions of dollars in damage (Kunz et al., 1991), and it is considered one of the main causes of loss in animal production (Byford et al., 1992). In Brazil, this parasite causes US$ 150 million in losses per year to the cattle business (Grisi et al., 2002).

The financial loss associated with the presence of this bovine parasite is caused by the host's intense stress resulting from the parasite's relentless and constant feeding off its blood, which interferes with animal weight gain. Steelman et al. (1991) estimated that, for every 100 flies on an animal, it is possible to expect 8.1 kg less in weight gain over a one-year period. Large fly populations also generate losses in the leather industry due to skin damage from multiple bites and secondary infections (Guglielmone et al., 1999).

Control of *H. irritans* is based almost exclusively on the use of chemical insecticides, a fact that inevitably leads to a selection of resistant individuals, lessening the efficiency of control methods (Barros et al., 2002). Treatment with these chemicals leaves residues in the meat reducing market value

* Corresponding author. Tel.: +55 16 32092652; fax: +55 16 32024275.
*E-mail address:* montecar@fcav.unesp.br (A.C. Monteiro).

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and causing rejection in export markets. Furthermore, it has had an impact on the insect's natural enemies (Cook and Gerhardt, 1977), such as parasitoids, predators and microorganisms responsible for reducing fly populations.

Each environment boasts a vast diversity of microorganisms. Of these, entomopathogenic fungi stand out because they occur naturally on more than 300 insect species, in their many different life cycle stages. These include many important insect pests (Alves, 1998). According to this author, the great variability of these fungi allows a selection of highly virulent isolates, specific or not, with characteristics that favor their use in biocontrol.

Many studies have reported the efficacy of these fungi on dipterans such as the Mediterranean fruit fly (*Ceratitis capitata*) (Castillo et al., 2000; Mochi et al., 2006) and the domestic fly (*Musca domestica*) (Barson et al., 1994). Although Steenberg et al. (2001) reported the natural occurrence of entomopathogenic fungi on adult horn flies in grazing areas, the first studies on the pathogenic effect of the fungi on these flies were only recently published (Angel-Sahagún et al., 2005; Lohmeyer and Miller, 2006), and many others aspects of this phenomenon require further investigation.

Due to the economic importance of horn flies and the implications of insecticide use on environmental and human health, the need for studies to establish methods for biological control is growing. Control of *H. irritans* populations should avoid financial loss, yet not cause environmental imbalance. The objective of this study was to evaluate the action of different entomopathogenic fungi species, isolates and concentrations on pupae and adults of *H. irritans* under laboratory conditions.

## 2. Materials and methods

### 2.1. Fungi

A description of the fungi species used in this study is found in Table 1. These entomopathogenic fungi were maintained in stock cultures at 4 °C, in test tubes containing a potato-dextrose-agar (PDA).

### 2.2. Fungal preparation

For the assays the isolates were cultivated in Petri dishes containing PDA and maintained in incubators at $27 \pm 0.5$ °C for 20 days. Isolate viability was assessed according to the method described by Francisco et al. (2006), and was consistently higher than 95%.

Spores from the colony surface were aseptically transferred to tubes containing a mixture (1:1) of 0.89% (p/v) NaCl solution and 0.1% (v/v) Tween 80® solution. After vigorous agitation with an electrical tube agitator, all suspensions were standardized using a Neubauer chamber.

For the *H. irritans* pupae assay, conidia concentrations obtained varied from 1.2 to $2.0 \times 10^8$ conidia $ml^{-1}$. For the adult assays, suspensions were held at a standard between 1.1 and $1.5 \times 10^8$ conidia $ml^{-1}$. In both assays, as well as in all of the isolates, $10^7$ and $10^6$ conidia $ml^{-1}$ concentrations were obtained from all suspensions by serial dilution.

### 2.3. Capture of H. irritans specimens

The flies used in the study were collected at a cattle farm located in the county of Borborema, São Paulo State, Brazil. For the pupal assays, adult flies were captured with a net passed over the backs of cattle or other areas of their bodies infested with flies. The flies were confined in plastic bags until eggs were produced. For obtaining pupae, the eggs were collected in plastic bags and transported to feces in environments close to saturated humidity. They were then accommodated in uncovered Petri dishes (60 mm × 15 mm). These dishes were then put into larger dishes (90 mm × 15 mm), covered, and kept at $27 \pm 0.5$ °C in an incubator. After five days of incubation the larvae were seen exiting the mass of feces, searching for a dry spot (in the larger dish) to pupate. The pupae formed were collected within 48 h, when their number was sufficient to start the bioassays.

For the adult assay, adult flies were captured as already described and maintained for 12 h in styrofoam boxes (340 mm × 225 mm × 306 mm). A window covered with thin netting (*voile*) was made in one side of each box and, on the side parallel to that, a circular opening was made and closed off with a sleeve of the same material for manipulating the flies. Another window was made in the top side of each box. It was closed off with nylon netting (1 mm) over which cotton imbibed with distilled water and cotton soaked with fresh bovine blood for the flies to feed off were placed and then covered with plastic to maintain humidity.

**Table 1**
Fungi species and different isolates used in the *Haematobia irritans* pupae and adult fly assays.

| Fungi | Isolates | Source (host insect) | Geographical area (state and country) |
|---|---|---|---|
| *Metarhizium anisopliae* | E9 | *Deois flavopicta* | Espírito Santo, Brasil |
| | IBCB425 | Soil | São Paulo, Brasil |
| | IBCB159 | Soil | Paraná, Brasil |
| *Beauveria bassiana* | JAB06 | *Atta sexdens sexdens* | São Paulo, Brasil |
| | JAB07 | *Musca domestica* | São Paulo, Brasil |
| | AM09 | *Deois incompleta* | Amazonas, Brasil |
| *Isaria fumosorosea* | IBCB133 | Soil | São Paulo, Brasil |
| | CB75 | Not identified | São Paulo, Brasil |
| *Isaria farinosa* | CG189 | Soil | Distrito Federal, Brasil |
| | CG195 | *Chlosyne lacinia saundersi* | Paraná, Brasil |