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Interaction and activity of nematophagous fungus *Duddingtonia flagrans* on *Haematobia irritans* (Diptera: Muscidae)

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***Haematobia irritans*, also known as the horn fly, is a "plague" that spreads rapidly among cattle herds, especially in the southeast of Brazil. The aim of this study was to evaluate the interaction and activity of nematophagous fungus *Duddingtonia flagrans* (AC001) on *H. irritans* (Diptera: Muscidae). The experiment was conducted using the nematophagous fungus (AC001), which is harmless to animals, humans, and the environment. At the beginning of the experimental trial, samples of adult *H. irritans* were collected manually, directly from the dorsal region of naturally infested cattle of the Nelore breed. The flies where divided into two groups: groups of adult flies treated with AC001 (treated group) and groups of flies that did not receive treatment (control group). During the trial, the experiment was monitored daily for five days and the results were recorded. The results showed that the AC001 fungal isolate grew, colonized, and consequently caused the death of the flies in the treated group, while in the control group, no interaction or growth was observed, and the flies remained alive. It was concluded that the fungus *D. flagrans* interacted with adult flies, taking into consideration a "possible attack" by chitinase enzymes, since the fungal isolate drew on the chitin contained in the exoskeletons of the insects. In addition, attention should be focused on new studies that can demonstrate that, in the future, biological control of the horn fly could be an effective and safe method when compared with other methods.**

Key words: Biological control, *Duddingtonia flagrans*, horn fly, Nelore, Brazil.

INTRODUCTION

The cattle herd in Brazil consists of approximately 212.3 million heads, an impressive number that puts the country in second place worldwide for the herd with the largest number of cattle, accounting for 22.5% of the

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world's total herd. There are various states that produce cattle of the Nelore breed, and the state of Espírito Santo currently has 2,295,624 heads, accounting for approximately 1.1% of the Brazilian herd (IBGE, 2014). However, livestock losses caused by ectoparasitic infestations are exorbitant and can reach an annual cost of approximately US$ 2.26 billion (Byford et al., 1992), and the situation is no different in the Brazilian state of Espírito Santo. Among the parasites that are harmful to cattle health is the horn fly (*Haematobia irritans*), which causes worldwide losses of approximately US$ 730 million and national livestock losses of US$ 150 million (Grisi et al., 2002).

In Brazil, the horn fly (*H. irritans*) is a veritable "plague" that ravages rural producers on a daily basis. This fly is a hematophagous dipteran of the phylum Arthropoda, family Muscidae, measuring between 3 and 5 mm in length, preferentially parasitizing bovines, day and night, except during a short egg-laying period. The horn fly's hematophagous activity is not its most damaging characteristic (Brito et al., 2005), but the painful (irritating) and incessant bites cause severe stress in the animals. Consequently, this "irritating and painful" activity of the fly is reflected in lower animal productivity, resulting in economic losses for the producer (Chagas et al., 2010). Bianchin et al. (2004) reported that the weight loss of the animals may reach 10%, while the bites directly damage leather quality (Saueressig, 1992; Guglielmone et al., 1999).

Control is not restricted to the use of "chemical insecticides"; however, parasitic resistance as a direct consequence of the abuse of these drugs is one of the aggravating factors (Barros, 2004). Several studies have been conducted to find a possible biological controller for *H. irritans*, but the literature on this subject is scarce (Mochi, 2009; Braga et al., 2016). The use of nematophagous "eating" fungi has been widely studied and in recent years, it has been found that growing these nematophagous fungi in chitin-based culture media could "mold" their predatory activity for combatting arthropods, demonstrating the use of chitin as a source of nutrition (Braga et al., 2013). On the other hand, some fungi that previously only destroyed infective larvae of helminth gastrointestinal parasites may very well produce extracellular enzymes such as chitinases, which promote the destruction of the exoskeletons of arthropods (Soares et al., 2014). The aim of this study was to evaluate the interaction and activity of nematophagous fungus *D. flagrans* on *H. irritans* (Diptera: Muscidae) in Nelore cattle in the Southeast region of Brazil.

MATERIALS AND METHODS

Location and animals

The experimental trial was conducted on a ranch that breeds Nelore cattle in the state of Espírito Santo, Brazil. The animals used in the experiment were of the Nelore breed and approximately 18 to 24 months of age. All the animals used were raised in *Brachiaria decumbens* pastures and were naturally infested with *H. irritans*.

Fungus

A fungal isolate of *Duddingtonia flagrans* (AC001) was used. This fungus came from the mycology collection of the Parasitology Laboratory of the Department of Veterinary Medicine of the Universidade Federal de Viçosa (UFV), in Viçosa, Minas Gerais. Then, in a continuous transfer to Petri dishes containing chitin-based culture medium, 2% chitin agar (CA), transplants of 3 cm in diameter were transferred to achieve growth and subsequent conidia production.

Trial

Specimens of adult horn flies were collected from the dorsal region of Nelore cattle with a fine-mesh wire net. The captured flies were then placed in Erlenmeyers flasks, well-sealed with fine webbing. Based on the methodology of Svedese et al. (2012), a source of food for the dipterans and cotton moistened with water to maintain humidity in the flask were added to promote survival conditions for the duration of the experiment. They were divided into two groups: groups of adults flies treated with AC001 (treated group) and groups that did not receive treatment (control group). Approximately 6 repetitions were performed per group. During the trial, the experiment was monitored daily for five days and the results were recorded.

In the groups of treated flies, 1 ml of fungal solution containing *D. flagrans* (AC001) conidia was sprayed according to the methodology modified by Braga et al. (2016). The control groups did not receive this treatment. The samples were observed daily, for five days, at the Parasitology Laboratory of The Universidade Vila Velha, for (a) interaction and/or fungal growth in both groups and (b) death of the flies in both groups. After this period, an evaluation of evolution was conducted in which it was possible to observe fungal growth and its action on the horn flies. In addition, to verify possible fungal growth, isolation to a Petri dish with agar-agar culture medium was performed for 7 days to confirm the presence or absence of the AC001 isolate by means of its characteristics (Braga et al., 2016).

RESULTS AND DISCUSSION

The experimental part of this work was conducted under laboratory conditions and at the end of the trial, it was demonstrated that there was interaction and/or growth, colonization of the *D. flagrans* (AC001) fungus on the horn fly specimens in the treated groups, which can be "viewed" as pioneer research. In the control groups, neither growth nor colonization of the fungus was observed under the same conditions. As previously mentioned, the "verification" of the interaction of the isolate tested was observed with the death of the arthropods (Figure 1a to c). The literature has shown that some fungi of the entomopathogenic group have been used in horn fly control experiments both in the laboratory and in the field (Mochi, 2009); however, this is the first report of interaction of a predator group fungus in the possible control of this arthropod. For an understanding of the different activities of entomopathogenic and nematophagous predator fungi, it is first and foremost

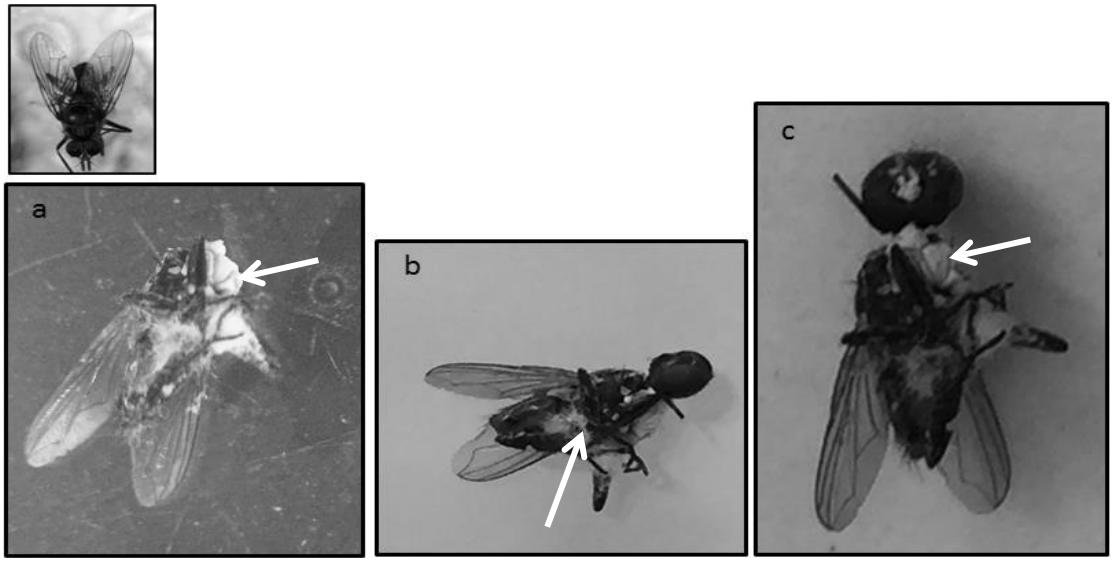

Figure 1. (a-c) Growth, colonization and destruction (white arrow) of hornflies by the fungus *Dudfingtonia flagrans* (AC001) in treated groups.

necessary to demonstrate that the action of predatory fungi is restricted to the infectious forms of helminths (Araújo et al., 2008; Silveira et al., 2017), although the production of enzymes that cause destruction of the "target (organism)" is not restricted to interaction with nematodes. Along this line of reasoning, Braga et al. (2013) isolated a chitinase enzyme during the interaction of *D. flagrans*, a nematophagous predator, with female equine ticks *Amblyomma cajenense* and on that occasion, the authors reported the production and characterization of that enzyme under laboratory conditions. However, as previously mentioned, the culture medium serves not only for fungal growth, but also as a "modulator" of enzymatic production. For example, rich casein-based media produce a greater quantity of proteases and chitin-based media produce a greater quantity of chitinase (Soares et al., 2013, Braga et al., 2014).

Braga et al. (2015) reported the production of extracellular enzymes, chitinases, by the fungus *D. flagrans,* in the presence of nematodes, suggesting that the infection is due to the fact that the eggs of the nematodes are rich in chitin. Thus, which confirms the fungal action on *H. irritans*, since the exoskeletons of the arthropods are made up of these carbohydrates. The authors suggest that the chitinase produced by the fungus *D. flagrans* was critical for the fungal colonization of the dipteran, emphasizing its entomopathogenic action. This fact corroborates the report of Braga et al. (2016) where the colonization occurs from the supplementation of the fungus in a medium based on chitin, a sugar present in insects like the horn fly.

According to Almeida et al. (2010), *H. irritans* has a parasite-host relationship with cattle, causing great damage to animal production. Weight loss in the animals and reduced milk production (Campbell et al., 2001) have already been documented in herds infested with this ectoparasite. Higher rates of infestation have been confirmed in animals from the cross-breeding of *Bos taurus* with *Bos indicus* (Bianchin et al., 2004, 2006). *H. irritans* prefers dark-haired bovines as hosts, but can be found in lighter-haired animals (Franks et al., 1964). Other factors related to horn fly infestation in cattle include body temperature, light intensity, carcass size, and age of the animals (Morgan, 1964; Steelman et al., 1996; Fordyce et al., 1996). Lima et al. (2002) observed a preference of the ectoparasites for certain regions of the host's body (scapular, costal, and interscapular) and suggested the existence of animals that are less-resistant to infestations.

Excessive use of chemical substances as an alternative for controlling these parasites has become unfeasible because it leads to the development of resistance to the active ingredient. The presence of this resistance in the parasite population leads to uncontrolled use by the producer, incurring high production costs, increasing the risk of human and environmental contamination and the chances of there being traces in the meat and milk (Barros, 2004; Chagas et al., 2010).

The interaction of the nematophagus fungus *D. flagrans* with horn flies stands out as a new biological control alternative due to its lack of toxicity both to the animals and to man, to its economic feasibility, and to its contribution to the reduction of resistant parasites. It is worth noting that, by properly controlling herd infestations, the productive gains will be more evident, benefitting producers in general (Chagas et al., 2010).

The fungus *D. flagrans* was effective in "colonizing and

killing" the horn flies and is an innovative biological control alternative. This horn fly control alternative could very soon contribute to decreasing the use of chemical compounds, reducing economic losses from Nelore cattle, and improving animal well-being, so important to the national economy. It is important to note that this fungus is environmental, non-toxic for handling, and does not leave residues on meat or cause any type of environmental pollution. However, the need for new research in the state of Espírito Santo in the Southeast region of Brazil must be emphasized, as this area has not yet been well-explored and the biological control of *H. irritans* in Nelore cattle is a pioneering study.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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