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Susceptibility of adult and larval stages of the horn fly, *Haematobia irritans*, to the entomopathogenic fungus *Metarhizium anisopliae* under field conditions

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ABSTRACT

The efficacy of *M. anisopliae* strain E9 as a biological insecticide for the adult and larval stages of *H. irritans* was assessed under field conditions. To assess larvicidal activity, nine heifers were randomly divided into three groups, which were maintained separated from each other. The first group ingested fungal spores encapsulated in alginate pellets. The second group ingested *in natura* spores that were grown on sterilized rice. In both groups, each animal received three meals a day, with each meal containing 2×10^{10} conidia. The third group received no treatment and was used as a control. Fecal samples from manure and whole dung pats were collected from each of the three separate pastures on the day that the animals were allocated and on days 1, 3, 6, 9 and 12 afterwards. The fecal samples were tested for the presence of fungal colony forming units (CFU), and the emergence of horn flies was observed in the dung pats. Significantly less ($P < 0.01$) adult horn flies were found in dung pats of the group treated with encapsulated fungi (11.7) than in those from the heifers treated with conidia *in natura* (27.9) or from the control group (29.5). The fecal samples of the treated animals presented significantly higher numbers of *M. anisopliae* CFUs then those from the untreated controls. We found that on day 9 fecal samples from animals given microencapsulated conidia had significantly higher CFUs than those from animals treated with conidia *in natura*. To assess adulticide activity, four heifers were sprayed with a suspension of 3×10^{10} conidia l^{-1} of *M. anisopliae*, and four control animals were sprayed with the same solution without conidial content. Four sprayings were done at five-day intervals, and all animals were photographed daily to observe the quantity of flies present. After the second spraying, we observed an average of 22.9 flies per animal; untreated heifers had an average of 43 flies per animal; thus, the treatment significantly ($P < 0.05$) decreases fly infestation. The results obtained from both tests show that *M. anisopliae* strain E9 has a pathogenic effect on *H. irritans* larvae in bovine manure when administered orally and on adult fly infestations when applied as a spray on the hosts.

1. Introduction

Entomopathogenic fungi are considered to be promising biological control agents for insect pests. Their efficiency, however, could be affected by many environmental factors, such as solar radiation, temperature and humidity (Inglis et al., 2008). According to Thomas and

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Jenkins (1997), environmental temperatures in particular can adversely affect the biological parameters of *Metarhizium anisopliae*.

The *Haematobia irritans* horn fly is a bovine parasite that constantly feeds off its host's blood for its entire adult life. Fertilized females deposit their eggs on fresh bovine feces, where the immature development of the fly occurs (larvae, pupae and eggs). Under favorable environmental conditions, the larvae will eclode after 1 or 2 days (Foil and Hogsette, 1994). The entire development of the larvae, from instar 1 to 3, occurs in 4–8 days (Lysyk, 1992); the following pupae stages develop in the ground (Barros, 2002) in 6–8 days until the adult emerges (Foil and Hogsette, 1994). This insect is an important external parasite (Barros et al., 2007; Li et al., 2007), and infestations are controlled almost exclusively by use of chemical insecticides that are applied directly onto the hosts by dipping or spraying. According to Byford et al. (1999), however, the development of parasite resistance to these products has become a problem of critical importance.

Strategies for controlling *H. irritans* infestations are usually directed at adult flies that infest the bovine hosts or the immature stages that are found in manure. During the day, the temperature on the surface of a bovine's body can vary from 28 to 40 °C (Monty and Garbareno, 1978), which could probably have a negative effect on biological control agents (Polar et al., 2005). The use of *M. anisopliae* for biologically controlling external parasites by spraying bovine hosts is a strategy still to be explored. Studies on the efficacy of this strategy for controlling the *Rhipicephalus (Boophilus) microplus* tick have delivered promising results (Correia et al., 1998; Polar et al., 2005; Alonso-Díaz et al., 2007); the control of *H. irritans* by this strategy has not yet been unexplored.

Entomopathogenic fungi can be nonviable in ruminants when administered orally, due to the complexity of the host digestive process. The acidic ruminal and abomasal pH (Merchen, 1998), the high temperatures due to fermentation (Andriguetto et al., 1981) and the microbial activity in the bovine rumen (Nogueira Filho et al., 2004) can compromise fungal survival. Encapsulation can protect conidia against the digestive aggressions, and the oral administration of microencapsulated conidia from fungi with nematocidal activity has been used previously to control bovine and ovine gastrointestinal parasites (Araújo et al., 2004; Graminha et al., 2005). Promising results have been obtained using sodium alginate to encapsulate conidia from entomopathogenic fungi, and techniques for the microencapsulation of mycelium have been described by Pereira and Roberts (1990).

To effectively control horn flies using entomopathogenic fungi, we need to develop methods that allow conidia to be administered orally but remain viable when eliminated in the fecal matter; additionally, we need to identify strains that affect adult flies when applied to the hosts externally.

The present study was done under field conditions. We assessed the susceptibility of *H. irritans* larvae to orally administered *M. anisopliae*, using conidia encapsulated in alginate pellets and conidia grown on rice *in natura*. We also evaluated the susceptibility of adult flies after hosts were externally given conidia in a solution as a spray.

2. Materials and methods

2.1. Fungi

M. anisopliae strain E9 was used in this study. The isolate was cultivated in Petri dishes containing a potato-dextrose-agar (PDA) medium incubated at 27 ± 0.5 °C for 20 days. Viability was measured as 100% according to the methods described by Francisco et al. (2005). Mass production was obtained with a modified methodology described by Alves and Pereira (1998).

2.2. Preparation of micro pellets

Micro pellets were prepared using the technique described by Graminha et al. (2005). Three different solutions were prepared. A bacteriostatic agent was prepared by dissolving 0.6 g of sodium benzoate in 10 ml distilled water. A sodium alginate solution was prepared by adding 6 g of sodium alginate gradually to 210 ml distilled water under constant manual agitation until hydration was complete. Finally, a conidia solution was prepared at a concentration that would allow each animal to receive 2×10^{10} *M. anisopliae* conidia. The dehydrated conidia was then added to a solution of 0.15 ml polysorbate 80 dissolved in 10 ml distilled water under constant agitation for several minutes until a homogeneous suspension was obtained; 6 g oatmeal was then added.

The bacteriostatic solution and the conidia suspension were mixed into the sodium alginate solution and the volume was then brought to 300 ml with distilled water. The resultant emulsion was then transferred to a funnel and dripped into a calcium chlorate solution (1000 ml); both solutions were under constant agitation (adapted from Fravel et al., 1985). Micro granules formed in the solution and were separated with a sieve, washed three times in distilled water and then transferred to a flat sieve where they were dried under forced ventilation at room temperature.

2.3. Control of H. irritans under field conditions

This study was carried out at Sítio Córrego do Pavão, Borborema county – São Paulo state, Brazil, located at 21°35′43.3″ South and 49°02′51.3″ West, approximate altitude 448 m. The area has a hot, subtropical climate and dry winters. Larvicidal studies lasted from April 10th to 22nd 2008, and the adulticide studies from April 10th to May 1st 2008. Environmental temperatures and humidity were registered at 9 a.m. and again at 3 p.m. daily during the entire experimental period using a digital thermal-hygrometer (Fig. 1A and B).

2.3.1. Larvae control in manure

Nine heifers of mixed breed, between two-and-a-half and four years of age and naturally infested by *H. irritans* were randomly distributed into three separate 300 m^2 pastures, which were 50 m apart from each other. Each closed off area was composed of *Brachiaria brizantha* grass and had a medium sized tree in its center. After