Veterinary Parasitology 212 (2015) 350–355

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

# Efficacy of the entomopathogenic fungi *Metarhizium anisopliae* in the control of infestation by stable flies *Stomoxys calcitrans* (L.), under natural infestation conditions

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ARTICLE INFO

*Article history:*
Received 17 March 2015
Received in revised form 22 June 2015
Accepted 1 July 2015

*Keywords:*
*Stomoxys calcitrans*
*Metarhizium anisopliae*
Natural infestation
Biological control

ABSTRACT

The objective of this study was to evaluate the efficacy of an isolate of *Metarhizium anisopliae* applied by aspersion to control of *Stomoxys calcitrans* flies in dairy cattle naturally infested. Was applied by aspersion an aqueous formulation of *M. anisopliae sensu lato* (Ma134), at a concentration of $1 \times 10^8$ conidia/ml, four times with seven day intervals, on a group of eight Holstein cows; a control group of eight Holstein cows, received a water solution with Tween 80 (0.1%). The average number of flies per animal was estimated one day before each application, and then daily counts were done in both groups. The effectiveness of the formulation was calculated using the Abbott's formula. At the same time, defensive behaviors of stamp/kicks and tail movements were evaluated daily, estimating relative frequency per hour. The Ma134 formulation had an infestation control efficacy of 73%, taking into consideration the four study weeks. The population reduction effect was observed since the first week post-application ($p < 0.05$), and the effect increased with the subsequent applications. Defensive behaviors were reduced beginning from the first application, reaching a reduction of 66% and 70%, respectively, during the four weeks of study. These results demonstrated the effectiveness of the formulation to control infestation by *S. calcitrans,* as well as reduce defensive behaviors which involves the infestation.



## 1. Introduction

The stable fly, *Stomoxys calcitrans* (L.), is recognized as the most important ectoparasite in cattle maintained in confined systems such as dairy farms and feedlots. Infestation by these ectoparasites derives in diverse negative effects on cattle health and productivity because of their hematophagous habits, which besides causing direct damage by their blood sucking, cause behavior alterations and irritation with consequent energy consumption in order to repel these insect attacks. Its effects are also evident in a decrease of weight gain and decrement in milk production, in addition to can transmit various pathogens (Foil and Hogsette, 1994; Cruz-Vázquez, 2012; Taylor et al., 2012). Infestation control represents an important challenge to cattlemen since the main alternative is insecticide application whose indiscriminate use has environmental consequences as well as the development of resistance in different populations of *S. calcitrans* (Kunz and Kemp, 1994; Cilek and Greene, 1994; Marcon et al., 1997; Pitzer et al., 2010; Salem et al., 2012), situation that limits its use. Entomopathogenic fungi represent a non-chemical alternative control method that has been scarcely studied for hematophagous flies; some studies have shown that the stable fly is susceptible to infection by strains of *Beauveria bassiana* and *Metarhizium anisopliae,* under laboratory conditions (Watson et al., 1995; Moraes et al., 2008, 2010; López-Sánchez et al., 2012). Nevertheless, there is insufficient information on its performance in naturally infested cattle.

The objective of this study was to evaluate the efficacy of an isolate of *M. anisopliae* applied by aspersion to control of adult stages of *S. calcitrans* in naturally infested dairy cattle.

## 2. Materials and methods

### 2.1. Study site

This study was carried out in the dairy cattle production unit of the Instituto Tecnológico El Llano Aguascalientes, which is located

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http://dx.doi.org/10.1016/j.vetpar.2015.07.003
0304-4017/

in the El Llano municipality of the State of Aguascalientes, Mexico. The site is at 2020 m above sea level and has a dry semi-warm climate with summer rains. The farm maintains Holstein cattle in a free confinement system, which is characterized by open pens, with dirt floors, shaded area, and concrete drinking and feeding troughs, providing 40 $m^2$ per animal; in the region, the stable fly infestation is seasonal, being more intense from the middle of summer to the middle of autumn (Cruz-Vázquez et al., 2000, 2004, 2007).

### 2.2. M. anisopliae isolate and mass production

*M. anisopliae sensu lato* 134 (Ma134), fungus was originally isolated from lodging pens in the dairy production unit of the Instituto Tecnológico El Llano Aguascalientes; this isolate has shown high pathogenicity for *S. calcitrans* adults when evaluated under laboratory conditions (López-Sánchez et al., 2012). The isolate was cultured on Sabouraud dextrose agar enriched with 1% yeast extract (Watson et al., 1995) containing 500 ppm chloramphenicol and incubated at 25 ± 1 °C for 21 days, in a 12:12 h light/darkness regime. Conidia were harvested by scraping and suspended in sterile distilled water containing 0.1% (v:v) Tween 80, and homogenized on a vortex mixer. Spore viability, which exceeded 98%, was determined by seeding 100 μl of conidial suspension on Sabouraud dextrose agar and colonies were counted 48 h later. Mass reproduction of the isolate was carried out on grains of rice in accordance with the methodology outlined by Ángel-Sahagún et al. (2010).

### 2.3. Test design

Two experimental groups were formed with eight lactating cows each that were maintained in different lodging pens; each one whit surface of 500 $mt^2$. The pens were separated each other by 50 m. All cows were identified with large numbers (15 × 15 cm) painted on their flanks so they could be observed at a distance. The supplied ration was made of corn silage, oats hay, and a commercial concentrated feed with 14% protein; ingredients were mixed to be offered as an integral ration that was supplied daily on two occasions. The animals did not receive any treatment to control fly infestation during the year prior to this experiment. The study was started on August 21, before the most important fly population peak of the season that occurs in the region (Cruz-Vázquez et al., 2000, 2004, 2007).

The treated group received the application of the aqueous formulation of Ma134 fungi adjusted to a concentration of $1 \times 10^8$ conidia/ml, with water plus Tween 80 (0.1%), by aspersion using a back sprayer with a cone-type nozzle and pressure of about 40 $lb/in^2$, on four occasions with seven day intervals between treatments, applying 5 L per animal on each occasion. The control group received a suspension of water plus Tween 80 (0.1%) with the same schedule and in the same manner than the treated group. Applications were carried out from 7 to 8 pm to avoid the exposure to sunlight. Application intervals were established based on *in vitro* experiences with the same isolate (López-Sánchez et al., 2012).

### 2.4. Estimation of S. calcitrans infestation

The average number of flies per animal (Infestation index) was estimated by direct counting of adult flies found resting or feeding on the front of the legs of the animals, by lateral observation, with the aid of binoculars when necessary (Thomas et al., 1989; Cruz-Vázquez et al., 2000, 2004, 2007) counting was done daily between 14:00 and 16:00 h, always by the same person from the day of the first application until the end of the study. This person was not aware the experimental status of the animals.

**Table 1**
Infestation index and efficacy of the Ma134 aqueous formulation applied on Holstein cows naturally infested with *S. calcitrans*.

| Study week | Treated group | Control group | Efficacy (%)[1] |
|---|---|---|---|
| Day 0 | 67 a | 65 b | NA |
| 1 | 56 a | 68 b | 17.65 |
| 2 | 38 a | 68 b | 44.12 |
| 3 | 29 a | 61 b | 52.46 |
| 4 | 18 a | 40 b | 55.0 |

Different literals on the line indicate statistical differences ($p < 0.05$).
[1] Reduction percentage of the average infestation index.
NA — does not apply.

### 2.5. Presence of defensive behavior

Behavior of individual cows was recorded during nine 5 min episodes every 15 min during the same observation periods. With this schedule, behaviors of the cows were assessed 252 times (28 days × 9 times per day). Defensive behaviors recorded included: stamp/kicks of the front or hind legs (which were recorded as one event when cows raised their legs or hit the ground), and tail movements (defined as the travel of the tail from its resting position to one side), if the tail re-crossed its resting position, this was recorded as another movement (Dougherty et al., 1993; Vitela et al., 2007). Observations were conducted between 14:00 and 16:00 h and were carried out by the same person, different from the one carrying out the fly count, and he was not aware the experimental status of the animals.

### 2.6. Analysis of information

Infestation index was estimated daily and per week for each group, on each of the four study weeks. These values were analyzed using ANOVA and Student's- *T* test ($p < 0.05$) in order to detect differences between groups each week. Efficacy of the Ma134 formulation was estimated applying Abbott's formula (Hillerton et al., 1984), in order to identify the percentage of reduction of the average infestation index for each evaluated week (Galindo-Velasco et al., 2015).

The sum of behaviors recorded during the nine 5 min daily observation episodes was transformed to behaviors per hour by a proportion relationship (Vitela et al., 2007). This value was obtained by daily and weekly behavior in each group, in each of the four study weeks. These values were subjected to Student's- *T* test ($p < 0.05$) in order to detect differences between groups in each week. The efficacy of the formulation Ma134 was estimated applying Abbott's formula (Hillerton et al., 1984), in order to identify the percentage of reduction of each one of the studied behaviors during each week of evaluation.

## 3. Results

Daily infestation index curve in the group of treated animals showed a reduction tendency from the first days after they received the first application of the Ma134 formulation as can be observed in Fig. 1. The group of cows treated with Ma134, showed 73% efficacy in infestation control, taking into consideration the four weeks of study duration, reducing the infestation average from 67 to 18 flies per animal. The population reduction was observed beginning on the first week of study, although it became more important as the other applications of the formulation were applied at 7, 14 and 21 days. The control group, began the study with an average infestation index of 65 flies/animal, maintaining these figures between 68 and 60 until the third week; even though during the fourth week it came down to 40, the amount was always above that of the treated group ($p < 0.05$) (Table 1).