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Efficacy of the entomopathogenic fungus *Metarhizium brunneum* in controlling the tick *Rhipicephalus annulatus* under field conditions

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ABSTRACT

High infectivity of entomopathogenic fungi to ticks under laboratory conditions has been demonstrated in many studies. However, the few reports on their use under field conditions demonstrate large variations in their success, often with no clear explanation. The present study evaluated the factors affecting the efficacy of the fungus *Metarhizium brunneum* against the tick *Rhipicephalus (Boophilus) annulatus*. It demonstrates how environmental conditions and ground cover affect the efficiency of the fungus under field conditions. During the summer, 93% of tick females exposed to fungus-contaminated ground died within 1 week, whereas during the winter, only 62.2% died within 6 weeks. Nevertheless, the hatchability of their eggs was only 6.1% during the summer and 0.0% during winter. Covering the ground with grass, leaves or gravel improved fungal performance. Aside from killing female ticks, the fungus had a substantial effect on tick fecundity. Fungal infection reduced the proportion of female ticks laying full-size egg masses by up to 91%, and reduced egg hatchability by up to 100%. To reduce the negative effect of outdoor factors on fungal activity, its conidia were mixed with different oils (olive, canola, mineral or paraffin at 10% v/v) and evaluated in both laboratory and field tests for efficacy. All tested oils without conidia sprayed on the sand did not influence tick survival or weight of the laid eggs but significantly reduced egghatchability. Conidia in water with canola or mineral oil spread on agarose and incubated for 18 h showed 57% and 0% germination, respectively. Comparing, under laboratory conditions, the effects of adding each of the four oils to conidia in water on ticks demonstrated no effect on female mortality or weight of the laid egg mass, but the percentage of hatched eggs was reduced. In outdoor trials, female ticks placed on the ground sprayed with conidia in water yielded an average of 175 larvae per female and there was no hatching of eggs laid by females placed on ground sprayed with conidia in water with canola or mineral oils.

1. Introduction

Ticks are important pests of farm animals, pets and wild animals, mainly due to their efficiency as vectors of a large variety of vertebrate pathogens. They are almost solely controlled by chemical acaricides, and the use of biological control agents is very limited. Entomopathogenic

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fungi (EPF) have been shown to be effective killers of several tick species. The presence of EPF on ticks collected in nature varies to a large extent according to region and season (Samish et al., 2008). Even though up to 50% of ticks collected in the field have been found contaminated with these fungi (Samsinakova et al., 1974), their influence on tick populations in nature is not clear.

The commercial use of EPF for controlling plant pests has seen much development (although still very little as compared to chemicals) in the last few years, whereas their use against animal pests is still in its infancy (Faria and Wraight, 2007). The success of fungi in killing ticks is strongly influenced by the interactions between specific tick species and stages and the pathogen isolate (Samish et al., 2008). Environmental conditions also play a highly important role in the success of EPF efficacy (Zimmerman, 2007; Fernandes et al., 2012). The microclimate around ticks in the off-host part of their cycle is strongly influenced by ground cover, where many of these ticks tend to rest.

Despite the large number of experiments demonstrating the high infectivity of EPF against different tick species and stages under laboratory conditions (Fernandes and Bittencourt, 2008; Samish et al., 2008), their use to control off-host *Rhipicephalus* ticks under field conditions has shown large variations in degree of control (Kaaya, 2000; Kaaya and Hassan, 2000; Bittencourt et al., 2003; Basso et al., 2005; Elkin et al., 2009; Ángel-Sahagún et al., 2010; Ojeda-Chi et al., 2010; Garcia et al., 2011). However, the environmental conditions responsible for these variations have never been studied. Recognizing the outdoor inhibitory factors and learning how to overcome them are often key to satisfactorily translating laboratory success in using microbial control agents into outdoor success. Furthermore, adding oil to conidial formulations is known to improve their performance, mainly under low humidity or UV irradiation (Moore et al., 1993; Lomer et al., 2001).

Gindin et al. (2001, 2002) reported that *Metarhizium brunneum* (previously *M. anisopliae*) strain 7 is especially virulent against *Rhipicephalus (Boophilus) annulatus* stages under laboratory conditions. The present report takes this one step further by applying conidia under semi-field conditions and recording the extent to which outdoor conditions influence fungal anti-tick efficiency.

2. Material and methods

2.1. Tick

Rhipicephalus (Boophilus) annulatus ticks were collected in 1984 from cattle in Israel and then fed every 2 months on Friesian calves. The off-host stages were incubated in the dark at 28 °C, 90% relative humidity (RH). The engorged female ticks were tested for fungal susceptibility within 24 h after repletion or kept at 14 °C and tested within the following 5 days.

2.2. Fungus

The *M. brunneum* strain 7 (previously *M. anisopliae*-7) used in all experiments in this report was isolated two decades ago from an unidentified beetle collected in Israel. The fungus was cultivated on Sabouraud dextrose agar (SDA) at 25 °C in the dark, while passing twice a year through engorged *R. annulatus* ticks. For field experiments, the fungus was cultivated on media made by adding 400 g "organic" wheat to 1 l water and then autoclaving for 40 min at 127 °C in plastic bags. The medium in each bag was inoculated with 25 ml of spore suspension (1×10^6 spore/ml with 0.01% v/v Triton X-100) and incubated at 28 °C for 2–3 weeks until sporulation. Then, conidia were collected from the wheat suspension with distilled water containing 0.01% Triton X-100 and filtered through Miracloth (Calbiochem, La Jolla, CA). Conidial concentrations were determined by hemocytometer. A concentration of 1×10^8 conidia/ml in distilled water containing 0.01% Triton X-100 was used for all experiments unless otherwise specified. The percentage of viable conidia was determined by counting germination on SDA 1 day prior to each bioassay. Only suspensions containing at least 95% viable conidia were used.

2.3. Climatological data

EPF are sensitive to environmental conditions, especially before penetrating their arthropod host. We therefore separated the data on temperature and RH into two parts: 5 days postinoculation (PI), i.e. the period during which fungal spores adhere, germinate and invade the host, and the following 25 days (Table 1). The data collected 200 cm above the ground were from a standard Stevenson instrument shelter. The temperature measurements collected 5 cm above the ground were from a thermocouple kept unshaded at that height (including direct irradiation). All data were provided by the Israel Meteorological Service.

The average minimal and maximal air temperatures (200 cm above the ground) during the winter (December–January) were 8.3–9.6 °C lower than during the summer (June). The unshaded thermocouples, 5 cm above the ground, recorded even larger differences between maximal summer and winter temperatures (Table 1).

2.4. Bioassay procedures

2.4.1. Effect of application period and soil cover on fungal anti-tick efficacy

Seasonal effects on fungal efficacy were determined in 5-l buckets (Ø 20 cm), with drainage holes in the bottom, filled to two-thirds their volume with sandy loam and placed in an open field. A 50-ml suspension of 1×10^8 *M. brunneum*-7 conidia/ml (about 1.6×10^7 conidia/cm^2) was sprayed on the soil surface of each bucket. Twenty engorged female ticks were scattered in each bucket 2 h after spraying and covered with a 2-cm layer of dry eucalyptus foliage. The buckets were covered with a light metal net to prevent tick predation by birds or rodents. The buckets were irrigated daily at midday with 50 ml water/bucket. The tests were performed in December–January (winter period in Israel) and June (summer period in Israel).

To determine the effect of soil coverage on fungal efficacy, three different covers were tested in the 5-l buckets: dry eucalyptus foliage (2–3 cm thick), gravel (2–3 cm thick) and growing grass (6–10 cm thick). The latter consisted of