Available online at www.sciencedirect.com
ScienceDirect
Veterinary Parasitology 158 (2008) 336–343
veterinary parasitology
www.elsevier.com/locate/vetpar

Pathogenicity of endophytic entomopathogenic fungi to *Ornithodoros erraticus* and *Ornithodoros moubata* (Acari: Argasidae)

Iñigo Zabalgogeazcoa, Ana Oleaga, Ricardo Pérez-Sánchez *

Instituto de Recursos Naturales y Agrobiología (IRNASA, CSIC), Cordel de Merinas, 40-52, 37008 Salamanca, Spain

Received 23 January 2008; received in revised form 12 March 2008; accepted 15 September 2008

Abstract

The argasid ticks *O. erraticus* and *O. moubata* are of great medical and veterinary importance because they are vectors of the African swine fever virus and several species of human relapsing fever borreliae. Biocontrol of these ticks using entomopathogenic fungi has not been previously reported. We examined the pathogenicity to different developmental stages of these two argasids of six strains of the fungal species *Beauveria bassiana* (strains Bb1764 and Bb2157), *Lecanicillium lecanii* (strains Ll586, Ll618 and Ll3047) and *Tolypocladium cylindrosporum* (strain Tc3398). Three strains, Bb1764, Bb2157, and Tc3398, caused in Spanish *O. erraticus* mean mortality rates between 34.4% and 62% in 14–28 days post-inoculation. Additionally, Bb2157 also induced in African *O. moubata* mean mortality rates of 31.9%. The remaining strains caused lower mortality rates and were not considered effective. This is the first study in which some strains of entomopathogenic fungi are found to be effective against argasid ticks of the genus *Ornithodoros*, and its results might justify further efforts towards the application of entomopathogenic fungal strains as anti-argasid biocontrol agents.

Keywords: Tick biocontrol; *Ornithodoros*; Entomopathogenic fungi; *Beauveria bassiana*; *Lecanicillium lecanii*; *Tolypocladium cylindrosporum*

1. Introduction

The argasid ticks *Ornithodoros erraticus* and *O. moubata* are reservoirs and vectors of important animal and human pathogens. In the Iberian Peninsula *O. erraticus* transmits the African swine fever virus (ASFv) (Basto et al., 2006), and several species of tickborne relapsing fever borreliae, such as *Borrelia hispanica* and *B. crocidurae* (Piesman and Gage, 2004). In Africa *O. moubata* is an important vector of the ASFv (Rennie et al., 2001) and of the causal agent of the African human relapsing fever, *Borrelia duttoni* (Piesman and Gage, 2004). Accordingly, control of these ticks would greatly improve the control of such diseases.

Current tick control is based on the use of acaricides, but these chemicals have serious drawbacks, including the development of resistance in ticks, toxicity, contamination of food products, and environmental pollution (Graf et al., 2004; Ostfeld et al., 2006). These disadvantages have stimulated the search for alternative methods to control ticks.

Biological control based on entomopathogenic fungi is a promising option. The ability of entomopathogenic fungi to penetrate ticks through their cuticle, thus avoiding the need to be ingested, the capability of a single fungal species or strain to kill several stages of

* Corresponding author at: Patología Animal, IRNASA, CSIC, Cordel de Merinas, 40-52, 37008 Salamanca, Spain.
Tel.: +34 923219606 fax: +34 923219609.
E-mail address: ricarpe@usal.es (R. Pérez-Sánchez).

0304-4017/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2008.09.019

the same tick, and the specific virulence of each fungal strain to one or a small group of ticks make them good candidates as biocontrol agents (Samish et al., 2001, 2004; Gindin et al., 2002; Pirali-Kheirabadi et al., 2007).

Among the entomopathogenic fungal species examined for pathogenicity against ticks in diverse laboratory and field studies, the most pathogenic were found to be *Beauveria bassiana* and *Metarhizium anisopliae* (Samish et al., 2004 and Ostfeld et al., 2006). Consequently, these two fungal species have received major attention and have been the object of subsequent studies (Alvares-Campos et al., 2005; Arruda et al., 2005; Hornbostel et al., 2005a,b; Polar et al., 2005a,b; Bahiense et al., 2006; Pirali-Kheirabadi et al., 2007). However, these studies have been focussed almost exclusively on the control of ixodid ticks, and have neglected the control of argasid ticks. The only exception seems to be the work by Sewify and Habib (2001), which studied the pathogenic effect of *M. anisopliae* on the argasid tick *Argas persicus*. These authors sprayed heavily infested poultry houses with a fungal spore suspension and observed that the argasid population disappeared in 3 weeks. Despite this interesting result, no further studies on the control of argasids with *M. anisopliae*, *B. bassiana*, or other entomopathogenic fungi have been published.

The objective of the present work was to test the pathogenicity of several strains of three entomopathogenic fungal species, *Beauveria bassiana*, *Lecanicillium lecanii*, and *Tolypocladium cylindrosporum*, to different developmental stages of the argasid ticks *O. erraticus* and *O. moubata*.

Lecanicillium lecanii (=*Verticillium lecanii*) is an entomopathogen with a wide host range which has been used as a biological control agent against agricultural insect pests (Shah and Pell, 2003). *L. lecanii* is known to naturally infect the ixodid ticks *Ixodes ricinus* and *I. scapularis* (Kalsbeek et al., 1995; Zhioua et al., 1999). However, its effect on argasid ticks is unknown.

Tolypocladium cylindrosporum is pathogenic to larvae of several mosquito genera, including *Anopheles* and *Aedes*, which contain vectors of human parasites causing important diseases such as malaria, yellow fever and dengue (Scholte et al., 2004). In addition, this fungus is known to be pathogenic to other insects such as black flies (*Simulium vittatum*), and *Galleria mellonella*, a honeybee pest (Bandani, 2004; Nadeau and Boisvert, 1994). However, it has not been previously reported as a tick pathogen.

2. Materials and methods

2.1. Ticks

The *O. erraticus* and *O. moubata* ticks used in this work came from two colonies maintained in our laboratory. The colony of *O. erraticus* was established from specimens captured in Salamanca, western Spain, and the colony of *O. moubata* was established from specimens obtained from the Institute for Animal Health, Pirbright, Surrey, UK. These ticks are fed regularly on rabbits, and kept at 28 °C and 80% relative humidity (RH).

2.2. Fungal strains and preparation of conidial suspensions

The isolates of the three species of fungi used for the experiments were endophytes obtained from asymptomatic grasses. The strains of *Beauveria bassiana* (Bb1764 and Bb2157), and *Lecanicillium lecanii* (Ll586, Ll618, and Ll3047) were isolated from plants of *Dactylis glomerata* (Sánchez Márquez et al., 2007), and the strain of *Tolypocladium cylindrosporum* (Tc3398) was obtained from a plant of *Holcus lanatus*.

To obtain conidia, fungal cultures grown on potato dextrose agar Petri plates were maintained in the dark at room temperature (22–25° C). Conidia from 3-week old cultures were released from the mycelium with a glass rod, after adding 5 ml of sterile water containing 0.01% Tween 80 to each plate. The conidial suspension from three plates was collected and centrifuged at 2000 × *g* for 5 min. The pellet was resuspended in 1 ml of sterile water and the concentration of conidia was estimated with a Bürker chamber. Suspensions of 10^7 or 10^8 conidia/ml were prepared in sterile water containing 0.01% Tween 80.

2.3. Bioassays

Two bioassays were carried out using a methodology adapted from that described by Samish et al. (2001) and Fernandes et al. (2003).

2.3.1. Bioassay 1

Five developmental stages (newly moulted unfed males, females, nymphs-4, nymphs-3, and nymphs-2) from both *Ornithodoros* species were treated with the two strains of *B. bassiana* (Table 1). Each treatment group was placed in a vial containing 2 ml of the corresponding conidial suspension (10^7 conidia/ml in 0.01% Tween 80). After 5 min, the excess suspension