Available online at www.sciencedirect.com
ScienceDirect
Veterinary Parasitology 148 (2007) 310–317
veterinary parasitology
www.elsevier.com/locate/vetpar

Control of the sheep scab mite *Psoroptes ovis in vivo* and *in vitro* using fungal pathogens

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Received 5 April 2007; received in revised form 1 June 2007; accepted 4 June 2007

Abstract

As part of a research programme designed to identify biological agents for the control of sheep scab, the pathogenicity of the fungus *Metarhizium anisopliae* to *Psoroptes* mites in the presence of sheepskin and wool was examined in the laboratory. No inhibitory effects of skin and wool were observed and high levels of infection were recorded. Subsequently the pathogenicity of formulations of both *M. anisopliae* and *Beauveria bassiana* to *Psoroptes ovis* was studied *in vivo*. For this, 36 batches of 20 adult female *Psoroptes* mites were confined in 25 mm diameter chambers which were attached to the backs of 6 scab-naive sheep. In some treatments, mites were exposed to the fungal pathogens for 48 h *in vitro* prior to being placed on the host, while other treatments involved mites with no prior exposure placed directly onto the skin of a host treated with a fungal pathogen. After 48 h on the host, mites were removed, incubated individually and all fungal infections were recorded. Fungal infection was observed in all treatments, except untreated controls. However, *B. bassiana* infected a significantly greater number of mites than *M. anisopliae* with all the formulations examined. Infection rates were highest when mites were exposed to dry conidia (>90%) and lowest with *M. anisopliae* in diatomaceous earth. Overall, the infection rate was not affected by whether or not the mites were given prior exposure to the conidia, before being placed on the sheep. The results demonstrate that *Psoroptes* mites can become infected by entomopathogenic fungi on the skin of sheep and provides a first demonstration of the potential of this technology for the control of sheep scab.

Keywords: *Psoroptes ovis*; Sheep scab; Fungus; *Metarhizium anisopliae*; *Beauveria bassiana*; Biological control

1. Introduction

In the UK, in 1989 when compulsory national plunge-dipping for the control of psoroptic mange in sheep (sheep scab) was reduced from two to one annual treatment, there were approximately 40 reported outbreaks of the disease in that year (French et al., 1999). In 1991, when the final compulsory dip was abolished, there were approximately 120 outbreaks (French et al., 1999). Following that year, sheep scab was treated by farmers on a case-by-case basis. However, by 1997 up to 3000 outbreaks were estimated to have occurred nationally (Lewis, 1997) and by 2004 a national prevalence of 9% of flocks was established, equating to approximately 6750 cases of scab per year (Bisdorff et al., 2006). This represents a steady and concerning increase. The disease appears to be most prevalent in Wales, Scotland and

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doi:10.1016/j.vetpar.2007.06.008

Northern England (Bisdorff et al., 2006), suggesting an association with upland farms, the movement of stock and the use of common grazing (O'Brien, 1999).

Throughout most of this period, the only insecticides registered for use in dips were the organophosphates propetamphos and diazinon, and the pyrethroids flumethrin and later high *cis*-cypermethrin. However, because of environmental concerns the pyrethroids are currently unavailable for scab control in the UK. Propetamphos was withdrawn for economic reasons and following temporary withdrawal to allow the development of safer packaging, diazinon remains the only available insecticide for use in dips in the UK, although pressure for its removal remains because of concerns over human exposure. Currently, only the injectable avermectins (moxidectin, ivermectin and doramectin) are available as therapeutic alternatives to dips, although their use is constrained by the relative application difficulty by farmers, lack of residual activity (ivermectin) and expense (Bates, 2004).

As a result, there has been considerable recent interest in the development of alternative approaches to scab treatment, particularly the use of entomopathogenic fungi (Brooks and Wall, 2001; Brooks et al., 2004). High levels of infection and mortality in *Psoroptes ovis* (Herring) (Acari: Psoroptidae) has been demonstrated *in vitro*, using the fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin (Deuteromycotina: Hyphomycetes) following immersion in a 1×10^7 conidia ml^{-1} suspension (Smith et al., 2000). Subsequently, the ability of mites to acquire a fatal infection following contact with a treated surface (Brooks and Wall, 2001) and the ability of infected mites to transfer infection to other in-contact individuals, were demonstrated (Brooks and Wall, 2005). However, preliminary attempts to use the fungal pathogen applied to infested sheep initially gave unpromising results (Brooks, 2004). One possible problem was that the lack of initial success *in vivo* might have been due to naturally occurring fungicides present in the sheep fleece. A second possibility is that the temperature at the sheep skin surface is too high; at the skin temperature found on a fully fleeced sheep, around 35 °C, most strains of *M. anisopliae* are close to their upper lethal limits (Brooks et al., 2004; Polar et al., 2005). A third alternative was that the method of application failed to deliver fungal spores to the skin surface. The aims of the work reported here, were to assess the first two possible obstacles to *in vivo* application by examining the pathogenicity of the two fungal pathogens, *M. anisopliae* and *B. bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes), when applied to sheep skin and undertaking quantitative trials of the pathogenicity of the fungus under carefully controlled experiments on live hosts.

2. Materials and methods

2.1. Sheep skin bioassay

The skin of a freshly killed lamb with no history of treatment for *P. ovis* was obtained from an abattoir. A series of 20 mm diameter circles were cut from the skin and the fleece was cut to a length of 20 mm. These discs of skin were placed into chambers constructed from 6 mm × 25 mm × 75 mm glass blocks. Each block had a 20 mm diameter hole drilled through its centre. The bottom of the block was sealed with a fine grade cotton cloth glued to the glass with epoxy resin (Araldite®, Bostik Findlay Ltd., Leicester, UK). Once a disc of skin and fleece, with its appropriate treatment, had been placed into the chamber created in these blocks, the chamber was sealed using a glass microscope slide with a 5 mm diameter hole drilled through its centre, which was also closed with cotton cloth. The hole in the upper glass slide was to allow for regulation of the humidity of the air within the chamber.

Four treatment groups were used: untreated fleece only, fleece to which conidia of *M. anisopliae* suspended in silicone oil had been added, fleece to which only silicone oil had been added and, as a positive control, cotton cloth to which conidia suspended in silicone oil had been added.

Treatments containing conidia used a suspension of 1×10^8 conidia ml^{-1} of *M. anisopliae* (isolate IMI386697) in silicone oil (dimethylpolysiloxane hydrolyzate, Sigma–Aldrich, Dorset, UK). The isolate had been cultured on potato dextrose agar plus yeast (PDAY). Conidia were collected from 10 to 14 day-old plates, by scraping a culture of sporulating *M. anisopliae* with a sterile loop, into 4 ml of silicone oil. The concentration of the conidial suspension was calculated using a haemocytometer and diluted further with silicone oil to produce the desired concentration. The silicone oil or silicone oil with its suspended conidia was applied using a household plant sprayer (Hozelock Group Ltd., Aylesbury, UK) fixed 20 cm directly above the skin or cloth using a clamp stand. This distance between the nozzle of the sprayer and the discs of skin or cloth was chosen to give an even application of the treatment across the skin or cloth and to simulate a possible application method for use on sheep. The sprayer delivered a standard 0.08 ml of conidia suspended in silicone oil to each surface resulting in the application of 8×10^6 conidia per disc.