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Control of the cattle louse *Bovicola bovis* with the fungal pathogen *Metarhizium anisopliae*

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Abstract

The effects of the entomopathogenic fungus *Metarhizium anisopliae* (Metschnikoff) were evaluated against the common louse parasite of cattle, *Bovicola bovis* (Piaget) (Trichodectidae: Ischnocera). Two different concentrations and formulations of conidial suspensions were applied to contained populations of adult female lice. *In vitro*, lice immersed in suspensions of *M. anisopliae* formulated in 0.03% Tween 80 developed infections; at the highest concentration (1×10^8 conidia ml^{-1}) a mean of 71% ($\pm$11.52%, 95% C.I.) of lice became infected. Lice exposed to the Tween 80 only *in vitro*, showed high levels of survival and zero infection. *In vivo*, fungal conidia were applied to louse populations contained in 7 cm diameter circular arenas glued to the backs of Holstein cattle, maintained in controlled climate conditions. Conidia were formulated in either Tween 80 or silicone oil. The treatment with *M. anisopliae* resulted in high levels of infection and there was no overall difference between the two formulations in the number of infections observed. At the highest concentration (1×10^8 conidia ml^{-1}) a mean of 73% ($\pm$15.57%, 95% C.I.) lice became infected. It is concluded that the strategic seasonal use of a fungal pathogen on cattle, applied in early winter, may be of value in suppressing the winter increase in abundance, preventing the population increasing to clinically significant levels.

Keywords: Biological control; Fungus; Lice; Cattle; *Bovicola bovis*; *Metarhizium anisopliae*; Pediculosis

1. Introduction

Lice are one of the most common parasites of domestic cattle; the most abundant and clinically important of which is the chewing louse, *Bovicola bovis* (Piaget). Herd prevalences of 75–90% have been widely reported (Milnes and Green, 1999; Nafstad and Gronstol, 2001; Colebrook and Wall, 2004). *B. bovis* feeds on the outer layers of hair shafts, dermal scales, and blood scabs. It is generally most abundant on the top of the head, especially the hair of the poll and forehead, the neck, shoulders, back, rump and occasionally the tail switch. However, as infestations increase, the lice may spread down the sides to cover the rest of the body, causing considerable irritation to the host animal. Infested cattle may show disrupted feeding patterns. The skin reaction can cause the hair to loosen and the cattle react to the irritation by rubbing or scratching, which results in patches of hair being pulled or rubbed off. Scratching may produce wounds or bruises and a roughening of the skin which may lead to secondary skin infections and skin trauma. Infestations by *B. bovis* are associated in particular with lesions, called light

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spot and fleck, which leave depressions in the papillary dermis 1–3 mm, and less than 1 mm, respectively. They are only detected once the hides have been tanned. The resultant losses from these lesions in the UK was estimated at £15–20 million per annum (Coles et al., 2003).

Although *B. bovis* may cause less individual damage than the various species of sucking lice, it is present in larger numbers and so can be extremely damaging. Poor control may be associated with a failure to detect or identify louse infestation in its initial stages and by the time clinical diagnosis is achieved the entire herd may be infested (Milnes and Green, 1999).

Louse control currently relies largely on the use of synthetic chemicals such as the organophosphate and pyrethroid insecticides or the broad spectrum macrocyclic lactone parasiticides. However, resistance to insecticides, concerns over the possible harmful effects of insecticides on human health and the environment and the need to ensure that meat and milk remain residue-free, have led to tighter regulation and pressure to reduce their use. Clearly therefore it will be of considerable value to identify safer, more sustainable, and environmentally appropriate approaches to the control of parasitic lice of livestock. Amongst candidate entomopathogens, microbial control agents may be particularly suitable for use in integrated control programmes because of their low toxicity, specificity, and because these organisms are unlikely to be affected by the concurrent application of synthetic neutrotoxic insecticides. For example, the toxins of various isolates of the bacterium *Bacillus thuringiensis* Berliner have been shown to be effective against a wide range of pest insect species (Gough et al., 2002) including *B. ovis* (Pinnock, 1994).

The use of fungal pathogens for the control of insect pests has been widely considered (Gillespie and Moorhouse, 1989). Approximately 750 species of fungi in 56 genera are known to be pathogens of arthropods (Kirk et al., 2001). There has been success in commercialising the entomopathogenic fungus *Metarhizium anisopliae* (Metchnikoff) as both Green Guard™ and Green Muscle® for the control of crop pests in Africa and Asia (Arthurs and Thomas, 2001). Both *M. anisopliae* and *Beauveria bassiana* (Balsamo) have also shown promising pathogenic activity against the ticks *Rhipicephalus appendiculatus* (Neumann) and *Amblyomma variegatum* Fabricius (Kaaya and Hassan, 2000) and *Boophilus annulatus* (Say), *Hyalomma excavatum* (Koch) and *R. appendiculatus* (Gindin et al., 2002) and also the mange mite *Psoroptes ovis* (Hering) (Brooks et al., 2004). The aim of the present study therefore, was to consider whether *M. anisopliae* might also be a valuable candidate pathogen for the control of *B. bovis* on cattle.

2. Materials and methods

2.1. Fungal culture

Isolates of the fungal pathogen *M. anisopliae* (isolate 4556 USDA-ARS) were obtained from the collection maintained by Richard Humber (Ithaca, NY, USA). This isolate was originally cultured from a *Boophilus* spp. tick. The fungus was cultured on Sabaroud dextrose agar plus yeast (Associate of Merck KGaA, Darmstadt, Germany) at 26 °C for 11–14 days. For each experiment a sub-plate was cultured. Spores were harvested by carefully scraping a sterile inoculating loop (5 mm diameter) over the surface of the plate. The conidia were then transferred to a sterile centrifuge tube (Fisher Scientific, Fair Lawn, New Jersey), containing 5 ml sterilised Tween 80 (0.05%) aqueous solution.

The concentration of conidia in suspension was determined using an Improved Neubauer Haemocytometer (Hausser Scientific, USA). The percent viable spores was determined for each experiment by growing the spores at 25 °C on potato dextrose agar (Difco™, Bectin, Dickinson and Company, Spaks, MD 21152, USA) with Benlate 50 (E I Dupont De Nemours & Co. Inc., Wilmington, DE, USA) fungicide to slow the growth of the spores, and counting the number of viable versus non-viable spores under a microscope after 24 h. The concentration of the conidial solution was then adjusted accordingly and diluted with autoclave sterile 0.03% Tween 80 or silicone oil (Fisher Scientific) to the required 1×10^6, 1×10^7 and 1×10^8 conidia ml^{-1} concentrations.

2.2. In vitro trials

B. bovis were obtained from Holstein cows maintained at the Agriculture and Agrifood Canada, Lethbridge Research Centre, Alberta, Canada. Lice were removed with forceps from the upper back, shoulders and rump of the cattle and placed in 15 cm diameter Petri dishes (Fisher Scientific). Ventilation holes of about 2 cm in diameter, covered by fine mesh, had been cut into the lids of these dishes. Dishes of lice were maintained at 33 °C, and ~55% relative humidity in an unilluminated incubator (NAPCO National Appliance Co., Portland, Oregon, USE, model 320-6). Lice were provided *ad libitum* with bovine skin scurf and hair supplemented with brewer's yeast. The scurf was

obtained by vacuuming the backs of Holstein cattle and collecting the resultant residue.

Only adult females were selected for use in experiments and were used for up to 2 weeks after collection. All trials were conducted between November 2004 and May 2005.

To determine the effect of conidial concentration, five adult female *B. bovis* were placed in a sterile microfuge tube (Rose Scientific Ltd., Edmonton, Alberta, Canada). Using a pipette (100–100 μl Oxford® benchmate™, Nichiyo, Japan) 1 ml of a 1×10^6, 1×10^7 or 1×10^8 conidia ml^{-1} suspension, formulated in 0.03% Tween 80, was added to each microfuge tube. The tube was inverted once and lice were left immersed in the conidial suspension for 15 s. For the controls, groups of five lice were either exposed to 1 ml of 0.03% Tween 80 or received no treatment at all. Three replicates of each treatment were carried out.

After treatment, the microfuge tubes were emptied onto autoclaved sterile cotton cloth in a sterile Petri dish. The groups of lice were transferred with forceps to sterile wells in a 24 well microtitre plate, with 0.01 g scurf and 0.001 g yeast. Prior to use, four air holes over each well had been melted into the lid with a hot mounting needle. The plates were sealed at the edge with Parafilm® (Pechiney Plastic Packaging, Chicago, USA) and incubated at 33 °C and ∼60% relative humidity, to approximate the cattle skin microclimate. The plates were monitored every 24 h for 14 days, live lice were counted and dead lice were removed.

2.3. Determining infection

Entire dead lice were cultured to determine whether they were infected by *M. anisopliae*. For this, lice were surface sterilised in 2% sodium hypochlorite solution (Javex, Colgate-Palmolive, Canada Inc., Toronto) for 15 s, then rinsed in filter sterilised (Nalgene® 0.2 μm PES syringe filter, Nalge Nunc International Corp., New York, USA) purite water for 15 s. Sterile 6 mm disks of Whatman® No. 1 filter papers (Whatman International Ltd, Maidstone, UK) were placed in the individual wells of UV sterilised microtitre plates (NUNCLON®, Nunc Postbox 280, Kamstrup, DK 400 Rosklide, Denmark). The disks were dampened with sterile water prior to adding a single dead louse into an individual well. The plates were sealed with Parafilm® and incubated at 26 °C. The lice were inspected daily for 3 weeks and infections were confirmed visually by the emergence of hyphae of *M. anisopliae* from the cadavers and sporulation. The infection was confirmed as *M. anisopliae* by morphological inspection. After this time dead lice with no signs of hyphal growth were considered to be not infected.

2.4. In vivo trials

Trials were conducted *in vivo* to evaluate the effects of two concentrations and two formulations of the fungal suspension of *M. anisopliae* against *B. bovis* infested cattle. For this, 7 cm diameter circular arenas were glued with contact cement (Brushable Contact Cement, Helmitin Helmiprene Inc., Toronto, Ontario) to the flanks of Holstein steers (12–18 months old) 24 h prior to treatment. The arenas were made from the rim of flexible plastic cups (Twinpak Inc., Calgary, Canada) with cloth Stockinette enclosing the arena, surrounded by mesh (fibreglass window screen, Lethbridge, Canada) coated in rubber cement (Ross Adhesives Conros Corp., Scarborough, Ontario) to form the attachment surface to the hide. To maximise the contact for the adhesive between the rubber mesh and the cattle hide, cattle were shaved with an electric razor (Oster animal clippers, #10 blade) to 2–4 mm around the area where the arena was to be attached. To facilitate retrieval of the lice after each trial, the area of hair enclosed by the arena was also shaved, but to a length of 8–10 mm. Six arenas were glued along the back of each cow, with three on each side of the midline. Cattle were allocated to trials at random. After each trial, the area where the arena had been was swabbed with 70% alcohol.

For each trial, two Holstein steers were maintained in individual crates in a walk-in controlled climate room at 15 °C, with an 16-h light:8-h dark cycle. The cattle were fed barley silage and loose grass hay and had *ad libitum* access to fresh water. The animals were cared for according to the guidelines of the Canada Council for Animal Care and with approval of the Lethbridge Research Centre Animal Care Committee (Protocol # 0438).

Two concentrations of *M. anisopliae* conidia were utilized: 1×10^7 and 1×10^8 conidia ml^{-1}. The condia were prepared in two formulations: conidia suspended in Tween 80 at 0.03% or in 100% silicone oil (Fisher Scientific). Individual cows were assigned to Tween 80 or silicone oil treatment. For each trial two arenas per cow received fungal treatment, two were negative controls in which the lice received no treatment and two were positive controls in which the lice received excipient but no conidia. Treatments were assigned to arenas at random on each cow.

Adult female *B. bovis* were selected at random from the stock-dish, maintained as described above.

Groups of 10 female lice were placed in sterile 5 cm diameter Petri dishes, then tipped into the arena 10 min prior to treatment. Fungal suspensions and excipient were then applied into the arenas using 50 ml spray bottles (Stafford Pharmacy, St. Edwards Boulevard, Lethbridge, Alberta, Canada). Prior to use spray bottles and spray pumps were sterilised with 70% alcohol and then thoroughly rinsed with sterile water. Four sprays were applied, directed at the top, right, bottom and then left of the centre of the arena, giving a total application of 0.2 ml per arena. Sprays were applied from a distance of approximately 10 cm. The cloth covers of the arenas were then closed with metal ties to prevent contamination. The negative controls were sealed first, then the positive control treatments were applied, and sealed, followed lastly by the fungal treatments.

Seven days after treatment, all lice were removed from each arena. Controls were removed first, followed by the excipient then fungal treatment arenas. The lice were collected into sterilised 5 cm diameter Petri dishes with dampened sterile filter paper to minimise static charge. The arena was removed to locate any lice not found in the initial search.

All of the recovered lice were submerged in sterile water for 2–3 min to ensure any live lice were killed. Lice were then surface sterilised in 2% sodium hypochlorite solution and incubated at 26 °C in sterile individual microtitre plate chambers, as described above. Lice were inspected every 24 h for 3 weeks after removal from the cattle and infections were confirmed visually by the emergence of *M. anisopliae* from cadavers, as described above. Five replicates of the 1×10^8 conidia ml^{-1} in Tween 80 and five replicates of the 1×10^8 conidia ml^{-1} in silicone oil, were conducted. Four replicates for the 1×10^7 conidia ml^{-1} in Tween 80 and four replicates for the 1×10^7 conidia ml^{-1} in silicone oil were conducted.

2.5. Statistical analysis

For the *in vitro* trial, the proportionate mortality was arcsin transformed and subjected to analysis of variance, in which excipient-type and conidial concentration were treated as fixed factors. Tukey multiple range tests were used to determine differences between within-factor groups. For the *in vivo* trial, because of the relatively smaller sample sizes and non-normal distribution of the data, which included large number of zero counts, non-parametric Kruskal–Wallis and Mann–Whitney *U*-tests were used to compare the effects of treatment and formulation on infection rate.

3. Results

3.1. In vitro

In the control groups of untreated lice or lice treated with Tween 80 alone survival was high, with over 70% of lice surviving for more than 2 weeks (Fig. 1). None of the lice that died in these groups developed fungal infections (Fig. 1). In contrast, for lice immersed in suspensions of *M. anisopliae* formulated in 0.03% Tween 80, more than 80% of all dead lice developed fungal infections (Fig. 1) and mortality and the proportion that became infected increased significantly with the concentration of conidia to which they were exposed (ANOVA, $F = 25.85$, $P < 0.001$, Fig. 1); a mean of 71% of lice (±11.52%, 95% confidence interval) became infected at the highest concentration of 1×10^8 conidia ml^{-1}. A Tukey HSD multiple range test showed that this was significantly higher than for the other two fungal concentration treatments, 1×10^6 and 1×10^7 conidia ml^{-1}, for which the number of infections were not significantly different from each other (means of 22 and 40%, respectively).

3.2. In vivo

There was a significant effect of treatment (Kruskal–Wallis, $H = 94.13$, $P < 0.001$). No lice became infected

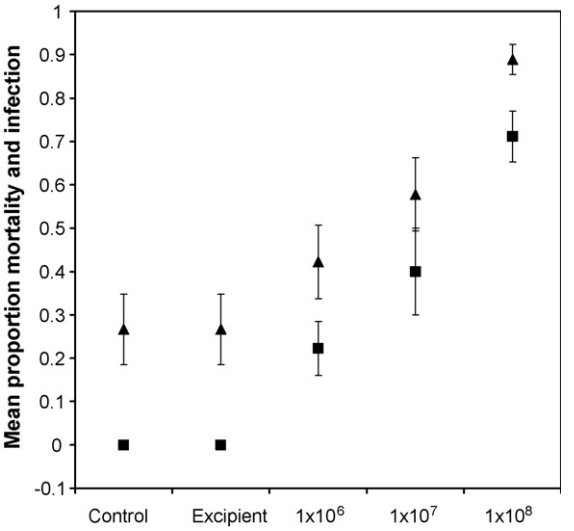

Fig. 1. Mean proportionate mortality (▲) of and mean proportion infected (■) (±95% confidence intervals) of *B. bovis* after immersion in *M. anisopliae* suspended in 0.03% Tween 80 at concentrations of 1×10^6, 1×10^7, or 1×10^8 conidia ml^{-1} for 15 s *in vitro*. For controls the lice were untreated or immersed in 0.03% Tween 80 only. Each group contained 15 lice and each treatment was replicated three times.

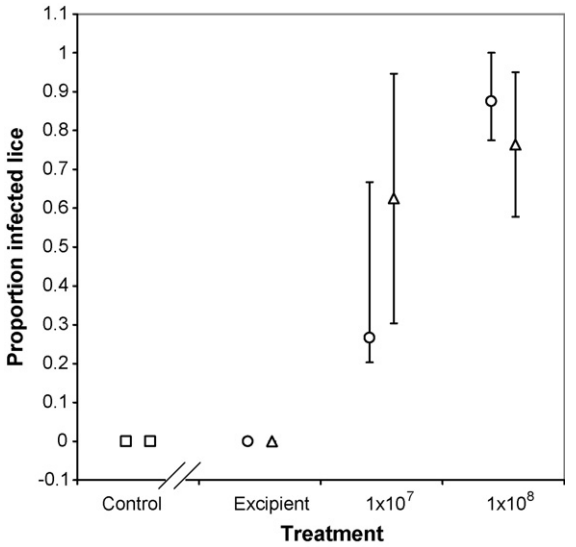

Fig. 2. Median proportion of lice infected (±interquartile range) 7 days after treatment with a spray application of *M. anisopliae* suspended in either 0.03% Tween 80 (○) or silicone oil (△) at concentrations of 1×10^7or 1×10^8 conidia ml^{-1} on cattle *in vivo*. For controls the lice were untreated (□) or treated with excipient only. Each group contained 20 lice and treatments were replicated five times for the 1×10^8 and four times for the 1×10^7 conidia ml^{-1} suspensions.

in either the untreated control or the excipient only controls whereas treatment with a suspension of *M. anisopliae* resulted in high levels of infection (Fig. 2). There was no overall difference in the number of lice infected when the fungus was formulated either in Tween 80 or silicone oil (Mann–Whitney $U = 0.10$, $P = 0.75$). Overall a mean of 73% (±15.57%, 95% C.I.) of treated lice became infected.

4. Discussion

The results of the present study demonstrate the entomopathogenic activity of *M. anisopliae* against *B. bovis in vitro* and, importantly, indicate that this pathogen is effective *in vivo* when used on cattle. At 1×10^8 conidia ml^{-1} mean infections of 71% were observed *in vitro* and 73% *in vivo*.

When used against the astigmatid scab mite *P. ovis*, *M. anisopliae* has shown considerable promise as a biological control agent. Following immersion of mites in a 1×10^8 conidia ml^{-1} suspension of conidia of *M. anisopliae* 77% of mites developed infections. After contact for 24 h with a surface treated with 1×10^8 conconidia ml^{-1}, 73% of the mites became infected. Dead infected mites are also able to act as sources of infection for live uninfected mites. These results suggest that *M. anisopliae* is a good candidate control agent for *Psoroptes* mites, however, formulation of conidia in a manner that can penetrate through sheep fleece to reach the skin and mites has proved problematic to date (Brooks, 2005).

More success has been achieved using *M. anisopliae* and *B. bassiana* against ticks on cattle. Samish et al. (2001) examined three isolates of *M. anisopliae* against the tick *R. sanguineus*. They found the most successful isolate caused 82.6 and 60% mortalities in engorged larvae and nymphs, respectively, and 92–100% mortality in adults and unfed larvae and nymphs. Another trial on the tick, *Ixodes scapularis*, with *M. anisopliae* induced 100% mortality in females and engorged larvae (Zhioua et al., 1997). In field experiments, Kaaya et al. (1996) treated the ears of Zebu cattle naturally infested with *R. appendiculatus* with spray suspensions of *M. anisopliae* or (*B. bassiana*,) and induced mortalities ranging from 76–85%. They also recorded reductions in fecundity (85–99%) and a significant reduction in egg hatchability (94–100%), with the fungus persisting for 1–3 weeks post-inoculation (Kaaya et al., 1996). Hornbostel et al. (2004) reported similar sublethal effects, with reductions in fecundity in the tick *I. scapularis* treated with *M. anisopliae*, indicating potential as a biocontrol agent, not only in respect of mortality.

The reasons why fungal pathogens appear to be pathogenic to arthropods when on cattle but much less so when applied to sheep, for example, is unknown, but may be associated with temperature. To be effective putative fungal biocontrol agents they must be able to operate at the relatively high temperatures and humidities found at the skin surface. The skin surface temperature of a fully fleeced sheep under UK conditions rarely falls below 30 °C and is frequently well above 35 °C (Wall et al., 1992) and this approaches the upper lethal temperature of most isolates of *M. anisopliae* tested to date (Brooks et al., 2004). Bovine skin temperature varies with ambient temperature, wind velocity, and amount of solar radiation, but has been shown to vary between 28 and 36 °C when ambient temperatures range from 7 to 27 °C, in the absence of solar radiation (Polar et al., 2005). Incident radiation will rapidly increase temperatures to above 40 °C (Colwell unpublished). However, at times when louse populations on cattle are growing the surface temperatures would be well within the tolerable limits for *M. anisopliae*.

Biological control approaches rarely result in pest eradication; they are used primarily with the aim of reducing a pest population below an acceptable threshold level. This mode of action may be particularly

appropriate for use with cattle lice. Louse infestation of cattle is often a chronic condition and cattle may harbour low, sub-clinical infestations for much of their lives showing few clinical signs. Highly seasonal fluctuations in abundance in temperate climates may occur with population peaks in winter and early spring (Craufurd-Benson, 1941; Michalski and Romaniuk, 2003) associated with the thickening of the winter coat and a decrease in light intensity and heat. The strategic seasonal use of a fungal pathogen, applied in early winter, may be of value in suppressing the winter increase in abundance, preventing the population increasing to clinically significant levels. This strategic approach may overcome one of the disadvantages of fungal pathogens, which is that they are slow acting and are unlikely to be effective for the immediate treatment and control of severe infestations.

Considerably more work is required, however, particularly in relation to the formulation of the fungal spores that is likely to best penetrate the hair coat and promote maximum longevity of the conidia on the skin. In the present trial the hair in the arenas where lice were placed was clipped prior to application. The ability of the formulation to penetrate the thicker winter coat and reach the sites where lice were present would be critical. Possible competition from other skin microflora and the presence of inhibitory skin secretions should also be evaluated. Studies to test the efficacy of the pathogen against other species of lice, particularly the sucking lice, will also be important so that broad spectrum activity is established. Nevertheless, this work has demonstrated the considerable potential of this control agent in a cattle parasite system.

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References

Arthurs, S., Thomas, M.B., 2001. Effects of temperature and relative humidity on sporulation of *Metarhizium anisopliae* var. *acridum* in mycosed cadavers of *Schistocerca gregaria*. J. Invertebr. Pathol. 78, 59–65.

Brooks, A., 2005. Entomopathogenic effects of the fungus, *Metarhizium anisopliae* (Metschnikoff) (Deuteromycontina: Hyphomycetes), on the sheep scab mites *Psoroptes ovis* (Hering) (Acari: Psoroptidae). Ph.D. Thesis, University of Bristol.

Brooks, A.J., Aquino de Muro, M., Burree, E., Moore, D., Taylor, M.A., Wall, R., 2004. Growth and pathogenicity of isolates of the fungus *Metarhizium anisopliae* against the parasitic mite, *Psoroptes ovis*: effects of temperature and formulation. Pest. Sci. 60, 1043–1049.

Colebrook, E., Wall, R., 2004. Ectoparasites of livestock in Europe and the Mediterranean region. Vet. Parasitol. 120, 251–274.

Coles, G.C., Hadley, P., Milnes, A.S., Green, L.E., Stosic, P.J., Garnsworthy, P.C., 2003. Relationship between lice infestation and leather damage in cattle. Vet. Rec. 153, 255–259.

Craufurd-Benson, H.J., 1941. The cattle lice of Great Britain. Part II: Lice populations. Parasitology 33, 331–358.

Gillespie, A.T., Moorhouse, E.R., 1989. The use of fungi to control pests of agricultural and horticultural importance. In: Whipps, J.M., Lumsden, R.D. (Eds.), Biotechnology of Fungi for Improving Plant Growth. Cambridge University Press, Cambridge, pp. 55–83.

Gindin, G.M., Samish, M., Zangi, G., Mishoutchenko, A., Glazer, I., 2002. The susceptibility of different species and stages of ticks to entomopathogenic fungi. Exp. Appl. Acarol. 28, 283–288.

Gough, J.M., Akhurst, R.J., Ellar, D.J., Kemp, D.H., Wijffels, G.L., 2002. New isolates of *Bacillus thuringiensis* for control of livestock ectoparasites. Biol. Control 23, 179–189.

Hornbostel, V.L., Ostfeld, R.S., Benjamin, M.A., 2004. Sublethal effects of *Metarhizium anisopliae* (Deuteromycetes) on engorged larval, nymphal, and adult *Ixodes scapularis* (Acari: Ixodidae). J. Med. Entomol. 41, 922–929.

Kaaya, G.P., Hassan, S., 2000. Entomogenous fungi as promising biopesticides for tick control. Exp. Appl. Acarol. 24, 913–926.

Kaaya, G.P., Mwangi, E.N., Ouna, E.A., 1996. Prospects for biological control of livestock ticks, *Rhipicephalus appendiculatus* and *Amblyomma variegatum*, using the entomogenous fungi *Beauveria bassiana* and *Metarhizium anisopliae*. J. Invertebr. Pathol. 67, 15–20.

Kirk, P.M., Cannon, P.F., David, J.C., Staplers, J.A., 2001. Ainsworth and Bisby's Dictionary of the Fungi, ninth ed. CAB International, Wallingford, UK.

Michalski, M., Romaniuk, K., 2003. Existence of mallophagans in milk cows during the alcove breeding period. Medycyna Weterynaryjna 59, 396–397.

Milnes, A.S., Green, L.E., 1999. Prevalence of lice on dairy cattle in England and the bordering counties of Wales. Vet. Rec. 145, 357–362.

Nafstad, O., Gronstol, H., 2001. Eradication of lice in cattle. Acta Vet. Scand. 42, 81–89.

Pinnock, D.E., 1994. The use of *Bacillus thuringiensis* for control of pests of livestock. Agric. Ecosys. Environ. 49, 59–63.

Polar, P., Aquino de Muro, M., Kairo, M.T.K., Moore, D., Pegram, R., John, S.-A., Roach-Benn, C., 2005. Thermal characterisations of *Metarhizium anisopliae* isolates important for the development of biological pesticides for the control of cattle ticks. Vet. Parasitol. 134, 159–167.

Samish, M., Gindin, G., Alekseev, E., Glazer, I., 2001. Pathogenicity of entomopathogenic fungi to different developmental stages of *Rhipicephalus sanguineus* (Acari: Ixodidae). J. Parasitol. 87, 1355–1359.

Wall, R., French, N., Morgan, K., 1992. Effects of temperature on the development and abundance of the sheep blowfly *Lucilia sericata*. Bull. Entomol. Res. 82, 125–131.

Zhioua, E., Browning, M., Johnson, P.W., Ginsberg, H.S., LeBrun, R.A., 1997. Pathogenicity of the entomopathogenic fungus *Metarhizium anisopliae* (Deuteromycetes) to *Ixodes scapularis* (Acari: Ixodidae). J. Parasitol. 83, 815–818.