final report

Project code: B. AHE.0193

Prepared by: Dr Diana Leemon

Agri-Science Queensland,
Department of Agriculture,
Fisheries and Forestry

Date published: April 2012

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Fungal biopesticide for cattle tick and buffalo fly control

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

Cattle ticks (*Rhipicephalus (Boophilus) microplus*) and buffalo flies (*Haemotobia irritans exigua*) are serious ectoparasites of cattle in Northern Australia. Current control strategies for both parasites rely heavily on extensive chemical treatments which are beset with the problems of resistance, residues and OH&S issues. Fungal biopesticides have emerged as realistic non-chemical control options for a range of pests in agriculture and a potential option for tick and buffalo fly control. This project aimed to assess the efficacy of a fungal biopesticide based on *Metarhizium anisopliae* in controlling all stages of cattle tick (*Rhipicephalus* (*Boophilus) microplus)* on cattle under field conditions and appraise the effect of the formulation on buffalo fly (*Haematobia irritans exigua*) populations on these cattle. The tick efficacy trials showed there are too many variables influencing the performance to guarantee a consistent high level of control of all on-animal tick stages with a *Metarhizium* based biopesticide. The activity range and efficacies obtained in these trials were too narrow to support future commercial consideration of a fungal biopesticide for tick control. However the fungal biopesticide did give good control of buffalo flies, particularly with a “pour on” style of treatment. These results indicate that a fungal biopesticide could provide excellent buffalo fly control with further development supported by more research in both the laboratory and field.

Executive summary

Cattle ticks (*Rhipicephalus (Boophilus) microplus*) and buffalo flies (*Haemotobia exigua irritans*) are serious pests of cattle in northern Australia. Important control options include chemical treatments (acaricides and insecticides) applied in strategic control programs along with breeding tick resistant cattle genotypes and pasture management. However there is scope for more control options to effectively manage these serious pests including a fungal biopesticide based on *Metarhizium anisopliae*.

DEEDI investigations found that some Australian isolates of *M. anisopliae* are extremely effective at killing ticks in the laboratory, with death occurring in 100% of ticks within two days (Leemon & Jonsson, 2008). However a series of field and pen trials have provided inconclusive evidence as to the commercial potential of a fungal biopesticide for tick control (Leemon et al, 2008, Leemon, 2010). Previous DEEDI investigations have also noted that buffalo flies are extremely susceptible to *Metarhizium* infection under laboratory conditions. During one of cattle tick trial it was found that buffalo flies netted from biopesticide treated cattle had high levels of *Metarhizium* infection and died faster after laboratory incubation than flies netted from untreated cattle. The current study was therefore undertaken to repeat investigations into the efficacy and commercial potential of a fungal biopesticide in controlling cattle ticks under field conditions and include an appraisal of the fungal biopesticide for buffalo fly control.

Two field trials were conducted in this study. The first trial aimed to investigate the therapeutic and short-term persistent (prophylactic) efficacy of a fungal biopesticide against artificial burdens of cattle tick on cattle under field conditions. Two dose rates of a *Metarhizium* formulation were compared to a negative 'untreated' control group and a positive control group treated with a common commercial acaricide. Cattle from the first trial were retained for the second trial in which the effect of the fungal biopesticide on buffalo fly populations and natural infestations of ticks on cattle were appraised. Two different *Metarhizium* formulations were applied to cattle, one a spray formulation applied once and the other a "pour-on" formulation applied three times a week for two weeks.

The results of the two trials showed that a *Metarhizium* based fungal biopesticide has potential for the control of buffalo flies but has limited efficacy against cattle ticks.

The results of the study suggest that the conditions required to achieve a tick control efficacy greater than 40% with a fungal biopesticide are too narrow to be practical for commercial development with the performance affected by too many variables. While some efficacy was apparent in both trials in this study, and in previous tick studies on animals (Leemon *et al*, 2008, Leemon, 2010), there was no evidence of the outstanding and consistent results required to justify further research into the commercial development of a fungal biopesticide for tick control on cattle.

Both the biopesticide spray and "pour-on" formulations resulted in huge decreases in buffalo fly populations on treated cattle and the fungal spores appeared to remain viable in the animal coat for up to two weeks after application. The spray formulation caused a rapid decrease in buffalo fly numbers with scarcely five flies per side seen on the sprayed animals after two days. This effect gradually decreased with time and fly numbers subsequently increased. However buffalo fly numbers on these animals did not reach an economic threshold (300+) until nearly three weeks after treatment. The "pour on" strategy was even more effective than the spray strategy giving greater control with less formulation. The number of buffalo flies on "pour on" treated cattle decreased to 1-50 flies /side /animal and stayed down for until at least a week after the last application. Throughout the trial the untreated cattle carried buffalo fly infestations of 500+ flies /side / animal.

The results of this project suggest that a fungal biopesticide for buffalo fly control has potential. A biopesticide would be an important addition to current control strategies, particularly those that utilise walk through buffalo fly traps, back rubbers and dust bags. However further research is required to optimise both the formulation and application strategies. It is recommended that further research into the development of a fungal biopesticide for buffalo fly control is supported through laboratory and field studies. The results of this study also indicate that a fungal biopesticide for tick control is subject to too many variables to deliver a consistent and high level of tick control on animals across a range of climatic regions and seasons. Therefore further development of a fungal biopesticide for cattle tick control, is not recommended.

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1 Background

The cost of cattle ticks (*Rhipicephalus microplus*) and buffalo flies (*Haemotobia exigua irritans*) to the Australian cattle and dairy industries has been estimated respectively at $146 million and $78.2 million (Sackett and Holmes, 2006). With the increased temperatures predicted by climate change modelling, the range of parasites such as these is likely to extend (White *et al*, 2003). Chemical treatments (acaricides) applied in strategic control programs are important control options for ticks along with breeding tick resistant cattle genotypes and pasture management. Insecticides, particularly ear tags, are currently relied on to control buffalo flies. However there has been increasing commercial, environmental and biological imperatives to find alternative controls. Fungal biopesticides offer a sustainable and promising alternative method of tick and buffalo fly control. Laboratory and animal studies by DEEDI have firmly established the potential for the fungus *Metarhizium anisopliae* in tick control and provided data that suggest a secondary effect of controlling buffalo flies is possible. Small field trials are required to obtain a proof of concept for the control of ticks and buffalo flies before engaging a commercial party.

DEEDI investigations found that some Australian isolates of *Metarhizium anisopliae* are extremely effective at killing ticks in the laboratory, with 100% tick mortality within two days (Leemon and Jonsson, 2008). These results compare very favorably with those from overseas laboratories, where 100% mortality in four days would be considered to be a good result. Field studies conducted on cattle in Queensland yielded positive, though variable results. A series of three pen trials conducted in 2003-2004 showed that while lethal doses of the fungal biopesticide can be applied to ticks on cattle, high temperature at the skin surface during mid-summer may limit the efficacy of a fungal biopesticide (Leemon *et al,* 2008). The third trial, conducted in extreme summer heat, was aborted when tick numbers on all animals, including the untreated controls, declined drastically. One trial in which the fungal biopesticide appeared to be quite effective took place under cooler ambient temperatures.

A recent pen trial was conducted to evaluate the knock-down and residual efficacy of the fungal biopesticide formulation against all tick stages on cattle (Leemon, 2010). This trial showed a reduced efficacy compared to previous studies. We believe that the pens insulated cattle from the cooling effects of breezes and night temperatures thereby maintaining a higher animal surface temperature which was not optimal for fungal growth and subsequent tick mortality.

Previous DEEDI investigations into the control of buffalo flies found that they are extremely susceptible to *Metarhizium* isolates under laboratory conditions. During a cattle tick trial conducted in 2004 the uptake of *Metarhizium* from treated cattle by buffalo flies was also investigated. Although only 6 animals out of 15 were treated with the *Metarhizium* based biopesticide a marked effect on buffalo flies was observed. Buffalo flies netted after treatment died much faster than those netted before treatment and *Metarhizium* was recovered from more than 90% of the buffalo flies following treatment. Such results suggest that further investigations into the potential of a *Metarhizium* based biopesticides are warranted.

Therefore this study was undertaken to repeat investigations into the efficacy of a fungal biopesticide in controlling cattle ticks under more natural conditions in a field trial and include an appraisal of the fungal biopesticide for buffalo fly control.

2 Project objectives

The primary aim of this project was to conduct field studies to evaluate the efficacy of a fungal biopesticide based on *Metarhizium anisopliae* in controlling all stages of cattle tick (*Rhipicephalus* (*Boophilus) microplus)* on cattle under field conditions and appraise the effect of the formulation on buffalo fly (*Haematobia irritans exigua*) populations on these cattle.

This study aimed to investigate the therapeutic and short-term persistent (prophylactic) efficacy of the fungal biopesticide against artificial burdens of cattle tick on cattle in a field situation. Two *Metarhizium* formulations, were compared to a negative 'untreated' control group and a positive control group treated with a common commercial acaricide. Secondary observations on buffalo fly present on untreated and treated cattle during the study were planned. However no buffalo fly were present during the first trial, therefore a second trial was conducted later in the fly season. Cattle from the first trial were retained for this trial. In Trial 2 the effect of the biopesticide on both buffalo fly populations and natural infestation of ticks on cattle were recorded. Samples of netted buffalo fly were regularly collected and incubated under laboratory conditions to assess mortality and uptake of *Metarhizium* spores.

3 Methodology

Two field studies designed around the recommendations from WAAVP guidelines[4 and 5] and APVMA GL20 and APVMA GL19[1] for establishing the knock-down and residual efficacy of products to be used for the control of cattle ticks and buffalo were conducted.

The trials are restricted randomized, controlled and unblinded studies to determine the comparative efficacy of two different dose rates of the fungal biopesticide.

Both studies were conducted in paddocks within the University of Queensland Vet Farm, 2436 Moggill Road, Pinjarra Hills, Queensland. Trial 1 was conducted from September to November, 2011, while Trial 2 was conducted from January to February, 2012. The studies were conducted under the APVMA Small-scale Trial Permit PER 7250 and with Animal Ethics approval (permit SVS/256/11/MLA) from the University of Qld animal ethics committee.

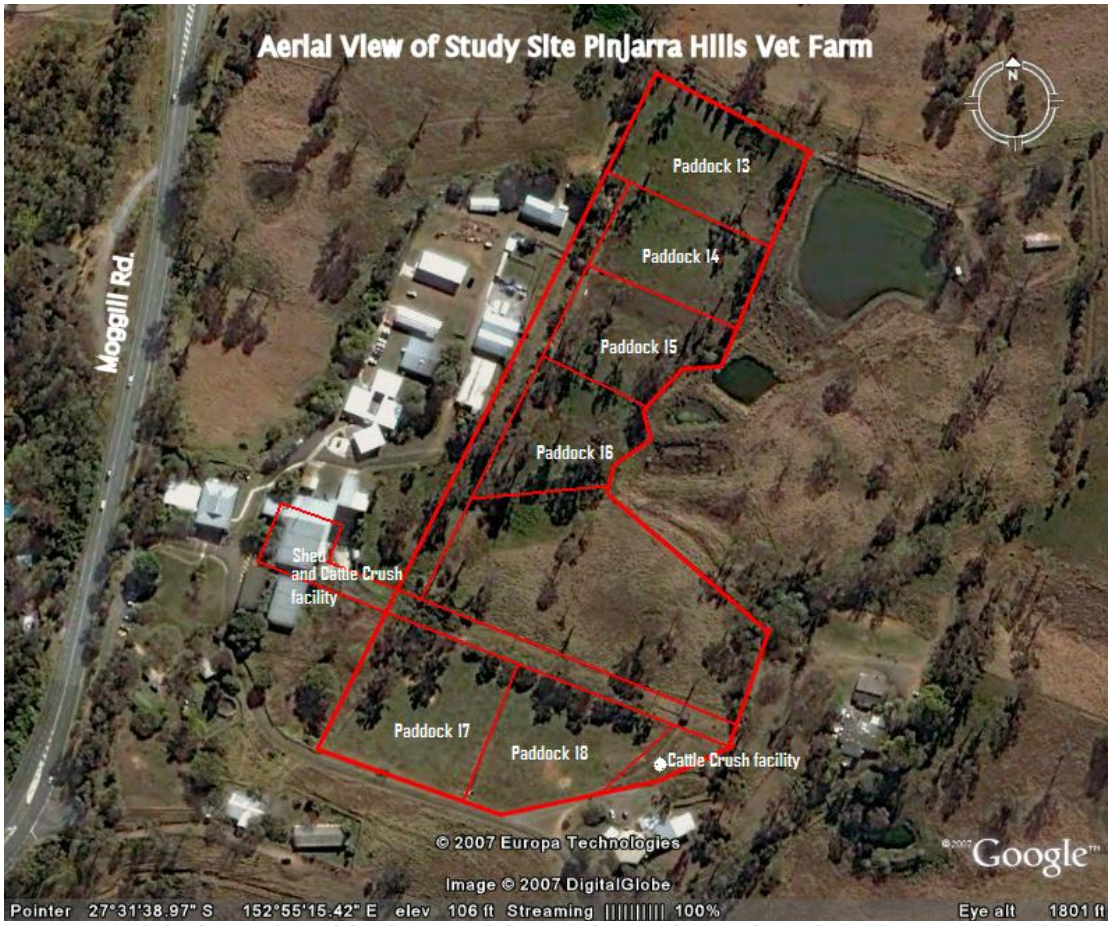

Figure 1. Aerial view of paddocks used in study at the University of Queensland farm at Pinjarra Hills

Paddocks 13, 14, 15, 17 and 18 (Figure 1, 2) were used for housing the cattle during both trials. Paddocks 13, 17 and 18 were approximately 85m x 75m (0.6375 ha) in area with sufficient shade provided by trees predominantly along the northern fence lines. Paddocks 13, 14 and 15 were approximately 80 m x 50 m (0.400 ha) in area with sufficient shade provided by trees predominately along the eastern fence lines and some larger shade trees growing throughout the paddocks. Water for each paddock was provided *ad libitum* via a water trough in each paddock connected to the Brisbane water supply. Double fencing separated each paddock with an approximate 1-2 metre buffer area. Natural or improved pasture was available for grazing supplemented with barley hay fed as required via hay racks.

During Trials 1 and 2 untreated cattle were housed in paddocks 17 and 18. During Trial 1 the treated groups were housed in paddocks 13 (positive control); 14 (low dose biopesticide) and 15 (high dose biopesticide). During Trial 2 the treated groups were housed in paddocks 13 (spray biopesticide) and 14 ("pour on" biopesticide).

Figure 2. Field paddocks used for Trials 1 and 2. Paddocks 18 and 17 are visible behind the heifers in the foreground (left). The north eastern aspect of paddock 18, paddocks 16 to 13 are in the far distance (though not visible) to the right (right)

The fungal biopesticide was evaluated for efficacy against the non-resistant field strain (NRFS) of cattle tick (*R. microplus)* by comparison with untreated control animals and animals treated with a standard commercial acaricide spray formulation in Trial 1. There were no buffalo fly (*Haematobia irritans exigua*) during Trial 1. Therefore Trial 2 was conducted to appraise the effect of the fungal biopesticide on buffalo fly. The effect of the biopesticide on natural infestations of cattle tick was also assessed during this trial. Observations on the presence of buffalo fly were made prior to treatment and following treatment. In addition samples of buffalo fly were netted then incubated under laboratory conditions to investigate fly mortality and uptake of *Metarhizium* spores.

3.1 Trial 1: Cattle tick control study

3.1.1 Animals

Twenty-four Hereford heifers (~ 9 months) were obtained from a tick free property in the Roma region of Qld. The weights of the animals ranged from 158 to 210 kg (average 186 kg) at the start of the trial. All heifers were vaccinated against clostridial diseases (Coopers 7 in 1 cattle vaccine) and tick fever (Trivalent vaccine) and drenched for worms (oxfendazole, Coopers Systamex). Heifers were vaccinated at the Centre for Advanced Animal studies (CAAS) at Gatton and held there until sero-conversion of the trivalent vaccine then transported to Pinjarra Hills.

In Trial 1 cattle were artificially infested with approximately 2,500 NRFS larvae of *R. microplus* on 14 separate occasions in the 24 days prior to treatment. At each infestation, a single tube containing viable active tick larvae (~0.125g or 2,500) was opened and manually dispersed along the back-line of the animal, extending from behind the point of the shoulders to the rump, using a small paintbrush (Figure 3). Infestation occurred three times a week for three weeks. This approach ensured that all tick stages were present at the time of treatment. To assess the prophylactic (persistent) efficacy of treatment, three infestations (days 2, 5 and d7) of 2,500 NRFS larvae per animal were conducted post treatment. Side counts of standard female ticks (4.5 – 8 mm) were recorded on days -4, -3, - 2 and -1 to enable randomisation of the animals into treatment groups (Figure 3).

Figure 3. Applying larval ticks to cattle (left), conducting side counts of standard ticks (right)

A stratified randomised complete block design method was used to allocate animals to treatment groups. On day -1, cattle were ranked from highest to lowest on the basis of total individual day -4, -3, -2, -1 pre-treatment tick counts and sequentially blocked into groups of four. The four highest ranked animals formed block 1, the next four highest animals formed block 2 continuing to the lowest four animals which formed block 6. From each block, individuals were randomly allocated to one of four treatment groups (Table 1). Groups were allocated to separate outside paddocks. Untreated control animals were kept as separated as possible from treated groups to minimise cross-contamination for the integrity of tick counting.

Table 1. Treatment groups

GROUP (and treatment order)	TREATMENT	Number
1	Untreated control	6
2	Positive control Taktic® EC (0.25%) commercial spray formulation	6
3	Low dose fungal biopesticide	6
4	High dose fungal biopesticide	6

3.1.2 Biopesticide formulation

Dried spore powder of *Metarhizium anisopliae* isolate ARIM16 was produced and supplied by a commercial company. The spores were sealed in plastic after production then shipped from the company to DEEDI. On receipt they were stored at 4°C until required for use. The spores were formulated in Codacide, an emulsifiable canola oil, manufactured by Microcide in the United Kingdom and supplied through Kendon, Melbourne, Australia. Different weights of spores were mixed into the codacide to give two different dose rates which were later diluted with water to give the final volume for the spray treatments as summarised in Table 2 below.

Table 2. Components of formulations and spray treatments

	Codacide oil (L)	Weight spores (g)	Concentration of spores in formulation (g/L)	Final volume (L) of spray treatment (codacide %)	Concentration of spores in spray treatment (sp/ml)
Low Dose	3	75	25	60 (5)	~5×10^7
High Dose	3	750	250	60 (5)	~5×10^8

3.1.3 Treatment application

On day 0 heifers were bought to the yards (Figure 4) in their treatment groups in the order shown in Table 1. The untreated control cattle were introduced to the crush first for the collection of standard ticks (see 3.1.6) from each animal.

All spray treatments were applied to cattle held in a crush using a tractor mounted Hardi PTO drive 95/10 spray unit. The spray unit operated at 6 bar, 1700 rev/min to deliver approximately 1.0 litre of formulation per minute through a Hardi – ISO Injet-01 fan nozzle. The time taken for the spray to saturate the animals was recorded to estimate that amount of spray treatment applied. The spray technique followed that recommended by the FAO (1984) standard operating procedure for hand spraying cattle for tick control. Spraying started with the rear underparts (Figure 4) moving to the flank, back and belly then front legs and axillae for both sides before finishing with the head and ears. By the end of the spray treatment all heifers were thoroughly wetted (Figure 4).

Figure 4. Yarded cattle before spray treatment (top left); spray application of fungal biopesticide to heifer (top right); biopesticide treated heifer (bottom left); closer view of biopesticide treated heifer (bottom right)

The positive control group was sprayed with Taktic® EC (amitraz) at the recommended dose according to the product label.

The biopesticide formulations were prepared before spraying by mixing the spores into the codacide oil (Figure 5) then diluting with tap water in the spray tank (Figure 5). Approximately 6-8 litres of fungal formulation were sprayed onto each of 6 animals in the biopesticide treatment groups (Figure 4). One group received the low dose (~5 × 10^7 spores/ml suspended in codacide oil (5%) and water). The second treatment group was

sprayed with the high dose (~5 × 10^8 spores/ml suspended in codacide oil (5%) and water) (Figure 4). After spraying standard ticks (see 3.1.6) and hair samples (see 3.1.8) were taken from each animal in the biopesticide treatment groups.

Figure 5. Mixing spores into codacide oil (left), Pouring fungal formulation into spray tank for dilution to spray strength (right)

Once the last group of heifers had gone through the yards, both the race and cattle crush were treated with 70% alcohol, to inactivate any residual fungal spores, then thoroughly hosed. Animals were carefully observed daily during the trial for any adverse effects from the tick infestation and fungal treatments.

3.1.4 Assessment of treatment efficacy

Side counts of standard tick (4.5–8 mm) were conducted on the same side (left) of the animals three times per week until day 29 (Figure 3). Counts were partitioned into 4 regions on each animal (Figure 6): The neck, which included the head, ear, neck and dewlap to the point of the sternum. The shoulder, which included the shoulder, outer and Inner foreleg from point of the sternum back to the start of the forebelly. The back, which included the rib and forebelly (to the front of the umbilicus. The rump, which included the rump, tail, escutcheon, outer and inner hind leg and rearbelly (including the umbilicus).

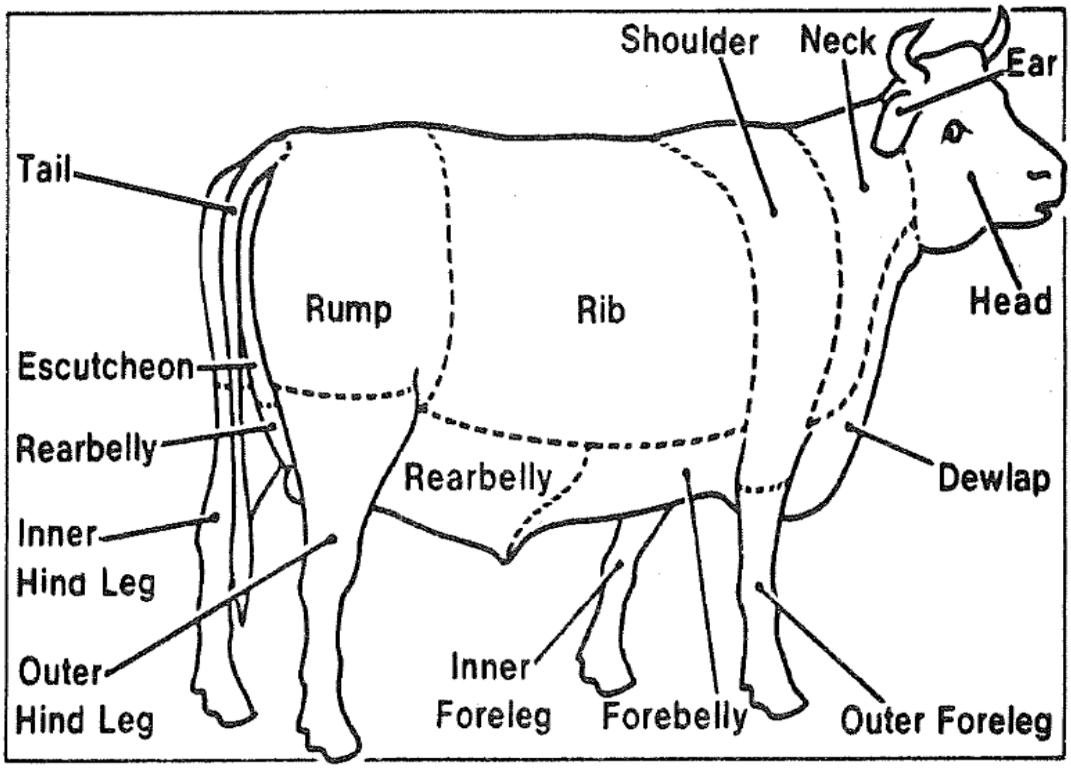

Figure 6. Pre-determined zones for tick counting (Extracted from Sutherst, *et al* (1978))

Percentage efficacy of the treatments on parasitic tick stages was calculated as outlined in the WAAVP guidelines for evaluating the efficacy of acaricides against ticks (Holdsworth *et al*, 2006) which follows:

Daily percentage (%) Efficacy = $100 - \left(\frac{TickCountTreatedGroup}{ADEQ} \times 100\right)$

Where ADEQ is the number of ticks expected in the treated group if left untreated.

ADEQ = $\frac{Total\ \mathrm{Pre}-TreatmentCountsTreatedGroup}{Total\ \mathrm{Pre}-TreatmentCountsControlGroup} \times$ Daily control count

3.1.5 Formulation check

Engorged female ticks (20 per sample) supplied from the Biosecurity Queensland tick culture were immersed for 1.5 min in samples of the formulation that were collected immediately before and during application to animals. After immersion ticks were blotted on absorbent paper, and then added to 24-well micro titre trays with water agar in the wells for incubation at 28^{o}C. Tick mortality was assessed over six days.

3.1.6 Application check

Twenty semi-engorged female engorged ticks between 4.5 and 8 mm were removed from each animal immediately after treatment for laboratory incubation at 28^{o}C in microtitre trays with water agar in the wells (Figure 7). Tick mortality was assessed over six days.

Figure 7. Collection of standard ticks from treated heifer for application check (left) ticks added to incubation trays for transport to and incubation in laboratory (right)

3.1.7 Temperature

The ambient temperatures and the surface temperatures of selected animals in the biopesticide treatment groups were recorded. Three data loggers (Tinytag View®, Hastings data loggers) recording the relative humidity and ambient temperature every 20 minutes were placed in different locations (paddock 13 in shade, paddock 14 on fence in open, under cover in the yards). Remote sensor data loggers (Tinytag Talk®, Hastings data loggers) were attached to the base of the tail of nine animals (three / treatment group) using sticky plaster (Figure 8). The sensors were secured in the coat along the backline of each animal. The data loggers recorded the surface temperature of these animals every 20 minutes for 9 days.

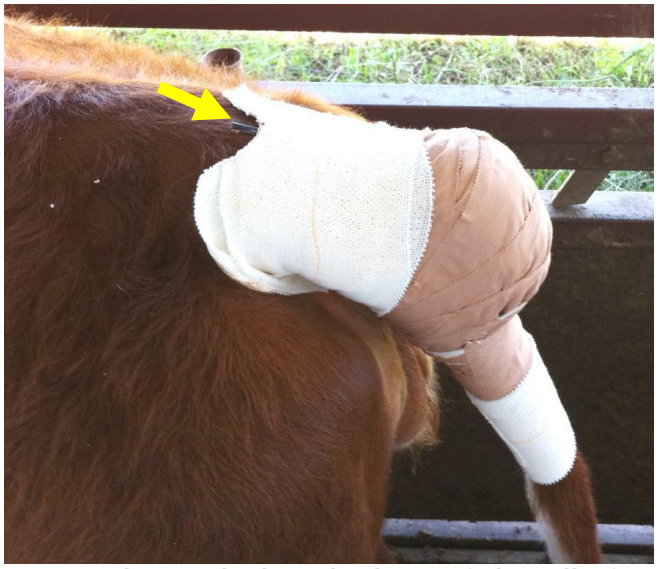

Figure 8. Data logger securely attached to the base of the tail of a heifer. Sensor probe arrowed

3.1.8. Spore viability in animal coats

Hair samples (~6 g/animal) were taken from the rump and flanks of each animal in the untreated and biopesticide treated groups. Samples were taken immediately after treatment (T_0) then weekly for 4 weeks (T_1; T_2; T_3; T_4) and stored at 4°C until required. Sub samples (0.2 g) of hair from three different animals were grouped into the one sample of 0.6 g which was added to 8 ml sterile 1.0% Tween 80 detergent then agitated for 10 min. The liquid was decanted off and diluted if required. Aliquots of 100 µl were spread across plates of CAD

selective agar. The agar plates were incubated at 28°C for 14 days to assess the emergence of *Metarhizium* colonies from viable spores.

3.2 Trial 2: Buffalo fly control appraisal and tick control assessment

3.2.1 Animals

Eighteen Hereford heifers selected from Trial 1 were held in two groups in paddocks 17, 18 and 13 (later14) over summer. Natural tick infestations were allowed to develop on the cattle and tick numbers were monitored twice weekly. When tick burdens increased to levels that could threaten an animal's welfare those animals were treated with Taktic® EC. Animals in paddock 17/18 were treated late December. Six animals held in paddock 13/14 were treated early January. The increase in the buffalo fly populations on the animals was also monitored to determine the optimal time to commence Trial 2. Taktic® EC was chosen for treating the tick burdens because it does not have any impact on buffalo flies and has a short withholding period.

Because of the necessary intervention required to control excessive tick burdens only six animals had ticks at the time of treatment. These animals were randomly allocated to either the control group or biopesticide spray group. All other animals were randomly allocated to one of the three treatment groups in Table 3.

Table 3. Treatment groups

GROUP (and treatment order)	TREATMENT	Number
1	Untreated control	6
2	Biopesticide 'pour – on" formulation	6
3	Biopesticide – spray formulation	6

3.2.2 Treatment formulation

Colonies of the *M. anisopliae* isolate ARIM16 recovered after one week in the coat of biopesticide treated cattle during Trial 1 were selected for spore production by DEEDI. Spores were produced via a biphasic process involving liquid culture followed by growth on solid rice media as described by Goettel (1984). The rice was dried at 20°C then harvested by shaking through 300 µm and 150 µm Endicott sieves. Harvested spores were stored at 4°C before formulating. A freshly produced batch of spores was also provided by a commercial company. However, based on QA results (see 3.3.3) only the spores produced by DEEDI were used.

Spores were suspended in the same oil, codacide, used in Trial 1. Two fungal biopesticide formulations were prepared one for spray application (Table 4) to cattle the other was a "pour on" equivalent that was painted onto cattle. The spray formulation was diluted with water to give the final spray volume.

Table 4. Spray formulation components

Codacide oil (L)	Weight spores (g)	Concentration of spores in formulation (g/L)	Final volume (L) of spray treatment (codacide %)	Concentration of spores in spray treatment (sp/ml)
6	375	62.5	60 (10)	~2.5×10^8

The "pour on" formulation consisted of 62.5 g/L spores suspended in codacide oil.

3.2.3 Treatment application

The spray treatment was applied in the same manner as for Trial 1 (3.1.3) on day 0.

The pour on formulation was painted onto delimited areas along the back at the shoulder and on the belly just behind the front legs (Figure 9) on both sides of each animal. Application was performed on the initial treatment day (day 0) then three times per week for three weeks (days 2,5,7,9,12 and 13). Initially 110 ml of formulation was applied to each animal with a soft 5 mm wide paint brush on days 0 and 2. This amount was reduced to 60 ml per animal for the rest of the applications when it was realised that we didn't require as much formulation per animal. The application site was also revised on the fifth application (Figure 9) because of varying degrees of "scurfing" seen in the shoulder area of most of the cattle. The "scurfing" appeared as pieces of opaque flaking material in the coat hair of the animals affected. There was no evidence of inflammation of the underlying skin or hair loss in the area. It is possible that the scurfy material resulted from oxidised oil residue.

Figure 9. Application sites of the "pour on" formulation on both sides of each animal

3.2.4 Assessment of treatment efficacy

3.2.4.1 Ticks

Side counts of ticks were conducted at days 5, 12 and 19 post treatment as per Trial 2.

3.2.4.2 Buffalo flies

The effect of the fungal biopesticide treatment on buffalo flies was visually assessed by a comparison of buffalo fly populations on all animals before and after treatment as well as a comparison of buffalo fly populations on treated versus untreated control animals post-treatment. Visual observations of buffalo flies were made on yarded animals on each scheduled day of assessment. Each group of cattle was brought to a holding yard, assessed for buffalo fly, and returned to its paddock, separately, to ensure no mixing of fly populations. Untreated cattle were assessed first in one holding yard (Figure 1: cattle crush facility). Both groups of treated cattle were taken to a different holding yard (Figure 1: shed and cattle

crush facility) except for the first two assessment days. Observations were made on the average number of buffalo flies per side on a group basis and classified as described in the Table 5. Observations of buffalo fly are considered as being qualitative only.

Table 5. Scoring of buffalo fly counts

Score	Number of buffalo flies per side
0	0 flies
+	1 – 50 flies per side (low infestation)
++	50-150 per side (Moderate infestation)
+++	150 – 300 per side (High infestation)
++++	>300 + flies per side (Very high infestation)

In addition the levels of *Metarhizium* infecting buffalo flies before and after treatment was investigated. Approximately 200-300 flies were sampled from each group of cattle, by sweeping a net (40 cm diameter hoop) over the backlines and under the bellies of yarded cattle. The netted flies were transported to the laboratory in a cage (44 cm L x 16 cm W x 12 cm H) made of tubon over a plastic frame placed inside an insulated cooler with ice bricks to lower the air temperature. For sustenance, a cotton wool pad soaked in bovine blood was provided for each cage of flies.

In the laboratory the groups of flies were separated into four replicates of approximately 50 flies and added to plastic containers (12cm diam. × 8cm H). Each container held a 90 mm filter paper, a small vial with a wick containing 10% sugar solution, and lid with a gauze insert. A cotton pad soaked in bovine blood was placed on the lid and replaced daily. All containers were incubated at 27°C and 80% RH for four days, after which the number of dead and living flies was recorded. Dead and live flies were recovered from each container and stored in separate vials at 4°C until required. To assess *Metarhizium* infection flies were surface sterilised (70% ethanol for 2 minutes, followed by sterile water rinse) blotted on dry sterile filter paper then incubated on water agar in Petri dishes for 10 days at 28°C (21 flies per plate, 3 replicate plates where available).

3.2.5 Spore viability in animal coats

Hair samples (~6g /animal) were taken from the back and belly application areas of the pour-on treatment group, and from the same areas on the spray treatment group. Samples were taken at one, two and three weeks after day 0, then 12 days after the last pour on application and stored at 4°C until required. Viable spores were recovered using the same method outlined for Trial 1 (3.1.8).

3.3 Quality assurance procedures

3.3.1 Spore viability and concentration

Spore viability was determined by suspending 0.01 g spores in 1ml of sterile distilled water containing 0.1% Tween 80, making a 1:10 dilution, then pipetting 20 μl onto a Petri dish containing potato dextrose agar (Difco) and covering with a 24 × 40 mm coverslip, then incubating at 25°C. Two coverslips per dish and 2 plates per spore batch were used. After 18 hr the number of germinated and ungerminated spores in four fields of view under each coverslip was determined at 400X.

Spore concentrations were determined using an improved Neubauer haemocytometer and the method outlined in (Goettel and Inglis, 1997).

3.3.2 Spore moisture

1 gram of spores was placed in each of 5 unlidded glass Petri dishes (90 mm diameter ea.). The Petri dishes were put into an oven at 100^{o}C for 24 hours, after which the spores were transferred to a desiccator for 3-4hours, to cool. The spores were then weighed again and difference in spore weight determined and expressed as percent moisture content.

3.3.3 Formulation check (Trial 2)

A formulation check was conducted as in 3.1.5 with spores produced by both DEEDI and supplied by a commercial company. The spores were formulated in either 10% codacide oil or 0.1% Tween 80 at a concentration of 2.5 × 108 spores/ml.

4 Results

4.1 Trial 1

4.1.1 Formulation check

When the virulence of formulation samples collected during spraying was checked using engorged adult ticks the results were quite poor (Figure 10). Tick mortality after immersion in the high dose only reached 65% after six days. While there was only 20% mortality in ticks immersed in the low dose over the same time. In previous animal trials (2003, 2004, 2010) the *Metarhizium* formulation usually caused 100% tick mortality within 48 hrs of immersion. It appeared that the viability or the spores had been compromised in some way. Investigations into the level of moisture in and viability of spores were initiated to understand what had happened to the spores.

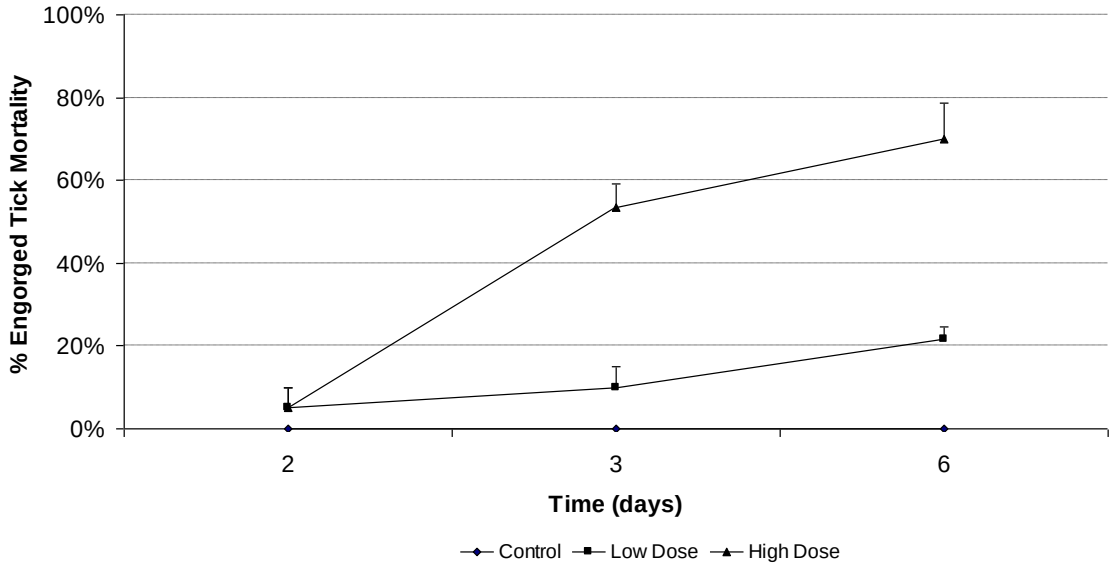

Figure 10. Mortality in engorged ticks immersed in the two different doses of the fungal biopesticide and incubated at 28°C

4.1.2 Application check

The mortality of laboratory incubated semi-engorged ticks sampled from animals immediately after spraying was higher over six days than that of ticks immersed in the formulation. The mortality in ticks sampled from high dose treated animals was 93 (±4) % after three days and 99(±1)% after six days (Figure 11). The mortality in ticks sampled from low dose treated animals was only 20(±6)% after three days but 90(±5)% after six days (Figure 11). These results suggest that despite possible problems with the formulation the

application was still thorough enough to deliver sufficient viable spores to ticks to cause high mortality after six days.

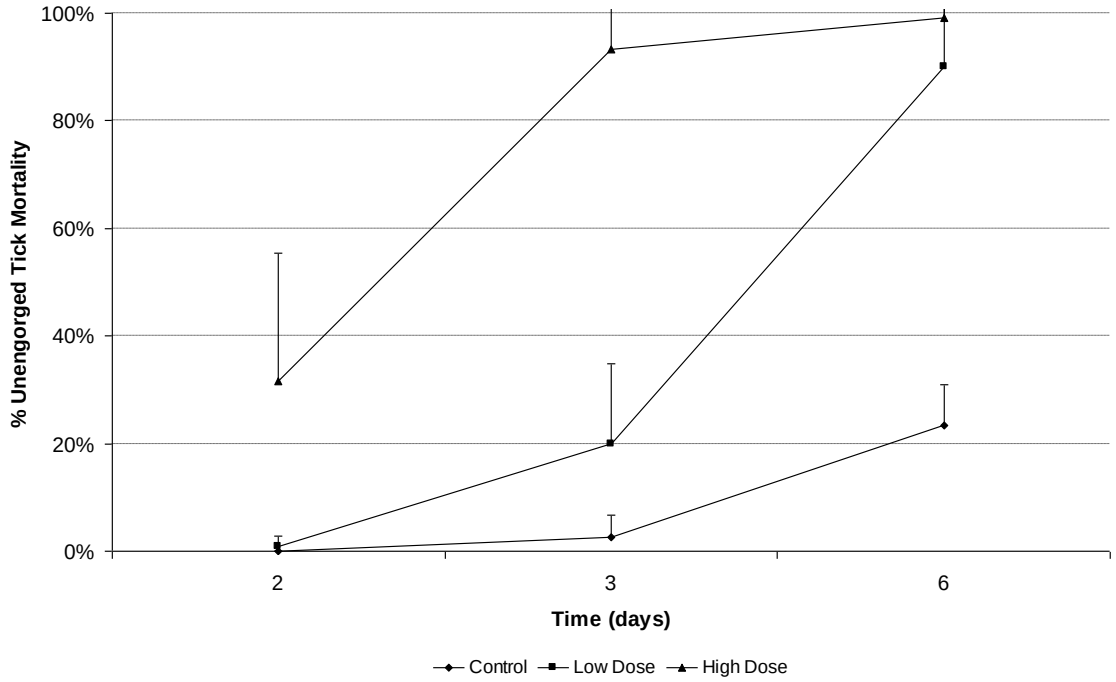

Figure 11. Mortality in standard ticks sampled from cattle in the control group and biopesticide treatment groups immediately after treatment. All ticks were taken to the laboratory for incubation at 28°C

4.1.3 Temperature

The weather during the trial was mild and thus should have provided suitable conditions for the germination and growth of ticks on animals. Across the three monitored sites the ambient temperatures ranged from 11°C to 33°C, with the temperature below 24°C for more than 60% of most of the 9 days following treatment. Correspondingly the surface temperatures recorded on selected heifers in the nine days after treatment averaged 32°C or lower for more than 60% of each day (Figure 12). Thus the temperature for more than 60% of each day was in the growth range (under 32°C) for the *Metarhzium* isolate used in the biopesticide formulation. It appears that when ambient temperatures dip below 24°C surface temperatures on cattle can be expected to drop to 32°C or lower under field conditions (Figure 12).

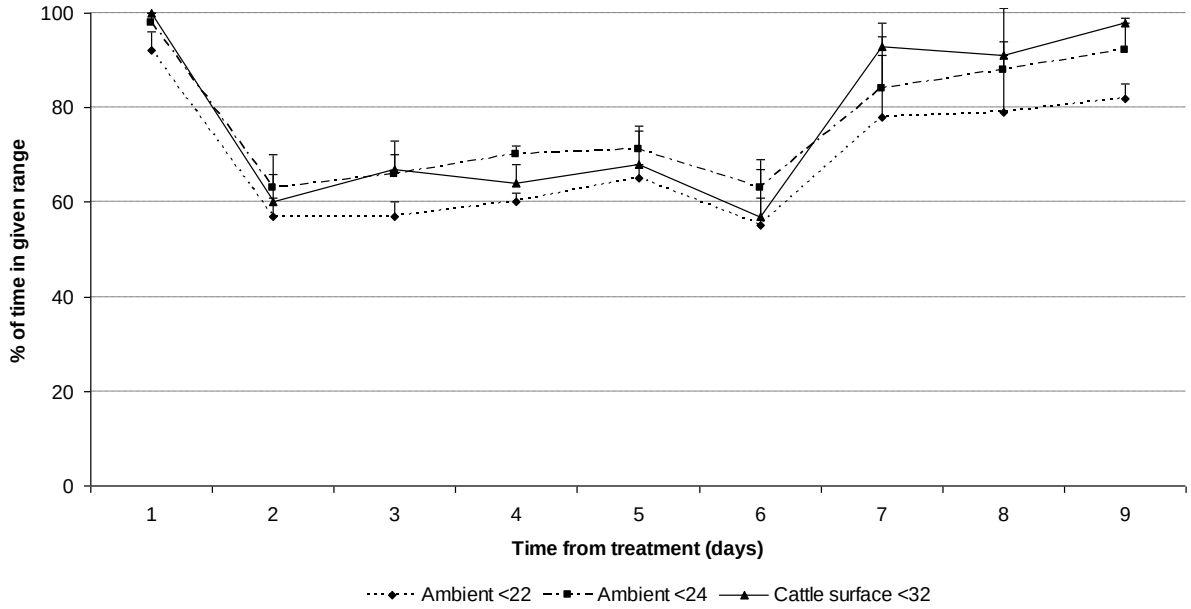

Figure 12. Daily percentage of time averaged surface temperatures on monitored animals were below 32°C and the daily percentage of time the averaged ambient temperatures were either below 22°C or 24°C

4.1.4 Assessment of treatment efficacy

4.1.4.1 Side counts of semi-engorged ticks

A very high level of control, averaging close to 100%, was achieved with all tick stages on the animals at the time of treatment with the positive control. In addition the prophylactic control of larval ticks applied after treatment was 100% (Figures 13, 14). The high level of control achieved with the positive control indicated that the method of spray application was very thorough. The same application procedure was used for the low and high dose formulations of the fungal biopesticide. However there was a wide variation in the level of tick control achieved with the fungal biopesticide formulations (Figures 13, 14).

The average efficacy of the low dose (Figure 14) varied from close to 80% (day 20) against ticks that were at the larval stage at treatment to 0% (day 27) for the prophylactic control of one group of larval ticks applied after treatment. The efficacy against adult and nymph tick stages varied between 20% and 40% (Figures 13, 14). After day 3 the efficacy of the high dose formulation was consistently lower than that achieved with the low dose formulations. The highest efficacy achieved with the high dose was 50% against the larval stage of ticks at treatment. No prophylactic control occurred with the high dose formulation (Figures 13, 14).

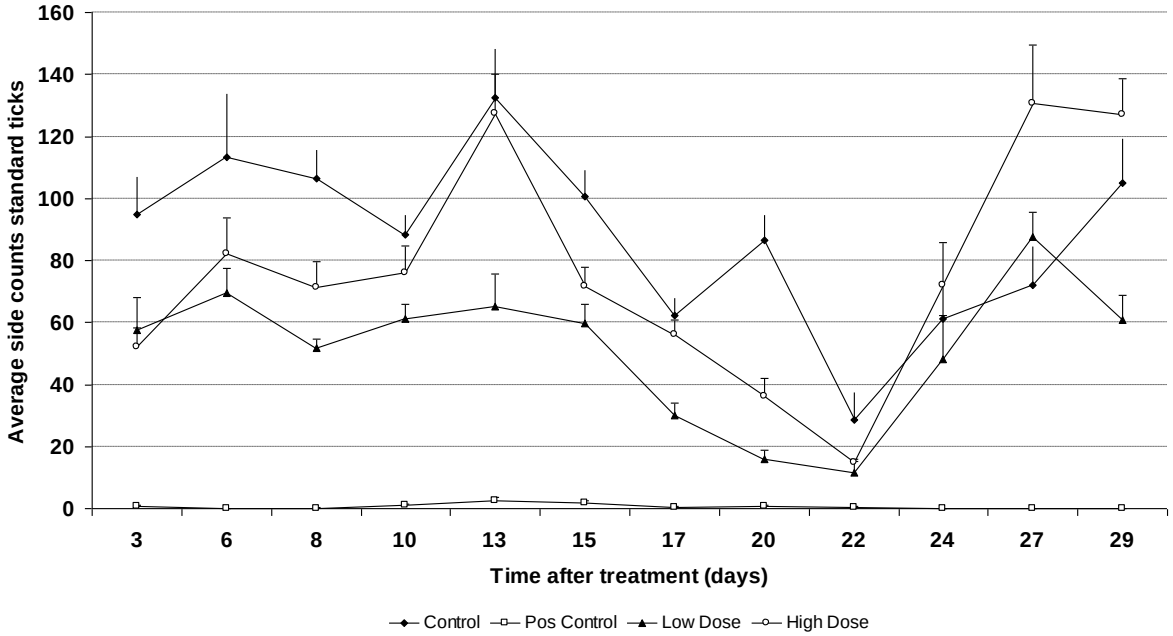

Figure 13. Average side counts of standard ticks from each of the four treatment groups

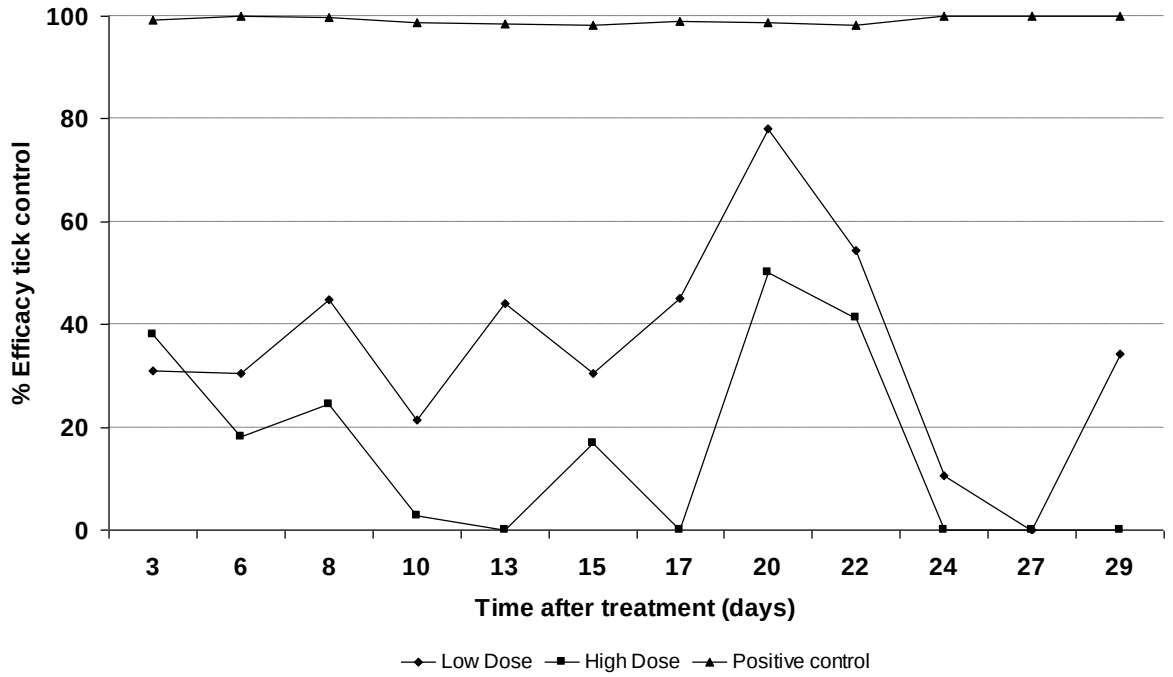

Figure 14. Average percent efficacy of tick treatments compared to untreated animals in the 29 days after treatment

4.1.5 Spore viability in cattle coats

A large number of viable *Metarhizium* spores were recovered from coat samples taken one week after the application of the formulation to the animal coats. However by two weeks post application no viable spores were recovered from the cattle coats.

4.2 Trial 2

4.2.1 Assessment of treatment efficacy

4.2.1.1 Side counts of semi-engorged ticks

For Trial 2 it was planned to artificially infest cattle with larval ticks in the weeks leading up to treatment to ensure all stages of ticks were present on cattle at treatment day. However this proved unnecessary as the heifers picked up large numbers of natural tick infestations from the paddocks at Pinjarra Hills. Between Trials 1 and 2 the heifers were held in two different paddocks. These paddocks had different levels of larval ticks which were influenced by which treatment group was in that paddock during Trial 1. Tick infestations from one paddock were so high that all cattle in that paddock had to be treated with Taktic™ EC three weeks before Trial 2 for welfare considerations. In the week before Trial 2 commenced a number of cattle in the other paddock also needed to be treated with Taktic™ EC. This left only six animals in the group of 18 for Trial 2 that had any tick burden at the time of treatment. These heifers were then randomly allocated with three going to either the untreated control group or three going to the spray treatment group. The heifers treated in the week prior to treatment were all allocated to the "pour on" treatment group because Taktic™ EC has no effect on buffalo fly. The remaining heifers were then randomly allocated to either the control or spray groups.

Although side counts were performed on all six heifers in both the untreated control and biopesticide spray groups, only three animals in each group had any ticks to count for the first two counts. Therefore only the counts on the three heifers in each group that had a tick burden on treatment day are given in Table 5. This compromised the power of the trial with only three replicates per treatment; but welfare considerations took precedent over gathering tick control data in Trial 2. The efficacy of the fungal biopesticide was 39% and 69% for the first two counts at days 5 and 12 post treatment (Table 6). At the third count (19 days) there was a huge increase in ticks on the treated animals compared to the untreated controls. The ticks being counted would have been larval ticks on treatment day. This result is possibly a reflection of the uncontrolled variability of tick infestations coming from the paddocks and that three animals per treatment group is not adequate in a cattle tick trial.

Table 6. Average side counts of standard ticks on untreated cattle and cattle sprayed with the biopesticide

Treatment		Time after spray treatment		
		5 days	***12 days***	***19 days***
Control	*n=3*	101 (±24)	32 (±10)	34 (±4)
Biopesticide Spray	*n=3*	62 (±11)	10 (±1)	132 (±35)
Efficacy		39%	69%	-288%

4.2.1.2 Buffalo fly assessment

Natural infestations of buffalo flies built up to extremely high levels on all heifers between Trials 1 and 2 so that by treatment day all groups had approximately 500+ flies per side (Figure 15). This very high number of flies on untreated control cattle continued to increase so by day 21 it was estimated these heifers were carrying 500-1000 flies per side (Table 7). In the last days of the trial some of the untreated heifers were beginning to develop lesions associated with extremely heavy buffalo fly infestations. No such lesions or even a hint of them were seen on any treated cattle. Moreover the condition of the treated cattle was much better than that of the untreated cattle.

The number of buffalo flies on heifers sprayed with the biopesticide dropped rapidly so that two days later there was approximately only five flies per side on each heifer in this group

(Table 7). However a linear decline occurred in the effect with fly numbers building to an economic threshold of approximately 300 flies per side by 19 days post treatment. Although there was a steady increase in fly numbers on the sprayed cattle the infestation never reached the levels seen on the untreated cattle.

Cattle treated with the “pour on” formulation showed a steady decline in buffalo fly numbers to approximately 1- 50 flies per side by 7 days after the first application (Table 7). This low number was maintained for much of the trial except for the counts in days 14 and 19. By 12 days after the last application (day 26) fly numbers recovered to approximately 150-300 flies per side. A number of heavy rain events occurred during the trial, which did not appear to affect fly numbers on untreated heifers, but may have had a negative effect on the biopesticide. It is possible that the number of flies recorded on cattle 5 days after the first application was also higher than actual. No flies were seen on these cattle when they were collected from their paddock and walked to the yards. But once in the yard a number of flies were noted. The untreated group had previously been in the yards, thus it is possible that flies disturbed from this group and left behind moved to the “pour on” group once they entered the yards. After this all treated cattle were moved to a different set of yards for assessment.

Table 7. Appraisal of buffalo fly populations of control and biopesticide treated cattle

Treatment	Fly rating	Assessment day									
		0	2	5	7	9	12	14	19	21	26
Control	**0** **1-50** **50-150** **150-300** **300+**	500+	500+	500+	500+	500+	500+	500+	500+	500+	500+
Spray	**0** **1-50** **50-150** **150-300** **300+**	500+	~5	✓	✓	✓	✓	✓	✓ ✓	✓ ✓	✓
“Pour On”	**0** **1-50** **50-150** **150-300** **300+**	500+	✓	✓	✓	✓	✓	✓	✓	✓	✓

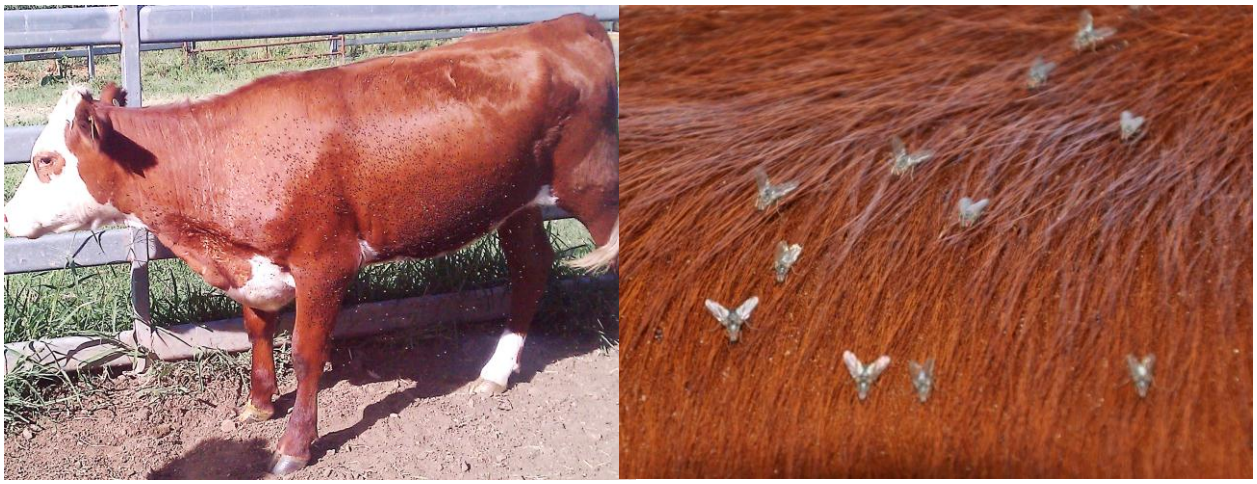

Figure 15. Very heavy infestation of buffalo flies (500+ per side) on untreated heifer (left). Close up of buffalo flies on the back of an untreated heifer (right)

Laboratory investigations were conducted with buffalo flies netted from the three treatment groups of cattle. It was hoped that these investigations would provide further evidence for

the uptake of lethal doses of *Metarhizium* from treated cattle. This effect was seen in a cattle trial conducted during 2004. However the very high levels of mortality seen in all incubated flies indicated there was something wrong with the assay conditions (Table 8). In previous assays with buffalo flies the mortality in untreated flies after 4 days was as low as 10% and 3%. The source of bovine blood used to feed the flies might account for the poor results.

Table 8. Average percent mortality (±SE) in buffalo flies netted from cattle groups and incubated for 4 days in the laboratory

Treatment	Collection days											
	Pre-treatment			*Post-treatment*								
	d-7	d-2	d0	d2	d5	d7	d9	d12	d14	d19	d21	d26
Control	22 (13)	28 (10)	84 (18)	88 (11)	77 (17)	77 (22)	77 (14)	47 (15)	66 (40)	99 (1)	100 (0)	73 (28)
Spray			86 (7)	50 (0)	36 (4)	55 (31)	73 (14)	50 (7)	79 (23)	96 (5)	99 (1)	51 (28)
"Pour on"				81 (6)	58 (13)	42 (0)	68 (11)	36 (10)	76 (4)	98 (1)	100 (0)	91 (16)

If buffalo flies die from a *Metarhizium* infection the fungus will grow out of the surface sterilised flies within 2–3 days of incubating on an agar plate (Figure 16, left). The fungus consumes the fly in a fuzz of mycelium before the characteristic green patches of *Metarhizium* spores appear (Figure 16, right). No *Metarhizium* was found in any flies netted before treatment (Table 9; d-7, d-2, d0), but *Metarhizium* did grow from many of the flies netted from biopesticide treated cattle. The level of *Metarhizium* detected was greatest in days 2, 5 and 7 after the initial treatment day. However the drop in *Metarhizium* in flies from the "pour on" group after day 7 is puzzling as the formulation was still being applied until day 14. The level of *Metarhizium* seen in flies from the untreated heifers is also interesting, but may indicate that some infected flies are blown across paddocks.

Table 9. Percentage *Metarhizium* infection in netted buffalo flies which were dead after 4 days laboratory incubation

Treatment	Collection days											
	Pre-treatment			*Post-treatment*								
	d-7	d-2	d0	d2	d5	d7	d9	d12	d14	d19	d21	d26
Control	0 (56)**	0 (63)	0 (84)	2.4 (84)	0 (82)	1.3 (77)	NA	2.4 (84)	4.8 (84)	0 (84)	1.2 (84)	0 (77)
Spray			0 (84)	66.7 (3)	29.6 (27)	6.3 (64)	NA	1.4 (72)	0 (75)	0 (84)	0 (84)	0 (65)
"Pour on"				20.5 (6)	12.2 (74)	50 (10)	NA	1.4 (72)	1.2 (84)	9.5 (84)	0 (63)	0 (84)

*** total number of dead flies incubated in each group*

Figure 16. *Metarhzium* infection emerging from incubated infected buffalo flies, early stage (left) and later stage with characteristic green sporulation (right)

4.2.2 Spore viability in cattle coats

Washes from cattle coat samples indicated that a large number of *Metarhizium* spores stayed viable in the coats of cattle in paddocks through summer for at least two weeks after the biopesticide was sprayed onto the coats (Figure 17, left bottom). Samples taken from cattle treated with the "pour on" formulation showed very large numbers of viable spores were recovered from coat samples taken two days after the last application (Figure 17, left top). Large numbers of viable spores were still recovered 12 days after the last application (Figure 17, right). While the number of viable spores giving rise to *Metarhizium* colonies was lower than for the spray application after two weeks, sampling bias could have affected the result. The "pour on" formulation was only applied to a limited area and hair samples were taken from the edge of this area. However the actual edge of the area was not well defined because there was little visual evidence of the oil application even three days after application. Therefore after 12 days it is possible that some samples were taken just out of the application area. In addition heavy rain events during the latter part of the trial resulted in long wet grass which could have helped wash some of the spores out of the belly coat. The simple formulation tested did not contain any additives to make it rain-fast.

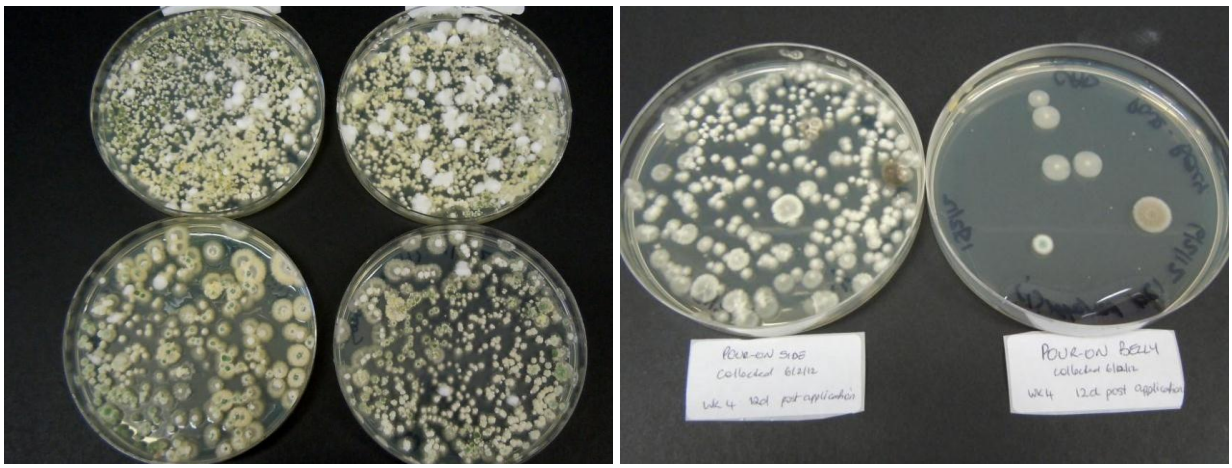

Figure 17. *Metarhizium* colonies growing from cattle hair sampled after treatment. Left: top - sampled two days after a "pour on" application; bottom - sampled two weeks after spray application. Right: sampled 12 days after the last "pour on" application. In each photo plates on the left are from samples taken at the shoulder/side and plates on the right are from samples taken from the belly

4.2.3 Quality Assurance (QA) procedures

4.2.3.1 Trial 1

The QA data provided by the commercial company stated the spore viability to be 87% and moisture to be 9.1%. However investigations by DEEDI found the spore viability to be 52% and moisture to be 44.3% (Table 10). This moisture level is exceptionally high and would contribute to a rapid loss of viability once the spores are exposed to oxygen and higher temperatures when removed from cold storage.

Table 10. QA data on spores provided by a commercial company for the biopesticide formulation in trial 1

Data Source	% Viability	% Moisture
Commercial company	87	9.1
DEEDI	52	44.3

4.2.3.2 Trial 2

The batch of spores produced by DEEDI had the high viability expected of freshly produced spores (Table 11). The moisture level of these spores was 15.8% which is higher than the ideal spore moisture for storage. However this was not a problem as the spores were to be used immediately. In addition the higher moisture level was deliberate because of concerns regarding the time required for imbibition prior to germination if the spores were too dry. The commercially produced spores had a low viability that appeared to be decreasing with time (Table 11). The different viability of the two different spore batches was reflected in the formulation check. The formulations (aqueous and oil) with the DEEDI produced spores resulted in 100% tick mortality after 24 hrs. However the aqueous and oil formulations with the commercially produced spores caused respectively 50% and 75% tick mortality after 24 hrs. Thus the spores produced by the commercial company failed the QA undertaken by DEEDI and only the DEEDI produced spores were used.

Table 11. QA on batches of spores provided by a commercial company and produced by DEEDI for the biopesticide formulation in Trial 2

Source of spores	Data source	% Viability	% Moisture
Commercial company	Commercial company	77	8.8
	DEEDI	55.6 (23/12/2011) 25.3 (5/1/2012)	6.5
DEEDI	DEEDI	95.4	15.8

5 Discussion

The results of these two cattle trials showed that a *Metarhizium* based fungal biopesticide shows potential for the control of buffalo flies but has limited efficacy against cattle ticks.
Both biopesticide spray and “pour-on” formulations resulted in huge decreases in buffalo fly populations on treated cattle and the fungal spores appeared to remain viable in the animal coat for up to two weeks after application. However, there were no clear trends to support good efficacy in tick control in either of the two trials though the viability of the biopesticide formulation assessed against ticks in Trial 1 was compromised and Trial 2 was conducted at a hotter time of year. If there was an obvious potential for commercial development clear trends should have been evident despite these problems.

Laboratory investigations revealed that when *Metarhizium* invades cattle ticks under optimal conditions it rapidly destroys the cuticle killing the ticks within 48 hrs (Leemon and Jonsson, 2012). This mechanism suggested a good potential for *Metarhizium* as a biopesticide for

ticks, as long as optimal conditions for the fungal invasion prevailed long enough for the fungus to grow rapidly. The potential limitation of the temperature and relative humidity in the microclimate around ticks on cattle could only be evaluated through field trials.

During Trial 1 the ambient conditions resulted in animal surface temperatures below 32°C for more than 60% of each day in the 9 days after treatment (average of 77%). Therefore it was expected that the temperature should have been suitable for the germination and growth of *Metarhizium* spores on ticks during Trial 1. However it was unfortunate the efficacy testing in Trial 1 was complicated by issues with the viability and virulence of the formulation. Both the application and formulation checks showed that the formulation took much longer (6 days instead of 2 days) to kill appreciable, but lower numbers of ticks compared to previous trials (Leemon *et al*, 2008, Leemon, 2010). Viable spores were recovered from the cattle coat one week after treatment but the number of colonies was low compared to the recovery from the cattle coats two weeks after treatment in Trial 2. Nonetheless, allowing for the reduced spore viability, with the spore concentrations being used some clear trend should have been apparent if suitable conditions for the biopesticide occurred within the cattle coats.

The lower efficacy for the high dose compared to the low dose in Trial 1 is counter-intuitive and of concern, because no logical explanation is apparent. Tick mortality in both the formulation and application checks, in which ticks were incubated in the laboratory under optimal conditions for temperature and humidity, showed a dose response (Figures 10, 11).

In Trial 2, recent salvage treatment of the majority of the cattle with amitraz meant that there were too few animals on which to assess the fungal tick efficacy.

Overall it can be concluded that despite promising efficacy of the fungal biopesticide in the laboratory and some efficacy in studies on animals (Leemon *et al*, 2008, Leemon, 2010) the results of this project failed to demonstrate the feasibility of a commercially viable fungal biopesticide for tick control on cattle.

Especially promising results were achieved with the fungal biopesticide control of buffalo flies on cattle when applied as either a “pour on” formulation or as a spray (Table 7). The spray formulation caused a rapid decrease in buffalo fly numbers. By two days after treatment barely five flies per side were seen on the sprayed heifers. This effect gradually decreased with time and buffalo fly numbers subsequently increased; although fly numbers on these animals did not reach an economic threshold (300+) until nearly three weeks after treatment. However the “pour on” strategy was more effective than the spray strategy giving greater control with less formulation. To spray treat six heifers, 375 g of spores was mixed into 6 litres of oil which was diluted and sprayed onto cattle at approximately 6-8 litres/heifer. However, only 3.12 litres of oil containing 195 g spores was applied to six heifers over two weeks via seven “pour on” applications. The 195 g spores was produced by DEEDI using just 4 kg of rice, suggesting that with scale up savings the production of a biopesticide for buffalo fly control could be an economical proposition.

Trial 2 was only an appraisal of the effect of the fungal biopesticide on buffalo fly rather than a full efficacy trial; therefore treatments using only the spore carrier (oil base) were not included. However we were aware that any reduction in buffalo fly populations on cattle might simply result from a repellent effect of the carrier. Therefore the way in which the “pour on” formulation was applied to cattle recognised the need to identify if the codacide oil had any repellent effect on flies. Thus the “pour on” was applied to delimited areas on the shoulder and belly so any repellent effect on flies could be monitored. This limited application still left a large area away from the oiled areas, particularly on the rump, for flies to land if they were repelled by the oil. Application to both the belly and shoulder allowed a comparison to be made between areas with different levels of direct exposure to sunlight

and UV during the day. UV is known to negatively affect the viability of fungal spores. In some coat samples more *Metarhizium* colonies grew from the belly samples than from the shoulder samples, but the reverse occurred in the sample taken 12 days after the last "pour-on" application. The effect of large amounts of rain and long wet grass on the number of spores in the belly coat can only be guessed at.

Neither the spray nor "pour on" formulations appeared to have any obvious repellent effect on buffalo flies. No flies were seen on cattle immediately after spraying when the coat was still wet. However by the next observation of the cattle (2 days later) their coats were dry and flies were observed on the cattle, albeit a very small number (~ 5 / side / animal). This low number of flies seemed more related to a real effect of the fungus than a repellent effect of the oil carrier. However any future trials should incorporate a positive control using only the oil base to provide data on any possible repellent effect of the carrier on buffalo flies. Flies were observed to land on areas newly treated with the "pour on" formulation, but they would soon move away to groom themselves. They appeared to react to contact with the wet oil. However by 2 -3 days after application of the "pour on" formulation the area treated was no longer wet and did not appear to be oily and flies were observed to land in the treated area and stay longer. Investigations into the viability of the fungal spores in cattle coats showed that large numbers of spores stayed viable in the coat for up to 12 days. In addition, low numbers of flies (1-50) were observed on cattle a week after the last "pour on" application. This suggests that the "pour on" formulation may give at least a week of protection and a future strategy for investigation might be a weekly "pour on" type application over a longer period of time. However further investigation of the number and timing of spray applications may also be worth considering.

The results of the laboratory investigations into the uptake of *Metarhizium* by buffalo flies netted from cattle were disappointing. The high rates of fly mortality across most of the samples are possibly related to a poor supply of bovine blood for fly feeding. In previous assays the average rate of fly mortality was much lower. This included incubating field netted flies which were kept alive in the laboratory for 7 days before the mortality began to rise. However in these assays the buffalo flies were fed twice daily with fresh blood collected from a known source of cattle at the DEEDI Animal Research Institute. Such a source of bovine blood was not available for feeding flies in Trial 2. Instead frozen bovine blood from a number of sources was used. Further investigations are required to develop a consistent assay system in which the mortality of untreated flies is minimised. These investigations should include an exploration of the effect of bovine blood from different sources, and regularity of feeding on fly mortality.

The isolation of *Metarhizium* from a greater number of buffalo flies netted from treated cattle than from untreated cattle (Table 9) supports the notion that flies were taking up lethal doses of *Metarhizium* spores from treated cattle. Therefore it is likely that the lower numbers of buffalo flies observed on treated cattle resulted from flies picking up spores from these cattle, becoming infected with *Metarhizium,* and dying. However the level of *Metarhizium* isolated from netted buffalo flies was lower than seen previously seen in the 2004 trial. Based on this study it was hoped that *Metarhizium* would be recovered from a high percentage of flies netted from treated cattle. However the lower amount of *Metarhizium* recovered might be accounted for by a difference in procedures in the two trials. In the 2004 trial heifers were held in small outdoor pens and buffalo flies were netted from animals *in situ.* In Trial 2 cattle were collected from their paddocks and walked at least 300m to yards for assessment and buffalo fly netting. It is possible that the many *Metarhizium* infected flies were left behind when the cattle were moved and thus not netted. During the 2004 trial more *Metarhizium* infected flies were netted. Little is known of the behaviour of buffalo flies in the early and mid stages of fungal infection. Laboratory investigations conducted by DEEDI with other flies (*Musca domestica* and *Lucilia cuprina*) revealed that these flies developed

severely ataxic movements as the fungal infection progressed. This suggests that if buffalo flies are similarly affected they might have difficulty staying with moving cattle. In this case obtaining evidence of large amounts of *Metarhizum* uptake by buffalo flies from cattle in the field would therefore be difficult. Behavioural studies with buffalo flies in the laboratory are needed to provide some insights.

Based on the results in this project, further investigations into a fungal biopesticide for buffalo fly control are warranted. These should encompass both laboratory investigations and field studies. Laboratory investigations are required to improve the *in vitro* incubation of flies and understand the process of *Metarhizium* infection of buffalo flies as noted above. The laboratory investigations should also include optimising the *Metarhizium* strain selected through screening field hardened isolates and investigating a range of formulation options to support different application strategies (such as: spray, pour on, back rubber or dust bag). Efficacy field studies which include cattle pre-ranked on buffalo fly infestations and a positive control of the carrier should then be conducted over a longer period of time than the current study.

6 Success in achieving objectives

The objectives of this project have been met. Two trials were conducted in which the efficacy of a *Metarhizium* based fungal biopesticide in controlling all stages of cattle tick on cattle under field conditions was thoroughly assessed. One trial also successfully appraised the effect of the *Metarhizium* formulation on buffalo fly populations on cattle.

7 Impact on meat and livestock industry – Now and in five years time

The results of this project suggest that a fungal biopesticide for buffalo fly control has potential. A biopesticide will be an important addition to current control strategies, particularly those that utilise walk through buffalo fly traps, back rubbers and dust bags. However further research is required to optimise both the formulation and application strategies.
The results of the project also suggest that a fungal biopesticide for tick control is subject to too many variables to deliver a consistent and high level of tick control on animals across a range of climatic regions and seasons. Therefore further progress towards the commercialisation of a fungal biopesticide for ticks is not supported by this project.

8 Conclusions and recommendations

The results of the tick control efficacy trials showed there are too many variables influencing the optimal performance to guarantee a consistent high level of control of all on-animal tick stages with a *Metarhizium* based biopesticide. These variables include: ambient temperature, time of day, time of year; relative humidity, animal coat length, and grass length (moisture in grass) and integrity of biopesticide manufacture. The activity range is too narrow and the efficacies obtained in these trials are too low for commercial consideration.

The appraisal of buffalo fly control with a fungal biopesticide gave positive results indicating that a fungal biopesticide could provide excellent buffalo fly control with further development, particularly of a “pour on” style of treatment. Thus further research in the laboratory and in the field is warranted to provide the information for this development.

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