Experimental and Applied Acarology (2006) 38:211–218
DOI 10.1007/s10493-006-0002-6
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Performance of a prototype baited-trap in attracting and infecting the tick *Amblyomma variegatum* (Acari: Ixodidae) in field experiments

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Received 15 December 2004; accepted in revised form 22 December 2005

Key words: Attraction-aggregation-attachment pheromone, Fungi, Vegetation

Abstract Investigations were commenced to study the potential use of the fungi, *Beauveria bassiana*, *Metarhizium anisopliae*, and the attraction-aggregation-attachment pheromone (AAAP) for the control of *Ambloyomma variegatum* as an environmentally friendly technology. The objective of the study was to develop and test a device, which could be used for pheromone and carbon dioxide delivery and infection of ticks with the fungi in an attempt to control the tick populations in the vegetation. Using a pheromone-baited device treated with the fungi mixture, 79% of the ticks released were attracted and exposed to the fungi and of these, 78% died during incubation in the laboratory. In another set of experiments, of the released ticks that were similarly exposed to fungi using the pheromone-baited device and left in the vegetation, 33.8% were recovered compared to recoveries of between 76 and 84% in the controls. These results were significantly different at the 5% level, an indication that the pheromone/fungi mixtures had significant effect in reducing the tick population in the field.

Introduction

The potential of semiochemical signals that mediate host-seeking behavior of the tropical bont (*Amblyomma*) ticks in the control of these ticks has been recognized since 1970s (Gladney et al. 1974; Rechav and Whitehead 1978). Two such signals mediate this behavior: host-derived carbon dioxide (CO_2), which marks the presence of a potential host and stimulates the ticks' searching behavior, and attraction-aggregation pheromones (AAAPs) produced by successfully feeding male ticks, which guide off-host male and female conspecifics to appropriate hosts and feeding sites (Hess and DeCastro 1986; Norval et al. 1989 a, b). Although some overlap occurs in the roles of these inter- and intra-specific signals in different tick populations, the presence of both are needed for optimal attraction of the ticks (Norval et al. 1987, 1989a; Barré et al. 1997; Maranga et al. 2003).

One approach to exploit the bont tick semiochemicals for its control has explored pheromone and acaricide-impregnated decoys placed on the host that provides natural source of CO_2 (Norval et al. 1994). High levels of control of *A. hebreum* have been demonstrated with impregnated PVC tags placed on the tails of cattle (Norval et al. 1996). Although the attraction of CO_2-activated *Amblyomma* ticks to AAAPs off-host has been demonstrated (Hess and DeCastro 1986; Norval et al. 1989a), their potential in the control of these ticks without the participation of cattle has not been explored.

The present study was designed to explore off-host livestock tick control tactics that integrate semiochemicals with entomogeneous fungi pathogenic to these ticks. Earlier studies demonstrated high pathogenicity of strains of the fungi *Beauveria bassiana* and *Metarhizium anisopliae* against the ticks *Rhipicephalus appendiculatus* and *A. variegatum* as well as large reductions in engorgement, fecundity and egg hatchability in surviving members of both species of these ticks (Kaaya et al. 1996). More recently, we found that a conidial mixture of the two fungi gave higher mortality of *A. variegatum* in the field compared to individual fungi (Maranga et al. 2005). In another study, we compared the attractive range of different doses of AAAP to *A. variegatum* dispensed from the centers of circular plots in the presence/absence of elevated levels of CO_2 and found that up to 90% of released ticks were attracted to the pheromone source in the presence of 500 g CO_2 (Maranga et al. 2003). On the basis of these results, the present study was initiated to assess the efficacy of a prototype trap baited with AAAP and CO_2 and treated with a mixture of *B. bassiana* and *M. anisopliae* conidia, to attract and infect *A. variegatum* adults released in experimental plots.

Materials and methods

Ticks

Two- to three-month-old unfed adult ticks of *A. variegatum* from the ICIPE rearing unit (from a colony that has been maintained since 1978) were used. They were kept in vials covered with cotton wool and then placed in aluminum tins, where relative humidity and temperature were maintained at 75% and 25 ± 1 °C, respectively.

Fungi

Isolates of *B. bassiana* and *M. anisopliae* were obtained from cultures that have been maintained in the Entomopathology Unit at the ICIPE by preserving them in mineral oil and liquid nitrogen. The *B. bassiana* isolate (ICIPE germplasm accession number 51) was originally obtained from the banana weevil, *Cosmopolites sordidus* (German), in Nairobi, while *M. anisopliae* isolate (ICIPE germplasm accession number 7) was isolated from the

migratory locust, *Locusta migratoria*, in Madagascar (Kaaya et al. 1993). Both fungi were periodically passaged through *A. variegatum* ticks to maintain their virulence.

Fungal cultures and formulations

The fungi were cultured on Sabouraud Dextrose Agar (SDA) (Mast Laboratories, Mersyside, U.K.) for 2–3 weeks at room temperature and harvested using 0.01% Triton X-100 as previously described (Kaaya 1989). Briefly, the fungal formulations were prepared as follows: the water formulations were prepared by first centrifuging the harvested conidia and decanting the supernatant after which distilled water was added to the conidia in the centrifuge tubes. The mixture was thoroughly mixed using a vortex mixer after which it was centrifuged and the supernatant decanted. This procedure of washing conidia was repeated three times and the conidia were then suspended in distilled water. One ml of the suspension was then added to 99 ml of distilled water and a drop of this suspension was placed on a Neubauer hemacytometer to count the conidia under a microscope at magnification 40×. The concentration of the conidia in 1 ml of the stock suspension was then calculated and diluted to 10^{10} conidia/ml for each fungi. The stock concentration of the oil formulation for each fungi consisted of 15% peanut oil, 1% emulsogen-1900T (Kenya Food Industries, Nairobi) and 84% of the aqueous conidial stock concentration. In preparing the conidial mixture of the oil formulation, 100 ml of the stock suspension *B. bassiana* conidia was added to 100 ml of the stock suspension of *M. anisopliae* both of oil formulation and equal conidia concentrations (10^{10} conidia/ml), and thoroughly mixed using a vortex mixer.

Pheromone-baited pathogen trap

The trap was made of aluminum material and consisted of a pheromone dispenser, dry ice container and contamination trays (Figure 1). The pheromone dispenser consisted a Petri dish (9 cm diameter) on which a Whatman's filter paper (9 cm diameter) was fixed on the bottom side using laboratory parafilm. The inner contamination tray measured 16 cm in diameter and was supported by aluminum anchor rods and an outer round measuring 6 cm in width (Figure 1). The outer tray was held in place by three aluminum bars (5 cm in length and 1 cm width) welded to the inner contamination tray. The inner tray was filled with 50 ml of the oil formulation which is a mixture of *B. bassiana* and *M. anisopliae* of a concentration of 1×10^{10} conidia/ml while the outer tray carried 200 ml of the same mixture. The dry ice container, which consisted of a cylindrical aluminum tube measuring 8 cm in diameter was welded at the center of the inner contamination tray (Figure 1).

A stock solution of the AAAP was prepared by dissolving *o*-nitrophenol (200 mg), methyl salicylate (100 mg) and nonanoic acid (800 mg) in hexane (1 ml). One microliter of this solution used in the trap contained the three components in approximate amounts produced by one attached feeding *A. variegatum* male (Schöni et al. 1984). The traps were baited with 6 μl of the solution (containing 1.2 mg *o*-nitrophenol, 0.6 mg methyl salicylate and 4.8 mg nonanoic acid) that was found previously to be optimally attractive in the field (Maranga et al. 2003). The components of the pheromone were obtained from Sigma-Aldrich Company (Ltd), UK.

Experimental site

Field experiments were carried out at the ICIPE's Mbita Point field station (Latitude; 0°25–30′ S, 34°10–15′ E) situated in Suba District, Kenya. The

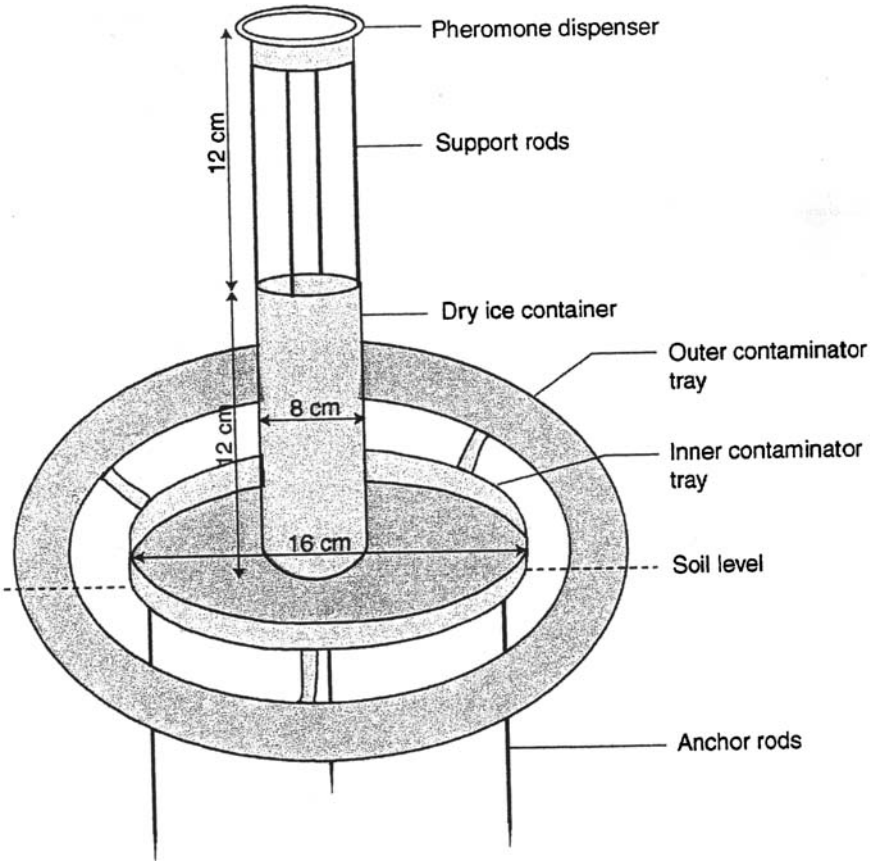

Figure 1. Prototype tick trap.

station has an altitude of 1240 m with annual rainfall of 1150 mm and temperatures ranging between 21.1 and 28.3 °C. Two paddocks, measuring about two acres which had previously been ploughed and planted with Rhodes grass, were used (Maranga et al. 2003). By the time the experiment commenced, the grass was about 50 cm. The experiments were carried out in 15 circular plots of 6 m radius. Starting from the center, the plots were marked with wooded pegs every 1 m up to 6 m in straight lines at 45° interval all round. A small circle of 10 cm radius was prepared at the center of the plot with all grass removed where the trap was placed. Each plot had a barrier of 5 m uncleared grass surrounding it.

Experiment 1

Groups of marked ticks (as in the method by Maranga et a1. 2003), 10 males and 10 females each were released at 1, 2, 3, 4 and 5 m distance from the center of each circular plot in the North, South, East and West positions in each of the 15 plots (making a total of 400 ticks/plot). These were then left for five days to acclimatize. On the sixth day, a pheromone trap treated with the oil formulation of the mixed fungi of a total concentration of 1×10^{10} conidia/ml and baited with AAAP (6 μl) and CO_2 (500 g) dry ice obtained from Carbacid Kenya, Nairobi), were set at the center of each of three randomly selected experimental plots. Sets of three control plots were similarly prepared with: (a) one set containing AAAP, CO_2 and oil formulation without fungi; (b) the second, with AAAP, CO_2 and distilled water; (c) the third with AAAP, CO_2 and empty traps; and (d) with AAAP and CO_2 only. The ticks that were attracted to the treatment and the controls were collected and placed in separate vials after which they were incubated in the laboratory at 28 ± 1 °C and 75% relative humidity and their mortality monitored and recorded for three weeks.

Experiment 2

The plots were cleared off ticks using AAAP and 500 g of CO_2 traps. New sets of marked ticks were then released and treatments with controls set as in experiment 1 above. The traps were then left in the field for three weeks after which sampling of the ticks was carried out using AAAP combined with 500 g of CO_2 in each of the plots.

Data analyses

Prior to ANOVA, data on ticks attracted to the traps and that of their subsequent mortality in the laboratory were subjected to arcsine transformation after which mortality data were analyzed using Analysis of Variance (ANOVA), (SAS 1988). Mean separation was done using the Student–Newman–Keul's

(SNK) test, ($p = 0.05$). Data on ticks collected in the field were similarly handled.

Results

ANOVA results on the levels of mortality of ticks incubated in the laboratory after exposure to different treatments show that the fungi-treated pheromone trap was highly effective in attracting and infecting the ticks in the field ($P < 0.001$; df = 4, $F = 3464.7$). In the fungi infected set, 77.8% of the ticks were killed compared to only about 1% in the controls (Table 1). There were no significant differences in the mean percentage number of ticks that were attracted to the different treatments (Table 1).

The difference between the mean percentage numbers of ticks recovered from the field arenas with fungi-treated and untreated traps were also highly significant (ANOVA, $p < 0.001$ df = 4, $F = 17.21$). The paddock with the fungi trap had the lowest number of live ticks recovered (33.8%), which was less than half the number of similar ticks recovered in the other paddocks (Table 2). There were no significant differences among the controls ($p > 0.05$).

Discussion

The results of this study have shown that semiochemicals-baited traps treated with a mixture of fungi is effective in attracting and infecting *A. variegatum* and result in high mortality of the exposed ticks that were incubated in the laboratory (77.8%; compared 0–1% in the control) (Table 1) and in a large reduction of ticks that were recovered (33.8% compared with 76.3–84.1% in the control) from the field plots three weeks after exposure (Table 2). The study represents the first attempt to evaluate the use of semiochemicals/fungi

Table 1. Mean percentage (±SE) of *Amblyomma variegatum* killed, due to infections cause by the mixture of *Beauveria bassiana* and *Metarhizium anisopliae* 3 weeks after exposing them to the fungi using the traps.

Treatment	Mean % of ticks	
	Exposed	Killed
AAAP + CO_2 + fungi	79.00 ± 1.39Aa	77.83 ± 1.26Aa
AAAP + CO_2 + oil formulation	85.00 ± 3.72Aa	1.00 ± 0.29Bb
AAAP + CO_2 + distilled water	83.00 ± 2.24Aa	0.67 ± 0.22Bb
AAAP + CO_2 + traps	86.00 ± 3.28Aa	0.08 ± 0.08Bb
AAAP + CO_2	77.00 ± 3.33Aa	0.00 ± 0.00Bb

Means within the same column followed by the same lowercase letter and those in the same row bearing the same uppercase letters are not significantly different $p = 0.05$ [Student–Newman–Keul's (SNK) test].

Table 2. Mean percentage (±SE) of *Amblyomma variegatum* recovered, 3 weeks after setting the traps in the paddocks.

Treatment	Mean % of ticks recovered
AAAP + CO_2 + fungi	33.77 ± 9.96b
AAAP + CO_2 + oil formulation	76.33 ± 2.04a
AAAP + CO_2 + distilled water	80.42 ± 4.07a
AAAP + CO_2 + traps	84.08 ± 2.56a
AAAP + CO_2	83.83 ± 2.62a

Means followed by the same letter are not significantly different $p = 0.05$ [Student–Newman–Keul's (SNK) test].

combination to control livestock ticks and to target off host population of ticks. Previous studies focused on the use of pheromone/acaricide mixtures for tick control on host (Gladney et al. 1974; Rechav and Whitehead 1978; Norval 1991, 1996). In another study, it was shown that spraying fungi in grazing pastures seeded with *Rhipicephalus appendiculatus* larvae significantly reduced the populations of this tick species on cattle (Kaaya and Hassan 2000). However, this approach is likely to be very expensive in view of the spatial scale of application that would be needed. Moreover, such an approach has high risks of non-target effects. The present approach is more target-oriented and likely to be more economical and environmentally friendly.

The use of fungi for the control of ticks has an advantage over acaricides because fungi can be more readily produced locally, and are likely to be more economical and with less side effects on other organisms. There is also a possibility of horizontal transfer of the fungi from infected to non-infected ticks, which may raise the level of mortality achieved in a control operation. In nature, pathogenic fungal transmission in air arthropod population is almost entirely by cross-contamination and fungi may cause natural epizootics and devastate populations (Burgess and Hussey 1971). Further studies are needed to establish if the bait technology developed in this study can be used as a basis of initial infection to effect lateral transmission of fungal pathogens within a tick population.

Acknowledgements

The authors wish to thank the German Academic Exchange Service (DAAD) for a fellowship to R.O. Maranga through ICIPE-based African Regional Postgraduate Programme in Insect Science (ARPPIS). We also acknowledge the technical assistance of Ms. Elizabeth Ouna, Mr. Fred Thuo and Mr. Patrick Muchisu.

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