



Entomogenous fungi as promising biopesticides for tick control

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Abstract. When ticks were sealed in nylon tetrapacks and infected with the entomogenous fungi, *Beauveria bassiana* and *Metarhizium anisopliae* and maintained in potted grass in the field, the fungal oil formulations (10^9 conidia per ml) induced 100% mortality in larvae of *Rhipicephalus appendiculatus* and *Amblyomma variegatum*, whereas mortalities in nymphs varied between 80–100% and in adults 80–90%. The aqueous formulations (10^9 conidia per ml) induced mortalities of 40–50% and reductions in egg hatchability of 68% (*B. bassiana*) and 48% (*M. anisopliae*) when sprayed on *Boophilus decoloratus* engorging on cattle. The strains of *B. bassiana* and *M. anisopliae* isolated from naturally infected ticks were also found to induce high mortalities in both *R. appendiculatus* and *A. variegatum* in tetrapacks placed in potted grass. Both aqueous and oil-based formulations were found to be effective, although the latter induced higher mortalities. These fungal strains in aqueous formulation (10^8 conidia per ml) suppressed on-host populations of adult *R. appendiculatus* by 80% (*B. bassiana*) and 92% (*M. anisopliae*) when sprayed on tick-infested grass once per month for a period of 6 months. The feasibility of using entomogenous fungi for tick control in the field is discussed.

Key words: *Rhipicephalus appendiculatus*, *Amblyomma variegatum*, *Boophilus decoloratus*, *Beauveria bassiana*, *Metarhizium anisopliae*, entomopathogen, biological control

Introduction

Ticks and tick-borne diseases (T&TBDs) are considered the greatest animal disease problem in Africa (Young *et al.*, 1988; DeCastro, 1997). Ticks are controlled mainly with acaricides applied on cattle by means of plunge dips or spray races. Although acaricides have been effective in suppressing tick populations and incidences of TBDs, their main disadvantages have been the high costs relative to values of cattle and cattle products, development of acaricide resistance in ticks, environmental and food contamination by acaricides and their residues (Norval *et al.*, 1992). To alleviate these problems, researchers have been developing new methods of tick control that can

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be applied alone or integrated with acaricides. These include the use of tick resistant animals (Sutherst, 1983; Jongejan *et al.*, 1989; Rechav *et al.*, 1990); use of tick-killing plants (Thompson *et al.*, 1978; Sutherst *et al.*, 1982; Norval *et al.*, 1983) and use of pheromones (Ziv *et al.*, 1981; Rechav and Whitehead, 1981; Sonenshine *et al.*, 1985; Norval *et al.*, 1991).

Little data is available in the literature on biological control of ticks, especially on the use of tick pathogens. Entomogenous fungi have been used successfully to control various agricultural and pasture pests. In Brazil, they have been sprayed with airplanes in large fields to control sugarcane pests (Gillespie and Claydon, 1989), whereas in Indonesia and Malaysia, they are used to control the rhinoceros beetle, a serious pest of oil palms (Munaan and Wikardi, 1986). In Australia, entomogenous fungi have been used to control the subterranean pasture pest, *Adoryphorus couloni* (Rath, 1992; Rath *et al.*, 1995a,b) and field tests have produced promising results with other Australian pests such as the plague locust, *Phaulacridium vittatum*, and the wingless grasshopper, *Chortoicetes terminifera* (Milner *et al.*, 1994; Hopper *et al.*, 1995). Entomogenous fungi have also been found to induce high mortality in tsetse flies (Kaaya, 1989; Kaaya and Munyinyi, 1995) and evidence of horizontal transmission from infected to uninfected flies has been demonstrated (Kaaya and Okech, 1990). Mycopesticides are currently being developed for control of the African locusts and grasshoppers by the Commonwealth Agricultural Bureau International (CABI) and the International Institute of Tropical Agriculture (IITA) (Lomer *et al.*, 1997).

Recently, we reported high mortality and reduced fecundity and egg viability in ticks experimentally-infected with the entomogenous fungi, *B. bassiana* and *M. anisopliae* (Kaaya *et al.*, 1996). This paper reports further observations, from laboratory and pilot field experiments, on the potential of entomogenous fungi for the control of some African tick species.

Materials and Methods

Animal and potted grass experiments

Entomogenous fungi *B. bassiana* and *M. anisopliae* were cultured on Sabourauds dextrose agar (SDA) plates and conidia harvested as already described (Kaaya *et al.*, 1996). Suspensions containing 10^9 conidia per ml were then prepared in water and sprayed on ticks engorging on cattle or applied by immersion on ticks sealed in nylon tetrapacks in case of potted grass experiments (Kaaya *et al.*, 1996; Kaaya and Mwangi, 1998).

Oil-based formulations

Oil-based formulations consisting of fungal conidia in 15% peanut oil + 1% Tween-80 (emulsifier) + 84% water were prepared and their efficacies determined and compared with those of aqueous formulations.

Efficacy tests of entomogenous fungi, Beauveria bassiana and Metarhizium anisopliae formulated in oil

The two fungal isolates, *B. bassiana* and *M. anisopliae* previously isolated from banana weevil, *Cosmopolites sordidus* and the migratory locust, *Locusta migratoria*, respectively, and used in our previous experiments in aqueous formulations (Kaaya *et al.*, 1996) were formulated in oil as described above at a concentration of 10^9 conidia per ml. All life stages of *R. appendiculatus* and *A. variegatum* were sealed in nylon tetrapacks (Kaaya *et al.*, 1996) larvae and nymphs 200×3 tetrapacks for each and adults 100×3 tetrapacks, immersed in the fungal suspension for 3 s (Kaaya, 1989), put in potted grass (9 cm diameter \times 8 cm height) and placed under a tree in the field for 4 weeks, after which mortalities were recorded. In another experiment, adult *R. appendiculatus* and *A. variegatum* (200 ticks per pot \times 3) were released freely in the potted grass, sprayed with the fungal oil formulation mentioned above, and the pots securely wrapped in nylon mosquito nets to prevent ticks from escaping. These pots were also placed in the shade under a tree for 4 weeks (mean day temperature 25–28°C; relative humidity 60–70%) after which the nylon nets were removed and all live ticks on the grass and dead ticks in the soil counted. Each experiment was repeated three times. The soil was sieved using a fine nylon sieve to ensure recovery of all the ticks. In both experiments, control ticks were treated with the oil emulsion without fungal conidia.

Determination of pathogenicity of Rusinga island tick-isolated strains of B. bassiana and M. anisopliae against R. appendiculatus and A. variegatum in vegetation

Engorged female *R. appendiculatus* and *A. variegatum* were collected from zebu cattle in Rusinga island and incubated (28°C and 93% rh) in the laboratory for egg production. A few ticks failed to lay eggs and died of fungal infection. One *A. variegatum* was found to be infected with *M. anisopliae* and another with *B. bassiana*, while one *R. appendiculatus* was found to be infected with *B. bassiana*. The fungi were isolated and tested for pathogenicity against ticks. One isolate of *B. bassiana* and one of *M. anisopliae*, both isolated from *A. variegatum* were cultured on SDA at room temperature

(24–25°C) for 2 weeks and conidia harvested using the standard procedure. Concentrations of 10^9 conidia per ml were then prepared in water or 15% peanut oil–water emulsions, each sealed in three nylon tetrapacks. All life stages of *R. appendiculatus* and *A. variegatum* (3×200 larvae; 3×100 nymphs and 3×100 adults) per tetrapack, immersed in the fungal suspension for 3 s (Kaaya and Okech, 1990; Kaaya and Munyinyi, 1995), placed in potted grass and left in the field for 6 weeks, after which tick mortalities were recorded and compared between two formulations. This experiment was repeated three times.

Assessment of pathogenicity of M. anisopliae and B. bassiana to Boophilus decoloratus feeding on cattle in the field

Beauveria bassiana and *M. anisopliae* previously isolated from banana weevil and locust, respectively (Kaaya *et al.*, 1996), were tested on *Boophilus decoloratus*, the vector of babesiosis and anaplasmosis. Aqueous formulations containing 10^9 conidia per ml were prepared and sprayed on the ticks engorging on Zebu cattle in farms at Muhaka on the southern coast of Kenya. After engorgement and dropping, the ticks were placed individually in nylon tetrapacks, sealed and left in the grass under a tree for 6 weeks, after which mortalities and egg viability were recorded.

Pasture experiments

Conidia of *M. anisopliae* and *B. bassiana* were produced on a substrate of ground maize and vermiculite (*M. anisopliae*) or rice (*B. bassiana*). The substrate was autoclaved for 1 h at 121°C and 15 lb/sq inch pressure, transferred to plastic buckets ($33 \times 25 \times 13$ cm) and inoculated with a 3-day-old culture of blastospores (50 ml). The cultures were incubated for 21 days at ambient conditions (20–26°C; 40–70% rh) (Maniania, 1993) and then allowed to dry for 5 days at room temperature before conidia were harvested by sieving. The conidia were stored in the refrigerator (4–6°C) until required for field spraying. Viability tests were conducted prior to each field experiment by culturing the conidia in SDA for 24 h and determining the percent germination. The field trials were carried out at ICIPE's Kuja river field site in western Kenya. The conidia were mixed thoroughly with water and 1% Tween-80 in 240 l metal drums to obtain a final concentration of 10^8 conidia per ml of water. These suspensions were then sprayed on grass once per month on 5 acre paddocks previously seeded with approximately 2×10^5 larvae of *R. appendiculatus*. The first paddock was sprayed with *B. bassiana*, the second with *M. anisopliae* and the third acted as control. Each paddock contained 5 zebu cattle. The control paddock was sprayed with 1% Tween-80 in

water. On-host tick populations were counted on all cattle (whole body count) monthly.

Data analysis

Analysis of variance (Anova) was used to test levels of significance in tick counts and mortalities.

Results

High mortalities were induced by oil formulations of both *B. bassiana* and *M. anisopliae* in all stages of *R. appendiculatus* and *A. variegatum*. Mortality

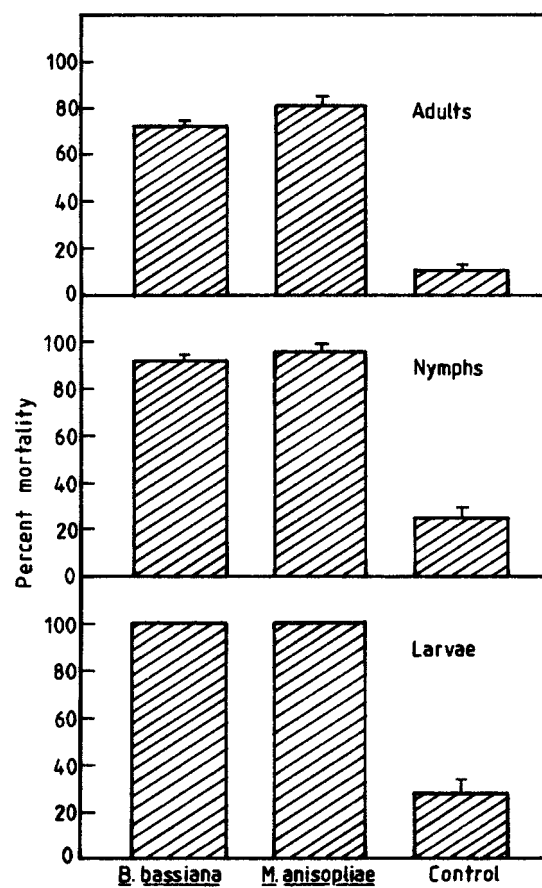


Figure 1. Mortality in *R. appendiculatus* treated with conidia of *B. bassiana* and *M. anisopliae* (10^9 conidia per ml) in 15% oil emulsion in tetrapacks placed in grass in the field. Means of 3 experiments (\pm SE) are presented.

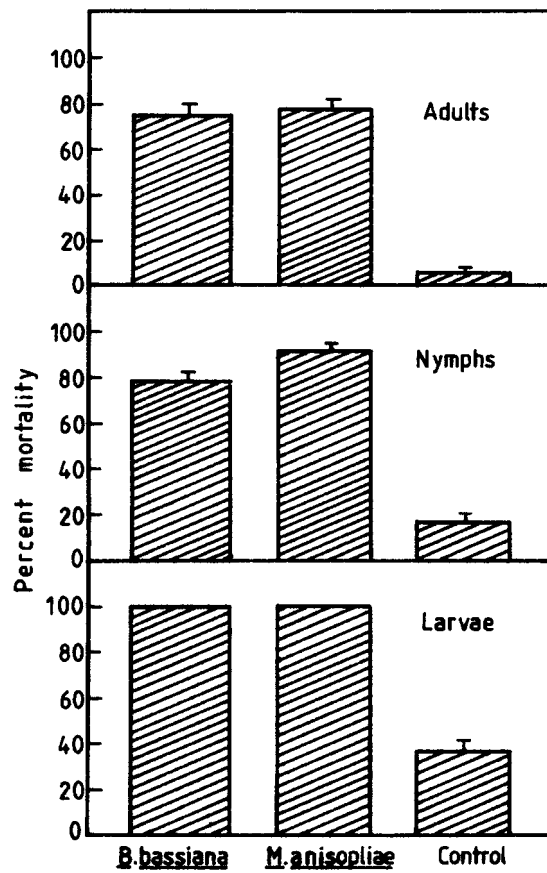


Figure 2. Mortality in *A. variegatum* treated with conidia of *B. bassiana* and *M. anisopliae* (10^9 conidia per ml) in 15% oil emulsion in tetrapacks placed in grass in the field. Means of 3 experiments (\pm SE) are presented.

rates were higher in larvae (100%) than in nymphs (80–95%) or adults (78–80%) (Figures 1 and 2). In adult *R. appendiculatus* and *A. variegatum* let free in potted grass and sprayed with the fungal conidia in oil emulsion, the mortality varied from 65–75% in *A. variegatum* to 75–80% in *R. appendiculatus*, compared to control mortality (from 5–15% (Figure 3)).

Both aqueous and oil formulations of the Rusinga island tick-isolated strains of *M. anisopliae* and *B. bassiana* induced high mortalities in both *R. appendiculatus* and *A. variegatum*, especially in larvae, where mortality was 100% in both tick species (Figures 4 and 5). The oil formulation, however, induced a higher ($P < 0.05$) mortality in both tick species in nymphs and adults than the aqueous formulation. In nymphs of both *R. appendiculatus* and *A. variegatum*, mortality was about 100% with oil and 80% with aqueous for-

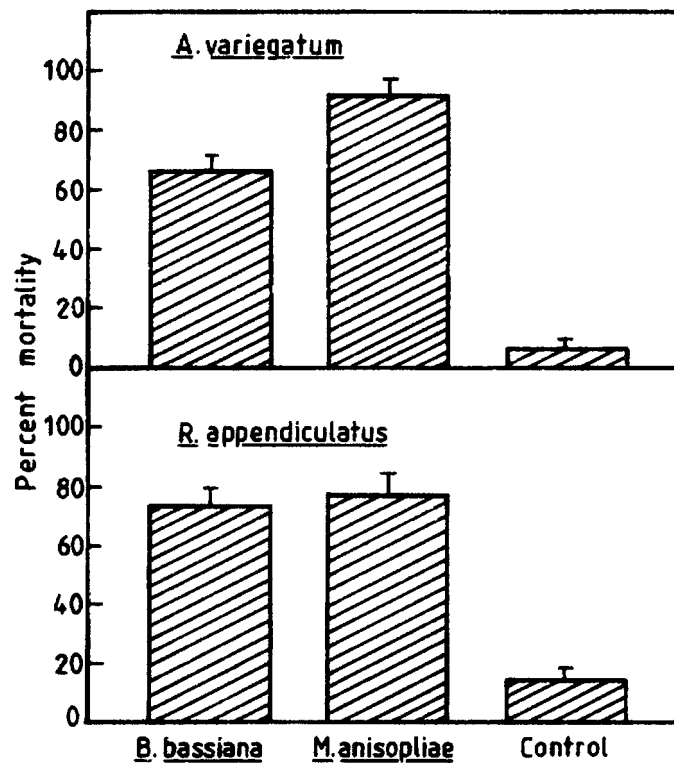


Figure 3. Mortality in adult *R. appendiculatus* and *A. variegatum* let free in grass and sprayed with conidia of *B. bassiana* and *M. anisopliae* (10^9 conidia per ml) in 15% oil emulsion. Mean of 3 experiments (\pm SE) are presented.

mulations, whereas in adults, mortality was 20–40% (aqueous) and 65–100% (oil), respectively (Figures 4 and 5).

Aqueous formulations of both fungi (*M. anisopliae* and *B. bassiana*) induced mortality in *B. decoloratus*, which was significantly higher ($P < 0.05$) than that in controls. Mortalities were 10%, 40% and 50% in control, *B. bassiana* and *M. anisopliae* groups, respectively. There were also significant reductions in viability of eggs produced by the surviving ticks. In the control group, egg viability was 98% compared with 30% and 50% in *B. bassiana* and *M. anisopliae*-treated groups, respectively.

The viability of the conidia used in field trials was found to vary from 86–90%. In paddocks sprayed with *B. bassiana* and *M. anisopliae* once per month, tick populations were much lower ($P < 0.05$) than in the control paddock from May to July 1998 (Figure 6). In July 1998, after 6 months of spraying, the mean numbers of adult *R. appendiculatus* on cattle had been reduced by 80% in *B. bassiana* and 92% in *M. anisopliae*-treated paddocks, compared to controls.

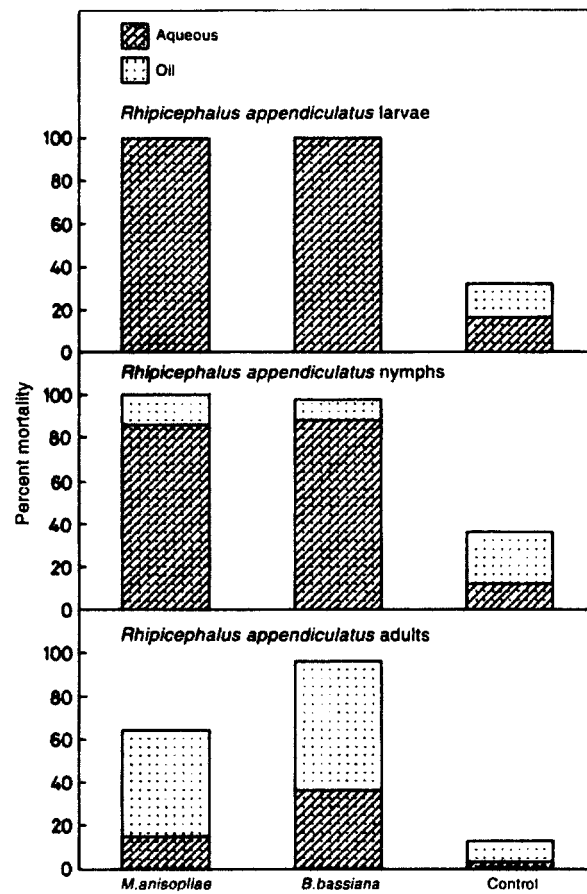


Figure 4. Mortality in larvae, nymphs and adults of *R. appendiculatus* caused by tick-isolated *B. bassiana* and *M. anisopliae* (10^9 conidia per ml) applied as aqueous and oil-based formulations in vegetation.

Discussion

The results with oil-based formulation were similar to those obtained in the previous experiments with aqueous formulation (Kaaya *et al.*, 1996) for larvae and nymphs, but much better for adults. The aqueous formulations in our previous experiments induced only 36–64% adult mortality compared to 78–80% in the present experiment. The oil-based formulation is therefore more effective against adult *R. appendiculatus* than the aqueous formulation. Results of pathogenicity of the tick-isolated strains of *B. bassiana* and *M. anisopliae* to *R. appendiculatus* and *A. variegatum* indicate that both fungi are tick pathogens with potential for biological control of ticks. Both *B. bassiana* and *M. anisopliae* induced mortalities and reductions in egg hatchability in

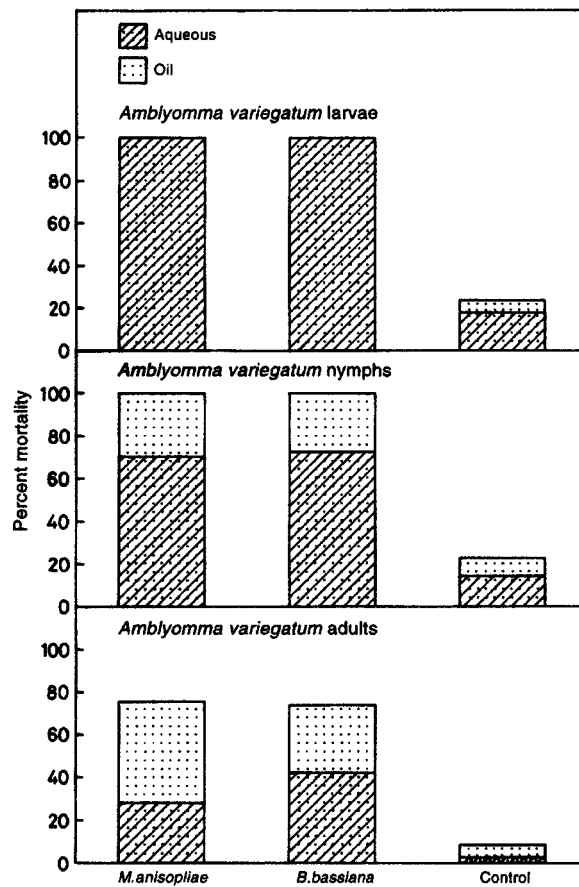


Figure 5. Mortality in larvae, nymphs and adults of *A. variegatum* caused by tick-isolated *B. bassiana* and *M. anisopliae* (10^9 conidia per ml) in aqueous and oil-based formulations in vegetation.

B. decoloratus. The impact of these fungi on populations of *B. decoloratus* is likely to be high when both the tick mortality and reductions in egg viability are considered; for instance in *M. anisopliae* where mortality of 53% and loss of egg viability of 52% were achieved, the combined effect on the next tick generation is likely to be significant. If there was also a reduction in fecundity (not examined) as observed in other tick species (Kaaya *et al.*, 1996), then the impact of fungi on *B. decoloratus* populations will be even higher.

Resistance develops faster in one-host ticks e.g. *B. decoloratus* due to their short generation time, than in 2- and 3- host ticks (Norval *et al.*, 1992). Development of acaricide resistance in *B. decoloratus* has infact been the main reason for introduction of new acaricides in Africa (Tatchell, 1984; Norval *et al.*, 1992). Entomogenous fungi may therefore be used instead of

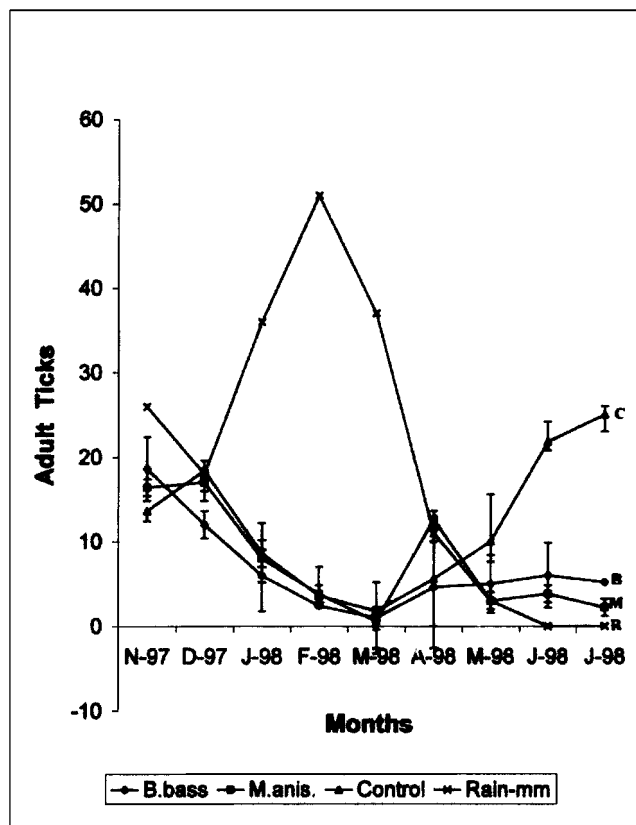


Figure 6. The impact of aqueous formulation of Rusinga island tick isolates of *B. bassiana* and *M. anisopliae* (10^8 conidia per ml) sprayed on pastures once per month on populations of adult *R. appendiculatus* on cattle. Mean tick counts from 5 cattle are presented.

or integrated with acaricides to control *B. decoloratus* and to alleviate the problem of resistance. Previous studies have shown that acaricides have no detrimental effect on fungi when mixed together (Kaaya *et al.*, 1996). Thus, fungi may be sprayed on cattle alone or in a mixture with acaricides to kill the acaricide-resistant ticks. Alternatively, since the larva is the only host-seeking stage in *B. decoloratus*, fungi may be sprayed on vegetation to kill the larvae as well as the engorged females that drop on the ground to lay eggs. The same integrated approach may be used to control the other tick species, e.g. *R. appendiculatus* and *A. variegatum*.

Despite using a fairly low concentration of conidia (10^8 instead of 10^9 conidia per ml), the fungi suppressed populations of *R. appendiculatus* on cattle by 80–92% when applied once per month in the field paddock experiment. Other investigators have applied concentrations as high as 2×10^9

conidia per ml of water or oil to control other arthropods and obtained positive results (Milner *et al.*, 1994). The excessive rains of El Niño in Kenya in 1998, which often occurred immediately after fungal application, must also have reduced the efficacy of the fungi. In future, a higher concentration e.g. 10^9 conidia per ml should be tested at different frequencies of application. Fungal isolates capable of inducing such high mortalities in all life stages of ticks are likely to reduce tick populations significantly and consequently the incidence of tick-borne and tick-associated diseases. Their use in tick control may therefore reduce the frequency of acaricide application and the use of curative drugs for TBDs. The reduction in frequency of acaricide application will reduce the cost of tick control as well as the rate of development of tick resistance to acaricides.

In intensive tick control programs in exotic and crossbreed dairy cattle in Africa, acaricides are applied as frequently as once per week (Norval *et al.*, 1992). Small-scale farmers raise most of these dairy cattle where the family provides labor. Although spraying pastures with fungi may appear to create more labor for the family, this may not be the case for the following reasons: Firstly, horizontal transmission of infection from fungus-infected to uninfected arthropods has been observed (Kaaya and Okech, 1990; Backer *et al.*, 1994). This often leads to fungal epizootic (Fargues and Remaudière, 1977), especially in moist environments. Non-target organisms may also serve as secondary hosts on which fungal inoculum is maintained and propagated, thus promoting later infections in the target host populations (Goettel and Johnson, 1992). This epizootic phenomenon may reduce the frequency of field applications, probably to only a few times in a year. Secondly, vegetation acts as a reservoir for ticks that attach on cattle. In a tick population model developed for *R. appendiculatus* at the International Center of Insect Physiology and Ecology (ICIPE), we observed that for each tick attached on cattle, there were as many as 1000–10,000 ticks in vegetation (Odulaja and Kaaya, unpublished).

Spraying ticks in vegetation will therefore have an advantage of killing a far greater number of ticks than spraying on cattle, thus reducing the numbers of ticks that would have attached on cattle and hence the frequency of acaricide application. Spraying vegetation will also have an advantage over spraying on cattle because it will kill ticks before they attach on cattle to suck blood and transmit diseases. All stages of *Boophilus* spp. (one host tick) develop on the host and therefore the tick spends a relatively longer period on the host than any individual stage of the 3-host ticks, which develop on separate hosts. While with *Boophilus* it may be possible to kill ticks on cattle by spraying with fungus, it may not be possible to kill adult *R. appendiculatus* or *A. variegatum* on host since the fungus takes 7–10 days to kill the

ticks (Kaaya *et al.*, 1996) and by that time most of the ticks, especially *R. appendiculatus*, will have engorged and dropped on the ground to lay eggs. Thus, the fungus may not prevent adult ticks from engorging and probably spreading diseases, although fecundity will most probably be reduced and consequently the size of the progeny.

If it were possible to produce fungi on local raw materials such as used barley from breweries and sugarcane stems from sugar factories, the fungal products for tick control would be cheaper and more affordable. Preliminary data (Kaaya, unpublished) indicated that these two substrates can support growth and sporulation of *B. bassiana* and *M. anisopliae* but further studies are needed to optimize the culture systems. Entomogenous fungi are known to be more specific to target organisms under field conditions, especially during epizootic (Goettel and Johnson, 1992) and this specificity evidently reduces the level of hazard to non-target organisms. Rath *et al.* (1995) observed that application of *M. anisopliae* for the control of the subterranean scarab, *Adoryphorus couloni* (Burmeister) in pasture had no adverse effects on non-target invertebrates. Mycopesticides are therefore likely to be environmentally safer than the conventional acaricides.

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References

- Backer, G.L., Milner, R.J., Lutton, G.G. and Watson, D.M. 1994. Preliminary field trial on the control of *Phaulacridium vittatum* (sjostedt Orthoptera: Acrididae) populations with *Metarhizium flavoviride* Gams and Rozsypal (Deuteromycotina: Hyphomycetes). *J. Austr. Entomol. Soc.* 33: 190–192.
- De Castro, J.J. 1997. Sustainable ticks and tick-borne disease control in livestock improvement in developing countries. *Vet. Parasitol.* 71: 69–76.
- Fargues, J. and Remaudiere, G. 1977. Consideration on the specificity of entomopathogenic fungi. *Mycopathologia* 62: 31–41.
- Gillespie, A.T. and Claydon, N. 1989. The use of entomogenous fungi for pest control and the role of toxins in pathogenesis. *Pesticide Sci.* 27: 203–215.
- Goettel, M.S. and Johnson, D.L. 1992. Environmental impact and safety of fungal biocontrol agents, In: *Biological Control of Locusts and Grasshoppers*, C.J. Lomer and C. Prior (eds), pp. 356–361 CAB International, Wallingford, UK.
- Hooper, G.H.S., Milner, R.J., Spurgin, P.A. and Prior, C. 1995. Initial field assessment of *Metarhizium flavoviride* Gams and Rozsypal (Deuteromycetina: Hyphomycetes) for control of *Chortoicetes terminifera* (Walker) (Orthoptera: Acrididae). *J. Aust. Ent. Soc.* 34: 83–84.

- Jongejan, F., Pegram, R.G., Zivkovic, D., Hensen, E.J., Mwase, E.T., Theilemans, M.J.C., Cosse, A., Niewold, T.A. Ashraf ElSaid and Uilenberg, G. 1989. Monitoring of naturally acquired and artificially induced immunity to *Amblyomma variegatum* and *Rhipicephalus appendiculatus* ticks under field and laboratory conditions. *Exp. Appl. Acarol.* 7: 181–199.
- Kaaya, G.P. 1989. *Glossina morsitans morsitans*: Mortalities caused in adults by experimental infection with entomopathogenic fungi. *Acta Trop.* 46: 107–114.
- Kaaya, G.P. and Okech, M.A. 1990. Horizontal transmission of mycotic infection in adult tsetse, *Glossina morsitans morsitans*. *Entomophaga* 35: 46–57.
- Kaaya, G.P. and Munyinyii, D. 1995. Biocontrol potentials of the entomogenous fungi *Beauveria bassiana* and *Metarhizium anisopliae* for tsetse flies, *Glossina* spp. at developmental sites. *J. Invert. Pathol.* 66: 237–241.
- Kaaya, G.P., Mwangi, E.N. and 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. Invert. Pathol.* 67: 15–20.
- Kaaya, G.P. and Mwangi, E.N. 1998. Control of livestock ticks in Africa: Possibilities of biological control using the entomogenous fungi, *Beauveria bassiana* and *Metarhizium anisopliae*. In: The second International Conference on tick-borne Pathogens at the Host-vector Interface: A Global Perspective, L. Coons and M. Rothschild (eds). August, 28–September 1, Kruger National Park, South Africa, pp. 5–16, United Litho.
- Lomer, C.J., Prior, C and Kooyman, C. 1997. Development of *Metarhizium* spp. for the control of grasshoppers and locusts. *Mem. Entomol. Soc. Canada* 171: 265–286.
- Maniania, N.K. 1993. Effectiveness of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. for the control of the stem borer *Chilo partellus* in maize in Kenya. *Crop Protect.* 12: 601–604.
- Milner, R.J., Hartley, T.R., Lutton, G.G and Prior, C. 1994. Control of *Phaulacridium vittatum* (sjostedt) (Orthoptera: Acrididae) in field cages using an oil-based spray of *Metarhizium flavoviride* Gams and Rozsypal (Deuteromycetina: Hyphomycetes). *J. Austr. Entomol. Soc.* 33: 165–167.
- Munaan, A. and Wikardi, E.A. 1986. Towards the biological control of coconut insect pests in Indonesia. In: Biological Control in the Tropics, M.Y. Hussein, and A.G. Ibrahim (eds), pp. 149–157, Penerbit Universiti Partanian, Malaysia, Serdang.
- Norval, R.A.I. 1983. Arguments against intensive dipping. *Zimbabwe Vet J.* 4: 19–25.
- Norval, R.A.I., Yunker, C.E., Duncan, I.M. and Peter, T. 1991. Pheromone/acaricide mixtures in the control of the tick *Amblyomma hebraeum*: Effects of acaricides on attraction and attachment. *Exp. Appl. Acarol.* 11: 233–240.
- Norval, R.A.I., Perry, B.D. and Young, A.S. 1992. The Epidemiology of Theileriosis in Africa, Academic Press London, pp. 301–342.
- Narval, R.A.I., Tebele, N., Short, N.J. and Clatworthy, J.N. 1983. A laboratory study on the control of economically important tick species with legumes of the genus *Stylosanthes*. *Zimbabwe Vet. J.* 14: 26–29.
- Rath, A.C. 1992. *Metarhizium anisopliae* for control of the Tasmanian pasture Scarab, *Adoryphorus couloni*. In: The use of pathogens in Scarab Pest Management, T.A. Jackson and T.R. Glare, (eds), pp. 217–227, Andover.
- Rath, A.C., Worledge, D., Koen, T.B. and Rowe, B.A. 1995a. Long-term field efficacy of the entomogenous fungus, *Metarhizium anisopliae* against the subterranean scarab, *Adoryphorus couloni*. *Biocontr. Sci. Tech.* 5: 439–451.
- Rath, A.C., Koen, T.B. and Anderson, G.C. 1995b. Field evaluation of the entomogenous fungus, *Metarhizium anisopliae* (DAT F-001) as a biocontrol agent for the redheaded

- pasture Cockchafer, *Adoryphorus couloni* (Coleoptera: Scarabaeidae). Aust. J. Agric. Res. 46: 429–440.
- Rechav, Y., Dauth, J. and Els, D.A. 1990. Resistance of Brahman and Simmentaler cattle to southern African ticks. Onderst. J. Vet. Res. 57: 7–12.
- Rechav, Y. and Whitehead, G.B. 1981. Can *Amblyomma* pheromones be used for control? In: Tick Biology and Control, G.B. Whitehead and D.J. Gibson (eds). Proc. Int. Conf. Grahamstown, 27–29 January 1981 pp. 209–210.
- Sonenshine, D.E., Taylor, D. and Corrigan, G. 1985. Studies to evaluate the effectiveness of sex-pheromone-impregnated formulations for control of populations of the American dog tick, *Dermacentor variabilis* (Say) (Acari: Ixodidae). Exp. Appl. Acarol. 1: 23–34.
- Sutherst, R.W. 1983. Management of arthropod parasitism in livestock In: Tropical parasitoses and parasitic zoonoses, J.P. Dunsmore (ed.), pp. 41–56, Murdoch Univ., Perth.
- Sutherst, R.W., Raymond, J.J. and Schnitzer, H.J. 1982. Tropical legumes of the genus *Stylosanthes* immobilize and kill cattle ticks. Nature 295: 320–321.
- Tatchell, R.J. 1984. Strategies for tick control following east coast fever immunization programs. In: Immunization against Theileriosis in Africa, A.D. Irvin (ed.), 1–5 October 1984, Proc. Joint Workshop, Nairobi ILRAD, pp. 110–113.
- Thompson, K.C., Rao, J. and Romero, T. 1978. Anti-tick grasses as the basis for developing practical tropical tick control packages. Trop. Anim. Hlth. Prod. 10: 179–182.
- Ziv, M., Sonenshine, D.E., Silverstein, R.M., West, J.R. and Gingher, K.H. 1981. Use of sex pheromone, 2,6-dichlorophenol, to disrupt mating by the American dog tick, *Dermacentor variabilis* (Say). J. Chem. Ecol. 7: 829–840.