Mycopathologia (2005) 160: 151–157
DOI: 10.1007/s11046-005-0120-4

Comparison of water, oils and emulsifiable adjuvant oils as formulating agents for *Metarhizium anisopliae* for use in control of *Boophilus microplus*

Perry Polar[1], Moses T.K. Kairo[2], Dave Moore[3], Rupert Pegram[4] & Sally-Ann John[1]
[1]*CAB International Caribbean and Latin America Regional Centre, Curepe, Trinidad and Tobago;* [2]*Center for Biological Control, College of Engineering Sciences, Technology and Agriculture, Texas A&M University, Florida, USA;* [3]*CAB International UK Centre, Bakeham Lane, TW20 9TY, Egham, Surrey, UK;* [4]*FAO-Caribbean Amblyomma Programme, Friars Hill Road, P.O. Box W 1572, St Johns, Antigua*

Received 10 March 2005; accepted in revised form 14 June 2005

Abstract

Studies were conducted to identify oil-based formulating agents (paraffinic oil, palm oil and emulsifiable adjuvant oils (EAOs)) for *Metarhizium anisopliae* that were superior to water with simple surfactants using a germination test and a bioassay against *Boophilus microplus*. Germination of conidia in all formulations, except 10% coconut EAO, produced more than 68% germination at 24 h and nearly 100% at 48 h. Coconut oil (average survival time (AST) = 4.6 ± 0.28 days) and 10% liquid paraffin EAO (AST = 4.4 ± 0.15 days) enhanced the pathogenicity of *M. anisopliae* to *B. microplus* relative to water (AST = 8.4 ± 0.42 days). *M. anisopliae* in 10% liquid paraffin EAO was the most effective formulation having a moderately high germination after 24 h and a low AST as well as a high AST in the control. In the second experiment, germination of conidia in 2% liquid paraffin EAO and 2% Cropspray was higher than in 2% Codacide oil at 24 h, however, all treatments reached 100% germination after 48 h. The ASTs of the EAO based *M. anisopliae* formulations (Average AST = 6.4 ± 0.54 days) were similar but lower that the ASTs of the controls (Average AST = 9.6 ± 0.28 days).

Key words: adjuvant, biological pesticide, cattle tick, coconut, formulation, paraffin, Trinidad and Tobago

Introduction

Metarhizium anisopliae (Metschnikoff) Sorokin is a common entomopathogenic fungus used in the development of biological pesticides for tick control [1–3]. Researchers, in these studies have only utilized water-based formulations containing simple surfactants such as Tween 80 [1] and Triton X-100 [3]. These formulations have been shown to be pathogenic to cattle ticks under laboratory conditions [1–3]. However, pathogenicity to *Boophilus microplus* Canestrini on cattle in pen trials has been variable. Corrieia et al. [4] did not notice any significant change in the *B. microplus* population with a single spray of *M. anisopliae* on stabled cattle, while de Castro et al. [5] recorded a decrease of over 50% in the *B. microplus* population in a similar experiment. This variability in performance may indicate that further improvements in formulation are required to develop an effective biological pesticide for the control of cattle ticks. The use of improved formulating agents requires investigation, as formulation is a key factor in improving performance of biological pesticides [6].

Many of the currently available biological pesticide products are wettable powders containing conidia [6]. Water is a useful formulating agent because it is non-toxic, readily available, cheap, and can be dispersed using simple hydraulic sprayers.

Water can readily suspend hydrophilic conidia and lipophilic conidia with the use of simple surfactants. However, several studies have shown that oil formulations can be more effective than water based formulations [7–10]. This possibly results from the greater adhesion of the conidia to the cuticle and the greater spreading of the conidia in to high humidity locations such as the inter-segmental membranes with an oil based formulation compared to a water based formulation [11, 12].

Oils (palm, vegetable, mineral, paraffinic) are inherently compatible with lipophilic conidia such as *Metarhizium* and make superior spray carriers [13, 14]. They are ideal for ultra low volume applicators that produce atomized droplets (50–100 μm) which do not evaporate before hitting the target [13, 14]. Oil formulations, however, may be more expensive than water. Further, vegetable oils may eventually clog spraying equipment [14].

Suspoemulsions are a mixture of suspensions (solid-in-liquid system) and emulsions (two-phase liquid-in-liquid system). The use of emulsifiable adjuvant oil (EAO) allows for the conidia and oil droplets to be suspended in a continuous, aqueous phase. This imparts some of the beneficial properties of an oil formulation in a less costly water based environment. Alves [15] investigated the effect of EAO based formulations on pathogenicity to *Tenebrio molitor* Linnaeus and various other biological pesticide parameters.

The first experiment was conducted to identify oil-based formulating agents (coconut oil, liquid paraffin and their EAOs) for *M. anisopliae* which were superior to water using a germination test and a bioassay against engorged female *B. microplus*. In the second experiment, *M. anisopliae* formulations using the most effective formulating agent from above and commercial EAOs were compared using a germination test and bioassay of engorged female *B. microplus*.

Materials and methods

Ticks

All ticks used in the experiment were fully engorged female *B. microplus* freshly picked from cattle at the Aripo Livestock Station, Trinidad.

Fungal isolates

M. anisopliae ARSEF3297 isolated from *Boophilus* sp. in Mexico was obtained from the United States Department of Agriculture (USDA), Agricultural Research Service, Collection of Entomopathogenic Fungi (ARSEF).

Conidia

Cultures of *M. anisopliae* were grown on Sabouraud Dextrose Agar (SDA) (Sigma) plates for 14 days. Conidia were harvested by scraping each plate with a glass rod and suspending the conidia in sterile distilled water with 0.2% Tween 80. The stock suspension was filtered through several layers of cloth mesh to remove mycelia and the concentration was checked with a haemocytometer (Hausser Scientific) and adjusted to 6×10^6 conidia/ml using sterile distilled water with 0.2% Tween 80.

One ml was used to inoculate 250 ml glass flasks (Kimax) containing 100 ml autoclaved MGS (3.0 g/l Peptone – Mycological (Oxoid); 0.3 g/l $MgSO_4$; 0.3 g/l KH2PO4; 0.3 g/l $NaCl_2$; 20 g/l glucose). The flasks were shaken on an orbital shaker (Cole Parmer) at 28 °C in the dark at 150 rpm for 3 days.

Seventy-five ml of 3-day-old inoculum from above was used to inoculate bags containing 1 kg of autoclaved rice. The rice was prepared by adding 300 ml of sterile distilled water and 20 ml of soybean oil to each kilogram of rice and autoclaving at 121 °C at 15 psi for 40 min. The bags were placed on shelves and incubated for 10 days at 25–30 °C. The bags were then opened and allowed to dry for 7 days. The conidia were harvested using a cyclone extractor developed by CAB International [16]. The conidia were dried to 5% humidity using silica gel and packaged in trilaminate foil bags (Flexible Packaging Ltd.) to prevent rehydration and loss of viability [16]. The bags were placed in a refrigerator at 10 °C to prevent loss of viability and used as required.

Experiment 1: Effects of M. anisopliae *in water, oils and emulsifiable adjuvant oils on conidial germination and Average Survival Time (AST) of* B. microplus

Formulation

One gram of *M. anisopliae* conidia at 5% humidity was assumed to contain 5×10^{10} conidia [17].

Batches of conidia (0.001 g) were weighed using a top loading digital balance scale (Adam Equipment) sensitive to four decimal places and left to re-hydrate for 30 min in a moisture chamber to avoid possible imbibition damage [18]. Fifty ml of *M. anisopliae* (1×10^6 conidia/ml) in water, coconut oil and liquid paraffin for the germination test were prepared by mixing conidia with either sterile distilled water with 0.2% Tween 80, coconut oil (Naisa) or liquid paraffin (V&S Pharmaceuticals Limited).

A coconut EAO was prepared by adding 1 ml of Triton X-100 to 9 ml of coconut oil in a test tube and vigorously shaking by hand for 5 min. Five ml of the coconut EAO was added to the conidia and mixed into slurry. Forty-five ml of sterile distilled water was added to produce *M. anisopliae* (1×10^6 conidia/ml) in 10% coconut EAO. *M. anisopliae* in 10% liquid paraffin EAO was prepared in a similar manner.

Control solutions containing sterile distilled water with 0.2% Tween 80, coconut oil, liquid paraffin, 10% coconut EAO and 10% liquid paraffin EAO were prepared in a similar manner without conidia. All formulations were shaken on an orbital shaker at 250 rpm for 30 min to ensure mixing and left to stand for 2 h to allow the conidia to be sensitized to any adverse effect caused by the formulations [19].

M. anisopliae formulations (1×10^8 conidia/ml) for bioassays were prepared in a similar manner using batches of 0.1 g of conidia. Control formulations were prepared in an identical manner.

Germination test

Sterile glass microscope slides (Hyline Enterprises Limited) with a drop of SDA on each end were prepared. Aliquots of 0.1 ml of each formulation were thinly spread over the SDA surface and the slides were placed in sterile plastic petri dishes (15 mm in height and 100 mm in diameter) (Phoenix Biomedical), wrapped with two rounds of Parafilm (American Can) and incubated at 28 °C. The germination of conidia was assessed 24 and 48 h. Conidia were viewed under a microscope at ×300 and a conidium was considered germinated when the germ tube was equal to at least half of the long axis of the conidium [18]. All the conidia in the field of view were counted to obtain at least a total of 300 conidia for each replicate [20]. The experiment was repeated three times.

Bioassay

A batch of 10 engorged female *B. microplus* was dipped for 10 s in the fungal inoculum of the respective treatments. The inoculum was drained and the ticks were placed on filter paper to drain-off excess inoculum. Controls were treated similarly but dipped into the respective control solutions. Ticks were placed in sterile plastic Petri dishes containing sterile moist filter paper and placed in an incubator at 28 °C. The ticks were observed daily for mortality over a period of 20 days. The 10 treatments were arranged in a completely randomized design and the experiment was replicated three times with freshly prepared inoculum.

Experiment 2: Effects of M. anisopliae *in emulsifiable adjuvant oils on conidial germination and on the Average Survival Time of* B. microplus

M. anisopliae (1×10^6 conidia/ml for germination test and 1×10^8 for bioassay) in 2% liquid paraffin EAO, 2% Newman's Cropspray 11-E (Loveland Industries Limited) and 2% Codacide oil (Microcide Limited) were prepared in a similar manner as above, however 1 ml of EAO was added to 49 ml of sterile distilled water.

Control solutions were prepared in a similar manner without conidia. A germination test and bioassay were conducted as described above, however, the duration of time for the bioassay was reduced to ten days.

Data analysis

The Average Survival Times (ASTs) and a log rank statistic were calculated using Kaplan–Meier analysis in the statistical package SPSS for Windows [21]. A Bonferroni correction was applied to the significance figures to correct for the likelihood of increased significance due to performing a number of pairwise comparisons. Each of the observed significance levels was multiplied by the number of pairwise comparisons before rejection or acceptance of the null hypothesis. The ASTs were presented along with standard errors.

The percentage germination was transformed using an arcsine transformation ($y = \sin^{-1}\sqrt{x/k}$ where $k = 100$ and x is the percentage germina-

tion). The data was analyzed by two way analysis of variance (two way ANOVA) using SPSS for Windows and the significant differences between treatments are presented. A Post Hoc Test ($LSD_{0.05}$) was calculated for the transformed formulation means. The means were back transformed and presented along with standard errors.

Results

Effects of M. anisopliae *in water, oils and emulsifiable adjuvant oils on conidial germination and Average Survival Time (AST) of* B. microplus

Formulation, time and interaction between formulation and time were significant ($p < 0.05$) for germination of the conidia. At 24 h, germination of conidia in water (95.4%) was significantly ($p < 0.05$) higher than in all other formulations (Table 1). Germination of conidia in liquid paraffin (91.6%) was significantly ($p < 0.05$) higher than germination of conidia in 10% liquid paraffin EAO (86.0%) and coconut oil (86.2%). Germination of conidia in 10% coconut EAO was lower (10.9%; $p < 0.05$) than all other treatments. All formulations exhibited nearly 100% germination after 48 h ($p > 0.05$) with the exception of conidia in 10% coconut EAO (59.2%).

This indicates that most of the formulating agents had minor detrimental effects on the speed of germination, however, 10% coconut EAO had the most pronounced effect. No germination was observed in the control solutions and as such, the data was not presented.

For *M. anisopliae* formulations, 10% liquid paraffin EAO (AST = 4.4 ± 0.15 days) and coconut oil (AST = 4.6 ± 0.28 days) produced significantly ($p < 0.05$) lower ASTs of *B. microplus* compared to water (AST = 8.4 ± 0.42 days) (Table 2). The ASTs of *B. microplus* were not significantly ($p > 0.05$) different between liquid paraffin (AST = 5.5 ± 0.48 days) and water, however, 10% coconut EAO (AST = 11.9 ± 0.56 days) produced the highest AST of *B. microplus*. This indicates that 10% liquid paraffin EAO and coconut oil enhanced the ability of *M. anisopliae* to produce mortality in *B. microplus* relative to water while the opposite effect was obtained by the use of 10% coconut EAO.

All the control formulations had significantly ($p < 0.05$) higher ASTs than their counterparts with *M. anisopliae* conidia. Ticks in the coconut oil control had a significantly ($p < 0.05$) lower AST (9.6 ± 0.80 days) in comparison to the other control formulations which were similar ($p > 0.05$) to each other (Average AST = 13.9 ± 0.72 days). This indicates that coconut oil may produce mortality in *B. microplus* and by extension, may have contributed to the mortality observed in ticks treated with *M. anisopliae* in coconut oil.

Effects of M. anisopliae *in emulsifiable adjuvant oils on conidial germination and on the Average Survival Time of* B. microplus

Formulation, time and interaction between formulation and time were significant ($p < 0.05$) for germination of the conidia. At 24 h, germination of conidia in 2% liquid paraffin EAO (99.4%) and 2% Cropspray (99.9%) were not significantly ($p > 0.05$) different but both were significantly ($p < 0.05$)

Table 1. The effect of *M. anisopliae* in water, oils and emulsifiable adjuvant oils on the germination of conidia

Formulation	Germination of conidia			
	Transformed		Back transformed %	
	24 h	48 h	24 h	48 h
Water	77.7 ± 0.33 A a	90.0 ± 0.31 A b	95.4	100.0
Coconut oil	68.2 ± 0.35 C a	88.4 ± 0.31 A b	86.2	99.9
Liquid paraffin	73.2 ± 0.34 B a	89.2 ± 0.31 A b	91.6	99.9
10% Coconut EAO	19.3 ± 0.66 D a	50.3 ± 0.41 B b	10.9	59.2
10% Liquid paraffin EAO	68.0 ± 0.44 C a	84.9 ± 0.28 A b	86.0	99.2

Formulation ($p < 0.05$), Time ($p < 0.05$), Formulation × Time ($p < 0.05$).
Means with the same upper case letter in the same column indicate the non-significance ($p > 0.05$) of germination between formulations.
Means with the same lower case letter in the same row indicates non-significance ($p > 0.05$) of germination over time.

Table 2. The effect of *M. anisopliae* in water, oils and emulsifiable adjuvant oils on the Average Survival Times (ASTs) of engorged female *B. microplus*

Formulation	Average Survival Times/days	
	Control	*M. anisopliae*
Water	13.4 ± 0.57 A a	8.4 ± 0.42 B b
Coconut oil	9.6 ± 080 B a	4.6 ± 0.28 C b
Liquid paraffin	11.8 ± 0.80 AB a	5.5 ± 0.48 BC b
10% Coconut EAO	15.2 ± 0.69 A a	11.9 ± 0.56 A b
10% Liquid paraffin EAO	15.2 ± 0.82 A a	4.4 ± 0.15 C b

Formulation ($p < 0.05$).
Means with the same upper case letter in the same column indicate the non-significance ($p > 0.05$) of AST of *B. microplus* between formulations. Means with the same lower case letter in the same row indicates non-significance ($p > 0.05$) of ASTs of *B. microplus* between similar formulations with or without *M. anisopliae*.

higher than germination in conidia in 2% Codacide oil (95.8%) (Table 3). The germination in all the formulations increased over time, and nearly 100% germination was observed after 48 h. None of the control formulations exhibited germination and thus the data was not presented. These results indicate that 2% Codacide oil may mildly reduce the speed of germination of conidia relative to 2% liquid paraffin EAO and 2% Cropspray.

There were no statistical differences ($p > 0.05$) between the ASTs of *B. microplus* treated with *M. anisopliae* in 2% liquid paraffin EAO, 2% Cropspray and 2% Codacide oil (Average AST = 6.4 ± 0.54 days) (Table 4). However, *M. anisopliae* in 2% Codacide oil tended towards a lower AST. The ASTs of the controls were not significantly ($p > 0.05$) different from each other (Average AST = 9.6 ± 0.28 days) but were significantly ($p < 0.05$) higher that the ASTs of their respective *M. anisopliae* counterparts. This indicates that EAOs had no negative effects on germination or mortality of *B. microplus* and were similar to each other.

Discussion

This study examined oil-based formulating agents as an alternative to water for the formulation of a biological pesticide for tick control. Pure paraffinic oils (e.g. Shelsol T, Ondina), pure vegetable oils (e.g. sunflower, soyabean), paraffin based EAOs (Cropspray, Ashlade) and vegetable-based EAOs (Cropspray, Cutinol) have been investigated as formulating agents of *M. anisopliae* [19]. In the first experiment, commercial liquid paraffin was used pure and to make an EAO due to the non-availability of paraffinic oils and paraffinic EOAs designed for agricultural use at the time of the experiment. Coconut oil was investigated previously as a formulating agent for *M. flavoviride* [12]. Indeed, Prior et al. [10] demonstrated that the conidia of *Beauveria bassiana* (Balsamo) Vuillemin in coconut oil were 36 times more pathogenic to the cocoa pest weevil *Pantorhytes plutus* (Oberth.) in comparison to a water formulation. From the literature reviewed, there is no reference to a coconut based EAO.

Table 3. The effect of *M. anisopliae* in emulsifiable adjuvant oils on germination of conidia

Formulation	Germination of conidia			
	Transformed		Back transformed %	
	24 h	48 h	24 h	48 h
2% Cropspray	88.4 ± 1.16 A a	90 ± 1.16 A a	99.9	100
2% Codacide oil	78.3 ± 1.16 B a	90 ± 1.16 A b	95.8	100
2% Liquid paraffin EAO	85.9 ± 1.16 A a	90 ± 1.16 A a	99.4	100

Formulation ($p < 0.05$), Time ($p < 0.05$), Formulation × Time ($p < 0.05$).
Means with the same upper case letter in the same column indicate the non-significance ($p > 0.05$) of germination between treatments. Means with the same lower case letter in the same row indicates non-significance ($p > 0.05$) of germination over time.

Table 4. The effect of *M. anisopliae* in emulsifiable adjuvant oils on Average Survival Times (ASTs) of engorged female *B. microplus*

Formulation	Average Survival Times/days	
	Control	*M. anisopliae*
2% Cropspray	9.7 ± 0.21 A a	5.8 ± 0.52 A b
2% Codacide oil	9.5 ± 0.30 A a	7.4 ± 0.57 A b
2% Liquid paraffin EAO	9.5 ± 0.33 A a	5.9 ± 0.52 A b

Formulation ($p > 0.05$).
Means with the same upper case letter in the same column indicate the non-significance ($p > 0.05$) of ASTs of *B. microplus* between formulations. Means with the same lower case letter in the same row indicates non-significance ($p > 0.05$) of ASTs of *B. microplus* with similar treatments with or without *M. anisopliae*.

In the study, *M. anisopliae* in a water formulation with Tween 80 produced the highest germination suggesting that most of the formulating agents had a mild initial inhibitory effect, however, as time progresses the adverse effects were lessened and virtually 100% germination was achieved after 48 h. Alves et al. [19] examined a range of water, oils and EAO-based formulations of *M. anisopliae* and the germination rates after 24 h (90–99%) were higher than those achieved in our study.

Monitoring the germination of conidia at 48 h was difficult due to the growth of the mycelia particularly in treatments where the initial germination was high. However, monitoring the germination after 48 h is useful to determine if a formulation agent has slowed the rate of germination or has inhibited germination [19].

Unlike other formulations in our study, *M. anisopliae* in 10% coconut EAO gave poor germination at 24 h (10.9%) and despite a 5-fold increase after 48 h (59.2%) was still lower than the other formulations. This was similar to a formulation in Alves et al. [19] containing water with 0.1% Etoken, a cationic surfactant containing polyoxyethelene tallow amine, which produced a germination rate of 28.8% after 24 h and 64.5% after 48 h.

It is unclear why 10% coconut EAO caused such poor germination since relatively high germination (86.2%) was obtained by *M. anisopliae* in coconut oil. It can be speculated that there was an interaction between the fatty acids of coconut oil and Triton X-100. Some fatty acids have been shown to be inhibitory to germination while others promote germination [22]. Coconut oil contains 8% caprylic (C-8) and 7% capric (C-10) acids [23] which have been demonstrated to inhibit germination of *M. anisopliae* [22]. Although a high proportion of stearic acid was shown to overcome the inhibition of germination caused by capric acid [22], coconut oil only contains 2% stearic acid [23].

The poor germination in 10% coconut EAO may have been responsible for the poor mortality when applied to *B. microplus*. Poor pathogenicity is understood to be correlated with poor germination.

M. anisopliae in 10% liquid paraffin EAO and coconut oil produced more mortality than their water-based counterpart. However, coconut oil caused higher mortality in the control, as such may have contributed to tick mortality in the *M. anisopliae* in coconut oil treatment. It has been observed that control mortality increases with vegetable oils as they age [12]. As such, *M. anisopliae* in 10% liquid paraffin EAO was considered to be the most effective formulation by virtue of having moderate germination at 24 h, no deleterious effect on germination after 48 h, a low AST and a high AST in the control.

Germination in the paraffinic EAO-based formulations (2% liquid paraffin EAO, 2% Cropspray) were initially higher at 24 h in comparison to rapeseed EAO-based formulation (2% Codacide oil). It is unlikely that statistical differences between the treatments would lead to a biological effect as the germination exceeded 95% in all treatments. There were no differences after 48 h.

No significant difference in the ASTs was observed in the *M. anisopliae* treatments. This suggests that there are no major differences in the performance of *M. anisopliae* formulations based on paraffinic or vegetable-based EAOs.

This study demonstrated that the effectiveness of *M. anisopliae* could be improved by formulation in oils and EAOs. Future work involved the assessment of EAO based formulations of *M. anisopliae* on *B. microplus* on cattle *in vivo*.

Acknowledgements

We thank the International Fund for Agricultural Development, the Food and Agriculture Organization of the United Nations, and CAB International Partnership Facility for funding this project. We also would like to thank Richard Humber from United States Department of Agriculture, Agricultural Research Service, Plant Protection Research Unit for providing isolate ARSEF3297 and staff of the Ministry of Agriculture, Land and Marine Resources, Aripo Livestock Station, Trinidad and Tobago for their assistance throughout the project.

References

1. Bittencourt VREP, Massard CL, de Lima AF. Acto do fungo *Metarhizium anisopliae* sobre a fase não parasitaria do ciclo biologico de *Boophilus microplus*. Revista Universidade Rural, Série Ciência da Vida 1994; 16: 49–55.
2. Frazzon APG, Junior IDV, Masuda A, Shrank A, Vainstein MH. *In vitro* assessment of *Metarhizium anisopliae* isolates to control the cattle tick *Boophilus microplus*. Vet Parasitol 2000; 94: 117–125.
3. Gindin G, Samish M, Alekseev E, Glazer I. The susceptibility of *Boophilus annulatus* (Ixodidae) ticks to entomopathogenic fungi. Biocontrol Sci Technol 2001; 11: 111–118.
4. Correia ACB, Fiorin AC, Monteiro AC, Veríssimo CJ. Effects of *Metarhizium anisopliae* on the tick *Boophilus microplus* (Acari: Ixodidae) in stabled cattle. J Invert Pathol 1998; 71: 189–191.
5. de Castro ABA, Bittencourt VREP, Daemon E, Viegas EDC. Eficácia do fungo *Metarhizium anisopliae* sobre o carrapato *Boophilus microplus* em teste de estábulo. Revista Universidade Rural, Série Ciência da Vida 1997; 19: 73–82.
6. Burges HD. Formulation of Mycoinsecticides. In: Burges HD ed. Formulation of Microbial Biopesticides: Beneficial Microorganisms, Nematodes and Seed Treatments: Dordrecht, Kluwer Academic Publishers, 1998: 131–185.
7. Agudelo F, Falcon LA. Mass production, infectivity, and field application studies with the entomopathogenic fungus *Paecilomyces farinosus*. J Invert Pathol 1983; 42: 124–132.
8. Bateman RP. The development of a mycoinsecticide for the control of locust and grasshoppers. Outlook Agric 1997; 26: 13–18.
9. Jenkins NE, Thomas MB. Effect of formulation and application method on the efficacy of aerial and submerged conidia of *Metarhizium flavoviride* for locust and grasshopper control. Pestic Sci 1996; 46: 299–306.
10. Prior C, Jollands P, Le Patourel G. Infectivity of oil and water formulations of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) to cocoa weevil pest *Pantorhytes plutus* (Coleoptera: Curculionidae). J Invert Pathol 1988; 52: 66–72.
11. Bateman RP, Carey M, Moore D, Prior C. The enhanced infectivity of *Metarhizium flavoviride* in oil formulations to desert locust at low humidities. Ann Appl Biol 1993; 122: 145–152.
12. Prior C, Carey M, Abraham YJ, Moore D, Bateman RP. Development of a bioassay method for the selection of entomopathogenic fungi virulent to the desert locust, *Schistocerca gregaria* (Forkål). J Appl Entomol 1995; 119: 567–573.
13. Bateman RP. Method of application of microbiological pesticide formulations for the control of grasshoppers and locust. Mem Entomol Soc Can 1997; 171: 69–81.
14. Wraight SP, Jackson MA, de Kock SL. Production, stabilization and formulation of fungal biocontrol agents. In: Butt TM, Jackson CW, Magan N, eds. Fungi as Biological Control Agents: Wallingford, CAB International, 2001: 253–287.
15. Alves RT. Development of mycoinsecticide formulations and application techniques appropriate for pest control [Dissertation] University of London, London, 1999: 225 pp.
16. Jenkins NE, Heviefo G, Langewald J, Cherry AJ, Lomer CJ. Development of mass production technology for aerial conidia for use as mycopesticides. Biocontrol News Inform 1998; 19: 21N–31N.
17. Blanford S, Thomas MB. Host thermal biology: the key to understanding host-pathogen interactions and microbial pest control? Agric For Entomol 1999; 1: 195–202.
18. Moore D, Langewald J, Obognon F. Effects of rehydration on the conidial viability of *Metarhizium flavoviride* mycopesticide formulations. Biocontrol Sci Technol 1997; 7: 87–94.
19. Alves RT, Bateman RP, Gunn J, Prior C, Leather SR. Effect of different formulations on viability and medium term storage of *Metarhizium anisopliae* conidia. Neotrop Entomol 2002; 31: 91–99.
20. Moore D, Bateman RP, Carey M, Prior C. Ultra-violet radiation damage to *Metarhizium flavoviride* conidia and the protection given by vegetable and mineral oils and chemical sunscreens. Ann Appl Biol 1993; 122: 605–616.
21. Norusis MJ, SPSS® Inc. SPSS® for Windows Advanced Statistics Release 6.0. SPSS® Inc. Chicago 1993.
22. Barnes S, Moore D. The effect of fatty, organic or phenolic acids on the germination of conidia of *Metarhizium flavoviride*. Mycol Res 1997; 10: 662–666.
23. USDA. USDA Nutrient Database for Standard Reference, Release 11, No 04047 (September 1996), 1996.

Address for correspondence: Perry Polar, CAB International Caribbean and Latin America Regional Centre, Curepe, Trinidad and Tobago
Phone: 1-868-662-4173; Fax: 1-868-663-2859
E-mail: p.polar@cabi.org