Experimental and Applied Acarology (2005) 36: 41–50
DOI 10.1007/s10493-005-0508-3 © Springer 2005

Pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* to the tobacco spider mite *Tetranychus evansi*

VITALIS W. WEKESA[1,2], NGUYA K. MANIANIA[2,*], MARKUS KNAPP[2] and HAMADI I. BOGA[1]
[1]*Jomo Kenyatta University of Agriculture and Technology (JKUAT), P.O. Box 62000, Nairobi, Kenya;* [2]*International Centre of Insect Physiology and Ecology (ICIPE), P.O. Box 30772-00100 GPO Nairobi, Kenya; *Author for correspondence (e-mail: nmaniania@icipe.org; phone: +254-20-861680; fax +254-20-860110)*

Received 28 October 2004: accepted in revised form 5 January 2005

Key words: *Beauveria bassiana*, Biological control, Fungi, *Metarhizium anisopliae*, Pathogenicity, *Tetranychus evansi*

Abstract. Seventeen isolates of *Metarhizium anisopliae* (Metschnikoff) Sorokin and two isolates of *Beauveria bassiana* (Balsamo) Vuillemin were evaluated for their pathogenicity against the tobacco spider mite, *Tetranychus evansi* Baker & Pritchard. In the laboratory all the fungal isolates were pathogenic to the adult female mites, causing mortality between 22.1 and 82.6%. Isolates causing more than 70% mortality were subjected to dose–response mortality bioassays. The lethal concentration causing 50% mortality (LC_{50}) values ranged between 0.7×10^7 and 2.5×10^7 conidia ml^{-1}. The lethal time to 50% mortality (LT_{50}) values of the most active isolates of *B. bassiana* and *M. anisopliae* strains varied between 4.6 and 5.8 days. Potted tomato plants were artificially infested with *T. evansi* and treated with *B. bassiana* isolate GPK and *M. anisopliae* isolate ICIPE78. Both fungal isolates reduced the population density of mites as compared to untreated controls. However, conidia formulated in oil outperformed the ones formulated in water. This study demonstrates the prospects of pathogenic fungi for the management of *T. evansi*.

Introduction

The tobacco spider mite, *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae), is a major pest of solanaceous crops including tomato, pepper, eggplant, tobacco and nightshade (Qureshi et al. 1969; Ramalho and Flechtmann 1979). It is the most important dry season pest of tomatoes in Eastern and Southern Africa. Current management of this pest in Africa heavily relies on the use of large quantities of synthetic acaricides (Knapp et al. 2003; Saunyama and Knapp 2003) with associated ecological problems, effects on non-target organisms, development of acaricide resistance, and health risks to farmers and consumers.

The use of biological control agents such as predators and pathogens as part of an integrated pest management (IPM) strategy could reduce the dependence on chemical control. Although predatory mites of the family Phytoseiidae have been used to control other species of spider mites, no such predator has been

identified for the management of *T. evansi*. Recently, the use of pathogenic fungi as biological control agents of Acari has been advocated (Chandler et al. 2000) and *Neozygites* sp. reported to regulate natural populations of *T. evansi* in Brazil (Humber et al. 1981). However, *Neozygites* sp. is an obligate parasite and cannot be mass-produced *in vitro*, thereby limiting the prospects for its use in inundative strategies for control of mites.

Pathogens that are not associated with a specific host in nature can be tested for pathogenicity in laboratory bioassays. For instance, isolates of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin have been reported to be pathogenic to various Acari (Mwangi et al. 1995; Alves et al. 2002; Shaw et al. 2002) and so may also be pathogenic to *T. evansi*. We evaluated the pathogenicity of *M. anisopliae* and *B. bassiana* isolates against *T. evansi* in order to select the most pathogenic. We also carried out a greenhouse trial using two formulations of the fungi to evaluate the field potential of the most pathogenic isolates.

Materials and methods

Mite culture

A stock culture of *T. evansi* was established in the laboratory at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya. The initial culture originated from mites collected from Mwea Irrigation Scheme, Kenya, in 2001. The mites were reared on tomato, *Lycopersicon esculentum* Mill. variety Cal-J, in a rearing room maintained at 25 ± 2°C, 60–70% r. h. and a photoperiod 12:12 L:D. To obtain fixed-age females for the bioassays, quiescent deutonymphs were collected from the mite culture and put on leaf discs. The newly emerged females were used for the experiments 1–2 days later.

Fungal pathogens

Two isolates of *B. bassiana* and 17 of *M. anisopliae* were obtained from the ICIPE Germplasm Centre and originated from different hosts (Table 1). The fungi were grown on Sabouraud Dextrose Agar (SDA) in Petri dishes and maintained at ambient temperatures of 23–30 °C. Conidia were harvested from 21-day-old surface cultures by scrapping and suspended in 10 ml sterile distilled water containing 0.05% Triton X-100 in universal bottles containing glass beads. The conidial suspension was vortexed for 5 min to produce a homogenous suspension. Spore concentrations were determined using a haemocytometer. The viability of conidia was determined before bioassays by spread-plating 0.1 ml of conidial suspension titrated to 3.0×10^6 conidia ml^{-1} on SDA plates. A sterile microscope cover slip was placed on each plate. Plates were incubated at 26 ± 2°C and examined after 15–18 h. Percentage

germination was determined from 100-spore counts for each cover slip. Each plate was replicated four times.

Bioassay

Ten millilitre (10 ml) of a single dose of 1.0×10^7 conidia ml^{-1} was sprayed onto both surfaces of tomato leaf discs (25 mm diameter) using the Burgerjon's spray tower (Burgerjon 1956) (INRA, Dijon, France), corresponding to a deposit of approximately 3.8×10^6 conidia cm^2. The leaf discs were then air-dried under the laminar flow cabinet (20 min) and placed on wet cotton wool in Petri dishes. Twenty adult *T. evansi* females were then placed onto the treated leaf discs and exposed for 4 days after which they were transferred to untreated leaf discs. The treated leaf discs were arranged in an incubator at 25 ± 2°C in completely randomised design. Each treatment was repeated eight times and included control leaf discs sprayed with sterile 0.05% Triton X-100. Mortality was recorded daily for 7 days and never exceeded 7.5% in the control. Dead mites were transferred to Petri dishes lined with moist filter paper for 14 days to observe mycosis. Mortality caused by fungi was confirmed by microscopic examination of spores on the surface of the mites.

The isolates *B. bassiana* GPK and *M. anisopliae* isolates ICIPE 78, MA/GPK and B. Matete caused over 70% mortality during screening and were selected for dose–mortality relationship experiments. Four concentrations were tested: 3.0×10^6, 1.0×10^7, 3.0×10^7 and 1.0×10^8 conidia ml^{-1}. All the experimental procedures remained the same as described above.

Lethal effects of formulating agents on fungal isolates and mites

The effects of carrier (corn oil and Silwet L-77, Loveland Industries, Inc., Greeley, CO, USA) on the viability of conidia and mites were tested with *M. anisopliae* isolate ICIPE 78 and *B. bassiana* isolate GPK. Conidia were harvested from plates, diluted to concentration of 3.0×10^6 conidia ml^{-1}, and then suspended in Silwet L-77, water and corn oil in centrifuge tubes. The viability of conidia was determined using the technique described earlier. For mites, Silwet L-77, corn oil and water were mixed in proportions of 1, 22 and 77% (v/v), respectively, and the treatments consisted of water alone, Silwet L-77 and water or emulsifiable oil. Twenty adult female *T. evansi* were placed on tomato leaf discs (25 mm diameter) and sprayed with the formulations described above for 5 s using a hand sprayer. The experiment was replicated 10 times. Mortality was recorded daily for a period of 4 days.

Production of inoculum

Metarhizium anisopliae isolate ICIPE78 and *B. bassiana* isolate GPK were produced on long white rice. Three kilogram of rice was weighed into poly-

ethylene bags and autoclaved for 40 min at 121 °C. Erlenmeyer flasks containing 250 ml of Sabouraud dextrose medium were inoculated with conidial suspensions of *M. anisopliae* or *B. bassiana* and incubated for 3 days in a shaker incubator at 28 °C and 100 rpm. The aerated rice bags were inoculated with the broth and maintained in an incubation room (23–27 °C, 35–60% RH) for 21 days. The substrate containing conidia/mycelia was transferred into plastic basins and allowed to dry for 5 days at room temperature before the conidia were harvested by sieving. The conidia were stored for two weeks in the refrigerator (4–6 °C) before being used in greenhouse sprays. After 24 h on SDA medium, 89–93% of conidia germinated.

Greenhouse experiment

Forty-eight potted tomato plants, variety Cal-J (30 days-old and supported by sticks), were artificially infested with *T. evansi* by placing five highly infested leaflets from the mite stock culture on each plant. The mites were allowed to establish and multiply for 45 days. Eight plants were randomly assigned to each treatment and mite density was estimated two days before treatment by picking two leaves per tomato plant; one from the top and another from the middle. All motile stages of mites were counted in the laboratory using a dissecting microscope. Leaf area was determined with a leaf area meter (Li-3100, Li-Cor, Lincoln, Nebraska, USA) to establish the density of mites per square centimetre.

Conidial suspensions titrated to 1.0×10^8 conidia ml^{-1} were prepared in water and Silwet L-77 or emulsifiable oil (water, corn oil and Silwet L-77) at concentrations described above. Treatments were sprayed together to run-off using a 1.5-l hand sprayer (Spraying Systems Co., Wheaton, IL, USA) outside the greenhouse to avoid contamination of neighbouring plants. Control treatments were sprayed with water and Silwet L-77 or water, oil and Silwet L-77. Each treatment consisted of two tomato plants replicated four times in completely randomised blocks. Population densities of *T. evansi* were again estimated at 7 and 14 days after spraying. Temperature and relative humidity in the greenhouse ranged between 13.0 and 34.9 °C (mean 23.9 °C), and from 8.5 to 95.1% (mean 51.8%), respectively.

Statistical analysis

Mortality data was corrected for natural mortality (Abbott 1925) and arcsine-transformed before ANOVA (SAS 1990). Means were separated by Student–Newman–Keuls test at ($p = 0.05$). Probit analysis was used to estimate the lethal time to 50% mortality (LT_{50}) and the lethal concentration causing 50% mortality (LC_{50}) (SAS 1990). Population counts for the greenhouse experiment were log transformed to normalise the means and transformed values were

Table 1. Viability of fungal isolates tested, percent mortality and LT_{50} values of adult female *T. evansi* after 7 days following exposure to tomato leaf discs treated at a concentration of 1.0×10^7 conidia ml^{-1}.

Fungal species	Isolate	Host or source	% Germination ± SE	Percent mortality ± SE[a]	LT_{50} in days[b] (95% fiducial limits)
Metarhizium anisopliae	ICIPE 78	*Temnoscoita niroplagiata* Qued. (Coleoptera)	96.0 ± 1.0	77.9 ± 4.7bc	4.8 (4.7–4.9)
	MA/GPK	Soil	95.0 ± 0.0	73.8 ± 4.8abc	4.7 (4.6–4.8)
	B. Matete	*Galleria melonella* (Linnaeus) (Lepidoptera)	95.0 ± 1.0	71.1 ± 4.8abc	5.0 (5.0–5.1)
	ICIPE 69	Soil	91.0 ± 1.0	64.4 ± 3.6bcd	5.3 (5.2–5.4)
	ICIPE 48	Earwigs (Forficulidae) (Dermaptera)	87.5 ± 3.0	62.4 ± 4.8bcd	5.7 (5.6–5.8)
	KITUI 13	Sandy soil	88.0 ± 1.0	59.1 ± 6.7cde	5.4 (5.3–5.6)
	ICIPE 20	Soil	92.0 ± 1.0	59.1 ± 3.9cde	5.8 (5.7–5.9)
	ICIPE 24	Soil	87.5 ± 1.0	58.4 ± 5.9cde	5.5 (5.4–5.6)
	ICIPE 18	Soil	86.0 ± 1.0	49.0 ± 4.1def	n.o.
	ICIPE 60	Soil	93.5 ± 1.0	46.3 ± 5.3def	n.o.
	ICIPE 41	Unknown	89.0 ± 1.0	45.6 ± 5.3def	n.o.
	ICIPE 62	Soil	96.0 ± 1.0	45.6 ± 4.0def	n.o.
	Green Muscle	*Schistocerca gregaria* Forskål (Caelifera)	95.0 ± 1.0	39.6 ± 4.0efg	n.o.
	MAT	Soil	91.5 ± 1.0	36.9 ± 5.5efg	n.o.
	ICIPE 32	*Amblyomma variegatum* Fabricius (Acari)	93.5 ± 1.0	32.2 ± 6.1fg	n.o.
	ICIPE 30	*Busseola fusca* Fuller (Lepidoptera)	92.0 ± 3.0	23.5 ± 3.0g	n.o.
	ICIPE 21	*S. gregaria*	90.5 ± 2.0	22.1 ± 2.3g	n.o.
Beauveria bassiana	Bb/GPK	Unknown	100.0 ± 0.0	82.6 ± 2.8a	4.6 (4.5–4.6)
	Kericho	*Cyclocephala* sp. (Coleoptera)	100.0 ± 0.0	56.4 ± 6.4cde	5.8 (5.7–6.0)
	Control			6.9 ± 1.3h	n.o.

[a]Means followed by the same letters are not significantly different (Student–Newman–Keuls test, $p > 0.05$).
[b]n.o. = 50% mortality was not observed in the bioassays.

Table 2. LC_{50} values for the most pathogenic fungal isolates against adult female *T. evansi* at 7 days post-treatment.

Fungal species	Isolate	LC_{50} (conidia ml^{-1}) (95% fiducial limits)	Slope (standard error)	χ^2 (*df*)
M. anisopliae	B. Matete	2.3×10^7 ($2.0–2.5 \times 10^7$)	2.41 ± 0.11	471.56 (1)*
	ICIPE 78	1.0×10^7 ($0.9–1.2 \times 10^7$)	2.24 ± 0.11	429.55 (1)*
	MA/GPK	0.7×10^7 ($0.6–0.8 \times 10^7$)	1.83 ± 0.10	322.10 (1)*
B. bassiana	Bb/GPK	1.1×10^7 ($1.0–1.2 \times 10^7$)	2.42 ± 0.11	464.52 (1)*

*$p < 0.001$.

subjected to analysis of variance using the ANOVA procedure of SAS (1990). Means were separated as above.

Results

In viability tests, 85–100% of spores germinated, and all fungal isolates were pathogenic to the adult females of *T. evansi* (Table 1). However, mortality varied between the isolates with *B. bassiana* GPK being the most pathogenic (82.6%) and ICIPE 21 the least (22.1%) ($F = 18.03$; $df = 19{,}140$; $p = 0.001$) (Table 1). The lethal time mortality values (LT_{50}) ranged from 4.6 to 5.8 days (Table 1), with LC_{50} values of the four most pathogenic isolates varying between 0.7 and 2.3×10^7 conidia ml^{-1} and *M. anisopliae* isolate MA/GPK having the lowest LC_{50} (Table 2).

The carriers used to formulate conidia consisting of water, oil and Silwet L-77 did not have any effect on mites ($F = 0.76$; $df = 2{,}27$; $p = 0.4755$) and germination of fungal conidia. The density of mites taken before treatment was not significantly different between treatments: middle leaf ($F = 1.23$; $df = 5{,}42$; $p = 0.3101$) and top leaf ($F = 1.48$; $df = 5{,}42$; $p = 0.2176$) (Table 3). At day 7 post-treatment, the density of mites on both top and middle tomato leaves was higher in the controls than in the fungus-treated plants: middle leaf ($F = 135.76$; $df = 5{,}42$; $p = 0.0001$) and top leaf ($F = 53.77$; $df = 5{,}42$; $p = 0.0001$) (Table 3). However, conidia of both isolates formulated as emulsifiable oil performed better than the ones formulated in water and Silwet L-77 only (Table 3). At day 14 post-treatment, the density of mites in controls treated with water/oil and *M. anisopliae* ICIPE78 formulated in water and Silwet L-77 was not different in both middle and top tomato leaves. However, the other treatments significantly reduced mite populations: middle leaf ($F = 121.75$; $df = 5{,}42$; $p = 0.0001$) and top leaf ($F = 70.96$; $df = 5{,}42$; $p = 0.0001$) (Table 3). Emulsifiable oil formulation of *B. bassiana* had significantly less mites on middle leaves than *M. anisopliae* formulated in the same way (Table 3).

Discussion

All fungal isolates tested were able to infect adult female *T. evansi* in the laboratory but there was considerable variation between the isolates. This

Table 3. Effect of different formulations of *B. bassiana* and *M. anisopliae* on the density of *T. evansi* on tomato plants in greenhouse.

Treatment	Top leaves (mites cm^{-2})[a]			Middle leaves (mites cm^{-2})[a]		
	Pre-treatment	7 days post-treatment	14 days post-treatment	Pre-treatment	7 days post-treatment	14 days post-treatment
Control (water/oil/Silwet)	70.7 ± 11.4a	57.7 ± 12.6b	48.5 ± 18.2b	52.9 ± 7.2a	44.1 ± 5.2b	31.6 ± 4.3b
Control (water/Silwet)	54.9 ± 12.5a	152.2 ± 57.6a	467 ± 122.1a	63.2 ± 6.4a	76.9 ± 9.7a	267.1 ± 61.8a
ICIPE78/water/Silwet	116.6 ± 23.0a	20.5 ± 7.7c	25.1 ± 4.7b	65.5 ± 6.0a	12.7 ± 2.7c	24.2 ± 5.2b
ICIPE78/water/oil/Silwet	68.2 ± 10.6a	1.0 ± 0.3e	1.4 ± 0.4c	63.5 ± 9.8a	2.0 ± 0.5e	2.3 ± 0.6d
Bb GPK/water/Silwet	81.2 ± 10.1a	4.4 ± 1.9d	2.2 ± 0.7c	47.1 ± 7.6a	7.6 ± 0.9d	5.3 ± 1.5c
Bb GPK/water/oil/Silwet	78.4 ± 17.2a	0.4 ± 0.2e	1.4 ± 1.1c	59.9 ± 8.5a	0.4 ± 0.1f	0.3 ± 0.1e

[a]Means within column bearing the same letter are not significantly different by Student–Newman–Keuls test ($p > 0.05$).

study identified four isolates, *B. bassiana* GPK, *M. anisopliae* B. Matete, *M. anisopliae* ICIPE78 and *M. anisopliae* GPK as superior. Many studies have reported intraspecific variations in pathogenic activity of fungal pathogens on other arthropod pests (see Maniania and Fargues 1984; Poprawski et al. 1985; Feng and Johnson 1990; Ekesi et al. 1998). This underlines the importance of strain selection as stressed by Soper and Ward (1981).

Shaw et al. (2002) reported that fungal isolates from non-acarine hosts were pathogenic to *Varroa destructor* Anderson and Trueman in the laboratory. Pena et al. (1996) found that fungal isolates originating from *Polyphagotarsonemus latus* Banks (Tarsonomidae) were more pathogenic to this mite species than those isolated from other hosts. Although strict adaptation of strains of *M. anisopliae* to the original host has been reported in case of scarabaeid beetles (Ferron et al. 1972), *M. anisopliae* and *B. bassiana* are ubiquitous pathogens recorded on many hosts (Veen 1968). The lethal concentration values obtained in this study were in the range of those reported by other workers on mite hosts such as *Tetranychus urticae* Koch and *V. destructor* (Alves et al. 2002; Shaw et al. 2002).

Conidia of *B. bassiana* isolate GPK and *M. anisopliae* isolate ICIPE 78 formulated as emulsifiable oil caused higher *T. evansi* mortality than the ones formulated in water. The enhanced infectivity of conidia formulated in oil formulations was first reported by Prior et al. (1988). Kaaya and Hassan (2000) found that oil-based formulations of conidia of *B. bassiana* and *M. anisopliae* were more effective against *Ripicephalus appendiculatus* Neumannn than the aqueous formulation. Batta (2003) also reported that conidia of *M. anisopliae* formulated in an invert formulation (water-in-oil formulation) with a coconut/soybean oil preparation were more effective against *T. urticae* than non-formulated conidia. Although aqueous formulation of Silwet L-77 has been shown to be toxic to the two spotted spider mite, *T. urticae*, and the Pacific spider mite, *T. pacificus* McGregor (Cowles et al. 2000; Tipping et al. 2003), this was not observed in our study.

In conclusion, our greenhouse results indicate that pathogenic fungi have great potential for management of *T. evansi*, particularly when formulated in oil. However, additional experiments are required to determine the timing and the frequency of applications and to improve on the formulations. The effects of tomato varieties on the viability and infectivity of the fungus also need to be investigated since the alkaloid tomatine, present in the foliage can cause complete inhibition of colony formation and growth of entomopathogenic fungi (Costa and Gaugler 1989).

Acknowledgements

We wish to thank Drs F. Schulthess and S. Ekesi, ICIPE, for their comments on the manuscript, Ms E.O. Ouna, Ms M. Kungu, Mr R. Rotich, and Mr C. Kyallo for their technical support. The study obtained financial support

from the German Federal Ministry for Economic Cooperation and Development (BMZ). The first author received support from ICIPE's Capacity Building Programme (DRIP) funded by the Government of the Netherlands.

References

Abbott W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Alves S.B., Rossi L.S., Lopes R.B., Tamai M.A. and Pereira R.M. 2002. *Beauveria bassiana* yeast on agar medium and its pathogenicity against *Diatraea saccharalis* (Lepidoptera: Crambidae) and *Tetranychus urticae* (Acari: Tetranychidae). J. Invertebr. Pathol. 81: 70–77.

Batta Y.A. 2003. Production and testing of novel formulations of the entomopathogenic fungus *Metarhizium anisopliae* (Metschinkoff) Sorokin (Deuteromycotina: Hyphomycetes). Crop Protect. 22: 415–422.

Burgerjon A. 1956. Pulvérisation et poudrage au laboratoire par des préparations pathogènes insecticides. Ann. Epiphyt. 7: 675–684.

Chandler D., Davidson G., Pell J.K., Ball B.V., Haw K. and Sunderland K.D. 2000. Fungal biocontrol of Acari. Biocontrol Sci. Technol. 10: 357–384.

Costa S.D. and Gaugler R.R. 1989. Sensitivity of *Beauveria bassiana* to solanine and tomatine: plant defensive chemicals inhibit an insect pathogen. J. Chem. Ecol. 15: 697–707.

Cowles R.S., Cowles E.A., McDermott A.M. and Ramoutar D. 2000. 'Inert' formulation ingredients with activity: toxicity of trisiloxane surfactant solutions to two spotted spider mites (Acari: Tetranychidae). J. Econ. Entomol. 93: 180–188.

Ekesi S., Maniania N.K., Onu I. and Löhr B. 1998. Pathogenicity of entomopathogenic fungi (Hyphomycetes) to the legume flower thrips, *Megalurothrips sjostedti* (Trybom) (Thysanoptera: Thripidae). J. Appl. Entomol. 122: 629–634.

Feng M.G. and Johnson J.B. 1990. Relative susceptibility of six strains of *Beauveria bassiana* on *Diuraphis noxia* Mordwilko (Homoptera: Aphididae). Environ. Entomol. 19: 785–790.

Ferron P., Hurpin B. and Robert P.H. 1972. Sur la spécifité de *Metarhizium anisopliae* (Metsch.). Sor. Entomophaga 17: 165–178.

Humber R.A., de Moraes G.J. and dos Santos J.M. 1981. Natural infection of *Tetranychus evansi* (Acarina: Tetranychidae) by a *Triplosporium* sp. (Zygomycetes: Entomophthorales) in Northeastern Brazil. Entomophaga 26: 421–425.

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

Knapp M., Wagener B. and Navajas M. 2003. Molecular discrimination between the spider mite *Tetranychus evansi* Baker & Pritchard, an important pest of tomatoes in southern Africa, and the closely related species *T. urticae* Koch (Acarina: Tetranychidae). Afr. Entomol. 11: 300–304.

Maniania N.K. and Fargues J. 1984. Spécificité des hyphomycètes entomopathogènes pour les larves de lépidoptères Noctuidae. Entomophaga 12: 451–464.

Mwangi E.N., Kaaya G.P. and Essuman S. 1995. Experimental infections of the tick *Ripicephalus appendiculatus* with pathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, and natural infections of some ticks with bacteria and fungi. J. Afr. Zool. 109: 151–160.

Pena J.E., Osborne L.S. and Duncan R.E. 1996. Potential of fungi as biocontrol agents of *Polyphagotarsonemus latus* (Acari: Tarsonemidae). Entomophaga 41: 27–36.

Poprawski T.J., Marchal M. and Robert P.H. 1985. Comparative susceptibility of *Otiorhynchus sulcatus* and *Sitona lineatus* (Coleoptera: Curculionidae) early stages to five entomopathogenic hyphomycetes. Environ. Entomol. 14: 247–253.

Prior C., Jollands P. and le Patourel G. 1988. Infectivity of oil and water formulations of *Beauveria bassiana* Deuteromycotina: Hyphomycetes) to the cocoa weevil pest *Pantorhytes plutus* (Coleoptera: Curculionidae). J. Invertebr. Pathol. 52: 66–72.

Qureshi A.H., Oatman E.R. and Fleschner C.A. 1969. Biology of the spider mite, *Tetranychus evansi*. Ann. Entomol. Soc. Am. 62: 898–903.

Ramalho F.S. and Flechtmann C.H.W. 1979. Niveis de infestacao de *Tetranychus (T.) evansi* em diferentes fases de desenvolvimento do tomateiro. Rev. Agricult. 54: 51–56.

SAS Institute 1990. SAS/STAT User's Guide. Version 6, 4th ed. Vol. 2. SAS Institute, Cary, NC.

Saunyama I.G.M. and Knapp M. 2003. The effects of pruning and trellising of tomatoes (*Lycopersicon esculentum* Mill) on red spider mite (*Tetranychus evansi* Baker and Pritchard) incidence and crop yield in Zimbabwe. Afr. Crop Sci. J. 11: 269–277.

Shaw K.E., Davidson G., Clark S.J., Ball B.V., Pell J.K., Chandler D. and Sunderland K.D. 2002. Laboratory bioassays to assess the pathogenicity of mitosporic fungi to *Varroa destructor* (Acari: Mesostigmata), an ectoparasite mite of the honeybee, *Apis mellifera*. Biol. Control 24: 266–276.

Soper R.S. and Ward M.G. 1981. Production, formulation and application of fungi for insect control. In: Papavizas G.C. (ed.), Biological Crop Production. Allanheld, Osmum, Montclair, New Jersey, pp. 161–180.

Tipping C., Bikoba V., Chandler G.J. and Mitcham E.J. 2003. Efficacy of Silwet L-77 against several arthropod pests of table grape. J. Econ. Entomol. 96: 246–250.

Veen K.H. 1968. Recherche sur les maladies due à *Metarhizium anisopliae* chez le criquet pèlerin. Mededelingen Landbouwhogeschool Wageningen, Nederland 68: 1–117.