Letters in Applied Microbiology

Letters in Applied Microbiology ISSN 0266-8254

ORIGINAL ARTICLE

Selection of promising fungal biological control agent of the western flower thrips *Frankliniella occidentalis* (Pergande)

S. Niassy[1], N.K. Maniania[1], S. Subramanian[1], L.M. Gitonga[2], D.M. Mburu[1], D. Masiga[1] and S. Ekesi[1]

1 International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya
2 Jomo Kenyatta University of Agriculture and Technology (JKUAT), Nairobi, Kenya

Keywords
Beauveria bassiana, biological control, genotyping, *Metarhizium anisopliae*, thrips.

Correspondence
Nguya K. Maniania, International Centre of Insect Physiology and Ecology (icipe), PO Box 30772-00100, Nairobi, Kenya.
E-mail: nmaniania@icipe.org

2011/1995: received 23 November 2011, revised 3 February 2012 and accepted 3 February 2012

doi:10.1111/j.1472-765X.2012.03241.x

Abstract

Aims: Larval stages of *Frankliniella occidentalis* are known to be refractory to fungal infection compared with the adult stage. The objective of this study was to identify promising fungal isolate(s) for the control of larval stages of *F. occidentalis*.

Methods and Results: Ten isolates of *Metarhizium anisopliae* and eight of *Beauveria bassiana* were screened for virulence against second-instar larvae of *F. occidentalis*. Conidial production and genetic polymorphism were also investigated. *Metarhizium anisopliae* isolates ICIPE 7, ICIPE 20, ICIPE 69 and ICIPE 665 had the shortest LT_{50} values of 8·0–8·9 days. ICIPE 69, ICIPE 7 and ICIPE 20 had the lowest LC_{50} values of $1{\cdot}1 \times 10^7$, $2{\cdot}0 \times 10^7$ and $3{\cdot}0 \times 10^7$ conidia ml^{-1}, respectively. *Metarhizium anisopliae* isolate ICIPE 69 produced significantly more conidia than *M. anisopliae* isolates ICIPE 7 and ICIPE 20. Internally transcribed spacers sequences alignment showed differences in nucleotides composition, which can partly explain differences in virulence.

Conclusion: These results coupled with the previous ones on virulence and field efficacy against other species of thrips make *M. anisopliae* isolate ICIPE 69 a good candidate.

Significance and Impact of the Study: *Metarhizium anisopliae* isolate ICIPE 69 can be suggested for development as fungus-based biopesticide for thrips management.

Introduction

The western flower thrips (WFT), *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), is a serious quarantine pest of horticultural crops worldwide (Lewis 1997; EPPO 2002). In addition to crop damages such as abscission of buds, abortion of flowers and malformation of pods, WFT are efficient vectors of tospoviruses (Lewis 1997). Synthetic chemical pesticides are widely used for control of thrips, despite their toxicity and hazardous effects to humans and the environment (Kirk and Terry 2003; Nderitu *et al.* 2008). In addition, the WFT have developed resistance to major groups of synthetic chemicals (Jensen 2004; Broughton and Herron 2009). There is therefore the prevailing need to develop ecologically sound and sustainable alternative for management of thrips. Entomopathogenic fungi are among the control strategies being developed (Ekesi and Maniania 2000a; Ekesi *et al.* 2007). For instance, Maniania *et al.* (2002) reported that application of *Metarhizium anisopliae* (Metschnikoff) Sorokin (Hypocreales: Clavicipitaceae) significantly reduced WFT in chrysanthemum crop, but the control of larval populations was much lower than for adults. Similar observations were made in laboratory bioassays by Vestergaard *et al.* (1995) and Ugine *et al.* (2005) with *M. anisopliae* and *Beauveria bassiana* (Balsmo) Vuillemin. The objective of this study was therefore to screen different fungal isolates of *M. anisopliae* and *B. bassiana* for selection of virulent isolate(s) against second-instar larval stage of WFT. We also considered other

parameters such as conidial production and evolutionary phylogenetic variability among the fungal isolates using molecular tools.

Materials and methods

Insect colony

Insects were obtained from the Mass Rearing Unit at the International Centre of Insect Physiology and Ecology (*icipe*). They were reared on French bean pods *Phaseolus vulgaris* (L.) var. *Samantha* at 25 ± 2°C, 60–80%, r.h. with a 12 L: 12 D photoperiod. Second-instar larval stage was used in the experiments.

Fungus

Fungal isolates were obtained from the *icipe*'s Arthropod Germplasm Centre (Table 1). They were cultured on Sabouraud Dextrose Agar (SDA) in 9-cm Petri dishes and incubated at 25 ± 2°C in complete darkness. Conidia were harvested by scrapping the surface using a spatula. Inocula were suspended in 10 ml sterile distilled water containing 0·05% Triton X-100 in universal bottles containing glass beads. Conidial suspensions were vortexed for 5 min to produce a homogenous suspension. Spore concentrations were determined using a haemocytometer. The viability of conidia was determined before any bioassay by spread-plating 0·1 ml of a 3×10^6 conidia ml^{-1} suspension onto 9-cm Petri dishes containing SDA medium. A sterile microscope cover slip was placed on each plate, and the plates were incubated in complete darkness at 25 ± 2°C and examined after 20 h. Percentage of germination of conidia was determined by assessing the number of germ tubes formed among 100 random conidia on the surface area covered by each cover slip under the light microscope (400×). Four replicate plates of the isolates were used.

Screening of fungal isolates

Ten millilitres of standard concentration of 1×10^7 conidia ml^{-1} was sprayed on pods of French beans using a Burgerjon spray tower (Burgejon 1956). Pods were allowed to dry for 5 min, after which they were transferred to 30-ml glass tubes. Twenty 2nd-instard larvae of WFT were then introduced gently in glass tubes using camel brush. In the control treatments, pods were sprayed with sterile distilled water containing 0·05% Triton X-100. Treatments were randomized, and the experiment was replicated four times over time.

Dose-mortality relationships were calculated for the most pathogenic isolates using five doses of inoculum: 3×10^6; 1×10^7; 3×10^7; 1×10^8 and 3×10^8 conidia ml^{-1}. Test insects were incubated at 25 ± 2°C and 90 ± 2% r.h. with a photoperiod of 12 L : 12 D. Mortality was recorded daily for 10 days. Dead insects were placed in a humid chamber to allow the development of

Table 1 List of fungal isolates and their origin tested against second-instar larvae of *Frankliniella occidentalis* and percentage of germination after 16 h on SDA plates at 25 ± 1°C

Fungal species	Isolates	Locality (country)	Source	Percentage of germination
Metarhizium anisopliae	ICIPE 7	Rusinga Island (Kenya)	*Amblyoma variegatum*	92·0 ± 1·6
	ICIPE 18	Mbita (Kenya)	Soil	92·8 ± 1·6
	ICIPE 20	Migori-Kenya	Soil	96·5 ± 0·8
	ICIPE 30	Kendu Bay (Kenya)	*Busseola fusca*	89·4 ± 1·2
	ICIPE 41	Migori (Kenya)	Soil	100
	ICIPE 69	Matete (DRC)	Soil	93·5 ± 0·6
	ICIPE 78	Ungoye (Kenya)	*Temnoschoita nigroplagiata*	90·7 ± 1·0
	ICIPE 84	(Senegal)	*Ornitacris turbida*	100
	ICIPE 625	Kabuti (Kenya)	Soil	100
	ICIPE 665	Ahero Plains (Kenya)	Soil	92·8 ± 1·3
Beauveria bassiana	ICIPE 279	Kericho (Kenya)	Coleopteran larvae	97·0 ± 0·7
	ICIPE 284	Mauritius	Soil	95·0 ± 0·7
	ICIPE 620	Kapsorok (Kenya)	Soil	100
	ICIPE 621	Motinet (Kenya)	Soil	100
	ICIPE 622	Kapiti sondu (Kenya)	Soil	100
	ICIPE 646	(Mauritius)	Soil	96·8 ± 0·9
	ICIPE 659	Kapmonyok (Kenya)	Soil	100
	ICIPE 664	Bungoma (Kenya)	Soil	100

mycosis on the surface of cadaver. Each treatment consisted of four replicates of 20 insects each and was repeated three times.

Conidial production

Fungal isolates with the lowest median lethal concentration (LC_{50}) values were compared for conidial production. Second-instar larvae of WFT were exposed for 24 h to fungus-treated French bean pods at the concentration of 1×10^8 conidia ml^{-1}, after which they were transferred onto sterile glass tubes containing clean pods. At 3, 6 and 9 days postinfection, five mycosed insects were collected and dried in an oven for 30 min at 30 ± 1°C and transferred individually into 2-ml cryogenic tubes containing 0·1 ml of sterile 0·05% Triton X-100. The tube was then vortexed for 5 min to dislodge conidia from the insect, and the number of conidia was determined using a haemocytometer (Hausser Scientific, Horsham, Pennsylvania, USA). The experiment was repeated four times.

Characterization of fungal isolates based on internally transcribed spacer (ITS) sequences

DNA extraction. Pure cultures of *M. anisopliae* isolates ICIPE 7, ICIPE 20 and ICIPE 69 were produced on SDA. Equal amounts (0·1 g) of conidia of each of the isolates were weighed in microcentrifuge tubes on a weighing balance (Mettler AT 261 Delta, Listers 2000, USA). DNA was extracted using a slight modification of the CTAB method described by Doyle and Doyle and resuspended in prewarmed sterile deionized water. The primer pairs n-SSU-1766-5 (ITS5) and nu-LSU-0041-3 (ITS4) (White *et al.* 1990) were used to amplify the ITS of the genomic DNA. PCR amplification reactions were carried out in a total volume of 20 μl containing PCR buffer (Genscript, Piscataway, NJ, USA), 2·5 mmol l^{-1} of each dNTP (Genscript), 0·2 μl of each primer, 2·5 mmol l^{-1} of $MgCl_2$, 0·5 units *Taq* DNA polymerase (Genscript) and *c.* 25 ng of genomic DNA. PCR amplification conditions involved initial denaturation at 94°C for 3 min, followed by 30 cycles of 94°C for 40 s, annealing temperature of 52°C for 40 s with an extension at 72°C for 1 min and final elongation at 72°C for 10 min. These reactions were carried out on a PTC-100 thermocycler (MJR Inc., Minneapolis, MN, USA). Negative controls without fungal DNA were run for each experiment to check for contamination of reagents.

DNA quantification and sequencing. The amplification products were separated by electrophoresis in 1% agarose gels containing ethidium bromide (3 μl), in 1× TAE buffer for 1 h at 70 V cm^{-1}. DNA was visualized under UV light and recorded using a Kodak Gel imaging system (Gel logic 200; Carestream Health, New Haven, CT, USA). The lengths of the amplicon products were estimated by comparison with 1-kb Smart DNA ladder (Noxo, Tallinn, Estonia). The PCR products were purified using QuickClean DNA gel extraction kit (Genscript) and sequencing outsourced.

Data analysis

Per cent mortality was corrected for control mortality (Abbott 1925) and normalized by arcsine transformation. Data were analysed using analysis of variance (ANOVA) using PROC GLM (SAS ver. 9.2.; SAS Inc., Cary, NC) at 95% level of significance. Means were separated using Student–Newman–Keuls (SNK). Median lethal time (LT_{50}) and LC_{50} were estimated using logistic regression. A Pearson correlation analysis was carried out to relate mortality rate with the conidial production.

DNA sequences of the most virulent fungal isolates were edited using BIOEDIT (ver. 7.0.5.3) (Hall 1999) and aligned using CLUSTAL W (ver. 2.012) (Larkin *et al.* 2001) software.

A Basic Local Alignment Search Tool (BLAST) was performed using NCBI, EMBL and Fungal Genome Search databases. The first best hit accession number was considered.

Results

Conidial viability of the isolates varied between 89 and 100% (Table. 1). Mortality in the controls was low and did not exceed 15% in all the experiments. All tested fungal isolates were pathogenic to the second-instar WFT at the concentration of 1×10^7 conidia ml^{-1}, causing mortalities of between 24 and 56% (Table 2). *Metarhizium anisopliae* isolates ICIPE 20 and ICIPE 69 caused the highest mortality and were significantly different from only two other isolates (ICIPE 30 and 78) and six of the *B. bassiana* isolates (ICIPE 646, 659, 284, 279, 664 and 622) ($F_{17,195} = 17{\cdot}37$, $P < 0{\cdot}001$). *B. bassiana* isolates ICIPE 664 and ICIPE 622 caused the lowest mortalities. *Metarhizium anisopliae* isolates ICIPE 7, ICIPE 20, ICIPE 69 and ICIPE 665 had the shortest LT_{50} values causing mortalities within 8·0–8·9 days as compared to the other fungal isolates (Table 2).

Among the seven isolates of *M. anisopliae* and one isolate of *B. bassiana* selected for lethal concentration response bioassays, *M. anisopliae* isolate ICIPE 69 had the lowest LC_{50} value followed by ICIPE 7 and ICIPE 20 (Table 3). However, there were significant differences between ICIPE 69 and ICIPE 20. There were also no significant differences between ICIPE 69 and ICIPE 7 and between ICIPE 7 and ICIPE 20 (95% confidence interval using fiducial limit overlapping). *Metarhizium anisopliae* isolate ICIPE 69 produced significantly more conidia than

Table 2 Virulence of fungal isolates against second-instar larvae *Frankliniella occidentalis*: Per cent mortality and LT_{50} values at the concentration of 10^7 conidia ml^{-1} 10 days post-treatment

Fungal species	Isolates	Mortality (% ±SE)	LT_{50} (days) (95% CI)	Slope (±SE)
Metarhizium anisopliae	ICIPE 20	56·2 ± 2·9a	8·5 (8·3–8·8)	4·7 ± 0·1
	ICIPE 69	55·9 ± 1·9a	8·2 (8·0–8·4)	4·1 ± 0·1
	ICIPE 7	51·2 ± 5·0ab	8·3 (8·0–8·5)	4·0 ± 0·1
	ICIPE 665	49·6 ± 3·0ab	8·4 (8·1–8·7)	3·7 ± 0·1
	ICIPE 18	44·1 ± 3·0abc	10·6 (10·1–11·2)	2·8 ± 0·1
	ICIPE 41	48·2 ± 3·5abc	9·2 (9·0–9·6)	4·0 ± 0·1
	ICIPE 625	48·3 ± 3·5abc	10·5 (10·1–10·6)	4·3 ± 0·1
	ICIPE 84	43·3 ± 2·5abc	11·3 (10·8–11·9)	3·5 ± 0·1
	ICIPE 30	40·4 ± 4·0bcd	11·8 (11·2–12·4)	3·7 ± 0·1
	ICIPE 78	40·9 ± 3·2bcd	11·1 (10·7–11·7)	3·6 ± 0·1
Beauveria bassiana	ICIPE 620	45·9 ± 2·1abc	10·2 (10·0–10·6)	4·7 ± 0·1
	ICIPE 621	44·6 ± 2·5abc	11·6 (11·1–12·2)	4·1 ± 0·1
	ICIPE 646	42·5 ± 2·5bc	10·5 (10·0–11·0)	3·4 ± 0·1
	ICIPE 659	38·0 ± 2·3bcd	12·9 (12·2–13·7)	3·9 ± 0·1
	ICIPE 284	35·0 ± 4·2cd	14·8 (13·9–16·0)	3·5 ± 0·1
	ICIPE 279	29·9 ± 2·8de	17·7 (16·2–19·6)	2·6 ± 0·1
	ICIPE 664	24·6 ± 2·0e	24·8 (21·9–28·7)	2·7 ± 0·1
	ICIPE 622	23·8 ± 1·4e	33·0 (27·8–40·7)	2·0 ± 0·1

Within column means followed by the same letters are not significantly different by Student–Newman–Keuls ($P < 0·05$).

Table 3 Lethal concentration values (LC_{50}) of selected fungal isolates against second-instar larvae of *Frankliniella occidentalis*

Species	Isolates	LC_{50} (95% CI) ($\times 10^8$ conidia ml^{-1})	Slope (±SE)
Metarhizium anisopliae	ICIPE 69	0·1 (0·0–0·1)	2·1 ± 0·1
	ICIPE 7	0·2 (0·1–0·2)	1·2 ± 0·0
	ICIPE 20	0·3 (0·2–0·3)	1·1 ± 0·0
	ICIPE 41	0·8 (0·6–1·0)	0·9 ± 0·0
	ICIPE 84	3·6 (2·5–5·8)	1·0 ± 0·0
	ICIPE 18	18 (8·1–58·0)	0·7 ± 0·0
	ICIPE 625	4·0 (2·8–6·4)	1·0 ± 0·8
Beauveria bassiana	ICIPE 620	14·4 (7·5–36·6)	0·8 ± 0·1

the other two isolates in all the three sampling dates ($F_{2,31} = 8·9$, $P < 0·0009$) (Fig. 1). The conidia production was significantly different between the sampling days 3, 6 and 9 days ($F_{2,31} = 18·9$, $P < 0·0001$, SNK) postinfection

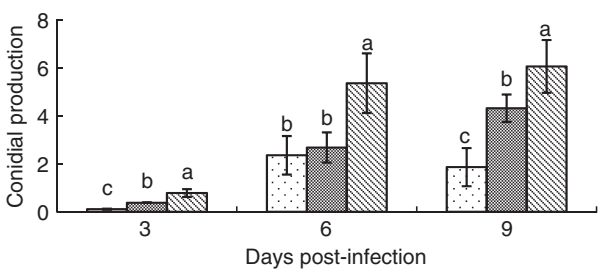

Figure 1 Mean conidial production ($\times 10^5$ conidia) of three isolates of *Metarhizium anisopliae* following infection of second-instar larvae of *Frankliniella occidentalis*. (□) ICIPE 7; (▩) ICIPE 20 and (▨) ICIPE 69.

(Fig. 2). A correlation between conidial production and mortality (Pearson $R = 0·65$, $P < 0·001$) was observed (Table 4).

ITS sequences alignment showed a difference of two base pairs on the isolate ICIPE 69, which is not present in ICIPE 7 and ICIPE 20. The latter two were identical at this locus on the other isolates. Restriction sites were identified on the ITS sequence *Eco*R1 and *Zho*I and were common for all the three *M. anisopliae* isolates. However, *Sfo*I restriction site was found to be specific to *M. anisopliae* ICIPE 69 (Fig. 2).

A Basic Local Alignment Search Tool on NCBI, EMBL and Fungal Genome Search indicated a low expect value(*E*) and similarity values ranging between 97 and 100% with *Metarhizium anisopliae* FJ545302, FJ609312 respectively on NCBI and EMBL. The Fungal Genome Search database identified the isolates as affiliated to *M. anisopliae* variety *anisopliae* AF136376 (Table 5).

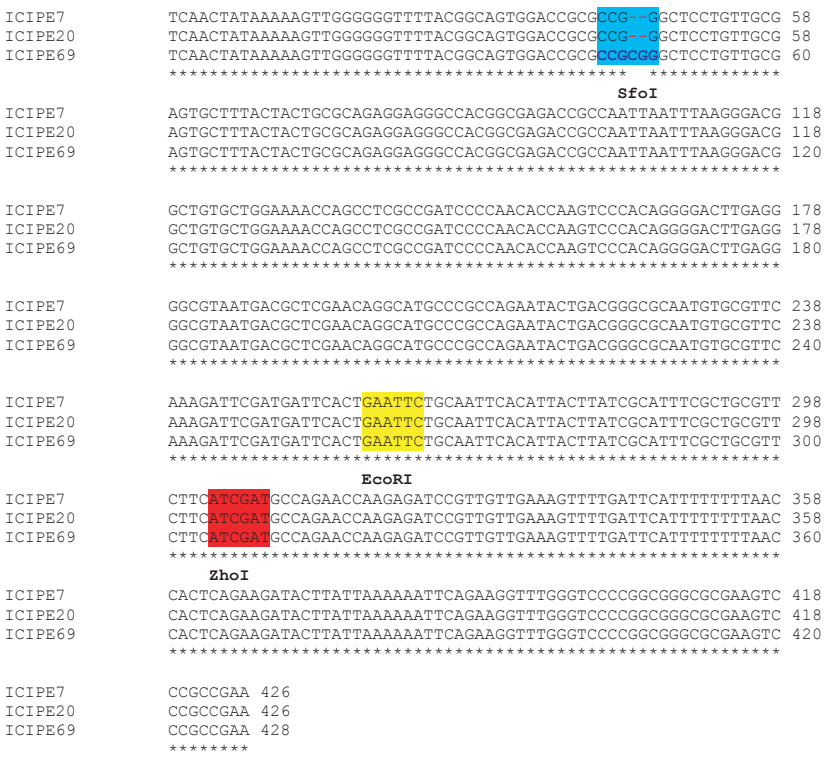

Figure 2 ITS4-ITS5 sequence alignments of DNA of three *Metarhizium anisopliae* isolates ICIPE 7, ICIPE 20 and ICIPE 69 showing the restriction sites *Eco*R1, *Zho*I and *Sfo*I. Sections of the two sequences marked and unmarked with asterisks indicate homology and divergence, respectively, between the sequences.

Table 4 Correlation between mortality and conidial production of three *Metarhizium anisopliae* isolates ICIPE 7, ICIPE 20 and ICIPE 69 applied on second-instar larvae of *Frankliniella occidentalis* at 1×10^8 conidia ml^{-1}

Isolates	Mean mortality (%)	Mean conidial production ($\times 10^5$ conidia)
ICIPE 7	55·5 ± 6·7	2·5 ± 0·9
ICIPE 20	50·8 ± 7·6	3·4 ± 1·2
ICIPE 69	70·9 ± 10·5	6·9 ± 2

Pearson: $R = 0{\cdot}65$; $P < 0{\cdot}0001$; $N = 36$.

Discussion

The aim of this study was to identify potential fungal candidate(s) for control of the larval stage of *F. occidentalis* that has been reported to be refractory to fungal infection (Vestergaard *et al.* 1995; Maniania *et al.* 2002; Ugine *et al.* 2005). All the fungal isolates tested were pathogenic to the second-instar larvae of WFT; however, mortality and LT_{50} values varied between the isolates. Such variations have already been reported for fungal pathogens in many groups of insects (Ekesi *et al.* 1998; Mburu *et al.* 2009; Migiro *et al.* 2010). Four isolates of *M. anisopliae* (ICIPE 20, ICIPE 69, ICIPE 7 and ICIPE 665) with LT_{50} between 8·0 and 8·8 days outperformed the other fungal isolates (Table 2). When seven isolates of *M. anisopliae* and one of *B. bassiana* were evaluated for the LC_{50} bioassays, only three isolates of *M. anisopliae* (ICIPE 7, ICIPE 20 and ICIPE 69) had the lowest LC_{50} ($1–3 \times 10^7$ conidia ml^{-1}) (Table 3). Virulence has always been one of the most important parameters considered for strain selection (Inglis *et al.* 2001), whereas parameters such as persistence, UV tolerance and conidial production have been overlooked. In this study, the three best fungal isolates (lower LC_{50} values) were compared for conidial production. The *M. anisopliae* isolate ICIPE 69 produced significantly more conidia than the other isolates, which may be an advantage in terms of inoculum dispersion in the habitat, mass production and subsequent commercialization. The virulence of isolate ICIPE 69 against adults of the legume flower thrips, *Megalurothrips sjostedti* Trybom (Ekesi *et al.* 1998), onion thrips, *Thrips tabaci* Lindeman (Maniania *et al.* 2003) and WFT (Maniania *et al.* 2002), coupled with high conidial production and tolerance to broad range temperature (Ekesi *et al.* 1999), makes it a suitable biopesticide candidate for thrips control. More-

Table 5 Basic Local Alignment Search Tool of *Metarhizium anisopliae* ICIPE 7, ICIPE 20 and ICIPE69 ITS4, ITS5 sequences using NCBI, EMBL and Fungal Genome Search databases

Isolates	Length (bp)	hit Accession number	Expect value (*E*)	Identity (%)	Species
NCBI					
ICIPE 7	426	FJ545302	0·0	100	*M. anisopliae*
ICIPE 20	426	FJ545302	0·0	100	*M. anisopliae*
ICIPE 69	428	FJ545302	0·0	99	*M. anisopliae*
EMBL					
ICIPE 7	426	FJ609312	$4{\cdot}3\ e^{-85}$	98	*M. anisopliae*
ICIPE 20	426	FJ609312	$5{\cdot}3\ e^{-86}$	98	*M. anisopliae*
ICIPE 69	428	FJ609312	$5{\cdot}3\ e^{-86}$	97	*M. anisopliae*
Fungal Genome Search					
ICIPE 7	426	AF136376	$6{\cdot}6\ e^{-87}$		*M. anisopliae* var. *anisopliae*
ICIPE 20	426	AF136376	$6{\cdot}6\ e^{-87}$		*M. anisopliae* var. *anisopliae*
ICIPE 69	428	AF136376	$5{\cdot}3\ e^{-86}$		*M. anisopliae* var. *anisopliae*

over, Ekesi and Maniania (2000b) have established that ICIPE 69 can significantly alter feeding, fecundity, fertility and longevity of *M. sjostedti*. Because larval stages of thrips are known to be acquisition agents and replication hosts of viral proteins (Whitfield *et al.* 2005), hence ICIPE 69 can play an important role in the control of tospovirus epizooties.

The results of the ITS gene sequence amplification showed two base pair differences in ICIPE 69, which alter the restriction site sequence for *Sfo*I. This restriction enzyme (*Sfo*I) can be used to identify ICIPE 69 by the RFLP technique (Fig. 2).

A Basic Local Alignment Search Tool (BLAST) in NCBI, EMBL and Fungal Genome Search showed homology over 95% with *M. anisopliae*. However, the nucleotide sequences of the three isolates suggest intra-specific genotypic variation (Freed *et al.* 2011; Mburu *et al.* 2011). Geographical and ecological features of the fungal isolates may explain the variation observed in this study (Meyling and Eilenberg 2007; Bischoff *et al.* 2009; Enkerli and Widmer 2010). For instance, *M. anisopliae* isolate ICIPE 69 originated from the Democratic Republic of Congo, whereas the other two originated from Kenya. In conclusion, our results reconfirm the efficacy of the *M. anisopliae* isolate ICIPE 69 and suggest its development as fungal biopesticide for thrips management.

Acknowledgements

We are grateful to Dr S. Dara, University of California, Davis, for reviewing the first draft of the manuscript, and Miss Barbara Obonyo for technical support. We also thank Joel Ltilitan Bargul for assistance with the molecular work. This study was jointly funded by the German Academic Exchange Services through the African Regional Postgraduate Programme in Insect Science (ARPPIS; http://www.icipe.org/arppis) of *icipe* and the Federal Ministry for Economic Cooperation and Development, Germany (BMZ) through the Thrips IPM Project.

References

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

Bischoff, J.F., Rehner, S.A. and Humber, R.A. (2009) A multilocus phylogeny of the *Metarhizium anisopliae* lineage. *Mycologia* **101**, 512–530.

Broughton, S. and Herron, G.A. (2009) Potential new insecticides for the control of western flower thrips (Thysanoptera: Thripidae) on sweet pepper, tomato, and lettuce. *J Econ Entomol* **102**, 646–651.

Burgejon, A. (1956) Pulverisation de poudrage au laboratoire par des preparations pathogens insecticides. *Ann Epiphyties* **4**, 677–688.

Ekesi, S. and Maniania, N.K. (2000a) *Metarhizium anisopliae*: an effective biological control agent for the management of thrips in horti and floriculture in Africa. In *Advances in Microbial Control of Insects Pests* ed. Upadhyay, R.K. pp. 165–191. New York: Kluwer Academic/Plenum publishers.

Ekesi, S. and Maniania, N.K. (2000b) Susceptibility of *Megalurothrips sjostedti* developmental stages to *Metarhizium anisopliae* and the effects of infection on feeding, adult fecundity, egg fertility and longevity. *Entomol Exp Appl* **94**, 229–236.

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

Ekesi, S., Maniania, N.K. and Ampong-Nyarko, K. (1999) Effect of temperature on germination, radial growth and virulence of *Metarhizium anisopliae* and *Beauveria bassiana*

on *Megalurothrips sjostedti*. *Biocontrol Sci Technol* **9**, 177–185.

Ekesi, S., Dimbi, S. and Maniania, N.K. (2007) The Role of entomopathogenic fungi in the integrated management of fruit flies (Diptera: Tephritidae) with emphasis on species occurring in Africa. In *Use of Entomopathogenic Fungi in Biological Pest Management* ed. Ekesi, S. and Maniania, N.K. pp. 239–274. Trivandrum, Kerala, India: Research Signpost Publishers.

Enkerli, J. and Widmer, F. (2010) Molecular ecology of fungal entomopathogens: molecular genetic tools and their applications in population and fate studies. *Biocontrol* **55**, 17–37.

EPPO (2002) Diagnostic protocols for regulated pests *Frankliniella occidentalis*. *EPPO Bull* **2**, 281–292.

Freed, S., Jin, F.-L. and Ren, S.-X. (2011) Determination of genetic variability among the isolates of *Metarhizium anisopliae* var. *anisopliae* from different geographical origins. *World J Microbiol Biotechnol* **27**, 359–370.

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.

Inglis, D.G., Goettel, S.M., Butt, M.T. and Strasser, H. (2001) Use of hyphomycetes fungi for managing insect pests. In *Fungi as Biocontrol Agents* ed. Butt, T.M., Jackson, C. and Magan, N. pp. 23–27. Wallingford: CABI Publishing.

Jensen, E.S. (2004) Insecticide resistance in the Western flower thrips, *Frankliniella occidentalis*. *Integr Pest Manag Rev* **5**, 131–146.

Kirk, W.D.J. and Terry, L.I. (2003) The spread of the Western flower thrips *Frankliniella occidentalis* (Pergande). *Agric Forest Entomol* **5**, 301–310.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M. *et al.* (2001) Clustal W and Clustal X version 2.0. *Bioinformatics* **23**, 2947–2948.

Lewis, T. (1997) *Thrips as Crop Pests*. Oxon, UK: CAB Wallingford.

Maniania, N.K., Ekesi, S., Lohr, B. and Mwangi, F. (2002) Prospect for biological control of the Western Flower Thrips, *Frankliniella occidentalis*, with the entomopathogenic fungus, *Metarhizium anisopliae* on Chrysanthemum. *Mycopathologica* **155**, 229–235.

Maniania, N.K., Sithanantham, S., Ekesi, S., Onu, I., Ampong-Nyarko, K., Baumgärtner, J., Löhr, B. and Matoka, C.M. (2003) A field trial of the entomopathogenous fungus *Metarhizium anisopliae* for control of onion thrips, *Thrips tabaci*. *Crop Prot* **22**, 553–559.

Mburu, D.M., Ochola, L., Maniania, N.K., Njagi, P.G.N., Gitonga, L.M., Ndung'u, M.W., Wanjoya, A.K. and Hassanali, A. (2009) Relationship between virulence and repellency of entomopathogenic isolates of *Metarhizium anisopliae* and *Beauveria bassiana* to the termite *Macrotermes michaelseni*. *J Insect Physiol* **55**, 774–780.

Mburu, D.M., Ndung'u, M.W., Maniania, N.K. and Hassanali, A. (2011) Comparison of volatile blends and gene sequences of two isolates of *Metarhizium anisopliae* of different virulence and repellency toward the termite *Macrotermes michaelseni*. *J Exp Biol* **214**, 956–962.

Meyling, N.V. and Eilenberg, J. (2007) Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: potential for conservation biological control. *Biol Control* **43**, 145–155.

Migiro, L.N., Maniania, N.K., Chabi-Olaye, A. and Vandenberg, J. (2010) Pathogenicity of entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* (Hypocreales: Clavicipitaceae) isolates to the adult pea leafminer (Diptera: Agromyzidae) and prospects of an autoinoculation device for infection in the field. *Environ Entomol* **39**, 468–475.

Nderitu, J.H., Kasina, J.M. and Nyamasyo, G.N. (2008) Management of thrips (Thysanoptera: Thripidae) on French bean (Fabaceae) in Kenya: economics of insecticide applications. *J Entomol* **5**, 148–155.

Ugine, T.A., Wraight, S.P. and Sanderson, J.P. (2005) Acquisition of lethal doses of *Beauveria bassiana* conidia by Western flower thrips, *Frankliniella occidentalis*, exposed to foliar spray residues of formulated and unformulated conidia. *J Invertebr Pathol* **90**, 10–23.

Vestergaard, S., Gillespie, A. T., Butt, T. M., Schreiter, G. and Eilenberg, J. (1995) Pathogenicity of the Hyphomycete Fungi *Verticillium lecanii* and *Metarhizium anisopliae* to the Western Flower Thrips, *Frankliniella occidentalis*. *Biocontrol Sci Technol* **5**, 185–192.

White, T.J., Bruns, T., Lee, S. and Taylor, J.W. (1990) *Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics*. New York: Academic Press.

Whitfield, A.E., Ullman, D.E. and German, T.L. (2005) Tospovirus-Thrips interactions. *Annu Rev Phytopathol* **43**, 9–89.