SHORT, NON-REFEREED PAPER

ENTOMOPATHOGENS ASSOCIATED WITH AFRICAN AND MAURITIAN SCARABAEIDAE AFFECTING SUGARCANE

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Abstract

A multi-party consortium, funded under the EU-Africa, Caribbean and Pacific Economic Partnership Agreement, was established in 2010 for research and development of bioinsecticides against white grubs (Scarabaeidae) affecting sugarcane. By January 2012, the Insect Pathogen Quarantine Facility of ARC-Small Grain Institute received and processed a total of 1,549 insect cadavers shipped from within South Africa (South African Sugarcane Research Institute), Swaziland (Swaziland Sugar Association), Tanzania (Tanganyika Plantation Company), Malawi (Sugar Corporation of Malawi), Zimbabwe (Zimbabwe Sugar Association Experiment Station) and Mauritius (Mauritius Sugar Industry Research Institute). These samples included 1,320 scarab cadavers (larvae and adults), 155 *Galleria mellonella* and 57 *Chilo sacchariphagus* larval cadavers used as bait (indirect) from soil-trapping systems; 17 cadavers were of unknown identity. Four species of entomopathogenic fungi were isolated, *viz. Metarhizium anisopliae*, *Beauveria bassiana*, *Beauveria brongniartii* and *Lecanicillium lecanii*. In addition, 21 white grub cadavers collected in Tanzania were infected with *Ophiocordyceps* sp. and one cadaver with an entomopathogenic nematode, *Steinernema* sp. Overall the most prevalent fungi isolated directly from the Scarabaeidae were *M. anisopliae* (59%) and *B. bassiana* (35%). A total of 36 *Metarhizium* and five *Beauveria* cultures were established from the soil-trapped cadavers. Notably, the South African material yielded mainly *Beauveria*, whereas cadavers from the other countries were infected primarily by *Metarhizium*. A large number of bacteria (557) were isolated, although the entomopathogenic status of these isolates remains unknown. Research on the fungal isolates has progressed to screening for virulence against a substitute coleopteran beetle, *Tenebrio molitor*, and preliminary results are discussed.

Keywords: entomopathogens, *Beauveria*, *Metarhizium*, Scarabaeidae, sugarcane, Africa, Mauritius

Introduction

A multi-party consortium, funded under the EU-Africa, Caribbean and Pacific Economic Partnership Agreement (EU-ACP), was established in 2010 for research and development of bioinsecticides against white grubs (Scarabaeidae) affecting sugarcane. By January 2012, the Insect Pathogen Quarantine Facility of the Agricultural Research Council - Small Grain Institute (ARC-SGI) received and processed a total of 1,549 insect cadavers shipped from within South Africa (South African Sugar Research Institute), Swaziland (Swaziland Sugar Association), Tanzania (Tanganyika Plantation Company), Malawi (Sugar Corporation of Malawi), Zimbabwe (Zimbabwe Sugar Association Experiment Station) and Mauritius

(Mauritius Sugar Industry Research Institute). The principal entomopathogens associated with scarabs include fungi, bacteria, microsporidia and nematodes (Jackson and Glare, 1992). As part of this EU-ACP project, collaborating institutions initiated direct collection of diseased scarabs as well as indirect collection via soil-trapping (Hatting, 2010) with *Galleria mellonella* or *Chilo sacchariphagus* (Lepidoptera: Pyralidae). The samples comprised 1,320 scarab cadavers (larvae and adults), 155 *G. mellonella* and 57 *C. sacchariphagus* larval cadavers used as bait (indirect) from soil-trapping systems; 17 cadavers were of unknown identity. This paper presents an overview of the entomopathogens isolated from this material as well as some preliminary bioassay results for 12 fungal isolates from South Africa, Tanzania and Mauritius.

Materials and Methods

Isolation and in vitro *culture*

Material received was characterised as either showing (1) signs of fungal mycosis, (2) signs (and odour) of bacterial infection, (3) no obvious signs of pathogen-induced mortality and/or (4) judged unusable due to desiccation or contamination by non-entomopathogenic entities, mainly mites and/or fungi such as *Aspergillus* spp. Fungal isolations were made directly from cadavers (showing overt mycosis) by collecting conidia from the conidiophores with a sterile needle and plating onto Sabouraud Dextrose Agar + 1% Yeast (SDAY; Biolab, Wadeville, Gauteng, South Africa) and/or Potato Dextrose Agar (PDA; Biolab Wadeville, Gauteng, South Africa). Cadavers without external fungal growth but with 'spongy' body tissue were surface-sterilized in 70% ethanol (2-3 seconds), rinsed in sterile water and submerged in 1% sodium hypochlorite for 30 seconds, followed by three washes in sterile water. Cadavers were blotted dry and transferred onto water agar in sealed petri dishes to facilitate the development of overt mycosis. Cadavers not showing any obvious signs of pathogen-induced mortality were surface-sterilised as above and placed on White Traps as described by Kaya and Stock (1997) to facilitate overt mycosis, septicaemia or emergence of entomopathogenic nematodes. Bacterial isolations were made by puncturing the cuticle and collecting a small quantity of body fluid for transfer onto Nutrient Agar (Biolab, Wadeville, Gauteng, South Africa). All of the above-mentioned were maintained at 22°C, in complete darkness, for 7-10 days. Cultures were then examined and, where necessary, sub-cultured to establish pure cultures for tentative identification before shipment to the National Collection of Fungi, ARC-Plant Protection Research Institute, Pretoria, South Africa.

Bacterial cultures were cryopreserved in 15% sterile glycerol at -80°C. The infective juvenile (IJ) stage of entomopathogenic nematodes was collected from White Traps and suspended in 50 ml of sterile distilled water before being transferred into 250 ml culture flasks and maintained at 15°C, in complete darkness.

Bioassays

Individual suspensions of 12 fungal isolates containing 3.75 x 10^7 conidia/ml were prepared in 8 ml of 0.01% aqueous Break-Thru® (Polyether-polymethylsiloxane-copolymer surfactant; Goldschmidt Chemical Corporation, Hopewell, VA, USA) solution. Groups of 30 three-day old adult *Tenebrio molitor* beetles were inoculated with 5 ml of each fungal suspension in a Burgerjon spray tower (Burgerjon, 1956) and incubated at 22°C, in complete darkness, for seven days. Beetles were provided with fresh cabbage leaves for the duration of the assay. Controls were sprayed with 5 ml 0.01% Break-Thru® solution only. During each spray event, a plate of water agar was positioned adjacent to the insects (five insects x six groups) for later quantification of the number of conidia deposited per mm^2. Dead insects were surface-

sterilised as described above and placed on water agar at 22°C, in complete darkness, to facilitate the development of overt mycosis.

Results

The number of cadavers and entomopathogens isolated from material received from collaborating institutions is presented in Table 1.

Table 1. Number of cadavers and entomopathogens isolated from material sent to ARC-SGI by collaborating institutions.

Country	# Cadavers	*B. bassiana*-group	*M. anisopliae*-group	Bacteria
South Africa	760[1]	31 (27[2] + 4[3])	25 (15 + 10)	196 (190 + 6)
Tanzania	322[4]	10 (10 + 0)	32 (27 + 5)	163 (146 + 17)
Swaziland	43	0	0	4 (4 + 0)
Malawi	75	0	1 (1 + 0)	54 (47 + 7)
Zimbabwe	154	0	4 (4 + 0)	69 (69 + 0)
Mauritius	195	1 (0 + 1)	38 (16 + 21 + 1[5])	71 (52 + 19)
Total	1,549	42 (37 + 5)	100 (63 + 36 +1[5])	557 (508 + 49)

[1]including four *Beauveria brongniartii*, one *Lecanicillium lecanii* and one *Paecilomyces lilacinus*-infected cadavers
[2]number isolated from Scarabaeidae
[3]number isolated from soil sample (insect-trapping method)
[4]including 21 scarab cadavers infected with *Ophiocordyceps* sp. and one cadaver infected with an entomopathogenic nematode (*Steinernema* sp.)
[5]unknown host

The percentage mortality recorded for the 12 fungal isolates assayed is presented in Figure 1. The mean conidial concentration applied was 493±86 conidia per mm^2.

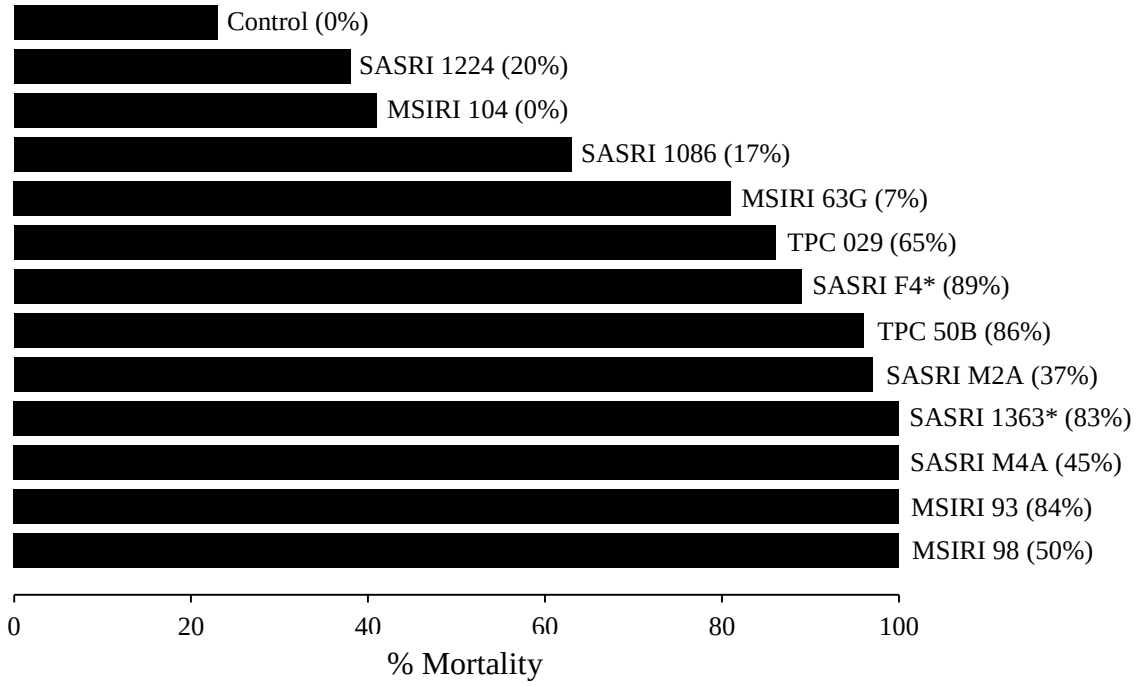

Figure 1. Mortality of *Tenebrio molitor* seven days post-inoculation with 10 strains of *Metarhizium anisopliae* and two strains of *Beauveria bassiana (% overt mycosis in parenthesis)**

Discussion

Overall the most prevalent fungi collected directly from the Scarabaeidae were *M. anisopliae* (59%) and *B. bassiana* (35%). Likewise, a total of 36 *Metarhizium* and five *Beauveria* cultures were established from the soil-trapped cadavers received. Notably, 74% of all *Beauveria* isolates originated from the South African material, whereas cadavers from the other countries were infected primarily by *Metarhizium*. The high prevalence of *Beauveria* in South African soils was also noted during soil-trapping surveys reported by Hatting (2004) and Goble (2009). Three strains of *Metarhizium* (SASRI M4A, MSIRI 93, MSIRI 98) and one *Beauveria* (SASRI 1363) showed superior virulence against the coleopteran substitute *T. molitor*. Additional assays continue at ARC-SGI in an attempt to identify the most virulent strains for potential development as bio-insecticide/s.

Although a large number of bacteria (557) were isolated, the entomopathogenic status of these isolates remains unknown. Further investigation is warranted, given the successful commercialisation of species such as *Bacillus popilliae, B. thuringiensis* (subsp. *japonensis* strain *buibui*) and *Serratia entomophila* in Europe, North America and/or New Zealand (Copping, 2009).

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