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RESEARCH ARTICLE

# Virulence of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* to sweet potato weevil *Cylas puncticollis* and effects on fecundity and egg viability

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## Abstract

The virulence of eight isolates of *Metarhizium anisopliae* and four isolates of *Beauveria bassiana* (Ascomycota: Hypocreales) to adult *Cylas puncticollis* (Coleoptera: Curculionidae) was tested in the laboratory. Insects were sprayed with a standard concentration of $1.0 \times 10^7$ conidia $mL^{-1}$ using Burgerjon's spray tower. All the isolates tested were pathogenic to *C. puncticollis*. Mortality varied between 77.5% and 84.2% with isolates of *B. bassiana* and between 62.5% and 89.2% with isolates of *M. anisopliae*, 26 days post-treatment. The lethal time to 50% mortality for the 12 isolates varied between 9.7 and 18.5 days. Four isolates, *M. anisopliae* International Centre of Insect Physiology and Ecology (ICIPE) 18 and ICIPE 62 and *B. bassiana* ICIPE 275 and ICIPE 114 were selected for dose–response mortality [lethal concentration to 50% mortality ($LC_{50}$)] bioassays. Five concentrations ($1.0 \times 10^6$, $3.0 \times 10^6$, $1.0 \times 10^7$, $3.0 \times 10^7$ and $1.0 \times 10^8$ conidia $mL^{-1}$) of both fungal species were used. *B. bassiana* ICIPE 275 was the most active isolate with $LC_{50}$ value of $0.7 \times 10^6$ conidia $mL^{-1}$. The effect of fungal infection on feeding, fecundity and egg viability of *C. puncticollis* adult females was also investigated under laboratory conditions. *M. anisopliae* isolate ICIPE 18 and *B. bassiana* isolate ICIPE 114 were tested for feeding experiment using six concentrations (0, $1.0 \times 10^6$, $3.0 \times 10^6$, $1.0 \times 10^7$, $3.0 \times 10^7$ and $1.0 \times 10^8$ conidia $mL^{-1}$). For reproduction potential (fecundity and egg viability) bioassays, five concentrations (0, $1.0 \times 10^6$, $3.0 \times 10^6$, $1.0 \times 10^7$ and $3.0 \times 10^7$ conidia $mL^{-1}$) of *M. anisopliae* isolate ICIPE 18 were used. Adult sweet potato weevils (SPWs) treated with *M. anisopliae* at the concentrations of $3.0 \times 10^7$ and $1.0 \times 10^8$ conidia $mL^{-1}$ consumed significantly less food than weevils in the control and *B. bassiana* treatments at all the concentrations, except at the higher concentration of $1.0 \times 10^8$ conidia $mL^{-1}$, 14 days post-treatment. Female weevils in the control treatments laid more eggs than fungus-treated females. Percentage egg viability differences between controls and fungus treatments were significant at all the concentrations tested, 10 days post-treatment. These results show that *B. bassiana* and *M. anisopliae* are pathogenic to SPWs and infection can reduce feeding, fecundity and egg viability.

## Introduction

The sweet potato weevil (SPW), *Cylas puncticollis* Boheman (Coleoptera: Curculionidae), is a major constraint to sweet potato, *Ipomoea batatas* (Lam.), production and utilisation in tropical Africa (Wolfe, 1991; Mutuura *et al.*, 1992). In East Africa, sweet potato is mostly grown as a subsistence crop by resource-poor female farmers who do not use agricultural inputs. Yield losses of up to 73% have been reported in Uganda as result of direct

infestation of SPW and diseases associated with weevil attack (Smit, 1997). Some of integrated SPW management techniques include host plant resistance, cultural and chemical control and mass trapping using sex pheromones (Smit & Odongo, 1997). Although some varieties are less susceptible than others to SPW, no reliable source of resistance has been identified. Suitable cultural control practices are site specific and depend on agro-ecological and socio-economic conditions and therefore cannot be generalised. Because SPW spends most of its life cycle underground, postplant application of synthetic chemical insecticide requires frequent applications to kill newly emerged adults. This approach is not cost-effective for subsistence farmers. Dipping cuttings in chemical insecticide before planting is effective but the synthetic chemical insecticides involved are highly toxic (Smit, 1997). A male sex pheromone for African *Cylas* spp. has been identified and developed for use in traps for mass trapping in field conditions (Smit & Odongo, 1997). Biological control using predators and parasitoids has not been considered because of the difficulty for the parasites to locate the target insect, whose most of the life cycle is underground inside the tuber. Entomopathogenic fungi, bacteria and nematodes, which are soil dwellers, offer greater potential as biological control agents of *Cylas* spp. However, among the pathogens, entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Ascomycota: Hypocreales) are the predominant fungal species isolated from *Cylas* spp. (Castineiras *et al.*, 1982; Allard, 1990; Allard & Rangi, 1995). In Rwanda, Burundi, Uganda and Kenya, *B. bassiana* is the predominant species infecting *Cylas* spp. (Allard & Rangi, 1995). In Taiwan, an isolate of *B. bassiana* naturally occurring in soils was reported to be very virulent to *C. formicarius* (Su *et al.*, 1988). Despite the association of entomopathogenic fungi–SPW, no attempt has been made to use them for control of *C. puncticollis*. In Cuba for instance, *B. bassiana* is used as a component of SPW Integrated Pest Management (Castellon *et al.*, 1993; CIP, 1995; Alcazar, *et al.*, 1997). Several products based on *B. bassiana* and *M. anisopliae* are available for managing adult coleopteran insect species such as coffee berry borer *Hypothenemus hampei* Ferrary, *Otiorrhynchus sulcatus* (F.) and Colorado potato beetle (CPB) *Leptinotarsa decemlineata* (Say) (Adane *et al.*, 1996; de la Rosa *et al.*, 1997; Rice & Cogburn, 1999). However, *B. bassiana* and *M. anisopliae* have not been investigated for the development of a biocontrol agent against SPW. Mitosporic entomopathogenic fungi have worldwide distribution, have a good safety record towards non-target organisms (Hokkanen & Lynch, 1995) and can be mass produced using low-input technology (Jackson *et al.*, 2000).

Infection by fungal pathogen can result in reduced feeding in insect pests (Fargues & Rodriguez-Rueda, 1980; Mohamed *et al.*, 1982; Thorvilson *et al.*, 1985; Moore *et al.*, 1992; Seyoum *et al.*, 1994; Thomas *et al.*, 1997; Ekesi & Maniania, 2000; Ekesi, 2001) and fecundity (Fargues *et al.*, 1991; Castillo *et al.*, 2000). We report here the results of a laboratory study to evaluate the virulence of isolates of *M. anisopliae* and *B. bassiana* to *C. puncticollis* adults to select candidate isolate(s) that could be considered as microbial control agents for this pest. Adult is the most vulnerable stage to target because the insect spends most of the life cycle as larvae inside the tuber. We also investigate the effect of fungal infection on feeding, fecundity and egg viability of *C. puncticollis.* Studying the effect of fungal infection on food consumption, fecundity and subsequent egg viability could provide relevant information for the management of SPW.

## Materials and methods

### Insect

Adult *C. puncticollis* was obtained from a colony maintained in the laboratory at the ICIPE, Nairobi, Kenya. The initial stock culture was obtained in 2004 from infested potato tubers in farmers' fields in Mwea Irrigation Scheme, Kenya. The weevils were reared on sweet potato var. 'yellow fleshed', highly susceptible to the pest and maintained at room temperature [23–30°C and 40–70% relative humidity (r.h.)] under a photoperiod of 12L : 12D. Fresh potato tubers were regularly supplied to the weevils. Adults of 2- to 3-weeks of both sexes were used in the experiments.

### Fungi

All the fungal isolates used in this study were obtained from the ICIPE's Arthropod Germplasm Centre, Arthropod Pathology Unit (Table 1). The fungus was grown on Sabouraud dextrose agar (SDA) medium at 26 ± 2°C under natural light. Conidia were harvested by surface scraping 21-day-old culture plates. Inocula were suspended in 10-mL sterile distilled water containing 0.05% Triton X-100 in universal bottles containing 3-mm glass beads. Conidial suspensions were vortexed for 5 min to produce a homogeneous suspension. Spore concentrations were quantified with a bright line haemocytometer. Serial dilutions were prepared to obtain the desired concentrations. Viability of conidia was determined before each bioassay by spread plating 0.1 mL of conidial suspension titrated at $3.0 \times 10^6$ conidia $mL^{-1}$ on SDA plates. Sterile microscope cover slips were placed on each plate and plates were incubated at 26 ± 2°C and


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**Table 1** Fungal isolates used against *Cylas puncticollis* and their viability

| Species/ Isolate | Origin of Isolates | Locality/Country | % Germination ± SE |
|---|---|---|---|
| *Metarhizium anisopliae* | | | |
| ICIPE 18 | Soil | Mbita, Kenya | 95.3 ± 1.3ab |
| ICIPE 20 | Soil | Migori, Kenya | 91.9 ± 2.0bc |
| ICIPE 30 | *Busseola fusca* (Lepidoptera) | Migori, Kenya | 88.3 ± 2.1cd |
| ICIPE 62 | Soil | Kinshasa, DRC | 92.7 ± 1.5bc |
| ICIPE 21 | *Schistocerca gregaria* (Orthoptera) | Port Sudan, Sudan | 88.6 ± 1.7cd |
| ICIPE 7 | Soil | Matete, DRC | 84.9 ± 1.8d |
| ICIPE 45 | Kales | JKUCAT, Kenya | 85.1 ± 1.4d |
| ICIPE 56 | Tree | Nairobi, Kenya | 84.5 ± 2.0d |
| *Beauveria bassiana* | | | |
| ICIPE 114 | White grub | Kericho, Kenya | 89.3 ± 2.8cd |
| ICIPE 275 | Soil | Homa Bay, Kenya | 98.8 ± 0.6a |
| ICIPE 59 | Soil | Busia, Kenya | 89.1 ± 2.2cd |
| ICIPE 51 | Soil | Mombasa, Kenya | 91.3 ± 3.3bc |

DRC, Democratic Republic of Congo; ICIPE, International Centre of Insect Physiology and Ecology; JKUCAT, Jomo Kenyatta University College of Agriculture and Technology.

examined after 15–18 h. Percentage germination was determined from 100 spore counts at ×40 magnification. Each plate was replicated four times.

### Treatments

A standard concentration of $1.0 \times 10^7$ conidia $mL^{-1}$ was used for the screening of all the fungal isolates. Ten millilitres of conidial suspension was directly sprayed on adult SPW using Burgerjon's (1956) spray tower (INRA, Dijon, France). The tower was fitted with an air-atomising nozzle connected to a regulator valve providing a constant airflow under 4 bar pressure, resulting to a deposit of approximately $3.8 \times 10^6$ conidia $cm^2$. Weevils in control treatments were sprayed with sterile distilled water containing 0.05% Triton X-100. Test insects were transferred to cages (20 × 15 × 15 cm) containing fresh sweet potato tubers that served as food. Treatments were arranged in complete randomised blocks and consisted of 20 weevils (mixed sex) per replicate and the experiment was replicated six times. Test insects were maintained at room conditions (23–30°C and 40–70% r.h.). Mortality was recorded every 2 days for 26 days. Fresh tubers were supplied every 4 days. Dead weevils were surface sterilised in 1% sodium hypochlorite and 70% alcohol solutions for 30 s, respectively, and rinsed three times in sterile distilled water. They were then transferred to Petri dishes lined with moistened filter paper, sealed with Parafilm and maintained in an incubator at 26 ± 2°C to allow the growth of the fungus on the cadaver. Mycosis was confirmed by microscopic examination of the hyphae and spores on the surface of the cadaver. Based on the results of mortality, lethal time mortality values and optimal sporulation of fungal isolates on the cadavers, four fungal isolates were selected for lethal-concentration-response experiments in which five concentrations were used: $1.0 \times 10^6$, $3.0 \times 10^6$, $1.0 \times 10^7$, $3.0 \times 10^7$ and $1.0 \times 10^8$ conidia $mL^{-1}$. All the experimental procedure remained the same as described earlier, except that mortality was recorded for 14 days instead of 26 days.

### Effect of fungal infection on feeding by *Cylas puncticollis*

To study the effect of fungal infection on feeding, one isolate with the lower lethal concentration to 90% mortality ($LC_{90}$) value (*M. anisopliae* isolate ICIPE 18) was compared with another isolate with higher $LC_{90}$ value (*B. bassiana* isolate ICIPE 114). Five conidial concentrations of $1.0 \times 10^6$, $3.0 \times 10^6$, $1.0 \times 10^7$, $3.0 \times 10^7$ and $1.0 \times 10^8$ conidia $mL^{-1}$ were used. The fungal suspensions were sprayed using the method described above. Weevils in control treatments were sprayed with sterile distilled water containing 0.05% Triton X-100. Test insects were transferred to plastic containers (16 × 10 × 6 cm) containing sweet potato tubers previously washed in a solution of 1% of sodium hypochlorite for 2–3 min, rinsed three times in sterile distilled water and dried in the oven for 5 min at 40°C. Insects were allowed to feed on treated potato tubers for the whole experimental period (14 days) after treatment. Insects were maintained in an incubator at 26 ± 2°C and 60–70% r.h. Treatments were complete randomised blocks and consisted of 20 weevils (mixed sex) and the experiment was replicated six times. To determine food consumption by weevils, sweet potato tubers were weighed before they were given to insects. The fresh weight of uneaten sweet potato tubers was recorded after feeding (14 days postinfection). Tubers were then dried in an oven at 80°C for 120 h and the dry weight taken. Correction factors for weight loss because of moisture content changes were derived from sweet potato tubers that were not exposed to insect feeding. The difference between the initial and the final dry weights of the tubers was used to calculate the feeding by the adult weevils as: percent dry matter = weight of the tuber after 120 h/initial weight of the tuber × 100 and percent dry food eaten = percent dry matter × (initial weight of the tuber − final weight of the tuber)/100 (Schroeder, 2004).

### Effect of fungal infection on fecundity and viability of eggs of *Cylas puncticollis*

The results of the experiment on fungal infection on feeding indicated that *M. anisopliae* isolate ICIPE 18 could

reduce feeding of SPW at all the concentrations and was therefore selected for this study. In addition, this isolate has been reported to be virulent to the mango seed weevil, *Sternochetus mangiferae* (Fab.) (S. Ekesi, unpubl. data). Fungal suspensions at concentration of $1.0 \times 10^6$, $3.0 \times 10^6$, $1.0 \times 10^7$ and $3.0 \times 10^7$ conidia $mL^{-1}$ were directly sprayed on 2- to 3-week-old adult SPW (five females and five males) using Burgerjon's spray tower as described earlier. Control weevils also consisted of five females and five males and were sprayed with sterile distilled water containing 0.05% Triton X-100. The experiment was replicated six times. Insects were placed into plastic containers (16 × 10 × 6 cm) containing sweet potato tubers (approximately 80 g) and maintained in incubator at 26 ± 2°C and 60–70% r.h. for 10 days. Tubers were removed every 2 days and replaced with new ones. The periderm of the tuber was gently peeled with a razor blade without removing it and the number of eggs recorded. The periderm was then returned back to the original position. The tubers were then placed in incubator for 5–8 days to allow eggs to hatch and were thereafter dissected to record the number of larvae.

### Statistical analysis

Percentage mortality data were arcsine transformed to normalise the data (Gomez & Gomez, 1984) after correcting for natural mortality (Abbott, 1925); angular values were then subjected to analysis of variance using the ANOVA procedure of SAS (SAS Institute, 1990). Means were separated by Student–Newman–Keuls (SNK) test at ($P = 0.05$). Lethal time and lethal concentration to 50% mortality ($LT_{50}$ and $LC_{50}$) and $LC_{90}$ mortality were estimated with repeated measures logistic regression using generalised estimating equations (Stokes *et al.*, 2000). All analyses were carried out using GENMOD procedure of SAS (SAS Institute, 1999–2001). A computerised general linear model for repeated measures was used to compare mean dry food weight eaten, fecundity and percent egg viability between infected and control adults, respectively, and their mean were separated across concentrations with SNK ($P = 0.05$) test using the ANOVA procedure of SAS (SAS Institute, 1999–2001).

## Results

### Pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* to adult *Cylas puncticollis*

In viability tests, germination of conidia ranged from 84.5% to 98.8% after 15–18 h (Table 1). Mortality in the controls was 16.7%. At standard concentration of $1.0 \times 10^7$ conidia $mL^{-1}$, both fungal species were pathogenic to adult *C. puncticollis*, causing mortality between 62.5% and 89.2% with *M. anisopliae* and between 77.5% and 84.2% with *B. bassiana* (Table 2). There was, however, significant difference between fungal isolates ($F = 23.3$; d.f. = 12,5; $P < 0.0001$). The $LT_{50}$ values ranged from 9.7 to 18.9 days with *M. anisopliae* isolates and from 12.6 to 17.6 days with *B. bassiana* isolates (Table 2). Slopes of the regression equation ranged from 1.24 to 2.08 and from1.98 to 2.46 for *M. anisopliae* and *B. bassiana* isolates, respectively (Table 2). Based on $LT_{50}$ values and optimal sporulation (visual observation) on the cadaver, *M. anisopliae* isolates ICIPE 18 and ICIPE 62 and *B. bassiana* isolates ICIPE 275 and ICIPE 114 were selected for dose-response mortality bioassays. Among the four isolates tested, *B. bassiana* isolate ICIPE 275 had the lowest $LC_{50}$ and $LC_{90}$ values of $0.7 \times 10^6$ and $3.3 \times 10^7$ conidia $mL^{-1}$, respectively. *M. anisopliae* isolate ICIPE 18 had $LC_{90}$ values of $3.8 \times 10^7$ conidia $mL^{-1}$ (Table 3).

### Effect of fungal infection by *Beauveria bassiana* and *Metarhizium anisopliae* on adult *Cylas puncticollis* feeding, fecundity and egg viability

Adult *C. puncticollis* treated with *M. anisopliae* isolate ICIPE 18 at all the concentrations and *B. bassiana* at the concentration of $1.0 \times 10^8$ conidia $mL^{-1}$ consumed significantly less food than weevils in the control ($F = 3.48$; d.f. = 5,10; $P = 0.0001$) at 14 days post-treatment

**Table 2** Virulence of *Metarhizium anisopliae* and *Beauveria bassiana* isolates against *Cylas puncticollis*. Percent mortality and $LT_{50}$ values[a]

| Fungal Isolate | % Mortality (±SE)[b] | $LT_{50}$ (days) (95% Fiducial Limits) | Slope (±SE) | $\chi^2$-test[c] |
|---|---|---|---|---|
| Control | 16.7 ± 1.1e | | | |
| *M. anisopliae* | | | | |
| ICIPE 62 | 89.2 ± 1.5a | 9.7 (9.1–10.2) | 2.07 ± 0.07 | 1096.1 |
| ICIPE 21 | 85.0 ± 1.3abc | 10.9 (10.3–11.5) | 1.86 ± 0.03 | 1071.1 |
| ICIPE 7 | 74.2 ± 3.0c | 14.2 (11.8–17.2) | 2.00 ± 0.09 | 1123.0 |
| ICIPE 18 | 85.8 ± 2.0ab | 10.9 (10.2–11.6) | 2.08 ± 0.12 | 1183.3 |
| ICIPE 20 | 80.0 ± 2.9abc | 13.4 (11.8–15.2) | 1.73 ± 0.08 | 1018.6 |
| ICIPE 30 | 75.8 ± 1.5bc | 12.1 (10.9–13.5) | 1.63 ± 0.07 | 860.3 |
| ICIPE 45 | 80.0 ± 5.2abc | 12.1 (10.7–13.7) | 1.84 ± 0.18 | 1015.6 |
| ICIPE 59 | 62.5 ± 5.0d | 18.5 (10.9–31.3) | 1.24 ± 0.05 | 664.6 |
| *B. bassiana* | | | | |
| ICIPE 56 | 79.2 ± 2.4abc | 17.1 (15.8–18.6) | 2.46 ± 0.05 | 1278.0 |
| ICIPE 114 | 84.2 ± 5.4abc | 12.5 (11.7–13.3) | 2.02 ± 0.24 | 1170.5 |
| ICIPE 275 | 80.8 ± 3.0abc | 13.0 (12.0–14.0) | 1.98 ± 0.14 | 1133.8 |
| ICIPE 51 | 77.5 ± 7.9bc | 16.4 (11.8–22.8) | 2.10 ± 0.22 | 1177.4 |

ICIPE, International Centre of Insect Physiology and Ecology.
[a]Means within columns followed by the same letters are not significantly different by Student–Newman–Keuls test at $P = 0.0001$. Means were arcsine transformed before analysis but values represent untransformed data.
[b]Mean of six replicates of 20 insects.
[c]d.f. = 1.


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**Table 3** Lethal concentration values of selected isolates of *Metarhizium anisopliae* and *Beauveria bassiana* against *Cylas puncticollis*[a]

| Fungal Isolate | $LC_{50}$ (95% Fiducial Limits) | $LC_{90}$ (95% Fiducial Limits) | Slope | $\chi^2$-test[b] |
|---|---|---|---|---|
| *M. anisopliae* | | | | |
| ICIPE 62 | $1.9 \times 10^6$, (1.5–2.4) $10^6$ | $7.1 \times 10^7$, (5.4–9.4) $10^7$ | 0.60 ± 0.04 | 355.9 |
| ICIPE 18 | $2.7 \times 10^6$, (1.9–3.8) $10^6$ | $3.8 \times 10^7$, (2.2–6.4) $10^7$ | 0.84 ± 0.04 | 590.7 |
| *B. bassiana* | | | | |
| ICIPE 114 | $2.3 \times 10^6$, (1.9–2.7) $10^6$ | $9.4 \times 10^7$, (5.8–15.3) $10^7$ | 0.59 ± 0.03 | 366.6 |
| ICIPE 275 | $0.7 \times 10^6$, (0.5–1.0) $10^6$ | $3.3 \times 10^7$, (1.6–6.9) $10^7$ | 0.57 ± 0.04 | 246.4 |

ICIPE, International Centre of Insect Physiology and Ecology; $LC_{90}$, lethal concentration to 90% mortality.
[a]Six replicates/treatment of 20 insects.
[b]d.f. = 1.

(Table 4). This difference may be explained by the fact that *B. bassiana* had higher $LC_{90}$ value than *M. anisopliae*.

Female weevils in the control treatments laid more eggs than fungus-treated females ($F = 60.1$; d.f. = 4,16; $P = 0.0001$). However, the number of eggs laid by the weevils treated at concentration of $3.0 \times 10^7$ conidia $mL^{-1}$ was generally lower than the one treated at the lowest concentration of $1.0 \times 10^6$ conidia $mL^{-1}$ at all days, except on the 4-day post-treatment (Table 5). The number of eggs decreased after 10 days post-treatment in all the treatments including the control (Table 5). There was a significant difference in percent egg viability between eggs laid by untreated and fungus-treated female weevils at all the concentrations ($F = 42.0$; d.f. = 4,16; $P = 0.001$). For example, 97.5% of eggs hatched in the controls and 71.5% at fungal concentration of $3.0 \times 10^7$ conidia $mL^{-1}$.

## Discussion

All the fungal isolates tested were pathogenic to adult *C. puncticollis*; however, there was variation in the virulence between the fungal isolates. Such variations have already been reported with different host species including coleopterans (Ferron *et al.*, 1975; Poprawski *et al.*, 1985; Ansari *et al.*, 2004; Mura *et al.*, 2006). This emphasises the need for strain selection (Soper & Ward, 1981). *M. anisopliae* and *B. bassiana* are ubiquitous pathogens recorded on many hosts (Veen, 1968) and can be, therefore, tested against insects that are not associated with them in nature and developed as biopesticides.

Feeding by adult *C. puncticollis* generally decreased following fungal infection, particularly with *M. anisopliae* and at some extent with *B. bassiana* at the higher concentration. Similar results have been reported by other workers on coleopteran insects. Ekesi (2001) reported a reduction in food intake by *Ootheca mutabilis* (Shalberg) following infection by *M. anisopliae* and *B. bassiana*. Fargues *et al.* (1994) found that CPB infected with *B. bassiana* reduced their food intake 3 days after treatment. The reduction in feeding in insects has been attributed to production of toxic substances by fungi and/or mechanical disruption of the insect structural integrity by hyphal growth. This reduction could partly offset the relatively slow speed of kill by fungal pathogens compared with conventional insecticides and may play an important role in field control.

Infection of adult female SPW by *M. anisopliae* significantly reduced their fecundity. Fargues *et al.* (1991) reported a reduction in fecundity of CPB treated with *B. bassiana*. Sikura *et al.* (1972) reported that CPB surviving infection by *B. bassiana* as larvae induced histological and cytological injuries to the ovaries, thus preventing follicle development or causing their degeneration and thereby reducing fecundity. Egg viability was dose dependent with the highest concentration of $3.0 \times 10^7$ conidia $mL^{-1}$ producing the lowest viability. Similar results have been reported on legume flower thrips, *Megalurothrips sjostedti* (Trybom), following infection with *M. anisopliae* (Ekesi & Maniania, 2000). However, Fargues *et al.* (1991) found no difference between fertility of eggs laid by untreated and fungus-treated females of CPB.

**Table 4** Effects of fungal infection by *Beauveria bassiana* and *Metarhizium anisopliae* on feeding of adult *Cylas puncticollis*[a]

| Treatment | Fungal Concentration (conidia $mL^{-1}$) | Mean Tuber Dry Weight (g ± SE) |
|---|---|---|
| Control | 0 | 15.6 ± 5a |
| *B. bassiana* ICIPE 114 | $1.0 \times 10^6$ | 13.3 ± 2.0ab |
| | $3.0 \times 10^6$ | 11.5 ± 1.8abc |
| | $1.0 \times 10^7$ | 11.0 ± 1.34abc |
| | $3.0 \times 10^7$ | 11.1 ± 1.4abc |
| | $1.0 \times 10^8$ | 6.9 ± 1.1cd |
| *M. anisopliae* ICIPE 18 | $1.0 \times 10^6$ | 9.5 ± 1.5bcd |
| | $3.0 \times 10^6$ | 9.4 ± 0.8bcd |
| | $1.0 \times 10^7$ | 9.0 ± 1.8bcd |
| | $3.0 \times 10^7$ | 5.7 ± 3.0d |
| | $1.0 \times 10^8$ | 5.2 ± 1.9d |

ICIPE, International Centre of Insect Physiology and Ecology.
[a]Six replicates/treatment of 20 insects. Means within columns followed by the same letters are not significantly different by Student–Newman–Keuls test at $P = 0.001$.

**Table 5** Effect of infection by *Metarhizium anisopliae* isolate International Centre of Insect Physiology and Ecology 18 on fecundity of adults *Cylas puncticollis*. Mean number of eggs (X ± SE) per five females at different time intervals[a]

| Fungal Concentration (conidia $mL^{-1}$) | Days After Treatment | | | | |
|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | 10 |
| Control | 20.7 ± 1.2a | 21.0 ± 1.1a | 22.0 ± 1.2a | 22.5 ± 1.0a | 16.3 ± 1.5a |
| $1.0 \times 10^6$ | 18.0 ± 0.6b | 16.7 ± 1.3b | 15.0 ± 0.5b | 15.3 ± 1.1b | 9.2 ± 0.7b |
| $3.0 \times 10^6$ | 15.7 ± 1.2b | 14.5 ± 1.1b | 13.8 ± 1.0bc | 12.0 ± 0.7bc | 7.5 ± 1.1b |
| $1.0 \times 10^7$ | 14.2 ± 0.6c | 14.7 ± 0.7b | 13.5 ± 1.0bc | 11.8 ± 1.1bc | 7.5 ± 0.4b |
| $3.0 \times 10^7$ | 13.8 ± 0.6c | 15.0 ± 1.0b | 12.2 ± 0.3c | 11.2 ± 0.5c | 4.7 ± 1.0c |

[a]Means within columns followed by the same letters are not significantly different by Student–Newman–Keuls test at $P = 0.0001$, X, mean of six replicates of 20 insects.

Entomopathogenic fungi could have great potential for control of SPW considering the aggregating behaviour of this insect. Fungus-infected insects could spread the pathogen among healthy insects and thereby initiate epizootics. In addition, the sex pheromone of SPW could be integrated in an autodissemination device whereby insects that are attracted to the system are contaminated with fungus before they return to the environment where they can contaminate other insects (Vega *et al*., 2000). An attractant trap for autodissemination of an entomopathogenic fungus into insect populations has been developed and tested in the field against the Japanese beetle, *Popillia japonica* Newman (Klein & Lacey, 1999). Finally, because split sweet potato tubers are attractive to weevils, they could be used as fungus-contaminated bait. A similar approach has been developed for banana weevil, *Cosmopolites sordidus* Germar (Nankinga & Moore, 2000).

In conclusion, this study has provided useful baseline data for strain selection against adult *C. puncticollis*. However, further work is needed to test the potential of the isolates, especially *M. anisopliae* ICIPE 18, as a biological control agent for SPW in the field.

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