Beauveria and *Metarhizium* against false codling moth (Lepidoptera: Tortricidae): A step towards selecting isolates for potential development of a mycoinsecticide

C.A. Coombes[1*], M.P. Hill[1], S.D. Moore[1,2], J.F. Dames[3] & T. Fullard[1]

[1]*Department of Entomology and Zoology, Rhodes University, P.O. Box 94, Grahamstown, 6140 South Africa*
[2]*Citrus Research International, P.O. Box 20285, Humewood, Port Elizabeth, 6013 South Africa*
[3]*Department of Biochemistry and Microbiology, Rhodes University, P.O. Box 94, Grahamstown, 6140 South Africa*

False codling moth, *Thaumatotibia leucotreta* Meyrick (1912) (Lepidoptera: Tortricidae), can cause both pre- and post-harvest damage to citrus fruit. Not only can this result in reduced crop yield, but more importantly because of the moth's endemism to sub-Saharan Africa, it is classified as a phytosanitary pest by many export markets. An entire consignment of citrus may be rejected in the presence of a single moth (Moore 2012). Since the bulk of citrus fruit production in South Africa is exported, the control of *T. leucotreta* is critical (Citrus Growers Association, South Africa 2012). Traditionally, control has been achieved through the use of chemical insecticides; however, residue restrictions, resistance development and concerns about environmental pollution have substantially reduced the dependence on chemical pesticides in citrus. Research on *T. leucotreta* control has therefore focused on the use of biological organisms (*e.g.* parasitoids and viruses), which are used as control agents within an integrated pest management (IPM) programme in citrus. These biological control agents, however, only targeted the above-ground life stages of the pest, not the soil-dwelling life stages (late fifth instars, prepupae, pupae), which is the subject of this contribution (Moore 2012).

Entomopathogenic fungi, ubiquitous soil microbes, are currently under investigation as control agents for use against *T. leucotreta* in the soil. Various entomopathogenic fungal isolates are known to infect late fifth instar *T. leucotreta* which pupate within the soil (Begemann 2008; Goble *et al*. 2010; Goble *et al*. 2011). These fungi were previously isolated from soil samples collected from various citrus orchards around the Eastern Cape Province, South Africa (Goble *et al*. 2010). In total, 62 isolates were identified, of which 21 were screened against the pest by exposing fifth instars to soil inoculated with fungal conidia in laboratory bioassays. Of these, 12 isolates caused 80 % mortality of the *T. leucotreta* test population exposed. However, the lethal concentration required to kill 50 % of the population (LC_{50}) has been determined for only four of these isolates (Goble *et al*. 2011)

Since isolate selection is a key first step in biopesticide development (Ravensberg 2010), this study aimed to: (i) examine the dose-response relationship (LC_{50} and LC_{90}) of a further five untested isolates (Table 1), four *Metarhizium anisopliae* Sorokin (Metschnikoff) *s.l.* and two *Beauveria bassiana* Balsamo (Vuillemin) *s.l.* isolates, against late fifth instar *T. leucotreta*; (ii) to compare the results obtained in this study with the LC_{50} and LC_{90} of the four previously tested isolates (Table 1); and (iii) to compare the relative performance of these new isolates against those used in two commercially available fungal based mycoinsecticides, namely Eco-Bb® (*B. bassiana* strain R444) (Plant Health Products, South Africa) and *M. anisopliae* strain ICIPE 69 (Real IPM, Kenya).

Fungal cultures were maintained on Sabouraud dextrose agar (SDA) supplemented with 1 ml/l dodine, 50 mg/l chloramphenicol and 50 mg/l rifampicin. Only two- to three-week-old fungal cultures were used in the assays. Conidia were harvested using a sterile glass rod; conidia were gently scraped off the plate and suspended in 20 ml of distilled water containing 0.01 % Tween™ 20. Sterile glass beads were added to help break up hyphal aggregates and spore clumps and vortex mixed for approximately 3 min to obtain a homogenous suspension. Fungal concentration was determined using a haemocytometer and the appropriate experimental dilutions were prepared from the stock suspension. Viability was measured according to (Inglis *et al*. (2012) and was >95 % for all isolates. Three concentrations were tested; 1×10^4, 1×10^5, and 1×10^6 conidia/ml, which provided soil concentrations of 1×10^3, 1×10^4 and 1×10^5 conidia/g soil, respectively. For each isolate, and each concentration, four replicates were conducted. A control treatment was included. All treatments were replicated concurrently. *Thuama-*

*Author for correspondence. E-mail: candicecoombes@gmail.com