Potential of Entomopathogenic Fungal Isolates for Control of the Soil-Dwelling Life Stages of *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae) in Citrus

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Short communications

Potential of entomopathogenic fungal isolates for control of the soil-dwelling life stages of *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae) in citrus

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Thaumatotibia leucotreta Meyrick (Lepidoptera: Tortricidae) is a key pest of citrus in South Africa. In addition to the fruit damage caused, export markets such as the United States, South Korea and China regulate *T. leucotreta* as a phytosanitary organism in addition to restricting the use of pesticides on exported fruit (Grout & Moore 2015; SA-DAFF 2015). The bulk of citrus in South Africa is exported (Citrus Growers' Association 2015). Thus, the control of *T. leucotreta* is crucial. Consequently, the citrus industry adopts a zero tolerance approach controlling the pest, being strongly reliant on integrated pest management (Moore & Hattingh 2012). Numerous control options are available, but are largely limited to use against the above-ground life stages of this pest: eggs, neonates and adults (Moore & Hattingh 2012; Grout & Moore 2015).

Isolates of the entomopathogenic fungal species *Metarhizium anisopliae* (Metchnikoff) Sorokin (G 11 3 L6 and FCM Ar 23 B3, referred to as Ma1 and Ma2 respectively) and *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (G Ar 17 B3, referred to as Bb1) have been identified as capable of inducing greater than 80 % mycosis of late fifth instar *T. leucotreta* upon their exposure to soil inoculated with these fungal spores under controlled laboratory conditions at a concentration of 1×10^7 conidia/ml (Goble *et al.* 2010, 2011; Coombes *et al.* 2015). These isolates originated from soil samples collected from conventional citrus orchards and isolated using the insect baiting technique (Goble *et al.* 2010). The possibility of applying these isolates in the field to control the soil-dwelling life stages (wandering late instars, prepupae and pupae) of *T. leucotreta* as a complementary strategy to above-ground control options was suggested (Coombes *et al.* 2015). However, environmental factors (abiotic and biotic) in the field are complex and have the potential to hinder fungal efficacy (Inglis *et al.* 1997; Jaronski 2010; Foster *et al.* 2011).

A trial to assess the efficacy of these isolates against *T. leucotreta* late fifth instars using plastic cages (5-l containers, 20 × 20 × 30 cm, with breathable mesh inserts) was conducted in an organic 22-year-old Palmer Navel orange citrus orchard (Eastern Cape, South Africa) (33 37′S 25 40′E). Soil texture was classified as loam (16 % clay, 38 % silt and 46 % sand) with a soil pH of 7.7. Average soil temperature and moisture within the upper 10 cm soil surface was measured as 21.3 °C and 36.5 %, respectively. The trial was initiated on 13 March 2014 and terminated one month thereafter.

The efficacy of each isolate was determined at three concentrations [2.5×10^{13} (low), 5×10^{13} (intermediate) and 1×10^{14} (high) spores/ha] and in the presence of a lucerne hay mulch at the intermediate concentration. A mulch treatment was included, as the orchard was mulched regularly with sheep's wool and lucerne. Mulch was added to each respective cage as a thin complete-cover-layer prior to fungal application. Controls and a treatment applied with a commercially produced fungal formulation (Broadband®, a.i. *B. bassiana* strain PPRI 5339) (BASF, South Africa), were included. The trial design was a completely randomised design replicated eight times. Cages were buried in the upper soil layers underneath the canopy of citrus trees (on the south-facing side and 1 m from an irrigation sprinkler) and filled with the soil removed during hole-digging. Fungi, with the exception of Broadband®, were mass-produced as dry aerial conidia by Agrauxine (Loches, France) and applied as an aqueous suspension (water supplemented with 0.01 %

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Breakthru® S240 a.i. 100 % polyether modified trisiloxane) using a handheld 1-l spray applicator. Broadband® (obtained from BASF, South Africa) was applied according to the manufacturer's instructions at the recommended field rate for use in citrus crops. Each treatment replicate was treated with 125 ml of the appropriate fungal suspension. A separate applicator was used for each fungal treatment including the commercial treatment and controls to avoid cross contamination. Viability, assessed according to Goble *et al.* (2011), was above 90 % for all isolates including Broadband®.

Thirty *T. leucotreta* fifth instars, obtained from River Bioscience (Addo, South Africa) and reared on an artificial maize-based diet (Moore *et al.* 2014), were added to each cage 24 h prior to pupation. The cages were sealed with mesh and monitored weekly for the presence of eclosed adults. The experiment was terminated two weeks after eclosion was first noted. Cages were brought back to the laboratory and the number of eclosed adults was counted. Pupal casings, which generally remained intact, were also counted for each cage to limit the possibility of underestimation as a result of predation or disintegration of adults. The higher of the two counts was taken as the total number of eclosed *T. leucotreta* adults.

Treatment effects were determined *via* non-parametric Kruskal-Wallis ANOVA ($P < 0.05$) due to the non-normality of the data even after arcsine transformation. If significant treatment effects were found, a multiple comparison of mean ranks was performed ($P < 0.05$). Natural mortality was high in all cages including the control. Thus, Abbott's formula (Abbott 1925) was used to compute the corrected mortality due to the application of each respective treatment. It should be noted that overt mycosis was not measured as it was impossible to locate deceased cadavers after the trial was terminated (one month post-initiation). The high natural mortality was suspected to be a result of the presence of predators within the cages, such as ants and spiders. Nevertheless, four outcomes were apparent (Fig. 1).

Firstly, in accordance with previous laboratory bioassays (Goble *et al.* 2011; Coombes *et al.* 2015), results were dose-dependent. All isolates applied at the highest rate resulted in significant *T. leucotreta* mortality, above 90 % (χ^2 = 41.29, d.f. = 12, $P < 0.0001$). This is a high level of control, but the feasibility of applying spores at this rate to larger areas is impractical owing to the cost of conidial production. Fungal application at the lowest rate failed to induce mycosis greater than 35.0 %. Thus, application at the intermediate rate (5 ×

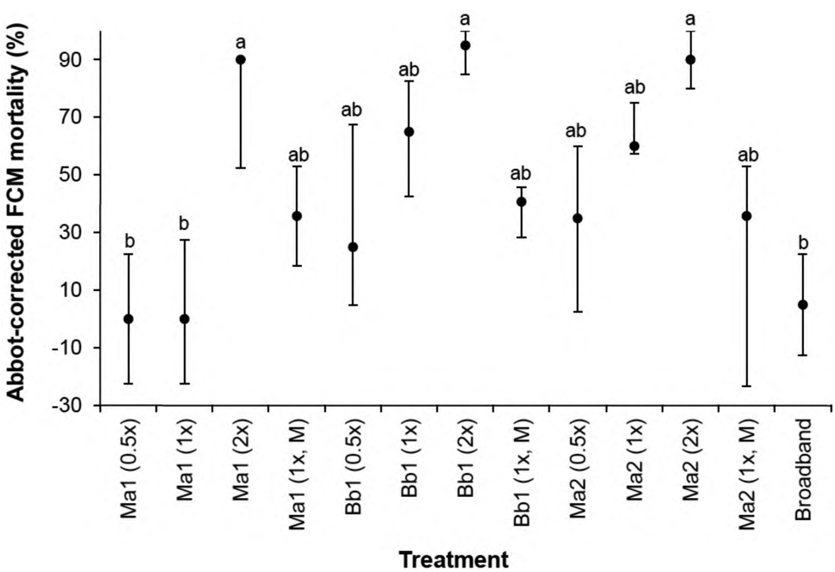

Fig. 1. Median percentage (of eight replicates) of Abbott-corrected *Thaumatotibia leucotreta* mortality, calculated for each treatment to which fungus was applied. Vertical bars denote the interquartile range. Letters indicate significantly different results (multiple comparison of mean ranks, $P < 0.05$). 0.5×, 1× and 2× refer to application rates equivalent to 2.5×10^{13}, 5×10^{13} and 1×10^{14} spores/ha, respectively. Treatments to which mulch was applied are represented by the letter M.

10^{13} spores/ha) appeared to be the most appropriate for future experimental field research. However, for commercialisation purposes, this rate is still likely too high to allow for economically feasible application (Mulock & Chandler 2000). As these isolates were chosen based on their extremely high level of virulence recorded in laboratory bioassays (>80 %), from a multitude of potential isolates (Goble *et al.* 2010, 2011; Coombes *et al.* 2015), bioprospecting is unlikely to identify more potent isolates. Formulation has been recorded to boost the efficacy of applied fungi (Vega-Aquino *et al.* 2010; Ekesi *et al.* 2011; Luz *et al.* 2016). Thus, it may be possible to synergise the virulence of these fungi through formulation permitting fungal application at a lower, more feasible rate whilst still maintaining the same level of efficacy.

Secondly, median percentage mortality at the intermediate rate was greater for isolates Bb1 and Ma2 than isolate Ma1. Corrected median mortality of 65 %, 60 % and 0 % was recorded, respectively. This questions the suitability of isolate Ma1 for field application. However, the failure of Ma1 to induce mortality may only be apparent under the conditions measured in this study. The variability in fungal efficacy amongst entomopathogenic fungal isolates exposed to differing environmental factors (*e.g.* moisture, temperature, pH and soil texture) is well documented (Padmavathi *et al.* 2003; Devi *et al.* 2005). Ekesi *et al.* (2003) evaluated the performance of four *M. anisopliae* isolates in inducing infection in puparia of *Ceratitis capitata.* Although mortality was higher in drier soil (–0.1 and –0.01 MPa) than wet soil (–0.0055 and –0.0035 MPa), under the latter conditions, two isolates were significantly more effective. Therefore, to conclude definitively that Ma1 is not suitable for field application is premature especially given that statistically, differences amongst treatments were limited due to a high level of variability amongst replicates.

Thirdly, Broadband® recorded a lower median mortality (5 %) than isolates Bb1 and Ma2 at the intermediate rate (Fig. 1). At the highest application rate, Broadband® median mortality was significantly lower than that recorded for all investigated fungal isolates. This supports the further research of these fungal isolates, specifically isolates Bb1 and Ma2, rather than simply using a currently commercially available product.

Lastly, mulching may influence fungal efficacy. Mulch cages treated with either Ma2 or Bb1 incurred median corrected mortalities of approximately 25 % less than that applied at the equivalent rate in the absence of mulch, whilst a higher median corrected mortality was recorded for Ma1 in the mulch treatment compared to the non-mulch treatment (Fig. 1). Mulches are typically used in agriculture to maintain soil moisture, prevent soil erosion and to promote soil productivity and plant growth (Li 2003; Ramakrishna *et al.* 2006). They may also increase biological activity and be used as a nutritive substrate on which fungi can sporulate (Brévault *et al.* 2007). Reasons for this observation were unclear, especially given the high variability in the data set. However, it is well known that entomopathogenic fungi can be adversely impacted by agricultural amendments and practices (Magara *et al.* 2003; Klingen & Haukeland 2006). As these isolates may be applied to citrus orchards in which mulch is used, these results suggest that compatibility should be established.

In conclusion, this study provides preliminary evidence for the potential use of these three laboratory-virulent isolates, particularly isolates Bb1 and Ma2, against *T. leucotreta* soil-dwelling life stages in the field.

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