Entomopathogenic fungi to control false codling moth in citrus orchards

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This article is a review of the research conducted on the use of entomopathogenic fungi (EPF) against false codling moth (FCM) soil-dwelling life stages in South African citrus orchards.

What are EPF and why use them?

EPF are common, soil-borne, micro-organisms that have been used extensively for the control of various agricultural pests globally. EPF are favourable control agents as they infect their hosts (target pests) via penetration of the cuticle, rather than ingestion, making them ideal for targeting insect life-stages which do not feed e.g. soil-dwelling life stages of FCM and sap-sucking insects such as mealybug, whitefly and scale insects. They also offer a more environmentally-safe alternative to chemical pesticides and have the potential to offer long-term pest control due to environmental recycling (Inglis et al. 2001; Shah & Pell 2003; Chandler 2017).

PHASE 1: EPF isolation and identification

EPF were isolated from soil samples collected from numerous citrus orchards and surrounding refugia in the Eastern Cape Province, South Africa. A total of 62 fungal strains were identified, mostly belonging to the two most common entomopathogenic fungal species, *Beauveria bassiana* and *Metarhizium anisopliae* (Goble et al. 2010).

PHASE 2: Identifying the most virulent strains

Of the 62 fungal strains identified, 21 were further screened for their pathogenicity and virulence towards the soil-dwelling life stages of FCM, specifically the late fifth instars, as they burrow into soil to pupate (Goble et al. 2011). Twelve of these caused FCM mortality of greater than 80% and were further investigated by Coombes et al. (2015). Based on dose-response bioassays, three of these strains: *Beauveria bassiana* G Ar 17 B3 (Bb1), *Metarhizium anisopliae* FCM Ar 23 B3 (Ma1) and *M. anisopliae* G 11 3 L6 (Ma2), were found to be the most virulent against FCM late fifth instars. Although these results were exceptionally positive, if these fungi are to be used in practice, it was imperative to determine whether they could perform as well in the field as they did in the laboratory, where conditions were optimal for infection, unlike conditions experienced in the field (Jaronski 2010). Thus, small-scale semi-field trials were initiated to establish the likelihood of good field performance.

PHASE 3: Semi-field performance: efficacy and persistence

Semi-field efficacy was assessed in an organic Navel orange orchard using plastic cages (5-l containers, 20×20×30 cm, with breathable material fine mesh inserts), which were partially buried in the soil underneath the canopy of the trees. The cages were filled with sand from the orchard and treated with each fungus (Bb1, Ma1, Ma2) at three concentrations (low: 2.5×10^{13}, intermediate: 5×10^{13} and high: 1×10^{14} conidia/ml). A control treatment, to which no fungus (only water and surfactant) was added, and the commercial fungal-based product, Broadband® (BASF, South Africa), was included. Thirty FCM fifth instars ready to pupate were introduced into each cage. The cages were sealed and checked one month thereafter for adult eclosion. All fungi recorded FCM mortality above 90% (corrected for control mortality) at this high application rate. This rate was concluded to be unfeasibly high for large-scale application. At the intermediate application rate, Bb1, followed by Ma1, were the most effective in reducing the number of eclosed FCM: 65% and 60%, respectively. Ma2 failed to cause FCM mortality, while Broadband® caused only 5% median corrected-mortality (Coombes et al. 2017).

All three fungi were also evaluated for their ability to persist within a citrus orchard. Persistence is considered an important factor in the success and failure of microbial pesticides and has implications for long-term pest control (Jackson 1999). Sterilised soil, housed in net bags, was inoculated with each fungus and buried in the upper soil surface underneath the canopy of citrus trees. For a period of six months, a representative bag for each fungus was removed monthly and the fungal density determined. All fungi were found to persist for the duration of the trial (Coombes et al. 2013).

The outcomes of these trials suggested that as soil treatments (1) all fungi were able to persist under field conditions, (2) Bb1 and Ma1 are more suited for field application, based on efficacy results, and (3) Bb1 and Ma1 were more effective than a currently available fungal-based commercial product (albeit not registered for this

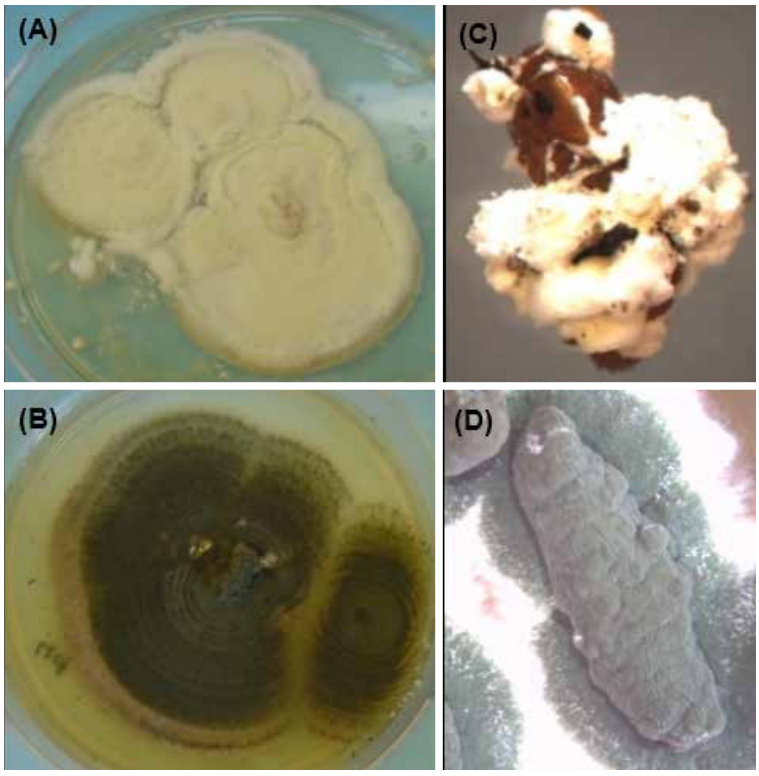

Figure 1: Bb1 (A) and Ma1 (B) on agar. Bb1 (C) and Ma1 (D) sporulating on FCM pupal cadavers after successful infection.

mode of application). This prompted large-scale field trials using the fungi Bb1 and Ma1 (Figure 1).

PHASE 4: Field performance: efficacy and persistence

To date, five field trials have been conducted. The results of two of these are presented (Coombes et al. 2016).

Field trials were initiated in a conventional citrus orchard in Sunday's River Valley (Eastern Cape Province). Field trial 1 occurred over the 2013/14 citrus growing season; field trial 2 (F2) over the 2014/15 citrus growing season in Navel orange blocks. The fungi were applied to the soil surface underneath the canopy of the trees using a spray machine and handguns (Figure 2), each to an area of approximately 1 ha in late October before a peak in FCM infestation. Fungi were applied at 5×101^3 conidia/ml, the rate at which the semi-field trials indicated were the most suitable (Coombes et al. 2017). Control blocks, where no fungus was applied, were included. Fruit drop surveys were conducted to determine whether the fungi were effective in reducing the FCM population according to Moore et al. 2015. Fruit were considered infested if FCM were found within the fruit or if characteristic features (tunneling and frass, Figure 3) of its presence were seen. The average number of FCM infested fruit was calculated (Coombes et al. 2016).

Figure 2: Fungi being sprayed on the soil surface underneath the canopy of the trees. (A) Bb1 and (B) Ma2 being added to the spray tank.

In field trial 1, Bb1 was found to maintain a high level of FCM suppression throughout the trial and reduced FCM infestation by 82% at the end of the trial, 30 weeks after the fungi were applied. In comparison, Ma1 only reduced FCM infestation by 28% (Figure 4a). In field trial 2, Ma1 was reported as more effective in reducing FCM infestation than Bb1. FCM infestation was reduced by 63% and 34%, respectively (Figure 4b) (Coombes et al. 2016).

During the course of these trials, soil samples were also taken to establish the persistence of the applied fungi. In field trial 1, persistence was greater and fungal density increased towards the end of the trial to a density similar to that immediately after application. This pattern was observed for both fungi and was suggested to be a result of FCM mortality from fungal infection, thus resulting in recycling of the infective fungal propagules in the soil environment. Unfortunately, fungal density assessed the next season suggested that applications each year would be necessary to maintain FCM control. In field trial 2, the persistence of Ma2 declined steadily, whereas Bb1 only increased slightly, but declined thereafter. The authors attributed this to the drier soil conditions experienced in this trial, suggesting that fungal recycling is dependent on the moisture of the soil. Nevertheless, both fungi were recovered at the end of the trials after only a single application at the start of the citrus growing season and always reduced FCM infestation (Coombes et al. 2016).

Conclusion

Both fungi, Bb1 and Ma1, were able to effectively reduce the level of FCM infestation following a single application to the soil surface at both field sites, with Bb1 performing very impressively in field trial 1. Discrepancies between field sites

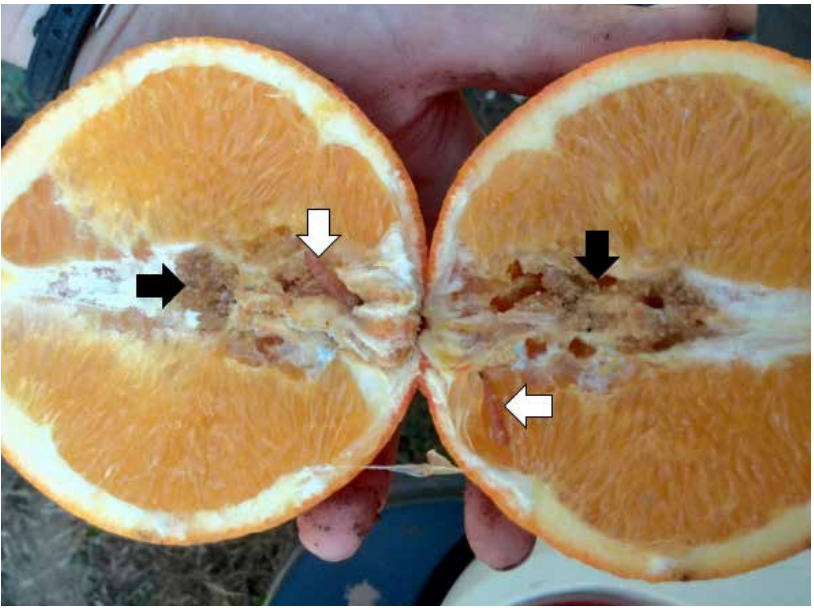

Figure 3: Dissected orange showing the presence of FCM (white arrows) and characteristic tunneling/frass (black arrows).

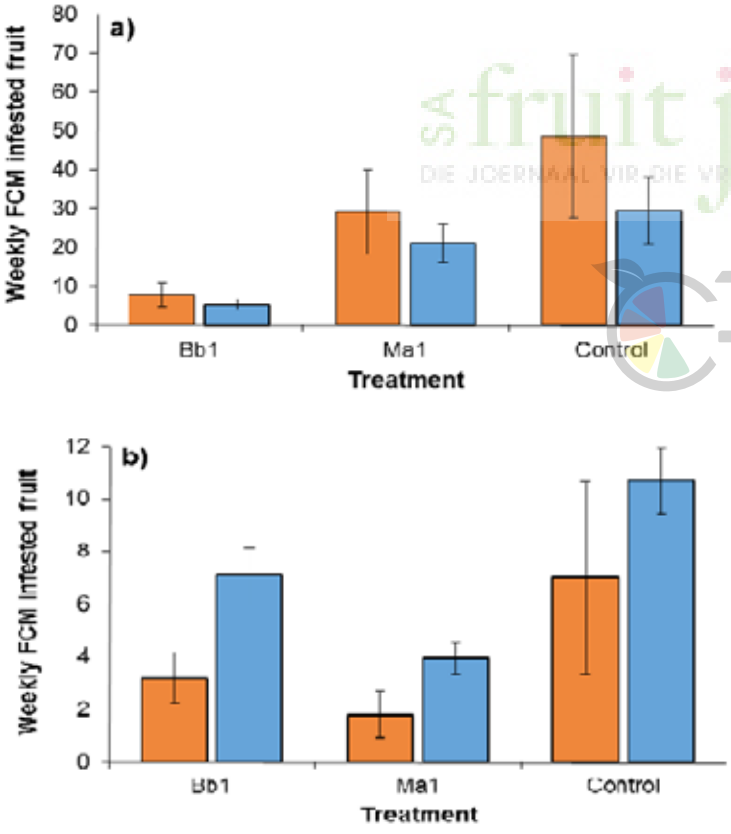

Figure 4: Mean (± SE) weekly FCM infested fruit (per 12 trees) recorded 15 (orange) and 30 (blue) weeks after fungal application, over a period of eight and 21 weeks respectively, at field trial 1.
(a) and over a period of five and 18 weeks, respectively at field trial 2
(b) Significant differences amongst treatments were found 30 weeks after treatment (Tukey's HSD test, P < 0.05), denoted by different letters (adapted from Coombes et al. 2016).

may suggest the level of expected variability of fungal efficacy in the field, a problem that can be addressed with formulation and a better understanding of the environmental factors which may negatively impact the performance of these fungi (Jaronski 2010; Chandler 2017). Thus, the future of these fungal entomopathogens in FCM control programmes is promising.

Opsomming

'n Opname wat in grond van sitrusboorde en aangrensende grond gedoen is het 62 verskillende isolate opgelewer, meestal van die spesies *Beauveria bassiana* en *Metarhizium anisopliae*. Laboratoriumproewe het die mees virulente isolate teen die grondwonende lewensstadiums van valskodlingmot (VKM) (volwasse larwes en papies) geïdentifiseer. Veldproewe in sitrusboorde met twee van die swamme het VKM vir die volle seisoen met tussen 28% en 82% onderdruk, met 'n enkele behandeling op die grond in die lente. Variasie in werking kan onder andere aan verskille in besproeingstegniek en grond vogtigheid toegeskryf word.

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