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Short Communication

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Short Communication

The infection efficacy of *Metarhizium* strains (Hypocreales: Clavicipitaceae) against the Queensland fruit fly *Bactrocera tryoni* (Diptera: Tephritidae)

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The Queensland fruit fly (Qfly), *Bactrocera tryoni* Froggatt, is a devastating pest of Australia's commercial fruit systems. Fruit fly mitigation is heavily centered around the use chemical insecticides, with limited investigation into microbial control alternatives. The wet tropics of northern Queensland is a highly biodiverse ecosystem containing many species of insect pathogenic fungi, but it is unclear whether any of these entomopathogens could contribute to Qfly management programs. In laboratory trials, we investigated the potential for Qfly microbial control by 3 locally sourced strains of entomopathogenic fungi comprising 2 species, *Metarhizium guizhouense* (Chen and Guo) and *Metarhizium lepidiotae* (Driver and Milner). Additionally, we evaluated 2 different inoculation methods to derive the most effective way to expose the flies to conidia—either through dry conidia or in a conidial suspension. All 3 strains were successful in causing Qfly mortality. *Metarhizium lepidiotae* resulted in the highest mean mortality over the trials, while *M. guizhouense* resulted in the highest mortality in a single replicate. Laboratory experiments revealed exposure through dry conidia to be the most effective method to inoculate the flies. These results suggest that fungal entomopathogens could be a viable pathway to Qfly suppression.

Key words: Metarhizium, fruit fly, biological control, pest

Introduction

Native to the tropical and sub-tropical north-eastern region of Australia, the Queensland fruit fly (Qfly), *Bactrocera tryoni* Froggatt is a key pest of Australian horticulture (Clarke et al. 2011). Larvae emerge from eggs beneath the fruit skin feed on the flesh, for up to 1 month, generally spoiling fruit before pupating in the topsoil (May 1958, Bateman 1968). Orchard level Qfly management typically includes organophosphate and neonicotinoid insecticides that currently are being phased out (Cremlyn 1978, Sproul et al. 2001, Hetherington 2005, Dodds et al. 2014), protein bait sprays, and male annihilation technique mass traps (Clarke et al. 2011, Vargas et al. 2015). These methods are often combined with other fruit fly management tactics of sterile insect technique and orchard sanitation (Allwood et al. 2002, Stonehouse et al. 2007, Vargas et al. 2015, Ekesi 2016). However, increasing chemical control restrictions, coupled with strict quarantine, and maximum residues limit regulations for market supply and exporting produce necessitates alternative control development. Qfly's tropical native range suggests co-occurrence with an immense reservoir of potential natural enemies that could act as biological control agents (Aung et al. 2008, McGuire and Northfield 2020). Here, we consider fungal entomopathogens as a prospect for Qfly management.

Pest control using fungal entomopathogens such as those belonging to the genus, *Metarhizium*, are commonly used in biological control regimes due to their potential efficacy in pest population suppression and long residual times when environmental conditions allow (Milner et al. 2003, Guerrero-Guerra et al. 2013). *Metarhizium* species are genetically diverse (Brunner-Mendoza et al. 2019), embody a cryptic phylogenetic species complex (Bischoff et al. 2009) and have different life-history strategies dependent on host availability and biotic and abiotic factors (Bidochka and Small 2005, Lovett and St. Leger 2015, McGuire and Northfield 2020). For example, when insect host availability is scarce, fungal species can exist in the soil profile (Rocha et al. 2013, Korosi et al. 2019),

grow endophytically in plants (Greenfield et al. 2016), and on leaf surfaces (Garrido-Jurado et al. 2015).

Here, we evaluated the potential for *Metarhizium* to cause mortality in Qfly. To our knowledge, Carswell et al. (1998) is the only prior study that has evaluated fungal entomopathogens against Qfly. A previous tropical Australian soil survey suggests commercial farms can support high *Metarhizium* prevalence (McGuire and Northfield, 2021), and the soil-dwelling Qfly pupal stage makes it a promising target species for these fungal pathogens. Three *Metarhizium* strains comprising 2 species, *Metarhizium guizhouense* and *Metarhizium lepidiotae*, were selected from soil on or surrounding agricultural farms in Far North Queensland, Australia within Qfly's native range. We conducted laboratory trials to evaluate the potential for aboveground control by infecting adult flies, with implications for soil application as well.

Methods and materials

The Qfly pupae obtained for this study were reared by the QLD Department of Agriculture fruit fly rearing facility, Cairns, primarily on carrot media containing dehydrated carrot granules, water, Nipagin (anti-fungal preservative), Torula yeast, and hydrochloric acid (33%). We fed adult flies sucrose, water, and yeast paste. Newly emerged flies were separated into their treatment replicates in groups of 20, contained in ventilated plastic containers (120 × 300 × 225 mm) and maintained at room temperature for 10 days prior to commencing the experiment and for its duration. Three *Metarhizium* strains were reared from soil samples from Ecoganic banana farms at South Johnstone (McGuire and Northfield 2021), Queensland, and maintained in petri-dishes (90 × 90 mm) on malt extract agar (MEA) at room temperature. The 3 *Metarhizium* strains evaluated were *M. guizhouense* (ARSEF:4303) (Mg1), *M. guizhouense* (ARSEF:7502) (Mg2), and *M. lepidiotae* (ARSEF:7412) (Ml3) in 2 different experiments. First, we evaluated the *Metarhizium* strains against Qfly under laboratory conditions over 2 trial periods (experiment 1). Second, we selected the isolate from experiment 1 resulting in highest mortality in a single replicate to evaluate the most effective inoculation method (experiment 2).

Laboratory trials: *Metarhizium* strain efficacy

The experiment was conducted twice with 4 replicate groups per treatment (Mg1, Mg2, Ml3, and control) each containing 20 flies. Qfly mortality was monitored and recorded at the same time each day by counting dead flies in each replicate until the trial ended after 14 days. Each strain was sub-cultured and maintained on MEA in petri-dishes for 4 weeks leading up to the experiment. To ensure Qfly inoculation, we followed a similar method to that outlined by Dimbi et al. (2003), where flies were inoculated through their confinement in a cylindrical apparatus (clear vinyl tube) lined with a velvet cloth and capped at both ends. The velvet cloth was inoculated with dry conidia (0.3 g) by scraping spores from MEA petri-dishes and placing onto the cloth. Twenty flies were transferred into the apparatus and kept there for 3 min while lightly agitating the tube. The flies were transferred to their respective replicate groups (4 replicate groups per treatment), and fed sucrose, yeast paste, and water. All inoculations were conducted at 4 °C to reduce Qfly activity. At the end of the trial, the dead flies were surface-sterilized in 1% sodium hypochlorite for 3 s and rinsed using 70% ethanol for 3 s, then rinsed 3 times with distilled water. Flies were transferred into petri-dishes lined with damp filter paper to promote sporulation and mycosis was confirmed by microscopic examination. For the replicate with the highest mean mortality, spores where cultured directly from the cadavers onto MEA plates containing 0.3g/liter of chloramphenicol to inhibit bacterial growth. Stock strain solutions were prepared, and 10^{-1} conidia/ml were aliquoted and spread onto Potato Dextrose Agar plates and examined after 24 h for germination. The percentage germination was quantified by counting 100 spores on each plate at ×40 magnification. Each plate served as a replicate with 4 replications per strain.

Laboratory trials: inoculation method efficacy (wet vs. dry conidia)

We compared 2 methods of inoculating Qfly with *Metarhizium*: (i) application through spores suspended in a carrier solution (0.01% Tween 80) and (ii) exposure to dry conidia. The selected isolate *M. guizhouense* (Mg1) was cultured straight from fly cadavers resulting from the comparison of *Metarhizium* strains, described above, onto full strength MEA petri-dishes (Supplementary Fig. S1). This strain was then sub-cultured from the conidia plated directly from Qfly and left for 4 weeks before conducting the inoculation experiment. The conidial suspension used in the wet treatments was prepared by scraping 10 large plates with a sterile spatula into a falcon tube before adding 50 ml of Tween 20 (0.1%) and vortexing for 30 s at a concentration of 4×10^7 spores/ml, determined using a hemocytometer. The conidial suspension was aliquoted onto each individual fly at 0.05 ml. The carrier solution was used to treat the control replicates. Inoculation using dry conidia followed the same protocol as outlined above in the Laboratory trials: *Metarhizium* strain efficacy section, as did the mortality monitoring and analysis. This experiment was repeated twice, each with 4 treatment replicates containing 20 flies—DT: dry treatment, WT: wet treatment, with control groups DC: dry control, and WC: wet control (Supplementary Fig. S1, exp. 2).

Statistical analysis

To examine survivorship/mortality in R (R Core Team 2020), we used a generalized linear mixed model with a binomial distribution and observation level random effect, where each data point receives a value informing a random effect that is then used to absorb extra variation driving overdispersion (Warton and Hui 2011, Harrison 2015). To evaluate treatment effects for experiment 1 (Mg1, Mg2, Ml3, Control) and experiment 2 (DT, WT, DC, WC) we used likelihood ratio tests using models fit with the function glmer in the R package lme4 (Bates et al. 2015), which allowed us to conduct Tukey's style tests evaluating all treatment comparisons via the multcomp package in R (Hothorn et al. 2008). Results were deemed significant when $P < 0.05$.

Results and discussion

Laboratory trials: *Metarhizium* strain efficacy

All 3 *Metarhizium* strains successfully infected Qfly, resulting in mortality and greater than 90% mean sporulation in all dead flies within treatment replicates (see Fig. 1B for sporulating Qfly). Sporulation did not occur in the control replicates. The Qfly mortality differed between fungal treatments (likelihood ratio test: χ^2 (3) = 147.54, $P <$ 0.001), with significantly higher mortality in treatments inoculated with *Metarhizium* strains when compared to the control group (Fig. 2). However, there was no significant difference in mean mortality between different *Metarhizium* strains (Fig. 2). The percent mortality over the 2 trial periods in the presence of *Metarhizium* strains ranged from 57% (Ml3) to 40% (Mg1 and Mg2), compared to 8% in controls (Fig. 2). Trial 2 demonstrated a similar trend in

variability, although the variance in mortality values for Mg1 was generally lower than trial 1, as was its efficacy in causing mortality in Qfly (Fig. 2). The treatment replicate with the highest infection efficacy was Mg1 at 100% mortality in trial 1. Given the potential for selection relating to virulence from a single host exposure, this Mg1 strain was cultured directly from the fly cadaver and used in the following experiment. The mean percentage conidial germination for Mg1, Mg2, and Ml3 was 72%, 78%, and 70%, respectively.

The *Metarhizium* pathogenicity (predominately *Metarhizium anisopliae*) against various fruit fly species have been reported in other studies, including *Bactrocera zonata* (Ibrahim et al. 2014, Gul et al. 2015, Hussein et al. 2018, Ahmad et al. 2022, El-Gendy et al. 2022), *Bactrocera cucurbitae* (Sookar et al. 2014, Hamzah et al. 2021, Iqbal et al. 2021), *Bactrocera dorsalis* (Faye et al. 2021, Melesse and Ferdu 2021, Wang et al. 2021, Wangkeeree and Suwanchaisri 2022), and *Ceratitis capitata* (Castillo et al. 2000, Ekesi et al. 2002, Quesada-Moraga et al. 2006, Beris et al. 2013, Soliman et al. 2020), with limited research focus on Qfly fungal entomopathogens (Carswell et al. 1998). The strains used in the current study originated from local banana farm soils, and local adaption by these strains may facilitate prolonged control due to climate suitability (McGuire and Northfield 2021). Thus, the apparent dominance of *Metarhizium* species within tropical soils (McGuire and Northfield 2020, 2021) warrant their consideration for the biological control of geographically similar pest species.

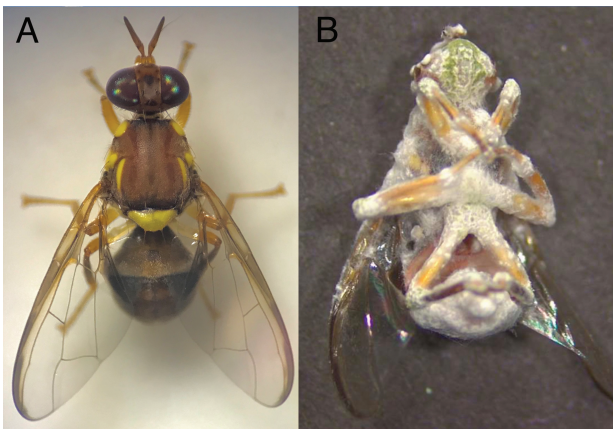

Fig. 1. Photograph of a "clean" Qfly before inoculation of Metarhizium strains A), and afterwards resulting in death and sporulation B).

Laboratory trials: inoculation method efficacy (wet vs. dry conidia)

We observed significant differences in Qfly mortality in the different inoculation methods (likelihood ratio test: χ^2 (3) = 325.75, $P < 0.001$), with significantly higher mortality in both treatments inoculated with Mg1 (DT: 88%, WT: 59%) compared to the relevant control treatment (DC: 11%, WC: 8%) (Fig. 3). Greater than 87% mean sporulation in all dead flies was achieved in the wet and dry treatments and no sporulation occurred in the control groups. Exposure of *Metarhizium* through dry conidia resulted in significantly higher mortality over the 2 trials compared to exposure through a conidial suspension (wet treatments), an increase in probability from 0.59 to 0.88 (Fig. 3). Within-treatment variability was similar between the 2 trials and Qfly mortality, and exposure from dry conidia generally resulted in less variability compared to the wet treatments (Fig. 3).

Dry conidia success could be attributed to its cell surface hydrophobicity, mediating adhesion to hydrophobic surfaces characteristic of insect epicuticle barriers rich in lipids (Ortiz-Urquiza and Keyhani 2013). Gindin et al. (2006) found application of *Metarhizium* in a dry powder, rather than aqueous suspension caused greater red palm weevil (*Rhynchophorus ferrugineus*) mortality over a shorter time period (Gindin et al. 2006). Faye et al. (2021) found traps inoculated with *Metarhizium acridum* dry conidia and the parapheromone methyl eugenol to lure and infect male *B. cucurbitae* flies significantly reduced *B. cucurbitae* in mango orchards, and spray application of *Beauveria bassiana* and *M. anisopliae* have improved *B. cucurbitae* field suppression (Hamzah et al. 2021). While the application of *Metarhizium* in situ demonstrates promise for Qfly mitigation, field studies are required to indicate this as a primary control or as a component of integrated pest management regimes. Effects on natural enemies are still unknown, limiting knowledge about how it integrates into conservation biological control by predators and/or parasitoids.

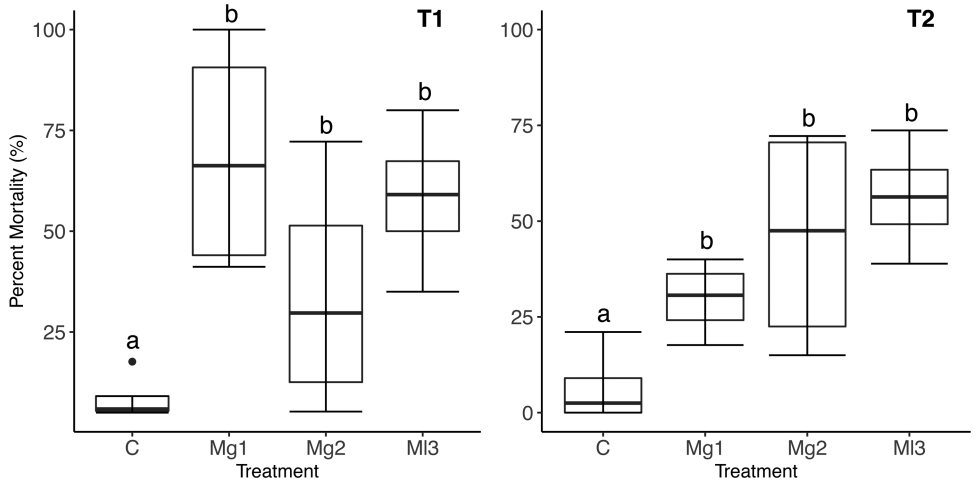

Fig. 2. Box-plot of experiment 1 laboratory of trials 1 (**T1**) and 2 (**T2**): mean percent mortality of *Bactrocera tryoni* at the end of the trial period (day 14) according to treatment type (control vs. Metarhizium strains: Mg1, Mg2, Ml3). "a" and "b" represent treatment differences as determined by Tukey style test. These plots illustrate the inter-quartile range between lower and upper box boundaries 25th and 75th percentiles, respectively.

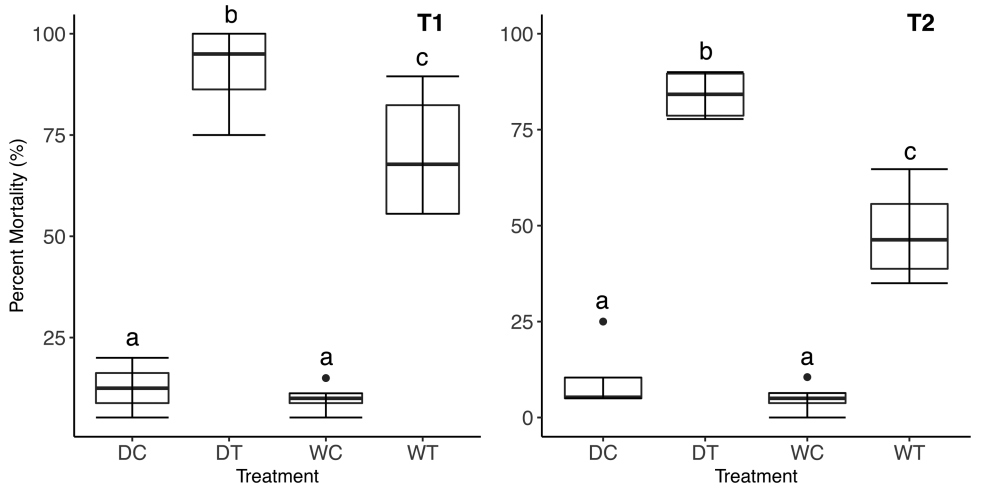

Fig. 3. Box-plot of experiment 2 laboratory of trials 1 (**T1**) and 2 (**T2**): mean percent mortality of *Bactrocera tryoni* at the end of the trial period (day 14) according to treatment type (DC: dry control, DT: dry treatment, WC: wet control, WT: wet treatment). "a," "b," and "c" represent treatment differences as determined by Tukey style test. These plots illustrate the inter-quartile range between lower and upper box boundaries 25th and 75th percentiles, respectively.

Nevertheless, the data here suggest significant potential to offer highly effective Qfly control tactics using entomopathogenic fungi.

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Author Contributions

Amy McGuire (Conceptualization-Equal, Data curation-Lead, Formal analysis-Lead, Investigation-Equal, Methodology-Equal, Project administration-Lead, Validation-Lead, Writing – original draft-Lead, Writing – review & editing-Equal), William Edwards (Conceptualization-Supporting, Data curation-Supporting, Formal analysis-Supporting, Investigation-Supporting, Methodology-Supporting, Resources-Lead, Supervision-Equal, Writing – original draft-Supporting, Writing – review & editing-Equal), Tobin D. Northfield (Conceptualization-Equal, Data curation-Supporting, Formal analysis-Equal, Investigation-Supporting, Methodology-Supporting, Supervision-Equal, Visualization-Lead, Writing – original draft-Equal, Writing – review & editing-Equal)

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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