# Entomopathogenic fungi for the control of Tephritid fruit flies

**Amy McGuire**

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College of Science and Engineering

James Cook University

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## Abstract

Safety and environmental concerns surrounding the use of insecticides against pestiferous insects in food systems has directed attention towards exploring biological control alternatives. Among these alternatives, entomopathogenic fungi, comprising diverse genera capable of infecting and killing insects, are becoming increasingly prominent in horticultural research focused on pest mitigation. Their global distribution, varying degrees of host specificity, and minimal impact on non-target organisms position them as promising candidates for the biological control of important pest species. This thesis aims to assess the potential of entomopathogenic fungi in controlling insect pest populations, with a focus on the Queensland fruit fly (Qfly), *Bactrocera tryoni* Froggatt, as the model organism in infection efficacy bioassays. Furthermore, the study contributes to the development of theoretical frameworks for the broader application of fungal entomopathogens against insect pests sharing similar life history strategies with fruit flies.

In Chapter 1 of this study, I conducted a comprehensive literature review on fungal entomopathogens, aiming to synthesize theory that illuminates the factors influencing their efficacy against fruit flies within the Tephritidae family. The analysis discerns intricacies in the pathogenicity of entomopathogenic fungi, noting considerable variations among strains with respect to the developmental stage and species of the fruit fly, environmental factors, and the application method. Furthermore, the review highlights relevant studies that demonstrate the successful field control of tephritid fruit flies through the application of fungal entomopathogens, emphasizing their compatibility within integrated pest management regimes.

In Chapter 2, my investigation centred on assessing the potential of infecting fruit flies with entomopathogenic fungi, which is a crucial step in considering their utilisation as biological control agents. Through laboratory experiments, I assessed the infection efficacy of

locally reared strains of *Metarhizium* against Qfly. I trialed three strains, comprising 2 species, *Metarhizium guizhouense* (strains Mg1 and Mg2) and *Metarhizium lepidiotae* (strain Ml3). All strains induced mortality in Qfly. *Metarhizium lepidiotae* achieved the highest mean mortality rate, while *M. guizhouense* demonstrated the highest mortality in a single replicate. Furthermore, experiments evaluating the efficacy of inoculation type revealed higher percentages of Qfly mortality when exposed to dry conidia compared to a conidial suspension. The notable contrast observed in conidia delivery - whether wet or dry application – underscores the critical nature of conducting thorough evaluations when determining the optimal delivery method for field application.

Chapter 3 involved an assessment of the potential for unidirectional selection between *Metarhizium* and Qfly. I strategically chose the strain (Mg1) that demonstrated the highest Qfly mortality (100%) in a single replicate during the infection efficacy experiments conducted in Chapter 2. Through serial passages of Mg1 through Qfly, elevated mortality rates were observed compared to the parent strain with no previous host exposure. This phenomenon indicates the possibility of enhancing the virulence of fungal entomopathogens by adapting strains to non-evolving insect hosts, offering a potential solution to the challenge of naturally low infectivity in pest populations. The findings in this chapter revealed an unexpected level of simplicity in cultivating more virulent isolates for targeted pest control.

Mathematical modelling could offer invaluable insights for optimising control parameters in the implementation of biological control agents within pest management programs. In Chapter 4, I advanced theoretical insights by adapting a modelling approach to predict the potential transmission dynamics of fungal entomopathogens from males exposed to auto-inoculation traps to females through mating with infected males. I adapted a susceptible-infected-recovered model to only include susceptible and infected compartments, as the assumption was that once infected, all cases eventually result in mortality. This model

adaption is exemplified by relevant insect pests with larval life-stages like fruit flies. The model parametrisation was informed by relevant literature on fruit fly biology, as well as infection and transmission dynamics involving entomopathogenic fungi. The model outputs revealed control optimisation at low to intermediate levels of virulence, with an increase in the rate of male transmission correlating with higher optimal virulence values. Furthermore, the presence of reproductive costs stemming from female infection altered the optimal virulence value. The nuanced interplay observed between pathogen virulence and host reproductive capacity during infection indicates optimal control strategies across varying levels of virulence.

The findings outlined in this thesis highlight entomopathogenic fungi as a promising and effective solution for managing tephritid fruit flies. By providing fresh insights into the control efficacy of these pathogens, this study complements previous research efforts and underscores a tangible pathway for the development of sustainable pest management strategies.

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## Introduction

The use of chemical pesticides to control crop pests is consistently under scrutiny due to adverse effects on human health, diverse beneficial non-target organisms, and the environment (Begum et al., 2017; Pretty et al., 1998). Furthermore, escalating restrictions on pesticides (Dominiak & Ekman, 2013a), coupled with the development of pesticide resistance in crop pests from repeated application (Hawkins et al., 2019), have contributed to an increasing demand for environmentally sustainable control alternatives. Entomopathogenic fungi, a group of soil-dwelling microorganisms, comprise a significant portion of the biopesticides market for the biological control of arthropods (Lacey et al., 2015) and have the capability to infect and kill across many insect orders (Araújo & Hughes, 2016). Serving as natural regulators of insect populations, pathogenic fungi contribute more than 60% of insect deaths in nature (Faria & Wraight, 2007). Host specificity exhibits significant variability among species and strains (Hajek & Butler, 2000), and those species and/or strains displaying high virulence against diverse insect pests are readily sought after for the control of important pest species (Mohammed et al., 2018; Murtaza et al., 2022).

Species within the genera *Beauveria* and *Metarhizium*, particularly *Beauveria bassiana* and *Metarhizium anisopliae*, are the most well studied and extensively utilised fungal groups for insect pest management (McGuire & Northfield, 2020a). Additionally, other noteworthy and frequently studied species are found in the genera *Isaria*, *Lecanicillium* and *Hirsutella* (Khan et al., 2012; Vega et al., 2009). The spores produced by these fungi possess a unique range of chemical properties that enable them to attach and penetrate the insect cuticle (Mannino et al., 2019). Once the cuticle is breached, the mycelium undergoes rapid proliferation, exhausting the host tissue water content and inducing cadaver mummification. In insects with soft exoskeletons, the fungus can readily emerge and engulf the host body with a dense layer of mycelium, producing a sporulating structure, often

triggered by high humidity conditions (Evans & Hywel-Jones, 1997). Depending on the fungal strain and number of infecting spores, it can take approximately 4-10 days to induce death (Irsad et al., 2023). This cycle continues as sporulating insect cadavers promote the dispersal of fungal entomopathogens back into the environment and between hosts (Evans & Hywel-Jones, 1997). Outside of infecting hosts, fungal entomopathogens can be found inhabiting the soil (McGuire & Northfield, 2021a), on plant surfaces as epiphytes (Nishi et al., 2021), or within plants as endophytes (Greenfield et al., 2016b). This versatility in ecological niches, both above and below ground, position them as ideal candidates for the control of insect pests with soil-dwelling life-stages, such as fruit flies.

Fruit flies (Diptera: Tephritidae) are among the most destructive horticultural pests in the world (Dias et al., 2018). Characterised by high invasiveness and wide host ranges, many of these species pose substantial threats to a wide array of fruit and vegetable crops throughout tropical and sub-tropical regions (Clarke et al., 2011; Vera et al., 2002). In the context of Australia, the Queensland fruit fly (Qfly), *Bactrocera tryoni* Froggatt (Diptera: Tephritidae), is the leading economically damaging horticultural pest (Drew, 1978; Sutherst et al., 2000). Initially endemic to tropical and sub-tropical coastal Queensland (Drew, 1989), the fly now occupies the entire east coast of Australia and has also invaded certain South Pacific Island nations (Drew, 1989; White & Elson-Harris, 1992). The wide geographic spread and ability to colonise new locations has led to its designation of a ‘climate generalist’ (Merkel et al., 2019). The continued expansion of horticulture across Australia facilitates the enlargement of Qfly’s distribution range and host breadth (Smith et al., 1988), a trend likely to intensify with climate change (Sultana et al., 2017).

Qfly is arguably the most destructive pest in Australia, mainly attributed its polyphagous nature, utilising over two hundred native and introduced plant species for oviposition (Drew et al., 1982; Hancock et al., 2000). Consequently, major restrictions are

imposed on both international and domestic trading due to the pest's pervasive impact (White et al., 2012). Climate warming and Qfly's highly polyphagous nature poses a significant threat to the concept of 'area freedom' within the Fruit Fly Exclusion Zone (Sutherst et al., 2000). This classification, crucial for international market access, is contingent on the exclusion of fruit fly breeding populations (Anon, 1997). Any compromise to 'area freedom' can result in substantial market losses, triggering the closure of international borders to trading and escalating control costs for commercial growers (Anon, 1993; Sutherst et al., 2000). In view of these market access restrictions and production losses, the effective management of Qfly is a core priority of Australia's horticultural industries (NFFSIC, 2010). However, chemical control is becoming increasingly difficult as restrictions on its use intensifies (Dominiak & Daniels, 2012).

Previous studies have demonstrated variable success in controlling numerous fruit fly species with fungal entomopathogens (Faye et al., 2021; Murtaza et al., 2022; Toledo et al., 2017; Yousef et al., 2018), with limited investigation against Qfly. This current study, presented in Chapters 2 and 3, marks the first examination of the efficacy of *Metarhizium guizhouense* and *Metarhizium lepidiotae* against Qfly. It is noteworthy, however, that with continued molecular advancements refining sequencing techniques and subsequent taxonomic revisions, earlier studies evaluating *M. anisopliae* may encompass a broader range of species not previously identified (Bischoff et al., 2009a). While Qfly served as the model organism of this thesis for assessing the infection efficacy of *M. guizhouense*, a comprehensive review of the literature (Chapter 1) broadened the scope of fruit fly control using fungal entomopathogens. This review incorporated various fruit fly species within the genus Tephritidae, providing a broader understanding of the potential applications of fungal entomopathogens in fruit fly management.

Tropical entomopathogenic fungal strains can exhibit distinct life-history preferences and environmental tolerances compared to their temperate counterparts, potentially due to a contrast in selection pressures – biotic verses abiotic factors – governing their evolved habitat and host affinity (McGuire & Northfield, 2020a). Consequently, the effectiveness of high-performance strains observed in studies with temperate climates does not necessarily guarantee success in tropical horticulture. To address this, I conducted laboratory trials evaluating the pathogenicity of three locally sourced strains of *M. guizhouense* from Qfly's endemic range to identify the most effective strain and inoculation method for inducing mortality (Chapter 2). The mortality data of Qfly obtained from Chapter 2 experiments hinted at the potential for optimising virulence through prior host exposure, raising the question of whether incremental improvements could be achieved in *Metarhizium* as a biopesticide through unidirectional selection.

Notably, a growing body of research is shedding light on the capacity to augment the biological control efficacy of entomopathogenic fungi through genetic engineering and adaptive techniques (Du et al., 2022; Quesada-Moraga & Vey, 2003; Song & Feng, 2011; Zhao et al., 2016). Locally reared strains may have limited tolerance to the environmental conditions of novel applications and naturally low virulence to novel hosts. However, those amenable to genetic modification hold the potential to enhance strain efficacy against the target pest or applied system, thereby overcoming limitations imposed by restricted sample sizes through fungal rearing techniques. This approach, involving the rearing of locally adapted strains tailored to the target system of application, holds promise for enhancing their virulence against an insect pest, thereby overcoming important control limitations such as field persistence and low host affinity. Serial passages of fungal entomopathogens through insect hosts have been documented to enhance virulence against various arthropods, including aphids (*Sitobion avenae*) (Adames et al., 2011), beetles (*Tribolium castaneum*) (Joop &

Vilcinskas, 2016; Rafaluk et al., 2017), planthoppers (*Nilaparvata lugen*) (Song & Feng, 2011), locusts (*Locusta migratoria*) and ticks (*Rhipicephalus microplus*) (Adames et al., 2011). However, there is no documentation evaluating this phenomenon in fruit flies. The research elucidated in Chapter 3 stands as the pioneering study showcasing the capacity to enhance the virulence of *M. guizhouense* against Qfly through adaptive techniques. The DNA from both the parent and serially passed *M. guizhouense* isolates was extracted and the whole genome sequencing was conducted to provide deeper insights into the genetic alterations following host exposure. However, as the genetic comparison analysis of different strains will be conducted through collaboration after the submission of this thesis, the results of that genetic component are not presented here.

Mitigating fruit fly infestation often involves integrated pest management, comprising a multitude of control tactics such as protein bait sprays and lure-and-kill mass trapping (Dias et al., 2018). Pheromone traps use female attractants to entice male flies in search of mates (Navarro-Llopis et al., 2008). Given the absence of effective female lures, this method relies on skewing the sex ratio and diminishing the male mating pool (El-Sayed et al., 2009a). The horizontal transmission, or autodissemination, of fungal entomopathogens involves the transfer of conidia from infected to susceptible individuals, achievable through mating and other pathways (e.g., direct contact with conidia deposited in the field and mycosed cadavers) (Furlong & Pell, 2001). Previous studies have demonstrated effective field control by adapting the pheromone-based lure-and-kill trapping technique to trap and release lured males after exposure to an inoculated medium with entomopathogenic fungi (Benvenuti et al., 2019; Faye et al., 2021; Maniania & Ekesi, 2013). Infected males subsequently mate with wild female flies, thereby transmitting the pathogen to the wider population. While some laboratory and field trials have shown success in demonstrating the potential for controlling pest populations through the autodissemination of fungal entomopathogens (Toledo et al.,

2017), there is a scarcity of theoretical frameworks describing crucial factors contributing towards its success. Therefore, a theoretical framework was developed in Chapter 4 to provide insights into important infection mechanisms driving the efficacy of a lure-and-infect trapping system for supressing fruit fly populations. When infection reduces female reproduction, the model outputs suggest low to intermediate virulence may be more effective in pest control strategies. This approach challenges the traditional preference for highly virulent strains, highlighting the importance of conducting comprehensive bioassays to guide the selection of important infection traits for effective pest management.

# Chapter 1. Review of fungal entomopathogens relating to tephritid fruit fly management

**Author Contributions:** The manuscript was written by Amy McGuire, and reviewed and edited by Tobin Northfield and William Edwards.

## 1.1. Abstract

Entomopathogenic fungi have been demonstrated to be effective against a diverse number of tephritid fruit flies. The discovery of numerous efficacious strains/species, advances in their mode of application, and an increasing demand for reducing pesticides has resulted in a surge of research development and commercial interest. We assessed factors influencing the pathogenicity and applicability of fungal entomopathogens in tephritid fruit fly management, and established current knowledge on their potential to control populations at various life stages, environmental conditions and in conjunction with other biological control agents. The management of fruit flies is challenging due to their wide host range and rapid adaption to various regions throughout the world. At present, tephritid fruit fly control relies on methods that predominately target the adult life stage, such as bait sprays and/or traps, the sterile insect technique and male annihilation. However, increasing research has exhibited effective control of preimaginal and female fruit flies' through soil inoculations and the autodissemination of fungal entomopathogens that facilitates horizontal transmission. The reviewed literature indicates discrepancies in fly mortality from the targeted life-stage, inoculation method and medium, and important abiotic factors influencing pathogenicity. The compatibility of fungal entomopathogens with other biological control agents and horticultural chemical products promote their incorporation into integrated pest managements regimes.

## 1.2. Introduction

Tephritid fruit flies (Diptera: Tephrititdae) represent one of the world's most destructive horticultural pest, with important species belonging to the genus *Bactrocera* (major fruit fly pest throughout Asia-Pacific region), *Anastrepha* (dominant throughout the Western Hemisphere) and *Ceratitis* (wide-spread throughout the Mediterranean region, southern Europe, the Middle East, Western Australia, South and Central America and Hawaii). With over 4000 species globally, approximately 350 are categorised as economically important (Plant Health Australia, 2018). In Australian horticulture, seven are of major concern (*Bactrocera tryoni, Bactrocera aquilonis*, *Bactrocera neohumeralis*, *Bactrocera jarvisi*, *Bactrocera musae*, *Ceratitis capitata*, and *Zeugodacus cucumis*) (Horticultural Policy Council, 1991; Plant Health Australia, 2018). Immediately to the North of Australia, the Oriental fruit fly (*Bactrocera dorsalis*) is the world's most damaging pest of tropical horticulture (Vargas et al., 2015). The risk of *B. dorsalis* incursions inside Australian borders and the migration of *B. tryoni* (currently occupying the eastern states and Northern Territory) and *C. capitata* (exclusive to Western Australia) into each other's territory has prompted extensive monitoring and control optimisation tactics nation-wide. To prevent fruit fly spread, strict quarantine regulations are in place for trading in international and domestic markets, limiting trade options for infested areas and exacerbating local economic impacts (White et al., 2012). In view of these market access restrictions and production losses, effective management of this pest is at the core of Australia's horticultural industries (NFFSIC, 2010).

Native to tropical and sub-tropical coastal Queensland, *B. tryoni* is the most significant biosecurity threat to Australia's $9 billion per annum horticultural industry (Plant Health Australia, 2018). Until recently, chemical control programs and post-harvest treatments for market access requirements of Australian states where *B.tryoni* is absent

predominately utilised organophosphates dimethoate and fenthion (Dodds et al., 2014; Dominiak & Ekman, 2013b; Hetherington, 2005; Sproul et al., 2001). However, the Australian Pesticides and Veterinary Medicines Authority prohibited the use of these products due to the negative impacts on human health and the environment (Australian Pesticides and Veterinary Medicines Authority, 2011, 2015). The group has listed Malathion (predominant toxicant in bait sprays) as an alternative (Australian Pesticides and Veterinary Medicines Authority, 2014) despite its restricted use in many countries and similar health concerns (Richmond, 2022). Research has been focused towards trialing a multitude of insecticidal alternatives for fruit fly, such as neonicotinoids (e.g. clothianidin, imidacloprid and thiacloprid), pyrethroids (e.g. alpha-cypermethrin) and spinosyns (e.g. spinosad) with varying degrees of efficacy (Reynolds et al., 2017; Yee & Alston, 2012). Nevertheless, restrictions on effective broad-spectrum and systemic insecticides are continually tightening. Additionally, tephritid fruit fly species are evolving resistance to a wide-array of chemical products (e.g. resistance to spinosad sprays reported in *B. oleae* (Kakani et al., 2010) and levels of resistance in *C. capitata* to malathion and lambda-cyhalothrin have resulted in field control failures (Arouri et al., 2015; Magaña et al., 2007)). Thus, there is growing need to investigate environmentally friendly approaches that reduce chemical dependencies.

In recent years, there has been an influx of research showcasing insect pathogenic fungi as an exciting prospect for global pest management. Second to parasitoids, fungal entomopathogens are the most-studied biological control agents of tephritid fruit flies (Dias et al., 2022). Fungal entomopathogens show promise in their control of important fruit fly species including *Bactrocera zonata* (Ahmad et al., 2022; El-Gendy et al., 2022; Gul et al., 2015; Hussein et al., 2018; Ibrahim et al., 2014), *Bactrocera cucurbitae* (Hamzah et al., 2021; Iqbal et al., 2021; Sookar et al., 2014a), *Bactrocera dorsalis* (Faye et al., 2021; Melesse & Ferdu, 2021; Wang et al., 2021; Wangkeeree & Suwanchaisri, 2022), *Ceratitis capitata*

(Beris et al., 2013; Castillo et al., 2000b; Ekesi et al., 2002; Quesada-Moraga et al., 2006; Soliman et al., 2020) and *Bactrocera tryoni* (Carswell et al., 1998). Despite encouraging results, horticultural adoption of fungal entomopathogens is limited, likely due to the low cost and control efficiency of chemical alternatives (Dominiak & Ekman, 2013b).

The commercial adoption of fungal entomopathogens in Australian farming systems remains limited. While substantial research has demonstrated the efficacy of fungal entomopathogens in achieving high fruit fly mortality rates under controlled conditions (Gava et al., 2020; Soliman, 2020; Tora & Azerefegn, 2021), large-scale field studies are comparatively scarce (Mantzoukas et al., 2022; Shaurub, 2023). Additionally, research on the cost-effectiveness of these fungi for fruit fly control remains insufficient (Shaurub, 2023), with inconsistent field performance and dependence on environmental factors such as temperature, humidity and rainfall further hindering adoption (Quesada-Moraga et al., 2024; Wraight et al., 2009). The limited availability of products and inadequate public sector support (Dias et al., 2022) are likely contributing to the slow integration of these biological agents into existing pest managements frameworks.

Ecological constraints also present challenges, including an incomplete understanding of interactions between entomopathogenic fungi and non-target organisms like natural predators and soil microbiota (Islam et al., 2021). Furthermore, the compatibility of these fungi with systems previously reliant on chemical pesticides remains underexplored (Dara, 2019). In some cases, slower insect mortality rates and sub-optimal virulence of certain entomopathogens, compared to synthetic chemical alternatives, limit their appeal (Islam et al., 2021). Additional barriers include production and storage issues; continuous culturing can lead to virulence attenuation (Nahar et al., 2008), while prolonged storage may reduce spore viability (Islam et al., 2021). Consequently, despite the environment and health risks associated with chemical pesticides, growers may continue to favour these methods over

biological alternatives. Addressing these challenges is crucial for optimising the performance of fungal entomopathogens in horticultural systems and facilitating their broader adoption as a sustainable, effective alternative to chemical pesticides.

This review seeks to highlight important factors contributing the pathogenicity and efficacy of entomopathogenic fungi in infecting tephritid fruit flies, building on previous research to promote greater adoption of these biological control agents.

### 1.3. Current fruit fly control practises: A brief overview

Various technologies have been developed for monitoring and controlling fruit flies throughout Australia and the Pacific. Chemical control involves cover sprays, protein bait sprays, and soil drenches (Vargas et al., 2015). Common post-harvest fruit treatments include hydrothermal methods, cold quarantine and gamma radiation to kill eggs or larvae developing within fruit (Grout, 2016). Cover sprays and soil drenches afford high crop protection, although they have economic and environmental implications due to heavy insecticidal use. Conversely, protein bait sprays that act as lures and kill both sexes significantly reduces the volume of insecticide required for control (Vargas et al., 2015). The male annihilation technique involves mass trapping by exploiting the attraction of males to parapheromone lures (e.g. methyl eugenol, cue-lure, raspberry ketone, and trimedlure) (Vargas et al., 2014). The sterile insect technique involves the mass-release of sterile flies as a form of 'birth control' in wild fruit fly populations (Enkerlin, 2021). Orchard hygiene and management can involve fruit bagging/wrapping, cultural control (e.g. removing fallen fruit) (Allwood, 1997), and the use of natural enemies, with particular emphasis on parasitoids (over predators), because they are typically relatively specialized (Dias et al., 2022; Gingrich, 1993). The

combined use of these methods is common in integrated pest management regimes across a wide range of targeted control scales.

Lure and kill trapping techniques represent one of the most commonly used methods for fruit fly management (Bateman et al., 1973; Smith, 2000). Lures acting as semiochemicals can involve odours such as pheromones or host plant volatiles (Stringer et al., 2019), mixed with a toxicant contained in a device or carrier, enables effective surveillance, and population suppression given sufficient trap densities (Domimak et al., 2003; Petacchi et al., 2003). There are no effective lures commercially available for female flies. Although, males are strongly attracted to the phytochemical compounds methyl eugenol, and raspberry ketone and its synthetic analog, cuelure (Bateman, 1972; Dominiak et al., 2011; Metcalf & Metcalf, 1992). Lures such as these are a key factor in fruit fly population suppression utilising the male annihilation technique (Dominiak et al., 2009). Devices are deployed containing the selected lure and toxicant; by attracting and killing males, it skews the sex ratio and females struggle to find prospective mates, ultimately reducing crop infestations (El-Sayed et al., 2009b). While this represents a commonly used and effective population suppression tool, used alone it has some control limitations stemming from the survival rate of male flies, facilitating continued mating and female reproduction (Stringer et al., 2017).

The sterile insect technique is used for regional management of the most important fruit fly pests which cannot be controlled on an orchard level without systematic insecticidal use (Enkerlin et al., 2015; Hendrichs et al., 2002). Millions of flies are sterilised (generally males) and mass-released into the field to mate with wild females, restricting reproduction, as they become incapable of producing viable offspring (Fanson et al., 2014; Knipling, 1955). Over generations, the fruit fly population is reduced, sometimes resulting in eradication (Krafsur, 1998). Sterile mass-released flies compete within leks and achieve mating success

with wild females, although they are generally less competitive than their wild counterparts due to the mass-rearing conditions in which they are held (i.e. irradiation effects on physiology and age of strains in colonies) (Cayol, 1999; Hendrichs et al., 2002). Solely used, this method also has limitations due to the competitiveness and ecological superiority of wild flies. However, it is worth noting that studies have shown an enhanced competitiveness of sterile flies' by improving the gut microbiota through bacterial inoculations (Ami et al., 2010; Wang et al., 2014; Zhang et al., 2021). For regions occupied by endemic pest species such as *B. tryoni*, the sterile insect technique is not employed, nor is it commercially available in Australia (Horticulture Innovation Australia, 2018).

Protein bait sprays have been used extensively worldwide in fruit fly management programs, employing the 'attract and kill' technique as an alternative to cover sprays (Fletcher & Bateman, 1983; Hargreaves et al., 1986). Female flies require sufficient protein for sexual development and egg production, so they are highly attracted to available sources (Hagen & Finney, 1950). This method involves the mixture of a protein attractant and toxicant, applied as 'spots' or 'squirts' on foliage susceptible to infestations, generally the lower canopy of the trees (Bateman, 1982; Smith et al., 1997). The application rates and scales vary from aerial sprays to ground-based spot sprays (Magaña et al., 2007; Prokopy et al., 1992). However, bait sprays might not be effective for high density populations, and requires frequent re-applications (i.e. weekly and after high temperature and rainfall events) (Vickers, 1997). Moreover, although this method is a safer alternative to cover sprays, on large scale applications there is still a considerable amount of toxicant entering the system on a regular basis, which is intensified as tephritid fruit fly species develop insecticidal resistance (Magaña et al., 2007; Vontas et al., 2011).

Various control techniques of fruit fly are often combined in integrated pest management regimes, particularly in an area wide management framework (Vargas et al.,

2015). For example, the male annihilation technique is often deployed before the sterile insect technique (Barclay et al., 2014; Steiner et al., 1970), complementing this method by contributing to the effective overflooding ratio when releasing sterile males, and reducing the competitiveness of wild mating males (Vreysen et al., 2006). However, simultaneous use could nullify control by the sterile insect technique due to the suppression of sterile flies by the male annihilation traps (Stringer et al., 2017). In 2000, the Hawaii Fruit Fly Area-Wide Pest Management program was implemented by the USDA-ARS to develop various suppression techniques for *Bactrocera cucurbitae*, *C. capitata* and *B. dorsalis* populations during a 6-year period. Substantial reductions in fruit infestations were achieved through a combination of field sanitation, protein bait sprays and/or traps, male annihilation with male lures and attractants, augmentative parasitoid releases and sterile insect releases – resulting in significant reductions in pesticide use by growers (Vargas et al., 2003; Vargas et al., 2010). Method integration in fruit fly management programs has demonstrated the importance of combining techniques which target different components of the fly's behavioural ecology, life stage and the chosen scale/systems of control.

### 1.4. Infection of fruit flies by entomopathogenic fungi at different life stages

Entomopathogenic fungi occupy a variety of life-history preferences. In addition to living as insect endoparasites, species thrive in the soil profile (serving as an immense reservoir of fungal inoculates), as well as persisting on leaf surfaces and growing endophytically inside plants. The persistence of entomopathogenic fungi in soil, within and on plant surfaces gives way to biological control tactics of similarly occurring pest species. The different life stages of tephritid fruit flies could provide an opportunity for targeted control using fungal entomopathogens – either within the soil profile for preimaginal stages (i.e. larvae and pupae), or above ground utilising spraying and/or trapping techniques for adults. Tephritid fruit flies deposit their eggs beneath the skin of the fruit (Fitt, 1990) and

hatch within two to three days of oviposition, larvae feed on the flesh and develop inside their host for up to one month. Infested fruit generally spoils and drops to the ground, facilitating pupation in the upper layer of the soil over approximately seven days (delayed in cool conditions). Teneral flies emerge and become sexually mature after two to three weeks; this is reduced to one week under favourable conditions (Bateman, 1968; Christenson & Foote, 1960; Fletcher, 1989; May, 1958). The potential to target the soil-dwelling life stage of tephritid fruit flies, or adults that emerge from the soil after pupating could significantly enhance population control.

Numerous studies have demonstrated positive dose-mortality relationships at all life-stages of *B. dorsalis, B. zonata and C. capitata* when inoculated with various genera of entomopathogenic fungi, such as *Metarhizium, Beauveria, Aspergillus, Verticillium, Aschersonia* and *Paecilomyces* (Aemprapa, 2007; El-Gendy et al., 2022; Hallouti et al., 2021; Murtaza et al., 2022; Soliman, 2020; Soliman et al., 2020; Tora & Azerefegn, 2021). Bioassays examining the comparative effects of infection by fungal entomopathogens at different life stages of *B. dorsalis, B. zonata, C. capitata, Anastrepha ludens and B. oleae* indicate adult susceptibility to be greater than preimaginal stages (Beris et al., 2013; De et al., 2002; Elghadi & Port, 2019; Gul et al., 2015; Hussein et al., 2018; Mahmoud, 2009; Soliman et al., 2020; Usman et al., 2021; Yousef et al., 2017). The characteristics of the cuticles of pre-pupal larvae and pupae of tephritids (i.e. thickness, density and degree of sclerotization), pupal age and inoculated medium (i.e. soil texture) could be important factors to consider when assessing the pathogenicity of entomopathogenic fungi. For example, when compared to adult flies, the cuticles of pre-pupating larvae and pupae are more sclerotized, potentially inhibiting direct penetration and thus reducing susceptibility (Quesada-Moraga et al., 2006). Furthermore, mortality was reduced with increased age of pupae for both *C. capitata* (Ekesi

et al., 2002) and *B. zonata* (Hussein et al., 2018) when treated with strains of *M. anisopliae* and *B. bassiana*.

Various methods are developed to expose preimaginal flies to fungal entomopathogens. Direct contact including spraying, dipping into a conidial suspension and body contact bioassays are common methods that have been employed to evaluate the efficacy of various entomopathogenic fungi (predominately *Beauveria* and *Metarhizium*) against preimaginal flies belonging to *B. dorsalis, C. capitata and A. ludens* (Beris et al., 2013; De et al., 2002; Hallouti et al., 2021; Quesada-Moraga et al., 2006; Soliman et al., 2020; Tora & Azerefegn, 2021). To better replicate in-field conditions for immature life-stages, *B. dorsalis, B. zonata, C. capitata and B. oleae* were buried or left to borrow in treated sand or soil columns inoculated with strains of *Metarhizium* and/or *Beauveria* (Gava et al., 2020; Hussein et al., 2018; Murtaza et al., 2022; Soliman, 2020; Usman et al., 2021; Yousef et al., 2017). The resulting preimaginal mortality outcomes for the studies previously cited are highly variable, with ranges reaching as low as 2% up to 100%. Potentially important factors contributing to this variability include fruit fly species (e.g. differences in cuticle composition), preimaginal stage, applied fungal entomopathogenic strain (i.e. variations in host range relating to virulence), concentration and inoculation methodology. In addition to reductions in adult emergence, infection but not death during preimaginal stages can result in significant deferred mortality in the newly emerged adults (Ekesi et al., 2002; Gava et al., 2020; Imoulan & Elmeziane, 2014; Marri et al., 2016; Yee & Lacey, 2005). Under laboratory conditions, soil inoculated with *B. bassiana* strains LCB53 and LCB289 caused 44.4 and 60.1% pupal mortality in *C. capitata*. When corrected to include the delayed mortality from infected adults that emerged from the treatments, mean mortality totalled 83.5% and 88.7% (Gava et al., 2020). Therefore, studies evaluating only preimaginal mortality might not represent the true infection potential of trialed strains.

### 1.5. Strain selection and prospects for field application in fruit fly suppression

Fungal isolates are commonly reared from soil through antibiotic-based selective media (Majchrowska-Safaryan & Tkaczuk, 2021), soil-baiting (Zimmermann, 1986) or cultured directly from insect cadavers (Jaber et al., 2016). Isolation of entomopathogenic fungi from soil is frequently based on insect bait methods using *Galleria mellonella* or *Tenebrio molitor* larvae due to their high susceptibility to infection (Kim et al., 2018; Senthilkumar et al., 2021). However, despite the low susceptibility of the Mexican fruit fly (*A. ludens*) larvae during soil-baiting experiments, Presa-Parra et al. (2020) was the first to reveal *M. robertsii* to be highly virulent against *A. ludens* in laboratory trails when applying spores isolated from the cadavers. Additionally, Hallouti et al. (2020) used *C. capitata* pupae as bait and reported greater diversity of soil reared species (10 fungal genera containing highly virulent strains) when compared to a similar study from the same region and forested habitat type using *G. mellonella* as bait (2 fungal genera reared from soil) (Imoulan et al., 2011). This raises the question of whether we are missing host specific strains by using non-target larvae for soil-baiting. Studies have also reported increased virulence by entomopathogenic fungi after serial passages through the insect host (Adames et al., 2011; Hayden et al., 1992; Joop & Vilcinskas, 2016; Quesada-Moraga & Vey, 2003). The potential to rear strains with greater host specificity and virulence through a natural affinity for the pest (thereby outcompeting inferior strains) or a co-evolutionary advantage through previous host exposure (i.e. culturing spores from infected cadavers for field application) are important factors to consider during strain selection.

Factors influencing fungal persistence when applied to soil include biodegradation, vertical percolation into deeper layers and physical weathering (Fargues et al., 1983; Vaenninen et al., 2000). Given that pupation of most fruit fly species occurs in the first 7cm of the soil profile (Hennessey, 1994), application of fungal entomopathogens in this soil layer

should maximise infection. The ingredients used during the formulation of fungal entomopathogens for field application could be critical to fungal viability and the efficacy of transmission, virulence and soil persistence. Ekesi et al. (2005) demonstrated the efficacy of three formulations of *M. anisopliae* against *C. capitata*, *C. fasciventris*, and *C. cosyra* through soil inoculations. The granular formulation resulted in greater persistence in soil treatments and infectivity against the *Ceratitis* spp. when compared to the aqueous and oil/aqueous (50:50) solutions (Ekesi et al., 2005).

Wild female fruit fly populations could be targeted for control through autodissemination of fungal entomopathogens. The method attracts males using food, visual or chemical lures to a source of entomopathogenic inoculum to then disseminate among the target population. Once infected, the fungus can take days to kill its host (dependent on pathogen virulence and host immunity) (Lu & St. Leger, 2016), allowing time to find a female mate and transmit the entomopathogen (potentially multiple times) before ensuing death in both sexes. The pheromone cuelure is a common male attractant for tephritid fruit flies, and can be used together with fungal entomopathogens in autodissemination traps. Lure feeding has been linked to sexual selection in tephritid fruit flies. Cuelure-fed *B. tryoni* males metabolize the substrate which is accumulated in the rectal gland as raspberry ketone, subsequently, it is emitted along with endogenous pheromones, increasing the males attractiveness to females (Kumaran et al., 2014). Therefore, females are more likely to mate with lure-fed males, and also experience increased fecundity, although a reduced with a life-span (Kumaran et al., 2013). In later work by Kumaran and Clarke (2014), offspring of cuelure-fed *B. tryoni* males demonstrated enhanced ability to locate lures compared to sons denied the attractant, thus improving their mating potential (i.e. ‘sexy-sons’ effect of lures). Raspberry ketone attractants have also been found to increase the rate of sexual development in immature flies (Akter et al., 2017a; Akter et al., 2017b). Understanding the physiological

influence of pheromones on male flies and the indirect effect on females could enhance biological control efficacy using fungal entomopathogens in place of a toxicant.

Numerous studies have confirmed the ability of fungal entomopathogens to be transmitted horizontally through mating in tephritid fruit flies, including *A. ludens* (Toledo et al., 2007; Toledo et al., 2014), *B. cucurbitae* (Onsongo et al., 2022; Sookar et al., 2014a), *B. zonata* (Sookar et al., 2014a), *C. capitata* (Beris et al., 2013; Dimbi et al., 2013b; Quesada-Moraga et al., 2008), *C. cosyra*, and *C. fasciventris* (Dimbi et al., 2013b). These studies have reported significant mortality through horizontal transmission (i.e., 83-100% in *C. capitata* (Dimbi et al., 2013b; Quesada-Moraga et al., 2008), 69-83% in *B. zonata*, 78-88% in *B. cucurbitae* (Sookar et al., 2014a), 72-85% in C. cosyra, 81-84% in *A. ludens* (Toledo et al., 2007) and 71-93% in *C. fasciventris* (Dimbi et al., 2013b)). Even contact through unsuccessful mating attempts has led to the transmission of spores from infected males to females – representing 15.4 and 21.6% transmission in the first day of attempted courtship in *A. ludens* for two *Beauveria* products (Toledo et al., 2007). Additionally, a contaminated fly could transmit a lethal dose to at least three mates before dying from infection (Sookar et al., 2014a). Infection in female flies has also lead to substantial drops in fecundity when compared to untreated flies (Castillo et al., 2000b; Dimbi et al., 2013b; Onsongo et al., 2022; Sookar et al., 2014a; Toledo et al., 2007).

There has been a recent emergence in field studies demonstrating considerable potential for fruit fly mitigation using fungal entomopathogens. Faye et al. (2021) evaluated the efficacy of the autodissemination approach for the management of *B. dorsalis* using attractant contaminant traps inoculated with *M. acridum* in citrus orchards, reporting a significant reduction in fruit damage increasing over time. Field suppression of *C. capitata* by *M. anisopliae* was achieved through a micro-sprinkler irrigation system below the mango canopy into the soil. A modified cage used to capture emerging *C. capitata* adults revealed

52-68% mean mortality (Gava et al., 2021). The application of *B. bassiana* and *M. anisopliae* underneath tree canopies in mango orchard field cage trials also resulted in significant mortality and reductions in *B. zonata* and *B. dorsalis* adult emergence (Usman et al., 2021; Wakil et al., 2022). Yousef et al. (2017) observed a reduction in the adult *B. oleae* population by 50-70% over four growing seasons when applying *M. brunneum* to the soil beneath the tree canopy. In a follow-on study, the field trialed strain was found to be compatible with use of a herbicide, with no impacts on soil persistence by *M. brunneum* (Yousef et al., 2018) – this supports other findings of minimal or non-toxic effects of herbicides towards fungal entomopathogens (Clifton et al., 2015; Sharma et al., 2021).

### 1.6. Environmental factors influencing the pathogenicity of entomopathogenic fungi against fruit flies

Important abiotic factors affecting the persistence and infectivity of entomopathogenic fungi include temperature, ultraviolet (UV) radiation and moisture. Temperature variations can have marked effects on fungal survival and pathogenicity, which largely depend on the fungal species/strain (Nussenbaum et al., 2013; Ouedraogo et al., 1997), host species (Dimbi et al., 2004) and the region of origin resulting in optimal temperature thresholds through local adaption (Alali et al., 2019). Strains obtained from warmer climates tend to perform better at high temperatures, while those sourced from cooler regions can have a greater infection efficacy at lower temperatures (Doberski, 1981; Thomas & Jenkins, 1997). The optimal temperature range for germination and mycelial growth of *M. anisopliae* was 25–30°C, and fruit fly susceptibility was influenced by temperature during infection and fly species (i.e. *C. capitate*, *C. fasciventris* and *C. cosyra*) (Dimbi et al., 2004). Suboptimal temperatures could significantly influence the efficacy of fungal entomopathogens for fruit fly control under field conditions. The ability for fungal entomopathogens to survive in the field is also influenced by solar radiation. While inter and

intraspecific variations in the effect of UV radiation occur among fungal entomopathogens (Braga et al., 2001; Huang & Feng, 2009), solar UV-A (320-400 nm) and UV-B (290-320 nm) can be detrimental to conidia viability (Moore et al., 1993). Fernández-Bravo et al. (2017) reported a significant reduction in the virulence of *M. brunneum* to *C. capitata* adults after exposing pure dry conidia to highly energetic UV-B solar radiation (280-315 nm).

The pathogenicity of entomopathogenic fungi when targeting preimaginal flies and newly emerged adults could be influenced by soil moisture. Quesada-Moraga et al. (2006) evaluated the pathogenicity of *B. bassiana* and *M. anisopliae* toward *C. capitata* pupae in sterilised soil at a constant temperature (25°C) under three moisture conditions (-0.1, 0.01 and -0.0055 MPa). At -0.01 MPa, all strains exhibited similarly low levels of pathogenicity, whereas significant differences in pupal mortality occurred between strains at -0.1 and -0.0055 MPa (Quesada-Moraga et al., 2006). Additionally, the effectiveness of entomopathogens in soil appears to be significantly influenced by the complex interplay and variations in soil moisture and temperature. At a temperature range between 20-30°C, Ekesi et al. (2003) demonstrated pupal mortality in *C. capitata* infected with *M. anisopliae* to be highest at water potentials of -0.1 and -0.0035MPa. In wet soils (-0.0035 MPa) there was a significant reduction in colony counts in two out of the four trailed strains of *M. anisopliae* at 25 and 30°C. Conversely, the survival of conidia in dry soil (-0.1MPa) was not affected by temperature. There were differences in mortality effects between fruit fly species at low temperatures (15 and 20°C) combined with water potentials of −0.1 and −0.0035 MPa. However, at higher temperatures and the same moisture levels, the strains were equally pathogenic across the three fruit fly species (Ekesi et al., 2003).

Soil physical and chemical properties have been shown to affect the pathogenicity and occurrence of entomopathogenic fungi. Hallouti et al. (2020) reported *C. capitata*–associated fungi were more abundant in soils with moderate pH (7.5-8), high sand and organic matter

content. High relative humidity negatively affected the abundance of these entomopathogens, and both factors (soil properties and relative humidity) directly influenced fungal infection percentages in *C. capitata* pupae (Hallouti et al., 2020). Gava et al. (2021) demonstrated greater mortality in *C. capitata* pupae when *B. bassiana* was applied to sandy soil versus clayey Utisol. Garrido-Jurado et al. (2011) reported no effect of soil texture or ionic strength on the pathogenicity of *M. anisopliae* and *B. bassiana* strains to *C. capitata* pupae. Quesada-Moraga et al. (2007) found soils with increasing organic matter content, heaviness in soil texture and acidity led to higher percentages of samples harbouring fungal entomopathogens. There can be variability in preference to soil properties between species and strains. For example, studies on the optimum pH range for entomopathogens indicate *M. anisopliae* is better adapted than *B. bassiana* to slightly acidic soils (Issaly et al., 2005; Padmavathi et al., 2003; Quesada-Moraga et al., 2007). Moreover, higher soil pH and exchangeable Ca-ions promoted the occurrence *B. bassiana* while inhibiting *M. robertsii* (Sharma et al., 2021).

### 1.7. Compatibility of entomopathogenic fungi with other biological control agents and pesticides in fruit fly management

The infection of parasitoids and predators by entomopathogens can be broadly grouped into the concept of intraguild predation, where two or more species that share a host or prey (potentially competing) engage in a trophic interaction with each other (parasitism or predation) (Rosenheim et al., 1995). The interaction between entomopathogenic fungi and other biological control agents when targeting pest populations can be synergistic, additive or antagonistic: additive interactions occur when natural enemies do not interact, so mortality is equivalent to the combined individual mortality caused by each agent; synergistic suggests an interaction where one agents causes the target organism to become more susceptible to the other; and antagonistic is an interaction where the agents are in competition of interfere with each other (Abbas, 2020; Northfield et al., 2017; Půža & Tarasco, 2023).

Under laboratory conditions, Usman et al. (2020) demonstrated additive interactions between entomopathogenic nematodes (*Steinernema carpocapsae* and *Steinernema riobrave*) and fungi (*B. bassiana*, *M. brunneum*, *Isaria javanica*, and *Isaria fumosorosea*) when applied against *Rhagoletis pomonella* pupae in all fungus–nematode combinations. Wakil et al. (2022) evaluated the effects of the combined applications of *B. bassiana* and *M. anisopliae*, and the entomopathogenic nematodes, *Heterorhabditis bacteriophora*, and *S. carpocapsae* against larvae, pupae and adults of *B. zonata* and *B. dorsalis* under laboratory, glasshouse and field cage conditions. Laboratory bioassays revealed synergistic interactions between *B. bassiana* and *H. bacteriophora*, when applied against *B. zonata* and *B. dorsalis* larvae and adults, between *B. bassiana* and *S. carpocapsae* against larvae of both species and *B. zonata* adults, and between *M. anisopliae* and *H. bacteriophora* against *B. zonata* larvae. However, under glasshouse and field cage conditions, there were only additive interactions between the combined treatments applied against both flies (Wakil et al., 2022). The results of these studies indicate *Bactrocera* fruit fly species could be effectively controlled through the combination of entomopathogenic fungi and nematodes.

The application of parasitoids for field control of fruit flies offer the advantage of high host specificity, self-perpetuating populations from generation to generation when released in systems with favourable environmental conditions (Dias et al., 2022; Vreysen & Robinson, 2011), and their ability to discriminate between parasitized and unparasitized fruit fly larvae potentially increasing field control efficacy (Devescovi et al., 2020). The most-studied hymenopteran parasitoid species in the biological control of fruit flies belong to the families Braconidae (Opiinae), Pteromalidae, Figitidae (Eucoilinae) *Diachasmimorpha longicaudata* (Ashmead), *Doryctobracon areolatus* (Szépligeti), *Fopius arisanus* (Sonan), *Utetes anastrephae* (Viereck) (Hymenoptera: Braconidae, Opiinae), and *Ganaspis pelleranoi* (Brèthes) (Hymenoptera: Figitidae, Eucoilinae) (Dias et al., 2022).

The risk of intraguild predation between parasitoids and entomopathogenic fungi depend on parasitoid susceptibility to infection as well as timing and method of fungal application. In field-cage trials targeting *C. capitata*, *C. fasciventris*, and *C. cosyra*, the direct soil application of *M. anisopliae* beneath tree canopies in Kenya had no adverse effects on the development of native braconid parasitoid species, *Psyttalia concolor* and *P. cosyrae* (emergence assessed from *Ceratitis* spp. pupae exposed to soil inoculations) (Ekesi et al., 2005). Martínez-Barrera et al. (2019) found no effect of *B. bassiana* on the survival and fecundity of parasitoid, *Coptera haywardi*. However, their simultaneous application against *Anastrepha obliqua* was not effective; low levels of *A. obliqua* mortality by *B. bassiana* (< 21.2%) in field-cage trials could be a contributing factor (Martínez-Barrera et al., 2020). Presa-Parra et al. (2021) evaluated the intraguild predation risk of *M. robertsii* and *M. anisopliae* when applied in conjunction with the braconid parasitoid, *D. longicaudata*, against *A. ludens* larvae. In laboratory assays, there were no adverse effects on parasitoid longevity of those that emerged from parasitized larvae exposed to *M. robertsii*. However, both species of *Metarhizium* caused moderate mortality to *D. longicaudata* adults. The authors thus suggested that to avoid infection of *D. longicaudata*, the fungal entomopathogen should only be applied to the soil or in infective devices to promote their compatibility (Presa-Parra et al., 2021).

The African weaver ant (*Oecophylla longinoda*), the Asian weaver ant (*Oecophylla smaragdina*) and the red fire ant (*Solenopsis invicta*) (Hymenoptera: Formicidae), are major predators of tephritid fruit flies (Heve et al., 2021). Other predators including mites, crickets, beetles and earwigs are known to prey on fruit fly larvae and/pupae in the soil, while spiders prey on adults (Garcia et al., 2020). It is possible that fungal entomopathogens may interfere with predation of adult fruit flies by the predatory ants listed above, or exposure may directly kill the ant colonies established on trees in orchards, although it has not been reported. The

Asian weaver ant workers (common in Australia and Asia) were more resistant to *Metarhizium* when they could secrete venom, which was strongly antimicrobial. Furthermore, behavioural differences varied phenotypically, with minor workers demonstrating lower self-grooming, higher allogrooming and lower up-regulation in response to threats by fungal entomopathogens than major workers (Tranter & Hughes, 2015). The inter and intra-specific differences in defence mechanisms to fungal infection and subsequent impacts on fruit fly predation, particularly by formicid fauna in orchards, is complex and requires further investigation to ensure the conservation of important predatory species.

Given the likelihood of exposure to pesticides by entomopathogenic fungi in the field (e.g., through integrated pest management or residual levels in the system at the time of fungal application), their compatibility in fruit fly suppression warrants investigation. Ekesi et al. (2011) reported an additive interaction between *M. anisopliae* applied to soil and GF-120 spinosad bait sprays in Mango orchards in Kenya when targeting *Bactrocera invadens.* In the 2006/2007 growing season, fruit damage application of *M. anisopliae* (38-46% fruit damage) or GF-120 (28-30% fruit damage) reduced fruit damage compared to untreated control orchards (52-59% fruit damage), while the combined application of *M. anisopliae* and GF-120 spinosad reduced fruit infestations the most (11-16% fruit damage) (Ekesi et al., 2011). In field-cage trials, Ekesi et al. (2005) evaluated the efficacy of *M. anisopliae* formulations (aqueous, oil/aqueous (50:50) and granular) and the chemical insecticide, diazinon, against three *Certaitis* fruit fly species. While all the fungal formulations and diazinon significantly reduced fruit fly emergence from soil, exposure of larvae to treated soil from the field at 183 and 366 days after treatments revealed the *M. anisopiae* formulations were more effective than the insecticide. Additionally, in *M. anisopiae* treated cages, a high number of previously released parasitoids eclosed, indicating no adverse effect on non-target natural enemies. Conversely in diazinion treatments, no parasitoids emerged from treated soil

sampled immediately after inoculation (no effect on parasitoid emergence by diazinion was reported 183 days post-inoculation) (Ekesi et al., 2005).

## 1.8. Conclusion

Entomopathogenic fungi exhibit efficient mortality of tephritid fruit flies at various life stages, environmental conditions and in conjunction with other biological control agents, representing a feasible alternative to chemical control methods. Ideally, soil physiochemical properties should be evaluated prior to soil inoculations in the field for environmental compatibility assays and subsequent effects on pathogenicity by entomopathogens. Numerous laboratory studies have confirmed the efficacy of fungal entomopathogens against fruit flies, but field studies remain comparatively underexplored. This review highlights the need for more field-based research to evaluate pest suppression effectiveness when scaling up from laboratory conditions, with a particular emphasis on the potential for horizontal transmission in pest management regimes. Additionally, further research is needed in fungal entomopathogens as components of integrated pest management regimes, target host specificity by species/strains of entomopathogens reared from soil by different bait species (i.e. using generalist larvae or larvae of the target pest), and non-target effects on the prevalent natural predators of tephritid fruit flies.

# Chapter 2. The infection efficacy of *Metarhizium* strains (Hypocreales: Clavicipitaceae) against the Queensland fruit fly *Bactrocera tryoni* (Diptera: Tephritidae)

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**Amy V. McGuire[1*], William Edwards[1], Tobin D. Northfield[1,2]**

[1] Centre for Tropical Environmental Sustainability Science, James Cook University, Cairns, QLD 4878, Australia.

[2] Tree Fruit Research and Extension Center, Washington State University, Wenatchee, WA, 98802, United States.

[*]**Corresponding author:** amy.mcguire@my.jcu.edu.au

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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).

## 2.1. Abstract

The Queensland fruit fly (Qfly), *Bactrocera tryoni* Froggatt, is a devastating pest of Australia's commercial fruit systems. Fruit fly mitigation is heavily centred 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 three locally sourced strains of entomopathogenic fungi comprising two species, *Metarhizium guizhouense* (Chen & Guo) and *Metarhizium lepidiotae* (Driver & Milner). Additionally, we evaluated two 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 three strains were successful in causing Qfly mortality. *Metarhizium lepidiotae* resulted in the highest mean mortality over the trials, while *M. guizhouense* resulted in 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.

## 2.2. 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 one month, generally spoiling fruit before pupating in the topsoil (Bateman, 1968; May, 1958). Orchard level Qfly management typically includes organophosphate and neonicotinoid insecticides that currently are being phased out (Cremlyn, 1978; Dodds et al., 2014; Hetherington, 2005; Sproul et al., 2001), 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; Ekesi, 2016; Stonehouse et al., 2007; Vargas et al., 2015). 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 & Northfield, 2020b). 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 (Guerrero-Guerra et al., 2013; Milner et al., 2003). *Metarhizium* species are genetically diverse (Brunner-Mendoza et al., 2019), embody a cryptic phylogenetic species complex (Bischoff et al., 2009c) and have different life-history strategies dependent on host availability and biotic and abiotic factors (Bidochka & Small, 2005; Lovett & St. Leger, 2015; McGuire & Northfield, 2020b). For example, when insect host availability is scarce,

fungal species can exist in the soil profile (Korosi et al., 2019; Rocha et al., 2013), grow endophytically in plants (Greenfield et al., 2016a) 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 & Northfield, 2021), and the soil-dwelling Qfly pupal stage makes it a promising target species for these fungal pathogens. Three *Metarhizium* strains comprising two species, *M. guizhouense* and *M. 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 above-ground control by infecting adult flies, with implications for soil application as well.

## 2.3. 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 × 225mm) 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 & Northfield, 2021a), Queensland, and maintained in petri-dishes (90 × 90mm) on malt extract agar (MEA) at room temperature. The three *Metarhizium* strains evaluated were *M. guizhouense* (ARSEF:4303) (Mg1), *M. guizhouense* (ARSEF:7502) (Mg2) and *M. lepidiotae* (ARSEF:7412) (Ml3) in two

different experiments. First, we evaluated the *Metarhizium* strains against Qfly under laboratory conditions over two 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).

### *2.3.1. Laboratory trials: Metarhizium strain efficacy*

The experiment was conducted twice with four 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 four 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. In the *Metarhizium* treatments (Mg1, Mg2 and Ml3), the velvet cloth was inoculated with dry conidia (0.3 g) by scraping spores from MEA petri-dishes and placing onto the cloth. The control treatment followed the same procedure as the *Metarhizium* treatments, but without the application of conidia to the cloth. Twenty flies were transferred into the apparatus and kept there for three minutes while lightly agitating the tube. The flies were transferred to their respective replicate groups (four 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-sterilised in 1% sodium hypochlorite for 3 seconds and rinsed using 70% ethanol for 3 seconds, then rinsed three 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/L 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 four replications per strain.

### *2.3.2 Laboratory trials: Inoculation method efficacy (wet versus dry conidia)*

We compared two methods of inoculating Qfly with *Metarhizium*: 1) application through spores suspended in a carrier solution (0.01% Tween 80) and 2) 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 (Figure S1). This strain was then sub-cultured from the conidia plated directly from Qfly and left for four 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 50ml of Tween 20 (0.1%) and vortexing for 30 s at a concentration of $4 \times 10^7$ spores/ml, determined using a haemocytometer. 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 section 2.1, as did the mortality monitoring and analysis. This experiment was repeated twice, each with four treatment replicates containing 20 flies – DT: dry treatment, WT: wet treatment, with control groups DC: dry control and WC: wet control (Figure S1, Exp. 2).

### *2.3.3. 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 (Harrison, 2015; Warton & Hui, 2011). 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 $P < 0.05$.

## 2.4. Results and Discussion

### *2.4.1. Laboratory trials: Metarhizium strain efficacy*

All three *Metarhizium* strains successfully infected Qfly, resulting in mortality and greater than 90% mean sporulation in all dead flies within treatment replicates (see Figure 2.1 for sporulating Qfly). Sporulation did not occur in the control replicates. The Qfly mortality differed between fungal treatments (likelihood ratio test: $\chi^2$ (3) = 147.54, $p < 0.001$), with significantly higher mortality in treatments inoculated with *Metarhizium* strains when compared to the control group (Figure 2.2). However, there was no significant difference in mean mortality between different *Metarhizium* strains (Figure 2.2). The percent mortality over the two trial periods in the presence of *Metarhizium* strains ranged from 57% (Ml3) to 40% (Mg1 and Mg2), compared to 8% in controls (Figure 2.2). Trial two 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 (Figure 2.2). The treatment replicate with the highest infection efficacy was Mg1 at 100% mortality in trial one. 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 *M. anisopliae*) against various fruit fly species have been reported in other studies, including *Bactrocera zonata* (Ahmad et al.,

2022; El-Gendy et al., 2022; Gul et al., 2015; Hussein et al., 2018; Ibrahim et al., 2014), *Bactrocera cucurbitae* (Hamzah et al., 2021; Iqbal et al., 2021; Sookar et al., 2014b), *Bactrocera dorsalis* (Faye et al., 2021; Melesse & Ferdu, 2021; Wang et al., 2021; Wangkeeree & Suwanchaisri, 2022) and *Ceratitis capitata* (Beris et al., 2013; Castillo et al., 2000b; Ekesi et al., 2002; Quesada-Moraga et al., 2006; 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 & Northfield, 2021a). Thus, the apparent dominance of *Metarhizium* species within tropical soils (McGuire & Northfield, 2020b, 2021a) warrant their consideration for the biological control of geographically similar pest species.

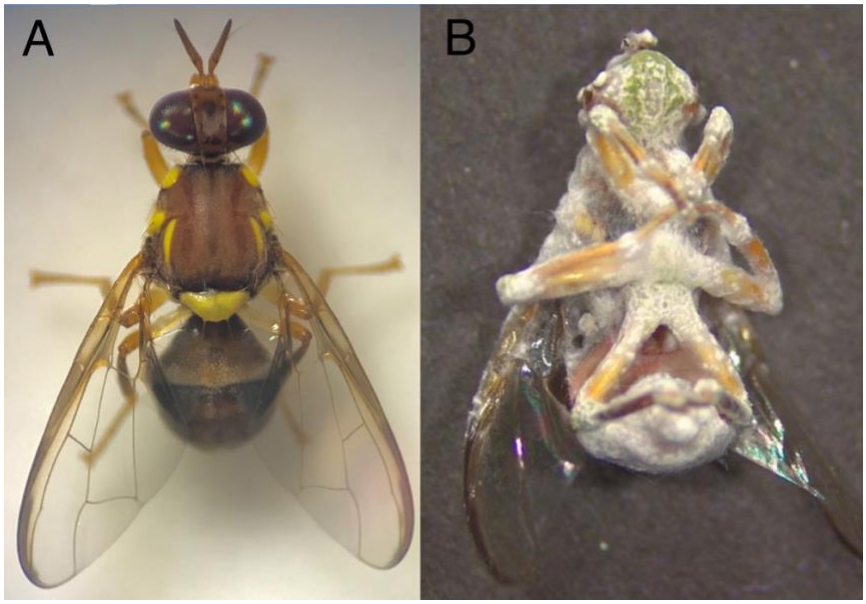


***Figure 2.1.*** *Photograph of a 'clean' Qfly before inoculation of Metarhizium strains (A), and afterwards resulting in death and sporulation (B).*

### *2.4.2. Laboratory trials: Inoculation method efficacy (wet versus dry conidia)*

We observed significant differences in Qfly mortality in the different inoculation methods (likelihood ratio test: $\chi^2$ (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%) (Figure 2.3). 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 two trials compared to exposure through a conidial suspension (wet treatments), an increase in probability from 0.59 to 0.88 (Figure 2.3). Within-treatment variability was similar between the two trials and Qfly mortality, and exposure from dry conidia generally resulted in less variability compared to the wet treatments (Figure 2.3).

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

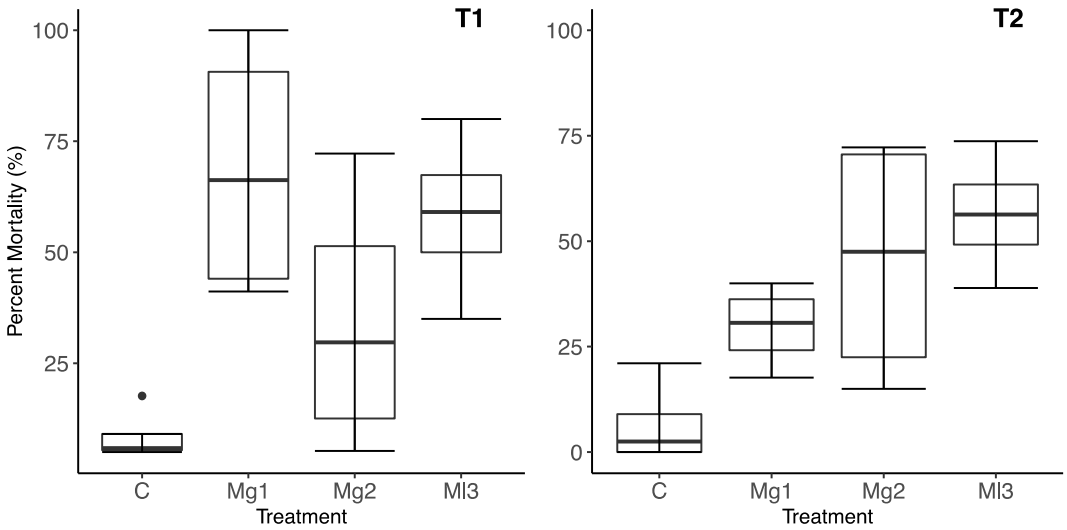


***Figure 2.2.*** *Box-plot of experiment 1 laboratory of trials one (T1) and two (T2) : mean percent mortality of B. tryoni at the end of the trial period (day 14) according to treatment type (control versus Metarhizium strains: Mg1, Mg2, Ml3). These plots illustrate the inter-quartile range (IQR) between lower and upper box boundaries 25$^{th}$ and 75$^{th}$ percentiles, respectively.*

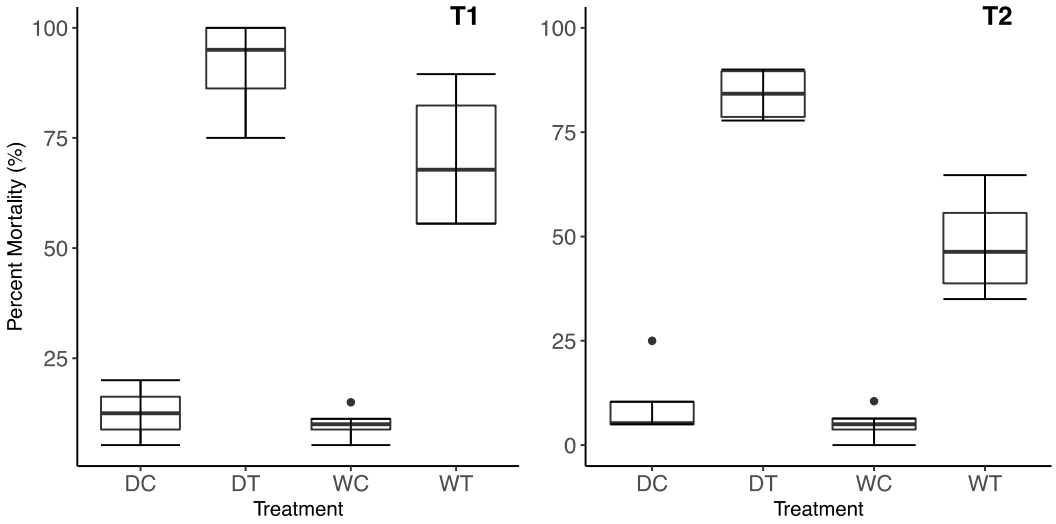


***Figure 2.3.*** *Box-plot of experiment 2 laboratory of trials one (**T1**) and two (**T2**) : mean percent mortality of B. tryoni at the end of the trial period (day 14) according to treatment type (DC: dry control, DT: dry treatment, WC: wet control, WT: wet treatment. These plots illustrate the inter-quartile range (IQR) between lower and upper box boundaries 25$^{th}$ and 75$^{th}$ percentiles, respectively.*

# Chapter 3. Enhanced virulence of *Metarhizium guizhouense* (Hypocreales: Clavicipitaceae) against the Queensland fruit fly (Diptera: Tephritidae) through directed selection

**Author Contributions:** Amy McGuire: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft. William Edwards: Supervision, Writing – review & editing. Tobin Northfield: Conceptualization, Methodology, Supervision, Writing – review & editing.

## 3.1. Abstract

Fungal entomopathogens exhibiting naturally high virulence are preferably sought for the biological control of important pest species. While entomopathogens are typically mass produced in laboratories, in nature virulence is often under selection from coevolution between host and pathogen, potentially playing out over short time scales. The potential for rapid evolution of some fungal entomopathogens could make them optimal candidates for unidirectional artificial selection, where strains are adapted to insect hosts to enhance virulence to improve the biopesticides in which they're used. We carried out a selection experiment, allowing for unidirectional selection to occur between *Metarhizium guizhouense* (Mg1) and the Queensland fruit fly (Qfly), *Bactrocera tryoni* Froggatt. After just one round of selection (isolate Mg1_1), we observed a significant increase in Qfly mortality compared to the parent strain (Mg1_0). The second serial passage through Qfly (isolate Mg1_2) maintained the virulence gains from the first round of selection, but there was no significant further improvements. The genetic variance and significance of host mortality between serially passed isolates is an important consideration when evaluating the effects and applicability of unidirectional selection on virulence. Optimising fungal pathogenicity through *In vivo* serial passages could overcome limitations in the efficacy of microbial control agents, particularly for locally adapted strains with naturally low virulence.

## 3.2. Introduction

Entomopathogenic fungi are gaining traction in coevolutionary studies and pest management programs, where generalist species such as *Metarhizium anisopliae* and *Beauveria bassiana* have been extensively evaluated for their efficacy against numerous key pests (McGuire & Northfield, 2020b; Peng et al., 2022; Sharma et al., 2023). The ongoing assessment of over 1000 species of entomopathogenic fungi for commercialisation as biopesticides represents a significant advancement in the tools available for sustainable pest management (Rajula et al., 2021). Nevertheless, despite their potential, the adoption of commercial entomopathogens lags behind chemical alternatives. This discrepancy can be attributed to factors such as the time required to effectively reduce insect pest populations, the need for adequate environmental conditions, and the inherent variability in virulence among strains, which exhibit differing degrees of host specificity (Lovett & St Leger, 2018).

Entomopathogenic fungi typically initiate infection by penetrating the insect cuticle after spores adhere to the host, thereby triggering internal hyphal growth (Sharma et al., 2023). Once the insect has died from infection and nutrients are exhausted (typically occurring within 6-14 days), the hyphae can readily emerge from the cadaver and produce spores that cover the host body (Bava et al., 2022; Charnley, 2003). To overcome host immunity, distinct infection pathways evolve, often subjecting the fungi and their insect hosts to intense selection pressures leading to intimate genetic adaptations, fostering tightly coupled arms races (Qu & Wang, 2018).

The process of infection, virulence and overcoming host immunity differs between generalist and specialist entomopathogenic fungi (Lovett & St Leger, 2018). Generalists appear to be better adapted to the environment, exhibiting high abundance in rhizospheric soil (McGuire & Northfield, 2020b). The mode of infection by generalists is effective across a diverse range of insects as the pathogen overpowers host immunity through numerous

enzymes and toxins. In contrast, fungal species that are highly specialised to their insect host (e.g., typically above-ground feeders in high abundance) generally do not produce toxins and can take longer to induce mortality; demonstrating fewer universal tools of killing than generalists (St. Leger & Wang, 2020). In the case of specialisation, the insect then becomes the principal source of selection pressure and virulence becomes a limiting factor when assessing its compatibility with other potential insect hosts (Kryukov et al., 2020). For example, *Metarhizium* strains with narrow host ranges may exhibit considerably less physiological adaptability, and require certain chemical and physical features of their specialised host cuticle to onset infection (Wang & St Leger, 2005).

Optimal entomopathogenic strains ideally demonstrate high virulence against the target pest and are compatible with the applied system of control (i.e., can persist within the prevalent climatic and environmental conditions). However, locally reared strains may still possess inadequate abiotic tolerances at the time of application and naturally low virulence. Yet, these strains, which are genetically modifiable, hold promise for improvements tailored to the target pest or applied system, thereby overcoming limitations posed by restricted sample sizes of fungal entomopathogens through rearing techniques. For example, directed evolution by continuous culture led to an increased tolerance to high temperatures by *M. anisopliae* (de Crecy et al., 2009). Additionally, previous studies have reported an increase in virulence by entomopathogenic fungi after repeated passages through the insect host (Adames et al., 2011; Hayden et al., 1992; Joop & Vilcinskas, 2016; Quesada-Moraga & Vey, 2003; Rafaluk et al., 2017; Song & Feng, 2011), while others report no change (Latch, 1976; Scully & Bidochka, 2005; Valero-Jiménez et al., 2017; Vandenberg & Cantone, 2004), and in some cases, virulence was reduced (Hussain et al., 2010; Tomilova et al., 2023; Vandenberg & Cantone, 2004). Leveraging the genetic manipulability of entomopathogenic fungi presents an opportunity to investigate the impact of laboratory-directed evolution on

virulence and explore the feasibility of strain improvements, beyond genetic engineering, to optimise pest control.

We evaluated the potential to evolve a more virulent strain of *Metarhizium guizhouense* against the Queensland fruit fly (Qfy), *Bactrocera tryoni* Froggatt. *Metarhizium guizhouense* belongs to the *Metarhizium anisopliae* complex (Bischoff et al., 2009b) and has been identified as having an intermediate host range (Hu et al., 2014; Xu et al., 2016), found occurring on various insect orders (i.e. *Coleoptera*, *Diptera, Lepidoptera*) (Mongkolsamrit et al., 2020) and in the soil (McGuire & Northfield, 2021b; Mongkolsamrit et al., 2020). Endemic to tropical north-eastern Queensland, Qfly is the most devastating pest of Australia's $9 billion/annum horticultural industry (Hort, 2016), as well as a devastating pest to fruit production in the areas it has invaded (Clarke et al., 2011; Drew et al., 1982; Sutherst et al., 2000). Adults deposit eggs beneath the skin of fruit, and once hatched, the larvae feed on the flesh, leading to premature fruit drop due to decay. Temperature plays a pivotal role in the population ecology and physiology of Qfly. Within its endemic thermal range, breeding is only constrained by host availability (Muthuthantri et al., 2010).

Given Qfly's prevalence in tropical regions, where conditions favour fungal persistence and proliferation, coupled with its high local infestation rates, we investigated if a locally adapted strain of *M. guizhouense* has potential to control this pest. We facilitated unidirectional selection with the goal of augmenting virulence. This research seeks to address key challenges in the accessibility and efficacy of microbial control by optimising fungal entomopathogen strains through targeted laboratory bioassays. Entomopathogenic fungi inhabit almost all natural and agricultural environments. In cases where reared strains exhibit suboptimal virulence, we aim to demonstrate that virulence can be enhanced efficiently to improve pest infection success. Specifically, this study focuses on rapid virulence selection through serial host passages, providing a practical strategy to overcome one of the major

limitations in broader adoption – low natural virulence. This work ultimately aims to support the greater use of fungal entomopathogens in pest management by presenting a feasible approach to boost their efficacy.

## 3.3. Methods and Materials

We obtained Qfly pupae from the QLD Department of Agriculture fruit fly rearing facility, Cairns. Adult flies were fed on 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 × 225mm) and maintained at room temperature for 10 days prior to commencing the experiment and for its duration. The strain used in the experiment, *Metahrizium guizhouense* (ARSEF:4303) (Mg1), was reared from soil samples collected from Ecoganic banana farms at South Johnstone, Queensland, and maintained in petri-dishes (90 × 90mm) on malt extract agar at room temperature. In previous experiments, (see Chapter 2, McGuire et al., 2023a) we introduced the parent strain (Mg1_0) to Qfly through a cylindrical apparatus containing a velvet cloth inoculated with dry conidia and confined them for three minutes at 4°C to slow their movement while lightly agitating the cylindrical tube. The treatment replicate with the highest mortality after 14 days was used to rear the next generation of Mg1 directly from the fly (Mg1_1). The Mg1_1 isolate was introduced to Qfly in the same manner as previously described, and the highest mortality replicate reared the third and final Mg1 generation to be evaluated in the selection experiment (isolate Mg1_2) (Chapter 2, McGuire *et al*., 2023).

### *3.3.1. Selection experiment*

The experiment was conducted twice, with four replicate groups per treatment group (Mg1_0, Mg1_1, Mg1_2 and control). Qfly mortality was monitored and recorded daily until the end of the trial period (14 days). Each generation of Mg1 was maintained on malt extract

agar in petri-dishes for four weeks leading up to the experiment. The inoculation method for Qfly followed a similar method outlined by Dimbi et al. (2003). Flies were inoculated by confining them in a cylindrical apparatus (clear vinyl tube) lined with a velvet cloth and capped at both ends. In the Metarhizium treatments (Mg1_0, Mg1_1 and Mg1_2), the velvet cloth was inoculated with dry conidia (0.3 g) by scraping spores from malt extract agar petri-dishes and placing the spores onto the cloth. The control treatment followed the same procedure as the *Metarhizium* treatments, but omitted the conidia application. Twenty flies were then transferred into the apparatus and kept there for three minutes while lightly agitating the tube. The flies were transferred to their respective replicate groups (four replicate groups per treatment), and fed on sucrose, yeast paste and water. All inoculations were conducted at 4°C to reduce Qfly activity. At the end of the trial, dead flies were surface-sterilised in 1% sodium hypochlorite for 3 seconds, followed by rinsing in 70% ethanol for 3 seconds, and then rinsed three times with distilled water. The flies were transferred into petri-dishes lined with damp filter paper to promote sporulation. Spores where cultured directly from the cadavers onto malt extract agar plates containing 0.3g/L of chloramphenicol to confirm infection by *Metarhizium* strains and inhibit bacterial growth.

We employed generalised linear mixed-effects models using the function glmer in the R package lme4 (Bates et al., 2015) with a binomial distribution to test for the main effect of treatment type (C, Mg1_0, Mg1_1, Mg1_2) and a fixed effect of trial. To evaluate the full treatment effect, a likelihood ratio test was used, and Tukey's style tests evaluate all treatment comparisons using the multcomp package in R (Hothorn et al., 2008). Results were deemed significant when $P < 0.05$.

### 3.4. Results

We observed a significant difference among altered levels of entomopathogenic fungal exposure to Qfly ($\chi^2$ (3) = 251.73, p < 0.001), with significantly higher mortality in

treatments inoculated with *Metarhizium* when compared to the control group (Figure 3.1, Table 3.1). The Mg1_1 isolate (selection round 1) resulted in the highest mean percent mortality between the two trials (92.8%), followed by isolate Mg1_2 (selection round 2) (89.1%), isolate Mg1_0 (parent strain) (62.1%) and the control group (18.1%). Comparisons of the mean mortality between the treatments revealed a significant difference between the parent strain (Mg1_0) and the isolates which had been serially passed through Qfly (Mg1_1 and Mg1_2) (Table 3.1). However, there was no significant difference in the mean mortality between isolates produced after one or two rounds of selection (Table 3.1). Additionally, mortality due to infection from serially selected isolates revealed a considerably lower variance when compared to the parent isolate, Mg1_0 (Figure 3.1).

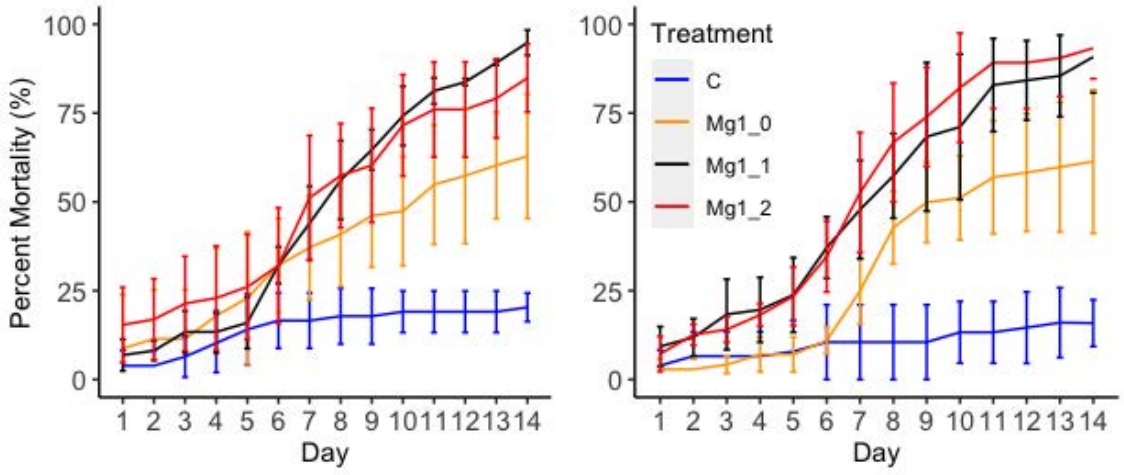


***Figure 3.1.*** *Experiment 1 laboratory of trials one (left plot) and two (right plot) mean percent mortality of B. tryoni at over a 14-day period according to treatment type (control, C (blue), and Metarhizium isolates: Mg1_0 (yellow), Mg1_1 (black), Mg1_2 (red)). Bars represent standard deviation of the mean.*

***Table 3.1.*** *Multiple comparisons of the mean mortality for treatments over two laboratory trials (Tukey contrast). There was a significant effect of treatment (χ2 (3) = 251.73, p<0.001) according to a likelihood ratio test on a binomial generalized linear model with a fixed effect of trial.*

| Treatment | Estimate | Standard error | *z* value | P |
|---|---|---|---|---|
| Mg1_0 -Control | -2.0020 | 0.2682 | -7.465 | <0.001 |
| Mg1_1 - Control | -4.0437 | 0.3766 | -10.736 | <0.001 |

| | | | | |
|---|---|---|---|---|
| Mg1_2 - Control | -3.6277 | 0.3442 | -10.538 | <0.001 |
| Mg1_1 - Mg1_0 | -2.0417 | 0.3557 | -5.740 | <0.001 |
| Mg1_2 - Mg1_0 | -1.6257 | 0.3212 | -5.062 | <0.001 |
| Mg1_2 - Mg1_1 | 0.4160 | 0.4158 | 1.000 | 0.745 |

### 3.5. Discussion

The present study demonstrated that serial passages of *M. guizhouense* through Qfly can lead to enhanced virulence. Prior to this study (Chapter 2, McGuire et al. 2023), we conducted a series of inoculation experiments, resulting in three isolates of *M. guizhouense* (Mg1) with different levels of exposure to Qfly. These isolates, used to evaluate the effects of unidirectional selection on virulence, demonstrated that isolates serially passed through Qfly (Mg1_1: one host transfer and Mg1_2: two host transfers) exhibited significantly higher mortality (a proxy for virulence) when compared to the parent strain (Mg1_0). Interestingly, there was no significant difference in morality between serially passed Mg1 isolates. The similarity in Qfly mortality data between serially passed isolates, and the significant difference between these treatments and the parent strain, Mg1_0, suggests that an initial mutation led to improved control rather than incremental changes in virulence from serial transfers. Remarkably, just one host transfer was sufficient to enhance the pathogenicity of *M. guizhouense* against Qfly, consistent with previous observations (Chapter 2, McGuire et al., 2023a). This discovery highlights a practical approach to overcoming naturally low virulence of some fungal strains, supporting their broader adoption in pest management programs.

Evolutionary arms races between pathogen and pest could derive optimal biological control agents. The expression of physiological and biochemical *In vivo* mutations depend upon whether they are deleterious, neutral or beneficial, shaping genetic variation within a population (Szűcs et al., 2019). The genetic variance relating to the virulence of fungal

entomopathogens between serially past and ancestral isolates represents an important research niche for optimising biological control. Consistent with our findings, studies have demonstrated a notable augmentation in fungal virulence after a single passage through the insect host. For instance, researchers have documented a marked increase in the virulence of *M. anisopliae* following a sole passage through hosts, mosquito larvae (*Culex pipiens*) (Daoust & Roberts, 1982) and cotton bollworm (*helicoverpa armigera*) (Nahar et al., 2008). After two passages through plant hopper nymphs (*Nilaparvata lugens*), Song and Feng (2011) noted no significant effect of additional host transfers, similar to our results. Notably, Daoust and Roberts (1982) reported a significant increase in virulence against *C. pipiens* after passage through an alternative host (brown planthopper, *Nilaparvata lugens*). Furthermore, enhanced virulence has been noted when isolates are cultured on selective media incorporating the insect host cuticle (Hayden et al., 1992). Conversely, reports have highlighted a de-adaption process on non-selective media following repeated sub-culturing, leading to the attenuation of fungal cultures (Daoust & Roberts, 1982; Nahar et al., 2008; Shah et al., 2007).

Noteworthy is the study by Tomilova et al. (2023) which observed minimal impacts on the virulence of *Metarhizium robertsii* when sub-culturing on non-selective media, yet noted a decrease in virulence after eight serial passages through G*alleria mellonella* larvae. They attributed the diminished virulence to a decline in lipolytic and proteolytic activities in *M. robertsii* reisolates, impairing its ability to penetrate the insect cuticle and trigger insect immune reactions. This phenomenon potentially facilitates evasion of the host's defence mechanisms, thereby favouring fungal survival and propagation (Tomilova et al., 2023). Reductions in virulence after serial host passages observed in other studies could be attributed to an initially highly virulent strain (Tomilova et al., 2023). This hypothesis could explain the significant change after a single passage in the present study, as the parent strain

(Mg1_0) exhibited sub-optimal virulence levels against Qfly, leaving room for adaption in its infection potential. This revelation underscores the necessity of understanding the initial virulence profiles among different fungal strains in an applied sense. In this context, it illuminates an extremely dynamic process where virulence can be modified through a multitude of pathways. The outcomes are intricately linked to the pathogenicity of the parent strain and the allocation of resources towards expressed genes that favour either virulence or propagation.

### 3.6. Conclusion

The pathogenicity of entomopathogenic fungi to insect pests stands as a crucial pre-requisite for their integration into biological control regimes. Serial host passages of fungal entomopathogens could offer an advantage over an evolutionary static host at the time of application. While continued application might eventually lead to resistance in the pest population, our experiments demonstrate a potential method for troubleshooting the strain to reclaim an edge in infection efficacy when needed.

# Chapter 4. Theory for optimizing attract-and-infect methods for pest management

**Author Contributions:** Amy McGuire: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft. William Edwards: Supervision, Writing – review & editing. Hazel Parry: Writing – review and editing. Tobin Northfield: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Supervision, Writing – review & editing.

## 4.1. Abstract

The attract-and-infect system in insect pest control is garnering attention amid growing interest in alternatives to chemical insecticides. By employing traps that capture males with lures and inoculate them with microbes before releasing them, known as autodissemination traps, pest managers can indirectly target the female portion of the population through horizontal transmission. We developed theory to assess the potential for pest suppression using entomopathogenic fungi. In particular, we focus on insects with larval life stages, with special reference to Tephritid fruit flies. The theory, developed by evaluating a susceptible-infected-recovered (SIR) model, suggests there is a necessary balance between pathogen-induced mortality that reduces the likelihood of infected males mating and the impacts of infection on female reproduction or mortality. When infection reduces female reproduction, high pathogen-induced mortality hinders the mating success of inoculated males, leading to high larval numbers. However, when there is no reduction in fecundity from infection, some mortality is needed to reduce population growth. If the pathogen-induced mortality is too high, however, infected males are not able to mate, reducing transmission to females, eventually leading to population growth. Thus, the key to attract-and-infect program success may depend on finding the right pathogen that strikes a balance between keeping virulence low enough to allow males to successfully mate, while being virulent enough to kill females, or at least reduce their reproduction.

## 4.2. Introduction

As we face a so-called 'chemically-limited future' for insect pest control (Hunt et al., 2011), producers will increasingly need to develop and rely on alternative management strategies to conventional chemical pesticides. Such approaches are generally more complex to administer, require a higher level of ecological knowledge to ensure effectiveness, and still have gaps in knowledge around when and how they can be best applied to achieve pest suppression (Collier & Van Steenwyk, 2004; Doerr et al., 2012). Mathematical modelling can help determine how the deployment of novel approaches could be optimised (Blackwood et al., 2012; Blackwood et al., 2018; Ghosh & Bhattacharya, 2010; Lampert & Liebhold, 2021), allowing for simulated experimentation than can accelerate and scale-up field-based trials.

One alternative to chemical controls, the use of entomopathogenic fungi as biological control agents, is being rapidly adopted in pest management regimes throughout the world (Lacey et al., 2015). Fungal entomopathogens are commonly found inhabiting the soil profile when not infecting an insect host (Konopická et al., 2022; Majchrowska-Safaryan & Tkaczuk, 2021), with the potential for field transmission to occur. Historically, research on entomopathogenic fungi are focused on field application in the same general method as a chemically based insecticide, with the assumption that field transmission is not common. However, there has been growing interest in the potential to control pest populations through horizontal transmission when infected insects mate with their uninfected counterparts (e.g. Dimbi et al., 2013a; Kreutz et al., 2004; Onsongo et al., 2022; Opisa et al., 2019; Paradza et al., 2021; Sookar et al., 2014c; Srei et al., 2019). Thus, there may exist an opportunity to engineer an epidemic of entomopathogenic fungi for insect vector control using a method, known as 'autodissemination'. This strategy involves momentarily trapping insects, which are then infected with an active compound or microbe such as a fungal entomopathogen. Subsequently, the infected insect acts as a vector, disseminating the compound or microbe

throughout the wider population (Dembilio et al., 2018; Hassemer et al., 2020; Toledo et al., 2017). Auto-dissemination offers a more targeted and sophisticated control mechanism, with parallels to other novel biological approaches. For instance, gene drive techniques facilitate the spread specific traits – such as sterility or resistance to disease – through populations by biasing inheritance (Hammond et al., 2016). Similarly, genetically modified endosymbionts like *Wolbachia* bacteria propagate through insect populations by altering reproduction and inducing cytoplasmic incompatibility, a form of reproductive parasitism (Mateos et al., 2020). Here, we reconsider epidemic theory, originally developed to understand how to reduce epidemic outbreaks, to determine when and how we can make an epidemic have the greatest impact on its host to optimize pest control.

Fungal disease epidemics within insect pest populations are dependent on complex bioecological factors in both the pathogen and host population (Sharma & Sharma, 2021). The development of theoretical frameworks aimed at unravelling the underlying mechanisms that shape the development and spread of an introduced pathogen among pest populations is paramount. This theoretical foundation is essential for the successful application of pest management techniques across diverse scenarios. Epizootics produced by fungal entomopathogens exhibit notable efficacy in supressing various agriculturally important insects, including grasshoppers, leafhoppers, aphids, and soil-dwelling pests (Bidochka et al., 2000). Many of these fungi are currently being exploited or developed for commercial crop protection with encouraging results, albeit with limited adoption (Rajula et al., 2020).

In the absence of reliable field data pertaining to pathogen transmission, mathematical modelling becomes an important method to evaluate dynamic characteristics within epidemiological ecosystems (Anderson & May, 1981; Keeling & Rohani., 2008). Most epidemiological models depend on the compartmentalization of hosts according to their disease status and assume an idealised susceptible population with random mixing (Anderson

& May, 1991; Bailey, 1957; Diekmann & Heesterbeek, 2000). These simple but fundamental models are based on the division of a host population into three compartments: susceptible (S), infected (I) and recovered (R), which is known as the SIR model (Anderson & May, 1979). In this framework, the susceptible compartment includes individuals who are vulnerable to infection, the infected compartment contains infected individuals that can transmit the disease to others, and the recovered compartment represents hosts who have overcome the infection and now assumed to be immune. The infection rate is proportionate to the number of susceptible and infected individuals (Anderson & May, 1979; Otto & Day, 2007). For more complex disease transmission scenarios, models have incorporated additional compartments and intricate flows between them. These adaptions account for parameters such as latency, loss of immunity, births and deaths (Bjørnstad et al., 2020; Keeling & Eames, 2005), and heterogeneous mixing assumptions (Volz & Meyers, 2007). Relevant models have demonstrated mathematical evidence for controlling a pest population by infectious disease (Sun & Chen, 2009; Wang & Song, 2008).

In this model analysis, we direct our attention towards introducing a fungal entomopathogen into a host population with the objective of reducing pest numbers by enabling horizontal transmission from infected males, initially captured in traps, to susceptible female flies during mating. The potential for this mode of transmission has been demonstrated in labrortory bioassays with two tephritid fruit fly species, *Bactrocera cucurbitae* and *Bactrocera zonata,* utilising *Metarhizium anisopliae* (Sookar et al., 2014). Their findings revealed significant mortality effects in both sexes attributed to transmission during mating. Notably, infected male flies were capable of transmitting a lethal dose to at least three mates before succumbing to infection (Sookar et al., 2014). Similarly, Faye et al. (2021) developed autoinoculation traps containing *Metarhizium acridum* and the parapheromone, methyl-eugenol, resulting in significant reductions in *Bactrocera dorsalis*

under field conditions within citrus orchards in Senegal. Analysis of recovery traps provided insights into the infection rate of *B. dorsalis* males exposed to the autoinoculation traps. The contamination rate displayed an escalating trend with greater trap densities (potential horizontal transmission was not monitored) (Faye et al., 2021).

Despite preliminary evidence for effective mating transmission of entomopathogens (Dimbi et al., 2013a; Khun et al., 2021; Maniania, 1998; Mkiga et al., 2020; Onsongo et al., 2022; Opisa et al., 2019; Sookar et al., 2014c; Toledo et al., 2007), there is little theory describing how this pest management approach could scale up to population levels. Moreover, field studies demonstrating successful control of pests through the autodissemination of fungal entomopathoegns are limited, and tracking pest mortality attributable solely to transmission via mating in the field presents significant challenges. These limitations highlight a need for a modelling approach to analyse critical infection parameters that could improve the effectiveness of this pest management strategy at a population level. Here, we developed a mathematical epidemiological model adapted from the SIR framework delineated above to evaluate infection dynamics within natural pest populations. We parameterized our model based on fruit flies, for which attract-and-infect systems have been commonly evaluated (Faye et al., 2021; Navarro-Llopis et al., 2015; Onsongo et al., 2022). We conducted an assessment with the model that enabled us to identify the important physiological factors influencing the efficacy of population control, due to entomopathogenic fungal transmission in pest populations.

### 4.3. Model description and analysis

The model is designed to evaluate the effect of pathogen transmission on pests that primarily cause economic damage in their larval stage, such as fruit flies. We define the total number of susceptible adults (uninfected), infected adults and immature pest hosts to be $S$, $I$ and $L$, respectively. We assume that immature hosts ($L$) cannot be infected (i.e., no transovarial

transmission), susceptible females ($S_f$) are only infected from exposure to infected males ($I_m$), and male infection is determined by transmission rates upon exposure to inoculated traps ($\beta_m$). We also assume that once infected, all cases eventually result in mortality with a given mortality rate due to infection α, i.e. the recovery rate $v$ is fixed at zero and is therefore not incorporated in the equations that follow, with an absence of recovered ($R$) individuals (Figure 4.1).

We include both density independent and dependent population control, by including a term $b$ that represents density independent mortality of adults (male and female), and a term $k$, that represents the single species carrying capacity when $b = 0$, which is applied to the larval stage only. We track populations of males ($S_m$ and $I_m$) and females ($S_f$ and $I_f$) separately and assumed that immature hosts ($L$) are born with an equal sex ratio. The reproductive rate of susceptible and infected females is defined by $r$ and $r$', respectively. Modifying the reproductive impact on female infection is represented by the equation $\gamma = r'/r$. The maturation rate from birth to adult for the immature individuals ($L$) of the population is $\mu$. The baseline adult mortality rate in the absence of infection, $b$, is constant for both sexes, whereas $b_L$ denotes the mortality rate of larvae. Finally, $\alpha_m$ and $\alpha_f$ represents mortality rate due to infection for males and females, respectively.

Our model is similar to a typical model of sexually transmitted disease (e.g. Anderson & May, 1991). Biologically, male infection occurs only by landing in pheromone-baited traps and female infection only through mating, but for simplicity we describe male transmission with a single parameter, $\beta_m$. In reality, the value of this parameter will increase with each, the number of traps applied, the effectiveness of each trap at capturing males, and the likelihood that a captured male becomes infected. Because females become infected through mating with infected males, the female transmission rate, $\beta_f$, is dependent on the mating rate, $\delta$, the

total fraction of infected males, $I_m$, and the probability of a female becoming infected given mating, $p_\delta$:

$$\beta_f = \delta p_\delta (I_m/(S_m+I_m))$$

The differential equations for infection dynamics are given by,

$$dL/dt = (1-L/k)(rS_f + r'I_f) - \mu L - b_L L \quad (1),$$

$$dS_m/dt = \mu(L/2) - \beta_m S_m - bS_m \quad (2),$$

$$dI_m/dt = \beta_m S_m - (\alpha_m + b)I_m \quad (3),$$

$$dS_f/dt = \mu(L/2) - \beta_f S_f - bS_f \quad (4),$$

$$dI_f/dt = \beta_f S_f - (\alpha_f + b)I_f \quad (5).$$

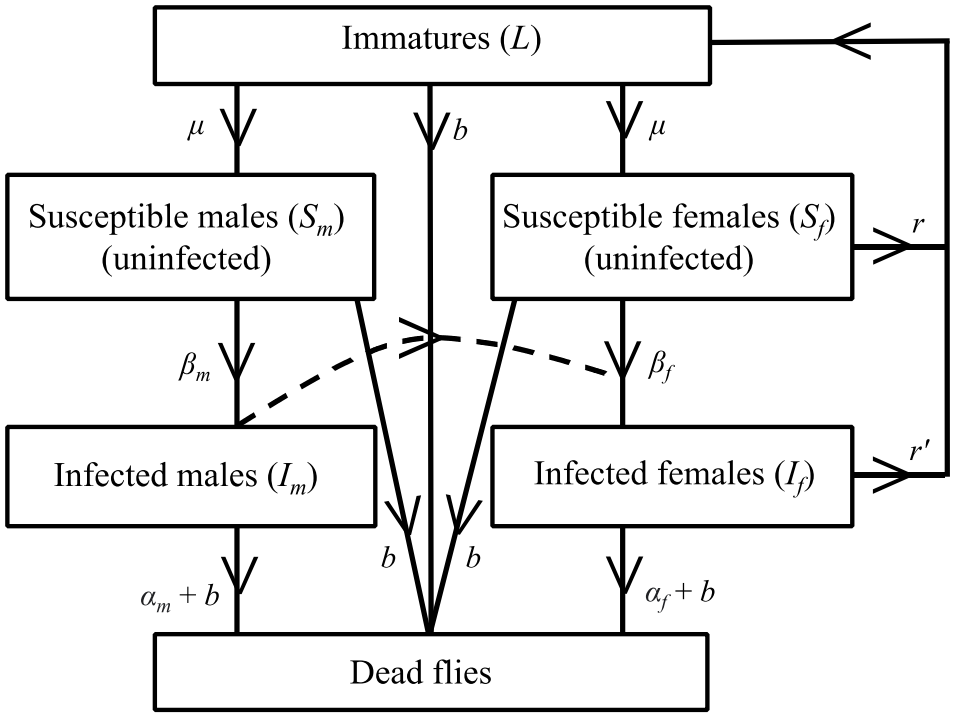


***Figure 4.1.*** *Structural outline of each population compartment and the infection dynamics driving final population densities. Solid lines illustrate the progression of the flies through life stages, ultimately leading to death from infection, or reproduction in one life stage that contributes to the number of individuals in another life stage. The dotted line represents the impact of infected males on female transmission rate ($\beta_f$), as the source of pathogen exposure.*

In our simulations, initial densities of susceptible males, susceptible females, and immatures were $1\times10^{-6}$. As the introduction of the pathogen is not expected to occur naturally, and is instead primarily introduced through trapping males, the infected portion of the population had an initial density of 0 (infected males and females). We selected parameter values (Table 4.1) related to life history characteristics outlined in fruit fly biology and reproduction studies across different species (Balagawi et al., 2023; Dimbi et al., 2013a), as well as field trapping data (Lance & Gates, 1994; Shelly, 2021) and infection and transmission dynamics pertaining to fungal entomopathogens (Dimbi et al., 2013a; McGuire et al., 2023b; Onsongo et al., 2022; Sookar et al., 2014c). We determined the initial parameter values (listed in Table 4.1) based on realistic relative rates. In cases where parameters are unknown, we used terms that led to realistic population abundances that allow for persistence of the pathogen, to determine when population regulation by autodissemination is effective versus ineffective. Parameter values related to ecological and behavioural characteristics (e.g., male transmission rate, mating frequency, and likelihood of infection) are more uncertain, as they are difficult to obtain parameter estimates for. While the male transmission rate has been simplified into a single parameter for modelling purposes, to establish a realistic initial value, we consider its dependence on trap capture rates and the probability of becoming infected once trapped (which are not explicitly modelled here). However, mark-recapture rate studies in fruit flies have documented trap rates ranging from 0 to 10% (Barry et al., 2002; Lance & Gates, 1994; Shelly, 2021; Shelly et al., 2014). To obtain even a value of 0.05 for male transmission ($\beta_m$), relatively high rates of infection in trapped males (e.g., 80-100%) are necessary to compensate for realistic trapping rates (e.g. 5-6%).

We determined realistic values based on previous literature (as denoted in Table 4.1) and consider the model sensitivity to variability in these model parameters. Starting with this

set of parameter values allows for an ideal scenario where control potential should be high. We then systematically varied some parameters from this parameter set to demonstrate the impacts of each on control. For the parameter values we considered, the population typically reached equilibrium in less than 500 timesteps, with each time step equivalent to a day. We present results from simulations run up to 1000 timesteps.

***Table 4.1.*** *Model parameter definitions, initial values and range evaluated. Initial values with superscript letters correspond to the reference used to estimate its value (listed below table). References [a]Balagawi et al. (2023), [b]Dimbi et al. (2013a), [c]Shelly (2021), [d]Giardina et al. (2017), [e]Mortality is calculated per day. A mortality of 0.1 is equivalent to 77% survivorship over a 14-day period (1-0.914)(McGuire et al., 2023b).*

| Parameter | Definition | Initial value | Range evaluated |
|---|---|---|---|
| $r$ | Reproductive rate (susceptible females) | 2 [a] | 2, 4 (Figure 4, 5) |
| $r$' | Reproductive rate (infected females) | 0.5 [b] | 0.001-2 (Figure 4 and 5) |
| $\mu$ | Maturation rate from birth for the immature individuals | 0.04 [a] | 0.04 (constant) |
| $b$ | Mortality rate in absence of infection | 0.05 [a] | 0.05 (constant) |
| $b_L$ | Mortality rate of larvae | 0.05 [a] | 0.05 (constant) |
| $\alpha_m$ | Mortality due to infection (males) | 0.1 [e] | 0–1 (Figure 4, 5) |
| $\alpha_f$ | Mortality due to infection (females) | 0.1 [e] | 0–1 (Figure 4, 5) |
| $\beta_m$ | Transmission rate of the pathogen in males | 0.05 [c] | 0–0.5 (Figure 2, 3A, 4, 5) |
| $p_\delta$ | Probability of a female becoming infected given mating | 0.75 [b] | 0–1 (Figure 3D) |
| $\delta$ | Mating rate | 0.8 [d] | 0–1 (Figure 3C) |
| $k$ | Carrying capacity | 1000 | 1000 (constant) |

## 4.4. Results and Discussion

Increasing the transmission rate of the pathogen in males from $\beta_m = 0$ (Figure 4.2A) (presumably through either increasing trap effort or efficacy) reduced equilibrium larval population densities by 14% at $\beta_m = 0.05$ (Figure 2B) and 56% at $\beta_m = 0.50$ for our particular parameter set (Figure 4.2C).

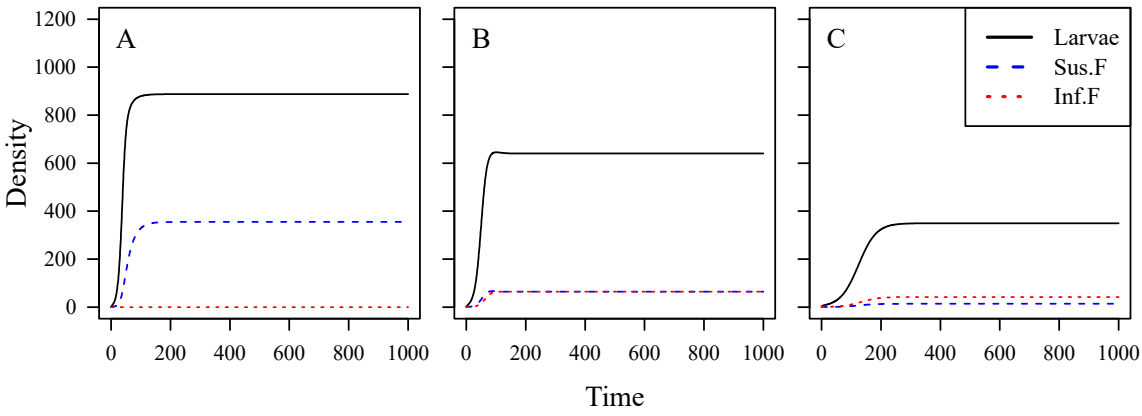


***Figure 4.2.*** *Population densities of larvae, susceptible females (Sus.F) and infected females (Inf.F) with increasing values of the male transmission rate ($\beta_m$) at 0 (A), 0.05 (B), and 0.50 (C).*

Increasing the male transmission rate from zero rapidly reduced larval density from around 600 to 400 individuals for transmission rates up to 0.2 (Figure 4.3A). However, there was a much more gradual rate of reduction in larval density as transmission rates exceeded 0.2 to a minimum of 312 individuals when transmission was 1.0 (Figure 4.3A). It's notable that larval populations never went extinct, presumably due to our assumption that we can only effectively trap and infect a relatively small proportion of the male population, ultimately limiting horizontally transmission of the pathogen to female flies. This finding indicates that while there is potential for a discernible level of population control, eradication is highly unlikely at achievable field rates of male transmission, as the probability of trapping greater than 30% of the population at each point in time, with close to 100% transmission for each trapping event is unlikely to be logistically feasible.

The larval density, influenced by the effects of infection on female reproduction (*r*'), increased as the relative reproductive rate of infected females relative to uninfected females ($\gamma = r'/r$ ) gradually increased from 0 (complete reproductive shut down) to 1 (no reproductive impact) (Figure 4.3B). Thus sterilization effects of the pathogen would be expected to reduce larval density, as has been shown in sterile insect release programs (Enkerlin, 2021). Larval densities exhibited a decline with rising rates of female transmission, either due to heightened mating frequency (Figure 4.3C), or the likelihood of female infection for each mating event (Figure 4.3D).

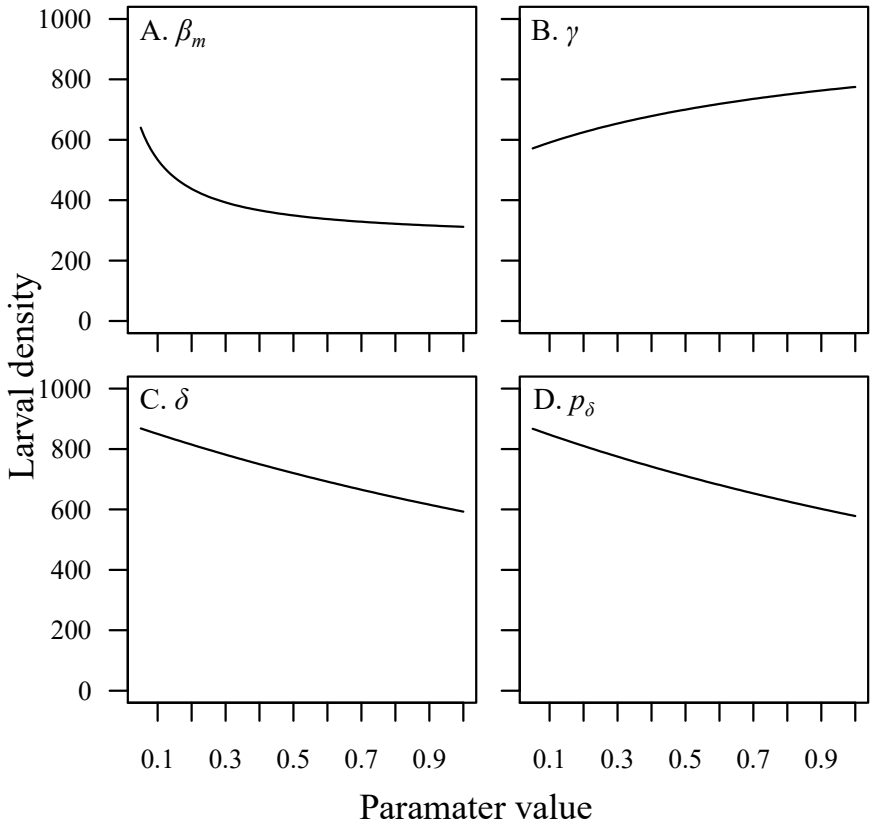


***Figure 1.3.*** *Larval population density in response to increasing male transmission rate, $\beta_m$ (A), as infected female reproduction relative to susceptible female reproduction, $\gamma = r'/r$ (B), the mating rate, $\delta$ (C) and the probability of a female becoming infected given mating $p_\delta$ (D). Unless specifically manipulated, the parameter values were $\beta_m = 0.05$, $r'/r = 0.25$, $\delta = 0.8$ and $p_\delta = 0.75$.*

When determining the optimal pathogen virulence level for pest control (i.e., that value that minimizes equilibrium larval abundance), we must consider effects mediated by virulence or simply by the reduction in oviposition rates among infected females. Therefore, we present the model results for four different reproductive rates by infected females (*r'*) to ascertain the combined effects of female and male virulence at both low ($\beta_m = 0.05$) (Figure 4.4A) and high ($\beta_m = 0.50$) (Figure 4.4B) rates of male transmission. The assessed rates were derived as follows: $r' = \gamma * r$ (where the uninfected female reproductive rate, *r*, denotes a constant rate of 2). These rates correspond to a 0%, 50%, 75% and a 99.99% reduction in infected female reproduction for $\gamma$ values of 1, 0.5, 0.25 and 0.005, respectively (Figure 4.4).

The impact of infection on female reproduction alters the virulence (i.e., pathogen-induced mortality) level that minimises larval densities, when virulence is identical for males and females (Figure 4.4). In general, when virulence is low and males can effectively infect females through mating, pathogen introduction can still be effective when it reduces female reproduction (Figure 4.4). Variations in optimal virulence (i.e., pathogen-induced mortality) arise from differences in the importance of killing females before they reproduce. If infection significantly diminishes female reproduction, increasing female virulence has little effect on larval abundance. Hence, control is improved by reducing overall virulence, enabling susceptible females to mate with infected males. This finding occurs because there is little need for the pathogen to kill females for effective control if it substantially reduces oviposition (Figure 4.4). Although we have not explicitly evaluated this here, when oviposition by infected females is completely halted, the system becomes similar to sterile insect technique management, and that theory is applicable (Enkerlin, 2021).

In general, elevating the male transmission rate from 0.05 (Figure 4.4A) to 0.5 (Figure 4.4B) increases the optimal virulence value across various infected female reproductive rates (*r*'). At high levels of virulence, the influence of infected female reproduction on larval

densities diminishes (Figure 4.4). Notably, the impact of altering infected female reproductive rates (*r*') on larval density is most significant at the lowest virulence value, α = 0.05 (Figure 4.4). This effect of reducing reproduction becomes particularly pronounced when male transmission is high (Figure 4.4B). For instance, at $\alpha = 0.05$, reducing *r*' by 50% ($\gamma = 0.5$) and 75% ($\gamma = 0.25$) resulted in respective reductions in larval density by 18% and 43%, whereas larvae were eradicated for virulence values between 0 and 0.2 when infect female reporduction (*r*') was reduced by 99.99% ($\gamma = 0.005$) (Figure 4.4B). In contrast, eradication was not attained at low male transmission rates (Figure 4.4A). Although altering *r*' at $\alpha = 0.05$ still led to reductions in larval density by 11.5%, 23% and 45% at $\gamma$ values of 0.5, 0.25 and 0.005, respectively (Figure 4.4A).

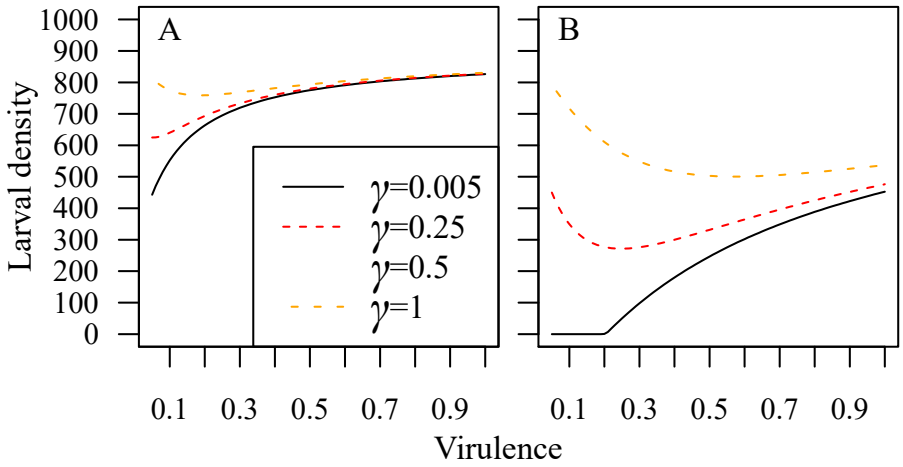


***Figure 4.4.*** *Larval population density in response to increasing male and female mortality due to infection, α, at different values of female reproduction due to infection, r' when altering γ = r'/r, at low (0.05, panel A) and high (0.5, panel B) male transmission rate. The black solid line represents 99.99% reduction in r', the red dotted line represents a 75% reduction in r'. the blue dotted line represents a 50% reduction in r' and the yellow dotted line represents 0% reduction in r'.*

To gain deeper insights into the mechanisms driving final larval densities amidst varying rates of male transmission ($\beta_m$) and infected female reproduction (*r*'), we present an

analysis of virulence effects for both males and females, both in combination and independently. This analysis was performed across scenarios of low and high male transmission, under two conditions: one with a reproductive impact (experiencing a 75% reduction in *r*' compared to *r*) and another without any reproductive impact. Across all scenarios, increasing male virulence ($\alpha_m$) alone invariably led to an escalation in larval density because lower male virulence allows more males to survive long enough to mate with females and transmit the pathogen (Figure 4.5). Conversely, a sole increase in female virulence ($\alpha_f$) consistently resulted in a reduction of larval density, as it diminishes oviposition by infected females either through direct mortality or infection-induced reproductive impacts (Figure 4.5). However, assuming the pathogen elicits similar effects on males and females ($\alpha_f + \alpha_m$), a balance emerges between independently modelled male and female virulence. This balance results in low optimal virulence for effective larval suppression when male transmission is low ($\beta_m$) (Figure 4.5A, C). In this scenario, equilibrium larval densities are lowest for very low (approximately 0.05) virulence, attributed to a reproductive impact during infection (Figure 4.5A), and slightly higher (0.2) virulence when infection has a negligible impact on reproduction (Figure 4.5C). As male transmission increases (Figure 4.5B, D), presumably either through increasing the number of trapped males or by enhancing the transmission success of inoculum in male flies, the optimal virulence proportionally rises to an intermediate level. Here, we observed an optimal value of 0.25 when there is a reproductive impact of infection (Figure 4.5B) and 0.58 when reproductive impacts are absent (Figure 4.5D).

The lower optimal virulence when infection impacts reproduction shows that it can be better to reduce the direct mortality effects of the fungus in favour of reducing population growth. Low virulence, in this context, fosters a greater likelihood of transmission of the sterilising pathogen. The increase in larval abundance with increasing virulence above the

level for optimal control is due to reduced mating with infected males, as the number of males decreases with increased mortality (Figure 4.5, blue dotted lines). Fewer infected males, due to their high mortality rates means lower numbers of infected females, leaving high numbers of susceptible females continuing to reproduce at high rates.

The higher optimal virulence range at higher male transmission rates emerges because high male transmission rates boost the numbers of infected males enough to mate with and infect females, even when some males die before mating. Thus, the benefits of direct mortality effects on larval suppression intensifies at higher male transmission rates. In practice, when trapping efforts are high, as are capture and infection rates, more virulent fungal strains may be ideal.

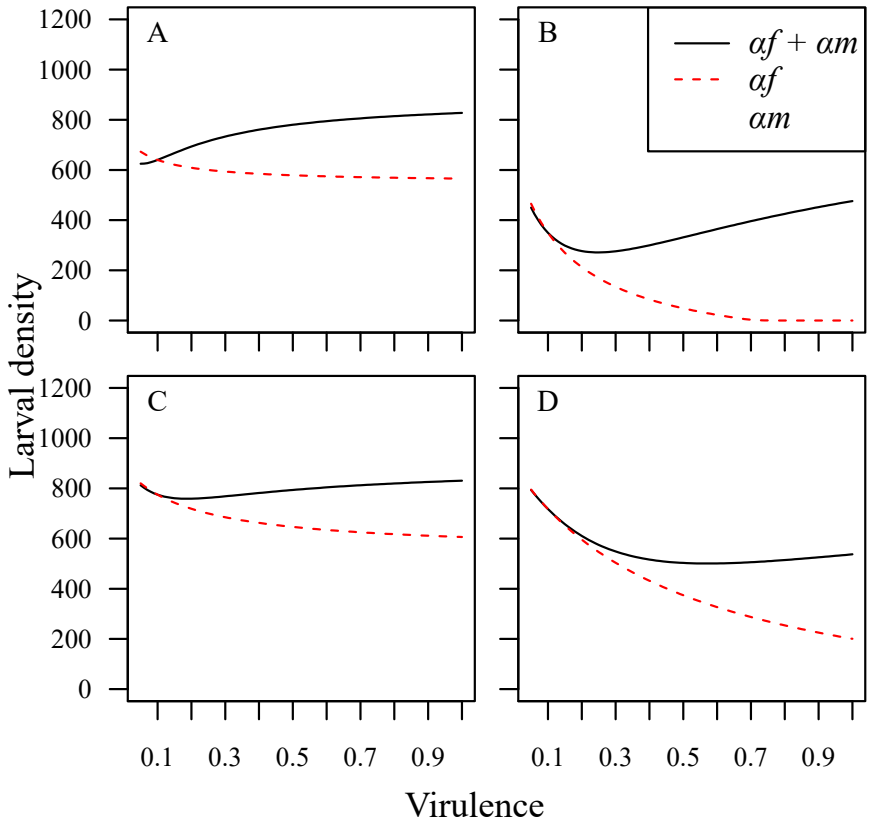


***Figure 4.5.*** *Larval population density in response to a combination in male and female virulence (αf +α m, black solid line), male virulence only (αm, blue dotted line) or female virulence only (αf, red dotted line) when reproduction is impacted by infection (75% reduction) at low (0.05, panel A) and*

*high (0.50, panel B) male transmission rates, and when there is no impact on infection (0% reduction) at low (0.05, panel C) and high (0.50, panel D) male transmission rates.*

Due to the physiological impacts of entomopathogenic fungal infection, it is reasonable to anticipate reductions in oviposition in infected female insect hosts. Reyes-Ramírez et al. (2019) found that female *Tenebrio molitor* beetles, after mating with *Metarhizium*-treated males, exhibited diminished egg production. This phenomenon was hypothesised to stem from a trade-off between the re-allocation of more resources into pheromones by infected males (terminal investment for survival) and the consequent lessening of spermataphore quality, giving rise to fewer and smaller eggs (Reyes-Ramírez et al., 2019). Significant reductions in fecundity and fertility of fungus-treated females have also been noted in studies involving fruit flies (Castillo et al., 2000a; Dimbi et al., 2013a; Sookar et al., 2014c) and house flies (Baker et al., 2018; Mullens, 1990). These effects could be attributed to the direct impact of infection by females through the invasive action of mycelia and/or the toxicity of fungal metabolites (Hajek & St. Leger, 1994). Given the role infection-induced reproduction plays on determining the ideal level of fungal virulence for larval control, reproductive effects of pathogen infection should be considered when designing autodissemination and horizontal transmission programs for pest management.

A key assumption in the parameterization of our model is that mating is random between females and either infected or uninfected males. While our current interpretation of the parameter $p_\delta$ is that it is the probability of infection given that a female has mated to an infected male, it could also be scaled to account for relative mating preference (which in our case we assume is one) for infected versus uninfected males. While we focus on general trends here, we must acknowledge that the spectrum of mating success when infected is likely complex and varies among insect hosts and fungal strains, emphasising the significance of preliminary examinations. To promote transmission, certain fungal entomopathogens alter

insect behaviour and neurological patterns to their benefit (Hughes et al., 2016). In a recent review, Hansen and De Fine Licht (2019) proposed two categories of host-altering outcomes on insect sexual behaviour by fungal entomopathogens. First, pathogen-induced alterations on the host may modify chemical or visual cues to promote the display of sexual behaviour towards potential mates. Second, fungal entomopathogens might directly change the sexual behaviour of the infected insect host to promote more frequent encounters with healthy mates (Hansen & De Fine Licht, 2019). This potential increase in attractiveness of infected individuals is an exciting prospect for pest mitigation, particularly by means of autodissemination and horizontal transmission.

A potential scenario that we do not consider here is the possibility that fungal spores covering infected males are passed to females during mating just after emerging from a trap with autodissemination. The model presented here could be adapted by including a new life stage for adult males that includes the time period just after emerging from a trap. Alternatively, a mass action approach could be used, where the assumption is that across the entire population the transmission rate is proportional to the period of a male's life where it has just emerged from the trap and the spores are still infective, but before the male is infected. Presumably this time period is quite short, and thus transmission rates across the population may be very low. Nonetheless, empirical estimation of longevity of each, spores on the surface of males, as well as the males themselves may help inform evaluation of this alternative transmission pathway.

### 4.5. Conclusion

There is a nuanced relationship between the pathogenicity of fungal entomopathogens and their impact on female reproduction when evaluating suppression. Examination of model outputs indicates that for an attract-and-infect system to be most effective, the

entomopathogen employed should display low to intermediate virulence, particularly when infection reduces reproduction. This benefit of not maximizing virulence arises from the need to balance the divergent impacts of male and female virulence on larval suppression. Consequently, this finding challenges the conventional assumption favouring highly virulent strains in applications targeting pest populations where transmission is not a primary concern. The potential to target wild insect pests, leveraging them as vectors within the population to facilitate their own control, offers an economical and straightforward alternative for local growers aiming to reducing insecticidal use. We advocate for comprehensive bioassays that include lethal and sublethal effects conducted on the target pest and fungal entomopathogen to guide the selection of specific infection traits (such as virulence, transmission and host-altering effects) when devising pertinent pest management strategies.

## Discussion

This thesis indicates the significant potential of fungal entomopathogens in the microbial control of tephritid fruit flies. The intricate dynamics of infection with the target pest necessitates thorough research to determine the optimal physiological impact on the pest. Unlike synthetic products, fungal entomopathogens offer a high degree of flexibility in their infection efficacy, allowing for customisation to the target pest and applied system. This versatility enables a diverse range of opportunities for implementing biological control strategies.

In addition to posing risks to human health, pesticides like malathion, commonly used to combat fruit flies, can significantly disrupt ecosystem function by indiscriminately harming non-target organisms (De Armas et al., 2020; Mulla & Mian, 1981). Conversely, microbial pest control utilising entomopathogenic fungi has emerged as a promising and environmentally friendly alternative to chemical-based solution in both organic and conventional farming systems (Bamisile et al., 2021). The findings of this thesis illuminate the potential of entomopathogenic fungi as highly effective biological control agents of tephritid fruit flies. This study marks the first to demonstrate the ability to control Qfly using *M. guizhouense* (strains Mg1 and Mg2) and *M. lepidiotae* (strain Ml3). Additionally, laboratory bioassays have confirmed that the application of dry conidia, as opposed to exposure through a conidial suspension, maximises infection in Qfly. The preference for dry conidia may be attributed to their hydrophobic nature, facilitating enhanced adherence to the hydrophobic surfaces of insect cuticles in the absence of a liquid barrier (Ortiz-Urquiza & Keyhani, 2013).

The ease of spore adherence to insect cuticles is a pivotal factor in the efficacy of fungal entomopathogens against insect pests (Evans & Hywel-Jones, 1997). Thus, beyond

assessing the strain pathogenicity of entomopathogens against target pests, it is equally crucial to evaluate the application method. For instance, although the application of dry conidia led to higher rates of Qfly mortality compared to a conidial suspension, deploying dry conidia in environments with exposure to high ultraviolet (UV) radiation may not be viable for some strains as it could jeopardize conidia viability (Fernandes et al., 2015). In such environments, incorporating a UV protectant in the form of a conidial suspension could be essential for sustaining field persistence and maximising the entomopathogens' infection potential (Inglis et al., 1995; Kaiser et al., 2019). Disregarding this assessment may lead to discrepancies between the efficacy of fungal entomopathogens applied in laboratory settings versus field conditions.

The genetic characteristics governing the infection dynamics of the evaluated strains remain understudied. While *M. guizhouense* has been classified as possessing an intermediate host range (Hu et al., 2014; Xu et al., 2016), studies elucidating the host range of *M. lepidiotae* are notably absent. The diversity in host preference among species of fungal entomopathogens and their comparative effects on insect pest mortality represents a significant and relatively unexplored research avenue. Specialist fungal species exhibit limitations in their pathogenicity against diverse insect pests (Sree & Joshi, 2015). In contrast, generalist species appear to possess a more versatile toolkit for infection, attributed to the production of diverse secreted proteins that allow them to infect a wide range of arthropod species (St. Leger & Wang, 2020). For studies prioritising local adaption through rearing techniques such as soil-baiting strains (McGuire & Northfield, 2021a), a nuanced understanding of each species' position on the spectrum of host specificity could inform its infection potential, contributing to a more comprehensive interpretation of mortality data in bioassays.

When strains of entomopathogenic fungi exhibit suboptimal infection traits, directed evolution presents itself as a promising solution. The coevolutionary dynamics between pathogens and their hosts involve rapid reciprocal adaptions, revealing a complex molecular arms race facilitated by their communication during the infection process (Vilcinskas, 2019). Interrupting communication to the insect could potentially result in unidirectional selection, wherein the pathogen evolves alongside an evolutionary static host (Adames et al., 2011). In the current study, a significant elevation in Qfly mortality was observed after just one passage through Qfly. This finding is in accordance with other studies that have demonstrated enhanced virulence after a sole host passage (Daoust & Roberts, 1982; Nahar et al., 2008). However, this is not always the case. Some isolates require numerous serial passages to exhibit enhanced virulence (Adames et al., 2011; Quesada-Moraga & Vey, 2003). In some instances, serial passages through the host can result in negligible effects (Scully & Bidochka, 2005) or even negative impacts on virulence (Tomilova et al., 2023). Additionally, studies have documented adverse impacts on virulence following repeated sub-culturing on non-selective artificial media (Daoust & Roberts, 1982; Nahar et al., 2008). Therefore, assessing virulence pathways by subjecting pathogens to host exposure or specific culture media could serve as a pivotal stage in infection efficacy bioassays. This holds particular significance for growers routinely cultivating and applying these pathogens in field settings, as continued culturing might lead to diminished suppression if virulence is compromised due to sub-culturing methods employed to sustain conidia viability.

In my bioassays, like many infection efficacy studies involving fungal entomopathogens for pest control, the primary objective was to identify a highly virulent strain capable of effectively managing tephritid fruit fly populations. However, the findings revealed in Chapter 4 introduced a novel perspective on strain selection based on their virulence levels for population control. Notably, the modelling outputs presented scenarios

where low to intermediate virulence proved optimal for larval suppression in some applications. Specifically, in the context of autodissemination traps, which are designed to capture males and subsequently release them, lower virulence levels enhance the transmission of the pathogen to the female population. This transmission is more effective if the pathogen does not rapidly eliminate males, thereby increasing their mating potential and subsequently reducing oviposition by diminishing the reproductive potential of females. The insights gleaned from this chapter offer a fresh perspective on the optimal virulence level of fungal entomopathogens for pest suppression. Additionally, it underscores the importance of considering the broader impact of infection on the fecundity of the target host females. This nuanced approach suggests that selecting strains with low to intermediate virulence could be beneficial in autodissemination systems, particularly when female infection results in reproductive costs. This discovery marks a significant advancement in our understanding of optimal virulence traits and goes beyond conventional approaches to pest management employing fungal entomopathogens.

In conclusion, the findings presented in this thesis provide compelling evidence supporting the use of entomopathogenic fungi for controlling of tephritid fruit flies and offer a promising alternative or complementary approach to conventional pest control methods. This approach has the potential to reduce the reliance on chemical pesticides, mitigate the development of pesticide resistance, and align with the growing demand for more environmentally sustainable horticultural practices. To achieve broader adoption of these fungi, further research and field trials are essential to refine their application and ensure their effectiveness across diverse environmental conditions and fruit fly life stages. Additionally, given the long-term reliance on chemical pesticides, understanding how fungal entomopathogens can integrate into existing pest management systems that have previously depended on chemical control is a crucial step towards their successful adoption. Addressing

these challenges will be key to enhancing integrated pest management strategies and promoting more sustainable agricultural practices that reduce chemical inputs while maintaining effective pest suppression.

**Appendix A**

**R code for susceptible-infected model**

```
rm(par.val)
par.val <- seq(0.05,1, by = 0.01)
out.matrix <- matrix(0,nrow=1,ncol=7)

for(i in par.val){

#parameter values
t=1000                  #time
a = 2                   #birth rate of susceptible females
gamma = 0.25
a.prime = gamma*a   #birth rate infected females
b=0.05                  #susceptible mortality rate
bM=0.05                 #mortality rate larvae
alpha.m=0.1             #mortality due to infection (males)
alpha.f=0.1             #mortality due to infection (females)
M=0.04                  #immature maturation rate
Bm=0.05                 #transmission rate of the pathogen in males
m = 0.8                 #mating rate
pm = 0.75               #probability of a female becoming infected given mating
k= 1000                 #carrying capacity

#after introducing pathogen
init <- c(L=1-1e-6,Sm=1-1e-6,Im=1-1e-6,Sf=1-1e-6,If=1-1e-6)

parameters <- c(t,a,a.prime,b,alpha.m,alpha.f,M,Bm,m,pm,k)
time <- seq(0,t,by=t/(2*length(1:t)))

eqn <- function(time,init,parameters){
  with(as.list(c(init,parameters)),{
```

```
    dL <- a*(1-L/k)*Sf+a.prime*If-M*L-bM*L
    dSm <- M*L/2-Bm*Sm-b*Sm
    dIm <- Bm*Sm-(alpha.m+b)*Im
    dSf <- M*L/2-m*(Im/(Sm+Im))*pm*Sf-b*Sf
    dIf <- m*(Im/(Sm+Im))*pm*Sf-(alpha.f+b)*If
    return(list(c(dL,dSm,dIm,dSf,dIf)))})}

#run the ordinary differential equation finder
out<-ode(y=init,times=time,eqn,parms=parameters)
out.df<-as.data.frame(out)
out.matrix<- rbind(out.matrix,c(i,tail(out,1)))
}
colnames(out.matrix)<- c('parameter.value','time.steps','dL','dSm','dIm','dSf','dIf')
```

## References: Introduction


Adames, M., Fernández-Ruvalcaba, M., Peña-Chora, G., & Hernández-Velázquez, V. M. (2011). Effects of passages through a suitable host of the fungus, *Metarhizium anisopliae*, on the virulence of acaricide-susceptible and resistant strains of the tick, *Rhipicephalus microplus*. *J Insect Sci, 11*(1), 21. doi:10.1673/031.011.0121

Anon. (1993). Proposal to establish a Fruit Fly Exclusion Zone in south east Australia: Agriculture Victoria, Melbourne.

Anon. (1997). Code of practice for management of Queensland fruit fly. *Report by the InterState Plant Health Regulation Working Group to the Plant Health Committee of the Standing Committee on Agriculture and Resource Management*. Canberra.

Araújo, J. P. M., & Hughes, D. P. (2016). Chapter One - Diversity of Entomopathogenic Fungi: Which Groups Conquered the Insect Body? In B. Lovett & R. J. St. Leger (Eds.), *Advances in Genetics* (Vol. 94, pp. 1-39): Academic Press.

Begum, A., Alam, S. N., & Jalal Uddin, M. (2017). Management of pesticides: purposes, uses, and concerns. In M. S. Khan & M. S. Rahman (Eds.), *Pesticide Residue in Foods: Sources, Management, and Control* (pp. 53-86). Cham: Springer International Publishing.

Benvenuti, C., Barzanti, G. P., Marianelli, L., Peverieri, G. S., Paoli, F., Bosio, G., . . . Roversi, P. F. (2019). A new device for auto-disseminating entomopathogenic fungi against *Popillia japonica*: a study case. *Bull. Insectology, 72*, 219-225.

Bischoff, J. F., Rehner, S. A., & Humber, R. A. (2009). A multilocus phylogeny of the *Metarhizium anisopliae* lineage. *Mycologia, 101*(4), 512-530. doi:10.3852/07-202

Clarke, A. R., Powell, K. S., Weldon, C. W., & Taylor, P. W. (2011). The ecology of *Bactrocera tryoni* (Diptera: Tephritidae): what do we know to assist pest management? *Ann. Appl. Biol., 158*(1), 26-54. doi:10.1111/j.1744-7348.2010.00448.x

Dias, N. P., Zotti, M. J., Montoya, P., Carvalho, I. R., & Nava, D. E. (2018). Fruit fly management research: A systematic review of monitoring and control tactics in the world. *Crop Protection, 112*, 187-200. doi:10.1016/j.cropro.2018.05.019

Dominiak, B. C., & Daniels, D. (2012). Review of the past and present distribution of Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) and Queensland fruit fly (*Bactrocera tryoni* Froggatt) in Australia. *Australian Journal of Entomology, 51*(2), 104-115. doi:10.1111/j.1440-6055.2011.00842.x

Dominiak, B. C., & Ekman, J. H. (2013). The rise and demise of control options for fruit fly in Australia. *J. Crop Prot., 51*, 57-67. doi:10.1016/j.cropro.2013.04.006

Drew, R., Hooper, G., & Bateman, M. (1982). *Economic fruit flies of the South Pacific Region*: Queensland Department of Primary Industries.

Drew, R. A. (1978). Taxonomy. In R. Drew, G. Hooper, & M. Bateman (Eds.), *Economic fruit flies of the South Pacific Region.* (pp. 1-94): Department of Primary Industries

Drew, R. A. (1989). The tropical fruit flies (Diptera: Tephritidae: Dacinae) of the Australasian and Oceanian regions. *Memoirs of the Queensland Museum, 26*.

Du, Y., Xia, Y., & Jin, K. (2022). Enhancing the biocontrol potential of the entomopathogenic fungus in multiple respects via the overexpression of a transcription factor gene MaSom1. *J. Fungi. , 8*(2), 105.

El-Sayed, A. M., Suckling, D. M., Byers, J. A., Jang, E. B., & Wearing, C. H. (2009). Potential of “lure and kill” in long-term pest management and eradication of invasive species. *J. Econ. Entomol., 102*(3), 815-835. doi:10.1603/029.102.0301

Evans, H. C., & Hywel-Jones, N. L. (1997). Entomopathogenic Fungi. In Y. Ben-Dov & C. J. Hodgson (Eds.), *Soft Scale Insects their Biology, Natural Enemies and Control* (Vol. 7, pp. 3-27): World Crop Pests.

Faria, M. R. d., & Wraight, S. P. (2007). Mycoinsecticides and Mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. *Biol Control, 43*(3), 237-256. doi:10.1016/j.biocontrol.2007.08.001

Faye, P. D., Bal, A. B., Ndiaye, N. M., Diop, F., Sangaré, Y. K., Haddad, C., . . . Niassy, S. (2021). Field efficacy of *Metarhizium acridum* (Hypocreales: Clavicipitaceae) in the control of *Bactrocera dorsalis* (Diptera: Tephritidae) in citrus orchards in Senegal. *Int. J. Trop. Insect Sci., 41*(2), 1185-1195. doi:10.1007/s42690-020-00306-5

Furlong, M. J., & Pell, J. K. (2001). Horizontal Transmission of Entomopathogenic Fungi by the Diamondback Moth. *Biol. control, 22*(3), 288-299. doi:10.1006/bcon.2001.0981

Greenfield, M., Gómez-Jiménez, M. I., Ortiz, V., Vega, F. E., Kramer, M., & Parsa, S. (2016). *Beauveria bassiana* and *Metarhizium anisopliae* endophytically colonize cassava roots following soil drench inoculation. *Biological Control, 95*, 40-48. doi:10.1016/j.biocontrol.2016.01.002

Hajek, A. E., & Butler, L. (2000). Predicting the host range of entomopathogenic fungi. In P. A. Follett & J. J. Duan (Eds.), *Nontarget Effects of Biological Control* (pp. 263-276). Boston, MA: Springer US.

Hancock, D., Hamacek, E., Lloyd, A., & Elson-Harris, M. M. (2000). *The distribution and host plants of fruit flies (Diptera: Tephritidae) in Australia*.

Hawkins, N. J., Bass, C., Dixon, A., & Neve, P. (2019). The evolutionary origins of pesticide resistance. *Biol Rev, 94*(1), 135-155. doi:doi.org/10.1111/brv.12440

Irsad, Shahid, M., Haq, E., Mohamed, A., Rizvi, P. Q., & Kolanthasamy, E. (2023). Entomopathogen-based biopesticides: insights into unraveling their potential in insect pest management. *Front Microbiol, 14*, 1208237. doi:10.3389/fmicb.2023.1208237

Joop, G., & Vilcinskas, A. (2016). Coevolution of parasitic fungi and insect hosts. *Zoology, 119*(4), 350-358. doi:10.1016/j.zool.2016.06.005

Khan, S., Guo, L., Maimaiti, Y., Mijit, M., & Qiu, D. (2012). Entomopathogenic fungi as microbial biocontrol agent. *Molecular Plant Breeding, 3*(7).

Lacey, L. A., Grzywacz, D., Shapiro-Ilan, D. I., Frutos, R., Brownbridge, M., & Goettel, M. S. (2015). Insect pathogens as biological control agents: Back to the future. *J. Invertebr. Pathol., 132*, 1-41. doi:10.1016/j.jip.2015.07.009

Maniania, N. K., & Ekesi, S. (2013). The use of entomopathogenic fungi in the control of tsetse flies. *J. Invertebr. Pathol., 112*, S83-S88. doi:10.1016/j.jip.2012.07.019

Mannino, M. C., Huarte-Bonnet, C., Davyt-Colo, B., & Pedrini, N. (2019). Is the insect cuticle the only entry gate for fungal infection? Insights into alternative modes of action of entomopathogenic fungi. *J. Fungi., 5*(2), 33.

McGuire, A. V., & Northfield, T. D. (2020). Tropical occurrence and agricultural importance of *Beauveria bassiana* and *Metarhizium anisopliae*. *Front. sustain. food syst., 4*. doi:10.3389/fsufs.2020.00006

McGuire, A. V., & Northfield, T. D. (2021). Identification and evaluation of endemic *Metarhizium* strains for biological control of banana rust thrips. *Biol. control, 162*, 104712. doi:h10.1016/j.biocontrol.2021.104712

Merkel, K., Schwarzmueller, F., Hulthen, A. D., Schellhorn, N., Williams, D., & Clarke, A. R. (2019). Temperature effects on "overwintering" phenology of a polyphagous, tropical fruit fly (Tephritidae) at the subtropical/temperate interface. *J. Appl. Entomol., 143*(7), 754-765. doi:10.1111/jen.12645

Mohammed, A. A., Kadhim, J. H., & Kamaluddin, Z. N. A. (2018). Selection of highly virulent entomopathogenic fungal isolates to control the greenhouse aphid species in Iraq. *EJBPC, 28*(1), 71. doi:10.1186/s41938-018-0079-3

Murtaza, G., Naeem, M., Manzoor, S., Khan, H. A., Eed, E. M., Majeed, W., . . . Ummara, U. E. (2022). Biological control potential of entomopathogenic fungal strains against peach Fruit

fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). *PeerJ, 10*, e13316. doi:10.7717/peerj.13316

Navarro-Llopis, V., Alfaro, F., Domínguez, J., Sanchis, J., & Primo, J. (2008). Evaluation of traps and lures for mass trapping of mediterranean fruit fly in citrus groves. *J. Econ. Entomol., 101*(1), 126-131. doi:10.1093/jee/101.1.126

NFFSIC. (2010). Draft national fruit fly strategy: Implementation action plan. *Plant Health Australia, Canberra, ACT, Australia*.

Nishi, O., Sushida, H., Higashi, Y., & Iida, Y. (2021). Epiphytic and endophytic colonisation of tomato plants by the entomopathogenic fungus *Beauveria bassiana* strain GHA. *Mycology, 12*(1), 39-47. doi:10.1080/21501203.2019.1707723

Pretty, J., Vorley, W., & Keeney, D. (1998). Pesticides in World Agriculture: Causes, Consequences and Alternative Courses In W. Vorley & D. Keeney (Eds.), *Bugs in the System* (1 ed., pp. 33). Oxon and New York: Earthscan.

Quesada-Moraga, E., & Vey, A. (2003). Intra-specific variation in virulence and In vitro production of macromolecular toxins active against locust among *Beauveria bassiana* strains and effects of in vivo and In vitro passage on these factors. *Biocontrol Sci Technol, 13*(3), 323-340. doi:10.1080/0958315031000110346

Rafaluk, C., Yang, W., Mitschke, A., Rosenstiel, P., Schulenburg, H., & Joop, G. (2017). Highly potent host external immunity acts as a strong selective force enhancing rapid parasite virulence evolution. *Environ. Microbiol., 19*(5), 2090-2100. doi:10.1111/1462-2920.13736

Smith, E. C., Chin, D., Allwood, A., & Collins, S. (1988). A revised host list of fruit flies (Diptera: Tephritidae) from the Northern Territory of Australia. *Queensland Journal of Agricultural and Animal Sciences, 45*(1), 19-28.

Song, T. T., & Feng, M. G. (2011). In vivo passages of heterologous *Beauveria bassiana* isolates improve conidial surface properties and pathogenicity to *Nilaparvata lugens* (Homoptera: Delphacidae). *J Invertebr Pathol, 106*(2), 211-216. doi:10.1016/j.jip.2010.09.022

Sultana, S., Baumgartner, J. B., Dominiak, B. C., Royer, J. E., & Beaumont, L. J. (2017). Potential impacts of climate change on habitat suitability for the Queensland fruit fly. *Scientific reports, 7*(1), 13025-13025. doi:10.1038/s41598-017-13307-1

Sutherst, R., Collyer, B., & Yonow, T. (2000). The vulnerability of Australian horticulture to the Queensland fruit fly, *Bactrocera (Dacus) tryoni*, under climate change. *Crop Pasture Sci., 51*, 467-480. doi:10.1071/AR98203

Toledo, J., Flores, S., Campos, S., Villaseñor, A., Enkerlin, W., Liedo, P., . . . Montoya, P. (2017). Pathogenicity of three formulations of *Beauveria bassiana* and efficacy of autoinoculation devices and sterile fruit fly males for dissemination of conidia for the control of *Ceratitis capitata*. *Entomol Exp Appl, 164*(3), 340-349. doi:10.1111/eea.12608

Vega, F. E., Goettel, M. S., Blackwell, M., Chandler, D., Jackson, M. A., Keller, S., . . . Roy, H. E. (2009). Fungal entomopathogens: new insights on their ecology. *Fungal Ecology, 2*(4), 149-159. doi:10.1016/j.funeco.2009.05.001

Vera, M. T., Rodriguez, R., Segura, D. F., Cladera, J. L., & Sutherst, R. W. (2002). Potential geographical distribution of the Mediterranean Fruit Fly, *Ceratitis capitata* (Diptera: Tephritidae), with Emphasis on Argentina and Australia. *Environ. Entomol., 31*(6), 1009-1022. doi:10.1603/0046-225x-31.6.1009

White, B., Sadler, R., Florec, V., & Dominiak, B. (2012). *Economics of surveillance: a bioeconomic assessment of Queensland fruit fly*. Conference (56th), February 7-10, 2012, Fremantle, Australia 125741, Australian Agricultural and Resource Economics Society.

White, I. M., & Elson-Harris, M. M. (1992). *Fruit flies of economic significance: their identification and bionomics*: CAB international.

Yousef, M., Alba-Ramírez, C., Garrido Jurado, I., Mateu, J., Raya Díaz, S., Valverde-García, P., & Quesada-Moraga, E. (2018). *Metarhizium brunneum* (Ascomycota; Hypocreales) Treatments Targeting Olive Fly in the Soil for Sustainable Crop Production. *Front Plant Sci, 9*, 1. doi:10.3389/fpls.2018.00001

Zhao, H., Lovett, B., & Fang, W. (2016). Genetically engineering entomopathogenic fungi. *Adv Genet, 94*, 137-163. doi:10.1016/bs.adgen.2015.11.001

## References: Chapter 1


Abbas, M. S. T. (2020). Interactions between entomopathogenic fungi and entomophagous insects. *Adv. entomol., 8*(03), 130.

Adames, M., Fernández-Ruvalcaba, M., Peña-Chora, G., & Hernández-Velázquez, V. M. (2011). Effects of passages through a suitable host of the fungus, *Metarhizium anisopliae*, on the virulence of acaricide-susceptible and resistant strains of the tick, *Rhipicephalus microplus*. *J Insect Sci, 11*(1), 21. doi:10.1673/031.011.0121

Aemprapa, S. (2007). Entomopathogenic fungi screening against fruit fly in Thailand. *Curr. j. appl., 7*(2-1), 122-126.

Ahmad, S., Sarwar, A., Shoaib, A., Javaid, A., Hanif, M. S., & Ali, Q. (2022). Sustainable management of guava fruit fly *Bactrocera zonata* (Tephritidae: Diptera) by entomopathogenic fungi. *Fresenius Environ. Bull., 31*(6), 5522-5527.

Akter, H., Adnan, S., Morelli, R., Rempoulakis, P., & Taylor, P. W. (2017a). Suppression of cuelure attraction in male Queensland fruit flies provided raspberry ketone supplements as immature adults. *PloS one, 12*(8), e0184086. doi:10.1371/journal.pone.0184086

Akter, H., Mendez, V., Morelli, R., Pérez, J., & Taylor, P. W. (2017b). Raspberry ketone supplement promotes early sexual maturation in male Queensland fruit fly, *Bactrocera tryon*i (Diptera: Tephritidae). *Pest Manag Sci, 73*(8), 1764-1770. doi:10.1002/ps.4538

Alali, S., Mereghetti, V., Faoro, F., Bocchi, S., Al Azmeh, F., & Montagna, M. (2019). Thermotolerant isolates of *Beauveria bassiana* as potential control agent of insect pest in subtropical climates. *PloS one, 14*(2), e0211457. doi:10.1371/journal.pone.0211457

Allwood, A. (1997). *Control strategies for fruit flies (Diptera: Tephritidae) in the South Pacific.* Paper presented at the Management of fruit flies in the Pacific. A regional Symposium ACIAR Proceedings No 76., Nadi, Fiji.

Ami, E. B., Yuval, B., & Jurkevitch, E. (2010). Manipulation of the microbiota of mass-reared Mediterranean fruit flies *Ceratitis capitata* (Diptera: Tephritidae) improves sterile male sexual performance. *ISME J, 4*(1), 28-37. doi:10.1038/ismej.2009.82

Arouri, R., Le Goff, G., Hemden, H., Navarro-Llopis, V., M'saad, M., Castañera, P., . . . Ortego, F. (2015). Resistance to lambda-cyhalothrin in Spanish field populations of *Ceratitis capitata* and metabolic resistance mediated by P450 in a resistant strain. *Pest Manag Sci, 71*(9), 1281-1291. doi:10.1002/ps.3924

Australian Pesticides and Veterinary Medicines Authority. (2011). *Dimethoate: residues and dietary risk assessment report*: Australian Pesticides & Veterinary Medicines Authority.

Australian Pesticides and Veterinary Medicines Authority. (2014). Alternatives for fruit fly control.

Australian Pesticides and Veterinary Medicines Authority. (2015). Fenthion chemical review. *Pesticides and Veterinary Medicines Authority*.

Barclay, H. J., McInnis, D., & Hendrichs, J. (2014). Modeling the area-wide integration of male annihilation and the simultaneous release of methyl eugenol-exposed *Bactrocera* spp. sterile males. *Ann. Entomol. Soc. Am., 107*(1), 97-112, 116.

Bateman, M. (1968). Determinants of abundance in a population of the Queensland fruit fly. In T. R. E. Southwood (Ed.), *Insect Abundance* (pp. 119-131). Oxford, UK: Blackwell Scientific Publications.

Bateman, M. (1982). Chemical methods for suppression or eradication of fruit fly populations. In R. A. I. Drew, G. H. S. Hopper, & M. A. Bateman (Eds.), *Economic*

*Fruit Flies of the South Pacific Region* (pp. 115-128). Brisbane: Watson Ferguson and Co.

Bateman, M., Insunza, V., & Arretz, P. (1973). The eradication of Queensland fruit fly from Easter Island. *Plant Protection Bulletin, FAO, 21*(5).

Bateman, M. A. (1972). The ecology of fruit flies. *Annu. Rev. of Entomol., 17*(1), 493-518. doi:10.1146/annurev.en.17.010172.002425

Beris, E. I., Papachristos, D. P., Fytrou, A., Antonatos, S. A., & Kontodimas, D. C. (2013). Pathogenicity of three entomopathogenic fungi on pupae and adults of the *Mediterranean* fruit fly, Ceratitis capitata (Diptera: Tephritidae). *J Pest Sci, 86*(2), 275-284. doi:10.1007/s10340-012-0468-4

Braga, G. U. L., Flint, S. D., Messias, C. L., Anderson, A. J., & Roberts, D. W. (2001). Effect of UV-B on conidia and germlings of the entomopathogenic hyphomycete Metarhizium anisopliae. *Mycological Research, 105*(7), 874-882. doi:10.1017/S0953756201004270

Carswell, I., Spooner-hart, R., & Milner, R. J. (1998). Laboratory susceptibility of *Musca domestica* L. (Diptera: Muscidae) and *Bactrocera tryoni* (Frogatt) (Diptera: Tephritidae) to an isolate of *Metarhizium anisopliae* (Metsch.) Sorokin. *Aust. J. Entomol., 37*, 281-284.

Castillo, M.-A., Moya, P., Hernández, E., & Primo-Yúfera, E. (2000). Susceptibility of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) to entomopathogenic fungi and their extracts. *Biocontrol, 19*(3), 274-282. doi:10.1006/bcon.2000.0867

Cayol, J.-P. (1999). Changes in sexual behavior and life history traits of tephritid species caused by mass-rearing processes *Fruit Flies (Tephritidae)* (pp. 861-878): CRC Press.

Christenson, L. D., & Foote, R. H. (1960). Biology of Fruit Flies. *Annu. Rev. Entomol., 5*(1), 171-192. doi:10.1146/annurev.en.05.010160.001131

Clifton, E. H., Jaronski, S. T., Hodgson, E. W., & Gassmann, A. J. (2015). Abundance of soil-borne entomopathogenic fungi in organic and conventional fields in the midwestern USA with an emphasis on the effect of herbicides and fungicides on fungal fersistence. *PloS one, 10*(7), e0133613. doi:10.1371/journal.pone.0133613

Dara, S. K. (2019). Interactions of entomopathogens with other pest management options. In M. A. Khan & W. Ahmad (Eds.), *Microbes for Sustainable Insect Pest Management : An Eco-friendly Approach* (Vol. 1, pp. 299-316). Cham: Springer International Publishing.

De, l., R., Lopez, F. L., & Liedo, P. (2002). Beauveria bassiana as a pathogen of the Mexican fruit fly (Diptera: Tephritidae) under laboratory conditions. *J Econ Entomol, 95*(1), 36-43. doi:10.1603/0022-0493-95.1.36

Devescovi, F., Bachmann, G., Nussenbaum, A. L., Cladera, J., & Segura, D. (2020). Host discrimination in the fruit fly parasitoid *Diachasmimorpha longicaudata*: evidence from virgin female behaviour and egg distribution patterns. *Bull. Entomol. Res., 111*, 1-9. doi:10.1017/S0007485320000589

Dias, N. P., Montoya, P., & Nava, D. E. (2022). A 30-year systematic review reveals success in tephritid fruit fly biological control research. *Entomol. Exp. Appl. , 170*(5), 370-384. doi:10.1111/eea.13157

Dimbi, S., Maniania, N. K., & Ekesi, S. (2013). Horizontal transmission of *Metarhizium anisopliae* in fruit flies and effect of fungal infection on egg laying and fertility. *Insects, 4*(2), 206-216. doi:10.3390/insects4020206

Dimbi, S., Maniania, N. K., Lux, S. A., & Mueke, J. M. (2004). Effect of constant temperatures on germination, radial growth and virulence of *Metarhizium anisopliae* to three species of African tephritid fruit flies. *BioControl, 49*(1), 83-94. doi:10.1023/B:BICO.0000009397.84153.79

Doberski, J. W. (1981). Comparative laboratory studies on three fungal pathogens of the elm bark beetle *Scolytus scolytus*: Effect of temperature and humidity on infection by *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces farinosus*. *J. Invertebr. Pathol., 37*(2), 195-200. doi:10.1016/0022-2011(81)90075-6

Dodds, K., O'Malley, K., Hetherington, S., & Kidston, J. (2014). Orchard Plant Protection Guide for Deciduous Fruits In NSW 2014–15 (pp. 2200–7539). New South Wales; Orange, Australia: Department of Primary Industries.

Domimak, B. C., Gilmour, A. R., Kerruish, B., & Whitehead, D. (2003). Detecting low populations of Queensland fruit fly '*Bactrocera tryoni*' (Froggatt) with McPhail and Lynfield traps. *Gen. Appl. Ent: The Journal of the Entomological Society of New South Wales, 32*, 49-53.

Dominiak, B., Clifford, C., & Nielsen, S. (2009). Queensland fruit fly (*Bactrocera tryoni* Froggatt) attraction to and chemical analysis of male annihilation blocks using three concentrations of cuelure at Dubbo, New South Wales, Australia. *Plant Prot. Q., 24*(4), 157.

Dominiak, B. C., & Ekman, J. H. (2013). The rise and demise of control options for fruit fly in Australia. *Crop Prot., 51*, 57-67. doi:10.1016/j.cropro.2013.04.006

Dominiak, B. C., Kerruish, B., Barchia, I., Pradhan, U., Gilchrist, A. S., & Nicol, H. I. (2011). The influence of mixtures of parapheromone lures on trapping of fruit fly in New South Wales, Australia. *Plant Prot. Q., 26*(4), 136-140.

Ekesi, S., Maniania, N., & Mohamed, S. (2011). Efficacy of soil application of *Metarhizium anisopliae* and the use of GF-120 spinosad bait spray for suppression of Bactrocera invadens (Diptera: Tephritidae) in mango orchards. *Biocontrol Sci. Technol., 21*, 299-316. doi:10.1080/09583157.2010.545871

Ekesi, S., Maniania, N. K., & Lux, S. A. (2002). Mortality in three African Tephritid fruit fly puparia and adults caused by the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*. *Biocontrol Sci Technol, 12*(1), 7-17. doi:10.1080/09583150120093077

Ekesi, S., Maniania, N. K., & Lux, S. A. (2003). Effect of soil temperature and moisture on survival and infectivity of *Metarhizium anisopliae* to four tephritid fruit fly puparia. *J. Invertebr. Pathol., 83*(2), 157-167. doi:10.1016/S0022-2011(03)00069-7

Ekesi, S., Maniania, N. K., Mohamed, S. A., & Lux, S. A. (2005). Effect of soil application of different formulations of *Metarhizium anisopliae* on African tephritid fruit flies and their associated endoparasitoids. *Biol. Control, 35*(1), 83-91. doi:10.1016/j.biocontrol.2005.06.010

El-Gendy, I. R., Zawrah, M. F. M., & El-Banobi, M. I. (2022). Virulence effect of *Metarhizium anisopliae* (Met.) and *Beauveria bassiana* (Bals.) fungi against the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). *Egypt J Biol Pest Control, 32*(1), 43. doi:10.1186/s41938-022-00545-3

El-Sayed, A. M., Suckling, D. M., Byers, J. A., Jang, E. B., & Wearing, C. H. (2009). Potential of "lure and kill" in long-term pest management and eradication of invasive species. *J. Econ. Entomol., 102*(3), 815-835, 821.

Elghadi, E., & Port, G. (2019). Use of entomopathogenic fungi for the biological control of the greater melon fly *Dacus frontalis* in Libya. In C. Press (Ed.), *Area-Wide Management of Fruit Fly Pests* (1 ed., pp. 251-266).

Enkerlin, W. (2021). Impact of fruit fly control programmes using the sterile insect technique. In V. Dyck, J. Hendirchs, & A. S. Robinson (Eds.), *Sterile insect technique* (pp. 979-1006). New York: CRC Press.

Enkerlin, W., Gutiérrez-Ruelas, J. M., Cortes, A. V., Roldan, E. C., Midgarden, D., Lira, E., . . . Arriaga, F. J. T. (2015). Area freedom in mexico from Mediterranean fruit fly (Diptera: Tephritidae): A review of over 30 years of a successful containment program using an integrated area-wide SIT approach. *Fla. Entomol., 98*(2), 665-681, 617.

Fanson, B. G., Sundaralingam, S., Jiang, L., Dominiak, B. C., & D'Arcy, G. (2014). A review of 16 years of quality control parameters at a mass-rearing facility producing Queensland fruit fly, *Bactrocera tryoni*. *Entomol. Exp Appl., 151*(2), 152-159. doi:10.1111/eea.12180

Fargues, J., Reisinger, O., Robert, P. H., & Aubart, C. (1983). Biodegradation of entomopathogenic hyphomycetes: Influence of clay coating on *Beauveria bassiana* blastospore survival in soil. *J. Invertebr. Pathol., 41*(2), 131-142. doi:10.1016/0022-2011(83)90212-4

Faye, P. D., Bal, A. B., Ndiaye, N. M., Diop, F., Sangaré, Y. K., Haddad, C., . . . Niassy, S. (2021). Field efficacy of *Metarhizium acridum* (Hypocreales: Clavicipitaceae) in the control of *Bactrocera dorsalis* (Diptera: Tephritidae) in citrus orchards in Senegal. *Int. J. Trop. Insect Sci., 41*(2), 1185-1195. doi:10.1007/s42690-020-00306-5

Fernández-Bravo, M., Flores-León, A., Calero-López, S., Gutiérrez-Sánchez, F., Valverde-García, P., & Quesada-Moraga, E. (2017). UV-B radiation-related effects on conidial inactivation and virulence against *Ceratitis capitata* (Wiedemann) (Diptera; Tephritidae) of phylloplane and soil *Metarhizium* sp. strains. *J. Invertebr. Pathol., 148*, 142-151. doi:10.1016/j.jip.2017.06.012

Fitt, G. P. (1990). Comparative fecundity, clutch size, ovariole number and egg size of *Dacus tryoni* and *D. jarvisi*, and their relationship to body size. *Entomol. Exp. Appl., 55*(1), 11-21. doi:10.1111/j.1570-7458.1990.tb01343.x

Fletcher, B. (1989). Life history strategies of tephritid fruit flies *World Crop Pests: Fruit Flies: Their Biology, Natural Enemies and Control* (Vol. 3B, pp. 195-208). Amsterdam, The Netherlands: Elsevier Science Publ.

Fletcher, B., & Bateman, M. (1983). *Combating the fruit fly problems in Australia: the current situation and future prospects.* Paper presented at the Fruit flies of economic importance. Proceedings of the CEC/IOBC International Symposium, Rotterdam, Athens, Greece, 16-19 November 1982.

Garcia, F. R., Ovruski, S. M., Suárez, L., Cancino, J., & Liburd, O. E. (2020). Biological control of tephritid fruit flies in the Americas and Hawaii: A review of the use of parasitoids and predators. *Insects, 11*(10), 662.

Garrido-Jurado, I., Torrent, J., Barrón, V., Corpas, A., & Quesada-Moraga, E. (2011). Soil properties affect the availability, movement, and virulence of entomopathogenic fungi conidia against puparia of *Ceratitis capitata* (Diptera: Tephritidae). *Biol. Control., 58*(3), 277-285. doi:10.1016/j.biocontrol.2011.05.017

Gava, C. A. T., da Silva, J. C., Simões, W. L., & Jordão Paranhos, B. A. (2021). Impact of soil texture on conidia movement and residual effect of entomopathogenic fungi applied through irrigation to control fruit-fly pupae in mango orchards. *Biol. Control, 163*, 104559. doi:10.1016/j.biocontrol.2021.104559

Gava, C. A. T., Tavares, P. F. d. S., Gonçalves, J. S., & Paranhos, B. A. J. (2020). Applying local entomopathogenic fungi strains to the soil can control *Ceratitis capitata* (Diptera: Tephritidae) Wiedemann adults. *Biocontrol Sci. Technol, 30*(2), 103-115. doi:10.1080/09583157.2019.1691716

Gingrich, R. (1993). Biological control of tephritid fruit flies by inundative releases of natural enemies *Fruit Flies* (pp. 311-318): Springer.

Grout, T. G. (2016). Cold and heat treatment technologies for post-harvest control of fruit flies in Africa. In S. Ekesi, S. A. Mohamed, & M. De Meyer (Eds.), *Fruit Fly Research and Development in Africa - Towards a Sustainable Management Strategy to Improve Horticulture* (pp. 465-473). Cham: Springer International Publishing.

Gul, H. T., Freed, S., Akmal, M., & Malik, M. N. (2015). Vulnerability of different life stages of *Bactrocera zonata* (Tephritidae: Diptera) against entomogenous fungi. *Pak. J. Zool., 47*.

Hagen, K. S., & Finney, G. L. (1950). A food supplement for effectively increasing the fecundity of certain tephritid species. *J. Econ. Entomol., 43*(5).

Hallouti, A., Ait Hamza, M., Zahidi, A., Ait Hammou, R., Bouharroud, R., Ait Ben Aoumar, A., & Boubaker, H. (2020). Diversity of entomopathogenic fungi associated with Mediterranean fruit fly (*Ceratitis capitata* (Diptera: Tephritidae)) in Moroccan Argan forests and nearby area: impact of soil factors on their distribution. *BMC Ecology, 20*(1), 64. doi:10.1186/s12898-020-00334-2

Hallouti, A., Benjlil, H., El Hamdaoui, A., Ait Hammou, R., Ait Hamza, M., Zahidi, A., . . . Boubaker, H. (2021). Evaluation of native entomopathogenic fungi isolates for microbial control of the Mediterranean fruit fly (*Ceratitis Capitata* (Diptera: Tephritidae)) pupae and adults. *SJB*, 153-164. doi:10.47752/sjb.44.153.164

Hamzah, A. M., Mohsin, A. u., Naeem, M., & Khan, M. A. (2021). Efficacy of *Beauveria bassiana* and *Metarhizium anisopliae* (Ascomycota: Hypocreales) against *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) under controlled and open-field conditions on bitter gourd. *Egypt J Biol Pest Control, 31*(1), 144. doi:10.1186/s41938-021-00490-7

Hargreaves, J., Cooper, L., & Murray, D. (1986). Studies on the stinging of passionfruit by Queensland Fruit fly, Dacus tryoni and its control by bait and cover sprays. *Queensland Journal of Agricultural and Animal Sciences 43*, 33-40.

Hayden, T. P., Bidochka, M. J., & Khachatourians, G. G. (1992). Entomopathogenicity of Several Fungi toward the English Grain Aphid (Homoptera: Aphididae) and Enhancement of Virulence with Host Passage of *Paecilomyces farinosus*. *J. Econ. Entomol., 85*(1), 58-64. doi:10.1093/jee/85.1.58

Hendrichs, J., Robinson, A. S., Cayol, J. P., & Enkerlin, W. (2002). Medfly area wide sterile insect technique programmes for prevention, suppression or eradication: the importance of mating behavior studies Hendrichs 2002. *Fla Entomol., 85*(1), 1-13, 13.

Hennessey, M. K. (1994). Depth of pupation of Caribbean fruit fly (Diptera: Tephritidae) in soils in the laboratory. *Environ. Entomol., 23*(5), 1119-1123. doi:10.1093/ee/23.5.1119

Hetherington, S. (2005). Integrated pest and disease management for Australian summerfruit. *The State of New South Wales, NSW Department of Primary Industries, Orange, NSW, Australia*.

Heve, W. K., Adjadeh, T. A., & Billah, M. K. (2021). Overview and future research needs for development of effective biocontrol strategies for management of *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) in sub-Saharan Africa. *Pest Manag. Sci., 77*(10), 4224-4237. doi:https://doi.org/10.1002/ps.6485

Horticultural Policy Council. (1991). *The impact of fruit flies on Australian horticulture: Report to the Minister for Primary Industries and Energy*. Retrieved from Canberra:

Horticulture Innovation Australia. (2018). Management Options. Retrieved from https://area-wide-management.com.au/about/management-options/ - 1519257028473-c7cadf21-bcaf

Huang, B.-F., & Feng, M.-G. (2009). Comparative Tolerances of Various *Beauveria bassiana* Isolates to UV-B Irradiation with a Description of a Modeling Method to Assess Lethal Dose. *Mycopathologia, 168*(3), 145-152. doi:10.1007/s11046-009-9207-7

Hussein, M. A., Khaled, A. S., Ibrahim, A. A., Soliman, N. A., & Attia, S. H. (2018). Evaluation of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* on peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera:Tephritidae). *Egypt. Acad. J. Biol. Sci, 10*(1), 59-68. doi:10.21608/eajbsf.2018.17020

Ibrahim, A., Soliman, N., El-Deen, M., Ramadan, N., & Farag, S. (2014). Susceptibility of the peach fruit fly, *Bactrocera zonata* (Saunders) and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) adults to the entomopathogenic fungi; *Metarhizium anisopliae* (Met.) and *Beauveria bassiana* (Bals.). *Egypt J Biol Pest Control, 24*(2).

Imoulan, A., Alaoui, A., & El Meziane, A. (2011). Natural occurrence of soil-borne entomopathogenic fungi in the Moroccan Endemic forest of *Argania spinosa* and their pathogenicity to *Ceratitis capitata*. *World J. Microbiol. Biotechnol., 27*(11), 2619-2628. doi:10.1007/s11274-011-0735-1

Imoulan, A., & Elmeziane, A. (2014). Pathogenicity of *Beauveria bassiana* isolated from Moroccan Argan forests soil against larvae of *Ceratitis capitata* (Diptera: Tephritidae) in laboratory conditions. *World J Microbiol Biotechnol, 30*(3), 959-965. doi:10.1007/s11274-013-1514-y

Iqbal, M., Gogi, M. D., Atta, B., Nisar, M. J., Arif, M. J., & Javed, N. (2021). Assessment of pathogenicity of *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii* and *Bacillus thuringiensis var. kurstaki* against *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae) via diet-bioassay technique under controlled conditions. *Int. J. Trop. Insect Sci., 41*(2), 1129-1145. doi:10.1007/s42690-020-00298-2

Islam, W., Adnan, M., Shabbir, A., Naveed, H., Abubakar, Y. S., Qasim, M., . . . Ali, H. (2021). Insect-fungal-interactions: A detailed review on entomopathogenic fungi pathogenicity to combat insect pests. *Microb. Pathog., 159*, 105122. doi: 10.1016/j.micpath.2021.105122

Issaly, N., Chauveau, H., Aglevor, F., Fargues, J., & Durand, A. (2005). Influence of nutrient, pH and dissolved oxygen on the production of Metarhizium flavoviride Mf189 blastospores in submerged batch culture. *Process Biochem., 40*(3), 1425-1431. doi:https://doi.org/10.1016/j.procbio.2004.06.029

Jaber, S., Mercier, A., Knio, K., Brun, S., & Kambris, Z. (2016). Isolation of fungi from dead arthropods and identification of a new mosquito natural pathogen. *Parasit Vectors, 9*(1), 491. doi:10.1186/s13071-016-1763-3

Joop, G., & Vilcinskas, A. (2016). Coevolution of parasitic fungi and insect hosts. *Zoology, 119*(4), 350-358. doi:https://doi.org/10.1016/j.zool.2016.06.005

Kakani, E. G., Zygouridis, N. E., Tsoumani, K. T., Seraphides, N., Zalom, F. G., & Mathiopoulos, K. D. (2010). Spinosad resistance development in wild olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae) populations in California. *Pest Manag. Sci., 66*(4), 447-453. doi:https://doi.org/10.1002/ps.1921

Kim, J. C., Lee, M. R., Kim, S., Lee, S. J., Park, S. E., Nai, Y.-S., . . . Kim, J. S. (2018). Tenebrio molitor-mediated entomopathogenic fungal library construction for pest management. *J. Asia Pac. Entomol., 21*(1), 196-204. doi:https://doi.org/10.1016/j.aspen.2017.11.018

Knipling, E. F. (1955). Possibilities of insect control or eradication through the use of sexually sterile males. *J. Econ. Entomol., 48*(4), 459-462. doi:10.1093/jee/48.4.459

Krafsur, E. (1998). Sterile insect technique for suppressing and eradicating insect population: 55 years and counting. *J. Agric. Entomol., 15*(4), 303.

Kumaran, N., Balagawi, S., Schutze, M. K., & Clarke, A. R. (2013). Evolution of lure response in tephritid fruit flies: phytochemicals as drivers of sexual selection. *Animal Behaviour, 85*(4), 781-789. doi:https://doi.org/10.1016/j.anbehav.2013.01.024

Kumaran, N., & Clarke, A. R. (2014). Indirect effects of phytochemicals on offspring performance of Queensland fruit fly, *Bactrocera tryoni* (Diptera: Tephritidae). *J. Appl. Entomol., 138*(5), 361-367. doi:10.1111/jen.12082

Kumaran, N., Hayes, R. A., & Clarke, A. R. (2014). Cuelure but not zingerone make the sex pheromone of male Bactrocera tryoni (Tephritidae: Diptera) more attractive to females. *J. Insect Physiol., 68*, 36-43. doi:https://doi.org/10.1016/j.jinsphys.2014.06.015

Lacey, L. A., Grzywacz, D., Shapiro-Ilan, D. I., Frutos, R., Brownbridge, M., & Goettel, M. S. (2015). Insect pathogens as biological control agents: Back to the future. *J. Invertebr. Pathol., 132*, 1-41. doi: 10.1016/j.jip.2015.07.009

Lu, H. L., & St. Leger, R. J. (2016). Chapter Seven - Insect Immunity to Entomopathogenic Fungi. In B. Lovett & R. J. St. Leger (Eds.), *Advances in Genetics* (Vol. 94, pp. 251-285): Academic Press.

Magaña, C., Hernández-Crespo, P., Ortego, F., & Castañera, P. (2007). Resistance to malathion in field populations of Ceratitis capitata. *J Econ Entomol, 100*(6), 1836-1843. doi:10.1603/0022-0493(2007)100[1836:rtmifp]2.0.co;2

Mahmoud, F. M. (2009). Susceptibility of the peach fruit fly, *Bactrocera zonata* (Saunders), (Diptera: Tephritidae) to three entomopathogenic fungi. *Egypt J Biol Pest Control, 19*, 169-`175.

Majchrowska-Safaryan, A., & Tkaczuk, C. (2021). Abundance of entomopathogenic fungi in leaf litter and soil layers in forested habitats in Poland. *Insects, 12*(2). doi:10.3390/insects12020134

Mantzoukas, S., Kitsiou, F., Natsiopoulos, D., & Eliopoulos, P. A. (2022). Entomopathogenic Fungi: Interactions and Applications. *Encyclopedia, 2*(2), 646-656.

Marri, D., Gomez, D. A. M. A., Wilson, D., Billah, M., Yeboah, S., & Osae, M. (2016). Evaluation of the efficacy of a commercial formulation of *beauveria bassiana* for the control of the invasive fruit fly *bactrocera dorsalis* (Diptera: Tephritidae). *Biopestic. Int. , 12*, 9-19.

Martínez-Barrera, O. Y., Toledo, J., Cancino, J., Liedo, P., Gómez, J., Valle-Mora, J., & Montoya, P. (2020). Interaction Between *Beauveria bassiana* (Hypocreales: Cordycipitaceae) and *Coptera haywardi* (Hymenoptera: Diapriidae) for the Management of *Anastrepha obliqua* (Diptera: Tephritidae). *J. Insect Sci., 20*(2). doi:10.1093/jisesa/ieaa010

Martínez-Barrera, O. Y., Toledo, J., Liedo, P., Gómez, J., Valle-Mora, J., Cancino, J., & Montoya, P. (2019). Does *Beauveria bassiana* (Hypocreales: Cordycipitaceae) affect the survival and fecundity of the parasitoid *Coptera haywardi* (Hymenoptera: Diapriidae)? *Environ. Entomol., 48*(1), 156-162. doi:10.1093/ee/nvy182

May, A. (1958). Fruit fly problem in southern and central Queensland. *Queensland Department of Agriculture and Stock, Division of Plant Industry Advisory leaflet, 84*, 153-159.

McGuire, A. V., & Northfield, T. D. (2020). Tropical occurrence and agricultural importance of *Beauveria bassiana* and *Metarhizium anisopliae*. *Front. Sustain. Food Syst., 4*(6). doi:10.3389/fsufs.2020.00006

Melesse, T., & Ferdu, A. (2021). Virulence of *Beauveria bassiana* and *Metarhizium anisopliae* Isolates against the oriental fruit fly *Bactrocera dorsalis* (Diptera: Tephritidae) hendel under laboratory conditions. *Ethiop. J. Agric. Sci., 31*(3), 53-67.

Metcalf, R. L., & Metcalf, E. R. (1992). *Plant kairomones in insect ecology and control*. New York: Chapman and Hall

Moore, D., Bridge, P. D., Higgins, P. M., Bateman, R. P., & Prior, C. (1993). Ultra-violet radiation damage to *Metarhizium flavoviride* conidia and the protection given by vegetable and mineral oils and chemical sunscreens. *Ann. Appl. Biol., 122*(3), 605-616. doi:10.1111/j.1744-7348.1993.tb04061.x

Murtaza, G., Naeem, M., Manzoor, S., Khan, H. A., Eed, E. M., Majeed, W., . . . Ummara, U. E. (2022). Biological control potential of entomopathogenic fungal strains against peach Fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). *PeerJ, 10*, e13316. doi:10.7717/peerj.13316

NFFSIC. (2010). Draft national fruit fly strategy: Implementation action plan. *Plant Health Australia, Canberra, ACT, Australia*.

Northfield, T. D., Barton, B. T., & Schmitz, O. J. (2017). A spatial theory for emergent multiple predator–prey interactions in food webs. *Ecol. Evol., 7*(17), 6935-6948. doi: 10.1002/ece3.3250

Nussenbaum, A. L., Lewylle, M. A., & Lecuona, R. (2013). Germination, radial growth and virulence to boll weevil of entomopathogenic fungi at different temperatures. *World Appl. Sci. J., 25*, 1134-1140. doi:10.5829/idosi.wasj.2013.25.08.1237

Onsongo, S. K., Mohamed, S. A., Akutse, K. S., Gichimu, B. M., & Dubois, T. (2022). The entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* for management of the Melon fly *Zeugodacus cucurbitae*: Pathogenicity, horizontal transmission, and compatability with cuelure. *Insects, 13*(10), 859.

Ouedraogo, A., Fargues, J., Goettel, M. S., & Lomer, C. J. (1997). Effect of temperature on vegetative growth among isolates of *Metarhizium anisopliae* and *M. flavoviride*. *Mycopathologia, 137*(1), 37-43. doi:10.1023/a:1006882621776

Padmavathi, J., Uma Devi, K., & Uma Maheswara Rao, C. (2003). The optimum and tolerance pH range is correlated to colonial morphology in isolates of the entomopathogenic fungus *Beauveria bassiana* – a potential biopesticide. *World J. Microbiol. Biotechnol., 19*(5), 469-477. doi:10.1023/A:1025151000398

Petacchi, R., Rizzi, I., & Guidotti, D. (2003). The 'lure and kill' technique in *Bactrocera oleae* (Gmel.) control: effectiveness indices and suitability of the technique in area-wide experimental trials. *Int. J. Pest Manag., 49*(4), 305-311. doi:10.1080/0967087031000155648

Plant Health Australia. (2018). The Australian Handbook for the Identification of Fruit Flies (Vol. Version 3.1). Canberra, ACT: Plant Health Australia.

Presa-Parra, E., Hernández-Rosas, F., Bernal, J. S., Valenzuela-González, J. E., Altúzar-Molina, A., & Birke, A. (2020). Occurrence, identification, and virulence of native fungal pathogens isolated from Mexican fruit fly (Diptera: Tephritidae) larvae from soils of three cropping systems. *J. Econ. Entomol., 113*(3), 1088-1096. doi:10.1093/jee/toaa012

Presa-Parra, E., Hernández-Rosas, F., Bernal, J. S., Valenzuela-González, J. E., Martínez-Tlapa, J., & Birke, A. (2021). Impact of *Metarhizium robertsii* on Adults of the Parasitoid *Diachasmimorpha longicaudata* and Parasitized *Anastrepha ludens* Larvae. *Insects, 12*(2), 125.

Prokopy, R. J., Papaj, D. R., Hendrichs, J., & Wong, T. T. Y. (1992). Behavioral responses of Ceratitis capitata flies to bait spray droplets and natural food. *Entomol. Exp. Appl., 64*(3), 247-257. doi:10.1111/j.1570-7458.1992.tb01615.x

Půža, V., & Tarasco, E. (2023). Interactions between Entomopathogenic Fungi and Entomopathogenic Nematodes. *Microorganisms, 11*(1), 163.

Quesada-Moraga, E., González-Mas, N., Yousef-Yousef, M., Garrido-Jurado, I., & Fernández-Bravo, M. (2024). Key role of environmental competence in successful use of entomopathogenic fungi in microbial pest control. *Journal of Pest Science, 97*(1), 1-15. doi:10.1007/s10340-023-01622-8

Quesada-Moraga, E., Martin-Carballo, I., Garrido-Jurado, I., & Santiago-Álvarez, C. (2008). Horizontal transmission of *Metarhizium anisopliae* among laboratory populations of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). *Biol. Control, 47*(1), 115-124. doi:10.1016/j.biocontrol.2008.07.002

Quesada-Moraga, E., Navas-Cortés, J. A., Maranhao, E. A. A., Ortiz-Urquiza, A., & Santiago-Álvarez, C. (2007). Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils. *Mycol. Res., 111*(8), 947-966. doi:10.1016/j.mycres.2007.06.006

Quesada-Moraga, E., Ruiz-García, A., & Santiago-Álvarez, C. (2006). Laboratory evaluation of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* against puparia and adults of *Ceratitis capitata* (Diptera: Tephritidae). *J. Econ. Entomol., 99*(6), 1955-1966. doi:10.1093/jee/99.6.1955

Quesada-Moraga, E., & Vey, A. (2003). Intra-specific variation in virulence and In vitro production of macromolecular toxins active against locust among *Beauveria bassiana* strains and effects of in vivo and In vitro passage on these factors. *Biocontrol Sci Technol, 13*(3), 323-340. doi:10.1080/0958315031000110346

Reynolds, O. L., Osborne, T. J., & Barchia, I. (2017). Efficacy of chemicals for the potential management of the Queensland fruit Fly *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). *Insects, 8*(2). doi:10.3390/insects8020049

Richmond, M. (2022). Use: Growth in Use, Present-Day Volume, and Projected Use of Parathion, Malathion, Diazinon, Tetrachlorvinphos, and Glyphosate. Switzerland: Springer International Publishing AG.

Rosenheim, J. A., Kaya, H. K., Ehler, L. E., Marois, J. J., & Jaffee, B. A. (1995). Intraguild Predation Among Biological-Control Agents: Theory and Evidence. *Biological Control, 5*(3), 303-335. doi:10.1006/bcon.1995.1038

Senthilkumar, M., Amaresan, N., & Sankaranarayanan, A. (2021). Galleria bait technique for the isolation of entomopathogenic fungi from soil. In M. Senthilkumar, N. Amaresan, & A. Sankaranarayanan (Eds.), *Plant-Microbe Interactions: Laboratory Techniques* (pp. 257-259). New York, NY: Springer US.

Sharma, L., Oliveira, I., Gonçalves, F., Raimundo, F., Singh, R. K., Torres, L., & Marques, G. (2021). Effect of soil chemical properties on the occurrence and distribution of entomopathogenic fungi in Portuguese grapevine fields. *Pathogens, 10*(2). doi:10.3390/pathogens10020137

Smith, D., Beattie, G. A., & Broadley, R. (1997). *Citrus pests and their natural enemies: integrated pest management in Australia*. Brisbane: Dept. of Primary Industries, Queensland.

Smith, E. (2000). *Internal and external fruit fly quarantine in Australia.* Paper presented at the Proceedings of the Indian Ocean Commission, Regional Fruit Fly Symposium, Flic en Flac, Mauritius, 5th-9th June, 2000.

Soliman, N. A. (2020). Toxicological and Biochemical Effects of *Beauveria bassiana* (Bals.) on Peach Fruit Fly, *Bactrocera zonata* (Saunders) Immature Stages. *j. plant prot. & pathol., 11*, 579-585. doi:10.21608/jppp.2020.133805

Soliman, N. A., Al-amin, S. M., Mesbah, A. E., Ibrahim, A. M. A., & Mahmoud, A. M. A. (2020). Pathogenicity of three entomopathogenic fungi against the Mediterranean

fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). *Egypt J Biol Pest Control, 30*(1). doi:10.1186/s41938-020-00235-y

Sookar, P., Bhagwant, s., & Allymamod, M. N. (2014). Effect of *Metarhizium anisopliae* on the fertility and fecundity of two species of fruit flies and horizontal transmission of mycotic infection. *J. Insect Sci., 14*(1). doi:10.1093/jis/14.1.100

Sproul, A., Broughton, S., De Lima, F., Hardie, D., & Monzu, N. (2001). The fight against fruit flies in Western Australia. . *Department of Agriculture, Western Australia; South Perth, Australia*(4504).

Steiner, L. F., Hart, W. G., Harris, E. J., Cunningham, R. T., Ohinata, K., & Kamakahi, D. C. (1970). Eradication of the oriental fruit fly from the Mariana Islands by the methods of male annihilation and sterile insect release. *J. Econ. Entomol., 63*(1), 131-135. doi:10.1093/jee/63.1.131

Stringer, L., Kean, J., Beggs, J., & Suckling, D. (2017). Management and eradication options for Queensland fruit fly. *Population Ecology*. doi:10.1007/s10144-017-0593-2

Stringer, L. D., Soopaya, R., Butler, R. C., Vargas, R. I., Souder, S. K., Jessup, A. J., . . . Suckling, D. M. (2019). Effect of Lure Combination on Fruit Fly Surveillance Sensitivity. *Scientific reports, 9*(1), 2653. doi:10.1038/s41598-018-37487-6

Thomas, M. B., & Jenkins, N. E. (1997). Effects of temperature on growth of Metarhizium flavoviride and virulence to the variegated grasshopper, Zonocerus variegatus. *Mycological Research, 101*(12), 1469-1474. doi: 10.1017/S0953756297004401

Toledo, J., Campos, S. E., Flores, S., Liedo, P., Barrera, J. F., Villasenor, A., & Montoya, P. (2007). Horizontal Transmission of *Beauveria bassiana* in Anastrepha ludens (Diptera: Tephritidae) Under Laboratory and Field Cage Conditions. *J. Econ. Entomol., 100*(2), 291-297. doi:10.1093/jee/100.2.291

Toledo, J., Campos, S. E., Flores, S., Liedo, P., Barrera, J. F., Villaseñor, A., & Montoya, P. (2014). Horizontal transmission of *Beauveria bassiana* in *Anastrepha ludens* (Diptera: Tephritidae) under laboratory and field cage conditions. *J. Econ. Entomol., 100*(2), 291-297. doi:10.1093/jee/100.2.291

Tora, M., & Azerefegn, F. (2021). Virulence of *Beauveria bassiana* and *Metarhizium anisopliae* isolates against the oriental fruit fly *Bactrocera dorsalis* (Diptera: Tephritidae) Hendel under laboratory conditions. *Ethiop. J. Agric. Sci., 31*(3), 53-67.

Tranter, C., & Hughes, W. O. H. (2015). Acid, silk and grooming: alternative strategies in social immunity in ants? *Behav. Ecol. Sociobiol. , 69*(10), 1687-1699.

Usman, M., Gulzar, S., Wakil, W., Wu, S., Piñero, J. C., Leskey, T. C., . . . Shapiro-Ilan, D. (2020). Virulence of Entomopathogenic Fungi to *Rhagoletis pomonella* (Diptera: Tephritidae) and Interactions With Entomopathogenic Nematodes. *J Econ Entomol, 113*(6), 2627-2633. doi:10.1093/jee/toaa209

Usman, M., Wakil, W., Piñero, J. C., Wu, S., Toews, M. D., & Shapiro-Ilan, D. I. (2021). Evaluation of locally isolated entomopathogenic fungi against multiple life stages of *Bactrocera zonata* and *Bactrocera dorsalis* (Diptera: Tephritidae): laboratory and field study. *Microorganisms, 9*(8), 1791.

Vaenninen, I., Tyni-Juslin, J., & Hokkanen, H. (2000). Persistence of augmented *Metarhizium anisopliae* and *Beauveria bassiana* in Finnish agricultural soils. *BioControl (Dordrecht, Netherlands), 45*(2), 201-222. doi:10.1023/A:1009998919531

Vargas, R., Leblanc, L., Pinero, J., & Hoffman, K. (2014). Male annihilation, past, present, and future (pp. 493-511).

Vargas, R. I., Jang, E. B., & Klungness, L. M. (2003). *Area-wide pest management of fruit flies in Hawaiian fruits and vegetables*. Retrieved from Japan: http://inis.iaea.org/search/search.aspx?orig_q=RN:39064898

Vargas, R. I., Pinero, J. C., & Leblanc, L. (2015). An overview of pest species of *Bactrocera* fruit flies (Diptera: Tephritidae) and the integration of biopesticides with Other biological approaches for their management with a focus on the Pacific region. *Insects, 6*(2), 297-318. doi:10.3390/insects6020297

Vargas, R. I., Piñero, J. C., Mau, R. F., Jang, E. B., Klungness, L. M., McInnis, D. O., . . . Wong, L. (2010). Area-wide suppression of the Mediterranean fruit fly, *Ceratitis capitata*, and the Oriental fruit fly, *Bactrocera dorsalis*, in Kamuela, Hawaii. *J. Insect. Sci. (Online), 10*, 135. doi:10.1673/031.010.13501

Vickers, R. (1997). Progress in developing an alternative to protein hydrolysate bait sprays. In A. Allwood & R. A. Drew (Eds.), *Management of Fruit flies in the Pacific* (pp. 117-120). Nadi, Fiji: ACIAR.

Vontas, J., Hernández-Crespo, P., Margaritopoulos, J. T., Ortego, F., Feng, H.-T., Mathiopoulos, K. D., & Hsu, J.-C. (2011). Insecticide resistance in Tephritid flies. *Pestic. Biochem. and Phys., 100*(3), 199-205. doi: 10.1016/j.pestbp.2011.04.004

Vreysen, M. J. B., Barclay, H. J., & Hendrichs, J. (2006). Modeling of preferential mating in areawide control programs that integrate the release of strains of sterile males only or both sexes. *Ann. Entomol. Soc. Am., 99*(3), 607-616, 610.

Vreysen, M. J. B., & Robinson, A. S. (2011). Ionising radiation and area-wide management of insect pests to promote sustainable agriculture. A review. *ASD, 31*(1), 233-250. doi:10.1051/agro/2010009

Wakil, W., Usman, M., Piñero, J. C., Wu, S., Toews, M. D., & Shapiro-Ilan, D. I. (2022). Combined application of entomopathogenic nematodes and fungi against fruit flies, *Bactrocera zonata* and *B. dorsalis* (Diptera: Tephritidae): laboratory cups to field study. *Pest Manag Sci, 78*(7), 2779-2791. doi:10.1002/ps.6899

Wang, D., Liang, Q., Chen, M., Ye, H., Liao, Y., Yin, J., . . . He, Y. (2021). Susceptibility of oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae) pupae to entomopathogenic fungi. *Appl. Entomol. Zool., 56*. doi:10.1007/s13355-021-00734-w

Wang, H., Jin, L., Peng, T., Zhang, H., Chen, Q., & Hua, Y. (2014). Identification of cultivable bacteria in the intestinal tract of Bactrocera dorsalis from three different populations and determination of their attractive potential. *Pest Manag. Sci., 70*(1), 80-87. doi:10.1002/ps.3528

Wangkeeree, J., & Suwanchaisri, K. (2022). Effectiveness of Beauveria and Metarhizium in the biological control of the fruit fly Bactrocera dorsalis (Hendel)(Diptera: Tephritidae). *Khon Kaen Agr. J., 50*(1), 301-309.

White, B., Sadler, R., Florec, V., & Dominiak, B. (2012). *Economics of surveillance: a bioeconomic assessment of Queensland fruit fly*. http://ageconsearch.umn.edu/record/125741/files/White_etal_Economics_of_Surveillance_AARES.pdf

Wraight, S. P., Lacey, L. A., Kabaluk, J. T., & Goettel, M. S. (2009). Potential for microbial biological control of coleopteran and hemipteran pests of potato. *Fruit, Vegetable, and Cereal, Science and Biotechnology, 3*(1), 25-38.

Yee, W. L., & Alston, D. G. (2012). Behavioral responses, rate of mortality, and oviposition of western cherry fruit fly exposed to malathion, zeta-cypermethrin, and spinetoram. *Journal of Pest Science, 85*(1), 141-151. doi:10.1007/s10340-011-0388-8

Yee, W. L., & Lacey, L. A. (2005). Mortality of different life stages of *Rhagoletis indifferens* (Diptera: Tephritidae) exposed to the entomopathogenic fungus *Metarhizium anisopliae*. *J. Entomol. Sci., 40*(2), 167-177. doi:10.18474/0749-8004-40.2.167

Yousef, M., Alba-Ramírez, C., Garrido Jurado, I., Mateu, J., Raya Díaz, S., Valverde-García, P., & Quesada-Moraga, E. (2018). *Metarhizium brunneum* (Ascomycota;

Hypocreales) Treatments Targeting Olive Fly in the Soil for Sustainable Crop Production. *Front Plant Sci, 9*, 1. doi:10.3389/fpls.2018.00001

Yousef, M., Garrido-Jurado, I., Ruíz-Torres, M., & Quesada-Moraga, E. (2017). Reduction of adult olive fruit fly populations by targeting preimaginals in the soil with the entomopathogenic fungus *Metarhizium brunneum*. *J Pest Sci, 90*(1), 345-354. doi:10.1007/s10340-016-0779-y

Zhang, Q., Cai, P., Wang, B., Liu, X., Lin, J., Hua, R., . . . Ji, Q. (2021). Manipulation of gut symbionts for improving the sterile insect technique: quality parameters of *Bactrocera dorsalis* (Diptera: Tephritidae) genetic sexing strain males after feeding on bacteria-enriched diets. *J Econ Entomol, 114*(2), 560-570.

Zimmermann, G. (1986). The 'Galleria bait method' for detection of entomopathogenic fungi in soil. *J. Appl. Entomol., 102*(1-5), 213-215. doi:10.1111/j.1439-0418.1986.tb00912.x

## References: Chapter 2


Ahmad, S., A. Sarwar, A. Shoaib, A. Javaid, M. S. Hanif, and Q. Ali. 2022. Sustainable management of guava fruit fly *Bactrocera zonata* (Tephritidae: Diptera) by entomopathogenic fungi. Fresenius Environ. Bull. 31: 5522-5527.

Allwood, A., E. Vueti, L. Leblanc, and R. Bull. 2002. Eradication of introduced *Bactrocera* species (Diptera: Tephritidae) in Nauru using male annihilation and protein bait application techniques, pp. 19-25. In C. R. Veitch and M. N. Clout (eds.), Turning the tide: the eradication of invasive species. IUCN SCC Invasive Species Specialist Group, Gland, Swisterland and Cambridge, UK.

Aung, O. M., K. Soytong, and K. Hyde. 2008. Diversity of entomopathogenic fungi in rainforests of Chiang Mai Province, Thailand, vol. 30.

Bateman, M. 1968. Determinants of abundance in a population of the Queensland fruit fly, pp. 119-131. In T. R. E. Southwood (ed.), Insect Abundance. Blackwell Scientific Publications, Oxford, UK.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67: 1 - 48.

Beris, E. I., D. P. Papachristos, A. Fytrou, S. A. Antonatos, and D. C. Kontodimas. 2013. Pathogenicity of three entomopathogenic fungi on pupae and adults of the *Mediterranean* fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). J Pest Sci 86: 275-284.

Bidochka, M. J., and C. L. Small. 2005. Phylogeography of *Metarhizium*, an insect pathogenic fungus, pp. 75-118, Insect-fungal associations: ecology and evolution. Oxford University Press, New York, NY.

Bischoff, J. F., S. A. Rehner, and R. A. Humber. 2009. A multilocus phylogeny of the *Metarhizium anisopliae* lineage. Mycologia. 101: 512-530.

Brunner-Mendoza, C., M. d. R. Reyes-Montes, S. Moonjely, M. J. Bidochka, and C. Toriello. 2019. A review on the genus *Metarhizium* as an entomopathogenic microbial biocontrol agent with emphasis on its use and utility in Mexico. Biocontrol Sci. Technol. 29: 83-102.

Carswell, I., R. Spooner-hart, and R. J. Milner. 1998. Laboratory susceptibility of *Musca domestica* L. (Diptera: Muscidae) and *Bactrocera tryoni* (Frogatt) (Diptera: Tephritidae) to an isolate of *Metarhizium anisopliae* (Metsch.) Sorokin. Aust. J. Entomol. 37: 281-284.

Castillo, M.-A., P. Moya, E. Hernández, and E. Primo-Yúfera. 2000. Susceptibility of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) to entomopathogenic fungi and their extracts. Biocontrol 19: 274-282.

Clarke, A. R., K. S. Powell, C. W. Weldon, and P. W. Taylor. 2011. The ecology of *Bactrocera tryoni* (Diptera: Tephritidae): what do we know to assist pest management? Ann. Appl. Biol. 158: 26-54.

Cremlyn, R. 1978. Pesticides. Preparation and mode of action, John Wiley & Sons., Chichester, UK.

Dimbi, S., N. K. Maniania, S. A. Lux, S. Ekesi, and J. K. Mueke. 2003. Pathogenicity of *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin, to three adult fruit fly species: *Ceratitis capitata* (Weidemann), *C. rosa var. fasciventris* Karsch and *C. cosyra* (Walker) (Diptera :Tephritidae). Mycopathologia 156: 375-382.

Dodds, K., K. O'Malley, S. Hetherington, and J. Kidston. 2014. Orchard Plant Protection Guide for Deciduous Fruits In NSW 2014–15, pp. 2200–7539. Department of Primary Industries, New South Wales; Orange, Australia.

Ekesi, S. 2016. Baiting and male annihilation techniques for fruit fly suppression in Africa, pp. 275-292. In S. Ekesi, S. A. Mohamed and M. De Meyer (eds.), Fruit Fly Research and Development in Africa - Towards a Sustainable Management Strategy to Improve Horticulture. Springer International Publishing, Cham.

Ekesi, S., N. K. Maniania, and S. A. Lux. 2002. Mortality in three African Tephritid fruit fly puparia and adults caused by the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*. Biocontrol Sci Technol 12: 7-17.

El-Gendy, I. R., M. F. M. Zawrah, and M. I. El-Banobi. 2022. Virulence effect of *Metarhizium anisopliae* (Met.) and *Beauveria bassiana* (Bals.) fungi against the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). Egypt J Biol Pest Control 32: 43.

Faye, P. D., A. B. Bal, N. M. Ndiaye, F. Diop, Y. K. Sangaré, C. Haddad, E. V. Coly, E. O. Dieng, and S. Niassy. 2021. Field efficacy of *Metarhizium acridum* (Hypocreales: Clavicipitaceae) in the control of *Bactrocera dorsalis* (Diptera: Tephritidae) in citrus orchards in Senegal. Int. J. Trop. Insect Sci. 41: 1185-1195.

Garrido-Jurado, I., M. Fernández-Bravo, C. Campos, and E. Quesada-Moraga. 2015. Diversity of entomopathogenic Hypocreales in soil and phylloplanes of five Mediterranean cropping systems. J. Invertebr. Pathol. 130: 97-106.

Gindin, G., S. Levski, I. Glazer, and V. Soroker. 2006. Evaluation of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* against the red palm weevil *Rhynchophorus ferrugineus*. Phytoparasitica 34: 370-379.

Greenfield, M., M. I. Gómez-Jiménez, V. Ortiz, F. E. Vega, M. Kramer, and S. Parsa. 2016. *Beauveria bassiana* and *Metarhizium anisopliae* endophytically colonize cassava roots following soil drench inoculation. Biol. 95: 40-48.

Guerrero-Guerra, C., M. d. R. Reyes-Montes, C. Toriello, V. Hernández-Velázquez, I. Santiago-López, L. Mora-Palomino, M. E. Calderón-Segura, S. D. Fernández, and C. Calderón-Ezquerro. 2013. Study of the persistence and viability of *Metarhizium acridum* in Mexico's agricultural area. Aerobiologia. 29: 249-261.

Gul, H. T., S. Freed, M. Akmal, and M. N. Malik. 2015. Vulnerability of different life stages of *Bactrocera zonata* (Tephritidae: Diptera) against entomogenous fungi. Pak. J. Zool. 47.

Hamzah, A. M., A. u. Mohsin, M. Naeem, and M. A. Khan. 2021. Efficacy of *Beauveria bassiana* and *Metarhizium anisopliae* (Ascomycota: Hypocreales) against *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) under controlled and open-field conditions on bitter gourd. Egypt J Biol Pest Control 31: 144.

Harrison, X. A. 2015. A comparison of observation-level random effect and Beta-Binomial models for modelling overdispersion in Binomial data in ecology & evolution. PeerJ 3: e1114.

Hetherington, S. 2005. Integrated pest and disease management for Australian summerfruit. The State of New South Wales, NSW Department of Primary Industries, Orange, NSW, Australia.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. Biom. J. 50: 346-363.

Hussein, M. A., A. S. Khaled, A. A. Ibrahim, N. A. Soliman, and S. H. Attia. 2018. Evaluation of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* on peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera:Tephritidae). Egypt. Acad. J. Biol. Sci 10: 59-68.

Ibrahim, A., N. Soliman, M. El-Deen, N. Ramadan, and S. Farag. 2014. Susceptibility of the peach fruit fly, *Bactrocera zonata* (Saunders) and the Mediterranean fruit fly,

*Ceratitis capitata* (Wiedemann) adults to the entomopathogenic fungi; *Metarhizium anisopliae* (Met.) and *Beauveria bassiana* (Bals.). Egypt J Biol Pest Control 24.

Iqbal, M., M. D. Gogi, B. Atta, M. J. Nisar, M. J. Arif, and N. Javed. 2021. Assessment of pathogenicity of *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii* and *Bacillus thuringiensis var. kurstaki* against *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae) via diet-bioassay technique under controlled conditions. Int. J. Trop. Insect Sci. 41: 1129-1145.

Korosi, G. A., B. A. L. Wilson, K. S. Powell, G. J. Ash, A. Reineke, and S. Savocchia. 2019. Occurrence and diversity of entomopathogenic fungi (*Beauveria* spp. and *Metarhizium* spp.) in Australian vineyard soils. J. Invertebr. Pathol. 164: 69-77.

Lovett, B., and R. J. St. Leger. 2015. Stress is the rule rather than the exception for *Metarhizium*. Curr. Genet. 61: 253-261.

May, A. 1958. Fruit fly problem in southern and central Queensland. Queensland Department of Agriculture and Stock, Division of Plant Industry Advisory leaflet 84: 153-159.

McGuire, A. V., and T. D. Northfield. 2020. Tropical occurrence and agricultural importance of *Beauveria bassiana* and *Metarhizium anisopliae*. Front. Sustain. Food Syst., 4.

McGuire, A. V., and T. D. Northfield. 2021. Identification and evaluation of endemic *Metarhizium* strains for biological control of banana rust thrips. Biol. Control 162: 104712.

Melesse, T., and A. Ferdu. 2021. Virulence of *Beauveria bassiana* and *Metarhizium anisopliae* Isolates against the oriental fruit fly *Bactrocera dorsalis* (Diptera:Tephritidae) hendel under laboratory conditions. Ethiop. J. Agric. Sci. 31: 53-67.

Milner, R. J., P. Samson, and R. Morton. 2003. Persistence of conidia of *Metarhizium anisopliae* in sugarcane fields: Effect of isolate and formulation on persistence over 3.5 years. Biocontrol Sci. Technol. 13: 507-516.

Ortiz-Urquiza, A., and N. O. Keyhani. 2013. Action on the surface: Entomopathogenic fungi versus the insect cuticle. Insects 4: 357-374.

Quesada-Moraga, E., A. Ruiz-García, and C. Santiago-Álvarez. 2006. Laboratory evaluation of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* against puparia and adults of *Ceratitis capitata* (Diptera: Tephritidae). J. Econ. Entomol. 99: 1955-1966.

R Core Team 2020. R: A language and environment for statistical computing computer program, version By R Core Team, Vienna, Austria.

Rocha, L. F. N., P. W. Inglis, R. A. Humber, A. Kipnis, and C. Luz. 2013. Occurrence of *Metarhizium* spp. in Central Brazilian soils. J. Basic Microbiol. 53: 251-259.

Soliman, N. A., S. M. Al-amin, A. E. Mesbah, A. M. A. Ibrahim, and A. M. A. Mahmoud. 2020. Pathogenicity of three entomopathogenic fungi against the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Egypt J Biol Pest Control 30.

Sookar, P., S. Bhagwant, and M. N. Allymamod. 2014. Effect of *Metarhizium anisopliae* on the fertility and fecundity of two species of fruit flies and horizontal transmission of mycotic infection. J Insect Sci 14: 100.

Sproul, A., S. Broughton, F. De Lima, D. Hardie, and N. Monzu. 2001. The fight against fruit flies in Western Australia. . Department of Agriculture, Western Australia; South Perth, Australia.

Stonehouse, J. M., J. D. Mumford, A. Verghese, R. P. Shukla, S. Satpathy, H. S. Singh, T. Jiji, J. Thomas, Z. P. Patel, R. C. Jhala, R. K. Patel, A. Manzar, T. M.

Shivalingaswamy, A. K. Mohantha, B. Nair, C. V. Vidya, V. S. Jagadale, D. B. Sisodiya, and B. K. Joshi. 2007. Village-level area-wide fruit fly suppression in India: Bait application and male annihilation at village level and farm level. J. Crop Prot. 26: 788-793.

Vargas, R. I., J. C. Pinero, and L. Leblanc. 2015. An overview of pest species of *Bactrocera* fruit flies (Diptera: Tephritidae) and the integration of biopesticides with Other biological approaches for their management with a focus on the Pacific region. Insects 6: 297-318.

Wang, D., Q. Liang, M. Chen, H. Ye, Y. Liao, J. Yin, L. H. Lu, Y. Lei, D. Cai, W. Jaleel, and Y. He. 2021. Susceptibility of oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae) pupae to entomopathogenic fungi. Appl. Entomol. Zool. 56.

Wangkeeree, J., and K. Suwanchaisri. 2022. Effectiveness of Beauveria and Metarhizium in the biological control of the fruit fly Bactrocera dorsalis (Hendel)(Diptera: Tephritidae). Khon Kaen Agr. J. 50: 301-309.

Warton, D. I., and F. K. C. Hui. 2011. The arcsine is asinine: the analysis of proportions in ecology. Ecology 92: 3-10.

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## References: Chapter 3


Adames, M., Fernández-Ruvalcaba, M., Peña-Chora, G., & Hernández-Velázquez, V. M. (2011). Effects of passages through a suitable host of the fungus, *Metarhizium anisopliae*, on the virulence of acaricide-susceptible and resistant strains of the tick, *Rhipicephalus microplus*. *J Insect Sci, 11*(1), 21. doi:10.1673/031.011.0121

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw., 67*(1), 1 - 48. doi:10.18637/jss.v067.i01

Bava, R., Castagna, F., Piras, C., Musolino, V., Lupia, C., Palma, E., . . . Musella, V. (2022). Entomopathogenic Fungi for Pests and Predators Control in Beekeeping. *Vet Sci, 9*(2). doi:10.3390/vetsci9020095

Bischoff, J. F., Rehner, S. A., & Humber, R. A. (2009). A multilocus phylogeny of the *Metarhizium anisopliae* lineage. *Mycologia, 101*(4), 512-530. doi:10.3852/07-202

Charnley, A. K. (2003). Fungal pathogens of insects: Cuticle degrading enzymes and toxins *Advances in Botanical Research* (Vol. 40, pp. 241-321): Academic Press.

Daoust, R. A., & Roberts, D. W. (1982). Virulence of natural and insect-passaged strains of Metarhizium anisopliae to mosquito larvae. *J. Invertebr. Pathol., 40*(1), 107-117. doi:10.1016/0022-2011(82)90042-8

de Crecy, E., Jaronski, S., Lyons, B., Lyons, T. J., & Keyhani, N. O. (2009). Directed evolution of a filamentous fungus for thermotolerance. *BMC Biotechnology, 9*(1), 74. doi:10.1186/1472-6750-9-74

Dimbi, S., Maniania, N. K., Lux, S. A., Ekesi, S., & Mueke, J. K. (2003). Pathogenicity of *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin, to three adult fruit fly species: *Ceratitis capitata* (Weidemann), *C. rosa var. fasciventris* Karsch and *C. cosyra* (Walker) (Diptera :Tephritidae). *Mycopathologia, 156*(4), 375-382. doi:10.1023/B:MYCO.0000003579.48647.16

Frazzon, A. P. G., Vaz Junior, I. d. S., Masuda, A., Schrank, A., & Vainstein, M. H. (2000). In vitro assessment of *Metarhizium anisopliae* isolates to control the cattle tick *Boophilus microplus*. *Veterinary parasitology, 94*(1), 117-125. doi:10.1016/S0304-4017(00)00368-X

Hayden, T. P., Bidochka, M. J., & Khachatourians, G. G. (1992). Entomopathogenicity of Several Fungi toward the English Grain Aphid (Homoptera: Aphididae) and Enhancement of Virulence with Host Passage of *Paecilomyces farinosus*. *J. Econ. Entomol., 85*(1), 58-64. doi:10.1093/jee/85.1.58

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biom. J., 50*(3), 346-363. doi:10.1002/bimj.200810425

Hu, X., Xiao, G., Zheng, P., Shang, Y., Su, Y., Zhang, X., . . . Wang, C. (2014). Trajectory and genomic determinants of fungal-pathogen speciation and host adaptation. *PNAS, 111*(47), 16796-16801. doi:doi:10.1073/pnas.1412662111

Hussain, A., Tian, M.-Y., He, Y.-R., & Lei, Y.-Y. (2010). Differential fluctuation in virulence and VOC profiles among different cultures of entomopathogenic fungi. *J Invertebr Pathol, 104*(3), 166-171. doi:10.1016/j.jip.2010.03.004

Joop, G., & Vilcinskas, A. (2016). Coevolution of parasitic fungi and insect hosts. *Zoology, 119*(4), 350-358. doi:10.1016/j.zool.2016.06.005

Kryukov, V. Y., Kryukova, N. A., Tomilova, O. G., Vorontsova, Y., Chertkova, E., Pervushin, A. L., . . . Yaroslavtseva, O. N. (2020). Comparative analysis of the immune response of the wax moth *Galleria mellonella* after infection with the fungi *Cordyceps militaris* and *Metarhizium robertsii*. *Microb. Pathog., 141*, 103995. doi: 10.1016/j.micpath.2020.103995

Latch, G. C. M. (1976). Studies on the susceptibility of *Oryctes rhinoceros* to some entomogenous fungi. *Entomophaga, 21*(1), 31-38. doi:10.1007/BF02372013

Lovett, B., & St Leger, R. J. (2018). Genetically engineering better fungal biopesticides. *Pest Manag Sci, 74*(4), 781-789. doi:10.1002/ps.4734

McGuire, A. V., Edwards, W., Northfield, & D, T. (2023). The infection efficacy of *Metarhizium* strains (Hypocreales: Clavicipitaceae) against the Queensland fruit fly *Bactrocera tryoni* (Diptera: Tephritidae). *J. Econ. Entomol., 116*(2), 627-631. doi:10.1093/jee/toad040

McGuire, A. V., & Northfield, T. D. (2020). Tropical occurrence and agricultural importance of *Beauveria bassiana* and *Metarhizium anisopliae*. *Frontiers in Sustainable Food Systems, 4*(6). doi:10.3389/fsufs.2020.00006

McGuire, A. V., & Northfield, T. D. (2021). Identification and evaluation of endemic *Metarhizium* strains for biological control of banana rust thrips. *Biological Control, 162*, 104712. doi:10.1016/j.biocontrol.2021.104712

Mongkolsamrit, S., Khonsanit, A., Thanakitpipattana, D., Tasanathai, K., Noisripoom, W., Lamlertthon, S., . . . Luangsa-ard, J. (2020). Revisiting *Metarhizium* and the description of new species from Thailand. *Stud. Mycol., 95*, 171-251. doi:10.1016/j.simyco.2020.04.001

Muthuthantri, S., Maelzer, D., Zalucki, M. P., & Clarke, A. R. (2010). The seasonal phenology of *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) in Queensland. *Australian Journal of Entomology, 49*(3), 221-233. doi:10.1111/j.1440-6055.2010.00759.x

Nahar, P. B., Kulkarni, S. A., Kulye, M. S., Chavan, S. B., Kulkarni, G., Rajendran, A., . . . Deshpande, M. V. (2008). Effect of repeated in vitro sub-culturing on the virulence of *Metarhizium anisopliae* against *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Biocontrol Sci. Technol., 18*(4), 337-355. doi:10.1080/09583150801935650

Peng, Z.-Y., Huang, S.-T., Chen, J.-T., Li, N., Wei, Y., Nawaz, A., & Deng, S.-Q. (2022). An update of a green pesticide: *Metarhizium anisopliae*. *All Life, 15*(1), 1141-1159. doi:10.1080/26895293.2022.2147224

Qu, S., & Wang, S. (2018). Interaction of entomopathogenic fungi with the host immune system. *DCI, 83*, 96-103. doi:10.1016/j.dci.2018.01.010

Quesada-Moraga, E., & Vey, A. (2003). Intra-specific variation in virulence and In vitro production of macromolecular toxins active against locust among *Beauveria bassiana* strains and effects of in vivo and In vitro passage on these factors. *Biocontrol Sci Technol, 13*(3), 323-340. doi:10.1080/0958315031000110346

Rafaluk, C., Yang, W., Mitschke, A., Rosenstiel, P., Schulenburg, H., & Joop, G. (2017). Highly potent host external immunity acts as a strong selective force enhancing rapid parasite virulence evolution. *Environ. Microbiol., 19*(5), 2090-2100. doi:10.1111/1462-2920.13736

Rajula, J., Karthi, S., Mumba, S., Pittarate, S., Thungrabeab, M., & Krutmuang, P. (2021). Chapter 4 - Current status and future prospects of entomopathogenic fungi: A potential source of biopesticides. In S. De Mandal & A. K. Passari (Eds.), *Recent Advancement in Microbial Biotechnology* (pp. 71-98): Academic Press.

Scully, L. R., & Bidochka, M. J. (2005). Serial passage of the opportunistic pathogen *Aspergillus flavus* through an insect host yields decreased saprobic capacity. *Can J Microbiol, 51*(2), 185-189. doi:10.1139/w04-124

Shah, F. A., Allen, N., Wright, C. J., & Butt, T. M. (2007). Repeated in vitro subculturing alters spore surface properties and virulence of *Metarhizium anisopliae*. *FEMS Microbiology Letters, 276*(1), 60-66. doi:10.1111/j.1574-6968.2007.00927.x

Sharma, A., Sharma, S., & Yadav, P. K. (2023). Entomopathogenic fungi and their relevance in sustainable agriculture: A review. *Cogent food agric., 9*(1), 2180857. doi:10.1080/23311932.2023.2180857

Song, T. T., & Feng, M. G. (2011). In vivo passages of heterologous *Beauveria bassiana* isolates improve conidial surface properties and pathogenicity to *Nilaparvata lugens* (Homoptera: Delphacidae). *J Invertebr Pathol, 106*(2), 211-216. doi:10.1016/j.jip.2010.09.022

St. Leger, R. J., & Wang, J. B. (2020). *Metarhizium*: jack of all trades, master of many. *Open Biology, 10*(12), 200307. doi:doi:10.1098/rsob.200307

Szűcs, M., Vercken, E., Bitume, E. V., & Hufbauer, R. A. (2019). The implications of rapid eco-evolutionary processes for biological control - a review. *Entomol. Exp. Appl., 167*(7), 598-615. doi:10.1111/eea.12807

Tomilova, O. G., Kryukov, V. Y., Kryukova, N. A., Tolokonnikova, K. P., Tokarev, Y. S., Rumiantseva, A. S., . . . Glupov, V. V. (2023). Effects of passages through an insect or a plant on virulence and physiological properties of the fungus *Metarhizium robertsii*. *PeerJ, 11*, e15726. doi:10.7717/peerj.15726

Valero-Jiménez, C. A., van Kan, J. A. L., Koenraadt, C. J. M., Zwaan, B. J., & Schoustra, S. E. (2017). Experimental evolution to increase the efficacy of the entomopathogenic fungus *Beauveria bassiana* against malaria mosquitoes: Effects on mycelial growth and virulence. *Evol. Appl., 10*(5), 433-443. doi:10.1111/eva.12451

Vandenberg, J. D., & Cantone, F. A. (2004). Effect of serial transfer of three strains of *Paecilomyces fumosoroseus* on growth in vitro, virulence, and host specificity. *J Invertebr Pathol, 85*(1), 40-45. doi:10.1016/j.jip.2003.12.004

Wang, C., & St Leger, R. J. (2005). Developmental and transcriptional responses to host and nonhost cuticles by the specific locust pathogen *Metarhizium anisopliae var. acridum*. *Eukaryot Cell, 4*(5), 937-947. doi:10.1128/ec.4.5.937-947.2005

Xu, Y.-J., Luo, F., Li, B., Shang, Y., & Wang, C. (2016). Metabolic conservation and diversification of *Metarhizium* species correlate with fungal host-specificity. *Front Microbiol, 7*. doi:10.3389/fmicb.2016.02020

## References: Chapter 4


Anderson, R., & May, R. (1991). *Infectious Diseases of Humans: Dynamics and Control*: Oxford University Press.

Anderson, R. M., & May, R. M. (1979). Population biology of infectious diseases: Part I. *Nature, 280*(5721), 361-367. doi:10.1038/280361a0

Anderson, R. M., & May, R. M. (1981). The population dynamics of microparasites and their invertebrate hosts. *Philosophical Transactions of the Royal Society of London Series B, 291*, 451-524. doi:10.1098/rstb.1981.0005

Bailey, N. T. J. (1957). *The Mathematical Theory of Epidemics*. London: Charles Griffin & Company.

Baker, D. K., Rice, S. J., Leemon, D. M., & James, P. J. (2018). Horizontal transmission of *Metarhizium anisopliae* (Hypocreales: Clavicipitacea) and the effects of infection on oviposition rate in laboratory populations of *Musca domestica* (Diptera: Muscidae). *Pest Manag. Sci., 74*(4), 987.

Balagawi, S., Drew, R. A. I., & Clarke, A. R. (2023). Comparative demography of a specialist and generalist fruit fly: Implications for host use and pest management. *Ann. Appl. Biol., 182*(3), 295-311. doi:10.1111/aab.12818

Barry, J. D., Dowell, R. V., & Morse, J. G. (2002). Comparison of two sterile Mediterranean fruit fly (Diptera: Tephritidae) strains released in California's Preventative Release Program. *J Econ Entomol, 95*(5), 936-944. doi:10.1093/jee/95.5.936

Bidochka, M. J., Kamp, A. M., & de Croos, J. N. A. (2000). Insect pathogenic fungi: from genes to populations. In J. W. Kronstad (Ed.), *Fungal Pathology* (pp. 171-193). Dordrecht: Springer Netherlands.

Bjørnstad, O. N., Shea, K., Krzywinski, M., & Altman, N. (2020). The SEIRS model for infectious disease dynamics. *Nature Methods, 17*(6), 557-558. doi:10.1038/s41592-020-0856-2

Blackwood, J. C., Berec, L., Yamanaka, T., Epanchin-Niell, R. S., Hastings, A., & Liebhold, A. M. (2012). Bioeconomic synergy between tactics for insect eradication in the presence of Allee effects. *Proceedings of the Royal Society. B, Biological sciences, 279*(1739), 2807-2815. doi:10.1098/rspb.2012.0255

Blackwood, J. C., Vargas, R., Fauvergue, X., & Cuddington, K. (2018). A cascade of destabilizations: Combining Wolbachia and Allee effects to eradicate insect pests. *J Anim Ecol., 87*(1), 59-72. doi:10.1111/1365-2656.12756

Castillo, M.-A., Moya, P., Hernández, E., & Primo-Yúfera, E. (2000). Susceptibility of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) to Entomopathogenic Fungi and Their Extracts. *Biol. Control, 19*(3), 274-282. doi:10.1006/bcon.2000.0867

Collier, T., & Van Steenwyk, R. (2004). A critical evaluation of augmentative biological control. *Biol. Control, 31*(2), 245-256. doi:10.1016/j.biocontrol.2004.05.001

Dembilio, Ó., Moya, P., Vacas, S., Ortega-García, L., Quesada-Moraga, E., Jaques, J. A., & Navarro-Llopis, V. (2018). Development of an attract-and-infect system to control *Rhynchophorus ferrugineus* with the entomopathogenic fungus *Beauveria bassiana*. *Pest Manag Sci, 74*(8), 1861-1869. doi:10.1002/ps.4888

Diekmann, O., & Heesterbeek, J. A. P. (2000). *Mathematical Epidemiology of Infectious Diseases: Model Building, Analysis and Interpretation*. Chichester: Wiley.

Dimbi, S., Maniania, N. K., & Ekesi, S. (2013). Horizontal Transmission of *Metarhizium anisopliae* in Fruit Flies and Effect of Fungal Infection on Egg Laying and Fertility. *Insects, 4*(2), 206-216.

Doerr, M. D., Brunner, J. F., & Granger, K. R. (2012). Incorporating organophosphate alternative insecticides Into codling moth management programs in Washington apple orchards. *J. Integr. Pest Manag., 3*(2), E1-E4. doi:10.1603/ipm09001

Enkerlin, W. (2021). Impact of fruit fly control programmes using the sterile insect technique *Sterile insect technique* (pp. 979-1006): CRC Press.

Faye, P. D., Bal, A. B., Ndiaye, N. M., Diop, F., Sangaré, Y. K., Haddad, C., . . . Niassy, S. (2021). Field efficacy of *Metarhizium acridum* (Hypocreales: Clavicipitaceae) in the control of *Bactrocera dorsalis* (Diptera: Tephritidae) in citrus orchards in Senegal. *Int. J. Trop. Insect Sci., 41*(2), 1185-1195. doi:10.1007/s42690-020-00306-5

Ghosh, S., & Bhattacharya, D. K. (2010). Optimization in microbial pest control: An integrated approach. *Appl. Math. Model., 34*(5), 1382-1395. doi: 10.1016/j.apm.2009.08.026

Giardina, T. J., Clark, A. G., & Fiumera, A. C. (2017). Estimating mating rates in wild Drosophila melanogaster females by decay rates of male reproductive proteins in their reproductive tracts. *Mol Ecol Resour, 17*(6), 1202-1209. doi:10.1111/1755-0998.12661

Hajek, A. E., & St. Leger, R. J. (1994). Interactions between fungal pathogens and insect hosts. *Annu. Rev. Entomol., 39*(1), 293-322. doi:10.1146/annurev.en.39.010194.001453

Hammond, A., Galizi, R., Kyrou, K., Simoni, A., Siniscalchi, C., Katsanos, D., . . . Nolan, T. (2016). A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector Anopheles gambiae. Nat Biotechnol, 34(1), 78-83. doi:10.1038/nbt.34

Hansen, A. N., & De Fine Licht, H. H. (2019). Why are there so few examples of entomopathogenic fungi that manipulate host sexual behaviors? *Fungal Ecology, 38*, 21-27. doi:10.1016/j.funeco.2018.09.004

Hassemer, M. J., Lopes, R. B., Borges, M., Alves, L. F. A., Withall, D. M., Pickett, J. A., . . . Blassioli-Moraes, M. C. (2020). Development of an attract-and-infect device for biological control of lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) in poultry houses. *Biol. Control, 149*, 104326. doi:10.1016/j.biocontrol.2020.104326

Hughes, D. P., Araújo, J. P. M., Loreto, R. G., Quevillon, L., de Bekker, C., & Evans, H. C. (2016). From so simple a beginning: The evolution of behavioral manipulation by fungi. *Adv. Genet, 94*, 437-469. doi:10.1016/bs.adgen.2016.01.004

Hunt, P., Thatcher, L., Downes, S., Binns, M., Gardinier, D., Vadakattu, G., & Walsh, T. (2011). Overcoming a chemically limited future for weed, pest and disease control in agriculture. ECOS 278. Retrieved from https://ecos.csiro.au/a-chemically-limited-future/

Keeling, M. J., & Eames, K. T. D. (2005). Networks and epidemic models. *J. R. Soc. Interface., 2*(4), 295-307. doi:doi:10.1098/rsif.2005.0051

Keeling, M. J., & Rohani., P. (2008). *Modeling Infectious Diseases in Humans and Animals*: Princeton University Press.

Khun, K. K., Ash, G. J., Stevens, M. M., Huwer, R. K., & Wilson, B. A. L. (2021). Transmission of *Metarhizium anisopliae* and *Beauveria bassiana* to adults of *Kuschelorhynchus macadamiae* (Coleoptera: Curculionidae) from infected adults and conidiated cadavers. *Sci Rep, 11*(1), 2188. doi:10.1038/s41598-021-81647-0

Konopická, J., Bohatá, A., Palevsky, E., Nermuť, J., Půža, V., & Zemek, R. (2022). Survey of entomopathogenic and mycoparasitic fungi in the soil of onion and garlic fields in

the Czech Republic and Israel. *JPDP, 129*(2), 271-281. doi:10.1007/s41348-021-00557-5

Kreutz, J., Zimmermann, G., & Vaupel, O. (2004). Horizontal transmission of the entomopathogenic fungus *Beauveria bassiana* among the Spruce Bark beetle, *ips typographus* (Col., Scolytidae) in the laboratory and under field conditions. *Biocontrol Sci Technol 14*(8), 837-848. doi:10.1080/788222844

Lacey, L. A., Grzywacz, D., Shapiro-Ilan, D. I., Frutos, R., Brownbridge, M., & Goettel, M. S. (2015). Insect pathogens as biological control agents: Back to the future. *J. Invertebr. Pathol., 132*, 1-41. doi: 10.1016/j.jip.2015.07.009

Lampert, A., & Liebhold, A. M. (2021). Combining multiple tactics over time for cost-effective eradication of invading insect populations. *Ecology Letters, 24*(2), 279-287. doi:10.1111/ele.13640

Lance, D. R., & Gates, D. B. (1994). Sensitivity of detection trapping systems for Mediterranean fruit flies (Diptera: Tephritidae) in Southern California. *J Econ Entomol, 87*(6), 1377-1383. doi:10.1093/jee/87.6.1377

Majchrowska-Safaryan, A., & Tkaczuk, C. (2021). Abundance of entomopathogenic fungi in leaf litter and soil layers in forested habitats in Poland. *Insects, 12*(2). doi:10.3390/insects12020134

Maniania, N. K. (1998). A device for infecting adult tsetse flies, *Glossina* spp., with an entomopathogenic fungus in the field. *Biol. Control, 11*(3), 248-254. doi:10.1006/bcon.1997.0580

Mateos, M., Martinez Montoya, H., Lanzavecchia, S. B., Conte, C., Guillén, K., Morán-Aceves, B. M., . . . Tsiamis, G. (2020). *Wolbachia pipientis* associated with Tephritid fruit fly pests: from basic research to applications. *Front Microbiol, 11*, 1080. doi:10.3389/fmicb.2020.01080

Mkiga, A. M., Mohamed, S. A., du Plessis, H., Khamis, F. M., Akutse, K. S., & Ekesi, S. (2020). *Metarhizium anisopliae* and *Beauveria bassiana*: Pathogenicity, Horizontal Transmission, and Their Effects on Reproductive Potential of *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae). *J. Econ. Entomol., 113*(2), 660-668. doi:10.1093/jee/toz342

Mullens, B. A. (1990). *Entomophthora muscae* (Entomophthorales: Entomophthoraceae) as a pathogen of filth flies. In D. A. Rutz & R. S. Patterson (Eds.), *Biocontrol of Arthropods Affecting Livestock and Poultry* (pp. 231–245): Westview, Boulder, CO.

Navarro-Llopis, V., Ayala, I., Sanchis, J., Primo, J., & Moya, P. (2015). Field efficacy of a *Metarhizium anisopliae*-based attractant-contaminant device to control *Ceratitis capitata* (Diptera: Tephritidae). *J Econ Entomol, 108*(4), 1570-1578. doi:10.1093/jee/tov157

Onsongo, S. K., Mohamed, S. A., Akutse, K. S., Gichimu, B. M., & Dubois, T. (2022). The entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* for management of the Melon fly *Zeugodacus cucurbitae*: Pathogenicity, horizontal transmission, and compatability with cuelure. *Insects, 13*(10), 859.

Opisa, S., du Plessis, H., Akutse, K. S., Fiaboe, K. K. M., & Ekesi, S. (2019). Horizontal transmission of *Metarhizium anisopliae* between *Spoladea recurvalis* (Lepidoptera: Crambidae) adults and compatibility of the fungus with the attractant phenylacetaldehyde. *Microb. Pathog., 131*, 197-204. doi:10.1016/j.micpath.2019.04.010

Otto, S. P., & Day, T. (2007). Princeton: Princeton University Press.

Paradza, V. M., Khamis, F. M., Yusuf, A. A., Subramanian, S., & Akutse, K. S. (2021). Virulence and horizontal transmission of *Metarhizium anisopliae* by the adults of the

greenhouse whitefly *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) and the efficacy of oil formulations against its nymphs. *Heliyon, 7*(11), e08277. doi:10.1016/j.heliyon.2021.e08277

Rajula, J., Rahman, A., & Krutmuang, P. (2020). Entomopathogenic fungi in Southeast Asia and Africa and their possible adoption in biological control. *Biol. Control, 151*, 104399. doi:10.1016/j.biocontrol.2020.104399

Reyes-Ramírez, A., Enríquez-Vara, J. N., Rocha-Ortega, M., Téllez-García, A., Córdoba-Aguilar, A., & Bertram, S. (2019). Female choice for sick males over healthy males: Consequences for offspring. *Ethology, 125*(4), 241-249. doi:10.1111/eth.12854

Sharma, R., & Sharma, P. (2021). Fungal entomopathogens: a systematic review. *EJBPC, 31*(1), 57. doi:10.1186/s41938-021-00404-7

Shelly, T. (2021). Distant-dependent capture probabilities of Mediterranean and Oriental fruit flies (Diptera: Tephritidae) in a food-based trap in a Hawaiian mango orchard. *Fla. Entomol., 104*(2), 117-123. doi:10.1653/024.104.0207

Shelly, T., Nishimoto, J., & Kurashima, R. (2014). Distance-dependent capture probability of male Mediterranean fruit flies in trimedlure-baited traps in Hawaii. *J. Asia Pac. Entomol. , 17*(3), 525-530. doi:10.1016/j.aspen.2014.05.001

Sookar, P., Bhagwant, S., & Allymamod, M. N. (2014). Effect of *Metarhizium anisopliae* on the fertility and fecundity of two species of fruit flies and horizontal transmission of mycotic infection. *J. Insect Sci., 14*(1), 100. doi:10.1093/jis/14.1.100

Srei, N., Lavallée, R., & Guertin, C. (2019). Horizontal transmission of the entomopathogenic fungal isolate INRS-242 of *Beauveria bassiana* (Hypocreales: Cordycipitaceae) in Emerald Ash Borer, *Agrilus planipennis* (Coleoptera: Buprestidae). *J. Econ. Entomol., 113*(1), 543-545. doi:10.1093/jee/toz256

Sun, S., & Chen, L. (2009). Mathematical modelling to control a pest population by infected pests. *Appl. Math. Model., 33*(6), 2864-2873. doi:10.1016/j.apm.2008.08.018

Toledo, J., Campos, S. E., Flores, S., Liedo, P., Barrera, J. F., Villasenor, A., & Montoya, P. (2007). Horizontal Transmission of *Beauveria bassiana* in Anastrepha ludens (Diptera: Tephritidae) Under Laboratory and Field Cage Conditions. *J. Econ. Entomol., 100*(2), 291-297. doi:10.1093/jee/100.2.291

Toledo, J., Flores, S., Campos, S., Villaseñor, A., Enkerlin, W., Liedo, P., . . . Montoya, P. (2017). Pathogenicity of three formulations of *Beauveria bassiana* and efficacy of autoinoculation devices and sterile fruit fly males for dissemination of conidia for the control of *Ceratitis capitata*. *Entomol. Exp. App., 164*(3), 340-349. doi:10.1111/eea.12608

Vargas, R., Leblanc, L., Pinero, J., & Hoffman, K. (2014). Male annihilation, past, present, and future. In T. Shelly, N. Epsky, E. Jang, J. Reyes-Flores, & R. Vargas (Eds.), *Trapping and the detection, control, and regulation of tephritid fruit flies* (pp. 493-511). Netherlands: Springer.

Volz, E., & Meyers, L. A. (2007). Susceptible–infected–recovered epidemics in dynamic contact networks. *Proc. R. Soc. B: Biol. Sci., 274*(1628), 2925-2934. doi:doi:10.1098/rspb.2007.1159

Wang, X., & Song, X. (2008). Mathematical models for the control of a pest population by infected pest. *Comput. Math. with Appl., 56*(1), 266-278. doi:10.1016/j.camwa.2007.12.015

## References: Discussion


Adames, M., Fernández-Ruvalcaba, M., Peña-Chora, G., & Hernández-Velázquez, V. M. (2011). Effects of passages through a suitable host of the fungus, *Metarhizium anisopliae*, on the virulence of acaricide-susceptible and resistant strains of the tick, *Rhipicephalus microplus*. *Journal of insect science (Online), 11*(1), 21. doi:10.1673/031.011.0121

Bamisile, B. S., Akutse, K. S., Siddiqui, J. A., & Xu, Y. (2021). Model application of entomopathogenic fungi as alternatives to chemical pesticides: Prospects, challenges, and insights for next-generation sustainable agriculture. *Front Plant Sci, 12*, 741804. doi:10.3389/fpls.2021.741804

Daoust, R. A., & Roberts, D. W. (1982). Virulence of natural and insect-passaged strains of Metarhizium anisopliae to mosquito larvae. *J. Invertebr. Pathol., 40*(1), 107-117. doi:https://doi.org/10.1016/0022-2011(82)90042-8

De Armas, F. S., Dionei Grutzmacher, A., Edson Nava, D., Antonio Pasini, R., Rakes, M., & de Bastos Pazini, J. (2020). Non-target toxicity of nine agrochemicals toward larvae and adults of two generalist predators active in peach orchards. *Ecotoxicol., 29*(3), 327-339. doi:10.1007/s10646-020-02177-5

Evans, H. C., & Hywel-Jones, N. L. (1997). Entomopathogenic Fungi. In Y. Ben-Dov & C. J. Hodgson (Eds.), *Soft Scale Insects their Biology, Natural Enemies and Control* (Vol. 7, pp. 3-27): World Crop Pests.

Fernandes, É. K. K., Rangel, D. E. N., Braga, G. U. L., & Roberts, D. W. (2015). Tolerance of entomopathogenic fungi to ultraviolet radiation: a review on screening of strains and their formulation. *Curr. Genet., 61*(3), 427-440. doi:10.1007/s00294-015-0492-z

Hu, X., Xiao, G., Zheng, P., Shang, Y., Su, Y., Zhang, X., . . . Wang, C. (2014). Trajectory and genomic determinants of fungal-pathogen speciation and host adaptation. *PNAS, 111*(47), 16796-16801. doi:doi:10.1073/pnas.1412662111

Inglis, G. D., Goettel, M. S., & Johnson, D. L. (1995). Influence of ultraviolet light protectants on persistence of the entomopathogenic fungus, *Beauveria bassiana*. *Biol Control, 5*(4), 581-590. doi:https://doi.org/10.1006/bcon.1995.1069

Kaiser, D., Bacher, S., Mène-Saffrané, L., & Grabenweger, G. (2019). Efficiency of natural substances to protect Beauveria bassiana conidia from UV radiation. *Pest Manag. Sci., 75*(2), 556-563. doi:https://doi.org/10.1002/ps.5209

McGuire, A. V., & Northfield, T. D. (2021). Identification and evaluation of endemic *Metarhizium* strains for biological control of banana rust thrips. *Biol. control, 162*, 104712. doi:https://doi.org/10.1016/j.biocontrol.2021.104712

Mulla, M. S., & Mian, L. S. (1981). *Biological and environmental impacts of the insecticides malathion and parathion on nontarget biota in aquatic ecosystems*, New York, NY.

Nahar, P. B., Kulkarni, S. A., Kulye, M. S., Chavan, S. B., Kulkarni, G., Rajendran, A., . . . Deshpande, M. V. (2008). Effect of repeated in vitro sub-culturing on the virulence of *Metarhizium anisopliae* against *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Biocontrol Sci. Technol., 18*(4), 337-355. doi:10.1080/09583150801935650

Ortiz-Urquiza, A., & Keyhani, N. O. (2013). Action on the surface: Entomopathogenic fungi versus the insect cuticle. *Insects, 4*(3), 357-374. doi:10.3390/insects4030357

Quesada-Moraga, E., & Vey, A. (2003). Intra-specific variation in virulence and In vitro production of macromolecular toxins active against locust among *Beauveria bassiana* strains and effects of in vivo and In vitro passage on these factors. *Biocontrol Sci Technol, 13*(3), 323-340. doi:10.1080/0958315031000110346

Scully, L. R., & Bidochka, M. J. (2005). Serial passage of the opportunistic pathogen *Aspergillus flavus* through an insect host yields decreased saprobic capacity. *Can J Microbiol, 51*(2), 185-189. doi:10.1139/w04-124

Sree, K. S., & Joshi, H. (2015). Comparative account of generalist and specialist species of the entomopathogenic fungus, *Metarhizium*. In K. S. Sree & A. Varma (Eds.), *Biocontrol of Lepidopteran Pests: Use of Soil Microbes and their Metabolites* (pp. 161-167). Cham: Springer International Publishing.

St. Leger, R. J., & Wang, J. B. (2020). *Metarhizium*: jack of all trades, master of many. *Open Biology, 10*(12), 200307. doi:doi:10.1098/rsob.200307

Tomilova, O. G., Kryukov, V. Y., Kryukova, N. A., Tolokonnikova, K. P., Tokarev, Y. S., Rumiantseva, A. S., . . . Glupov, V. V. (2023). Effects of passages through an insect or a plant on virulence and physiological properties of the fungus Metarhizium robertsii. *PeerJ, 11*, e15726. doi:10.7717/peerj.15726

Vilcinskas, A. (2019). Evolutionary ecology of parasitic fungi and their host insects. *Fungal Ecology, 38*, 12-20. doi:https://doi.org/10.1016/j.funeco.2018.04.007

Xu, Y.-J., Luo, F., Li, B., Shang, Y., & Wang, C. (2016). Metabolic conservation and diversification of *Metarhizium* species correlate with fungal host-specificity. *Front Microbiol, 7*. doi:10.3389/fmicb.2016.02020

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## Chapter 2. The infection efficacy of *Metarhizium* strains (Hypocreales: Clavicipitaceae) against the Queensland fruit fly *Bactrocera tryoni* (Diptera: Tephritidae)