Reproductive trade-offs in the click beetle, *Agriotes obscurus,* exposed to the fungal pathogen *Metarhizium brunneum*

by

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Abstract

Several of the more pathogenic fungal species that infect insects have been developed as biological control agents. Adult insects can respond to potentially lifespan-reducing pathogen challenges by fighting infection, allocating resources to resistance over other activities. Alternatively, they can allocate resources to maximizing fecundity in response to early death, the terminal investment hypothesis. The click beetle *Agriotes obscurus* is an agricultural pest, and the fungus *Metarhizium brunneum* is being developed as a control agent. I examined the impact of *M. brunneum* challenge on *A. obscurus* reproduction and whether this changed under different nutritional conditions in beetles of varying ages. Beetles reduced their preoviposition period in response to fungal-induced decreases in lifespan when they were older, resulting in maintained fecundity, or under starved conditions, although fecundity could not reach the level of fed beetles. These results suggest that *M. brunneum* should be used early in the season when resources are abundant.

Keywords: *Agriotes obscurus*; *Metarhizium brunneum*; terminal investment hypothesis; reproductive trade-offs

This thesis is dedicated to Mom, Dad, Sarah, Marianne and Mylo. Thank you all for your patience and understanding!

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EPF	Entomopathogenic fungi
TIH	Terminal investment hypothesis

Chapter 1.

Introduction

The term entomopathogenic fungi (EPF) describes fungi that infect and exploit insects and represents around 1000 species in over 100 genera (Hajek & Shapiro-Ilan, 2017). Many EPF shorten the lifespan of their host, and several of the more pathogenic species of fungi, such as *Beauveria bassiana* and *Metarhizium* spp*.,* have been exploited for insect pest control (Gouli et al., 2013; Kim et al., 2013). The main interest in these species is their capacity to kill a target host in a relatively short time, while the impact of fungal challenge on other components of life history, such as reproduction, have received less attention (Rangel et al., 2015; Yii et al., 2016). Fungi infect insects through the cuticle and do not need to be ingested, meaning that infection can occur at multiple life stages, including adults at reproductive age (Santi et al., 2010; Yu et al., 2015). Infection with a pathogen, such as a fungus, can elicit an immune response, which is costly to the host (Joop & Vilcinskas, 2016; Sahar et al., 2016). After the fungus penetrates the cuticle and reaches the hemolymph of the insect, hemocytes aggregate around spores in an attempt to remove them (Sahar et al., 2016). In addition, the hemocytes also release antimicrobial peptides, such as gallerimycin, to directly kill the spores, as well as phenoloxidase, which is involved in melanization of the cuticle in response to injury (Joop & Vilcinskas, 2016; Sahar et al., 2016; Yokoi et al., 2015).

In adult insects, trade-offs occur both between reproductive traits (e.g. egg size and egg number, or current and future reproduction) and between reproductive traits and other processes such as immunity and growth (Koch & Meunier, 2014; Simmons, 2011; Zhang & Hood, 2016). Individuals with a high expectation of future reproduction (reproductive value) moderate allocations to current reproduction, while those with little to no reproductive value, due to approaching the end of their lifespan or an increased risk of death, maximize current reproduction (Duffield et al., 2017; Williams, 1966; Zhang & Hood, 2016). Survival risks such as pathogen exposure also elicit costly immune responses in the host; since resources are often limited, this decreases resources available to allocate elsewhere (Anggraeni et al., 2011; Wang et al., 2017). When pathogen-challenged individuals facing a shorter lifespan allocate resources to favour

reproduction, such as by decreasing preoviposition period (time to start egg laying), or increasing or maintaining oviposition period (time spent laying eggs), oviposition rate (number of eggs laid per day) or fecundity, compared to longer-lived, unchallenged individuals, the shift in direction of reproductive parameters is referred to as terminal investment (Clutton-Brock, 1984; Duffield et al., 2017). Individuals that carry out this behaviour are said to follow the expectations of the terminal investment hypothesis (TIH) (Duffield et al., 2017).

1.1. Entomopathogenic fungi and their impact on fitness in adult insects

Here, I review the effects of EPF exposure on fecundity and related parameters in adult insects. The universal effect of exposure to EPF on an adult insect is a reduction in lifespan, a phenomenon noted in 96% of the studies surveyed (Table A.1). The effects of reduced lifespan on reproduction vary among both insect and fungal species, and insects may respond in a way that either reduces, increases or maintains their reproductive output compared to unexposed individuals. Excellent reviews of EPF have been written, describing their production, use, mode of action, interactions with the host, diversity, persistence, safety and non-target effects (Araújo & Hughes, 2016; Butt et al., 2016; Hajek & Shapiro-Ilan, 2017; Kaya & Vega, 2012; Lacey et al., 2015; Lu & St. Leger, 2016; Miranda-Hernández et al., 2016; Qu & Wang, 2018; Scholte et al., 2006; Zimmermann, 2008). Here I examine whether, and to what degree, reductions in lifespan resulting from exposure to the common EPF *Metarhizium acridum, M. anisopliae, M. brunneum, M. robertsii* and *Beauveria bassiana* correspond to alterations in reproductive parameters in adult insects. Insect parasitoids were not included since their association with the EPF often involves interaction with their insect host.

1.1.1. Decreased Fecundity with Exposure to EPF

Individuals may respond to fungal-induced decreases in lifespan with a reduction in fecundity, due to either energy allocations to defence or fungal depletion of resources. In either case, we expect decreases in fecundity to result from decreases in the number of eggs laid per day (oviposition rate), as well as reductions in oviposition period (the time spent laying eggs), which could be due to increases in preoviposition period (the time until the start of egg laying), decreases in lifespan or both. Which parameters are

altered may depend on the insect's reproductive strategy or schedule relative to lifespan or may simply be dependent on the resources available.

The majority (65%) of studies that reported a decrease in fecundity in exposed insects observed reduced oviposition rates compared to unexposed individuals (Table A.1). It has been suggested that individuals that have reduced their daily fecundity have done so due to allocations to immunity or a lack of resources, while fluctuations in daily fecundity relative to that of controls may indicate a shift in resources between processes or a change in resource level (Baverstock et al., 2006). Although most studies reported reduced daily fecundity in infected insects compared to controls throughout the oviposition period, several noted changes in relative daily fecundity over the course of the oviposition period. For example, *M. anisopliae*-infected red palm weevils *(Rhynchophorus ferrugineus)*, Asian longhorned beetles (*Anoplophora glabripennis),* and German cockroaches *(Blattella germanica)* each displayed oviposition rates equal to those of controls for the first few days of egg laying, but then oviposition rate declined (Dembilio et al., 2010; Hajek et al., 2008; Quesada-Moraga et al., 2004). This reduction in oviposition rate may indicate a shift in strategy; in the beginning, infected individuals allocate resources to reproduction to maintain reproductive output, and then later allocate resources to immunity at the cost of reproduction or run out of resources faster than control individuals as the infection spreads (Baverstock et al., 2006). Alternatively, any fecundity-reducing effects of infection may not take effect until later in the oviposition period, as which point oviposition rate decreases. To test whether the observed differences are due to differences in strategy in reproductive timing in response to infection and reduced lifespan, or are just due to lack of resources as a result of infection, future studies could compare the effect of pathogen infection and immune induction under varying nutritional scenarios. This would allow us to determine whether changes over time may indicate shifts in allocation between reproduction and immunity or are merely due to lack of time or resources.

Several studies observed reduced fecundity as well as an increase in preoviposition period compared to controls. Mediterranean fruit flies (*Ceratitis capitata)* infected with either *M. anisopliae* or *B. bassiana* and Moroccan locusts *(Dociostaurus maroccanus)* infected with both *M. anisopliae* and *B. bassiana* experienced both decreases in lifespan and increases in preoviposition period, resulting in a decrease in fecundity (Quesada-Moraga et al., 2006; Valverde-Garcia et al., 2019). An increase in

preoviposition period may be an indication of early allocation of resources to immunity, or it may simply be a result of declining resources; in either case, it may result in a decrease in oviposition period and a reduction in fecundity if not combined with an increase in oviposition rate. Reduced oviposition period was observed in red palm weevils *(R. ferrugineus)* exposed to *M. anisopliae* and tarnished plant bugs *(Lygus lineolaris)* exposed to *B. bassiana* (Gindin et al., 2006; Ugine, 2011). In both cases, the insects did not change their overall oviposition rate and experienced reduced fecundity compared to controls.

The widespread use of EPF in biological pest control indicates that EPF infection is expected to decrease insect fecundity, along with lifespan. Although adult insects experienced decreases in fecundity after fungal infection, the variation in resource allocation, indicated by the changes in oviposition rate, suggest that it might also be possible to allocate resources in such a way that approaches or results in overall increases or maintenance of fecundity. Next, I will examine studies where infected insects do not show a decline in fecundity compared to unchallenged insects and determine when and how the reproductive schedule is altered to achieve that outcome.

1.1.2. Increased or Maintained Fecundity with Exposure to EPF

When facing a reduction in lifespan, insects may increase or maintain current fecundity to offset the decreased opportunity for future reproduction; this behaviour is characteristic of terminal investment and was reported in 26% of the papers surveyed. Ways in which insects could alter reproduction to achieve maintained or increased fecundity would be to decrease the preoviposition period, while maintaining the oviposition period, in order to maximize the time available for laying eggs, as well as increasing the oviposition rate to maximize how many eggs are laid to offset any decreases in the time available. The ability of individuals to alter their reproductive schedule is likely to vary with the resources available, and it is not clear whether some traits are easier to manipulate than others or whether multiple traits can be altered.

An increase in daily fecundity, especially when employed early in the adult stage or soon after infection, is expected to allow short-lived, infected insects to achieve a similar or higher level of fecundity compared to longer-lived unchallenged insects. This was the case in Giehr et al.'s (2017) study on ants, where females with *M. brunneum*-

induced reductions in lifespan increased their daily fecundity, resulting in higher overall fecundity than longer-lived, uninfected controls. Similarly, increases in initial daily fecundity compared to unexposed insects were observed in locusts (*Locustana pardalina* and *Schistocerca gregaria*) exposed to *M. acridum* (Arthurs & Thomas, 2000; Blanford & Thomas, 2001), in tomato leaf miners *(Tuta absoluta)* exposed to *M. anisopliae* (Pires et al., 2009) and in mosquitoes *(Aedes aegypti)* exposed to *B. bassiana* (Darbro et al., 2012). These initial increases were followed by decreases in oviposition rate, which may indicate a later allocation of resources to immunity after terminal investment. These differ from the changes in oviposition rate observed in Section 1.1.1 in that in these cases, daily fecundity was higher than that of the controls, which resulted in an equal level of overall fecundity to controls. Interestingly, the initial increase observed in locusts was due to a decrease in preoviposition period, while that observed in the tomato leaf miner occurred without alterations in either preoviposition or oviposition period. In all cases the initial increase in daily fecundity resulted in a level of overall fecundity equal to that of controls (Arthurs & Thomas, 2000; Blanford & Thomas, 2001; Darbro et al., 2012; Pires et al., 2009). The increase in initial fecundity observed in mosquitoes was in contrast with another experiment in the same study where the infected mosquitoes were observed to increase daily fecundity later, rather than earlier, in their oviposition period (Darbro et al., 2012). The two trials only differed in the orientation of the outdoor cage the mosquitoes were housed in (north-facing versus south-facing), and in both cases the infected mosquitoes laid an equal number of eggs compared to the uninfected mosquitoes (Darbro et al., 2012). The move towards an increased oviposition rate later in the oviposition period observed by Darbro et al. (2012) may indicate a shift in allocation from immunity to egg laying, the opposite of what was observed by Dembilio et al. (2010), Hajek et al. (2008) and Quesada-Moraga et al. (2004) (see Section 1.1.1). Pires et al. (2009) and Pereira et al. (2011) found that *M. anisopliae*-infected tomato leaf miners *(Tuta absoluta)* and *M. anisopliae*-infected obscure mealybugs *(Pseudococcus viburni)*, laid eggs for an equal period of time as uninfected insects, resulting in equal fecundity, despite experiencing both a reduction in lifespan and no changes in preoviposition period. It is possible that long-lived insects may not always lay eggs over their entire adult period, so short- and long-lived individuals may have similar oviposition periods.

Liu & Bauer (2008) reported that emerald ash borers *(Agrilus planipennis)* exposed to *B. bassiana* had equal fecundity compared to unexposed insects despite a reduced lifespan. However, they did not report on any reproductive parameters that impact fecundity, so it is not clear how the insects achieved this. Reporting on multiple reproductive parameters helps to shed more light on what the insects can and do manipulate, what trade-offs occur, and how trade-offs impact fecundity.

The strategies for terminal investment vary, but the result is an optimization of current reproduction to partially or fully offset the loss of future reproduction resulting from a shortened lifespan. Terminal investment is the use of a reproductive strategy resulting from one or multiple triggers of reduced reproductive value, and the number of papers that document evidence of terminal investment in response to EPF exposure in insects is relatively small (Duffield et al., 2017). Pathogen exposure reduces lifespan and resources for fecundity, triggering terminal investment, but the insect's propensity to terminally invest will ultimately depend on its overall state and expectation of future reproduction, which is also impacted by intrinsic factors such as age (as a proxy for remaining lifespan) and nutrition, as well as extrinsic factors such as temperature (Clutton-Brock, 1984; Duffield et al., 2017).

There may also be an effect of social status on the propensity to terminally invest; although Giehr et al. (2017) found *Cardiocondyla obscurior* ant queens laid more eggs per day after infection with *M. brunneum*, a similar study on queenless workers of a longer-lived species of ant, *Temnothorax crassispinus,* found a decrease in the number of eggs per day after infection with *M. brunneum* (Giehr & Heinze, 2018). In addition, some studies observed terminal investment behaviours that did not result in increased or maintained fecundity compared to controls. An example of this was observed by Ugine (2011), who that found tarnished plant bugs *(Lygus lineolaris)* exposed to *B. bassiana* at 32°C, the highest temperature tested, laid a higher initial number of eggs per day but achieved a lower fecundity compared to controls at the same temperature. Such findings suggest that terminal investment behaviours may not always result in increased or maintained fecundity.

1.1.3. Conclusions

In examining the effect of EPF exposure on insects, increase in oviposition rate appears to be a commonly reported terminal investment behaviour that results in increased or maintained fecundity. Although variation in oviposition rate over the course of the oviposition period is suggestive of terminal investment, of those insects that showed fluctuations in oviposition rate, only those that were able to achieve a higher oviposition rate over the controls at some point in time achieved an increased or maintained fecundity compared to the controls. However, we do not know whether individuals varied in their ability to increase their oviposition rate over that of the controls due to differences in specific intrinsic or extrinsic factors, or a combination of multiple factors. As an extrinsic factor, temperature can affect egg laying rate, which could contribute to differences. Since the studies surveyed used a range of insect species with different optimal temperatures, we know little about the impact of different temperature conditions. Further studies, with insects tested at multiple temperatures, are needed to determine what effect, if any, temperature has on egg laying rate as a terminal investment behaviour, and how this impacts overall fecundity.

Another reproductive parameter determining fecundity was oviposition period, with individuals more likely to achieve a fecundity equal to that of the controls if they were able to achieve a similar oviposition period length. Although oviposition period is expected to be dependent on both preoviposition period and lifespan, Pires et al. (2009) and Pereira et al. (2011) both found oviposition periods of infected insects to be equal to those of controls, despite reductions in lifespan and with no changes in preoviposition period. As suggested earlier, this may be attributed to species-specific timing for egg laying, and whether the oviposition period depends only on lifespan, and less indicative of a shift in reproductive timing to achieve a level of fecundity. In general, studies that examine both preoviposition period and oviposition period can help us to determine whether changes or maintenance of oviposition period are due to or oviposition timing within lifespan or alteration of reproductive schedule in response to pathogen exposure. In studies that look at the effect of EPF exposure on insect reproduction, examination of the parameters preoviposition period, oviposition period and eggs per day, along with their interactions, as well as the effects of abiotic factors such as temperature, can help us to achieve a comprehensive view of whether variations in reproductive parameters

occur in response to exposure indicative of reproductive strategies or are incidental consequences of other factors, such as temperature, age and nutrition.

1.2. Role of life history trade-offs in the application of EPFs

In terms of insect pest management, the vast majority of studies on EPF focus on their ability to kill their target hosts or hosts. However, if adults are the targeted life stage, we also need to know whether fungal infection with the proposed EPF species does actually result in reductions in fecundity and thus population growth in the target insect. To determine how EPF exposure impacts fecundity and the relationships between reproductive parameters, we need to examine the effect of exposure on preoviposition period, oviposition period and oviposition rate for each species individually. To better understand the effect of EPF exposure on an adult target insect, I examine the response to fungal exposure in the click beetle, *Agriotes obscurus*, a major pest of many root crops and a system where targeting adults is more feasible than attempting to control the long-lived, subterranean larvae.

1.3. The study system, the click beetle *Agriotes obscurus*

Wireworms, the larval stage of click beetles (Coleoptera: Elateridae), are common and problematic in many crop systems. In particular, the dusky wireworm, *Agriotes obscurus,* is a pest of root crops on the west and east coasts of Canada, including the Fraser Valley and Vancouver Island in British Columbia, as well as the United States (Vernon & Tóth, 2007; Vernon & van Herk, 2013). The larval stage of *A. obscurus* is called the dusky wireworm, while its adult form is a click beetle referred to as the dusky wireworm beetle or the dusky click beetle. *A. obscurus* larvae live in the soil, eating seeds, roots and soil organic matter up until pupation; the larval stage is the life stage responsible for agricultural crop damage (Barsics et al., 2013). The agricultural crop plant roots they eat include wheat, corn, onion, sugar beets, potatoes and strawberries (Traugott et al., 2015; Vernon & van Herk, 2013). *A.obscurus* crop damage results in substantial economic losses, especially in staple crops such as wheat and potato (Traugott et al., 2015)

1.3.1. Life Cycle

The larval stage of *A. obscurus* lasts between three and five years, depending on diet and region (Kabaluk, 2014; Sufyan et al., 2014; Vernon & van Herk, 2013). In Agassiz and Chilliwack, British Columbia, larvae are most active in June, August and September (Vernon & van Herk, 2013). *A. obscurus* pupation takes place in August or September; the pupal stage lasts around two weeks, after which they emerge as adult beetles (Sufyan et al., 2014). Adult beetles overwinter in the soil until they emerge on the soil surface in early spring, usually around March or at a time when the temperature is above 10˚C (Kabaluk, 2014).

Males emerge on the soil surface just before females; the females emit a pheromone which the males use to find suitable mates (Zacharuk, 1958). After mating, a female stores the spermatozoa in her copulatory pouch; egg development lasts for a few days before eggs are laid (Zacharuk, 1958; Sufyan et al., 2014). Females lay their eggs in moist soil, either singly or in clutches of up to 39 eggs, which hatch into larvae in around three weeks (Sufyan et al., 2014). Adults die just a few months after egg laying; during the adult stage, the diet of *A. obscurus* is made mainly of grasses (Kabaluk, 2014; Vernon & van Herk, 2013).

1.3.2. Historical Biological Control and Persistence

Wireworms initially became a problem in Europe and North America during the first and second World Wars, when large areas of grassland containing wireworm species were converted into fields used for potato and cereal crops to provide more food for humans (Vernon & van Herk, 2013). At this time, wireworms were not easily controlled by the available insecticides, so the focus was on the study of wireworm biology to understand whether other methods of control, such as biological control using parasitoids, predators and pathogens could be used (Thomas, 1940; Vernon & van Herk, 2013). During World War II, synthetic chemicals, such as organochlorines and organophosphates, were developed and found to be highly effective for killing pests, including wireworms, so the study of wireworm biology and the development of biological control methods were abandoned (Vernon & van Herk, 2013). However, the broad environmental and health impacts of these effective broad-spectrum chemicals led to their deregistration, resulting in the return of wireworms as a worldwide problem in the

1990's after decades of effective management (Parker & Howard, 2001; Vernon et al., 2016). This brought about a renewed need to study their biology in order to develop new, more sustainable methods of management, such as attract-and-kill strategies, which involve biological control (Kabaluk et al., 2015). Current methods of biological control for *A. obscurus* include the use of nematodes and entomopathogenic fungi such as *Metarhizium brunneum* (Arrington et al., 2016; Kabaluk, 2014; Morton & Garcia-del-Pino, 2017).

1.4. The fungal pathogen *Metarhizium brunneum*

Metarhizium brunneum, an EPF from the order Hypocreales, occurs naturally in soil and is one of several species of fungi responsible for green muscardine disease in insects (Bischoff et al., 2009; Gouli et al., 2011). *M. brunneum* is known to infect insects from multiple orders, and several strains of *M. brunneum* are registered for commercial use in the control of various insect species (Aydin et al., 2018; Bischoff et al., 2009; Cossentine et al., 2011; Eckard et al., 2014). The infection cycle is illustrated in Figure 1.1 and begins when a spore of *M. brunneum* makes contact with the cuticle of a host; the fungal hyphae grow from the spore, penetrate the host cuticle and produce blastospores in the hemocoel (Gouli et al., 2011; Hajek & St. Leger, 1994; Jaronski, 2014; Lacey et al., 2015). The host dies soon after the hyphae reach the hemocoel, usually several days after the initial spore contact; the host can die of mechanical damage, fungal depletion of host nutrients or by the toxic destruxins produced by the fungus (Hajek & St. Leger, 1994; Lacey et al., 2015). After host death, the hyphae grow back towards the cuticle, where spores are produced and dispersed by passive transmission (Gouli et al., 2011; Hajek & St. Leger, 1994; Jaronski, 2014; Lacey et al., 2015). *M. brunneum* needs insect hosts to complete its life cycle, but its spores can survive for years in the soil awaiting a new host (Brandl et al., 2017; Jaronski, 2010). Although *M. brunneum* is a common choice as a biological control agent, it shares the EPF characteristic of taking several days to kill its host, giving an adult host several days to reproduce before death, although their behaviour may be altered by fungal infection (Hajek & St. Leger, 1994; Kabaluk, 2014).

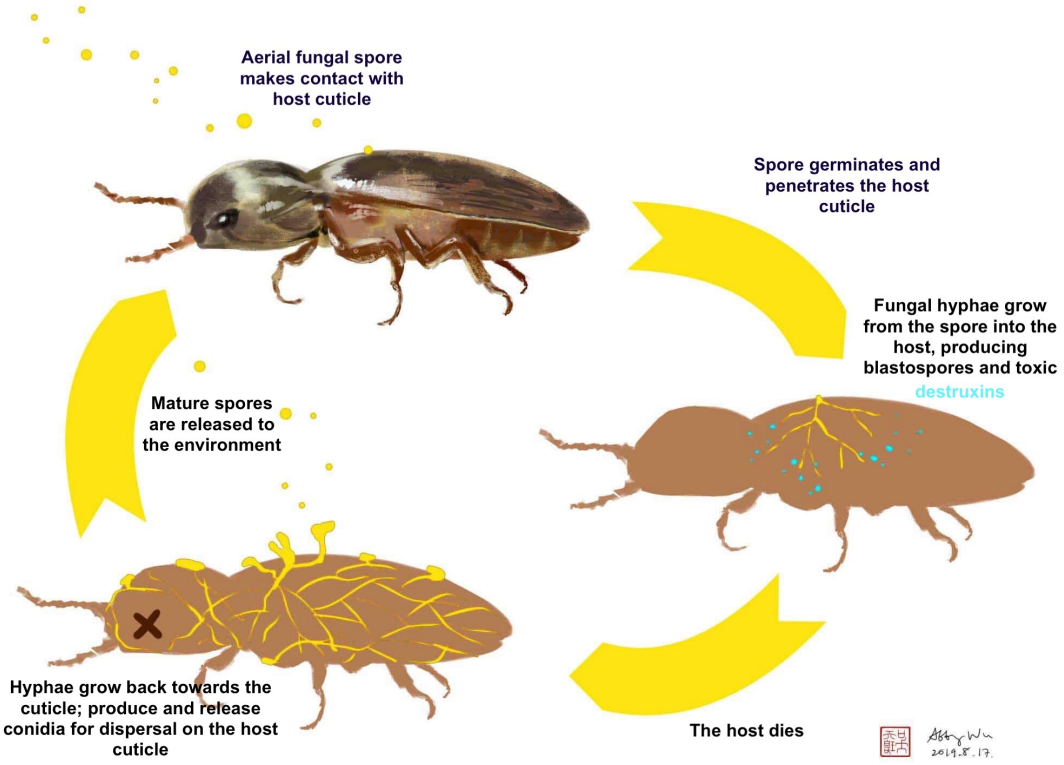

Figure 1.1. Diagram of entomopathogenic fungus infection cycle (Figure by Abby Wu)

1.5. The System: The Effect of *Metarhizium brunneum* on Lifespan and Reproduction in *Agriotes obscurus*

A new strategy for *A. obscurus* population suppression is the use of *M. brunneum* alongside a synthetic female pheromone in an attract-and-kill system for adult male click beetles (Kabaluk, 2014; Kabaluk et al., 2015). Exposure to *M. brunneum* causes adult *A. obscurus* mortality rates of 30%, 44.3% and 93.3% in 4, 8 and 18 days, respectively, but the effect of exposure on host reproduction before mortality is not known (Kabaluk, 2014). To better understand the effectiveness of this system in suppressing *A. obscurus* populations, we need know whether challenge with *M. brunneum* actually reduces click beetle fecundity, as well as how this might change over the season and be impacted by varying resource conditions. The information obtained from this study can be used to inform the use of the proposed attract-and-kill system, as well as similar biological control strategies using *M. brunneum.*

1.6. Research Objectives

To date, relatively few studies have examined reproductive trade-offs, particularly in relation to terminal investment, in insects in response to exposure to live entomopathogenic fungi, and still fewer have looked at this with the added challenge of suboptimal nutrition conditions. The aim of this thesis is to examine the impact of *M. brunneum* exposure on reproduction in adult click beetles and whether this is impacted by varying nutritional conditions. I will do this by:

1. Investigating the impact of exposure to the fungal entomopathogen *M. brunneum* on longevity and multiple reproductive behaviours in female *A. obscurus.*

2. Determining whether starvation alters the effect of the fungal pathogen on adult female longevity and reproductive behaviour.

Examination of each of these factors together will help us to better understand the effect of pathogens on populations of wild insects, which will have important implications for both the ecology and the management of insects.

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Chapter 2.

Modification of reproductive schedule in response to pathogen exposure in a wild insect: Support for the terminal investment hypothesis

2.1. Abstract

Trade-offs in the time and energy allocated to different functions, such as reproductive activities, can be driven by alterations in condition, often in response to extrinsic factors such as pathogens or parasites. When individuals are challenged by a pathogen, they may either passively reduce reproduction as a cost of increasing defence mechanisms, or alternatively, modify reproductive activities so as to minimize the fitness costs of earlier death; a behaviour consistent with the terminal investment hypothesis (TIH). The TIH predicts that individuals with decreased likelihood of future reproduction will maximize current reproductive effort, which may include shifts in reproductive timing. I examined how wild female adult click beetles *(Agriotes obscurus)* responded after exposure to their natural fungal pathogen *Metarhizium brunneum*. Unsurprisingly, field-collected beetles exposed to a high concentration of *M. brunneum* died earlier and in greater numbers than those exposed to a low concentration. Using a multi-variate approach, I examined the impact of different levels of pathogen challenge on a suite of reproductive behaviours. Fungal-induced reductions in lifespan corresponded to changes in the reproductive schedule, characterized by a decrease in preoviposition period. This allowed total fecundity to be maintained compared to uninfected beetles. These changes are consistent with the TIH and indicates that there is a threshold for the response that relates to the level of the threat to survival.

2.2. Introduction

Individuals are frequently faced with competing demands from the activities that they have to perform, necessitating trade-offs in the energy or time allocated to them (Duffield et al., 2017; Hale et al., 2005; Rivalan et al., 2005). Trade-offs can become more pronounced in response to changes in intrinsic factors, such as age or energetic state (Katz & Naug, 2015), or exposure to extrinsic factors, such as pathogens or

parasites (Giehr et al., 2017). Maintaining or upregulating resistance mechanisms to fight off pathogen challenge usually has costs. In insects, the costs of resisting infection can negatively affect other activities such as growth, development, competitive ability, sperm production and fecundity (e.g. Cotter et al., 2004; Kraaijeveld & Godfray, 1997; Quesada-Moraga et al., 2004; Simmons, 2011). Alternatively, pathogen exposure may result in increased investment in reproduction to counteract the potential reduction in lifespan; the terminal investment hypothesis (TIH). According to the TIH, an individual's current reproductive effort is inversely related to its future reproductive potential (Clutton-Brock, 1984; Duffield et al., 2017; Williams, 1966). Behaviours consistent with the TIH have been observed in both males and females and in a variety of animals, including birds and amphibians (An & Waldman, 2016; Marzal et al., 2008) and recent studies have explored the potential for terminal investment in insects (Duffield et al., 2017, 2018).

Although numerous studies have looked at the impact of immune challenge on one or more aspects of reproductive effort in insects (e.g. Adamo, 1999; Cotter et al., 2010), few have examined the potential for terminal investment using active pathogens introduced naturally to individuals at reproductive age (Duffield et al., 2017). This is in part because the impact of pathogen growth can potentially be confounded with the costs of immunity and it can be difficult to disentangle the costs and benefits accrued to either party. However, there is a need to investigate natural host-pathogen systems if we want to predict what the consequences of pathogen exposure will be for wild populations. In a more applied context, such as species conservation or biological control of pests, it is important to know whether pathogen exposure actually alters population growth and how this is related to the risk of infection. In addition, the threshold for terminal investment is likely to vary with both host condition and the level of intensity of the threat and thus it is important to start examining reproductive behaviours under a range of realistic scenarios.

I examined the effect of different levels of pathogen exposure on reproduction in the click beetle *(Agriotes obscurus)*. The beetles come from wild, genetically variable populations and I used a fungus to which the beetle is naturally exposed, *Metarhizium brunneum.* I measured different aspects of reproductive effort and determined whether any patterns supported the TIH or are more in line with the costs of pathogen exposure and possible infection. A multivariate approach which examines covariation can

determine how a combination of reproductive traits responds to pathogen challenge and which are the drivers of any observed changes (Duffield et al., 2017). The TIH predicts that behaviours that would increase reproductive output should be favoured, such as decreasing the preoviposition period and/or lengthening the oviposition period or increasing reproductive investment by increasing egg size or quality. Behaviours that are more likely to result from the costs of fighting infection include an increase in preoviposition period (delayed development), and a decrease in oviposition period, resulting in declines in fecundity. Here I show that challenge with a fungal pathogen reduces the insect's lifespan, which in turn corresponds to changes in reproductive timing so that total fecundity is maintained relative to unexposed beetles.

2.3. Methods

2.3.1. Insects

I collected *A. obscurus* adults from a grass pasture in Chilliwack, in the Fraser Valley in British Columbia (49.3764° N, 121.8159° W) using pitfall traps from May to June 2016. Beetles were stored together at 7 °C in the dark and fed fresh apple until ten days before the experiment, when males and females were separated.

2.3.2. Fungal treatment

Metarhizium brunneum LRC112 was isolated from an infected *A. obscurus* larva near Agassiz, in the Fraser Valley in British Columbia (Kabaluk et al., 2007). The isolate was amplified and tested for viability on potato dextrose agar prior to use in the experiment (Kabaluk, 2014). Before pathogen challenge, each beetle was photographed and measured using a Motic DM-143-FBGG dissecting microscope and the Motic Images Plus 2.0 program. Beetles were then dipped in 1 ml of either 10^2 or 10^8 fungal conidia/mL of 0.03% Tween 20 in sterilized distilled water for 10 secs and allowed to blot dry. Control beetles were dipped in 0.03% Tween 20 in distilled water. An attempt was made to measure fungal dose received, but it was not possible to obtain a value, likely due to too much time passing between fungal exposure and attempted measurement. Thirty randomly chosen male/female pairs per treatment were placed in 2-oz Solo cups with sterilized soil and wheat grass clippings (Sufyan et al., 2014). Beetles were kept at 20 °C with a 14:10 L:D cycle.

2.3.3. Monitoring

Beetles were checked for mortality every two days. They were then transferred to new containers so any eggs laid could be counted. The lengths of up to five eggs per female per day were photographed and measured as described above. Dead beetles were surface sterilized in a 1% sodium hypochlorite solution for 30 secs, washed twice in sterilized water and placed in a 0.5-oz Solo cup containing moist paper to determine cause of death. Cadavers were examined after a minimum of 14 days. Brown or green sporulation confirmed *M. brunneum* infection, while white spores or no sporulation were classified as death from an unknown cause. A sub-sample was examined under a compound microscope to confirm cause of death.

2.3.4. Statistical Analysis

M. brunneum-induced mortality of females and the number of pairs that laid eggs relative to fungal concentration were analyzed using generalized linear models (GLM) with a binomial distribution and a logit link function. Only female beetles that laid eggs were included in further analyses. In order to simultaneously analyse a series of dependent measures, a multivariate analysis of covariance (MANCOVA) was carried out with fungal concentration as the independent variable and female lifespan (days alive post treatment), preoviposition period (days between treatment and first egg), fecundity (total eggs laid) and egg size (average length per female) included as dependent variables and female area (length x width) as a covariate. Dependent variables were transformed where necessary to satisfy the assumption of normality. Lifespan and preoviposition period were square root transformed and fecundity was natural log transformed. Oviposition period could not be normalized and was not included in the MANCOVA. Female area did not significantly influence the outcome and was removed and the analysis was re-run as a multivariate analysis of variance (MANOVA). The following MANOVA assumptions were met: observations of the dependent variables were independent, the dependent variables were transformed where necessary to meet the assumption of normality, the covariance matrices were equal and the dependent variables tested in the MANOVA were not highly correlated (greater than 0.60) (Finch, 2005). In order to examine the contribution of the different dependent variables, the data were further investigated using Roy-Bargmann stepdown analysis, with variables listed in the sequence given above, starting with the factor which I considered least likely to be

influenced by other factors (Roy & Bargmann, 1958). This technique examines the significance of each variable in turn, but only for residual variance after the previous variable has been included in the model. I also carried out univariate analyses of variance (ANOVA) due to their widespread use; however, correlations among the dependent variables indicate that they should be interpreted with caution (Tabachnick et al., 2007). The alpha level for the stepdown procedure was set at 0.012 (0.05 divided by the number of tests) to reduce the inflated Type I error rate due to multiple testing. When fungal treatment was significant, differences between concentrations were determined using Tukey HSD tests.

I ran Kendall's rank correlations on the untransformed data to further examine the relationship among the different components of reproduction, specifically the correlation between lifespan and reproductive variables. Since the correlations were nonparametric, oviposition period was included in the pairwise correlations. The alpha level was adjusted using a Bonferroni adjustment to 0.005 (Bray & Maxwell, 1985). To determine how multiple variables affected fecundity, I modelled the effect of lifespan, preoviposition period and the number of eggs laid per day on the natural log of fecundity, and their interactions with a GLM and compared models using AIC values. Eggs laid per day was the average of the number of eggs per laying event per female and was natural log transformed (this provide the lowest AIC value). All dependent variables were mean centred to alleviate collinearity among the independent variables. The means and 95% confidence intervals for all parameters of interest were calculated. All data were analyzed using R Version 3.5.1 (R Core Team, 2018), except the generalized linear models for M. brunneum mortality and egg laying rates, which were analyzed using JMP® (Version 13 SAS Institute Inc., Cary, NC, 1989-2019).

2.4. Results

Fungal treatment influenced mortality ($\chi^2 = 25.09$, $p < 0.001$), with more females dying from fungal infection at the high concentration compared to the low (Table 2.1). No control females died from fungal infection. Fungal exposure had no effect on the number of pairs that laid eggs ($\chi^2 = 0.297$, $p = 0.862$).

Table 2.1. Influence of *Metarhizium brunneum* challenge on adult click beetle (*Agriotes obscurus*) mortality, longevity and reproductive measures

	treatment		
	control	***low (1 x 10^2 conidia/mL)***	***high (1 x 10^8 conidia/mL)***
fungal mortality	*0%*	*17.2%*	*80.0%*
percent laying eggs	*48%*	*55%*	*53%*
days until death	*11.90 ± 1.82*	*11.34 ± 1.81*	*8.33 ± 1.78*
preoviposition period (days)	*6.69 ± 1.76*	*5.27 ± 1.64*	*3.75 ± 1.59*
oviposition period (days)	*4.92 ± 1.85*	*5.73 ± 1.73*	*3.75 ± 1.67*
eggs laid per female	*10.46 ± 5.04*	*10.67 ± 4.70*	*7.31 ± 4.54*
eggs laid per female per day of oviposition	*5.46 ± 2.96*	*5.25 ± 2.76*	*5.61 ± 2.67*
average egg length per female (µm)	*534.75 ± 29.88*	*538.41 ± 27.81*	*529.69 ± 26.93*

(Mean ± 95% confidence interval). Sample sizes: mortality, percent laying eggs and days until death: 29 control, 29 low, 30 high; oviposition and egg data: 13 control, 15 low, 16 high.

The MANOVA indicated that fungal concentration strongly influenced female lifespan and the reproductive traits tested in the overall model ($F_{2,41}$=2.96, p = 0.006). The stepdown analysis showed that female lifespan is the primary factor that distinguishes among the treatments (Table 2.2). Beetles challenged with the high concentration of fungus did not live as long as those challenged with the low concentration (p = 0.006) or unchallenged beetles (p < 0.001); there was no difference in lifespan between the low concentration and unchallenged beetles (p = 0.085) (Table 2.1). Preoviposition period, total fecundity and egg size did not distinguish among the treatments beyond any impact of female longevity (Tables 2.2 and 2.3). Fungal concentration was significant for lifespan regardless of the ordering of the variables in the stepdown procedure, whereas preoviposition period was not significant ($F_{2,41}$=2.79;

p=0.07) when included prior to lifespan, confirming that differences in preoviposition period can be explained by differences in lifespan (Koslowsky & Caspy, 1991).

Table 2.2. Stepdown analyses showing the effect of exposure to the fungal pathogen *Metarhizium brunneum* on reproductive components of adult female click beetles *Agriotes obscurus*

independent variable	*dependent variable*	*univariate F*	*p*	*df*	*stepdown F*	*p*	*df*
fungal concentration	*lifespan*	*14.71*	***< 0.001***	*2, 41*	*14.71*	***< 0.001***	*2, 41*
	preoviposition period	*2.79*	*0.073*	*2, 41*	*0.024*	*0.98*	*2, 40*
	fecundity	*0.42*	*0.66*	*2, 41*	*0.58*	*0.57*	*2, 39*
	average egg length	*0.11*	*0.90*	*2, 41*	*0.59*	*0.56*	*2, 38*

***Bold** indicates significance at p < 0.012. Univariate statistics are shown for comparison.*

Fungal concentration only affected lifespan, so lifespan was used as an indicator of the effect of fungal concentration on the dependent variables. The effect of changes in lifespan on the reproductive variables was further investigated using pairwise correlations. The results of the pairwise correlations (Table 2.3) indicate that female preoviposition period increased by one day for every two day increase in lifespan. Preoviposition period was negatively associated with oviposition period, such that a one day increase in preoviposition period corresponded to a three day decrease in oviposition period, however the correlation was only marginally significant when using the conservative Bonferroni-adjusted p-value. Fecundity decreased with increases in preoviposition period but increased with increases in oviposition period. None of the variables tested had an effect on egg size. The GLM resulted in the model ln(Fecundity) = 0.181 + 0.023 [Lifespan] - 0.048 [Preoviposition Period] + 0.419 [ln(Eggs per Day)], showing a positive correlation between lifespan and preoviposition period, indicating that the negative impact of a decrease in lifespan on total fecundity can be offset by a decrease in preoviposition period.

Table 2.3. Kendall's pairwise correlations between reproductive components (untransformed) of adult female click beetles *Agriotes obscurus* after exposure to the fungus *Metarhizium brunneum*

	female lifespan			*preoviposition period*			*oviposition period*			*fecundity*		
	z	***tau***	***p***	***z***	***tau***	***p***	***z***	***tau***	***p***	***z***	***tau***	***p***
pre-oviposition period	*3.4*	*0.42*	***< 0.001***									
oviposition period	*1.1*	*0.14*	*0.126*	*-2.7*	*-0.33*	*0.008*						
fecundity	*0.4*	*0.044*	*0.70*	*-1.9*	*-0.22*	*0.056*	*3.1*	*0.37*	***0.002***			
average egg length	*-0.2*	*-0.017*	*0.88*	*-1.0*	*-0.11*	*0.31*	*0.8*	*0.089*	*0.44*	*0.02*	*0.0022*	*0.98*

Bold *indicates significance at p < 0.005.*

2.5. Discussion

I looked at multiple measures of reproductive behaviour in wild, adult female *A. obscurus* in response to challenge with their natural fungal pathogen *M. brunneum* and the subsequent decrease in lifespan. Each beetle was exposed to fungus at a set concentration, but the actual dose (fungal spores per beetle) received may vary between beetles within a treatment. Pathogen challenge reduced beetle lifespan, but they were able to maintain a similar level of fecundity with decreases in lifespan by a shift in reproductive schedule by shortening their preoviposition period, without sacrificing egg quality, at least in terms of size. These results support the terminal investment hypothesis. Maintenance of or increases in fecundity are necessary observations for terminal investment, but the route by which this can be achieved may vary. A recent study that examined the effect of fungal (*M. brunneum*) challenge on insect reproduction in queen ants within the framework of terminal investment found no alteration in the

timing of egg laying, but did record a rise in total fecundity due to an increase in the number of eggs laid per day (Giehr et al., 2017). However, this response was not seen in a later study on a longer-lived ant species, possibly due to a higher potential for future reproduction (Giehr & Heinze, 2018). A decrease in preoviposition period with reduced longevity has also been reported by Hendry et al. (2016) in pea aphids after ingestion of a live bacterium *Pseudomonas syringae,* which is common on plants and can kill aphids. Pea aphids challenged at a high concentration also produced more offspring per individual compared to those challenged at lower concentrations, but not the controls. These few recent studies suggest that the means of achieving fecundity alteration are not consistent across species and are likely to vary with other aspects of host ecology or condition.

I found no evidence for a change in egg size in response to fungal challenge, although egg size can vary in beetles (Ekbom & Popov, 2004; Kojima, 2015). Few studies have examined changes in egg size in insects in response to pathogens, but an experiment with mealy bugs and *Metarhizium sp.*, also found no change (Pereira et al., 2011). Interestingly, another study with beetles (*Tenebrio molitor*) did find a reduction in egg size when healthy females were mated with fungus-infected males, which the authors suggest is due to infected males producing reduced resources or the females choosing to invest less (Reyes-Ramírez et al., 2019). My results suggest that strategies to manipulate reproductive output or quality may not include alterations in egg size, at least for this system. This may be due to a trade-off between egg size and number with a threshold hinging on a variable I did not test, such as host age or nutrition. Alternatively, it could be that changes in reproductive timing are less costly or risky than altering investment in egg size or quality.

Pathogens are expected to have a negative effect on both the survival and reproduction of their hosts as resources are used for host defence or recovery or are utilized by the pathogen for replication. Indeed, numerous studies have demonstrated that fungal infection in adult insects results in reduced fecundity (e.g. Gindin et al., 2006; Quesada-Moraga et al., 2004, 2006; Ugine, 2011), and decreased rates of egg hatch (Quesada-Moraga et al., 2004, 2006). One of the intriguing aspects of my results, and terminal investment in general, is what cue makes the difference between a host adopting a pathogen resistance or a terminal investment strategy (see Duffield et al., 2017 for a discussion of dynamic thresholds). I found that adult click beetles exposed to

a fungal pathogen exhibited behaviours consistent with the TIH, but this appeared to be concentration dependent i.e. the likelihood increased with the increased probability of fatal infection or reduction of lifespan. How terminal investment links to pathogen virulence and mortality risk remains to be elucidated and more research is needed on exploring the thresholds that trigger this behaviour, and how it is modulated by changing conditions. My insects were also in the latter half of their natural lifespan, which may have made them more sensitive to their potentially reduced residual reproductive value. In insects, adult life stages vary in how much, or even whether, they feed, and thus nutrition in either the larval/nymphal or adult stage could also have an impact on the strategy adopted. All these factors need to be investigated if we are to understand pathogen-mediated changes in reproduction.

In summary, I show that wild caught insects exposed to their natural pathogen can modify their reproductive timing by starting to lay their eggs earlier, thereby compensating for the negative impacts of pathogen exposure on reducing their lifespan. These results support the terminal investment hypothesis and indicate that this behaviour could occur in natural insect populations. This gives us insight into how pathogens can alter reproductive trade-offs in insects, as well as indicating that pathogen exposure might not always reduce population growth.

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Chapter 3.

The impact of starvation and pathogen exposure on terminal investment in a wild insect

3.1. Abstract

Terminal investment describes the alteration of reproductive parameters towards increased current reproduction when the opportunity for future reproduction is reduced. Future reproduction may be decreased by suboptimalities in intrinsic factors, such as nutritional state, and extrinsic factors, such as pathogen exposure. Intrinsic and extrinsic factors are both expected to have an impact on the propensity to terminally invest. When combined, suboptimalities in intrinsic and extrinsic factors are expected to lower the threshold for and induce terminal investment. I investigated behaviours indicative of terminal investment in wild click beetles *(Agriotes obscurus)* exposed to the fungal entomopathogen *Metarhizium brunneum* under both starved and fed conditions. Beetles experienced reductions in lifespan in response to both starvation and exposure to fungus at the high concentration. When controlling for the effects of nutrition, I found beetles reduced their preoviposition period with fungal-induced decreases in lifespan, but this was not sufficient to maintain their fecundity compared to uninfected individuals. In starved beetles, preoviposition period decreased with lifespan, but fecundity did not change with lifespan, suggesting terminal investment in response to reductions in lifespan caused by both starvation and infection. Although fed beetles laid more eggs than starved beetles, they did not reduce their preoviposition period with changes in lifespan, resulting in decreases in the number of eggs laid with decreases in lifespan. These results indicate that nutritional conditions and fungal exposure each have an effect on inducing terminal investment in *A. obscurus* exposed to *M. brunneum.*

3.2. Introduction

Resources are limited in natural systems, and individuals must allocate energy to a range of different activities (Braendle et al., 2011; Jacot et al., 2004). This can result in trade-offs where one activity or function is favoured over another, or a single function is favoured at one point in time over another; a strategy to maximize overall fitness under

the given conditions (Braendle et al., 2011; Hau & Wingfield, 2011; Simmons, 2011). Reproduction is both costly and vital to individual fitness, and individuals allocate resources to optimize reproductive output under the given conditions (Edward & Chapman, 2011; McKean & Lazzaro, 2011). Under varying or suboptimal conditions, reproduction can be subject to trade-offs, for example with immunity or growth, or between reproductive components, such as egg size and number, or in reproductive timing (Benowitz et al., 2013; Clutton-Brock, 1984; McKean & Lazzaro, 2011).

Reproductive value is a measure of an individual's future reproduction (Williams, 1966). When the reproductive value is low, individuals are expected to allocate resources to current reproduction, altering reproductive parameters in an attempt to maintain or increase current fecundity compared to longer-lived individuals; this is referred to as the terminal investment hypothesis (TIH) (Clutton-Brock, 1984; Duffield et al., 2017). Behaviours consistent with the TIH have been observed in a variety of species with decreases in lifespan, and thus reproductive value, due to intrinsic factors, such as older age or decreases in nutritional resources (Decamps et al., 2007; Heinze & Schrempf., 2012) and extrinsic factors, such as pathogen exposure (Bowers et al., 2015; Marzal et al., 2008).

Pathogen challenge poses both a cost of immune system activation and a risk of reduced lifespan and decreased reproductive value, making it an extrinsic factor commonly expected to drive trade-offs between survival and reproduction (Jacot et al., 2004; Siva-Jothy & Thompson, 2002). In particular, the effect of pathogen exposure on reproductive trade-offs is an emerging field of interest (Duffield et al., 2018; Giehr & Heinze, 2018; Giehr et al., 2017). Several studies have found that pathogen-challenged insects increased or maintained reproductive output compared to healthy insects, a behaviour predicted by the TIH, but whether and to what degree this occurs depends on the level of pathogen challenge, and thus survival threat (Duffield et al., 2017; Giehr et al., 2017; Marzal et al., 2008). In addition, less is known about the impact of alterations in intrinsic factors, such as nutrition and age, on the effect of pathogen challenge on terminal investment, although there is some evidence to suggest an additive effect in triggering terminal investment (Duffield et al., 2017, 2018).

In their 2017 review, Duffield et al. proposed that an individual's intrinsic factors, including age or nutritional state, along with extrinsic factors such as pathogen

challenge, influence an individual's reproductive value and determine their likelihood of terminal investment. Intrinsic factors set the threshold for terminal investment, while extrinsic factors determine whether the threshold is reached. Individuals at an older age or a low nutritional state have a lower threshold for terminal investment than younger individuals or those at a high nutritional state. A few of the studies that have examined the effect of intrinsic condition on propensity to terminally invest have confirmed that increasing age, and decreasing reproductive value, triggers terminal investment in insects. Heinze and Schrempf (2012) found that *Cardiocondyla obscurior* ant queens laid more eggs per week with increasing age. Similarly, Duffield et al. (2018) found older male *Gryllodes sigillatus* crickets called more than young males, although this was only observed at a high level of infection cue induced by heat-killed *Escherichia coli.* The results of several other studies suggest that nutrition may also impact reproductive trade-offs (Edward & Chapman, 2011). In particular, Dong et al. (2008) found that starved *Nasonia vitripennis* ectoparasitic wasps experienced a decrease in preoviposition period compared to those fed on sucrose; starved individuals also experienced decreases in both oviposition period and fecundity compared to fed insects. In general, individuals with poor nutrition and a high level of pathogen exposure are expected to terminally invest, while those with adequate nutrition and no pathogen exposure are unlikely to terminally invest. A poor nutritional state can also negatively affect disease resistance, increasing the risk of death and potentially driving trade-offs toward terminal investment when poor nutritional state and pathogen exposure are combined (Siva-Jothy & Thompson, 2002).

In this study, I manipulated the nutritional state of the dusky wireworm beetle, *Agriotes obscurus* both before and after challenge with two concentrations of the fungal pathogen *Metarhizium brunneum* in order to determine whether and how differences in nutritional state altered the effect of pathogen challenge on reproductive timing and output. The larval form of *A. obscurus* is an agricultural crop pest that occurs with and is known to be infected and killed by *M. brunneum* under both natural conditions and artificial application (Kabaluk et al., 2007). When applied to populations in field trials, the time between exposure and death lasts for a week or more (Kabaluk et al., 2007). A similar, concentration-dependent mortality response is seen in adult beetles (Kabaluk, 2014). *M. brunneum* is being developed for the management of *A. obscurus* populations, particularly the adults, which are easier to treat than the subterranean larvae. Therefore,

it is important to determine how fungal treatment impacts reproduction and whether this is likely to result in longer term population suppression, in addition to the factors that might influence it. In Chapter 2, I found that *A. obscurus* adults altered their reproductive schedule to maintain their fecundity level when lifespan was reduced by *M. brunneum* challenge, indicating terminal investment. Here I examine whether nutritional state alters the beetles' reproductive behaviour and how this interacts with pathogen exposure. I expect female *A. obscurus* adults to change their reproductive schedule in response to reductions in lifespan resulting from pathogen exposure (terminal investment), consistent with my previous findings. In addition, I predict that a lack of nutritional resources will lower the threshold for terminal investment in response to pathogen exposure such that starved individuals exposed to a pathogen are more likely to terminally invest than fed individuals with no fungal exposure. A combination of starvation and fungal exposure would induce a stronger alteration in reproductive timing and output than either factor alone; this would be characterised by more reproductive parameters altered favouring increased reproduction. Exploration of this system will help us to better understand the effect of pathogen exposure on wild insect populations, which will have important implications for both the ecology and the management of insects.

3.3. Methods

3.3.1. Insect collection and storage

A. obscurus adults were collected from a pasture in Agassiz, British Columbia (49.1422° N, 121.4555° W) using pitfall traps between April 24 to May 4, 2017. The traps were checked at least once every three days. All beetles were stored at 8°C in 24h dark conditions and were housed in containers separated by date of catch and fed on fresh apple. Beetles were stored for a maximum of two months before fungal exposure. Beetles caught on each day were sexed at varying intervals after capture, split into male and female groups of up to five individuals and stored in 2-oz Solo cups with a small amount of moistened sterilized soil (Sufyan et al., 2014). At this time, beetles were fed on wheat grass clippings instead of apple. Two weeks before fungal exposure, beetles were separated into individual 2-oz Solo cups with moist soil and provided with one apple piece instead of grass clippings. One week before fungal exposure, beetles were moved to 20°C with a 12:12 L:D cycle. One hundred and fifty males and 150 females

were randomly selected to be in the "starved" group and two days before fungal exposure, their food was removed. The remaining beetles (150 of each sex) were allocated to the "fed" group and continued to be fed with apple.

3.3.2. Fungal treatment

Metarhizium brunneum LRC112 was isolated from an infected dead larva of *A. obscurus* near Agassiz, British Columbia in 1999 (Kabaluk et al., 2007). The isolate was amplified and tested for viability on potato dextrose agar prior to use in the assay (Kabaluk, 2014). Starved and fed females were each split randomly into three groups of fungal exposure: control, low and high concentration. Each female was dipped in 1 ml of a suspension of either 10^4 (low) or 10^8 (high) conidia/mL in 0.03% Tween 20 in sterilized distilled water for 10 secs and allowed to blot dry. Untreated control beetles were dipped in 0.03% Tween 20 in sterilized distilled water. Each female was placed in a 2-oz Solo cup already containing a male of the same nutritional status (fed with fed, starved with starved) and a small amount of moistened sterilized soil. Individuals in the fed group were also given a piece of apple. Fifty pairs of beetles were used in each of the six treatments. Beetles were kept at 20°C with a 12:12 L:D cycle.

3.3.3. Monitoring

Beetles were checked for mortality every day, transferred to new containers and any eggs were counted and photographed using a Motic DM-143-FBGG dissecting microscope and Motic Images Plus 2.0. Fed beetles were provided with a new apple piece every day. Dead beetles were surface sterilized by dipping in a 1% sodium hypochlorite solution for 30 secs, followed by three dips in sterilized water. After drying, the beetle was placed in a 3-cm Petri dish containing a moist cotton pad and examined for probable cause of death after a minimum of 14 days. Beetles showing signs of green or brown sporulation were recorded as having died of *M. brunneum* infection, while those that showed white or no sporulation were recorded as having died of unknown causes.

3.3.4. Statistical Analysis

A generalized linear model (GLM) with a binomial distribution and logit link function was used to determine if there were any differences in *M. brunneum*-induced mortality and the number of beetles that laid eggs, with the independent variables fungal concentration, nutrition status, female size (length multiplied by width) or their interactions. Only female beetles that laid eggs with a preoviposition period of greater than one day were included in further analyses. A multivariate analysis of covariance (MANCOVA) was run, testing the effect of fungal concentration, nutrition status, female size (length multiplied by width) and their interactions on lifespan (days of survival after exposure to the pathogen or control), preoviposition period (number of days between exposure to the pathogen or control and observation of the first egg), fecundity (number of eggs laid) and egg size (average length per female). The assumptions of observation independence and equal covariance matrices were met, and dependent variables were transformed to meet the assumption of normality (Finch, 2005). Histograms were used to examine the distribution of the data for each of the factors of interest and data were transformed when needed to satisfy the assumption of normality. Lifespan and preoviposition period were transformed with natural logs, while fecundity was transformed using a square root transformation. Oviposition period could not be normalized and was highly correlated with fecundity so it was not included in the MANCOVA. The MANCOVA results were further investigated using both Roy-Bargmann stepdown analysis with variables listed in the sequence given above, starting with the factor that I considered least likely to be influenced by others (Roy & Bargmann, 1958), and univariate analysis of covariance (ANCOVA). Both analyses were used to determine the effect of independent variables on dependent variables assuming the dependent variables are related (stepdown) or unrelated (ANCOVA). The stepdown analysis and ANCOVAs only included factors and interactions that were significant in the MANCOVA. The alpha level for each step of the stepdown analysis was set at 0.05, divided by the number of tests to reduce the inflated Type I error rate due to multiple testing (Tabachnick et al., 2007). When fungal concentration or nutrition was significant, differences between groups were determined using Tukey HSD tests.

I ran Kendall's partial correlations on untransformed reproductive parameters to further examine the relationships between the parameters and between the parameters and lifespan. Partial correlations were run for all fungal concentrations while controlling

for nutrition status and female size to determine the effects of the dependent variables on one another. The same procedure was carried out for each level of nutrition (starved and fed), controlling for female size to determine the effect of variations in lifespan (including the impact of fungal exposure) within each level of nutrition. The alpha level was adjusted using a Bonferroni adjustment to 0.005, which was 0.05 divided by the number of correlations (Bray & Maxwell, 1985). Eggs laid per day was calculated as number of eggs per laying event divided by the length of the event in days and the average number of eggs per event were averaged for each female. All data were analyzed using R version 3.5.1 (R Core Team, 2018), except the GLMs, means and 95% confidence intervals, which were analyzed and calculated using JMP® (Version 14 SAS Institute Inc., Cary, NC, 1989-2019).

3.4. Results

Fed beetles were more likely to lay eggs than starved beetles, with 87.5% of fed beetles and 72.0% of starved beetles laying eggs ($\chi^2 = 7.16$, $p = 0.0074$). Fungal exposure had no impact on whether the beetles laid eggs ($\chi^2 = 0.19$, $p = 0.91$) (Table 3.1). Smaller females were more likely to lay eggs than larger ones ($\chi^2 = 12.5$, $p = 0.0004$). No interactions were significant (fungal concentration*nutrition status: $\chi^2 = 0.47$, $p = 0.79$, fungal concentration*female area: $\chi^2 = 1.43$, $p = 0.49$, nutrition status*female area: $\chi^2 = 1.43$, $p = 0.23$, fungal concentration*nutrition status*female area: $\chi^2 = 0.79$, $p = 0.67$).

The MANCOVA indicated that the reproductive measures were influenced by fungal concentration ($F_{2,161}=13.11$, $p < 0.001$), nutrition status ($F_{1,161}=10.88$, $p < 0.001$) and female size ($F_{1,161}=3.84$, $p = 0.0053$), although their interactions were not significant (fungal concentration*nutrition status: $F_{2,161}=0.89$, $p = 0.52$; fungal concentration*female size: $F_{2,161}=0.89$, $p = 0.52$; nutrition status*female size: $F_{1,161}=0.85$, $p = 0.49$, fungal concentration*nutrition status*female size: $F_{2,161}=1.30$, $p = 0.24$). To understand which reproductive traits varied with the two independent variables (fungal concentration and nutrition), I performed stepdown analyses to determine the effect of each independent variable in the context of influential dependent variables on reproductive parameters, starting with lifespan, the variable least likely to be influenced by the other variables. (Table 3.2). Stepdown analysis was not carried out for female size because the

MANCOVA indicated that female size did not interact with fungal concentration or nutrition, the variables of interest.

Table 3.1. Influence of *Metarhizium brunneum* challenge and nutrition status on adult mortality, longevity and reproductive parameters

	treatment					
	control		***low (1 x 10^4 conidia/mL)***		***high (1 x 10^8 conidia/mL)***	
	fed	***starved***	***fed***	***starved***	***fed***	***starved***
fungal mortality	*33.3%*	*16.0%*	*32.6%*	*14.9%*	*84.0%*	*73.9%*
percent laying eggs	*87.5%*	*72.0%*	*87.0%*	*76.6%*	*88.0%*	*67.4%*
days until death	*31.48 ± 3.18*	*24.36 ± 3.12*	*30.26 ± 3.25*	*24.06 ± 3.21*	*14.56 ± 3.12*	*13.87 ± 3.25*
preoviposition period (days)	*7.03 ± 1.67*	*8.12 ± 1.69*	*9.69 ± 1.64*	*9.26 ± 1.77*	*7.03 ± 1.60*	*7.48 ± 1.90*
oviposition period (days)	*19.11 ± 2.67*	*7.53 ± 2.71*	*18.81 ± 2.64*	*6.13 ± 2.84*	*6.03 ± 2.57*	*4.15 ± 3.05*
eggs laid per female	*54.14 ± 9.15*	*24.12 ± 9.29*	*58.06 ± 9.03*	*18.29 ± 9.73*	*30.87 ± 8.79*	*13.11 ± 10.42*
eggs laid per female per day of oviposition	*8.50 ± 2.46*	*8.67 ± 2.5*	*9.21 ± 2.43*	*9.09 ± 2.61*	*8.99 ± 2.37*	*7.26 ± 2.80*
average egg length per female (µm)	*550.8 ± 11.0*	*547.2 ± 12.2*	*558.2 ± 10.9*	*547.0 ± 13.4*	*564.2 ± 11.1*	*559.0 ± 14.8*

(Mean ± 95% confidence interval). Sample sizes: mortality, percent laying eggs and days until death: 48 control fed, 50 control starved, 46 low fed, 47 low starved, 50 high fed, 46 high starved; oviposition data: 35 control fed, 34 control starved, 36 low fed, 31 low starved, 38 high fed, 27 high starved; egg size data: 34 control fed, 28 control starved, 35 low fed, 23 low starved, 34 high fed, 19 high starved.

Table 3.2. Stepdown analyses showing the effect of exposure to the fungus *Metarhizium brunneum* and nutritional level on reproductive parameters in adult female *Agriotes obscurus*

independent variable	***dependent variable***	***univariate F***	***p***	***df***	***stepdown F***	***p***	***df***
fungal concentration	*lifespan*	*58.10*	*< 0.001*	*2, 196*	*58.10*	***< 0.001***	*2, 196*
	preoviposition period	*3.77*	*0.025*	*2, 196*	*3.58*	*0.030*	*2, 195*
	fecundity	*7.95*	*< 0.001*	*2, 196*	*0.03*	*0.97*	*2, 194*
	egg length	*2.38*	*0.096*	*2, 168*	*3.18*	*0.044*	*2, 165*

nutrition status	*lifespan*	*11.37*	*< 0.001*	*1, 196*	*11.37*	***< 0.001***	*1, 196*
	preoviposition period	*0.15*	*0.70*	*1, 196*	*0.22*	*0.64*	*1, 195*
	fecundity	*60.72*	*< 0.001*	*1, 196*	*47.41*	***< 0.001***	*1, 194*
	egg length	*1.72*	*0.19*	*1, 168*	*0.63*	*0.43*	*1, 165*

Bold *indicates significance at p < 0.0125.*

Fungal Challenge

Beetles exposed to the high fungal concentration lived for a shorter period of time (15.43 ± 2.67 days) than those in the low (30.21 ± 2.63 days) ($p < 0.001$) and control groups (31.04 ± 2.59 days) ($p < 0.001$), as indicated by the stepdown analysis and Tukey HSD tests (Table 3.2). There was no difference between the control and low concentration ($p = 1.00$). Beyond lifespan, there was no direct effect of fungal concentration on the reproductive traits examined.

To better understand how the reproductive traits correlated with changes in lifespan, with decreases in lifespan acting as a proxy for the effects of fungus, under the influence of fungal concentration alone, I ran partial correlations examining the relationships between reproductive traits and controlling for both nutritional status and female size (Table 3.3). Decreases in lifespan corresponded to decreases in preoviposition period, oviposition period and fecundity when controlling for nutrition and female size, but there was no correlation between lifespan and egg size.

Table 3.3. Kendall's pairwise partial correlations between untransformed reproductive components of adult female click beetles *Agriotes obscurus* at all fungal concentrations, controlling for nutrition status and female size

all beetles controlling for nutrition status and female size												
	female lifespan			***preoviposition period***			***oviposition period***			***fecundity***		
	z	***tau***	***p***	***z***	***tau***	***p***	***z***	***tau***	***p***	***z***	***tau***	***p***
pre-oviposition period	*3.4*	*0.16*	***< 0.001***									
oviposition period	*9.5*	*0.45*	***< 0.001***	*-2.2*	*-0.11*	*0.027*						

fecundity	*5.2*	*0.25*	***< 0.001***	*0.069*	*0.0033*	*0.94*	*9.0*	*0.43*	***< 0.001***			
average egg length	*-0.41*	*-0.021*	*0.68*	*0.73*	*0.038*	*0.46*	*-0.97*	*-0.050*	*0.33*	*-0.091*	*-0.0047*	*0.93*

Bold *indicates significance at p < 0.005.*

Nutrition Status

Individuals that were starved lived for a shorter period of time (22.91 ± 2.62 days) than those that were fed (28.08 ± 2.41 days) ($p < 0.001$). Individuals in the fed group also laid considerably more eggs (47.3 ± 5.4 eggs) than those that were starved (18.9 ± 5.9 eggs) ($p < 0.001$). To better understand how decreases in lifespan corresponded to alterations in reproductive traits within each level of nutrition (i.e. how fungus-related decreases in lifespan impacted reproductive traits within the starved group compared to within the fed group), I ran separate partial correlations between reproductive traits in fed and starved female beetles (Tables 3.4 & 3.5). For both partial correlations, I controlled for female size, which did not differ with nutrition status (average beetle size: 0.272 ± 0.005 cm^2 fed versus 0.275 ± 0.006 cm^2 starved), but I did not control for fungal concentration.

Table 3.4. Kendall's pairwise partial correlations between untransformed reproductive components of fed adult female click beetles *Agriotes obscurus*, controlling for female size

fed beetles controlling for female size												
	female lifespan			***preoviposition period***			***oviposition period***			***fecundity***		
	z	***tau***	***p***	***z***	***tau***	***p***	***z***	***tau***	***p***	***z***	***tau***	***p***
pre-oviposition period	*1.7*	*0.11*	*0.090*									
oviposition period	*10.0*	*0.65*	***< 0.001***	*-2.4*	*-0.15*	*0.018*						
fecundity	*4.7*	*0.31*	***< 0.001***	*-0.78*	*-0.051*	*0.43*	*6.4*	*0.42*	***< 0.001***			
average egg length	*0.50*	*0.033*	*0.62*	*1.4*	*0.091*	*0.17*	*-0.63*	*-0.042*	*0.53*	*-0.43*	*-0.029*	*0.66*

***Bold** indicates significance at p < 0.005.*

When beetles were fed, fecundity declined as lifespan decreased, but there was a weak positive relationship between lifespan and preoviposition period (Table 3.4). Reductions in lifespan correlated with reductions in both oviposition period and fecundity, while reductions in oviposition period correlated with reductions in fecundity.

Table 3.5. Kendall's pairwise partial correlations between untransformed reproductive components of starved adult female click beetles *Agriotes obscurus*, controlling for female size

starved beetles controlling for female size												
	female lifespan			***preoviposition period***			***oviposition period***			***fecundity***		
	z	***tau***	***p***	***z***	***tau***	***p***	***z***	***tau***	***p***	***z***	***tau***	***p***
pre-oviposition period	*3.4*	*0.24*	***< 0.001***									
oviposition period	*3.2*	*0.23*	***0.001***	*-0.33*	*-0.023*	*0.74*						
fecundity	*2.4*	*0.17*	*0.015*	*1.5*	*0.11*	*0.13*	*6.3*	*0.45*	***< 0.001***			
average egg length	*-1.4*	*-0.11*	*0.17*	*-0.33*	*-0.027*	*0.74*	*-0.83*	*-0.069*	*0.40*	*0.090*	*0.074*	*0.93*

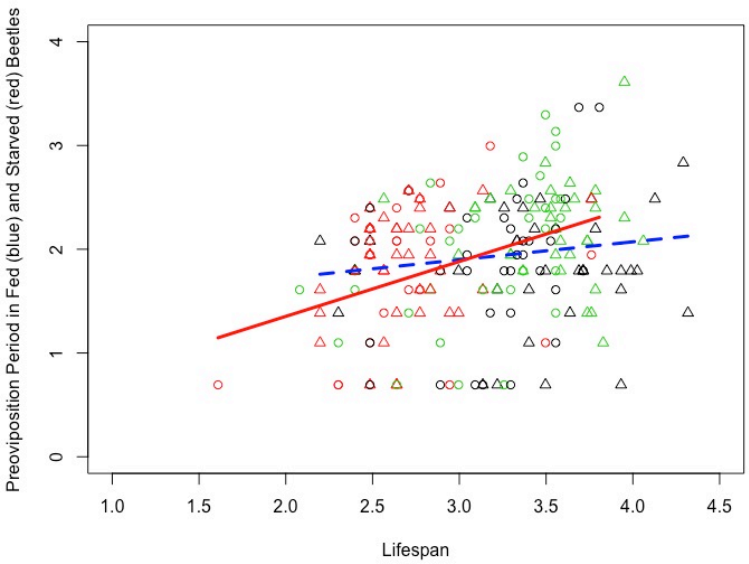
***Bold** indicates significance at p < 0.005.*

In contrast, when beetles were starved, declines in lifespan corresponded to decreases in preoviposition and oviposition period, but the positive relationship between lifespan and fecundity was weak (Table 3.5). Thus overall, fed beetles had a higher fecundity than starved beetles, but fed beetles laid fewer eggs with decreases in

lifespan, while starved beetles did not, likely due to the reduction in preoviposition period in starved but not fed beetles (Figure 3.1).

Fungal concentration influenced the likelihood of post-mortality sporulation (i.e. fungal mortality) ($\chi^2 = 82.7$, $p < 0.001$), with more females cadavers sporulating at the high concentration compared to the low and the control (high compared to low and control: $\chi^2 = 35.6$, $p < 0.001$) (79.2%, 23.7% and 24.5% respectively). More female cadavers also sporulated in the fed treatment than in the starved treatment (50.7% and 34.3% respectively) ($\chi^2 = 9.79$, $p = 0.0018$). However, there was no effect of female size ($\chi^2 = 1.88$, $p = 0.17$) or any of the interactions tested on likelihood of post-mortality sporulation (fungal concentration*nutrition status: $\chi^2 = 0.47$, $p = 0.79$, fungal concentration*female area: $\chi^2 = 1.43$, $p = 0.49$, nutrition status*female area: $\chi^2 = 0.14$, $p = 0.71$, fungal concentration*nutrition status*female area: $\chi^2 = 0.79$, $p = 0.67$).

a)



b)

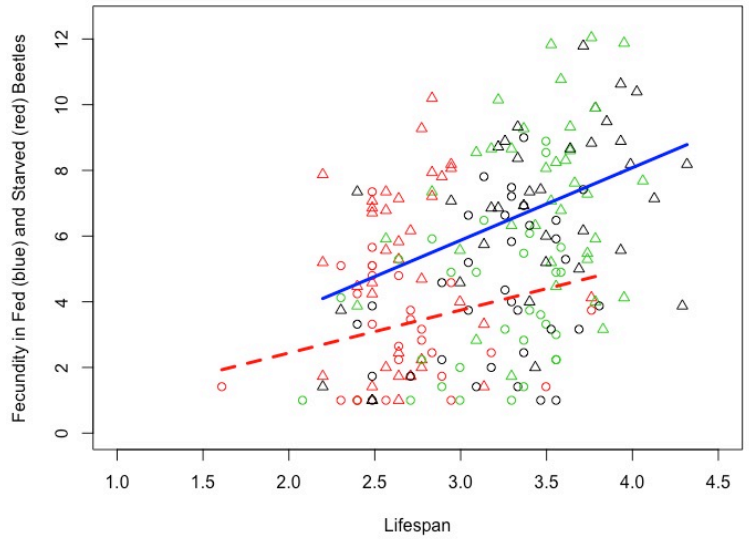


Figure 3.1. **Scatterplots of the correlations between natural log of lifespan (in days) and a) natural log of preoviposition period (in days) and b) square root of fecundity (in number of eggs) on fed (blue and triangles) and starved (red and circles) adult female click beetles *Agriotes obscurus*. Solid line = significant correlation. Dotted line = non-significant correlation. Black shapes = control; green shapes = low; red shapes = high.**

3.5. Discussion

When controlling for the effect of nutrition, *A. obscurus* females exposed to *M. brunneum* experienced decreases in both preoviposition period and fecundity with decreases in lifespan, and thus increases in fungal exposure, showing evidence of terminal investment but not fecundity compensation. Starved beetles lived for a shorter period of time and laid fewer eggs overall than fed beetles. In addition, starved beetles reduced their preoviposition period while maintaining fecundity with reductions in lifespan induced by fungal exposure. These results suggest that starvation-induced reductions in lifespan lowered the threshold for terminal investment in response to fungal exposure, which resulted in the alteration of multiple parameters in favour of increased reproduction, including fecundity compensation. Fed beetles, in contrast, lived for longer and laid more eggs, indicating a higher reproductive value, and did not show evidence of terminal investment, even when challenged with fungus.

Pathogen exposure and immune challenge are expected to trigger terminal investment both alone and in combination with starvation or poor nutrition (Duffield et al. 2017). Insects have fewer resources to allocate to immunity under starved conditions, and, in agreement with my study, pathogen and immune challenged insects have been shown to experience reductions in lifespan under nutrient-poor conditions compared to nutrient-rich conditions (Lee et al., 2008; Moret & Schmid-Hempel, 2000; Vogelweith et al., 2011). Similar to my study, several studies have shown insects exposed to live pathogens experience a longer lifespan when fed a high-quality diet than when starved or fed a low-quality diet. For example, Miller & Cotter (2018) found burying beetles *(Nicrophorus vespilloides)* exposed to entomopathogenic bacteria *Photorhabus luminescens* experienced a higher rate of survival when fed a high-fat diet than when fed a low-fat diet. In addition, Di Pasquale et al. (2013) found honeybees *(Apis mellifera)* infected with the microsporidian parasite *Nosema ceranae* survived for longer when fed than when starved. As with my study, both studies observed increases in lifespan in well-fed insects affected by live pathogens or parasites compared to poorly-fed or starved insects. In general, insects exposed to an immune system activation, rather than a live pathogen, are more likely to live longer with increases in nutritional resources because their lifespan is not limited by a pathogen that can use the resources to survive and continue to infect the host. In other instances, the reduction in lifespan resulting from

starvation is expected to induce terminal investment, and, although this phenomenon is not well-studied, this would be expected to occur when the individual is older and already at a higher propensity to terminally invest and more likely to die of the infection. In the current study, fungal-induced decreases in lifespan resulted in declines in preoviposition period; this resulted in the maintenance of fecundity, but only in starved beetles. My results confirm that starvation and pathogen exposure, both of which reduce reproductive value, each have an effect in triggering terminal investment. Although the beetles in this study were not at a significantly advanced age, they were close to the end of their reproductive lifespan and thus could have an increased propensity to terminally invest.

In this study, when controlling for the effect of nutrition, beetles at the higher fungal concentration had a reduced average lifespan, and reductions in lifespan corresponded to decreases in preoviposition period and fecundity. These results differ from those of Chapter 2, where beetles at the high concentration experienced a reduced lifespan, but fecundity was not affected by lifespan. Notably, the beetles in Chapter 2 were older than those in this study, and age has been observed to drive reproductive trade-offs towards terminal investment in insects in several studies. For example, Heinze & Schrempf (2012) and Shoemaker et al. (2006) observed *Cardiocondyla obscurior* ants and *G. texensis* Texas field crickets, respectively, to show increases in oviposition rate, an indication of increased reproductive effort, with increasing age. Increases in reproductive effort with increasing age have also been observed in *Hetaerina americana* American rubyspot damselflies, *Achroia grisella* lesser wax moths and *Ostrinia scapulalis* European corn borer moths (González-Tokman et al., 2013; Lafaille et al., 2010; Thanda Win et al., 2013). Although the beetles in Chapter 2 laid fewer eggs per day than those in Chapter 3, the fecundity compensation achieved in Chapter 2, but not in Chapter 3, in response to infection alone suggests an increase in reproductive effort in the older beetles after pathogen exposure. Although age was not the only difference between the two groups of beetles, it may have been a contributing factor in the differences in likelihood for terminal investment between the two studies.

The likelihood of cadaver sporulation in each of the three fungal treatment groups was similar to that of Chapter 2, despite the fungal concentration of the low treatment (10^4 spores per ml) being higher than in the earlier experiment (10^2 spores per ml). It has previously been observed that susceptibility to pathogen infection varies more widely

between individuals when exposed to lower compared to higher pathogen concentrations and that this variation is highly dependent on the level of genetic variation in the population (Hughes & Boomsma, 2004; King & Lively, 2012). The variation present at lower concentrations but absent at higher concentrations suggests that there may be a threshold concentration (dose) for widespread fatal infection within a population, past which point variation does not occur. This has been noted to occur in systems such as the *Beauveria bassiana-Mylabris pustulata* (blister beetle) system (Devi & Rao, 2006), while more logistic relationships between fungal concentration and host death have been observed in other systems, such as the *Metarhizium anisopliae-Blattella germanica* (German cockroach) system (Quesada-Moraga et al., 2004). This variation suggests that the likelihood of dying of infection is logistic with fungal dose in some systems, while a threshold exists for likelihood of dying of infection in other systems. Which pattern occurs for a specific system likely varies with both fungal and insect species, and this study would need to be repeated with a wider range of fungal concentration to determine which pattern is followed by the *Metarhizium brunneum-Agriotes obscurus* system. In addition, sporulation occurred in the control group in this study but not in Chapter 2, which could be attributed to the different trap locations. The beetles in this study were caught in the area where the fungus was originally isolated, so they may have had exposure to the fungus before capture. In contrast, the beetles in the study in Chapter 2 were caught in an area where the fungus had not been applied. If previous exposure did occur, it would be evenly distributed throughout the treatment groups and does not affect the results of this study.

Interestingly, the cadavers in the fed group were more likely to sporulate than those in the starved group. If we view the likelihood of sporulation as an indication of succumbing to infection, similar effects of nutrition have been observed in other studies. Although there is no clear single method by which this occurs, there are several ways that adequate nutrition can correspond to a higher likelihood of succumbing to pathogen infection. Individuals fed on a high-quality diet may use the surplus energy and nutrition to increase growth rate, which may occur at the cost of immunity, leading to a higher likelihood of pathogen infection. This was observed by Krams et al. (2015), who found greater wax moth *(Galleria mellonella)* larvae to favour growth rate over immunity when fed on a high-quality diet compared to those fed a diet of average quality. Another possible reason for this trend is that the stress of poor nutrition induces the production of

heat-shock proteins, which would aid in minimizing the damage inflicted by invading pathogens (Kangassalo et al., 2015). Alternatively, living in a nutrition-poor environment may act as a signal for disease risk, which would cause individuals to allocate resources to immunity as a response (Kangassalo et al., 2015). Since the latter two methods were speculative suggestions made by Kangassalo et al. (2015) to explain their observation that greater wax moths infected with *B. bassiana* were more likely to die of fungus when fed a high-quality diet than those fed a low-quality diet, and, to the best of my knowledge, have not been examined specifically, it is not clear whether the mechanisms involved would vary with insect species or nutritional resource provided. Additionally, pathogens may have a higher level of productivity in a well-fed host due simply to a higher level of nutrition available to the pathogen. This was observed by Tseng & Myers (2014), who found that the baculovirus AcMNPV produced more viral occlusion bodies within its host, the cabbage looper *(Trichoplusia ni)*, when the host had a longer period of daily access to food. Similarly, they reviewed several other studies on insects which observed increased potential parasite fitness (i.e. increased production of transmission stages) with higher insect host food level or quality. Each of the suggested methods present a reasonable possible cause for the observed trend, but since the insect species, pathogen used and level and quality of nutritional resource provided vary widely between studies, further and more specific study would be needed to determine whether the method or strategy varies with any of these factors. Since the beetles in my study showed an increased likelihood of succumbing to infection in agreement with all of the suggested possible methods, further study could be used to examine which method appears to be the most likely cause of the observed difference in this system.

Since reducing preoviposition period allowed starved beetles to avoid decreases in fecundity with reductions in lifespan, it is perhaps surprising that this strategy was not also observed in fed beetles, despite fed and starved beetles having a similar lifespan when exposed to *M. brunneum* at the high concentration. Although this has not specifically been studied in *A. obscurus,* there is evidence that the rate of egg maturation in insects is subject to trade-offs in resource allocation, and that the level of early investment in reproduction is negatively correlated with lifespan (Jervis & Ferns, 2004; Jervis et al., 2007; Rosenheim et al., 2000). This is mediated through molecular cues such as the release of juvenile hormone, which promotes both oocyte development and immunosuppression, favouring reproduction over immune activity, and occurs in insects

including *Drosophila melanogaster* (Schwenke & Lazzaro, 2017). Fed beetles at the high concentration had a relatively high fecundity and a short lifespan, suggesting a trade-off between reproduction and immunity such as that mediated by juvenile hormone, but since the strategy did not appear to involve investment in early egg maturation, it may not have involved juvenile hormone specifically. However, this is likely mediated by another molecular cue, which likely differed between fed and starved beetles. Since fed beetles were more likely to sporulate and had a longer oviposition period than starved beetles, the strategy for fed beetles may have been to extend oviposition closer to the end of their lifespan, rather than start laying eggs early, but with a cost to immunity. Although reduction of preoviposition period can be used to achieve fecundity compensation, it is not necessarily the most efficient use of resources for every scenario.

Insects, including beetles, are expected to manipulate egg size as an indication of resource allocation to and investment in offspring and fitness, but in the current study, egg size did not differ with fungal concentration or nutrition status (Caley et al., 2001; Ekbom & Popov, 2004; Fox & Czesak, 2000; Kojima, 2015; Reyes-Ramírez et al., 2019; van de Pol & Verhulst, 2006). The lack of alteration in egg size with fungal exposure was also found in Chapter 2, but since other studies have found poor nutrition to correspond to larger egg sizes in insects, my observed lack of change in egg size with differences in nutrition was surprising. For example, Vijendravarma et al. (2009) found fruit flies, *Drosophila melanogaster*, laid heavier eggs when fed a poor-quality diet as larvae. In addition, Braby & Jones (1995) found that the tropical satyrine butterfly *Mycalesis terminus* was able to maintain average egg weight later in life when fed on rotting fruit, in contrast to decreases in egg weight with age when fed on honey. However, neither study noted a change in egg number to correspond to changes in egg size, so it is possible that egg sizes change in some systems, while egg number changes in others. In addition, starvation may have different effects on egg size and number than those of poor nutrition. My findings indicate that the terminal investment strategies and trade-offs used by *A. obscurus* may not involve alterations in egg size, at least with changes in the variables tested in this system, although egg number was altered. Additional studies have found insect egg size varied with other factors, such as age (see Giron & Casas, 2003), colony size (see Matsuura & Kobayashi, 2010) and temperature (see Fischer et al., 2003; Seko & Nakasuji, 2006), so further studies examining changes in egg size in this system might focus on these factors. Since the study of wireworm reproduction is

still relatively new, and little is known about their reproductive physiology, further study would be necessary to confirm that egg size is not a manipulated reproductive parameter for *A. obscurus* under any conditions, and whether diet quality, rather than starved compared to fed conditions, has an impact on egg size.

Terminal investment is the result of trade-offs resulting from changes in reproductive value, and whether and how it occurs depends on both extrinsic and intrinsic factors and their effect on lifespan (Clutton-Brock, 1984; Duffield et al., 2017; Williams, 1966). Factors such as age, nutritional status and pathogen exposure, either individually or in concert with one another, may affect the propensity of an individual to terminally invest, and how reproductive parameters are altered as part of terminal investment, which differs with system and factors manipulated (Duffield et al., 2017). Further study is needed to better understand the conditions under which terminal investment will occur, and how this will differ between systems and study species. For this system, I observed that terminal investment occurred with fungal-induced reductions in lifespan in starved beetles, as well as a suggestion that pathogen-induced terminal investment is more likely in older individuals. The effect of age on pathogen-induced terminal investment needs further study, the results of which will add to our knowledge of the species and system and can guide future management efforts.

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Chapter 4.

Concluding Discussion

In Chapter 2, I found that older *Agriotes obscurus* beetles responded to reductions in lifespan resulting from exposure to *Metarhizium brunneum* by shifting their reproductive schedule to start laying eggs earlier (reduced preoviposition period), allowing them to maintain a similar level of fecundity to unexposed beetles. This result was similar to the reduced preoviposition period observed by Arthurs & Thomas (2000) and Blanford & Thomas (2001) in their studies on the effects of *M. acridum* on the locusts *Locustana pardalina* and *Schistocerca maroccanus*, respectively. However, terminal investment in insects in response to EPF is a rare observation compared to terminal investment in response to immune activation by bacterial or heat-killed agents (Duffield et al., 2017). This is in part due to the fact that EPF exposure is generally examined in the context of potential biological control, and relatively few studies examine multiple reproductive parameters that are required to identify terminal investment effort.

Next, in Chapter 3, I found that, for starved younger beetles, reductions in lifespan corresponded to reductions in preoviposition period but not with changes in fecundity. In contrast, the fecundity of fed beetles declined with lifespan, and preoviposition period did not change with decreases in lifespan. This indicates that starved beetles carried out terminal investment while fed beetles did not. When controlling for the effect of nutrition, beetles reduced their preoviposition period in response to *M. brunneum*-induced reductions in lifespan, indicating terminal investment, but also showed reduced fecundity compared to unexposed individuals.

My findings demonstrate that female *A. obscurus* beetles exhibit behaviours predicted by the TIH in response to a risk of reduced lifespan, as well as reductions in lifespan when subjected to starvation, but the effects of additional intrinsic and extrinsic factors still need to be considered. The effects of both age and temperature conditions on terminal investment, both in response to fungal-induced reductions in lifespan and under starved conditions, are in need of further study. In field conditions, click beetles emerge in March and mate and lay eggs until June, so age, variations in temperature, and food quality in addition to resource availability are important avenues of further study

for this system. In addition, few studies have looked at whether terminal investment, EPF exposure or both have any effect on offspring fitness of the exposed individuals, an important consideration when planning the use of EPF for population suppression. Of the studies that have found an effect of EPF exposure on fecundity and went on to look at the effects on offspring, both Hajek et al. (2008) and Dembilio et al. (2010) found that Asian longhorned beetles *(Anoplophora grabripennis)* exposed to *M. anisopliae* and red palm weevils exposed to *B. bassiana,* respectively, showed a reduction in the viability of eggs laid and a decrease in the survival of the resulting offspring compared to controls. In contrast, Gindin et al. (2006) found no effect of parental exposure to *M. anisopliae* on offspring larval weight in red palm weevils and Baverstock et al. (2006) found no effects of exposure to *B. bassiana* on fecundity in offspring of pea aphids *(Acyrthosiphon pisum).* The relatively few studies that look at offspring effects, and the limited results from these studies suggest that much still needs to be examined to better understand the effect of EPF exposure on insect populations in the long-term.

The results of this thesis have several implications for the proposed use of *M. brunneum* as a biological control agent for the management of *A. obscurus* populations. The beetles in these studies adjusted their reproductive schedule under several conditions, which might imply that fungal treatment may not be effective in reducing population density. Further work should be carried out under more realistic conditions to examine changes in *A. obscurus* fecundity after treatment with *M. brunneum,* as well as the effect of parental exposure on egg viability and offspring fitness, including alterations of resources provided to offspring and vertical transmission of EPF, and the potential for immune priming, which could increase their resistance (Moreau et al., 2012; Shikano et al., 2016; Zanchi et al., 2011). Modelling studies should be carried out to better understand long-term population effects of *M. brunneum* on *A. obscurus* in the field, as well as the effects of additional control methods, such as application of nematodes and synthetic chemical Insecticides. More detailed research is needed to understand whether and under what circumstances its application will result in population suppression or potential population increase.

In general, EPF or other lifespan-reducing agents considered for use as biological control agents against insect pests may have population suppressing qualities, but also run the risk of inducing terminal investment with alterations in intrinsic and extrinsic conditions. A comprehensive view of the reproductive and offspring effects of a

proposed biological control agent on its target host is necessary to allow us to estimate the long-term population effects of its implementation. This would include studies on the effect of the biological control agent on the species and its offspring under various conditions of temperature, nutrition and age, as well as trans-generation and modelling studies to determine the long-term effects of the agent on the population and how it varies under different conditions.

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Appendix A.

Table A.1. Observed reproductive parameters in insects exposed to entomopathogenic fungi

Species	*Common Name*	*Order*	*Hemi-metabolous (HM) or holo-metabolous (HL)*	*Lab-Reared or Wild*	*Fungal Species*	*Reduced Life-span?*	*Fecundity*	*Pre-oviposition Period*	*Oviposition Period*	*Daily Fecundity*	*Reference*
Blattella germanica	*German cockroach*	*Blattodea*	*HM*	*Lab-reared*	*Metarhizium anisopliae*	*Yes*	*Decreased*			*No change, then decreased*	*Quesada-Moraga et al., 2004*
Lygus hespergus	*Western tarnished plant bug*	*Hemiptera*	*HM*	*Wild*	*Beauveria bassiana*	*Yes*	*Decreased*			*Decreased in non-sporulated bugs, no change in sporulated bugs*	*Noma & Strickler, 2000*
Pseudococcus viburni	*Obscure mealybug*	*Hemiptera*	*HM*	*Wild*	*Metarhizium anisopliae*	*Yes*	*No change*		*No change*		*Pereira et al., 2011*

Species	Common Name	Order	Hemi-meta-bolous (HM) or holo-meta-bolous (HL)	Lab-Reared or Wild	Fungal Species	Reduced Life-span?	Fecundity	Pre-oviposition Period	Oviposition Period	Daily Fecundity	Reference
Lygus lineolaris	*Tarnished plant bug*	*Hemiptera*	*HM*	*Lab-reared*	*Beauveria bassiana*	*Yes*	*Decreased*		*Decreased*	*No change overall, high temp subset increased initially*	*Ugine, 2011*
Acyrthosiphon pisum	*Pea aphid*	*Hemiptera*	*HM*	*Lab-reared*	*Beauveria bassiana*	*Not stated*	*Decreased*			*Decreased*	*Baverstock et al., 2006*
Acyrthosiphon pisum	*Pea aphid*	*Hemiptera*	*HM*	*Lab-reared*	*Metarhizium anisopliae*	*Yes*	*Decreased*				*Parker et al., 2014*
Locustana pardalina	*Brown Locust*	*Orthoptera*	*HM*	*Wild*	*Metarhizium acridum*	*Yes*	*No change*	*Decreased*		*Increased initially*	*Arthurs & Thomas, 2000*
Dociostaurus maroccanus	*Moroccan locust*	*Orthoptera*	*HM*	*Lab-reared*	*Metarhizium acridum+ Beauveria bassiana*	*Yes*	*Decreased*	*Increased*			*Valverde-Garcia et al., 2019*

Species	*Common Name*	*Order*	*Hemi-metabolous (HM) or holo-metabolous (HL)*	*Lab-Reared or Wild*	*Fungal Species*	*Reduced Life-span?*	*Fecundity*	*Pre-oviposition Period*	*Oviposition Period*	*Daily Fecundity*	*Reference*
Schistocerca gregaria	*Desert locust*	*Orthoptera*	*HM*	*Lab-reared*	*Metarhizium acridum*	*Yes*	*No change*	*Decreased*		*Increased initially*	*Blanford & Thomas, 2001*
Agrilus planipennis	*Emerald ash borer*	*Coleoptera*	*HL*	*Wild*	*Beauveria bassiana*	*Yes*	*No change*				*Liu & Bauer, 2008*
Anoplophora glabripennis	*Asian longhorned beetle*	*Coleoptera*	*HL*	*Wild*	*Beauveria bassiana*	*Yes*	*Decreased*			*Decreased*	*Dubois et al., 2004*
Adalia bipunctata	*Two-spotted ladybug*	*Coleoptera*	*HL*	*Lab-reared*	*Beauveria bassiana*	*Yes*	*Decreased, no change (conc-dependent)*			*Decreased*	*Roy et al., 2008*

Species	*Common Name*	*Order*	*Hemi-metabolous (HM) or holo-metabolous (HL)*	*Lab-Reared or Wild*	*Fungal Species*	*Reduced Life-span?*	*Fecundity*	*Pre-oviposition Period*	*Oviposition Period*	*Daily Fecundity*	*Reference*
Diabrotica virgifera virgifera	*Western corn rootworm*	*Coleoptera*	*HL*	*Lab-reared*	*Beauveria bassiana*	*Yes*	*Decreased, no change (dependent on age exposed)*			*Decreased*	*Mulock & Chandler, 2001*
Rhynchophorus ferrugineus	*Red palm weevil*	*Coleoptera*	*HL*	*Lab-reared*	*Beauveria bassiana*	*Yes*	*Decreased*			*No change, then decreased*	*Dembilio et al., 2010*
Rhynchophorus ferrugineus	*Red palm weevil*	*Coleoptera*	*HL*	*Lab-reared*	*Metarhizium anisopliae*	*Yes*	*Decreased*		*Decreased*	*No change*	*Gindin et al., 2006*
Cylas puncticollis	*Sweet potato weevil*	*Coleoptera*	*HL*	*Lab-reared*	*Metarhizium anisopliae*	*Yes*	*Decreased*			*Decreased*	*Ondiaka et al., 2008*

Species	***Common Name***	***Order***	***Hemi-metabolous (HM) or holo-metabolous (HL)***	***Lab-Reared or Wild***	***Fungal Species***	***Reduced Life-span?***	***Fecundity***	***Pre-oviposition Period***	***Oviposition Period***	***Daily Fecundity***	***Reference***
Anoplophora glabripennis	*Asian longhorned beetle*	*Coleoptera*	*HL*	*Lab-reared*	*Metarhizium anisopliae*	*Yes*	*Decreased*			*No change first week, decreased second week*	*Hajek et al., 2008*
Tenebrio molitor	*Mealworm beetle*	*Coleoptera*	*HL*	*Lab-reared*	*Metarhizium robertsii*	*Yes*	*Decreased*				*Reyes-Ramírez et al., 2019*
Musca domestica	*Housefly*	*Diptera*	*HL*	*Wild*	*Beauveria bassiana, Metarhizium anisopliae*	*Yes*	*Decreased*				*Farooq & Freed, 2016*
Aedes aegypti	*Mosquito*	*Diptera*	*HL*	*Lab-reared*	*Beauveria bassiana*	*Yes*	*No change*			*Increased initially*	*Darbro et al., 2012*

Species	***Common Name***	***Order***	***Hemi-metabolous (HM) or holo-metabolous (HL)***	***Lab-Reared or Wild***	***Fungal Species***	***Reduced Life-span?***	***Fecundity***	***Pre-oviposition Period***	***Oviposition Period***	***Daily Fecundity***	***Reference***
Ceratitis capitata	*Mediterran-ean fruit fly*	*Diptera*	*HL*	*Lab-reared*	*Metarhizium anisopliae, Beauveria bassiana*	*Yes*	*Decreased*	*Increased*			*Quesada-Moraga et al., 2006*
Ceratitis capitata	*Mediterran-ean fruit fly*	*Diptera*	*HL*	*Lab-reared*	*Metarhizium anisopliae*	*Yes*	*Decreased*			*Decreased*	*Castillo et al., 2000*
Anopheles gambiae	*Mosquito*	*Diptera*	*HL*	*Lab-reared*	*Metarhizium anisopliae*	*Yes*	*Decreased*				*Scholte et al., 2006*
Temnothorax crassispinus	*Ant*	*Hymen-optera*	*HL*	*Wild*	*Metarhizium brunneum*	*Yes*	*Decreased*			*Decreased*	*Giehr & Heinze, 2018*
Cardiocondyla obscurior	*Ant*	*Hymen-optera*	*HL*	*Lab-reared*	*Metarhizium brunneum*	*Yes*	*Increased*			*Increased*	*Giehr et al., 2017*
Tuta absoluta	*Tomato leaf miner*	*Lepidoptera*	*HL*	*Lab-reared*	*Metarhizium anisopliae*	*Yes*	*No change*		*No change*	*Increased initially*	*Pires et al., 2009*

Species	***Common Name***	***Order***	***Hemi-metabolous (HM) or holo-metabolous (HL)***	***Lab-Reared or Wild***	***Fungal Species***	***Reduced Life-span?***	***Fecundity***	***Pre-oviposition Period***	***Oviposition Period***	***Daily Fecundity***	***Reference***
Chrysoperla rufilabris	*Green lacewings*	*Neuroptera*	*HL*	*Lab-reared*	*Beauveria bassiana*	*Yes*	*Decreased*			*Decreased*	*Portilla et al., 2017*

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