# Consequences of Host Quality Variation for the Behaviour, Life Histories and Ecological Interactions of Insects at Higher Trophic Levels

A thesis submitted by

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## Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged. I was the lead researcher for all research reported in the papers included in this thesis listed below.

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## Abstract

Species interactions have caught ecologists' attention since the field of ecology first developed. Direct effects between species are readily recognised as the interaction is explicit and easy to detect. In contrast, indirect effects are masked under direct effects and difficult to detect. However, ecologists have been attempting to quantify and understand the role of indirect effects on community structure. In Chapter 1, I reviewed the literature, clarifying the basic definitions of direct and indirect effects, and then apply these concepts to components in my study systems. I employed two different study systems to examine direct (host seed-bean beetle system) and indirect (plant pathogen-plant-insect system) effects in this thesis. In Chapter 2, I explored the direct effects of host quality and maternal effects on preference and performance of two bean beetle species, *Callosobruchus maculatus* and *C. analis*. They showed similar and consistent preference, but their performance was species dependent and influenced by both host and maternal effects. In Chapter 3, I investigated the direct effects of host quality and mating status on *C. maculatus* reproductive costs. I found that host quality affects beetle longevity, and this effect also depends on mating status, which then differs between treatments and sexes. In Chapter 4, I examined the indirect effects emanating from the plant pathogen *Botrytis cinerea* on two aphid species, *Aphis fabae* and *Acyrthosiphon pisum*. I found that *A. fabae* experiences indirect negative effects while *Ac. pisum* gains indirect positive effects from *B. cinerea* infection. In Chapter 5, I investigated the indirect effects of *B. cinerea* infection on *A. fabae* and their natural enemies and ant mutualists in the field, where levels of urbanisation varied. My results suggest that the population of *A. fabae* is influenced by infection, while the population of aphid natural enemies and ant mutualists are instead dependent on urbanisation. In Chapter 6, I investigated the effects of plant pathogens on insect herbivores' natural enemies using meta-analysis technique. I found no overall effects of plant pathogens on insect natural enemies, but if considered by type of pathogen, I found fungal pathogens cause consistent indirect negative effects, while bacterial pathogens show indirect positive effects on insect natural enemies. No significant effects of viral pathogens were found. In my last Chapter (Chapter 7), I discuss the main findings, contributions and further recommendations of my study.

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# Chapter 1 Introduction

## 1.1 General introduction

"You are what you eat" is the classic phrase presenting the premise that how healthy you are depends on the food you consume. For humans, this phrase is true, but being what we are according to what we eat is not quite measurable in a short time scale, as we have a long-life span, a long time before reaching our reproductive phase, and we tend to have few offspring and great variation in food choices. However, this phrase is certainly appropriate for short-lived animals such as insects which have a brief life cycle which greatly contrasts with ours.

In terms of ecology, organisms can interact with one another directly or indirectly (Moon et al., 2010), and this leads to flows of complex interactions between species, and these interactions can be affected by diet. Typically, ecologists consider direct interactions when studying species interactions while indirect interactions are overlooked (Werner and Peacor, 2003). For example, plants interact with primary consumers (herbivores) which in turn are consumed by predators. This food chain results in energy transfer from producers to the organisms at higher trophic levels. However, by reducing numbers of herbivores, plants indirectly benefit from the presence of predators. These indirect effects can also arise from changes in plant quality measured in terms of the plant's nutritional and chemical composition, which directly affects herbivores and indirectly affects the predators consuming them (Bush et al., 1997; Omacini et al., 2001; Utsumi and Ohgushi, 2009; Al-Naemi and Hatcher, 2013; Rizvi and Raman, 2017).

Host plant quality can affect life histories of insect herbivores via two main avenues: nutritional and defensive components (Awmack and Leather, 2002). Plant nutrition can be modified by the application of fertiliser (Facknath and Lalljee, 2005; Muller et al., 2005; Sarfraz et al., 2009) and influenced by genetic variation (Collins et al., 2001; Wetzel et al., 2016). Likewise, plant defensive compounds vary across genotypes (Züst and Agrawal, 2017) and also depend on previous infection or attack (known as induced resistance) (Hammerschmidt, 2014). At the same time, while there have been many studies focusing on how variation in plant quality can result in indirect effects at higher trophic levels, very few studies investigate how the presence of factors such as plant pathogens, which can induce changes in plant quality, also affect organisms at higher trophic levels.

To sum up, plants are the foundation of food chains, and variation in host plant quality, whether constitutive or caused by external factors such as pathogen attack, may affect primary consumers and in turn can be relayed to higher trophic levels, examples of which will be explored in this thesis.

## 1.2 Role of top-down and bottom-up effects in population ecology

Top-down effects result from the control of lower food-web elements by the upper-level predators while bottom-up effects occur where primary producers or the addition of limited nutrients influences interactions at higher trophic levels (Pace et al., 1999). For example, the presence of fish predators reduced mayfly nymph grazing and therefore resulted in increased algal abundance (McIntosh and Townsend, 1996). This shows the indirect consequences of top-down effects influencing the abundance of an organism in the lower part of the food chain, which can be considered a trophic cascade because there is at least one intermediate species (see below).

The consequences for other species of willow defences being induced by the swift moth, *Endoclita excrescence*, provides an example of a bottom-up effect as boring resulting in the emergence of lateral shoots with leaves containing more nitrogen, which in turn increases both the abundance and species richness of other herbivores. The increase of herbivore abundance also caused the increase of both abundance and species richness of predators (Utsumi and Ohgushi, 2009).

### 1.3 Direct effects, indirect effects and trophic cascades

There is some confusion about the definitions of terms used in interaction studies; what are direct effects, indirect effects and trophic cascades? A direct effect is the result from the physical interaction between two species without intermediate species, while an indirect effect is the result of the interaction between two species which requires the presence of at least one intermediate species (Wootton, 1994; Ripple et al., 2016). For example, when a caterpillar consumes leaves this is considered a direct effect, but if the caterpillar consumes leaves and induces change (in either nutritional or physiological qualities) of the host plant, which in turn affects expression of pathogenic fungus infection, this is considered an indirect effect, in which the plant acts as an intermediate species.

A trophic cascade is the indirect effect of a predator spreading downwards thorough food webs across multiple trophic levels, and they can be activated by consumptive interactions between predators and prey and non-consumptive effects due to perceived predation risk by prey (Ripple et al., 2016). From this definition a trophic cascade is strictly considered to originate from predators, but recently this definition has been amended so that not only predators but also infectious agents can be originators of the cascade (Buck and

Ripple, 2017). Thus, indirect interactions originating from plant pathogens and relayed to herbivores and further trophic levels can be considered as a trophic cascade.

In addition, a trophic cascade must involve an indirect effect in which one species affects another species through one or more mediator species. This definition does not include bottom-up effects because researchers intend to discriminate between trophic processes in which the distribution and abundance of species is determined by negative or positive effects between predators and consumers (Ripple et al., 2016). While top-down effects can alter the abundance and productivity of plants, which in turn activates the alteration of bottom-up effects of plants in the trophic food chain, these responding bottom-up influences are considered 'knock-on effects' (Ripple et al., 2016). For example, the top-down effect of the grey wolf *Canis lupus* on elk *Cervus elaphus* can increase berry production (a trophic cascade) which in turn increases berry consumption by grizzly bears *Ursus arctos* (a knock-on effect; Ripple et al., 2014).

Although Ripple et al. (2016) and Buck and Ripple (2017) did not consider bottom-up effects as a trophic cascade, I argue that in plant pathogen-plant-insect system I see a trophic cascade in the form of bottom-up effects, which previous studies did not consider. Following Kagata and Ohgushi's (2006) inclusion of bottom-up trophic cascades, I argue that plant pathogens modify host plant quality and in turn affect herbivorous insects and their natural enemies, in which plant pathogens can be originators of a bottom-up trophic cascade.

## 1.4 Importance of direct and indirect effects in applied ecology

### 1.4.1 Managing insect pests

A knowledge of direct and indirect effects can be applied to pest management and can enhance the effectiveness of pest control. The direct effects used to control insect pests

usually involve predation, parasitism and infection, for example, predation by natural enemies (e.g. coccinellid beetles, reduviid bugs and lacewings), parasitism by parasitoid wasps and infection by bacteria, viruses, fungi, nematodes and protozoa (van Driesche et al., 2008). This direct effect provided by various biocontrol agents causes straightforward effects resulting in a reduced population of insect pests. The use of indirect effects also aims to decrease pest populations, but through a more complicated process. For example, enhancement control of the pea aphid *Acyrthosiphon pisum* combines the use of two predators, the foliar foraging ladybeetle *Coccinella septempunctata* and the ground foraging beetle *Harpalus pennsylvanicus* (Losey and Denno, 1998). The underlying process is the foliar predator activates the dropping behaviour of the aphids which in turn benefits the predation of ground foraging beetles (Losey and Denno, 1998). This result illustrates how knowledge of indirect effects can be applied to a pest management programme.

### 1.4.2 Unintended consequences

Sometimes the use of biocontrol agents is not as successful as expected because of interactions between other organisms and the agents or even between the agents themselves (van Driesche et al., 2008). These may result in negative effects reducing the effectiveness of pest control. For example, intraguild predation happens when two predators compete for the same prey, and one of them can feed upon the another (asymmetrical) or they feed upon each other (symmetrical) (Muller and Brodeur, 2002). The coexistence of fire ants *Solenopsis invicta* with cotton aphids *Aphis gossypii* contributed to the high mortality of ladybeetles and lacewing larvae in a greenhouse experiment (Kaplan and Eubanks, 2002). Another example is the intraguild predation interaction between biocontrol agents. The percentage of second-instar green lacewings *Chrysoperla carnea* consumed by fourth-instar Harlequin ladybirds

*Harmonia axyridis* was not significantly different from the percentage of consumed aphids (Wells et al., 2017).

Another concern is the effect of biocontrol agents on non-target organisms. *Cactoblastis* are moths native to South America introduced to Australia to control prickly pear cacti. It was successful in controlling the cacti in Australia, so the moths were imported to the Caribbean, and inadvertently distributed to Florida and the southern part of the USA and Mexico where native cacti were under threat (Cory and Myers, 2000). Similarly, the flowerhead weevil *Rhinocyllus conicus* was introduced from Europe to the USA to control thistles *Carduus* spp. but later the native thistles *Cirsium* spp. also came under threat (Cory and Myers, 2000; Louda and Arnett, 2000).

### 1.4.3 Failures of IPM

The unsuccessful outcomes of integrated pest management may arise from factors relating to biocontrol agents. For example, generalist predators have a broad spectrum of prey; therefore, they consume a variety of insects which may include non-target organisms or even other predator agents (intraguild competition) (van Driesche et al., 2008; Snyder and Ives, 2009). Furthermore, generalist predators have long generation times compared with pests, so they cannot overcome pests via density-dependent responses to increasing pest populations (Snyder and Ives, 2009).

However, a critical factor influencing the success of biocontrol may also come from the interaction between plant pathogens and biocontrol agents. Infection by barley yellow dwarf luteovirus (BYDV) had detrimental effects on the parasitoid *Aphidius ervi* mediated by its host aphid, *Sitobion avenae.* The effects include longer developmental time, a delay in starting pupation, increased death before pupation and fewer parasitoid eggs laid on infected

aphids (Christiansen-Weniger et al., 1998). In addition, in some cases biocontrol agents themselves cause worse consequences to host plants. For example, parasitoids facilitate the spread of plant pathogens because the parasitized aphids tend to increase their movement (Christiansen-Weniger et al., 1998), and parasitoids provoke aphids scattering via dropping behaviour (Hodge and Powell, 2008).

### 1.5 Host seed quality

Bean seeds (legumes) vary in nutritional value (Messina, 2014). For insect seed predators, nutrition is not the only factor determining preference. Olfactory (Ajayi et al., 2015), chemical cues (volatile and surface chemicals) (Gokhale et al., 1990), the curvature of host seeds (Gokhale et al., 1990), seed size (Cope and Fox, 2003), the existence of larvae inside the seeds (Guedes and Yack, 2016) and the chemical markers left by other females (Yamamoto, 1990) all affect host preference. Seed quality not only affects host preference, but also offspring performance (Messina et al., 2018). This cannot only be explained by the difference in nutrition, but also the existence and the differences of plant secondary metabolites (Desroches et al., 1997; Rêgo et al., 2020). These represent the direct effects of host plant quality on insect life histories. Host preference is not necessarily fixed and innate, and experience, whether as an ovipositing female, or as a developing larva, can alter host choice decisions.

### 1.6 Insect learning

Learning is the acquirement of neuronal representation of new information including spatial environmental configurations, information from olfactory, visual and auditory organs, relations between perceived stimuli and environment, and the manipulation of novel food associated with movement (Dukas, 2008). Numerous studies have shown that insects have

the ability to learn across taxa including fruit flies (Diptera), grasshoppers (Orthoptera), and parasitoid wasps, bees and ants (Hymenoptera) (Folkers, 1982; Raubenheimer and Tucker, 1997; Morris and Fellowes, 2002; Farina et al., 2005; Franks and Richardson, 2006).

The ability to learn in insects varies across individuals in the same species based on genetic variation associated with variation in fitness through natural selection (Dukas, 2008). An artificial selection study of proboscis extension in blow fly *Phormia regina* response to sucrose with water or saline condition demonstrated that the results of selection are genetic correlates (McGuire and Hirsch, 1977), and another study showed that the associative learning of grasshoppers, *Schistocerca americana*, does improve their fitness (Dukas and Bernays, 2000).

There are two main types of insect learning, non-associative and associative learning, in which the latter means conditioned learning associated with neutral stimulus. Non-associative learning consists of sensitisation and habituation in which the former is the increase of response to a stimulus through repeated exposure, while the latter is the decrease of response through the exposure of repeated stimulus (Jones and Agrawal, 2017; Little et al., 2019).

If associative learning results in a positive reward it is called ‘appetitive learning’, but if it causes negative punishment it is called ‘aversive learning’ (Jones and Agrawal, 2017; Little et al., 2019). Indeed, associative learning can be distinguished into three categories, classical conditioning (Pavlovian), differential conditioning and operant conditioning (Little et al., 2019).

- Classical conditioning is the learning induced by a neutral stimulus that causes reflexive response (Little et al., 2019).
- Differential conditioning is the learning with two or more stimuli are used, in which one conditioned stimulus is paired with unconditioned stimulus (e.g. reward) and another stimulus is not paired (American Psychological Association, 2018).
- Operant conditioning is the learning that an individual associates the particular behaviour and the consequence of that behaviour, which leads to reinforcement or punishment (McLeod, 2018).

However, it is predicted that non-associative learning (sensitisation and habituation) is more common in the environment with low variation, while associative learning should be more common in environments with high variation, in different stimuli (Jones and Agrawal, 2017). This may point to the crucial role of the surrounding environment that influences insect learning, which is closely tied to their survival and fitness in a particular environment.

### 1.6.1 Ovipositional learning

Oviposition in insects can be influenced by previous ovipositional experience, and foraging experience either during larval or adult stages, which may cause different outcomes depending on particular life stage (Jones and Agrawal, 2017).

Adult ovipositional experience influences host selection and acceptance as shown in in the moth *Helicoverpa armigera* (Cunningham et al., 1998). Ovipositional experience also can be learnt through associative learning as demonstrated in the fruit fly *Drosophila melanogaster* to oviposit their eggs on fruit juice and avoid quinin-contaminated fruit juice

(Mery and Kawecki, 2002), and the pipevine swallowtails *Battus philenor* which learn to associate leaf shape with leaf chemistry that is suitable for oviposition (Papaj, 1986).

The influence of larval experience on adult oviposition is still contentious (Jones and Agrawal, 2017), and some argued that the benefits of larval learning are limited as the benefits will be occur only when the larval environment is better than the combination of genetic determination and adult environment (Janz et al., 2009). The phenomenon that insects prefer to oviposit on host plants they consume as larvae is called Hopkins' host selection principle (HHSP) (Barron, 2001). The mechanism of HHSP consists of preimaginal conditioning (the ability to retain larval experience through metamorphosis), chemical legacy (the chemicals in larval environment are retained in haemolymph through metamorphosis) and imaginal conditioning (the learning of adults as they emerge from the host plant) (Jones and Agrawal, 2017).

### 1.7 Transgenerational effects

Transgenerational effects are a nongenetic inheritance that occurs when the environment experienced by parents influences the life histories of offspring, and this can be transmitted through epigenetic, cytoplasmic, somatic, nutritional, environmental and behavioural variation (Bonduriansky and Day, 2009; Bell and Hellmann, 2019). The environment experienced by parents can occur before offspring are born (prefertilisation), or after offspring are born (passed through parental behaviour), which can be in either short lived (e.g. facing predators) or long lived environments (e.g. a dry breeding season) (Bell and Hellmann, 2019).

Maternal effects are a subset of transgenerational effects and have received much attention due to the intimate connection between mother and offspring during embryonic and

juvenile development in most animals (Ho, 2014). Maternal effects have been described in many insect taxa (Mousseau and Dingle, 1991). These effects are sometime critically important to the survival of offspring by determining offspring performance (Mbande et al., 2020; Tougeron et al., 2020), altering offspring behaviours to avoid predators (Keiser and Mondor, 2013), and resistance to pathogens and pesticides (Brevik et al., 2018; Schulz et al., 2019). Maternal diet/nutritional status can affect offspring performance, for example, with the cockroach *Diploptera punctata*, maternal nutritional status influences the investment in embryos resulting in different development rate and body size of male offspring (Holbrook and Schal, 2004).

## 1.8 Plant pathogens

Plant pathogens are important to human food security, and in term of economic damage they cause global losses of around US$220 billion annually (Agrios, 2005). Moreover, plant diseases have impacts on human livelihood and society as shown in the great famine in Ireland which began in 1845, and was caused by the potato blight disease *Phytophthora infestans*. This event contributed to hundreds of thousands people dying from starvation and disease associated with hunger, and forced 1.5 million people to emigrate from Ireland (Schumann and D'Arcy, 2012).

### 1.8.1 Can plant pathogens influence ecological interactions and biocontrol?

There is an excellent example of a serial study investigating the influence of a plant pathogen on oviposition behaviour of the Light brown apple moth *Epiphyas postvittana* feeding upon vines *Vitis vinifera*. Host plant infection by *Botrytis cinerea* has negative impacts on the moth. Gravid females avoid laying eggs on moderately and intensely infected vine leaves (Rizvi et al., 2015). Choice experiments showed that moths preferred to oviposit

on uninfected leaves, but the moth larvae which fed on infected leaves exhibited better survival rates, pupation times, pupal mass and fecundity when compared with larvae fed on uninfected leaves (Rizvi et al., 2016). The identification of volatiles emanating from infected vine leaves showed a reduction of several chemicals which were also present in uninfected leaves, and new synthesis was found only on *Botrytis*-infected leaves. This may be the cause of gravid moth deterrence (Rizvi and Raman, 2017). These studies show that infection by a plant pathogen can alter plant traits which in turn influences the interactions between host plants and insects.

Similarly, there also are several studies which indicate that *Botrytis* infection of host plants influences the interaction between host plants and insects. For example, the larvae of the European grapevine moth *Lobesia botrana* fed on infected vines *V. vinifera* in France showed higher survival rates and faster development in field experiments. In the laboratory, larvae fed with an artificial diet containing the mycelium of *B. cinerea* exhibited faster development, higher survival rate and fecundity, and better synchronisation of emergence (Mondy and Corio-Costet, 2004). In contrast, negative effects can still be observed from different insect herbivores interacting with *Botrytis*-infected plants. For example, *Rhodobium porosum* fed on the rose plant *Rosa hybrida* infected by *B. cinerea* showed a reduction in growth rate compared with uninfected plants (Desneux et al., 2012). *Aphis fabae* also showed decreases in development rate, survival, and fecundity on infected broad bean *Vicia faba* due to lower nitrogen concentrations in infected leaves (Al-Naemi and Hatcher, 2013).

Plant pathogens not only transmit their effects to herbivores but also on to the herbivores' natural enemies in both negative and positive ways. A recent study using meta-

analysis technique showed consistent indirect negative effects from fungal pathogens, while bacterial pathogens cause indirect positive effects via chewing herbivores on natural enemies of insect herbivores (Srisakrapikoop et al., 2020). Moreover, the powdery mildew fungus *Podosphaera plantaginis* influenced the population dynamics of the parasitoid *Cotesia melitaearum* mediated by the Glanville fritillary butterfly *Melitaea cinxia.* The results from a six-year study indicated that the plant pathogen facilitated the parasitoids' ability to colonise the fragmented landscape by changing the parasitoids' sex ratio to one which was highly female biased, which enhanced the population's intrinsic rate of increase and ability to found new populations (van Nouhuys and Laine, 2008).

### 1.8.2 Effects of plant pathogen-insect interaction on higher trophic levels

There are several studies that show that the effects of endophytic fungi are not restricted to three food chain systems [producer (host plants), primary consumer and secondary consumer], but are also transmitted to higher trophic levels (Omacini et al., 2001; Chaneton and Omacini, 2007). Moreover, Ngah et al. (2018) shows that asymptomatic infection of lettuce by the plant pathogen *Botrytis cinerea* can mediate negative effects through aphid traits including host plant preference, and parasitoids through lower mummification and host preference. Moreover, the effects of this asymptomatic plant pathogen affect the species richness of the aphid's natural enemies in the field.

Combining Omacini et al. (2001), Chaneton and Omacini (2007) and Ngah et al. (2018) together, there remains a question as to whether the effects of a plant pathogen can be mediated through to a higher trophic level which is not a consumptive trophic level.

### 1.8.3 Plant pathogenic fungi-herbivore interactions and beyond

The study of indirect interactions among plants, pathogens and herbivores is termed a tripartite system, where both pathogens and herbivores affect their shared host plant but not directly affecting each other (Hatcher et al., 2004). Prior to the study of tripartite systems, scientists had studied the interactions between plants and plant pathogens or plants and herbivores separately (Hatcher, 1995). Each paired interaction, plant-pathogen, plant-herbivore and plant pathogen-herbivore, has its own direct interaction. With the first two paired interactions it is known that in most cases plants do not benefit from these interactions. In contrast, it generally would be more difficult to consider direct interactions between plant pathogens and herbivores. The interactions between plant pathogens and herbivores can be both positive and negative to one another (Table 1).

**Table 1** The direct effects of plant pathogens on herbivores and vice versa.

| *Direct effect of plant pathogens on herbivores* | | | | | |
|---|---|---|---|---|---|
| Effect | Type of effect | Type of pathogen | Type of herbivore | Detail | Reference |
| Food source | Positive | Fungus | Thrips | As a food source for insects | Yarwood, 1943 |
| Enhance insects' digestion | Positive | Fungus | Cerambycid beetles, siricid woodwasps, stonefly nymphs | Fungal enzymes such as cellulase help insect's digestion | Martin, 1992 |
| Facilitate establishment | Positive | Fungus | Mites | As a food source for gravid female mites | Laemmlen and Hall, 1973 |
| Mycotoxin | Negative | Fungus | Beetles | Cause mortality | Pfliegler et al., 2020 |
| Synergise plant allelochemicals | Negative | Fungus | Butterfly larvae | Inhibit some enzymes that detoxify plant metabolites | Dowd, 1989 |
| *Direct effect of herbivores on plant pathogens* | | | | | |
| Effect | Type of effect | Type of pathogen | Type of herbivore | Detail | Reference |
| Facilitate infection by wound and sugar secretion | Positive | Fungus | Mites, beetles, aphids | Create opening routes for infection and help plant pathogens to survive before penetrating host cells | Laemmlen and Hall, 1973; Bergstrom et al., 1982 |
| Facilitate growth by frass | Positive | Fungus | Butterfly larvae | As a medium for plant pathogens | Christensen and Wilcoxson, 1966 |
| Grazing | Negative | Fungus | Slugs, snails | Decrease fungal population | Ramsell and Paul, 1990 |

Returning to the tripartite study, herbivores have indirect effects on plant pathogens, and the outcome can be positive or negative. A positive indirect effect is generally considered to result from herbivores causing stress to plants or by reducing plant vigour (Russin et al., 1986), and these changes benefit plant pathogens. A negative indirect effect is caused by induced resistance in plants by insect infestation (Hatcher, 1995) which will be discussed later.

Likewise, there are both positive and negative indirect effects of plant pathogen infection on herbivores caused by changes in both plant quality and quantity (Hatcher, 1995). A positive indirect effect can be a result of changes in plant quality, for example increased nitrogen concentration in infected plant tissues caused selective feeding by the slug *Arion ater* (Ramsell and Paul, 1990), and weight increase in adult *Aphis fabae* fed on broad bean leaves infected by *Uromyces viciae-fabae* compared with those reared on uninfected leaves (Pruter and Zebitz, 1991).

In contrast, infection of cotton *Gossypium hirsutum* by *Verticillium dahliae* caused negative indirect effects on spider mite *Tetranychus urticae* populations via both quantity (reduction in leaf quantity) and quality (change in nutrition and induced resistance) (Karban et al., 1987). The chrysomelid beetle *Gastrophyla viridula* received negative effects from feeding on *Rumex* infected by *Uromyces rumicis* throughout its life cycle, ranging from longer development time and higher mortality compared with those on feeding uninfected plants (Hatcher et al., 1994) which is consistent with the reduction in nitrogen content of infected leaves (Hatcher et al., 1995). Recently, *Myzus persicae* feeding on lettuce plants with asymptomatic infection by *Botrytis cinerea* were also shown to suffer from reduced fecundity, off-plant survival time and size (Ngah et al., 2018).

The effects of plant pathogens are not restricted to only transmitting their effects to herbivores, but they can also affect the herbivores' natural enemies in both negative and positive ways. The blight pathogen *Phytophthora infestans* infecting potato plants caused a lower intrinsic rate of population increase and survival probability of the peach-potato aphid *Myzus persicae* compared with those on water inoculated plants. This led to a lower percentage of aphid parasitism by the biocontrol agent parasitoid *Aphidius colemani* on

blight inoculated plants (Lazebnik et al., 2017). In contrast, plant pathogens may benefit natural enemies. The peanut plant *Arachis hypogaea*, infected with the white mould fungus *Sclerotium rolfsii*, were preferred by the beet armyworm moth *Spodoptera exigua* as oviposition sites compared with the healthy plants, and the parasitoid *Cotesia marginiventris* landed more often on infected plants damaged by larval moths than healthy plants damaged by the larvae (Cardoza et al., 2003).

The indirect effects of pathogens can also affect the population level of natural enemies. The powdery mildew fungus *Podosphaera plantaginis* influenced the population dynamics of the parasitoid *Cotesia melitaearum* mediated by changes in the larvae of the Glanville fritillary butterfly *Melitaea cinxia*. The fungus infection caused increased developmental time and may weaken the immunity of the larvae, which is thought to be beneficial to the parasitoids. A positive indirect effect from the plant pathogen facilitated the parasitoids' ability to colonise the fragmented landscape by changing their sex ratio to one which was highly female biased, which enhanced the population's intrinsic rate of increase and ability to found new populations (van Nouhuys and Laine, 2008).

In summary, indirect interactions between plant pathogens and herbivores can occur in both positive and negative directions via changes in plant quantity and quality, and this effect passes beyond primary consumers to their natural enemies. The outcomes also depend on the species involved in the interaction.

### 1.9 Plant induced resistance

Plant resistance can be both passive and active processes (Hammerschmidt, 2014). The former is constitutively expressed, and the latter is expressed when plants are induced by attack or infection. Induced resistance is an active process against herbivores or pathogens

which can be local or systemic after some initial inducing treatment (Hammerschmidt, 2014). Thus, in some cases indirect effects are the result of induced resistance.

The backbone of signals for induced resistance are salicylic acid (SA) which is typically activated by piercing/sucking herbivores and biotrophic pathogens, and jasmonic acid (JA) which is typically activated by chewing herbivores and necrotrophic pathogens (Pieterse et al., 2014; Stout, 2014). However, these two main pathways are not completely independent, they interact each other and lead to different outcomes. This interaction, termed crosstalk, can fine-tune responses to attackers (Reymond and Farmer, 1998). When plants are attacked by single attacker they have to optimise SA and JA pathways in response (Spoel et al., 2003); however, the crosstalk between SA and JA may be neutral (Schenk et al., 2000), synergistic (Mur et al., 2006; van Wees et al., 2000), or antagonistic (Mur et al., 2006) depending on the concentration of SA and JA (Mur et al., 2006), timing (Koornneef et al., 2008) and the sequence of SA and JA produced (Leon-Reyes et al., 2010).

The antagonistic interaction between SA and JA is considered a trade-off in which SA can suppress JA signaling, causing down-regulation of JA responsive genes (Spoel et al., 2003) as well as JA which in turn can also suppress the SA pathway (Brooks et al., 2005). Thus, in nature if plants are first infected by SA-inducing pathogens, plants will be more resistant to the subsequent pathogens which are sensitive to SA. On the other hand, this also suppresses the JA pathway, and makes plants are more susceptible to JA-sensitive pathogens. Vice versa, if plants are first attacked by JA-inducing pathogens, plants will be more susceptible to SA-sensitive pathogens (Leon-Reyes et al., 2010).

Induced resistance can relay negative effects to herbivores' natural enemies. Fewer syrphid fly eggs were found on tomato plants induced with jasmonic acid compared with

control plants, which was correlated with a 17% reduction in aphid abundance. The results also showed that induction hinders the effect of parasitism of aphelinid parasitoids on *Spodoptera exigua* caterpillars (more caterpillars survived in the induction treatment) as well as fewer parasitoids emerging from the induction treatment as a result of the lower mass of caterpillar feeding on induced plants (Thaler, 2002). Likewise, tobacco cutworm *Spodoptera litura* showed longer developmental times and reduced adult body weight when fed on soybean *Glycine max* infested by the soybean cyst root nematode *Heterodera glycines* compared with controls. Induced resistance still transmits its effects to its parasitoid *Meteorus pulchricornis*, which shows an increased developmental time, and reduced hind tibia length and fecundity (Li et al., 2017) suggesting that the infestation of the root nematode can induce plant resistance against the above ground herbivore.

## 1.10 Study systems

In this thesis, I employed two different study systems: a beetle and a plant pathogen system. The beetle system allowed me to examine the direct effect of host quality on a higher trophic level, and at the same time I can inspect maternal effects which I could not do with the latter system. On the other hand, the plant pathogen system allowed me to investigate indirect effects originating from a plant pathogen to higher trophic levels both in the laboratory and field environments.

### 1.10.1 Bruchid beetles (Bean beetles)

#### 1.10.1.1 Economic importance, life cycle and reproduction

Bruchid beetles are currently classified in order Coleoptera, Family Chrysomelidae (leaf beetles) and subfamily Bruchinae; however, in the past these beetles were previously classified in their own Family Bruchidae (Majka and Langor, 2011). There are around 1,400

species of beetles in this subfamily worldwide, but about 30 species are considered to be pests (Pintilioaie et al., 2018). Almost all of them exclusively feed on seeds during their larval development (Pintilioaie et al., 2018) and are most frequently found on the Fabaceae, but the beetles can also be found on Arecaceae (e.g. palm), Convolvulaceae (e.g. morning glory), Malvaceae (e.g. mallow) and about other 30 plant families (Southgate, 1979; Kingsolver, 2002). Several species of *Callosobruchus*, *Acanthoscelides*, *Zabrotes*, *Caryedon* and *Bruchus* are considered to be storage pests (Tuda, 2007).

As mentioned above and from their common name, these beetles are specialised to exploit hosts in the bean family (Fabaceae) which are an important protein source for both humans and livestock. However, recently there was a report that a number of species of bean beetles also can develop on ornamental plants (Yus-Ramos et al., 2014). Therefore, bean beetles are considered as important pests causing huge economic losses; for example, *Callosobruchus maculatus* can cause the yield loss of black gram up to 90% (Soundararajan et al., 2012), though the yield loss depends on species and biotype of the beetles, and the cultivar of the hosts (Mishra et al., 2017).

With most bean beetles, females oviposit their eggs on pods and/or seeds, then the hatched larvae penetrate into the host substrate and develop inside the host until they emerge as adults (Southgate, 1979). These beetles are monophagus or oligophagus within the restricted subtribes or tribes of plant taxa, though some can feed on hosts from different subfamilies (Tuda, 2007). Temperature, relative humidity and host species all affect developmental time of the beetles (Beck and Blumer, 2014). As this group of beetles is relatively diverse, their life histories are also considerably different, for instance, some species of bean beetles pupate inside the hosts with/without a cocoon inside the hosts, while

some pupate outside the hosts, or some species as adults require feeding (e.g. *Bruchus pirosum*) (Southgate, 1979) while some do not require food (e.g. *Callosobruchus maculatus*) to complete their life cycle (Beck and Blumer, 2014).

1.10.1.2 Host location by bruchid beetles

There are three main host location steps after adult bruchid beetles emerge. Take off from the host, landing on the new host for feeding, and host location for oviposition, which is seen with *Acanthoscelides obtectus* (Pouzat, 1981). However, these steps can be different for some species which do not require feeding during adult stage, so I will focus on host location for the bean beetles.

Like many insects, bean beetles require olfactory cues to locate distant hosts and they also need to employ contact chemoreception and tactile receptors to explore the suitability of hosts for oviposition (Pouzat, 1981). The effects of host odours vary with host plant strain and the physiological age of the bean beetles, in which the species with faster ovarian development tend to respond to host odours sooner after emergence. The olfactory receptors (sensilla) are mostly located on antennae (Pouzat, 1981). Evidence from GS-MS analyses suggested that synthetic 2-ethyl hexanol is attractive to the *Callosobruchus maculatus* females (Ajayi et al., 2015). This is an example confirming that female bean beetles utilise plant volatiles to locate their hosts.

Contact chemoreception also plays a part in host recognition to detect chemicals left on the seed coat which can be detected by both taste sensilla and taste receptors on the ovipositor (Pouzat, 1981). *C. maculatus* can distinguish and lay more eggs on glass beads coated with French bean extract compared with untreated glass beads (Gokhale et al., 1990). It is not only plant secondary substances that can be detected by gravid bean beetles; they

also can detect the chemicals left on oviposited beans by other females. This cocktail of chemicals is called a biological conditioning substance (BCS), and females prefer to oviposit on beans with no or less-BCS present (Yamamoto, 1990).

The concept of BCS perceived by contact chemoreception may be confirmed by olfactometer studies showing that with olfactory cues alone the egg-carrying seeds still attracted bean beetles. In contrast, seeds infested by larvae have a repellent effect to females (Ignacimuthu et al., 2000; Babu et al., 2003). Though these studies did not compare egg-carrying seeds and larvae infested seeds, the results imply that the beetles cannot detect BCS by employing olfactory senses.

Tactile receptors are located on the ovipositor and have a crucial role in shape recognition (Pouzat, 1981). In some species (e.g. *Callosobruchus chinensis*), the suitable curvature (physical stimuli) alone is enough to induce the females to oviposit even though the substrate is glass beads, but the presence of plant chemicals (chemical stimuli) on the substrate also gives additive effects to oviposition (Gokhale et al., 1990).

To sum up, bean beetles employ olfactory senses to locate hosts at a distance. Once they reach the hosts, chemoreception and tactile receptors are used in turn to explore the host surface to determine the most suitable hosts. Some species primarily rely on chemical cues while some heavily rely on physical cues, but it seems that employing both cues would contribute to the best result of host location.

### 1.10.2 *Botrytis*

#### 1.10.2.1 Introduction and its importance

*Botrytis* (teleomorph: *Botryotinia*) is a genus of plant pathogenic fungi which belongs to phylum Ascomycota (Williamson et al., 2007). The nomenclature of fungi is quite complicated because they have a teleomorph (sexual reproductive stage) and an anamorph (asexual reproductive stage); therefore, dual nomenclature has arisen from the different reproductive patterns, particularly with the ascomycetes which rarely present the teleomorph stage in laboratories (Guarro et al., 1999).

*Botrytis* is an important fungal pathogen which has a wide distribution, ranging from tropical to cold areas, and causes damage to plants at both pre- and post-harvest periods in various types of plants including vegetables, orchard products, ornamental plants, stored and transported products (Elad et al., 2004). Some species such as *B. cinerea* have more than 200 host plant species (Williamson et al., 2007), while some species have high host specificity, especially in monocots (Elad et al., 2004). Normally, *Botrytis* is a necrotrophic pathogen causing blossom blights, fruit rots, leaf spots and bulb rots (Staats et al., 2005). However, *Botrytis* sometimes acts as an systematic endophyte and causes host plants (lettuce) not to show symptoms (Sowley et al., 2010). This latent infection will not show any symptoms until the plants reach senescence, for example, in grape (Keller et al., 2003). Thus, latent infection results in ripe fruit damage.

#### 1.10.2.2 Conidia and factors influencing infection and damage

Conidium (plural: conidia), an asexual spore of fungus, is viewed as the most crucial dispersion propagule for *Botrytis* species and spores can be dispersed by wind, rain and insects. However, conidia are not tolerant in the field and their viability depends on microbial

activity, moisture, temperature and sunlight exposure (Holz et al., 2004), of which UV light is the most important environmental factor determining conidia survival (Rotem and Aust, 1991). Once the conidia reach plant surfaces there are two factors determining the aggressiveness of *Botrytis*. First, nutrient supplements, when conidia are inoculated with water they cause no symptoms in many plants, for example fruits of plum and nectarine (Fourie and Holz, 1998), while adding glucose with $KH_2PO_4$ or Na-ATP into the inoculation causes lesions (van den Heuvel and Waterreus, 1983). Second, the density of conidia, as at low density the conidia might not release phytotoxic metabolites and proteins to trigger plant cell death (Shaw et al., 2016).

#### 1.10.2.3 Infection routes into host plants

Once *Botrytis* is present on plant surfaces, there are three routes to infect host plants (Holz et al., 2004).

1. Penetration through specialised host structures: Most of these structures are related to flowering organs, for example, infection of the pistil through stigma (McNicol and Williamson, 1989), stamens through filaments (de Kock and Holz, 1992), pedicel (Holz et al., 2003) and receptacle area (Keller et al., 2003). Besides flowering organs, *Botrytis* is capable of infecting the fluid-secreting glands of chickpea, *Cicer arietinum*, which secrete exudates to defend the plant against insects (Holz et al., 2004).

2. Penetration through undamaged host tissues and natural openings: *Botrytis* conidia can infect into plant leaves via stomatal openings, the epidermis near guard cells and epidermal cells on the abaxial surface (Hsieh et al., 2001), and through micro-fissures and lenticels (Holz et al., 2004).

3. Penetration through wounds: Many studies show that fresh wounds inoculated with *Botrytis* cause infection (Holz et al., 2004).

#### 1.10.2.4 Control of *Botrytis*

To control *Botrytis*, approaches include the avoidance of damage due to saprophytic infections, environmental modification, using host resistance, fungicides (Shaw et al., 2016) and microbial agents (Elad and Stewart, 2004). Although there are many practical ways to manage *Botrytis*, chemical control is still the major method applied to use in economic crops against this fungus (Leroux, 2004). There are five mechanisms of botryticides: fungicides affecting fungal respiration, anti-microtubule toxicants, compounds affecting osmoregulation, fungicidal toxicity reversed by amino acids and sterol biosynthesis inhibitors. However, multi-drug resistance occurs in *Botrytis cinerea* associated with the change in functions of transporter membrane proteins (Leroux, 2004).

### 1.10.3 Aphids

#### 1.10.3.1 Economic importance

Aphids are phloem-feeders and considered as a group of insect pests, causing considerable yield losses in many crop plants. These losses will be greater if the damage caused by viruses transmitted by aphids are accounted for (van Emden, 2013). As phloem-feeders, this causes a decrease in plant reserve for plant development, and aphids' saliva increases host respiration rate which in turn accelerates the plant reserve (carbohydrate) catabolism (van Emden, 2013). Furthermore, the honeydew which aphids excrete can lead to fungal infection (usually *Cladosporium*), and a reduction in photosynthesis because honeydew on leaves obscures sunlight (van Emden, 2013). Aphids also cause a reduction of

plant roots, and when considered with plasmolysis of plants by sap-sucking aphids, there is no doubt that is why intensely infested plants are usually wilted (van Emden, 2013).

Aphids are known as vectors of plant viruses (van Emden, 2013). There are two types of viruses carried by aphids, stylet-borne and circulative. Circulative viruses need to complete their cycle inside the host body and then are accumulated in salivary glands before transmission, whereas stylet-borne viruses are found only at the stylet and do not pass through inside the vector's body (van Emden, 2013). Thus, the latter type of viruses has short infective time on aphids and do not appear after aphids moult (van Emden, 2013). Some aphid species such as the black bean aphid *Aphis fabae* can be a vector for more than 30 plant viruses on various host plants (Blackman and Eastop, 2000).

1.10.3.2 Life cycle and reproduction

Aphids are hemimetabolous insects classified in the Family Aphididae. The most intriguing aspect of aphids is their reproduction. Female aphids can produce their progeny directly without fertilisation in a system which we call parthenogenesis, and most aphid species are viviparous (Davis, 2012). All progeny are genetically identical to their mother, and together form a clonal lineage, or clone (Davis, 2012). However, aphids do not rely on only asexual reproduction (parthenogenesis) but also on sexual reproduction. This is called cyclical parthenogenesis, which means the seasonal alteration in reproduction modes between parthenogenesis and sexual reproduction (Davis, 2012). The life cycle of aphids begins with emergence in the spring from diapausing, frost-resistant overwintering eggs. Then, female aphids establish their colonies by producing progeny via parthenogenesis until the autumn, when the asexual females produce sexual males and females to mate. After that, the sexual females lay diapausing, frost-resisting eggs before winter (Dixon, 1985).

#### 1.10.3.3 Factors influencing cyclical parthenogenesis

There are several factors influencing the alternation between asexual and sexual reproduction in aphids. Photoperiod is a determining factor for asexual mother aphids to produce either asexual or sexual progeny during embryogenesis (Davis, 2012). During the short night period, an asexual-promoting signal from the mother's brain results in the asexual cycle, while during the long night period the signal is hindered, resulting in sexual reproduction (Davis, 2012). For some species of aphids living underground, photoperiod does not seem to affect cyclical parthenogenesis. However, these aphids may use cues from host plants to synchronise their reproduction with the growth and development of their host plants (Dixon, 1985). This phenomenon which organisms change phenotype (from the same genotype) in response to particular environmental factors is called polyphenism (Simpson et al., 2011).

#### 1.10.3.4 Advantages and disadvantages of sex and asexual reproduction

The advantages of asexual reproduction for aphids are the quick increase of aphid numbers in a short time, allowing them to exploit ephemeral habitats (Dixon, 1985) with less cost compared with sexual reproduction. However, why do aphids still maintain sexual reproduction which is costlier? This because sexual reproduction allows aphids to reproduce cold-resistant diapausing eggs to survive the harsh winter (Simon et al., 2002). Moreover, sex can purge mutation accumulation during parthenogenesis, increase genetic diversity, enhance ability to escape parasites and reduce intraspecific competition from increased success of dispersal (Simon et al., 2002).

1.10.3.5 Polymorphism and factors determining polymorphism

Polymorphism is a phenomenon found in many aphid species. They can be in winged (alate) or unwinged (apterous) morphs. This phenomenon shows the greatest development during host alternation when the aphids are induced by many factors to reproduce winged-form progeny (Dixon, 1985). These factors are:

1. Crowding: Apterous female vetch aphids *Megoura viciae* which experienced crowding in limited space gave birth of alate aphids even though these aphids then were reared individually while individual apterous females confined in equal space size gave birth to apterous aphids. This showed that crowding response results from maternal mediation (Lees, 1967).

2. Host quality: 24 hours old *Dysaphis devecta* aphid nymphs were reared on control plants and nitrogen deprived plants for 2-8 weeks, and the results showed that the mature aphids in alate morph were found on the nitrogen deprived plants. This showed the post-natal stimulus which influences morph determination in this aphid (Forrest, 1970).

3. Day-length: The maturation of the green spruce aphid *Elatobium abietinum* into alate morphs, coincided with the emergence of new spruce leaves, and the dominant factor influencing in alate production morph is day-length (Dixon, 1985).

4. Natural enemies: When a large aphid colony develops on a primary host plant, there are some alate aphids which can escape from natural enemies. These winged aphids then move to secondary host plants where the new colonies have time to establish

without quick disturbance from the natural enemies (Dixon, 1985; Hazell et al., 2005).

1.10.3.6 *Aphis fabae*

Black bean aphid *Aphis fabae* (Scopoli, 1763) are insects in the Order Hemiptera, Family Aphididae. The alate form is 1.3 – 2.6 mm in size, while the apterous form is 1.5 – 3.1 mm in size (Blackman and Eastop, 2000). The body is an oval shape with black colouration and individuals in old colonies have apparent white wax patches on the abdomen. Antennae are very much shorter than the body, while the tapered-black siphunculi are conspicuously longer than the bluntly finger-shaped cauda on the edge of the anus (Alford, 1999).

This aphid's life cycle begins with the hatching of an overwintering egg on spindle tree (*Euonymus europaeus*), which is its primary host (Figure 1). In spring, the parthenogenetic female aphid hatching from overwinter egg is called a fundatrix, and it gives birth to parthenogenetic females on the primary host called fundatrigenia. Fundatrigenia have an alate form to disperse to secondary host plants in the summer. Virginoparae are the parthenogenetic females on the secondary host plant and they give birth to males (in autumn) and parthenogenetic females. When the autumn comes, the parthenogenetic female bears the female sexual morph (gynopara), which mates with males to produce overwintering eggs (van Emden, 2013).

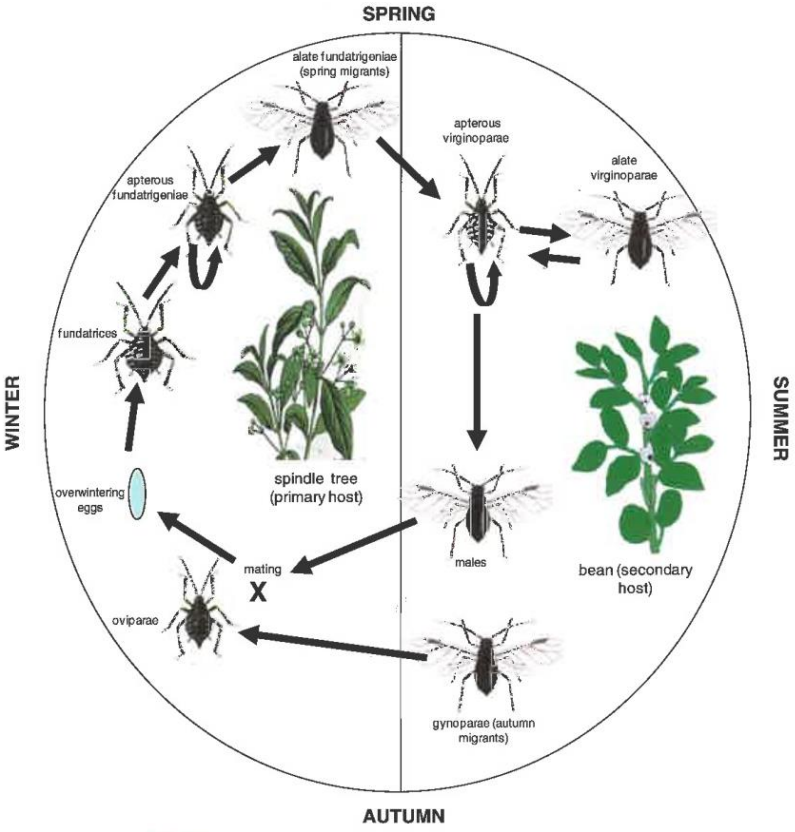


**Figure 1** The life cycle of black bean aphid *Aphis fabae* on its primary and secondary host plants (figure from van Emden, 2013).

The black bean aphid is very polyphagous, feeding on many economically important crops, especially broad bean, *Vicia faba*. It is considered as a vector of more than 30 viruses in many plants such as pea, bean, beet, potato, tomato, tobacco, tulip, cucurbit and crucifer. It is an important pest, not only as a vector for viruses with a wide host range but also has a wide distribution from the temperate zone in the northern hemisphere to America and Africa (Blackman and Eastop, 2000). Moreover, this aphid species is often tended by ants (Alford, 1999).

1.10.3.7 *Acyrthosiphon pisum*

The pea aphid *Acyrthosiphon pisum* (Harris, 1776) is an insect in the Order Hemiptera, Family Aphididae. The pea aphid is considerably large in size (alate form is 2.3 – 4.3 mm, apterous form is 2.5 – 4.4 mm) with green or pink and slender appendages (Blackman and Eastop, 2000). The colour polymorphism (green or pink) is determined by

genetic factors (Caillaud and Losey, 2010), especially the *tor* gene encoding carotene dehydrogenase which is significantly up-regulated in the red morph (Zhang et al., 2018), but a study suggested a facultative endosymbiont of the genus *Rickettsiella* influences body colour of pea aphid (Tsuchida et al., 2010).

Pea aphids feed mostly on plants in the Family Fabaceae and can be a vector of more than 30 virus diseases. Pea aphids in temperate regions can be holocyclic (parthenogenesis with sexual reproduction in autumn), but in warmer regions it can be anholocyclic (entirely asexual reproducing by parthenogenesis) (Blackman and Eastop, 2000).

### 1.11 Research questions and objectives

In this thesis, I investigate how the effects of variation in host plant quality relay through higher trophic levels through behavioural and fitness effects. *Callosobruchus* spp. are a serious pest of storage crops; however, the effects of different host quality and maternal effects on offspring performances have not been well addressed. This provides an excellent model system for examining direct effects of variation in host plant quality, which results from differences between host plant species. Plant pathogens are also ubiquitous in both natural and agricultural environments, but their effects on higher tropic levels in insect community structure has received relatively little attention. The presence of plant pathogens allows us to vary host plant quality within a host species.

I used two models to explore my questions, and where possible I used two herbivore species to examine how consistent results were across species. In the first model I used bean beetles *Callosobruchus maculatus* and *C. analis* to explore how host (bean seed) quality influences oviposition preference, offspring performance and reproductive cost of the beetles (Chapters 2 and 3). This investigated the bottom-up direct effects originating from the plant

itself through a higher trophic level (insect herbivore). The second model used symptomatic infection by the plant pathogenic fungus *Botrytis cinerea* on broad bean *Vicia faba* to investigate bottom-up indirect effects originating from the pathogen through black bean aphid *Aphis fabae* and pea aphid *Acyrthosiphon pisum* life histories in the laboratory (Chapter 4), and through to higher trophic levels in the field (Chapter 5). Finally, using meta-analysis, I examine if the type of plant pathogen infecting plants affects interactions at higher tropic levels (Chapter 6).

# Chapter 2 Differing effects of parental and natal host on the preference and performance of the stored product pests *Callosobruchus maculatus* and *C. analis*

## 2.1 Introduction

*Callosobruchus* Pic 1902 (Coleoptera: Chrysomelidae) is one of the most important genera of stored product pests, causing serious damage and economic loss to a wide range of legumes and non-leguminous crops (Tuda et al., 2005). There are approximately 20 species in this genus (Tuda et al., 2006). Pest species include the cosmopolitan cowpea weevil *C. maculatus* (Fabricius, 1775), and *C. analis* (Fabricius, 1781), which is widespread across the tropics and subtropics (Beck and Blumer, 2014).

Both species lay eggs on the surface of a bean; after the eggs hatch the larvae bore into the bean where they develop and pupate (Giga and Smith, 1991). The beetles develop and spend their entire larval and pupal life inside an individual bean seed (Giga and Smith, 1991; Tuda et al., 2005). The adult beetles emerge from the host and require no food or water to complete their life cycle (although providing nutrients can influence longevity and fecundity; Moller et al., 1989), so adult traits are influenced by the natal host (Beck and Blumer, 2014). Seed resources are limited, and therefore host species (and hence female choice) affects offspring traits, such as development time, larval mortality, emergence mass, size (e.g. pronotum width or elytral length), rate of adult emergence, adult longevity, and fecundity (Timms, 1998; Paukku and Kotiaho, 2008; Mainali et al., 2015; Hosamani et al., 2018; Messina et al., 2018).

Oviposition choice can be influenced by olfactory (Ajayi et al., 2015) and chemical cues emanating from hosts (volatile and surface chemicals) (Giga and Smith, 1985; Gokhale

et al., 1990), the curvature of host seeds (Gokhale et al., 1990), seed size (Cope and Fox, 2003), vibration from larvae already inside the seeds (Guedes and Yack, 2016) and chemical markers deposited by other females (Giga and Smith, 1985; Yamamoto, 1990). Bean beetles are responsive to these cues, affecting host preference (Messina, 2004; Messina et al., 2018). Most studies show that females preferred their natal host when given a choice (Messina and Slade, 1997; Boeke et al., 2004; Paukku and Kotiaho, 2008; Rova and Björklund, 2011; Bergeron et al., 2019), suggesting a learned response, but some studies did not find a preference (Mainali et al., 2015; Bergeron et al., 2019). Many studies have demonstrated the capability of herbivorous insects to learn during oviposition (Jones and Agrawal, 2017) although the presence of such a learned response in *Callosobruchus* is not clear.

While studies generally focus on female fitness traits (typically measured as fecundity), little is known as to how host quality may affect male traits. Most bean beetle species show sexual size dimorphism, with females being larger than males (Guntrip et al., 1997; Savalli and Fox, 1999). In terms of male mate choice, male *C. maculatus* show no preference between females differing in size (Holme, 2019; Kirschke et al., 2019), but when two males compete for mating, larger males have an advantage (Savalli and Fox, 1998). However, it is still not clear if natal host, by affecting the traits of emerging adults, also affects mate choice in *Callosobruchus*.

Studies have shown the existence of transgenerational effects in some insect taxa. These effects can affect offspring performance (Mbande et al., 2020; Tougeron et al., 2020), change offspring predator avoidance behaviours (Keiser and Mondor, 2013), and alter resistance to pathogens and pesticides (Brevik et al., 2018; Schulz et al., 2019). For herbivorous insects transgenerational effects can be induced by variation in host plant quality

(Mousseau and Fox, 1998). Although transgenerational effects are transmitted from the parental generation, where the environment experienced by the mother can influence offspring life histories, the environment is usually dynamic and the environment for the parental generation can change and consequently differ for the offspring generation. Therefore, the environment directly experienced by the offspring may be more important in determining their fitness.

*Callosobruchus maculatus* and *C. analis* both attack a range of legume hosts, and show notable differences in competitive behaviour, in particular showing scramble (*C. maculatus*) and contest (*C. analis*) forms of competition (Giga and Smith, 1983), where with the former multiple adults emerge from a bean (but each is smaller) and with the latter form only one adult emerges from a bean (Toquenaga and Fujii, 1991). This difference in behaviour may have profound effects on weevil life histories, causing different species to react differently on the same host.

In this laboratory study, and using these two economically important stored product pests, I ask: 1) if host preference shows evidence of transgenerational or maternal effects (parental host effects), and if these can be modified by experience (learning; natal host effects); 2) if performance is influenced by parental (transgenerational effects) and/or natal (current environment) hosts; and 3) if these effects are consistent across pest species with differing life histories.

## 2.2 Materials and methods

*Callosobruchus maculatus* and *C. analis* were cultured separately on either mung beans or lentils in a culture room at 28 ± 2°C with 40% relative humidity and constant light (termed culture room). This produced four cultures of the beetle; *C. maculatus* reared on

mung (CmM), *C. maculatus* reared on lentil (CmL), *C. analis* reared on mung (CaM) and *C. analis* reared on lentil (CaL). CmM, CmL, CaM and CaL have been cultured on their hosts since 2010, 2016, 2011 and 2013, respectively. Twenty mated females of each species from each culture were placed together in a 90 mm Petri dish containing a single layer of the same bean from which they had emerged. They were left for two hours to lay eggs in the culture room to produce beans harbouring a single egg. Each mung bean and lentil harbouring a single egg was then selected and stored in a 1 ml perforated Eppendorf tube before the beetles hatched. All subsequent experiments used only the beetles emerging from the beans harbouring a single egg to exclude the effect of larval competition.

### 2.2.1 Oviposition experience experiments

After the beetles emerged from the seeds, any remaining seeds were discarded. Each beetle was sexed and a single male and female from the same culture were then placed in a 1 ml perforated Eppendorf tube for 48 hours to mate before experiments. All the following experiments were conducted in a controlled environment (CE) room at 28°C with 60% relative humidity.

#### 2.2.1.1 No choice experiment (first oviposition, naïve)

A single mated female was put into a 90 mm Petri dish containing 50 evenly dispersed seeds of either mung bean or lentil (termed the focal hosts). This was replicated with 32 females from each of the four cultures for both of the focal hosts, producing eight treatments in total. The beetles were left in the dark (mimicking storage conditions) to oviposit eggs for six hours (a time frame found to be suitable through personal observation). The number of eggs laid on each bean was then counted to determine host acceptance rate, and emergence rate was recorded three weeks after oviposition to allow all adults to emerge. This experiment

also created an oviposition experience for the females on the given focal host (i.e., same or different to their natal host). Each female was kept individually in a 1 ml perforated Eppendorf in the CE room before being used in the choice experiment 24 hours later.

2.2.1.2 Choice experiment (second oviposition, experienced)

Each female used in the no choice experiment was put into individual 90 mm Petri dishes containing 100 evenly dispersed seeds (50 mung beans and 50 lentils; focal host) and allowed to oviposit for a further six hours in the dark. The number of eggs laid on each bean was then counted. Host acceptance rate and emergence rate were recorded as previously described.

2.2.1.3 Oviposition preference: quality or quantity?

Previous studies showed that *C. maculatus* oviposits more eggs on larger seeds (Cope and Fox, 2003; Yang et al., 2006; Paukku and Kotiaho, 2008); therefore, we performed a second choice experiment with 30 different mated females from each of the four cultures, under the same conditions as previously described. This time the females were offered fifteen small seed mung beans and fifteen lentils and allowed to oviposit for two hours. The number of eggs laid on each host were counted. Thirty randomly selected seeds of lentil and small seed mung bean were measured under a high-performance stereomicroscope (Leica MZ9.5), and a significant difference in size between lentil [5.18 ± 0.06 mm (n = 30)] and small seed mung bean [4.38 ± 0.06 mm (n = 30)] was confirmed (Wilcoxon rank sum test: W = 879.5, $n_1$ = 30, $n_2$ = 30, $P < 0.001$). Note that the size of mung bean used in other experiments is 5.56 ± 0.07 mm (n = 30).

2.2.2 Development and survival time

Each bean from the no choice experiment harbouring a single egg (eight groups [CmLL, CmLM, CmML, CmMM, CaLL, CaLM, CaML and CaMM. The first two letters represent species where Cm = *C. maculatus* and Ca = *C. analis*. The third and fourth letters represent parental host and natal host, respectively. L = lentil, M = mung bean.]) was transferred into a 1 ml perforated Eppendorf tube with a unique code. These beans were kept in the CE room and were checked once a day, starting three weeks after oviposition, to record emergence date from which development time was calculated. The emerged beetles were kept in the 1 ml perforated Eppendorf tubes without resources and moved into a laboratory at room temperature where they were checked every twelve hours until death, to calculate adult survival time.

2.2.3 Performance

2.2.3.1 Size and sex of offspring from no choice experiment

The dead beetles from the survival experiment were sexed and the pronotum width and right elytron length were measured under a high-performance stereomicroscope (Leica MZ9.5).

2.2.3.2 Male-male competition

Two 24-hour old virgin males of the same species but emerging from different hosts (e.g., CmM vs CmL and CaM vs CaL), were placed in a 1 ml perforated Eppendorf tube at room temperature which contained a single 24-hour old virgin female that emerged from either a mung bean or lentil. Males were marked with a permanent marker to distinguish host origin. The two males were put into the Eppendorf tube at the same time to reduce bias. The

beetles were continually observed until a successful mating occurred. The host origin of the successful male was recorded.

2.2.3.3 Copulation time and male fitness

Twenty-four-hour old bean beetles were used in this experiment. A virgin female from each of the four cultures was mated with a virgin male that had emerged from the same or a different host (8 groups in total) in a 1 ml perforated Eppendorf tube at room temperature. Copulation time is defined here as the time since the male beetle started palpating the female until the time they separated. After copulation, each female was put in a 90 mm Petri dish containing a single layer of the same host bean that she had emerged from and was allowed to oviposit in the dark CE room at 28°C 60% relative humidity until she died. Forty-five days after the copulation, the Petri dishes were put in a freezer to prevent a subsequent generation emerging after the first-generation offspring of that original female. The number of bean beetles (less one; the original female) in each Petri dish was counted.

2.2.4 Data analyses

2.2.4.1 Oviposition experience, development and survival time

All analyses were performed using R 4.0.4 (R Core Team, 2021). Host acceptance rate and mean number of eggs oviposited on each host were calculated for no-choice and choice experiments. Emergence rate obtained from beans in no-choice experiment was calculated as well as sex ratio which was analysed using *G*-tests with expected 1:1 sex ratio.

The number of eggs oviposited in the no-choice experiment for each species was modelled with zero-inflated negative-binomial model (ZINB) using *pscl* package (Zeileis et al., 2008) with natal and focal hosts as independent variables. Models were generated from a global model from dredge function in *MuMIn* package (Barton, 2019). Development and

survival time were modelled with accelerated failure time models (AFT) using *survival* package (Therneau, 2021) with parental host, natal host, sex and right elytron length as independent variables. Models were selected with stepwise method using *MASS* package (Venables and Ripley, 2002) and validated by deviance residuals analysis (Achilonu et al., 2019). All models were selected based on Akaike's Information Criteria (AIC) (Burnham and Anderson, 2003). Collinearity was not detected as VIF values ranged from 1.00 to 2.28.

2.2.4.2 Performance

Three-way ANOVA was performed to examine the effect of parental host, natal host and sex on pronotum width and right elytron length of the offspring from each species. Copulation time and offspring number (male fitness) of each species were compared between host origin (from lentil or mung bean) of females and males using two-way ANOVA and the Scheirer-Ray-Hare test (non-parametric two-way ANOVA) from the *rcompanion* package (Mangiafico, 2021), respectively. Mating times for *C. maculatus* and *C. analis* were $\log_{10}$ and square root transformed respectively to meet normality assumptions. Competition between males from different natal hosts was tested using a *G*-test with expected mating success taken as equal. I hypothesised that size may attribute to mating success; therefore, I extracted the size data from the no choice experiment (only individuals that came from the same bean type for both parental and natal hosts (no host switching e.g. CmLL, CmMM, CaLL and CaMM)). Then a *t*-test was performed to quantify the differences in size between males and females of each species from lentil and mung bean in the male-male competition experiment.

## 2.3 Results

### 2.3.1 Oviposition experience experiment

In the no choice experiment, both bean beetles species more readily accepted and oviposited more eggs on mung bean regardless of natal host (Table 2). My results showed that the prior oviposition experience (no choice experiment) did not influence subsequent oviposition preference (choice experiment) in both bean beetles species as the percentage of host acceptance and numbers of oviposited eggs were higher on mung bean regardless of experience (Table 3). Both bean beetle species oviposited more eggs on mung bean as a focal host [*C. maculatus*: lentil 6.73 ± 1.06 (n = 60), mung 22.40 ± 1.10 (n = 54); *C. analis*: lentil 2.78 ± 0.66 (n = 58), mung 24.00 ± 1.36 (n = 56)], but bean beetles emerging from lentils laid significantly more eggs than those emerging from mung bean (*C. maculatus*: lentil 17.4 ± 1.45 (n = 60), mung 10.5 ± 1.40 (n = 54); *C. analis*: lentil 17 ± 1.91 (n = 59), mung 9.13 ± 1.39 (n = 55); Table 4).

**Table 2** Percentage and mean ± SE number of eggs oviposited on focal hosts (no choice experiment) grouped by natal host of the bean beetles. Number of ovipositing individuals is given in parentheses and N is the sample size.

| Species and natal host | Focal host | N | % Acceptance | Mean ± SE no. egg |
|---|---|---|---|---|
| *C. maculatus* (Lentil) | Lentil | 31 | 87.10 (27) | 10.89 ± 1.62 |
| | Mung | 29 | 100 (29) | 25.90 ± 1.20 |
| *C. maculatus* (Mung) | Lentil | 29 | 51.72 (15) | 7.33 ± 2.00 |
| | Mung | 25 | 100 (25) | 18.32 ± 1.58 |
| *C. analis* (Lentil) | Lentil | 30 | 53.33 (16) | 8.50 ± 1.54 |
| | Mung | 29 | 100 (29) | 29.90 ± 1.53 |
| *C. analis* (Mung) | Lentil | 28 | 21.43 (6) | 4.17 ± 1.45 |
| | Mung | 27 | 96.30 (26) | 18.35 ± 1.46 |

**Table 3** Percentage host acceptance and mean ± SE number of eggs oviposited by females in the choice experiment. Number of individuals that oviposited eggs is given in parentheses and N is the sample size.

| Species and natal host | Experience | N | Focal host | % Acceptance | Mean ± SE no. egg |
|---|---|---|---|---|---|
| *C. maculatus* (Lentil) | Lentil | 31 | Lentil | 32.26 (10) | 0.45 ± 0.13 |
| | | | Mung | 100 (31) | 19.65 ± 1.77 |
| | Mung | 29 | Lentil | 6.90 (2) | 0.07 ± 0.05 |
| | | | Mung | 100 (29) | 12.24 ± 0.74 |
| *C. maculatus* (Mung) | Lentil | 29 | Lentil | 10.35 (3) | 0.10 ± 0.06 |
| | | | Mung | 100 (29) | 22.69 ± 1.54 |
| | Mung | 25 | Lentil | 8.00 (2) | 0.12 ± 0.09 |
| | | | Mung | 100 (25) | 12.88 ± 0.75 |
| *C. analis* (Lentil) | Lentil | 30 | Lentil | 3.33 (1) | 0.10 ± 0.10 |
| | | | Mung | 100 (30) | 32.27 ± 1.32 |
| | Mung | 29 | Lentil | 0 (0) | 0 |
| | | | Mung | 100 (29) | 16.34 ± 1.12 |
| *C. analis* (Mung) | Lentil | 28 | Lentil | 7.14 (2) | 0.07 ± 0.05 |
| | | | Mung | 100 (28) | 23.68 ± 1.77 |
| | Mung | 27 | Lentil | 3.70 (1) | 0.04 ± 0.04 |
| | | | Mung | 100 (27) | 18.11 ± 1.00 |

**Table 4** Summary of coefficients and model selection based on AIC for models predicting number of eggs oviposited by bean beetles in no choice experiment with zero-inflated negative-binomial model (ZINB). Only models with the lowest AIC are shown.

| Species | Dependent variable | Independent variable | Coefficient value ± SE | *P*-value |
|---|---|---|---|---|
| *C. maculatus* | Egg number | Intercept | 2.37 ± 0.105 | <0.001 |
| | | Focal host (Mung) | 0.90 ± 0.125 | <0.001 |
| | | Natal host (Mung) | -0.37 ± 0.125 | 0.003 |
| *C. analis* | Egg number | Intercept | 2.09 ± 0.111 | <0.001 |
| | | Focal host (Mung) | 1.32 ± 0.125 | <0.001 |
| | | Natal host (Mung) | -0.52 ± 0.102 | <0.001 |

Emergence rate was high for all treatments (>88% emergence) regardless of parental or natal host, except for CmM (from mung), which performed very poorly on lentil with only 16% successfully emerging (Table 5). The sex ratio did not differ between cultures, except for CaL and CaM on lentil, which showed significant female (67%) and male (66%) bias, respectively (Table 6). In addition, when the beetles were given choices between small seed mung bean and lentil, both beetle species still preferred to oviposit on mung bean, suggesting that host species (quality), rather than host size (quantity), mattered (Table 7).

**Table 5** The emergence rate of *C. maculatus* and *C. analis* offspring from parental and natal hosts (no choice experiment). Number of emerged and un-emerged individuals is given in parentheses and N is the sample size.

| Parental host | Natal host | N | % Emerged | % Un-emerged |
|---|---|---|---|---|
| *C. maculatus* (Lentil) | Lentil | 63 | 92.06 (58) | 7.94 (5) |
| | Mung | 62 | 90.32 (56) | 9.68 (6) |
| *C. maculatus* (Mung) | Lentil | 58 | 15.52 (9) | 84.48 (49) |
| | Mung | 60 | 100 (60) | 0 (0) |
| *C. analis* (Lentil) | Lentil | 64 | 95.31 (61) | 4.69 (3) |
| | Mung | 62 | 96.77 (60) | 3.23 (2) |
| *C. analis* (Mung) | Lentil | 46 | 93.48 (43) | 6.52 (3) |
| | Mung | 60 | 88.33 (53) | 11.67 (7) |

**Table 6** Percentage of emerged female and male *C. maculatus* and *C. analis* offspring from parental and natal hosts (no choice experiment). Number of female and male individuals is given in parentheses and N is the sample size. *G* test for deviation from 1:1 sex ratio. Significant results are represented in bold.

| Parental host | Natal host | N | Emerged | | G value | *P*-value |
|---|---|---|---|---|---|---|
| | | | % Female | % Male | | |
| *C. maculatus* (Lentil) | Lentil | 57 | 47.37 (27) | 52.63 (30) | 0.32 | 0.574 |
| | Mung | 56 | 42.86 (24) | 57.14 (32) | 2.31 | 0.126 |
| *C. maculatus* (Mung) | Lentil | 9 | 44.44 (4) | 55.56 (5) | 0.22 | 0.636 |
| | Mung | 60 | 41.67 (25) | 58.33 (35) | 3.38 | 0.066 |
| *C. analis* (Lentil) | **Lentil** | **61** | **67.21 (41)** | **32.79 (20)** | **15.39** | **<0.001** |
| | Mung | 60 | 43.33 (26) | 56.67 (34) | 2.15 | 0.142 |
| *C. analis* (Mung) | **Lentil** | **41** | **34.15 (14)** | **65.85 (27)** | **8.69** | **0.003** |
| | Mung | 53 | 47.17 (25) | 52.83 (28) | 0.34 | 0.560 |

**Table 7** Percentage and mean ± SE of eggs oviposited on focal hosts (between small seed mung bean and lentil) in relation to female natal host. N = 20 for each natal and focal host combination.

| Natal host | Focal host | % Acceptance | Mean ± SE no. egg |
|---|---|---|---|
| *C. maculatus* (Lentil) | Lentil | 45 | 1.25 ± 0.40 |
| | Mung | 100 | 15.60 ± 1.11 |
| *C. maculatus* (Mung) | Lentil | 30 | 0.55 ± 0.23 |
| | Mung | 100 | 7.95 ± 0.76 |
| *C. analis* (Lentil) | Lentil | 60 | 1.50 ± 0.39 |
| | Mung | 100 | 12.00 ± 0.93 |
| *C. analis* (Mung) | Lentil | 15 | 0.15 ± 0.08 |
| | Mung | 100 | 6.60 ± 0.90 |

2.3.2 Performance

Overall, the effect of parental host, natal host and sex was significant for both pronotum width and right elytron length of both species (Table 8). Generally, females were larger than males for both species (Figure 2), and the offspring were large when parental host was lentil or natal host was mung bean for *C. analis* (Figure 3). A significant two-way interaction between parental and natal hosts was detected only on *C. maculatus* (Table 8) where the beetles were larger from mung bean as a natal host, but the performance was very poor when beetles came from mung bean as a parental host and lentil as a natal host (Figure 4).

**Table 8** The effect of parental host, natal host and sex on pronotum width and right elytra length of *C. maculatus* and *C. analis* offspring analysed using three-way ANOVA. Only significant terms are reported.

| | Source | F | *P*-value |
|---|---|---|---|
| *C. maculatus* | | | |
| Pronotum width | Parental host | $F_{1,170} = 57.53$ | <0.001 |
| | Natal host | $F_{1,170} = 35.86$ | <0.001 |
| | Sex | $F_{1,170} = 31.34$ | <0.001 |
| | Parental host: Natal host | $F_{1,170} = 32.68$ | <0.001 |
| Right elytron length | Parental host | $F_{1,168} = 87.75$ | <0.001 |
| | Natal host | $F_{1,168} = 67.52$ | <0.001 |
| | Sex | $F_{1,168} = 41.97$ | <0.001 |
| | Parental host: Natal host | $F_{1,168} = 58.73$ | <0.001 |
| *C. analis* | | | |
| Pronotum width | Parental host | $F_{1,202} = 100.85$ | <0.001 |
| | Natal host | $F_{1,202} = 9.80$ | 0.002 |
| | Sex | $F_{1,202} = 157.52$ | <0.001 |
| Right elytron length | Parental host | $F_{1,205} = 92.41$ | <0.001 |
| | Natal host | $F_{1,205} = 18.16$ | <0.001 |
| | Sex | $F_{1,205} = 219.80$ | <0.001 |

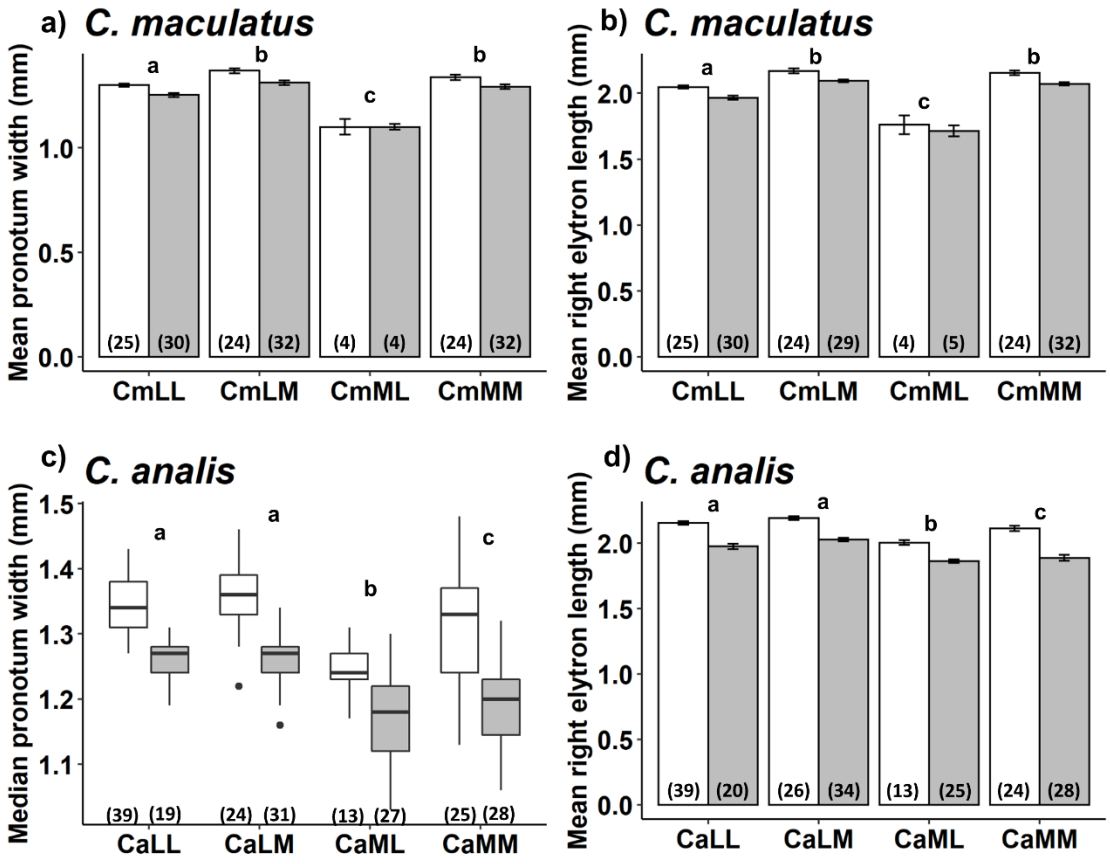


**Figure 2** Mean ± SE of *C. maculatus* (a) pronotum width and (b) right elytron length. Median and interquartile range of *C. analis* (c) pronotum width and mean ± SE of *C. analis* (d) right elytron length. The white bars represent female, and the grey bars represent male. The letters above bar represent significant difference between groups. The first two letter represents species where Cm = *C. maculatus* and Ca = *C. analis*. The third and fourth letters represent parental host and natal host, respectively. L = Lentil, M = Mung bean. The number of replicates is given in parentheses.

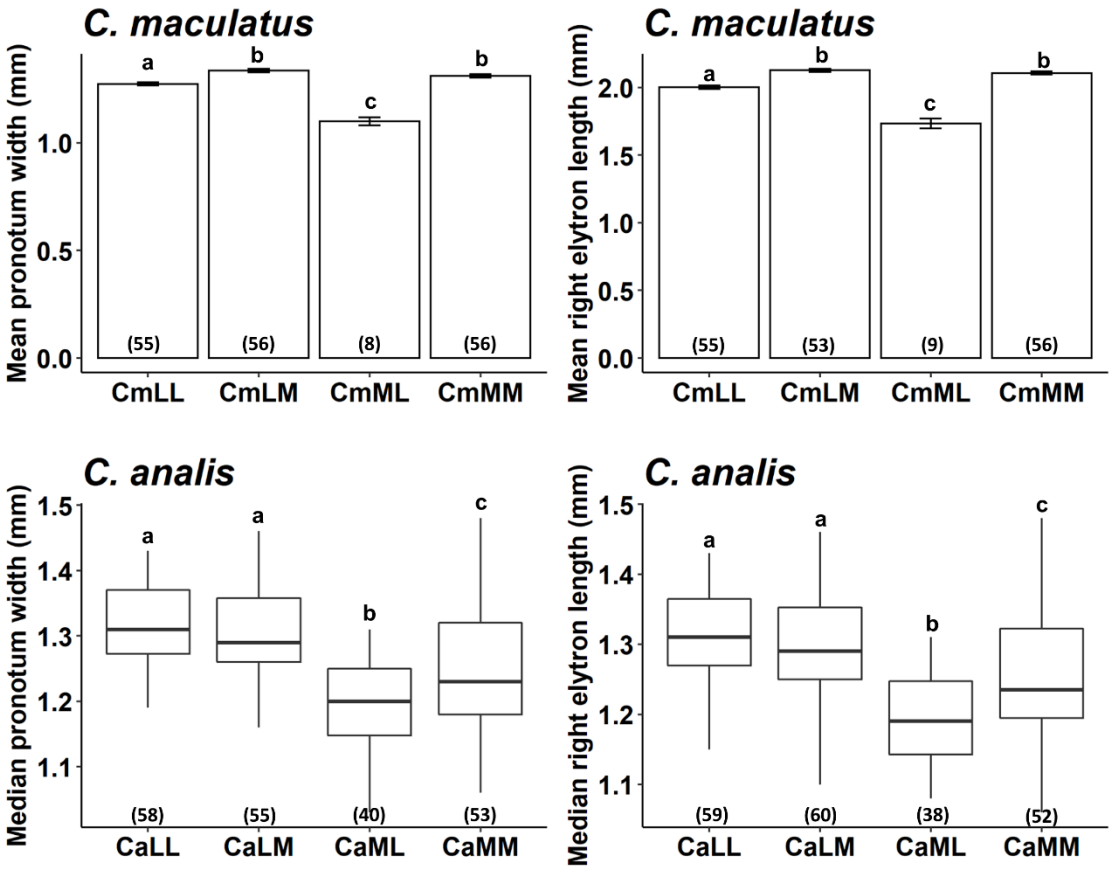


**Figure 3** Mean ± SE of *C. maculatus* (a) pronotum width and (b) right elytron length. Median and interquartile range of *C. analis* (c) pronotum width and (d) right elytron length. The letters above bar represent significant difference between groups. The first two letter represents species where Cm = *C. maculatus* and Ca = *C. analis*. The third and fourth letters represent parental host and natal host, respectively. L = Lentil, M = Mung bean. The number of replicates is given in parentheses.

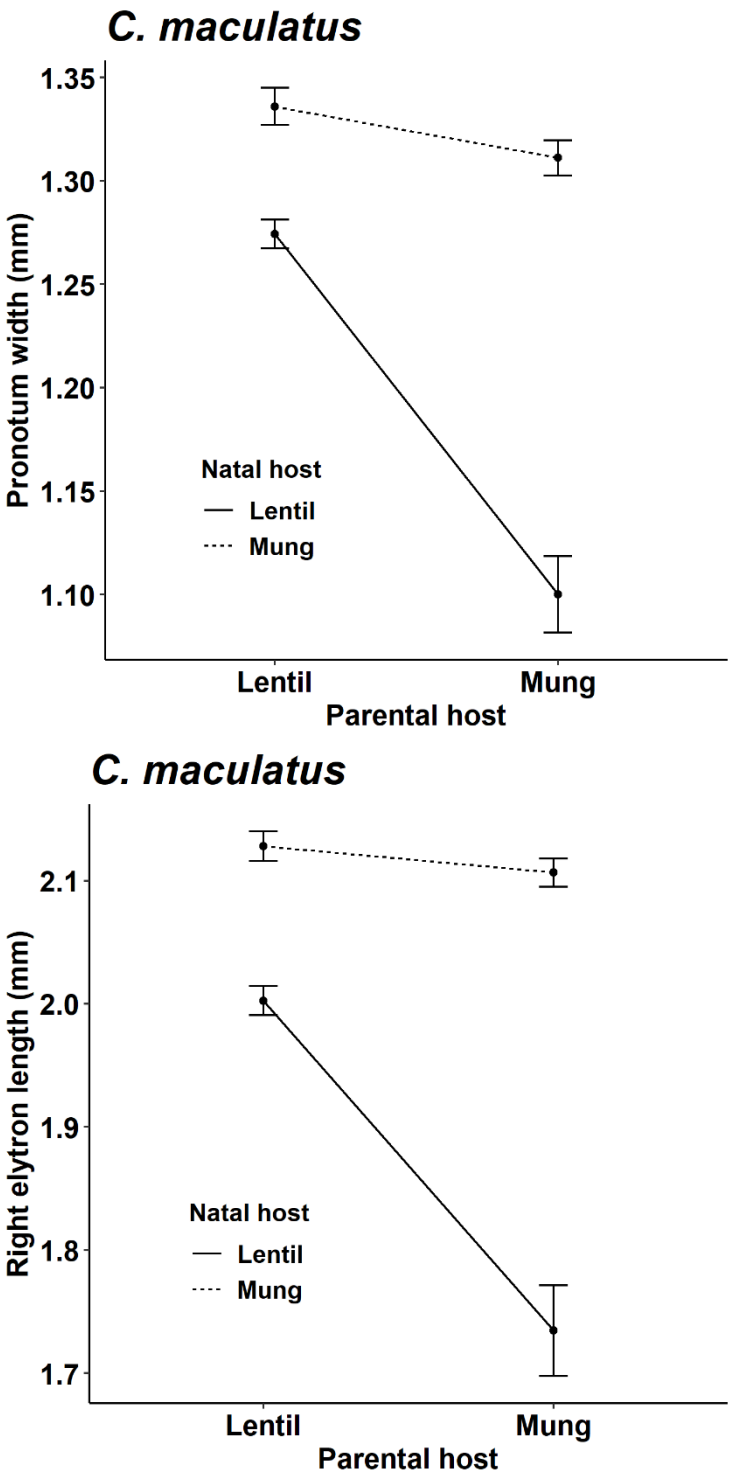


**Figure 4** The interaction plots between parental host and natal host on pronotum width (above) and right elytron length (below) of *C. maculatus*. Solid lines represent lentil as a natal host and dashed lines represent mung bean as a natal host.

Males from lentil were more successful in competition for mates against males from mung bean (Table 9). I hypothesised that size may influence the outcome of mating success where larger males are more likely to outcompete smaller rivals; however, I found a reverse size trend between the two bean beetle species in both sexes. *Callosobruchus maculatus* was larger on mung bean (compared with *C. maculatus* from lentil), whereas *C. analis* was larger on lentil (compared with *C. analis* from mung bean) (Figure 5). Thus, in this study size may not fully explain the outcome of male-male competition. No significant terms were detected for *C. analis* (lentil: 516.62 ± 18.35 seconds (n = 60); mung: 522.61 ± 20.08 seconds (n = 60), Figure 6). Male *C. maculatus* from lentil had a significantly longer mating times than males which emerged from mung bean (lentil: 629.32 ± 28.78 seconds (n = 60), mung: 522.84 ± 20.86 seconds (n = 60), $F_{1,116} = 10.14$, $P = 0.002$). This was the only significant factor affecting mating time and mating times for lentil reared *C. maculatus* were longer than in *C. analis*. My results also showed that male natal host did not affect male fitness in terms of offspring number in either species (*C. maculatus*: $H_{1,115} = 0.47$, $P = 0.495$; *C. analis*: $H_{1,116} = 3.1$, $P = 0.078$), but female natal host (from lentil or mung bean) did significantly affect fecundity (*C. maculatus*: $H_{1,115} = 29.32$, $P < 0.001$; *C. analis*: $H_{1,116} = 21.6$, $P < 0.001$). Females from mung bean had more offspring than females from lentil (*C. maculatus*: lentil: 44.37 ± 4.31 (n = 60), mung: 77.59 ± 2.97 (n = 59), Wilcoxon rank sum test: W = 751.5, $n_1 = 60$, $n_2 = 59$, $P < 0.001$; *C. analis*: lentil: 59.62 ± 4.96 (n = 60), mung: 92.40 ± 1.89 (n = 60), Wilcoxon rank sum test: W = 914.5, $n_1 = 60$, $n_2 = 60$, $P < 0.001$).

**Table 9** Percentage of male bean beetles from different hosts succeeding in mating with female bean beetles from different hosts. The expected mating success is 1:1 and deviation from expected is shown. Number of trials is given in parentheses.

| Female and natal host | Percentage of male mating success | | *G* value | *P*-value |
|---|---|---|---|---|
| | Males from lentil | Males from mung | | |
| *C. maculatus* (Lentil) | 77.78 (21) | 22.22 (6) | 19.93 | <0.001 |
| *C. maculatus* (Mung) | 48.15 (13) | 51.85 (14) | 0.074 | 0.785 |
| *C. analis* (Lentil) | 72.72 (24) | 27.28 (9) | 15.28 | <0.001 |
| *C. analis* (Mung) | 76.67 (23) | 23.33 (7) | 20.08 | <0.001 |

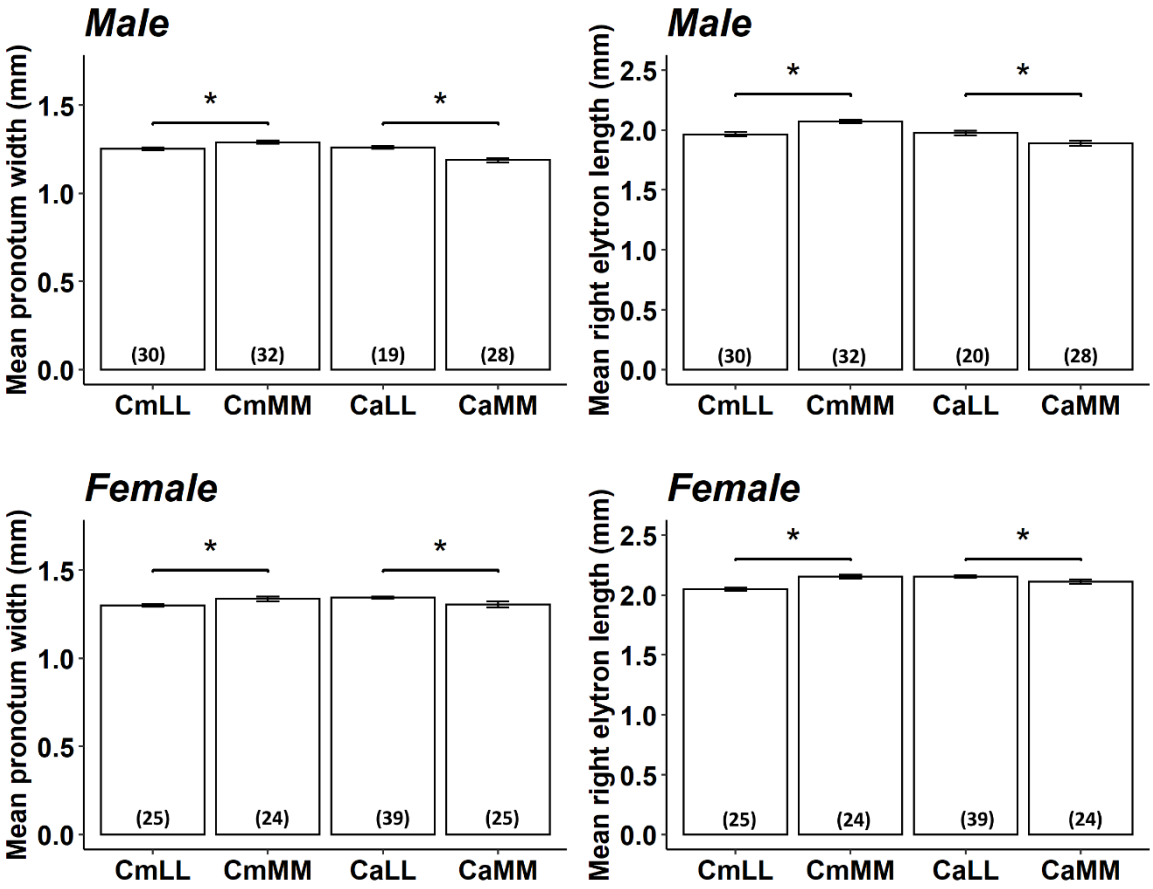


**Figure 5** Mean ± SE of male (a) pronotum width, (b) right elytron length and female (c) pronotum width and (d) right elytron length. The asterisk above represents the significant difference between groups. The first two letter represents species where Cm = *C. maculatus* and Ca = *C. analis*. The third and fourth letters represent parental host and natal host, respectively. L = Lentil, M = Mung bean. The number of replicates is given in parentheses.

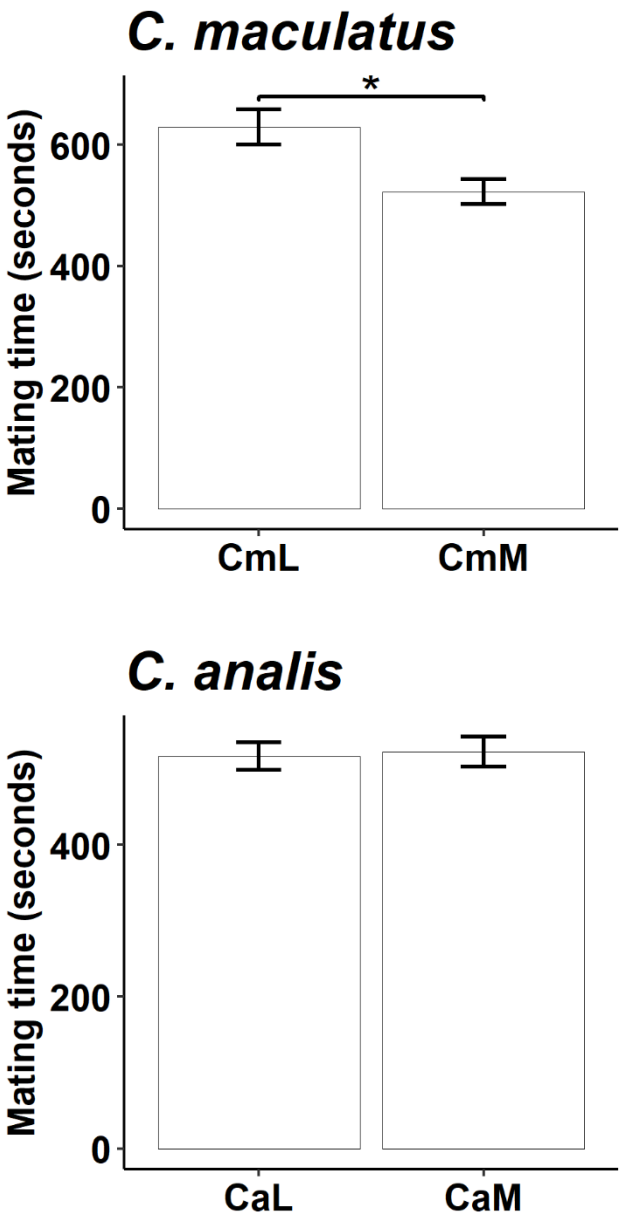


**Figure 6** Mating time in seconds of males of *C. maculatus* (above) and *C. analis* (below) that emerged from the two different hosts (mung and lentil). *Callosobruchus maculatus* reared on mung (CmM), *C. maculatus* reared on lentil (CmL), *C. analis* reared on mung (CaM) and *C. analis* reared on lentil (CaL). The asterisk above represents the significant difference between groups.

2.3.3 Development and starvation resistance time

*Callosobruchus maculatus* with mung bean parental host had a longer development time (Table 10, 11) compared to beetles with a lentil parental host, and they showed a greater difference between different natal hosts. Beetles from lentil natal hosts had a longer development time (54.70 ± 2.59 days), compared with those from mung natal hosts mung (29.40 ± 0.44 days). I found no effects of parental or natal hosts on development time of *C. analis* (Table 10).

**Table 10** Summary of coefficients and model selection based on AIC for models predicting development time with accelerated failure time models (AFT). Only models with the lowest AIC are shown. The significant terms are designated in bold. PtHost = Parental host, NtHost = Natal host, Ely = Right elytron length, (M) = Mung bean.

| Species | Dependent variable | Independent variable | Coefficient value ± SE | *P*-value | Model distribution |
|---|---|---|---|---|---|
| *C. maculatus* | Development time | Intercept | 3.88 ± 0.166 | <0.001 | Lognormal distribution |
| | | **PtHost(M)** | **0.78 ± 0.214** | **<0.001** | |
| | | **NtHost(M)** | **-0.48 ± 0.189** | **0.011** | |
| | | Sex(Male) | -0.30 ± 0.152 | 0.053 | |
| | | **Ely** | **-0.26 ± 0.082** | **0.002** | |
| | | **PtHost(M): Ely** | **-0.36 ± 0.101** | **<0.001** | |
| | | **NtHost(M): Ely** | **0.23 ± 0.092** | **0.014** | |
| | | Sex: Ely | 0.13 ± 0.073 | 0.075 | |
| *C. analis* | Development time | Intercept | 3.41 ± 0.115 | <0.001 | Loglogistic distribution |
| | | PtHost(M) | 0.13 ± 0.246 | 0.586 | |
| | | NtHost(M) | 0.27 ± 0.307 | 0.376 | |
| | | Sex(Male) | -0.21 ± 0.219 | 0.346 | |
| | | Ely | -0.03 ± 0.054 | 0.601 | |
| | | **PtHost(M): NtHost(M)** | **0.83 ± 0.420** | **0.049** | |
| | | PtHost(M): Sex(Male) | 0.15 ± 0.394 | 0.706 | |
| | | NtHost(M): Sex(Male) | 0.43 ± 0.414 | 0.298 | |
| | | PtHost(M): Ely | -0.05 ± 0.122 | 0.714 | |
| | | NtHost(M): Ely | 0.11 ± 0.140 | 0.432 | |
| | | Sex(Male): Ely | 0.09 ± 0.109 | 0.420 | |
| | | Pthost(M): NtHost(M): Sex(Male) | -0.98 ± 0.581 | 0.091 | |
| | | **Pthost(M): NtHost(M): Ely** | **-0.41 ± 0.198** | **0.040** | |
| | | PtHost(M): Sex(Male): Ely | -0.08 ± 0.203 | 0.703 | |
| | | NtHost(M): Sex(Male): Ely | -0.20 ± 0.197 | 0.320 | |
| | | PtHost(M): NtHost(M): Sex (Male): Ely | 0.45 ± 0.286 | 0.114 | |

**Table 11** Mean ± SE development and survival time of *C. maculatus* and *C. analis* offspring in relation to their parental and natal hosts. The number of replicates is given in parentheses.

| Parental host | Natal host | Development time (days) | Survival time (days) |
|---|---|---|---|
| *C. maculatus* (Lentil) | Lentil | 28.30 ± 0.201 (56) | 37.70 ± 1.170 (56) |
| | Mung | 27.60 ± 0.174 (56) | 37.30 ± 1.100 (56) |
| *C. maculatus* (Mung) | Lentil | 54.70 ± 2.590 (9) | 37.80 ± 2.540 (9) |
| | Mung | 29.40 ± 0.439 (58) | 39.40 ± 1.310 (58) |
| *C. analis* (Lentil) | Lentil | 28.40 ± 0.130 (60) | 44.50 ± 1.010 (61) |
| | Mung | 27.60 ± 0.153 (58) | 41.60 ± 1.170 (60) |
| *C. analis* (Mung) | Lentil | 29.50 ± 0.196 (31) | 47.70 ± 1.110 (41) |
| | Mung | 27.60 ± 0.246 (52) | 37.60 ± 1.420 (53) |

Interactions between parental host and size, and between natal host and size were detected in *C. maculatus* (Table 10). Smaller beetles took longer to emerge, but the slope of the relationship between size and development rate varied with both parental and natal host. Generally, larger beetles had a shorter development period before reaching adulthood, and there were minor host effects for larger beetles. However, where the parental host was mung bean, and beetles were smaller, the reduction in development rate was considerable, suggesting a significant transgenerational effect. There was little relationship between size and development rate where mung bean was the natal host, suggesting that development rate was optimised in the higher quality host, while a negative relationship between size and development rate was seen when the natal host was lentil (Figure 7).

In *C. analis*, there was a marginally significant two-way interaction of parental-natal host (Table 10). *Callosobruchus analis* had a shorter development time when the natal host was mung bean, but if their parental host was lentil then the development time was shorter than those whose parental host was mung bean (Figure 8). A significant three-way interaction of parental-natal-elytron length was also detected for *C. analis* (Table 10). Generally larger individuals tended to have a shorter development time. However, offspring whose parental host was lentil and natal host was mung, smaller individuals had shorter development time, suggesting a compensatory benefit of a better-quality natal host bean (Figure 8).

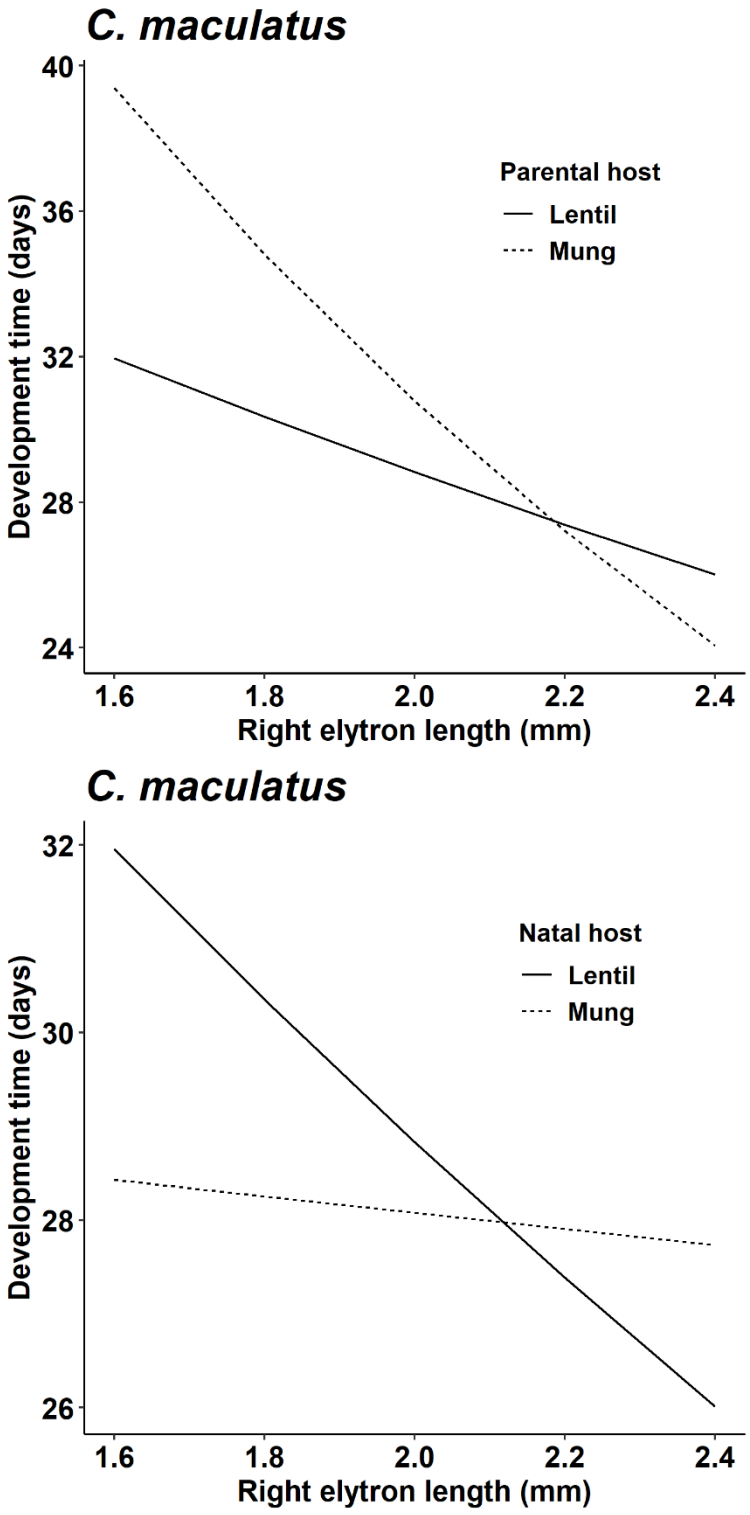


**Figure 7** The interaction plots between parental host and right elytron length (above) and natal host and right elytron length (below) on development time of *C. maculatus*.

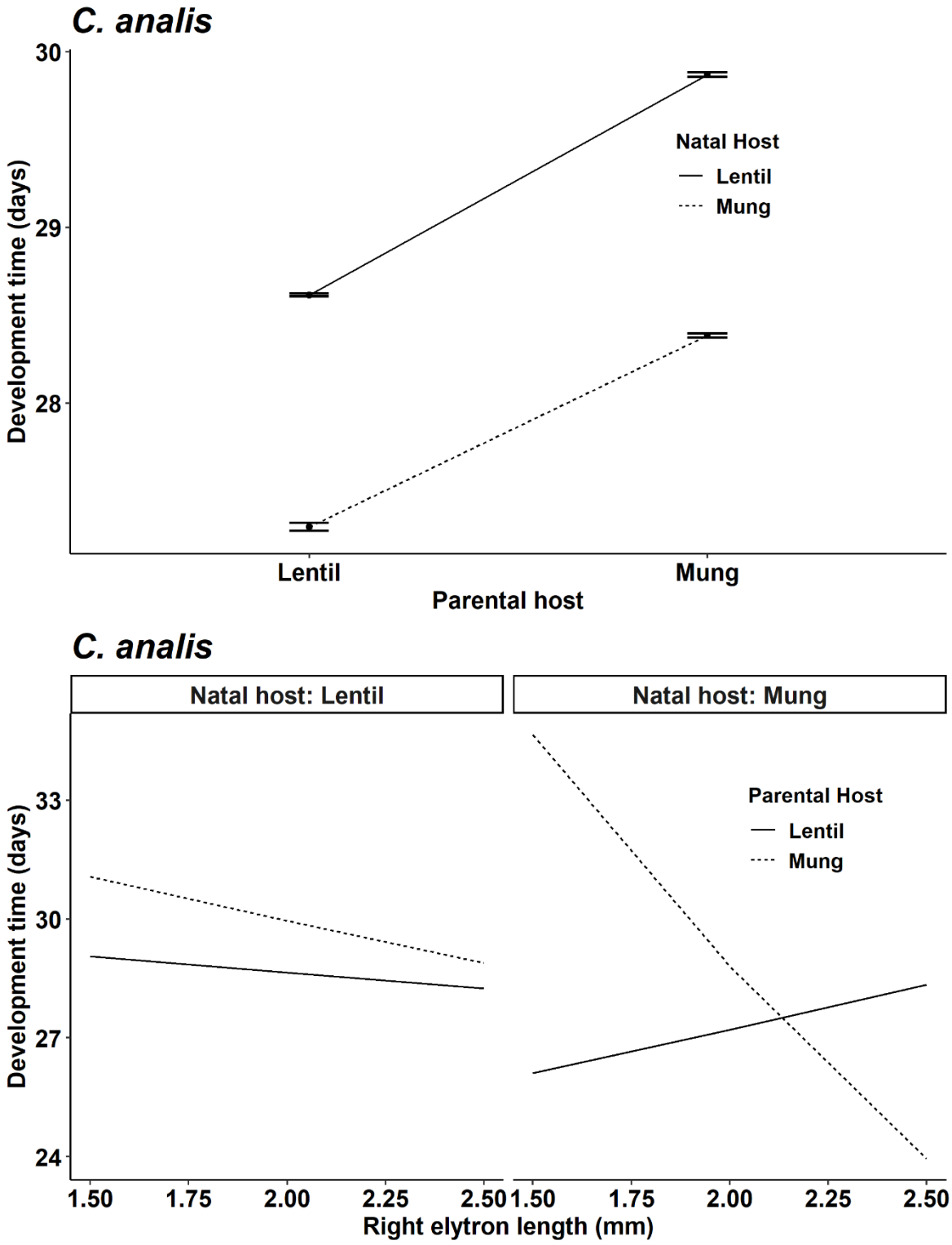


**Figure 8** The interaction plots between parental host and natal host (above) and right elytron length, parental host and natal host (below) on development time of *C. analis*.

*Callosobruchus maculatus* with mung bean as a parental host had greater adult longevity [lentil 37.50 ± 0.80 days (n = 112), mung 39.2 ± 1.18 days (n = 67)] (Table 12). In addition, significant interaction terms were detected as larger males had longer survival times when compared with the same size of females in the *C. maculatus* model (Figure 9). *C. analis*, the interactions between parental and natal hosts, and between natal host and right elytron length were significant (Table 12, Figure 10). Lentil natal host resulted in longer lived offspring, regardless of parental host (except for very large individuals from mung bean natal host which lived longer), but if parental host was mung the survival time was longer compared to lentil parental host.

**Table 12** Summary of coefficients and model selection based on AIC for models predicting survival time with accelerated failure time models (AFT). Only models with the lowest AIC are shown. The significant terms are designated in bold. PtHost = Parental host, NtHost = Natal host, Ely = Right elytron length, (M) = Mung bean.

| Species | Dependent variable | Independent variable | Coefficient value ± SE | *P*-value | Model distribution |
|---|---|---|---|---|---|
| *C. maculatus* | Survival time | Intercept | 3.16 ± 0.393 | <0.001 | Weibull distribution |
| | | **PtHost(M)** | **0.09 ± 0.034** | **0.005** | |
| | | NtHost(M) | -0.02 ± 0.057 | 0.719 | |
| | | **Sex(Male)** | **-1.14 ± 0.521** | **0.029** | |
| | | Ely | 0.25 ± 0.196 | 0.201 | |
| | | NtHost(M): Sex(Male) | -0.14 ± 0.075 | 0.072 | |
| | | **Ely: Sex(Male)** | **0.63 ± 0.265** | **0.018** | |
| *C. analis* | Survival time | Intercept | 12.09 ± 13.274 | 0.362 | Gaussian distribution |
| | | **PtHost(M)** | **5.93 ± 1.929** | **0.002** | |
| | | **NtHost(M)** | **-50.90 ± 17.530** | **0.004** | |
| | | **Ely** | **15.55 ± 6.351** | **0.014** | |
| | | **PtHost(M): NtHost(M)** | **-5.61 ± 2.494** | **0.024** | |
| | | **NtHost(M): Ely** | **22.75 ± 8.36** | **0.007** | |

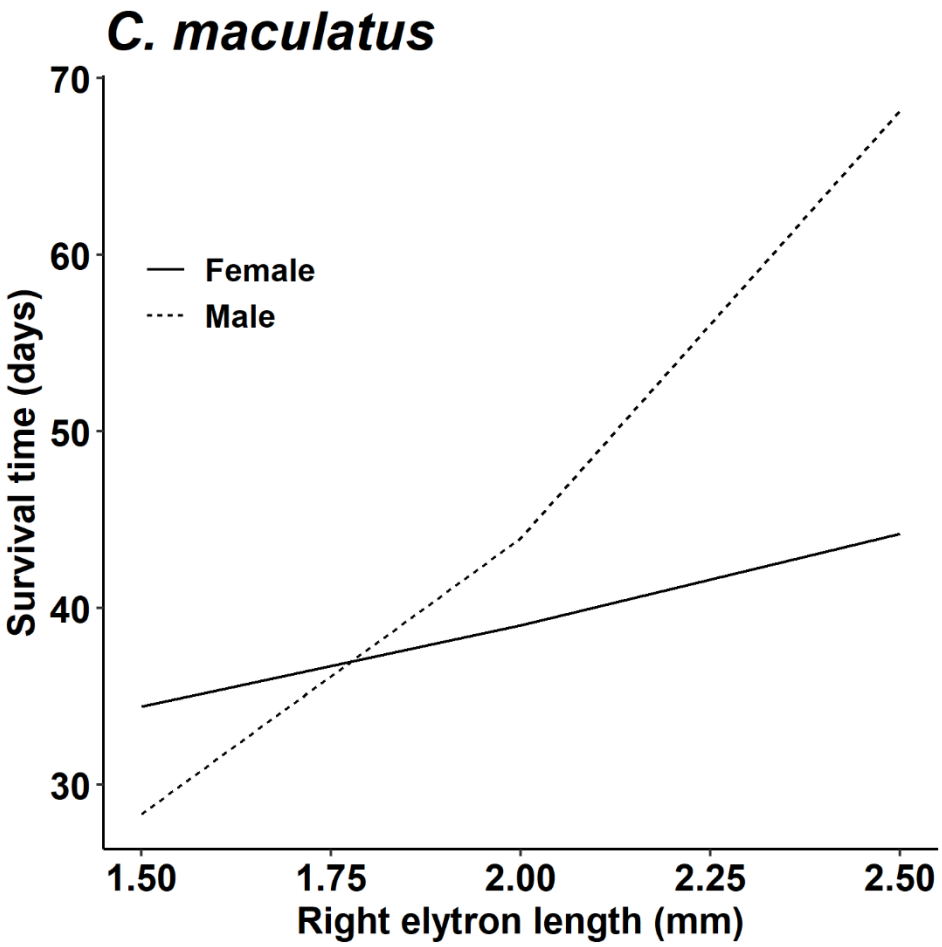


**Figure 9** The interaction plots between sex and right elytron length on adult longevity of *C. maculatus*.

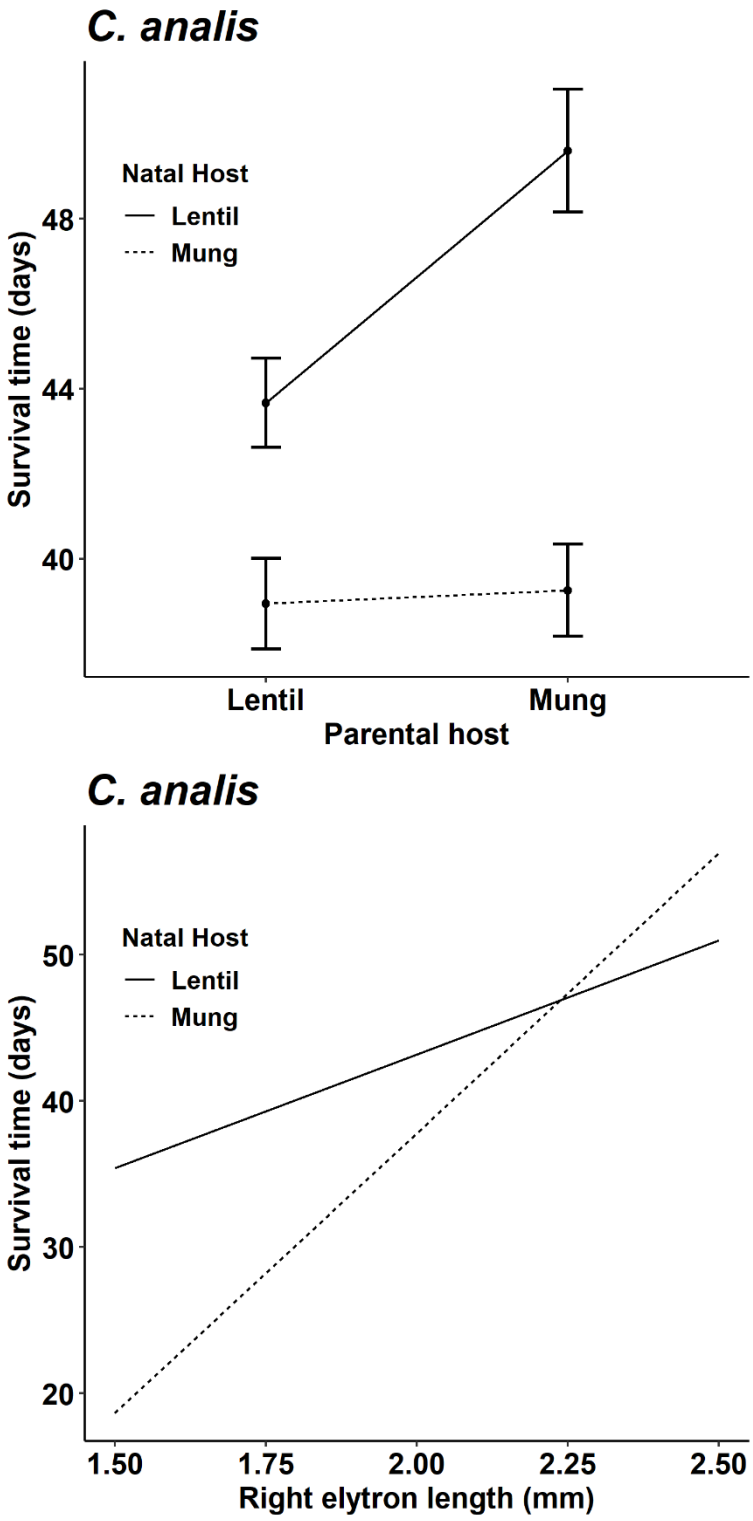


**Figure 10** The interaction plot between parental host and natal host (above), and natal host and right elytron length (below) on adult longevity of *C. analis*.

## 2.4 Discussion

I examined the effects of parental host, natal host and oviposition experience on the fitness parameters of two related, economically significant pests of stored products, *C. maculatus* and *C. analis*. Parental and natal host greatly affected many life history traits (fecundity, host acceptance, emergence rate, development time, survival time, size, sex ratio, mating competition and mating time), and these responses differed between the beetle species (see results summary table, Appendix 1).

I found no effects of natal host and oviposition experience on oviposition preference, with both preferring mung bean, but oviposition experience with lentil did increase the chance of acceptance of lentil for future oviposition. Females of both species emerging from lentil laid more eggs when the better host (mung bean) was provided. However, host switching by *C. maculatus* from better (mung bean) to poor (lentil) quality hosts had a negative effect on fitness (emergence number, development time and size). Parental and natal hosts influenced development time and survival time, and this was affected by beetle size. Generally, larger individuals developed more quickly, with the exception of *C. analis* whose parents came from lentil and their natal host was mung bean, where smaller individuals had a quicker development rate.

Larger individuals had increased adult longevity, irrespective of species. Females were larger than males, and the effect of parental and natal hosts affected offspring size. In *C. analis*, parental and natal host influenced observed offspring sex ratio. Males from lentil tended to outcompete males from mung bean in mating competition, with the exception of male *C. maculatus* emerging from lentil spent longer mating than males from mung bean.

No effect of natal host was found on male fitness, but was observed in female fecundity instead.

Previous work has considered ovipositional experience of *C. maculatus*, but the study did not include the effects of natal host (Chiu and Messina, 1994). In no choice and choice experiments both beetle species, regardless of their natal hosts, clearly preferred to oviposit more eggs (in terms of focal host) on mung bean over lentil. Previous studies have suggested that *C. maculatus* preferred larger beans within (Cope and Fox, 2003; Yang et al., 2006) and between species (Paukku and Kotiaho, 2008). However, I found that both beetle species preferred ovipositing on smaller sized mung beans compared with lentils (which were larger in size compared to the small seed mung). Seed size is not the only factor determining bean beetle oviposition preference, which is also affected by chemical signals (Pouzat, 1981), smoothness (Sulehrie et al., 2003), curvature of seeds (Gokhale et al., 1990) and the presence of other eggs (Otake and Dobata, 2018). My results suggest oviposition preference in bean beetles is not transgenerational as host quality comes before host quantity.

Lentil is considered to be an inferior host to mung bean (Messina et al., 2009). In terms of natal host, the two beetle species from lentil laid more eggs than those from mung bean when a better host (mung bean) was provided. This result is consistent with a previous study showing that *C. maculatus* fecundity was enhanced on lentil (Messina and Jones, 2009). Poor early life nutrition can lead to thrifty phenotype (Hales and Barker, 2001), the characteristic which helps organisms to perform best under poor resource conditions by promoting fat storage and high glucose blood levels. This may lead to the accumulation of lipid storage in insects (Barrett et al., 2009; Jehrke et al., 2018) which is important in

programmed cell death of fat cells contributing to ovary maturation and fecundity (Aguila et al., 2013). This may explain why beetles from lentil had higher fecundity.

A previous study showed that the effect of experience depended on the beetle strains and played little or no role in oviposition (Chiu and Messina, 1994). My study also found no effect of oviposition experience on subsequent oviposition preference. I speculate that in bean beetles contact chemoreception and tactile receptors are more reliable tools to examine host quality compared with oviposition experience, although in other beetle species the experience can influence oviposition preference, but this comes with employing different cues (Lyu et al., 2018). My result indicates that oviposition preference in bean beetles is innate and cannot be modified by experience.

Responses differed between species when they switched to a poorer natal host (lentil); where *C. maculatus* suffered from switching to the inferior host in terms of emerging adult, *C. analis* had no such response. This result is consistent with survival time results in this study, as *C. analis* who were larger survived for longer compared with *C. maculatus* with lentil as a natal host. In addition, the performance of *C. maculatus* offspring were also affected when the parental host was mung bean and natal host was lentil (Figure 4). The poor performance seen in *C. maculatus* resulting from switching to the inferior host (lentil) may be linked to reduced expressions of genes which help detoxify plant secondary metabolites (Rêgo et al., 2020).

In both beetle species, having lentil as the parental host resulted in larger offspring. Again, mothers already adapted to a poor quality host may generate offspring tolerant to poor quality hosts (Amarillo-Suárez and Fox, 2006), so the offspring tend to have improved fitness. In terms of natal host, mung bean is a better-quality host as emerged adults were

larger than those from lentil. Overall, females were larger than males, following the general trend in insects (Stillwell et al., 2010; Teder, 2014).

In the absence of local mate competition and haplodiploidy, a 1:1 female: male sex ratio is favoured through natural selection (Trivers and Willard, 1973; King, 1987). I found a deviation in sex ratio from 1:1 only in *C. analis* when the natal host was lentil. Differences in parental host yielded different sex biases; where the parental host was lentil, the offspring had a female bias, while where mung bean was the parental host I saw a male bias. There is no evidence that bruchid bean beetles can directly control their offspring sex ratio, but deviations from a 1:1 ratio can occur through intraspecific competition and differential mortality between sexes (Cipollini, 1991; Ishihara and Shimada, 1993; Reece et al., 2005).

This study found males that emerged from lentil were generally more successful in gaining matings than those from mung bean regardless of species. Even though larger males tend to achieve more mating success by outcompeting other male competitors during direct conflict (Andersson, 1994), smaller males can gain more mating success through better aerobatics in acquiring females (Mclachlan and Allen, 1987) or better morphological compatability during mating (Weissman et al., 2008). A similar study system found that larger male *Sitophilus oryzae* are preferred by females, demonstrated by reduced pairing time and increased mating time (Holloway and Smith, 1987). Higher mating success in larger male bean beetles can be explained only in the case of *C. analis* where males from lentil were larger, but not for *C. maculatus* where males from mung bean were larger. In this study, *C. maculatus* from mung bean seemed to be less active than those from lentil (pers. obs.) which provided an opportunity for the smaller males to mate.

Nutritional quality may influence male fitness in terms of number of offspring as shown in studies in other insects (Fricke et al., 2008; Morimoto and Wigby, 2016). In this study I found no difference in the number of offspring sired by the two male bean beetle species which emerged from different hosts. Male size is not likely to be a good proxy of male fitness (Savalli and Fox, 1999). *Callosobruchus maculatus* males that previously mated still provide many more sperm than females need (Eady, 1995). Hence, it seems that host quality could not account for a difference in offspring number among treatments as in this study I used unmated males.

Egg size varies both within and among females of *C. maculatus*, and individuals from larger eggs develop faster and emerge as larger adults (Fox, 1994), which is consistent with my results that larger individuals of both species developed faster (with the exception of *C. analis* where the parental host was lentil and natal host was mung bean). Developmental times were also found to be influenced by host; where mung bean was a parental host development rate was slower in *C. maculatus*. The effect of maternal rearing host on offspring is still unclear (Amarillo-Suárez and Fox, 2006); therefore, I remain cautious about the interpretation of the interactions between parental host-size and natal host-size on development time of the two beetle species.

*Callosobruchus analis* that developed in mung bean as a natal host had reduced development times, whereas lentil as parental host reduced development time on mung bean. Mothers who are pre-adapted to poor food quality may change resource allocation to eggs resulting in offspring better able to tolerate poor quality food (Amarillo-Suárez and Fox, 2006). Thus, when offspring are reared on better quality food, they might in turn perform better. Interestingly, in *C. analis* whose parental host was lentil and natal host was mung

bean showed the reverse result where smaller individuals developed faster. I currently have no explanation for this, though a previous study suggested that the size of *C. maculatus* is not determined by maternal effects (Fox, 1993a).

Studying adult lifespan is more complicated than I expected. In this study I found a significant effect of parental host on adult longevity for both beetle species. However, a previous study found genetic architecture in lifespan is different between males and females of *C. maculatus* where male lifespan is largely affected by maternal effects, while female lifespan is more affected by dominant long-life alleles (Fox et al., 2004). Fox et al. (2004) also failed to detect the effect of rearing hosts on lifespan, which may be due to the use of two closely related hosts (*Vigna radiata* and *V. unguiculata*). For *C. maculatus*, I found that males had a more reduced lifespan than females, and offspring lived longer when the parental host was mung bean. For *C. analis*, emerging from lentil increased adult longevity (except for very large individuals with mung bean as their natal host, which survived longer), but having mung bean as parental host resulted in increased survival time. While body size alone cannot fully explain the variance in the differences in lifespan between the sexes, it is likely that this arises from variation in metabolism and energy expenditure between the sexes (Fox et al., 2003). It is also unlikely that body size has co-evolved with longevity, but rather it is the environment influencing body size that also influences longevity (Fox et al., 2003).

Overall, my results indicate that differences in host quality can influence life histories of bean beetles, but responses differ between species, making the drawing of generalities difficult (Srisakrapikoop et al., 2021). I expected that the effects of host quality may be different between species with different life histories (scramble or contest). In my experimental design used only seed harbouring single egg, so different life histories in terms

of larvae competition cannot explain my results. However, in nature with the competition, this trait may attribute to results which are different from this study. Bean beetles preferred high quality over poor quality hosts with profound effects of parental and natal hosts on offspring life history. Switching from poor to high quality hosts generally improves offspring performance but when switching from high to poor quality hosts, effects are species dependent. My study provides insights into how host quality can affect offspring performance and potentially play a role in sexual selection through male-male competition. Understanding on how these pests respond and adapt to novel hosts provides us with a better understanding of both the fundamental biology of the system, and also how this knowledge can be applied to challenges in pest management.

# Chapter 3 Costs of mating in the bean beetle *Callosobruchus maculatus* are affected by host quality experienced during development

## 3.1 Introduction

The amount of resources invested in reproduction frequently differs between male and female insects (Bonduriansky, 2001; Kelly, 2018). This variation can affect mate preference which in turn can benefit offspring either directly (offspring quality and quantity), or indirectly (improved offspring quality resulting from the genetic contribution of the partner) (Ihle et al., 2015). Mating with a high-quality partner can therefore increase the immediate (resource determined) and longer term (genetic) benefits for offspring. However, mating does not only affect the quantity and quality of offspring, but it can also affect the fitness of those mating.

Mated male damselflies can experience reduced immunity (Honkavaara et al., 2009) and mating can affect immunity in both sexes of mealworm (Rolff and Siva-Jothy, 2002). Reduced longevity in mated males is found across insect taxa such as in beetles (Kotiaho and Simmons, 2003), parasitoid wasps (Burton-Chellew et al., 2007), flies (Papadopoulos et al., 2010) and thrips (Li et al., 2014) due to the costs of courtship, competition, sperm production and the energy costs expended during mating itself (Li et al., 2014). A decrease in longevity can also be observed in mated females of species such as seed bugs (Shuker et al., 2006) and thrips due to male harassment and physical injuries from resistance behaviour (Li et al., 2014). However, other studies suggest that females can live longer after mating due to proteins secreted from male accessory glands, which does not appear to be a nutritive effect (Villarreal et al., 2018). Evidently, mating can be costly, and the resources available to invest

in mating and reproduction will depend on the physiological status (i.e., reserves) of the individuals involved.

Host plant quality can affect adult insect fitness both in terms of fecundity and longevity (Leather et al., 1998; Morrill et al., 2000; Cárcamo et al., 2005; Moreau et al., 2006; Luo et al., 2018; Hong et al., 2019). Surprisingly, while many studies have shown the existence of reproductive costs in both sexes across insect taxa (e.g., Smith, 1958; Partridge and Farquhar, 1981; Service, 1989; Sheeba et al., 2000; Parker et al., 2013; Brent, 2018; Li et al., 2021), these have not taken the effects of larval host plant quality into account. In this study I combine both perspectives.

*Callosobruchus maculatus* (Coleoptera: Chrysomelidae) is a cosmopolitan pest species (Utida, 1981). Larvae develop inside the seeds of legumes and non-leguminous crops (Tuda et al., 2005) causing serious economic losses. This bean beetle species exhibits sexual dimorphism in which females are larger in size than males (Savalli and Fox, 1999) and the sexes can be distinguished by differences in visible characteristics (Beck and Blumer, 2014). *Callosobruchus maculatus* males transfer a large ejaculate (nuptial gift or spermatophore) averaging more than 5% of their body mass during their first mating, and this provides nutritional benefits to females (Fox et al., 1995) used in somatic maintenance and egg production (Thornhill 1976; Fox, 1993b). In *C. maculatus*, males that have mated show a decrease in longevity (Paukku and Kotiaho, 2005), while mated females which have not oviposited exhibit an increase in longevity compared with virgin females and mated females that have oviposited (Rönn et al., 2006).

In insects, females are generally larger in size as selection favours larger females with greater fecundity (Stillwell et al., 2010; Teder, 2014); hence, size is generally used as a proxy

for female fitness. On the other hand, in *C. maculatus* the relationship between male size and female fecundity is unclear as some studies showed a positive relationship (Savalli and Fox, 1999; Paukku and Kotiaho, 2005), while other studies found no such relationship (Fox et al., 2006; Małek et al., 2019). It is speculated that larger males may contribute to greater female fecundity by providing a larger ejaculate (Savalli and Fox, 1999; Małek et al., 2019). This leads to the hypothesis that mating with larger males may also increases the lifespan of mated female, but this remains uncertain (Paukku and Kotiaho, 2005; Małek et al., 2019). Although adult *C. maculatus* can complete their life cycle without requiring food (Beck and Blumer, 2014) and it is clear that food provision can increase longevity and fecundity (Moller et al., 1989), it is still unknown if food provision and larval host plant quality interactively affect beetle longevity and fecundity.

Given that both the larval host and mating status may affect fitness in *C. maculatus*, this study investigates the interaction between host quality (high quality: mung bean or poor quality: lentil), mating status (mated or unmated) and adult food provision (yes or no) on the costs of reproduction in *C. maculatus*.

## 3.2 Materials and methods

Experimental populations of *Callosobruchus maculatus* had been cultured separately on mung beans and lentils since 2010 and 2016, respectively. Fifteen mated females from each host were separately allowed to oviposit on the host on which they have been reared in a Petri dish containing a single layer of mung beans or lentils for three hours at 28 ± 2°C with 40% relative humidity to produce seeds harbouring a single egg for experimental use. Seeds with a single egg were individually transferred into a perforated 0.5 ml Eppendorf tube and allowed to develop under the same conditions with constant light for the first seven days,

and then they were moved into an incubator at 35°C with 40% relative humidity in darkness until adults emerged. After the adults emerged, the remaining seeds were discarded, and the adults were sexed and allowed to rest individually in their Eppendorf tube for 24 hours before experiments commenced.

### 3.2.1 Unmated experiment

In this unmated (V) treatment, virgin females and males from mung bean and lentil were kept individually in perforated 0.5 ml Eppendorf tube and were moved into a controlled environment room at 28°C 60% relative humidity with constant light. These beetles were observed daily to record days to death. The right elytron length of the dead beetles was then measured under a high-performance stereomicroscope (Leica MZ9.5).

### 3.2.2 Mated experiment

Each virgin female from mung bean or lentil was allowed to mate once in a perforated 0.5 ml Eppendorf tube with a virgin male which had emerged from the same or a different host (providing four groups in total: mung female with mung male; mung female with lentil male; lentil female with lentil male; lentil female with mung male) at room temperature in the laboratory. After mating, each female and male were kept separately in a controlled environment room at 28°C 60% relative humidity with constant light in one of three treatments. 1) Mated (M) treatment: mated females and males were kept individually in a 0.5 ml perforated Eppendorf tube. 2) Mated and oviposited (MO) treatment: each mated female was allowed to oviposit in a 90 cm Petri dish containing a single layer of beans of the host species from which she had emerged. 3) Mated, oviposited and fed (MOF) treatment: this treatment was similar to 2) except 10% sucrose solution in a 0.5 ml Eppendorf tube plugged with cotton wool was provided *ad libitum*. The beetles in all treatments were checked daily

to record time to death. In the experiments where oviposition was allowed, seeds were replaced with new seeds every three weeks to prevent the emergence of offspring. Right elytron length of both sexes in 1) and females in 2) was measured under a high-performance stereomicroscope (Leica MZ9.5). The numbers of eggs oviposited on each bean in 2) and 3) were recorded.

### 3.2.3 Data analyses

All analyses were performed using R 4.1.0 (R Core Team, 2021). All survival time models in this study were modelled with accelerated failure time models (AFT) using *survival* package (Therneau, 2021). The survival time of females and males in all treatments was tested with treatment, natal host and sex as factors. Pairwise comparison was conducted with *emmeans* packages (Lenth, 2021). In V treatment, the survival time of female and male was modelled with natal host, sex and size (right elytron length) as factors. In M treatment, female survival time was modelled with natal host of female and male, and size of female and male. I included male size in the model because I hypothesised that larger males produce larger nuptial gifts (Savalli and Fox, 1999; Małek et al., 2019), and this may influence female longevity. Mated male survival time was tested with natal host of female and male, and male size. I also investigated whether there is sex differential mortality in mated individuals, so I modelled survival time of mated females and males with natal host and sex.

Female survival time in MO treatment was modelled with natal host of female and male, and number of oviposited eggs. Female survival time in MOF treatment was also tested with natal host of female and male, and number of oviposited eggs. In MO and MOF treatments, females that did not oviposit were excluded from the analyses. To quantify the effect of host quality and mating, survival time of females in MO treatment were compared

with survival time of males in M treatment fitted with natal host and sex. Models fitted with *survival* package were selected with stepwise method using *MASS* package (Venables and Ripley, 2002) and validated by deviance residuals analysis (Achilonu et al., 2019). To investigate whether providing food to mated females interacts with natal host and in turn affects female fecundity, the number of eggs in MO and MOF treatments was modelled with zero-truncated model with negative binomial distribution using *VGAM* package (Yee, 2021) with natal host of female and male, and treatment as factors. The females that had not oviposited were excluded from the analysis. Then the model was selected with stepwise method. All models in this study were selected based on Akaike's Information Criteria (AIC) (Burnham and Anderson, 2003). Collinearity was not detected as VIF values ranged from 1.00 to 1.74.

## 3.3 Results

The results from survival analysis of all treatments showed that individuals from mung bean [36.18 ± 0.86 days (n = 260)] lived longer than those from lentil [27.40 ± 0.72 days (n = 277)], females [32.93 ± 0.80 days (n= 357)] also lived longer than males [29.11 ± 0.70 days (n = 180)]. There was a significant difference in survival time between treatments and a significant interaction term between natal host and treatments (Table 13). Survival time was shortest in the MO treatment and longest in the MOF treatment, while there was no significant difference between the V and M treatments (Table 14, Figure 11). The results from pairwise comparison of the interaction between treatments and natal host showed three non-significant differences between M treatment Lentil – V treatment Lentil ($P$ = 0.993), MOF treatment Lentil – MOF treatment Mung ($P$ = 0.911) and M treatment Mung – V treatment Mung ($P$ = 0.995) (Figure 12).

**Table 13** Summary of coefficients and model selection based on AIC for models predicting survival time of all treatments combined with accelerated failure time models (AFT) with Weibull distribution. Only models with the lowest AIC are shown.

| Dependent variable | Independent variable | Coefficient value ± SE | *P*-value |
|---|---|---|---|
| Survival time | Intercept | 3.38 ± 0.025 | <0.001 |
| | Host (Mung) | 0.37 ± 0.030 | <0.001 |
| | Sex (Male) | -0.12 ± 0.024 | <0.001 |
| | Treatment | - | <0.001 |
| | Host: Treatment | - | <0.001 |

**Table 14** Mean ± SE of survival time of each treatment. Superscript alphabets indicated significant differences between groups calculated from post-hoc Tukey test. Number of samples is given in parentheses.

| Dependent variable | Treatment | Mean ± SE |
|---|---|---|
| Survival time | Unmated (V) | $31.57 \pm 0.94^{a}$ (120) |
| | Mated (M) | $30.94 \pm 0.64^{a}$ (240) |
| | Mated and oviposited (MO) | $15.64 \pm 0.45^{b}$ (85) |
| | Mated, oviposited and fed (MOF) | $48.40 \pm 1.36^{c}$ (92) |

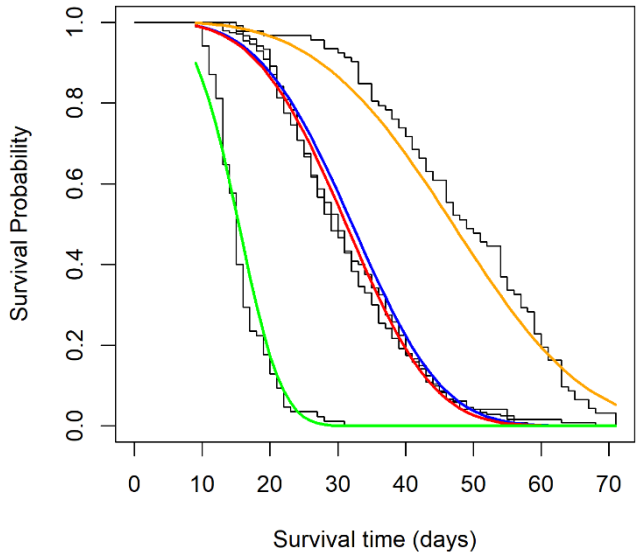


**Figure 11** Survival curves fitted from accelerated failure time models (AFT) with Weibull distribution of *C. maculatus* according to unmated (V; blue), mated (M; red), mated and oviposited (MO; green) and mated, oviposited and fed (MOF; orange) treatments.

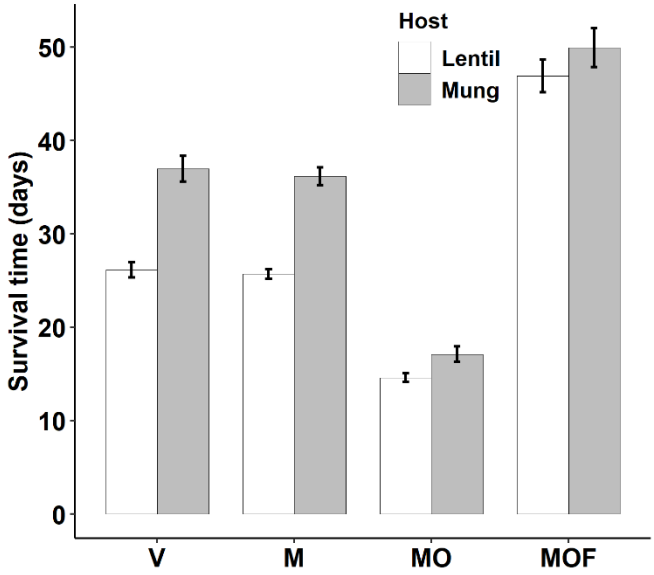


**Figure 12** Mean ± SE of survival time (days) of *C. maculatus* according to natal host and treatment. V = unmated, M = mated, MO = mated and oviposited and MOF = mated, oviposited and fed treatments.

In the V treatment, I found that natal host [mung 34.94 ± 1.12 days (n = 55), lentil 26.15 ± 0.82 days (n = 60)], sex [female 31.91 ± 1.10 days (n = 56), male 28.88 ± 1.12 days (n = 59)] and size influenced survival time (Table 15). In the M treatment (females), only the interactions between female natal host and female size, and between female natal host and male size, were significant (Table 16). Interaction plots reveals that female survival time increased along with female size, but the effect was more prominent in mung bean as the female natal host (Figure 13). Females from lentil seemed to receive greater benefit in terms of longevity when mated with larger males (Figure 13). In the M treatment (males), male longevity increased with male size and those who mated with females from mung also had greater longevity (Table 17). A significant interaction between female natal host and male size was detected as larger males that had mated with females emerging from lentil generally lived longer than those mated with females from mung bean (Figure 14). Overall, in the M

treatment, individuals from mung bean [mung 36.18 ± 0.95 days (n = 120), lentil 25.70 ± 0.52 days (n = 120)], and female [females 32.89 ± 0.90 days (n = 120), males 28.99 ± 0.86 days (n = 120)] had longer survival time (Table 18).

**Table 15** Summary of coefficients and model selection based on AIC for models predicting survival time of unmated treatments (V) with accelerated failure time models (AFT) with Weibull distribution. Only models with the lowest AIC are shown.

| Dependent variable | Independent variable | Coefficient value ± SE | *P*-value |
|---|---|---|---|
| Survival time | Intercept | 2.15 ± 0.583 | <0.001 |
| | Host (Mung) | 0.16 ± 0.006 | 0.013 |
| | Sex (Male) | -0.12 ± 0.055 | 0.030 |
| | Elytron length | 0.64 ± 0.291 | 0.028 |
| | Host: Sex | - | 0.138 |

**Table 16** Summary of coefficients and model selection based on AIC for models predicting survival time of females in mated treatments (M) with accelerated failure time models (AFT) with lognormal distribution. Only models with the lowest AIC are shown.

| Dependent variable | Independent variable | Coefficient value ± SE | *P*-value |
|---|---|---|---|
| Survival time | Intercept | 19.66 ± 11.274 | 0.081 |
| | Female host (Mung) | 0.90 ± 1.416 | 0.523 |
| | Female elytron length | -8.65 ± 5.628 | 0.124 |
| | Male elytron length | -8.47 ± 5.648 | 0.134 |
| | Female host: Female elytron length | - | 0.003 |
| | Female host: Male elytron length | - | <0.001 |
| | Male elytron length: Female elytron length | 4.47 ± 2.819 | 0.115 |

**Table 17** Summary of coefficients and model selection based on AIC for models predicting survival time of males in mated treatments (M) with accelerated failure time models (AFT) with Weibull distribution. Only models with the lowest AIC are shown.

| Dependent variable | Independent variable | Coefficient value ± SE | *P*-value |
|---|---|---|---|
| Survival time | Intercept | 1.61 ± 0.742 | 0.030 |
| | Female host (Mung) | 2.16 ± 0.857 | 0.012 |
| | Male host (Mung) | -1.75 ± 1.184 | 0.141 |
| | Male elytron length | 0.83 ± 0.385 | 0.031 |
| | Male host: Male elytron length | - | 0.088 |
| | Female host: Male elytron length | - | 0.017 |

**Table 18** Summary of coefficients and model selection based on AIC for models predicting survival time of all individuals in mated treatments (M) with accelerated failure time models (AFT) with Weibull distribution. Only models with the lowest AIC are shown.

| Dependent variable | Independent variable | Coefficient value ± SE | *P*-value |
|---|---|---|---|
| Survival time | Intercept | 3.37 ± 0.025 | <0.001 |
| | Host (Mung) | 0.38 ± 0.029 | <0.001 |
| | Sex (Male) | -0.10 ± 0.029 | <0.001 |

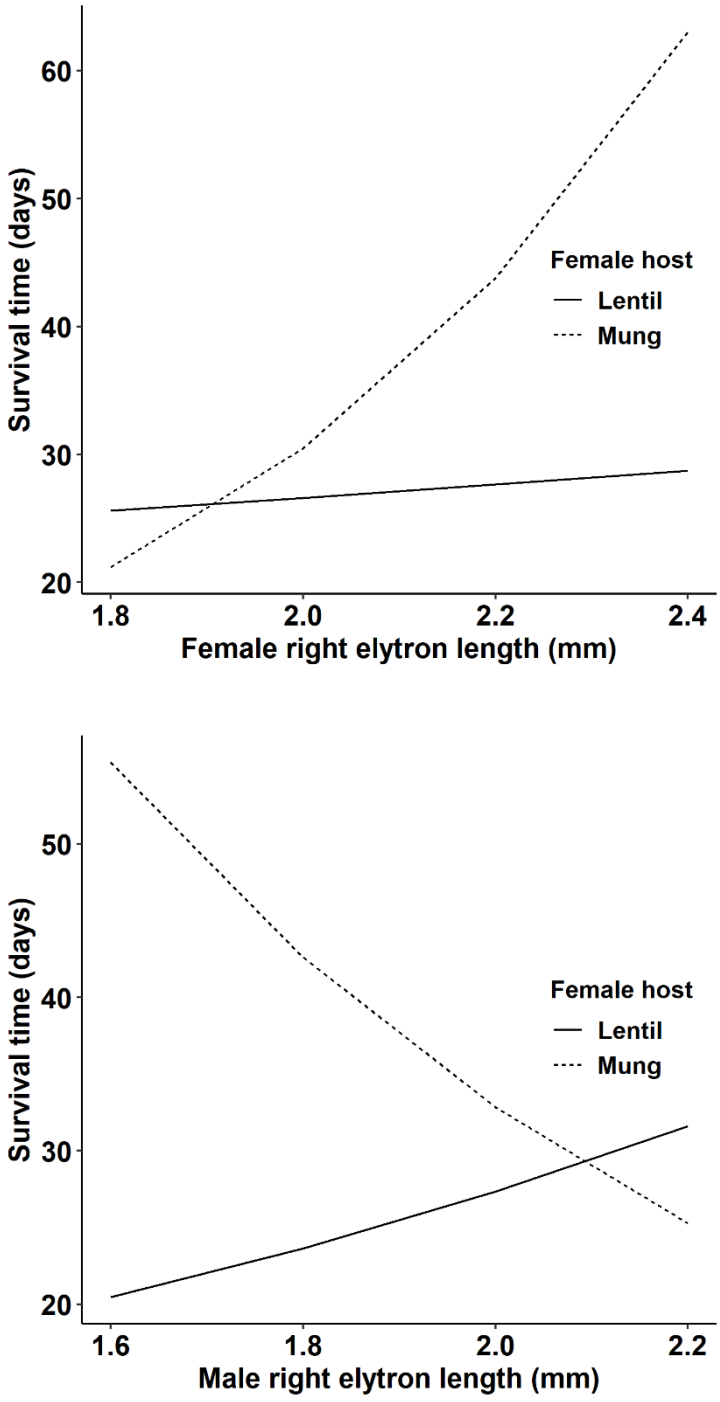


**Figure 13** The interaction plots between female natal host and female right elytron length (above) and female natal host and male right elytron length (below) on survival time of female *C. maculatus* in mated treatment.

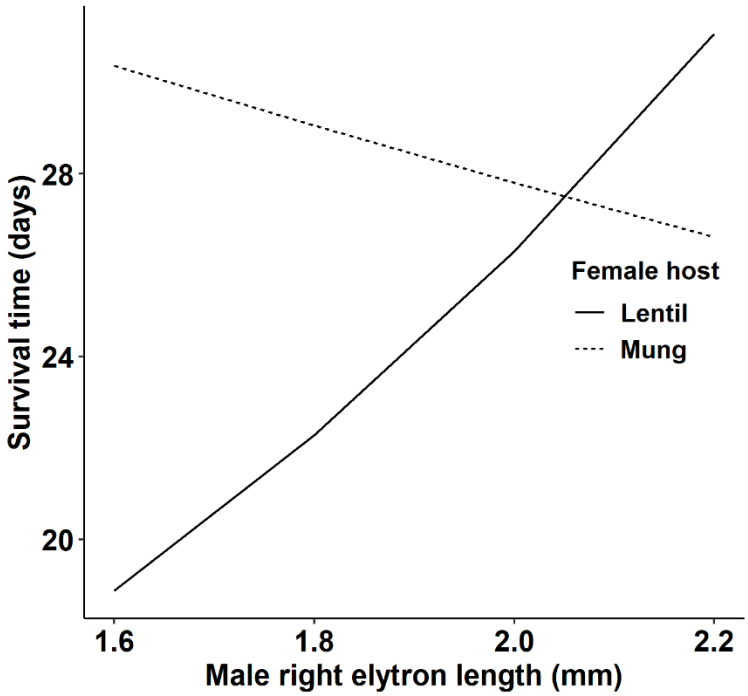


**Figure 14** The interaction plots between female natal host and male right elytron length on survival time of male *C. maculatus* in mated treatment.

Results showed that only oviposited egg number and female natal host [mung 16.36 ± 0.69 days (n = 33), lentil 14.60 ± 0.45 days (n = 50)] significantly influenced female longevity in the MO treatment (Table 19) and only oviposited egg number (negatively) influenced female survival time in the MOF treatment (Table 20). Comparing the survival time after mating of both sexes when females were allowed to oviposit (M males vs MO females), showed that overall individuals from mung bean lived longer [mung 26.38 ± 1.09 days (n = 94), lentil 20.14 ± 0.60 days (n = 117)], males [28.19 ± 0.80 days (n = 116)] lived longer than females [16.48 ± 0.43 days (n = 95)] after mating, and the interaction between natal host and sex was significant (Table 21). Pairwise comparison of the interaction between natal host and sex showed only one non-significant difference between lentil and mung females ($P$ = 0.114) (Figure 15). Comparison between the MO and MOF treatments showed

that feeding [MO 44.92 ± 3.12 (n = 85), MOF 59.67 ± 3.71 (n = 91)] affected female fecundity, but not host quality (Table 22).

**Table 19** Summary of coefficients and model selection based on AIC for models predicting survival time in mated and oviposited treatments (MO) with accelerated failure time models (AFT) with Weibull distribution. Only models with the lowest AIC are shown.

| Dependent variable | Independent variable | Coefficient value ± SE | *P*-value |
|---|---|---|---|
| Survival time | Intercept | 2.92 ± 0.043 | <0.001 |
| | Host (Mung) | 0.11 ± 0.041 | 0.011 |
| | Egg number | -0.004 ± 0.001 | <0.001 |

**Table 20** Summary of coefficients and model selection based on AIC for models predicting survival time in mated, oviposited and fed treatments (MOF) with accelerated failure time models (AFT) with gaussian distribution. Only models with the lowest AIC are shown.

| Dependent variable | Independent variable | Coefficient value ± SE | *P*-value |
|---|---|---|---|
| Survival time | Intercept | 55.98 ± 2.693 | <0.001 |
| | Host (Mung) | 3.68 ± 2.256 | 0.100 |
| | Egg number | -0.13 ± 0.032 | <0.001 |

**Table 21** Summary of coefficients and model selection based on AIC for models predicting survival time between mated and oviposited (MO) females and mated (M) males with accelerated failure time models (AFT) with loglogistic distribution. Only models with the lowest AIC are shown.

| Dependent variable | Independent variable | Coefficient value ± SE | *P*-value |
|---|---|---|---|
| Survival time | Intercept | 2.71 ± 0.032 | <0.001 |
| | Host (Mung) | 0.12 ± 0.052 | 0.024 |
| | Sex (Male) | 0.47 ± 0.046 | <0.001 |
| | Host: Sex | - | 0.016 |

**Table 22** Summary of coefficients and model selection based on AIC for models predicting oviposited egg number in mated and oviposited (MO) and mated, oviposited and fed (MOF) treatments with zero-truncated model with negative binomial distribution. Only models with the lowest AIC are shown.

| Dependent variable | Independent variable | Coefficient value ± SE | *P*-value |
|---|---|---|---|
| Oviposited egg number | Intercept1 | 3.88 ± 0.109 | <0.001 |
| | Intercept2 | 0.31 ± 0.112 | 0.006 |
| | Host (Mung) | -0.21 ± 0.132 | 0.119 |
| | Treatment (MOF) | 0.29 ± 0.132 | 0.028 |

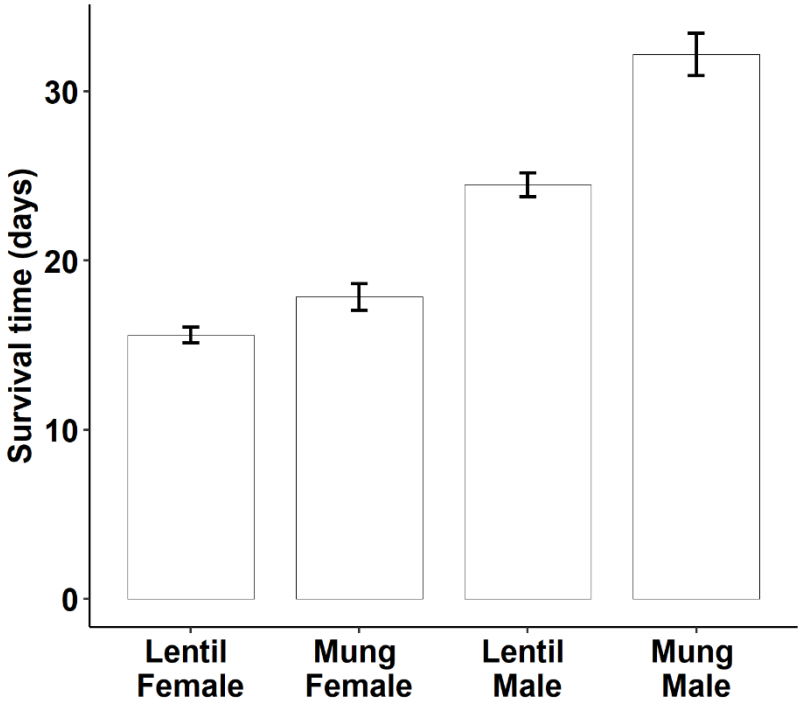


**Figure 15** Mean ± SE of survival time (days) of *C. maculatus* according to natal host and sex. This bar chart derives from the comparison in survival time between mated and oviposited (MO) females and mated (M) males.

## 3.4 Discussion

In this study I investigated the effects of natal host and mating status on the cost of reproduction of both male and female *C. maculatus*. Overall, I found that individuals from mung beans and female beetles (except the comparison between MO females and M males) survived longer than those originating from lentils or were male. I also found that larger individuals tended to live longer than smaller ones. Mated females originating from lentils lived longer when mated with larger males, but this was not so for females emerging from mung beans. Larger males that mated with females from lentils also lived longer, but this was not seen with those mating with females emerging from mung beans. Mating caused a reduction of lifespan for MO females compared with those from the V and M treatments, so my results showed that oviposition is costly in terms of adult female survival. Food provision for adults removed differences in longevity resulting from changes in host quality and enhanced female fecundity, though the effect of food provision still did not fully offset the cost of oviposition.

I consistently found that mung bean is a superior host and results in a longer lifespan for *C. maculatus* than lentil. Dry mung beans and lentils contain similar amounts of carbohydrate, protein and lipid per 100 gram (USDA, 2019a, 2019b), thus nutrient content does not seem to be a factor causing different in host quality for *C. maculatus*. Previous studies showed that host switching *C. maculatus* from mung bean to lentil leads to poor performance in emerging adults (Messina et al., 2009), and this can be explained by the low capacity to detoxify plant secondary metabolites in *C. maculatus* (Rêgo et al., 2020). My study suggested that although my study *C. maculatus* line has thrived on lentil, their fitness

has been reduced, perhaps as a result in investing resources in responding to plant secondary metabolites compared with those in the mung bean line.

Generally, in this study females lived longer than males. In *Callosobruchus* spp., females are larger in size than males (Paukku and Kotiaho, 2008; Małek et al., 2019), so females may have higher energy reserves and this may result in longer lifespan. I also found that larger size for both males and females is associated with increased longevity (Paukku and Kotiaho, 2005; Małek et al., 2019). However, when I compare the longevity of MO females and M males, males lived longer than the females. This suggests that oviposition is a energy demanding activity for adults as mated females need to invest their energy in ovipostion and in egg maturation as *C. maculatus* emerge with some matured eggs in their ovaries (Wilson and Hill, 1989). In nature, mated males may die sooner than mated famales as *C. maculatus* are polygamous (Fricke and Maklakov, 2007); therefore, multiple mating in males can shorten their lifespan (Paukku and Kotiaho, 2005), but multiple matings increases female longevity in starvation conditions (Fox, 1993b).

The interaction between male size and female natal host on male longevity showed that larger males lived longer when mated with females from lentil but not with females from mung bean. Larger males provide larger spermatophores, but the relative mass of spermatophore does not change with male body mass (Małek et al., 2019). Although I found that males from mung bean as a natal host are larger, the effects of natal host played no role (Table 17) in determining the longevity of mated males (as the relative spermatophore mass is constant). To the best of my knowledge, no study has examined if male bean beetles can adjust ejaculate size according to female quality, and further work would be required to consider this knowledge gap. The interaction between female size and natal host on mated

female longevity supports the hypothesis that larger individuals live longer, even though the effect of natal host remains. An interaction between female natal host and male size on the longevity mated females was detected in this study. A previous study found that larger males produced larger nuptial gifts, and larger gifts resulted in increased female longevity (Małek et al., 2019). However, this explanation can only explain why females from lentil lived longer when mated with larger males. The opposite outcome was found with females from mung bean, which exhibited shortened lifespan when mated with larger males. This was unexpected and is worthy of further exploration.

It was not surprising that individuals in the MO treatment had the shortest survival time as they spent a lot of energy on the oviposition process (Cury et al., 2019). I found no difference in longevity between individuals in the V and M treatments, though individuals in the V treatment lived slightly longer. I also observed that some M females oviposited some of their eggs on Eppendorf tubes even though no beans were provided. This result is inconsistent with a previous study finding that single mating is enough to increase female lifespan compared with unmated females (Rönn et al., 2006). My result also suggested that although males ejaculate size decreases over consecutive matings (Rönn et al., 2008), the first ejaculation did not affect males' longevity compared with virgin males. In nature multiple matings may yield different results.

Food provision increased female fecundity and maximised female lifespan in this study, which is consistent with a previous study (Moller et al., 1989), but I failed to detect an interaction of food provision with host quality. Nevertheless, this study showed that food supply can remove the effect of host quality on *C. maculatus* longevity but cannot alleviate

the cost of oviposition as the number of eggs oviposited has a negative effect on female longevity.

This study therefore demonstrates that larval host quality influences longevity of *C. maculatus*, but this is also affected by another key life history event, mating. Individuals emerging from mung beans lived longer, and females also had a longer lifespan, associated with their larger size. Females that have oviposited have reduced adult longevity, and the cost of oviposition cannot simply be offset by food provision. I also showed a cost of mating associated with male size, but this effect differed depending on the females' natal host. My study illustrates how host quality and mating can interact to affect the survivorship of a globally important insect pest, and this may play an important role in determining the population dynamics of *C. maculatus*. Excluding any potential food sources for adults in storage warehouse is also a good cultural method preventing the worse infestation of this pest species.

# Chapter 4 Aphids show interspecific and intraspecific variation in life history responses to host plant infection by the fungal pathogen *Botrytis cinerea*

## 4.1 Introduction

*Botrytis* spp. are globally important fungal plant pathogens, causing disease in >1,400 plant species (Elad et al., 2016), including many economically important crops (Elad et al., 2004). *Botrytis cinerea* is an aggressive necrotrophic fungus that destroys host plants with necrotic lesions (Shaw et al., 2016) and is perhaps the most notorious species of this genus, causing dramatic losses in both pre- and postharvest crops (Dean et al., 2012). *Botrytis cinerea* has been ranked as the second most important fungal pathogen in terms of its scientific and economic value (Dean et al., 2012).

Aphids are among the most important crop pests in temperate regions (van Emden and Harrington, 2007), causing both direct damage to host plants, and indirect damage through acting as vectors of plant viruses and by the production of honeydew which can result in fungal infection and reduce photosynthesis (van Emden, 2013). Aphids show both between and within species variation in their life history responses to environmental factors such as host plant quality (Service, 1984; Stacey and Fellowes, 2002a), temperature (Stacey and Fellowes, 2002b; Stacey et al., 2003), and crowding (Hazell et al., 2005). Such variation will have economic and ecological consequences, affecting which species or genotypes are likely to benefit from such changes (Thompson, 1988; Bolnick et al., 2011; Des Roches et al., 2018).

Fungal plant pathogen infection can alter host plant quality as experienced by herbivores by inducing biochemical defense responses inside the host plant. These

biochemical responses can also negatively affect herbivorous insects (Fernandez-Conradi et al., 2018; Ederli et al., 2021) and indeed may also affect species at higher trophic levels (Ngah et al., 2018; Srisakrapikoop et al., 2020). Nevertheless, the effect of plant pathogen infection may also benefit some insect herbivores (Tack and Dicke, 2013), and herbivores may differ in their responses to host plants infected by different plant pathogens (e.g. positive effects found for *Aphis fabae* feeding on *Vicia faba* infected by *Uromyces viciae-fabae*, but negative effects are seen when *V. faba* is infected by *B. cinerea*; Al-Naemi and Hatcher, 2013).

What is not clear is whether different species of aphid or aphid genotypes differ in the life history consequences of feeding on the same host plant species infected by the same plant pathogen. I addressed these questions using two aphid species (the black bean aphid *Aphis fabae* and the pea aphid *Acyrthosiphon pisum*), and three clones of the latter species, asking if host plant infection status influenced the size, fecundity, maturation time and off-plant survival of the study aphids.

## 4.2 Materials and methods

*Botrytis cinerea* Pers.: Fr (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel) was cultured on malt extract agar and incubated at 20°C under conditions of 12 h UV light : 12 h dark (LD 12:12) to encourage the fungus to produce spores.

The host plants, *Vicia faba* L. (Fabaceae, cv. Sutton dwarf), were individually grown in 1 L pots with peat compost (Clover®, UK). When the plants had 5 true leaves, they were divided into two treatment groups. Plants in the infected group were treated with a 0.1 ml suspension of one-month old *B. cinerea* ($10^6$ conidia/ml) on the adaxial surfaces of the leaves using a paint brush. Uninfected plants were treated with distilled water in a similar manner.

Plants were then kept individually in a sealed polythene bag at 20°C for 48 h to encourage spore germination.

A single black bean aphid *Ap. fabae* Scop. (Hemiptera: Aphididae) was collected from opium poppy *Papaver somniferum* L. (Papaveraceae), while three pea aphid *Acyrthosiphon pisum* Harris (Hempitera: Aphididae) clones were collected from bird's-foot trefoil *Lotus corniculatus* L. (Fabaceae), from three widely separated locations, all in the University of Reading Whiteknights campus, UK. The two aphid species were identified and confirmed following Blackman and Eastop (2000). Aphid cultures were maintained as a monoclonal culture in separate insect cages and provided with either uninfected or infected *V. faba* plants for more than three generations before the experiments started to avoid confounding maternal effects. All work was conducted in a controlled environment room at 20$^{o}$C, LD 16:8, 60% RH.

4.2.1 *Aphis fabae* experiment

For the black bean aphid experiment, treatments comprised of five uninfected or five infected plants, each of which held eight aphids. Each aphid was confined individually in a clip cage (20 mm in diameter; Noble, 1958) attached to individual leaflets (total 40 aphids/infection status; aphids were transferred from culture plants of the same infection status to avoid confounding maternal effects). Aphids were left to produce nymphs for 24 hours, then all apart from one nymph were removed, which was allowed to grow to maturity and produce offspring.

Time to maturity was recorded and the number of offspring produced was then recorded every second day for ten days. During each visit nymphs were removed to prevent competition. The intrinsic rate of increase (rm) was calculated from the formula rm = [c ln

(Md)]/D, where c is a constant (0.738), Md is the number of offspring produced by the adult aphid in the D days of reproduction (Wyatt and White, 1977).

Separately, 80 7-day-old aphids (40 from each treatment) were randomly selected from cultures and transferred into individual Petri dishes without food or water and monitored every 8 hours until death to yield off-plant survival time. Another 80 7-day-old aphids (40 from each treatment) were used to measure hind tibia length (Nicol and Mackauer, 1999) under a high-performance stereomicroscope (Leica MZ9.5).

4.2.2 *Acyrthosiphon pisum* experiment

For the pea aphid experiment, ten adult apterous pea aphids from each of the three clones were randomly selected from each of the base culture colonies feeding on uninfected and infected plants (60 aphids in total). Each aphid was placed into an individual clip cage (40 mm in diameter) directly onto an individual plant of the same colony infection status. Fecundity and intrinsic rate of increase were recorded in the same manner as described above, except the number of offspring were recorded every other day for fourteen days.

In addition, aphid off plant survival time, hind tibia size, days to maturity and the intrinsic rate of increase were also recorded again in a similar manner as for *Ap. fabae*. A total of 180 nymphs (30 from each clone and treatment) were allowed to grow for seven days, reaching the $4^{th}$ instar stage. When the individuals were transferred into a Petri dish without food or water, they were monitored every 12 hours until death. Forty 7-day-old aphids (from each clone and treatment) were used to measure hind tibia length under a high-performance stereomicroscope (Leica MZ9.5).

### 4.2.3 Data analyses

All statistical analyses were performed on R 4.0.3 (R Core Team, 2020). For the *Ap. fabae* experiment, as the data are not normally distributed, Wilcoxon rank sum tests were used to test for differences in hind tibia length and off-plant survival time between infected and uninfected plants. Initial examination of the data showed that the effect of nested data could be ignored as the variances resulting from different plants were very close to zero, and then the intrinsic rate of increase, fecundity and maturation time data could also be analysed by using Wilcoxon rank sum tests.

In the *Ac. pisum* experiment the hind tibia length, off plant survival time, maturation time and intrinsic rate of increase data were analysed using analysis of variance of aligned rank transformed using the *ARTool* package (Wobbrock et al., 2011) as data were not normally distributed. Fecundity data were analysed by analysis of variance (ANOVA) using *car* package (Fox and Weisberg, 2019). Post hoc analyses with Tukey tests were analysed using the *emmeans* package (Lenth, 2019) or with Mann-Whitney U test to examine clonal variation within infection status group.

## 4.3 Results

*Aphis fabae* feeding on plants infected by *B. cinerea* had significantly shorter hind tibia length (W = 1148, $n_1$ = 40, $n_2$ = 39, $P < 0.001$; Figure 16a), reduced off-plant survival time (W = 509, $n_1$ = 40, $n_2$ = 39, $P = 0.007$; Figure 16b), slower development rates (W = 402, $n_1$ = 38, $n_2$ = 33, $P = 0.016$; Figure 16c), reduced intrinsic rate of increase (W = 880, $n_1$ = 38, $n_2$ = 33, $P = 0.004$; Figure 16d) and lower fecundity (W = 880, $n_1$ = 38, $n_2$ = 33, $P = 0.004$; Figure 16e).

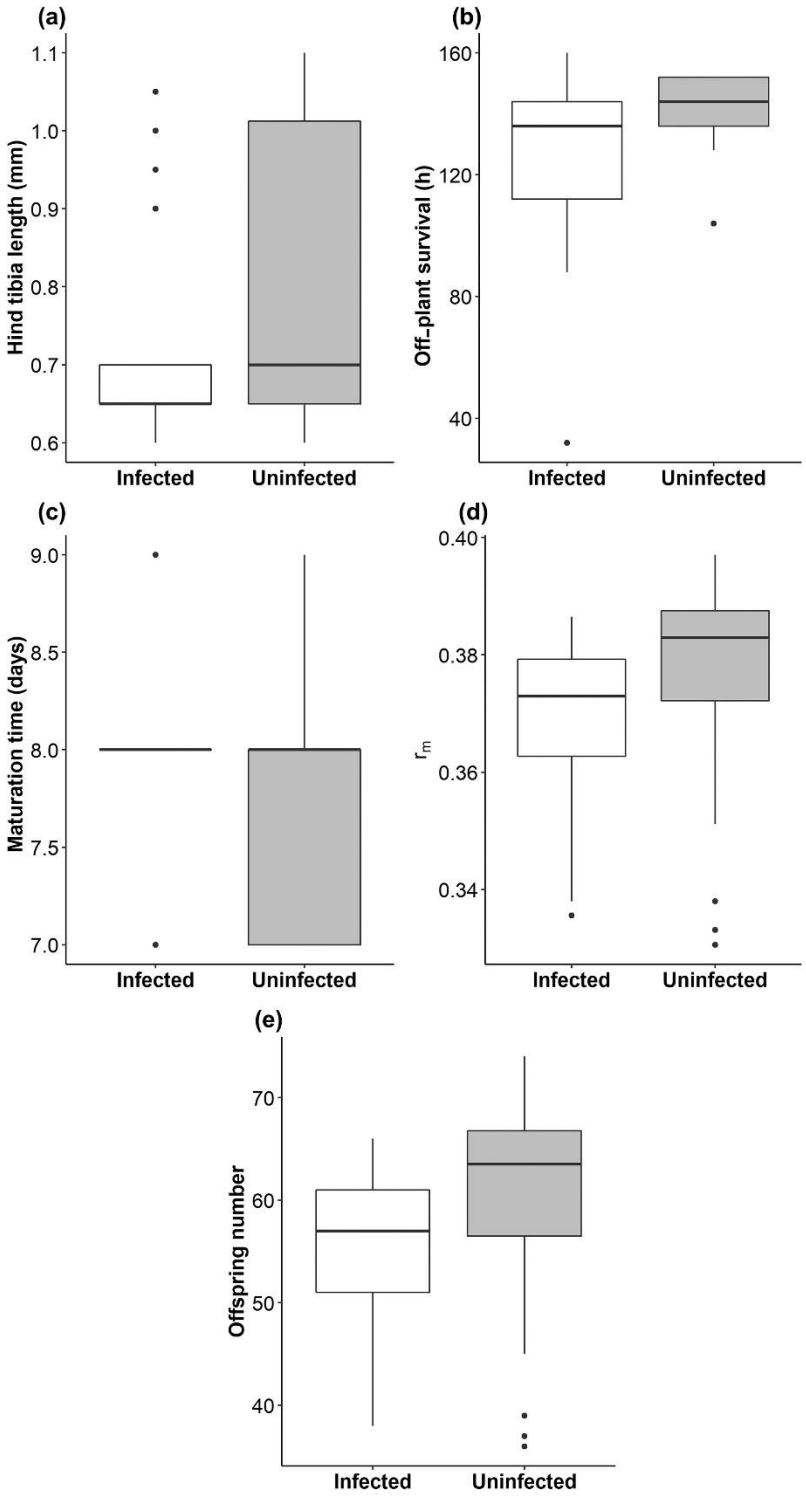


**Figure 16** Medians, interquartiles and range of (a) hind tibia length, (b) off plant survival time, (c) maturation time, (d) intrinsic rate of increase and (e) fecundity of *Ap. fabae* feeding on *B. cinerea* infected and uninfected host plants.

Pea aphids feeding on plants infected by *B. cinerea* had significantly longer hind tibia lengths than those on uninfected plants ($F_{1,226} = 49.17$, $P < 0.001$; Figure 17a). There was no overall effect of aphid clone on hind tibia length ($F_{2,226} = 2.01$, $P = 0.137$), but the interaction term was significant ($F_{2,226} = 3.87$, $P = 0.022$). There was no effect of host plant infection status on off-plant survival time ($F_{1,173} = 1.35$, $P = 0.246$), but this differed between clones ($F_{2,173} = 61.49$, $P < 0.001$; Figure 17b), and there was a significant interaction effect of clone and host plant infection status on off-plant survival ($F_{2,173} = 4.68$, $P = 0.011$). Time to maturity differed among clones ($F_{2,54} = 34.82$, $P < 0.001$; Figure 17c), and differed between plant infection status ($F_{1,54} = 10.82$, $P = 0.002$). The interaction term was significant ($F_{2,54} = 13.73$, $P < 0.001$). The intrinsic rate of increase differed between pea aphid clones ($F_{2,38} = 4.09$, $P = 0.025$; Figure 17d) and was significantly higher on infected plants ($F_{1,38} = 34.95$, $P < 0.001$). The interaction term was significant ($F_{2,38} = 7.20$, $P = 0.002$). The fecundity of aphids feeding on infected plants was significantly higher than those feeding on uninfected plants ($F_{1,40} = 13.95$, $P < 0.001$; Figure 17e), but there was no clone ($F_{2,40} = 1.30$, $P = 0.283$) nor interaction effect ($F_{2,38} = 1.04$, $P = 0.364$).

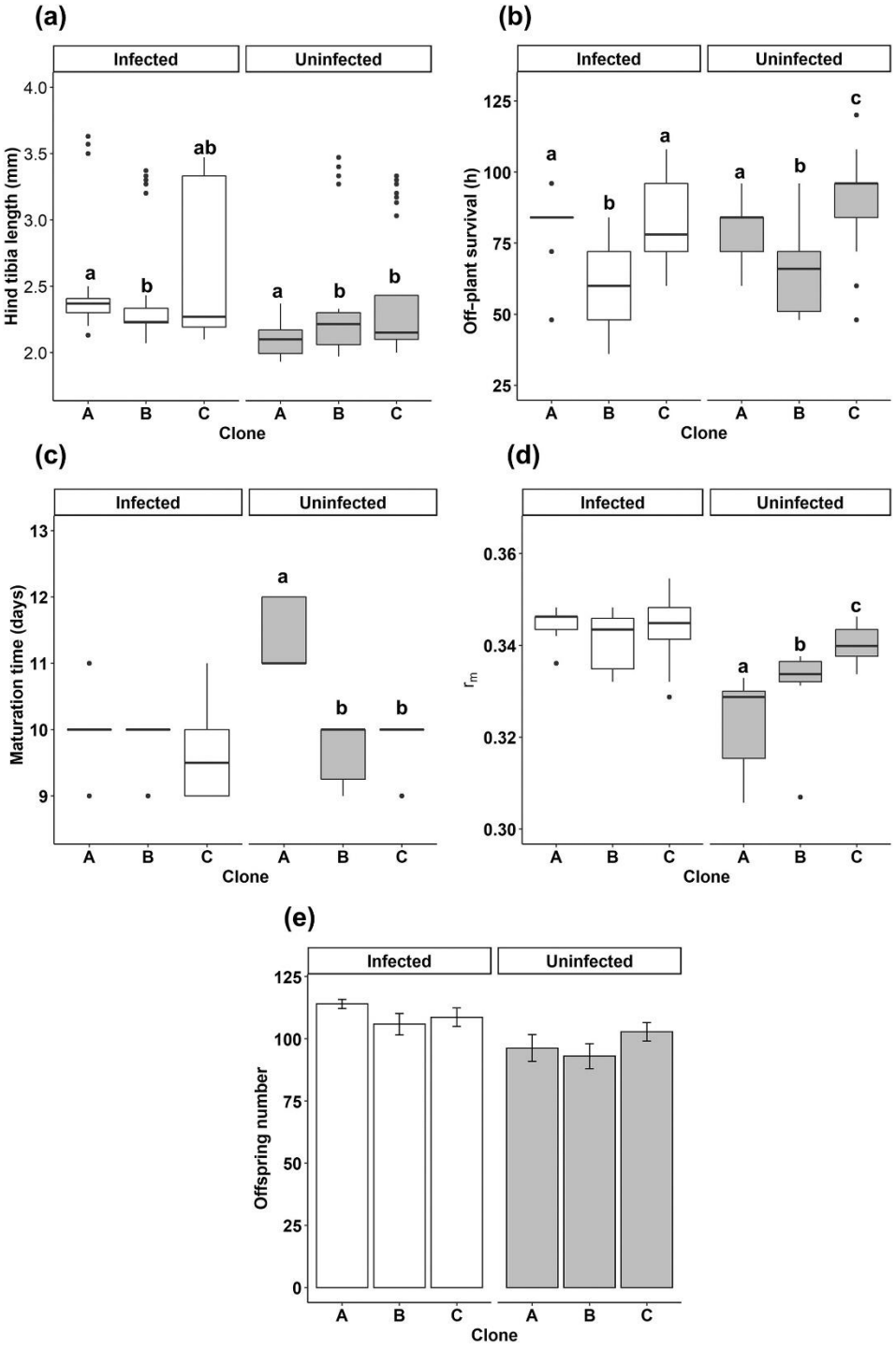


**Figure 17** Medians, inter-quartiles and range of (a) hind tibia length, (b) off-plant survival time, (c) maturation time, (d) intrinsic rate of increase, and (e) mean ± SE fecundity for three *Ac. pisum* clones (A, B and C) feeding on *B. cinerea* infected and uninfected host plants. The letters above the bars show traits where a significant difference ($P < 0.05$) was found within infection status group.

## 4.4 Discussion

There is increasing evidence showing that plant pathogens play important roles in determining the interaction between insects and their host plants, and indeed these effects can ramify through communities (Grunseich et al., 2020; Srisakrapikoop et al., 2020). In this study, I find that two aphid species respond in different directions to the same plant pathogen-host system. Host plant infection by *B. cinerea* caused negative indirect effects on *Ap. fabae* for all measured parameters, while infection caused positive indirect effects on *Ac. pisum*, and here the magnitude of these effects differed between aphid clones. The indirect effects of plant pathogen infection therefore differ both between and within aphid species.

*Aphis fabae* feeding on uninfected plants performed better than those feeding on infected plants in all measured parameters, which is consistent with a previous study with a similar system (Al-Naemi and Hatcher, 2013). *Acyrthosiphon pisum* expressed the reverse pattern, benefitting from feeding on infected plants, and these effects were consistent, but differed in magnitude, across clones. The mechanisms underpinning the between species differences are not clear, given that both are generalist aphid species, which feed in a similar manner, and the host plant/pathogen treatment was controlled. The ultimate cause of this variation is worthy of more detailed study, as it suggests that the population dynamics of different aphid species may be differentially affected by pathogen infection.

While the causes of the differences in response are unclear, I note that the indirect effects of *B. cinerea* on aphid life histories may be transmitted in two (non-independent) pathways, either via a change in host nutrition and/or in host defense. The former is caused by a change in nitrogen content, which is decreased by fungal infection (Dulermo et al., 2009; Al-Naemi and Hatcher, 2013), and the latter is induced resistance, where the plant responds

to infection via two signaling routes, the Salicylic Acid (SA) and Jasmonic Acid (JA) pathways (Pieterse et al., 2014). The SA pathway is usually employed by plants to respond to sucking/piercing herbivores (including aphids), while the JA pathway is upregulated in response to necrotrophic pathogens and chewing herbivores (Pieterse et al., 2014; Stout, 2014), and these pathways are considered to trade-off against each other (Spoel et al., 2003; Brooks et al., 2005).

In this study, the plants had been first infected by *B. cinerea* before aphids were introduced, so it is likely that the JA pathway was triggered, suppressing the SA pathway (Leon-Reyes et al., 2010). Theoretically, aphids should benefit from this, but here *Ap. fabae* showed reduced performance when feeding on infected plants, perhaps because aphids are not only affected by outcomes of suppressing the SA pathway (Thaler et al., 2010), but also by the elicited JA pathway (Thaler et al., 2001; Goggin and Cooper, 2005). Aphid species may therefore differ in their responses to changes in nutrition and plant defenses. In contrast, variation in the strength of within species responses between aphid clones is expected, given the importance of both genetic and endosymbiotic factors (Stacey et al., 2003; Hazell and Fellowes, 2009; Heyworth et al., 2020).

This work is of considerable applied interest, as I show that the effects of infection by an economically very important plant pathogen on the life histories of two related and also economically important aphid pest species differ. While feeding on *B. cinerea*-infected host plants benefits pea aphids (albeit the strength of this varies across clones), black bean aphids are detrimentally affected by infected host plants. The cause of this difference between species is unclear. This may be the result of differences in the direct physiological effects of host plant quality on the developing aphids, or an indirect effect mediated by

differences in other factors, such as the composition of the aphid's endosymbiont community. The links between plant pathogens and insect herbivores are rarely simple; elucidating the mechanisms that result in such unexpected contrasting effects would be of considerable value.

# Chapter 5 Urbanisation and plant pathogen infection interact to affect the outcome of ecological interactions in an experimental multitrophic system

## 5.1 Introduction

With over half of the world's people now living in towns and cities, urban areas have rapidly expanded to accommodate growing populations (Goddard et al., 2010). Urban growth is associated with habitat loss and degradation, increased habitat heterogeneity, fragmentation, disturbance, the emergence of novel habitats and introduced species, and an increase in impervious surfaces (Goddard et al., 2010; Kowarik, 2011). These factors in turn greatly affect the abundance, distribution and patterns of ecological interactions between organisms, such as insects (Rocha and Fellowes, 2018, 2020; Rocha et al. 2018). Insects provide excellent model systems for examining how urbanisation affects ecological interactions between predators and prey, hosts and parasitoids, plants and herbivores, hosts and pathogens, and between mutualistic species. Insects also lend themselves to more powerful experimental approaches to explore the effects of urbanisation on ecological interactions.

The key drivers that alter insect community structures vary across urban areas. In heavily urbanised temperate areas, host plant scarcity and fragmentation drives a reduction in overall insect abundance, with specialist predators and parasitoids particularly affected (Peralta et al., 2011; Bennett and Gratton, 2012; Turrini et al., 2016; Rocha and Fellowes, 2018, 2020). The diversity of host plants tends to reach a peak in suburban settings with high proportions of private domestic gardens, where ornamental species are widely planted (Thompson et al., 2003; McKinney, 2008; Čepelová and Münzbergová, 2012). As a result, suburban areas can have the remarkable diversity of insect herbivores (Owen and Owen,

1975; Raupp et al., 2010), in turn driving increased numbers of natural enemies. As we reach peri-urban areas, a wide range of suitable habitats and less fragmented areas predominate, with increasing numbers of butterfly species (Tzortzakaki et al., 2019), before we move into heavily altered agricultural land, where insect diversity can decline.

It is not simply habitat availability that affects the structure of insect communities. Urbanisation can have more subtle effects, modulating changes in insect (particularly herbivore) abundance through changes in host plant quality (Raupp et al., 2010). For example, urban heat island effects, the introduction of exotic plants, increased soil nitrification, pollution and plant drought stress (Raupp et al., 2010; Long et al., 2019) can change plant quality and therefore result in both positive and negative effects on insect herbivores (Leather et al., 1998; Herms, 2002; Huberty and Denno, 2004) and in turn their natural enemies (Vollhardt et al., 2019; Zhu et al., 2020).

In many terrestrial ecosystems, plant pathogen infection can be an important direct (by reducing plant diversity and abundance) and indirect (by changing host plant quality and therefore influencing herbivore host choice and fitness) driver of insect community structure (Srisakrapikoop et al., 2020). For example, the fungal pathogen *Hymenoscyphus fraxineus* causes devastating ash dieback on European ash (*Fraxinus excelsior*) and this effect cascades to associated species (Hultberg et al., 2020). Furthermore, plant pathogen presence and infection are also likely to be affected by urbanisation given the effects of air pollution on plant susceptibility and pathogen population dynamics (Heagle, 1973; Manning, 1975; Bearchell et al., 2005). What is unstudied is whether urbanisation alters the effects of plant pathogen infection on insect community structure.

*Botrytis* species are among the most important plant pathogens, and they have a worldwide distribution (Williamson et al., 2007). *Botrytis cinerea* is a widespread necrotrophic pathogen causing soft rot in host plants and infects more than 200 species of host plants, causing pre and post-harvest damage (plant disease and rotting) to many commercial plants, including ornamentals, fruits, and vegetables, as well as stored and transported agricultural products (Elad et al., 2004; Williamson et al., 2007). It is suggested that *Botrytis cinerea* growth rates are reduced by some air pollutants, including $SO_2$ (Couey and Uota, 1961; Xue and Yi, 2018) and ozone (Violini, 1995), and so infection may be less frequent in urban areas.

To date, how urbanisation affects ecological interactions in systems than include plant pathogens has not been studied. To address this gap in knowledge, I performed a field experiment to investigate the respective and possibly interacting effects of urbanisation and plant pathogen infection on interactions between an insect herbivore, its host plant, natural enemies and mutualists.

## 5.2 Materials and methods

### 5.2.1 Study sites

The three study sites were located on grounds belonging to the University of Reading, Berkshire, UK. The London Road campus (UoRL), Whiteknights campus (UoRW) and Centre for Dairy Research (CEDAR), representing urban, suburban and rural habitats, respectively. The urban site is located in Reading town centre (51°26'58.0"N, 0°57'43.6"W) and is surrounded by impervious surfaces made up of buildings and roads. The planted area is predominantly grass lawn, with ornamental shrubs and some large deciduous trees. The UoRW (51°26'13.1"N, 0°56'31.6"W) site is in mature suburbs and is surrounded by a mix of

impervious surfaces and domestic gardens and mixed-use green space. The campus landscape is composed of meadows, urban woodland and diverse ornamental planting. CEDAR (51°24'46.3"N, 0°54'37.6"W) is largely dairy farmland situated south of the suburbs and is largely surrounded by agricultural pastureland and hedgerows. The location of the study sites is shown in Figure 18.

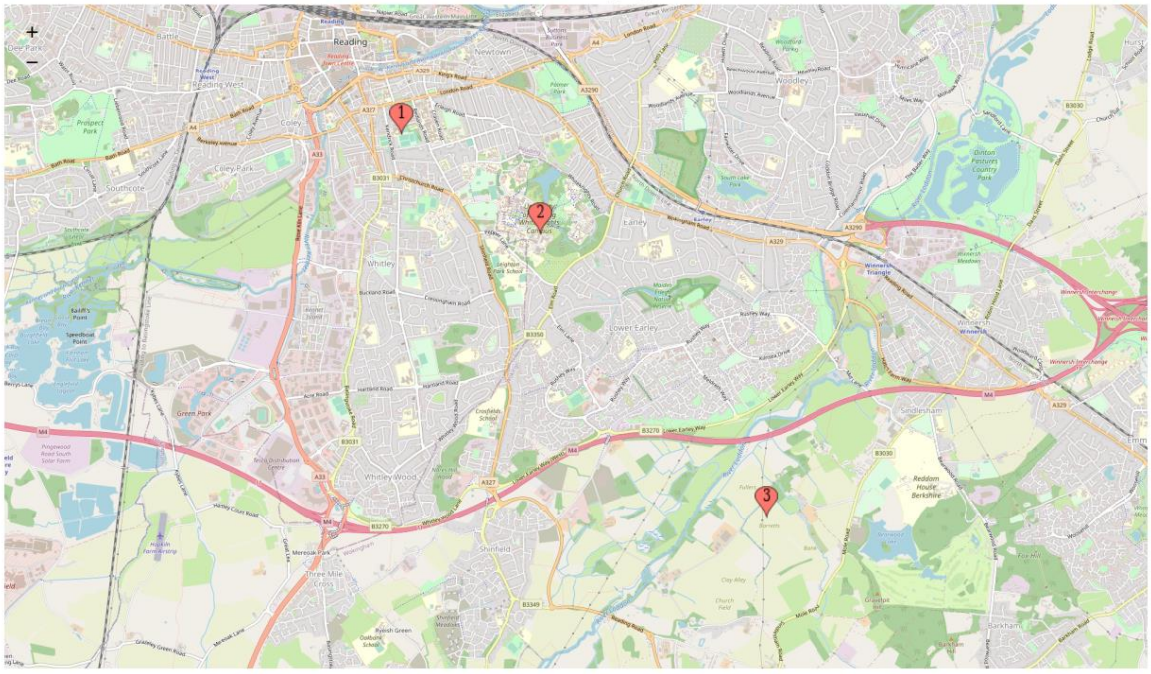


**Figure 18** Location of study sites in Greater Reading, England. The map was obtained from www.mapcustomizer.com. Pinned location number 1, 2 and 3 represent the London Road campus (UoRL), Whiteknights campus (UoRW) and Centre for Dairy Research (CEDAR), respectively.

5.2.2 Study system

A single Black bean aphid, *Aphis fabae*, was collected in July 2018 from the University of Reading Whiteknights campus. A monoculture was reared and maintained on Broad bean, *Vicia faba* (Fabaceae, cv. Sutton Dwarf) in a laboratory with ambient temperature and light. A monoclonal culture was used to avoid the variation in predator-prey interactions seen between aphid clones (Hazell and Fellowes, 2009).

*Botrytis cinerea* pepper isolate (Denby et al., 2004) was cultured on apricot halves in grape juice (Del Monte®) and incubated at 20°C with 12 hours of UV light and 12 hours of dark cycle to encourage the fungus to produce spores.

*Vicia faba* (Fabaceae, cv. Sutton Dwarf) plants were grown individually in 1 L pots with pot/bedding peat compost (Clover®) in a controlled environment (CE) room 20°C with 16:8 L:D light cycle at 60% RH. When the plants had four true leaves, they were divided into two groups. One group of 150 plants was left uninfected (treated only with water) and the other 150 were inoculated with 0.1 ml of $10^5$ conidia/ml of *B. cinerea* suspension on adaxial surface with a paint brush to produce the infected group. Each plant was kept in an individually sealed polythene bag at 20°C for 48 h to encourage spore germination. Three adult aphids were placed on every uninfected and infected plant and were then kept in the CE room for seven days before moving them to the study sites.

The study was conducted during May 2019. One hundred plants (50 infected and 50 uninfected) were randomly selected for each site and then each plant was randomly placed within a grid one meter apart at the site. Plants in the CEDAR and UoRL sites were placed in the field on the same day, and for logistical reasons plants in UoRW were placed in the field the next day. Each plant was enclosed by a cylinder (50 cm in height) made of chicken

wire (25 mm mesh) to prevent access by vertebrate herbivores. The aphids and plants were left to acclimatise for three days before data collection began. Data were collected five times every four days, rotating from CEDAR, UoRL, and UoRW, with the fourth day taken as a break. The numbers of aphids, ants, predators and mummified aphids were recorded on each day of counting (session). Predators were identified using appropriate keys (Rotheray, 1989, 1993; Roy and Brown, 2018). Mummified aphids were collected and kept separately in an Eppendorf tube. Emerged parasitoids were sorted into morphospecies and sent to the Natural History Museum London for formal identification.

The chlorophyll content (a measure of plant quality: Curran et al., 1990; Filella et al., 1995) of each plant was measured once using a chlorophyll content meter (Hansatech Instruments, Model CL-01) in the field at the end of the experiment, before the above ground parts of the plants were harvested. All the plants were removed on the same day from all sites a month after they were placed *in situ*. They were put in a hot air oven at 70°C until they reached constant mass. Each plant was weighed to get the above ground plant dry mass.

### 5.2.3 Data analyses

All statistical analyses were performed using R 4.0.3 (R Core Team, 2021). Some plants and some aphid colonies died before the end of data collection; these replicates were excluded from the data analyses, yielding 91 plants at CEDAR (46 uninfected and 45 infected plants), 87 plants at UoRL (45 uninfected and 42 infected plants), and 90 plants at UoRW (44 uninfected and 46 infected plants).

#### 5.2.3.1 Aphid abundance

Aphid abundance was analysed using a generalised linear mixed model (GLMM) with negative-binomial distribution family (nbinom2) with a log link, using the *glmmTMB*

package (Brooks et al., 2017). Infection status, site, number of ants and natural enemies were used as fixed effects and session was treated as a random effect.

5.2.3.2 Ant and natural enemy abundance

To deal with excessive zeros in the ant and natural enemy models, a zero-inflated generalised linear mixed model (ZIGLMM) in the *glmmTMB* package (Brooks et al., 2017) was adopted with a negative binomial distribution family (nbinom2) for the count part, and with a binomial distribution with a logit link for the binary part. Session was also used as a random effect for the count part, while session and site were used for the binary part. Adding the binary part did enhance model fit by reducing the AIC value. Zeros can be accounted for in both the count part (true zeros), and binary part to explain the probability of false zeros which may occur from design, survey and observer errors (Zuur et al., 2009).

5.2.3.3 Chlorophyll content and above ground plant dry mass

Plant chlorophyll content was compared between uninfected and infected groups using independent-sample *t*-tests. Plant above ground dry mass was analysed using cumulative data set consisting of the total number of aphids recorded. Plant dry mass was modelled using a generalised linear model (GLM) with a Gamma distribution with the identity link with infection status, site and the cumulative numbers of natural enemy and aphid as fixed effects in a global model.

The aphid and plant above ground dry weight models were generated by dredge function in *MuMIn* package (Barton, 2019) from the global models. Model selection was then based on Akaike's Information Criteria (AIC) by comparing all candidate models (Burnham and Anderson, 2003). This was not done for ant and natural enemy models due to the complex structure of ZIGLMM containing two parts to model excessive zeros. Instead,

these models were built by adding all independent variables, then dropping the non-significant terms and adding the ecologically meaningful interactions to see if the interaction terms did improve AIC values.

For all models with multiple candidate models the best models were determined by delta AIC within 2 units ($\Delta_i < 2$) from the lowest AIC (best model) were considered as candidate models. Akaike weights were also calculated, which can suggest the overall importance of a model. A higher weighting signifies a greater probability the model is the best model (Anderson et al., 2000).

In all of the analyses, *multcomp* (Hothorn et al., 2008) or *emmeans* (Lenth, 2021) packages with post-hoc Tukey tests were used to determine the significant differences between means of dependent variables across study sites. Independent variables were checked for collinearity through checking variance inflation factors (VIF). If VIF values were higher than three it indicated there was collinearity between independent variables (Zuur et al., 2007). From my data, collinearity was not detected as VIF values ranged from 1.02 to 1.22.

## 5.3 Results

During the study, 398,586 aphids, 2,039 ants and 861 insect natural enemies (746 predators (86.64%) and 115 parasitoids (13.36%)) were recorded. All ants attending aphids were black garden ants (*Lasius niger*). Hoverfly larvae (Diptera: Syrphidae) were the most abundant predators (75.91%), followed by parasitoids (13.45%; Hymenoptera), seven-spot ladybirds (4.21%; *Coccinella septempunctata*), harlequin ladybirds (3.74%; *Harmonia axyridis*), common flower bugs (1.75%; *Anthocoris nemorum*), fourteen-spot ladybirds (0.58%; *Propylea quattuordecimpunctata*), lacewings (0.23%; Family: Chrysopidae) and

rove beetles (0.12%; Coleoptera: Staphylinidae). In terms of natural enemy diversity, UoRW had the most diversity, followed by CEDAR and UoRL, respectively (Table 23).

**Table 23** Natural enemy diversity found at each study site. (UoRW = University of Reading Whiteknights campus; UoRL = Reading London Road campus; CEDAR = University of Reading Centre for Dairy Research).

| Site | Family | Species |
|---|---|---|
| Rural-CEDAR | Diptera: Syrphidae | *Syrphus ribesii* (Linnaeus) |
| | | *Episyrphus balteatus* (De Geer) |
| | Coleoptera: Coccinellidae | *Harmonia axyridis* (Pallas) |
| | | *Coccinella septempunctata* (Linnaeus) |
| | | *Propylea quatuordecimpunctata* (Linnaeus) |
| | Neuroptera: Chrysopidae | *Chrysoperla carnea* (Stephens) |
| Suburban-UoRW | Diptera: Syrphidae | *Syrphus ribesii* (Linnaeus) |
| | | *Episyrphus balteatus* (De Geer) |
| | | *Epistrophe eligans* (Harris) |
| | Coleoptera: Coccinellidae | *Harmonia axyridis* (Pallas) |
| | | *Coccinella septempunctata* (Linnaeus) |
| | | *Propylea quatuordecimpunctata* (Linnaeus) |
| | Coleoptera: Staphylinidae | *Tachyporus* sp. |
| | Hemiptera: Anthocoridae | *Anthocoris nemorum* (Linnaeus) |
| | Hymenoptera: Aphidiinae | *Praon volucre* (Haliday) |
| | | *Lysiphlebus fabarum* (Marshall) |
| | Charipinae | *Alloxysta* sp. |
| Urban-UoRL | Diptera: Syrphidae | *Syrphus ribesii* (Linnaeus) |
| | | *Episyrphus balteatus* (De Geer) |
| | Coleoptera: Coccinellidae | *Harmonia axyridis* (Pallas) |
| | | *Propylea quatuordecimpunctata* (Linnaeus) |
| | Hymenoptera: Aphidiinae | *Praon volucre* (Haliday) |

### 5.3.1 Aphid abundance

Although there were two candidate models, the first model showed the lowest AIC with substantial Akaike weight support (Table 24). Aphid numbers on infected plants were significantly lower than those on uninfected plants, and there was a positive relationship between aphid abundance and ant numbers. There was also an effect of site on the number of aphids in which the suburban site (UoRW) had higher aphid number than the other two sites (Table 25). The significant interaction term between natural enemies and site suggests that the effect of natural enemies depends on study site, which is consistent with the natural enemy model where natural enemy abundance was different across the study sites. In addition, the mean aphid number on different plant infection status in three different sites over five sampling sessions is shown (Figure 19).

**Table 24** Summary of coefficients and model selection based on AIC for models predicting aphid abundance with GLMM with negative-binomial distribution family, and session as a random effect. Only models with $\Delta_{AIC} < 2$ units are shown. The model in bold is considered as the best model. W is the Akaike weight. (Ants = ant number; Inf = infection status; NE = natural enemies; Site = study sites). An asterisk signifies the coefficients and *P*-values are derived when compared with a reference level for each categorical variable. (Note that the coefficients of the interaction terms with one categorical variable with more than two levels cannot be yielded).

| Dependent variable | Independent variables | Coefficient value ± SE | *P*-value | AIC | $\Delta_{AIC}$ | W |
|---|---|---|---|---|---|---|
| **Aphids** | **Intercept** | **5.387 ± 0.339** | **< 0.001** | **15742.4** | **0** | **0.723** |
| | **Ants** | **0.028 ± 0.005** | **< 0.001** | | | |
| | **Inf (Uninfected)** | **0.124 ± 0.048*** | **0.0104*** | | | |
| | **NE** | **0.018 ± 0.030** | **0.5550** | | | |
| | **Site (urban-UoRL)** | **-0.103 ± 0.055*** | **0.0619*** | | | |
| | **Site (suburban-UoRW)** | **0.365 ± 0.052*** | **<0.001*** | | | |
| | **NE:Inf (Uninfected)** | **-0.039 ± 0.019*** | **0.0402*** | | | |
| | **Inf:Site** | **-** | **0.0242** | | | |
| | **NE:Site** | **-** | **<0.001** | | | |
| Aphids | Intercept | 5.387 ± 0.339 | < 0.001 | 15744.3 | 1.91 | 0.277 |
| | Ants | 0.030 ± 0.007 | < 0.001 | | | |
| | Inf (Uninfected) | 0.124 ± 0.048* | 0.0103* | | | |
| | NE | 0.018 ± 0.030 | 0.5461 | | | |
| | Site (urban-UoRL) | -0.108 ± 0.057* | 0.0606* | | | |
| | Site (suburban-UoRW) | 0.363 ± 0.052* | <0.001* | | | |
| | Ants:Inf (Uninfected) | -0.003 ± 0.010* | 0.7695* | | | |
| | NE:Inf (Uninfected) | -0.040 ± 0.019* | 0.0383* | | | |
| | Inf:Site | - | 0.0323 | | | |
| | NE:Site | - | <0.001 | | | |

**Table 25** Mean ± SE of aphid number (over five sessions) for each of the three study sites. Superscript alphabets indicated significant differences between groups calculated from post-hoc Tukey test.

| Dependent variable | Site | Mean ± SE |
|---|---|---|
| Aphids | Rural-CEDAR | 291.00 ± 11.93[a] |
| | Suburban-UORW | 329.50 ± 12.54[b] |
| | Urban-UORL | 313.20 ± 15.13[a] |

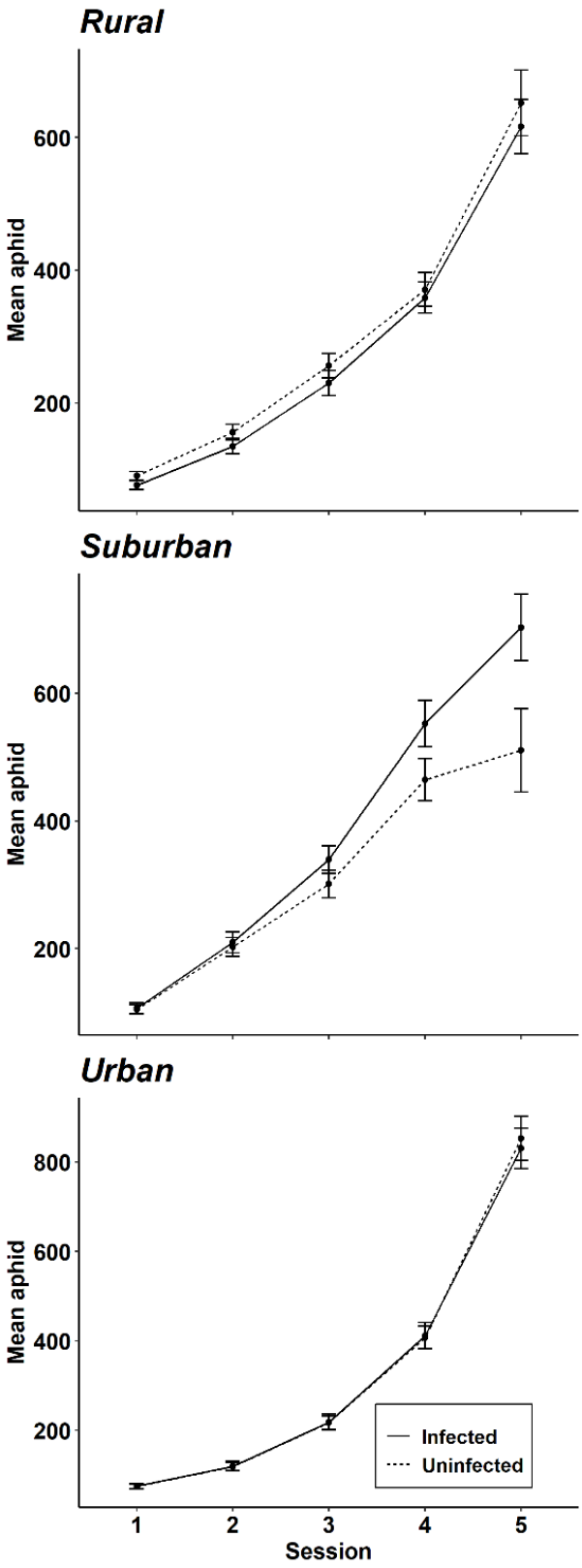


**Figure 19** Mean ± SE aphid numbers on plants with infected or uninfected status in three different study sites over five sampling sessions.

### 5.3.2 Ant abundance

At the rural site, ants were found tending aphids on 3 out of 91 plants (3.30%), at the suburban site on 66 out of 90 plants (73.33%), and at the urban site on 82 out of 87 plants (94.25%). The best model included the number of aphids, site, number of natural enemies, interaction between site and number of natural enemies, and interaction between site and number of aphids as independent variables (Table 26). Aphids and natural enemy numbers were positively associated with ant numbers, while the number of ants was significantly different across the study sites, ant abundance was highest at the urban site (UoRL) followed by suburban (UoRW) and rural (CEDAR) (Table 27). Again, the significant interaction term between site and natural enemies also showed that the effect of natural enemies on ant abundance depends on site.

**Table 26** Summary of coefficients of the best models selected based on the AIC for models predicting ant abundance with ZIGLMM with negative binomial distribution family (nbinom2), session as a random effect and site and session in binary part. Only models with $\Delta_{AIC} < 2$ units are shown. (Aphids = aphid number; NE = natural enemies; Ants = ant number; Site = study sites). An asterisk signifies the coefficients and *P*-values are derived when compared with a reference level for each categorical variable. (Note that the coefficients of the interaction terms with one categorical variable with more than two levels cannot be yielded).

| Dependent variable | Independent variables | Coefficient value ± SE | *P*-value | AIC |
|---|---|---|---|---|
| Ants | Intercept | -3.650 ± 1.141 | 0.0014 | 3019.656 |
| | Aphids | 0.002 ± 0.000 | < 0.001 | |
| | NE | 1.360 ± 0.534 | 0.0110 | |
| | Site (urban-UoRL) | 4.865 ± 1.101* | < 0.001* | |
| | Site (suburban-UoRW) | 3.867 ± 1.107* | 0.0005* | |
| | Site:NE | - | 0.0357 | |
| | Aphids:NE | -0.007 ± 0.000 | 0.0119 | |

**Table 27** Mean ± SE of ant numbers recorded on each plant for each of the three study sites. Superscript alphabets indicated significant differences between groups calculated from post-hoc Tukey tests.

| Dependent variable | Site | Mean ± SE |
|---|---|---|
| Ants | Rural-CEDAR | $0.011 \pm 0.005^{a}$ |
| | Suburban-UORW | $1.135 \pm 0.118^{c}$ |
| | Urban-UORL | $3.705 \pm 0.219^{b}$ |

5.3.3 Natural enemy abundance

Site, ant number and the interaction between aphid and site were included in the best model, in which ant abundance had a negative influence on natural enemy numbers (Table 28). The number of natural enemies varied across the sites, with the suburban site (UoRW) having the highest number of natural enemies followed CEDAR and UoRL (Table 29) sites. Again, the effect of aphids on natural enemy abundance also depended on site, which is consistent with the result from the aphid model that aphid abundance varied across the study sites.

**Table 28** Summary of coefficients of the best models selected based on the AIC for models predicting natural enemy abundance with ZIGLMM with negative binomial distribution family (nbinom2), session as a random effect and site and session in binary part. Only models with $\Delta_{AIC} < 2$ units are shown. (Aphids = aphid number; NE = natural enemies; Ants = ant number; Site = study sites). An asterisk signifies the coefficients and *P*-values are derived when compared with a reference level for each categorical variable. (Note that the coefficients of the interaction terms with one categorical variable with more than two levels cannot be yielded).

| Dependent variable | Independent variables | Coefficient value ± SE | *P*-value | AIC |
|---|---|---|---|---|
| NE | Intercept | -1.693 ± 0.745 | 0.0230 | 1818.543 |
| | Site (urban-UoRL) | 0.977 ± 0.340* | 0.0040* | |
| | Site (suburban-UoRW) | 1.132 ± 0.270* | <0.001* | |
| | Ants | -0.056 ± 0.027 | 0.0386 | |
| | Aphids:Site | - | 0.0258 | |

**Table 29** Mean ± SE of natural enemy numbers recorded on each plant for each of the three study sites. Superscript alphabets indicated significant differences between groups calculated from post-hoc Tukey tests.

| Dependent variable | Site | Mean ± SE |
|---|---|---|
| Natural enemies | Rural-CEDAR | $0.408 \pm 0.043^{a}$ |
| | Suburban-UORW | $1.077 \pm 0.108^{c}$ |
| | Urban-UORL | $0.386 \pm 0.046^{b}$ |

5.3.4 Chlorophyll content and above ground plant dry mass

There was no significant difference found in chlorophyll content between uninfected (31.5 ± 0.57 units) and infected (31.2 ± 0.53 units) plants ($t_{257} = 0.34$, $P = 0.74$). However, above ground plant dry mass did vary across study sites. The mass at the rural site (CEDAR) was significantly higher than that recorded at the other two sites (Table 30). The best candidate model for above ground plant dry mass included aphid number, infection status, site, interaction between aphid number and infection status, and interaction between site and infection status (Table 31). Results suggested that plant above ground dry mass was negatively correlated with aphid abundance, and that plant dry mass was higher in infected plants than uninfected. The plant dry mass with different infection status in three different sites was shown in Figure 20.

**Table 30** Mean ± SE of plant above ground dry mass (grams) for each of the three study sites. Superscript alphabets indicated significant differences between groups calculated from post-hoc Tukey test.

| Dependent variable | Site | Mean ± SE |
|---|---|---|
| Plant above ground dry mass | Rural-CEDAR | $4.692 \pm 0.150^{a}$ |
| | Suburban-UORW | $3.699 \pm 0.170^{b}$ |
| | Urban-UORL | $3.645 \pm 0.128^{b}$ |

**Table 31** Summary of variable coefficients and model selected based on the AIC for predicting plant above ground dry mass with GLM with Gamma distribution family. Only the model with the lowest AIC and highest weight AIC is shown. W is the Akaike weight. (Plants = plant above ground dry mass; Aphids = aphid number; Inf = infection status; NE = natural enemies; Site = study sites). An asterisk signifies the coefficients and *P*-values are derived when compared with a reference level of each categorical variable. (Note that the coefficients of the interaction terms with one categorical variable with more than two levels cannot be yielded).

| Dependent variable | Independent variables | Coefficient value ± SE | *P*-value | AIC | W |
|---|---|---|---|---|---|
| Plants | Intercept | 6.851 ± 0.404 | <0.001 | 848.31 | 0.234 |
| | Aphids | -0.001 ± 0.0002 | <0.001 | | |
| | Inf (Uninfected) | -1.640 ± 0.492* | 0.0010* | | |
| | NE | 0.128 ± 0.084 | 0.1296 | | |
| | Site (urban-UoRL) | -1.055 ± 0.342* | 0.0023* | | |
| | Site (suburban-UoRW) | -1.103 ± 0.416* | 0.8044* | | |
| | Aphids:Inf | - | 0.0035 | | |
| | Site:Inf | - | 0.0460 | | |
| | Site:NE | - | 0.1110 | | |

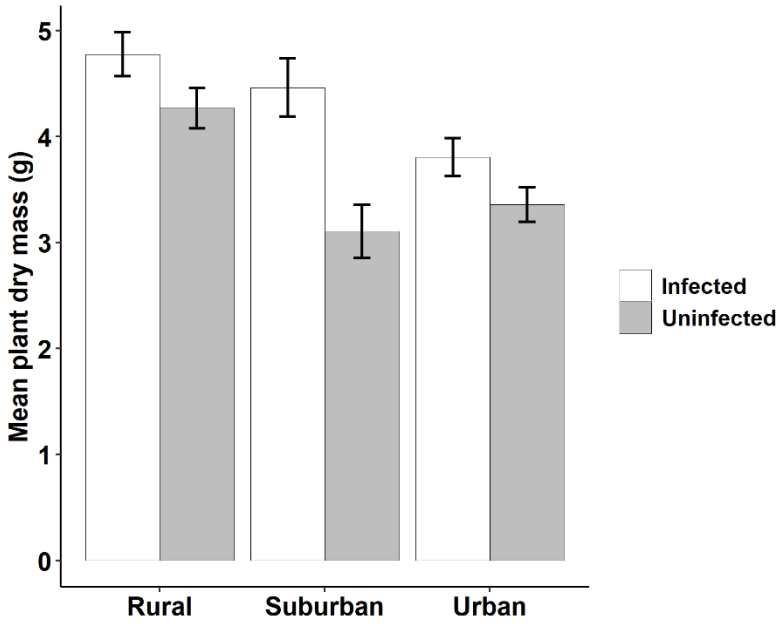


**Figure 20** Mean ± SE of plant dry mass between infected and uninfected treatments in three different sites.

5.4 Discussion

The abundance and diversity of insects is affected by urbanisation, but the majority of studies are observational studies of single (or limited numbers of) species. If I am to understand how urbanisation affects insect ecology, then experimental studies of interacting species provide a powerful approach, but there are very few such studies to date. Here, I use replicated colonies of aphids on experimental plants that are either infected or uninfected by a ubiquitous plant pathogen, to examine how urbanisation affects the recruitment of natural enemies and mutualists, which in turn will feed back and affect aphid abundance and hence plant traits.

I found that natural enemy abundance was greatest in the suburban site, and aphid numbers were lowest on uninfected plants, most likely because of increased rates of attack. In turn, the differences in size between infected and uninfected plants was greatest in the

suburban site, reflecting differences in aphid numbers. Numbers of natural enemies were lowest, and the presence of mutualist ants highest, in the urban site, leading to the largest aphid colony sizes as top-down regulation was limited. There was no effect of plant infection on aphid numbers here. Similarly, although ant numbers were minimal in the rural site, natural enemies were not abundant, again leading to little difference in aphid numbers between infected and uninfected plants.

*Aphis fabae* abundance was affected by symptomatic infection of *B. cinerea* with aphid colony sizes on infected plants being smaller than those found feeding on uninfected plants. This result is consistent with previous studies showing that the symptomatic infection and asymptomatic infection of *B. cinerea* had negative effects on aphid population size (Al-Naemi and Hatcher, 2013; Ngah et al., 2018). These negative effects may arise from changes in plant quality due to the infection resulting in altered nitrogen concentration (nutritional change), and changes in plant induced defenses (Hatcher, 1995; Al-Naemi and Hatcher, 2013).

However, this general pattern was affected by urbanisation, as more aphids were found on infected plants in the suburban site. This may be explained by the higher number of natural enemies in the suburban site, which showed a preference for attacking aphids on uninfected plants (Ngah et al., 2018). This hypothesis is supported by the experimental data, as the aphid populations on uninfected plants in the suburban site was starting to fall due to predation, unlike the other two sites, with lower levels of predation and no difference between aphid numbers on infected and uninfected plants.

Some ant and aphid species form mutualistic relationships, both of which receive reciprocal benefits (Stadler et al., 2002; Styrsky and Eubanks, 2007; Nielsen et al., 2010;

Novgorodova and Kryukov, 2017; Senft et al., 2017). I found a positive relationship between ant numbers and aphid density, as reported in many previous studies (e.g. Kaplan and Eubanks, 2005; Yoo et al., 2013). The abundance of ants was positively correlated with natural enemy numbers, as both are driven by aphid colony size. Ant abundance differed between sites, with the highest abundance present in urban followed by the suburban and then rural sites, and such patterns have been previously reported (Rocha and Fellowes, 2018, 2020).

At the same time, mutualistic ants reduce rates of insect natural enemy attack experienced by aphid colonies, and these ants also affect other insects sharing the same host plant, such as unattended aphids, pollinators, and other herbivorous insects (Kaplan and Eubanks, 2005; Mooney and Agrawal, 2008; Oliver et al., 2008; Yoo et al., 2013; Assunção et al., 2014; Adachi and Yano, 2017). In this study, most insect natural enemies were predators rather than parasitoid wasps and the number of natural enemies was negatively associated with increased numbers of ants. Hoverfly larvae were the most abundant of the aphids' insect natural enemies. The presence of ants reduces the survival rate of hoverfly larvae and alters adult hoverfly oviposition behaviour (Amiri-Jami et al., 2017). This study reinforces the evidence that urbanisation can affect ecological patterns by modifying the likelihood of finding mutualistic interactions.

Site as a proxy for urbanisation had a strong effect on natural enemy interactions with other insects. Insect predators and parasitoids are more abundant in less fragmented habitats with more complex vegetation, and nectar and pollen resources, which are important food sources for some adult insects (Root, 1973). This explains why natural enemy abundance was greatest at the suburban site which had greater plant diversity than the urban site

(surrounded by impervious surfaces) and the rural site (surrounded by grass pasture). Previous studies also found an increase in natural enemy abundance with increasing vegetation complexity in urban areas (Langellotto and Denno, 2004; Thomson and Hoffmann, 2009; Martin et al., 2015; Rocha et al., 2018).

The effects of symptomatic infection also affected the host plants. Increased herbivore (aphid) numbers were negatively associated with plant dry mass, and aphid numbers on infected plants were significantly lower than those feeding on uninfected plants. Infection by *B. cinerea* had a (albeit likely short term) positive indirect effect on the host plant by inhibiting aphid population growth. Above ground plant dry mass was highest in the rural site, where aphid abundance was lowest.

It is important to note a caveat with this study. For sound logistical reasons (access, time and security of experimental plants) this study was completed at three sites, clearly differentiated by location (heavily urbanised, suburban, rural). Following Davies and Gray (2015), I am careful to acknowledge this issue, and defend my approach as it provides insight into how the possible (and ecologically reasonable) effects of plant pathogen infection on tritrophic and mutualistic interactions may vary with urbanisation. The great value in this study is in its novelty, and the understanding that emerges from the work, which I hope will encourage further consideration of this topic.

This is the first study that investigates the effects of plant pathogen infection and urbanisation simultaneously, introducing further complexity into an experimental approach to understanding the effects of urbanisation on a complex web of interactions. My study showed that both factors have roles in altering insect community structure. While urbanisation is evidently the dominant factor determining changes in ecological dynamics,

food webs are complex, and it is only by examining key interactions that we will be able to understand how and why urbanisation alters the structure of ecological communities.

# Chapter 6 Meta-analysis suggests differing indirect effects of viral, bacterial, and fungal plant pathogens on the natural enemies of insect herbivores

## 6.1 Introduction

Plant pathogens are exceptionally common in terrestrial ecosystems, and have considerable economic impact in agroecosystems. Despite this, they have received surprisingly little attention from ecologists studying their wider ecological effects. For example, plant pathogens may alter host plant quality as perceived by insect herbivores, but what are the indirect consequences for species at higher trophic levels, such as the herbivores' insect predators and parasitoids?

Studies show that plant pathogens can alter host plant quality as perceived by insect herbivores by altering nutritional value (Dulermo et al., 2009), volatiles (Desurmont et al., 2016), defensive compounds (Stout et al., 2006) and appearance (Mauck et al., 2015), and these affect herbivore life histories via both individual performance and preference (Martini et al., 2014; Liu et al., 2018; Ngah et al., 2018). Changes in plant quality affect the dynamics of insect herbivores and their natural enemies (Leather et al., 1998; Müller et al., 2005), and pathogen infection can cause similar changes. The effects of plant pathogens on insect herbivores are a plant-mediated effect, which can be categorised as an indirect effect where it is the result of the interaction between two species, with at least one intermediate species in the chain (Ripple et al., 2016). The results of studies of the indirect effects of plant pathogens on insect natural enemies are inconsistent. Some studies found positive (de Oliveira et al., 2014; Martini et al., 2014) effects of infection on natural enemy preference and performance, while others show negative consequences of plant pathogen infection

(Belliure et al., 2008; Ngah et al., 2018). An overall understanding of how plant pathogen infection may influence the indirect interactions between plants and the natural enemies of their insect herbivores remains unexplored, a knowledge gap that this paper begins to address.

One key link between plants and the natural enemies of their herbivores comes from the effects of plant defenses on their insect herbivores. Plants have to defend themselves against attack, and there are two primary hormonal signaling pathways that facilitate defense: the salicylic acid (SA) and the jasmonic acid (JA) pathways (Pieterse et al., 2014). The SA signaling pathway is activated against piercing-sucking herbivores, biotrophic pathogens and viruses, while the JA signaling pathway is activated against chewing herbivores, necrotrophic pathogens and bacteria (Moreira et al., 2018). As a result, infection by a pathogen, or attack by a herbivore, may result in differing defenses being elicited, defenses which affect more than one group of plant natural enemies.

The preference–performance hypothesis contends that insect herbivore performance matches their host plant preferences (Jaenike, 1978), a suggestion which has received support (Singer et al., 1988; Gripenberg et al., 2010), albeit not always (Valladares and Lawton, 1991; Clark et al., 2011). Few studies have considered similar effects at higher trophic levels, where there is some support (Steiner et al., 2007; Ngah et al., 2018) and also contradictions (Rostás et al., 2006; Xu et al., 2014; de Oliveira et al., 2016; Joffrey et al., 2018). This inconsistency is worth addressing.

Here I consider the indirect effects of pathogens on insect natural enemies. It is important to note that plant pathogens may modify the phenotypic responses of infected plants and their herbivores, which then influence herbivore–enemy interactions. These

effects can be classified into consumptive and non-consumptive pathways; with the former the effects are transmitted through the consumption of herbivores, while with the latter the effects are transmitted through changes in appearance and/or volatiles from plants and herbivores as perceived by natural enemies.

Furthermore, natural enemies may differ in their responses due to differences in life histories. For example, parasitoid development is intimately associated with individual hosts, and so the quality of a single host may have a substantial effect on an individual parasitoid's fitness. In contrast, insect predators may be able to compensate for poor quality hosts by consuming more prey.

The objectives of this study were (1) to investigate the overall effect of plant pathogen infection on natural enemies; and (2) to examine if pathogen type, herbivore feeding guild, natural enemy guild (predator or parasitoid), natural enemy responses (performance and preference) and their interactions affect natural enemies. To answer these questions, we systematically reviewed the literature and used metanalytical techniques.

## 6.2 Materials and methods

### 6.2.1 Data collection

I searched for published articles reporting the indirect effects of plant pathogen infection on insect natural enemies in the Web of Science database (ISI) by applying the following search terms "(Plant or tree) and (predator* or *parasitoid* or natural enem*) and (preference or performance or choice or indirect effect*) and (fung* or oomyc* or bacteri* or virus or plant pathogen* or plant infection) not *mycorrh* not endophyt* not *symbio* not entomopathogen*". The search was refined by showing only the studies published in articles, proceedings papers, reviews and book chapters with no restriction in date range. The

search yielded 423 papers and the other 25 papers were found from references in the reviews, book chapters and in press articles (see the PRISMA flow diagram, Appendix 2).

The studies included in the meta-analysis had to meet the following criteria: they (1) report natural enemy performance or preference toward either healthy versus pathogen-infected plants or insect herbivores feeding on healthy versus pathogen-infected plants; (2) report plant, insect herbivore, pathogen at species level and natural enemy to at least at family level and (3) report the mean and variability (standard deviation, standard error or confidence interval) with sample size. When necessary, ImageJ was used to extract mean and variability from figures. This yielded 216 case studies from 29 studies (note that three studies came from a PhD thesis (Ngah, 2018)). The references of the primary studies included in this study are shown in Appendix 3.

### 6.2.2 Moderators

For each case study, the following moderators were extracted according to the pathogen, insect herbivore, type and process of insect natural enemy response; pathogen types (bacterial, fungal or viral); insect herbivore feeding guild (chewing or piercing-sucking); experimental conditions (laboratory or field); type of natural enemy (predator or parasitoid); type of natural enemy response (performance or preference); mechanisms of the indirect effects originating from plant pathogens that are relayed to natural enemies (consumptive via herbivore (CH), non-consumptive via herbivore (NH), non-consumptive via plant (NP) and non-consumptive via herbivore and plant (NHP)).

The definition of preference in this study is any trait exhibited by natural enemies where more than one stimulus is given at the same time and the insects make a choice, whereas performance is any trait exhibited by natural enemies where no choice is given. For

mechanisms, the indirect effect can (a) originate from plant pathogens relayed to natural enemies mediated by the consumption of insect herbivore prey (CH), or (b) through indirect effects mediated by changes in appearance and/or volatiles emanating from the herbivore (NH), the host plant (NP) or both herbivore and plant together (NHP). These mechanisms were categorised based on the dissemination processes (Figure 21) of the effects and experimental design of primary studies. The cases where natural enemy performance was measured after the consumption of insect herbivore prey (e.g., longevity, development time, fecundity, weight, size) were categorised as CH, whereas in some cases the natural enemy performance (no choice given) or preference (choice given) were measured through non-consumptive traits (e.g., proportion of parasitized hosts, time spent around herbivores, number of antennation events, time to first attack) were categorised as NH. Studies conducted only with the host plant were classified as NP, while the cases where insect herbivores are present on host plants were classified as NHP. I differentiated NHP from NP as in some measured responses, both herbivore and host plant were present, so it is uncertain that the indirect effects affecting the natural enemies come only from changes in the herbivore, the plant or both. All of these processes can help elucidate the mechanism by which the indirect effects from plant pathogens can be relayed to natural enemies.

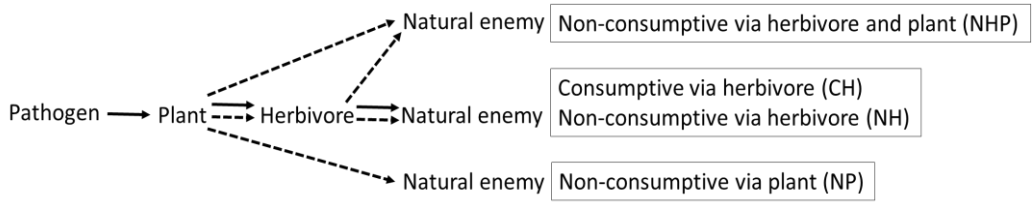


**Figure 21** The dissemination processes of indirect effects originating from plant pathogens relaying to natural enemies. A filled arrow represents a direct effect and a dashed arrow represents an indirect effect.

To avoid the confounding effect of the non-independence of data resulting from bias due to the nested experimental design in the same studies, each published article was given a *Study ID*, *Case ID* and *System ID* (Fernandez-Conradi et al., 2018). Each published article was given an identifier (*Study ID*), in which each study can contain more than one measured response. Each measured response was a response variable of a natural enemy towards a paired treatment (healthy versus infected plants, or insect herbivores feeding on healthy versus infected plants), in which each measured response was given a unique ID (*Case ID*). In addition, *System ID* was given to the combination of plant pathogen, plant, insect herbivore and insect natural enemy.

6.2.3. Data analyses

All statistical analyses were performed in R 4.0.2 (R Core Team, 2020) using the *metafor* package (Viechtbauer, 2010). The effect size of each measured response was calculated using Hedges'*d* metric and its variance (Hedges, 1981) (effect size calculation refers to Appendix 4). All models were performed using multilevel linear mixed-effect models (with Restricted Maximum Likelihood (REML)) to avoid confounding effects from non-dependent data as most studies contained more than one measured response, and these measured responses from a single study tended to be correlated. As measured responses from the same study system were likely to be correlated, *System ID* was used as a random factor as well as *Case ID* nested in *Study ID* was used as another random factor (Fernandez-Conradi et al., 2018).

Grand mean effect size was calculated using the complete data set. Then subgroup analyses were performed. First, the effect of different experiment conditions (laboratory versus field) was tested, and found no influence of different experiment conditions on effect

size. Therefore, data from laboratory and field experiments were pooled for the following subgroup analyses. Note that in subgroup analyses some levels of moderators were combined with other levels of moderator or excluded from the subgroup analyses as appropriate. One study with oomycete as a plant pathogen was assigned into the fungus group as well as the feeding guild; a study with thrips as an insect herbivore was assigned into the chewing group. Two field studies, one in which the insect herbivore was a seed predator caterpillar (the indirect effect arises from change in plant phenotype by infection rather than the effect from herbivore feeding; (Biere et al., 2002)) and another with a mixed type of herbivores (a field study in which insect herbivores naturally occurred without manipulation; (Mauck et al., 2015)) were excluded from the subgroup analyses where feeding guild was a moderator. Additionally, in some subgroup analyses there were unbalanced measured responses as there were no measured responses for some levels of some moderators (Appendix 5).

Publication bias was tested by (1) inspection of funnel plots, (2) conducting cumulative meta-analysis (Leimu and Koricheva, 2004), (3) calculation of fail-safe number (Rosenberg, 2005) and 4) detecting the relationship between effect sizes and journal impact factor (Murtaugh, 2002). For sensitivity analysis, Cook's distances were considered and studentised deleted residual values greater than |3.0| were excluded from the analysis (Viechtbauer and Cheung, 2010).

## 6.3 Results

I can identify 40 primary studies examining the indirect effects of plant pathogen infection on insect natural enemies, but only 29 primary studies that reported mean and variability were suitable for inclusion in the study. These consisted of 18 plant species, 15 insect herbivore species, 22 plant pathogen species (bacteria (3), fungus (10) and virus (9))

and 19 natural enemy species (parasitoid (11), predatory mite (2), predatory bug (2), lacewing (2) and ladybird (2)).

The grand mean effect size (± 95% CI) after outliers were removed ($k$ = 213) was not significant ($P$ = 0.187) with −0.21 ± CI (−0.51, 0.10). This indicated that there was no indirect effect of plant pathogens on insect natural enemies. However, the significance and large amount of residual heterogeneity indicated that other moderators which were not included in the initial model may influence effects on natural enemies ($Q_E$ = 2213.36, $df$ = 212, $P$ < 0.0001). The estimated means for laboratory ($k$ = 183, −0.12 ± (−0.47, 0.22)) and field ($k$ = 29, −0.3924 ± (−1.02, 0.24)) studies were not significantly different from each other ($P$ = 0.46) and experimental condition was not a significant moderator ($Q_M$ = 1.96, $df$ = 2, $P$ = 0.38), so data from laboratory and field studies were pooled.

The indirect effects of plant pathogens on natural enemies were significantly different between pathogen types ($Q_M$ = 11.43, $df$ = 3, $P$ = 0.0096). Only fungal pathogens caused significant negative indirect effects on natural enemies, while the effects from bacteria and viruses were not significant (Figure 22a). However, there was a significant effect of the interaction between insect herbivore feeding guild and pathogen type (pathogen*feeding guild: $Q_M$ = 28.13, $df$ = 6, $P$ < 0.0001). The interaction between bacterial pathogen infection and chewing insect guild caused a significant positive indirect effect on natural enemies, while the interactions for both chewing and piercing-sucking insect guilds with fungal pathogens were negatively significant (Figure 22b). There was also a significant interaction between type of responses and pathogen type ($Q_M$ = 12.40, $df$ = 5, $P$ = 0.03), in that the fungal pathogen caused significant indirect negative effects on natural enemy performance and preference (Figure 22c).

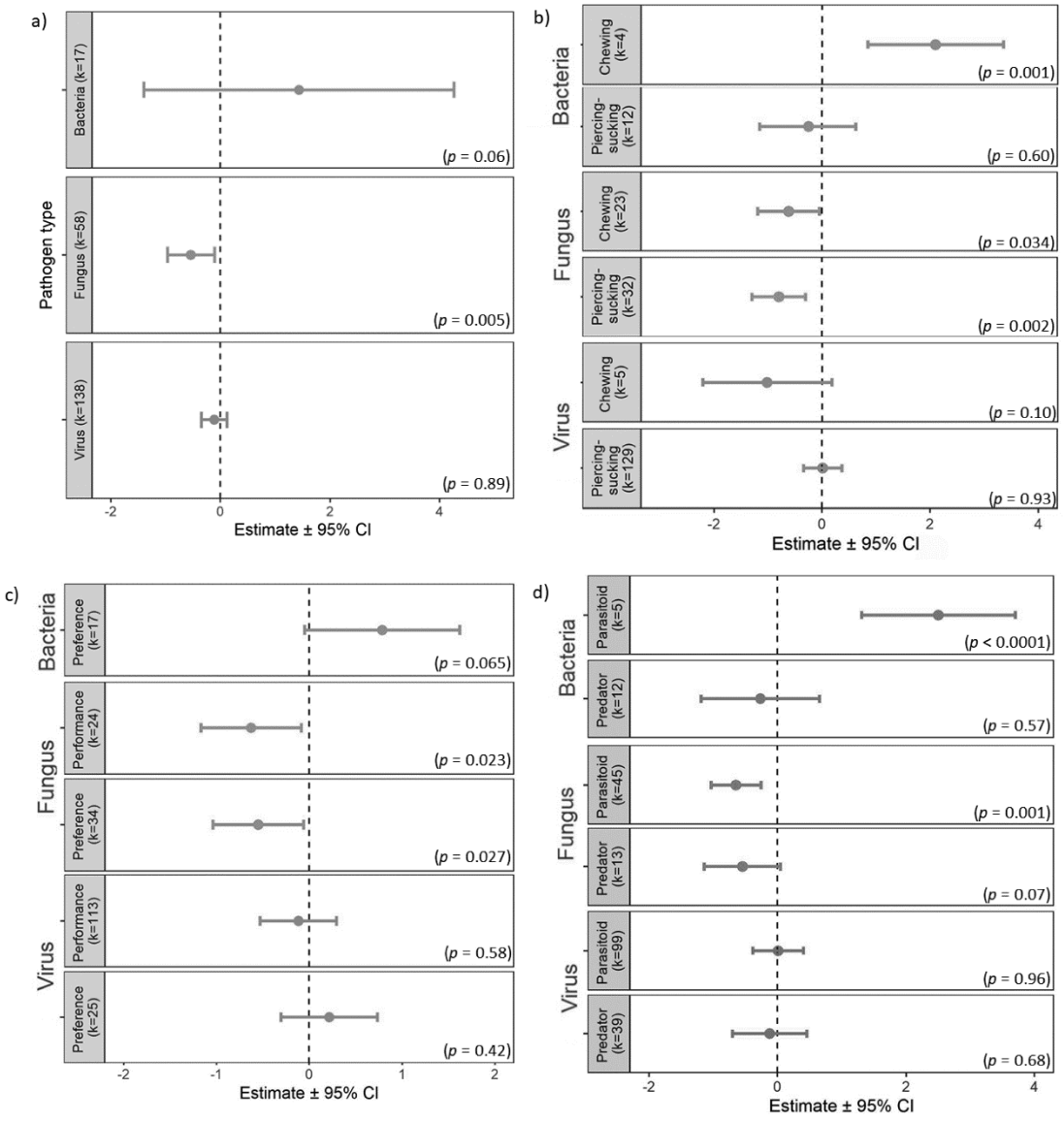


**Figure 22** Natural enemy responses toward the indirect effects from plant pathogen infection by (a) pathogen type (virus, fungus, or bacteria), (b) interaction of insect herbivore feeding guild (piercing-sucking or chewing) and pathogen type, (c) interaction of type of natural enemy response (preference or performance) and pathogen type and (d) interaction of pathogen type and natural enemy type (predator or parasitoid). Circles and error bars represent the estimates and corresponding 95% CI. The vertical dashed line at zero represents the null hypothesis (no difference in natural enemy response between control and infected treatments).

When considering the overall indirect effects of plant pathogens on natural enemy types, natural enemy type as a moderator was not significant ($Q_M$ = 1.72, *df* = 2, *P* = 0.42; estimated mean for predator: *k* = 65, −0.22 ± (−0.67, 0.23); parasitoid *k* = 148, −0.20 ± (−0.54, 0.14)). However, I found a significant interaction between pathogen type and natural enemy type ($Q_M$ = 28.68, *df* = 6, *P* < 0.0001) where a positive indirect effect was found in the Parasitoid × Bacteria interaction (2.51 ± (1.31, 3.70), *P* < 0.0001), and a negative indirect effect was found in the Parasitoid × Fungus interaction (−0.65 ± (−1.04, −0.26), *P* = 0.0011) (Figure 22d).

I also examined the interaction effect between pathogen types and mechanisms (pathogen × mechanism) on how the indirect effects from plant pathogens are passed to natural enemies. This interaction was significant ($Q_M$ = 30.88, *df* = 10, *P* = 0.0006) where the negative effects from fungal pathogens via CH and NHP were significant (Fungus × CH: −0.72 ± (−1.33, −0.11), *P* = 0.021; Fungus × NHP: −0.53 ± (−1.04, −0.02), *P* = 0.04). The positive indirect effect from viruses via NP was also significant (Virus × NP: 7.66 ± (2.60, 12.72), *P* = 0.003).

Finally, the pathways by which the indirect effects from plant pathogens are relayed, and their effect on natural enemies, were examined through the three-way interaction between pathogen type, mechanism and response type (pathogen × mechanism × response). This three-way interaction was significant ($Q_M$ = 29.53, *df* = 14, *P* = 0.0089) and the significant terms were similar to the previous model (pathogen × mechanism). The significant terms were Fungus × CH × Performance (−0.71 ± (−1.35, −0.06), *P* = 0.032) and Virus × NP × Preference (7.58 ± (2.49, 12.66), *P* = 0.0035).

Publication bias was detected in a funnel plot with visual assessment (Appendix 6), but the Rosenberg's fail-safe number of 11,095 exceeded the critical conservative value of 5 × k +10 = 1100, suggesting that my results are robust (Rosenberg, 2005). There was evidence of temporal change in cumulative meta-analysis (Appendix 7). The early studies of indirect effects from plant pathogens on natural enemies revealed the effects tended to be positive with large variance. However, results became negative over time with smaller variance. I found no correlation between effect sizes and journal impact factor (Spearman's rho = −0.0043, $P = 0.95$).

## 6.4 Discussion

Forty primary studies that investigated the indirect effects of plant pathogens on natural enemies were identified, of which the first was published in 1998 (Christiansen-Weniger et al., 1998). This suggests that during the last 22 years there has been relatively few studies that have considered the indirect effects of plant pathogen infection on the natural enemies of insect herbivores. Given that (a) plant pathogens are widespread, ecologically and economically important, and affect their quality as host plants for herbivores, and (b) that indirect effects, such as those found in tritrophic systems, are ecologically important and ubiquitous, then I suggest that the intersection of these two observations is likely to be of considerable interest to pure and applied ecologists. Overall, I found no significant effect of pathogen infection on the insect natural enemies of plant herbivores, but when I consider the pathogen types separately, I find that only fungal plant pathogens cause indirect negative effects on natural enemies. Viral pathogens show no consistent effect, but bacterial pathogens appear to benefit the enemies of chewing herbivores. Breaking this down to consider parasitoids and insect predators, I found no clear indirect effects of plant pathogens

on predators, but host plant infection by fungal plant pathogens has a negative effect and infection by bacterial pathogens has a positive effect on parasitoids. It should be noted that any meta-analysis is only as good as the data used in the synthesis. I find some evidence of publication bias, which given the number of available studies is perhaps unsurprising. I therefore conservatively see these results as indicative of the effects of plant pathogens on interactions at higher trophic levels, but further work is required to build on this evidence base.

The negative influence of fungal plant pathogens on insect natural enemies is consistent with a previous study (Fernandez-Conradi et al., 2018). I found that both the preference and performance of insect natural enemies was affected by fungal pathogen infection, and that natural enemies of both piercing-sucking and chewing herbivores were negatively affected. Insect herbivores feeding on fungus-infected plants are likely to suffer from changes in plant nutritional value (Dulermo et al., 2009; Al-Naemi and Hatcher, 2013), plant defensive compounds from pathogen infection (induced resistance) (Stout et al., 2006) and possibly mycotoxins resulting from fungal infection (Logrieco et al., 1998). All of these lead to poorer performance of the herbivores, which in turn is relayed to natural enemies due to poorer prey/host quality.

In contrast to the effects of fungal pathogens, bacterial pathogens can cause significant positive indirect effects on natural enemies via chewing insect prey. I note however, that this result comes from four measured responses, all resulting from one primary study (Ponzio et al., 2016). The four measured responses were preference studies with plant (NP) and herbivore and plant (NHP) mediated effects. The study found similar plant volatile responses between herbivory and infection, and infection alone was enough to induce a

change in the natural enemy's behaviour (Ponzio et al., 2016). Further studies are needed to confirm the generality of this finding. In addition, I suggest that further studies examining the effect of bacterial plant pathogens on natural enemy preference (where the non-significant trend was to a positive effect) and performance (where I lack studies) would be of considerable value. Finally, I found no consistent effect of viral plant pathogen infection on the preference and performance of insect natural enemies. Unlike the situation with bacterial plant pathogens, this was not due to a lack of studies.

Indeed, if I consider parasitoids and insect predators separately, a pattern emerges. While again acknowledging that there are few studies, I found no significant indirect effect of plant pathogens on insect predators. In contrast, and given that parasitoid fitness is tightly associated with host quality (and indeed host plant quality, e.g., Desurmont et al., 2016; Ngah, 2018; Ngah et al., 2018), I found an effect of plant pathogen infection on parasitoids. However, while fungal plant pathogens had a negative effect, bacterial plant pathogens [although this evidence comes from just two primary studies (Martini et al., 2014; Ponzio et al., 2016)] had a positive effect, attracting parasitoids to infected plants. This is an area which would greatly benefit from further study. Additionally, while I find an overall effect of fungal plant pathogen infection on natural enemies, I suggest that different fungal pathogen life styles (endophyte, biotrophic and necrotrophic pathogens) may contribute to different responses of insect herbivores (Fernandez-Conradi et al., 2018). Fungal infection can harm insect herbivores by both direct effect from mycotoxin produced (Logrieco et al., 1998) and indirect effects mediated by host plants from changing plant nutrition (Al-Naemi and Hatcher, 2013), relocation and sink source of nutrients, and changing in plant defense responses (Eberl et al., 2019). All of these lead to detrimental effects on herbivore

performances such as smaller herbivore size leading to smaller natural enemy size (Ngah et al., 2018). Likewise, my result indicated that fungal infection changes natural enemy preference by repelling them from infected plants.

Generally, natural enemies often employ chemical cues associated with insect herbivory such as herbivore-induced plant volatiles (HIPVs) to locate potential prey/host (Ponzio et al., 2013). Very few studies show that natural enemies substantially prefer herbivore-damaged plants over fungus-infected plants. However, when herbivore-damaged plants were compared with plants with both herbivory and fungal infection, then these two treatments were not statistically different (Rostás et al., 2006; Desurmont et al., 2016). Fungal infection can reduce the production of green leaf volatiles (GLVs), glucosinolate derivatives and terpenoids after herbivory (Desurmont et al., 2016), and these volatiles are important in attracting natural enemies (Mumm and Dicke, 2010).

I hypothesised that different pathogen types may have different pathways to relay the indirect effects of disease to insect natural enemies. I found that fungal infection contributes indirect negative effects to natural enemies via CH and NHP. The effect of fungal pathogens on natural enemies via the CH mechanism is straightforward, as feeding on poor quality hosts contributes to detrimental effects on the natural enemy. One study found that aphids feeding on fungus-infected plants were less resistant to starvation and the parasitoids emerged from the aphids feeding on the infected plants also showed reduced starvation resistance (Ngah et al., 2018). The evidence of fungal infection affecting natural enemies via NPH can be explained by changes in plant volatiles.

Viral plant pathogen infection significantly caused an indirect positive effect on natural enemies (parasitoid) via NP (albeit from one measured response (Liu et al., 2018))

and this means virus-infected plants attract natural enemies. I speculate that this attraction may arise from changes in plant volatiles even though I have no supporting evidence as the experimental design of the primary study did not include plant volatile analysis (Liu et al., 2018). If I were to speculate, I would suggest that given that viral pathogens frequently rely on insect vectors for transmission, it would not be surprising if changes in volatiles which attract insect herbivores, and hence their enemies, were selected for (Dáder et al., 2017). The presence of virus-infected plants is likely to be a reliable cue to indicate the existence of potential hosts to natural enemies (Mauck et al., 2010). It is not only volatiles that are changed in virus-infected plants, but also their colour (Mauck et al., 2015).

Although plant pathogen, plant, insect herbivore and natural enemy interactions are ubiquitous in both natural and agricultural ecosystems, there have been surprisingly few studies addressing how plant pathogen infection may indirectly affect the preference and performance of insect natural enemies. My meta-analysis suggests that the indirect effects of plant pathogens on insect natural enemies varies with both pathogen type, natural enemy type, and through the route by which the effect is transmitted. The indirect effects of infection by fungal plant pathogens were negative, and the limited number of studies using bacterial plant pathogens suggested a positive effect on parasitoid preference, although two primary studies showed a positive preference effect. Host plant infection by viral pathogens did not show such effects, although a transmission pathway was detected. It is therefore not simply that being a diseased plant alters the preference and performance of associated parasitoids and insect predators. Instead, it is an interaction between the causative agent of disease, combined with herbivore and enemy traits, which determines the ecological outcome. I hope that this meta-analysis highlights the knowledge gap that exists in my understanding of the

ecological consequences of plant disease. Further work will help us understand if the conclusions that can be drawn from the limited number of available studies are robust. I suggest that studies in this neglected area will be of considerable interest to both ecologists and pest managers.

# Chapter 7 General discussion

## 7.1 Main findings and contributions to the field

Plants are the foundation of energy flow in every terrestrial ecosystem, where photosynthesis harvests energy from sunlight and uses this to synthesise organic molecules, which are in turn accessible to heterotrophs (Gough, 2011). Plants vary in quality (the availability of appropriate nutritional resources) to their consumers; therefore, heterotrophs such as insect herbivores which have short generation times are likely to be affected by the food they consume. This work I investigated both direct and indirect effects of host plant quality relaying through to higher trophic levels using insect model systems in the laboratory and field.

In Chapter 2, I explored the direct (through consumption) and indirect (maternally transmitted) effects of two different hosts varying in quality (high: mung bean, poor: lentil) (Messina and Jones, 2009) simultaneously on the preference and performance of two bean beetle species, *Callosobruchus maculatus* and *C. analis*. In terms of preference, I measured oviposition preference using no-choice experiment and choice experiment designs. I also switched host for females to oviposit and measured offspring performance.

The major findings in Chapter 2 showed that oviposition preference is consistent in that mung bean is the preferable host regardless of natal host and previous oviposition experience. This finding supports a previous study although the effect of natal host was not considered (Chiu and Messina, 1994). Although the beetles have been separately cultured on different hosts for many generations, they still prefer ovipositing on mung bean. I also tested if seed size influences oviposition by given a choice between small seed mung bean and

lentil, and the result still showed a consistent preference toward mung bean. This suggests that bean beetles employ other cues to make oviposition decisions and this decision is innate. This result contrasts with previous studies showing that bean beetles prefer larger seeds when different seed plant species are provided (Paukku and Kotiaho, 2008; Rova and Björklund, 2011). This inconsistency may be a result of the previous studies using related host species, as mung bean and black-eyed beans belong to the same genus. The results from host switching on offspring performance are species dependent.

Generally, all host switching combinations yielded good offspring performance except for *C. maculatus* when their parental host was mung and natal was lentil, which showed a very low emergence rate. This result is also observed in a previous study where *C. maculatus* switched its host from mung bean to lentil showed a < 2% emergence rate in early generations (Messina et al., 2009). This did not happen with *C. analis*. Parental host and natal host also affected offspring traits such as development time, survival time and size. In addition, host quality also affected mating success and offspring sex ratio in some treatments. This Chapter is the first work which simultaneously investigates the effects of host plant quality and maternal effects and observed the effects across generations of two bean beetle species as well as their oviposition preference. Understanding the responses of bean beetles to different host quality may help us understand how the indirect effects from host plants can be relayed to higher trophic levels (bean beetle natural enemy) which is useful in pest management programmes.

In Chapter 3, I asked if host plant quality affected the costs of reproduction in *C. maculatus* and if this interacted with mating status. It was found that mung bean is a superior host, and resulted in longer longevity in both sexes, and overall females generally live longer

than males, with the exception of females that have oviposited. Larger individuals live longer, and I found an interaction between the size of male mate and the host origin of the focal female on female longevity. This suggests that host plant quality interacts with other factors to influence insect lifespan. The results from this Chapter also illustrate that oviposition is a costly activity which can shorten female lifespan, and this effect depends on the number of oviposited eggs. The more eggs laid, the shorter her lifespan. Food provision can remove the effect of host quality; however, it cannot remove the effects of oviposition on female longevity.

In Chapter 4, I focused on the indirect effects originating from the presence of the plant pathogen *Botrytis cinerea* infecting broad bean *Vicia faba* on two aphid species, the black bean aphid *Aphis fabae* and the pea aphid *Acyrthosiphon pisum*. A previous study found indirect negative effects of *B. cinerea* infection on *A. fabae* (Al-Naemi and Hatcher, 2013), but the effects remain unexplored for other aphid species. I also used different clones for *Ac. pisum*, allowing an investigation of the effects of host plant quality on different herbivore genotypes.

Consistent with previous work, *B. cinerea* infection causes negative indirect effects on *A. fabae* in all measured traits. However, I found the opposite result of infection with *Ac. pisum*, which exhibited indirect positive effects, and these effects varied in magnitude across different clones. My findings in this Chapter have demonstrated an unexpected result where different aphid species response differently to the same plant-pathogen system. This may lead to more complications for modelling the effect of infection in pest management programmes.

In Chapter 5, I investigated how the indirect effects of *B. cinerea* infection on *A. fabae* (aphid herbivore) and their enemies may be affected by their environment, in this case urbanisation. This experiment allowed me to assess not only the indirect effects of plant quality mediated by pathogen infection on experimental aphid colonies, but also for interactions at higher trophic levels (aphid natural enemies and ant mutualists). In line with the results in Chapter 4, it was found that *B. cinerea* infection caused indirect negative effects on *A. fabae* populations compared with those on uninfected plants, and this in turn causes indirect positive effects on plant biomass on infected plants. Aphid population size was not only affected by infection but also by natural enemies which in turn are influenced by the level of urbanisation. Aphid population sizes decreased on uninfected plants in suburban area where the landscape surrounded by variety of flowering plants, which supports a greater diversity and abundance of natural enemies and natural enemies prefer attacking prey/hosts on uninfected plants (Ngah et al., 2018).

The abundance of aphid natural enemies and ant mutualists were heavily influenced by level of urbanisation. Results from this Chapter show the importance of the surrounding landscape as natural enemies' population was highest in suburban area where there is high vegetation complexity, and the lowest natural enemies' population in the rural area predominated by a monoculture of farmland. Ant mutualist (*Lasius niger*) numbers were highest in the urban area, followed by suburban and rural areas, respectively. This finding is consistent with previous studies (Rocha and Fellowes, 2018, 2020).

I believe this is the first study that examines the indirect effects originating from a plant pathogen on higher trophic levels in multiple levels of urbanisation, which naturally and widely occurs in today's environments. Plant pathogens are ubiquitous, but they are

usually neglected. *Botrytis cinerea* has a wide distribution range (Elad et al., 2004) and a study revealed undistinguishable strains which were genetically similar between non-agricultural and agricultural areas (Bardin et al., 2018). Although Bardin et al. (2018) did not emphasise the distribution of *B. cinerea* within an urban setting, it did indicate that the fungus should be found across urbanised areas since it can survive in environments outside agricultural areas even in the absence of host plants. However, my study shows that although plant pathogens seem modest, they are an important component in environments to shape and determine the structure of insect communities, and they are deserving of consideration by urban ecologists.

In my final data Chapter (Chapter 6), although plant pathogens are pervasive and well known, the consensus effects of them on insect natural enemies have not been explored. I used meta-analysis technique to quantify the indirect effects of three types of plant pathogen (viral, bacterial and fungal) on the natural enemies of insect herbivores. I found that only fungal pathogens affect natural enemies when the type of pathogen was considered alone. Including the feeding type of herbivore, fungal pathogens show consistent indirect negative effects on natural enemies via chewing and piercing-sucking herbivores while bacteria relay their effects only to chewing herbivores. Likewise, fungal pathogens cause indirect negative effects on herbivores' natural enemies both in terms of preference and performance. Considering the type of natural enemy, parasitoids are evidently affected by the presence of plant pathogens, where fungal pathogens cause indirect negative effects while bacterial pathogens cause indirect positive effects. While the numbers of studies are limited, this is the first work to draw together studies of the effects of plant pathogens on insect natural enemies and to utilise a metanalytical approach to understand their effects. While clearly of

wide ecological interest, it would be beneficial to bring this knowledge into integrated pest management.

I tried to explore the relevant studies regarding to the indirect effects of plant pathogens on insect herbivores' natural enemies, but only forty studies were found. The first study that is relevant to this topic was published in 1998 (Christiansen-Weniger et al., 1998). Thus, throughout more than twenty years this topic has been overlooked by ecologists (Srisakrapikoop et al., 2020). This limits sample size, though in this Chapter I still find significant effects of plant pathogens on insect herbivores' natural enemies. It is clear that fungal pathogens consistently cause indirect negative effects on insect natural enemies, and viruses show non-significant effects. However, there is a very limited number of studies and low sample size considering the indirect effects originating from bacterial pathogens. Even I found significant results from bacterial pathogens in some aspects, more studies are required to increase the power of meta-analysis on the effects of bacterial pathogens on insect natural enemies.

## 7.2 Further research and recommendations

Insects and plants have together inhabited the Earth for at least 400 million years, and more 50% of insect species feed on plants (Bernays, 1992). Thus, they have co-evolved to protect themselves and overcome their rival defence mechanisms. Plants can protect themselves from both insect herbivores and plant pathogens by activating chemical defence systems (Pieterse et al., 2014; Stout, 2014). These imply how plant pathogens, plants and insect herbivores are closely related.

Historically, direct effects (e.g. competition, herbivory and predation) have received much attention because ecologists believe this is a primary force shaping population and

community structure (Moon and Moon, 2011), while indirect effects were considered to be unimportant (Vandermeer, 1969) and have been overlooked by community ecologists (Werner and Peacor, 2003). The underestimation of indirect effects may arise from the complexity and the difficulty to detect and quantify their role in communities (Menge, 1995; Strong, 1997). However, the exponential increase of studies of indirect effects (trophic cascades) in the literature began in the 1990s (Ripple et al., 2016). This reflects the increase of ecologists' attention toward indirect effects since then.

In this thesis, I examined both direct and indirect effects of plant quality on higher trophic levels. In the bean beetle system, although this system has been used to study the effects of host quality on beetle preference and performance (Boeke et al., 2004; Paukku and Kotiaho, 2008; Messina and Jones, 2009; Mainali et al., 2015; Bergeron et al., 2019), the effects of host plant quality on higher trophic levels (natural enemies) have not received much attention. Therefore, it is interesting to examine the direct effects of host plant quality on bean beetles, which in turn relay the effects of host plant to bean beetle's natural enemy. This would be very useful in pest management.

On the other hand, the indirect effects of plant pathogens on species at higher trophic levels have received little attention. In the past twenty years there are just forty studies relevant to this topic (Srisakrapikoop et al., 2020). My results revealed significant effects of plant pathogens on insect natural enemies; however, in some points there still lack of the volume of studies to provide a strong consensus conclusion. This indicates there is still a large knowledge gap in terms of the indirect effects of plant pathogens on higher trophic levels to explore.

The field of urban ecology has been largely expanded in the past twenty years (Pataki, 2015). The effects of urbanisation on insects also has been explored (Raupp et al., 2010; Egerer et al., 2017; Rocha and Fellowes, 2018). Insects are tied together with host plants; therefore, their richness, abundance and distribution are shaped by plant communities (Raupp et al., 2010). However, plant pathogens have been overlooked even though they pervade terrestrial environments. In this thesis showed that plant pathogens have a role in shaping insect community structure. I suggest that further studies should be conducted at larger scales with different urbanisation gradients to quantify the effects of plant pathogens on insect community and structure in urban settings.

Originally, my work had focused on a tritrophic system, investigating the direct and indirect effects of symptomatic infection of the widespread plant pathogen *Botrytis cinerea* on species at higher trophic levels (the host plant, insect herbivores and their natural enemies). This reflects differences in host plant quality (resulting from plant pathogen infection) that may affect the life history of insect pests and their biocontrol agents when different genotypes of plants are infected, and is of interest as the ecological consequences of infection by plant pathogens are relatively infrequently considered.

Unfortunately, due to challenges with culturing and issues related to access to study sites and facilities due to the COVID-19 pandemic, I had to adapt my questions and study system as I progressed. This explains the switch to studying bean beetles, which also proved to be a more tractable system for studies such as these. However, I still focused on the direct and indirect effects of host plant quality in a tritrophic system using pests. This switch in focal plant/insect was for practical reasons, but conceptually, the questions are naturally linked. I studied the direct effects of natal host beans (mung beans and lentils, which differ

in host quality) on the oviposition preference of two bean beetle species (the stored product pests *Callosobruchus maculatus* and *C. analis*), which when combined with oviposition experience, may influence later oviposition decisions and the subsequent fitness of offspring.

Nevertheless, while the change in study system was not planned, the bean beetle system did allow me to ask interesting questions, which may have not been possible with the original aphid system. Together, these studies show that host plant variation can have considerable effects on interactions at higher trophic levels, mediated by changes in both host life history and behaviour, but also with consequences for the host/prey preferences of natural enemies. Such variation will inevitably complicate my ability to understand ecological interactions in nature, where hidden abiotic and biotic variation results in quantitatively different ecological outcomes.

The overall contribution of this thesis is to investigate how the effects of host plant quality (bottom-up effects) pass through direct and indirect interactions to shape community structure at higher trophic levels. These are some of the basic concepts of interactions between organisms. I believe this thesis is also the first work that draws out the consequences of the indirect effects of plant pathogens on insect natural enemies. The presence of plant pathogens may perhaps turn the tide of the success of pest management. These effects allow pest managers and ecologists to understand this complex network of interactions in crop systems, which lead to beneficial outcomes both in terms of agriculture and ecology.

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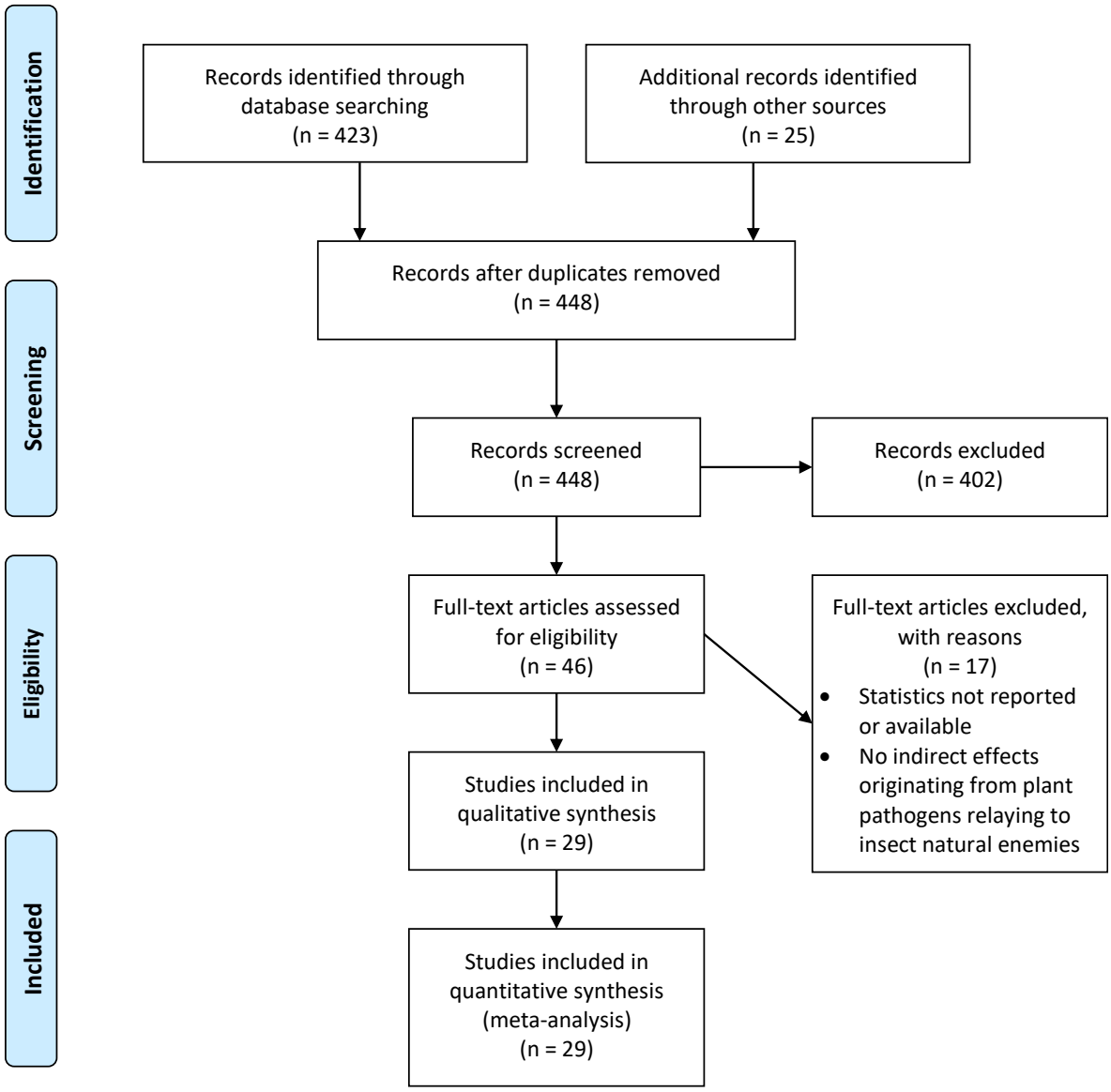
# Appendix

**Appendix 1** Results summary from Chapter 2

| Topic | Natal host | Focal host |
|---|---|---|
| Preference | Both species lentil natal host laid more eggs when mung was present compared with those mung natal | Both species laid more eggs on mung |
| | Smaller mung is preferred over bigger lentil | |
| Experience | No effect of experience | Lentil experience increased %acceptance in subsequent oviposition |

| Topic | Parental host | Natal host | Result |
|---|---|---|---|
| Emergence *C. mac* | Mung | Lentil | Low emergence, long dev, and very small |
| Sex ratio *C. analis* | Lentil | Lentil | Female bias |
| | Mung | Lentil | Male bias |
| Dev time *C. mac* | Mung | - | Longer dev time |
| | - | Mung | Dev quicker |
| | Parental*Size | Natal*Size | Larger dev quicker |
| Dev time *C. analis* (Parental*Natal) | Lentil | Mung | Dev quicker |
| Dev time *C. analis* (Parental*Natal*Size) | - | - | Larger dev quicker |
| | Lentil | Mung | Smaller dev quicker |
| Surv time C. mac | Mung | - | Lived longer |
| | Sex | | Male died quicker |
| | Size*Sex | | Larger male lived longer than female |
| Surv time *C. analis* (Parental*Natal) | Mung | Lentil | Lived longer |
| Surv time *C. analis* | Size | | Larger lived longer |
| | Size*Natal | | Very large mung natal lived longer |
| Size both species | Sex | | Female larger |
| Size *C. mac* | - | Mung | Bigger |
| | Mung | Lentil | Very small |
| Size *C. analis* | Lentil | Mung | Bigger |
| Male mating competition | - | Lentil | Outcompete except female from mung natal |
| Size (to explain male mating competition) | - | Mung (*C. mac*) | Male bigger |
| | | Lentil (*C. analis*) | |
| Mating time | - | Lentil (*C. mac*) | Only male origin is sig, and *C. mac* male lentil spent longer time than the rest |
| Male fitness both species | - | Mung | No effect of male, but female natal mung laid more eggs |




**Appendix 2** PRISMA flow chart for our data set

**Appendix 3** List of primary studies included in this quantitative synthesis

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**Appendix 4** Effect size and calculation

For each case study, we calculated effect size using the Hedges'*d* metric and its variance (Hedges, 1981):

$$d = J\frac{\overline{x} - \overline{x}_{\text{control}}}{\sqrt{\sigma}}$$

where $x_{treatment}$ refers to mean natural enemy response on infected plants and $x_{control}$ to mean natural enemy response on healthy, control plants, with

$$J = 1 - \frac{3}{4(n_{\text{treatment}} - n_{\text{control}} - 2) - 1}$$

where $n_{\ treatment}$ and $n_{\ control}$ are the sample sizes for infected and control plants and with

$$\sigma_{\text{pooled}} = \frac{(n_{\text{treatment}} - 1){\sigma^2}_{\text{treatment}} + (n_{\text{control}} - 1){\sigma^2}_{\text{control}}}{n + n - 2}$$

where σ refers to the variance of natural enemy response.

Hedges' *d* was preferred to other metrics of effect size such as the log-response ratio because it is corrected for bias due to small sample size and enables having control or experimental means equal to zero (Koricheva et al., 2013). For several natural enemy response variables reported in primary studies (*i.e.*, development time, mortality, time to mummification), positive values indicated lower performance on infected plants than on control plants. For these studies, $d_i$ was multiplied by –1 to make interpretations consistent across studies. Negative values therefore indicate that natural enemies avoided or performed worse on infected plants as compared to control plants. Positive values indicate better performance on infected plants. As a rule of thumb, it is commonly accepted that $d <$ 0.2, 0.5 and 0.7 correspond to small, moderate and large effect sizes.

In some papers, several experimental conditions were compared to the same control (e.g., plants infected by different pathogen species or strains compared to the same control plant). Non-independent effect sizes may underestimate sampling variance, which was therefore corrected to account for multiple comparison to the same control using the following equation:

$$v = \frac{1}{n_{\text{treatment}}} + \frac{1}{n_{control}} + \frac{d^2}{2N}$$

where *d* is the Hedges' effect size, and *N* the total sample size of the corresponding study.

Effect sizes and their corresponding variances were calculated in R using the '*metafor*' package (Viechtbauer, 2010; R Core Team, 2020).

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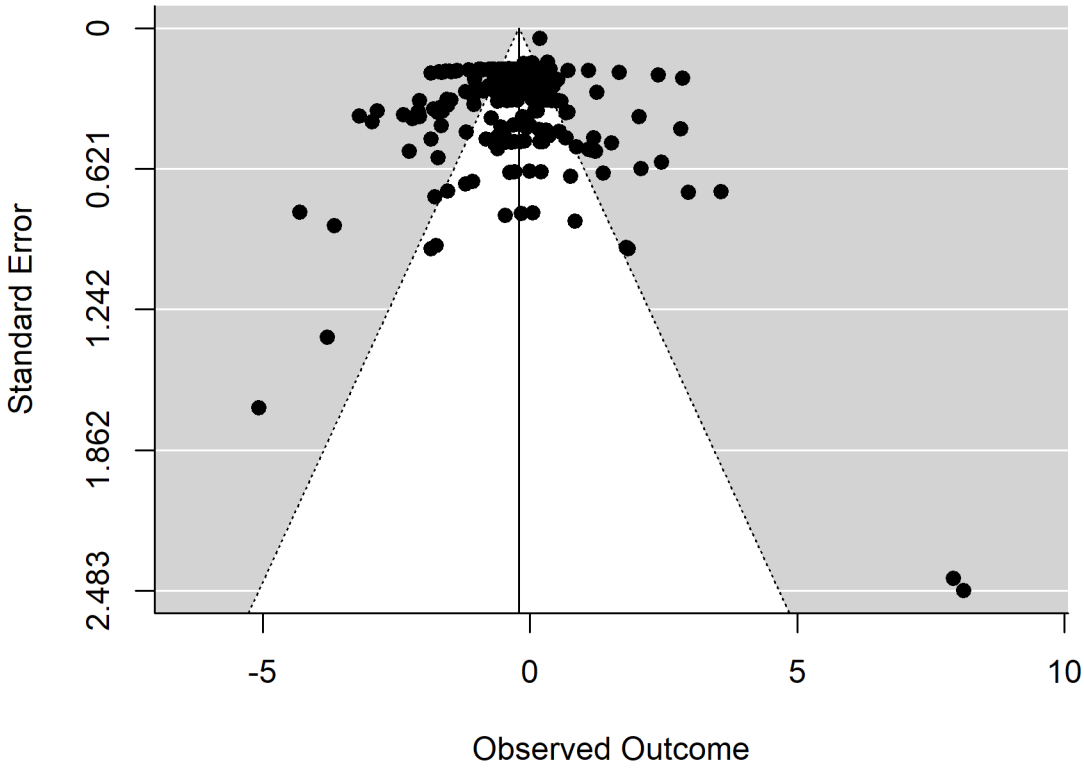
**Appendix 5** The number of case studies following given moderators; CH: consumptive effect via herbivore, NH: non-consumptive effect via herbivore and NHP: non-consumptive effect via herbivore and plant.

| **Pathogen type** | **Type of response** | **Mechanism** |
|---|---|---|
| Bacteria (n = 17) | Performance (n = 0) | CH (n = 0) |
| | | NH (n = 0) |
| | | NP (n = 0) |
| | | NHP (n=0) |
| | Preference (n = 17) | NH (n = 0) |
| | | NP (n = 8) |
| | | NHP (n = 9) |
| Fungus (n = 59) | Performance (n = 25) | CH (n = 14) |
| | | NH (n = 1) |
| | | NP (n = 9) |
| | | NHP (n = 1) |
| | Preference (n = 34) | NH (n = 0) |
| | | NP (n = 6) |
| | | NPH (n = 28) |
| Virus (n = 140) | Performance (n = 115) | CH (n = 102) |
| | | NH (n = 8) |
| | | NP (n = 0) |
| | | NHP (n = 5) |
| | Preference (n = 25) | NH (n = 13) |
| | | NP (n = 1) |
| | | NHP (n = 11) |

**Appendix 6** Funnel plot showing the relationship between individual effect size and the standard error.



**Appendix 7** Temporal trend in combined effect size through cumulative meta-analysis