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Linking aboveground and belowground insect herbivore interactions: a case study with the vine weevil (*Otiorhynchus sulcatus*)

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Abstract

Interactions between insect herbivores and host plants are fundamental, shaping both ecosystem functions and community structure. One aspect of insect-plant interactions that has received considerable attention recently is the indirect linkages between aboveground and belowground insect herbivores via a shared host plant. To date, the relationship between a maternal insect aboveground and her soil dwelling offspring has been largely overlooked. This study aimed to examine the interactions between the adult insect and soil dwelling larvae of the vine weevil (*Otiorhynchus sulcatus*) with reference to the preference–performance hypothesis, using an agronomically important host plant, red raspberry (*Rubus ideaus*).

A meta–analysis of aboveground–belowground insect herbivore interactions highlighted that belowground Coleopteran herbivores positively impacted aboveground Homoptera and that general predictions from conceptual models in the literature regarding the direction of interactions between insects were correct, but not statistically significant. In addition it was found that aboveground insect herbivores negatively influenced the survival of belowground herbivores. The preference–performance hypothesis was not supported in the findings for the vine weevil on raspberry. Instead a conflict between larval development and adult egg laying behaviour was observed, with a significant reduction in larval mass recorded when maternal adults fed on the same host plant. Larval mass was decreased by 19% after prior conspecific root feeding, but maternal weevils did not distinguish between plants with and without larvae for oviposition. Significant differences between larval performance (abundances and mass) on the raspberry cultivars Glen Rosa and Glen Ample were not correlated with adult oviposition aboveground. Instead, in some instances, maternal egg laying was correlated with foliar nitrogen content, suggesting that

this may be an influential factor in the oviposition behaviour of adult vine weevils. Significant differences seen in larval performance in the laboratory were not reflected in the field, with adult vine weevil populations on Glen Rosa and Glen Ample showing no significant differences in terms of abundance.

The findings from this study suggest that mother–offspring relationships in an aboveground–belowground context warrant further consideration. In particular, the identification of potential conflict between mother and offspring highlights another factor influencing aboveground–belowground relationships that could consequently influence terrestrial ecosystems.

Preface

Four of the chapters in this thesis (2, 4, 5 & 6) have been presented in a format appropriate for peer–review journals. Therefore some repetition in the introductions and recurrent methodologies was unavoidable. The co–authors in these chapters have made contribution to these chapters benefiting the extent of their authorship.

Co–authors for these papers were: Dr S. N. Johnson (Chapters 2, 4, 5 & 6), Professor S. E. Hartley (Chapters 2, 4, 5 & 6), Dr R. M. Brennan (Chapters 4 & 5), Dr K. MacKenzie (Chapters 4 & 5), Dr T. H. Jones (Chapter 2) and Dr J. Koricheva (Chapter 2).

Citations, references and text in this thesis are set out in accordance with the stipulations laid down by The British Ecological Society for publication in their journals.

Declaration

I hereby declare that this thesis has not been and will not be, submitted in whole or in part to another University for the award of any other degree.

Signature:……………………………………

Katherine Elizabeth Clark

June 2010

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For Neil xx

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Chapter One – Introduction

1.1. Insect–plant interactions

Interactions involving herbivorous insects and host plants are amongst the most well studied associations between terrestrial species. With over 50 % of existing insect species feeding on plants, approximately 25 % of all species on earth (Masters & Brown, 1997), it is doubtful if there are many plant species not exposed to at least one insect herbivore. Many insect–plant interaction studies address a phytophagous insect's preference of host plant, with both behavioural and chemical factors playing key roles in the final choice. Insect herbivores are particularly selective in choosing a host plant, with less than 10 % of herbivorous insect species feeding on three plant families or more (Bernays & Graham, 1988). Accurate host selection is clearly vital for specialist herbivores and host recognition is often based on the chemical composition of plants, particularly the presence of distinctive secondary metabolites. Host plant nutritional quality is also an important factor influencing an insect's decision. However, there are several other non–chemical considerations in host plant selection, such as the presence of competitors and/or the absence of enemies, and the physical attributes of the host plant, such as the shelter it provides.

1.2. Host plant selection

When considering the wealth of information available regarding host plant suitability, insects must respond accordingly, optimising both their own survival and that of their offspring. Several factors may influence the choice of host plant including the quality of the food source available, the suitability for offspring, prior plant experience (whether at a juvenile or adult stage), plant semiochemicals, plant physiology and the presence or absence of enemies or competitors. Which factors are most influential or important in the resulting selection of host plant by an insect herbivore, has been a long–standing area of debate.

Since Ehrlich and Raven's (1964) classic paper addressing the chemistry behind insect–plant interactions, plant secondary chemistry has received considerable attention. However, some (Bernays & Graham, 1988) have argued that too much emphasis has been placed on plant chemistry and that other fundamental plant characteristics (e.g. plant height, leaf area and leaf hairs) and environmental situations (e.g. presence of enemy free space, light intensity, moisture levels and competition) are of equal importance.

Visual cues, incorporating shape, colour and physical plant properties form part of the behavioural suite of stimuli influencing an insect's decision to select a host plant. For example, many insects have shown preferences for host plants with certain colours (e.g. Prokopy et al., 1983, Traynier, 1986) and/or leaf shapes (Dill et al., 1993) demonstrating the importance of visual choice (reviewed by Prokopy & Owens, 1983). In addition, physical plant properties including the presence of leaf hairs (e.g. Yencho & Tingey, 1994, Ranger & Hower, 2002), surface waxes (see Eigenbrode & Espelie, 1995) and leaf toughness (e.g. Foggo et al., 1994, Peeters et al., 2007) have all been shown to affect the behaviour of insects in terms of feeding and oviposition.

Plant semiochemicals play an important role in host plant decisions by insects, acting as attractants or deterrents in relation to both insect feeding and oviposition. Several phagostimulants have been documented for insect species in relation to different host plants. Equally, many deterrents have also been categorised (see summary tables in Schoonhoven et al., 2005). In some instances, both feeding stimulants and deterrent compounds can be found in the same host plant, confounding the response of the insect (Eriksson et al., 2008). Secondary plant compounds are also often non–uniformly distributed throughout plants in relation to either herbivore attack, environmental conditions or plant growth, thus insect herbivores must respond accordingly, selecting not

only a suitable host plant, but also a particular plant part. For instance, colonising gall aphids (*Pemphigus betae*) do not settle randomly on leaves of their host plant (*Populus angustifolia*) (narrowleaf cotton–wood), but instead choose to form galls at the base of the leaf, where phenolic concentrations are lowest (Zucker, 1982). Plant semiochemicals can also be highly influential in an adult insect's selection of host plant for oviposition, again either promoting the choice of plant or providing a deterrent (e.g. Jones & Finch, 1987, Huang & Renwick, 1993, Morris *et al.*, 2000, Derksen *et al.*, 2007). For instance, the peach tree borer (*Synanthedon exitiosa* (Say)) was found to oviposit in response to semiochemicals present in a gum frass mixture produced by conspecific larvae already developing on the plant (Derksen *et al.*, 2007). In contrast, the large cabbage white butterfly (*Pieris brassicae*) was deterred from ovipositing on cabbage leaves that either had eggs present, or had had eggs removed from them, suggesting the presence of a deterrent volatile cue (Rothschild & Schoonhoven, 1977).

Plants are often considered sub–optimal as a food source for insects, primarily due to the limited nitrogen in plant tissues available to insect herbivores (McNeill & Southwood, 1978, Mattson, 1980). Nitrogen and phosphorus are considered particularly limiting for insect herbivores, due to the low nutrient content in plants in comparison to the requirements of insects (Huberty & Denno, 2006). Nitrogen–rich proteins are one of the major components of an insect body, thus juvenile insects require a good source of nitrogen–rich food to develop. Adult insect fecundity and reproduction can be adversely influenced by limited nitrogen availability (Joern & Behmer, 1997, Colasurdo *et al.*, 2009). The optimal foraging hypothesis (Emlen, 1966, MacArthur & Pianka, 1966), states that a predator will forage in such a way to maximise its energy intake per unit of time. Thus, insect herbivores are expected to search for and select host plants such that they maximise the energy gain from the plant, whilst minimising the energy expended in doing so.

Consequently, it would be expected that adult insects will select host plants that maximise their fitness and subsequently the fitness of future generations. Several theories consider the role of plant nutrients, such as nitrogen, in host plant selection.

The plant vigour hypothesis (Price, 1991) predicts that plants which grow vigorously and develop to a size larger than average should be favoured by herbivores, in particular herbivores that feed on new growth (flush feeders) are likely to benefit from vigorous plant growth. Higher nutritional quality, increased resource quantity and reduced induced defence compounds are suggested as explanations for the preference (Price, 1991). Several feeding guilds have demonstrated a positive relationship between plant vigour and subsequent insect fitness, including chewers, leaf–miners, sap–suckers, stem–borers and leaf–miners (Cornelissen *et al.*, 2008).

Another theory that may explain host plant selection by phytophagous insects is the plant stress hypothesis (White, 1969), which proposes that when plants become stressed they are more vulnerable to herbivore attack. Elevated amino acids and reduced protein synthesis within the plant make it a more nutritious option for herbivores that are otherwise generally limited to a diet low in nitrogen. White (1969) originally proposed the hypothesis as an explanation for why insect outbreaks suddenly occur on trees, when previously the insect abundance was relatively low. Several studies have since investigated the influence of plant stress on insect herbivores, but the results have proved inconclusive. In particular, insect responses to plants exposed to drought stress have shown that different insect feeding guilds do not respond consistently (Waring & Cobb, 1992), thus making it difficult to determine how insect herbivores may perceive stressed plants.

However, several other factors may influence an insects' behaviour. Host plant suitability is affected by the nutritional quality of the plant, but sometimes, insect herbivores appear to disregard plants that provide them with optimal resources. Instances where an herbivorous insect has selected an 'inferior' host plant have often been deemed 'mistakes', but may actually be the result of an underlying benefit that is not apparent to the investigator.

Insect herbivores have been shown to select host plants that are inferior in terms of nutritional quality or offspring development, but provide a refuge from enemies, a so called 'enemy free space'. Larvae of the potato tuber moth (*Phthorimaea operculella*) were found to have higher survival on a novel host (the tomato) than on their original host (the potato), indicating that fewer enemies attacked the larvae on tomato. However, in achieving this 'protection' the fitness of the larvae was lower on tomato than it was on potato (Mulatu *et al.*, 2004). The generalist caterpillar *Estigmene acrea* (Salt marsh moth) has superior growth rates when feeding on a pure diet of *Viguiera dentata* (Sunflower goldeneye), in comparison to feeding solely on *Senecio longilobus* (Threadleaf groundsel) or a mixture of the two plants, although this exposes the caterpillar to a higher rate of parasitism (Singer *et al.*, 2004). By feeding on the nutritionally inferior *Senecio longilobus* in addition to *Viguiera dentata*, the caterpillar is able to accumulate toxins from the *Senecio longilobus* improving it's survival against parasitic attack, thus effectively providing a defence mechanism (Singer *et al.*, 2004).

It has previously been hypothesised that polyphagous insects are at a disadvantage in selecting host plants in comparison with specialist insects, due to their inability to choose between several host plant options (Levins & MacArthur, 1969). The neural limitation hypothesis (Bernays, 1998, Bernays, 2001) states that insects have limited capabilities to process information, thus specialist insects make superior host plant choices in comparison

to generalist insects, who are faced with more complex decisions. Consequently, polyphagous insects encountering several host plants have a higher probability of making a poor decision than specialist insects. In addition, specialist insects have been shown to be at an advantage in terms of the speed in which they select a host plant, thus limiting searching time, which if elongated may increase their exposure to predators. For example, when the host plant decisions of both a specialist and generalist population of the aphid *Uroleucon ambrosiae* (Brown ambrosia aphid) were investigated, aphids from the specialist population were more efficient at host–finding, host–selection, host–acceptance and host–settling (Bernays & Funk, 1999).

Herbivorous insects have also been found to demonstrate learning behaviour, with generalist insects selecting to preferentially feed on a particular plant following a feeding or oviposition experience (Jaenike, 1988, Prokopy & Papaj, 1988, Zhang *et al.*, 2007); such preference is termed 'induced preference' (Jermy *et al.*, 1968). Prior experience may not always result in the selection of that particular host plant again, but can influence an insect's selection of host plant, for instance, sixth instar *Schistocerca americana* reared on different diets, subsequently ranked plants differently according to plant palatability (Howard & Bernays, 1991). Additionally, aversion learning (Dethier, 1980), where insects avoid particular plants after previously having had a bad experience as a result of feeding on the plant can also affect host plant selection. The Hopkins host–selection principle hypothesises that adult insects will exhibit a preference for host plants that they themselves were raised on as larvae (see Barron, 2001). In a conditioning experiment, *Mandula sexta* larvae were exposed to electric shocks in response to ethyl acetate, which on later exposure the larvae avoided (Blackiston *et al.*, 2008). Interestingly, the avoidance conditioning was transferred through pupal moults, but did not pass to the adult.

Despite the numerous theories proposed for the selection of host plants by herbivorous insects, a major aspect of insect host plant selection relates to offspring fitness, where the oviposition decisions of maternal insects can be critical to the survival of future generations. Both insect fecundity and egg quality are influenced by host plant suitability, which subsequently can affect larval development (in terms of size and time) as well as later adult fecundity (Awmack & Leather, 2002). The selection of a host plant by a maternal insect for oviposition and the resulting performance of offspring developing on the chosen host plant have frequently been considered in relation to the preference–performance hypothesis.

1.3. Preference–performance hypothesis

In insect–plant interactions, the association between maternal choice of host plant for offspring and the subsequent performance of progeny has often been investigated in relation to the preference–performance hypothesis (PPH). Also known as the optimal oviposition theory (Jaenike, 1978), the naïve adaptionist theory (Courtney & Kibota, 1990) or the 'mother knows best' principle (Valladares & Lawton, 1991), the PPH was first proposed by Jaenike (1978). The hypothesis states that maternal insects will preferentially lay eggs on plants that optimise the survival and performance of their offspring. Specifically, the PPH applies to insects with offspring that have little or no capability to move and as such are dependent on the host plant choice of the mother. To optimise insect fitness, the PPH predicts a positive correlation between oviposition preference and offspring performance.

Numerous studies examining oviposition preferences and resulting larval performance have documented positive correlations that are consistent with the PPH (e.g. Craig *et al.*, 1989,

Heisswolf *et al.*, 2005, Staley *et al.*, 2009). However several maternal insects have also been found to make poor oviposition decisions, giving a weak preference–performance relationship between mother and offspring (e.g. Rausher, 1979, Scheirs *et al.*, 2004, Digweed, 2006, Gripenberg *et al.*, 2007). In a recent meta–analysis considering adult oviposition decisions in relation to offspring performance, support for the PPH was found, with maternal adult insects preferentially selecting plants that benefited offspring performance (Gripenberg *et al.*, 2010). However, considerable attention has focussed on examples of 'bad motherhood' choices, with variations of insect host plant selection theories being proposed to explain why the maternal insect does not select a host plant that maximises offspring survival and development (Mayhew, 2001).

One such theory is a special case of the optimal foraging hypothesis, first proposed by Jaenike (1986). The hypothesis states that maternal adult insects will maximise their own fitness by selecting host plants with optimal nutrition, regardless of their suitability for subsequent offspring performance. Recently, this hypothesis has received more attention, with several studies reporting stronger correlations between insect oviposition and feeding behaviour than between maternal oviposition and offspring performance (Scheirs *et al.*, 2000, Scheirs *et al.*, 2004). For example, the oligophagous grass miner (*Chromatomyia nigra*) showed a strong correlation between feeding and oviposition, but only a weak correlation between oviposition and offspring performance measures (Scheirs *et al.*, 2000).

Enemy free space has also been suggested as an explanation for poor PPH links. Frequently, studies investigating the PPH are conducted in controlled environments, often without the presence of predators who may be influential in the mother–offspring relationship (Thompson, 1988a, Thompson, 1988b) For example, the leaf beetle *Oreina elongata* was found to oviposit equally on *Adenostyles alliariae* (Asteraceae) and *Cirsium*

spinosissimum (Asteraceae) when natural enemies were absent, but preferentially oviposited on *C. spinosissimum* when enemies were introduced (Ballabeni *et al.*, 2001). Higher egg survival on *C. spinosissimum* suggests that this plant provides a refuge for the leaf beetle larvae (Ballabeni *et al.*, 2001).

It has been previously suggested that polyphagous insects are incapable of making complex decisions between several host plants due to limitations in their neural capacity (Levins & MacArthur, 1969, Bernays, 2001). Specialist insects are therefore hypothesised to make superior host plant decisions than generalists, as they are faced with simpler decisions and a greater selection pressure to make the correct choice. It could therefore be hypothesised that polyphagous insects will exhibit weaker links between adult oviposition and offspring survival as a result of poorer host plant choices. This theory was recently tested quantitatively by Gripenberg *et al.* (2010), who found that oligophagous insects showed stronger PPH linkages than polyphagous insects.

Adult insect preference for plants or plant modules that exhibit more vigorous growth and consequently provide better nutrition for larvae is hypothesised by the plant vigour hypothesis (Price, 1991). In particular, Price (1991) hypothesised that insects with larval stages which exhibited superior performance on plants growing more vigorously would have a strong PPH link, as ovipositing adults would select such plants to maximise offspring performance (e.g. Heisswolf *et al.*, 2005). However, a recent meta–analysis (Cornelissen *et al.*, 2008) demonstrated that insect herbivores did not show a strong preference–performance link on vigorously growing plants.

At present, the majority of research investigating the PPH has focused primarily on insect herbivores with life–cycles that take place wholly aboveground. Equally though, the

hypothesis could be applied to insects with life stages that reside belowground (Johnson *et al.*, 2006). In fact, it may be more important for this group of organisms because soil dwelling larvae have limited mobility to relocate and are thus highly dependent on the mother's choice of host plant. Much of the current literature addressing aboveground–belowground insect herbivore interactions principally focuses on interactions between different insect species (see section 1.4.), but does not consider interactions between mother and offspring, which may exhibit different relationships that could be instrumental in shaping aboveground–belowground insect herbivore interactions. Hence, incorporating insects with belowground life stages into the PPH may uncover alternative mechanisms that are important in driving insect–plant interactions, especially with respect to host plant choices.

1.4. Aboveground–belowground insect interactions

It is now widely accepted that aboveground and belowground mechanisms in terrestrial ecosystems are linked indirectly via plant–mediated interactions (van der Putten *et al.*, 2001, Wardle *et al.*, 2004, De Deyn & van der Putten, 2005, van der Putten *et al.*, 2009). Intrinsic links between aboveground and belowground communities (often facilitated by plants) have been found between microbes (Wardle *et al.*, 2005) mycorrhizal fungi and insects (Gange, 2001), insect herbivores (Masters *et al.*, 1993) and herbivores (Kaplan *et al.*, 2008). In particular, interactions between insect herbivores aboveground and belowground have received considerable attention, in an attempt to interpret the mechanisms underpinning such relationships (reviewed by Blossey & Hunt-Joshi, 2003, Johnson *et al.*, 2008b).

Masters *et al.* (1993) proposed the first conceptual model addressing plant mediated interactions between aboveground and belowground insect herbivores. The model

hypothesised that aboveground insect herbivores benefited indirectly from the belowground feeding of insects on plant roots. In particular, the model proposed that the removal of fine roots by insect herbivores would decrease water and nutrient uptake by the host plant. Consequently this deficiency would lead to elevated amino acids and carbohydrates in the plant foliage, promoting the performance of aboveground insect herbivores (see Brodbeck & Strong, 1987, Huberty & Denno, 2004). In contrast, the model predicted that aboveground herbivores negatively impacted belowground herbivores by indirectly reducing root biomass. The model neatly conceptualises aboveground–belowground insect herbivore interactions, but its basis on only a few studies and early successional plants resulted in queries over its general applicability (Blossey & Hunt-Joshi, 2003).

More recently, studies of aboveground and belowground insect herbivores have addressed how insects feeding on one part plant could induce plant defence compounds, with potential consequences for other insects utilizing a different part of the same plant (Bezemer *et al.*, 2003, Soler *et al.*, 2007). Bezemer *et al.* (2002) proposed that belowground insect herbivory enhanced defence compounds in aboveground foliage, detrimentally affecting aboveground insect herbivores. The "defence induction hypothesis" (Bezemer *et al.*, 2002) was the first to suggest that root feeding insects may negatively impact aboveground insect herbivores, providing an alternative mechanism to the "stress response hypothesis" proposed by Masters (1993).

The extrapolation of aboveground–belowground insect herbivore interactions to incorporate additional trophic levels is a natural extension to the primarily pairwise interactions that have thus far been studied. For example, foliar herbivory by cabbage white larvae (*Pieris brassicae* L.) was found to negatively impact the performance of the

cabbage root fly (*Delia radicum* L.) and subsequently influence the performance of its parasitoid *Trybliographa rapae* (Soler *et al.*, 2007). Such indirect interactions between insect herbivores highlight the important role that aboveground–belowground relationships play in structuring ecosystem communities (van der Putten *et al.*, 2001, Wardle *et al.*, 2004).

Currently, many of the studies that have investigated the links between aboveground–belowground insect herbivores have used unrelated insect species, despite the fact that maternal adults and offspring may utilise the same host plant. The potential to expand the PPH to include insects with both aboveground and belowground life stages has already been highlighted (Section 1.3.). Understanding the relationship between an adult insect feeding aboveground and its soil dwelling offspring could provide new insights into aboveground–belowground insect interactions.

1.5. Thesis objectives and outline

The overall aim of this thesis was to investigate the interactions between an adult insect aboveground and its soil dwelling larvae belowground using the vine weevil (*Otiorhynchus sulcatus*) feeding on red raspberry (*Rubus idaeus*). In particular, the interactions examined tested the preference–performance hypothesis in an aboveground–belowground context. Having briefly reviewed the literature regarding host plant selection by herbivorous insects, the preference–performance hypothesis and aboveground–belowground insect interactions in this chapter, **Chapter Two** aims to quantitatively review the current literature investigating insect herbivore interactions aboveground and belowground. Specifically the chapter tested the following hypotheses:

1. is there a general pattern for interactions between aboveground and belowground insect herbivores?

2. do aboveground and belowground insect herbivore interactions differ depending on whether the study is conducted within the laboratory or the field?

3. which insect performance parameters are affected by aboveground–belowground interactions?

4. does insect order influence the direction of interactions between aboveground and belowground insect herbivores?

5. does the life history strategy of the host plant mediating the interactions between aboveground and belowground insect herbivores affect the outcome?

In **Chapter Three**: 'Understanding vine weevil (*Otiorhynchus sulcatus*) dynamics in a protected red raspberry (*Rubus idaeus*) crop system', the main aim of the chapter was to consider the population dynamics of the vine weevil on red raspberry in a commercially equivalent cropping system. A review of the biology of the vine weevil is presented in the introduction of the chapter. In particular the chapter aimed to:

1. examine the seasonal changes in vine weevil abundances with respect to two different raspberry cultivars: Glen Ample and Glen Rosa,

2. investigate the population dynamics of vine weevils with respect to different initial egg densities,

3. estimate temporal changes in the population size using basic capture–mark–recapture calculations.

Chapter Four, 'Oviposition and feeding behaviour by the vine weevil (*Otiorhynchus sulcatus*) on red raspberry (*Rubus idaeus*): effects of cultivar and plant nutritional status'. The aim of this study was to investigate whether adult weevil oviposition (both preference and egg laying capacity) and feeding behaviour would be influenced by the chemical composition

and growth characteristics of nine different raspberry cultivars. In particular this study aimed to:

1. test whether vine weevil adults preferentially oviposited or fed on particular raspberry cultivars when given a choice between all nine cultivars,

2. determine whether egg laying capacity and feeding behaviour varied between cultivars in the absence of other cultivars,

3. assess whether differences in nutritional status between cultivars affected feeding and/or oviposition behaviour.

Chapter Five, 'Preference–performance relationships in aboveground–belowground life cycles: a laboratory and field study with the vine weevil (*Otiorhynchus sulcatus*)'. The objective of this study was to investigate vine weevil behaviour and performance, both aboveground and belowground, on two contrasting raspberry cultivars: Glen Ample and Glen Rosa. The specific aims of this study were:

1. to determine how the two cultivars affected different larval performance traits (abundance and body mass), and establish whether these traits were related with each other,

2. to determine whether either, or both, performance traits influenced oviposition behaviour by adults,

3. assess whether these differences were reflected in the field over a two–year period.

Chapter Six, 'Does mother know best? The preference–performance hypothesis and parent–offspring conflict in aboveground–belowground herbivore lifecycles'. The aim of this study was to characterise the underlying factors influencing the performance of a belowground insect herbivore and to subsequently determine whether this was linked to

the oviposition behaviour of the parent residing aboveground. The study specifically aimed to:

1. determine whether maternal insects feeding aboveground affected offspring performance belowground,

2. assess how maternal feeding affected root traits (e.g. root biomass), root nutritional quality (carbon and nitrogen concentrations) and secondary metabolites (phenolics), and assess whether these changes were related to larval performance,

3. establish whether prior feeding by conspecific larvae improved root suitability and performance of larvae that subsequently fed on the roots,

4. test whether maternal insects preferentially laid eggs on plants that are likely to be beneficial for their offspring (e.g. by having larger root biomass), and determine whether egg laying behaviour was linked to maternal feeding behaviour.

Chapter Seven, 'Discussion'. This chapter discusses the results presented in the thesis in a wider context. The possibility of the behaviour manipulation of insect pests in biological control is considered as well as the potential response of vine weevils to climate change and increasingly tougher chemical control legislations. The experimental approach to insect–plant interactions in aboveground–belowground systems is examined, in addition to the methodologies used in investigating insect host plant choice. Finally host plant choice and the preference–performance hypothesis in an aboveground–belowground situation is considered in terms of host plant suitability, insect diet breadth and vine weevil clonality.

Chapter Two – Testing for patterns in above– and belowground insect herbivore interactions: a meta–analysis approach

2.1. Abstract

Despite the growing literature investigating interactions between aboveground (AG) and belowground (BG) insect herbivores, underlying patterns have been difficult to identify due to a high degree of context dependency. In this study, the literature relating to AG and BG insect herbivore interactions is reviewed quantitatively using meta–analysis. The database consisted of 44 studies, encompassing over 30 different insect and 20 different plant species from laboratory and field studies. The study specifically addressed whether there were overall directions in AG and BG insect interactions, and considered whether the nature (e.g. direction and strength) of these interactions was influenced by particular features of each study system. The most statistically significant result from the meta–analysis demonstrated that BG Coleopteran (chewing) herbivores positively influenced AG Homopteran (sucking) insects. Performance parameter was important in respect that AG herbivores had a significantly negative influence on BG herbivore survival, more so than for any other parameter. The overall directions of AG–BG insect herbivore interactions supported the direction of the interactions previously theorised in the literature, but without reaching statistical significance. Separating studies into field or laboratory experiments or according to host plant lifespan (annual versus perennial) did not affect the significance of the interactions between AG and BG insect herbivores. The meta–analysis identified specific gaps in the current literature on AG–BG insect herbivore interactions, and in particular highlighted the limited range of BG insect herbivores (usually Coleopteran) included in study systems. This quantitative approach to addressing interactions between AG and BG insect herbivores provides direction for future research.

2.2. Introduction

Recent decades have seen a growing acknowledgement that many of the aboveground (AG) and belowground (BG) processes operating in terrestrial ecosystems are indirectly linked to each other through plant–mediated mechanisms (van der Putten *et al.*, 2001, Wardle *et al.*, 2004, De Deyn & van der Putten, 2005, van der Putten *et al.*, 2009). Such plant–mediated linkages between AG and BG organisms can have a wide range of influences on the community dynamics of microbes (Wardle *et al.*, 2005), plants (van Ruijven *et al.*, 2005) and herbivores (Kaplan *et al.*, 2008). In particular, the relationship between AG and BG insect herbivores has received particular attention, in an attempt to characterise the underpinning mechanisms (reviewed by Blossey & Hunt-Joshi, 2003, Johnson *et al.*, 2008b). Despite the number of studies addressing interactions between AG and BG insects, the identification of consistent patterns and generalities has so far proved difficult, potentially due to the wide range of study systems and experimental approaches used (Blossey & Hunt-Joshi, 2003)

A conceptual model proposed by Masters *et al.* (1993) considered the indirect interactions of foliar– and root–feeding insects. The model proposed that AG foliar–feeding insects were positively influenced by BG root–feeding insect herbivores, whereas BG insects were adversely affected by AG insects. The model hypothesised that the removal of fine roots by insect herbivores resulted in reduced water and nutrient uptake by the host plant, which consequentially led to elevated amino acids and carbohydrates within the plant foliage (see also Brodbeck & Strong, 1987, Huberty & Denno, 2004). AG insect herbivores benefited from the increased nutritional levels within the foliage, resulting in improved performance. In contrast, the model proposed that AG herbivory indirectly reduced root biomass, adversely influencing root–feeding insect herbivores. Whilst the model provides a concise

approach to AG – BG insect interactions, its general applicability has been questioned due to its reliance on the limited number of studies available at the time and its emphasis on early successional plants (Blossey & Hunt-Joshi, 2003).

More recent studies (Bezemer *et al.*, 2003, Soler *et al.*, 2007) have reported how AG and BG insects can interact by systemically inducing plant defence compounds which consequentially influence the other insect herbivore (Bezemer & van Dam, 2005). Results from such studies have sometimes had contradictory outcomes to those predicted by Masters *et al.* (1993). The nature of the research literature available has made it fragmented and sometimes inconsistent, making it difficult to predict how AG–BG interactions shape terrestrial ecosystems. Researchers, however, are already incorporating added trophic complexity into AG–BG insect herbivore systems (Poveda *et al.*, 2005, Soler *et al.*, 2005), so it is particularly timely to try and identify the key patterns that underpin such complex interactions.

This study aims to provide the first quantitative review of this research area by adopting a meta–analysis approach to investigate interactions between AG and BG insect herbivores via their shared host plant. Previous reviews have so far been qualitative, generalising trends in AG and BG insect interactions by vote counting (Blossey & Hunt-Joshi, 2003, Johnson *et al.*, 2008b), which does not take into account the magnitude of the effects and the variation in sample size and statistical power among the studies. Meta–analysis has significant advantages over vote–counting and other qualitative review methods as it enables estimation of the magnitude of the effect across several independent studies as well as the analysis of the various sources of variation (Gurevitch & Hedges, 2001). In addition, by taking into account variation in sample size, meta–analysis may allow the identification of trends even when the results of individual studies are not statistically significant. In

particular, meta–analysis helps to answer questions in particular research areas where individual studies show conflicting results (Arnqvist & Wooster, 1995). For example, Gurevitch *et al.* (1992) addressed some of the main controversies relating to field–competition experiments and illustrated that a quantitative summary analysis of existing datasets can help direct, and indeed change, prevailing opinions.

The aim of this meta–analysis was to address the following questions:

1. Is there a general pattern for interactions between AG and BG insect herbivores?

2. Do AG and BG insect herbivore interactions differ depending on whether the study is conducted within the laboratory or the field?

3. Which insect performance parameters are affected by AG–BG interactions?

4. Does insect order influence the direction of interactions between AG and BG insect herbivores?

5. Does the life history strategy of the host plant mediating the interactions between AG and BG insect herbivores affect the outcome?

2.3. Methods

2.3.1. The database

Initially, keyword searches were conducted in the Web of Science (ISI) electronic database (1945 – 2010) to find studies that investigated the relationships between AG and BG insect herbivores. The keywords 'shoot', 'leaf' 'root', 'aboveground', 'belowground' and 'insect' were used in different combinations to maximise the number of studies captured by the search. Reference lists of the captured studies were examined for further relevant studies. In addition, the database was enlarged by Web of Science searches of studies that cited some of the principal papers within this research area (notably Masters *et al.*, 1993, Bezemer *et al.*, 2002, Blossey & Hunt-Joshi, 2003). Data reported in postgraduate theses and unpublished data kindly provided by authors were also included in the database; data was obtained by contacting the authors directly. The final database consisted of 44 studies (**Table 2.1**).

Studies were required to meet a basic set of criteria to be incorporated into the database. The criteria were designed to ensure that the interaction between AG and BG insect herbivores was clearly discernable from any other treatments or factors in the study. The criteria were:

1. Studies had to have two treatments, one where only one insect from the pairwise interaction was present on the host plant and one where both insects were present.

2. Studies where measurements were taken on higher trophic level insects, in response to the primary AG – BG interaction were excluded.

3.   Studies where a pair of insects were studied in response to different host plants were treated separately, as host plant can influence the interaction that occurs between the insects.

4.   For studies where measurements were repeated over time, the final measurements were used to prevent pseudoreplication.

5.   Studies had to provide sufficient statistical information to allow calculation of effect sizes. This consisted of either sample sizes, means and standard errors/standard deviations for both the control and experimental groups, or statistics such as the F–statistic that could have the effect size calculated from them using the MetaWin statistical calculator (Rosenberg *et al.*, 2000). A high proportion of the data was presented graphically and the imaging software Image J (Abramoff *et al.*, 2004) was used to enlarge and digitalize the figures in order to obtain accurate numerical values (Borowicz, 2001, Leimu *et al.*, 2006).

A range of performance parameters and abundance measures were recorded in the studies to determine the influence of BG insect herbivores on AG insect herbivores, and *vice versa.* Performance parameters included relative growth rate (RGR), survival, fecundity, development time and longevity.

2.3.2. Meta–analysis

In meta–analysis the choice of how to calculate effect size is primarily based on the form in which the studies report their findings, although other considerations also influence this decision (Osenberg *et al.*, 1999). For this meta–analysis, Hedges' *d* (**Equation 2.1**) (Hedges

& Olkin, 1985) was used as the effect size, as the majority of studies reported means, standard errors and sample sizes:

$$d = \left(\frac{\overline{Y}_e - \overline{Y}_c}{\sqrt{\dfrac{(N_e - 1)(s_e)^2 + (N_c - 1)(s_c)^2}{N_e + N_c - 2}}} \right) \left(1 - \frac{3}{4(N_e + N_c - 2) - 1} \right) \qquad \textbf{(Equation 2.1.)}$$

Here, $\overline{Y}_c$ is the mean insect performance on the control group of plants, which for this study represents the treatment with only one insect type (AG or BG) present on the host plant and $\overline{Y}_e$ is the mean insect performance on the experimental group of plants, which represents the treatment where both AG and BG insects are present. The sample size and standard deviation of the control and experimental group is given by N_c and s_c and N_e and s_e respectively. Hedges' d is a more robust effect size measurement in comparison to other similar effect sizes when sample size is small (Rosenberg *et al.*, 2000). The MetaWin statistical calculator was used to convert other forms of statistics such as the F–statistic into Hedges' d where possible.

The influence of BG insect herbivores on AG insect herbivores, or *vice versa*, was quantified by calculating the effect size for each of the pairwise interactions. A positive effect size indicated that the presence of AG insects had a beneficial effect on the performance of BG insects and *vice versa*, similarly negative effect sizes indicated detrimental interactions between the insects. Larger effect sizes demonstrate a stronger influence between the two insects, with an effect size of 0.2 considered to be small, 0.5 moderate and 0.8 large (Cohen, 1988). For development time, the sign of the effect was reversed as an increase in development time between the control and experimental groups indicated a negative effect (i.e. increased development time is a detrimental response).

For several of the studies, more than one performance parameter was measured for the same experiment (e.g. abundance, development time and RGR). To prevent pseudoreplication, after checking that there were no significant statistical differences between herbivore effects on performance parameters, they were pooled using meta–analysis to produce a single effect size for that particular study (see Kaplan & Denno, 2007).

A mixed–effect model was used, as recommended by Gurevitch and Hedges (1999). This model assumes that the variation between the studies originates from both sampling error and random variation, which is most applicable for ecological data. To test whether effect sizes were significantly different from zero, where zero demonstrates that there is no interaction between the AG and BG insects, 95% bias–corrected bootstrap confidence intervals were calculated with 4999 iterations (Adams *et al.*, 1997). The interaction between the insects was considered to be statistically significant if the confidence intervals did not encompass zero. All analyses were conducted using MetaWin 2.1.3.4. (Rosenberg *et al.*, 2000).

To ascertain how performance parameters of AG and BG insect herbivores were influenced by one another, effect sizes were calculated for the performance parameters measured in each study. Subsequent analyses were conducted using the pooled effect sizes across the performance parameters as described previously. To investigate whether the strength of AG–BG interactions varied depending on insect order, host plant lifespan or type of experiment, studies were divided into corresponding groups (e.g. annual or perennial plants, laboratory and field experiments) and the between–group heterogeneity was inspected using a chi square test statistic Qb (Hedges & Olkin, 1985). This allowed

determination of whether there were significant differences between the effect sizes for different categories.

2.3.3. Publication bias

Publication bias in the literature selected was assessed using the funnel plot technique (Light & Pillemer, 1989). Effect sizes for AG and BG insect interactions were plotted against sample size. To illustrate that there is no publication bias, plots should show symmetry around the mean effect size for each group and no correlation between effect size and sample size (number of repetitions for each study) should be present. The exclusion of studies with non–significant results or weak effects will result in a gap in the funnel. A significant correlation between effect size and sample size can indicate that studies reporting large effects were more likely to be published than ones with small effects. Spearman's rank correlations were calculated between effect sizes and sample sizes for AG and BG insect interactions.

2.4. Results

There was no significant difference between how AG insects influence BG insects and how BG insects influence AG insects (Qb=2.532 df=1, p=0.1116). The influence of BG insect herbivores on AG insect herbivores was weakly positive, but not statistically significant (**Figure 2.1**). AG insect herbivores had a small negative influence upon BG insect herbivores, however the result was also not statistically significant (**Figure 2.1**). There was no significant difference between field and laboratory studies in the magnitude of the effect of AG herbivores on BG herbivores (Qb=0.0114, df=1, p=0.9149), although a statistically significant negative influence on BG insect herbivores was detected when experiments were conducted under field conditions (**Figure 2.2**), however this was measured on only two studies. There was also no statistical differences in the effect size between field and

laboratory studies assessing effects of BG herbivory on AG herbivores (Qb=0.4093, df=1, p=0.5223), both effects in this case were not significant (**Figure 2.2**).

Effects of AG herbivores on BG herbivores and *vice versa* did not depend on the performance parameter measured (AG→BG: Qb=3.934, df=3, p=0.2687, BG→AG: Qb=1.814, df=4, p=0.7699). However, survival of BG insect herbivores was the only performance parameter that was significantly negatively influenced by AG insect herbivores (**Figure 2.3 b**). The effect of AG herbivores on BG herbivore RGR was marginally significant (**Figure 2.3 b**). BG insect herbivores did not significantly influence any of the performance parameters measured in AG insects (**Figure 2.3 a**).
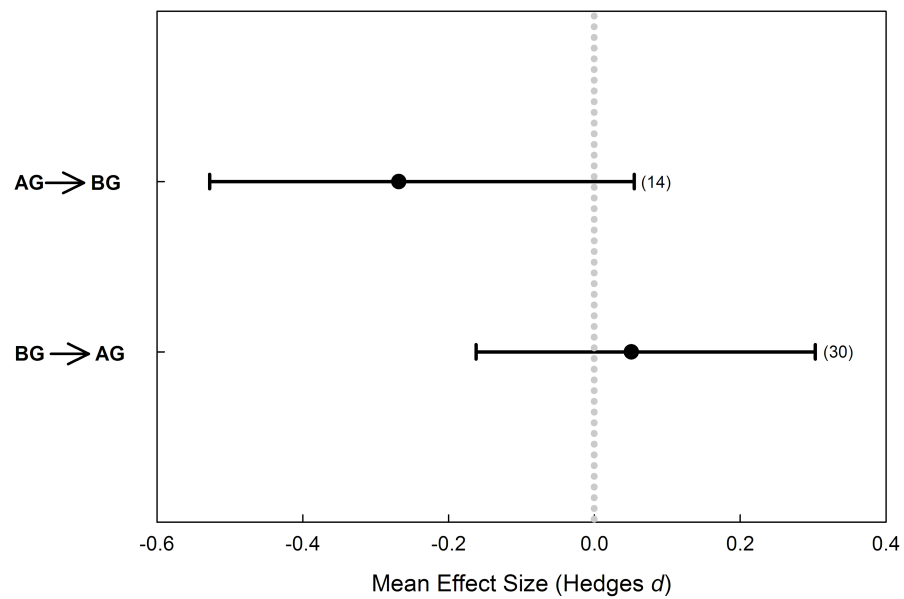
Figure 2.1. Overview of AG–BG insect herbivore interactions. Mean effect size with 95% CIs are reported for the influence of AG insects on BG insects (AG➜BG) and for the influence of BG insects on AG insects (BG➜AG). Effects are considered significant if their associated CIs do not overlap zero (marked by the dotted line). Numbers in brackets represent the number of studies included in the analysis.
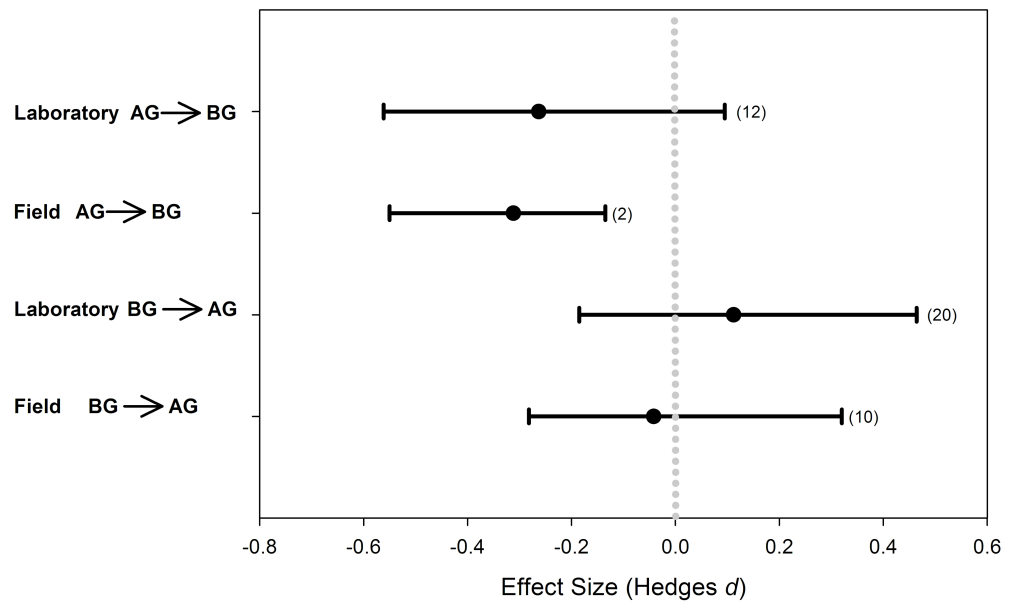
Figure 2.2. Influence of AG insect herbivores on BG insect herbivores (AG→BG) in field and laboratory studies and the influence of BG insect herbivores on AG insect herbivores (BG→AG). Effects are considered significant if their associated CIs do not overlap zero (marked by the dotted line). Numbers in brackets represent the number of studies included in the analysis.
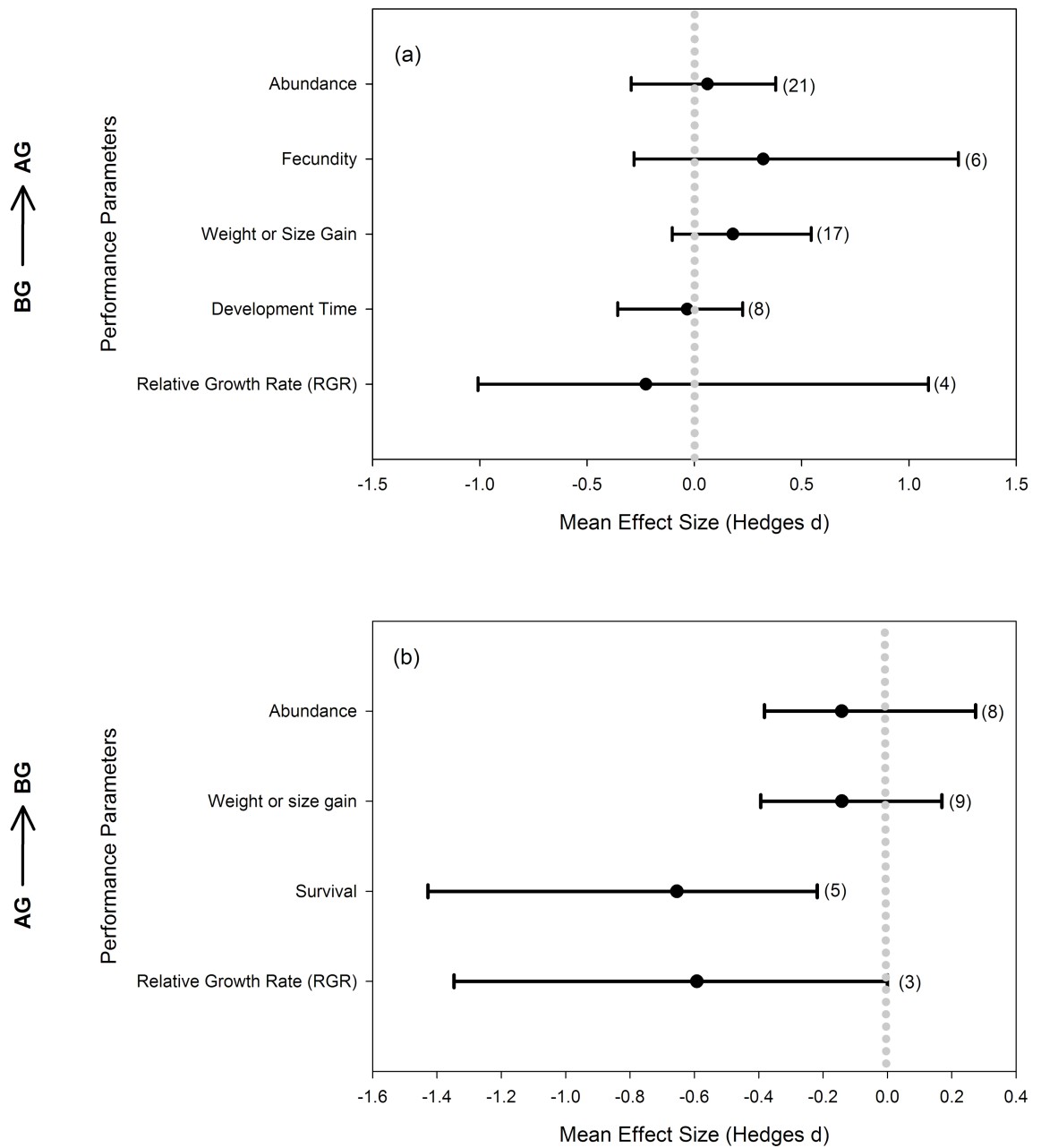
Figure 2.3. Performance parameters associated in AG-BG insect herbivore interactions. (a) Influence of AG insect herbivores on BG insect herbivores (AG→BG) and (b) Influence of BG insect herbivores on AG insect herbivores (BG→AG). Effects are considered significant if their associated CIs do not overlap zero (marked by the dotted line). Numbers in brackets represent the number of studies included in the analysis.

In 22 out of 29 studies investigating the influence of BG insect herbivores on AG insect herbivores, the BG insect was from the order Coleoptera (e.g. larvae of the click beetle (*Agriotes lineatus*)). To investigate how BG insects influenced AG insects within different orders, only BG insects within the order Coleoptera were considered. Insects from the order Coleoptera had a large, significantly positive effect on the performance of AG Homoptera (e.g. Peach–potato aphid (*Myzus persicae*) but did not significantly influence AG insects from other orders (**Figure 2.4**). However these non–significant results may be due to smaller numbers of studies representing these orders. The differences between effects on different AG insect orders was not significant (Qb=3.8062 df=4, p=0.2832). The influence of AG insects on Coleoptera BG could not be investigated due to the limited number of studies.

Plant life history (annual/perennial) had no significant effect on interactions between BG and AG herbivores (AG→BG: Qb=0.3516, df=1, p=0.5532, BG→AG: Qb=0.0078, df=1, p=0.9294). AG insect herbivores did however have a statistically significant negative influence on BG insect herbivores when both insects were feeding upon an annual plant (**Figure 2.5**).

Scatter plots of effect size plotted against sample size of all the data, categorized into AG and BG insect interactions, produced characteristic funnel shapes (Funnel Plots) (**Figure 2.6**). This indicated that studies with smaller sample sizes showed more variation around the mean effect size than studies with larger sample sizes and there was no obvious correlation between sample size and effect size (r_s=-0.192, p=0.081). These results suggest that there is little publication bias in the meta–analysis.
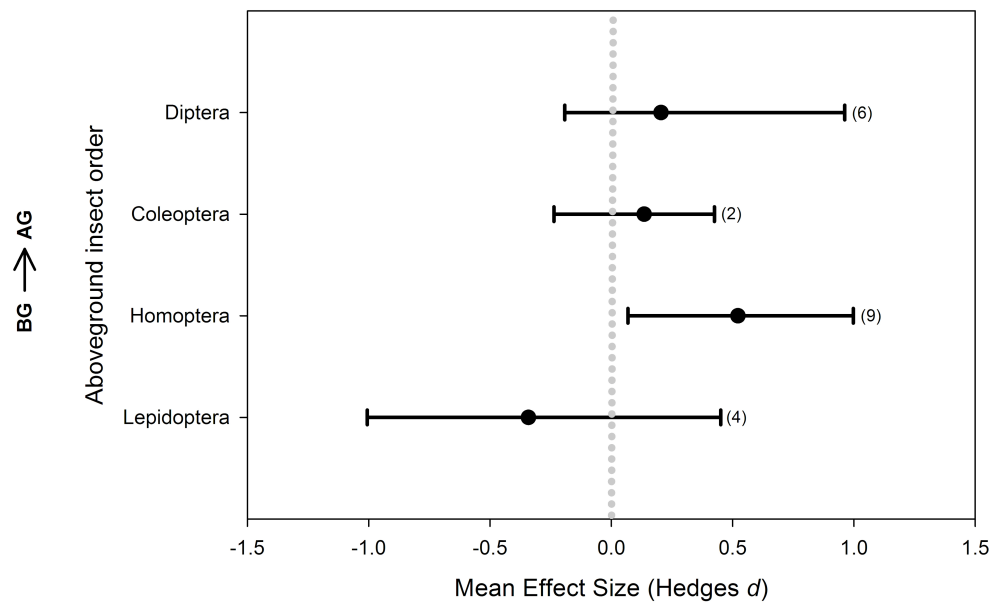
Figure 2.4. Effect of BG Coleopteran on AG insect orders (BG→AG). Effects are considered significant if their associated CIs do not overlap zero (marked by the dotted line). Numbers in brackets represent the number of studies included in the analysis.
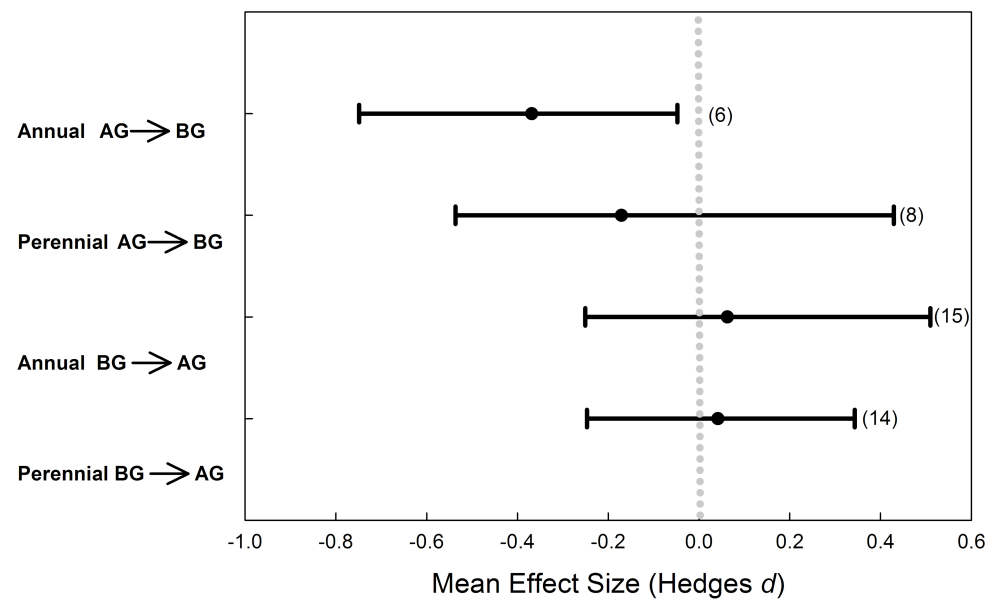
Figure 2.5. Influence of annual vs perennial host plants in AG and BG insect herbivore interactions. Effects are considered significant if their associated CIs do not overlap zero (marked by the dotted line). Numbers in brackets represent the number of studies included in the analysis.
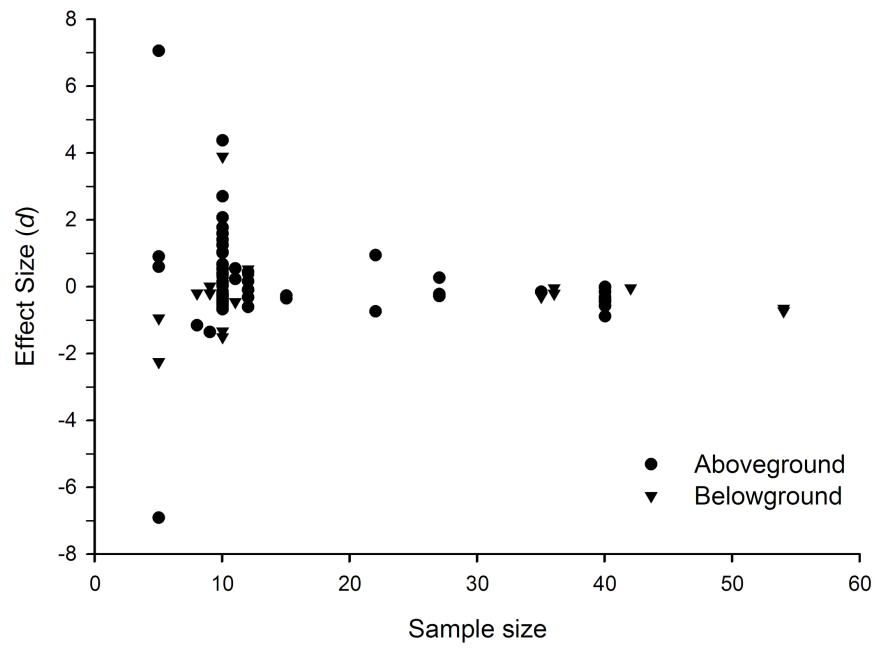
Figure 2.6. A funnel plot of effect size (*d*) and sample sizes. The black circles represent the influence of BG insect herbivores on AG herbivores (BG→AG), the black triangle represents the influence of AG insect herbivores on BG insect herbivores (AG→BG).

2.5. Discussion

2.5.1. Patterns in overall effect and direction

The study of AG and BG insect herbivore interactions has expanded rapidly over the last 20 years, with growing numbers of studies investigating increasingly complex interactions. Despite this increased interest, there are still relatively few studies that take a systematic approach to investigating these interactions with none taking a meta–analysis approach to assessing the generality of any patterns in these interactions and testing whether the outcome of these relationships is conditional on the nature of the insects and the shared host plant.

Several mechanisms proposed in the literature suggest that the effect of BG insect herbivores on AG insect herbivores is generally positive (Masters *et al.*, 1993). The present meta–analysis found that the positive direction of the effect was predicted, but was not demonstrated with statistical significance. Similarly, a negative effect of AG insect herbivores on BG insect herbivores was indicated, but without statistical significance. In addition, analyses showed that AG→BG interactions were not significantly different to BG→AG interactions. Masters *et al.* (1993) indicated the same directional responses in their conceptual model based on a small number of early studies, hypothesising that AG insect herbivores were positively influenced by elevated amino acid and carbohydrates in the foliage, whilst BG herbivores were negatively influenced due to a decrease in root quantity. Whilst our results do support the direction of the interactions hypothesised by the model, they do not provide statistically significant support for them.

2.5.2. Patterns in insect orders

In considering data for the meta–analysis it was impossible to separate feeding guild from insect order; AG insects in the order Homoptera were always classed as phloem feeders and the overall majority of BG insects in the order Coleoptera as chewing insects. The response of insect herbivores in different feeding guilds is perhaps one key area where generalisations can potentially be made regarding AG and BG insect herbivore interactions.

The principal AG feeding groups reported in the literature show varied responses when exposed to root herbivores; leaf–chewing insects react negatively, leaf–mining insects exhibit a neutral response and phloem feeders are positively influenced (Johnson *et al.*, 2008b). Both a 'stress response hypothesis' (amino acid and carbohydrate enriched foliage) and a 'defence induction hypothesis' (induction of secondary plant compounds) (Bezemer *et al.*, 2002) have been proposed to explain the response of AG insect herbivores to root feeders. Phloem feeders in particular, have been shown to react favourably to BG herbivory (Gange & Brown, 1989), with the response often attributed to the 'stress response hypothesis'. Our analyses demonstrated that insects from the order Homoptera were positively influenced by Coleoptera feeding BG. Although it should be highlighted that the majority of studies investigating AG Homoptera used aphids. Differences in insect feeding mechanisms, which may or may not overlap with insect order, have been highlighted as producing a range of plant responses, which impact on other insect herbivores sharing the plant (Bezemer & van Dam, 2005). Thus it is important to acknowledge that Homoptera may respond differently to other insect orders feeding BG, but may also react in different ways to other AG feeders to changes in plant nutrition and defence.

2.5.3. Future Directions

Recently, AG–BG experiments have expanded from so–called 'pair–wise' insect interactions and directed attention to more complex trophic interactions at a community level (Soler *et al.*, 2005, Soler *et al.*, 2007). Incorporating this additional layer of complexity will provide further insights into the relationships between AG and BG trophic groups, however they should be built upon a clear understanding of the principal basic processes in AG–BG insect interactions. Over 50% of the studies analysed as part of this meta–analysis used a BG insect from the order Coleoptera. However, BG insect herbivores are represented by many orders including six of the major orders: Diptera, Homoptera, Lepidoptera, Hemiptera, Isoptera and Coleoptera (Brown & Gange, 1990). Widening the range of insect orders studied BG would increase our understanding of AG–BG interactions and the generality of mechanisms that underpin them. Whilst incorporating plant–mediated responses and additional trophic levels into AG–BG insect interactions will provide greater knowledge of the topic, there is the possibility, that extrapolation of patterns seen will be limited to a few insect orders.

It has been previously acknowledged that predicting AG–BG interactions is difficult due to a high degree of context dependency in individual study systems (Wardle *et al.*, 2004) and that to disentangle the different aspects of these systems, spatial and temporal scales in terms of both the host plant and insect herbivores need to be investigated (Bardgett *et al.*, 2005, Bezemer & van Dam, 2005). The main problem in attempting to address these numerous relationships is the small number of studies that measure both the plant response, and the AG and BG insect responses to the interaction. This meta–analysis provides the first step in summarising the effects of insect herbivores AG and BG on one another via a host plant, allowing statistical generalisations to be formed and widening the focus away from particular case studies. Other reviews, such as Kaplan *et al.* (2008) seek to

address different aspects of AG – BG interactions, such as induced plant responses to AG and BG herbivory.

2.6. Conclusions

The overall direction of the effects of AG herbivores on BG herbivores and *vice versa* agreed with those hypothesised in the literature, but the majority of the effects were weak and not statistically significant. The strongest result from the analyses showed that AG Homoptera insects were positively influenced by BG Coleoptera herbivores. Performance parameters, experimental set up and host plant life span did not significantly affect magnitude or sign of AG–BG insect interactions. With growing interest in this field further generalisations may be possible, involving species spanning a wider range of functional groupings, so developing our knowledge and understanding of the basic mechanisms that underpin increasingly complex trophic interactions incorporating AG and BG insect herbivores.

Table 2.1. Studies used for the meta-analysis that investigated AG and BG insect herbivore interactions. Order: C = Coleoptera, D = Diptera, L = Lepidoptera and H = Homoptera. N/A is used when a mixture of plants or root insects were within the study.

Reference	AG insect herbivore	Order	Plant species	Annual or perennial	BG insect herbivore	Order	Performance parameter measured	Performance measured on AG or BG insect?
Agrawal (2004)	*Liriomyza asclepiadis*	D	*Asclepias syriaca*	Perennial	*Tetraopes tetrophthalmus*	C	Abundance (population)	AG
Bezemer *et al.* (2003)	*Spodoptera exigua*	L	*Gossypium herbaceum*	Perennial	*Agriotes lineatus*	C	RGR	AG
Bezemer *et al.* (2003)	*Spodoptera exigua*	L	*Gossypium herbaceum*	Perennial	*Agriotes lineatus*	C	RGR	BG
Clark (**Chapter Six**)	*Otiorhynchus sulcatus*	C	*Rubus ideaus* (c.v. Glen Ample)	Perennial	*Otiorhynchus sulcatus*	C	Abundance (population)	BG
Clark (**Chapter Six**)	*Otiorhynchus sulcatus*	C	*Rubus ideaus* (c.v. Glen Ample)	Perennial	*Otiorhynchus sulcatus*	C	Weight/size gain	BG
Erb *et al.* (2009)	*Spodoptera littoralis*	L	*Zea mays* (c.v. Delpriom)	Annual	*Diabrotica virgifera virgifera*	C	RGR	AG
Gange and Brown (1989)	*Aphis fabae*	H	*Capsella bursa-pastoris*	Annual	*Phyllopertha horticola*	C	Development time	AG
Gange and Brown (1989)	*Aphis fabae*	H	*Capsella bursa-pastoris*	Annual	*Phyllopertha horticola*	C	Weight/size gain	AG
Gange and Brown (1989)	*Aphis fabae*	H	*Capsella bursa-pastoris*	Annual	*Phyllopertha horticola*	C	RGR	AG
Gange and Brown (1989)	*Aphis fabae*	H	*Capsella bursa-pastoris*	Annual	*Phyllopertha horticola*	C	Fecundity	AG

Gange and Brown (1989)	*Aphis fabae*	H	*Capsella bursa-pastoris*	Annual	*Phyllopertha horticola*	C	Longevity	AG
Gerber *et al.* (2007)	*Ceutorhynchus alliariae*	C	*Alliaria petiolata*	Perennial	*Ceutorhynchus scrobicollis*	C	Abundance (population)	AG
Hunt-Joshi and Blossey (2005)	*Galerucella calmariensis*	C	*Lythrum salicaria*	Perennial	*Hylobius transversovittatus*	C	Survival	AG
Hunt-Joshi and Blossey (2005)	*Galerucella calmariensis*	C	*Lythrum salicaria*	Perennial	*Hylobius transversovittatus*	C	Weight/size gain	AG
Hunt-Joshi and Blossey (2005)	*Galerucella calmariensis*	C	*Lythrum salicaria*	Perennial	*Hylobius transversovittatus*	C	Abundance (population)	AG
Hunt-Joshi and Blossey (2005)	*Galerucella calmariensis*	C	*Lythrum salicaria*	Perennial	*Hylobius transversovittatus*	C	Survival	BG
Hunt-Joshi and Blossey (2005)	*Galerucella calmariensis*	C	*Lythrum salicaria*	Perennial	*Hylobius transversovittatus*	C	Weight/size gain	BG
Hunt-Joshi and Blossey (2005)	*Galerucella calmariensis*	C	*Lythrum salicaria*	Perennial	*Hylobius transversovittatus*	C	Abundance (population)	BG
Johnson *et al.* (2009)	*Rhopalosiphum padi*	H	*Hordeum vulgare*	Annual	*Agriotes* sp.	C	Fecundity	AG
Johnson *et al.* (2009)	*Rhopalosiphum padi*	H	*Hordeum vulgare*	Annual	*Agriotes* sp.	C	Weight/size gain	AG
Johnson *et al.* (2009)	*Rhopalosiphum padi*	H	*Hordeum vulgare*	Annual	*Agriotes* sp.	C	Fecundity	AG
Johnson *et al.* (2009)	*Rhopalosiphum padi*	H	*Hordeum vulgare*	Annual	*Agriotes* sp.	C	Weight/size gain	AG
Johnson *et al.* (2009)	*Rhopalosiphum padi*	H	*Hordeum vulgare*	Annual	*Agriotes* sp.	C	Survival	BG
Johnson *et al.* (2009)	*Rhopalosiphum padi*	H	*Hordeum vulgare*	Annual	*Agriotes* sp.	C	Weight/size gain	BG
Johnson *et al.* (2009)	*Rhopalosiphum padi*	H	*Hordeum vulgare*	Annual	*Agriotes* sp.	C	Survival	BG
Johnson *et al.* (2009)	*Rhopalosiphum padi*	H	*Hordeum vulgare*	Annual	*Agriotes* sp.	C	Weight/size gain	BG
Johnson *et al.* (Personal communication)	*Nematus olfaciens*	Hy	*Ribes nigrum*	Perennial	*Otiorhynchus sulcatus*	C	Abundance (population)	AG

Johnson *et al.* (Personal communication)	*Nematus olfaciens*	Hy	*Ribes nigrum*	Perennial	*Otiorhynchus sulcatus*	C	Weight/size gain	AG
Johnson *et al.* (Personal communication)	*Cryptomyzus galeopsidis*	H	*Ribes nigrum*	Perennial	*Otiorhynchus sulcatus*	C	Abundance (population)	AG
Johnson *et al.* (Personal communication)	*Cryptomyzus galeopsidis*	H	*Ribes nigrum*	Perennial	*Otiorhynchus sulcatus*	C	Weight/size gain	AG
Johnson *et al.* (Personal communication)	*Nematus olfaciens*	Hy	*Ribes nigrum*	Perennial	*Otiorhynchus sulcatus*	C	Abundance (population)	BG
Johnson *et al.* (Personal communication)	*Nematus olfaciens*	Hy	*Ribes nigrum*	Perennial	*Otiorhynchus sulcatus*	C	Weight/size gain	BG
Johnson *et al.* (Personal communication)	*Cryptomyzus galeopsidis*	H	*Ribes nigrum*	Perennial	*Otiorhynchus sulcatus*	C	Abundance (population)	BG
Johnson *et al.* (Personal communication)	*Cryptomyzus galeopsidis*	H	*Ribes nigrum*	Perennial	*Otiorhynchus sulcatus*	C	Weight/size gain	BG
Johnson *et al.* (Personal communication)	*Amphorophora idaei*	H	*Rubus ideaus* (c.v. Glen Rosa)	Perennial	*Otiorhynchus sulcatus*	C	Abundance (population)	AG
Johnson *et al.* (Personal communication)	*Amphorophora idaei*	H	*Rubus* ideaus (c.v. Glen Clova)	Perennial	*Otiorhynchus sulcatus*	C	Abundance (population)	AG
Johnson *et al.* (Personal communication)	*Amphorophora idaei*	H	*Rubus ideaus* (c.v. Glen Clova	Perennial	*Otiorhynchus sulcatus*	C	Weight/size gain	AG

Masters (1992)	*Megoura viciae*	H	*Mixed*	N/A	General root herbivores	N/A	Abundance (population)	AG
Masters (1992)	*Megoura viciae*	H	*Mixed*	N/A	General root herbivores	N/A	Abundance (population)	AG
Masters (1992)	*Megoura viciae*	H	*Mixed*	N/A	General root herbivores	N/A	Abundance (population)	AG
Masters (1992)	*Megoura viciae*	H	*Mixed*	N/A	General root herbivores	N/A	Abundance (population)	AG
Masters and Brown (1992)	*Chromatomyia syngenesiae*	D	*Sonchus oleraceus*	Annual	*Phyllopertha horticola*	C	Weight/size gain	AG
Masters and Brown (1992)	*Chromatomyia syngenesiae*	D	*Sonchus oleraceus*	Annual	*Phyllopertha horticola*	C	RGR	BG
Masters (1995)	*Myzus persicae*	H	*Cirsium palustre*	Annual	*Phyllopertha horticola*	C	Weight/size gain	AG
Masters (1995)	*Myzus persicae*	H	*Cirsium palustre*	Annual	*Phyllopertha horticola*	C	Fecundity	AG
Masters (1995)	*Myzus persicae*	H	*Cirsium palustre*	Annual	*Phyllopertha horticola*	C	RGR	AG
Moran and Whitham (1990)	*Hayhurstia atriplicis*	H	*Chenopodium album*	Annual	*Pemiphigus betae*	H	Abundance (population)	BG
Moran and Whitham (1990)	*Hayhurstia atriplicis*	H	*Chenopodium album*	Annual	*Pemiphigus betae*	H	Weight/size gain	BG
Moran and Whitham (1990)	*Hayhurstia atriplicis*	H	*Chenopodium album*	Annual	*Pemiphigus betae*	H	Abundance (population)	BG
Poveda (2005)	*Aphids*	H	*Sinapis arvensis*	Annual	*Agriotes* sp.	C	Abundance (population)	AG
Poveda *et al.* (2005)	*Aphids*	H	*Sinapis arvensis*	Annual	*Agriotes* sp.	C	Abundance (population)	AG
Salt *et al.* (1996)	*Aphis fabae fabae*	H	*Cardamine pratensis* L.	Perennial	*Pemphigus populitransversus*	H	Abundance (population)	AG
Salt *et al.* (1996)	*Aphis fabae fabae*	H	*Cardamine pratensis* L.	Perennial	*Pemphigus populitransversus*	H	Abundance (population)	BG

Simelane (2006)	*Aphis fabae*	H	*Lantana camara* L. (Verbenaceae)	Perennial	*Longitarsus bethae*	C	Survival	BG
Simelane (2006)	*Aphis fabae*	H	*Lantana camara* L. (Verbenaceae)	Perennial	*Longitarsus bethae*	C	Development time	BG
Soler *et al.* (2005)	*Pieris brassicae*	L	*Brassica nigra* L.	Annual	*Delia radicum*	D	Development time	AG
Soler *et al.* (2005)	*Pieris brassicae*	L	*Brassica nigra* L.	Annual	*Delia radicum*	D	Weight/size gain	AG
Soler *et al.* (2007)	*Pieris brassicae*	L	*Brassica nigra* L.	Annual	*Delia radicum*	D	Survival	BG
Soler *et al.* (2007)	*Pieris brassicae*	L	*Brassica nigra* L.	Annual	*Delia radicum*	D	Weight/size gain	BG
Soler *et al.* (2009)	*Phyllotreta spp.*	C	*Brassica nigra* L.	Annual	*Delia radicum*	D	Abundance (population)	AG
Soler *et al.* (2009)	*Brevicoryne brassicae*	H	*Brassica nigra* L.	Annual	*Delia radicum*	D	Abundance (population)	AG
Soler *et al.* (2009)	*Myzus persicae*	H	*Brassica nigra* L.	Annual	*Delia radicum*	D	Abundance (population)	AG
Soler *et al.* (2009)	*Pieris rapae*	L	*Brassica nigra* L.	Annual	*Delia radicum*	D	Fecundity	AG
Staley *et al.* (2007)	*Stephensia brunnichella*	L	*Clinopodium vulgare* L.	Perennial	*Agriotes* sp.	C	Abundance (population)	AG
Staley *et al.* (2007)	*Stephensia brunnichella*	L	*Clinopodium vulgare* L.	Perennial	*Agriotes* sp.	C	Development time	AG
Staley *et al.* (2007)	*Stephensia brunnichella*	L	*Clinopodium vulgare* L.	Perennial	*Agriotes* sp.	C	Weight/size gain	AG
Staley *et al.* (2007)	*Stephensia brunnichella*	L	*Clinopodium vulgare* L.	Perennial	*Agriotes* sp.	C	RGR	BG
Staley *et al.* (2008)	*Chromatomyia syngenesiae*	D	*Sonchus asper*	Annual	*Agriotes* sp.	C	Weight/size gain	AG
Staley *et al.* (2008)	*Chromatomyia syngenesiae*	D	*Sonchus oleraceus*	Annual	*Agriotes* sp.	C	Weight/size gain	AG

Staley *et al.* (2008)	*Chromatomyia syngenesiae*	D	*Sonchus arvensis*	Perennial	*Agriotes* sp.	C	Weight/size gain	AG
Staley *et al.* (2008)	*Chromatomyia syngenesiae*	D	*Sonchus palustris*	Perennial	*Agriotes* sp.	C	Weight/size gain	AG
Staley *et al.* (2008)	*Chromatomyia syngenesiae*	D	*Sonchus asper*	Annual	*Agriotes* sp.	C	Development time	AG
Staley *et al.* (2008)	*Chromatomyia syngenesiae*	D	*Sonchus oleraceus*	Annual	*Agriotes* sp.	C	Development time	AG
Staley *et al.* (2008)	*Chromatomyia syngenesiae*	D	*Sonchus arvensis*	Perennial	*Agriotes* sp.	C	Development time	AG
Staley *et al.* (2008)	*Chromatomyia syngenesiae*	D	*Sonchus palustris*	Perennial	*Agriotes* sp.	C	Development time	AG
Tindall and Stout (2001)	*Spodoptera frugiperda*	L	*Oryza sativa* L.	Annual	*Lissorhoptrus oryzophilus*	C	Abundance (population)	BG
Tindall and Stout (2001)	*Spodoptera frugiperda*	L	*Oryza sativa* L.	Annual	*Lissorhoptrus oryzophilus*	C	Weight/size gain	BG
van Dam *et al.* (2005)	*Pieris rapae*	L	*Brassica nigra* L.	Annual	*Delia radicum*	D	Weight/size gain	AG
van Dam *et al.* (2005)	*Pieris rapae*	L	*Brassica nigra* L.	Annual	*Delia radicum*	D	Development time	AG
van Dam *et al.* (2005)	*Pieris rapae*	L	*Brassica nigra* L.	Annual	*Delia radicum*	D	Weight/size gain	AG
S. Wurst (personal communication)	*Myzus persicae*	H	*Plantago lanceolata*	Perennial	*Agriotes lineatus*	C	Abundance (population)	AG

Chapter Three – Vine weevils (*Otiorhynchus sulcatus*) as a model herbivore with an aboveground–belowground lifecycle: a field study of population dynamics in a protected raspberry (*Rubus idaeus*) crop system

3.1 Introduction

The underlying basis of this thesis is to investigate the relationship between the vine weevil (*Otiorhynchus sulcatus* F.) (Coleoptera: Curculionidae) and the agronomically important crop species red raspberry (*Rubus idaeus*). In this chapter a review of vine weevil biology in the context of both aboveground and belowground interactions is presented, incorporating some of the more recent studies on the vine weevil since the last published review in 1992 (Moorhouse *et al.*, 1992a). Additionally, a field study monitoring a vine weevil population in a protected cropping system over two years is investigated. To date, no work has considered vine weevil population dynamics in a field environment in the UK, despite its highly destructive impact on crops. Consequently, this study could aid understanding the relationship between the vine weevil and red raspberry in their natural growing environment.

Anecdotal observations by fruit growers have indicated an increase in vine weevil numbers in soft fruit crops, probably due to the limited options now available to control this highly polyphagous pest. Soft fruit growers are increasingly dependent on protected cropping systems, with 80% of soft fruit available in UK supermarkets grown under protected tunnels (British Summer Fruits, 2009). However, little research has investigated the influence that protected systems may have on the population dynamics of insect pests (Bylemans *et al.*, 2003, Gordon *et al.*, 2006, Johnson *et al.*, 2010b).

In particular, limited information has been collected relating to the increase in abundance of vine weevil populations in a protected cropping system. The weevils' high fecundity means that only a few adult weevils can colonise a previously 'weevil–free' area. Furthermore, under suitable conditions this initial population then has the ability to grow

to an economically damaging level. Further investigation into the build up of weevil populations in a polytunnel environment would enable greater understanding of weevil dynamics in their typical agricultural environment and could consequently assist in the development of alternative control methods.

The experiment presented in this chapter is designed to represent a scenario where vine weevil adults have infested previously uninfected polytunnels containing two different raspberry cultivars (Glen Ample and Glen Rosa). The adults have laid eggs on the plants for eight weeks prior to being discovered and removed. The weevils were not uniformly distributed and thus some plants have received more eggs than others, with some receiving none. The experiment then monitors the impact of this scenario on a subsequent vine weevil population. To set up the experimental scenario in a realistic way, egg numbers were calculated using figures from Cram (1965b) and Moorhouse *et al.* (1992b) detailing the average number of eggs laid in field conditions by adult vine weevils, giving an average number of 15 eggs laid per adult per week. Viable egg percentages vary greatly depending on the host plant, thus, an average egg viability was calculated from the responses of black vine weevil to eight red raspberry cultivars (Cram & Daubeny, 1982), which gave an average of 20% of eggs laid by vine weevil adults being viable. The numbers of eggs applied to each plot are outlined in **Table 3.1**. The number of viable eggs corresponds to the number of eggs applied to each plot.

Table 3.1. Calculation for eggs applied per treatment plot

Treatment	Number of eggs laid per weevil per week	Number of weeks	Number of weevils	Total eggs laid	Percentage of viable eggs	Number of viable eggs
No eggs inoculated	15	8	0	0	20	0
Low egg inoculation	15	8	2	240	20	48
High egg inoculation	15	8	10	1200	20	240

Capture–mark–recapture techniques are frequently used in animal populations to obtain estimates of both population size and survival rates, by capturing, marking and releasing animals, which are then recaptured several times across a sampling period (Pradel, 1996). Obtaining accurate estimates of invertebrate populations is particularly useful in understanding insect abundances in relation to insects as both pests and biocontrol agents (Holland & Smith, 1999). In this study, the aim was to use mark–recapture techniques to calculate simple population estimates of the adult weevil population throughout 2008 and 2009, thus providing information on how quickly vine weevil populations could escalate in a protected raspberry cropping system.

To better understand the factors potentially influencing vine weevil population dynamics on raspberry, a review of their life–cycle is presented.

3.2. Red raspberry

The red raspberry (*Rubus idaeus*) is thought to have originated in the Ide mountains, Turkey (Jennings, 1988). It is generally accepted that the Romans are responsible for the initial development of raspberry as a crop. Today, both the European red raspberry (*R. idaeus* subsp. *vulgatus* Arrhen) and the American red raspberry (*R. idaeus* subsp. *strigosus* Michx) are

grown in temperate environments around the world. Raspberry production in the UK is worth £121 million, with 1,634 hectares of raspberries cultivated, yielding 13,400 tonnes of fruit in the 2008/2009 season (DEFRA, 2009) although demand for soft fruit still outstrips production.

Raspberries are perennial plants. The principal stem of a raspberry plant is vertical and shrubby, and referred to as a cane. Canes are biennial, with principal growth occurring in the first year and fruit following in the second year. The growth cycle commences when a developing axillary bud belowground pushes through the soil surface; leaves then develop around the growing point forming a rosette. The shoot grows throughout spring and summer to between two and three metres high. In late summer, the reduction in daylight and falling temperatures, trigger the plant to enter a period of dormancy. Flowering usually occurs simultaneously with the onset of dormancy. It is not until the following spring, that vegetative canes (primocanes) become fruiting canes (fructocanes).

Raspberry berries are not actually berries, but are termed as aggregate fruits, where several drupelets (which each contain a single seed) are gathered around a central core. Fruit development is relatively fast, between 30 and 36 days for most raspberry cultivars. Once a plant has reached the fruiting stage in the second year of development, it can then potentially continue to produce fruit for a further 15 years.

3.3. Vine weevil biology

The vine weevil (*Otiorhynchus sulcatus* F.) (Coleoptera: Curculionidae) is a highly polyphagous pest. Adults feed on plant leaves causing notching around the leaf margin, whilst the soil–dwelling larvae feed on plant roots causing reduced plant vigour and potentially death

(Penman & Scott, 1976b, La Lone & Clarke, 1981, Moorhouse *et al.*, 1992a). A variety of names have been attributed to the vine weevil including 'cyclamen borer', 'strawberry root weevil' and 'taxus weevil'. In addition, they have previously been referred to as *Brachyrhynchus sulcatus* in the US.

Otiorhynchus sulcatus originated from Europe (Smith, 1932, Moorhouse *et al.*, 1992a), however they are now distributed worldwide. Vine weevils have been documented in the US, Canada, Australia and Japan, probably spreading via plant shipments, where soil is infested with either eggs or larvae (Smith, 1932). Temperature has been found to be an influential factor in the insect's distribution, with no weevils located in areas where temperatures dropped below –6 °C in January (Stenseth, 1987) hence, they are mainly found in temperate environments.

3.3.1. Life–cycle

Vine weevils have four distinct stages in their life–cycle: eggs, larvae, pupae and adults. Vine weevils are parthenogenetic and unfertilised eggs develop into new female individuals without the need for males. Consequently, the vine weevil is an excellent study species for investigating parent–offspring relationships in an aboveground–belowground system, as offspring are essentially clones of the mother. The life–cycle takes one year to complete under field conditions, however each life stage is not discrete and all four stages may be present concurrently (Shread, 1972). Development and emergence timings of each stage are difficult to predict due to variation in abiotic factors (e.g. temperature, plant nutrition, light intensity and moisture) that are known to influence insect reproduction and development. Moorhouse *et al.* (1992a) summarised the life–cycle under field conditions, which has been adapted in **Figure 3.1**.

Figure 3.1. Vine weevil lifecycle in field conditions in the Northern hemisphere.
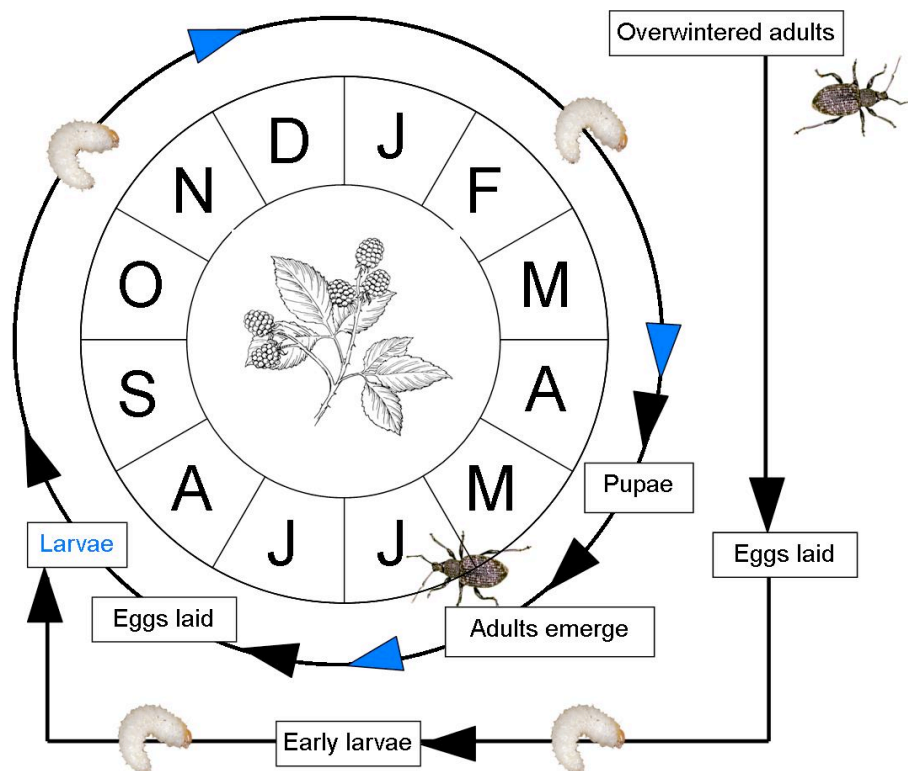
Adults

Characterised by their short, broad rostrums (typical in broad nosed weevils) adult vine weevils are between 8.5 mm and 11.5 mm in length, with randomly distributed yellow tufts of hair present on the elytra (**Figure 3.2**). On emergence, the weevil is brown in colour with a very soft exoskeleton. The colour alters to a dull black within a day, with the exoskeleton taking several days to harden. During this period adult weevils are particularly susceptible to damage. The elytra are weakly fused together, preventing the weevil from flying, hence the term 'wingless weevil'.

After emergence, vine weevils enter a non–reproductive period often termed the pre–oviposition period, which can vary in length in accordance with temperature (Son & Lewis, 2005) and plant nutrition (Cram, 1965a). As nocturnal insects, the adults hide in leaf litter

and dark crevices during the day and begin feeding shortly after dark. Their notching of leaf edges is often one of the principal indications of an infestation. Heaviest feeding is seen in the first ten weeks after eclosion (Doss & Shanks, 1985) with adults commencing feeding the night after emergence. If disturbed, weevils will drop from the foliage where they are feeding and freeze, a term know as death–feigning.

Eggs have been located around the base of the plant, in plant stem crevices and the underside grooves between plant leaf veins (personal observation) and adults have been seen ovipositing on the ground and foliage (Smith, 1932). Similarly, the number of eggs laid by an individual weevil varies considerably. In field conditions (Cram, 1965a) reported that on average a weevil produced 525 eggs over a 27 month period, whilst at optimal conditions of 21 ºC weevils had a per capita egg production of 1094.1 in 37 weeks (Son & Lewis, 2005).

 Adult weevils have been known to survive for over two years in a controlled environment, although average life expectancy is 46.5 weeks (Moorhouse *et al.*, 1992b). Survival in external conditions is considerably lower, with adult weevils reported to survive for 30 days at -3 ºC (Stenseth, 1987). Overwintering of adults has also been observed, with Garth and Shanks (1978) estimating a survival rate of 42.5% in Washington, USA.

Eggs

Eggs are subspherical in shape with a maximum diameter of 0.8 mm (Smith, 1932). After oviposition, they are creamy white in appearance and relatively soft, which alters to a chestnut brown colour one to three days later when the egg melanises and becomes harder. Eggs that do not undergo this change have previously been found to be non–viable and decompose within 15–20 days of oviposition (Smith, 1932).

Temperature plays an important role in the development of an egg to a larva. Eggs laid in field conditions are less viable than those laid in controlled temperature environments (Moorhouse *et al.*, 1992b). Successful egg development occurs between 12-29 °C (Montgomery & Nielsen, 1979), however development time also decreases with increasing temperatures, with a maximum viable temperature of 27 °C (Son & Lewis, 2005). At 11 °C an egg takes approximately 47 days to develop, compared to 9.4 days at 27 °C (Son & Lewis, 2005).

Larvae

The subterranean larvae have a creamy translucent body covered in small hairs, whilst their heads are a distinct brown colour (**Figure 3.3**). Due to their semi–transparent bodies, larvae can appear coloured in accordance with their food source (e.g. strawberries result in a pink tinge). Early instar larvae are relatively active with straight bodies, however as the larvae mature into later instars, the thoracic segments thicken causing the body to form a C shaped appearance (Smith, 1932, Moorhouse *et al.*, 1992a).

At present the exact number of larval instars is in doubt. La Lone and Clarke (1981) identified six distinct instars, however, Smith (1932) reported two groups of larvae, one with six instars and the other with seven. Head capsule measurements have been used to help identify larval instars, although substantial variation in size is still seen (**Table 3.2**). Larval development time varies in accordance with temperature, taking 84 days indoors and 211 days in field conditions (La Lone & Clarke, 1981). During development larvae feed predominantly on host plant roots, with early instars feeding mainly on fine roots, whilst later instars (fourth plus) can girdle plant stems belowground (La Lone & Clarke, 1981). The majority of vine weevils overwinter at the larval stage in soil cells 15–25 cm below the

ground (Smith, 1932).   An elevation in temperatures during spring, stimulates the development process to recommence and larvae continue to feed until pupation.

Table 3.2. Vine weevil larvae head capsule widths (data provided by M. Gaffney).

	Instars (mm)							Comments
	1	2	3	4	5	6	7	
Smith (1932)	0.32	0.41	0.58	0.72	0.95	1.17	1.54	
La Lone and Clarke (1981)	0.32	0.42	0.59	0.78	0.99	1.40		Did not identify a 7th instar
Tom and Fischer (unpublished data)	0.35	0.48	0.62	0.78	0.97	1.20	1.52	
Lola – Luz (2004)	0.32	0.43	0.55	0.78	1.09	1.37	1.75	Instars 1-3 from lab, instars 4-7 from field
Gaffney (unpublished data)	0.32	0.44	0.58			1.31	1.62	Values for 4th and 5th instar missing

Pupae

Pupae are initially creamy white in colour, however this changes five days prior to emergence, when the colour turns to rusty brown. Pupation can last between three and forty weeks, again depending predominantly on temperature (Smith, 1932).

Figure 3.2. Adult vine weevil *(photo credit – SCRI © 2010)*

Figure 3.3. Vine weevil larva *(photo credit – SCRI © 2010)*

3.3.2. Host plants

Adults

Vine weevil adults are highly polyphagous. Smith (1932) originally identified over 77 species of host plant, which has subsequently been expanded to over 150 (Warner & Negley, 1976). However, it was highlighted that many studies defined a host plant as one on which either adult or larvae were observed feeding, rather than establishing whether the plant could support the entire weevil life–cycle (Fisher, 2006). For example, on the basis of reproductive success, vine weevil adults are restricted to one gymnosperm genus (*Taxus* sp.) and a wide range of angiosperm plants in the two subclasses of the Dicotyledonae: Dilleniidae and Rosidae (van Tol *et al.*, 2004b).

Host plants have previously been demonstrated to influence the duration of the pre–oviposition period (Cram, 1980), fecundity and oviposition (Penman & Scott, 1976a, Nielsen & Dunlap, 1981, Cram & Daubeny, 1982, Hanula, 1988, van Tol *et al.*, 2004a) and egg viability (Shanks, 1980) of vine weevils. Host plant preference has also been hypothesised as differing in relation to geographical location, with preferences differing between continents and countries (Fisher, 2006). For example, strawberry has in some studies not been selected as a preferred host, but has also been highlighted as a crop frequently damaged by vine weevils (see Fisher, 2006).

The mechanism driving host plant selection by vine weevils is not fully understood, but has been attributed to physical responses to chemical stimuli detected by the weevils' antennae (Moorhouse *et al.*, 1992a). In particular contact chemoreception is thought to enable weevils to detect oviposition stimulants once they have alighted on a host plant (Hanula, 1988). In addition, mechanically damaged leaf volatiles (van Tol & Visser, 2002) and the

presence of conspecific frass (van Tol *et al.*, 2004b) have been found to attract adult weevils to potential host plants. Plant nutritional status, in particular leaf nitrogen concentration, has also influenced host plant choice, with weevils demonstrating a preference for elevated foliar nitrogen (Hesjedal, 1984). Physical plant properties, such as leaf pubescence (Doss & Shanks, 1988, Cowles, 2004) have also been proposed as a factor influencing the vine weevil adult's choice of host plant, but less focus has been directed towards these variables.

Larvae

Vine weevil larvae are also polyphagous, an essential attribute due to their limited capability to relocate to an alternative host plant belowground. The root feeding larvae are responsible for the majority of damage inflicted on plants, causing reduced plant vigour, stunted growth and even death (La Lone & Clarke, 1981, Moorhouse *et al.*, 1992a). In fact relatively few larvae (2-8) feeding on the roots of a strawberry plant can result in economic loss (Penman & Scott, 1976b).

To date, much of the research investigating vine weevil preferences in relation to host plants, has focussed on adult weevils and only a few studies have examined larval development and survival with respect to different plant species (e.g. Hanula, 1988, Moorhouse *et al.*, 1993b, Cowles, 2004, Fisher, 2006). Overlooking larval responses to host plants has probably arisen from their limited mobility belowground and consequently their inability to change host plants. However, studying the development and the survival of the larvae is equally important in gaining understanding of the life–cycle dynamics of the vine weevil.

Strawberry (*Fragaria✗ananassa*) has been demonstrated to enhance both the establishment of vine weevil populations and larval survival in comparison with Norway spruce (*Picea abies*), white spruce (*Picea glauca*), yew (*Taxus baccata*) and rhododendron (*Catawaba rhododendron*) (Fisher, 2006). Furthermore, larvae feeding on azalea (*Rhododendron kiusianum*) produced adult weevils with low survival and smaller body masses in comparison to adults developing from larvae feeding on strawberry (*Fragaria✗ananassa* Duchesne) or Japanese yew (*Taxus cuspidata*) (Hanula, 1988). After artificial inoculation with eggs, larval populations failed to develop on *Campanula isophylla*, poinsettia (*Euphorbia pulcherrima*), *Hypoestes sanguinolenta* and Winter cherry (*Solanum capsicastrum*), although a 70% survival rate was recorded on Cyclamen (*Cyclamen persicum*), highlighting that some plants are unsuitable larval hosts (Moorhouse *et al.*, 1993b).

3.4. Aims of the study

Field surveys were undertaken in 2008 and 2009 to investigate the seasonal and between year changes in a vine weevil population on red raspberry in a protected cropping system over two years. In particular the surveys aimed to:

1. examine the abundances of vine weevils on two different raspberry cultivars: Glen Ample and Glen Rosa,

2. investigate the population dynamics of vine weevils with respect to different initial egg densities,

3. estimate the population size at each survey date using basic capture–mark–recapture calculations,

4. document the presence of other invertebrates.

3.5. Materials and methods

3.5.1. Field site

The experiment was conducted within six separate and adjacent polytunnels at SCRI, Dundee, UK (56°447'N, 3°012'W). Each tunnel (22 x 8 x 3.3 m; length: width: height) was covered with Luminance THB polythene film (BPI, London, UK) and contained three raised beds of *c.* 24 plants covered with polythene mulch. Three tunnels had been planted with Glen Ample and three with Glen Rosa in July 2005. The tunnels had previously been checked for vine weevil occurrence, with none being found, however in addition predatory nematodes (*Steinernema kraussei*) (Nemasys, Becker Underwood, Littlehampton, UK) were also applied to the soil to eradicate any larvae present. Subsequent nematode densities were not monitored, but a sufficient length of time was left between the application of nematodes and eggs to prevent them influencing the experiment. The tunnels were left uncovered in all three years of the experiment from October until June, according to commercial practice.

3.5.2. Experimental design

Plants in each row were separated into plots of four and enclosed using a correx barrier dug into the ground at the base (i.e. each row contained six plots) (**Figure 3.4**). Barrier glue (Agralan, Swindon, UK) was applied to the inside of the surround and to the wires supporting the plants, to help prevent walking insects from escaping. Vine weevil eggs were applied to plants within a plot at three different densities: no eggs inoculated (0 eggs), low egg inoculation (48 eggs) and high egg inoculation (240) eggs. Treatments were distributed throughout the plots using a repeated 3 x 3 Latin square design (**Figure 3.5**). Eggs were collected from a culture maintained at SCRI at 17 °C ± 2 °C and 16:8 L:D

photoperiod. Due to the large number of eggs required, eggs were applied to the tunnels on four separate dates during Summer 2007 (27th July, 23rd August, 21st September and 28th September). Eggs were added to the soil around the base of each plant in a plot. The number of eggs applied on each date was in accordance with Table 3.1. and was distributed across the plots and tunnels equally, to prevent any one tunnel receiving eggs considerably earlier than another.

3.5.3. Vine weevil survey

Vine weevil adults were surveyed every 14 days (± 2 days) at night (2200-0100) from mid May until mid October in 2008 and 2009. Weevils were dislodged onto white beating trays (110 cm x 86 cm) (Watkins and Doncaster, Cranbrook, UK) held either side of the plants by shaking the two middle plants in each plot five times. Weevils were placed in labelled containers for marking on the following day. In 2008, weevils were marked with different coloured nail varnish depending on the date caught, recaptures were recorded but did not receive any additional marks. In 2009, weevils were marked with different coloured number discs (E. H. Thorne (Beehives) Ltd, Market Rasen, UK) applied with nail varnish allowing individual identification (**Figure 3.6**). Several marked weevils were also kept in culture to check for any influences of the marking process and no adverse influences were observed. Once marked, weevils caught from the tunnels were returned to the base of the plants where they had been captured.

3.5.4. Survey of other invertebrates

An additional survey of other invertebrates occurring within the polytunnels was conducted in conjunction with the weevil survey in 2008. Easily identifiable invertebrates that were displaced onto the beating trays whilst shaking the plants for weevils were counted and

recorded within each sampling plot. Invertebrates not identified were collected in sample vials containing 40% ethanol and identified later in the laboratory using field guides. Invertebrates were identified to species level where possible. However, in some instances grouping of invertebrates (e.g. spiders) was more appropriate than individual species.

Figure 3.4. Plots constructed in polytunnels using correx. (*photo credit – K. Clark © 2007*)

Figure 3.5. Schematic of polytunnels detailing cultivar and egg distribution.

Figure 3.6. Marked vine weevil adult. (*photo credit – SCRI © 2010*)

3.5.5. Statistical analysis for field survey

Differences between the numbers of weevils caught on the two cultivars (Glen Ample and Glen Rosa) and between the three initial egg density treatments (no eggs inoculated, low egg inoculation and high egg inoculation) were analysed using analysis of variance (ANOVA's) that incorporated both tunnel and row as factors and cultivar, egg density and cultivar*egg density as variables. Preliminary examination of the data highlighted that early on in the season the limited numbers of weevils caught was resulting in a type I error in the data analysis (i.e. rejecting the null hypothesis when it is true). Consequently in both years, survey data was only included in the analysis after the population exceeded 100 weevils caught on one sampling occasion. For each sampling year, an ANOVA was performed on the total number of weevils caught in each plot for that year. In addition, for each sampling date in each year, an ANOVA was conducted using the same factors and variables, but using only the weevil counts per plot for that sampling date. Repeat measures analysis was not used due to the complex experimental design, with several factors required in the analyses to account for any differences between the tunnels and rows, whilst also investigating any differences between the cultivars and the different initial egg inoculations.

3.5.6. Statistical analysis for capture–mark–recapture

Simple population estimates were calculated using the capture–mark–recapture data collected in both 2008 and 2009. The calculations used were based on the assumption of an open population, allowing for the possibility that the population could alter through time due to births, deaths, immigration or emigration. For the 2008 data an adaptation of Bailey's triple catch method, Bailey's correction factor (**Equation 3.1)** was used to estimate the size of the weevil population at each sampling date (Bailey, 1951, Southwood & Henderson, 2000). The correction factor was more appropriate for the data reported here,

due to the small numbers of recaptures obtained throughout 2008. Bailey's triple catch method allowed for the analysis of grouped data as was conducted in 2008. The method is typically used for the estimate of a population at time two, when there has been one prior sampling occasion at time one and one subsequent sampling at time three, however, a series of the estimates can be used to estimate the population size over a longer sampling period (Southwood & Henderson, 2000). In 2009, weevils were individually marked, providing more detail in the data, thus an alternative estimate was used to estimate the weevil population at each sampling date, the Jolly-Seber estimate (**Equation 3.2**) (Seber, 1982, Southwood & Henderson, 2000).

Notation: Bailey's triple catch method

N_2= the estimate of the population at time 2 (t=2).

a_2= the number of newly marked animals captured at t=2.

n_2 = the total number of animals captured at t=2.

r_{21} = the number of animals captured at t=2 that were marked at t=1.

r_{31} = the number of animals captures at t=3 that were marked at t=1.

r_{32} = the number of animals captured at t=3 that were marked at t=2.

$$\hat{N}_2 = \frac{a_2(n_2 + 1)r_{31}}{r_{21}r_{32}}$$

(**Equation 3.1**)

Notation: Jolly-Seber method

$\hat{N}_i$ = the estimate of the population on day i (i = 1,2,….,k;).

$\hat{M}_i$ = the estimate of the population on day i (i = 1,2,….,k;).

r_i = the total number of animals recaptured on day i (i = 1,2,….,k;).

n_i = the total number of animals captured on day i (i = 1,2,….,k;).

a_i = the total number of animals released into the population after marking on day i (i = 1,2,….,k).

R_i = the number of marked animals released on day i (i = 1,2,….,k) and then subsequently recaptured.

Z_i = the number of marked animals captured before day i (i = 1,2,….,k), not captured on day i, but subsequently captured on another sampling day.

$$\hat{N}_i = \frac{\hat{M}_i n_i}{r_i} \qquad \text{(\textbf{Equation 3.2})}$$

$$\hat{M}_i = \frac{a_i Z_i}{R_i} + r_i \qquad \text{(\textbf{Equation 3.3})}$$

3.6. Results

3.6.1. Vine weevil abundance in relation to the two raspberry cultivars

Overall, the number of weevils captured on Glen Ample (per plant) was not significantly different ($F_{1,4}$=0.21, p=0.669) in 2008 than the number captured on Glen Rosa. No significant differences between the mean number of weevils on Glen Ample and Glen Rosa were detected on specific sampling dates in 2008 (**Figure 3.7**) (**Table 3.3**). In 2009, the mean number of weevils caught was not significantly different on Glen Ample than on Glen Rosa ($F_{1,4}$=1.25, p=0.327). No significant differences between the number of weevils caught on Glen Rosa and Glen Ample were detected at any of the sampling dates in 2009 (**Figure 3.8**) (**Table 3.3**).

3.6.2. Vine weevil abundance in relation to the three initial egg densities

Overall, the number of weevils captured in the different treatment plots was not significantly different in 2008 ($F_{2,78}$=0.27, p=0.766). Additionally, no significant differences between the mean number of weevils in the different treatment plots were detected on specific sampling dates in 2008 (**Figure 3.9**) (**Table 3.4**). In 2009, the mean number of weevils caught was not significantly different between the three egg densities ($F_{2,78}$=1.81, p=0.170), and no significant differences between the three treatments were detected at individual sampling dates (**Figure 3.10**) (**Table 3.4**).

3.6.3. Vine weevil abundance in relation to the interactions between cultivar and initial egg densities

No significant interaction between egg density and cultivar was found for the total number of weevils caught throughout 2008 ($F_{2,28}$=0.26, p=0.771). In addition, no significant interactions were found for the mean number of weevils caught at specific sampling dates in 2008 (**Figure 3.11**)(**Table 3.5**). In 2009, no significant interaction was detected between egg treatment and cultivar for the total number of weevils captured ($F_{2,28}$=1.50, p=0.230). No significant interactions were detected at individual sampling dates in 2009 (**Figure 3.12**)(**Table 3.5**).
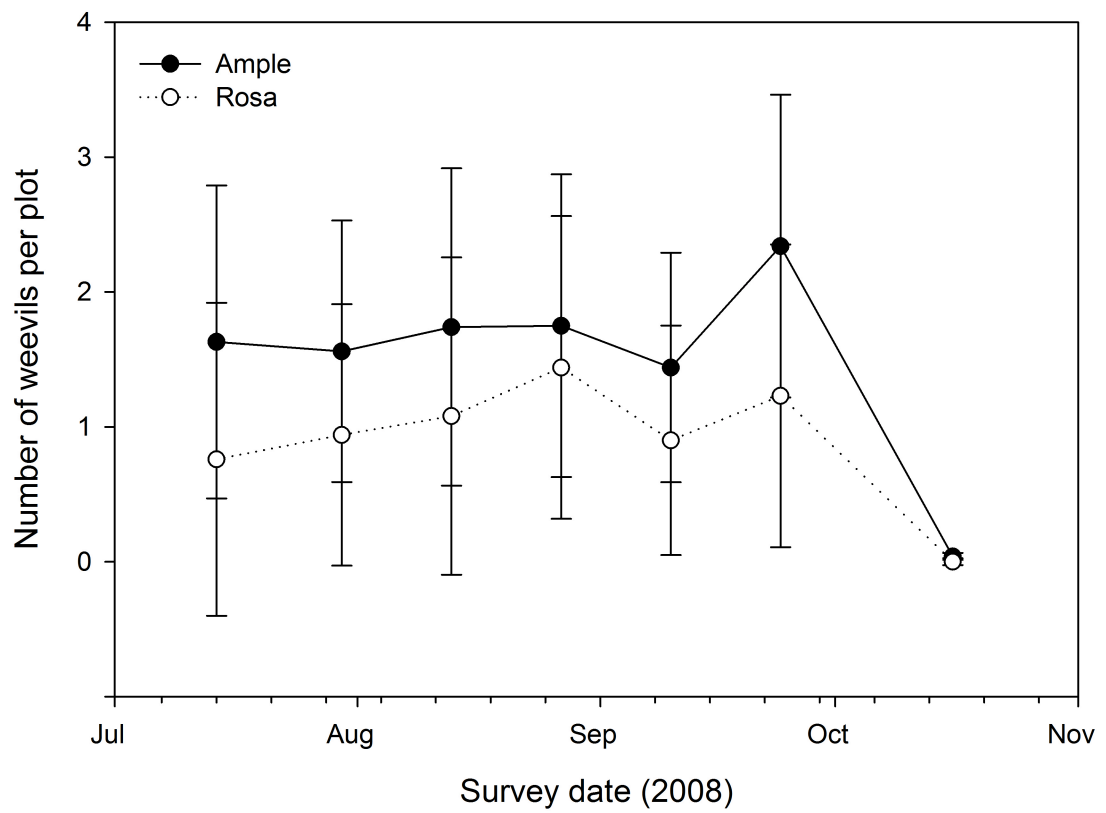
Figure 3.7. Number of weevils caught per plot on Glen Rosa and Glen Ample, predicted means ± SE from the data analysis, taking into account the experiment and analysis structure.
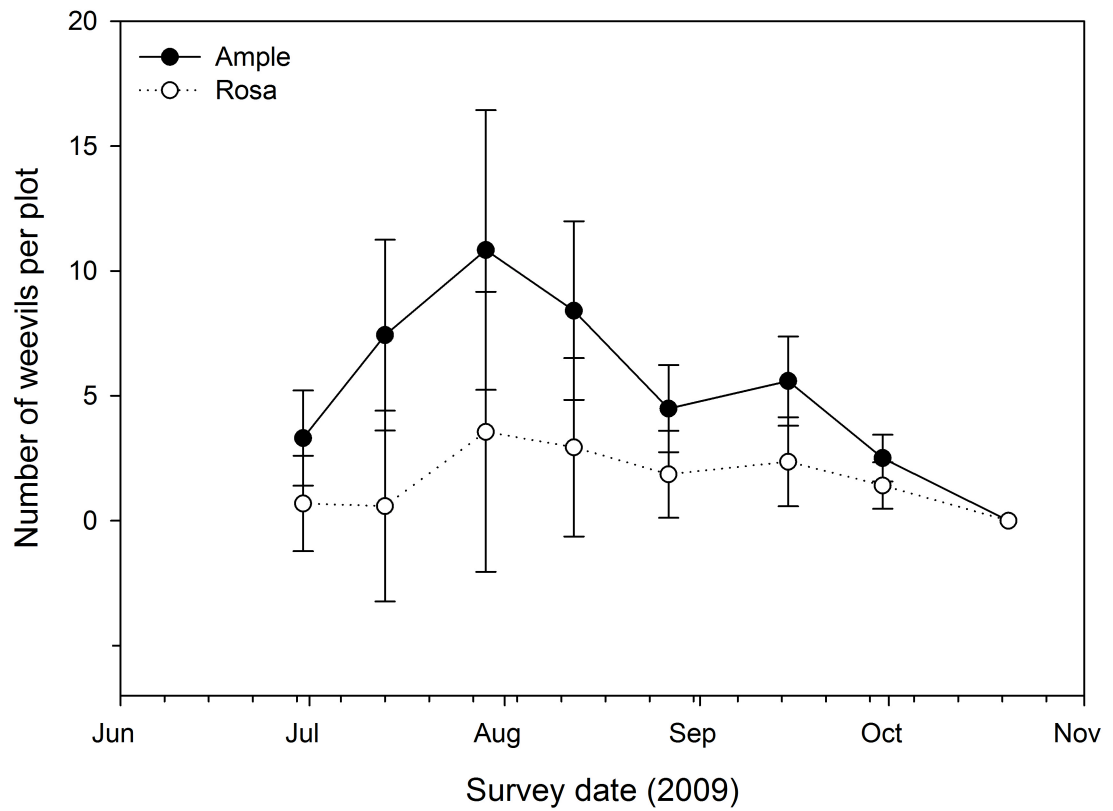
Figure 3.8. Number of weevils caught per plot on Glen Rosa and Glen Ample, predicted means ± SE from the data analysis, taking into account the experiment and analysis structure.

Table 3.3. Summary of statistical analyses comparing the number of weevils caught in Glen Ample and Glen Rosa plots. Analysed with ANOVA's that incorporated both tunnel and row as factors and cultivar, egg density and cultivar*egg density as variables.

2008			2009		
Survey date	$F_{1,4}$	P	Survey date	$F_{1,4}$	P
			30 June	0.94	0.388
14 July	0.28	0.624	13 July	1.61	0.274
30 July	0.20	0.676	29 July	0.85	0.410
13 August	0.16	0.711	12 August	1.17	0.340
27 August	0.04	0.852	27 August	1.14	0.346
10 September	0.20	0.678	15 September	1.64	0.269
24 September	0.48	0.525	30 September	0.69	0.452
16 October	1.07	0.360	20 October	No weevils	
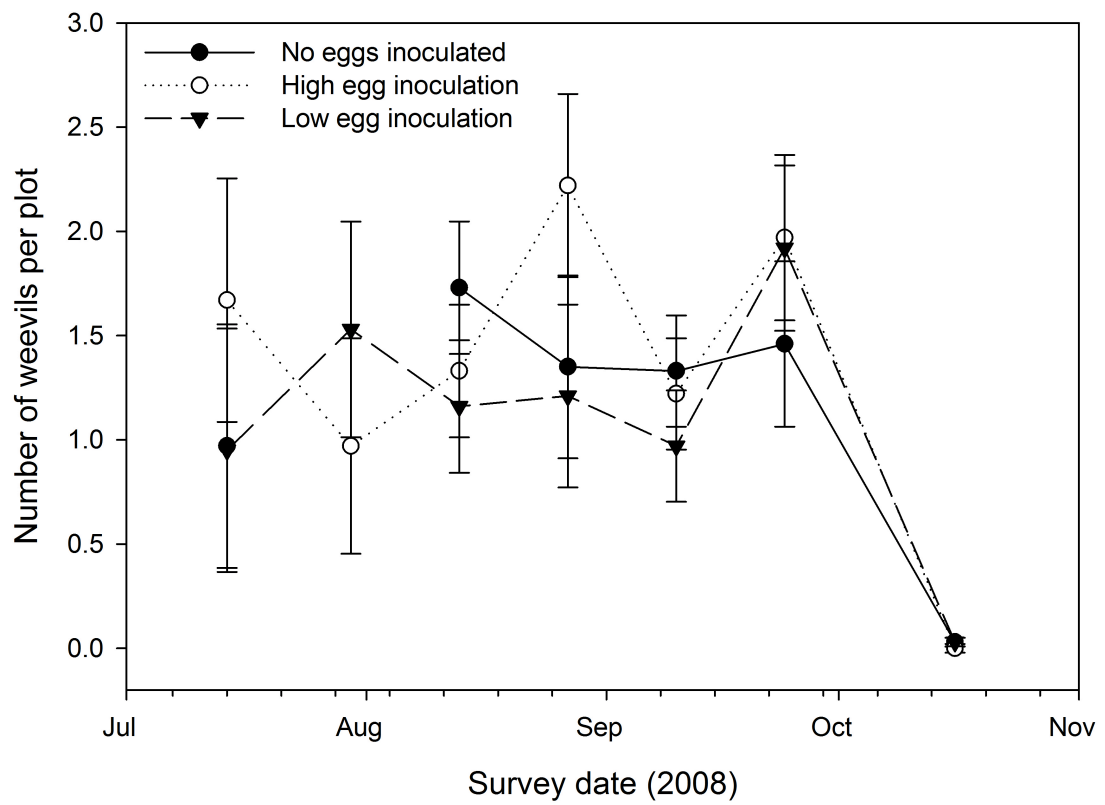
Figure 3.9. Number of weevils caught per plot in relation to the three egg inoculations, predicted means ± SE from the data analysis, taking into account the experiment and analysis structure.
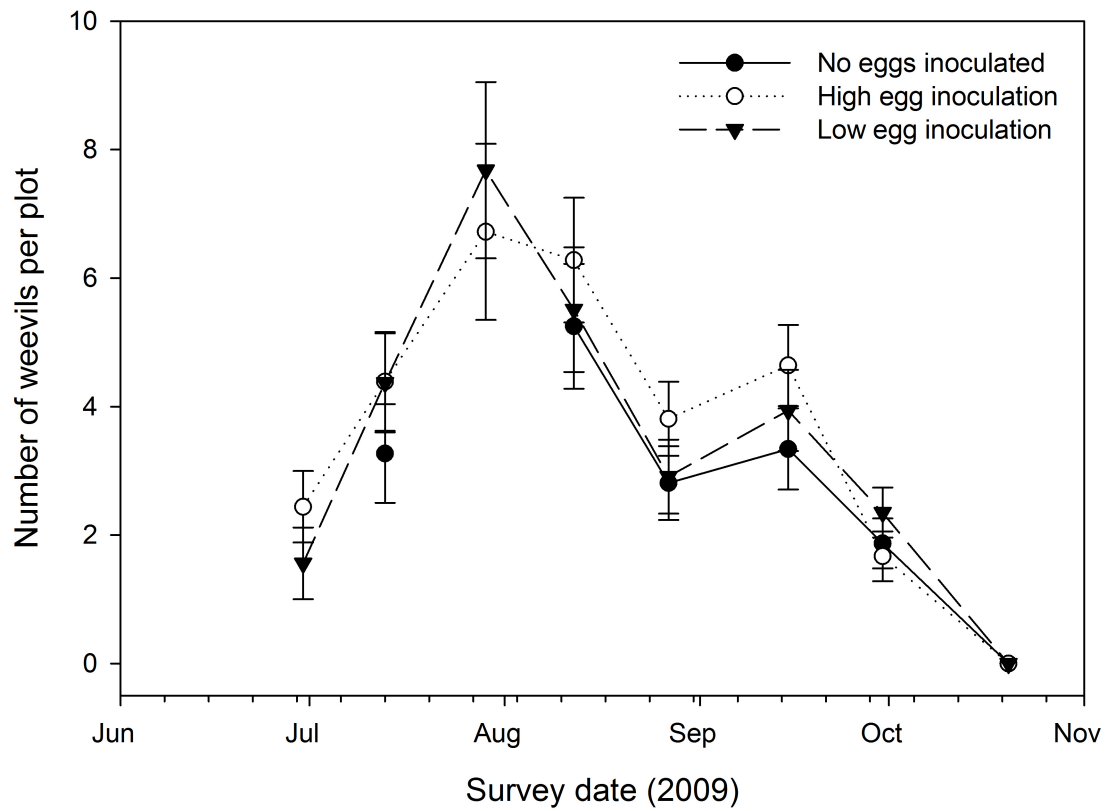
Figure 3.10. Number of weevils caught per plot in relation to the three egg inoculations, predicted means ± SE from the data analysis, taking into account the experiment and analysis structure.

Table 3.4. Summary of statistical analyses comparing the number of weevils caught in plots with different initial egg treatments (no eggs inoculated, low egg inoculation and high egg inoculation). Analysed with ANOVA's that incorporated both tunnel and row as factors and cultivar, egg density and cultivar*egg density as variables. [+]Denotes sampling dates where plots with no eggs inoculated were not sampled due to polytunnel management, thus the comparison is between the high egg inoculation and low egg inoculation plots only.

2008				2009			
Survey date	F	df	P	Survey date	F	df	P
				30 June	1.27	1,45	0.266
14 July	0.49	2,78	0.616	13 July	0.69	2,78	0.505
30 July[+]	0.59	1,45	0.447	29 July	0.24	1,45	0.625
13 August	0.83	2,78	0.442	12 August	0.30	2,78	0.739
27 August	1.55	2,78	0.219	27 August	0.91	2,78	0.407
10 September	0.48	2,78	0.620	15 September	1.06	2,78	0.351
24 September	0.51	2,78	0.604	30 September	0.80	2,78	0.451
16 October	0.68	2,78	0.511	20 October	No weevils		
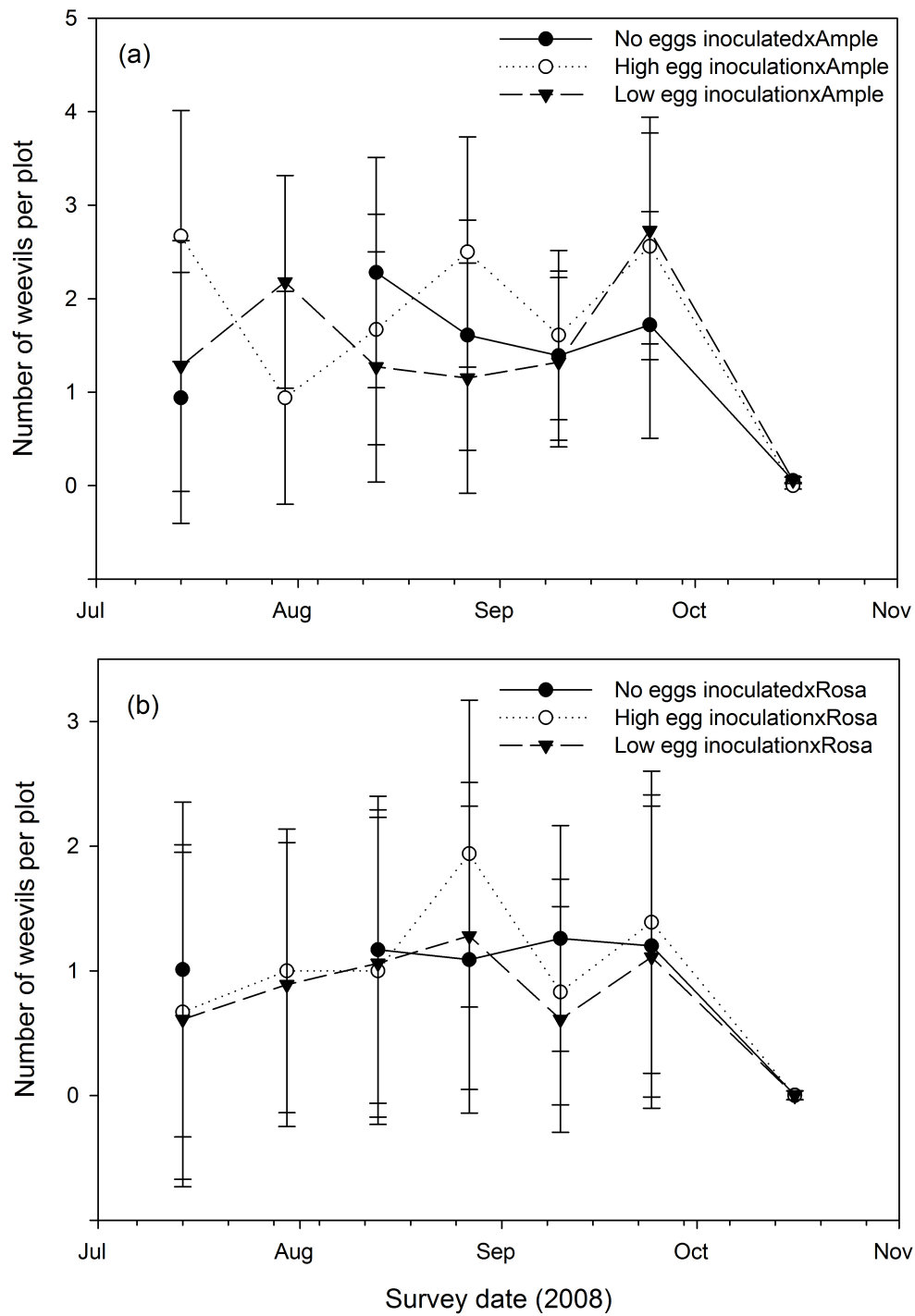
Figure 3.11. Number of weevils caught per plot in relation to raspberry cultivar and three egg inoculations, predicted means ± SE from the data analysis, taking into account the experiment and analysis structure.
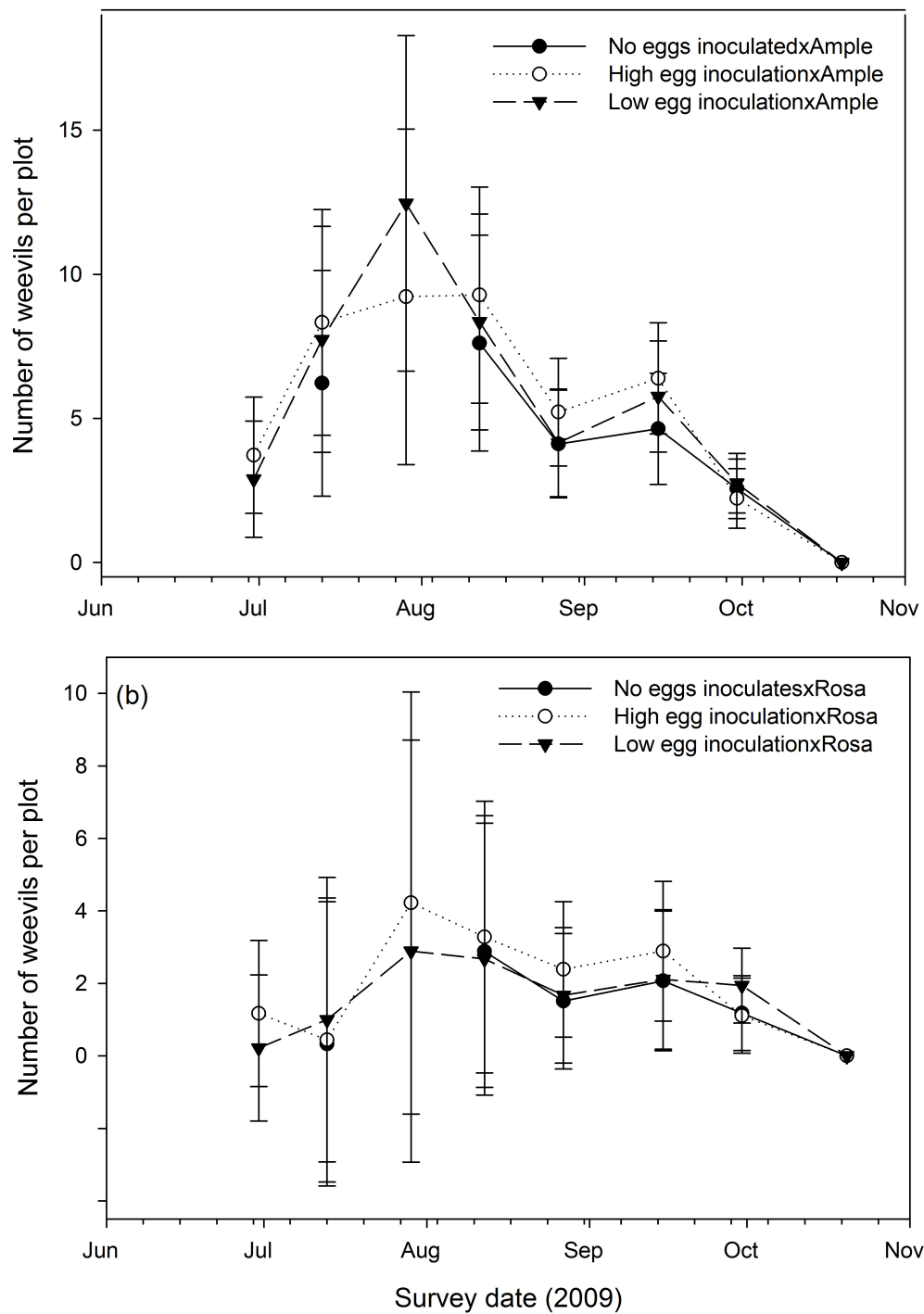
Figure 3.12. Number of weevils caught per plot in relation to raspberry cultivar and three egg inoculations, predicted means ± SE from the data analysis, taking into account the experiment and analysis structure.

Table 3.5. Summary of statistical analyses comparing the interaction between initial egg treatment (no eggs inoculated, low egg inoculation and high egg inoculation) and cultivar (Glen Ample and Glen Rosa) on the number of weevils caught. Analysed with ANOVA's that incorporated both tunnel and row as factors and cultivar, egg density and cultivar×egg density as variables. [+]Denotes sampling dates where plots with no eggs inoculated were not sampled due to polytunnel management, thus the comparison is between the high egg inoculation and low egg inoculation plots only.

2008				2009			
Survey date	F	df	P	Survey date	F	df	P
				30 June	0.01	1,25	0.942
14 July	0.49	2,28	0.616	13 July	0.69	2,28	0.505
30 July[+]	0.85	1,25	0.363	29 July	1.39	1,25	0.245
13 August	0.83	2,28	0.442	12 August	0.30	2,28	0.739
27 August	1.55	2,28	0.219	27 August	0.91	2,28	0.407
10 September	0.48	2,28	0.620	15 September	1.06	2,28	0.351
24 September	0.51	2,28	0.604	30 September	0.80	2,28	0.451
16 October	0.68	2,28	0.511	20 October	No weevils		

3.6.4. Capture–mark–recapture

In 2008, 894 weevils were captured and marked across 12 sampling dates, whilst in 2009 2,753 weevils were captured and marked across 11 sampling dates. In 2008, weevil numbers (greater than one) were first detected at the start of July, whilst in 2009 they were seen from the middle of May, with four of the weevils caught actually being marked from the previous year. In 2008, the maximum number of weevils caught was towards the end of the sampling season in September, just prior to the population crash. In 2009, the peak of the weevil population was detected earlier at the start of August. It is evident from the total number of weevils caught in 2008 and 2009, the population expanded considerably over just one year. The population estimates calculated in 2008 (**Table 3.6**) show the adult weevil population to be estimated between 2,660 and 3,472, from an initial egg inoculation of 10,368 viable eggs. During 2009, the population rises considerably (**Table 3.7**) and reaches an estimated population maximum of 44,099 weevils. The recapture rates of marked weevils was typically lower than 5% for the majority of sampling dates in 2008 and 2009, which is lower than ideally required for reliable mark-recapture population estimates.

3.6.5. Survey of other invertebrates

Earwigs (e.g. *Forficula auricularia*), caterpillars (e.g. *Pieris rapae*), spiders (e.g. *Pisaura mirabilis*), sawfly larvae (e.g. *Dolerus aerieus*) and raspberry beetles (*Byturus tomentosus*) were the most abundant invertebrates identified within the tunnels. *Byturus tomentosus* populations reached peak abundances at the end of June/ beginning of July, with the abundances on Glen Rosa being consistently higher than that on Glen Ample until the population crashed to zero in the middle of August (**Figure 3.13 a**). Sawfly larvae also reached higher levels on Glen Rosa than on Glen Ample, showing the maximum peak at the beginning of July (**Figure 3.13 b**). The spider population peaked on Glen Rosa at the end of June, but

crashed quite dramatically at the end of July. A similar pattern can be seen on Glen Ample, however, the number of spiders recorded is considerably lower than on Glen Rosa (**Figure 3.13 c**).   Earwig populations reached peak populations at the end of August (**Figure 3.13 d**), with higher numbers being recorded on Glen Ample than Glen Rosa.

Table 3.6. Summary of the number of weevils caught and marked during the 2008 sampling season and subsequent population estimates. [+] Denotes sampling dates where plots with no eggs inoculated were not sampled due to polytunnel management, thus the comparison is between high egg inoculation and low egg inoculation plots only.

Date	Total number of weevils caught	Total number of marked weevils in population	Number of marked weevils recaught	New weevils	New weevils in 'no eggs inoculated' plots	Number of marked weevils available to catch	Percentage of marked weevils caught (%)	Population estimate Bailey's triple catch
07 May	1	0	0	1	0	0		
21 May	1	1	0	1	0	1	0.00	0
04 June[+]	1	2	0	1	0	2	0.00	0
18 June	0	3	0	0	0	3	0.00	0
02 July[+]	47	3	0	47	0	3	0.00	0
14 July	129	50	2	127	34	50	4.00	3,302
30 July[+]	86	177	7	79	0	143	4.90	2,749
13 August	151	256	11	140	0	256	4.30	2,660
27 August	170	396	15	155	0	396	3.79	2,761
10 September	123	551	18	105	0	551	3.27	3,472
24 September	183	656	24	159	0	656	3.66	0
16 October	2	815	0	2	0	815	0.00	

Table 3.7. Summary of the number of weevils caught and marked during the 2009 sampling season and subsequent population estimates. [+]Denotes sampling dates where plots with no eggs inoculated were not sampled due to polytunnel management, thus the comparison is between high egg inoculation and low egg inoculation plots only.

Date	Total number of weevils caught	Total number of marked weevils in population	Number of marked weevils recaught	New weevils	New weevils in 'no eggs inoculated' plots	Number of marked weevils available to catch	Percentage of marked weevils caught (%)	Population estimate Jolly-Seber
22 May	26			26	9	0		
03 June[+]	8	26	2	6	0	17	11.76	8
18 June	44	32	1	43	7	32	3.13	44
30 June[+]	146	75	4	142	0	59	6.78	5,475
13 July	435	217	6	429	121	217	2.76	10,948
29 July[+]	515	646	12	503	0	509	2.36	7,725
12 August	614	1,149	35	579	0	1,149	3.05	9,122
27 August	337	1,728	34	303	0	1,728	1.97	11,953
15 September	418	2,031	37	381	0	2,031	1.82	44,099
30 September	210	2,412	15	195	0	2,412	0.62	210
20 October	0	2,607	0	0	0	2,607		
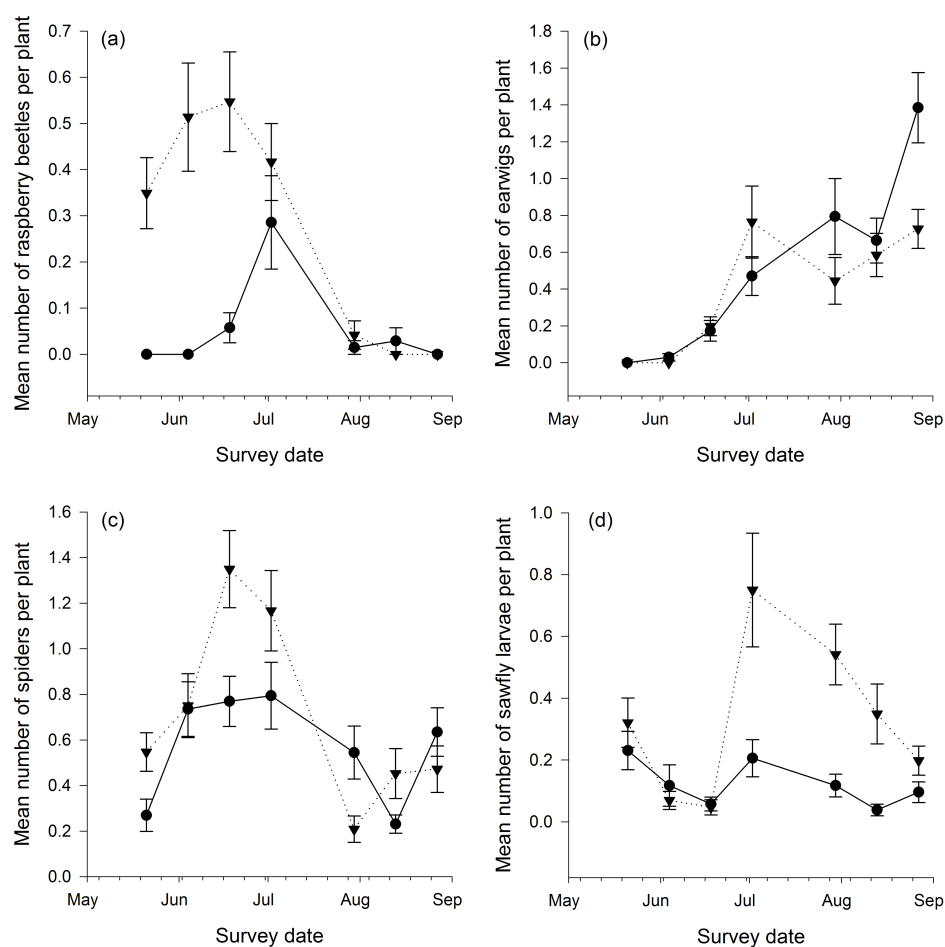
Figure 3.13. Invertebrate population dynamics within raspberry tunnels over the 2008 field season, (mean ± SE) number of insects per plant: (a) Raspberry beetle, (b) Earwigs, (c) Spiders and (d) Sawfly larvae ---▼--- represents insects sampled on Glen Rosa, —●— represents insects sampled on Glen Ample plants.

3.7. Discussion

Limited research to date has addressed the dynamics of invertebrates (both beneficial and detrimental) in protected cropping systems (Bylemans *et al.*, 2003, Gordon *et al.*, 2006), although covered tunnels are becoming an increasingly integral part of agricultural practices. In particular, the soft fruit industry produces a high percentage of supermarket berries under the protection of polythene tunnels (British Summer Fruits, 2009). Demands for residue free fruit from consumers and increasing legislative controls on insecticides, mean that alternative methods are being sought to control insect pests (Copping, 2008). Thus, it is particularly pertinent to investigate the population dynamics of the vine weevil, an economically damaging pest species (Moorhouse *et al.*, 1992a, Cowles, 2004, Alford, 2007), whose control has been reduced by the withdrawal of chemical insecticides. To date, no studies have considered the population dynamics of vine weevils in either an external field situation or within a protected cropping system, such as polytunnels. This study aimed to determine how vine weevil populations change over a two-year period in a protected raspberry cropping system, in relation to both two different raspberry cultivars and three different initial egg densities.

3.7.1. *Vine weevil dynamics in relation to cultivar and initial egg densities*

The abundance of vine weevil adults on the two raspberry cultivars did not differ significantly in either of the two sampling years. The egg laying capacity and preferences of vine weevil adults has previously been found to differ between raspberry cultivars (Cram & Daubeny, 1982), which potentially could influence subsequent population sizes. However, a recent study, which used whole raspberry plants rather than excised leaves (**Chapter 4**), found no significant differences in egg laying preferences or capacity and hypothesised that the use of excised leaves in preferential experiments could inadvertently influence the

oviposition behaviour of vine weevil adults. Consequently, a lack of preference in vine weevil egg laying behaviour between raspberry cultivars could result in similarly sized adult weevil populations developing in raspberry tunnels.

The development (abundance and mass) of vine weevil larvae has also been shown to differ significantly between the two raspberry cultivars (Glen Ample and Glen Rosa), with larvae on Glen Ample being more numerous, but smaller in size than larvae on Glen Rosa (data not presented in this Chapter, see **Chapter 5**). However, this difference in larval performance was not reflected by differences in adult population size in the field (data not presented in this Chapter, see **Chapter 5**). These results indicate that potentially neither egg laying preferences nor preferential larval performance alone are responsible for the population dynamics of vine weevils in a field environment. It should however be highlighted, that the preferences/performances exhibited by vine weevil adults and larvae in relation to raspberry cultivars were seen in controlled temperature situations and consequently may not directly reflect a field situation.

Initial egg densities did not generally influence the resulting abundance of vine weevil adults in 2008 or 2009, suggesting that initial egg density inoculations did not influence the subsequent population size. No previous work on vine weevils has considered the influence of egg densities on the resulting larvae, or adult population. To date, most work considering weevil development from eggs, has focussed on either the influence of host plant (e.g. Hanula, 1988, Fisher, 2006) or temperature (e.g. Moorhouse *et al.*, 1992b, Son & Lewis, 2005). In addition, field experiments considering the development of vine weevils from eggs have nearly all addressed the efficiency of either chemical (e.g. Cross & Burgess, 1997) or biocontrol (Lola-Luz *et al.*, 2005, Lola-Luz & Downes, 2007) of subsequent vine weevil larvae, rather than the resulting adult population dynamics.

The lack of significant differences in weevil numbers between either of the raspberry cultivars or the initial egg densities, suggest that other factors are more likely to be influencing vine weevil populations. However, as conditions were kept as similar as possible throughout the tunnels in terms of temperature, plant husbandry and control for leaf rust, it was difficult to select other factors that may have affected the weevil numbers and could be easily tested.

3.7.2. Population dynamics

Overall, the number of weevils caught in 2009 was higher than in 2008, indicating that weevils developing from eggs in 2008 were remaining in the tunnels to feed and oviposit, thus expanding the population. Interestingly in 2009, four weevils were caught at the start of the season that had been marked the previous year. Whilst this is a relatively small number of weevils when considered in relation to the overall population size, it does highlight the fact that vine weevils are capable of overwintering in an outdoor Scottish environment. Blackshaw (1984, 1987) proposed that early egg laying seen in Northern Ireland could be a result of overwintering adult weevils, however the proximity of the site to glasshouses meant the adults could have dispersed from there rather than surviving in the field. Overwintering adult weevils are capable of laying eggs earlier in the season, than newly emerged adults from larvae that have overwintered in the soil. Potentially, the provision of polythene mulch around the base of the plants may have provided the adult weevils with added protection to survive through the winter.

The capture–mark–recapture estimates of population size indicate that even with relatively low initial egg inoculations, vine weevil populations may be capable of growing

considerably in just two field seasons. However, the low percentage recaptures obtained in both 2008 and 2009 (**Table 3.6. and Table 3.7.**) demonstrate that the weevil population was likely to have been under-sampled, which may have resulted in the population estimates over-estimating the actual population size. Ideally a percentage recapture rate of greater than 20% on each sampling occasion would have yielded more accurate population estimates for the study, and Roff (1973) suggests that in the case of most mark-recapture study results over 50% of the population requires marking to gain modest accuracy in population estimates.

Plant damage was not quantified as part of the survey, however it was noticed that some plants exhibited reduced plant vigour in plots where consistently high numbers of weevils were captured. Vine weevil larvae have previously been shown to affect plant performance and even cause death in relatively young plants (Penman & Scott, 1976b, La Lone & Clarke, 1981). However, little work has addressed how a large population of vine weevils in an established crop may affect plant and subsequent crop performance.

3.7.3. Survey of other invertebrates

Peak population abundances for both damaging and non–damaging invertebrates were higher on Glen Rosa than Glen Ample, occurring from mid June to August. Glen Rosa has previously been found to be more resistant to some insect pests than Glen Ample (e.g. the large raspberry aphid, *Amphorophora idaei* (McMenemy *et al.*, 2009), whilst Glen Ample exhibits more vigorous growth than Glen Rosa (N. Jennings, personal communication), which may provide a more nutritious and attractive food source for insect herbivores in accordance with the plant vigour hypothesis (Price, 1991). Invertebrate populations peaked at different points throughout the growing season, in line with their own life–cycles. For

instance, raspberry beetle numbers peaked in mid–June, as they emerge shortly before flowering commences and then feed primarily on the growing tips of young primocane.

Other invertebrates which were recorded in the tunnels, but not at large enough numbers to make any statistical analyses viable included: clay coloured weevils (*Otiorhynchus singularis*), raspberry moths (*Lampronia rubiella*), ladybirds (e.g. *Adalia bipunctata*), lacewings (e.g. *Chrysoperla carnea*), bees (e.g. *Bombus lucorum*) and wasps (e.g. *Vespula vulgaris*). Large raspberry aphid (*Amphorophora idaei*) numbers were too high to record on several sampling dates (numbers in excess of several hundred per plant), thus an accurate representation of their population dynamics was difficult to ascertain from the data.

3.8. Conclusions

Vine weevil population dynamics were not found to differ significantly between either of the two cultivars or the initial egg densities, indicating that other factors not quantified as part of this research were more influential in determining weevil numbers. The population of weevils was found to grow considerably in just two field seasons, demonstrating the potential for a damaging population to arise from quite low initial egg inoculations. Additionally, overwintering adult weevils were detected, suggesting that protected cropping systems may allow weevils to survive a winter and consequently contribute to the following year's population. Future work aimed at ascertaining the principal factors determining how weevil populations develop in a protected cropping system, would help provide further understanding of vine weevil biology. This area of research is particularly pertinent in developing our knowledge of how aboveground and belowground insects interact via a host plant in field conditions and the influences these interactions may then have in agricultural systems.

Chapter Four – Oviposition and feeding behaviour by the vine weevil (*Otiorhynchus sulcatus*) on red raspberry (*Rubus idaeus*): effects of cultivars and plant nutritional status

4.1. Abstract

1. The vine weevil (*Otiorhynchus sulcatus*) is a major pest in horticultural crops worldwide, with root feeding larvae causing significant economic damage by reducing plant vigour.

2. Adult oviposition choices are an important determinant of plant damage as the larvae are relatively immobile. Many studies testing the effects of plant chemical composition on the feeding and oviposition behaviour of adult vine weevil adults have used excised plant material, but here weevil oviposition and feeding behaviour on nine raspberry cultivars was investigated using whole plants, ensuring choices reflected differences in cultivar appearance and chemical composition and were not influenced by the effects of excision on plant tissue.

3. In choice experiments, adult weevils showed no significant egg laying preference for any of the nine raspberry cultivars. Similarly, the egg laying capacity of the weevils (1.91-4.32 eggs day^{-1}) did not differ significantly between the cultivars in a no–choice situation i.e. when cultivars were offered singly. However, Glen Moy consistently received fewer eggs than the other cultivars in both choice (51% less) and no-choice (39% less) experiments.

4. Adult weevils significantly preferred to feed on particular cultivars in the choice experiment, with Tulameen having the highest probability of being eaten. In the no–choice experiment, weevils consumed significantly different quantities of leaf material from certain raspberry cultivars (ranging from 0.22 cm^2-1.03 cm^2 day^{-1}). In particular, Glen Moy, Glen Rosa and a wild accession had a significantly greater quantity of leaf material eaten by the adult weevils in comparison to most other cultivars.

5. Some leaf mineral element concentrations were found to be significantly correlated in relation to egg laying (Zn, Mg, Fe) and/or leaf consumption (K and Fe), although the results were not consistent between the choice and no–choice situations.

6. Foliar nitrogen concentration was strongly correlated with the number of eggs laid in both choice and no–choice situations, with greater numbers of eggs laid on plants with higher leaf nitrogen concentrations. This suggests that foliar nitrogen may be an influential factor underpinning the egg laying behaviour of vine weevils.

4.2. Introduction

The vine weevil (*Otiorhynchus sulcatus* F.) (Coleoptera: Curculionidae) originates from Europe, but is a significant pest in both horticultural and nursery crops worldwide (e.g. Moorhouse *et al.*, 1992a). The adult insect is particularly polyphagous and has been recorded feeding on over 150 different plant species (Smith, 1932, Warner & Negley, 1976). Adult weevils feed aboveground causing characteristic notching on leaf margins and lay eggs which subsequently fall into the soil where they develop into larvae. Root feeding larvae are also polyphagous and cause considerable damage by reducing plant vigour and growth, potentially resulting in plant death (Penman & Scott, 1976b, La Lone & Clarke, 1981, Moorhouse *et al.*, 1992a). Although the larvae are predominantly responsible for most of the plant damage, their limited mobility belowground means they are reliant on the host plant choice of the maternal weevil. Determining egg laying preferences by adult weevils between host plants could therefore be very important for limiting the damage caused by this pest.

Several studies have investigated vine weevil oviposition in relation to a wide range of host plants (Shanks, 1980, Nielsen & Dunlap, 1981, Hanula, 1988, Fisher, 2006). Certain species have been found to be unsuitable hosts (e.g. *Campanula isophylla* and *Euphorbia pulcherrima* (for larval development) and *Thuja occidentalis* (egg laying and mortality) are unsuitable as hosts (Moorhouse *et al.*, 1993b, van Tol *et al.*, 2004a). However, the majority of this research has used a diverse selection of plant species rather than considering whether weevils are able to detect subtle differences between cultivars or varieties of the same species. In particular, only a minority have addressed whether vine weevils can distinguish between horticultural cultivars (Cram & Pearson, 1965, Cram & Daubeny, 1982, Cowles, 2004), despite the economic consequences of vine weevil herbivory in such high

value crops (Alford, 2007). Additionally, in most studies vine weevil behaviour has been investigated with excised leaves rather than whole plants. Mechanically damaged leaves have previously been found to be attractive to vine weevils through volatile emissions (van Tol *et al.*, 2002) and thus using excised leaves may inadvertently influence feeding and oviposition of vine weevils. They may also have differences in their chemical composition, for example, water stress resulting from leaf excision can elevate ethylene production, which is not seen in whole plants exposed to drought situations (Morgan *et al.*, 1990). In addition, preferences tested with excised leaves cannot account for the effects that different plant biometrics or growth may have on behaviour. For instance the gall–inducing midge (*Harmandia tremulae*) preferentially oviposits on young leaves in trembling aspen (*Populus tremuloides*) and gall density is positively correlated with leaf length (Morrison & Quiring, 2009).

Host plant quality for insect herbivores is influenced by a range of nutritional aspects including: nitrogen (N), carbon (C) and other elemental minerals that can have positive, negative or neutral influences on insect herbivores (Awmack & Leather, 2002). The higher N and phosphorus (P) content in insects compared to plants, results in a constant struggle to gain adequate nutrition and thus N and P are often deemed to be the most limiting factors in insect development (Mattson, 1980, White, 1993, Elser *et al.*, 2000, Huberty & Denno, 2006). However, other mineral nutrients that are often overlooked can have significant influences on insect performance. For example, potassium (K) had a negative influence on the performance of the tobacco hornworm (*Manduca sexta*) (Stamp, 1994), whilst magnesium (Mg) was found to positively influence gall density and gall success for the eastern spruce gall adelgid (*Adelges abietis*) (McKinnon *et al.*, 1999). Other elemental minerals have also been found to affect insect herbivores including calcium (Ca) (e.g.

Scutareanu & Loxdale, 2006), zinc (Zn) (e.g. Alyokhin *et al.*, 2005) and iron (Fe) (Thangavelu & Bania, 1990).

The aim of this study was to investigate whether adult weevil oviposition (both preference and egg laying capacity) and feeding behaviour would be influenced by the chemical composition and growth characteristics of nine different raspberry cultivars. The cultivars were chosen to represent a range of geographical origin and genetic diversity (see **Table 4.1**). The germplasm covered both commercially cultivated types and a wild Scottish accession of *Rubus idaeus*. Manipulation of plant physiology and chemistry was kept to a minimum by using whole plants for the experiments rather than excised leaves. No–choice situations were used to test whether weevil egg laying and feeding would be affected by the chemical composition and plant characteristics of the raspberry cultivars. Choice situations were used to determine whether vine weevils would preferentially oviposit or feed on particular cultivars. In particular this study aimed to:

1. test whether vine weevil adults preferentially oviposited or fed on particular raspberry cultivars when given a choice between all nine cultivars,

2. determine whether egg laying capacity and feeding behaviour varied between cultivars in the absence of other cultivars, and

3. assess whether differences in nutritional status between cultivars affected feeding and/or oviposition behaviour.

Table 4.1. Background of raspberry genotypes used in experiments.

Raspberry Cultivar	Origin	Parentage	Summer season*	Commercial availability	Spines on canes	Cane Vigour+	Fruit Size^ (g)
Glen Ample	Scotland	SCRI 7326E1 x SCRI 7412H16	Mid season	Commercial	None	Vigorous	4.6
Glen Clova	Scotland	11/510 x SCRI S29/122	Early	Previously commercial	Spines	Vigorous	3.6
Glen Magna	Scotland	Meeker x SCRI 7719B11	Late	Amateur market	Spines (at base of cane)	Very vigorous	5.1
Glen Moy	Scotland	SCRI 688/12 x SCRI 6815/113	Early	Previously commercial	None	Low	3.5
Glen Rosa	Scotland	7326E1 x 7412H16	Mid season	Amateur market	None	Moderate	4.2
Malling Jewel	England	Prussen x EM23/50	Mid season	Previously commercial	Spines	Vigorous	3.8
Octavia	England	Glen Ample x Malling Hestia	Late	Commercial	Spines	Vigorous	4.9
Tulameen	Canada	Nootka x Glen Prosen	Mid season	Commercial	Spines	Vigorous in tunnel	4.0
Wild	Scotland	Scotland – Latitude 57.1755338, Longitude -4.7981994	–	–	Spines	–	–

* Early Season: Early June – Middle of August, Mid season: 3rd week in July – Middle of August, Late Season: Early August onwards

+Cane vigour primarily defined by plant height.

^ Fruit size measured from open field plots at SCRI.

4.3. Methods and materials

4.3.1. Plants and insects

Six week old plants (*c.* 3cm high with 9 leaflets) of nine raspberry cultivars (see **Table 4.1**) were grown in plastic pots (BEF Growers Number 5) containing a 2:1 mixture of insecticide–free compost (peat–sand–perlite mix containing 17N:10P:15K; William Sinclair Horticulture Ltd, Lincoln, UK) and sand (Silver sand, J. Arthur Bowers, Lincoln, UK). Plants were grown in a greenhouse at optimum conditions (15 – 20 ºC, 16:8 L: D photoperiod). All experiments were conducted in controlled temperature environments at 21 ºC ± 2 ºC and 16:8 L: D photoperiod. Ovipositing adult weevils were obtained from a culture maintained at 17 ºC ± 2 ºC and 16: 8 L: D photoperiod at SCRI. Adults in the culture were originally collected as teneral adults from a field site (56º447'N, 3º012'W) with a mixture of raspberry, strawberry and blackcurrant (see Johnson *et al.*, 2010a for full details). Weevils were thus all of a similar age (*c.* 8 weeks old) when used in the experiments. Weevils were fed on a mixture of strawberry (*Fragaria x ananassa*) cultivars as a precaution to limit any effects of prior experience of raspberry cultivars influencing their behaviour, although, weevil oviposition preferences have previously been shown to be unaffected by their previous exposure (as adults or larvae) to different host plants (Hanula, 1988).

4.3.2. Experimental setup

1. Choice experiment

One plant of each raspberry cultivar was randomly placed into one of sixteen mesh cages (62 (l) cm x 62 (w) cm x 45 (h) cm), i.e. each cage contained nine plants, with one plant of each cultivar in each cage (**Figure 4.1**). Plants were grouped according to size to minimise

any influence that plant biometrics may have on the vine weevil. A fine mesh circular collar with washed gravel (Coarse grit, J. Arthur Bowers, Lincoln, UK) (~2-6 mm) was placed around the stem of each plant to enable the recovery of weevil eggs at the end of the experiment (see Johnson *et al.*, 2010b for details). One ovipositing adult weevil was introduced into each cage. Plants were harvested three weeks after the introduction of the weevil. Weevils were removed from cages and plant biometrics were recorded (plant height, plant mass, number of leaves, maximum root length, root mass and leaf area). Eggs were recovered from the plants by immersing the gravel in a saturated KCl solution and gently agitating so that the eggs floated to the surface (as described in Johnson *et al.*, 2010b); additionally leaves and stems were checked for any eggs oviposited whilst the insect was in the plant canopy.

Total leaf consumption per plant was calculated using a LI-3100C area meter (LI-COR Inc., Lincoln, Nebraska, USA) and digitally scanned leaf areas. Digital images were analysed to determine eaten leaf areas (Johnson *et al.*, 2010b). After digitally scanning, only eaten leaves were snap frozen in liquid nitrogen for subsequent chemical analysis, thus the number of replicates is not the same as the number of plants, as some plants were not eaten.

2. No–choice experiment

Eight plants of each of the nine cultivars were placed into individual mesh cages (52 cm high, 12.5 cm diameter), constituting 72 cages in total. Plants were of a similar size to those offered to vine weevils in the choice experiment and were also similarly sized for each cultivar, so that the weevils were been offered an equal amount of plant material. Plants were then treated and harvested as described in the choice experiment.
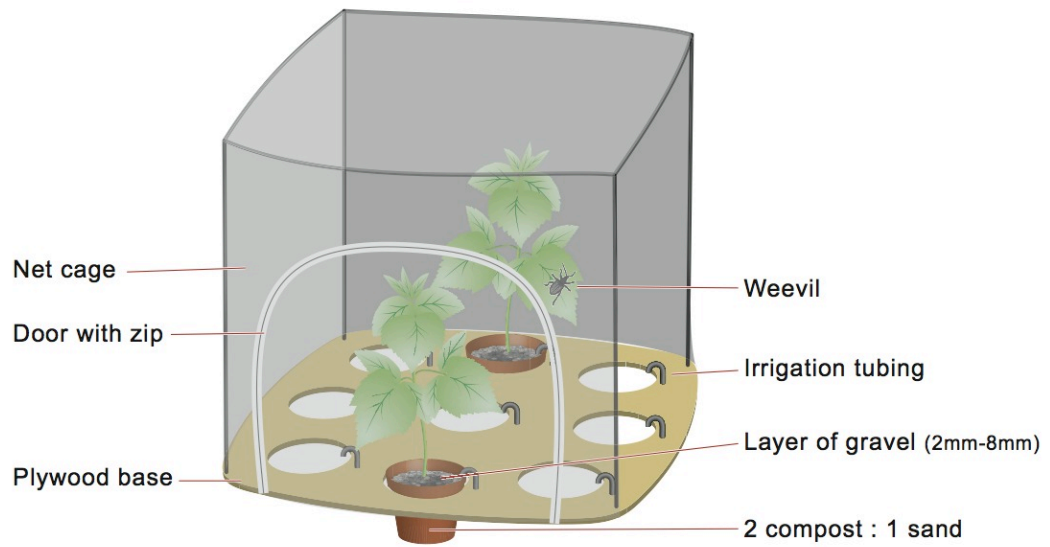
Figure 4.1. Schematic of mesh cage used for choice experiment with nine raspberry cultivars.

4.3.3. Plant chemical analysis

Frozen leaf samples were milled to a fine powder for all further chemical analyses. The %N (nitrogen) and %C (carbon) concentrations of 2 mg samples were determined by a combination of the Dumas and Pregl methods and were carried out using an Exeter Analytical CE440 Elemental Analyser. The percentage of carbon and nitrogen in the sample was calculated by comparison with known standards.

Measurement of other mineral elements was carried out by acid digesting leaf samples (0.05 g) for 20 min at 180 ºC in 3 ml of 15.8 M HNO_3 (Anistar grade, VWR International, Poole, UK) followed by oxidation with 1 ml of H_2O_2 for 20 min at 180 ºC in closed vessels within a MARS-Xpress microwave oven (CEM, Buckingham, UK). Digested samples were diluted to 50 ml using de–ionised water. Total mineral contents of calcium (Ca), phosphorus (P), magnesium (Mg), zinc (Zn), iron (Fe) and potassium (K) in the digested leaf samples were determined by inductively–coupled plasma mass spectrometry (Elan DRC-e, Perkin–Elmer, Beaconsfield, Bucks, UK).

4.3.4. Statistical analysis

Egg data for the choice experiment was analysed using a generalised mixed model with a Poisson error structure and log link function. Cultivar was designated as a fixed effect in the model and cage and cage interacting with plant as random effects, which also allowed for comparisons between the cultivars to determine which were significantly different in terms of the number of eggs laid. For the no–choice experiment the egg data was analysed using a generalised linear model with a Poisson error structure and log link function, which again allowed for determination of which cultivars were significantly different from one another. For the choice experiment, the quantity and number of plants eaten by the weevil

was very low, thus rather than modelling leaf area directly, the model considered the probability of a weevil eating a particular cultivar out of the choice of nine cultivars. Each plant cultivar was scored as having being eaten or not, and the data were then. The probabilities were analysed using a generalised mixed model with a binomial error structure and logit link function, from which the probability of each cultivar being eaten was predicted. For the no–choice experiment, leaf areas were analysed using a generalized linear model with a normal error structure and identity link function. Significant differences between the amount of leaf material eaten for each cultivar was compared using the model, by re-ordering the cultivars and re-running the analyses, to allow multiple t-test comparissons to be conducted. Plant biometrics were checked in each of the models to establish their significance in determining either egg laying or feeding behaviour. Any biometrics which were determined to be significant were investigated further using Spearman's rank correlations. The relationship between egg laying and feeding behaviour and plant nutrition were investigated using Spearman's rank correlations, Pearson's product moment correlations and linear regressions as appropriate. All statistical analyses were conducted in GenStat (version 12, VSN International, UK).

4.4. Results

4.4.1. Egg laying and egg laying capacity

Neither the oviposition preferences of adult vine weevils in choice experiments (F=1.55, df=8, p=0.157) (**Figure 4.2 a**) nor their egg laying capacity in no–choice experiments ($F_{8,65}$=1.26, p=0.280) (**Figure 4.2 b**) differed significantly between cultivars. None of the plant biometrics quantified significantly affected egg laying behaviour (results not shown) in the no–choice experiment; however, leaf area eaten did significantly influence egg laying (F=7.05, df=1, p=0.010) in the choice experiment. In the choice experiment the number of eggs laid was positively correlated with the quantity of leaf eaten (r_s=0.387, df=115, p=0.001), but this pattern was not seen in the no–choice experiment (r_s=0.059, df=64, p=0.637).

4.4.2. Feeding behaviour

When given a choice, the probability of a weevil eating was significantly higher on some raspberry cultivars than others (F=3.23, df=8 p=0.001). The probability of a particular cultivar being eaten was most notably higher for Glen Ample, Glen Rosa and Tulameen (**Figure 4.3 a**). In addition, raspberry cultivars significantly influenced the quantity of leaf area eaten when the weevils were given no–choice ($F_{8,57}$=3.58, p=0.002) (**Figure 4.3 b**). No plant biometrics were influential in determining the probability of a weevil eating a particular raspberry cultivar in choice tests (results not shown). However, total leaf area ($F_{1,64}$=17.24, p<0.001), number of leaves ($F_{1,64}$=5.47, p=0.022) and plant mass ($F_{1,64}$=7.82, p<0.007) affected the amount of leaf area eaten by the weevil in no–choice tests. All three variables were positively correlated with the quantity of leaf eaten: plant mass (r_s=0.453,

df=64, p=0.001), number of leaves (r_s=0.307 df=64, p=0.012, and total leaf area (r_s=0.589, df=64, p<0.001).
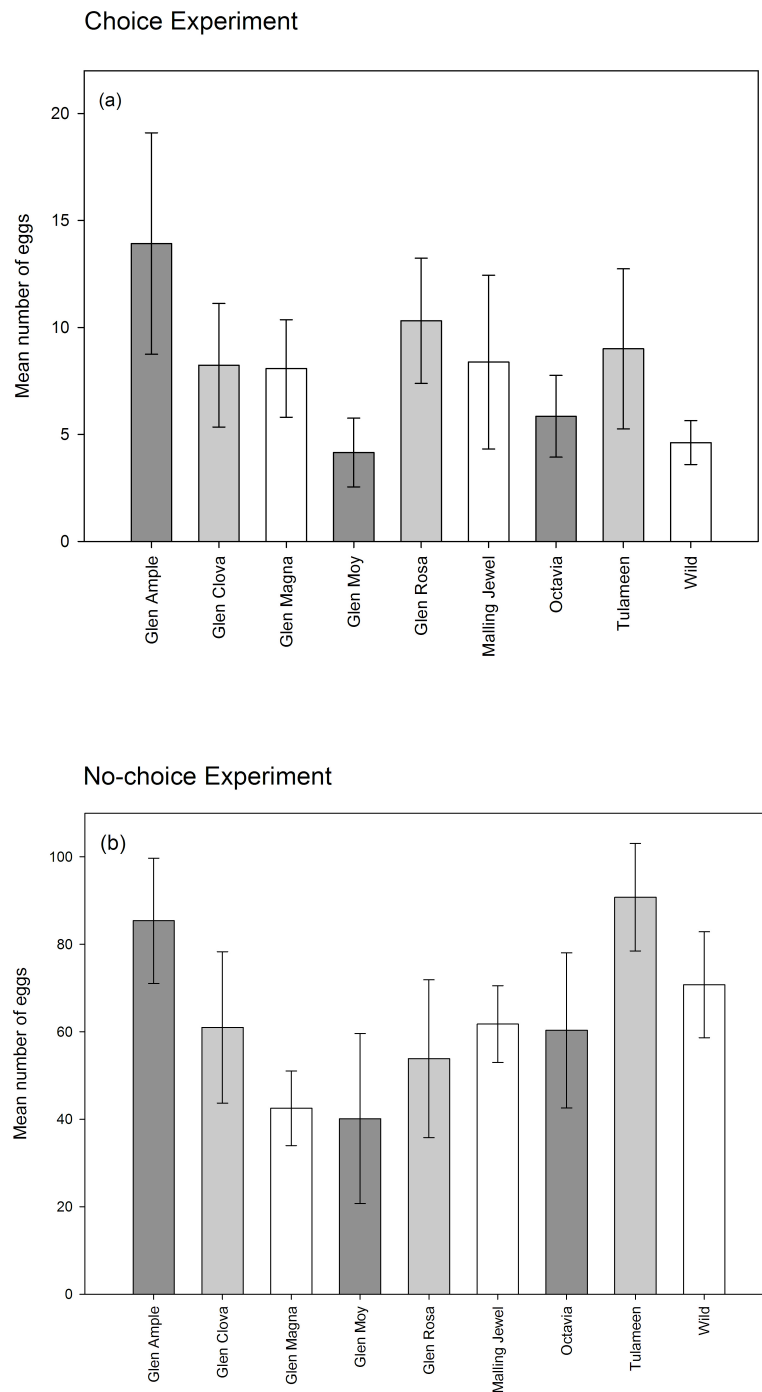
Figure 4.2. Egg laying behaviour and egg laying capacity. (a) Choice experiment: number of eggs laid in relation to nine raspberry cultivars, mean (± SE) shown, N = 13.

(b) No–choice experiment: egg laying capacity in relation to nine raspberry cultivars, mean (± SE) shown, N = 6-8.

Choice Experiment

No-choice Experiment
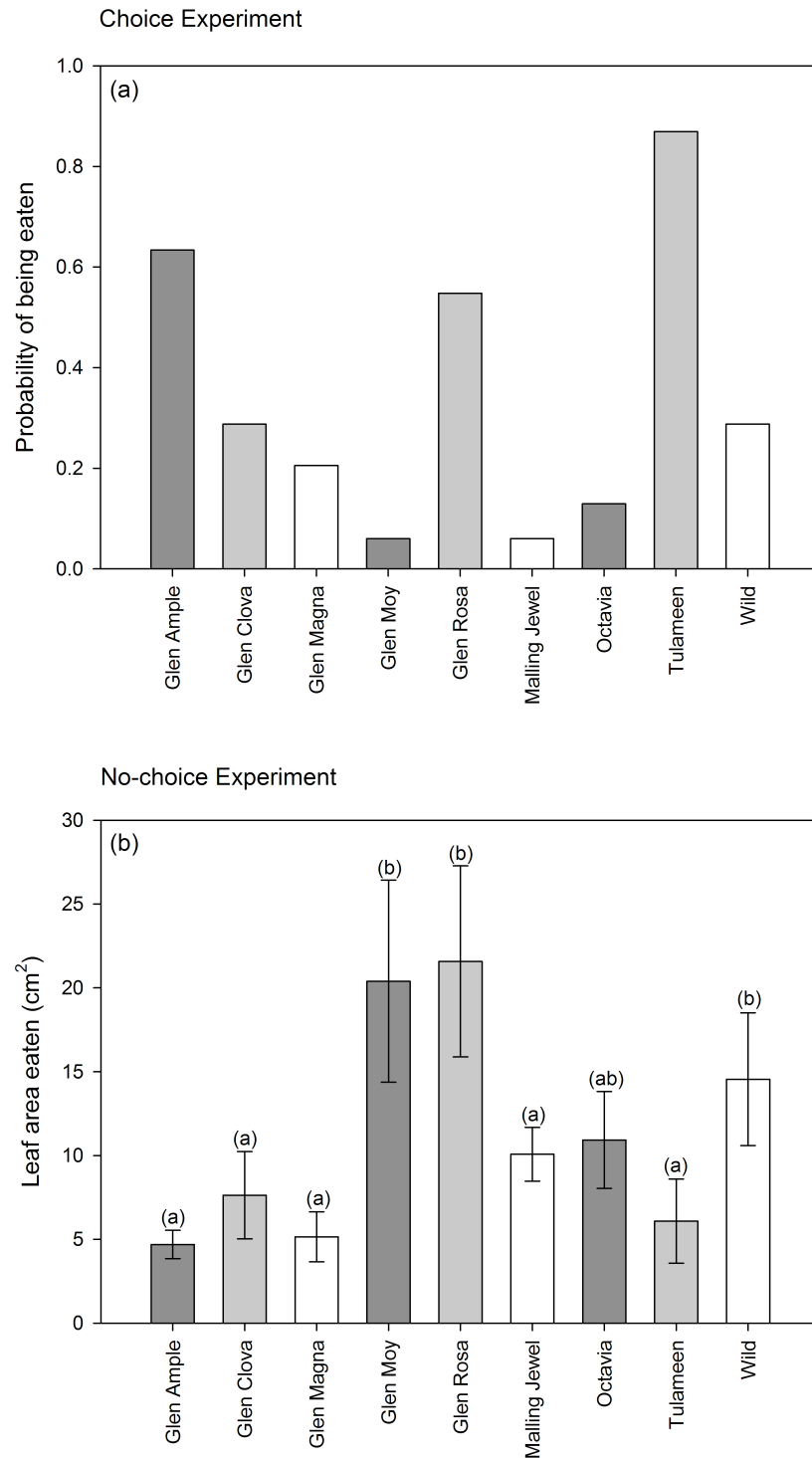
Figure 4.3. (a) Probability of a raspberry cultivar being eaten by a weevil in the choice experiment, shown, N = 13 (b) Mean leaf area eaten in relation to raspberry cultivar in no–choice experiment, mean (± SE) shown, N = 6-8. Differences between cultivars analysed using a generalized linear model with normal errors and identity link function. Bars accompanied by the same lowercase letter are not significantly different at the 5 % significance level.

4.4.3. Relationships between weevil feeding, oviposition behaviour and plant nutrition

In both choice and no–choice experiments, the number of eggs laid was positively correlated with leaf nitrogen concentrations (**Figure 4.4**). There were several weak positive correlations between the number of eggs laid and different leaf mineral element concentrations in each experiment, however, these relationships were not consistent between experiments (**Table 4.2**). Foliar nutritional quality in terms of carbon and nitrogen was not related to the quantity of leaf eaten in either experiment (**Table 4.2**). Leaf consumption was weakly correlated with some other leaf mineral concentrations, but as with the number of eggs laid the results were not consistent between the choice and no– choice experiments (**Table 4.2**).

Choice Experiment

No-choice Experiment
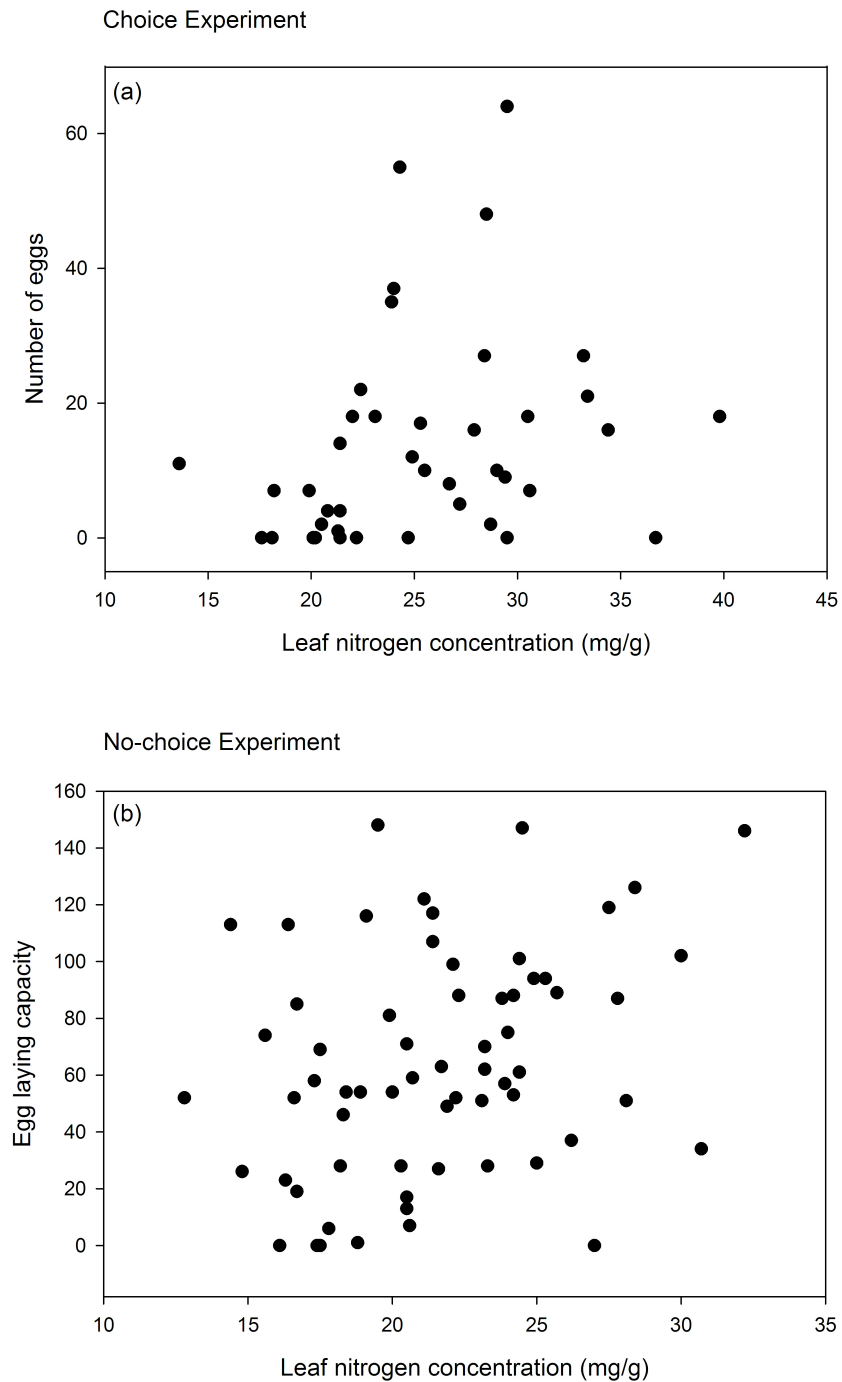
Figure 4.4. (a) Number of eggs laid in relation to leaf nitrogen concentration when weevils were given a choice of raspberry cultivar N=41. (b) Egg laying capacity in relation to leaf nitrogen concentration when weevils were not given a choice of raspberry cultivar, N=64.

Table 4.2. Summary of correlations for eggs laid and leaf area eaten in relation to leaf nutritional quality. Significant relationships highlighted in bold where $p<0.05$. Where possible data were transformed ([1]log or [2]square root) prior to analysis. Correlations were calculated using Spearman's rank correlation or Pearson's product moment correlation. Choice experiment, n= 41, No–choice experiment n=64

Experiment	Weevil Response	Correlations														
		N		C		Ca		P		Mg		Zn		Fe		K	
		r_s	p	r_s	p	r_s	P	r_s	p	r_s	P	r_s	p	r_s	p	r_s	p
Choice	Eggs	**0.385**	**0.013**	0.063	0.693	0.279	0.077	0.113	0.481	0.219	0.169	0.387	**0.012**	0.206	0.196	-0.225	0.157
	Leaf area eaten	0.188	0.240	0.087	0.587	-0.148	0.356	-0.125	0.437	-0.217	0.174	0.122	0.446	-0.256	0.107	0.349	**0.025**
		N		C		Ca		P[1]		Mg		Zn[1]		Fe		K	
		r	p	r	p	r	P	r	p	r	P	r	p	r_s	p	r	p
No–choice	Eggs	**0.310**	**0.013**	0.076	0.551	0.096	0.452	0.135	0.287	0.251	**0.045**	0.109	0.392	0.273	**0.029**	-0.115	0.368
	Leaf area eaten[2]	0.076	0.551	-0.203	0.107	0.234	0.063	0.112	0.378	0.224	0.075	0.207	0.100	0.255	**0.042**	-0.126	0.320

4.5. Discussion

Vine weevil egg laying behaviour and egg laying capacity did not significantly differ between the nine raspberry cultivars investigated. This result is contrary to a previous study (Cram & Daubeny, 1982), where a significant difference in egg laying by vine weevils was seen between raspberry cultivars, although only some of the cultivars were the same as presented here. The differences in the results between the two studies may reflect differences in the behaviour of vine weevils when presented whole plants (as in this study) compared with excised leaves (as in Cram and Daubeny's (1982) study). Excising leaves may have caused the production of volatiles in response to plant damage, as well as other changes in foliar composition and appearance, all of which may have affected the behaviour of the vine weevil (van Tol & Visser, 2002).

One characteristic of the raspberry cultivars which clearly did influence vine weevil behaviour in both the choice and no–choice situations was leaf nitrogen content. Vine weevil egg laying preferences and egg laying capacity were both found to be positively correlated with foliar nitrogen concentrations. This has not previously been reported for raspberry, but similar patterns have been observed in strawberry (Hesjedal, 1984). However, other studies have shown no such correlation between egg laying and foliar nitrogen content (Maier, 1981), suggesting that other factors may also influence vine weevil oviposition. A correlation between foliar and root nitrogen content in raspberry has not previously been reported, but a positive correlation could well influence preference-performance linkages in aboveground-belowground systems, with aboveground adult insects preferentially selecting host plants with a high foliar nitrogen content, which then would relate to an elevated root nitrogen content for their subsequent offspring, conversely the lack of a relationship between aboveground foliar nitrogen content and root nitrogen

content, could provide an explanation for the lack of a PPH linkage. Whilst vine weevil larvae feed belowground and would not directly benefit from elevated foliar nitrogen content, adult weevils may associate the increased nitrogen as an indication of an agronomically superior host plant. In particular, early instar larvae are susceptible to nitrogen availability, which can affect both growth rates and survival (see White, 1993 and references therin). Therefore, according to the preference performance hypothesis (Jaenike, 1978) maternal oviposition should favour host plants which optimise nitrogen availability for offspring.

Adult weevil feeding significantly differed between the nine raspberry cultivars investigated. When given a choice, weevils preferred to feed on Tulameen and avoided feeding on Glen Moy, whilst in a no–choice situation adult weevils consumed a high quantity of leaf material on Glen Moy and Glen Rosa. As the nutritional content of the foliage was not correlated with weevil feeding, this suggests that other plant properties may be influential. In the no–choice experiment, vine weevil feeding was found to be positively correlated with plant size (plant mass, leaf area and number of leaves), thus weevils may select to feed on plants with more vigorous growth in accordance with the plant vigour hypothesis (Price, 1991), although further investigation would be required to determine the factors influencing their elevated consumption on faster growing plants. Potentially, the elevated weevil feeding seen on larger plants in the no-choice experiment could indicate that they chose to feed on plants that provided them with superior shelter and a place to hide. However, given that in the choice experiment they showed no significant preferences for larger plants, it is unlikely that this is the situation. Alternatively, leaf hairs were found to be one of the factors determining the acceptance of strawberry cultivars for vine weevil feeding, although it was proposed that another undetermined property was also important

(Cowles, 2004). Potentially, subtle differences in plant defences between the cultivars may affect the feeding behaviour of the weevils.

In some polyphagous insects where the relationship between adult oviposition and offspring performance is not strongly linked as predicted by the preference–performance hypothesis (Jaenike, 1978), it has been suggested that egg laying behaviour is predominantly influenced by the nutritional status of the host plant for the maternal insect (Mayhew, 2001, Scheirs & De Bruyn, 2002). In this instance, adult feeding takes precedence over egg laying, hence eggs tend to be laid where the adults feed. In this particular study, when vine weevils were given a choice of raspberry cultivars, leaf consumption and egg laying behaviour were positively correlated. However, the quantity of leaf material consumed was not related to foliar nitrogen content, unlike egg laying, although a significant relationship between foliar nitrogen and plant consumption has previously been reported in strawberries (Cowles, 2004). Thus, although eggs were laid where weevils fed in the choice experiment, the nitrogen content of leaves did not dictate where the weevils fed.

Foliar mineral element concentrations were shown to be significant in relation to egg laying (Zn, Mg, Fe) and leaf consumption (K and Fe), but, inconsistent results between the choice and no–choice experiments make it difficult to determine whether particular elements underpinned weevil behaviour. Ascertaining how plant minerals influence fecundity of insect herbivores can prove complicated, due to the varying ways in which insects utilise them, in addition to the varying relationships that minerals may have with other plant properties (e.g. with levels of defensive compounds) and with one another (Awmack & Leather, 2002).

4.6. Conclusions

Vine weevil egg laying capacity and preference were not found to differ between the nine raspberry cultivars; in contrast feeding preferences were significantly different between the cultivars. Foliar nitrogen content was the most significant factor in determining egg laying capacity and preferences, but was not influential in feeding behaviour. Plant growth characteristics in the no–choice experiment showed a relationship with weevil feeding behaviour, but a range of plant minerals could not be clearly related to feeding and oviposition preferences, suggesting that other plant properties not recorded in this study (e.g. plant volatiles and plant defences, both physical and chemical) may have significant influences on weevil feeding preferences.

Chapter Five – Investigating preference–performance relationships in aboveground–belowground life–cycles: a laboratory and field study with the vine weevil (*Otiorhynchus sulcatus*)

5.1. Abstract

To date, the preference–performance hypothesis has principally considered insect herbivores with aboveground life–cycles, although the hypothesis could be equally relevant to insects with life stages occurring both aboveground and belowground. Moreover, most studies to date have focussed on either laboratory or field experiments, with little attempt made to relate the two. In this study, the preference–performance hypothesis was examined in an aboveground–belowground context using the vine weevil (*Otiorhynchus sulcatus* F.) (Coleoptera: Curculionidae) and two cultivars of the host plant red raspberry (*Rubus idaeus*), Glen Rosa and Glen Ample. A two–year field study (2008-2009) was also undertaken to characterise the population dynamics of adult vine weevils on the two raspberry cultivars. Vine weevil larval performance (abundance and mass) differed significantly between Glen Rosa and Glen Ample, with Glen Rosa resulting in 26% larger but 56% fewer larvae compared to Glen Ample. Larval abundances were significantly and positively correlated with root nitrogen and magnesium concentrations, but negatively correlated with root iron concentrations. The two cultivars were not significantly different in concentrations of these minerals however. Adult weevils did not preferentially select either of the two cultivars for egg laying (laying 3.08 and 2.80 eggs per day on Glen Ample and Glen Rosa respectively) suggesting that there was no strong preference–performance relationship between adult vine weevils and their belowground offspring. Whilst larval development in laboratory experiments showed significant differences between the two raspberry cultivars, populations of adult vine weevils were similar on both cultivars in 2008 and 2009. Our results highlight that performance differences detected in controlled experiments may not necessarily result in significant differences at a population level in the field.

5.2. Introduction

In insect–plant interactions, the selection of a host plant by a maternal insect can be a highly influential factor in parent–offspring relationships, where host plant suitability can affect both parental fecundity and offspring performance (Bernays & Chapman, 1994). The interaction between maternal choice of host plant and subsequent offspring performance is often addressed in relation to the preference–performance hypothesis (PPH), which has received renewed interest recently (Gripenberg *et al.*, 2010 and references therein). The PPH was first proposed by Jaenike (1978) and states that maternal insects will preferentially lay eggs on host plants that optimise the survival and performance of their offspring. The hypothesis particularly refers to insects whose larvae have limited or no ability to relocate, and are dependent on the maternal selection of host plant. In order to optimise offspring performance, the hypothesis predicts a strong association between the egg laying preferences of the mother and offspring performance (Mayhew, 2001).

Many studies examining maternal oviposition preferences and offspring performance support the PPH (e.g. Craig *et al.*, 1989, Heisswolf *et al.*, 2005, Staley *et al.*, 2009). Equally though, linkages between egg laying preferences and offspring performance can be weak or absent (e.g. Rausher, 1979, Scheirs *et al.*, 2004, Digweed, 2006, Gripenberg *et al.*, 2007). The presence of weak PPH linkages has led to a range of alternate hypotheses examining why maternal insects do not select the optimal host plant. These include: optimal foraging, where maternal insects select host plants with superior nutritional quality without accounting for the suitability for offspring performance (Scheirs *et al.*, 2000), and enemy free space (Thompson, 1988a, Thompson, 1988b), where preference–performance linkages are more strongly influenced by natural enemies (normally not incorporated in experiments).

To date, much of the research examining the PPH has incorporated insects with aboveground life–cycles. Equally though, the hypothesis could be applied to maternal insects living aboveground that have soil–dwelling offspring with little capacity to relocate between root systems (Johnson *et al.*, 2006). Although an increasing amount of literature has demonstrated interactions between aboveground and belowground insects on a shared host plant (van der Putten *et al.*, 2001, Wardle *et al.*, 2004, van der Putten *et al.*, 2009), all of these have used unrelated insect species and not considered the relationship between a maternal insect and her belowground offspring.

Here we consider the PPH in an aboveground–belowground context in relation to two raspberry (*Rubus idaeus*) cultivars using the vine weevil (*Otiorhynchus sulcatus* F.) (Coleoptera: Curculionidae) as a model species. Vine weevils are parthenogenetic and unfertilised eggs develop into new females without the need for males, thus, the vine weevil is a highly suitable study species for investigating parent–offspring relationships in an aboveground–belowground context, as offspring are genetic clones of the adult. The adult weevil is highly polyphagous feeding on over 150 different plant species (Smith, 1932, Warner & Negley, 1976). However, it is the root feeding larvae that cause most plant damage, decreasing plant vigour and growth and potentially causing death (Penman & Scott, 1976b, La Lone & Clarke, 1981, Moorhouse *et al.*, 1992a). Indeed, vine weevil larvae cause an estimated £8 million of damage to soft fruit production every year (HDC, 2003); the limited ability of the larvae to move belowground, means they are restricted to the host plant choice of the maternal weevil. Adult weevils live aboveground where they lay eggs both on the plant and soil surface which subsequently develop into root feeding larvae. The life–cycle has four distinct stages: eggs, larvae, pupae and adults, however, each stage may occur concurrently (Shread, 1972), thus adults can be feeding on plants aboveground, which are already exposed to root feeding larvae belowground.

The majority of studies investigating vine weevil preferences in relation to host plants have examined the relationship in terms of adult weevil oviposition and feeding behaviour (e.g. Shanks, 1980, Maier, 1981, Nielsen & Dunlap, 1981, Cram & Daubeny, 1982, van Tol *et al.*, 2004a). However, relatively few studies have considered the influence of host plants on larval performance. Strawberry (*Fragaria×ananassa*) has been demonstrated to enhance both the establishment of vine weevil populations and larval survival in comparison with Norway spruce (*Picea abies*), white spruce (*Picea glauca*), yew (*Taxus baccata*) and rhododendron (*Catawaba rhododendron*) (Fisher, 2006). Additionally, larvae feeding on azalea (*Rhododendron kiusianum*) were smaller and had poorer survival on reaching adulthood compared to adults developing on strawberry (*Fragaria×ananassa* Duchesne) or *Taxus cuspidata* (Hanula, 1988). Such examples provide evidence that the developmental stage of vine weevil larvae can be affected by the host plant species. However, these laboratory studies were conducted in controlled environments and so the relevance of any such infestations to field populations is unknown.

Like foliar feeding insects, root feeding vine weevil larvae are likely to be influenced by the nutritional status of their host plant, where minerals have been shown to have beneficial, detrimental or neutral influences on insect herbivores (Awmack & Leather, 2002). Nitrogen (N) and phosphorus (P) are often deemed the most limiting factors in insect development (Mattson, 1980, White, 1993, Elser *et al.*, 2000, Huberty & Denno, 2006) due to their low concentrations in plants in comparison to insects. However, other minerals have been shown to significantly affect insect performance but are often overlooked. These include: calcium (Ca) (Scutareanu & Loxdale, 2006), potassium (K) (Stamp, 1994), magnesium (Mg) (McKinnon *et al.*, 1999), Zinc (Zn) (Alyokhin *et al.*, 2005) and iron (Fe) (Thangavelu & Bania, 1990).

The objective of this study was to investigate vine weevil behaviour and performance, both aboveground and belowground, on two contrasting raspberry cultivars: Glen Ample and Glen Rosa. Glen Rosa has previously been found to be generally more resistant to some insect pests than Glen Ample (e.g. the large raspberry aphid, *Amphorophora idaei* (McMenemy *et al.*, 2009)). Glen Ample exhibits more vigorous growth than Glen Rosa, whilst Glen Rosa is particularly susceptible to leaf rust, which may deter insect feeding (N. Jennings, personal communication). The specific aims of this study were

1. to determine how the two cultivars affected different larval performance traits (abundance and body mass), and establish whether these traits were related with each other,

2. to determine whether either, or both, performance traits influenced oviposition behaviour by adults and,

3. to assess whether these differences were reflected in the field over a two–year period.

It was hypothesised that:

1. vine weevil larvae feeding on Glen Ample would show improved performance (in terms of either abundance or body mass, or both) compared to larvae on Glen Rosa,

2. adult vine weevils would preferentially lay more eggs on the cultivar that resulted in greatest larval performance (whether this was abundance, body mass, or both) and

3. field populations of adult vine weevils would be higher on the cultivar that increased larval performance and that was preferentially selected by ovipositing adults.

5.3. Methods and materials

5.3.1. Plants and insects

Raspberry plants (*cv*. Glen Ample and Glen Rosa) were grown in plastic pots (BEF Growers Number 5) containing a 2:1 mixture of insecticide free compost (peat–sand–perlite mix containing 17N:10P:15K; William Sinclair Horticulture Ltd, Lincoln, UK) and sand (Silver sand, J. Arthur Bowers, Lincoln, UK). Plants were grown in a greenhouse at optimum conditions (15 – 20 ºC, 16:8 L: D photoperiod). All experiments were conducted in controlled temperature environments at 21 ºC ± 2 ºC and 16:8 L: D photoperiod.

Ovipositing adult weevils were used from cultures maintained at 17 ºC ± 2 ºC and 16:8 L: D photoperiod fed on a mixture of strawberry cultivars. Melanised vine weevil eggs used in experiments were collected from the cultures ensuring egg viability (Smith, 1932).

5.3.2. Larval performance

Ten plants (*c*. 3 cm high with 9 leaflets) of each cultivar were treated with 30 vine weevil eggs (inserted into a small hole close to the plant stem). After five weeks, plants were harvested. Roots were carefully teased apart to recover larvae. Individual larvae were weighed on a microbalance (accuracy ± 0.01 mg). Root biometrics (root mass and maximum root length) were measured after washing and roots were then snap frozen in liquid nitrogen and stored at -18 ºC for subsequent chemical analyses.

Frozen root samples were milled to a fine powder for all chemical analyses. The %N (nitrogen) and %C (carbon) concentrations of 2 mg samples were determined by a combination of the Dumas and Pregl methods and were carried out using an Exeter

Analytical CE440 Elemental Analyser. The percentage of carbon and nitrogen in the sample was calculated by comparison with known standards.

Measurement of other mineral elements was carried out by acid digesting root samples (0.05 g) for 20 min at 180 °C in 3 ml of 15.8 M HNO_3 (Anistar grade, VWR International, Poole, UK) followed by oxidation with 1 ml of H_2O_2 for 20 min at 180 °C in closed vessels within a MARS-Xpress microwave oven (CEM, Buckingham, UK). Digested samples were diluted to 50 ml using de–ionised water. Total mineral contents of calcium (Ca), phosphorus (P), magnesium (Mg), zinc (Zn), iron (Fe) and potassium (K) in the digested leaf samples were determined by inductively–coupled plasma mass spectrometry (Elan DRC-e, Perkin-Elmer, Beaconsfield, Bucks, UK).

5.3.3. *Paired oviposition experiment with two raspberry cultivars*

For the paired choice experiment, twenty Glen Ample (*c.* 9 cm high with 14 leaflets) plants were paired with twenty Glen Rosa (*c.* 8 cm high with 14 leaflets) plants according to size. Each plant pair was placed into a mesh cage (45 cm (h), 45 cm (w) 28.3 cm (d)). A fine mesh circular collar was placed around the stem of all plants and then covered with washed gravel (Coarse grit, J. Arthur Bowers, Lincoln, UK) (~2-6 mm) to allow the retrieval of vine weevil eggs at the end of the experiment (see Johnson *et al.*, 2010b).

One ovipositing weevil was introduced into each cage. Plants were harvested three weeks after the addition of the weevils. Weevils were recovered from the cages and plant biometrics were recorded (plant height, plant mass, number of leaves, leaf area and root mass), no adult weevil biometrics were recorded. Eggs were recovered from the gravel by immersing it in a saturated KCl solution and gently stirring so that the eggs floated to the

surface (as described in Johnson *et al.*, 2010b); additionally leaves and stems were checked for any eggs oviposited whilst the insect was in the plant canopy.

Leaf consumption was calculated using a LI-3100C area meter (LI-COR Inc. Lincoln, Nebraska, USA) and digitally scanned leaf areas. Digital images were analysed to determine eaten leaf areas (see Johnson *et al.*, 2010b for details).

5.3.4. *Field experiment*

To investigate whether any laboratory differences in vine weevil larval performance and adult vine weevil feeding and oviposition behaviour would translate to differences in population sizes in a field environment, an experiment was conducted using the same two raspberry cultivars in a protected cropping system.

The experiment was conducted in six separate and adjacent polytunnels at SCRI, Dundee, UK (56°447'N, 3°012'W). Protected cropping systems now provide 80% of UK soft fruit sold through supermarkets (British Summer Fruits, 2009) and thus reflect the most realistic field environment for vine weevils feeding on raspberry. Each tunnel (22 x 8 x 3.3 m; length: width: height) was covered with Luminance THB polythene film (BPI, London, UK) and contained three raised beds of *c*. 24 plants covered with polythene mulch. Three tunnels had been planted with Glen Ample and three with Glen Rosa in July 2005. In all three years of the experiment the tunnels were left uncovered from October until June, according to commercial practice.

Plants in each row (3 rows per tunnel) were separated into plots of four and enclosed using a correx barrier dug into the ground at the base (i.e. each row contained six plots). Full

details of the experimental field set up are given in Chapter Three, but in summary, vine weevil eggs collected from culture were applied to plants at regular intervals so that each row received 576 eggs in total (equivalent to *c.* 24 eggs per plant). Eggs were added to the soil around the base of each plant in a plot.

Vine weevil adults were surveyed every 14 days (± 2 days) at night (2200-0100) from mid May until mid October in 2008 and 2009. Weevils were dislodged onto white beating trays (110 cm x 86 cm) (Watkins and Doncaster, Cranbrook, UK) held either side of the plants by shaking the two middle plants in each plot five times. Weevils were placed in labelled containers for counting on the following day after which they were returned to the base of the plants where they had been captured.

5.3.5. Data analysis

Larval mass was analysed by analysis of variance (ANOVA), with plant as a blocking factor. Larval survival was analysed with a generalised linear model with a Binomial error structure and logit link function. The number of larvae recovered from each plant was analysed with a two–sample t–test. Relationships between root mineral element concentrations and larval performance were analysed using Pearson's product moment correlation. Egg laying and feeding behaviour of adult weevils in relation to the paired cultivar experiment were analysed using paired t–tests and appropriate data transformations performed to address non–normally distributed data. Egg laying in relation to feeding behaviour was examined using Spearman's rank correlations.

Preliminary examination of the field survey data highlighted that early on in the season the limited numbers of weevils caught was resulting in a type I error in the data analysis (i.e.

rejecting the null hypothesis when it is true). Consequently in both years, survey data was only included in the analysis after the population exceeded 100 weevils caught on one sampling occasion. Differences between the numbers of weevils caught on the two cultivars (Glen Ample and Glen Rosa) were analysed using ANOVA's that incorporated both tunnel and row as factors and cultivar as a variable. Generalised linear models and mixed models, which are usually highly suitable for field population analysis (Johnson *et al.*, 2003) were also considered, but poorly described the data (results not shown). For each sampling year the test was performed on the total number of weevils caught in each plot for that year. In addition, for each sampling date in each year, an ANOVA was conducted using the same factors and variables, but using only the weevil counts per plot for that sampling date. In all cases, data conformed to normality (Shapiro–Wilks test) and had low heteroscedasticity when residual diagnostic plots were examined (Sokal & Rohlf, 1995). Repeated measures were not used due to the complex experimental design, with several factors required in the analyses to account for any differences between the tunnels and rows, whilst also investigating any differences between the cultivars. All analyses were conducted in Genstat (version 12, VSN International, UK).

5.4. Results

5.4.1. Larval performance

Vine weevil larvae were significantly more abundant on Glen Ample than on Glen Rosa (t_{18}=2.50, p=0.022) (**Figure 5.1 a)**, with larval survival in terms of the original inoculation with eggs considerably higher (p=0.052) on Glen Ample (18%) than on Glen Rosa (8%). In contrast, larval mass was significantly higher on Glen Rosa than on Glen Ample ($F_{1,65}$=1.14, p=0.001) (**Figure 5.1 b**).

Overall, the number of larvae recovered per plant was positively correlated with root nitrogen concentrations (**Figure 5.2**; **Table 5.1**). Root carbon and root nitrogen concentrations were not significantly different between the two cultivars (C: (t_{16}=0.90, p=0.384), N: (t_{16}=0.22, p=0.831)), nor did larval performance show any relationship with root carbon concentrations. The concentration of magnesium in the roots was positively correlated with the number of larvae recovered per plant (**Table 5.1**), but was not significantly different between Glen Ample and Glen Rosa (t_{17}=0.65, p=0.527). The number of larvae recovered was additionally negatively correlated with the concentration of iron in the roots (**Table 5.1**), but there was no difference in iron root concentrations between Glen Ample and Glen Rosa (Mann–Whitney U test U=25.0, p=0.113). Larval abundance was not correlated with any other root mineral concentrations and larval masses showed no relationships with root mineral concentrations (**Table 5.1**). Root biometrics (root mass and maximum root length) were not significantly correlated with either the number of larvae recovered per plant or larval mass (results not shown).

(a)
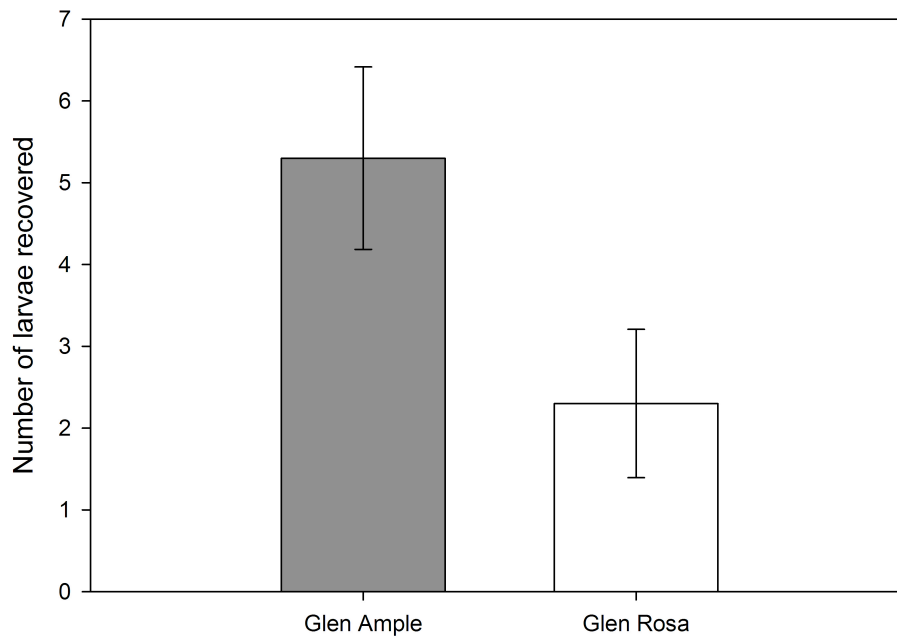
(b)
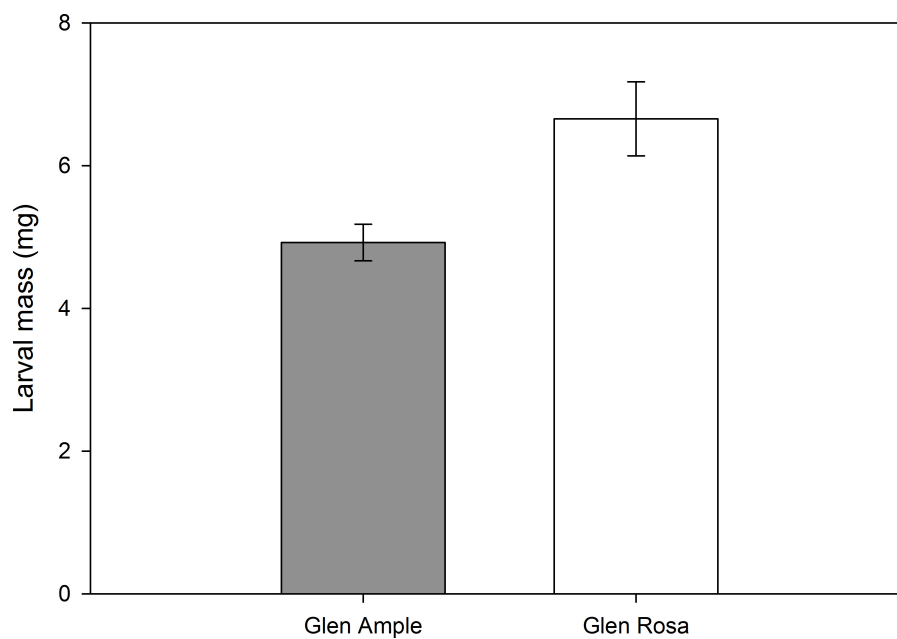
Figure 5.1. Differences in vine weevil larval performance on Glen Rosa and Glen Ample; (a) number of larvae recovered per plant and (b) average larval mass. Mean values ± SE shown, (a) Glen Ample n=53, Glen Rosa n=23, (b) n=10. Larval number data log+1 transformation prior to analysis, larval masses log transformation prior to analysis.
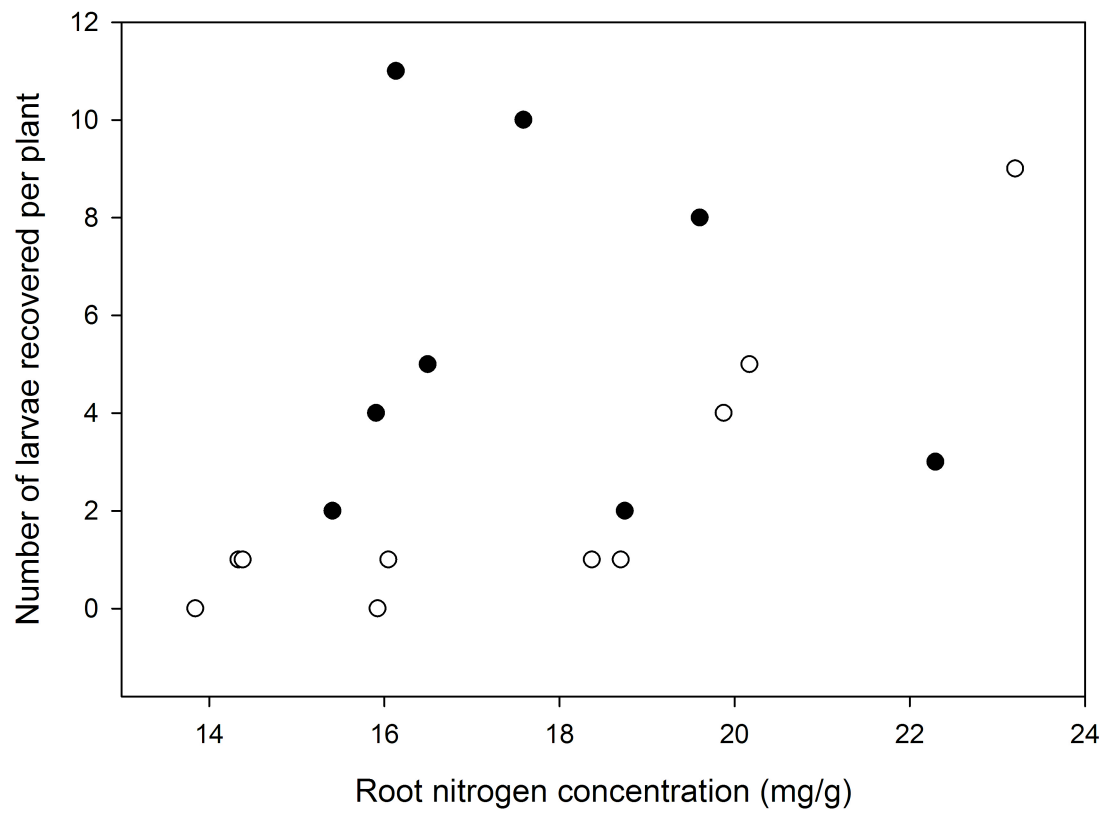
Figure 5.2. Relationship between root nitrogen concentrations and number of weevils per plant, Glen Rosa (○) and Glen Ample (●).

Table 5.1. Summary of correlations for larval mass and abundance in relation to root nutritional quality. Significant relationships highlighted in bold where $P<0.05$. Correlations were calculated using Pearson's product moment correlations or Spearman's rank correlations as appropriate. Larval mass, n= 17, larval abundance n=19.

Weevil Response	Correlations															
	N		C		Ca		P		Mg		Zn		Fe		K	
	r	P	r	p	r	p	R	P	r	P	R	p	r_s	p	r	p
Larval mass	-0.300	0.259	-0.204	0.449	-0.122	0.640	0.129	0.621	-0.037	0.888	-0.051	0.845	0.061	0.815	-0.386	0.126
Larval abundance	**0.501**	**0.034**	-0.079	0.755	0.367	0.126	0.265	0.273	**0.635**	**0.004**	-0.259	0.294	**-0.566**	**0.009**	0.162	0.506

5.4.2. Paired oviposition experiment on two raspberry cultivars

Adult weevils laid an equal number of eggs on Glen Ample and Glen Rosa when allowed to choose between the plants (t=0.92, df=19, p=0.369, n=20). Oviposition was not related with any of the plant characteristics quantified (data not shown) and nor was it related to adult feeding in terms of leaf area eaten (r_s=0.067, df=38, p=0.681) or proportion of plant eaten (r_s=0.012, df=38, p=0.943). Adult weevil feeding preferences between Glen Ample and Glen Rosa were not detected in terms of either the leaf area consumed (t_{19}=0.71, p=0.488) or proportion of plant eaten (t_{19}=0.58, p=0.566).

5.4.3. Field experiment

In 2008, 894 weevils were captured across 12 sampling dates, with 844 caught across the 7 sampling dates used in this analysis. In 2009 2,753 weevils were caught across 11 sampling dates, with 2,675 weevils caught across the 8 sampling dates used in this analysis. Overall in 2008, the number of weevils captured per plot on Glen Ample was not significantly different ($F_{1,4}$=0.21, p=0.669) than the number captured per plot on Glen Rosa. No significant differences between the mean number of weevils caught on Glen Ample and Glen Rosa were detected on specific sampling dates in 2008 (**Figure 5.3 a**). In 2009, the mean number of weevils caught per plot was not significantly different on Glen Ample than on Glen Rosa ($F_{1,4}$=1.25, p=0.327). No significant differences between the number of weevils caught on Glen Rosa and Glen Ample were detected at any of the sampling dates in 2009 (**Figure 5.3 b**).

(a)
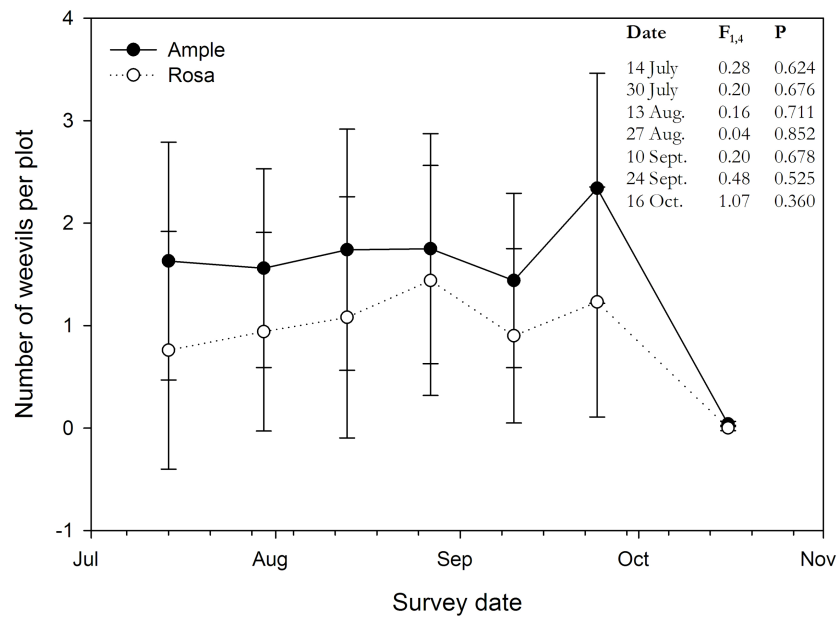
(b)
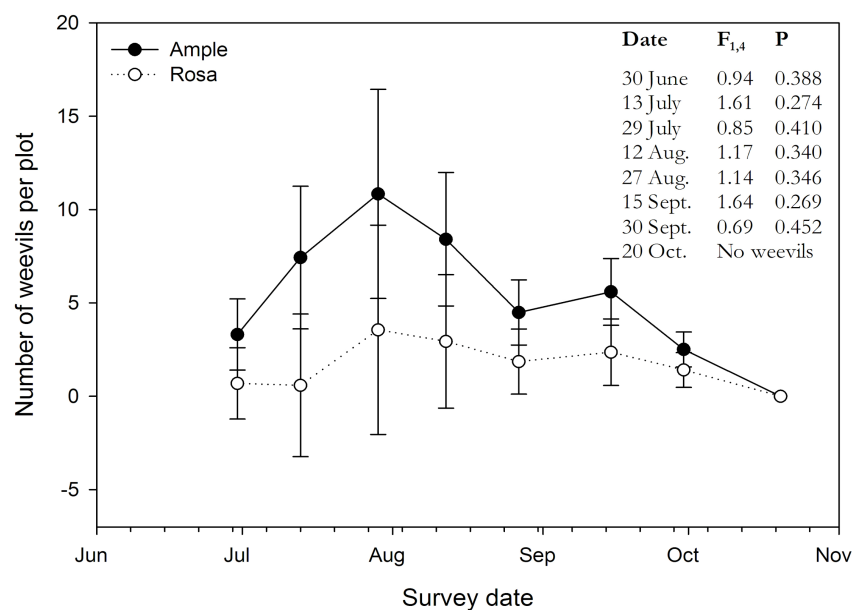
Figure 5.3. (a) Number of weevils captured per plot on Glen Ample and Glen Rosa in 2008, predicted mean values ± SE from data analysis (b) Number of weevils captured per plot on Glen Ample and Glen Rosa in 2009, predicted mean values ± SE from data analysis

5.5. Discussion

Vine weevil larval performance (abundance and masses) differed significantly between Glen Rosa and Glen Ample, with Glen Rosa having fewer, but heavier larvae than Glen Ample. Larger offspring are often deemed to show superior performance in comparison to smaller offspring (Stearns, 1992), which would suggest that larvae developing on Glen Rosa would be at an advantage. However, whilst the larvae on Glen Ample were smaller in terms of mass than those on Glen Rosa, they were more abundant, potentially indicating on this host plant that greater numbers are a better survival strategy than large individuals. The specific reasons for these differences between Glen Rosa and Glen Ample were not established in this study, but potentially indicate an intriguing life–history trade–off for root feeding larvae.

Overall, larval abundance was found to be positively correlated with root nitrogen concentrations, which is consistent with the reliance of young insect larvae on a nitrogen rich source (White, 1993). Potentially, root nitrogen concentration could play an important role in the performance of vine weevil larvae on host plants. Additionally, larval abundance was positively correlated with root magnesium concentrations, but negatively correlated with root iron concentrations. Foliar magnesium concentrations have previously been demonstrated to be positively correlated with adult vine weevil leaf consumption (D. Coyle, personal communication), although its influence in weevil nutrition is uncertain. Iron content in rice plants was found to detrimentally impact the growth and development of the white backed planthopper (*Sogatella furcifera*) (Hovarth), causing lower nymphal survival and prolonged nymphal development (Rath, 2004). Consequently, root mineral concentrations may be influential in determining the development of vine weevil larvae.

In our study, the presence of a trade off in larval performance parameters between the two raspberry cultivars may have complicated the decision of the adult weevil. The results showed no evidence of any link between adult weevil oviposition and the performance of vine weevil larvae belowground. The inability of maternal adult insects to select a host plant for oviposition, which maximises the survival and development of subsequent offspring, has been considered several times in relation to the preference–performance hypothesis (Jaenike, 1978, Denno *et al.*, 1990, Price, 1991, Scheirs & De Bruyn, 2002). A subtle decision between increased abundance or larval masses may simply prove too complex for the highly polyphagous vine weevil. The neural restraints hypothesis (Levins & MacArthur, 1969, Bernays, 2001) states that insects have limited capabilities to process information, consequently, generalist insects are believed to make poorer decisions regarding their choice of host plants in comparison to specialist insects, due to difficulties in assessing multiple host plant options. In addition, adult insects may exhibit a trade–off between the number of eggs and the size of eggs laid in response to environmental conditions, host plant quality or adult survival (Roff, 1992). For example, the butterfly *Bicyclus anynana* laid more, smaller eggs at lower temperatures, but fewer, bigger eggs at higher temperatures (Fischer *et al.*, 2003), with subsequent larvae developing from the larger eggs having a higher survival rate than larvae from smaller eggs.

Typically, the preference–performance hypothesis is studied in a controlled environment absent of factors that may influence the relationship between mother and offspring, for instance the presence of enemies (Thompson, 1988a, Thompson, 1988b). In this study, the preference–performance hypothesis was not investigated directly in the field, (due to difficulties in the collection of both eggs and larvae) however, the build up of a vine weevil population on the two cultivars was monitored for two consecutive years. Despite larval masses being significantly larger on Glen Rosa than Glen Ample in the laboratory, this did

not translate to any significant differences on population size between the two cultivars. Similarly, elevated larval abundances on Glen Ample were not represented in field population size. These results highlight that significant differences obtained in controlled performance experiments, do not necessarily extrapolate to field environments, which has also been noted in other study systems (e.g. Staley & Hartley, 2002).

Chapter Six – Does mother know best? The preference–performance hypothesis and parent–offspring conflict in aboveground–belowground herbivore lifecycles

6.1. Abstract

1. A substantial amount of research on host plant selection by insect herbivores is focussed around the preference–performance hypothesis (PPH). To date, the majority of studies have primarily considered insects with aboveground lifecycles, overlooking insect herbivores that have both aboveground and belowground life stages, for which the PPH could be equally applicable.

2. This study investigated the factors influencing the performance of the root–feeding vine weevil (*Otiorhynchus sulcatus*) larvae and whether this was linked to the oviposition behaviour of the maternal adult living aboveground.

3. Maternal insects feeding aboveground reduced root biomass by 34% and increased root carbon by 4%. Larvae feeding on plants subjected to aboveground herbivory had reduced mass. Irrespective of the presence of maternal herbivory, larval mass was positively correlated with root biomass.

4. Larval mass was also reduced by conspecific larvae, previously feeding on roots (19% reduction). However, the mechanism underpinning this effect remains unclear, as in contrast to maternal herbivory aboveground, prior larval feeding did not significantly affect root biomass or root carbon concentrations.

5. Maternal insects did not distinguish between plants infested with larvae and those that were free of larvae, in terms of their egg laying behaviour. Conversely, maternal insects tended to lay eggs on plants with smaller root systems, a behaviour which is likely to negatively affect offspring performance.

6. The PPH is not supported by our findings for vine weevil feeding on raspberry (*Rubus idaei*), and in fact our results suggest that there is the potential for strong parent–offspring conflict in this system.

6.2. Introduction

Parental care that maximises offspring survival and fitness is seen in numerous species, although the task of providing for offspring can prove detrimental to later adult survival and fecundity and hence a parent–offspring conflict may evolve (Trivers, 1974). For insect herbivores, host plant selection by parental insects can play an important role in the parent–offspring relationship. Host plant suitability can influence reproductive output in terms of both the quality and quantity of eggs, in addition to playing an important role in growth, development and potential fecundity of larval offspring (Awmack & Leather, 2002). In insect–plant interactions, the relationship between maternal choice of host plant for offspring and the resulting survival and development of the progeny has often been considered in the context of the preference–performance hypothesis (PPH). This hypothesis, also known as the optimal oviposition theory (Jaenike, 1978), the naïve adaptionist theory (Courtney & Kibota, 1990) or the 'mother knows best' principle (Valladares & Lawton, 1991) was first proposed by Jaenike (1978). The PPH states that female insects will preferentially oviposit on plants which maximise the survival and performance of their larvae. In particular, the hypothesis relates to insects whose larvae have little or no ability to relocate, and are thus reliant on the host plant choice of the mother. To maximise insect fitness, the hypothesis predicts a positive correlation between oviposition preference and offspring performance.

Several studies investigating oviposition preferences and larval performance have reported positive correlations consistent with the PPH (e.g. Craig *et al.*, 1989, Heisswolf *et al.*, 2005, Staley *et al.*, 2009). However, many insects have also been found to make poor oviposition decisions, resulting in a weak preference–performance linkage between adult and offspring (e.g. Rausher, 1979, Scheirs *et al.*, 2004, Digweed, 2006, Gripenberg *et al.*, 2007). Such 'bad motherhood' decisions have received considerable attention, with a range of explanations

proposed for why the adult insect does not choose the optimal host plant (discussed by Mayhew, 2001, Gripenberg *et al.*, 2010). Possible explanations include: optimal foraging, where maternal insects prefer host plants with better nutritional status to maximise their fitness, irrespective of later offspring development (Scheirs *et al.*, 2000); enemy free space, where studies are performed without the presence of predators, which may influence the mother–offspring relationship (Denno *et al.*, 1990) and insect neural capacity, where polyphagous insects which are presented with an array of plant choices make poorer decisions than more specialised herbivores due to an inability to process multiple options (reviewed by Bernays, 2001).

Currently, the majority of studies investigating the PPH have focussed on insects with lifecycles that take place aboveground, although the hypothesis could equally be applied to aboveground insects with soil–dwelling larvae that have limited mobility (Johnson *et al.*, 2006). Typically, early instar larvae feeding belowground are too small to move substantial distances to find food, consequently, the decision of the mother insect may be critical to larval survival and development. Mechanisms that underpin such interactions are largely unknown, but it has been hypothesised that roots are preconditioned for later generations of larvae by initial 'pioneer' larvae, so maternal insects are most likely to oviposit on such plants to maximise offspring fitness (Hausmann & Miller, 1989, Baur *et al.*, 1996, Degen *et al.*, 1999). For example, the onion maggot (*Delia antiqua*) preferentially oviposits on onion plants that have been exposed to moderate larval feeding, as the damaged onion bulbs are more susceptible to attack than healthy bulbs (Hausmann & Miller, 1989).

Here we consider the PPH in an aboveground–belowground context using the vine weevil (*Otiorhynchus sulcatus* F.) (Coleoptera: Curculionidae) as a model herbivore. Adult vine weevils live aboveground where they feed on leaves and lay eggs both on the plant foliage

and the soil surface. Eggs in the soil hatch and the resulting soil–dwelling offspring feed on plant roots. Vine weevils are parthenogenetic and unfertilised eggs develop into new female individuals without the need for males. Consequently, the vine weevil is an excellent study species for investigating potential parent–offspring relationships in an aboveground–belowground system, as offspring are essentially clones of the mother. The life–cycle has four distinct stages: eggs, larvae, pupae and adults, however, each stage may occur concurrently (Shread, 1972), thus adults can be feeding on plants aboveground, which are already exposed to root feeding larvae belowground.

The objective of this study was to determine how the performance of a belowground insect herbivore was affected by either aboveground maternal herbivory or prior belowground herbivory by conspecific larvae, characterise the factors underlying these interactions and ascertain whether the maternal oviposition choices optimised performance of belowground offspring. The study specifically aimed to:

1. determine whether maternal insects feeding aboveground affected offspring performance belowground,

2. assess how maternal feeding affected root traits (e.g. root biomass), root nutritional quality (carbon and nitrogen concentrations) and secondary metabolites (phenolics), and assess whether these changes were related to larval performance. Phenolics, which are present in nearly all terrestrial plants so are encountered by most insect herbivores, have been shown to deter insect herbivory (Zucker, 1982, Ikonen *et al.*, 2002) and reduce insect performance (McKinnon *et al.*, 1999, Fisher *et al.*, 2000, Eleftherianos *et al.*, 2006),

3. establish whether prior feeding by conspecific larvae improved root suitability and performance of larvae that subsequently fed on the roots,

4. test whether maternal insects preferentially laid eggs on plants that are likely to be beneficial for their offspring (e.g. by having larger root biomass), and determine whether egg laying behaviour was linked to maternal feeding behaviour.

6.3. Materials and methods

6.3.1. Plants and insects

Raspberry plants (*cv.* Glen Ample) were grown in plastic pots (BEF Growers Number 5) containing a 2:1 mixture of insecticide free compost (peat–sand–perlite mix containing 17N:10P:15K; William Sinclair Horticulture Ltd, Lincoln, UK) and sand (Silver sand, J. Arthur Bowers, Lincoln, UK). Plants were grown in a greenhouse at optimum conditions (15 – 20 °C, 16:8 L: D photoperiod). All experiments were conducted in controlled temperature environments at 21 °C ± 2 °C and 16:8 L: D photoperiod.

Ovipositing adult weevils were used from cultures maintained at 17 °C ± 2 °C and 16:8 L: D photoperiod fed on a mixture of strawberry cultivars. Vine weevil eggs used in experiments were collected from the cultures once the egg had melanised ensuring egg viability (Smith, 1932).

6.3.2. Impact of aboveground maternal herbivory on belowground larval offspring performance

Seventy raspberry plants were treated with 40 vine weevil eggs (inserted into a small hole in the compost close to the plant stem) and placed into individual mesh cages (52 cm high, 12.5 cm diameter). Larvae take approximately 21 days to emerge from eggs, so after this time 35 of the cages were selected at random, into which six vine weevil adults were

introduced (Aboveground herbivory treatment). The remaining 35 cages received no adult weevils (No aboveground herbivory treatment). A fine mesh circular collar was placed around the stem of all plants and then covered with washed gravel (Coarse grit, J. Arthur Bowers, Lincoln, UK) (~2-6 mm) (Johnson *et al.*, 2010b). This prevented eggs from entering the soil in those cages containing adult weevils.

After three weeks, adult weevils were removed from the cages and plant biometrics (plant height, number of leaves and plant mass) were measured. Leaf samples were snap frozen in liquid nitrogen and stored at -18 ºC for subsequent chemical analyses. Roots were teased apart carefully to recover larvae. Individual larvae were weighed on a microbalance (accuracy ± 0.01 mg). Roots were weighed after washing and then snap frozen.

6.3.3. *Impact of previous belowground herbivory on larval performance*

Fifty raspberry plants were grown in rhizosphere tubes (90 cm (h), 5 cm (w), 2.5 cm (d)), similar to those described in Johnson and McNicol (2010). Twenty five of the plants were inoculated with 40 vine weevil eggs (Pioneer larvae) and 25 received none (No pioneer larvae). Plants were placed at random in the growth room, where a special frame had been constructed to hold them upright.

After four weeks, all plants were harvested. Plants with pioneer larvae had their root systems examined for larvae to ensure that larvae had developed. Subsequently, the plants had their roots washed carefully to ensure all larvae were removed. Roots without pioneer larvae were also washed to ensure comparability. Plants were immediately returned to the rhizotubes after washing, where they were inoculated with 40 vine weevil eggs. After six

weeks the plants were harvested as in experiment one, with individual larval mass and number of larvae recorded for each rhizotube.

6.3.4. Maternal oviposition choices in relation to belowground feeding

Seventy raspberry plants were paired according to size. Each pair of plants was placed in mesh cages (45 cm (h), 45 cm (w) 28.3 cm (d)). One plant in each pair was inoculated with 40 vine weevil eggs (Belowground herbivory) and the other plant received no eggs (No belowground herbivory).

After four weeks, a fine mesh circular collar with washed gravel (as in experiment one) was applied to enable the recovery of weevil eggs at the end of the experiment. One ovipositing adult weevil was introduced into each cage. Plants were harvested two weeks after the addition of adult weevils. Weevils were removed from cages and plant biometrics were recorded (plant height, mass, number of leaves, root mass, maximum root length and leaf area). Eggs were recovered from the gravel by submerging it in a saturated KCl solution and gently agitating so that eggs floated to the surface (see Johnson *et al.*, 2010b), additionally leaves and stems were checked for any eggs oviposited whilst the insect was in the plant canopy.

Leaf consumption was calculated using a LI-3100C area meter (LI-COR Inc., Lincoln, Nebraska, USA) and digitally scanned leaf areas. Images were analysed to determine how much foliage had been consumed by weevils (Johnson *et al.*, 2010b). After scanning, leaves were snap frozen in liquid nitrogen for subsequent chemical analysis.

6.3.5. Carbon and nitrogen analysis

Plant material (leaves and roots) from the experiments were freeze dried and then milled to a fine powder for all further chemical analyses. Nitrogen and carbon concentrations were analysed using flash combustion and chromatographic separation of approximately 1.500 mg of ground and homogenised plant material, and were calibrated against a standard compound ($C_{26}H_{26}N_2O_2S$) using an elemental combustion system (Costech Instruments, Milan, Italy).

6.3.6. Phenolic analysis

Phenolic analysis was conducted using the enzymatic method described by Stevanto *et al.* (2004), which has increased precision over the more commonly used Folin–Ciocalteu method (Waterman & Mole, 1994) and in addition is not influenced by substances such as ascorbate, citrate and sulphite (Stevanato *et al.*, 2004). Phenolics were extracted in a 10:1 ratio from 50 mg of freeze dried plant material by incubating in 0.5 ml 50% methanol at 80 °C for 2.5 hours. The aqueous phase was removed and cleared by centrifugation. A 1 ml enzymatic reaction was conducted using 50 µl of the supernatant mixed with 740 µl 100 mM potassium phosphate buffer (pH 8.0), 100 µl 30 mM 4-aminophenazone, 100 µl 20 mM hydrogen peroxide and 1 U horseradish peroxidase dissolved in 10 µl potassium phosphate buffer. The reaction was incubated at room temperature for 15 min and absorbance read at a wavelength of 500 nm. Absorbance readings were converted to catechin equivalents using a standard curve produced by serial dilution (0 – 10 mg ml^{-1} catechin). All chemicals were obtained from Sigma-Aldrich (Dorset, UK).

6.3.7. Statistical analysis

Larval mass was analysed by analysis of variance (ANOVA), with a hierarchical structure for cage and plant. Larval survival was analysed with a generalised linear model with a Binomial error structure and logit link function. Oviposition and leaf consumption was analysed using a paired t–test. Differences in plant root mass and chemical composition were analysed using t–tests or, where data transformation was inappropriate, Mann Whitney U tests. Root mass and root chemical compositions were included as covariates in determining larval masses using analysis of co–variance (ANCOVA's). Relationships between variables were investigated using Spearman's rank correlations and Pearson's product moment correlations as appropriate. Where necessary, data were transformed prior to analysis as indicated in figure and table legends. Transformations were chosen to give residual diagnostic plots which best fitted a normal distribution and showed least heteroscedasticity (Sokal & Rohlf, 1995). All analysis was carried out in GenStat (version 12, VSN International, UK).

6.4. Results

6.4.1. Impact of aboveground maternal herbivory on belowground larval offspring performance

Individual larval mass was significantly reduced when the larvae fed on plants exposed to aboveground maternal feeding (**Figure 6.1 a**). Larval survival was not significantly affected between treatments ($F_{1,60}$=14.29, p=0.156), although was generally lower when adult weevils were present. Aboveground herbivory caused a significant reduction in root mass and significantly elevated root carbon concentrations (**Figure 6.1 b**). Root nitrogen and root phenolic concentrations were not significantly influenced by the presence of aboveground herbivory (nitrogen: Mann Whitney U, p=0.095; phenolics: t–test, t_{61}= -0.91, p=0.367). When included as a covariate, root phenolic concentrations were significant (ANCOVA, $F_{1,57}$ =5.64, p=0.021), but nitrogen concentrations were not. Root mass and root carbon concentrations were not incorporated as covariates, as they already differed significantly between treatments (see **Figure 6.1 b**).

Larval mass was significantly and positively correlated with root mass, both in plants with and without aboveground herbivory (**Figure 6.2**). Larval mass was not significantly correlated with root carbon concentration in plants with or without aboveground herbivory (aboveground herbivory: r_s=-0.274, df=28, p=0.143, no aboveground herbivory: r_s=-0.325, df=28, p=0.074). Interestingly, larval mass was positively correlated with root phenolic concentrations (r=0.3167, p=0.0137). Larval survival had a close to significant positive correlation with root mass (r_s=-0.245, df=60, p=0.056), but was not significantly correlated with any of the root chemistry measurements that were quantified.
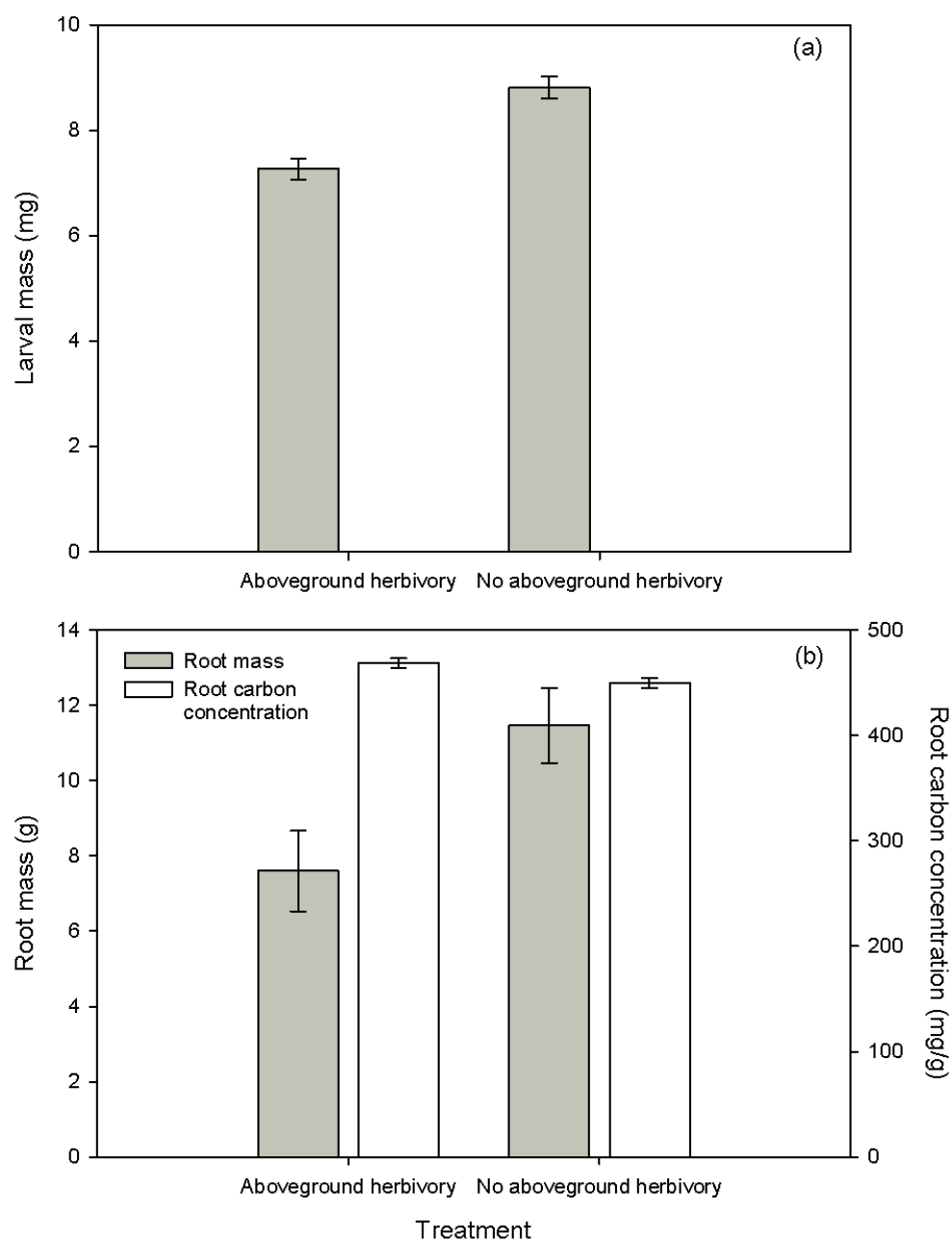
Figure 6.1. (a) Individual larval mass in relation to aboveground maternal herbivory, mean (± SE). Data transformed using square root, prior to analysis. $F_{1,657}$=5.75, p=0.015. aboveground herbivory: n=289, no aboveground herbivory: n=370. (b) Mean root mass and mean root carbon concentration in relation to aboveground herbivory, (± SE). Mean root mass analysed using Mann Whitney U, p=0.008, n=31). Root carbon concentration data transformed with arcsine transformation and analysed using Mann Whitney U, p=0.018, n=32.
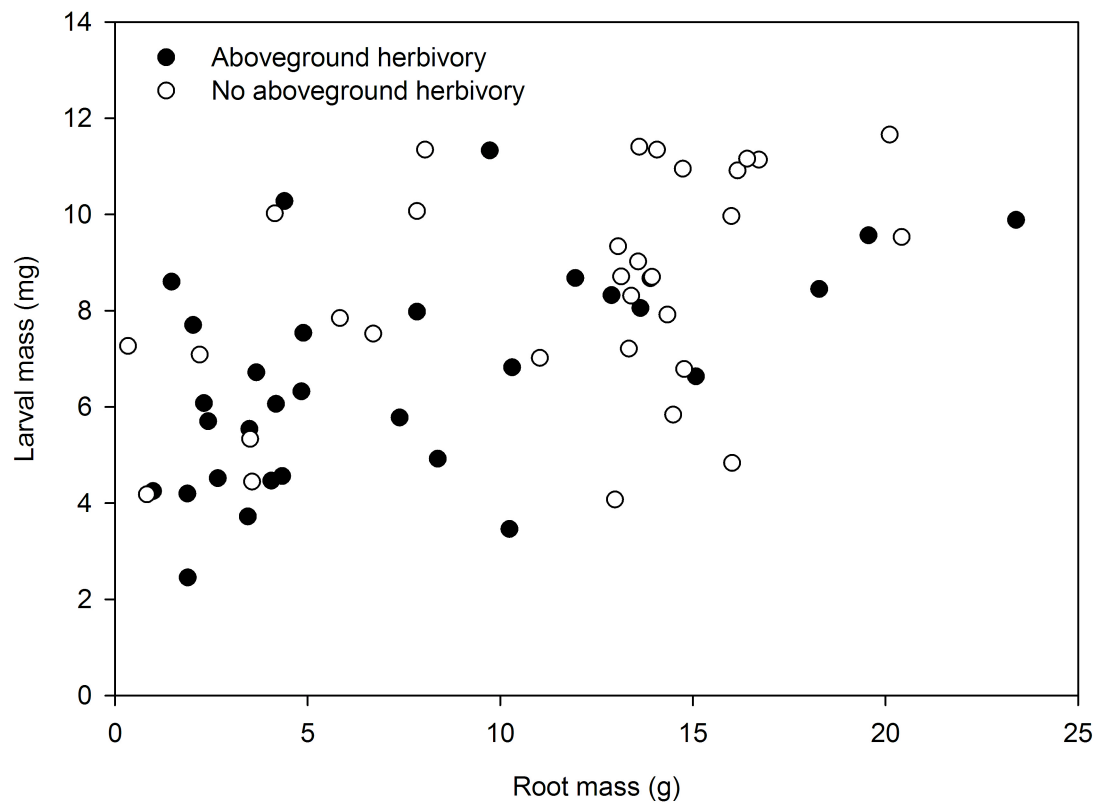
Figure 6.2. Mean individual larval mass in relation to root mass. No aboveground herbivory (○) and Aboveground herbivory (●). No aboveground herbivory (r_s=0.463, df=29, p=0.009), aboveground herbivory (r_s=0.571, df=29, p<0.001).

6.4.2. Impact of previous belowground herbivory on larval performance

Individual larval mass was significantly reduced when larvae fed on roots previously exposed to conspecific feeding (**Figure 6.3**). There was no significant difference in larval survival between the two treatments ($F_{1,40}$=0.53, p=0.471, n=21). Belowground herbivory by pioneering larvae did not significantly reduce root mass (t_{40}=1.34, p=0.188), carbon (t_{38}=-0.44, p=0.666) or nitrogen concentrations (t_{38}=-0.89, p=0.381), but phenolic concentrations tended to be lower in the roots of plants with prior belowground herbivory, although not sufficiently to meet the 95% confidence level (t_{38}=1.77, p=0.084). Root carbon, nitrogen and phenolic concentrations and root mass were not significant covariates in determining larval masses (results not shown). Larval mass was not significantly correlated with root carbon (r=-0.091, p=0.581) or nitrogen (r= -0.031, p=0.853) concentrations, but there was a positive relationship between larval mass and phenolic concentration in the roots, which came close to statistical significance (r=0.2970, p=0.066).

6.4.3. Maternal oviposition choices in relation to belowground feeding

The presence of belowground herbivory did not affect aboveground oviposition, with adults laying equally on plants with and without belowground herbivory (**Figure 6.4 a**). Only root mass was related to egg laying behaviour, with a significant negative correlation between the root masses of plants exposed to belowground herbivory and the number of eggs laid (**Figure 6.4 b**), although root mass was not significantly affected by belowground herbivory (t_{15}=0.48, p=0.637). Oviposition was not related to any other plant characteristic or aspect of plant chemical composition that was quantified (data not shown).

Oviposition was not affected by feeding preferences, with no relationship between eggs laid and leaf area eaten (r_s=0.186, p=0.147) or proportion of the plant eaten (r_s=0.169,

p=0.189). Belowground herbivory did not affect feeding preferences in terms of leaf material consumed (t_{30}=-0.11, p=0.912) or the proportion of the plant eaten (t_{30}=0.40, p=0.692).
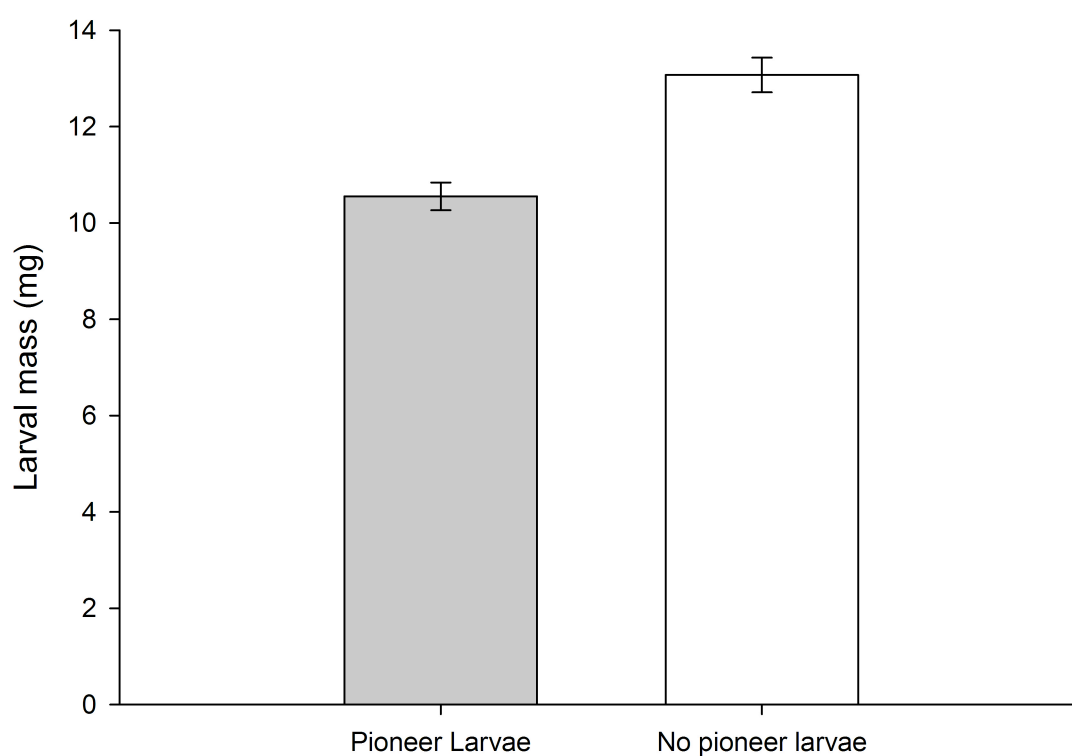
Figure 6.3. Individual larval mass in relation to prior belowground conspecific herbivory, mean ($\pm$ SE). Data transformed using square root, and analysed using ANOVA. $F_{1,417}$=5.40, p=0.026. Pioneer larvae n=215, No pioneer larvae n=242.
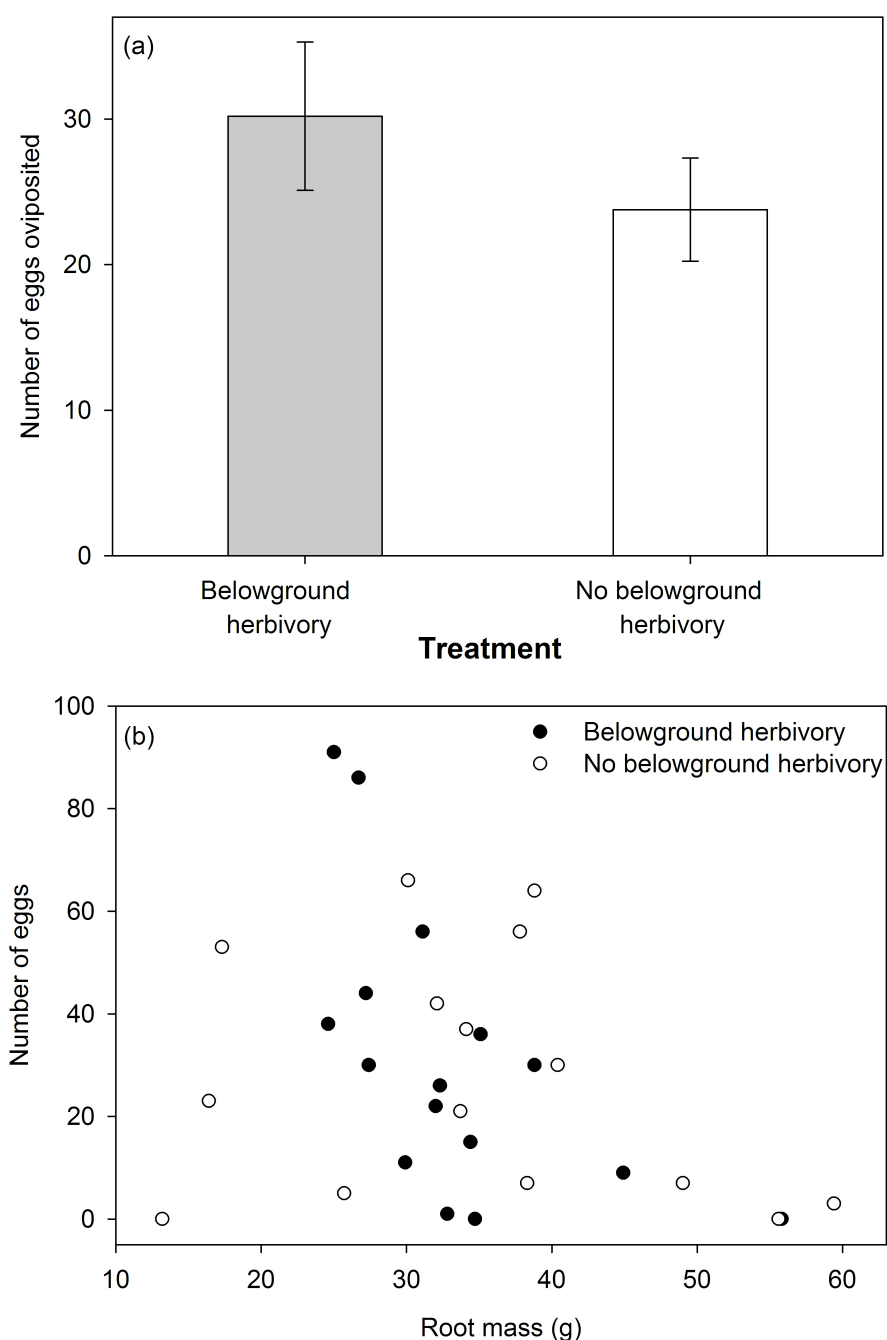
Figure 6.4. (a) Number of eggs oviposited in relation to belowground herbivory in choice experiment, mean ($\pm$ SE). Data transformed using square root, and analysed using two sample paired t–test (t_{28}=-1.31, p=0.200). (b) Eggs laid in relation to root mass. Belowground herbivory (●) and no belowground herbivory (○). Belowground herbivory (r_s = -0.705, df=13, p=0.003), no belowground herbivory (r_s = -0.175, df=13, p=0.532).

6.5. Discussion

A strong link between oviposition choice and offspring performance is assumed by the preference–performance hypothesis (PPH) literature (Jaenike, 1978, Mayhew, 1997), although, several studies have demonstrated weak relationships (Courtney, 1981, Berdegue *et al.*, 1998) and alternative hypotheses have been proposed to explain insect oviposition choices. The findings of this study suggest that in contrast to the predictions of the PPH, there was no discernible link between preference and performance in the case of adult vine weevils and their larvae. Larval mass was shown to be significantly reduced in response to both feeding by the mother and conspecific larvae. Furthermore, adult oviposition aboveground tended to be on plants with smaller root systems, which were detrimental to larval performance, highlighting a further conflict between larval development and adult oviposition.

6.5.1. Impact of aboveground maternal herbivory on belowground larval offspring performance

Previous studies investigating the interactions between aboveground and belowground insect herbivores via a shared host plant have reported both positive and negative outcomes for both of the insects (e.g. Simelane, 2006, Erb *et al.*, 2009). Often the result can be very context dependent, with host plant, insect feeding guild or performance parameter measured influencing the direction of the interaction (e.g. Hunt-Joshi & Blossey, 2005, Staley *et al.*, 2007). As such, making hypotheses regarding the overall direction of aboveground and belowground insect interactions is very difficult. In this study, feeding by adult weevils aboveground was found to be detrimental to larval development belowground, highlighting that negative interactions between aboveground and belowground insect herbivores of the same species can potentially lead to a parent–offspring conflict.

Larval mass and larval survival were found to be positively correlated with root biomass (albeit more weakly for survival), which was detrimentally affected by aboveground maternal herbivory. A reduction in root biomass is suggested as one of the principal mechanisms resulting in impaired performance of belowground insect herbivores when exposed to aboveground insect herbivory (Masters *et al.*, 1993), which is consistent with our findings. Additionally, increased root carbon as a result of aboveground maternal feeding could indicate the presence of higher levels of structural carbohydrates in the roots, making them more difficult and energetically costly to consume (see Hochuli, 1996). The reallocation of carbon to roots may be a response of the plant to aboveground herbivores, thus protecting their resources, but subsequently influencing belowground herbivores (Henkes *et al.*, 2008). Root phenolic concentrations were not affected by aboveground herbivory, suggesting that the phenolics detected were primarily induced by larval feeding. Larval mass was positively correlated with root phenolic concentrations indicating that vine weevil larvae were not negatively influenced by root phenolic content. The positive relationship between phenolic concentrations and larval mass may be incidentally correlated with another, but unmeasured beneficial root property. However, phenolics have also been shown to be beneficial for some insects (Bernays & Woodhead, 1982, Bernays *et al.*, 1991) and act as phagostimulants (Simmonds, 2001). In some instances, insects have been shown to use phenolics for cuticle sclerotization, enabling them to conserve amino nitrogen when feeding on woody plants (such as raspberry) (Bernays & Woodhead, 1982). The positive relationship between weevil performance and root phenolic content has also been reported in other plant taxa (*Ribes nigrum*) (Johnson *et al.*, 2010a).

6.5.2. Impact of previous belowground herbivory on larval performance

Insects that have been shown to preferentially oviposit on plants exposed to belowground conspecific herbivory are believed to be able to detect optimal rhizosphere conditions for their subsequent offspring (Baur *et al.*, 1996, Degen *et al.*, 1999). Prior feeding of offspring on the root system is thought to facilitate feeding for subsequent generations (Hausmann & Miller, 1989). However, the mechanisms underpinning the selection of plants with superior root characteristics are currently unknown. This study suggested that previous feeding on roots by vine weevil larvae negatively impacted the larval mass in the next generation, thus rejecting, in this species at least, any beneficial effects of 'pioneer larvae' reported elsewhere (Johnson *et al.*, 2006).

Root mass was not reduced by prior larval feeding, possibly because losses in root mass were relatively low or because the plant was able to compensate for such losses. Additionally, root carbon was not significantly affected by prior root herbivory, in contrast to the difference seen in root carbon concentrations when the plants were exposed to aboveground herbivory. This result may indicate that aboveground herbivory is influencing the raspberry plant to a greater degree than belowground herbivory, or that the plant is better able to respond to aboveground herbivores than root feeders, and alters its resource allocations accordingly (Henkes *et al.*, 2008).

6.5.3. Maternal oviposition choices in relation to belowground feeding

In this study, pioneer larvae negatively impacted later generations, hence, under the PPH adult vine weevils would be expected to avoid ovipositing on plants already exposed to weevil larvae. However, no difference in egg laying was detected when adult weevils were allowed to choose between plants exposed to and not exposed to larvae. In addition, adult

weevils were not deterred from feeding on plants with belowground conspecific progeny even though their presence aboveground caused reduced larval development. The conflict between adult oviposition behaviour and offspring survival was further demonstrated by the adult insects laying higher numbers of eggs on plants exposed to belowground herbivory with smaller root mass, despite larval mass being positively correlated with root mass.

Previous studies have found correlations between egg laying and feeding behaviour (Scheirs *et al.*, 2000, Janz *et al.*, 2005), indicating that insects oviposited where they fed. These correlations were not considered to be a consequence of limited insect mobility, but a response to adult insects maximising their feeding on nutritionally superior host plants even if the plant was not advantageous to offspring development. No correlation between leaf area consumed and eggs oviposited was found in this study, suggesting that weevils were not ovipositing directly where they fed and that foliar nutrition was unlikely to be influencing their egg laying behaviour.

Insects with a wide host range such as vine weevils may be incapable of making subtle decisions in terms of host plant suitability. Studies investigating the neural response hypothesis have shown polyphagous insects suffer in their decision making ability in comparison to monophagous insects due to the diversity of choices they encounter (Bernays, 1998, Bernays & Funk, 1999, Janz, 2003). Thus, polyphagous insects may make poorer choices when choosing host plants due to their inability to distinguish between them.

6.6. Conclusions

Despite the limited mobility of the soil–dwelling larvae of vine weevils, and hence their reliance on adult host choices, this study did not detect any preference–performance linkage in this aboveground–belowground system. Negative impacts of aboveground insects on belowground insect herbivores have previously been highlighted between different insect species (e.g. Bezemer *et al.*, 2003, Hunt-Joshi & Blossey, 2005), but conflict between maternal feeding aboveground and offspring performance belowground highlights a neglected, but potentially very significant, aspect of plant–insect interactions.

Chapter Seven – Discussion

7.1. Introduction

This thesis aimed to investigate the preference–performance hypothesis in an aboveground–belowground context. Previously, the majority of studies considering the preference–performance hypothesis have focussed on insect herbivores whose life–cycle is completed aboveground, but could equally be applicable to insect herbivores with both above and belowground life stages. The parthenogenetic vine weevil (*Otiorhynchus sulcatus*) was chosen as the study species, as offspring are clones, sharing a common genotype with the mother, thus ensuring a known relatedness in a parent–offspring context. In this chapter the findings of the research are discussed in a wider context.

7.2. Behavioural manipulation to augment insect pest control

In addition to being a highly suitable study species for examining fundamental interactions between related aboveground and belowground insect herbivores, the vine weevil is an economic pest with a declining number of options for control. Understanding the behaviour of this important pest species could potentially assist in the development of alternative control methods. Recently, the soft fruit industry has faced a considerable challenge in continuing to produce high quality fruit whilst dealing with a reduction in the level of chemical control available for managing insect pests and diseases (91/414 EEC). With the introduction of further legislation in 2011, where it is expected further chemical control will be withdrawn from use, the development of new pesticides for soft fruit is very unlikely (Gordon *et al.*, 2006, Gordon, 2008). In addition, demand for residue free fruit by consumers is driving insect pest management strategies towards biocontrol methods (Copping, 2008). The sole use of biocontrol methods has proven expensive for growers, as frequently several methods are required to control a range of insect pests, whereas previously one pesticide might have targeted them all. In addition bicontrol methods are

frequently not as effective in controlling the pests as the previous chemical control. Consequently, integrated pest management strategies are now being sought to combine the use of the few insecticides left for use with developing biocontrol methods. By furthering our understanding of insect behaviour, in particular considering the driving mechanisms behind oviposition decisions, a new area of biocontrol may be possible, by deterring adults from feeding and ovipositing on crop plants. Insect pests may be attracted to either less valuable bait plants, so called trap–cropping (reviewed by Hokkanen, 1991) or lured to pheromone baited traps (e.g. Mitchell *et al.*, 2004, Knight & Light, 2005).

In the case of the vine weevil, insecticidal control was the principal method for controlling vine weevil populations for a considerable number of years. Prior to its ban in 1989, the organochlorine insecticide Aldrin was commonly incorporated into growing media, providing a simple and effective (over 100% in polyanthus (Blackshaw, 1983)), control method. The removal of several insecticides over the last couple of decades and the lack of replacements (either chemical or biological) that provide the same level of control has undoubtedly led to an increase in vine weevil numbers, although this has not been empirically proven. Amongst the biocontrol alternatives, entomopathogenic nematodes (*Steinernema* spp. and *Heterorhabditis* spp.) (Bedding & Miller, 1981, Evenhuis, 1982, Willmott *et al.*, 2002, Lola-Luz *et al.*, 2005) and entomogenous fungi (e.g. *Metarhizium anisopliae*) (Moorhouse *et al.*, 1993a, Bruck & Donahue, 2007) have previously been demonstrated as effective biocontrol methods against vine weevil larvae, potentially even working together synergistically (Ansari *et al.*, 2008, Ansari *et al.*, 2010), although supplementation with (albeit more modest) insecticidal treatments may also be required. However, most of these methods have not reported the level of control required by fruit growers. It is therefore essential to also consider alternative approaches such as manipulating vine weevil oviposition and feeding behaviour. This can only be achieved by greater understanding of

vine weevil oviposition and feeding behaviour in the context of its aboveground–belowground environment, which is the underlying rationale of this thesis. In terms of vine weevil manipulation the results presented in Chapter Four highlight that weevil adults show no preferences for ovipositing on nine raspberry cultivars; however the adults did show a preference for feeding on Glen Rosa, Glen Moy and a wild accession. Such information could be potentially important for growers selecting cultivars, helping them minimise the risk to their crops by vine weevils.

7.3. Reductionist versus realism approaches in aboveground–belowground research

In the last couple of decades there has been growing recognition that many aboveground and belowground insect herbivores interact with one another indirectly through plant mediated mechanisms (van der Putten *et al.*, 2001, Wardle *et al.*, 2004, De Deyn & van der Putten, 2005, van der Putten *et al.*, 2009). However, despite the increasing number of studies in this area, it is still frequently acknowledged that the effect of an aboveground insect herbivore on a belowground insect herbivore via a shared host plant and *vice–versa* can be highly dependent on both the insect species and host plant under consideration, as well as the performance parameter being measured (e.g. Staley *et al.*, 2008). Consequently, predicting the relationship between aboveground and belowground insect herbivores is particularly difficult.

In Chapter Two, the first quantitative review of literature in this area of research is presented. In performing the meta–analysis it was highlighted that the majority of studies considering interactions between aboveground and belowground insect herbivores use insects from the orders Coleoptera and Homoptera, thus very much limiting our

understanding of interactions between other insect orders. It is easy to understand why insects from these two orders are frequently chosen, as they make particularly good study species, which are easy to culture and manage in experimental situations. However, in order to fully extend our knowledge of how aboveground insect herbivores influence belowground insect herbivores and *vice–versa*, many more studies are required that utilise a greater range of insect species representing several feeding guilds.

It could be argued that it may be more important to grasp an understanding of fundamental interactions, before expanding aboveground–belowground research into incorporating higher trophic levels (Soler *et al.*, 2007) or considering interactions at a community level (De Deyn & van der Putten, 2005). However, it can equally be argued that aboveground–belowground interactions should not be examined in such a narrow context, as in reality it is never just a simple case of pairwise interactions and trophic complexity should be included. Both approaches can be seen to have advantages and disadvantages (**Table 7.1**).

Simple pairwise interactions are invaluable for examining insect herbivore interactions aboveground and belowground as they enable direct comparisons between study systems. This is especially useful when considered the mechanisms underpinning the interactions in terms of plant nutrition and secondary metabolites. The inclusion of several trophic levels at the preliminary stages of investigating an interaction may make it impossible to untangle the main factors in the study system. Additional levels of complexity should ideally be added after the basic interactions are fully understood, as it is then possible to consider the influence of these other factors and how they have affected the existing interaction and mechanisms. Blossey and Hunt Joshi (2003) highlighted that simple pairwise experiments, investigating the influence of belowground root feeders on aboveground insect herbivores,

often used early successional plants in short term experiments and could thus not be extrapolated to real life situations where later successional plants have had time to establish and be exposed to a community of insect root herbivores. Recent reviews have also called for research between aboveground and belowground organisms to be considered in relation to spatial and temporal scales (Bardgett *et al.*, 2005) and that the tendency for studies to be limited to the short term and individual species, rather than multiple species, limits our understanding of how such interactions may influence community structure and ecosystem functioning. Van der Putten *et al.* (2009) highlight one of the challenges facing research in this area is the limited empirical capacity available to fully explore all the permutations in aboveground–belowground interactions, especially when more than one trophic level is considered, and the need to incorporate mathematical modelling into the area to assist in the unravelling of patterns above and below the soil. In practice, a combination between the two approaches would be ideal, starting with the simple pairwise experiments to identify mechanisms and basic interactions and then extrapolating these to incorporate higher trophic levels which can ultimately reflect linkages seen at a community level.

Table 7.1. – Advantages and disadvantages of realistic versus reductionist experimental approaches in aboveground–belowground insect herbivore interactions.

	Pairwise interactions – Reductionist	Multitrophic interactions - Realistic
Advantages	Simple experimental setup	More realistic to 'real world'
	Allows for easier comparisons in how plant nutrition and/or plant defences and/or plant volatiles are influenced by each study species	Easier to implement in the field as there is little or no requirement to exclude other species
	Enables easier comparisons between individual insect species to be made. This can provide useful information regarding how insects from certain feeding guilds or orders aboveground may differently affect insects from certain feeding guilds and orders belowground and *vice–versa*	
Disadvantages	Not realistic – does not account for other species that may also influence the interaction	May require pairwise interactions to establish the driving mechanisms and influences of study species on one another
	More complicated to implement in field experiments, as would require the exclusion of other species	More complicated experimental setup
	Often short term experiments on early successional plants	

7.4. How do you measure choice in insect research?

A considerable proportion of the studies addressing insect oviposition and feeding behaviour have been conducted using choice and no–choice bioassays, to ascertain how adult vine weevils react to particular host plants (e.g. Cram, 1980, Hanula, 1988, van Tol *et al.*, 2004a). Choice experiments are essential in insect–plant research to unravel insect behaviour on certain plant species. Bioassays may take the form of whole plants, plant parts or excised leaves offered to the insect(s) in either choice or no–choice situations. No–choice situations are thought to be more representative of agricultural systems, as different plant species are rarely available to an insect in such close proximity as is offered in a choice experiment (Schoonhoven *et al.*, 2005). However, this often depends on the crop system, for instance, soft fruit growers often grow a mixture of species and varieties in relatively close proximity. Choice experiments are more commonly employed when insect behaviour is being considered in relation to plant volatiles or semiochemicals (Schoonhoven *et al.*, 2005).

Both choice and no choice experiments have previously been employed when considering vine weevil feeding and oviposition behaviour, although more frequently no–choice studies have been used to assess the impact of host plant on: weevil fecundity, the length of the pre–oviposition period, survival and feeding preferences (e.g. Cram & Daubeny, 1982). When considering insect host plant choice, paired choice studies have been considered preferable to studies with multiple options, as results may be unclear and difficult to analyse (Raffa *et al.*, 2002). However there are many examples where multiple choice experiments have proved useful (e.g. Murray & Clements, 1993, Murray & Clements, 1994, Parker & Howard, 2000, Johnson *et al.*, 2008a). This technique can be especially valuable for screening a wider variety of plants and determining whether any elicit any particularly

strong repellent or attractant properties towards insect pests. Thus, it can be particularly useful as a starting examination in insect–plant interactions, to broadly determine whether a certain host plant is considerably more or less suitable than others. As part of the early research in this thesis, nine raspberry cultivars were examined for their suitability as host plants for vine weevils (**Chapter Four**) in both choice and no–choice experiments.

The analysis of multiple-choice oviposition and feeding experiments can be problematic, as the presentation of simultaneous multiple host plant options to one insect can be viewed as being non-independent and thus it is important that appropriate statistical analyses are conducted (Horton, 1995). For the analysis of feeding preference experiments several methods have been proposed for the analysis of data (Roa, 1992, Manly, 1993, Prince *et al.*, 2004), although each have their own subtleties and opinion on the correct procedure to follow. The incorporation of control plants into longer running feeding experiments, where plant biometrics (height, leaf area etc…) may change over the duration of the experiment has been highlighted (Roa, 1992), suggesting that conventional methods of determining the amount of plant material consumed at the end of an experiment, may not account for different plant development. Roa (1992) suggested the use of multivariate analysis when analysing data where multiple food options had been presented to the sea urchin (*Tetapygus niger*), although the requirement for the method to have equal numbers of replicates in both the control and treatment groups may limit the use of such a method, as replicates may have to be removed from an experiment for some reason, leaving an unbalanced experimental design. In analysing the data presented in Chapter Four, generalised linear mixed models and generalised linear models provided a suitable approach to the data analysis, by allowing unequal sample sizes to be considered and allowing the error structure to be non-normal where appropriate.

Horton (1995) highlighted the importance of sample size when using paired t-tests to analyse paired-choice assays, showing that the significance of results was being influenced by low numbers of replicates. It is therefore intuitive to consider the number of replicates required to provide good statistical power prior to the start of an experiment, however equally practical constraints (e.g. space, resources and time) may limit the ideal number of replicates required, as was the situation in conducting experiments for this thesis. Whilst alternative methods have previously been proposed for the analysis of multiple choice feeding and oviposition experiments, there is no strong consensus on the best way to analyse such data and indeed varying approaches to the data analyses are often taken (e.g. van Tol *et al.*, 2004a, Soler *et al.*, 2005)

Previous studies documenting vine weevil behaviour in terms of preferences of host plant for oviposition or feeding have largely been conducted using excised leaf material rather than whole plants. However, it is widely acknowledged that excised leaf material may lead to unreliable results, as plant damage alters both the plant's physiology and chemical composition. For example, water stress resulting from leaf excision can increase ethylene production in beans (*Phaseolus vulgaris* L.), cotton (*Gossypium hirsutum* L.) and miniature rose (*Rosa hybrida* L., *cv.* Bluesette), which is not seen when the whole plants are exposed to a drought situation (Morgan *et al.*, 1990); this could consequently influence insect performance or preference. Similarly, leaf eating chrysomelid beetles (*Diabrotica adelpha* (Harold)) offered leaf discs from corn (*Zea mays* L.), beans (*Phaseolus vulgaris* L.) and squash (*Cucurbita maxima* L.) showed different feeding preferences to those exhibited when offered whole plants (Risch, 1985). In the case of the vine weevil, volatiles from mechanically damaged leaves have been found to be attractive (van Tol *et al.*, 2002). Conversely, the related clay coloured weevil (*Otiorhynchus singularis*) avoided mechanically damaged plants (Gordon & Gordon, 1992). The use of excised plant material may therefore give

inaccurate results regarding weevil feeding and oviposition behaviour. By using whole plants for all experiments in this thesis, the aim was to eliminate any influence on the vine weevil that may have arisen from either differences in plant physiology or chemistry resulting from leaf or plant part excision. By taking this approach, our results highlighted that vine weevil oviposition on nine different raspberry cultivars was not significantly different (**Chapter Four**), although previously vine weevil adults had been shown to discriminate between excised leaves from different raspberry cultivars (Cram & Daubeny, 1982), highlighting the importance of using whole plant systems in preference and performance experiments. In addition, the feeding preferences of vine weevil feeding were different between the choice and no–choice experiments, demonstrating the importance of performing both types of experiment when investigating insect behaviour.

7.5. Host plant selection and the preference–performance hypothesis in an aboveground–belowground context

The research presented in this thesis highlights that vine weevil adults do not preferentially select host plants for oviposition that best maximise the survival of their larval offspring and that consequently no evidence was found of a positive preference–performance link between adult and offspring vine weevils in this thesis. The findings go on to highlight that in fact a parent–offspring conflict may occur between the aboveground adult weevil and the root feeding larvae, with adult weevils feeding on plants, despite the fact that this negatively impacts the root system on which their offspring are feeding.

Several factors influence an insect's selection of host plant including plant chemistry, in the form of secondary metabolites, host nutritional quality, physical plant attributes and environmental factors such as the presence of competitors and/or the absence of enemies

(these are discussed more fully in **Chapter One**). Consequently, there are potentially several factors underpinning why adult vine weevils do not select host plants that maximise the development and survival of their offspring and some of these are considered in more detail below.

7.5.1. *Accurately determining host plant suitability*

Probably due to the inherent lack of mobility in soil dwelling larvae of aboveground insect herbivores and potentially also because of the difficulties of working with belowground insects, relatively few studies consider the host plant preferences of belowground larvae and then relate these back to adult preference aboveground. In the case of the vine weevil, only a handful of studies have considered larval performance on particular host plants (Hanula, 1988, Cowles, 2004, Fisher, 2006), despite the fact that it is the larval stage of this insect herbivore that causes most plant damage (La Lone & Clarke, 1981). This thesis highlights the importance of considering the influence of a host plant on root–feeding larval offspring and any interactions they may have with the maternal insect aboveground.

The relationship between vine weevils (both adults and larvae) and their host plants has been discussed in some detail in Chapter Three. However, the number of host plants reported by the literature as acceptable host species is, to some degree, debatable (Fisher, 2006). Many studies examining host plant suitability for vine weevils have simply defined a host plant as one on which either the adult or larvae were observed feeding, instead of ascertaining whether the plant could support the development of the entire weevil life–cycle. Host plant acceptance is defined by Schoonhoven *et al.* (2005) to occur when an insect demonstrates either sustained feeding or oviposition. However, several studies with the vine weevil have reported foliage damage, but then subsequently shown the plant to be unviable as a host (Cram, 1970, Nielsen & Dunlap, 1981, Hanula, 1988, van Tol *et al.*,

2004a). This contradiction between adult feeding and plant suitability may arise from the weevil examining the palatability of the plant, as part of its selection procedure and thus not actually indicate its acceptance of the plant as a host. However, vine weevils may also oviposit on some unsuitable plant species for larval development when there is a lack of viable alternatives and in these instances a poor correlation between maternal choice and offspring performance may well be observed.

7.5.2. Diet breadth: monophagous insects versus polyphagous insects

One of the theories that has previously been proposed to explain weaker links between a maternal insect's choice of host plant and the subsequent survival and performance of her offspring relates to the neural capacity of insects. Levins and Macarthur (1969) were the first to suggest that polyphagous insects were more likely to make poor host plant decisions than monophagous insect herbivores due to their limited neural capacity. Consequently, when polyphagous insects are faced with a complex decision between several host plants they may make poor selections. In contrast, monophagous insects are finely tuned into their particular host plant and in essence their decision is more sharply contrasted, in terms of host plant/non host plant, thus monophagous insects have simpler decisions to make and are less likely to select a poor host plant.

Naturally, there are exceptions to the neural capacity hypothesis. Recently, a meta–analysis investigating the preference–performance hypothesis showed that oligophagous insects exhibited stronger linkages between mother and offspring than polyphagous insects, but that monophagous insects demonstrated a preference–performance link of the same strength as that for polyphagous insects (Gripenberg *et al.*, 2010). Equally, polyphagous insects have demonstrated preferences for certain host plants, the polyphagous comma

butterfly (*Polygonia c–album*) preferentially selected for hosts in Urticales over hosts in Salicales and this preference was well correlated with larval performance (Nylin, 1988).

The neural restraints hypothesis has previously been considered using insect herbivores that complete their life–cycles aboveground. However, potentially this could be extended to ascertain whether polyphagous or monophagous maternal adult insects residing aboveground differ in selecting suitable plants for their subsequent belowground offspring. In this thesis, the absence of a preference–performance linkage between adult vine weevils and their soil–dwelling offspring may have resulted from the fact that they are highly polyphagous. The lack of a strong relationship between vine weevils and their host plants is likely to have influenced their ability to detect whether a plant is suitable for offspring survival and development. Potentially, the subtle differences between raspberry cultivars that were examined in Chapters Four and Five would be more likely to be detected by a monophagous feeder on red raspberry, such as the clay coloured weevil (*Otiorhynchus singularis*).

7.5.3. Clonality and adaptation in host plant selection behaviour

Asexuality is considerably more common in invertebrates than vertebrates (Scholtz *et al.*, 2003) and is best studied in insects, where over 900 species have been identified as being asexual or having asexual forms (Normark, 2003). Out of the 900 insect species recognised, nearly 200 are weevil species (Normark, 2003), exhibiting parthenogenesis (true clonality), thus they are direct clones of the adult insect, unless a random mutation occurs. The genus *Otiorhynchus* contains more than 60 parthenogenetic species of vine weevil, all of which are significant pests in both agriculture and forestry (Stenberg & Lundmark, 2004).

True clonality is thought to benefit insect species by increasing the fecundity of insects and enabling an individual insect to colonise a new environment (Stenberg & Lundmark, 2004).

Potentially the reproductive mechanism of vine weevils could be responsible for the parent offspring conflict that has been observed as part of this thesis (**Chapter Six**). Adult weevils were found to show no discrimination between plants for feeding, which were exposed to belowground root feeding offspring, despite the fact that aboveground herbivory was found to detrimentally affect larval performance. Adult vine weevils have also been shown to preferentially feed on strawberry plants with belowground larvae, although the impact of aboveground feeding on belowground larval performance was not directly quantified (Gange, 2001). Vine weevils are unable to adapt by sexual reproduction meaning that weevil genotypes are not mixed, so any beneficial traits for choosing host plants can not be selected for, and, similarly any potentially damaging mutations that could influence poor host plant selections can not be removed from the gene pool.

7.6. Future research issues for the vine weevil

7.6.1. Protected cropping systems

Over the last decade soft fruit growers have become increasingly dependent on protected cropping systems for the production of their crop. Polytunnels (also referred to as Spanish tunnels or high tunnels) are now a common sight across the UK, facilitating longer growing seasons and higher quality berries in comparison to unprotected soft fruit production (Demchak, 2009). Currently over 80% of soft fruit sold in UK supermarkets is produced under protected cropping systems (British Summer Fruits, 2009) and this percentage is very likely to increase as consumer demand for high quality berries rises.

Interestingly, whilst the use of tunnels is now relatively widespread in the soft fruit industry, little research has been conducted investigating the dynamics of insects (both beneficial and detrimental) in a tunnel environment (Bylemans *et al.*, 2003, Gordon *et al.*, 2006, Johnson *et al.*, 2010b (Appendix 1)). Temperatures, light intensity and humidity are all altered in a tunnel environment, which may directly, or indirectly (by altering host plant quality) influence insect herbivores. For example, raspberry plants grown within a polytunnel environment were shown to have reduced foliar nitrogen in comparison to plants grown in the field (Johnson *et al.*, 2010b (Appendix 1)). Consequently, adult vine weevils feeding on plants in the tunnels consumed significantly more foliage than weevils in the field, suggesting that the weevils were demonstrating compensatory feeding due to the reduced leaf nitrogen concentration. If other insect herbivores were to demonstrate similar behaviour, which is not unlikely considering nitrogen is often a limiting factor in insect development (White, 1993), then potentially plants grown in tunnel environments may be exposed to a higher degree of foliar herbivory than in the field. In addition, Johnson *et al.* (2010b) (**Appendix 1**) demonstrated that conditions in the tunnels shortened the pre–oviposition period of newly emerged adult vine weevils, thus allowing them to lay significantly more eggs than in the field. Understanding the life–cycles, behaviour and population dynamics of insects in a protected cropping environment may assist in controlling detrimental pests and promote the effective use of predatory insects as biocontrols.

In Chapter Three, a vine weevil population was monitored over a two year period in a protected cropping system, examining how weevil numbers accumulate from a relatively low initial egg inoculation on two different raspberry cultivars. In the second year of surveying, adult vine weevil numbers were estimated to reach a maximum of *c.* 32,000.

This indicates the potential of vine weevil numbers to accumulate relatively quickly, to a potentially economically damaging level if left uncontrolled.

Vine weevils typically survive the winter period as larvae, however adult weevils have also been recorded as overwintering (Blackshaw, 1984, Blackshaw, 1987), emerging very early on in the following season, where they are then capable of laying eggs earlier than recently eclosed adult weevils, thus essentially starting the weevil life–cycle earlier in the year. A few marked adult weevils from the previous year were recaptured in the polytunnels in 2010, indicating that they are capable of survival in outdoor conditions in Scotland. Potentially, the polythene mulch surrounding the base of the plants as weed control offered the adults added protection from the elements and may highlight that such practices could serve to increase vine weevil populations and potentially other insects in soft fruit crops.

7.6.2. Climate change

Throughout Europe, warmer winters have been demonstrated to promote the emergence of some insect species several weeks earlier than previously documented, (e.g. aphids (Malloch et al., 2006, Harrington et al., 2007, Thackeray et al., 2010), thus elongating the season in which they can inflict damage on host plants. For the vine weevil, elevated winter temperatures have the potential to allow more vine weevil adults to survive through the winter in field conditions, consequently emerging in the early spring ready to lay eggs. Previously, weevils have not been located in areas where winter temperatures fell below -6 °C in January (Stenseth, 1987). The presence of adults early on in the growing season of soft fruits, could lead to increased plant damage aboveground, as the adults would feed on newly developing leaves and buds.

Whilst warmer temperatures in winter may benefit several insect species, extreme temperatures in summer may have an adverse effect. Several insect species demonstrate improved performance and fecundity as temperatures rise; however frequently a maximum temperature is reached, after which insect performance is negatively affected (eg. Karlsson & Wiklund, 2005, Wang *et al.*, 2009). In the case of the vine weevil, temperatures in excess of 27 ℃ have been found to detrimentally affect adult weevil life span and the viability of eggs produced (Son & Lewis, 2005). Thus, whilst warmer temperatures in the winter may assist vine weevil populations, particularly high temperatures in the summer may have the opposite effect. Interestingly, elevated temperatures produced by a polytunnel environment combined with a warmer climate, may adversely impact vine weevil populations, however many other variables (e.g. humidity, light temperature, host plant nutrition) would need to be considered in conjunction with temperature, to accurately predict how vine weevils may respond to climate change.

7.7. Future Directions

The work presented in this thesis highlights the potential importance that parent–offspring relationships can play in aboveground–belowground insect herbivore interactions. The results presented and subsequent discussions illustrate that theories relating to aboveground insect herbivore host plant selection may also be applicable to explaining the selection of host plants by insects with a soil dwelling larval stage in their life–cycle. However, there are significant contrasts between considering an insect life–cycle that completes solely aboveground and one in which one of the life stages develops belowground (**Table 7.2**). Consequently, this opens up many new possibilities for investigation in both the preference–performance hypothesis and in aboveground–belowground insect herbivore interactions in general.

A natural extension to the work presented in this thesis would be to conduct similar experiments using a specialist insect herbivore with an aboveground–belowground insect life–cycle. One insect herbivore already mentioned in this discussion is the clay coloured weevil, which is a specialist herbivore of the red raspberry, although there are undoubtedly many other suitable insect herbivores and study systems. However, by conducting work with the clay coloured weevil and raspberry, comparisons could be made between the clay coloured weevil and the vine weevil in terms of their responses to raspberry cultivars and conspecific herbivory. Equally, the experiments could be combined to ascertain the reaction of clay coloured weevil adults to herbivory belowground by vine weevil larvae and *vice–versa*. It would also be interesting to investigate the accuracy and speed of their responses to host plants, thus testing the neural capacity hypothesis in this study system.

Table 7.2. – Contrasting host plant selection theories in aboveground and aboveground–belowground insect herbivore lifecycles.

Theory	Aboveground only	Aboveground–belowground
Visual cues	Visual cues aboveground may indicate an unsuitable host plant for offspring e.g. high number of leaf trichomes that impair larval feeding.	Adult insects are unable to visually select root systems that are optimal for offspring.
Semiochemical	Plants semiochemicals, either attractants or deterrents are more likely to be the same for adults and offspring feeding on the same plant tissue.	Physical separation of adult and offspring can lead them exposed to different semiochemicals, although some may be common to roots and shoots.
Nutritional value	Adults and larvae are able to feed from plant parts with similar or the same nutritional value.	Roots are known to have lower nitrogen and higher carbon than aboveground plant parts, thus adult and offspring may not receive the same nutrition. Additionally the influences of aboveground and/or belowground herbivory in the plant may affect how the plant allocates resources, thus impacting the spatially separated herbivores differently (Bardgett *et al.*, 1998).
Plant vigour hypothesis	Adult insects may select plants that show high quality new growth that maximises offspring performance.	Disparities can occur between root and shoot vigour, depending on where plants allocate their resources. The presence of aboveground herbivores may reduce root biomass for belowground feeders (as seen in Chapter 6), or not (Soler *et al.*, 2007), which could be viewed as a compensatory response of the plant to divert resources to the root system. However, in either circumstance, the influence of herbivores on aboveground–belowground plant resources is complicated to predict.

Table 7.2. continued.

Theory	Aboveground only	Aboveground–belowground
Induction of plant defences	The presence of an insect herbivore aboveground can strongly induce a chemical defence response in leaves that will equally impact on larvae also feeding on aboveground plant material (Kaplan *et al.*, 2008).	Foliar herbivory aboveground produces a strong response in similar aboveground plant tissues, but only generates a weak defence response in plant roots. Conversely, root herbivory has been demonstrated to produce an equal chemical response in both leaves and roots (reviewed by Kaplan *et al.*, 2008), demonstrating that the impact of induced plant defences on root– and foliar–feeding insects is not symmetrical in aboveground–belowground interactions.
Enemy free space	Adult and larvae often exposed to same predators and/or competitors.	Adult and larvae exposed to very different predators and/or competitors (e.g. parasitoid wasps aboveground and soil–dwelling nematodes belowground), thus adults might select plants that avoid enemies and/or competitors for them, but are worse for offspring.

To strengthen the preference–performance findings presented in this thesis, additional experiments documenting the survival and performance of adult weevils on plants exposed to belowground offspring herbivory could be conducted. However, considerable time would be required to determine survival (if measured in terms of weevil lifespan) as adult vine weevils are capable of living for over one year in laboratory conditions. Additionally, larvae feeding on the roots of plants in such long running experiments would be developing and feeding more significantly as the study progressed, thus not exposing the adult weevils to a constant treatment.

In the field experiment, damage to plants was not empirically recorded as part of the survey, however it was noted that plants where high numbers of weevils were recovered exhibited poorer plant vigour in comparison to plants where lower numbers of weevils were recorded. The tunnels used for this research were additionally used for other research activities and thus it was not feasible to destructively sample plants, but for future work it would be interesting to see whether adult weevil numbers aboveground correlated with larval numbers in the root system. Consequently, these data could then be linked with plant performance parameters (e.g. plant vigour, nutritional quality of foliage and berry weight and quality) and would provide a fuller picture of the driving factors in vine weevil population dynamics in a polytunnel environment. Additionally the research would widen our knowledge regarding the interactions between aboveground and belowground insect herbivores in a field environment over several years, as many studies addressing aboveground–belowground interactions are either controlled temperature experiments or are limited to one study year in field conditions.

A considerable amount of research has considered how insect herbivores aboveground react to elevated carbon dioxide (eCO_2) concentrations (reviewed by Bezemer & Jones,

1998), in a response to the prediction of elevated CO_2 as a result of climate change. However, a recent review (Staley & Johnson, 2008) highlighted that only two studies have investigated the impact eCO_2 may have on belowground insect herbivores (Salt *et al.*, 1996, Johnson & McNicol, 2010). A recent study considering the influence of eCO_2 on vine weevil larvae found that larval abundance and body mass was significantly reduced when feeding on the blackcurrant (*Ribes nigrum*) roots of plants grown in eCO_2 compared to plants grown at atmospheric CO_2 concentrations (Johnson *et al.*, 2010a (Appendix 2)). The study highlights that impaired root growth at eCO_2 was closely related with the performance of vine weevil larvae, but that root water concentration may also play a role in larval development, indicating that climate change may influence vine weevil populations in the future in many different ways.

7.8. Concluding remarks

The findings presented in this thesis emphasise the importance of incorporating insects with aboveground–belowground life–cycles into the current theory relating to insect host plant choice. It demonstrates the requirement for further studies to be undertaken investigating the indirect linkages between belowground insect herbivores and aboveground insect herbivores and for such studies to include a wider range of insect species than has thus far been utilised. The accumulation of a significant vine weevil population from a relatively low initial egg inoculation shows the potential of vine weevils to colonise new areas, where they are capable of causing considerable economic damage. No evidence of a preference–performance linkage was found between maternal adult vine weevils and their soil–dwelling offspring, most probably because of the highly polyphagous nature of the vine weevil. A conflict between maternal weevils and larval offspring was evident, whereby adult weevils fed on plants with vine weevil larvae already present,

effectively reducing larval performance by altering host plant quality. The identification of potential conflicts occurring between related aboveground and belowground insect herbivore species may be an important factor in considering such relationships and how they shape terrestrial ecosystems.

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Appendix One

Co-authored manuscript in press in *Agricultural and Forest Entomology*.

This is a placeholder for the following publication. The full paper cannot be displayed here due to copyright restrictions.

Johnson, S.N., Petitjean, S., Clark, K.E. & Mitchell, C. (2010) Protected raspberry production accelerates onset of oviposition by vine weevils (*Otiorhynchus sulcatus*). *Agricultural and Forest Entomology,* **12**, 277-283.

Appendix Two

Co-authored manuscript accepted for publication in *Global Change Biology*.

This is a placeholder for the following publication. The full paper cannot be displayed here due to copyright restrictions.

Johnson, S.N., Barton, A.T., Clark, K.E., Gregory, P.J., McMenemy, L.S. & Hancock, R.D. (2010) Elevated atmospheric CO_2 impairs the performance of root-feeding vine weevils by modifying root growth and secondary metabolites. *Global Change Biology*, 17, 688-695.