Distributions and interactions of insect herbivores as influences on host plant density and performance

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

(Zoology)

THE UNIVERSITY OF BRITISH COLUMBIA

(VANCOUVER)

July 2012

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ABSTRACT

Biological control programmes to reduce the density of invasive weeds often introduce multiple species of insect herbivores before reductions of the weed population occur. The factors leading to successful control need to be identified to improve success rates and reduce the number of insect introductions. These factors may be traits of the biocontrol agent-plant system, or may be external influences such as the presence of other biocontrol agents or environmental conditions.

A characteristic that varies among insect herbivores is their distribution among plants. I developed a simulation model that demonstrates how insects distributed in direct proportion to plant density cause the fastest rate of host plant population decline. This agrees with observations of a successful species, *Larinus minutus*, compared to an unsuccessful one, *Urophora affinis*, in the diffuse knapweed (*Centaurea diffusa*) biocontrol system.

Using the successful biocontrol agent, *L. minutus*, I experimentally tested whether adult as well as larval feeding increased control of diffuse knapweed. My results suggest that agents with both adult and larval feeding can control plants in a wider range of environments.

With a meta-analysis I tested if the presence of other biocontrol agents alters the relationship that a natural enemy has with the host-plant, and showed that the reduction in plant performance caused by one natural enemy is, on average, independent of the second. I also show that the occurrence of non-independent interactions between natural enemies can be predicted by a small range of enemy or plant attributes. (direct interactions and attack to reproductive plant parts). My experiment using the root herbivore, *Cyphocleonus achates* and the aboveground herbivore *L. minutus* showed
that interactions between the two species can increase the reduction in seed produced by the plant, potentially enhancing biocontrol.

My thesis demonstrates that attributes of the biocontrol agents themselves and their interactions with traits of other agents can alter plant performance or plant population decline. Research into attributes associated with successful, and unsuccessful, biocontrol will improve success rates.


**Preface**

I designed the study in Chapter 2, in collaboration with J.H. Myers. I conducted and analysed the field observational study, and developed and analysed the model. J.H. Myers also contributed to interpreting results and writing the manuscript. This article has been published:


DOI: 10.1111/j.1365-2745.2012.01971.x.

Chapters 3 and 5 were designed by myself and J.H. Myers. I collected and analysed the data and wrote the manuscripts. J.H. Myers helped with writing and editing the manuscripts. Chapter 3 has been submitted.

Chapter 4 was conceived of by myself and J.H. Myers. I collated and analysed the data with assistance from D.S. Srivastava. I wrote the manuscript with assistance and guidance from both D.S. Srivastava and J.H. Myers.
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ACKNOWLEDGEMENTS

There are a large number of people without whom this thesis never would have been completed. First off, I would like to thank Judy Myers, for all her support and encouragement over the past five years. Judy has given me the opportunity explore interesting ideas and questions while providing me with the structure and resources to be achieve what I set out to. Diane Srivastava has been a highly valuable co-supervisor, challenging me to think critically how best to present my work. Her input has been particularly valuable for Chapter Four.

My committee members, Jake Goheen and Letitica Aviles also provided invaluable advice; thank you.

Outside UBC, advice from Jenny Cory (SFU) and Rob Bourchier (AgCanada) have proved very useful on numerous occasions. Steve Heard (UNB) provided invaluable input on Chapter Two.

I would also like to thank the numerous people in the Biodiversity Centre and Department of Zoology UBC who have provided advice throughout the past few years; there are simply too many of you to list! But, in particular, I would like to acknowledge the advice I’ve received by presenting my ideas at FLORUM – thanks to Mark Vellend and Bill Harrower for their organisation of this over the years. And also thanks to all those who have all organised and participated in the various seminar series and BDG over the years – attending these has enriched the learning experience I have had here in the Department.

Over the past four summers, I have had the pleasure to be hosted by Linda Edwards and Brian Mennel in Cawston, and also Tamara Richardson in the final season. I absolutely could not have had a better place from which to base myself; your hospitality
was amazing and truly appreciated. Coming in from the field to incredible food, wine and
excellent company made field work infinitely more enjoyable.

I have had a number of fantastic field and lab assistants: Alana Phelps, Chris
Thoreau, Adrian MacKay, Ishwarya Chaitanya, Kelsie Hardman, Aimee Gibson,
Elizabeth Yue, Alice Feng, Ada Sin and Kelsey Dawes. My parents, Bob and Alison, my
sister, Cielle, and my husband, Rich, have also helped me in the field on various
occasions.

The Nature Trust of British Columbia allowed the experiments to be conducted
on their land.

I would like to thank Karen Needham, Don Griffiths and Linda Jennings at the
Beaty Biodiversity Museum for allowing access to their microscopes. The ZCU, Alice Liou
and other administrative staff have helped to make the Zoology Department an enjoyable
and productive working environment. David Kaplan provided greenhouse space and
expertise.

A number of people kindly provided data for Chapter Four: Betty Owor; James
Legg; David Knochel; Katja Poveda; Esther Gerber; Tamaru Hunt-Joshi; Charles
Mitchell; Jennifer Rudgers; Kristen Hladun; Rick Karban; Ken Puliafito; Pete Turner;
Lincoln Smith; Scott Johnson; Nick Barber; Peter McEvoy. I would also like to thank all
those who looked unsuccessfully!

I have been lucky to have had a very large number of great lab mates through
being associated with both the Myers and Srivastava labs. In particular, I’d like to thank
Michelle Tseng for all her advice. Kathryn Turner (Rieseburg lab), Caroline Jackson and
Tom Deane all deserve thanks for sharing their knowledge of the diffuse knapweed and
sites.
A very large number of friends and colleagues have contributed to this thesis in a variety of ways through scientific discussions, company at conferences, or by helping me take a break from science! A big thank you to all of those with whom I have played board-games with, gone out to the pub after Friday beers with, had dinner/lunch/coffee with, gone snow-shoeing or hiking with; you've all made the time in Vancouver most enjoyable and will be the key people I'll miss (although many of you have already moved on).

And finally, big thank you to my family and especially to my husband, Rich. You guys have been my biggest cheerleaders throughout the whole thesis, in particular Rich who has always been there for me.
CHAPTER ONE: GENERAL INTRODUCTION

1.1 Why plants become invasive

Invasive species are a major threat to biodiversity world-wide (Vitousek et al. 1997) and can have major economic implications (Pimental et al. 2000). A recent meta-analysis showed that while plots containing an invasive plant had greater plant productivity, native plant growth, abundance, fitness and diversity was lower (Vilà et al. 2011). Invasive species lower plant species diversity in small areas (plots), although on a larger scale (regions), they increase diversity as the presence of exotic species increases the overall species richness of the area (Fridley et al. 2007; Stohlgren et al. 2008, but see Guo et al. 2012). However, global beta-diversity decreases as areas become homogenised (Stohlgren et al. 2011).

A large number of hypotheses have been put forward to explain why plants might become invasive (reviewed by Catton et al. 2009). The different hypotheses are often tested using comparisons between native and invasive species or between non-invasive and invasive species, or between an invasive species in its native range vs. in its invasive range. These different comparisons give different insights into the invasion process (van Kleunen et al. 2010).

One of the more commonly tested hypotheses to explain the success of exotic plants is the Enemy Release Hypothesis (ERH). It proposes that plants become invasive as they are released from top-down control by their specialist natural enemies in the introduced range (Keane & Crawley 2002). This hypothesis states that in their native ranges plant populations are regulated by specialist natural enemies (primarily insects and plant pathogens). When they arrive in the introduced range, they leave behind their natural enemies. In the introduced range they are only attacked by generalist enemies,
but to a lesser extent than the native plants of the introduced range. Thus the reduction in specialist attack gives the introduced plant a competitive advantage over the natives, leading to invasiveness.

The ERH predicts that 1. richness or load of insect herbivores and plant disease on the invasive plant will be lower in its introduced ranges than in its native range and 2. the impact of natural enemies on the invasive plants will be lower than on other (native) plants in the invaded community. Two recent reviews confirm the first the prediction (Colautti et al. 2004; Liu & Stiling 2006). Evidence for the second was equivocal but there was a dearth of studies testing both predictions on the same invader (Colautti et al. 2004). A remaining conundrum is that many introduced plants do not become invasive even though the same associations with natural enemies are expected.

The ERH though, is one of the few hypotheses that addresses both how plants become invasive and suggests a viable mechanism to control the invasion – biological control. In biological control, specialist natural enemies from the native range of the plant are introduced to the invasive range in the hope of controlling the plant. The natural enemies are generally arthropods (insects or mites) or plant pathogens and are always specialists to avoid attack to non-target plants. Often manipulation of disturbance regimes and plant competition as well as enemy regimes is necessary to achieve successful control (e.g. McEvoy et al. 1993).

1.2 Application of the Enemy Release Hypothesis: Biological control

The Enemy Release Hypothesis (ERH; Keane & Crawley 2002) is the premise of biological control. While biological control has had some high profile successes (e.g. control of prickly pear cactus by Cactoblastis in Australia; Myers & Bazely 2003), some programmes have not been successful despite the introduction of multiple species (e.g.
Hill et al. 2000). While multiple species are often introduced, control is generally attributable to one agent (Denoth et al. 2002). Some attempts have been made to understand what has led to biological control successes and failures (e.g. van Klinken & Raghu 2006; Myers 2000). Biocontrol failure can occur due to both technical reasons (e.g. taxonomy poorly defined, too few individuals introduced) as well as biological (e.g. not adapted to the environment, heavily predated by native species). Understanding the biological reasons for successes and failures allows us to more generally consider the role top-down control has in structuring plant communities.

While ERH is the basis for biological control, successful biological control does not necessarily confirm that enemy release was the key mechanism behind the invasion. Biological control agents are introduced without their natural enemies, potentially experiencing enemy release themselves. Myers (2001) suggested enemies that are rare in their native range due to the presence of natural enemies might be more successful biocontrol agents as they are more likely to be released from top-down control when introduced and the plant is less likely to have evolved defences to them.

In summary, improving biological control success rates requires an improved knowledge of when and how insects and plant pathogens exert top-down control on plants.

1.2.1 Variation in levels of top-down control

Not all enemies provide top-down control and top-down control does not explain the abundance of all plant species (e.g. MacDonald & Kotanen 2010). Variation in levels of top-down impacts will be due to myriad factors, some of which I discuss here. For the purposes of this mini-review, I separate factors that might influence the level of top-down control as intrinsic or extrinsic to the enemy-plant system. I define intrinsic factors
as those that are attributes or traits of either the herbivore or the plant and extrinsic factors is an outside influence to the enemy-plant system. It could be either an abiotic factor or another species in the community. Top-down control by herbivores might be correlated with certain plant traits or certain types of enemies might be more likely to exert top-down control. However, these factors are likely to interact so that plants with certain traits might be more susceptible to particular herbivore types. Intrinsic factors will also interact with extrinsic ones such that traits effects may vary depending on environmental context (both abiotic and biotic).

Plant damage, plant performance, plant fitness and plant population density.

The effect of natural enemies on plants may be measured at a number of different levels, this is primarily an issue of scale (Levin 1992). First, plant damage can be measured as the amount of tissue an insect eats (e.g. an insect ate five leaves), and this may be translated to changes in plant performance (e.g. changes in survival or amount of plant tissue between damaged versus undamaged plants). Plant performance measures incorporate both the amount of damage and the response of the plant (whether it is able to compensate for damage). The impact of the herbivore can next be evaluated as the influence on the potential or actual plant fitness – the contribution to the gene pool of the next generation. This is typically measured through relative seed-set, which again incorporates the ability of the plant to compensate for damage (Strauss & Agrawal 1999). Of interest to biological control is whether the spread of the plant population is reduced and plant population declines as a result of the damage received. This is more complex than the other levels as numerous factors can buffer the population against declines (e.g. Garren & Strauss 2009).

Here I discuss factors at all these levels with a focus on the population level.
Intrinsic factors

Plant attributes

Attributes of plants influence whether they can be controlled by natural enemies. Crawley (1989a) suggests that plants that are particularly difficult to control using insect herbivores have 1. long growing period, 2. reserves of carbohydrate that are inaccessible to the herbivore, 3. high powers of regrowth, 4. ability to replace flowers or fruit following defloration, 5. low food quality and/or 6. large seed-banks.

Graminoids, forbs and algae were the functional groups most affected by herbivory in a meta-analysis (Bigger & Marvier 1998) whereas there were only marginal effects on woody plants. There was no effect of herbivory on mosses.

The differences among the various functional groups may vary when only a particular type of herbivory is considered. In a second meta-analysis, woody plants showed worse performance with insect root herbivores than herbaceous plants (Zvereva & Kozlov 2012). Within herbaceous plants, performance of grasses was worse than forbs, but there were no differences between annuals and perennials. A third meta-analysis that considered sap-sucking arthropods (Zvereva et al. 2010) found significantly greater reduction in the performance of seedlings compared to saplings or mature trees and they had greater impacts on Angiosperms than Gymnosperms. Blossey & Hunt-Joshi (2003) suggested that root herbivores might influence the structure of later-successional communities more than early successional ones as later successional plants allocate relatively more biomass to roots than early successional ones.

The impact of a particular herbivore can vary with life-history stage of the plant. For example, black-tailed deer and California hares reduced the growth rate and survival of juvenile plants but had no effect on older ones (Warner & Cushman 2002); the impact
of attack to the plant is higher in earlier life-history stages (seeds and seedlings). However, if plants are not seed-limited this may have no effect on the plant population (Myers & Risely 2000) and reductions in seedling density can also be compensated for at the population-level (e.g. Garren & Strauss 2009).

Within a population, plants will vary in susceptibility to attack by different herbivores (e.g. Maddox & Root 1987) and different pathogens (e.g. Campanella et al. 2009). Differences in resistance to various herbivores can interact with microhabitat (e.g. Johnson & Agrawal 2007). Inbreeding depression can also be related to increased attack rates (e.g. Delphia et al. 2009).

Enemy attributes

Herbivores with certain attributes might be more likely to provide top-down control. While Crawley (1989b) hypothesised that vertebrates would have a greater influence on plant performance, a meta-analysis by Bigger & Marvier (1998) showed that on average neither mammalian (both large e.g. moose, elk and small mammals e.g. rabbits, gophers) nor crustacean herbivory affected plant biomass, but bird, insect, molluscan and fish herbivory significantly reduced plant biomass. Comparisons of mammalian and insect herbivory on the same plant species suggest that insect herbivory has a greater impact (e.g. Amsberry & Maron 2006). Kotanen & Rosenthal (2000) suggest that tolerance to insect and vertebrate herbivores might be quite different as insect herbivores have a much wider range of feeding modes than vertebrate herbivores.

Within the insect herbivores, some species might have behavioural and/or life-history traits that increase their ability to inflict top-down control on plants, e.g. small body size and high intrinsic rates of natural increase (Crawley 1989a). Crawley (1989a) points out that Coleoptera have been involved in more biocontrol successes than any other group. However, Coleoptera are the most speciose insect order. Therefore, all else
being equal, we would expect them to be introduced and successful as biocontrol agents more often than other insect orders. It is unclear whether the increased success of Coleoptera remains once we account for the relative size of the orders. Coleoptera do have a number of features that may increase the likelihood of success. Coleoptera tend to feed as both larvae and adults, often on different parts of the plant (Ch3), and they are often relatively larger and tend to be comparatively mobile at least as adults (both factors suggested as beneficial by Crawley 1989a).

Myers et al. (1988) suggested that enemies that are able to kill their host outright should make particularly good biocontrol agents. However, this will only reduce weed populations if there is no compensation at the population level (see Maron & Crone 2006).

Various authors have suggested that different feeding modes or feeding on different plant structures might be associated with differences in plant performance and top-down control. Species that form galls that act as metabolic sinks have been suggested as optimal biocontrol agents (Dennill 1988, Harris & Shorthouse 1996). Galls may be formed on all parts of the plant. Harris & Shorthouse (1996) suggested that the most effective gallers are those that induce galls by severing vascular tissue and persist over most of the growing season.

Root herbivores are becoming increasingly popular as biocontrol agents and there is some suggestion that they are particularly able to influence plant community composition in late-successional habitats (Blossey & Hunt-Joshi 2003). Sheppard et al. (1995) suggests they may be particularly good at controlling plants with tap-roots.

Maron & Crone (2006) found that floral and pre- & post dispersal seed-predators caused similar reductions to λ of plant population to other types of herbivory, despite predictions that seed-predators would often have minimal effects (e.g. Myers & Risley
Seed-feeders likely can only exert top-down control if the plant is seed-limited.

Some insect behavioural traits, such as mating, foraging and predator avoidance behaviour, may influence top-down control and thus biocontrol success (Mills & Kean 2010). Egg laying in clusters has been suggested as a positive trait of biological control agents (Cappuccino 2000) as has pattern of insect distribution in response to plant density (Ch 2).

*Extrinsic factors*

Abiotic factors

Various factors extrinsic to the herbivore-plant system may influence top-down control of a plant by a natural enemy. Extrinsic factors can also explain why an introduced herbivore may provide top-down control in parts of the host plant range but not in others.

The amount of damage or reduction in plant performance that natural enemies cause is influenced by a range of abiotic factors including water availability, soil type, elevation, CO$_2$ and N, plant habitat (e.g. forest edge vs. interior), light availability, temperature. Timing of herbivory can also influence the impact to the plant. Abiotic factors can interact to affect the performance of both the natural enemy and the plant.

A meta-analysis by Massad & Dyer (2010) showed that increasing CO$_2$, light availability and nutrients all increased insect herbivory, particularly that of generalists while increasing heavy metals decreased herbivory. However, particularly among specialists, responses to other factors were highly variable.
Biotic factors

The classic Hairston, Smith & Slobodkin (1960) paper concludes that herbivores rarely control plant populations as they themselves are under control by higher trophic levels (i.e. a trophic cascade). Trophic cascades can reduce herbivore damage to plants and potentially disrupt biocontrol (e.g. Hunt-Joshi et al. 2005, Swope & Satterthwaite 2012).

Plant mutualists such as pollinators may reduce or mitigate damage inflicted to the plant by the herbivore, however, enemies of mutualists can increase control. For example, the invasion of the honey-bee parasite varroa mite into New Zealand is predicted to improve control of broom by Bruchidius villosus if commercial honey-bee hives are suitably managed. Varroa may cause pollinator limitation so that the additional impact of B. villosus reaches the threshold for population suppression (Paynter et al. 2010).

Increasing performance of a natural enemy may lead to increasing top-down control, while decreasing performance may have the opposite effect. These changes in enemy performance may be dependent on other species in the community. For example, mycorrhizal fungi have mixed effects on herbivorous insects, the direction and magnitude of change in insect performance depends on the identity of the fungi (AM vs. ECM) and feeding guild and diet breadth of the insect (Koricheva et al. 2009). Fungal endophytes show similar patterns (e.g. Bultman & Bell 2003).

Recent evidence suggests that various members of the soil community may influence the damage herbivores inflict on the plant (e.g. decomposers, Poveda et al. 2005; soil microbes, Gera Hol et al. 2010). The surrounding plant community may also influence the natural enemy community on a plant (e.g. Bezemer et al. 2006).
Attack by one enemy may alter performance or attack by a second. This can occur by one species can change rates of colonisation by a second (e.g. Tack et al. 2012) or by altering the level of damage inflicted by the second species (e.g. Strauss 1991; Smith & Mayer 2005). Attack by one insect species generally reduces performance of a second insect sharing the host plant (Kaplan & Denno 2007). These interactions between natural enemy species can lead to changes in plant performance which may cascade through to changes in the level of control observed. Fournier et al. (2004) suggested that we might expect that competition between natural enemies might reduce the level of control observed from that predicted by the actions of both natural enemies alone while increases in control might occur when one enemy facilitates the second. Interference competition between insects has been shown to decrease the overall impact on the plant in a biological control context (e.g. Smith & Mayer 2005; Crowe & Bourchier 2006). Synergistic effects may also occur (e.g. Masters & Brown 1992) but are less common and may be primarily associated with situations where neither species has an effect alone but they significantly reduce plant performance when together (e.g. Paynter et al. 2006). An ability to predict what types of species interact to increase or decrease control would enable prioritisation or avoidance of species with these traits as biocontrol agents, improving rates of biocontrol success.

1.3 Study system: Diffuse knapweed and its biological control agents

My study system is the invasive plant, diffuse knapweed, and the suite of insects introduced as part of a biological control programme against it.

Diffuse knapweed (Centaurea diffusa Lam., Asteraceae) is a major weed in western North America. The genus Centaurea may be the most abundant noxious weed in the western United States (LeJeune & Seastedt 2001). Eurasian in origin, diffuse
knapweed has colonised up to 1.4 million hectares of rangelands in western North America (Seastedt et al. 2005). It is prickly, unpalatable to live-stock and able to out-compete a range of native species (Seastedt et al. 2005). It is a biennial or short-lived perennial species, that typically spends the first year or two as a “rosette” (Fig. 1.1a), followed by bolting and flowering (Fig. 1.1b). *Centaurea diffusa* is obligatorily out-crossing (Harrod & Taylor 1995).

A biological control programme for diffuse knapweed was initiated in 1970. Over the next thirty years, 12 insects were introduced for the control of diffuse knapweed and nine of these are now established in British Columbia, Canada (Myers 2007). In my thesis, I have studied biological control agents common in the southern Okanagan of British Columbia (Fig. 1.1).

1. *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) was the last of these biocontrol agents to be introduced, and has been attributed with causing the decline of diffuse knapweed in Colorado (Seastedt et al. 2005) and British Columbia (Myers et al. 2009). *Larinus minutus* adults (Fig. 1.1h) overwinter in the soil at the base of plants. In spring they emerge and feed on the stems and leaves of the bolts. This damage may kill the plant in dry years. In July-August during flowering, they lay eggs in the flower. The developing larvae consume all seeds in the seed-head (Fig 1.1g), and adults emerge from the seed-heads in autumn.

2. *Urophora affinis* Frauenfeld (Diptera: Tephritidae) females (Fig. 1.1e) oviposit in the seed-heads prior to flowering and the larvae form galls in the seed-heads (Fig 1.1f) reducing seed output. Larvae pupate and overwinter in the seed-head. While reductions in seed-output were often large, it did not reduce knapweed populations, as knapweed is not seed-limited (Myers & Risley, 2000). When *U.*
affinis and L. minutus co-occur in the seed-head, L. minutus destroys U. affinis (Crowe & Bourchier 2006; Smith and Mayer 2005).

3. Cyphocleonus achates Fahraeus (Col.: Curculionidae) was first introduced to British Columbia in 1987 (Myers 2007). Adults graze on the leaves (Fig 1.1b; Stinson et al. 1994) and lay eggs on rosette plants by boring a small hole in the root crown, laying one egg and covering the hole with plant debris (Steinger & Müller-Schärer 1992). Larvae attack the central vascular tissue of the roots of rosette plants (Fig 1.1c; Steinger & Müller-Schärer 1992); this increased plant mortality and reduced the height and biomass of con-generic spotted knapweed, Centaurea stoebe subsp. micranthos (Corn et al. 2006). Cyphocleonus achates appears to be increasing in the southern Okanagan (pers. obs.), at least partially due to redistribution by local weed co-ordinators. As this increase has mostly occurred after the decline of diffuse knapweed, it is difficult to know if it contributed to the decline. It has been credited with declines of spotted knapweed populations in Montana (Story et al. 2006).

I worked in three sites in the southern Okanagan of British Columbia (Fig 1.2); all of the sites occur in the bunch-grass biogeoclimatic zone (Nicholson et al. 1991). The vegetation type at White Lake (49°19'13" -119°37'46", Fig 1.2a) is sagebrush (Artemisia tridentata and Artemisia tripartita) substeppe. Diffuse knapweed invaded the site in the 1960s when the National Research Council’s Dominion Radio Astrophysical Observatory (who own the land and administer it in conjunction with the Nature Trust) installed a radio telescope, the installation of which created a disturbance that precipitated the invasion. The site is lightly grazed by cattle. The introduction of Larinus minutus has been associated with a decline in knapweed abundance (Myers et al. 2009) but unfortunately it has been replaced by the invasive cheat-grass, Bromus tectorum,
rather than native vegetation (Stephens et al. 2009), it would be interesting to further pursue the factors leading to succession of one weed by another rather than by native vegetation.

The second site is a meadow above Vaseux Lake (49°17’46", 119°31’34", Fig 1.2b), knapweed abundance has also declined here following introduction of *L. minutus* (Myers et al. 2009). This site is dominated by various native and introduced grasses including bluebunch wheatgrass (*Pseudoregneria spicatus*), needle-and-thread grass (*Heterostipa comata*), Sandberg bluegrass (*Poa secunda*), cheatgrass (*Bromus tectorum*) and red three-awn (*Aristida longiseta*) (Jackson 2008). The site was severely burnt in 2003 and all plants present have established since then. The site is not grazed by cattle but is regularly visited by Californian big-horn sheep and white-tailed deer. These vertebrates will occasionally damage knapweed bolts in the spring.

My third site, Anarchist Mountain (49°00’50", 119°16’57", Fig 1.2c) is at 1115m asl, higher than the other sites at 550 and 380 m asl respectively. At this site, biological control of diffuse knapweed has not been successful (Myers et al. 2009), and the site is dominated by the weed. It is grazed by cattle in the late summer or autumn although grass availability is very limited.

### 1.4 Thesis objectives and overview

In my thesis, I ask a series of questions about factors that could influence reductions in plant performance through top-down control by their natural enemies. I ask about two intrinsic factors in Chapters 2 & 3, and an extrinsic factor, the presence of other natural enemies, in Chapters 4 & 5.

In Chapter 2, I consider the insect response to plant density and how this might influence top-down control. Using a simulation model, I compare whether insects that are distributed on patches of various size in patterns consistent with resource
concentration (Root 1973), resource dilution (Otway et al. 2005) or the ideal free distribution (Kennedy and Gray 1993) influence the rates of plant population decline and persistence. I then illustrate my results using *L. minutus* and *U. affinis* on diffuse knapweed at White Lake and Vaseux Lake.

In Chapter 3, I conduct a series of experiments to compare the damage caused by adult and larval feeding by *L. minutus*. The cumulative stress hypothesis states that each additional species increases the level of stress experienced by the plant until it reaches a threshold where plant performance decreases sufficiently to cause plant population decline. I hypothesise that having an extra feeding mode (i.e. adult as well as larval feeding) might increase the level of damage experienced by the plant above that of one feeding mode alone. I examine this idea at two sites to determine whether extrinsic factors influence the patterns observed.

In the next two chapters, I investigate how additional natural enemies sharing a host-plant might alter plant performance using two different techniques. In Chapter 4, I conduct a meta-analysis to assess whether attributes of the species involved can allow us to predict when the natural enemies operate non-independently.

In my final data chapter (Chapter 5) I conduct a factorial experiment to consider whether interactions between an above- and belowground herbivore might be associated with non-independent effects on plant fitness. We might predict that facilitation between natural enemies could increase levels of damage while competition would decrease damage. I tested whether plants that were attacked both by *C. achates* and *L. minutus* had different plant performance than that predicted from the damage by each species alone, and whether positive or negative interactions between the natural enemies occurred.
Figure 1.1 Diffuse knapweed and common biocontrol agents in the Okanagan Valley, British Columbia, Canada. In its first year diffuse knapweed forms a rosette plant. It overwinters in this form and bolts in the spring of its second year if it is large enough and conditions are good. Species are: A. small rosette of diffuse knapweed; B. adult Cyphocleonus achates; C. larval C. achates in a diffuse knapweed root. diffuse knapweed roots are rarely attacked by multiple C. achates; D. flowering diffuse knapweed. Flowers are mostly white but can occasionally be purple; E. adult Urophora affinis; F. galls formed by larval U. affinis in seed-head; G. damage to seed-head caused by larval Larinus minutus; H. adult L. minutus feeding on the pollen of the flower. In the background you can see a stem damaged by this species.
Figure 1.2 Study sites. A is White Lake, B is Vasuex Lake, sites at which biocontrol has been successful. Anarchist Mountain is shown in C, biological control has not been successful here. In C all of the yellow-brown plants are diffuse knapweed.
CHAPTER TWO: RESOURCE CONCENTRATION BY INSECTS & IMPLICATIONS FOR PLANT POPULATIONS

2.1 Summary

1. The distribution of herbivores among plant patches may be an important factor determining plant population persistence. The Resource Concentration Hypothesis proposes that herbivores are more abundant per unit plant at higher host plant densities and this has been found to occur in many systems. However, the opposite pattern, resource dilution, in which the herbivores are more abundant in low density patches and situations in which the number of insect herbivores per unit plant remains constant, also occur.

2. We developed a simulation model to explore how the distribution of insects per plant affects plant population decline and persistence. We varied the numbers of plants per patch and the distribution pattern, i.e. whether insects were found in a resource concentration distribution (where insects per plant was greater in high densities patches), a resource dilution distribution (where insects per plant was greater in low densities patches) or a distribution in which insect abundance increased linearly with plant density (where insects per plant was constant).

3. Resource concentration resulted in longer persistence of plant populations. Plant populations declined more rapidly with either weak resource dilution or directly proportional insect distribution patterns. As the intensity of resource concentration increased, the decline in plant population density was reduced and plant persistence increased due to increasing variance in insect load. Under strong resource dilution,
increasing variance in the insect load had also lead to a reduction in plant population decline and an increase in plant persistence.

4. We complement our model with field data from the diffuse knapweed, *Centaurea diffusa* biocontrol system. We compared the relationship with plant density of a successful biocontrol agent, *Larinus minutus*, and an unsuccessful one, *Urophora affinis*. *Larinus minutus* density was directly proportional to plant density while *U. affinis* showed a resource concentration pattern with higher rates of attack in high-density patches.

5. *Synthesis*: Patterns of insect distribution with host plant density will alter the extent to which patches of differing plant densities decline or persist. Resource concentration promotes persistence of the insect–plant system because increased herbivore pressure in high-density patches leads to negative density-dependent plant growth. Weak resource dilution and a distribution of insects that is directly proportionate to plant density can accelerate plant population decline. Strong resource dilution leads to positive density dependence with higher population growth in large patches. Our simulation model and field data demonstrate that the relationship between insect distribution and plant densities can influence plant population dynamics, and has implications for choices of weed biological control agents.

### 2.2 Introduction

It is now commonly acknowledged that insects can influence the abundance of their host plants; however, our understanding of when insects are able to influence plant abundance remains poor (reviewed by Maron & Crone 2006). Spatial variation in herbivore load via insect aggregation on individual host plants has been suggested as a potential regulation mechanism for the populations of both plants and insects (Myers et
Such aggregations may arise through a range of mechanisms that mediate the response of insects to plant density e.g. sensory biases (visual vs. olfactory), dispersal biases, food requirements and diet breadth, competitive exclusion and predation (Kunin 1999).

The density of herbivorous insects may be related to the density of their host plants in a variety of ways (Rhainds & English-Loeb 2003). The Resource Concentration Hypothesis states that more dense or larger plant patches will recruit more herbivores per unit plant (Root 1973), and this effect will be strongest for specialist herbivores. Some herbivores have been documented to follow a resource concentration type distribution (e.g. Kèry et al. 2001; Ostergard & Ehrlén 2005; Sholes 2008). However, in other systems, (e.g. Kunin 1999; Elzinga et al. 2005; Fagan et al. 2005), the reverse pattern has been observed. In these cases, plants in smaller or less dense patches experience greater levels of herbivory per unit plant, a distribution termed ‘resource dilution’ (Otway et al. 2005).

These differences in density-dependent relationships could potentially influence the persistence of the insect–plant system. Under a resource concentration distribution, we predict that the higher density of specialist herbivores in large patches should increase herbivore damage and decrease plant reproduction or survival. This would allow smaller patches to grow in size or density (negative density dependence, i.e. higher plant population growth rates at low densities) and reduces the likelihood of extinction. Resource dilution could establish the opposite pattern (positive density dependence, i.e. greater plant population growth rates at high densities) as the increased number of specialist herbivores in small patches will reduce the number of plants in these patches making them more extinction-prone.

Previous models by Heard & Remer (2008) and Myers (1976) considered patterns of insect distributions on plant but not the influence of this on host-plant
populations. Here we evaluate the effect of contrasting patterns of insect distribution on the host-plant populations. To investigate this, we created a simulation model for a hypothetical insect–plant system in which the insects were pre-dispersal seed predators. Our model assumes that insects are able to reduce the populations of their host plant; this is the basic premise of the enemy release hypothesis (Keane & Crawley 2002) and weed biological control. Patches had different numbers of plants, and insects were differentially distributed across those patches following either a resource concentration pattern, a resource dilution pattern or directly proportional with the number of plants in the patch. We varied the strength of resource concentration or dilution. For example, as the resource concentration pattern strengthens, increasing numbers of insects occur in the larger patches and fewer in the smaller ones. We predicted that increasing variance in insect load (insects per plant) between patches would increase persistence times (less extinction). Although our model is based on pre-dispersal seed predators we predict that the model is applicable to other herbivore guilds that indirectly reduce plant reproduction. We then illustrate our simulation results with an observational study of the diffuse knapweed biocontrol system and consider the distributions of two different insect species with contrasting levels of weed control.

2.3 Methods

2.3.1 Model details

Plant populations

We created a simulation model in R (R Development Core Team, 2008) to test whether the insect distribution patterns that are driven by plant density alter the extent of plant population persistence and rate of population decline. Our modelled herbivores are pre-dispersal seed-predators that attack the seed-head (or other type of fruiting
body) and destroy a set proportion of seeds. Insects are able to move freely between plant patches. However, as plant recruitment is local to the patch, the model is not spatially explicit. The modelled plants are annuals.

The only density-dependent mechanisms present in the model are (i) the maximum number of plants in a given patch is limited by a carrying capacity, described below, and (ii) insect reproduction in a given patch is limited by the availability of seed-heads for offspring development. No additional density-dependent plant reproduction factors exist in the model to ensure that changes in the plant population due to insect herbivory are clearly evident and not altered by plant density-dependent mechanisms.

We started the model with twenty plant patches, with the number of plants in each patch drawn from the exponential distribution with a rate of 0.01 (resulting in a mean patch size of 100 plants). Using the exponential distribution gives many small and a few large patches (left skew), a distribution of patch sizes often seen in invading populations (Moody & Mack 1988; Bishop 2002; Müllerová et al. 2005). We define the total population as the sum of all plants in all patches, and each patch is a sub-population. The initial number of plants in the patch sets the patch carrying capacity, which is the maximum number of plants the patch can support. In each patch, plants can be of varying sizes – the number of seed-heads per plant was taken from a normal distribution with a mean of 75 and SD of 10, censored so that minimum seed-heads per plant equals zero. We then calculate the total number of seed-heads in each patch.

*Insect distribution in relation to plant patch size*

We started the model with 10 insects per plant in the total population. The insects are then distributed across the patches following power relationships as shown in Fig. 2.1a and described below. Fig. 2.1b shows how these total numbers convert to insect load. This form was chosen because it best matches the verbal description of Root (1973),
it asymptotes to zero (insect load cannot be negative nor can insects be present on nonexistent plants), and we can distinguish between weak and strong resource dilution. This is the same form as the relationship between weevil and sawfly densities on figwort *Scrophularia nodosa* (Andersson & Hambäck 2011). Andersson & Hambäck (2011) predicted a curvature of -0.5 based on scaling of olfactory information with patch size and suggest that departures from this value indicate differences in insect search behaviour (they reported curvature values of -0.15 and -0.53 for sawflies and weevils on figwort respectively). The curvature parameter determines whether the distribution follows a resource concentration, resource dilution pattern or is directly proportional to plant densities. If the curvature is one, this equation gives a direct proportional pattern between plant density and insect load (Fig. 2.1) or the ideal free distribution (Kennedy & Gray 1993). For resource concentration, curvature is greater than 1, and insect load is lower in small plant patches, but higher in large patches, compared to the direct proportional pattern. The further the curvature value is from one, the stronger the pattern. When the curvature is less than 1, resource dilution occurs and plants in small patches have a higher insect load and plants in large patches have a lower load compared to the direct proportional pattern. Weak dilution occurs when the curvature parameter is between 0 and 1, while a negative curvature parameter represents strong resource dilution. Under weak resource dilution, a small patch has a lower total abundance of insects than a large patch, but plants within a small patch will have a higher individual load. Under strong resource dilution, a small patch has both more insects overall and a higher load than a large patch (Fig. 2.1).

The number of insects in a given patch *i* at time *t* (*x*_\*\_j, \*t) is calculated as:

\[ x_{i,t} = \sum_{i} x_{i,t} \ast (y_{i,t} \theta / \sum_{i} y_{i,t} \theta) \]  

(1)
where \( y_{i, t} \) is the number of plants in patch \( i \) at time \( t \), \( \sum_i x_{i, t} \) is the total number of insects present in the total population at time \( t \), summed over all patches and \( \theta \) is the curvature parameter. Tables of all symbols used are available in Appendix A1.

We hypothesised that as the absolute distance of the curvature parameter from 1 increased the variance in insect numbers and load among the patches would increase. We calculated the variance of insect number and load on a standardised population using curvature parameters ranging from 3 to -1 to determine how variance changes, and used those curvature values subsequently in model simulations.

**Insect reproduction and damage to plants**

We modelled insect reproduction using this equation:

\[
x_{i, t+1} = \min\{x_{i, t} r x, (s_{i, t} * (1-e))\}
\]

(2)

where each patch produces \( x_{i, t} \) insects and \( \sum_j x_{j, t+1} \) represents the total number of insects in the second generation. Insect reproduction in each patch is determined by their reproductive potential, \( r \), the number of seed-heads available in that patch, \( s_{i, t} \), and the proportion of seed-heads escaping attack, \( e \). Each insect can produce eight offspring (\( r x \); values used by Heard & Remer (2008) and Myers (1976) are similar) if seed-heads are available for those offspring. Any offspring produced beyond the number of available seed-heads die – a seed-head can produce only one insect. This was done primarily to simplify the model but is analogous with the situation in many insect species where oviposition pheromones deter a second oviposition to reduce cannibalism (Dip.: Tephritidae, Prokopy et al. 1978; Lep.: Pieridae, Dempster 1997; Col.: Coccinellidae, Martini et al. 2009).

In the field, even when insect densities are high, some seed-heads will not be attacked. The proportion of seed-heads that escape attack characterises the search
efficiency of the insect. Escape from herbivory may occur due to mismatches between plant and insect phenology (Singer & Parmesan 2010, Bourchier & Crowe 2011). We refer to this variable as seed-head escape, $e$, and each generation is sampled from a uniform distribution ranging from 0–20% of the seed-heads in a patch. While these seed-heads are unavailable for insect reproduction, they produce seeds. A seed-head will also escape attack if there are insufficient insects to attack all seed-heads. Thus the total number of seed-heads in a patch that escape insect attack, $T_{i,t}$, is:

$$T_{i,t} = s_{i,t} e + (s_{i,t}^* (1-e)) - x_{i,t+1}$$

(3)

Plant reproduction (flowering plant to flowering plant) is a function of the number of seed-heads in a patch ($s_{i,t}$; determined by the number of plants in a patch), the number of seed-heads that escape attack ($T_{i,t}$), the impact of an insect when it attacks a seed-head ($a$) and a reproduction constant ($r_y$). When no herbivory occurs, average plant reproduction is 7.5 flowering plants for each flowering plant (essentially $\lambda$). This is in line with Heard & Remer (2008; four plants plant$^{-1}$ year$^{-1}$), Myers & Risley (2000; 6.3–0.53 knapweed plants plant$^{-1}$ year$^{-1}$) after including density dependent plant reproduction and initial patch size density of 1–9 plants m$^{-2}$, and Rees & Paynter (1997; 25 scotch broom plants plant$^{-1}$ year$^{-1}$) if sites were available for plant colonisation. The number of new plants produced by the patch $i$ ($p_{i,t+1}$) is calculated by:

$$p_{i,t+1} = ((s_{i,t} - T_{i,t}) r_y (1 - a)) + (T_{i,t}^* r_y)$$

(4)

The insect impact parameter ($a$) determines the proportion of seed-heads (5%) in the patch that are able to reproduce when attacked. When seed-heads are not attacked, each seed-head produces one seed. If $a = 1$, then no attacked seed-heads reproduce; if $a = 0$, insect attack has no effect. As the modelled plants were annuals, all plants in the previous generation ($\Sigma_i p_{i,t}$) were removed from the model.
For the next generation, all of the insects produced ($\Sigma_i x_{i, t+1}$) were re-allocated across the plant patches as described above (Eqn 1).

Output

To consider short-term (transient) dynamics, we ran the model for 10, 20 and 30 generations, redistributed the insects across the plant populations each generation and conducted 1000 simulations. We report the plant population size (total number of plant individuals summed across all patches, referred to as the total population) and number of plant patches (= patches) present after 10, 20 and 30 generations. A patch was present if it had at least one plant.

We then ran the model 1000 times for 1000 generations and considered persistence time, i.e. the number of generations required for plant populations to go extinct. Insect populations become extinct in the generation immediately after plant extinction. We report the proportion of runs in which the plant populations become extinct and the mean time to extinction of those populations.

We varied key parameters (plant reproduction, insect impact, insect reproduction and seed-head escape) to assess how dependent our results were on each variable. We chose the parameters due to a priori assumptions about their influence on rates of population decline. These parameters had a single value for the main analysis. For each parameter, we endeavoured to cover the range of values that produced a result between complete extinction after 1000 generations and no decline in the plant population. We ran the model 100 times for both 30 generations and 1000 generations to obtain varying curvature values and assessed the same responses as above.

To determine how different patterns of insect distribution alter the density dependence of plant reproduction, we removed plant carrying capacity and ran the model for 1000 simulations under varying curvature values for just two generations. For
each simulation, we calculated the change in plant population in each patch by dividing the patch population in Generation 2 with the patch population in Generation 1 (>1 plant population increases, <1 plant population decreases). We used the nlme package in R (Pinheiro et al. 2009) to calculate the average slope of the relationship between the relative change in plant population (Gen. 2/Gen. 1) and the number of plants in each patch in Generation 1. Simulation was treated as a random effect. We used 95% confidence intervals to assess whether the slope was significantly different from zero. Positive slopes occur when large patches produce more offspring per plant than small patches (positive density dependence). Negative slopes indicate negative density dependence and occur when small patches produce more offspring per plant than large patches.

2.3.2. Observational study

Study system & sites

Diffuse knapweed (Centaurea diffusa Lam., Asteraceae) is a biennial or short-lived perennial invasive weed in North America from Colorado to British Columbia. In its second or third year, it produces up to 1500 seed-heads (median 60; this study) and dies after seed production. Here, we document the distribution of two common seed-head herbivores that oviposit in the seed-heads: Larinus minutus Gyllenhal (Col.: Curculionidae) and Urophora affinis Frauenfeld (Dip.: Tephritidae). Urophora affinis larvae form galls in the seed-head while L. minutus larvae eat the entire contents of the seed-head. Larinus minutus eliminates U. affinis when they co-occur in a seed-head (Crowe & Bourchier 2006). Neither species moves between seed-heads as larvae. The introduction of U. affinis was not associated with apparent changes in knapweed abundance, while the recent decline of diffuse knapweed has been attributed to L. minutus (Myers et al. 2009).
Our two study sites were in the southern Okanagan Valley of British Columbia, Canada – a meadow above Vaseux Lake (49°17’45.97”, -119°31’34.21”, 384 m asl) and a meadow near the White Lake Observatory (49°19’12.65”, -119°37’46.43”, 549 m asl). At both sites, diffuse knapweed has declined overall (Myers et al. 2009) but dense patches are still present, resulting in a wide distribution of patch sizes.

**Density measurements**

In July 2008, we chose 80 flowering plants at each of the two sites. To select plants we ran a series of arbitrarily selected transects along the site. Focal plants along transects were chosen by blindly taking 10–20 paces then identifying the nearest neighbour. This design ensured that we had a good representation of all density levels across the meadow. We measured the number of flowering plant stems in a 1 m² quadrat surrounding the focal plant.

We harvested half the focal plants in early August and the second half in mid-September, to determine test the predict of Kunin (1999) that an inferior competitor might alter their pattern with density to escape from a superior competitor. In August, seed-heads often contain both species. By September however, *L. minutus* had consumed *U. affinis* galls and larvae.

**Seed-head herbivores**

To determine the level of seed-head attack by the larvae, we assessed sufficient seed-heads on each plant to achieve an accuracy of 90% (E = 0.1) and confidence interval (Z) of 1.645 (90% confidence) for each plant under the binomial distribution with finite population sizes (Krebs 1999). In September, some insects had emerged but either their damage or gall remained. In these cases, we scored the species as present.
Data analysis

For seed-head herbivores, the appropriate measure of insect load is the number of insects per seed-head (although for other feeding guilds, other definitions are more appropriate, e.g. Otway et al. 2005). We counted every insect in the seed-heads. In some cases more than one insect per seed-head was present, i.e. insect load was greater than one. This occurred either because the seed-head contained multiple *U. affinis* galls (Harris 1980) or because several *L. minutus* (eggs or small larvae) were sometimes present in August, although by September only one was ever present. If seed-heads could support only one insect, insect load would equal the proportion of seed-heads infested.

We determined whether the *L. minutus* and *U. affinis* loads on a plant were related to plant density using Spearman’s rank correlation in R (R Development Core Team 2008) as data did not meet the assumptions of normality and homoscedascity.

As we calculated insect load on the plant rather than total number of insects in the patch, a positive slope in response to density is indicative of resource concentration, a negative slope indicates resource dilution, and slope of zero indicates a direct proportional response between insect and plant densities.

2.4 Results

2.4.1 Model

Variance in insect load and numbers increased with both the strength of resource concentration or resource dilution (Fig. 2.2). As expected, variance in load was lowest when insect load was directly proportional to plant density (i.e. curvature equals 1).

Both the number of plant patches and the total population declined over time following the introduction of insect herbivores with all insect distribution patterns (Figs. 2.3 & 2.4). Total population decline was greatest when insect pattern was either directly
proportional to plant density or followed a weak resource dilution pattern (Fig. 2.3). The reduction in the number of patches was greater under the direct proportional pattern and resource dilution (both weak and strong) than resource concentration; this difference became greater over time (Fig. 2.3) but at high resource concentration all patches remained after 30 generations when curvature was greater than 1.75. More extreme curvature values (i.e. increasing strength of the pattern) were associated with less reduction in the total population.

Persistence of the insect–plant system was greater with resource concentration (Fig. 2.4). When curvature equalled 2, all populations survived, so we did not run the model for curvatures of 2.5 or 3. Plant populations persisted longer as the curvature parameter increased from 1 or decreased from 0, (Fig. 2.4).

Resource concentration and dilution caused opposing patterns of plant density dependence (Fig. 2.5). Strong resource dilution caused positive density dependence as more plant offspring were produced in the larger plant patches leading to a positive relationship between rate of change in patch population and patch size. Resource concentration caused negative density dependence as more offspring per plant were produced in smaller patches. No density dependence in plant reproduction was evident when insects responded in a weak resource dilution or direct proportional pattern (i.e. slopes were not significantly different from zero, Fig. 2.5).

Varying the key parameters demonstrated that the differences we observed between the insect distribution patterns were not dependent on the parameters chosen. Resource concentration always lead to a higher total population, more plant patches and longer persistence times, while directly proportional and weak resource dilution patterns were associated with a lower plant population and shorter persistence times. When seedhead escape or plant reproduction was high (above 20% or 0.12 respectively) or insect impact low (below 0.93), insects did not affect plant populations regardless of the
pattern. In the short term, increasing insect impacts, decreasing seed-head escape or decreasing plant reproduction strengthened the effect of the different patterns (curves were steeper). In the long term, however, intermediate levels of these values had the strongest effect. Variation in insect reproductive capacity had relatively little effect on the plant population parameters. Full results are presented in Appendix A2.

2.4.2 Observational field study

*Larinus minutus* load was negatively corrected with the density of knapweed flowering stems at White Lake in August (Table 2.1), a pattern consistent with resource dilution. At Vaseux Lake and at White Lake in September, the number of *L. minutus* per plant was not related to plant density (Table 2.1).

*Urophora affinis* loads increased with increasing density of knapweed flowering stems in both months at White Lake and in August at Vaseux Lake (Table 2.1). In September at White Lake, no pattern with density was observed.

2.5 Discussion

The relationship between the density of insects and their host plants can influence the rate of decline of plant populations and the persistence of plant patches. We have shown that a resource concentration pattern stabilises the number of plant patches and slows the rate of population decline. Similarly, strong resource dilution can slow the rate of plant population decline although the number of patches decreases with time. In contrast, a weak resource dilution or direct proportional pattern of insect herbivores in response to plant density accelerates plant population decline.

The consistency of our overall findings with different parameter sets shows that this outcome is robust as long as the insects can reduce plant population size. The strength of this relationship increases as insect impact increases or overall plant
reproduction decreases (either via the number of seeds produced or the number of seed-heads that escape insect attack). Changes in insect reproduction have relatively little effect on the strength of the pattern as this is primarily limited by the number of available seed-heads.

We know of only two models that previously addressed the effects of spatial aggregation of herbivores on their host-plants, and neither explicitly tested the resource concentration hypothesis nor the effects of a resource dilution pattern (Myers 1976; Heard & Remer 2008). Myers’ (1976) model asked whether aggregated distributions of eggs could regulate insect populations, but it did not include variation in plant population density. She showed that clumping could stabilise the insect–plant system while over-dispersion could lead to over-exploitation and extinction. In Heard & Remer’s (2008) model, increased egg clumping in response to plant rarity led to inverse density dependence of plant reproduction. They concluded that the plant–insect system can be stabilised if insect oviposition responds to plant abundance so that eggs are aggregated on rare plants. Our results agree with these conclusions.

Both the models of Heard & Remer (2008) and Myers (1976) focused on how insect behaviour might result in different patterns of insect distribution. In contrast, we evaluate the effect of contrasting patterns of insect distribution on the host-plant population, regardless of the mechanism/s generating those patterns. Because the variance of insect load between patches increases with the levels of resource concentration or dilution, allowing some plants escape herbivore attack (Heard & Remer 2008), both resource concentration and dilution can reduce the extent of plant population decline.

These ideas have not been well-explored for any enemy–victim system, although Nicholson-Bailey host–parasitoid models have been used to investigate the impact of
aggregation behaviour of the consumer on the host (for reviews, see Briggs & Hoopes 2004 and Mills & Getz 1996). They do not however, explicitly compare resource concentration and resource dilution mechanisms. Resource dilution, in particular, is neglected (Briggs & Hoopes 2004) even though negative density dependence is common in host–parasitoid systems (approximately 25% of reviewed cases showed evidence of negative density dependence (Walde & Murdoch 1988)). Similar to our results these models show that increasing the level of host density-dependant aggregation stabilises populations (Rohani et al. 1994), and results in a trade-off between the persistence of the system and the ability of the parasitoid to suppress the density of the host (Mills & Getz 1996).

Our field results were consistent with the findings from the model. We found that the less effective biological control agent, *Urophora affinis*, followed a resource concentration pattern. In contrast, the more effective agent, *Larinus minutus* followed either a directly proportional or resource dilution pattern. In September at Vaseux Lake, no relationship existed between *U. affinis* load and plant density, and overall *U. affinis* numbers had declined, likely due to competition with *L. minutus*. This confirms Kunin’s (1999) view that competitive exclusion between insect species can alter the relationship of insects with plant density.

While our model uses the number of plants per patch as the density measure, in the field we had to use density in the area surrounding the focal plants as diffuse knapweed patches were impossible to delineate accurately. Overall, a discrepancy exists in the literature between observational studies that tend to measure plant density and experimental studies that manipulate patch size. Rhairds & English-Loeb (2003) manipulated both plant density and patch size in experiments with tarnished plant bug (*Lygus lineolaris*, Hem.: Miridae) on strawberry plants. Adult bugs responded to neither
patch size nor patch density, however, the nymphs responded positively to patch density in the first generation and to patch size in the subsequent two generations. This suggests that responses to the two variables are likely to be similar. Different insect species locate plants using different mechanisms (e.g. active searching vs. passive settlement). As such, we might expect that some settlement or oviposition behaviours may be sensitive to patch size while others are sensitive to plant density. Further comparisons between the responses to patch size and density are needed.

Differences in the rates of plant population decline will have community level implications: herbivorous insects may function as classic keystone species if they have strong top-down effects on their host plant (Carson et al. 2004). A reduction in a community dominant will result in an increase in other members of the plant community (Carson & Root 2000). However, as a resource dilution pattern will reduce smaller patches, leaving larger ones, it may not promote species co-existence to the same extent. Long et al. (2003) experimentally demonstrated that the specialist herbivore Trirhabda virgata (Col.: Chrysomelidae) responded in a resource concentration pattern to patches of its host, meadow goldenrod (Solidago altissima). Attack by the beetle led to a decline of the host and allowed other plants in the community to increase. The effect on dominant plants by specialist herbivores following a resource concentration pattern has never been experimentally compared with that of specialist herbivores following resource dilution patterns.

Our results suggest that the pattern of insect distribution with plant density will alter the extent to which patches of differing plant density will decline, and thus release non-dominant plant species from competition. Our study also has implications for weed biological control. The goal of weed biological control is to reduce populations to levels where they no longer require additional control (MacFadyen 1998). Complete
eradication of well-established invasive species is rarely successful (Myers et al. 2000) and there has been only one putative case of eradication via biological control (coconut moth in Fiji; Kuris 2003). Therefore, an ideal weed biological control agent would be one that can rapidly reduce the weed populations but that can also persist long-term. However, these attributes may trade-off. One way this could be achieved is if an agent had dual strategies whereby it follows a weak resource dilution or directly proportional pattern when plants are at relatively high densities, but alters its strategy to one of resource concentration at lower host plant densities. For example, Capman et al. (1990) showed that the response of the common sooty-winged skipper butterfly to patch size depended on whether the patches were at low or high densities. Egg clumping is a mechanism that could lead to resource concentration or dilution, and this has previously been suggested as a desirable trait of biological control agents (Myers 1976; Cappuccino 2000; Heard & Remer 2008). The effect of egg clumping on plant populations, however, is likely to depend on whether clumping is on high- or low-density hosts.

In conclusion, we show that response by insect herbivores to plant density can have major effects on the rate of decline and persistence of the host–plant populations (and, by extension, on the insect–plant system). Such effects are likely to influence the structure of plant communities and should be considered in the evaluation of potential weed biocontrol agents.
**Table 2.1:** Spearman’s rank correlation coefficient results of the relationship between the density of diffuse knapweed bolts and its seed-head herbivore load. RC = resource concentration and RD to resource dilution.

<table>
<thead>
<tr>
<th></th>
<th>Spearman’s rho</th>
<th>P-value</th>
<th>Pattern</th>
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<tbody>
<tr>
<td><strong>Larinus minutus load</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White Lake August 2008</td>
<td>-0.48</td>
<td>0.0016</td>
<td>RD</td>
</tr>
<tr>
<td>White Lake September 2008</td>
<td>-0.16</td>
<td>0.34</td>
<td>no pattern</td>
</tr>
<tr>
<td>Vaseux Lake August 2008</td>
<td>-0.097</td>
<td>0.56</td>
<td>no pattern</td>
</tr>
<tr>
<td>Vaseux Lake September 2008</td>
<td>0.045</td>
<td>0.79</td>
<td>no pattern</td>
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<tr>
<td><strong>Urophora affinis load</strong></td>
<td></td>
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</tr>
<tr>
<td>White Lake August 2008</td>
<td>0.41</td>
<td>0.0086</td>
<td>RC</td>
</tr>
<tr>
<td>White Lake September 2008</td>
<td>0.55</td>
<td>0.0004</td>
<td>RC</td>
</tr>
<tr>
<td>Vaseux Lake August 2008</td>
<td>0.58</td>
<td>0.0001</td>
<td>RC</td>
</tr>
<tr>
<td>Vaseux Lake September 2008</td>
<td>0.16</td>
<td>0.32</td>
<td>no pattern</td>
</tr>
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</table>
Figure 2.1 Patterns of insect distribution modelled: directly proportional (curvature $\theta = 1$) to plant population, resource concentration (curvature = 2) and resource dilution (weak: curvature = 0.5; strong: curvature = -0.1). The total number of insects (A) was calculated as described in the text and was the same under all three distributions. We then calculated the corresponding insect load (= insects per plant; B) by dividing the total number of insects by the number of plants in the patch.
Figure 2.2 Variance in insect load (insects per plant) and numbers (log) on plant patches following redistribution under different curvature parameters. Total plant population was 275 in 10 patches containing 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 plants respectively. The total number of insects distributed among the plants was 2750 (10 insects per plant when curvature = 1). When curvature = 1 there is no variation in the number of insects per plant in the different plant patches, and when curvature = 0 there is no variation in insect load across in the patches (variance = 0). Above the x-axis s.RD refers to strong resource dilution, w.RD to weak resource dilution, DP refers to the directly proportional pattern and RC to resource concentration.
Figure 2.3 Mean (± SD) total plant populations (top) and total number of plant patches (bottom) remaining after 10, 20 and 30 generations under a range of curvature parameters. The mean value of each run is represented by the closed black symbol, while each simulation (1000) is represented by the open grey symbol. Curvature parameter values >1 indicate resource concentration, =1 indicate a directly proportional relationship with plant density, 0–1 weak resource dilution and <0 strong resource dilution. Above the x-axis s.RD refers to strong resource dilution, w.RD to weak resource dilution, DP refers to the directly proportional pattern and RC to resource concentration.
Figure 2.4 Proportion of simulations where plant populations persisted beyond 1000 generations (left) and mean (± SD) time to extinction of runs where all populations became extinct (right). The mean value of each run is represented by the closed black symbol, while each simulation (1000) is represented by the open grey symbol; at a curvature of 2, extinction of the plant population occurred in one simulation only and no extinctions occurred when curvature equalled 2.5 or 3. Above the x-axis s.RD refers to strong resource dilution, w.RD to weak resource dilution, DP refers to the directly proportional pattern and RC to resource concentration.
Figure 2.5 Impact of the curvature value on density dependence of plant reproduction. Mean slope of relationship (with 95% confidence intervals) between the relative change in plant population (Gen. n+1/Gen. n) and the number of plants in each patch in Generation n is presented. A positive slope refers to positive density dependence (plants in larger patches produce more offspring) while negative slopes indicate negative density dependence. If the slope does not differ from zero, no density dependence was observed. Above the x-axis s.RD refers to strong resource dilution, w.RD to weak resource dilution, DP refers to the directly proportional pattern and RC to resource concentration.
CHAPTER THREE: INDEPENDENT INFLUENCES OF TWO LIFE-STAGES OF THE WEEVIL, *LARINUS MINUTUS*, ON ITS HOST-PLANT *CENTAUREA DIFFUSA*.

3.1 Summary

Adults and larvae of the same species feeding in different ways on the same host-plant might have the similar impacts as the feeding of two separate species. We tested the impacts of the two feeding stages of *Larinus minutus*, a successful biological control agent of diffuse knapweed, *Centaurea diffusa*, in the southern Okanagan of British Columbia, Canada.

At one site, observational associations between adult and larvae damage were recorded, but no association between the damage types was found. We experimentally assessed plant-mediated competition between the life-history stages but found no evidence for it. At two sites and in two years we manipulated adult and/or larval herbivory and measured the impact of the different types of herbivory on the plant. We found no evidence for synergistic or antagonistic interactions between the life-history stages, but a different life-history stages had no impact at each site. This could be particularly important in biological weed control where the use of one species with two feeding modes could potentially increase the damage to plants without the costs and risks inherent in introducing a second agent. This apparent redundancy of different feeding modes may lead to reduced plant performance in a wider range of environments than insects with only one feeding mode.
3.2 Introduction

The multiple or cumulative stress hypothesis proposes that the more natural enemies inflicting stress to a plant, the greater the likelihood that the stress level will reach a threshold at which the plant will succumb and the population decline (Harris 1981; van Driesche et al. 2008). It remains controversial however as to whether multiple species are more effective than single species for successful control (Denoth et al. 2002). Despite this, McEvoy & Coombs (1999) extend the cumulative stress hypothesis to ask not only how many species, but also how many different feeding guilds are required for reduction of host-plant densities, and that attacking plant parts can be very different from attacking life-cycle transitions. For some insect species, particularly beetles, adults and larvae feed on different plant parts and/or at different times. Crawley (1989a) notes that the Coleoptera, which tend to have multiple feeding modes, are responsible for the largest number of weed biocontrol successes. Thus it is of interest whether a single species that attacks the host-plant in more than one way can be more effective than species for which only a single life-stage attacks the plant. The intra-specific interactions between multiple feeding modes of a single species might be similar to the interactions between different species such as a leaf-feeder and a root-feeder. Alternatively, the impact on a plant following a particular type of damage might depend on environmental conditions; for example root feeding can be more damaging in conditions of water-stress (Zvereva & Kozlov 2012). Therefore multiple feeding modes may increase the stress to the host-plant under a wider range of environmental conditions.

A plant’s response to one natural enemy may alter the frequency or intensity of its interaction with a second species. A range of mechanisms might influence these interactions. For example, Masters et al. (1993) suggested that root feeding might promote foliar feeders, as the removal of root biomass reduces water absorption and
stresses the plant thus improving food quality for foliar feeders as water stress can lead to the accumulation of amino acids and carbohydrates within the leaves. An opposing example is that of Kaplan et al. (2007) who found that potato leaf-hopper feeding lengthened the development time of Colorado potato beetles on the same plant, thus increased their susceptibility to predation and reduced the beetle population. Overall, plant-mediated interactions between insects sharing a host might be positive (e.g. Juenger & Bergelson 1998), negative (e.g. Hufbauer & Root 2002, James et al. 1992) or neutral (e.g. Gerber et al. 2007) for the plant. Fournier et al. (2006) hypothesised that if one natural enemy facilitates another, overall plant damage could be greater than predicted from the effects of the natural enemies alone. Alternatively if the natural enemies interfere with each other, the damage to the plant might be reduced. To evaluate the overall impact of the two natural enemies on a plant, we must evaluate the interactions between both natural enemy species and their shared host plant (Swope & Parker 2010).

Similar interactions may occur intra-specifically when different life-history stages of the same species feed on different parts of the host-plant or at different times. Kaplan & Denno (2007) showed that intra-specific competition between insects can be as strong as inter-specific competition. However, as different life-history stages more consistently share a host plant, selection might favour resistance or tolerance of the later-feeding stage to defences induced by the plant in response to the earlier-feeding stages.

An example of an insect species in which two life stages attack the host plant is the weevil, Larinus minutus Gyllenhall (Col.: Curculionidae) that is associated with the successful control of diffuse knapweed, Centaurea diffusa Lam. (Asteraceae) (Myers et al. 2009). From spring to mid-summer, adult L. minutus feed on vegetative parts of knapweed plants. This feeding damage can be severe and may kill plants, particularly in dry years. When plants flower in mid-late July, the weevils oviposit in the flower-heads.
Larval feeding on maturing achenes prevents seed production by that flower-head. Therefore, in this situation the early feeding by adult beetles could potentially reduce, or alternatively improve, the quality of the plant for larvae through a compensatory response. We tested the hypothesis that interactions between two life-history stages would be similar to interactions between two different species. First, we carried out observations on the association of adult and larval damage, then manipulated either adult herbivory or both larval and adult herbivory and measured insect and plant responses. These experiments were done at two sites where knapweed densities have declined following the introduction of *L. minutus* and one site where no plant population decline has been observed in the presence of *L. minutus*. We tested three specific hypotheses:

H1. **Adult weevil feeding damage to the plant would:**

H1a: reduce plant quality which would lead to

H1.B: reduced oviposition in seed-heads with high levels of adult herbivory, thus

H1.C: fewer subsequent larvae.

H2. **The reductions in plant performance due to adult and larval feeding would be independent of each other.** Adult feeding does not change the frequency or intensity of larval feeding.

H3. **The reduction of knapweed performance due to both adult and larval feeding will be greater at the site (White Lake) that has experienced the greatest plant population decline than at Anarchist Mountain where the plant population has not declined.**
3.3 Methods

3.3.1 Study sites

The recent decline of diffuse knapweed in the low elevation southern Okanagan of British Columbia has been attributed to *L. minutus* (Myers et al. 2009) and is typified at two of our study sites, a meadow above Vaseux Lake (49°18’02", -119°31’48", 350m asl) and a meadow near the White Lake Observatory (49°19’13", -119°37’46", 550m asl). At our third site, a pasture near the summit of Anarchist Mountain (49°00’50", -119°16’57", 1115m asl), knapweed has not declined following the introduction of *L. minutus* (Myers et al. 2009).

3.3.1 Observational study

To evaluate the impact of feeding by *L. minutus* adults on diffuse knapweed, 200 bolted plants were tagged and their height measured in mid-June (14-15th) 2008 at Vaseux Lake. In July, towards the end of flowering, adult *L. minutus* feeding damage was assessed on the plants using a four-point scale (Table 3.1). All plants had some damage (1 was the lowest score recorded).

In September, we harvested 189 of the 200 plants (11 plants lost their tags and/or died), counted the number of seed-heads on each plant, assessed seed-heads for the presence and developmental state of *L. minutus* (egg, larvae, pupae, adult or characteristic damage indicating beetle emergence). We assessed sufficient seed-heads to achieve an accuracy of ± 10% with 90% confidence intervals (precision) under the binomial distribution with a finite population size (Krebs 1999). We recorded plant height and number of seed-heads to determine whether the level of *L. minutus* damage was correlated with changes in growth rate or reproduction measured as the number of seed-heads produced.
3.3.2 Impact of adult *L. minutus* herbivory on larvae

To manipulate adult herbivory levels, we covered the entire plant in a nylon-tulle bag (90cm h * 45cm w). The bags were tied around the base of the plant so that *L. minutus* adults could not enter and excess fabric was nailed into the ground (Fig. 1 – treatments with triangles). Bagging was done on 29-31 May 2009 prior to emergence of *L. minutus* from its over-wintering refuge. Height and number of stems of all plants were recorded.

We established the herbivory treatments when reasonable numbers of *L. minutus* had emerged (16-17 June 2009). Treatments were randomly assigned but we ensured that there were no differences in initial height and number of stems between treatments (height: F<sub>2,72</sub> = 1.99, p = 0.15; no. stems: F<sub>2,72</sub> = 0.48, p = 0.62). The three treatments were: 1) high herbivory in which we added eight *L. minutus* adults per stem into each bag; 2) low herbivory with two adults per stem; and 3) no herbivory (control). We assessed the plants for actual levels of herbivory by *L. minutus* on 9 and 29 July, and 17 August 2009, levels of herbivory were consistent with levels found in the field.

As plants started to flower (9 July 2009), we exposed an arbitrary subset of the flowers on the plant to *L. minutus* adults. To do this, we cut small holes in the bags and poked through a stem containing a cluster of flowers, then tied off the flowers to prevent weevil movement in and out of the bag. Once flowering was complete (17-18 August 2009), we placed pieces of nylon tulle over the seed-head to catch insects that had developed in the seed head and were emerging. We missed insects that emerged very early, but earlier bagging would have prevented the oviposition of late eggs. The seed-heads were bagged until 17 September at which time the insects were collected and seed-heads brought in the lab to allow any additional insects to emerge.
Once weevil emergence from seed-heads had ceased, we dissected up to 50 seed-heads per plant and recorded whether the seed-head had been infested.

The femur and tibia on the right hind leg of each insect that emerged from the bag was measured as they correlate with overall body size, hence fecundity (Honěk 1993). We measured the femur and tibia length of the right hind leg of each insect twice and took the average of the two measurements. Legs were measured using the program LASmontage (Leica Microsystems) and a Leica M205C microscope with a Leica DFC490 camera attached. We conducted a repeatability assessment by re-measuring approximately 10% of the legs at a later date.

3.3.3. Impact of adult vs larval L. minutus on diffuse knapweed experiments

Experiment 1: White Lake 2009.

To determine the relative effects of stem herbivory versus seed-head herbivory inflicted on diffuse knapweed by L. minutus, we separated adult attack from larval attack using bagging treatments that exploited differences in phenology between the two feeding modes in a 2×2 factorial design. Treatments (16 replicates of each) were: 1) adult L. minutus only; 2) larval L. minutus only; 3) both adults and larvae and 4) control with no insects present (Fig. 3.1, top row).

On 29 May 2009, we measured the height, then bagged 64 single-stemmed plants (16 per treatment) prior to L. minutus emergence from its overwintering refuge. The entire plant was covered in a nylon tulle bag of 90 cm h × 45 cm w and fastened to the base of the plant. Excess fabric was nailed into the ground around the plants.

Four adult L. minutus were added to the bags encasing plants allocated to adult herbivory treatments on 17 June 2009.
Just prior to flowering, we poked clusters of flowers through small holes cut in the bags on plants allotted to the larval damage treatment. These flowers were available to female beetles for oviposition, Fig. 3.1. We tied off the base of the flower cluster to prevent beetle movement in and out of the bag. For plants allotted to the adult-only treatment flowers were bagged independently from the rest of the plant. Exposure and bagging of flower clusters was conducted on 7 and 8 July 2009. Enclosed flowers of all plants were hand pollinated on 20 and 29 July. The level of *L. minutus* damage to the stem was recorded at this time. Plants were harvested on 16 September 2009. In this and all subsequent experiments, the levels of adult and larval damage were within the range observed in the field.

**Experiment 2: White Lake 2010.**

The experimental design was similar to Experiment 1, but the plants were double-bagged with an inner bag constraining the adults (in adult feeding treatments) feeding on stems, flower clusters were poked through this inner bag (Fig. 3.1, bottom row). An outer bag covered the plant, such that the flower clusters were between the inner and outer bags. Ovipositing weevils (in larval treatments) were then placed between the inner and outer bags. All treatments only received pollen via hand-pollination unlike in Experiment 1. Unfortunately, adult *L. minutus* were able to leave the inner bag and travel to the outer one but not move in the opposite direction. Our final treatments (and number of replicates) were 1. control – both life-history stages absent (n=11); 2. adults absent, larvae present (n=11); and 3. both adults and larvae present (n=22).

Plant height was measured and plants were bagged on 12 May, four adult *L. minutus* were added to the treatment bags on 20 May and again on 3 June (eight insects total). The two addition dates were to simulate the increasing numbers of insects that emerge from the soil over this period. Damage to the stem was regularly assessed throughout the season.
Flowers were exposed to larval \textit{L. minutus} damage from 29 July when six additional insects were added to the outer bag. Flowers were hand-pollinated on 29 July and 9 August. Plants were harvested on 7 September 2010.

\textit{Experiment 3: Anarchist Mountain 2010.}

This experiment was set up in the same manner as the experiments at White Lake with four treatments, Fig. 1: 1) adult \textit{L. minutus} only; 2) larval \textit{L. minutus} only; 3) both adults and larvae and 4) control with no insects present. The bagging method was the same as in Experiment 2 (White Lake 2010), but here the exclusion of larvae was successful. There were 15 replicates per treatment, however, one plant in the larvae-only treatment disappeared.

Plants were bagged and plant height measured on 3 June 2010, prior to \textit{L. minutus} emergence that occurs later at this site due to the higher elevation. Adult \textit{L. minutus} were added to the bags on 16 June and 3 July (four at each time point). The insects for the 16 June addition came from Vaseux Lake due to cold temperatures and heavy rain at Anarchist Mountain.

Weevils for oviposition were added to the larval treatment bags on 29 July. We hand-pollinated flowers on 29 July and 10 August, and harvested plants on 8 September 2010.

\textit{Response variables}

For Experiments 2 and 3, we measured the final height of the plant to calculate growth. We counted the number of fully developed seed-heads from each plant for all three experiments and assessed the number of viable seeds and presence of weevils in 25 seed-heads. We extrapolated from the 25 seed-heads assessed to determine the number of seeds produced by the plant. We also measured the width of the 25 seed-heads to
determine seed-head size, which gives an indication of potential seed-head level reproduction prior to pollination and *L. minutus* larval damage.

### 3.3.4 Data analysis

All data were analysed using R (R Development Core Team 2009).

*Observational study*

The level of damage to the plants was treated as an ordered categorical factor in all analyses. We recorded plant height at the beginning and end of the observational period and calculated the growth of the plant and the number of seed-heads produced by plants that experienced different levels of adult herbivory. Growth was calculated as:

\[
\frac{(\text{Plant height final} - \text{Plant height initial})}{\text{Plant height initial}}
\]

in all analyses. Growth was analysed using linear regression. We assessed if initial plant height (square-root transformed) was a predictor of *L. minutus* damage. We counted the number of developed seed-heads and assessed whether this was influenced by the level of adult damage (none, low, medium or high) using (log-transformed) ANOVA. Initial plant height was included as a co-variate since plant height is a key influence on the number of seed-heads produced, and *L. minutus* preferentially attacked larger plants (see 3.5 Results).

We assessed the proportion of seed-heads infested by *L. minutus* in relation to the level of adult *L. minutus* damage that occurred in July (at flowering) with ANOVA.

We then assessed whether fewer second generation *L. minutus* on more heavily attacked plants reached adulthood. We conducted a Spearman’s correlation co-efficient (data did not meet the assumptions of ANOVA and could not be transformed to do so) to determine if there was a correlation between the proportion of insects that had completed development by September and the level of damage.
Adult impact on larvae

We used ANOVA to test whether there were differences in the number of larval *L. minutus* on plants with varying levels of adult damage (categorical factor of none, low or high).

Repeatability of the leg length measurements was done following Whitlock & Schuler (2009). As repeatability of the tibia measurement was higher, we used this measurement in all subsequent analyses. The size for each individual was based on the mean of the two leg measurements and the mean size of all individuals collected from a plant was determined (plant is the unit of replication). From these data, we used ANOVA to determine if the mean tibia length of the insects varied with different levels of adult damage to the plant from which the offspring adults emerged.

Impact on plants: Experiments 1 and 3

We tested whether the response variables were affected by the presence of larval and adult *L. minutus* using the multiplicative model (Sih et al. 1998; Fournier et al. 2006). We conducted GLMs using either the Gaussian (normal) distribution and log-transformation of the response variables, or Poisson distribution with a log-link function. Log-transformation had the additional advantage of reducing heteroscedasticity and non-normality.

We graphed the log-response ratio (a commonly used metric for effect size) with 95% confidence intervals for each response. Log-response ratios were calculated following Morris et al. (2007) who adapted the log-response ratios of Hedges et al. (1999) for factorial experiments.

Impact on plants: Experiment 2

Due to the loss of the adult only treatments, we used ANOVA to determine if there were differences between the three treatments, and then used *a priori* contrasts to
compare treatments of interest. First, we compared the control treatment with the other two treatments to test for larval impact. We then compared treatment 2 (adults absent, larvae present) with treatment 3 (adults present, larvae present) to test for adult impact. We calculated log response ratios for this experiment following Hedges et al. (1999).

3.5 Results

3.5.1 Observational study.

Adult *L. minutus* preferentially attacked plants that were larger at the beginning of the growing season (ANOVA with square root transformation, $F_{2, 198} = 21.43, p < 0.001$), but feeding reduced plant growth (Fig. 3.2, $F_{2, 179} = 9.05, p = 0.0002$).

The number of seed-heads on plants did not differ with the level of adult *L. minutus* damage after adjusting for plant height as a co-variate (Height $F_{1, 186} = 98.34, p < 0.0001$, *L. minutus* herbivory level $F_{2, 186} = 2.04, p = 0.13$).

The level of adult feeding damage did not affect the number of *L. minutus* larvae in seed-heads in July (Fig. 3.2; $F_{2, 186} = 1.28; p = 0.28$), nor did it alter the proportion of offspring that had reached adulthood by September (Fig. 3.2; Spearman’s rho = 0.055; $p = 0.47$).

3.5.2 Impact of adult *L. minutus* feeding damage on larvae

The frequency of *L. minutus* larval damage did not vary with adult damage. Adult damage did not influence the percentage of seed heads attacked by larvae (mean ± SEM - High = 63.8% ± 2.8, low = 67.0% ± 2.5, control = 69.3% ± 2.8; $F_{2, 70} = 1.05, p = 0.35$).

The mean size of weevils emerging from seed heads also did not differ with levels of adult herbivory as indicated by the mean tibia length (mean ± SEM - High = 851 µm ±
22.7, low = 852 μm ± 13.9, control = 860.6 μm ± 17.2; F_{2,64} = 0.083, p = 0.92). Repeatability of the tibia measurement was 88% (or 12% measurement error).

3.5.3 Impact of adult vs larval L. minutus on diffuse knapweed in experiments.

Overall there was no evidence for synergistic or antagonistic interactions between adult and larval L. minutus on diffuse knapweed plants (Table 3.2, Fig. 3.3) although in all experiments one of the two stages had no significant impact on the plant. In two of three experiments (White Lake 2010 and Anarchist Mountain) adults reduced the number of developed seed-heads on the plant (Tables 3.2 & 3.3). At Anarchist Mountain, this translated into a significantly reduced number of seeds per plant but that was not the case at White Lake (Fig. 3.3).

At White Lake, larvae had a greater impact than the adults on the number of seeds per plant. This was primarily achieved through a reduction in the number of seeds per seed-head (Tables 3.2 & 3.3; Fig. 3.3).

Neither adults nor larvae had any impact on the size of the seed-heads in any of the experiments (Fig. 3.3, Table 3.2 & 3.3).

3.6 Discussion

Larinus minutus reduced seed production at both sites but a different life-history stage was responsible for this reduction at each site. Adult feeding was associated with reduced plant growth in the observational study at Vaseux Lake. At White Lake in 2010, the number of seed-heads produced by the plants was significantly reduced by adult feeding. The reduction in the number of seed-heads was only marginally significant at Anarchist Mountain but led to a significant decrease of seeds per plant there. This reduction in seeds per plant due to adult reduction in the number of seed-heads, did not
occur at White Lake. Larval feeding reduced the number of seeds produced per plant at White Lake, but had no effect at Anarchist Mountain. Neither adult nor larval feeding affected the size of the seed-heads.

The presence of one natural enemy on a plant may alter the frequency and/or feeding intensity of another species on the plant. We hypothesised that adult feeding damage would reduce the plant quality and subsequent larval development and feeding damage. This hypothesis was rejected: the presence of adult *L. minutus* on the plant did not alter the number of seed-heads attacked by larval *L. minutus* (oviposition rates did not differ) and the growth of larvae, indicated by the size they attained as adults. This suggests that adult feeding either caused no induced chemical changes to the plant, or those changes did not affect the larvae.

We predicted that the overall effects of both life-history stages on the plant would be independent of each other. We did not find any evidence for synergistic nor antagonistic interactions: the interaction terms in Experiments 1 and 3 were non-significant. However many of the variables measured were non-significant, particularly for the adult stage. This lack of response to herbivore attack is common (although our ability to detect an effect is dependent on our statistical power). For example, root herbivory had no effect on any of the eight biomass and reproductive traits measured on *Sinapis arvensis* and leaf herbivores only reduced flowering time and anther length (Poveda *et al.* 2005b). Gerber *et al.* (2007) found that the shoot herbivore *Ceutorhynchus alliariae* affected only shoot height of *Alliaria petiolata* while the root herbivore *C. scrobicollis* was detrimental to four of the five traits measured. This lack of response may suggest that insect densities were not sufficiently high to damage the plant, i.e. the plant was able to compensate for the damage caused, however, Gerber *et al.* (2007) did not find increasing damage with increasing density which they ascribed to strong intraspecific competition.
We predicted that the impact of the insects would be greater at White Lake as plant population decline has been greater here; comparison of the effect sizes (Fig. 3.3) between the two sites shows that this was not confirmed. The differences between the two sites, and the two years at White Lake highlight that insect-plant interactions vary with environmental conditions – the differences at White Lake are likely driven by greater rainfall in 2010 (total rainfall May-July: 2009 – 85mm; 2010 – 139mm; Environmental Canada Penticton A weather station). Larval impacts were greatest at White Lake at the moister, higher elevation Anarchist Mountain, adults had a greater influence on overall seed output. A mechanism to account for this is not known.

One caveat of many herbivory experiments is there may have been some side effects of the cage treatments on both the plants and the insects.

It has been suggested that an advantage of introducing con-generics is that each species would be successful in slightly different environments, thus providing control in a greater range of environmental conditions (Jackson & Myers 2008). Our results suggest that two feeding modes might act in a similar manner. Damage by insects is often context-dependant and strongly interacts with resource levels (Hawkes & Sullivan 2001; Zvereva & Kozlov 2012). However, the performance of different guilds of herbivores varies under conditions of water stress (Huberty & Denno 2004). Huberty & Denno’s (2004) meta-analysis found that overall performance of sap-feeders on water-stressed plants was worse while the overall performance of borers was better and leaf miner performance was neutral with respect to water-stress. If a species consists of two feeding guilds that do better under different conditions (i.e. have slightly different niches), we might expect that they are able to damage the plant under a great range of environmental conditions. An additional impact of adult beetles that was not monitored in this study is the feeding of adults on rosettes and seedlings. This could contribute to
the negative impact of the adults on the plants and would most likely be independent from larval damage to seed production.

Overall, we found that the impact of two different feeding stages of *L. minutus* was similar to that of two different species. We were unable to find any examples in the literature where both a stem/leaf feeding species and a seed feeding species were manipulated and plant performance was measured (but see Hoffman & Moran (1998) for a comparison of a stem weevil and a flower-feeding weevil at the population level). However, Hladun & Alder (2009) and Barber *et al.* (2011) looked at the impacts of adult (above-ground) and larval feeding (below-ground) of *Acalymma vittatum* on *Cucurbita moschata* and *Cucumis sativus* respectively. If we compare their results to studies where different insect species fed above and belowground (e.g. Poveda *et al.* 2003, 2005a,b; Hunt-Joshi *et al.* 2004; Gerber *et al.* 2007), their studies indicate that feeding damage by two life-history stages is no different than that done by different species. In a majority of cases, there was no evidence for synergistic or antagonistic effects but for many variables one or both herbivores had no effect.

The importation of each additional weed biological control agent presents a certain level of risk and there is a financial cost involved in host-plant testing, rearing, release and distribution of additional species. While the benefits of weed biological control are considered to outweigh the risks and generally show acceptable financial returns (McFadyen 1998), reducing both the expense and the risk is optimal. Use of species that feed both as adults and larvae may reduce both the financial cost and also the risks to non-target plants and the risk of unexpected ecological consequences (e.g. Pearson & Callaway 2006) when compared to the importation of two biological control agents.
Table 3.1 Level of adult *Larinus minutus* adult damage in diffuse knapweed scored in the observational study

<table>
<thead>
<tr>
<th>Score</th>
<th>Damage status</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No <em>Larinus minutus</em> adult damage</td>
</tr>
<tr>
<td>1</td>
<td>Damage low, consists of nibbles to the stems</td>
</tr>
<tr>
<td>2</td>
<td>Obvious feeding on stems but less than 50% of stem attacked</td>
</tr>
<tr>
<td>3</td>
<td>Severe attack, more than 50% of stem attacked</td>
</tr>
</tbody>
</table>
Table 3.2 ANOVA results from the factorial experiments at White Lake in 2009 and Anarchist Mountain in 2010. Growth was not measured at White Lake in 2009. Symbols in front of the F statistic indicate statistical functions used: * = Gaussian distribution with logged transformed variables; ¶ = Poisson distribution with log-link function; and § = Quasipoisson with log-link function

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Experiment 1 White Lake 2009</th>
<th>Experiment 3 Anarchist Mountain 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult Larva Adult x larval</td>
<td>Adult Larval Adult x larval</td>
</tr>
<tr>
<td>Growth</td>
<td>* F_{1,59} = 0.07; ( p = 0.79 )</td>
<td>* F_{1,50} = 0.02; ( p = 0.89 )</td>
</tr>
<tr>
<td></td>
<td>* F_{1,59} = 3.0; ( p = 0.087 )</td>
<td>* F_{1,50} = 0.07; ( p = 0.79 )</td>
</tr>
<tr>
<td></td>
<td>* F_{1,59} = 0.055; ( p = 0.82 )</td>
<td>* F_{1,50} = 0.003; ( p = 0.96 )</td>
</tr>
<tr>
<td>No developed seed-heads</td>
<td>* F_{1,59} = 0.07; ( p = 0.79 )</td>
<td>* F_{1,55} = 3.5; ( p = 0.07 )</td>
</tr>
<tr>
<td></td>
<td>* F_{1,59} = 0.087</td>
<td>* F_{1,55} = 0.32; ( p = 0.10 )</td>
</tr>
<tr>
<td></td>
<td>§ F_{1,53} = 3.7; ( p = 0.057 )</td>
<td>§ F_{1,55} = 2.8; ( p = 0.10 )</td>
</tr>
<tr>
<td>Mean SH size</td>
<td>* F_{1,59} = 0.08; ( p = 0.78 )</td>
<td>* F_{1,55} = 0.75; ( p = 0.39 )</td>
</tr>
<tr>
<td></td>
<td>* F_{1,59} = 3.8; ( p = 0.54 )</td>
<td>§ F_{1,55} = 8.1; ( p = 0.007 )</td>
</tr>
<tr>
<td></td>
<td>* F_{1,59} = 3.7; ( p = 0.057 )</td>
<td>§ F_{1,55} = 1.89; ( p = 0.94 )</td>
</tr>
<tr>
<td>Seeds per seed-head</td>
<td>* F_{1,59} = 0.80; ( p = 0.38 )</td>
<td>§ F_{1,53} = 0.59; ( p = 0.44 )</td>
</tr>
<tr>
<td></td>
<td>* F_{1,59} = 4.0; ( p = 0.051 )</td>
<td>¶ F_{1,53} = 1.9; ( p = 0.17 )</td>
</tr>
<tr>
<td></td>
<td>* F_{1,59} = 1.1; ( p = 0.30 )</td>
<td>¶ F_{1,53} = 0.80; ( p = 0.44 )</td>
</tr>
<tr>
<td>Seeds per plant</td>
<td>* F_{1,59} = 0.58; ( p = 0.45 )</td>
<td>§ F_{1,53} = 8.1; ( p = 0.006 )</td>
</tr>
<tr>
<td></td>
<td>* F_{1,59} = 6.4; ( p = 0.014 )</td>
<td>§ F_{1,53} = 1.89; ( p = 0.17 )</td>
</tr>
<tr>
<td></td>
<td>* F_{1,59} = 0.74; ( p = 0.39 )</td>
<td>§ F_{1,53} = 0.007; ( p = 0.94 )</td>
</tr>
</tbody>
</table>
Table 3.3 ANOVA results from the 2010 White Lake experiment. Treatments were A. no Larinus minutus present of either stage; B. adults absent, larvae present; C. both life-history stages present. The *a priori* contrasts compare A with B+C to measure the effects of larvae, and B with C to measure the effect of the adults.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Treatments</th>
<th>Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Presence of larvae</td>
<td>Presence of adults</td>
</tr>
<tr>
<td>Growth</td>
<td>$F_{2,46} = 0.033, \ p = 0.97$</td>
<td>$F_{1,46} = 0.05, \ p = 0.82$</td>
</tr>
<tr>
<td>No developed seed-heads</td>
<td>$F_{2,46} = 3.64, \ p = 0.034$</td>
<td>$F_{1,46} = 0.9, \ p = 0.35$</td>
</tr>
<tr>
<td>Mean SH size</td>
<td>$F_{2,46} = 1.04, \ p = 0.36$</td>
<td>$F_{1,46} = 0.6, \ p = 0.44$</td>
</tr>
<tr>
<td>Seeds per seed-head</td>
<td>$F_{2,46} = 4.71, \ p = 0.14$</td>
<td>$F_{1,46} = 7.69, \ p = 0.008$</td>
</tr>
<tr>
<td>Seeds per plant</td>
<td>$F_{2,46} = 7.36, \ p = 0.002$</td>
<td>$F_{1,46} = 14.72, \ p = 0.0004$</td>
</tr>
</tbody>
</table>
Figure 3.1 Experimental set up at White Lake in 2009 (Experiment 1) and at White Lake and Anarchist Mountain in 2010 (Experiments 2 and 3). The star indicates the treatment absent in Experiment 2. The treatments indicated by the triangles provide an idea of the set up used in Experiment 4 at Vaseux Lake.
Figure 3.2 The relationship between the level of damage on the plant caused by adult *L. minutus* in the observational study at Vaseux Lake and (left) mean (± SEM) plant growth during the growing season; (centre) the proportion of seed-heads with larval *L. minutus* damage; and (right) the mean proportion of the second generation *L. minutus* that reached adulthood by September. Growth was calculated as (Plant height final – Plant height initial) / Plant height initial
Figure 3.3 Effect sizes for each measured response from the experiments at White Lake and Anarchist Mountain. A: Growth of plants, B: No. of seed-heads developed, C: seed-head size, D: seeds per seed-head, E: seeds per plant. Log response ratios for the factorial experiments (White Lake 2009 and Anarchist Mountain) were calculated following Morris et al. (2007), and following Hedges, Gurevitch & Curtis (1999) for the White Lake
2010 experiment. For the main effects, if the confidence intervals cross zero, there is no impact of that treatment on the measured response. If the mean and confidence intervals are above zero, the plant’s response is positive (plant performance has improved), if below zero, significant damage to the plant has occurred. For the interactions (A x L), if the response ratio is greater than zero then more damage has occurred than predicted; below zero, less damage has occurred than predicted by the main effects.
CHAPTER FOUR: PREDICTING THE EFFECT OF MULTIPLE NATURAL ENEMIES ON PLANT: A META-ANALYSIS

4.1 Summary

Although plants are attacked by a variety of insects and pathogens that can reduce their performance, there is relatively little understanding of how multiple enemies interact. If natural enemies operate independently, the total influence on growth and reproduction can be predicted from the impact of each alone. If the reductions are non-independent then the plant will do either better or worse than expected from that predicted by reductions in plant performance caused by each enemy alone. Here we review studies that measured the performance of plants attacked by multiple enemies to better understand the overall effect multiple enemies have on their host plant and to evaluate the frequency and occurrence of non-independent effects.

Our vote counting analysis showed that three quarters of plant responses were independent, in most of the remainder plant performance was less than predicted from each natural enemy alone. Results from the meta-analysis were similar and showed that on average reductions in plant performance were independent. Multiple attack reduced plant performance less than expected when (1) involved direct interactions (2) enemies attacked fruits and flowers, (2) were Dipterans, and (4) involved host plants in the family Asteraceae or (5) involved weedy plants. However, once statistical associations between these variables was accounted for, only interaction type and attack to reproductive plant parts remained significant.
In weed biological control, multiple natural enemies are often introduced to reduce host plant density. Interactions between the agents may influence plant performance thus overall control. Understanding of what types of agent combinations interact to either increase or decrease plant performance will improve the selection of effective agents, thus biological control success.

4.2 Introduction

How plants cope with multiple natural enemies has major implications for plant populations and communities as during their lifetime almost all plants will be attacked by multiple natural enemies (e.g. Thompson 1998) that can reduce their survival, growth and reproduction (Maron & Crone 2006). The role of interactions among these multiple enemies in determining the total impact on plants is not well understood (Strauss & Irwin 2004). Fournier et al. (2006) suggested that if one enemy facilitates a second, combined attack should reduce plant performance more than predicted from the sum of individual attacks, such that the plant performance is worse than expected. This might occur if attack by the first species improved the nutritional quality of the plant (e.g. Johnson et al. 2009) or caused changes to plant phenology leading to increased attack by the second species (e.g. Masters et al. 2001). However, if one natural enemy has a negative effect on the second, then their combined exploitation is predicted to be less than the sum of their individual effects and plant performance is better than expected. This could be due to plant defences induced in response to the first species that have a negative effect on the second (e.g. Bezemer et al. 2003), through interference competition (e.g. Juenger & Bergelson 1998), or via changes in plant phenology that reduce attack by the second (e.g. Milbrath & Nechols 2004). As negative interactions appear to be the norm for phytophagous insects (Kaplan & Denno 2007), we might
expect a prevalence of cases in which reductions in plant performance is less than expected from the sum of the reductions caused by attack of individual species. Negative interactions may also occur between arthropods and plant pathogens (e.g. Fournier et al. 2006), and between plant pathogens (e.g. Power 1996).

How plants cope with multiple natural enemies is of particular interest in weed biological control where the goal is to reduce populations of the target weed. In weed biological control, multiple agents are often introduced to control a single weed (Denoth et al. 2002), and agents must interact with generalist species. There are two broad rationales for introducing multiple biological control agents (Swope & Parker 2010). First, the lottery model predicts that the more biological control agents introduced, the more likely it is that a successful agent will be included, which is supported by the observation that successful control is generally attributable to one agent (Denoth et al. 2002). Second, the cumulative stress hypothesis predicts that the impact on the plant will be greater with more agents, as small amounts of damage by individual species accumulate. If multiple biological control agents are introduced, it would be optimal to import agents that will interact synergistically (greater than expected reduction in plant performance) and suboptimal to import strongly antagonistic agents. McEvoy & Coombs (1999) suggested that negative interactions between agents might be a key reason for the occurrence of “revenge effects” where control is made less effective or more risky. Biological control agents must also interact with native natural enemies, ideally to minimise plant performance. If we were able to predict which types of agents interact to promote the impact on the weed, we may be better able to select appropriate species for biological control introductions.

Two studies have previously collated evidence for non-independent effects of natural enemies on plant performance (Morris et al. 2007; Hatcher 1995) and reached
opposing conclusions. The meta-analysis by Morris et al. (2007) found high levels of variance among studies (but did not attempt to explain this) but overall natural enemies had independent impacts on plant performance. On the other hand, the vote-counting study of Hatcher (1995) found weak antagonistic effects on the shared host of insect herbivores and plant pathogens. Our study differs from these by specifically considering taxa that are used for biological control (arthropods and plant pathogens), by examining more studies and testing a number of hypotheses (Table 4.1, Appendix B2) to explain the variance between studies. Kaplan & Denno (2007) found that the presence of one herbivorous insect significantly (albeit weakly) reduced the amount of damage caused by the second. However, unlike the other reviews and our analysis, Kaplan & Denno (2007) consider the amount of damage to the plant rather than plant performance, and they did not consider the individual and combined effects of both insects on the plant.

In this study, we ask three questions. First, we asked how prevalent are non-independent effects on the plant of two enemies or how often does the effect of one enemy depend on the presence of the second. To do this, we determined the frequency of non-independent reductions in performance and whether they were less or greater than that predicted (vote-counting approach).

We then undertook a meta-analysis to ask whether, on average, two natural enemies that share a plant independently reduce plant performance. Finally, we ask if there are experimental factors, or attributes of plants or of the natural enemy associated with non-independence (Table 4.1; Appendix B2). By identifying attributes of insects and pathogens associated with non-independent impacts on host plants, we may be able to improve biological control success rates by improving selection of agents when multiple species are necessary.
4.3 Methods

4.3.1 Data collection

We searched for papers that:

1. Involved two or more natural enemies on a focal plant species. We considered potential biological control agents, i.e. plant pathogens (fungal, viral and bacterial) and herbivorous insects and mites (collectively referred to as “insects”).

2. Considered the effects of both enemies separately and together in a factorial experiment, observational studies were not included.

3. Measured changes in plant performance (i.e. changes to plant vegetative structures, reproductive output or mortality) or changes in plant density.

   We initially used a wide range of search terms in Google Scholar to find data papers (including “herbiv*”, “interspecific”, “competition”, “direct interactions”, “indirect interactions”) but found little consistency in keywords used. After identifying a number of data papers and reviews in this manner, we found subsequent papers by exhaustively searching the citation network of each paper (i.e. papers that were cited by the focal paper or cited it). Papers were collected until 20-Jan-2011.

   If factorial experiments tested more than two enemies, we considered them as a series of $2 \times 2$ interactions. There were too few studies to consider three-way interactions. We included studies that manipulated guilds of natural enemies (e.g. below-ground herbivores or defoliators) as guilds were typically dominated by one species. This increased our sample size when testing potential correlates. We verified that studies based on guilds and species did not differ (Table 4.1).
Almost all papers measured multiple responses to determine the effects of both species on the plant. Each response was recorded. If the same response was measured multiple times, we took an overall measure (preferentially) or the final measurement.

4.3.2 Prevalence of non-independent interactions: vote counting

We used only studies in which experiments were analysed using a 2-way ANOVA. We first categorised responses based on the model used (additive or multiplicative, see Appendix B1). Next, we further categorised responses as independent (non-significant interaction term), synergistic (greater than expected reduction in plant performance) or antagonistic (less than expected reduction in plant performance). Antagonistic interactions were subdivided into low and high antagonism. Low antagonism is less reduction in plant performance than predicted but more than either species alone – similar to “equivalency” of Hatcher (1995). High antagonism is less reduction in plant performance than either species alone (≈ “inhibitory” of Hatcher 1995).

When studies tested natural enemy effects under different environmental conditions, we recorded only the natural enemy treatments from the ANOVA. If separate ANOVAs were conducted on each level of the additional factor (e.g. cultivars analysed separately) we used the control condition or, if no control condition was indicated, we took the first presented.

As studies invariably took more than one measurement of plant performance, we determined the proportion of responses in each category for each species combination. We used a t-test to determine if the proportion of independent responses found differed depending on the null model used (multiplicative or additive, Appendix B1).
4.3.3 **Overall response and prediction of occurrence of non-independent effects: meta-analysis**

*Data collation & data structure*

Data were obtained from (in order of preference) the authors, tables in the paper or, by extraction from graphs using Plot Digitizer (Huwaldt 2010).

Some studies incorporated multiple species combinations, often under different conditions and almost all measured multiple responses. To incorporate this data structure, we ran mixed models using the R package nlme (R Development Core Team 2011; Pinheiro *et al.* 2011) with nested error terms: each measured response was nested within treatment condition, treatment condition was nested within species combination, and species combination was nested within study.

*Effect size calculation*

We used log-response ratios for interactions under a multiplicative model (Darling and Côté 2008). Many ecological meta-analyses use log-response ratios, as they require fewer parameters than Hedge’s D more studies can be incorporated (Hedges *et al.* 1999). Also Hedge’s D is inherently an additive model; use of these leads to the fundamental problem of testing for a reduction in plant performance of greater than 100% (explained in the Appendix B1) but see Morris *et al.* (2007).

For each plant performance variable (e.g. number of seeds), we calculated the difference between the reduction predicted if reductions were independent and the actual reduction in plant performance.

The actual reduction in plant performance (“log RR.SpAB”) was calculated as:

\[
\log \text{RR.SpAB} = \log \left( \frac{\text{SpAB}}{\text{control}} \right)
\]
where “SpAB” is the mean of the response variable when both natural enemy
species (A & B) were present.

To calculate the null reduction in plant performance, we first calculated the
response ratio with each species alone ("RR.SpA" & "RR.SpB"):

$$RR.SpA = \frac{SpA}{control}$$

where “SpA” is the mean of the response variable with only Species A present. A
similar formula was used for Species B.

We then calculated the predicted reduction in plant performance ("log RR.null")
following Darling and Côté (2008):

$$\log RR.null = \log [1-((1-RR SpA) + (1-RR SpB) - (1-RR SpA) * (1-RR SpB))]$$

We took the difference between the predicted and observed values as our test
statistic ("diff.RR"). Positive values indicate a greater than predicted reduction in plant
performance and negative values indicate the reduction was less than predicted:

$$diff.RR = \log RR.null - \log RR.SpAB$$

We could not calculate predicted values under an additive null model due to the
combination of plant overcompensation and predictions of >100% reduction of plant
performance. However, Darling and Côté (2008) found no difference between the two
models. Differences between the two models are greatest where reductions are greater
(Sih et al. 1998). As natural enemies often have weak effects on plants, we suspect we are
in the range where model choice matters little. Our vote counting results also showed
minimal differences between the two models. We compared the two models using
published data and found that choice of model changes the overall conclusions in ~5% of
cases (in Appendix B1).
Overall impact & response variables

We tested whether the difference between the actual and predicted log-response ratio was different from zero.

Then we tested a range of variables which we had an *a priori* reason to believe might correlated with the presence of non-independent interactions (Table 4.1 & Appendix B2). Many of the predictors have been shown to influence competitive interactions between herbivorous insects (Denno *et al.* 1995; Kaplan & Denno 2007), thus might predict when non-independent effects occur (Fournier *et al.* 2006). There has been little consideration of factors predicting interactions between plant pathogens, or between pathogens and insects.

We tested the variables in two ways. First, if the 95% confidence intervals of the difference do not cross zero, there is an association with non-independent effects. If above zero, impacts are synergistic (greater than predicted reduction in plant performance); if below, antagonistic (less than predicted reduction in plant performance). Second, to determine whether the variables correlated with non-independent interactions occurred, we compared models with the explanatory variable to the null model using log-likelihood ratios (L). If the log-likelihood test yields a significant *p*-value, the model containing the variable fits the data better than the null model. Note that while two groups of a variable may both have non-independent effects, the inclusion of the variable may not explain much variance if both subsets have similar effects.

Following identification of significant variables, we looked for situations where there was a non-random association between the two significant variables using either a Chi-Square or Fisher’s exact test, e.g. were reproductive plant parts more commonly attacked when the plant was an Aster? Where a significant association was found, we
reran the model incorporating both variables to tease out what is driving the significant responses observed.

4.4 Results

4.4.1 Literature search

In total, we found 68 papers meeting our criteria, giving us 75 unique enemy-enemy-plant combinations. Our data-set contained 45 unique insect-insect-plant combinations, 23 pathogen-insect-plant combinations and seven pathogen-pathogen-plant combinations. Not all studies could be incorporated into both analyses; see Appendix B3.

4.4.2 Prevalence of non-independent interactions: vote counting

On average, 79% of responses in each species combination had independent effects on plant performance under a multiplicative model, and 74% had independent effects under an additive model (Fig. 4.1). This difference was not significant ($t = 0.47; \text{df} = 45; p = 0.64$).

However, in 35 of the 138 measurements, neither natural enemy individually had a significant impact on the response measured, which precluding the possibility of non-independent effects. Removal these from the analysis the proportion of independent responses decreases by approximately 2.5%.

4.4.3 Overall response and prediction of non-independent effects: meta-analysis

When two species attacked a plant, there was no overall difference in plant performance predicted from their effects alone under the multiplicative model ($F_{1,235} = 1.55; p = 0.215$; Fig. 4.2).
We tested whether experimental factors or attributes of either the plants or natural enemies correlated with non-independent reductions in plant performance. We asked two questions: is the variable associated with non-independent effects and does it explain the variance? Of the 32 variables we examined, only seven significantly explained the variance, or contained levels with significant antagonistic effects of multiple enemies (Table 4.1). Only one variable had significant synergistic effects. Here we highlight significant results – full results are presented in Appendix B4.

The independence of multiple enemy effects depended on whether both natural enemies attack the same plant part at the same time (direct interactions) or differed in plant part or timing (indirect interactions). Direct interactions were significantly antagonistic (Fig. 4.3a) whereas indirect interactions were independent (Fig. 4.3a). However, separating direct and indirect interactions only marginally explained the variance (Fig. 4.3a, Table 4.1).

Multiple enemy effects depended on which part of the plant was attacked. Attack to fruits and flowers were significantly associated with antagonistic interactions (Fig. 4.3b), and inclusion of these predictor variables significantly explained variance in multiple enemy effects (Table 4.1). If both species attacked above-ground parts of the plant (neither attacked the roots), they had significant antagonistic effects on the reduction in plant performance (Fig. 4.3d), but this was not a significant predictor of multiple enemy effects (Table 4.1).

When one or both enemies was a Diptera, antagonistic effects occurred and was a significant predictor of multiple enemy effects (Fig. 4.3c; Table 4.1).

Multiple enemy effects were also influenced by the type of host plant. Biennial plants were associated with antagonistic effects, although plant life-history (i.e. annual, biennial or perennial) only marginally explained variance in multiple enemy effects.
This result is driven by Smith & Mayer (2005). Asteraceae were significantly associated with antagonistic effects (Fig. 4.3e) whereas Ranunculaceae were significantly associated with synergistic effects (Fig. 4.3e), although, overall, the 21 plant families did not significantly explain the variance in multiple enemy effects (Table 4.1). Plants categorised as weeds were associated with antagonistic effects (Fig. 4.3f), but this did not explain the variance in multiple enemy effects (Table 4.1).

Weeds were more commonly Asteraceae (Table 4.2) and were significantly associated with direct interactions and inversely associated with attack to roots (Table 4.2). When incorporated into a model with direct interactions, weeds were no longer significantly associated with antagonism (interaction type: $F_{1,43} = 3.43$, $p = 0.07$; weed: $F_{1,43} = 1.01$, $p = 0.32$). Both Asteraceae and Diptera were significantly associated with attack to reproductive structures (Table 4.2). When incorporated into a model with reproductive structures, neither Asteraceae nor Diptera retained their significant association with antagonism (Diptera: reproductive parts: $F_{1,44} = 4.85$, $p = 0.033$; Diptera: $F_{1,20} = 2.82$, $p = 0.11$. Asteraceae: reproductive parts: $F_{1,44} = 4.56$, $p = 0.038$; Asteraceae: $F_{1,19} = 2.47$, $p = 0.13$). Species attacking roots were less likely to interact directly (Table 4.2). Root attack loses its significance when incorporated into a model with interaction type (interaction type: $F_{1,43} = 3.39$, $p = 0.073$; root attack: $F_{1,43} = 0.79$, $p = 0.38$).

**4.5 Discussion**

Insect herbivores and plant pathogens can sometimes have large and obvious impacts on plant performance e.g. outbreaks of forest defoliators or bark beetles. However, more often, reductions to plant performance is not obvious except in agricultural situations. On average, multiple plant enemies reduce plant performance
independently. This confirms the result of an earlier meta-analysis (Morris et al. 2007), but contrasts with conclusions of non-independent, antagonistic effects of multiple enemies on plant performance (Hatcher 1995) and damage (Kaplan & Denno 2007) in two other literature reviews.

Several reasons could explain why our results did not support the prediction that multiple enemies would generally have antagonistic effects on plant performance. First, strong effects of herbivores on each other’s fitness may not translate into non-independent effects on plant performance; Kaplan & Denno (2007) documented much stronger effects of multiple insect herbivores on each other’s fitness than on the amount of damage done by each species, which they attributed to greater effects of plant quality than quantity in mediating interactions. Second, the prevalence of independent effects of multiple natural enemies may reflect their small influences on their host plants and the ability of plants to compensate for this damage. However, removal of responses where neither natural enemy had significant effects alone had little impact on the vote-counting results. Finally, the prevalence of antagonistic effects of multiple enemies on reductions in plant performance may depend on the characteristics of the natural enemies and the plants. Indeed, non-independent interactions were reasonably common (22% - 26% of responses, predominantly antagonistic), depended on a few attributes of the plants and natural enemies.

Attack by directly interacting natural enemies (i.e. occurring on the same plant part at the same time) reduced plant performance less than predicted, whereas those that interacted indirectly (i.e. through changes in plant quality) had independent effects on plant performance. This agrees with traditional competition theory that predicts the strongest competitive interactions when species use resources concurrently (Kaplan & Denno 2007), i.e. occur in the same niche. For example, rust mite damage to the leaf
epidermal cells reduced the size of powdery mildew colonies (Fournier et al. 2006). Although herbivores affect each other when spatio-temporally separated (e.g. Kaplan & Denno 2007; Denno et al. 1995), these indirect interactions do not translate into non-independent reductions in plant performance. Interactions involving attack to roots were independent, whereas some antagonism occurred between natural enemies that both attacked above-ground parts (Fig. 3). There is little evidence for competitive interactions between above- and below-ground herbivores (Clark 2010) and attack to roots was associated with fewer instances of direct interactions.

Antagonistic interactions were significantly associated with attack to reproductive structures (flowers and fruits). This could not be attributed to increased levels of interference competition (e.g. Juenger & Bergelson 1998; Smith & Mayer 2005) as attack to reproductive structures was not associated with direct interactions. Optimal defence theory (Zangerl & Rutledge 1996) states that defence compounds should be concentrated in more valuable plant parts such as reproductive structures. Defence compounds generated in response to one species may reduce performance of both enemies. However, if defence compounds are concentrated in valuable reproductive parts, this reduction in enemy performance may be more pronounced for species attacking these parts. A reduction in enemy performance may reduce their effect, thus plant performance is greater than predicted. Another potential mechanism is herbivore-induced changes in flowering/fruiting phenology although changes in phenology can also synchronise the plant and enemy causing synergistic reductions in plant performance (e.g. Masters et al. 2001).

Antagonistic effects were also found when species combinations included a weedy plant, an Aster or a Dipteran. Both Asteraceae and Diptera were associated with attacks to reproductive plants parts but there were no associations with direct interactions. This
suggests that the association with antagonistic interactions with Asteraceae and Diptera is because reproductive parts of Asteraceae were more commonly attacked, and Diptera more commonly attacked reproductive plant parts. Weedy plants were associated with attack to reproductive structures, highlighting their popularity of predispersal seed predators in biological control programmes. Synergistic effects occurred in the two studies involving the Ranunculaceae (Paynter et al. 2006; van Ruijven et al. 2005). With only one natural enemy species, van Ruijven et al. (2005) found an increase in plant performance (over-compensation) and Paynter et al. (2006) observed no change in plant cover. Both studies found a reduction in plant performance with both enemies present as predicted by the cumulative stress hypothesis. Synergistic effects might occur where the plant can compensate for one enemy but not both.

4.5.1 Implications for weed biological control

Many biological control programs release multiple enemies – up to 25 have been released against a single target (Denoth et al. 2002). Such a strategy is often justified by the cumulative stress hypothesis. Our results may appear to support this general strategy, in that the independent effects of most multiple enemies led to an overall reduced plant performance. However, we caution that in most cases successful control (i.e. plant population decline) is attributable to one biological control agent (Denoth et al. 2002). Furthermore, increasing the number of enemies increases the chance that some interactions will be non-independent, simply due to sampling effects (McEvoy & Coombs 1999).

The likelihood of non-independent effects can be predicted to some degree by considering the location of attack and whether the enemies directly interact. These factors were associated with the taxonomy of both the natural enemy and host plants.
Direct interactions were associated with antagonistic interactions, supporting earlier calls to exclude direct competitors from biological control programs (Story et al. 1991). Attack to reproductive structures was also associated with antagonistic interactions. It has been suggested that pre-dispersal seed predators might be poor biological control agents as weeds are rarely seed limited (Myers & Risley 2000); here we show that they also interact with other agents in an antagonistic manner. Root herbivores do not interact directly with above-ground species highlighting their value in biological control programs (Blossey & Hunt-Joshi 2003).

We caution that our results are relevant only for predicting plant performance; it is less well understood how natural enemy attack to an individual plant scales up to plant population declines (Maron & Crone 2006) and/or reductions in rates of spread. This reflects the larger problem of a lack of data on the long-term impacts of natural enemies on plant populations. As well as considering the effects individual species have on plant populations, future work should consider the overall impact of multiple enemies (e.g. Miller et al. 2009) and their interactions.

### 4.5.2 Conclusions

Natural enemies sharing a host-plant tend reduce plant performance independently. Approximately one quarter of responses are non-independent; generally antagonistic. Non-independent responses can be predicted to some degree based on attributes of the enemies or host plants involved. The low frequency of non-independent interactions likely results from the weak impacts natural enemies have on their host plant. Our results suggest that increasing the number of natural enemies in biological control programs is a double-edged sword - although on average the net performance of
plants may decrease, so does the likelihood of plant performance increasing due to the inclusion of a species pair with strong antagonistic interactions.
Table 4.1 Experimental, plant and natural enemy attributes tested for their ability to predict non-independent interactions. \( L \) is the statistic for the log-likelihood ratio test.

<table>
<thead>
<tr>
<th>Test</th>
<th>Variable &amp; levels</th>
<th>Significance in meta-analysis</th>
<th>All levels independent?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Predictive Ability</td>
<td>( L )</td>
<td>( p )</td>
</tr>
<tr>
<td>A</td>
<td>Response measured (density / reproductive / vegetative / survival)</td>
<td>0.75</td>
<td>0.861</td>
</tr>
<tr>
<td>B</td>
<td>Method of enemy manipulation (added / removed)</td>
<td>1.22</td>
<td>0.270</td>
</tr>
<tr>
<td>C</td>
<td>Guild or species manipulated</td>
<td>1.13</td>
<td>0.288</td>
</tr>
<tr>
<td>D</td>
<td>Experimental location (greenhouse / common garden / field)</td>
<td>0.69</td>
<td>0.707</td>
</tr>
<tr>
<td>E</td>
<td>Unit of replication (plot containing multiple plants / individual plant)</td>
<td>0.075</td>
<td>0.785</td>
</tr>
<tr>
<td>F</td>
<td>Length of experiment (no. days)</td>
<td>0.012</td>
<td>0.914</td>
</tr>
<tr>
<td>G</td>
<td>Plant life-history (annual / biennial / perennial)</td>
<td>4.69</td>
<td>0.096</td>
</tr>
<tr>
<td>H</td>
<td>Economic status (model organism / crop / weed / native)</td>
<td>2.46</td>
<td>0.482</td>
</tr>
<tr>
<td>I</td>
<td>Plant functional group (forb / grass / shrub / tree / vine)</td>
<td>0.49</td>
<td>0.975</td>
</tr>
<tr>
<td>J</td>
<td>Woody or non-woody</td>
<td>0.10</td>
<td>0.752</td>
</tr>
<tr>
<td>Test</td>
<td>Variable &amp; levels</td>
<td>Significance in meta-analysis</td>
<td>Predictive Ability</td>
</tr>
<tr>
<td>------</td>
<td>-----------------------------------------------------------------------------------</td>
<td>------------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>K</td>
<td>Plant family</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>Enemy grouping (two arthropods / an arthropod and a plant pathogen / two plant pathogens)</td>
<td>18.77</td>
<td>0.547</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>Direct interaction (same plant part, same time) \ Indirect interaction</td>
<td></td>
<td>0.23</td>
</tr>
<tr>
<td>N</td>
<td>No. of generalists present (0, 1, 2)</td>
<td>3.25</td>
<td>0.072</td>
</tr>
<tr>
<td>O</td>
<td>Both enemies in the same order</td>
<td>0.982</td>
<td>0.612</td>
</tr>
<tr>
<td>P</td>
<td>Both enemies in the same family</td>
<td>0.03</td>
<td>0.862</td>
</tr>
<tr>
<td>Q</td>
<td>Both enemies in the same feeding guild</td>
<td>0.006</td>
<td>0.936</td>
</tr>
<tr>
<td>R</td>
<td>No. of chewers present (0, 1, 2)</td>
<td>1.64</td>
<td>0.441</td>
</tr>
<tr>
<td>S</td>
<td>No. of sap-suckers present (0, 1, 2)</td>
<td>1.79</td>
<td>0.409</td>
</tr>
<tr>
<td>T</td>
<td>No. of miners present (0, 1, 2)</td>
<td>0.26</td>
<td>0.879</td>
</tr>
<tr>
<td>U</td>
<td>Reproductive plant parts attacked present</td>
<td>4.24</td>
<td>0.039</td>
</tr>
<tr>
<td>V</td>
<td>Leaf attacker present</td>
<td>0.85</td>
<td>0.356</td>
</tr>
<tr>
<td>W</td>
<td>Root attacker present</td>
<td>2.64</td>
<td>0.105</td>
</tr>
<tr>
<td>Test</td>
<td>Variable &amp; levels</td>
<td>Significance in meta-analysis</td>
<td>Predictive Ability</td>
</tr>
<tr>
<td>------</td>
<td>------------------------</td>
<td>------------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>X</td>
<td>Stem attacker present</td>
<td></td>
<td>0.0004</td>
</tr>
<tr>
<td>Y</td>
<td>Coleoptera present</td>
<td></td>
<td>0.009</td>
</tr>
<tr>
<td>Z</td>
<td>Lepidoptera present</td>
<td></td>
<td>0.84</td>
</tr>
<tr>
<td>AA</td>
<td>Diptera present</td>
<td></td>
<td>4.22</td>
</tr>
<tr>
<td>BB</td>
<td>Hemiptera present</td>
<td></td>
<td>1.53</td>
</tr>
<tr>
<td>CC</td>
<td>Fungi present</td>
<td></td>
<td>0.059</td>
</tr>
<tr>
<td>DD</td>
<td>Virus present</td>
<td></td>
<td>0.35</td>
</tr>
</tbody>
</table>
Table 4.2 Fisher’s tests for association between species combinations for the significant variables.

<table>
<thead>
<tr>
<th></th>
<th>Direct interactions</th>
<th>Reproductive structures attacked</th>
<th>Roots attacked</th>
<th>Asteraceae</th>
<th>Diptera</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weeds</strong></td>
<td><strong>p = 0.0004</strong></td>
<td>p = 0.20</td>
<td><strong>p &lt; 0.0001</strong></td>
<td><strong>p = 0.041</strong></td>
<td><strong>p = 1</strong></td>
</tr>
<tr>
<td>Direct interactions</td>
<td></td>
<td>p = 0.12</td>
<td><strong>p &lt; 0.001</strong></td>
<td>p = 0.58</td>
<td>p = 0.52</td>
</tr>
<tr>
<td>Reproductive structures attacked</td>
<td>-</td>
<td>-</td>
<td><strong>p = 0.035</strong></td>
<td><strong>p = 0.004</strong></td>
<td></td>
</tr>
<tr>
<td>Roots attacked</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>p = 1</td>
<td>p = 0.32</td>
</tr>
<tr>
<td>Asteraceae</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td><strong>p = 0.0027</strong></td>
</tr>
</tbody>
</table>
Figure 4.1 Mean proportion (± 95% confidence intervals) of different response types (independent, synergistic, low antagonism and high antagonism) from each study in each plant – natural enemy pair group in responses measured using a two-way ANOVA under A) the additive model and B) the multiplicative model (Appendix B1, Sih et al. 1998).
**Figure 4.2** Observed reduction in plant performance when two species are present on a plant compared with that predicted when the two species are alone on the plant compared with that predicted under the multiplicative model (Sih *et al.* 1998) as determined by the log response ratio (log RR).
Figure 4.3 Predictor from the meta-analysis showing some association with non-independent impacts on plant performance. A: direct vs. indirect interactions; B: attack to reproductive plant parts (i.e. fruits and flowers); C: attack by Diptera; D: attack to roots; E: attack to Asteraceae and Ranunulaceae; and F: attack to plants of differing economic status.
CHAPTER FIVE: DO INTERACTIONS BETWEEN INSECTS EXPLAIN THEIR COMBINED EFFECT ON THE SHARED HOST-PLANT

5.1 Summary

Belowground herbivores reduce plant performance and may influence aboveground herbivores. Few studies have simultaneously considered both the impact of a root herbivore on an aboveground herbivore, and their combined effects on plant performance.

We used two insects introduced for the biological control of diffuse knapweed to test 1. whether colonisation rates of either insect changes due to the presence of the other insect species; 2. if a root herbivore, *Cyphocleonus achates*, influences the impact of the seed and stem-feeding weevil, *Larinus minutus* and; 3. whether the reduction in plant performance caused by each species is independent of the other species.

Neither changes in colonisation rates nor competitive interactions were apparent between the two insect species. Both insects reduced plant performance; for most metrics, reduction by one herbivore was independent of the second. However, there was a synergistic reduction in the number of seeds produced per plant when both herbivores were present.

We suggest that synergistic reductions in plant performance in response to attack by above- and belowground herbivores might occur when two criteria are met. Firstly, both herbivores reduce plant performance when they occur alone, and second, the two species do not compete.
5.2 Introduction

There is substantial interest in the plant-mediated effects that belowground herbivorous insects can have on the aboveground insects and *vice versa*. Research over the past two decades has shown that the effects of belowground herbivores can be variable; the effects on a specific aboveground herbivore may be positive (e.g. Masters *et al.* 1993), negative (e.g. Bezemer *et al.* 2003) or neutral (e.g. Staley *et al.* 2008). However, despite significant case studies, a recent meta-analysis showed that the overall effect is relatively minor (Clark 2010). This is surprising given the strength of plant-mediated competition found overall (Kaplan & Denno 2007).

Like aboveground herbivores, root herbivore attack reduces plant performance (Blossey & Hunt-Joshi 2003), therefore can influence plant community diversity (Wardle *et al.* 2003). Overall, when two aboveground species attack a plant, a marginally significant trend occurs towards less than predicted plant damage (Chapter 4). In contrast, combinations involving a root herbivore typically show independent effects of the two herbivores (Chapter 4). The resulting interactions between above- and belowground herbivores can alter community diversity through differential impacts on dominant and sub-dominant plants (van Ruijven *et al.* 2005).

Very few studies, however, have integrated the competitive effects on insects and the overall impact on the plant of attack by both above- and belowground herbivores. On the rare occasion that the two processes have been considered within one study (e.g. Gange & Brown 1989; Staley *et al.* 2007), they have been considered as two separate processes. Linking the two processes may shed light on the mechanisms behind competition or non-independent impacts on plant performance, and explain some of the variation seen between studies.
Interactions between spatially and temporally separated insects must be mediated by the plant. The presence of one species may alter either host-plant quality (e.g. increase in defence compounds or decrease in plant nutritional composition) or host-plant quantity (e.g. amount of tissue produced). These changes may affect a second species attacking the plant. Plant performance responses can be difficult to predict: a lack of change in plant performance with increasing herbivore damage suggests a non-linear plant response to damage (Strauss & Agrawal 1999) which could be due to over-compensation by the plants. Plants may be able to compensate for damage up to a point and only succumb to attack once a damage threshold is reached (i.e. multiple stress hypothesis; Harris 1981; van Driesche et al. 2008). It has been hypothesised (Fournier et al. 2004) that facilitation between herbivores will lead to synergistic reductions in plant performance, while competitive interactions might lead to lower reductions in plant performance.

To investigate the effect of belowground herbivores on aboveground herbivory, we focussed on diffuse knapweed, *Centaurea diffusa*, and two insects introduced to control the weed. The aboveground herbivore, *Larinus minutus* (Coleoptera: Curculionidae) has been associated with the decline of diffuse knapweed in our study area (Myers et al. 2009). The adults feed on the stems and leaves of the plant and larvae develop in the seed-head. *Cyphocleonus achates* (Col.: Curculionidae) is a root herbivore that has been credited with population reductions of the con-gener, spotted knapweed *Centaurea stoebe* subsp. *micranthos* (Corn et al. 2006). In our study area, populations of *C. achates* have increased in recent years (AEAS pers. obs.), following the initial decline of diffuse knapweed (Myers et al. 2009).
Biocontrol systems are ideal for asking questions about insect herbivore interactions because they provide a simple experimental system where all players are known, dominance of the focal plant is well-established and, as the insects are introduced without their natural enemies, we can assume that interactions are most likely plant-mediated rather than enemy-mediated (e.g. Kaplan et al. 2007). Additionally, the increasing popularity of root herbivores in biocontrol introductions (Blossey & Hunt-Joshi 2005) underlines the need to understand how root herbivores affect focal weeds and interact with previously introduced biocontrol agents.

We considered three aspects of potential interactions between herbivore species and their shared host plant: herbivore colonisation rates, impacts on plants and influence of each species on the development of the other. Colonisation rates determine the frequency with which the species interact in the field. No change in colonisation rate suggests that the presence of one herbivore does not influence the presence of the second (Strauss & Irwin, 2004). Therefore, we considered whether the presence of C. achates or L. minutus altered plant attractiveness to oviposition by females of the other species.

In terms of the impacts on the plant, if two herbivores operate independently, then the reduction in plant performance of both species on the plant will be equivalent to that of the sum of each herbivore species alone. Independent effects are most common, particularly with above- and belowground herbivores (Chapter 4).

We have contrasting predictions regarding changes in the size or development rates on the aboveground herbivore. If species compete, we would expect reduced development through either simple resource limitation (reduction in host quantity) or due to systemic defences produced by the host-plant (reduction in plant quality, Bezemer et al. 2003). However, Masters et al. (1993) suggests that the performance of above-
ground herbivores should increase as plant quality improves due to an increase in soluble amino acids and carbohydrates aboveground caused by water stress as removal of roots by the root herbivore acts in a similar manner to drought.

In the following experiments, we evaluate the interactions between *Cyphocleonus achates* and *Larinus minutus* and impacts on their shared host, diffuse knapweed. *Larinus minutus* is attributed with controlling diffuse knapweed in this area: understanding the impacts that *C. achates* has on the *L. minutus* – diffuse knapweed system will also allow us to determine if *C. achates* might be positive or detrimental for the control of this invasive species.

### 5.3 Methods

This study was carried out in a meadow above Vaseux Lake in the southern Okanagan Valley of British Columbia, Canada (49°17′ 46″W, 119°31′ 34″N) from spring 2010 to autumn 2011. This study followed the occurrence of high levels of invasion of *C. achates* into cages established for another experiment. Thus, while the density of *L. minutus* in the cages was controlled, that of *C. achates* was not.

#### 5.3.1 Plant establishment

Three to six diffuse knapweed seeds were planted into potting soil in 260 mL polypropylene Ray Leach “Cone-tainers”™ (Stuewe & Sons, Oregon, USA) lined with tea-filters on the 29-30 March 2010 and maintained in the UBC Horticulture Greenhouse. Seedlings were subsequently reduced to the largest per cone.

When plants were at the 4-8 leaf stage, we transplanted the seedlings into the field (27-29 April 2010). Eight seedlings were planted in each 50 × 50 cm plot. Seedlings
were planted together with the tea-filter and associated potting mix to maintain root integrity.

Plants were watered at least daily until 1 May 2010 and regularly through to early June. May 2010 was an abnormally wet month (May 2010 rainfall = 76 mm, mean for May 2000-2010 = 38 mm Penticton A weather station), improving the establishment of the seedlings.

In early June (1-3) 2010, prior to the emergence of insects, we erected cages (50 × 50 × 100 cm) over the plots; for details of the cages see Myers et al. (2009). Cages were removed for the winter in September 2010 and re-erected in May 2011, and all plants were harvested in September 2011.

5.3.2 Insect establishment & collection

*Cyphocleonus achates* is increasing in the southern Okanagan. We presume they entered the cages either as reproductive females still present in the field in mid-September 2010 when the cages were removed. Alternatively, the flightless insects may have forced their way under the base of the cages encouraged by the presence of healthy plants as very few rosettes were present in the vicinity of the experiment.

*Larinus minutus* were added to the half of the 30 field cages each year. In 2010, six seed-head weevils per cage were added on the 4-5 July. In 2011, seven seed-head weevils per bolting plant and two per rosette plant were added to cages on the 7 June. This rate is similar to that used in other studies (Crowe & Bourchier 2006, Jackson 2008, Myers et al. 2009, Knochel et al. 2010). Insects were collected at both Vaseux Lake and at nearby White Lake. In mid-August, we placed organza bags over seed-head clusters of randomly selected plants in cages containing *L. minutus* to collect the emerging insects.
The insects were distributed in a split-plot design with *L. minutus* treatments (present or absent, n = 15 of each) allocated to plots (=cages), and plants within the plots had different levels of *C. achates* attack (present or absent).

**5.3.3 Data collection**

We harvested all plants in the experiment in September 2011, thus plants were in the field from May 2010 to September 2011. In each plot we recorded the number of surviving plants, the number that bolted, and the height of each stem. In some plots, more than eight plants were found. For each plant, we recorded the presence and identity of root herbivores.

Plants were dried for ~72 hrs at ~70°C and aboveground biomass was measured. We counted all seed-heads and assessed up to 25 seed-heads per plant for seeds and *L. minutus* attack. We recorded the life-history stages of the insects (larvae, pupae, teneral adults, adults gone but damage remaining). From the number of seed-heads and number of seeds and *L. minutus* in 25 seed-heads, we calculated expected number of seeds and number of seed-head weevils per plant.

The *L. minutus* adults were removed from the organza bags and the length of the right hind femur and tibia measured as they correlate with overall body size, hence fecundity (Honěk 1993). If after harvest, additional adult weevils emerged from the seed-heads, they were collected in the paper-bag holding the plant and were also measured. We measured the length of each leg part twice and then re-measured 20 insects at a later date to determine repeatability of the measurements.
5.3.4 Data analysis

All analyses were conducted in R (R Development Core Team, 2011). We used the nlme (Pinheiro et al. 2009) and lme4 (Bates et al. 2011) libraries to conduct the mixed-effects models.

For plant performance measurements, we tested whether the presence of *C. achates* altered levels of damage caused by *L. minutus* using a multiplicative null model (Sih et al. 1998). We converted the usual additive effects in linear models by either log-transforming the data (when using the normal distribution) or using a log-link function under the Poisson distribution. Variables were back-transformed for graphical presentation. For all analyses, we used mixed-effects models with cage and, where appropriate, plant nested within plot as random effects. To determine significance of the fixed effects and interactions, we sequentially removed terms from the model and compared reduced models to full models with Chi-square tests.

To assess changes in colonisation rates in the presence of the second species, we first assessed the uncontrolled *C. achates* attack was higher on plants which had previously been attacked by *L. minutus*. We used number of plants in the plot as a co-variate as denser patches may attract more insects (resource concentration, Root 1973) and included plot as a random effect. To assess changes in *L. minutus* larval colonisation rates within the cages, we determined whether the number of seed-heads containing *L. minutus* of the 25 seed-heads per plant evaluated was different depending on whether *C. achates* was present in the root of the plant. For this assessment we used the number of seed-heads on the plant as a co-variate, again to account for resource concentration effects, and included *L. minutus* treatment in the model to confirm that *L. minutus* damage varied between the treatments. We used plant nested within plot and plot as
random effects. Using plot as a random effect meant that we are comparing the colonisation rate of *L. mintus* on plants with and without *C. achates* attack within the cages. We were unable to assess whether adult *L. minutus* feeding damage varied with *C. achates* attack, as level of adult attack was only assessed at the plot level.

To assess the impact of *C. achates* on *L. minutus*, we looked at development rates, insect size and number of offspring produced on the plant. To assess changes in development rates, we took only those seed-heads attacked by *L. minutus* and considered reaching adulthood as a binary variable calculated separately for each insect under the binomial distribution with plant and plot as error terms. We were unable to assess whether *L. minutus* damage influenced *C. achates* size or development as all had completed developed when the plants were removed and we were unable to collect *C. achates* as they emerged from the root.

For the insect size data, we assessed repeatability of the measurements within a measurement period, took the means of the measurements taken at each time and calculated the repeatability across the two measurement periods. We took the average of the two measurements for each insect and used a mixed-effects model with plant and plot as error terms to calculate whether there were differences in the size of *L. minutus* on plants which were attacked by *C. achates*.

### 5.4 Results

We consider three aspects of the interactions between the belowground herbivore *C. achates* and the above-ground *L. minutus*. These aspects are 1. whether the presence of one herbivore alters colonisation rates of the second species; 2. whether herbivore damage to the plant by each herbivore is independent of the second and finally; 3.
whether the presence of *C. achates* altered *L. minutus* development rates, size (competitive effects) or the number of *L. minutus* produced overall.

### 5.4.1 Colonisation rates

Colonisation rates of diffuse knapweed by *C. achates* and *L. minutus* were independent of the presence of the other species (Table 5.1). *C. achates* attack rates were higher when there were more plants present in the plot, while *L. minutus* tended to attack plants with more seed-heads.

### 5.4.2 Damage to plant

**Bolting rates**

The presence of *C. achates* was significantly positively associated with the proportion of rosette plants bolting (Table 5.2). Bolting rate was unaffected by the number of plants in the plot or by the presence of *L. minutus*, with none of the interactions being significant (Table 5.2).

**Damage to vegetative structures**

Plants with *C. achates* attack were significantly shorter (lower maximum plant height) and lighter (Fig. 5.1, Table 5.3), while *Larinus minutus* reduced the number of stems present on a plant (Fig. 5.1, Table 5.3). No significant interactions were observed between the herbivores.

**Damage to reproductive structures**

*Larinus minutus* significantly reduced the number of seed-heads that produced seeds and the number of seeds per plant (Fig. 5.1, Table 5.4). *Cyphocleonus achates* reduced the number of seed-heads on the plant and the number of seeds per plant, but
did not reduce the proportion of seed-heads producing seed. Significant interactions occurred between the two insect species for the number of seed-heads and the number of seeds per plant. Interactions were such that *C. achates* only reduced the number of seeds per plant in the presence of *L. minutus* and only reduced seed-heads per plant in the absence of *L. minutus* (Fig. 5.1).

### 5.4.3 Effect on *Larinus minutus*

The proportion of *L. minutus* that reached adulthood did not vary with *C. achates* presence (Fig 5.2a; Chi = 0.0013, *p* = 0.97). Neither did *L. minutus* size vary as indicated by the tibia and femur lengths (Fig 5.2b; femur: *F*₁,₁₈ = 0.60; *p* = 0.45, tibia: *F*₁,₂₀ = 0.78, *p* = 0.39). Repeatability of the tibia and femur measurement between the two measurement periods were 98.7% and 99.8% respectively.

### 5.5 Discussion

When *Cyphocleonus achates* and *Larinus minutus* were both present on diffuse knapweed, overall seed reduction was greater than that predicted by their independent effects alone; the two species interact in a synergistic manner to reduce plant fitness. As the same number of *L. minutus* were added to the cage in each year and colonisation rates were constant across the treatments, this effect is likely due to increasing per capita effects of the insects. Impacts of each species on the vegetative structures were independent of the presence of the other: *L. minutus* reduced the number of stems per plant which likely occurred due to feeding on the rosettes, and *C. achates* reduced both plant height and above-ground biomass, consistent with previous studies on spotted knapweed (Steinger & Müller-Schärer 1992; Corn *et al.* 2006; Knochel *et al.* 2010). The reduction in the number of stems with *L. minutus* attack has not been previously
reported, we suspect it is due to adult feeding on rosettes. The reduction in the number of developed seed-heads is due to adult feeding damage (Chapter 3) while the seed reduction due to larval feeding has been extensively reported (Kashefi & Sobhian 1998; Smith & Mayer 2005; Crowe & Bourchier 2006). Knochel et al. (2010) found that spotted knapweed plants attacked by both L. minutus and C. achates had fewer seed-heads than unattacked plants, but neither species significantly reduced flowering alone. However, these authors did not test whether plant performance reductions were independent (i.e. they analysed data using a one-way rather than two-way ANOVA, see Chapter 4).

We found no negative impact of C. achates presence on L. minutus development rate or insect size. Therefore, the recent increase in C. achates densities should not reduce the success of L. minutus, as the biocontrol agent responsible for recent knapweed declines (Myers et al. 2009); and may even increase the rate of decline. We found no evidence for changes in the colonisation rates for either species in the presence of the second. Cyphocleonus achates was associated with an increased bolting rate; we believe this was because they require larger rosettes for development (Stinson et al. 1994) and larger rosettes are more likely to bolt (Powell 1990).

Fournier et al. (2006) suggested that a synergistic reduction in plant performance could occur when natural enemies have positive effects on each other (i.e. the presence of one insect leads to an increase in the performance of the second). We extend this to suggest that synergistic reduction in plant performance may also occur if 1. both species are damaging when alone and 2. neither species has a negative effect on the other. However, examples of synergistic reductions are rare, probably because herbivores often have little or no impact on plant performance (e.g. Poveda et al. 2005, Staley et al. 2007.
for belowground herbivores and Gerber et al. 2007, Johnson et al. 2009 for aboveground herbivores). One example of synergism involves tansy ragwort defoliation by the cinnabar moth and root damage by ragwort flea beetle. Both insects individually reduced ragwort performance and, when together, there was a synergistic reduction in performance (James et al. 1992). However, population modelling suggests that the best control strategy is to use just the flea beetle alone as cinnabar moth impacts were inconsistent (Dauer et al. 2012). Aphids and wireworms on barley plants caused a synergistic decline in the total fresh mass of the plant after 35 days (but this effect was not apparent after 45 days) and both species did better in the presence each other (Johnson et al. 2009). Another example is that of Masters & Brown (1992) who found synergistic reductions in plant performance between leaf miners and chafer larvae on Sonchus oleraceus but the above-ground herbivore reduced the growth rate of the root herbivore, but the root herbivore increased the growth rate of the above-ground species. Here we suggest that the negative impacts of the leaf miner on the root herbivore were mitigated by the positive impact of the root herbivore on the leaf miner.

One major caveat on these results, and all studies that consider reductions in plant performance caused by herbivores, is that we have a poor understanding of when and how reductions in plant fitness scale up to declines in plant populations (Crawley 1989a; Maron & Crone 2006). In a majority of cases, successful biocontrol of invasive plants is attributable to one agent (Denoth et al. 2002), including the reduction of diffuse knapweed (Myers et al. 2009). While many authors assume that an increase in damage will lead to an increase in control (e.g. Gerber et al. 2007; Knochel et al. 2010), this may be a faulty assumption – Hunt-Joshi et al. (2004) found little additional impact of root
herbivory and suppression of *Lythrum salicaria* did not increase in combined leaf and root herbivory treatments in a four-year investigation of root and leaf herbivore impacts.

Abiotic and biotic factors could potentially influence interactions. As plants and insects in our experiments were caged, pollinators had no access to the plants. While we hand-pollinated to compensate for this, herbivory can alter pollination rates (e.g. Strauss *et al*. 1996, Poveda *et al*. 2003); we may thus be missing a key aspect in the interaction between above- and belowground species. Secondly, we conducted this experiment over two relatively wet years (May-July 2010 and 2011 were 133% and 115% of normal precipitation respectively; Environmental Canada Penticton A weather station). While the rain in 2010 improved survivorship of the transplanted seedlings, it may also have altered interactions between the species (e.g. Staley *et al*. 2007). Jackson (2008) found a non-significant trend towards increased damage by *Larinus* in drought conditions. Finally, as some potting mix was transplanted along with the seedlings, nutrient levels are likely to have been above ambient. We do not believe that this is likely to have altered the relative performance of plants in our experiment; Knochel *et al*. (2010) manipulated soil N and insect herbivory on spotted knapweed and found no significant interactions on any plant performance metric measured. And further, we did not study the effects of treatments throughout the plant life cycle to include recruitment from seeds.

To optimise reduction in plant performance for weed biological control, interactions between species must be considered as well as the impact of each species on the host plant. If our results are found to be consistent across more studies, this would support an increased use of root herbivores in biocontrol particularly if multiple species are to be introduced. Antagonistic interactions between herbivores have been suggested as a reason for biocontrol failure (McEvoy & Coombs 1999). Competition between insect
species is common (Kaplan & Denno 2007) and may lead to a less than expected reduction in plant performance if the performance of the most damaging biocontrol agent is reduced. New introductions should be tested for interactions with already-established agents.

Belowground herbivores are likely to have disproportionate effects on aboveground systems if they cause synergistic reductions of plant performance, thus they may be even more important than previously thought for biological control and in the structuring of plant communities. Synergistic reductions in performance of a plant that is a competitive dominant has the potential to accelerate increases in plant diversity. This study highlights that linking various impacts of belowground herbivores on aboveground biota and the host-plant can enhance understanding of the role insect herbivores have in the maintenance of biodiversity.
Table 5.1 Results from statistical analysis of *Cyphocleonus achates* and *L. minutus* colonisation rates. Response variables are under the binomial distribution where the plant (in the case of *C. achates*) or seed-head for (*L. minutus*) can be attacked (1) or not attacked (0). For the *C. achates* analysis, plot was used as a random effect, and plot and plant nested within plot were used as random effects in the analysis of *L. minutus* attack. Significance of the fixed effects and interactions between them was conducted by comparing models without the variable to models with the variable. Variables were dropped in the order presented.

<table>
<thead>
<tr>
<th>Attack by <em>Cyphocleonus</em></th>
<th>Chi-sq</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. minutus</em> treatment × no plants</td>
<td>0.21</td>
</tr>
<tr>
<td><em>Larinus minutus</em> treatment</td>
<td>0.81</td>
</tr>
<tr>
<td>Number of plants in plot</td>
<td>4.23</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Attack by <em>Larinus</em></th>
<th>Chi-sq</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. minutus</em> trmt × <em>C. achates</em> x no. seed-heads</td>
<td>0.66</td>
</tr>
<tr>
<td>No. seed-heads × <em>L. minutus</em> treatment</td>
<td>0.0</td>
</tr>
<tr>
<td>No. seed-heads × <em>C. achates</em></td>
<td>0.11</td>
</tr>
<tr>
<td><em>C. achates</em> × <em>L. minutus</em> treatment</td>
<td>0.19</td>
</tr>
<tr>
<td><em>Cyphocleonus achates</em></td>
<td>0.66</td>
</tr>
<tr>
<td>No. seed-heads</td>
<td>5.03</td>
</tr>
<tr>
<td><em>Larinus minutus</em> treatment</td>
<td>25.78</td>
</tr>
</tbody>
</table>
Table 5.2  Results from statistical analysis of the proportion of plants that bolted with different types of insect attack. Response variables are under the binomial distribution where the plant has bolted (1) or is still a rosette (0). Plot was used as a random effect. Significance of the fixed effects and interactions between them was conducted by comparing models without the variable to models with the variable. Variables were dropped in the order presented.

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Chi-Sq</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. plants × <em>L. minutus</em> × <em>C. achates</em></td>
<td>0.53</td>
<td>0.47</td>
</tr>
<tr>
<td>No. plants × <em>L. minutus</em></td>
<td>0.11</td>
<td>0.74</td>
</tr>
<tr>
<td><em>C. achates</em> × <em>L. minutus</em></td>
<td>0.35</td>
<td>0.55</td>
</tr>
<tr>
<td>No. plants × <em>C. achates</em></td>
<td>0.77</td>
<td>0.38</td>
</tr>
<tr>
<td>Number of plants</td>
<td>0.65</td>
<td>0.42</td>
</tr>
<tr>
<td><em>Larinus minutus</em></td>
<td>1.72</td>
<td>0.19</td>
</tr>
<tr>
<td><em>Cyphocleonus achates</em></td>
<td>26.47</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
Table 5.3 Results from statistical analysis of damage to plant vegetative structures with different types of insect attack. All response variables used plot as a random effect. Maximum bolt height and aboveground biomass were log-transformed for testing under the multiplicative model and were normally distributed. Number of stems was Poisson-distributed and a log-link function was used; we determined significance of the fixed effects and interactions by comparing models without the variable to models with the variable; variables were dropped in the order presented.

<table>
<thead>
<tr>
<th>Number of stems on plant</th>
<th>Chi-Sq</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. achates × L. minutus</em></td>
<td>0.393</td>
<td>0.531</td>
</tr>
<tr>
<td><em>Cyphocleonus achates</em></td>
<td>0.150</td>
<td>0.698</td>
</tr>
<tr>
<td><em>Larinus minutus</em></td>
<td>3.992</td>
<td>0.0457</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Maximum bolt height (height of tallest stem on each plant)</th>
<th>df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cyphocleonus achates</em></td>
<td>1, 137</td>
<td>21.37</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td><em>Larinus minutus</em></td>
<td>1, 28</td>
<td>2.94</td>
<td>0.097</td>
</tr>
<tr>
<td><em>C. achates × L. minutus</em></td>
<td>1, 137</td>
<td>0.64</td>
<td>0.42</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Aboveground biomass</th>
<th>df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cyphocleonus achates</em></td>
<td>1, 137</td>
<td>7.97</td>
<td>0.0055</td>
</tr>
<tr>
<td><em>Larinus minutus</em></td>
<td>1, 28</td>
<td>1.28</td>
<td>0.267</td>
</tr>
<tr>
<td><em>C. achates × L. minutus</em></td>
<td>1, 137</td>
<td>2.197</td>
<td>0.141</td>
</tr>
</tbody>
</table>
Table 5.4 Results from statistical analysis of damage to plant reproductive structures with different types of insect attack. The proportion of seed-heads that produced seeds was analysed under the binomial distribution where seeds produced = 1 or no seeds produced = 0. All response variables used plot as a random effect and plant nested within plot was used as an error term for the proportion of seed-heads that produced seed. For both analyses, significance of the fixed effects and interactions between them was determined by comparing models without the variable to models with the variable, variables were dropped in the order presented.

<table>
<thead>
<tr>
<th>Number of seed-heads on plant</th>
<th>Chi-Sq</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. achates × L. minutus</td>
<td>112.12</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Larinus minutus</td>
<td>3.367</td>
<td>0.0665</td>
</tr>
<tr>
<td>Cyphocleonus achates</td>
<td>437.41</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Proportion of seed-heads that produced seeds</th>
<th>Chi-Sq</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. achates × L. minutus</td>
<td>0.0865</td>
<td>0.7687</td>
</tr>
<tr>
<td>Cyphocleonus achates</td>
<td>0.8832</td>
<td>0.3473</td>
</tr>
<tr>
<td>Larinus minutus</td>
<td>6.34</td>
<td>0.012</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Seeds per plant – total reproductive output</th>
<th>Chi-Sq</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. achates × L. minutus</td>
<td>45.871</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Larinus minutus</td>
<td>5.253</td>
<td>0.02191</td>
</tr>
<tr>
<td>Cyphocleonus achates</td>
<td>85.077</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
Figure 5.1 Interaction between *C. achates* and *L. minutus* impacts on vegetative (A-C) reproductive (D-F) plant responses (mean ± SEM). Solid circles and dashed lines represent no *C. achates* attack and solid triangles and dotted lines represent plants attacked by *C. achates*. In panel (A) number next to the points refers to the number of plants in each treatment and is the same in all panels.
Figure 5.2 Influences of *Cyphocleonus achates* presence on *Larinus minutus* A: development to adult stage; B: length of the femur and tibia (mean ± SEM).
CHAPTER SIX: GENERAL CONCLUSIONS

6.1 Thesis overview & future directions

My thesis research considers factors that influence either reductions of plant performance, or top-down control of plant populations by natural enemies. In Chapters 2 & 3 I considered two intrinsic factors, the distribution of insects in relation to densities of their host-plant, and whether insect species that have two feeding modes are more damaging to plants than those with only one feeding mode. Then in Chapters 4 & 5, I consider whether the presence of additional natural enemies alters the amount of damage the enemy inflicts. I used the diffuse knapweed biological control system as my study system in Chapters 2, 3 and 5.

In Chapter 2 I developed a model that showed that insect response to host plant density has the potential to alter plant population decline and persistence of the insect-plant system. Greatest declines were observed when insects were distributed linearly with plant density, while resource concentration patterns (where insects were disproportionately found in large plant patches) lead to greater persistence of the insect-plant system. I found that *L. minutus* distributions were in direct proportion to plant density while *U. affinis* distribution followed a resource concentration pattern. An ideal biological control agent might be one with a dual strategy i.e. responded in direct proportion to plant density when plant populations were large but then followed a resource concentration type pattern when populations are lower.

An interesting extension to the model would be to scale it up to the community level and consider whether differences in insect response to plant density can alter the co-existence of different plant species. We might expect that in plant-insect communities
where insects all follow a resource concentration pattern, the plant species diversity in each patch is higher than where insects all follow a resource dilution pattern. However, resource dilution might lead to greater beta diversity as different plant species come to dominate each patch.

In the future, it would be good to test if this pattern holds (successful biocontrol agents following a directly proportional pattern) on a wider range of biological control species and under a wider range of plant population densities. Undertaking an experiment similar to that of Long et al. (2003), with multiple insect species which distribute themselves differently and have similar impacts on plants, would be the ideal experiment but is unlikely to be feasible, although artificial herbivory could be employed.

Chapter 3 considers whether adults and larvae of the same species (*L. minutus*) that feed in different ways on the same host-plant have similar impacts to the feeding of two separate species. I made observational associations between adult and larval damage and did not find an association between the damage types. I looked for evidence of plant-mediated competition between the life-history stages of *L. minutus* experimentally, but did not find any. I also conducted experimental manipulation of adult and/or larval feeding damage at two sites and in two years and measured plant performance. No synergistic nor antagonistic interactions occurred, but at each of the two sites a different life-history stage was responsible for reduction in the number of seeds produced by the plant. This makes the species effective in a wider range of environments than a species with only one feeding mode. An additional worthwhile study would be to investigate if species that can feed as adults as well as larvae have a different numerical response to an increase in host density.
Prior to recommending that biocontrol practitioners focus on species with multiple feeding modes, we need to establish whether species with multiple feeding modes are more frequently involved in biocontrol success and/or provide control over a wider range of environments. While Crawley (1989a) stated that Coleoptera were more often successful, many additional introductions in the past 23 years should be evaluated. However, with these types of analyses, there are issues with defining “success” for a programme or introduction (Denoth et al. 2002) which is likely to be exacerbated if we want to compare across the range of an invader. Answering this questions will require experiments similar to mine but will require a wider range of taxa (both with and without multiple feeding modes).

Chapter 4 involved a meta-analysis in which I asked whether natural enemies (arthropods and plant pathogens, i.e. potential biocontrol agents) tended to have independent effects on plant performance and what attributes of the experiments, plants or natural enemies might predict when non-independent effects occur. Overall reduction in plant performance caused by one natural enemy was independent of plant performance reductions caused by the second. Antagonistic (less decline in plant performance than predicted) interactions accounted for approximately 16-22% of interactions and were associated with biennial plants, plants in the family Asteraceae, insects in the order Diptera, direct interactions between the enemies and feeding on fruit or flowers. Synergistic interactions were rare at 4.3-4.6% of interactions, and were only associated with the Ranunculaceae.

While reductions in plant performance appear to be independent, for biological control, it is necessary to get a better understanding of whether independent reductions in plant performance will scale up to independent effects at the population level. This is
not likely to be achieved in the near future given the lack of experimental data at the population level, particularly as effects can vary in both space and time. My analysis showed slight trends towards antagonism for a number of additional variables. If more studies become available over the next few years, it would be interesting to include them to increase the sample size and see whether any of these factors becomes significant.

In my final chapter, I look at the interactions between *C. achates* and *L. minutus* and whether the reductions in plant performance caused by both species were independent. *Cyphocleonus achates* reduced the maximum height of the plants, the above-ground dry weight and the number of seed-heads, while *L. minutus* decreased the number of stems on the plant and the number of seeds produced by each seed-head. Overall, the presence of both species resulted in a greater reduction in the number of seeds than that predicted from their individual effects – there was a synergistic reduction in plant performance.

This experiment was not what I had planned. I had planned to consider interactions between *L. minutus* and another root herbivore, *Sphenoptera jugoslavica* (Col.: Buprestidae). However, plots were invaded by *C. achates* such that little evidence of *S. jugoslavica* was found; I was able to confidently identify only 12 plants as having *S. jugoslavica* attack (an additional 12 plants had root herbivore attack but the insect could not be identified). While root herbivore attack on bolted plants was the same regardless of *S. jugoslavica* treatment ($F_{1,59} = 0.29$, $p = 0.59$), root herbivore attack on plants that did not bolt was higher in the *S. jugoslavica* treatments ($F_{1,42} = 5.24$, $p = 0.027$) suggesting possible interactions between the root herbivores. As such, I used only the plants in the no *S. jugoslavica* treatments. It would be interesting to formally investigate
this pattern as it suggests that increasing *C. achates* in the southern Okanagan might reduce populations of *S. jugoslavica* as it appears to be the competitive dominant.

Additionally, it would be of interest to repeat this type of study in other systems to further consider the association between competition between natural enemies and whether they have independent effects on the reduction of plant performance, particularly in an above- and below-ground context. This two processes have been considered separately. Linking different processes will help us get a better understanding of the processes structuring communities.

**6.2 Changes in plant performance vs. changes in the plant population.**

In most chapters of my thesis, with the exception of Chapter 2, I considered changes in plant performance. Implicitly we assumed in Chapters 3-5 (as do all studies that consider reductions in plant performance caused by herbivores) that a reduction in plant performance will likely reduce plant populations. However, there is little understanding of when and how reductions in plant performance scales up to declines in plant populations (Crawley 1989a; Maron & Crone 2006). Herbivores can reduce plant abundance if they 1. directly kill plants or seeds and 2. this mortality causes a reduction in the number of individuals in subsequent generations (Maron & Crone 2006). There are numerous cases where the first criteria applies but not the second due to compensatory density-dependence (Maron & Crone 2006).

A common way of scaling up from plant damage to changes in the plant population is through the use of population models (e.g. McEvoy & Coombs 1999; Miller *et al.* 2009) and these can be used to optimise management strategies (Shea *et al.* 2010).
While modelling whether changes in the performance of diffuse knapweed result in changes in plant populations was beyond the scope of this thesis, it is unlikely to have provided us with a robust prediction. Diffuse knapweed is known to have strong compensatory density-dependence; Powell (1990) showed that reducing plant density increases survivorship and bolting rates, and Myers & Risley (2000) developed a model of diffuse knapweed showing how compensation reduced the impact of seed predators. Incorporating density-dependence into matrix models of plant populations is rarely done as obtaining estimates is difficult and incorporating density-dependence makes calculating sensitivities difficult as growth rate is no longer exponential (Maron & Crone 2006). Therefore measuring $\lambda$ in one generation may not yield an accurate prediction of population change. However, some simulation models (e.g. Rees and Paynter 1997) have successfully incorporated density-dependence, I did not have appropriate data to build similar models.

### 6.3 Implications for weed biological control

To make weed biological control programs more successful, top-down control of plants by their natural enemies must be better understood. Top-down control is likely to occur only with certain combinations of plants and natural enemies, and only under certain conditions. An improved understanding of the attributes of plants and enemies that predict the success of biological control will improve success rates. While we have identified two attributes of insect species that are likely to be associated with success (distribution in response to plant density and multiple feeding modes), there are likely to be many more. However, different plants have different traits, therefore different types of insects will be successful. More research into both successful and unsuccessful biocontrol introductions is needed to determine attributes of both the plants and the
agents that are associated with successful control. While, in the future, we may be able to provide a biocontrol practitioner a list of traits that describe the ideal agent for their particular weed, it may not be possible to find the perfect species (see Waage (1990) for a discussion of similar ideas in insect biological control). An additional complication is that some of the attributes listed by van Klinken & Raghu (2006) are likely to be in contradiction at least in some situations (e.g. new associations are better than co-evolved ones vs. most effective agent will come from most climatically similar parts of range).

We have long known that external factors interact with the insect-plant system and that they can influence top-down effects, thus biological control. For example, biocontrol of salvinia in Papua New Guinea required fertilisation (increasing NPK) of the weed for the weevil to exert control (Room & Thomas 1985). The salvinia-weevil system seems to exist in two stable states (Schooler et al. 2011), the first where salvinia densities are low, have high N, high growth rates and weevils exert control. The second stable state is where densities are high, growth is low and weevils are unable to exert control. Potentially, fertilisation tipped the system from the second stable state to the first.

However there appears to have been more focus on changing abiotic factors than biotic ones, particularly with the need to understand the effects of climate change. I showed using meta-analysis that on average, additional natural enemies do not alter the reduction in plant performance. In contrast, in the experiment I conducted asking the same question, the interactions between C. achates and L. minutus led to a synergistic reduction in seed output of diffuse knapweed. The meta-analysis showed that there was variation in the response and my study highlights this variation (although it was not included in the meta-analysis). I suggest that the discrepancy between these results might be due to the lack of competition between C. achates and L. minutus or due to
experimental factors such as the period of observation. Lack of competition might be particularly evident between root herbivores and herbivores on above-ground parts of the plant (Clark 2010). Testing how new agents interact with already established agents (particularly common ones) may enable us to optimise control by focusing introductions on species which are unlikely to interact with other species to decrease potential control.

6.3.1 Recommendations for knapweed control

Diffuse knapweed is not yet under control in some parts of its range, either because insects have not been released there (or migrated naturally) or due to environmental conditions which appear to be sub-optimal for the insects; control appears to be less successful at higher elevations. This seems to be because at higher elevations there is a degree of mis-match between insect and plant phenology.

In areas where *L. minutus* has not been released, I recommend its release. It has been shown to be successful (Myers *et al.* 2009) and there are no reports of non-target effects. *Cyphocleonus achates* would appear to be a good addition if deemed necessary. It has been credited with the decline of spotted knapweed (Corn *et al.* 2006) and my results show that at Vaseux Lake it interacted with *L. minutus* to reduce seed production beyond that expected from the two species alone. *Urophora affinis* should not be redistributed further (although it may have reached its maximum extent) as it has been unsuccessful at controlling diffuse knapweed and it has been alleged that unintended knock-on effects occurred with its introduction (Pearson & Callaway 2006)

Harris (2011) suggests introducing more insects, particularly more root feeders for diffuse and spotted knapweed. I disagree with this idea as control has been observed with *L. minutus* and *C. achates*. The observations at Vaseux Lake of potential interactions between *S. jugoslavica* and *C. achates* suggests that the root herbivores
could compete to reduce overall control. Even if we disregard the control observed, failure to successfully control a weed with the introduction of 13 agents would suggest that natural enemy release is not the mechanism behind success of the target weed and other approaches need to be considered. It would be better use of knapweed control dollars to determine if there are ways in which we can improve control of knapweed with the agents already present, as discussed above. Improving control with the current suite of agents also decreases the risk of non-target attack.

Diffuse knapweed has the potential to invade elsewhere (e.g. the central Patagonian plateau of Argentina and Central Otago in New Zealand). If successful invasions occur, agents that have been shown to successfully control diffuse knapweed in North America (i.e. *L. minutus* and to a lesser extent *Cyphocleonus achates*) should be the first species considered; a key factor in predicting biocontrol success is that the agent is successful elsewhere (McFadyen 1998). Assessment of attack on non-target plants would still be needed and might render these agents unacceptable.
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Myers, J.H. & C. Risley (2000) Why reduced seed production is not necessarily translated into successful biological weed control. *Proceedings of the X*
International Symposium on Biological Control of Weeds (ed N.R. Spencer), pp 569-581. Montana State University, Bozeman, Montana, USA.


Shea, K., E. Jongejans, O. Skarpaas, D. Kelly & A.W. Sheppard (2010) Optimal management strategies to control local population growth or population spread may not be the same. Ecological Applications 20, 1148-1161.


APPENDIX A:

Chapter Two: Resource concentration by insects and implications for plant populations

Appendix A1: List of variables and parameters used in the model

Table A1.1 Variables used in the model

<table>
<thead>
<tr>
<th>Name &amp; explanation</th>
<th>Starting conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of insects in ( x_{i,t} ) at time ( t )</td>
<td>10 insects per plant in patch prior to redistribution of the insects</td>
</tr>
<tr>
<td>Number of plants in ( y_{i,t} ) at time ( t )</td>
<td>20 populations drawn from the exponential distribution with a mean of 100</td>
</tr>
</tbody>
</table>
Table A1.2 Parameters used in the model

<table>
<thead>
<tr>
<th>Name &amp; explanation</th>
<th>Value used</th>
<th>Range used in sensitivity analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curvature – determines the pattern of insect distribution</td>
<td>$\theta$</td>
<td>Range of -1 – 3</td>
</tr>
<tr>
<td>Insect reproduction $r_x$</td>
<td>8</td>
<td>2 – 32</td>
</tr>
<tr>
<td>Number of seed-heads on the plants $s_{i,t}$</td>
<td>Each generation, drawn from normal distribution with a mean of 75 per plant (SD = 10) but constrained so that minimum value = 0</td>
<td>–</td>
</tr>
<tr>
<td>Seed-heads that are unavailable for insect attack $e$</td>
<td>Uniform random value between 0 – 20% of seed-heads are unavailable for attack by insects</td>
<td>0-10% – 0-30%</td>
</tr>
<tr>
<td>Number of seed-heads that escape attack $T_{i,t}$</td>
<td>Calculated from the available seed-heads, the proportion unavailable for attack and the number of attacking insects (Eqn5)</td>
<td>–</td>
</tr>
<tr>
<td>Insect impact – equates to the proportion of seeds in seed-head destroyed if an insect is present in the seed-head $a$</td>
<td>0.95</td>
<td>0.93 – 0.97</td>
</tr>
<tr>
<td>Plant reproduction – proportion of seed-heads that will successfully produce one adult plant $r_y$</td>
<td>0.10</td>
<td>0.08 – 0.12</td>
</tr>
</tbody>
</table>
Appendix A2: Impact of varying parameters

We varied some of the key model parameters to test whether the overall results were robust to changes in these parameters.

Insect impact which equates to the percentage of the seeds in the seed-head destroyed was varied from 93-97% of seeds destroyed, Figs A2.1 & A2.5. We varied the number of insect offspring produced from 2 – 32, Figs A2.2 & A2.6, and the plant reproduction parameter from 0.08 – 0.12 which equates to between 6 and 9 flowering plants produced per flowering plant, when the number of seed-heads is at the mean of 75 (Figs A2.3 & A2.7). Finally, we varied the seed-head escape parameter, which indicates the search efficiency of the insects, from 0-5% of seed-heads escape attack to 0-20% of seed-heads escape attack (Figs A2.4 & A2.8). We ran the model for 30 generations and assessed the total plant population and the number of plant patches, then ran the model for 1000 generations and calculated proportion of populations that went extinct and the time taken for those populations to go extinct. In all Figs, the value indicated by the * is the value used for our main analyses.

While the absolute values alter with different parameter sets, the overall patterns of interest are consistent. Increasing variance between patches allows some patches to escape attack. Resource concentration leads to greater persistence of the insect-plant system while weak resource dilution leads to faster rates of population decline.
Short term

Insect impact

- 0.93
- 0.94
- 0.95 *
- 0.96
- 0.97

Figure A2.1: Impact of varying insect impact, i.e. the proportion of seeds insects destroy, on the total plant population (left) and number of plant patches (right) after 30 generations.

Insect reproduction

- 2
- 4
- 8 *
- 16
- 32

Figure A2.2: Impact of varying the maximum number of offspring produced by each insect, on the total plant population (left) and number of plant patches (right) after 30 generations.
Figure A2.3: Impact of varying the maximum number of flowering plants produced by each plant on total plant population i.e. proportion of the mean seed production per plant of 75 seeds that survive to reproduce; 6 to 9 plants (left) and number of plant patches (right) after 30 generations.

Figure A2.4: Impact of varying the maximum percentage of seed-heads that escape attack on total plant population (left) and number of plant patches (right) after 30 generations.
Long term

**Figure A2.5**: Impact of varying insect impact, i.e. the proportion of seeds insects destroy, on the percentage of populations that persisted (left) and the number of generations taken for populations to go extinct (right) within 1000 generations.

**Figure A2.6**: Impact of varying the maximum number of offspring produced by each insect, on the percentage of runs in which populations survived (left) and the number of generations taken for populations to go extinct (right) within 1000 generations.
Figure A2.7: Impact of varying the maximum number of flowering plants produced by each plant (i.e. proportion of the mean seed production per plant of 75 seeds that survive to reproduce; 6 to 9 plants) on the percentage of populations that persisted (left) and the number of generations taken for populations to go extinct (right) within 1000 generations.

Figure A2.8: Impact of varying the maximum percentage of seed-heads that escape attack on the percentage of populations that persisted (left) and the time taken for populations to go extinct (right) within 1000 generations.
Appendix B: Chapter Four: Predicting the effects of multiple natural enemies on plants: a meta-analysis

Appendix B1: Statistical issues in the identification of non-independent interactions

One-way vs. two-way ANOVA

In a number of cases, a one-way ANOVA has been used to analyse data generated from factorial experiments of two natural enemies. The ANOVA has typically been followed by a post-hoc test (e.g. Bonferroni or Tukey's HSD). In these studies, researchers were asking whether the addition of a second enemy increased overall damage. While these are factorial experiments, they have not been analysed as such; therefore interactions between natural enemies (i.e. independence or non-independence) cannot be statistically determined. While Hatcher’s (1995) definitions for additive (= independence under an additive model, see below), synergistic, equivalent and inhibitory effects have been used following a one-way ANOVA, this is an inappropriate extension when raw data are available and two-way ANOVAs can be used (see Section 4.3.2).

Effect of different statistical models for finding non-independent interactions

Independent interactions are identified by a non-significant interaction term in a two-way ANOVA (Fournier et al. 2006; Gerber et al. 2007; Hladun & Alder 2009) – it is the null model. The alternatives, synergism and antagonism are identified by a
significant interaction term followed by inspection of the summary data (means and variance).

Two basic models are used to test the effects of multiple enemies. This has been appreciated in the predation literature (Sih et al. 1998) but has been mostly ignored in the herbivory/plant pathogen literature, with the notable exceptions of Fournier et al. (2006) and Willis et al. (1993).

The two models are described as the additive and multiplicative models, and both test the same null hypothesis: i.e. that the two natural enemies act independently. Under the additive model the null expectation is:

\[ Sp_{AB} = Sp_A + Sp_B \]  \hspace{1cm} (1)

where \( Sp_A \) and \( Sp_B \) are the amount of biomass removed by Species A and Species B respectively and \( Sp_{AB} \) is the amount removed when both species are present. However, if Species A removes 60% of the biomass and Species B removes 70%, the expectation is that 130% of biomass will be removed (Sih et al. 1998, Fournier et al. 2006). This conundrum is solved by using the multiplicative risk model (Soluk & Collins 1988) where the null expectation is:

\[ Sp_{AB} = Sp_A + Sp_B - (Sp_A \times Sp_B) \text{ or} \]

\[ (1 - Sp_{AB}) = (1 - Sp_A) \times (1 - Sp_B) \]  \hspace{1cm} (2)

thus the expectation for the example above is that 88% of biomass will be removed, a more realistic figure. The additive risk model is generated by conducting a two-way ANOVA without transforming the data; to generate the multiplicative model, data must be log-transformed prior to analysis. Log-transformations have typically been used to achieve homoscedascity and normality rather than due to the underlying model. If \( Sp_{AB} \) is small, which happens when either a natural enemy has little or no impact on the plant when alone, and is often the case, the two models generate similar predictions.
We contacted authors to obtain raw data from which we could determine whether conducting the analysis under the multiplicative or additive models altered the categorisation of the response,

In total, we received data from 15 studies for this analysis, providing us with 26 species combinations. Papers from which we received raw data are listed in the Appendix B2.

We re-ran analyses under both the multiplicative and additive models. If necessary, we added 1 to the response variable. We ignored additional treatment conditions, and where three different herbivores were measured in a factorial design, we extracted data to treat them as a series of two-way interactions. Additionally, we did not consider whether the data met the assumptions of the ANOVA model. Therefore, our results cannot be compared directly to the results of the authors.

There were fewer cases of non-independence in the data obtained from authors than in the complete dataset; 10.3% of responses were non-independent under both models. In 5.1% of cases, the overall conclusions of whether interactions were independent depended in the model used (assuming an alpha of 0.05). The average change in F-value was 1.38 (SD = 4.04) while the average change in p-value was 0.16 (SD = 0.19).

This suggests that while an appreciation of the different model types is necessary, conclusions of individual papers are unlikely to change substantively with use of a different model. It is necessary however, to clearly state the model used. We recommend choosing the null model based on the natural history of the study system.
Appendix B2: Justification for the inclusion variables tested for association with non-independent interactions

Here we outline the reason for the inclusion of the variables we tested for association with non-independent interactions. The letters match the letters in Table 1 of the main text. Many of these are factors which influence competition between enemies or alter the elves of damage that the species inflict.

A: Density variables might be less likely to show a non-independent response than other variables as plant density is influenced by more factors.

B: Enemies are typically removed from the plants using either insecticide or fungicide. Pesticides may have phyto-toxic effects, and plants may have generated defence compounds in response to attack. Zvereva & Kozlov (2012) noted that the average impact of removing below-ground insects was more variable than adding them.

C: Manipulation of guilds may be more frequently associated with non-independence than the manipulation of species, if responses are associated with enemy traits due to sampling effects.

D: Field conditions are more variable and these could obscure non-independent interactions. Greenhouse experiments were associated with stronger negative effects of sap-suckers than field experiments (Zvereva et al. 2010).

E: Plots containing other species might allow generalists to host-switch avoiding competition causing fewer antagonistic interactions.

F: We predicted that longer experiments might allow more time for non-independent effects to be observed. However, length of experiment was inversely associated with sap-sucker damage (Zvereva et al. 2010). We calculated the number of days the
experiment ran for, if no day was given, we assumed the experiment started/finished on 15th of the month.

G: Longer-lived plants may have different strategies to deal with attack than shorter-lived plants.

H: This might primarily have an influence via the enemies selected i.e. research bias (Gurevitch & Hedges 1999). Enemies of crop plants and weeds tended to be pests and biological control agents respectively. Zvereva & Kozlov (2012) noted that responses to below-ground herbivores were strongest in agricultural and weed control studies. As economic status can vary, we followed the author’s perception.

I & J: Plant functional group or woodiness might explain differences in the occurrence of non-independent interactions. Competition between insects was more frequently reported between forb- and grass-feeders, than between tree and shrub feeders (Denno et al. 1995).

K: Some plant families may be more likely to respond non-independently than others.

L: Different combinations of natural enemy types (insects vs. plant pathogens) might show differences in responses. Insect herbivores can increase susceptibility to plant pathogens and vice versa (Agrawal 2005, Thaler et al. 1999) or a pathogen elicits biochemical defences protecting the plant from insect attack (Karban et al. 1987). While different metabolic pathways are used to enhance resistance to insect or pathogen attack, the induction of one pathway can influence induction of the other (Paul et al. 2000).

M: As direct interactions are associated with stronger competition (Kaplan & Denno 2007), they might be associated with increased antagonistic interactions when compared with indirect interactions.
N: All biological control agents are specialists. Therefore, in order to use generalists to predict traits of biological control, we need to test whether being a generalist or specialist matters. We might expect differences in the plant responses as generalists and specialists inflict different selective pressures (van der Meijden 1996). Additionally, biological control agents must interact with native generalists. While Denno et al. (1995) theorised that specialists might incur increased competition, they did not find any evidence to support this, however, Zvereva et al. (2010) found that generalist sap-suckers reduced plant performance to a greater extent than specialists.

O & P: We tested whether non-independent interactions were more likely to occur when enemies were in the same order or in the same family. Theoretically, increasing taxonomic similarity should lead increased competition. Kaplan and Denno (2007) found that relatedness was associated with competition for sapsuckers but not chewers. The colonisation ability of plant pathogen depended on the relatedness of competing strains (Koskella et al. 2006).

Q: Theoretically, sharing a feeding-guild should lead to greater competition, however little support has been found (Kaplan & Denno 2007).

R, S & T: Inter-specific competition occurs frequently between insects in all guilds except chewers (Denno et al. 1995).

U, V, W & X: Attack of more valuable plant parts (i.e. reproductive parts – fruits and flowers) might be associated with an increase in defence compounds (Zangeral & Rutledge 1996), leading to antagonistic interactions. Stem borers, seed and fruit feeders were more frequently involved in competitive interactions than leaf feeders (Denno et al. 1995).
Y, AA, BB, CC & DD: Some groups of species might be more often involved that other – e.g. Hemiptera were more often involved in competitive interactions than other orders (Denno et al. 1995).
Appendix B3: Studies in analyses

Table B3.1 gives details of all papers used in the various analyses. Papers were excluded from the vote-counting due to: 1. use of one-way rather than two-way ANOVA (see Appendix B1, e.g. Knochel et al. 2010), 2. unclear whether data were logged or not (see Appendix B1; e.g. Hunt-Joshi et al. 2004).

Papers were excluded from the meta-analysis if we were unable to determine the means of all four treatments (control, SpA only present, SpB only present, both species present) from either the paper or via contact with the authors (e.g. Hufbauer and Root 2002).

Poveda et al. (2005a, b) were treated as the same study. Two responses had to be removed from the data set as the actual response when both species were present was zero. They were both from James et al. (1992): realised fecundity and percent viable achenes. Additionally, we removed the growth rate response from Shivas & Scott (1993), as growth with Species A was negative.
**Table B3.1:** Species involved in all studies used in various analysis in paper.

<table>
<thead>
<tr>
<th>Species A taxonomy</th>
<th>Species B taxonomy</th>
<th>Plant species</th>
<th>Plant family</th>
<th>In meta-analysis</th>
<th>in vote counting?</th>
<th>Supplied data for Box Source</th>
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<td>Contarinia watti</td>
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<td>Mimulus guttatus</td>
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Appendix B4: Full results

None of the various attributes of the experimental set-up that we considered were correlated with the occurrence of non-independent interactions (Fig. B4.1) i.e. there was no difference from the null model. The lack of association with the manipulation of species or guilds allowed us to include studies where guilds of natural enemies were manipulated into later analysis.

None of the models containing the plant variables were significantly different from the null model (Figs B4.2 & B4.3), thus these did not improve our predictive ability. Biennial plants did show some antagonism (Fig. 4.2A), however most biennial plants were knapweeds (Centaurea spp.). Asteraceae were involved in antagonistic interactions and Ranunculaceae in synergistic ones (Fig. 4.3).

Whether the natural enemies were insects or plant pathogens or a combination of both did not improve the predictability of non-independent interactions, (Fig. B4.4A). Direct interactions were antagonistic (confidence intervals did not cross zero) but including this factor in the model does not improve the predictability of non-independent interactions (Fig. B4.4B).

Separating species into specialists (all biological control agents are specialists) and generalists does not improve the predictive power (Fig. B4.4C), neither group is more likely to be involved in synergistic or antagonistic interactions. Thus we can use both groups of species to further investigate where non-independent interactions occur.

Enemies that were in the same family or order or in the same feeding guild were no more likely to result in non-independent interactions than more distantly related enemies (Fig. B4.5A-C). The presence of insects in a particular feeding guild did not improve the model (Fig. B4.5D-F).

Attack on fruits and flowers, i.e. reproductive organs was associated with antagonistic interactions and was a significant predictor of non-independent interactions (Figs B4.6A). Species combinations without root herbivores were antagonistic (confidence intervals do not include zero), but the presence of this factor in the statistical model did not improve its predictive ability (Fig. 4.6C).
Attack to leaves and stems (Fig B4.6B & D) were not associated with non-independent interactions.

Antagonistic interactions were significantly more likely to occur if one or both natural enemies were Diptera (Fig. B4.7C) but no other taxonomic group predicted the occurrence of non-independent interactions (Fig. B4.7).

**Figure B4.1** Attributes of experiments as correlates of the presence of non-independent impacts of natural enemies on plant performance. A: type of response tested, B: type of natural enemy manipulation (addition vs. removal); C: whether species or guilds of natural enemies were manipulated; D: the location of the experiment; E: the unit of replication and F: length of the experiment in days with 95% confidence intervals.
Figure B4.2 Plant attributes as correlates of non-independent impacts on plant performance from two natural enemies. A: plant life-history status; B: plant economic status; C: plant functional group; D: plant woodiness.

Figure B4.3 Plant family as a correlates of the presence of non-independent impacts of natural enemies on plant performance.
Figure B4.4 Natural enemy type (A), interaction type (B) and number (C) of generalists or specialists as correlates of the presence of non-independent impacts of natural enemies on plant performance. Direct interactions are where natural enemies attack the same plant part (e.g. leaves) at the same time whereas indirect interactions involve spatial and/or temporal separation between the natural enemies.

Figure B4.5 Taxonomic similarity of enemies and enemy feeding guilds as correlates of the presence of non-independent impacts of natural enemies on plant performance. Whether natural enemies were in the same order (A), family (B) or feeding guild (C). Number of chewing insects (D), sap-suckers (E) and miners (F) on the plant.
**Figure B4.6** Plant part attacked as a correlate of the presence of non-independent impacts of natural enemies on plant performance. A: reproductive plant parts; B: leaves; C: roots and D: stems.

**Figure B4.7** Natural enemy taxonomy as a correlate of the presence of non-independent impacts of natural enemies on plant performance. A: Coleoptera; B: Lepidoptera; C: Diptera; D: Hemiptera; E: Fungi; F: Virus.