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# Food Web Ecology of a Leafminer-Parasitoid Community

by

Eleanor Jane Blitzer

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Committee in charge:

Professor Steve C. Welter, Chair
Professor Nick J. Mills
Professor Wayne P. Sousa

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# Abstract

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The importance of competition in phytphagous insect communities is a highly controversial and unresolved issue. This debate was resolved, in part, by the recognition that species may compete indirectly as well as directly. I first test the importance of direct competition on the fitness of *L. helianthi* and *C. platyptera* with a series of laboratory experiments which manipulate the densities of each species. I focus on the leafminers *Liriomyza helianthi* and *Calicomyza platyptera* because these two leafminer species co-occur spatiotemporally, are closely related, and share a cryptic feeding niche, and, are therefore predicated to compete and provide a model system for testing the assumptions of competition theory.

*Liriomyza helianthi* and *C. platyptera* along with their community of hymenopteran parasitoids provide a model system for studying apparent competition in the field. Apparent competition, one indirect pathway for competition, is defined as a negative effect of one species on the population growth rate or abundance of another species, mediated through the action of shared natural enemies. I surveyed populations of *L. helianthi* and *C. platyptera*, along with their community of parasitoids, over two years at seven sites in the Central Valley of California. Quantitative food webs for each year were used to visualize host-parasitoid interactions. I found the same dominant parasitoids were shared by both species of herbivore. Parasitism of *C. platypera,* was significantly positively correlated with *L. helianthi* leaf-miner density and parasitism levels.

Indirect interactions can extend herbivore competition across space and time. Apparent competition has generated much recent attention, but studies that test the mechanisms that lead to apparent competition are still rare. I use experimental data to test hypotheses on the mechanisms leading to asymmetric apparent competition. *Liriomyza helianthi* populations emerge approximately one month (two parasitoid generations) before *C. platyptera* and have a higher population density throughout the season. Experimental removal of *L. helianthi* populations in the early summer leads to a 50% reduction in parasitism of *C. platyptera*. Because parasitoids are known to be important mortality agents in this system, I conclude that emergence asynchrony between the two herbivore species significantly impacts populations of *C. platyptera*.

Land-use intensification has led to a mosaic landscape which juxtaposes human-managed and natural areas. In such human-dominated and heterogeneous landscapes, spillover across habitat types, especially in systems which differ in resource availability, may be an important ecological process structuring communities. While there is much evidence for spillover from natural habitats to managed areas, little attention has been given to flow in the opposite direction. Here we synthesize studies from five functionally important trophic groups, herbivores, pathogens, pollinators, predators, and seed dispersers, and discuss evidence for spillover from

managed to natural systems in all five groups.  I argue that this effect has been underestimated in the past. The most frequently documented examples of spillover from managed to natural systems report pathogen and vertebrate predator spillover, with a smaller number of examples reporting spillover of pollinators and herbivores.

# Dedication

To my parents for your unconditional love and support on this, and every other journey.

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# Introduction

How communities are organized is a fundamental question in ecology. My dissertation builds on existing research that develops and tests hypotheses on the mechanisms that shape ecological communities. I present insights from two perspectives on community structure. In the first section I use a model system, a dipteran leafminer-parasitoid community, native to California's Central Valley, to look at the relative strengths of both direct (Chapter 1) and indirect (Chapter 2 and 3) competition as structuring mechanisms. In the second section (Chapter 4) I review evidence for the spillover of herbivores, pathogens, pollinators, predators, and seed dispersers from managed to natural habitats. These two different ways of looking at community structure offer the opportunity to test specific mechanisms that shape ecological communities, and to set these findings in the context of existing research on the importance of movement as a structuring mechanism across trophic groups.

From Darwin's time to ours competition has played a central role in the thinking of community ecologists (Darwin 1859, Volterra 1926, Lotka 1932, Gause and Witt 1935, Hairston et al. 1960, MacArthur and Levins 1964, Kaplan and Denno 2007). Research on interspecific and intraspecific competition among phytophagous insects has a particularly long and controversial history. For decades, research on competition among herbivorous insects was based on the classical assumption that coexistence could only be achieved through niche partitioning of resources. Much of this research was based on findings from observational studies. In the 1980s two critiques challenged the importance of competition in structuring insect communities. First, growing evidence of positive interspecific associations led some to question the prevailing view that interspecific competition was common in insect communities (Lawton 1982, Lawton 1984, Jermy 1985). Second, a number of studies were published which appeared to validate the conclusions of Hairston et al's "world is green" hypothesis, which argues that, since defoliation is rare, it is unlikely that resources impose the main constraint on herbivore population size (Lawton and Strong 1981, Connell 1983, Schoener 1983, Strong et al. 1984).

The dispute over the importance of competition arose partly due to a lack of manipulative studies, but also because ecologists rarely examined indirect interactions, which are often complicated to measure. Recent research showing that species may influence each other indirectly as well as directly implies that indirect interactions may be among the key mechanisms that structure phytophagous insect communities (Denno et al. 1995, Kaplan and Denno 2007). Indirect competition among herbivores is now well documented (van Veen et al. 2006, Kaplan and Denno 2007) and indirect interactions via natural enemies or shared host plants are likely to explain some of the discrepancies which caused arguments in past decades (Kaplan and Denno 2007).

In the core section of this dissertation (Chapters 1-3), I contribute to our understanding of ecological structure by using a model system to investigate both direct and indirect interspecific competition. Unlike much of the previous research in this field, I measure both direct and indirect competition, and am thus able to assess their relative importance. I begin by testing, in the lab, the strength of direct resource competition between two species of leafminer (Chapter 1). The leafminer species I focus on, *Liriomyza helianthi* Spencer (Diptera: Agromyzidae) and

*Calicomyza platyptera* (Thomson) (Spencer) co-occur spatiotemporally, are closely related, and share a cryptic feeding niche, and, are therefore predicated to compete and provide a model system for testing the assumptions of competition theory.

In Chapter 2 I use observational data and quantitative analyses to investigate the strength of apparent competition between *L. helianthi* and *C. platyptera* in the field. I follow populations of both species of leafminer, along with all associated parasitoids, over two seasons in 8-9 field sites in the Californian Central Valley. I use quantitative methods, including quantitative food webs, quantitative food web overlap, and generalized linear mixed effect models to determine whether these two leaf-miner species interact indirectly through their shared natural enemies. Based on patterns observed in the field, along with the results of quantitative models discussed in Chapter 2, I develop hypotheses on the importance of indirect competition in my system. In Chapter 3 I report findings from a large-scale manipulative field study that tests these hypotheses. By manipulating leafminer densities in the field I show that emergence asynchrony between herbivores leads to asymmetric apparent competition.

In the final section of my dissertation I move away from the leafminer-parasitoid model system and look at spillover as a mechanism structuring communities for five functionally important trophic groups (Chapter 4). For this chapter I define spillover simply as the movement of organisms from one distinct habitat type, or population (in the case of pathogen spillover), to another. I am interested primarily in how this movement influences species interactions and assemblage structure in recipient habitats. I assess the evidence that indicates a potential for spillover effects, by reviewing the literature for five trophic groups, including both antagonisms (herbivory, disease, and predation) and mutualisms (pollination, seed dispersal), across the managed-natural system interface.

The four chapters of this dissertation deal with community structure on a number of levels; from lab experiments evaluating the importance to interspecific competition, to large scale field manipulations, to a wide ranging literature review. This body of work provides evidence that will help advance ecological theory on competition as well as encourage future empirical research on the movement of organisms across the managed system- natural habitat interface.

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# Chapter 1

# Experimental evidence of resource competition between two species of dipteran leafminers

## Abstract

The role of competition in regulating phytophagous insect communities has had a long and controversial history. Traditional competition theory predicts that the likelihood and intensity of competition between two organisms will increase with increasing density, spatiotemporal co-occurrence, and phylogenetic similarity. Research on indirect interactions involving plants (induced defenses), and natural enemies (apparent competition) has dramatically increased in the past 15 years. This new body of work has led to changes in theoretical predictions on community structure and indirect interactions between phytophagous insects. *Liriomyza helianthi* Spencer (Diptera: Agromyzidae) and *Calicomyza platyptera* (Thomson) (Spencer) (Diptera: Agromyzidae), co-occur spatiotemporally, are closely related, and share a cryptic feeding niche, and, therefore, are predicated to compete. We assess the importance of direct competition on the fitness of *L. helianthi* and *C. platyptera* with a series of laboratory experiments which manipulate the densities of each species. We find no evidence for competition between *C. platyptera* and *L. helianthi* at densities recorded in field populations. However, at high densities, we are able to detect significant, negative effects of competition on the fitness of both leafminer species.

## Introduction

The role that competition plays in regulating phytophagous insect communities has received special scrutiny (Darwin 1859, Hairston et al. 1960, MacArthur and Levins 1964, Strong et al. 1984, Denno et al. 1995, Kaplan and Denno 2007). After many years in which herbivores were presumed not to interact competitively (Lawton and Strong Jr 1981, Stiling et al. 1984, Strong et al. 1984), direct competition for resources by herbivores have now been well documented, but with a number of caveats (Denno et al. 1995, Kaplan and Denno 2007).

Although traditional competition theory predicts that the likelihood and intensity of competition between two organisms will increase with increasing density, spatiotemporal co-occurrence, and phylogenetic similarity, a recent review on the subject finds little evidence in phyphagous insect communities to support these traditional paradigms (Kaplan and Denno 2007). Research on indirect interactions involving plants (induced defenses), and natural enemies (apparent competition) has dramatically increased in the past 15 years (Chaneton and Bonsall 2000, Ohgushi 2005, van Veen et al. 2006). This new body of work, along with the development of modern meta-analytical tools, has led to changes in the theory predicting interspecific competition (Kaplan and Denno 2007). The evidence we report here, on direct intraspecific and interspecific competition, is part of a larger study in which we also looked at indirect apparent competition mediated through shared natural enemies (Blitzer and Welter 2011, submitted). We are therefore in the unique position to discuss the results of this study in the context of a system in which the importance of both direct and indirect competition have been evaluated.

Leaf-mining insects have often been selected as model systems in competition experiments. Although leaf-mining insects are associated with species-rich parasitoid communities, intraspecific competition has been observed in a number of species (Askew and Shaw 1974, Quiring and McNeil 1984, Stiling et al. 1984, Potter 1985, Bultman and Faeth 1986, Auerbach et al. 1995). Much of the previous research on intraspecific and interspecific leafminer competition has focused either on natural systems of univoltine leafminers on perennial vegetation (Askew and Shaw 1974, Faeth and Simberloff 1981, Quiring and McNeil 1984, Stiling et al. 1984, Potter 1985, Bultman and Faeth 1986, Auerbach and Simberloff 1989, Auerbach et al. 1995, Eber 2004, Gripenberg and Roslin 2008), or on multivoltine, agriculturally important species at high levels that would not be expected in natural systems (Oatman 1960, Parrella 1983, Petitt and Wietlisbach 1992). Here, the study system we focus on is multivoltine, with 4-6 generations per year, as well as being a non-pest species.

We test the importance of direct competition on the fitness of *Liriomyza helianthi* Spencer (Diptera: Agromyzidae) and *Calicomyza platyptera* (Thomson) (Spencer) (Diptera: Agromyzidae) with a series of laboratory experiments that manipulate the densities of each species. These two leafminer species co-occur spatiotemporally, are closely related, and share a cryptic feeding niche, and, are therefore predicated to compete and provide a model system for testing the assumptions of competition theory (Kaplan and Denno 2007).

## Methods and materials

### *Study system*

*Liriomyza helianthi* (sunflower leafminer) is a specialist serpentine leafminer found exclusively on *Helianthus annuus* (annual sunflower) and *Xanthium strumarium* (common cocklebur) (Gratton and Welter 2001). *L. helianthi* females lay their eggs in the leaves of the host plants, the eggs hatch and larvae feed in the upper mesophyll layer of the leaf creating characteristic serpentine mines visible on the surface of the leaf. Larvae progress through three instars in approximately 3-4 days under laboratory conditions and then cut an exit hole on the surface of the leaf and fall from the leaf to pupate. Adults emerge 10-14 days later.

*Calycomyza platyptera* (blotch leafminer) occurs sympatrically with *L. helianthi* in the field and, although it has been recorded on a wider range of host plants, feeds exclusively on *H. annuus* and *X. strumarium* at all of the sites we have studied in central and northern CA. Both are native to the Californian Central Valley. Adults of *C. platyptera* lay their eggs in the host plant leaves and larvae feed on the upper mesophyll of the leaf to create a whitish blotch mine which is larger and easily discernable from *L. helianthi* mines at low densities. In the lab *C. platyptera* progress through three instars in 8-12 days and then pupate inside the mine. *Calycomyza platyptera* adults emerge within 9-14 days. The egg to adult cycle time is 3-4 weeks for *L. helianthi* and 4-5 weeks for *C. platyptera*.

### *Laboratory colony procedures*

Colonies of both species of leafminer were established in 2006 from adult files reared from field collections of leaves. Field collected adult files were added during the summer of 2007 and 2008 whenever possible. Leafminer colonies are maintained at temperatures of 25 ± 1°C with relative humidity of 65 ± 5% and a photoperiod of 16:8 (L:D) hours. Leafminers are reared on a commercial variety of *Helianthus annuus* (cult. var “small black”, Turner Seeds, Breckenridge, TX, USA). For each species of leafminer, a tray of 10 six leaf stage (30 cm) *H. annuus* plants are placed in an oviposition cage with adults and exposed for 2-3 days depending on desired colony output (cycling plants through more quickly produces more adult flies). Trays of plants are then removed from the oviposition cages and moved to greenhouse benches to allow larvae to develop for 2-3 days. Procedures for both species are the same up to this point.

When *L. helianthi* mines have matured to the third instar the infested plants are placed on their sides in trays lined with laboratory bench paper. *L. helianthi* larvae exit the leaves and fall on the paper to pupate. Pupae are then collected with a wet paintbrush and placed inside emergence cages. Adult *L. helianthi* are added from emergence to oviposition cages twice weekly. *Calycomyza platyptera* infested plants are left upright for three to five more days allowing larvae to pupate in the leaves. After pupae have developed inside each mine all infested leaves are clipped and placed inside emergence cages. Adult *C. platyptera* flies are added from emergence to oviposition cages twice weekly.

### *Competition experiments*

Competition experiments were conducted in the greenhouse over five 3-4 week periods in November to February, 2008. *Helianthus annuus* plants at the eight to nine leaf stage (50 cm) were selected and two fully expanded leaves from each plant were individually caged in one liter clear plastic cups with foam lids. Each caged leaf was randomly assigned one of three treatments: (1) *L. helianthi* alone, n=16 (2) *C. platyptera* alone, n=16, or (3) *L. helianthi* + *C.platyptera*, n=16. Each leaf was also randomly assigned either a high or low number of adult female flies to achieve a range of larval densities within each treatment level. Due to differences in fecundity, the initial densities used in the experiments were slightly different between *L. helianthi* and *C. platyptera*. Initial densities for *L. helianthi* included two (low density), or four (high density) female adult flies. Initial densities for *C. platyptera* included four (low density), or eight (high density) female adult flies. Forty-eight hours after emerging from pupae, adult female flies were placed, along with equal numbers of male adult files to ensure mating success, in each enclosure. Over a period of three months we completed five trials with a total of 48 leaves.

Adult flies were checked two hours after being placed inside cups and any dead flies were removed and replaced. Adult flies were allowed to oviposit on caged leaves for 24 hours and were then removed. For a two week period, leaves were checked every 48 hours and the number of mines of each species of fly recorded until all larvae had pupated or it became impossible to count individual mines due to mine overlap. *Liriomyza helianthi* larvae were allowed to fall from the leaf to the bottom of the plastic cup to pupate. *Calycomyza platyptera* pupae were removed from leaves after all larvae pupated. Pupae were counted and weighted with a Mettler MT5 balance (Mettler Toledo Inc Columbus, OH, USA). Pupal weight is a known to predict adult leafminer fitness and it commonly used as a proxy for fitness in leafminer communities (Parrella 1983, West 1985).

### *Image analysis*

All leaves were scanned with a flatbed desktop scanner and the images analyzed using ASSESS 2.0 to measure leaf and mine areas and to calculate proportion leaf area mined for each leaf (Lamari 2002). This software, written for plant disease quantification analysis, calculates differences in pixel color, hue, and intensity (Fig. 1). This technique works well for calculating proportion of leaf area mined if mined pixels are different colors than background leaf pixels.

### *Statistical analysis*

Data from all trials were pooled after Tukey's HSD test found no significant differences ($P > 0.05$) in pupal weight, number of pupae, number of mines, or leaf area mined among comparisons of experimental trials. We used linear mixed effect models to examine the effects of leafminer density (number of pupae/leaf, fixed), and treatment (one species alone or both species together) on leafminer fitness (pupal weight). Plant and leaf effects were included as random effects. All analyses were conducted in the R statistical package (R Development Core Team 2008). Mixed effect models were run using the lme4 package in R (Bates et al. 2010).

## Results

Our initial number of adult flies achieved a range of larval densities for each competition treatment. The upper range of densities in our experimental treatments was higher than naturally occurring population densities observed in the field. Within a series of field studies over two years in Yolo county, CA (Chapter 3), we found population densities never exceeded 40 mines per leaf. Therefore, we first analyzed a subset of our data that included all experimental replicates below the upper limit of leafminer population levels, as well as repeated the same analysis including all densities.

We collected data on three measures of leafminer density or resources consumed; number of pupae per leaf, number of mines per leaf, and proportion of leaf area mined. These measures are closely correlated for both species (Spearman's rank correlation $P < 0.001$; Fig. 2). Because at very high densities we were unable to reliably count individual mines, there are a few instances where we have a few more pupae than mines. We analyzed each dependent variable separately, but found that patterns were not different depending on which measure of leafminer population density or resource consumption was used (Fig 2). Therefore, we only show the competition results using number of pupae per leaf as the independent variable.

### *Competition at field population density*

For all treatment levels, at population densities that we typically observe in the field we find no statistically significant relationship between leafminer density (total number of pupae per leaf) and pupal weight for either *L. helianthi* (P = 0.275, d.f. = 6, Fig. 3A) or *C. platyptera* (P = 0.380, d.f. = 6, Fig. 3A). Similarly, we found no differences between treatment (one species alone or both species together) for either *L. helianthi* (P = 0.180, d.f. = 6, Fig. 3A) or *C. platyptera* (P = 0.371, d.f = 6, Fig. 3A), but the sample size for the multiple species combinations at ≤ 40 mines per leaf was low. Thus, at levels that are ecologically relevant in the field for both leafminer species, there was no evidence for any type of direct competition either within or between species.

### *Competition at higher densities*

We are interested not only in the effects of competition at field population levels, represented by the subset of the lower range of densities, but also if we could detect a fitness cost due to competition at high extremes of population density. While other factors such as natural enemy impacts appear to be limiting the leafminers under field conditions, the question does arise as to what would be the significance of the interactions between the two species, if the indirect interactions via shared natural enemies was not present (Gratton and Welter 2001).

When we include the higher range of densities in our analysis of pupal weight we found a statistically significant effect of intraspecific competition on both *L. helianthi* (P = 0.001, d.f. = 19 Fig. 3B) and *C. platyptera* (P = 0.007, d.f. = 16, Fig. 3B) fitness. When all densities are included we still find no statistically significant effect of treatment of one species alone versus both species together for *L. helianthi* (P = 0.170, d.f =19) or for *C. platyptera* (P = 0.135, d.f. =16). The slopes of regression lines for *L. helianthi* and *C. platyptera* pupal weight on density are significantly different from each other (Fig 3B) (t = 3.402, P = 0.002, d.f. = 26). However, even though we observed a stronger negative relationship between *L. helianthi* fitness and density than between *C. platyptera* density and fitness, the difference was not dramatic. At the most extreme density of 150 pupae per leaf, pupal weight of *L. helianthi* would be predicted to be 20% less than the value at 1 pupa per leaf, whereas the pupal weight of *C. platyptera* would be reduced by 17%.

## Discussion

In a series of laboratory experiments we found no evidence for competition between *C. platyptera* and *L. helianthi* at densities recorded in field populations.  However, at higher densities, we are able to detect significant, negative effects of competition on the fitness of both leafminer species.  The effects we observed are likely due to exploitative or interference competition mechanisms that have already been shown to structure leafminer communities at high densities (Auerbach and Simberloff 1989, Hespenheide 1991, Faeth 1992).  While we were able to detect negative effects of competition at high densities in the lab, direct resource competition is not an important structuring mechanism for this leafminer community in natural systems, presumably due to the suppressing effects of natural enemies on overall population densities in the field (Gratton and Welter 2001).

For each of the intraspecific competition treatment levels (*L. helianthi* alone or *C. platyptera* alone) we achieved a range of larval densities.  But, for the interspecific competition treatment levels (*L. helianthi* + *C. platyptera*), we were never able to manipulate the densities so that we had low levels of *L. helianthi* occurring with high levels of *C. platyptera*.  Therefore, although we are able to evaluate the effect of interspecific competition of *L. helianthi* on *C. platyptera* we are unable to evaluate the opposite effect, of *C. platyptera* on *L. helianthi*

Traditional competition theory predicts that competitive interactions are reciprocal, necessitate spatial and temporal co-occurrence, and increase in intensity as the density, phylogenetic similarity, and niche overlap of competing species increase (Connell 1983, Schoener 1983, Denno et al. 1995).  The pair of herbivores we chose to study fit many of the traditional criteria for predicting competition (Denno et al. 1995, Kaplan and Denno 2007). *Liriomyza helianthi* and *C. platyptera* co-occur spatially and temporally, they are phylogenetically similar, and share an overlapping, cryptic feeding niche.  Kaplan and Denno (2007) re-visited these predictions in light of increasing work on plant and natural enemy mediated indirect interactions.  This review found that although competition is common among phytophagous insects, interactions were highly asymmetrical, similar in magnitude within vs. between feeding guilds (chewers vs. sap-feeders), and were unaffected by the quantity of resources removed (% defoliation) (Kaplan and Denno 2007).

The results of our study, however, fall somewhere in between the traditional view of phytophagous insect competition and the more recent review of the topic.  In agreement with traditional competition theory, the competitive interactions we observe do increase with the quantity of resources removed (proportion leaf area mined). However, we only observe reduced fitness effects on *C. platyptera* and *L. helianthi* at extremely high resource removal levels not actually observed in the field (Fig. 3B).   Because of overlap in mines we are unable to distinguish proportions of leaf area mined by species when both are present together, however, the number of pupae per leaf is highly correlated with area of the leaf mined by species (Fig. 2). In agreement with more recent thinking on insect interspecific competition (Kaplan and Denno 2007), field experiments have revealed that indirect interactions between *L. helianthi* and *C. platyptera* are strong even at very low densities, but, that these interactions are indirect and mediated by natural enemies (Chapter 3).  In the field, we find that temporal asynchrony leading to asymmetric apparent competition, mediated through shared natural enemies, is structuring this community.

The asymmetry of competitive interactions, across trophic levels and taxonomic groups, has had strong support for decades (Lawton and Hassell 1981, Connell 1983, Schoener 1983, Strong et al. 1984, Denno et al. 1995, Kaplan and Denno 2007).  Because we could not generate the extreme populations with *C. platyptera*, we couldn't directly test the symmetry of the direct interspecific competition that we observe at high densities. However, our field studies did show that indirect interactions between *L. helianthi* and *C. platyptera* are indeed asymmetrical (Chapter 3).

The disturbance regimes of agricultural systems often exclude natural enemies which in turn allows herbivore populations to reach much higher densities than observed in undisturbed native habitats (Bianchi et al. 2006). Although the leafminer species we focus on are not found in agricultural settings, important species in the *Liriomyza* genus can reach densities where direct intraspecific and interspecific competition play an important role in community structure (Parrella 1983, 1987). But, not all agricultural systems are highly disturbed or hostile to natural enemies. Conclusions about the importance of direct competition in leafminer communities by researchers working exclusively in either agricultural or natural habitats might vary dramatically, yet both groups would be correct. The relative strength of direct versus indirect competition is context dependent, and will vary between systems and over time. Insight into the importance of direct or indirect competition in a particular community will come through a careful understanding of natural history and rigorous testing of potential structuring mechanisms.

In conclusion, the effects of competition we were able to detect between *L. helianthi* and *C. platyptera* at high densities in the laboratory are unlikely to play an important role in structuring natural communities of these leafminers. This study supports current evidence that a new paradigm which accounts for indirect interactions is required to describe the organization of phytophagous insect communities.

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**Fig. 1** Example of ASSESS image analysis. Area shaded white is area used to calculate proportion area mined.

**Fig 2.** Correlation of three density metrics; total mines per leaf, total number of pupae per leaf, and proportion of leaf area mined. Data here are pooled across both species of leafminer. **A.** correlation between total pupae/leaf and total mines/leaf **B.** correlation of total pupae/leaf and proportion of leaf area mined **C.** correlation of total mines/leaf and proportion of leaf area mined. $R_s$ reports Spearman rank correlations values.

**Fig. 3 A.** Relationship between *L. helianthi* and *C. platyptera* fitness (pupal weight) and total leafminer density (pupae per leaf) for densities observed in the field.

**B.** Relationship between *L. helianthi* and *C. platyptera* fitness (pupal weight) and total leaf-miner density (leaf area mined) for all densities.

**Fig. 1**

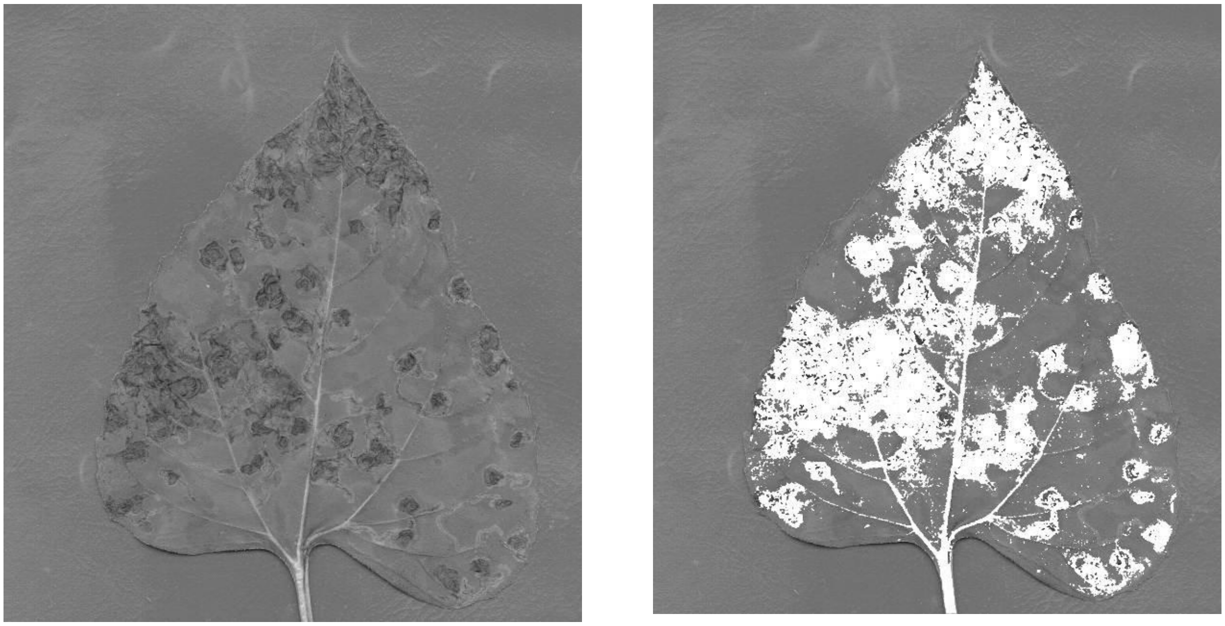


**Fig. 2**

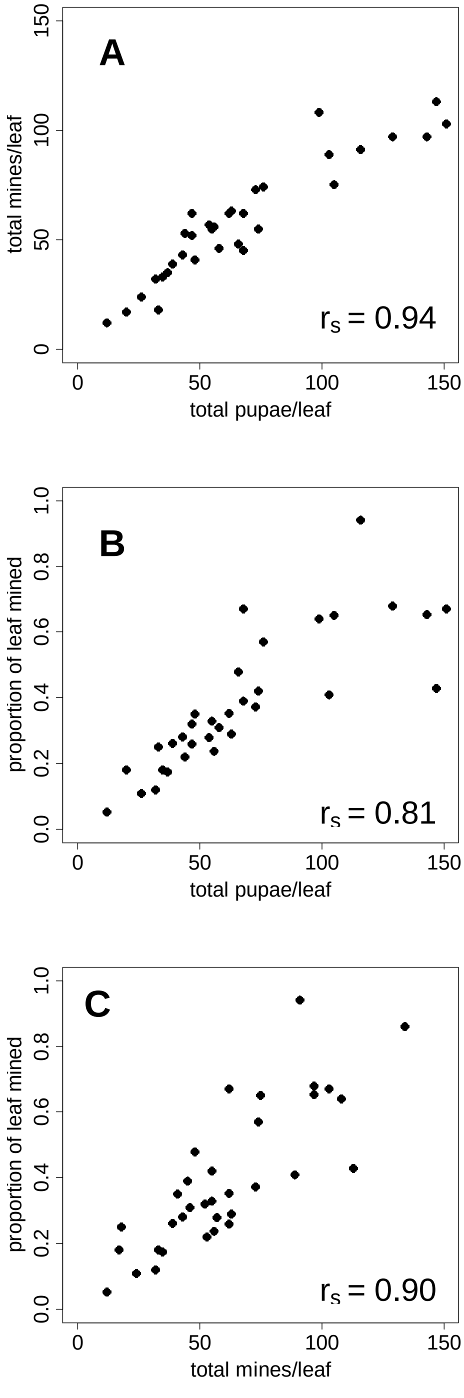
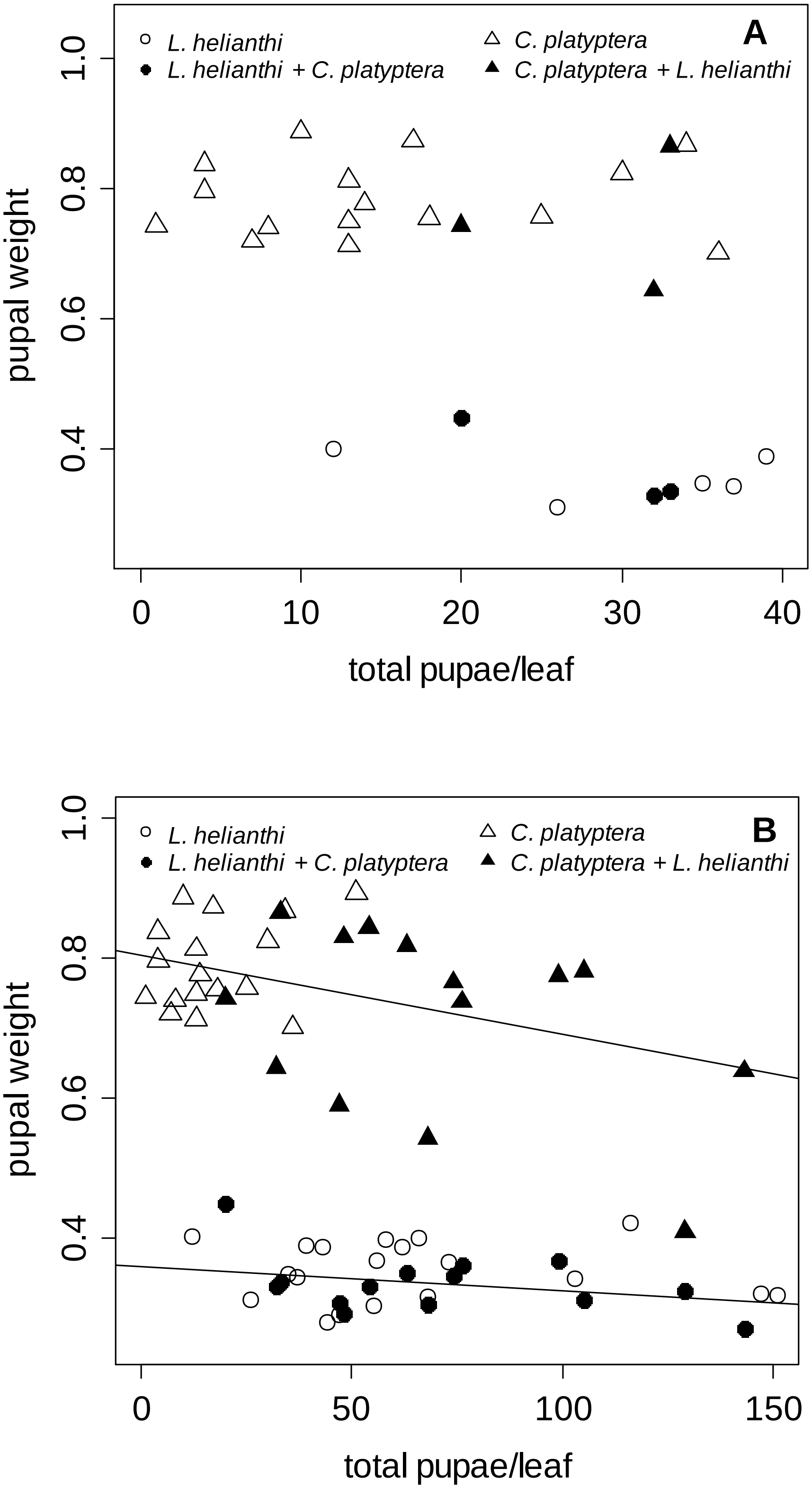

A
total mines/leaf
0
50
100
150
total pupae/leaf
0
50
100
150
$r_S = 0.94$
B
proportion of leaf mined
0.0
0.2
0.4
0.6
0.8
1.0
total pupae/leaf
0
50
100
150
$r_S = 0.81$
C
proportion of leaf mined
0.0
0.2
0.4
0.6
0.8
1.0
total mines/leaf
0
50
100
150
$r_S = 0.90$

**Fig. 3**


A
L. helianthi
L. helianthi + C. platyptera
C. platyptera
C. platyptera + L. helianthi
pupal weight
0.4
0.6
0.8
1.0
total pupae/leaf
0
10
20
30
40
B
L. helianthi
L. helianthi + C. platyptera
C. platyptera
C. platyptera + L. helianthi
pupal weight
0.4
0.6
0.8
1.0
total pupae/leaf
0
50
100
150

# Chapter 2

# Shared parasitism and temporal asynchrony structure a natural leafminer parasitoid community

## Abstract

Indirect interactions have generated much theoretical attention but empirical data on the scenarios that predict the importance of these interactions are still relatively scarce. One such indirect interaction, apparent competition - defined as a negative effect of one species on the population growth rate or abundance of another species, mediated through the action of shared natural enemies – although predicted to be important, rarely has been quantified in field studies. *Liriomyza helianthi* and *Calycomyza platyptera* along with their community of hymenopteran parasitoids provide a model system for studying apparent competition in the field. We surveyed populations of *L. helianthi* and *C. platyptera*, along with their community of parasitoids, over two years at seven sites in the Central Valley of California. Quantitative food webs for each year were used to visualize host-parasitoid interactions. We found the same dominant parasitoids were shared by both species of herbivore. Parasitism of *C. platypera* was significantly positively correlated with *L. helianthi* leafminer density and parasitism levels. At all sites *L. helianthi* emerged approximately one month before *C. platyptera* and had a higher population density throughout the season. We conclude that temporal asynchrony between the two herbivore species may drive the suppression of the late-season leafminer, *C. platyptera,* through apparent competition.

## Introduction

Competition is recognized as a major structuring force in natural communities (Begon et al. 2006). At times, this thesis has been controversial, especially within communities of phytophagous insects (Hairston et al. 1960, Lawton and Strong Jr 1981, Lawton and Hassell 1981, Strong et al. 1984, Denno et al. 1995, Kaplan and Denno 2007). The dispute arose partly due to a lack of manipulative studies, but also because frequently ecologists were not considering indirect interactions, which are often especially complicated. This debate was resolved, in part, by the recognition that species may influence each other indirectly as well as directly. Indirect competition for resources among herbivores is now well documented (van Veen et al. 2006, Kaplan and Denno 2007). A recent review on the topic concludes that indirect interactions via natural enemies or shared host plants are likely to explain some of the discrepancies which caused arguments in past decades (Kaplan and Denno 2007). Here we define indirect competition as an interaction between two species mediated by a third species in the community.

Apparent competition is one type of indirect interaction known to structure some herbivore communities (Chaneton and Bonsall 2000). First coined by Holt in 1977, the term refers to the negative effect of one species on the population growth rate or abundance of another, mediated through the action of shared natural enemies (Holt 1977). Theory predicts that apparent competition is capable of producing many of the same patterns as resource competition. In principle, two species that share the same natural enemy cannot coexist if exploited equally and symmetrically, just as we would not expect to see two species exploiting the same limiting resource in the same manner ((Nicholson and Bailey 1935, Holt 1977, Holt and Lawton 1993). The dominant apparent competitor is expected to be the species that can maintain itself under pressure from high long-term average natural enemy density (Holt and Lawton 1994). But, in fact, we often do observe shared natural enemies co-occurring in the field. This may be because natural enemies themselves are limited by factors other than the supply of hosts, that the range of natural enemies attacking the host competitors is different, or that an inferior apparent competitor can find a refuge in space or time (van Veen et al. 2006). The dynamics of natural enemies are often tightly coupled with those of their hosts, so that communities dominated by natural enemy effects are often harder to analyze than those communities dominated by resource competition where resources are often uncoupled from the dynamics of competitors (van Veen et al. 2006).

Natural enemies can extend interactions among herbivores across time and space, allowing even spatially and temporally disconnected species to compete (Kaplan and Denno 2007). Qualitative food web studies were a common way to study such communities in the past, but traditional food web studies suffered from the failure to quantify the frequency or even to detect the presence of interspecific interactions (Cohen et al. 1993, Tylianakis et al. 2007). Reviews on this subject document the recent resurgence of interest in the topic of indirect interactions and the need for more field level studies. Both reviews also highlight the importance of asymmetric interactions in structuring communities (Chaneton and Bonsall 2000, van Veen et al. 2006). Asymmetric linkages can determine the abundance and spatial distribution of alternative prey.

It is now possible to use quantitative food webs to measure the frequency of each trophic interaction (Memmott et al. 1994, Paniagua et al. 2009). Quantitative food webs have proven useful in various applications. They have recently allowed ecologists to generate and test hypotheses related to the effects of indirect interactions in host-parasitoid communities (Morris et al. 2004), the effects of introduced aliens on native food webs (Henneman and Memmott 2001), and the effects of tropical forest fragmentation on species interactions (Tylianakis et al.

2007). Empirical studies documenting apparent competition in the field are still quite rare, but recent work shows that if we ignore these links we can miss the underlying structure of whole communities (van Veen et al. 2006, Tylianakis et al. 2007)

We investigate the strength of apparent competition in the field between two species of dipteran leafminers mediated through their shared natural enemies in their natural setting. Over a two-year period we follow populations of *Liriomyza helianthi* Spencer (Diptera: Agromyzidae) and *Calycomyza platyptera* (Thomson) Diptera: Agromyzide, along with all associated hymenopteran parasitoids. These data are used to construct quantitative food webs and to determine whether these two leafminer species interact indirectly through their shared natural enemies, and if so, whether the interaction is symmetric. In addition, we look to see whether the two species were temporally connected by their shared natural enemies, as hypothesized by Kaplan and Denno (2007), such that early season populations of one species might drive the population trends of the second species later in the season.

# Methods

*Study system*

Leafminers are a diverse and varied group of insects. These insects are so named for their larval feeding habit: feeding inside the leaf lamina and building a characteristic mine which normally appears a shade lighter than the leaf tissue (Gratton and Welter 2001). Leaf mining behavior has evolved many times and includes numerous species of Diptera, Lepidoptera, Coleoptera, and Hymenoptera. Nearly all leafminers are attacked by parasitoids, and mortality often exceeds 50% (Spencer 1981, Hawkins and Mills 1996, Gratton and Welter 2001). *Liriomyza helianthi* feeds exclusively on sunflowers (*Helianthus annus*) and cocklebur (*Xanthium strumarium*). *Calycomyza platyptera* occurs sympatrically with *L. helianthi* and utilizes the same two host plants at our field sites (Spencer 1990).

Our study sites are located in wildlife refuges in the Sacramento River delta region of the Californian Central Valley. Sunflowers and cocklebur are often found in distinct patches of disturbed habitat surrounded by large areas of bunchgrass. These sites remain flooded until early June, after which both species germinate. Both species flower mid-July and go through senescence in late September.

*Liriomyza helianthi* larvae progress through three instars in 5-6 days, then cut a crescent-shaped exit hole and fall to the soil for pupation. Adult flies emerge in 10-14 days and return to the host plants to mate and oviposit. *Calycomyza platyptera* has a longer, 8-10 day, larval stage. *Calycomyza platyptera* pupate inside the mine, and adult flies emerge from the leaf 8-10 days after pupating. Both species of leafminers are multivoltine during the summer months with egg to adult life cycle of 3-5 weeks. Both species are native to the Central California Valley, and they host at least 16 different species of hymenopteran parasitoids, though only six species of wasps are numerically common (Spencer 1981, Gratton and Welter 2001).

Leafminers and their associated parasitoids provide a model system for the study of apparent competition in the field. This complex of plants, leafminers, and parasitoids was selected, first, because potential for competition and parasitism are high among cryptic species, and these leafminer species lack significant generalist predators (Askew and Shaw 1974, Gratton and Welter 2001). Second, leafminers are relatively easy to sample and rear, and the permanent, species-specific mines that each produce facilitate associating parasitoids with their correct hosts

(Lewis et al. 2002). Third, both species of leafminer studied complete their larval development within a single leaf, making quantitative assessments of host and parasitoid populations much easier than with more mobile hosts (Lewis et al. 2002). Finally, these leafminer species have short life-cycles allowing for timely studies over several generations each season.

### *Leafminer and parasitoid census*

From June to September of 2008 at eight sites, and in 2009 at nine sites, densities of *L. helianthi* and *C. platyptera*, along with their associated parasitoids, were surveyed once per week. Host plant (*H. annuus* and *X. strumarium*) densities at sites were 8.8 ± 1.6 (mean ± SE) plants per $m^2$. The minimum distance between sites was 550 m, which is far relative to the dispersal ability of Agromyzid leafminers and their parasitoids. Maximum distance between sites was 5 km. Studies on *Diglyphus* movement show mean distance flown is 21.5 m for females and 18.0 m for males (Jones and Parrella 1986, Parrella 1987). Sunflower and cocklebur plant abundance was estimated by recording the number of plant units per $m^2$ at 3-m intervals along a 10-m transect at each site. Transects were placed to pass through a representative section of the whole site. Estimates of plant density per $m^2$ allowed us to translate all the relative estimates of leafminer and natural enemy densities to absolute figures with units per $m^2$.

Six plants at each site were haphazardly chosen. All leaves were counted and all leaves with mines were removed, placed in plastic bags on ice, and returned to the laboratory. All mines were examined with a dissecting microscope and scored as alive, exited, parasitized, or dead. All mines except those with clear exit markings were reared until either an adult fly or adult parasitoid emerged or until enough time had passed to reasonably allow for any adults to emerge (six weeks). For rearing, the stems of each leaf were cut under water and then placed inside small plastic tubes (25 x 95 mm Azer scientific, Morgantown, USA) filled with water and covered with parafilm (Parafilm® M SPI supplies). Each leaf and tube were then placed inside a 1 liter clear plastic cup and fitted with a lid screened in fine mesh to allow airflow, but sufficiently small to prevent adult parasitoids escaping. After one week, most leaves had dried, and all vials were removed and leaves were transferred to a 0.5 liter cup with the same lid. All cups were checked weekly, and all emerging adult parasitoids and flies were recorded. All adult parasitoids were placed immediately in 95% EtOH for identification.

### *Data analysis*

To compare *L. helianthi* and *C. platyptera* fly densities and parasitism rates we used paired two sample t-tests. Data was first arcsine-square-root transformed to meet the residual assumptions of the statistical tests.

We also used statistical models to examine whether the proportion of individuals parasitized in a given parasitoid generation time ($t+1$) was influenced by densities and parasitism rates of heterospecific and conspecific individuals in the previous parasitoid generation ($t$). We considered each generation time-step to last two weeks, which corresponds to development time of our most common parasitoids (Minkenberg 1989, Murphy and LaSalle 1999, Tran et al. 2007).

To account for the non-normal error structure and spatial structure in the sampling design, we used generalized linear mixed models (the lmer4 package in R; (Pinheiro and Bates 2000, Zuur et al. 2009). We assumed a quasibinomial distribution for the error structure. To deal with between-site temporal autocorrelation, site was included as a random effect nested within week. Model simplification followed an information-theoretic approach (Anderson and Burnham 2002). A saturated model was defined containing all biologically meaningful predictor variables, and parameters were removed in a step-wise manner to identify the model with the lowest possible Akaike Information Criterion (AIC) value for the given parameter set. We ran separate models for each leafminer species. In our saturated models parasitism rate at generation $t$ +1 is the response variable. Fixed effect predictor variables included: *C. platyptera* host density and parasitism at generation $t$ and *L. helianthi* host density and parasitism at time $t$. Random effect predictor variables included site and week. Only parameters included in our best-fit models are reported.

### *Quantitative food webs and parasitoid overlap*

Rearing data from all sites and dates were combined to construct food webs showing all trophic interactions for 2008 and 2009 for the two leafminer species and any parasitoids reared from either species. Food webs were drawn using the bipartite package (van Veen et al. 2006, Dormann et al. 2009, Alarcón 2010, Gagic et al. 2011) in R (R Development Core Team 2008).

### *Data analysis*

A quantitative version of traditional predator overlap graphs was developed by Mueller et al. (1999) which allows us to assess the potential for indirect interactions, $d_{ij}$, by determining what fraction of parasitoids attacking species $i$ are likely to have developed on species $j$. The magnitude of $d_{ij}$ represents the potential importance of one species as a source of natural enemies attacking another. $d_{ij}$ is defined as:

$$d_{ij} = \sum_k \left[ \frac{\alpha_{ik}}{\sum_l \alpha_{il}} \frac{\alpha_{jk}}{\sum_m \alpha_{mk}} \right],$$

where $\alpha_{ik}$ is the strength of the link between host $i$ and parasitoid $k$; the first quantity in square brackets is the fraction of parasitoids of host $i$ that belong to species $k$, the second quantity is the fraction of parasitoids of species $k$ that develop on host species $j$, the outer summation is taken over all parasitoids (Mueller et al. 1999). When parasitoid species attacking $i$ develop commonly on host $j$ then $d_{ij}$ approaches 1 and the indirect effect of host $j$ on $i$ is predicated to be greater than when parasitoids attacking leafminer $i$ develop on species $i$ (Mueller et al. 1999*)*.

## Results

### *Population census*

*Liriomyza helianthi* and *C. platyptera* showed marked differences in phenology and population density throughout the sampling period in both years studied. *Liriomyza helianthi* populations emerged much earlier, in the first week of July in 2008 and 2009 (Fig 1a and b), compared to early August for the *C. platyptera* (Fig 1c and d). In 2008 both species reached peak abundance at approximately the same point in mid-September. Populations of both species crashed in late September both years. In 2008 the total *L. helianthi* population was three times higher than *C. platyptera* (t = 2.45, df = 6, p = 0.04), and in 2009 it was 12 times higher (t = 2.33, df = 7, p = 0.04). In 2008 the mean density of *L. helianthi* flies per $m^2$ was 37.42 ± 8.8 (mean ± SE), compared to a mean of 13.44 ± 3.3 *C. platyptera* per $m^2$. In 2009 we found 31.14 ± 6.8 *L. helianthi* flies per $m^2$, and 2.6 ± 0.53 *C. platyptera* flies per $m^2$.

Although the two species of leafminer showed a temporal disconnect and large differences in population density, overall parasitism was high both years and relatively similar for both species. In 2008 average parasitism rate (across all sites) was 43.9 ± 5.4% for *L. helianthi* and 48.3 ± 6.4 % for *C. platyptera*. In 2009 the average parasitism rates was 53.6 ± 3.6% for *L. helianthi* and 65.5 ± 5.5% for *C. platyptera* (Fig. 1). In 2008 we found no differences in total levels of parasitism between host species (t = 1.68, df = 6, p = 0.20). In contrast, in 2009 total parasitism in *C. platyptera* populations was significantly higher than parasitism of *L. helianthi,* (t = 1.68, df = 7, p = 0.02) despite the much lower densities of the *C. platyptera*.

*Statistical modeling*

Generalized linear mixed effect models found that *L. helianthi* parasitism rates at parasitoid generation *t* +1 were significantly correlated with conspecific (*L. helianthi)* leafminer host densities at parasitoid generation *t* both years (Table 1). *Calycomyza platyptera* parasitism rates at *t* + 1 were associated with *C. platyptera* host density at generation *t* in 2009, but not in 2008.

To test for potential indirect interactions we looked at whether parasitism rates of each species of leafminer in generation *t* +1 were dependent not only on conspecific population density and parasitism rates at generation *t*, but also on heterospecific host density and parasitism rates at generation *t*. Models show an asymmetry in the potential effects of apparent competition between *L. helianthi* and *C. platyptera* species. In 2008 *C. platyptera* parasitism rate at *t* +1, was significantly correlated only with *L. helianthi* leafminer population density and parasitism at generation *t*, and not correlated with conspecific density or parasitism (Table 1). In 2009 *C. platyptera* parasitism rate was significantly correlated with *L. helianthi* parasitism rates at generation *t* along with conspecific leafminer density at generation *t*. In contrast, *L. helianthi* leafminer parasitism at *t* + 1 was unrelated to *C. platyptera* density and parasitism at *t*, and only correlated with conspecific parasitism and density (Table 1).

All data were analyzed using overall parasitism rates. To determine if we should exclude rare parasitoid species we repeated the same analysis with a subset of data using only the two most common shared parasitoid species and found no difference between these and our reported results.

*Quantitative food webs*

We reared a total of 3,273 parasitic Hymenoptera. As revealed in the quantitative food webs (Fig. 2), the larval parasitoids *Neochrysocharis arizonensis* Crawford and *Diglyphus begini* (Crawford) were the dominant parasitoid species both years and together accounted for 79 and 81 % of total parasitism in 2008 and 2009 respectively. The larval parasitoids *Chrysocharis ainsiliei* Crawford (7 % in 2008, 4 % in 2009), and *Closterocerus utahensis* (Crawford) (4% in 2008, 8% in 2009), were the next most common parasitoid species. These four species together account for over 90% of parasitized *L. helianthi* and *C. platyptera* hosts.

The remaining parasitoids reared (none of which amounted to greater than 3% of total parasitism) included individuals in the genera *Baryscapus, Diaulinopis, Pnigalio,* and *Cirrospilus*, (family: Eulophidae) reared from both *L. helianthi* and *C. platyptera*; *Thinodytes* species (family: Pteromalidae) reared primarily from *C. platyptera* hosts, and *Opius dimidiatus* (Ashmead) (family: Braconidae) reared primarily from *L. helianthi* hosts (Fig. 2). Due to small sample size, difficulty in identifying these species, and revisions needed of the relevant keys we only identified the rare Chalcidoid species to the genus level.

Summary quantitative food webs showed that the two most common parasitoids (*N. arizonensis* and *D. begini*) (Fig. 2) were shared relatively equally by both host leafminer species, relative to their overall host populations. Because *L. helianthi* densities were higher in 2009 and hence represented a greater proportion of the potential host pool, the 'potential for apparent competition' was stronger in 2009 than 2008 (Table 2). When looking at each species individually, in 2008, *N. arizonensis* accounted for 64% of the *L. helianthi* parasitism, and 69% of *C. platyptera* parasitism. *D. begini* accounted for 14% of *L. helianthi* parasitism and 12% of *C. platyptera* parasitism. In 2009 we found a similar pattern, *N. arizonensis* accounted for 67% of *L. helianthi* parasitism, and 66% of *C. platyptera* parasitism; *D. begini* accounted for 16% and 8% of parasitism of *L. helianthi* and *C. platyptera* respectively. For the other common genera, (*C. ainsliei* and *C. utahensis*) there were only minor differences in symmetry between host species. Therefore, even at peak densities, where populations of *L. helianthi* were three to ten times those of *C. platyptera*, the dominant parasitoids appear not to be discriminating between host species.

## Discussion

While apparent competition is being looked at more frequently across systems, two questions that remain unresolved are "How frequently are indirect interactions structuring communities?" and secondly, "Why might these interactions be systematically overlooked and underestimated?" (Wootton 1994, 2002) It may be that it is the very nature of indirect interactions which make it so difficult to detect them. Such inherent factors for apparent competition would include increases in the needed scale of the examination beyond the planned physical or temporal scope of the study. Similarly, linkages between different functional groups that might not be expected to share natural enemies or groups that might require different types of expertise, e.g. predators and pathogens, might also be more easily overlooked. Apparent competition has already been shown to link herbivores across landscapes (Thies and Tscharntke 1999), seasons (Murphy et al. 1998) and taxonomic groups (Morris et al. 2004).

Our system provides clear evidence of the impact of natural enemies on two herbivores that are temporally asynchronous. *Liriomyza helianthi* populations emerge approximately one month before *C. platyptera* populations each year (Fig 1). By the time *C. platyptera* populations arrive in the field, the first generation of parasitoids has emerged from *L. helianthi* hosts. Because we see no discrimination by the dominant parasitoid species, and no evidence of resource competition in the laboratory (Chapter 1) as well as inferred from the pattern of field parasitism rates, we hypothesize that the early season build up, and eventual spill-over, of parasitoids from *L. helianthi* hosts to *C. platyptera* hosts leads to the suppression of the blotch leafminer throughout the season. Generalized linear mixed effects modeling and calculations of the quantitative measure $d_{ij}$ (the importance of species $j$ as a source of parasitoids attacking species $i$) support the hypothesis we draw. In our system, just studying single species interactions, single season, or even multi-trophic direct interactions, would have missed the apparent competition between these two species because of the temporal asynchrony between the two populations of leafminers.

The fact that spatial, temporal and taxonomic factors can all mediate indirect interactions demonstrates the complexity of population dynamics. Studies at the field level could miss the landscape level patterns that drive trophic dynamics of natural enemies (Tscharntke et al. 2005) and pollinators (Kremen et al. 2002) in agricultural systems. Studies that only last one season would miss the temporal connection between seasons as a key factor driving herbivore mortality in vineyards (Murphy et al. 1998). Studies that focus on only one or two taxonomic groups might miss the connection between diverse families of herbivores connected by a natural enemy communities in the tropics (Morris et al. 2004).

The type of predator and the ease of detection of the predator's impact on its prey is another factor that might lead to overlooking apparent competition. Because of the ease of associating parasitoids with their correct host, given that many parasitoids need to be reared from their host prior to identification or counting, all of the previous examples and much of the experimental work in terrestrial habitats look at parasitoid-host systems. However, there is no reason to think such interactions are absent in systems where generalist predators play important structuring roles. Because it is often difficult to ascribe mortality to a specific predator, which kills many more prey during its lifetime yet often leaves few clear markings on the prey, generalist predator linkages would be more likely to be overlooked. Yet, one might easily hypothesize that in fact generalists would be much more likely to be linked through apparent competition. New molecular techniques are being developed that allow researchers to identify prey remains in the guts, feces, and regurgitates (King et al. 2008), and may lead to a better understanding of the role of generalist predators in complex trophic interactions.

These types of linkages are important in structuring not only invertebrate communities, but across a diverse variety of systems and taxonomic groupings. Examples from fisheries show that direct and indirect contributions of benthivory must be considered to understand trophic dynamics in lakes (Vander Zanden and Vadeboncoeur 2008). Indirect interactions play a central role in structuring habitat-segregated vertebrate communities that share a predator (Oliver et al. 2009). Plant community makeup can be strongly influenced when two species compete through shared herbivore enemies (Rand 2003). A shared pathogen mediates the indirect interactions between two tree species and is a major driver of transmission of *Phytophthora ramorum* (sudden oak death) (Cobb et al. 2010).

Understanding indirect interactions such as apparent competition can also have practical applications for ecologists and conservation biologists as well (Memmott 2009). Management of endangered butterflies in the genus *Maculinea* must take into account how apparent competition, mediated by larval stages of butterflies between plant and ant host species can drive community dynamics in this fragile system (Mouquet et al. 2005). Decreases in native skink populations in New Zealand have been tied to apparent competition with introduced rabbits through shared predators (Norbury 2001). Understanding indirect interactions is also important when designing conservation biological control programs. Natural enemy populations often lag behind pest outbreaks in agricultural systems and are unable to provide adequate control. Researchers exploiting alternative prey as a temporal bridge between natural enemies and pest species have been successful in a number of systems (Doutt and Nakata 1973, Murphy et al. 1998, Pfannenstiel and Unruh 2003, Langer and Hance 2004).

Our findings build on a growing body of ecological research that shows the importance of indirect interactions for community dynamics (Wootton 1994, 2002, Holt and Barfield 2003, Cronin 2007, Kaplan and Denno 2007, Tylianakis et al. 2007, Tylianakis 2009, Orrock et al. 2010). Ecologists have discovered that the drivers of herbivore population regulation are often cryptic and complicated. Although the study of indirect interactions presents significant challenges, this remains a key area for further research.

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**Table 1.** Results of generalized linear mixed model analyses for *C. platyptera* and *L. helianthi* proportion parasitized at parasitoid generation *t+1*. One parasitoid generation time step corresponds to 14 days. Models included the following predictor variables: (1) *L. helianthi* host leafminer density ($m^2$) at parasitoid generation *t* (Pinheiro and Bates) *L. helianthi* parasitism rate at parasitoid generation *t* (3) *C. platyptera* leafminer density ($m^2$) at parasitoid generation *t* and (4) *C. platyptera* parasitism rate at generation *t*. Only final model results are shown. Bolded rows indicate statistical significance at $p < 0.05$.

**Table 1**

| | estimate | standard error | z value | Pr ( >\|z\| ) |
|---|---|---|---|---|
| 2008 | | | | |
| (1) *C. platyptera leafminer proportion parasitized (t +1)* | | | | |
| ***L. helianthi* leafminer density (*t)*** | **0.040** | **0.016** | **2.568** | **0.010** |
| ***L. helianthi* leafminer parasitism (*t*)** | **6.781** | **1.773** | **3.824** | **<0.001** |
| | | | | |
| *L. helianthi leafminer proportion parasitized (t+1)* | | | | |
| ***L. helianthi* leafminer density (*t*)** | **0.051** | **0.016** | **3.293** | **<0.001** |
| *L. helianthi* leafminer parasitism (*t*) | -0.031 | -0.025 | 1.240 | 0.215 |
| | | | | |
| 2009 | | | | |
| (1) *C. platyptera leafminer proportion parasitized (t+1)* | | | | |
| ***C. platyptera* leafminer density (t)** | **-0.189** | **0.097** | **-1.948** | **0.051** |
| ***L. helianthi* leafminer parasitism (*t*)** | **0.011** | **0.004** | **2.530** | **0.011** |
| | | | | |
| *L. helianthi leafminer proportion parasitized (t+1)* | | | | |
| ***L. helianthi* leafminer density (*t*)** | **0.008** | **0.002** | **4.557** | **<0.001** |
| ***L. helianthi* leafminer parasitism (*t*)** | **-0.003** | **0.001** | **-2.388** | **0.017** |
| | | | | |

**Table 2**. Potential for apparent competition $d_{ij}$ between *L. helianthi* and *C. platyptera* each year. Following Tack et al (2010), the entries in each column (Dijak and Thompson) are measurements of the proportion of parasitoid individuals attacking species *i* (row) that have developed on each of the host species *j* (columns).

**Table 2**

| **2008** | | |
|---|---|---|
| | *C. platyptera* | *L. helianthi* |
| *C. platyptera* | 0.42 | 0.58 |
| *L. helianthi* | 0.38 | 0.62 |

| **2009** | | |
|---|---|---|
| | *C. platyptera* | *L. helianthi* |
| *C. platyptera* | 0.22 | 0.78 |
| *L. helianthi* | 0.13 | 0.87 |

## List of figures

**Figure 1.** Density (mean ± SE) and percent parasitism of (A) *L. helianthi* density per $m^2$ in 2008 (B) *C. platyptera* density ($m^2$) in 2008 (C) *L. helianthi* density ($m^2$) in 2009 (D) *C. platyptera* density ($m^2$) in 2009.

**Figure 2**. Parasitoid communities of L. helianthi and C. platyptera in 2008 and 2009. Summary quantitative food webs across sites for 2008 and 2009. For each web, lower bars represent host (leafminer) density and upper bars represent parasitoid density. Linkage width indicates frequency of each trophic interaction. As a summary, the webs show interaction data pooled across all sites each year.

**Figure 1**

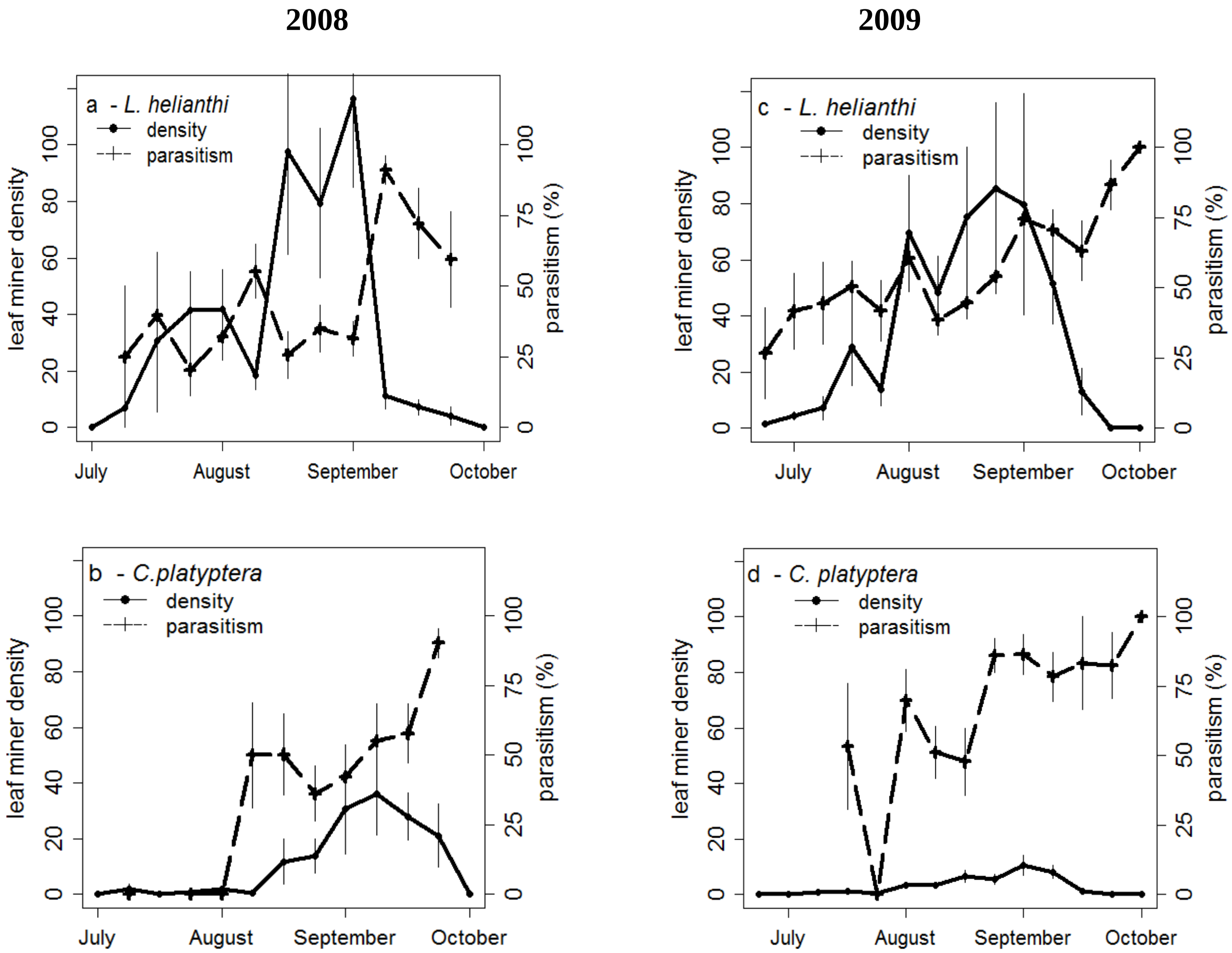
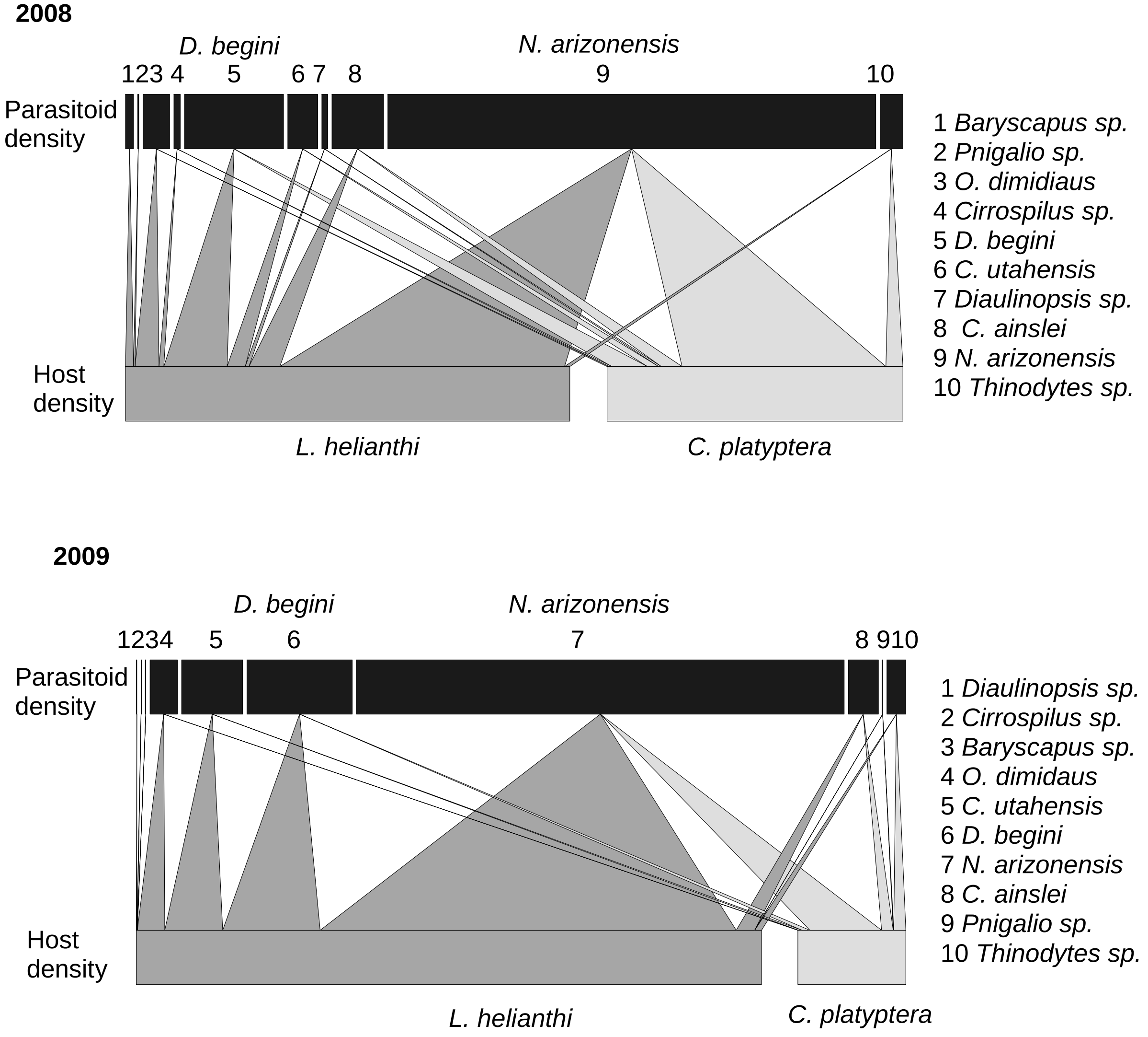

2008
2009
a - *L. helianthi*
density
parasitism
b - *C.platyptera*
c - *L. helianthi*
d - *C. platyptera*
leaf miner density
parasitism (%)
0
20
40
60
80
100
0
25
50
75
100
July
August
September
October

**Figure 2**


2008
D. begini
N. arizonensis
123 4 5 6 7 8 9 10
Parasitoid density
Host density
L. helianthi
C. platyptera
1 Baryscapus sp.
2 Pnigalio sp.
3 O. dimidiaus
4 Cirrospilus sp.
5 D. begini
6 C. utahensis
7 Diaulinopsis sp.
8 C. ainslei
9 N. arizonensis
10 Thinodytes sp.
2009
D. begini
N. arizonensis
1234 5 6 7 8 910
Parasitoid density
Host density
L. helianthi
C. platyptera
1 Diaulinopsis sp.
2 Cirrospilus sp.
3 Baryscapus sp.
4 O. dimidaus
5 C. utahensis
6 D. begini
7 N. arizonensis
8 C. ainslei
9 Pnigalio sp.
10 Thinodytes sp.

# Chapter 3

# Emergence asynchrony between herbivores leads to asymmetric apparent competition in the field

## Abstract

Apparent competition can extend interactions among herbivores across space and time. Apparent competition – defined as a negative effect of one species on the population growth rate or abundance of another species, mediated through the action of shared natural enemies – has generated much recent attention, but studies that test the mechanisms that lead to apparent competition are still rare. Here, we use experimental data to test hypotheses on the mechanisms leading to asymmetric apparent competition. We use observational data from a two-year population census and quantitative food webs of two leafminers, *Liriomyza helianthi* and *Calycomyza platyptera*, along with all associated parasitoids, at seven sites in the Californian Central Valley to generate hypotheses. Previous studies reveal that *Liriomyza helianthi* populations emerge approximately one month (two parasitoid generations) before *C. platyptera* and have a higher population density throughout the season. Quantitative food webs show that the parasitoid species *Neochrysocharis arizonensis* and *Diglyphus begini* are the dominant parasitoids shared by both species of leafminer. Experimental removal of *L. helianthi* populations in the early summer leads to a 50% reduction in parasitism of *C. platyptera*. Because parasitoids are known to be important mortality agents in this system, we conclude that emergence asynchrony between the two herbivore species significantly impacts populations of *C. platyptera*.

## Introduction

Apparent competition can extend herbivore interactions across time and space, allowing even spatially and temporally distant species to influence one another (Holt and Lawton 1994, Kaplan and Denno 2007). Apparent competition is one example of this kind of interaction. The term apparent competition refers to a type of indirect interaction where one species negatively affects the population growth rate or abundance of another species via the action of a shared predator (Holt 1977, Holt and Lawton 1993). Although the theory behind apparent competition has received much attention (Holt 1977, Jeffries and Lawton 1984, Cohen et al. 1993, Holt and Lawton 1993, Wootton 1994, 2002, Holt and Barfield 2003), and a number of studies now demonstrate its importance in structuring insect communities (Chaneton and Bonsall 2000, Lewis et al. 2002, van Veen et al. 2006, Tylianakis et al. 2007), studies that rigorously evaluate the mechanisms that lead to this indirect interaction under field conditions are still quite rare. In this paper we experimentally evaluate two mechanisms: asynchrony in seasonal emergence time leading to asymmetric population growth between species, and differential preference for a host species on the part of natural enemies.

The outcomes of the studies which have used large scale field manipulations to test for indirect interactions are variable, finding both negative (Van Nouhuys and Hanski 2000, Morris et al. 2004, Cronin 2007) and positive (Tack et al. 2010) effects of shared natural enemies on host herbivore species. Morris et al (2004) and Cronin (2007) found that herbivores utilizing spatially separated host plants can interact through shared natural enemies. In a study at higher trophic levels, Van Nouhuys and Hanski (Pinheiro and Bates) found that spatially separated primary parasitoid species can interact through a shared hyperparasitoid. In contrast, Tack et al. (2010) found that herbivores with overlapping parasitoid communities can have independent population dynamics and even apparent mutualisms between host species.

With one exception (Tack et al. 2009), all previous manipulative studies of apparent competition in natural systems have looked at spatial separation of herbivores or primary parasitoids on different hosts. But communities separated temporally can be connected through shared enemies as well (Holt and Lawton 1994, Langer and Hance 2004). Although not discussed in the context of indirect interactions, the importance of spatially separated alternative hosts in the agricultural literature has long been recognized (Starý 1993, Valladares and Salvo 1999, Langer and Hance 2004). One previous study from an agricultural system shows that differences in temporal distributions of herbivores can lead to asymmetric apparent competition with overall mortality levels being driven by the presence of one early-season species (Langer and Hance 2004).

Indirect interactions are often known to be asymmetric (Connell 1983, Schoener 1983, Chaneton and Bonsall 2000, van Veen et al. 2006). Less commonly addressed are the factors, such as differences in emergence times that generate the asymmetry in these interactions. Insect emergence times are strongly temperature dependent, and can be effected by climate change (Walther et al. 2002). Identifying the mechanisms that are likely drivers of indirect interactions is essential if we want to predict and model the impacts of climate change on community-wide interactions (Van der Putten et al. 2010).

It is now possible to use quantitative food webs to measure the frequency of each trophic interaction (Memmott et al. 1994, Paniagua et al. 2009, van Veen et al. 2009), and quantitative food webs have proven useful in various applications. They have recently allowed ecologists to generate hypotheses related to the effects of indirect interactions in host-parasitoid communities (Morris et al. 2004, Tack et al. 2010), the effects of introduced aliens on native food webs (Henneman and Memmott 2001), and the effects of land use gradients on species interactions in tropical forests (Tylianakis et al. 2007). Further empirical work which tests mechanisms that drive indirect interactions may also help determine the utility of quantitative food webs as tools for predicting indirect interactions.

As theoretical work and these studies reveal, indirect interactions through shared natural enemies can have both positive and negative outcomes (Godfray et al. 1994, Holt and Lawton 1994). Here we focus on temporal dynamics as a structuring mechanism that may lead to indirect interactions. We use experimental data to test whether emergence asynchrony between herbivores, under field conditions, leads to asymmetric apparent competition. Quantitative food webs constructed from observational data (chapter 2) are used to generate hypotheses that we experimentally test by manipulating leafminer densities in the field. Lastly, we test for differential preference by the most common parasitoid species for the two leafminers as an alternative mechanism to explain the observed mortality rates and population densities of *C. platyptera*.

## Methods

### *Study System*

Leafminer-parasitoid communities have often served as model systems in studies of apparent competition. This is due to a number of characteristics of these communities: high parasitism rates, ease in associating parasitoids with their correct hosts, and short life cycles that are relatively similar to that of their natural enemies (Askew and Shaw 1974, Hawkins et al. 1997). Here, we focus on the leafminers *Liriomyza helianthi* Spencer (Diptera: Agromyzidae) and *Calicomyza platyptera* (Thomson) (Spencer) (Diptera: Agromyzidae), and their associated parasitoids as a model system for the study of apparent competition in the field.

This complex of plants, leafminers, and parasitoids was selected, first, because parasitism is high, and these leafminer species lack significant generalist predators (Askew and Shaw 1974, Gratton and Welter 2001). Second, we see no evidence of direct competition between *L. helianthi* and *C. platyptera* in laboratory experiments (Blitzer and Welter *in prep*). Third, these leafminers are relatively easy to sample and rear, and the permanent, species-specific mines that each produce facilitate associating parasitoids with their correct hosts (Lewis et al. 2002). Fourth, both species of leafminer studied complete their larval development within a single leaf, making quantitative assessments of host and parasitoid populations much easier than with more mobile hosts (Lewis et al. 2002). Finally, these leafminer species have short life-cycles allowing for timely studies over several generations each season.

*Liriomyza helianthi* feeds exclusively on sunflowers (*Helianthus annuus*) and cocklebur (*Xanthium strumarium*). *Calycomyza platyptera* occurs sympatrically with *L. helianthi* and utilizes the same two host plants at our field sites (Spencer 1990, Gratton and Welter 2001). *Liriomyza helianthi* larvae progress through three instars in 5-6 days, then cut a crescent-shaped exit hole and fall to the soil for pupation. Adult flies emerge in 10-14 days and return to host plants to mate and oviposit. *Calycomyza platyptera* has a longer (8-10 day) larval stage, pupate inside the mine, and adult flies emerge directly from the leaf after pupating. Both species of leafminers are multivoltine during the summer months with egg to adult life cycles of 3-5 weeks. Both species are native to the Californian Central Valley in the United States, and they host at least 16 different species of hymenopteran parasitoids, though only six are numerically common (Spencer 1981, Gratton and Welter 2001). *Liriomyza helianthi* and *C. platyptera* are the only leafminer species found at our field sites.

Our study sites were located in the Yolo Wildlife Refuge in the Sacramento River delta region of the Californian Central Valley, USA. Sunflowers and cocklebur are the dominant vegetation in disturbed low-lying areas and at these sites are found either in large (3,000 – 5,000 $m^2$) patches or as smaller (250 - 500 $m^2$) raised, distinct habitat islands which are products of waterfowl management (Figure 1). Both habitat types are surrounded by areas of low, thick, dry grass cover (*Bromus, Avena*, *Digitaria* and *Poa* spp.). All sites remain flooded until early June, after which flood waters recede and both species germinate. Both plant species begin flowering mid-July and go through senescence in late September

### *Apparent competition experiment*

Based on our results from statistical modeling, quantitative food webs, and parasitoid overlap graphs (Chapter 2) we conducted a species removal experiment in the field to assess whether early season emergence of *L. helianthi* drives parasitism of *C. platyptera* through shared natural enemies. This experiment was conducted on isolated vegetation “islands” that are 200-500 $m^2$ raised areas of disturbed ground, ideal germination areas for wild sunflower plants, surrounded by mowed dry grasses (*Bromus*, *Avena*, *Digitaria* and *Poa* spp), and isolated from other islands by ≥ 70 m. Islands were spread throughout a 140 ha section of our field site (Appendix A). The grass surrounding the islands was mowed twice each summer, but islands were left un-mowed as waterfowl management strategy. In October, fields are flooded and islands are surrounded by 20-50 cm of water.

In 2009 and 2010 we selected 16 islands and randomly assigned each to one of two treatments: (1) early season removal of *L. helianthi*, n = 8 or (2) control, no removal, n = 8. Our removal treatment ran from as soon as we observed *L. helianthi* at our field sites (late June), until we began to observe *C. platyptera* (approximately one month). We spent two hours per removal island, per week, removing all leaves with evidence of *L. helianthi* mining. We are able to distinguish *L. helianthi* mines at an early stage from any other leaf damage, or leafminer species. On control islands we simulated the effects of our treatment by similarly disturbing the islands without removing any leaves. All *L. helianthi* infested leaves were brought back to our lab and destroyed.

After emergence of *C. platyptera* in the field we stopped weekly maintenance of removal treatments. We surveyed all islands’ leafminer populations to confirm that treatments had successfully reduced *L. helianthi* densities on removal islands. To survey island *L. helianthi* populations we walked a transect down the center of each island and haphazardly choose five plants from each side of the transect. For each plant we counted and recorded numbers of leaves and *L. helianthi* mines per plant.

We used sentinel larvae to measure differences in *C. platyptera* parasitism between treatment and control islands. A *C. platyptera* colony was established in the summer of 2007, and augmented each following summer with larvae collected from the field. In the lab, we infested 80 sunflower plants with *C. platyptera* larvae from our lab colony. Eggs laid on plants were allowed to develop for four to six days, resulting in plants infested with second and third instar

*C. platyptera* larvae. Four to five infested sunflower plants were placed in the center of each island at approximately 8:00 am and returned to the lab at 5:00 pm the following day, exposing larvae to field parasitism levels for two daylight periods. In 2009 we exposed 77 ± 6 sentinel larvae per island, 1156 larvae total. In 2010 we exposed 27 ± 3 sentinel larvae per island, 441 larvae total. Due to the attractiveness of plants grown in the greenhouse to grasshoppers, in 2010 plants infested with sentinel larvae were covered with nylon mesh netting to prevent grasshopper predation (Ace weave, Nylon Net Co. Memphis, TN). Mesh size was approximately 3.1 mm in diameter and eulophid parasitoids that attack *C. platyptera* have cross sectional diameters of < 1 mm. Previous work in our system with the same mesh material showed no cage effect of mesh on leafminer parasitoids (Gratton and Welter unpublished results). After the exposure period, plants with sentinel larvae were returned to the lab and reared until adult flies or parasitoids emerged.

To test that island vegetation patches were independent of one another we conducted a mark-capture study following (Jones et al. 2006) using protein-specific enzyme-linked immunosorbent assays to examine leafminer movement between islands.

### *Data analysis*

We used t-tests to examine *L. helianthi* density on treatment (removal) versus control islands. To account for the non-normal error structure and overdispersion we used generalized linear models with a quasibinomial error structure to examine the effects of the early season emergence of *L. helianthi* on parasitism of *C. platyptera* sentinel larvae (Zuur et al. 2009, Bates et al. 2010). Due to year-to-year variation in island vegetation structure we choose islands and randomly assigned treatments for manipulation independently in 2009 and again in 2010 and therefore analyzed each year separately. Our models included *C. platyptera* proportion parasitized in 2009 and 2010 as the response variables and treatment (removal of *L. helianthi* or control) as the explanatory variable.

### *Parasitoid host preference/suitability*

Another mechanism that could be driving patterns of herbivore population densities is parasitoid host preference. If the parasitoids in our system show a host preference for either *L. helianthi* or *C. platyptera* larvae, this could potentially explain the dominance of one apparent competitor over the other. To test for a parasitoid preference between *L. helianthi* and *C. platyptera* larvae we used sentinel larvae to conduct a field experiment in the summer of 2009. Sixty greenhouse grown sunflower plants were exposed to laboratory colonies of *L. helianthi* and *C. platyptera* adults for 24 hours. Eggs laid on plants were allowed to develop for four to six days, resulting in plants infested with second and third instar leafminer larvae. Larvae per plant were counted and assigned to replicates to achieve as equal a distribution of larvae per replicate as possible. Each replicate consisted of three plants infested with *C. platyptera*, (59 ± 8.5 per replicate) and three plants infested with *L. helianthi* (76 ± 11 per replicate).

This experiment took place at 10 replicate vegetation patches in the Yolo Bypass Wildlife Refuge. Sites were of approximately the same size and vegetation density. Each group of six infested plants was brought out to the field at 8:00 am and returned to the lab the following evening, exposing sentinel larvae to field parasitism levels for two daylight periods. All plants in this experiment were covered with the same mesh as described previously to protect against grasshopper predation. All larvae and pupae were reared until an adult leafminer or parasitoid emerged.

### *Data analysis*

To examine differences in proportion parasitized between leafminer species we used the Wilcoxon rank sum test in R (Zuur et al. 2009).

## Results

### *Apparent competition experiment*

Our removal of *L. helianthi* mines in the field for one month at the beginning of the summer was successful. We significantly reduced *L. helianthi* population density in removal versus control vegetation islands (t = 6.1, df = 8, $p < 0.005$). In 2009, three days before we brought out sentinel larvae, removal islands had 0.019 ± 0.006 (mean ± SE) *L. helianthi* mines/leaf and control islands were ten-fold greater density at 0.19 ± 0.027, *L. helianthi* mines/leaf (Figure 2a). In 2010, three days before beginning the sentinel larvae experiment, removal islands had 0.011 ± 0.002 *L. helianthi* mines/leaf and control islands had a four-fold greater density at 0.048 ± 0.007, *L. helianthi* mines/leaf (Figure 2b).

In 2009 parasitism of *C. platyptera* sentinel larvae was twice as high in control versus removal islands (15.4 ± 6.1% vs. 7.3 ± 3.7 %), but due to high variation in the data this trend was non-significant (p =0.4, df = 14) (Figure 2c). In 2010 we saw the same pattern. The proportion of parasitized *C. platyptera* sentinel larvae was three times lower on *L. helianthi* removal islands than control islands in 2010 (Figure 2d), and in this year the difference was significant ($p < 0.004$, df = 15). While some movement was observed between islands in our ELISA experiment, (< 10% of individuals captured in un-marked islands were marked) inter-island movement was not perceived as important for these experiments and was further supported by the success of the early season removal results (Figure 2a and 2b).

### *Parasitoid host preference/suitability*

We found no evidence that the dominant parasitoids in this system, *D. begini* and *N. arizonensis*, show any preference for either species of leafminer (Wilcoxon rank sum, W = 59, p = 0.278, Fig 3). These two species of parasitoids make up 90% of parasitoid individuals reared from this experiment, and the only parasitoid species used in this analysis.

## Discussion

Our results show that emergence asynchrony between two herbivores can lead to asymmetric apparent competition in the field mediated by natural enemies. Combined quantitative evidence from food webs, parasitoid overlap, and statistical modeling (Chapter 2), informed our hypothesis that parasitism rates of *C. platyptera* are strongly influenced by the early season build-up of shared parasitoids on *L. helianthi*. No support was observed for the alternative explanation that differential preference of natural enemies caused the observed patterns. In addition to these findings, additional experiments show that direct competition between herbivores is not important in our system (Chapter 1).

We found that *C. platyptera* larvae on vegetation islands with early season populations of *L. helianthi* have two to threefold higher parasitism rates than those on islands without early season populations of *L. helianthi*. The most common parasitoid species that attack both leafminers have egg to adult developmental times which are approximately half that of the herbivores. *Diglyphus begini* developmental time from egg stage to adult is approximately two weeks (Allen and Charlton 1981). While the developmental time of *N. arizonensis* is not known, based on our rearing records and developmental times of congeneric species (Murphy 1984), we believe it to be approximately 14 days as well. By the time *C. platyptera* leafminers emerge each summer in areas with early season populations of *L. helianthi*, the most common shared natural enemies will be emerging from their third generation of the season.

In a separate laboratory experiment not reported here we find no evidence of direct resource competition at population densities we observe in the field (Chapter 1). Therefore, the asymmetric interactions between *L. helianthi* and *C. platyptera* do not appear to be explained by direct competition. This finding is not surprising as parasitoids are known to be the dominant agents of mortality in leafminer communities in general (Jeffries and Lawton 1984, Connor and Taverner 1997, Hawkins et al. 1997) and our species complex in particular (Gratton and Welter 2001).

Furthermore, the pattern we observe cannot be explained due to a differential preference on the part of the two dominant parasitoids, *D. begini* and *N. arizonensis*, for one species of host. Results from our test of this alternative mechanism showed no evidence of any host preference on the part of parasitoid species in the field. Although the two dominant parasitoids, *D. begini* and *N. arizonensis*, are known to be generalists, both *L. helianthi* and *C. platyptera* can each host specialized natural enemies (Parrella 1987). Theoretically, differential parasitism by a shared parasitoid for one species of host can impact the strength and direction of indirect interactions (Bonsall and Hassell 1999). For example, parasitoid host preference for a native herbivore facilitated the invasion of a congeneric exotic species in California vineyards (Settle and Wilson 1990). Differential parasitoid preference for *C. platyptera* would have led to even stronger asymmetrical effects of apparent competition on this leafminer species. In a scenario where parasitoids might have demonstrated a preference for *L. helianthi*, we would have expected to find reduced parasitism on *C. platyptera*.

The appropriate spatial scale at which to measure indirect interactions has been the subject of debate in many recent studies on the topic (Cronin 2007, Tack et al. 2010). In both the observational and manipulative sections of this study we choose to use spatially separated local communities as our unit of replication. Our ELISA mark-capture experiment provided evidence that our treatment of vegetation island patches as functionally independent was acceptable for this trial. Yet, we do see some movement (10% of captured individuals in unmarked islands were marked with egg protein) among islands, and we cannot consider them to be independent populations over long timeframes. The possibility that the differences in *L. helianthi* populations on treatment versus control islands (a four to tenfold difference when sentinel larvae experiment was initiated) could homogenize over time was one reason we used a snap shot of *C. platyptera* parasitism on sentinel larvae. Larger, landscape scale manipulations of *L. helianthi* populations would allow us to follow these effects on *C. platyptera* populations over multiple generations at ecologically appropriate scales.

Quantitative food webs are powerful tools for displaying snapshots of complicated communities and for generating hypothesis on possible indirect interactions, but must be used with caution when used independently to make predictions (Tack et al. 2010). Apparent competition is not the only outcome possible for communities of herbivores with shared natural enemy communities. Field and laboratory studies also demonstrate neutral and positive indirect interactions between herbivores with overlapping natural enemies (Settle and Wilson 1990, Tack et al. 2010). This variety is one reason quantitative food webs alone may not be sufficient to make predictions about indirect interactions (Tack et al. 2010). In our system, however, predictions based on our quantitative food webs led to similar conclusions as those obtained with manipulative experimentation. The detailed understanding of our system's natural history as provided by the quantitative food webs allowed us to identify a key mechanism responsible for the outcome we find. More studies are needed which combine natural history data and direct empirical tests of possible mechanisms.

Understanding indirect interactions such as apparent competition can have practical applications for ecologists and conservation biologists (Memmott 2009). Temporal separation between host species has important implications for conservation biological control, climate change modeling, and conservation biology. Natural enemy populations often lag behind pest outbreaks in agricultural systems and are unable to provide adequate control. Researchers exploiting alternative prey as a temporal bridge between natural enemies and pest species have been successful in a number of systems (Doutt and Nakata 1973, Murphy et al. 1998, Pfannenstiel and Unruh 2003, Langer and Hance 2004). Insect emergence times are strongly temperature dependent, and can be affected by climate change (Walther et al. 2002). Changes in climate are likely to impact the mechanisms among species that drive indirect positive or negative interactions (Walther et al. 2002, Yang and Rudolf 2010).

Our findings build on a growing body of ecological research that shows the importance of indirect interactions for community dynamics (Wootton 1994, Cronin 2007, Kaplan and Denno 2007, Tylianakis 2009, Tack et al. 2010). One common thread in these studies is that both a detailed understanding of life history traits of organisms involved as well as rigorous field experimentation are needed to disentangle the mechanisms behind the observed patterns (Tack et al. 2010). The results presented here provide an example of using detailed, quantitative natural history data to generate hypotheses then testing these hypotheses with field-scale manipulation. Only with the careful study of mechanisms such as these that lead to apparent competition will we advance with a more predictive theory on indirect interactions.

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## List of figures

**Figure 1**

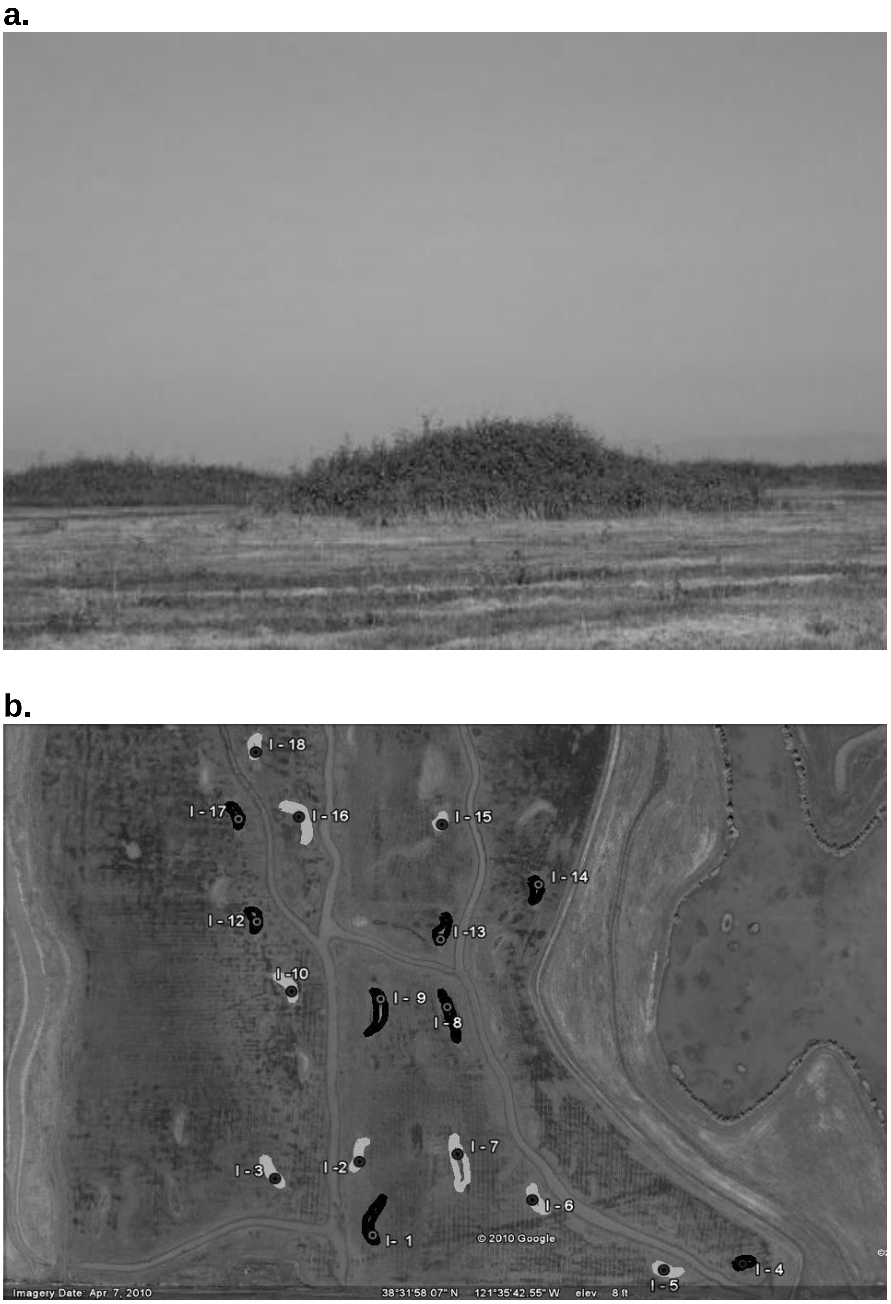
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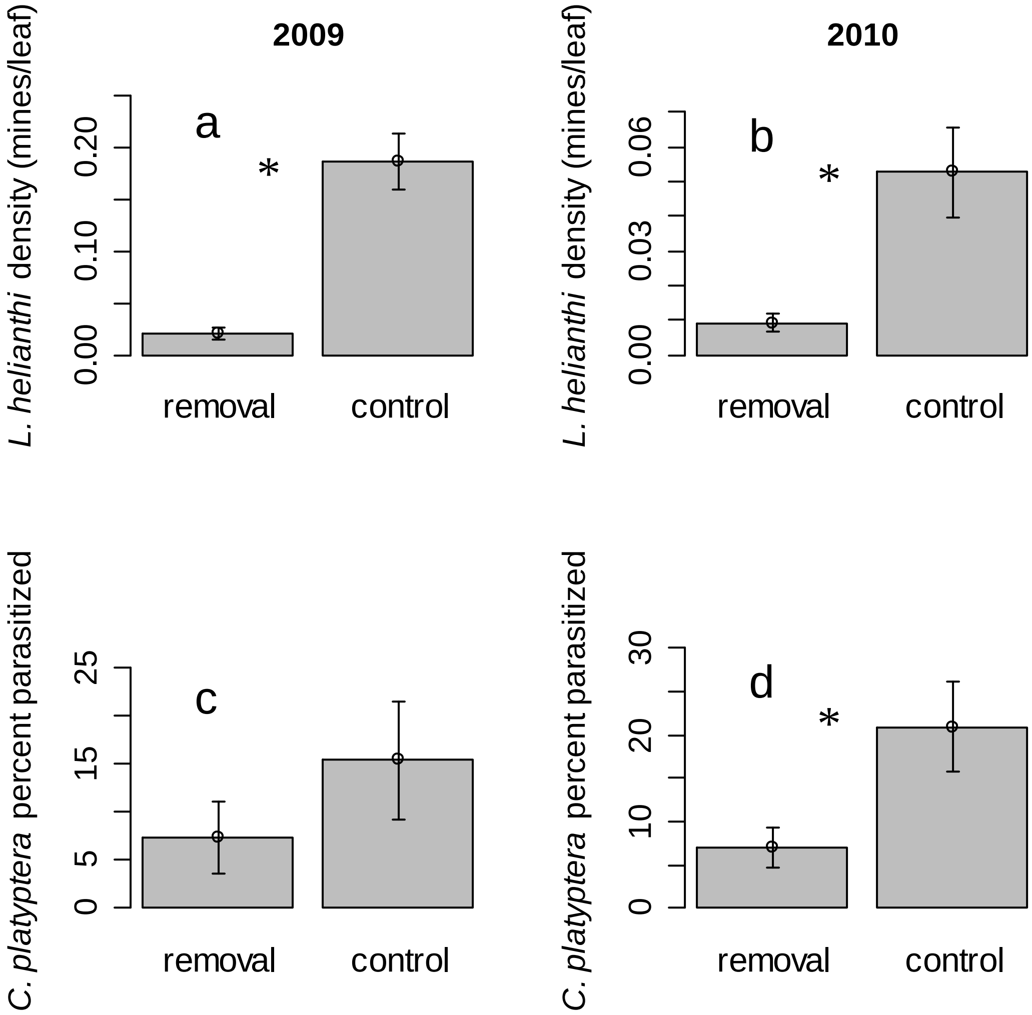
**Figure 2**



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L. helianthi density (mines/leaf)
C. platyptera percent parasitized
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0.00
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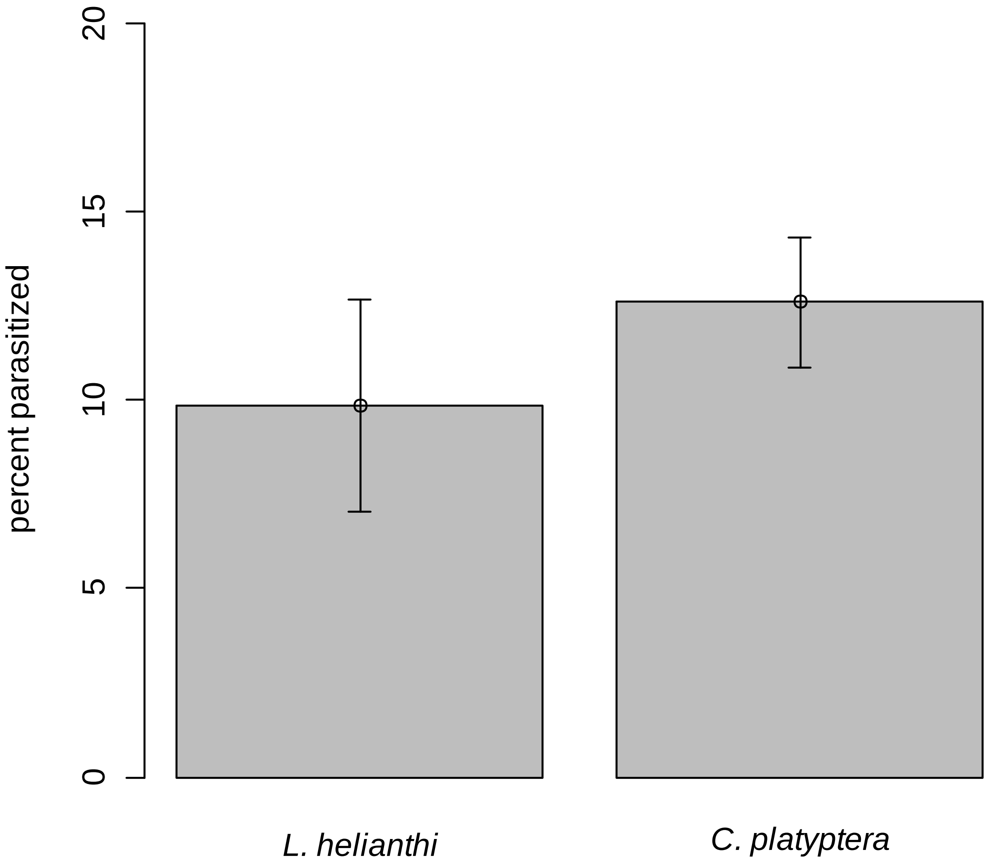
**Figure 3**



# Chapter 4

# Functionally important spillover of organisms across the managed system-natural habitat interface—a review

## Abstract

Land-use intensification has led to a mosaic landscape which juxtaposes human-managed and natural areas. In such human-dominated and heterogeneous landscapes, spillover across habitat types, especially in systems which differ in resource availability, may be an important ecological process structuring communities. While there is much evidence for spillover from natural habitats to managed areas, little attention has been given to flow in the opposite direction. Here we synthesize studies from five functionally important trophic groups, herbivores, pathogens, pollinators, predators, and seed dispersers, and discuss evidence for spillover from managed to natural systems in all five groups. We argue that this effect has been underestimated in the past. The most frequently documented examples of spillover from managed to natural systems report pathogen and vertebrate predator spillover, with a smaller number of examples reporting spillover of pollinators and herbivores. We conclude that in the ecology and conservation literature the valuation of mutual influences between managed systems and natural habitat is largely biased towards positive effects of natural areas, and negative effects of managed areas, whereas in agronomy, pest spillover from natural habitats has been well documented. As habitat modification continues, resulting in increasingly fragmented landscapes, the likelihood and size of any spillover effect will only increase. Timely empirical studies are needed in each focal group as well as across disciplines to fully understand the effects of this phenomenon

# Introduction

Land-use intensification at small and large scales over the last century is the major driver of global environmental change in terrestrial ecosystems (Sala et al. 2000). Intensification of land use causes landscape fragmentation leading to further losses in biodiversity and associated functions (Vitousek et al. 1997, Tilman et al. 2001). Ecological research focused on landscape effects has increased over the last decade, but has mostly (with the exception being studies looking at avian nest predation) dealt with landscape composition, not configuration, *i.e.*, the geometry of landscapes including corridor and edge effects. However, edge effects in particular are likely to become increasingly important as habitat loss increases the extent and types of edges, and thus the probability of dispersal of organisms across habitats. Recent work shows that edge influences on beetle communities can penetrate long distances into adjacent habitats (>1km), suggesting that edge effects likely have much more pervasive impacts than has been generally appreciated, even for small bodied organisms such as insects (Ewers and Didham 2008).

Although ecological coupling via exchanges of energy and organisms among different habitats can have important impacts on a variety of ecosystem functions (McCoy et al. 2009), the mechanisms that determine how different habitats affect one another are still poorly understood. The fundamental interconnectedness of different habitat types and ecosystems that are linked by movement of energy and organisms, and its implication for food web dynamics, are becoming increasingly clear. Examples include the importance of resource spillover from marine environments in determining community composition in coastal terrestrial systems (Polis et al. 1997) or 'predation shadows', cast by organisms with complex life histories (e.g. dragonflies or frogs), connecting spatially distinct ecosystems (McCoy et al. 2009). The literature on edge effects coupled with insights from work on the food web consequences of cross-boundary subsidies (Polis et al. 1997, Nakano et al. 1999), provide a conceptual framework to think about how mass movement between habitats, *i.e.*, across-habitat spillover, affects trophic interactions in complex landscape mosaics.

Recent reviews (Tscharntke et al. 2005b, Rand et al. 2006) have argued that spillover of generalist natural enemies, such as predators and herbivores, from managed to natural habitats is likely to be an important process affecting prey populations in adjacent natural systems. A previous review of the literature for insect predators revealed that such processes have been understudied, especially when compared with the relatively abundant literature examining flow in the opposite direction, *i.e.*, from natural to agricultural habitats (Rand et al. 2006). The general importance of agricultural to natural habitat spillover in influencing the strength and magnitude of other functionally important trophic interactions, has not been rigorously evaluated.

Here we assess the evidence that indicates a potential for spillover effects, by reviewing the literature across a broad range of functionally important trophic interactions, including both antagonisms (herbivory, disease, and predation) and mutualisms (pollination, seed dispersal), across the managed-natural system interface. For the purpose of this review, we define spillover simply as the movement of organisms from one distinct habitat type, or population (in the case of pathogen spillover) to another. We are interested primarily in how this movement influences function in recipient habitats. Although potentially important, plant spillover, and its influence on function, is beyond the scope of this review. We summarize the literature examining spillover in the relatively well investigated direction from natural to managed systems. We note that the meaning of natural and managed are context and site specific, especially when comparing highly-managed European landscapes to North America. For our purposes we define natural habitats as areas that are not intensively managed (eg. native forests, marshes, and semi-natural habitats such as hay meadows, and woodlots), and managed habitats as agricultural and other intensively managed systems (e.g. annually harvested systems dominated by cultivated plants). Finally, we focus on the functional importance of spillover effects in the opposite direction. As the number of publications explicitly addressing the latter is low, we could not prepare a quantitative review, but discuss all studies we do find. We conclude by weighing the balance of evidence for or against the suggestion from previous work (Rand et al. 2006) that the importance of spillover from managed to adjacent natural habitats has been understudied (Table 1).

## Focal groups

### Herbivores

*Spillover from natural to managed systems*

Studies documenting herbivore movement from natural areas to adjacent agricultural fields are increasingly common. Much of the older agricultural literature discusses semi-natural habitat as a “reservoir” of pests. Some examples include aphid density in barley patches adjacent to natural grass meadows (Gravesen and Toft 1987); Colorado potato beetle in non-crop plants near potatoes in Michigan (Mena-Covarrubias et al. 1996); Dipteran leafminers in weedy native vegetation in un-managed patches adjacent to tomato fields in Florida (Schuster et al. 1991, Oliveira and Fontes 2008); and scale insects moving from native plant hosts to cultivated cassava on the edges of fields in Brazil (Jefferies and Drent 2006).

*Spillover from managed to natural systems*

Although currently only a few studies have investigated the movement of herbivores from managed to natural systems, ecologists now recognize the potential detrimental effects of spillover of large populations of herbivores subsidized on agricultural crops to native plants in increasingly small semi-natural habitat fragments. The first study which directly measured this phenomenon investigated the effects of dispersal of adult corn-rootworm beetles, *Diobrotica baraberi*, from corn to native sunflower species in endangered tallgrass prairie ecosystems (McKone et al. 2001). The authors consistently found higher densities of the corn root worm, *D. baraberi* a common agricultural pest, in native sunflowers in natural prairie patches near the edge of corn fields. Although the abundance of *D.baraberi* was greatest at the corn edge (*D.baraberi* abundance was reduced by 80% 60 m from corn edge), no part of prairie patches studied were completely free of *D. baraberi*. Pollen feeding by *D.baraberi* was found to reduce seed set of native sunflower species, suggesting that this agricultural pest may interfere with the reproduction of native sunflowers and other late flowering composites (McKone *et al.* 2001). In Newfoundland, Canada, a more recent example looked at the threat rare, native, non-agricultural

Brassicaceae species face from *Plutella xylostella* (diamondback moth) spillover from agricultural Brassicaceae (Squires et al. 2009). *P. xylostella* is a global pest of broccoli and cabbage and is one of the most problematic agricultural pests worldwide (Squires et al. 2009). In the study area, typical agricultural host plants such as cabbage occur only in small patches, thus when *P. xylostella* arrives by wind dispersal from large agricultural Brassicaceae field further south, (*P. xylostella* is an annual migrant in Canada during the growing season) their populations are bolstered by on rare, native, and non-agricultural Brassicaceae species. *P. xylostella* larva were first seen on *Braya longii* and *Braya fernal* in 1995. In sites studied (Squires et al. 2009) 45% to 50% of *B. longii* and *B. fernaldii* fruit were damaged by *P. xylostella* herbivory, leading to a 60% decrease in seed set among plants with damaged fruit. The loss of seeds is of particular importance because *B. longii* and *B. fernaldii* have long-lived seed banks and large plants contribute disproportionately to the seed bank. *P. xylostella* larvae were the only insect observed feeding on *B. longii* and *B. fernaldii* leaves or fruit. *B. longii* mortality is linked to previous leaf and fruit damage, presumably because *P. xylostella* feeding weakens the plant so that death is more probable in subsequent years. Kaiser et al. (2008) gives an example of movement of a pest species that could impose a risk of degrading natural habitats. In Mauritius, an introduced pest species of cultivated coffee, *Prophantis smaragdina* (coffee berry moth), has been found feeding on *Bertiera zaluzania* with detrimental effects on the reproductive success of this endangered endemic plant (Kaiser et al. 2008). The authors of this study report that it is unlikely that *B. zaluzania* is the only endemic Mauritian Rubiaceae affected by this pest species.

Although, the three studies discussed above are the only examples found where the functional consequences of spillover were explicitly measured, there is ample evidence that spillover effects is likely widespread. Examples include aphids, of which at least 10% show host-plant alternation (Mueller and Godfray 1999) and a number of broadly distributed pest species show documented feeding on native plants in the U.K. (Mueller *et al.* 1999); grasshoppers with cyclical movement from grassland into winter wheat fields in the early spring, and then back into native grassland systems once the crop matures (Gillespie and Kemp 1996); the sunflower moth, *Homoeosoma electellum* Hulst, which attacks both native and agricultural *Helianthus* species (Chen and Welter 2002); and the tarnished plant bug, (*Lygus rugulipennis*) recorded on 437 plants in 57 families (Holopainen and Varis 1991).

There are also a number of examples providing evidence of the effects of managed herbivores spilling over to affect native plants in the biological control literature. Rhinocyllus conicus, a Coleopteran thistle specialist introduced to control exotic species, has been found to spillover from its weedy invasive host species onto multiple non-target native species, resulting in increased attack and reduced seed production of native thistles (Louda et al. 1997, Louda 1998, Rand and Louda 2004). *Cactoblastis cactorum,* introduced for weed biological control in Australia, has since invaded North America and now threatens endangered native cacti (Stiling et al. 2004). Invertebrate herbivores are not the only group that could be influenced by landscape change. In forest habitats seed recruitment and survival of native tree species may be affected by spillover of vertebrate seed predators. In Spain large oak forests have been reduced to small fragments within a matrix of agricultural fields. In the fall, after fields are ploughed, small mammals migrate from the barren fields to over-winter in small forest stands and are responsible for high acorn consumption in these areas (Santos and Telleria 1997). In central Chile, native granivores are more abundant in forest fragments surrounded by managed pine plantations than in the continuous forest areas (Donoso et al. 2004).

In conclusion, there is much evidence for herbivore spillover from natural to managed systems. However, while much anecdotal evidence suggests that spillover from managed to natural systems is also common, few studies have explicitly examined the functional consequences of such spillover.

## Pathogens

### *Spillover from natural to managed systems*

Current epidemiological theory is increasingly concerned with understanding disease ecology in multiple host systems (Power and Mitchell 2004). Scientists, as well as the general public, pay close attention to disease outbreaks affecting humans, such as acquired immunodeficiency syndrome (AIDS), or H5N1 influenza (bird flu) (Wolfe et al. 2007), but little attention is paid to disease outbreaks in vertebrate wildlife which do not have an impact on humans (Otterstatter and Thomson 2007), and even less to outbreaks in invertebrates and plants.

Disease movement from semi-natural land to domesticated animals and plants is well documented (Daszak et al. 2000, Power and Mitchell 2004). In the epidemiological literature, spillover is explicitly discussed, but it lacks the inherently spatial perspective that we use to define spillover in the other functional groups reviewed. In the case of pathogens, managed refers to actively managed host populations, and natural systems to non-managed natural populations. Native plant and animal populations are often cited as reservoirs of disease. A study in 2001 found that 77% of pathogens infecting mammalian livestock, and 90% of the pathogens infecting domestic carnivores, were generalists and could attack multiple hosts (Cleaveland et al. 2001). Examples include badgers in the United Kingdom as a reservoir for tuberculosis that infects cattle (Donnelly et al. 2003, Woodroffe et al. 2005), wild cervids as a reservoir in North America for three important diseases of livestock: bovine tuberculosis, chronic wasting disease, and brucellosis (Conner et al. 2008), and wild warthogs in Africa as a reservoir of swine fever in domesticated pigs (Plowright et al. 1969).

The agronomy literature contains many important examples of “weeds” in natural habitats serving as reservoirs for crop diseases (Wisler and Norris 2005). Cultivated legumes in Africa are under increasing threat from a parasitic plant (*Orobanche* sp.) which is commonly associated with wild legumes (Patto et al. 2008). Sweet potato feathery mottle virus, globally the most common pathogen of sweet potato, is found in 22 species of natural host plants growing near sweet potato fields in East Africa (Tugume et al. 2008). Another common example is *Berberis* serving as an alternative host for rust fungus attacking cereals in Europe (Newton and Johnson 1937). Weeds on the border of tobacco fields are often infected with tomato spotted wilt virus and *Impatiens* necrotic spot virus are thought to be reservoirs of disease (Martinez-Ochoa et al. 2003), and there are many more examples (Wisler & Norris 2005).

In contrast, pathogen spillover can have a positive effect on biological control in agricultural systems when the organisms attacked are pest insects rather than the crop plants. In fact, recent advances in conservation biological control have focused on the potential benefits of managing extra-field habitats to promote spillover of important entomopathogens into cropping systems (Pell et al. 2008). For example, a modeling study by (Carruthers and Soper 1987) demonstrated that providing alternative hosts outside of crop fields, could be a major factor contributing to greater control of onion maggot by the disease *Entomopthora muscae*. Similarly, the build up of economically unimportant aphid species in meadows and weedy field margins can serve as important reservoirs of pathogens regulating aphid populations in adjacent cropping systems (Keller and Suter 1980, Powell et al. 1986).

### *Spillover from managed to natural systems*

A recent prominent example of disease movement in the managed to natural direction which has received attention looks at the spillover of pathogens from domesticated to wild pollinators. Interest in this case is particularly high because of reported declines in bee species and the ecosystem services they provide (Kremen et al. 2002, Biesmeijer et al. 2006, Otterstatter and Thomson 2007). Commercially reared bumble bees are used commonly as greenhouse pollinators of at least 20 crops (Velthuis and van Doorn 2006). Infected commercial bumble bees can escape from greenhouses and forage on food sources that they share with wild *Bombus* species, infecting the wild species with *Crithidia bombi*, an intestinal parasite (Lipa and Triggiani 1988, Otterstatter and Thomson 2007). In Canada evidence for *Bombus* spp. decline has been quantified, and although it is likely that there are a number of driving factors, pathogen spillover from commercial colonies is thought to be a major culprit (Colla and Packer 2008). An experiment on the same system examined the prevalence of four pathogens in wild bumble bee populations at locations near and distant to commercial greenhouses in southern Ontario, Canada. Bumble bees collected near commercial greenhouses were more frequently infected by those pathogens capable of being transmitted at flowers (*Crithidia bombi* and *Nosema bombi*) than bees collected at sites away from greenhouses (Colla et al. 2006). Scientists in Australia have argued successfully to prevent the importation of *Bombus* as greenhouse pollinators due to the risk of disease transfer from *Bombus* to managed and wild *Apis* species (gov. report, personal comm. with S. Cunningham). Australia's high levels of endemic species pose special concerns when thinking about spillover. In Australia, where many agricultural crops rely on high levels of pollination from wild *Apis mellifera*, spillover of the invasive *Varroa destructor* from domesticated bee colonies is a concern, but viewed by bee keepers as inevitable (Cook et al. 2007).

Pathogen spillover in vertebrates also provides well-documented examples of how anthropogenic change has negatively impacted wildlife, although pathogen effects on wildlife are under researched (Daszak et al 2000). Endangered African wild dog and Ethiopian wolf populations have been seriously threatened by rabies, and although we are missing direct evidence, the authors argue that spillover from domestic dogs is almost certainly the cause (Gascoyne et al. 1993, Sillero-Zubiri et al. 1996, Knobel et al. 2008, Lembo et al. 2008). Lembo *et al.* (2008) adds evidence to the spillover argument by showing that the reservoir of rabies in the Serengeti ecosystem is a complex multi-host community where domestic dogs are the only population essential for persistence, although other carnivores contribute to the reservoir as non-maintenance populations (Lembo et al. 2008). After a 2000-2002 Foot and Mouth Disease outbreak in domestic livestock in Mongolia, a study in 2001 found foot and mouth disease in over half of the Mongolian gazelles sampled and authors believe spillover of the virus from domestic animals was the likely cause of the outbreak (Nyamsuren et al. 2006).

Studies of pathogen spillover from domesticated to wild plants are relatively rare. In a review on pathogen spillover, Power and Mitchell (2004) argue that this is due in part to the relative lack of data on pathogens in natural plant populations (Jarosz and Davelos 1995). In California, the invasion of native grasslands by Eurasian grasses provides an example of pathogen outbreaks brought by anthropogenic change. Herbarium records document the presence of barley and cereal yellow dwarf viruses (B/CYDV) in samples from the time period of historical invasion, suggesting that apparent competition mediated by B/CYDV may have facilitated grassland invasion (Malmstrom et al. 2007). In the same system, a recent experimental study found that grazing by vertebrates increases the density of host plants infected with aphid-vectored B/CYDV four-fold, which translates to higher viral prevalence (Borer et al. 2009). The prevalence of some strains of B/CYDV in wild grasses is highly dependent on their proximity to crop hosts (Remold and Power 1995).

Collectively, the pathogen literature provides perhaps the best data documenting the importance of anthropogenically mediated spillover from domesticated to wild plants and animals. Nonetheless, in most cases, we are still lacking quantitative data which unequivocally shows spillover as the mechanism.

## Pollinators

### *Spillover from natural to managed systems*

Managed and wild pollinators can provide effective pollination services in many cropping systems (Klein et al. 2007). Blueberry and watermelon crops are well known examples illustrating the importance of native pollinators. Watermelon is visited by over 30 species of native bees in addition to *Apis mellifera* in California (Kremen et al. 2002) and at least 47 species in New Jersey (Winfree et al. 2007). Blueberries are more effectively pollinated by native bumblebees than honeybees (Watanabe 1994, Cook et al. 2007). In each of these cases pollinators are moving from semi-natural land fragments to agricultural fields. Movement of pollinators from natural to managed agricultural landscapes has been documented on five continents across a wide range of habitat and managed landscapes (Ricketts et al. 2008). Many native tropical plants are also insect pollinated (Bawa 1990, Liow et al. 2001) and similar patterns of native tropical rainforests providing pollination services to agricultural crops have been observed (Blanche et al. 2006). Examples include rainforest habitats providing sources of pollinating bees for coffee plantations in Indonesia (Klein et al. 2003), Costa Rica (Ricketts 2004), and Brazil (De Marco and Coelho 2004); pollinating beetles for atemoya (*Annona squamosa* L. X *Annona cherimola* Mill); hybrid flowers in Queensland Australia (Blanche and Cunningham 2005); and stingless bees in Longan orchards in Queensland (Blanche et al. 2006). Another study from the tropics found that pollinator richness and abundance were correlated with landscape complexity and that species richness only was positively related to seed numbers in pumpkins (Hoehn et al. 2008). This study is a demonstration of the biodiversity-ecosystem function relationship among pollinators and plants along a gradient of land-use intensity.

### *Spillover from managed to natural systems*

Although the importance of the movement of pollinators from semi-natural land to more heavily managed agricultural land has been well documented in recent reviews, (Aizen and Feinsinger 1994b, Aizen and Feinsinger 2003, Klein et al. 2007, Ricketts et al. 2008), we are still missing an important piece of the puzzle; there are remarkably few studies on the effects of the movement of pollinators in the other direction. It seems likely that pollinator movement through the mosaic landscape of crop and noncrop habitats will have an impact on the sexual reproduction of wild plants in embedded natural habitat fragments. We know that many pollinating species cannot complete their life cycles in agricultural fields. Many bee species use nesting sites in semi-natural habitats and forage for pollen and nectar resources in spatially separated areas which often include a mixture of disturbed and un-disturbed habitats (Williams and Kremen 2007). A number of studies show that pollinators visit crop habitats for foraging, but that they must return to natural habitats to complete their reproductive cycle because of the frequent disturbance regime in agricultural fields (Hayter and Cresswell 2006, Greenleaf et al. 2007, Holzschuh et al. 2007, Kremen et al. 2007, Winfree et al. 2007). From these studies we can infer that pollinators are using, and maybe even relying on, resources from crop fields, and then returning to natural habitat fragments. The role of supplementary resources from agricultural habitats in promoting bee populations, and potential benefits of these resources to wild plant populations have rarely been measured. A recent study from Kenya, suggests that bee species-rich farmland might have a positive effect on nearby rain forest fragments by acting as a "pollinator rescue" that supports pollination services inside the natural forest (Hagen and Kraemer 2010). The authors measured network properties (network size and specialization indices) and bee species turnover at 18 sites in a tropical forest-agriculture mosaic in Western Kenya. The largest networks, highest diversity, and largest abundances of bees were found at the forest edge and in the farmland which hosted higher amounts of flowers and a more homogeneous distribution of resources in space and time when compared to forest sites (Hagen and Kraemer 2010).

Such spillover effects involving pollinators could result from the interactions of managed honey bees (brought in to provide pollination services to agricultural crops) with wild plants in semi-natural areas adjacent to fields. For example, a study which monitored both wild pollinators and managed honeybee visits on native plants found that the managed honeybees visited 24 of the 43 species of native plants found on the edges of agricultural land (Tuell et al. 2008) possibly contributing to increases in plant fitness. In some cases, honeybee populations from modified habitats may partially compensate for the declines of native pollinators in natural habitat fragments. For example, (Aizen and Feinsinger 1994b) found that while the frequency and richness of native pollinators visiting two native tree species declined with decreasing fragment size, the frequency of wild honeybee visits increased, such that total visitation frequency varied little with fragmentation. Similarly, at least four out of ten self-incompatible native forest plant species studied were heavily visited by honey bees in small habitat fragments, which may have compensated for declining visitation by native pollinators (Aizen and Feinsinger 1994a). These studies strongly suggest that some native plant species may benefit from the spillover of bees from managed systems into habitat fragments.

Additionally, mass flowering of crops could potentially facilitate pollination in semi-natural land if the crops serve as "magnet plants". Recent studies found that mass-flowering of rape increases the growth of bumble bee colonies (Westphal et al. 2009). This concentrates pollinators in the vicinity of mass-flowering agricultural fields, facilitating spillover into natural areas (Andrea Holzschuh et al., unpubl. data), and potentially benefiting native plants. The magnet crops might also provide resource subsidies that would allow increases in local pollinator population size the following year. Similarly, a high density of arable fields supporting high weed cover, as often found in organic agriculture, can increase bee populations on a landscape scale, thereby enhancing potential pollination in near-natural fallows (Holzschuh et al. 2008).

Alternatively, flower visitation, pollen transfer, and seed set of natural plants could be adversely affected by competition for pollinators with managed crops similar to the competition between native and invasive plants (Lopezaraiza-Mikel et al. 2007, Aizen et al. 2008). During the typical periods of mass flowering of crops, this competition between natural plants and agricultural crops could lead to a lack of pollination services in natural habitats and could be especially severe for rare plants in conservation areas adjacent to agricultural fields (Steffan-Dewenter and Westphal 2008). Furthermore, agricultural habitats may differentially benefit certain pollinator species, such as honey or bumblebees, resulting in competition with native solitary bees which could be more efficient pollinators of some wildland plants, but evidence is scarce (Steffan-Dewenter and Tscharntke 2000).

Overall, these examples provide clear evidence that pollinators are moving across crop non-crop habitat boundaries. Agricultural and other managed landscapes are sure to affect the pollinator-plant interactions of adjacent natural habitats. We therefore need to understand how mass-flowering crops and the introduction of managed pollinators to agricultural fields affect pollinator interactions and pollination services in wild habitats.

## Predators

### *Spillover from natural to managed systems*

Crop habitats can be hostile environments for many natural enemies and predators of insect herbivores (Kleijn et al. 2001, Meek et al. 2002), which as a result often rely on surrounding natural areas for their persistence. A number of reviews find evidence that such natural and semi-natural areas provide alternative energy sources and host species, as well as over-wintering and nesting habitats, thereby promoting populations of important natural enemy species moving into agricultural fields (Landis et al. 2000, Tscharntke et al. 2005a, Bianchi et al. 2006). Proximity to habitat edges may also then have an impact on insect herbivores in less productive semi-natural habitats as natural enemies spillover from more productive habitats (Rand et al. 2006, McCoy et al. 2009).

Despite many studies looking at the effects of landscape composition and proximity to edges on natural enemy abundance, composition or diversity, comparatively few actually measure the functional implications of such spillover, i.e. effects on pest suppression and crop injury levels (Bianchi et al. 2006). Examples of recent studies that investigate the relationship that the presence of non-crop habitats has on pest control services include increased parasitism rates of *Plutella xytostella* in Brussels sprouts near forest habitats in the Netherlands (Bianchi et al. 2008). Similarly, (Gardiner et al. 2009) found that both the abundance of generalist coccinellid predators and biological control services (the relative suppression of soybean aphids) increased with increasing landscape diversity and decreased crop cover.

Natural areas can also have negative impacts on managed systems. Well known examples of vertebrate predators moving from natural to managed systems to find prey include foxes on chickens (Lloyd 1980), and eagles on lambs (Avery and Cummings 2004).

### *Spillover from managed to natural systems*

Rand et al. (2006) argue that spillover of subsidized natural enemies across managed-to-natural habitat edges is likely to be an important process affecting prey species. Avian nest predation is one area which has a large body of literature assessing these dynamics. Although there have been no fewer than six review papers (Paton 1994; Andren 1995; Hartley & Hunter

1998; Soderstrom 1999; Lahti 2001; Chalfoun *et al.* 2002) on the subject in the last two decades, this literature has not succeeded in producing a consensus as to what effect being near a habitat edge has on nest predation. Some nest predation studies mention spillover of predators as the suggested mechanism, but none have actually quantified this suggestion (Lahti 2001, 2009). For this reason, and because this literature has been thoroughly reviewed in other publications, we have not included these studies here. Few studies from other groups since 2006 have looked into this phenomenon by directly measuring the movement of natural enemies from agricultural to semi-natural areas, and none have attempted to measure the direct and/or indirect impact this movement of predators and parasitoids has on herbivores or plants in these natural habitat fragments. One study measures spider activity density in wheat fields and adjacent natural semi-desert in Israel. The authors argue that their data showed a strong preference among sheetweb spiders for agricultural habitat which supports the conclusion that in these semi-desert habitats, sheetweb spiders are moving from wheat fields to adjacent semi-natural desert lands (Pluess et al. 2008). Another study found that wheat-dominated landscapes appear to augment generalist predators (ladybird beetles and spiders) of nettle aphids resulting in dramatically higher natural enemy–prey ratios and reduced aphid density in adjacent nettle patches (Rand and Tscharntke 2007).

### Seed dispersers

#### *Spillover from natural to managed systems*

In deforested landscapes, seed dispersal is a critical factor for tree succession (Holl 2002, Marcano-Vega et al. 2002). Low immigration of forest seeds is considered the overriding factor impeding reestablishment of forest in disturbed areas (Da Silva et al. 1996, Duncan and Chapman 1999, Ingle 2003). In fragmented, human-dominated landscapes, frugivorous birds and bats are the most important seed dispersal vectors, because habitat types such as grassland or early successional vegetation poses less of a barrier to them than to other vectors such as mammals (Ingle 2003). Seed input into disturbed habitats, such as abandoned pastures, usually steeply declines with distance from source habitats (Howe and Westley 1997) and dispersal vectors such as many birds do not cross open pasture unless attractive trees are in sight (Martínez-Garza and González-Montagut 2002, White et al. 2004). However, much remains unclear about dispersal when small patches of forested habitats are embedded in a matrix of agricultural land, including the efficiency of birds as dispersers in a mosaic landscape and the role of small forest fragments as seed source and refuge for birds (Lozada et al. 2007).

#### *Spillover from managed to natural systems*

We found no studies which explicitly measured spillover of seed dispersers from managed to natural systems, but we discuss studies that highlight the potential for spillover effects in this group. Similar to pollinators subsidized by agricultural crops, seed dispersers may profit form landscape matrix quality (Murphy and Lovett-Doust 2004). A diversity of land-use systems can have an impact on seed dispersers, especially in the tropics (Garcia and Banuelos 2003). Examples include studies showing that bats are less abundant in tropical forest fragments surrounded by pastures as compared to fragments embedded in a more structurally complex cocoa and coffee agricultural area (Estrada and Coates-Estrada 2002). Similarly, in agricultural landscapes of Ecuador, abandoned and managed coffee sites are much more common than forest patches, and they provide a refuge for plant and bird species (Lozada et al. 2007). In such a mosaic landscape, where the vast majority of natural forest has been replaced by agricultural land, agroforestry systems offer birds stepping-stones and thereby reduce reproductive isolation, enhancing tree recruitment (Lozada et al. 2007). Remnant trees in pastures are often used as perches and foraging canopies which can foster seed dispersal from the pasture to forest (Galindo-Gonzalez et al. 2000, Laborde et al. 2008, Herrera and Garcia 2009). Seed dispersal by avian frugivores is more frequent among patches connected by linear habitat patches than in isolated patches (Tewksbury et al. 2002). In human-dominated landscapes, patterns of plant recruitment and plant community succession are strongly influenced by seed spillover across all kinds of habitats, but we lack detailed and quantitative landscape-scale studies which measure spillover.

## Discussion

Our review focuses on highlighting the potential functional importance of spillover effects from managed to natural systems. In all five functional categories reviewed (pollinators, seed dispersers, pathogens, herbivores and predators), we found ample indirect evidence for influential cross-border effects. While the studies are too diverse to be formally used in a quantitative review, the evidence supporting important managed to natural system spillover is far from anecdotal.

We found that studies of spillover from managed to natural systems have been generally underrepresented relative to those examining flow in the opposite direction (Table 1). Although we did not complete an exhaustive quantitative analysis of spillover in the natural to managed direction, the numbers we report in Table 1, for this direction, were taken from review papers for each group. For each group we counted all studies from the most recent published review that show spillover. Numbers for this direction are therefore conservative, but prove our point, and we refer to the reviews for more detailed discussions of studies in this direction. Numbers of studies in the managed to natural direction are the results of computer searches using ISI Web of Knowledge and Google Scholar, for all studies examining spillover in this direction for each focal group. No limits were imposed with respect to the year of publication or specific journals.

In an ideal scenario, ecosystems would have never become fragmented in the first place, and in this pristine condition natural areas would not be expected to benefit from managed lands. However, most natural ecosystems have become fragmented to a greater or lesser extent due to conversion for human uses. In highly managed and fragmented landscapes (i.e. Europe), there are potential positive effects of spillover from managed systems to natural habitat fragments and we argue that these effects have been generally underappreciated in the conservation and ecology literature. As landscapes become increasingly heterogeneous, managing human-modified habitats to minimize negative effects while maximizing their potential contributions to biodiversity conservation and functional interactions in remaining natural habitats is essential.

Perhaps not surprisingly, all of the examples of positive effects of managed on natural habitats we found were confined to the two mutualisms, pollination and seed dispersal in highly fragmented landscapes. In both cases the available evidence suggests that managed habitats could be important sources of mutualists providing services to wildland plant species in the face of natural habitat loss. We found no studies examining the potential benefits of antagonists spilling over from managed to natural systems. Potentially interesting research along these lines might, for example, address the role of cropping systems as potentially important sources of herbivores spilling over and suppressing weedy plant species in adjacent natural habitats. As we noted earlier, *Brassica* crops share herbivores with wild *Brassica* species, which can have detrimental effects on rare native brassicas in Canada (Squires et al. 2009). However, many *Brassica* species in North America are weedy or invasive, such that similar herbivore spillover could actually be beneficial in these cases. Similar arguments may hold true for other antagonists in crops that are likely to be particularly detrimental to exotic, rather than native, species in natural habitats.

Thus, while it is clear that studies that examine spillover effects from managed to natural systems are rare across the board, there does seem to be a bias towards studies focused on the negative effects. One explanation for this bias may be that managed systems are often highly disturbed and thought to support only few, disturbance resistant, species. As a consequence they are not generally regarded as meaningful sources of organisms potentially moving over into natural systems. However managed biota can be rich. Indonesian homegardens, for example, may include 100-200 crop species and noncrop plants within arable fields and include endangerd species (Gabriel et al. 2006). Furthermore managed systems are often highly productive, thereby providing resources that may be used by non-agricultural species (Westphal et al. 2003).

Although many studies support the conservation view that natural systems provide benefits to managed systems, especially as sources of pollinators and biological control enemies, many cases of natural disservices were also found. The high value humans place on agricultural products likely leads to an immediate reporting and investigation of negative effects of spillover from natural to agricultural systems. This is evidenced by the long tradition of agronomic research looking at wildland plants as potential reservoirs of pathogens or herbivorous pests important in cropping systems, as well compensation payments to farmers for large predator and herbivore conservation. In contrast, little attention is paid to the wholesale collapse of wildlife populations due to increased contact with managed systems (e.g. wildebeest).

## Conclusions

1. Land-use intensification has led to a mosaic landscape which juxtaposes human-managed and natural areas. In such human-dominated and heterogeneous landscapes, movement across habitat types, especially in habitats which differ in resource availability, may be an important way communities are structured.
2. We found that studies of spillover from managed to natural systems have been generally underrepresented relative to those examining flow in the opposite direction. In particular, the potential positive effects of managed habitats for adjacent natural systems in fragmented landscapes remain largely unstudied. There are numerous examples of studies looking at spillover in the natural to managed direction, perhaps not surprising, as this is likely to impact human enterprises.
3. Much work has been done looking at both positive effects (eg. importance of natural habitat in maintaining pollination and biological control services) as well as negative effects (natural habitats and wildland plants serving as reservoirs of crop diseases and pests), suggesting a difference in traditional subject-area focus, with positive effects more likely to be discussed by conservation biologists and ecologists, and negative by agronomists.

4. As humans continue to modify their landscapes the likelihood and size of any spillover effect will only increase. Empirical studies are now crucial to provide quantitative evidence in each focal group as well as across disciplines to fully understand the effects of this phenomenon on natural communities.

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**Table 1**. Studies found for each focal group in the natural to managed and managed to natural directions. Although we did not complete an exhaustive quantitative analysis of spillover in the natural to managed direction, the numbers we report in Table 1 for this direction are conservative. The + symbol is meant to represent the idea that there are likely many more studies than we have listed, but that even these conservative numbers support our conclusions. We refer to reviews for more detailed lists of studies in this direction.

| **Focal group** | **natural → managed number of studies** | **managed → natural number of studies** |
|---|---|---|
| Pollinators | 30+<br>see Ricketts et al 2008 | 3<br>unpublished Holzschuh, Aizen 1994a and 1994b |
| Seed dispersers | 30+<br>see Duncan and Chapman 1999; Holl et al 2000; Ingle 2003; Lozada et al 2007 | 5<br>Estrada & Coates-Estrada 2002, Galindo-Gonzalez et al. 2000; Laborde et al. 2008; Herrera & Garcia 2009, Tewksbury et al. 2002 |
| Pathogens | 14+<br>see Power & Mitchell 2004 and Daszak et al 2000 | 8<br>Lipa & Triggiani 1988; Otterstatter & Thomson 2007; Colla & Packer 2008; Gascoyne *et al.* 1993; Sillero-Zubiri *et al.* 1996; Knobel *et al.* 2008; Lembo *et al.* 2008; Nyamsuren *et al.* 2006 |
| Herbivores | 50+<br>see Norris & Korgan 2000 | 3<br>McKone et al. 2001, Squires et al. 2009, Kaiser et al. 2008 |
| Predators | 30+<br>see Bianchi et al 2006; Landis et al 2000 | 3<br>Pluess et al. 2008, Rand & Tscharntke 2007, Rand & Louda 2006 |

## Conclusions

This dissertation is comprised of two sections. The main section investigated the strength of both direct (resource) competition and indirect (apparent) competition between two species of leaf-mining flies. I show that direct competition between *C. platyptera* and *L. helianthi* does not play an important role in structuring natural communities of these leafminers (Chapter 1). However, at high densities, I was able to detect significant, negative effects of direct competition on the fitness of both leafminer species. At high per-leaf densities, pupal weights of *L. helianthi* are predicted to be 20% less than at low per-leaf densities, and pupal weight of C. platyptera would be reduced by 17%. While we are able to detect negative effects of competition at high densities in the lab, direct resource competition is not an important structuring mechanism for this leafminer community in natural systems, presumably due to the suppressing effects of natural enemies on overall population densities in the field.

Observational studies of natural communities of *C. platyptera* and *L. helianthi* in the field (Chapter 2) support my hypothesis that natural enemies are driving the dynamics of this system. Overall parasitism was high both years and relatively similar for both species. The larval parasitoids *Neochrysocharis arizonensis* Crawford and *Diglyphus begini* (Crawford) were the dominant parasitoid species both years, for both species. *Liriomyza helianthi* and *C. platyptera* showed marked differences in phenology and population density throughout the sampling period in both years studied. *Liriomyza helianthi* leafminer populations emerge approximately one month before *C. platyptera* populations each year. By the time *C. platyptera* populations emerge in the field, the first generation of parasitoids has emerged from *L. helianthi* hosts. Because I see no discrimination by the dominant parasitoid species, and no evidence of resource competition in the laboratory (Chapter 1), I hypothesize that the early season build up, and eventual spill-over, of parasitoids from *L. helianthi* hosts to *C. platyptera* hosts leads to the suppression of *C. platyptera* populations throughout the season.

In Chapter 3, I test the hypothesis developed in Chapter 2, that asynchrony in herbivore emergence times leads to asymmetric apparent competition. I use a species removal experiment in the field to assess whether early season emergence of *L. helianthi* drives parasitism of *C. platyptera* through shared natural enemies. The experimental removal of *L. helianthi* populations in the early summer leads to a 50% reduction in parasitism of *C. platyptera*. This is the first demonstration of herbivore emergence times leading to asymmetric apparent competition in a natural system.

Results from this section of the dissertation lead to a clearer understanding of how complex ecological communities are structured, and has practical applications for ecologists and conservation biologists (Memmott 2009). Temporal separation between herbivore species (Chapters 2 and 3) has important implications for conservation biological control, climate change modeling, and conservation biology. Natural enemy populations often lag behind pest outbreaks in agricultural systems and are unable to provide adequate control. Researchers exploiting alternative prey as a temporal bridge between natural enemies and pest species have been successful in a number of systems (Doutt and Nakata 1973, Murphy et al. 1998, Pfannenstiel and Unruh 2003, Langer and Hance 2004). Insect emergence times are strongly temperature dependent, and can be affected by climate change (Walther et al. 2002). Changes in climate are likely to impact the mechanisms among species that drive indirect positive or negative interactions (Walther et al. 2002, Yang and Rudolf 2010).

In Chapter 4, I discuss my finding that studies on the spillover of herbivores, pathogens, pollinators, predators, and seed dispersers, from managed to natural systems have been generally under-represented relative to those examining flow in the opposite direction. In particular, the potential positive effects of managed habitats for adjacent natural systems in fragmented landscapes remain largely unstudied. The conclusions of this section help identify future directions for research on food web dynamics of fragmented landscapes. Land-use intensification at small and large scales over the last century is the major driver of global environmental change in terrestrial ecosystems (Sala et al. 2000). Intensification of land use causes landscape fragmentation leading to further losses in biodiversity and associated functions (Vitousek et al. 1997, Tilman et al. 2001). As humans continue to modify their landscapes the likelihood and size of any spillover effect will only increase. Empirical studies are now crucial to provide quantitative evidence in each focal group as well as across disciplines to fully understand the effects of this phenomenon on natural communities.

Although indirect interactions are being looked at more frequently across systems, they are still likely underestimated and/or overlooked by ecologists. In this dissertation, evidence from the main section on leafminer direct and indirect competition shows the importance of indirect interactions (apparent competition) on community structure. In Chapters 2 and 3, if I had looked only at the herbivores I would have wrongly concluded that the two species were not influencing one another. In the last section of this dissertation I conclude that although there is evidence to suggest that functional movement across managed- natural system border is likely, very few studies have actually measured it. But, here (Chapter 4), I am making the case for direct effects of functional movement; I believe that the indirect effects of functional spillover (i.e. apparent competition, apparent mutualisms) are also likely to play a large role in community structure. It may be that it is the very nature of indirect interactions which makes it so difficult to detect them. Such inherent factors would include increases in the needed scale of the examination beyond the planned physical or temporal scope of the study. This dissertation shows that the drivers of community structure are often cryptic, operating over large spatial scales, and at high levels of community complexity. The study of indirect interactions is a key area for further research and, although challenging, will provide greater insight into how natural communities are structured

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