TRITROPHIC INVESTIGATION OF THE EVOLUTION OF HOST PLANT USE IN AN INSECT HERBIVORE: A CASE STUDY WITH A LEAFMINER (*AMAUROMYZA FLAVIFRONS*)

by

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Abstract

Herbivorous insects are characterized by a great diversity of host-plant associations, yet the processes driving host range evolution are not fully understood. In my dissertation, I investigated ecological factors influencing the host plant use in an introduced leafmining fly, *Amauromyza flavifrons,* from tritrophic perspectives.

Amauromyza flavifrons has two host plants, *Silene latifolia* and *Saponaria officinalis* in the study locality. When given a choice female flies prefer to oviposit on *Si. latifolia* over *Sa. officinalis*. Consistent with the preference-performance hypothesis, larval survival was greater on *Si. latifolia* than on *Sa. officinalis* in the absence of natural enemies, indicating that *Si. latifolia* is a higher quality host for larval development.

Parasitism was also lower in *Si. latifolia* than *Sa. officinalis* patches, supporting the enemy-free space (EFS) hypothesis. Ephemeral patches of *Si. latifolia* provided *A. flavifrons* with EFS probably because parasitoid populations could not accumulate, since *A. flavifrons* population crashes in the middle of the summer. Parasitism was not random, but selectively removed leafminers with prolonged larval development period. Thus,parasitism is likely to play a significant role in shaping the evolution of life history traits, as well as oviposition preference of *A. flavifrons*.

Despite higher mortality on *Sa. officinalis*, the density of *A. flavifrons* in the field was higher in *Sa. officinalis* than *Si. latifolia* patches. *Sa. officinalis* formed denser and seasonally more stable patches than *Si. latifolia*, suggesting that females searching for

oviposition sites may locate *Sa. officinalis* patches more easily than *Si. latifolia* patches.

Amauromyza flavifrons population as a whole continues to use both *Si. latifolia* and *Sa. officinalis* because specialization on either host may be less adaptive. I hypothesized that, although *Si. latifolia* is a better host for larval survival, females frequently accept *Sa. officinalis* due to its ease of host location. The pattern of host plant use in *A. flavifrons* is, therefore, shaped by overall trade-offs in fitness functions driven by multiple ecological forces.

Chapter 1

Introduction

Herbivorous insects are extremely species-rich, making up more than a quarter of all described species on earth (May 1990). Specialization on a diverse plant resource is a likely mechanism for this rapid divergence (Jaenike 1990). Specialized populations may become reproductively isolated if the host species occur in different geographic regions (Peterson & Denno 1998), or if insects mate assortatively on host plant species (Hawthorne & Via 2001, Carroll & Boyd 1992, Feder et al 1988). Although extreme specialization is thought to be an evolutionary 'dead end' (Kelley & Farrell 2000), host expansion can re-fuel the speciation process by providing necessary variation on which populations can become further specialized (Janz et al 2006). Thus, the processes of diversification in herbivorous insects may be driven by host expansion and specialization repeated over evolutionary timescales (Janz et al 2006).

What processes, then, drive the evolution of host range in herbivorous insects? Host range is primarily constrained by the ability of insects to detoxify plant defense chemicals, as herbivores generally use host plant species that are taxonomically and/or chemically similar (Ehrlich & Raven 1964, Jaenike 1990). The realized host range of the insect herbivore in a given locality, however, may be much narrower, due to ecological interactions with their surrounding environment (Futuyma & Moreno 1988).

Several factors have been proposed to influence the pattern of host plant use in herbivorous insects (Fig. 1.1). First, the preference-performance hypothesis (PPH) predicts that females should prefer to oviposit on host species that are chemically and nutritionally suitable for larval development and survival (Thompson 1988). Second, females might oviposit on more abundant and persistent hosts because their host searching efficiency increases with host availability (Mayhew 1997). Third, intra-specific competition could reduce larval performance on densely populated hosts, thereby favoring host range expansion to include less populated host species (Rausher 1986). Fourth, the enemy-free space (EFS) hypothesis predicts that females should preferentially oviposit on host species on which larvae can better escape from predation or parasitism (Bernays & Graham 1988). Finally, female oviposition preference might be driven to maximize adult performance (i.e. longevity and egg production) rather than offspring performance (Scheirs & De Bruyn 2002).

The aim of this dissertation was to investigate multiple ecological processes influencing the evolution of diet breadth in herbivorous insects. Using a tritrophic system involving host plants (*Silene latifolia* and *Saponaria officinalis*: Caryophyllaceae), the leafminer (*Amauromyza flavifrons*: Agromyzidae), and parasitoids of *A. flavifrons*, I tested three major hypotheses, including preference-performance, host plant availability, and enemy-free space hypotheses, which might explain the pattern of host plant use in *A. flavifrons*.

Amauromyza flavifrons is an oligophagous leafminer that feeds on multiple genera in the family Caryophyllaceae. It represents an ideal system for testing hypotheses on diet breadth evolution for several reasons. First, the leafmining habit allows accurate measurements of host use pattern and the performance of individual larvae (e.g. survival, parasitism, larval development, etc) because larvae are confined within a single mine

(Koricheva & Haukioja 1994). Their fates in the field are easily estimated by rearing field collected larvae in the laboratory. Second, the ease of maintaining a laboratory population makes it suitable for laboratory experiments, which can complement field observations. Third, *A. flavifrons* system in North America comprises a relatively simple food web because *A. flavifrons* is essentially the only herbivore on their host plants (Blair & Wolfe 2004, Uesugi, personal observation). Both *A. flavifrons* and their host plant species were introduced from Europe, leaving behind other specialist herbivores. Yet, *A. flavifrons* is heavily attacked by multiple species of parasitic wasps, which may or may not be also introduced, making the test of enemy-free space hypothesis highly relevant. Finally, although the ecological interactions within the introduced range are relatively new, *A. flavifrons* population has been in this study area for approximately 100 generations (3-4 generations per year), suggesting that the population may have become adapted to the local environment. The distinct leaf mines belonging to *A. flavifrons* appear on the herbarium specimens of *Si. latifolia* and *Sa. officinalis* from the early 1980's in Michigan (Uesugi, personal observations), suggesting that *A. flavifrons* population has been established by then.

Using this system, I first tested the preference-performance hypothesis (PPH), predicting that females should prefer host plants that are more suitable for larval survival and development (Chapter 2). Because *A. flavifrons* females must make oviposition choices between host plant species, as well as among individual plants within each host species, PPH was tested at both inter- and intra-specific levels. I expected to obtain stronger support for PPH at the species level because females might be better able to discriminate between species-level host quality differences relative to within-species differences. Although not tested specifically, this chapter also provided evidence that intra-specific competition could negatively affect larval performance.

In Chapter 3, I examined the natural pattern of host plant use by *A. flavifrons* in host patches of *Si. latifolia* and *Sa. officinalis*. The resource concentration hypothesis (Root 1973) predicts that herbivore density would be greater in larger and denser patches because they are more apparent and easier to locate by dispersing females. Females may also stay longer in such patches, resulting in the accumulation of herbivores. I applied this hypothesis to predict that host use would be enhanced for the host plant species that form larger, denser and temporally stable patches.

The enemy-free space (EFS) hypothesis (Bernays & Graham 1988) was tested in Chapter 4 by comparing the levels of parasitism on *A. flavifrons* feeding on *Si. latifolia* and *Sa. officinalis* plants. Variation in parasitism is often attributed to the differences in plant chemical, nutritional, or morphological traits between host plant species (Feder 1995, Freese 1995, Gruenhagen and Perring 2001, Mira and Bernays 2002, Oppenheim and Gould 2002, Koller et al 2007, Obermaier et al 2008). However, when host species grow in distinct patches, spatial heterogeneity in abundance of parasitoids may further modify the benefit of EFS. I investigated the relative importance of plant characteristics and spatial heterogeneity in providing EFS. This was done by testing the EFS hypothesis at two spatial scales: in a common garden where both host species were interspersed, and in natural patches distributed across the landscape where host species grew separately. I predicted spatial heterogeneity to confer EFS because host species differ in patch characteristics, which could result in parasitoid abundance variation among patches.

Previous studies of EFS have considered only the number of individuals killed by natural enemies, ignoring which individuals are being eliminated (Berdegue et al 1996, Murphy 2004). In Chapter 5, I examined whether parasitoids could impose strong selection pressure on the life history traits of *A. flavifrons* that facilitate enemy avoidance. Selection on life history traits was investigated by estimating selection gradients on four traits—development time, adult body size, mine size and host plant quality—that were expected to influence parasitoids' prey searching behavior.

Together, this research attempts to provide a comprehensive examination of multiple ecological forces driving the evolution of diet breadth in a local population of *A. flavifrons*. By coupling field observations with laboratory and field experiments, this study also attempts to explore the mechanisms responsible for generating such selective forces.

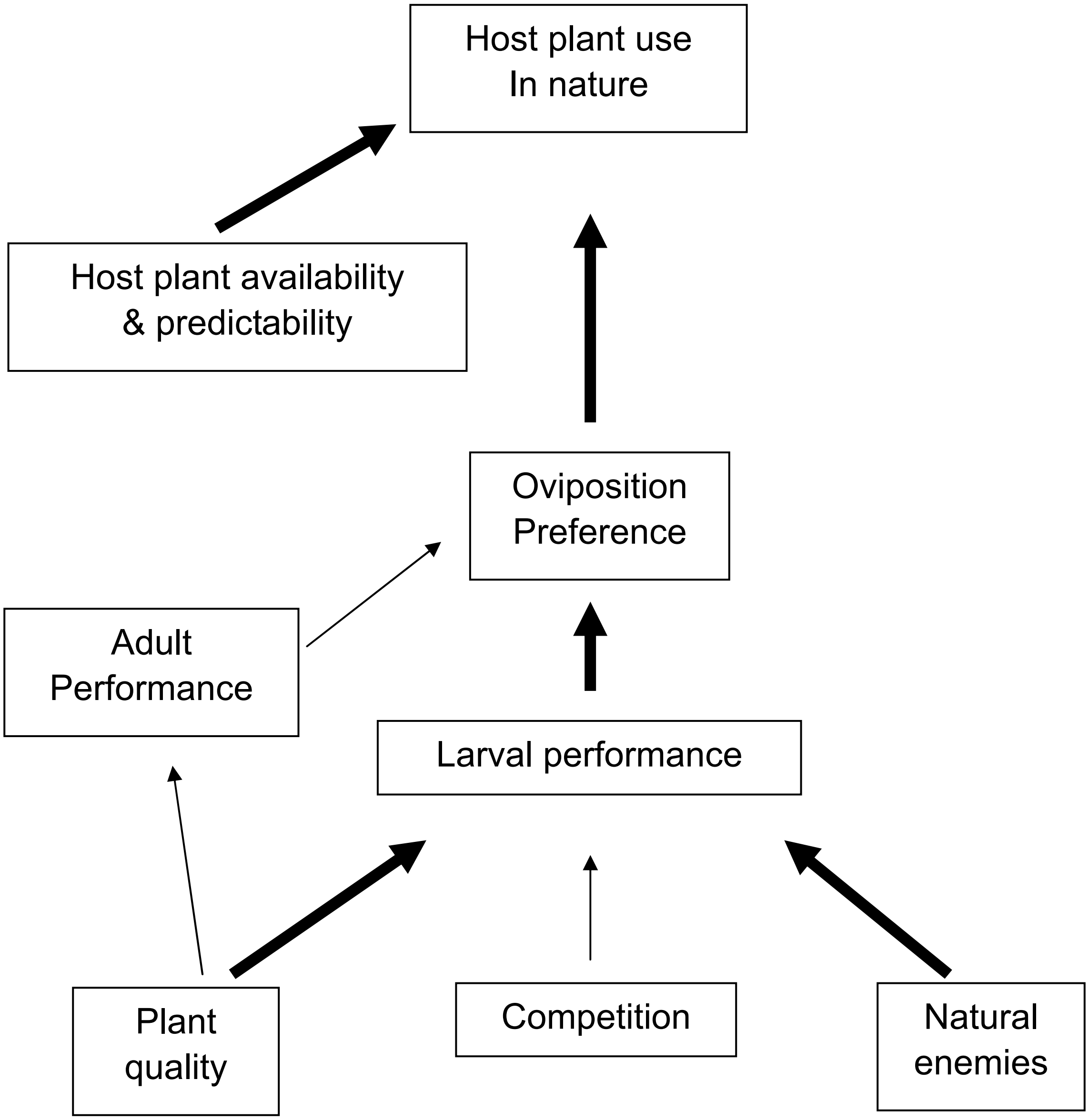


Figure 1.1: Proposed ecological factors influencing the pattern of host plant use by herbivorous insects. Host plant use is determined by local availability and predictability of the host plant species, as well as the oviposition preference of the females. Female preference, in turn, reflects the performance of their offspring, which is affected by plant quality as food, intra-specific competition, and the presence of natural enemies. Plant quality also affects oviposition preference via adult performance. Thick arrows indicate processes examined specifically in this study.

Chapter 2

Plant quality affects oviposition preference and larval performance of the leafminer *Amauromyza flavifrons* at the inter- and intra-specific levels

ABSTRACT

The preference-performance hypothesis (PPH) predicts that females should preferentially oviposit on host plants that enhance the performance of their offspring. In generalist insects, females must make such oviposition choices among host plant species, as well as among individual plants within host species. However, the PPH has rarely been tested at both inter- and intra-specific levels for the same herbivore species.

Here, I use *A. flavifrons Amauromyza flavifrons* (Agromyzidae, Diptera) to test the PPH at inter- and intra-specific levels. Oviposition preference and larval performance were measured on two host plant species, *Silene latifolia* and *Saponaria officinalis*, each growing at varying levels of soil nitrogen to generate intra-specific variation in host quality.

At the inter-specific level, oviposition preference favored *Si. latifolia* over *Sa. officinalis*, and this direction of preference was associated with increased larval survival on *Si. latifolia*. At the intra-specific level, preference and performance corresponded weakly on *Si. latifolia,* but were negatively associated on *Sa. officinalis*. Overall, the PPH was best supported at the inter-specific level, suggesting that the evolution of host

discrimination might be shaped by stronger selection at the species level, or that females are better able to discriminate between species-level host quality differences relative to within-species differences.

INTRODUCTION

Selection of oviposition sites by herbivorous insects can have significant consequences for the performance of their offspring. Because plant nutritional and chemical quality is a key determinant of larval performance (Awmack & Leather 2002), the preference-performance hypothesis (PPH) predicts that females should preferentially oviposit on high quality plants that maximize larval growth (Jaenike 1978). The PPH has traditionally been tested at one of two hierarchical levels: 1) at the inter-specific level, by comparing preference and performance among multiple host plant species, or 2) at the intra-specific level, by comparing among individual plants within a single host plant species.

At the inter-specific level, females often exhibit ranked preferences among host species, and in some cases, the rank order corresponds to the larval performance (Videla et al 2006, Travers-Martin 2008). More commonly, mismatches between preference and performance have been observed (Thompson 1988, Mayhew 1997, Courtney & Kibota 1990), potentially because females might prefer low quality hosts if they are more abundant (West & Cunningham 2006), or if they provide enemy-free space (i.e. spatial refuge from predation or parasitism: Bernays & Graham 1998).

Two hypotheses have been proposed to explain host use at the intra-specific level. The plant stress hypothesis (PSH) predicts that females should prefer stressed plants because they have a higher ratio of nutrients to chemical defenses, providing a better condition for larval growth (White 1984). In contrast, the plant vigor hypothesis (PVH) predicts that female preference and larval performance should be greater on vigorous plants because their nutritional quality is greater (Price 1991). Recent meta-analyses have tested these hypotheses. Patterns of larval performance appear to contradict the PSH, with plant stress causing negative effects on larval performance (Huberty & Denno 2004). Support for PVH is more equivocal: herbivore females generally prefer to oviposit on vigorous plants, but larval performance was not consistently higher on such plant hosts (Cornelissen et al 2008). This lack of correlation between preference and performance may occur if females cannot properly assess the host plant suitability for larval development, due to temporal change in plant quality (Gripenberg et al 2008), or if different plant chemicals influence the preference and performance of the insects (Wise et al 2008).

In generalist insects that feed on multiple host species, females must make oviposition choices at both hierarchical levels (Rausher 1983), yet the PPH has rarely been tested for a single herbivore species at both levels simultaneously (but see Koricheva & Haukioja 1994). Koricheva and Haukioja (1994) suggested that the relationship between preference and performance might differ at various levels of investigation if it is shaped by selective pressures that differ in strength. For example, natural selection may favor strong host discrimination at the inter-specific level to minimize the time lost in searching inappropriate host species (Papaj & Rausher 1983). In addition, generalist herbivores may be limited in their information processing ability so that they can discriminate among host species but not among individual plants (Bernays & Funk 1999, Talsma et al 2008). Thus, the PPH may be better supported at the inter-specific level than the intra-specific level.

The degree of correspondence between oviposition preference and larval performance at both inter- and intra-specific levels can be tested with *A. flavifrons*, *Amauromyza flavifrons* (Agromyzidae, Diptera). Leafminers are relevant for testing the

PPH because the oviposition choice of females is likely to strongly influence the performance of their larvae, which are sedentary and unable to move between hosts (Koricheva & Haukioja 1994, Gripenberg 2008). *Amauromyza flavifrons* is particularly appropriate for testing PPH at different hierarchical levels, because it is a generalist herbivore that feeds on multiple species of host plants within the family Caryophyllaceae (Spencer 1990). In the study area, *A. flavifrons* is most commonly found on two host species, *Silene latifolia* and *Saponaria officinalis,* which form separate monospecific patches across the landscape. Consequently, female *A. flavifrons* are confronted with oviposition decisions at both inter-specific (among plant patches) and intra-specific (within a patch) levels.

Here, I test the relationship between oviposition preference and larval performance in a common garden experiment. To test the PPH at both inter- and intra-specific levels, *Si. latifolia* and *Sa. officinalis* plants were grown under low and high nitrogen treatments to inflate within-species variation in plant quality. Oviposition preference was estimated using a natural population of *A. flavifrons* that had recently colonized the experimental plot. To measure the effect of plant quality alone, two measures of larval performance—larval survival and feeding efficiency—were estimated for a subset of host plants within enclosures, which prevented parasitism of *A. flavifrons* larvae. By contrasting patterns of host preference and larval performance among host species and nitrogen treatments, I address three questions:

1) Do females oviposit more frequently on host plant species or individual plants on which larvae perform better?
2) Which plant traits might mediate oviposition choice and larval performance between host species or between individual plants?

3) How do preference-performance relationships differ at inter- and intra-specific levels of oviposition decisions?

METHODS

Study system

Amauromyza flavifrons is an introduced leafminer from Europe. Within the introduced range from southern Michigan, USA, *A. flavifrons* is commonly found on two plant species, *Silene latifolia* and *Saponaria officinalis*, which are also introduced from Europe. Plants in the family Caryophyllaceae are known to contain saponins (Jia et al 2002), a group of secondary chemicals that have distinctive foaming characteristics. Saponins often serve as defensive compounds against many generalist herbivores (Adel 2000, Agerbirk et al 2003, Agrell et al 2004, Golawska 2007), but their effect on saponin specialists, such as *A. flavifrons*, is not well understood.

Field Experiment

The common garden experiment was conducted in July 2007 at Matthaei Botanical Gardens (MBG) in Dixiboro, Michigan (42˚29'N, 83˚66'W). Plants used in the experiment were grown in pots in the greenhouse at MBG two months prior to the experiment. All *Sa. officinalis* plants originated from vegetative growth from a natural patch near MBG, and *Si. latifolia* plants were grown from seeds collected from another patch. For both host species, individual plants were divided into low and high nitrogen treatments to artificially inflate variation in plant quality within each species. The low and high nitrogen treatments, respectively, received 5 ml of 0.009 mol and 1.17 mol ammonium nitrate weekly.

In early July, these plants were exposed to laboratory-reared females for oviposition. Six potted plants of the same species and nitrogen treatment were placed in a plastic cage where eight 3 to 4 day-old mated females were released for 6 hrs to allow oviposition. Plants were then kept in the greenhouse under mesh cover for four days before they were exposed in the field. This was done so that larger experimental larvae could be distinguished from larvae subsequently oviposited by the wild population of *A. flavifrons* (Scheffer 1995). Most leaf mines were initiated by the fifth day and the number of initial mines was counted on each plant. To avoid direct competition among larvae, a single larva per leaf was allowed to develop and the rest were killed by a needle. Half of the plants were bagged with fine mesh (caged treatment) to examine larval performance in the absence of natural enemies. The other half was exposed to the natural population of leafmining fly (exposed treatment) to examine the mean oviposition preference of the females in the population.

A total of 83 *Sa. officinalis* (16 high nitrogen/caged, 17 low nitrogen/caged, 24 high nitrogen/exposed, 26 low nitrogen/exposed) and 69 *Si. latifolia* (13 high nitrogen/caged, 17 low nitrogen/caged, 17 high nitrogen/exposed, 22 low nitrogen/exposed) plants were placed in an open field under shade cloth at MBG in a completely randomized design. The shade cloth shielded plants from direct sunlight and created a homogeneous environment between caged and exposed treatments. Ambient temperature marginally increased in the caged relative to the exposed treatment (mean difference = 0.3 ˚C, t=2.2, P=0.35), and no differences in plant quality measures, including leaf C: N ratio, water content, specific leaf area (SLA), and saponin concentrations were observed between caging treatments (*Si. latifolia*: $t < 1.5$, $P > 0.14$, *Sa. officinalis*: $t < 1.2$, $P > 0.24$). Thus, environments for larval development were approximately equivalent between caged and exposed plants of the same species and nitrogen treatments.

All leaves were checked daily for leafminer development. Each leaf containing a larva at the last stage of development was individually collected in a Petri dish, where larva pupated. Collected leaves were brought to the laboratory and immediately photographed to estimate mine size at the time of leaf collection, which was used as an approximation of the amount of food resources consumed (Low 2009). Adobe Photoshop software was used to calculate mine area which served as the proxy of mine size.

Larvae pupated within Petri dishes and were reared to adults at room temperature (25˚C) in the laboratory. Days from oviposition to pupation was defined as larval development time. Body size of the eclosed adult fly was estimated by measurements of thorax length, which was calculated with ImagePro program (Media Cybernetics Inc.), using the margin of the pronotum and scutelum as landmarks (Partridge & Fowler 1993). After all experimental larvae had pupated, the remaining leaves were checked for newly emerged mines a under dissecting scope to estimate the frequency of oviposition by the wild population of female flies.

Plant quality analysis

To examine how plant quality influences larval performance and oviposition preference, the following traits were measured for each plant: C: N ratio, water content, specific leaf area (SLA), and saponin concentration. To estimate the mean water content and SLA of each plant, I randomly collected five fully expanded leaves from the upper half of each plant, scanned individual leaves to calculate the leaf area, and measured wet and dry weights. The remainder of un-mined leaves were dried in a 50 ˚C oven and powdered to use for the subsequent chemical analyses.

Leaf C: N ratio was measured using a Perkins Elmer CNO analyzer. The presence of saponins in leaf samples were first qualitatively tested using the distinct foaming property of saponins (Bazzaz et al 1997). In a test tube, 20 mg of dried and powdered leaf sample was added with 1 ml of distilled water, and shaken vigorously for 30 seconds. The height of foam was measured after 10 min. This qualitative analysis resulted in characteristic foaming in *Sa. officinalis* samples, but very little in *Si. latifolia*. Thus, a quantification of saponins was conducted solely for *Sa. officinalis*.

The majority of saponins in *Sa. officinalis* have quillaic acid as an aglycone (Jia et al 1998, Rochd et al 2004). Thus, the concentration of quillaic acid saponins was measured here. Powdered leaf samples (200mg) were extracted with MeOH, and hydrolyzed with 2 ml of HCl at 80 ˚C for 8 hours to yield quillaic acid. The solution was neutralized by adding 1 mol KOH, and extracted with EtOAc (5 ml x 3). Dried compounds were re-suspended in MeOH containing the cardenolide digitoxin as an internal standard. Quillaic acid concentrations were estimated by reverse-phase high-performance liquid chromatography (HPLC) on a Waters Acquity UPLC with mass spectrometer (Waters Corporation, Milford, MA, USA), following methods in de Roode et al (2008). Saponin extract from *Quillaja saponaria* (Sigma-Aldrich) was used to generate standard curves relating the concentration of saponins and quillaic acid. Five concentrations of saponin extract (ranging from 1 to 8 mg/ml HCl) were hydrolyzed and analyzed with HPLC as described above. Using the standard curves, I estimated the amount of quillaic acid saponins in the leaf samples per gram of dry weight.

Data Analysis

Oviposition frequency was estimated on each exposed plant as the number of new mines that appeared after plants were brought to the field to distinguish from experimental ones. Although the number of eggs were not counted directly, the mine counts are likely to reflect the number of eggs oviposited, since leafminer eggs hatch successfully even on plant species that are toxic for larval development (*Beta vulgaris*: Uesugi 2008). All mines including very small ones were detected under the dissecting scope, thus this method of assessing oviposition frequency is believed to be accurate. Because plants differed in available leaf areas for oviposition, oviposition frequency was expressed as the expected number of mines per 1000 cm^2 leaf area. Hereafter, I define this measure as ‘oviposition preference’.

Two measures of larval performance, larval survival rate and feeding efficiency of surviving individuals, were estimated on caged plants to determine the adverse effect of plant quality. Only plants from the caged treatment were used, because larvae on exposed plants were severely attacked by parasitoids, which can affect survival rate (see Chapter 3) as well as efficiency through non-random parasitism (see Chapter 4). The survival rate was estimated per plant, and calculated as the proportion of experimentally initiated larvae that survived to the adult stage. Feeding efficiency was calculated as

$$\text{Efficiency} = \frac{\text{thorax length (mm)}}{(\text{larval development time (days)})(\text{mine size }(\mathrm{cm}^2))}.$$

This variable estimates the final body size achieved by an individual controlling for the time spent feeding and the amount of resource consumed. Although this measure of efficiency differs from conventional measure of leaf-use efficiency in leafminers (calculated as larval mass / frass mass: Koricheva & Haukioja 1994), it incorporates multiple aspects of larval performance, and thus simplifies a measure of resource use in *A.*

flavifrons. Feeding efficiency was calculated for each surviving individual and averaged for each plant. Since feeding efficiency did not differ between females and males (t = 0.76, P = 0.45), both sexes were pooled to calculate the means.

To test the PPH at the inter-specific level, oviposition preference, larval survival and feeding efficiency were compared between host plant species after controlling for the effect of soil nitrogen level (nitrogen treatment) and the density of experimental larvae. The density was included in the analyses because it varied among individual plants regardless of plant species and nitrogen treatments, and because the presence of other larvae on the plant might affect both oviposition behavior and larval performance.

Oviposition frequency was examined in exposed plants using a Generalized Linear Model (GLM) with Poisson distribution. Survival was tested in caged plants using a GLM with binomial distribution. Feeding efficiency was similarly tested for caged plants, but was analyzed using a General Linear Model (LM) because the variable was normally distributed. In all analyses, host plant species, nitrogen treatment and density were modeled as fixed factors. The measures of plant traits were compared between the host species and nitrogen treatments using a LM. To assess whether the host species were similarly affected by nitrogen treatments, species x nitrogen interactions were also included.

To examine how *A. flavifrons* respond to within-species variation in plant traits, I examined oviposition, survival and efficiency separately for each host species. Because plant traits (C:N ratio, SLA, water content) were expected to be correlated with each other, Principal Component Analysis (PCA) was used to obtain independent axes describing the largest variability among plant samples. PCA was conducted separately for the host species, but caged and exposed plants within each species combined. This method provided principal component axes shared between the exposed and caged plants, so that their effects on the preference and performance could be compared indirectly. The scores of the first and the second principal components (PC1 and PC2) were then used in multiple regression analysis as predictors of the oviposition preference, survival and efficiency. As in the between-species comparisons, the density of experimental larvae was also included as predictors, since the presence of other larvae on a plant may alter plant quality due to induced responses (Karban & Baldwin 1997). In *Sa. officinalis*, quillaic acid saponin concentration was also included in the model. The oviposition frequency was analyzed with a GLM with Poisson distribution, the survival was tested with a GLM with binomial distribution, and the efficiency was tested with a LM. All analyses were conducted in R version 2.6.1 (The R Foundation for Statistical Computing).

RESULTS

Inter-specific comparisons

Oviposition preference was 301 % higher on *Si. latifolia* than on *Sa. officinalis* when nitrogen treatment and density were held constant (Table 2.1, Fig. 2.1a), suggesting a strong preference for the former host species. A significant, but smaller effect of nitrogen treatment (30 % increase on high compared to low nitrogen treatment) was also observed. Oviposition frequency was not affected by the density of experimental larvae.

Larvae also survived better on *Si. latifolia* than on *Sa. officinalis* (Table 2.1, Fig. 2.1b). The probability of survival was predicted to be 0.73 and 0.56 on *Si. latifolia* and *Sa. officinalis*, respectively, when nitrogen treatment and density were held constant. Survival was also strongly influenced by the nitrogen treatment and larval density (Table 2.1), suggesting that the conditions in which plants grow could obscure the effect of host plant species differences. Feeding efficiency did not differ between host plant species, but marginally increased in the high nitrogen treatment, and significantly decreased with larval density (Table 2.1, Fig. 2.1c).

Plant traits varied between host species and between nitrogen treatments (Table 2.2, Fig. 2.2). Leaf C: N ratio and water content were greater in *Sa. officinalis* than in *Si. latifolia*, but the nitrogen treatments generated greater variation within each species, resulting in a large overlap between the host species (Fig 2.2a,c). Specific leaf area (SLA) was greater in *Si. latifolia* than *Sa. officinalis*, indicating that *Si. latifolia* has thinner leaves (Fig 2.2b). The high nitrogen treatment increased SLA in both species, but the effect of nitrogen treatment was smaller compared to the effect of host species. Finally, the presence of saponins was detected in *Sa. officinalis* from the distinct foaming in the aqueous solution, but only marginal foaming was detected in *Si. latifolia* (Fig. 2.2d). The foam height did not differ between nitrogen treatments, but the concentration of quillaic acid saponins measured in *Sa. officinalis* were greater in the low nitrogen treatment than the high nitrogen treatment (Fig. 2.2e).

Intra-specific comparisons

The principal component analysis for *Si. latifolia* plants produced the first and second axes ($PC1_{sl}$ and $PC2_{sl}$) that accounted for 63.0 and 21.2 % of the trait variability. $PC1_{sl}$ was positively associated with C: N ratio and water content, and negatively associated with SLA. $PC2_{sl}$ was negatively associated C: N ratio and SLA (Table 2.3, Fig. 2.3a). $PC1_{sl}$ had slightly negative effects on oviposition preference, larval survival, and feeding efficiency, but none of the effects were significant (Table 2.4, Fig.2.4a). $PC2_{sl}$ had positive effects on all leafminer parameters, but only the efficiency was marginally significant. The density of experimental larvae had negative effect on all, and the effect was significant on feeding efficiency.

In *Sa. officinalis*, the first and second axes ($PC1_{so}$ and $PC2_{so}$) accounted for 74.7 and 17.3 % of the trait variability. Similar to *Si. latifolia*, $PC1_{so}$ was positively associated with C: N ratio and water content, and negatively associated with SLA. $PC2_{so}$ was negatively associated with water content and SLA in *Sa. officinalis* (Table 2.3, Fig. 2.3b). $PC1_{so}$ had significantly negative effect on oviposition frequency, but positive effect on survival (Table 2.5, Fig 2.4b). $PC2_{so}$ had non-significant positive effects on oviposition and efficiency, but had significantly negative effect on survival. Larval density increased oviposition preference and reduced survival rate and efficiency, but the effect was significant only for the survival. Quillaic acid saponins affected oviposition negatively, and survival and efficiency positively, but these effects were not significant.

DISCUSSION

Inter-specific comparisons

The preference-performance hypothesis (PPH: Jaenike 1978) predicts that females should preferentially oviposit on host plants that enhance offspring performance. At the inter-specific level, PPH was supported, since females oviposited more frequently on *Si. latifolia*, on which larvae survived better, compared to *Sa. officinalis*. There was no correspondence between oviposition preference and larval feeding efficiency, which did not differ between host plant species. Considering that mortality due to plant defense can be as high as 40 % on *Sa. officinalis*, strong discrimination against the host seems adaptive.

While the nitrogen treatment was associated with oviposition preferences, plant species identity was the most important predictor. This indicates that *Si. latifolia* is a preferred host to *Sa. officinalis* across a wide range of plant growth conditions, and that host discrimination in *A. flavifrons* occurs primarily at the species level. Such hierarchical preferences have been previously observed in other generalist insects (Courtney & Kibota 1990).

Larval survival was also strongly influenced by host variability within species, including soil nitrogen level and larval density. Consequently, while *Si. latifolia* will generally promote larval survival, differences in larval survival between host species can potentially be obscured by intra-specific plant variation. However, such an effect might be experimentally exaggerated, as soil nitrogen levels were artificially manipulated in this study. Field collected plants of *Si. latifolia* and *Sa. officinalis* had similar leaf C: N ratios as experimental plants in the high nitrogen treatment (see Fig. 4.1, Chapter 4), suggesting that survival difference between host species should generally be greater in natural conditions (see Fig.2.1b).

Host species differed in saponin concentrations and specific leaf area (SLA), variables that could potentially be used by *A. flavifrons* females as cues for host discrimination. The concentration of total saponins (or foam height) was much greater in *Sa. officinalis* than in *Si. latifolia*, which raises the possibility that saponins may function as a deterrent for the ovipositing females. Although the effect of saponins on oviposition behavior is currently unclear in herbivorous insects, saponins are known to reduce feeding activities in pea aphids (Golawska 2007), and might represent a deterrent in *A. flavifrons*, where females feed on plant sap prior to oviposition.

High SLA in *Si. latifolia* might also indicate reduced mechanical defense in this host (Agrawal & Fishbein 2008). For example, plant species in the Myrtaceae family are more likely to be mined by leafminers if they have thinner leaves, presumably because thinner leaves have a thinner epidermis layer, which makes it easier for females to ovipuncture (Sinclair & Hughes 2008).

Intra-specific comparisons

On *Si. latifolia*, oviposition preference and larval performance showed a positive association. Multiple regression coefficients typically exhibited similar signs between preference and performance (Fig 2.4a). Although the trend was weak, preference and performance favored nutrient-rich plants (low C: N ratio and water content), which is consistent with the predictions of the plant vigor hypothesis (PVH: Price 1991). However, the plant variables examined here were relatively poor predictors of oviposition preference, suggesting that females do not strongly discriminate among individual *Si. latifolia* plants.

Feeding efficiency on *Si. latifolia* was negatively associated with larval densities on individual plants. Direct larval competition was unlikely to explain this pattern because each leaf contained a single larva, thereby eliminating direct interference or exploitative competition between individuals. However, increased herbivory can potentially induce plant chemical defense or reduce plant quality by triggering a reallocation of nitrogen away from the leaves (Karban & Baldwin 1997). These factors might indirectly influence the growth of larvae on densely populated plants. Despite the negative effect of density, females did not discriminate between plants based on larval density, possibly because *A. flavifrons* females could not detect or respond to this variable.

Oviposition preferences on *Sa. officinalis* did not correspond with larval performance (Fig.2.4b). Instead, females showed a strong preference for high-nitrogen plants, which were detrimental for larval survival. This paradoxical oviposition choice can be explained if female behavior maximizes female performance at the expense of offspring performance (Scheirs & DeBruyn 2002). For example, Scheirs et al (2003) found that females of grass miners, *Chromatomia nigra* and *C. milii*, preferentially fed, oviposited, and survived longer on host plants with higher protein contents. The larval performance, however, did not correspond with the oviposition choice. Because female *A. flavifrons* feed on plant sap prior to oviposition, the nutritional value of the sap could influence their feeding behavior. Feeding frequency, in turn, can be correlated with the oviposition in females (Scheirs et al 2003, A. Uesugi, unpublished data). Females preferred plants rich in nitrogen, which could fulfill their nutritional requirements for egg production. Low water content was possibly preferred because essential nutrients in the sap were less diluted (Slanky and Weeler 1992). While the present study did not test whether feeding preferences improved female performance, several studies in agromyzid flies suggest that this is a likely possibility (Scheirs et al 2000, 2003, 2004).

The negative effect of leaf nitrogen on larval survival was surprising because nitrogen is an essential nutrient for larval growth (Awmack & Leather 2002). The adverse effect of nitrogen in *Sa. officinalis* suggests that the host is potentially defended by nitrogen-based defense metabolites or plant defensive proteins (Awmack & Leather 2002), which were not measured in this study. The contrasting effects of nitrogen on larval survival between *Sa. officinalis* and *Si. latifolia* suggest that plant species respond differently to nitrogen elevation (Awmack & Leather 2002), which makes it difficult for ovipositing females to predict plant quality suitable for the performance of their offspring (Wise et al 2008).

Saponaria officinalis also responded to herbivory with induced defense (Karban & Baldwin 1997), as evident from the negative effect of density on larval survival. This consequence of "indirect" competition is more severe on *Sa. officinalis* than on *Si. latifolia,* since it not only reduced the larval growth, but also increased mortality. Although the specific compounds increasing the larval mortality are not known, the results suggest that *Sa. officinalis* is chemically better defended against *A. flavifrons* compared to *Si. latifolia*.

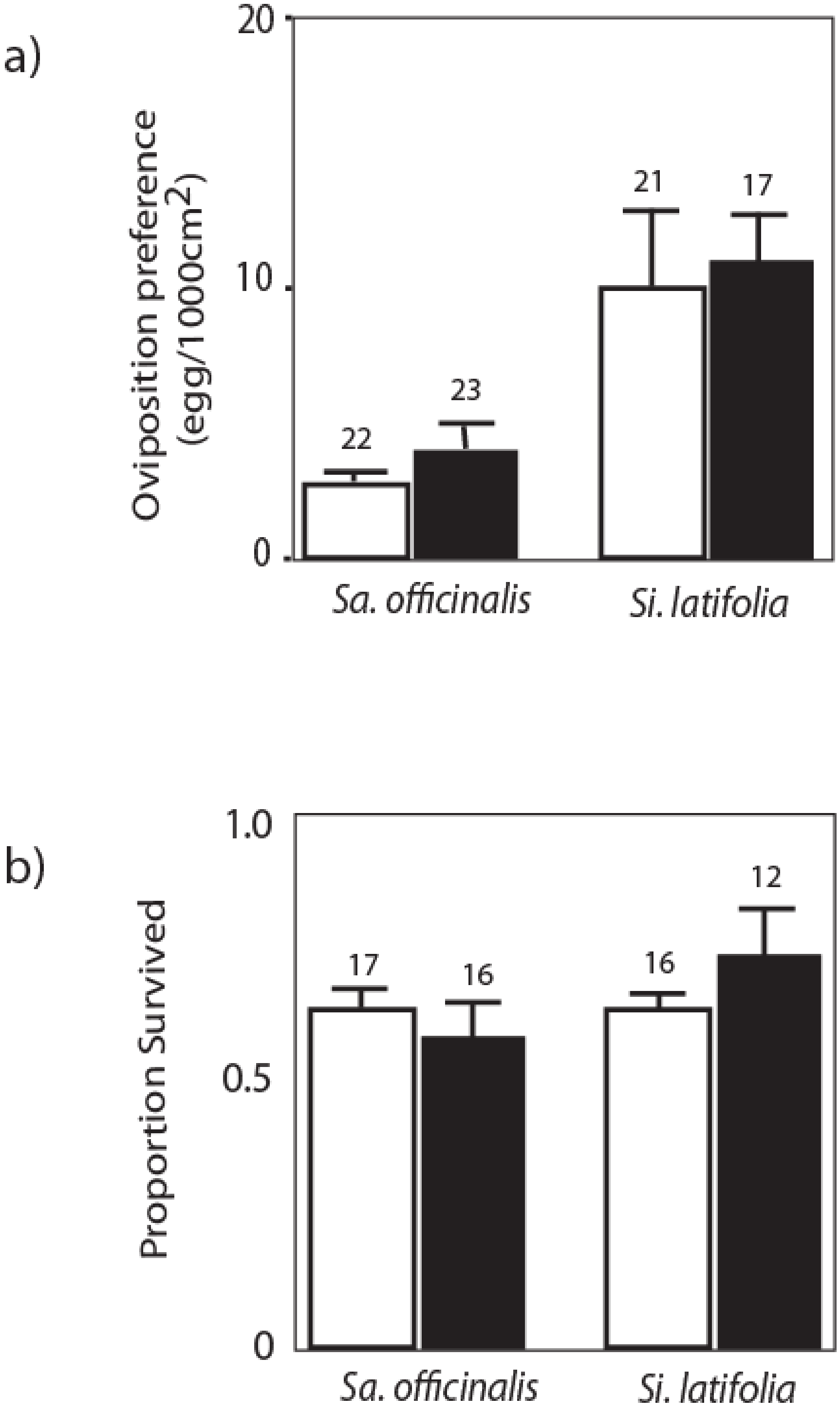
Levels of quillaic acid saponins, which are often used as defense chemicals against generalist herbivores (Adel 2000, Agerbirk et al 2003, Golawski 2007), did not influence patterns of oviposition or larval performance on *Sa. officinalis*. This lack of response suggests that saponins are not effective against herbivores that are adapted to saponins, such as *A. flavifrons* (Travers-Martin & Muller 2008). Alternatively, it is possible that within-species variation in saponins was too small to generate a strong effect on *A. flavifrons*. Saponins exhibited much more pronounced differences between *Si. latifolia* and *Sa. officinalis*, and might have stronger influence on oviposition behavior and larval performance at the inter-specific level. Future studies will be necessary to evaluate this possibility.

Conclusion

The common garden experiment revealed that *A. flavifrons* females preferentially oviposit on *Si. latifolia* relative to *Sa. officinalis*. The inter-specific comparisons showed that this oviposition preference was positively correlated with larval survival, supporting the PPH. At the intra-specific level, the association between preference and performance was weak on *Si. latifolia*, and negative on *Sa. officinalis*. Thus, the PPH was better supported at the inter-specific rather than intra-specific level.

This pattern may reflect neurological constraints on female oviposition behavior (Bernays & Funk 1999). In general, the ability to discriminate between conspecific plants decreases with increases in diet breadth (Janz & Nylin 1997). In generalist insects that feed on multiple host species, females might be capable of making adaptive choices at the host species level, but might not be able to differentiate within-species variation in plant quality due to limited ability to process finer scale information (Bernays & Funk 1999). The lack of strong discrimination among *Si. latifolia* plants, as well as oviposition “mistakes” made on *Sa. officinalis*, might be the result of neurological limitation of these insects.

Natural selection may also favor stronger host discrimination at the species level so that females will not waste their time searching for hosts on poor quality species (Papaj & Rausher 1983). Selection on females to discriminate against *Sa. officinalis* may be exacerbated due to the negative consequence of female choice on larval survival. On *Sa. officinalis,* females chose plants that were detrimental to their offspring, thereby exaggerating survival differences between larvae on the two host species. Thus, preference-performance correlation at the intra-specific level might indirectly influence oviposition preference at the inter-specific level. However, more studies examining the PPH at both inter- and intra-specific levels are necessary to test this hypothesis.


a)
Oviposition preference (egg/1000cm^2)
20
10
0
22
23
21
17
Sa. officinalis
Si. latifolia
b)
Proportion Survived
1.0
0.5
0
17
16
16
12
Sa. officinalis
Si. latifolia

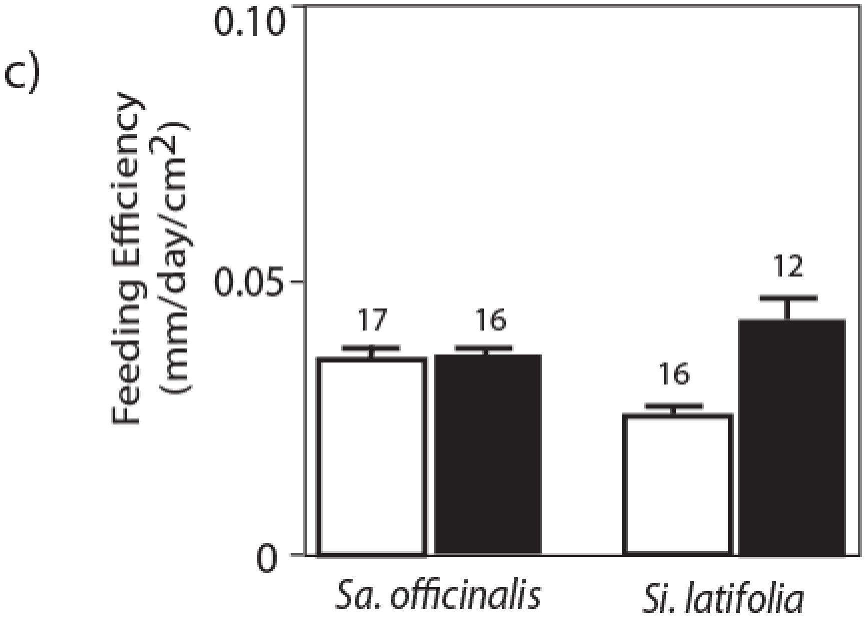
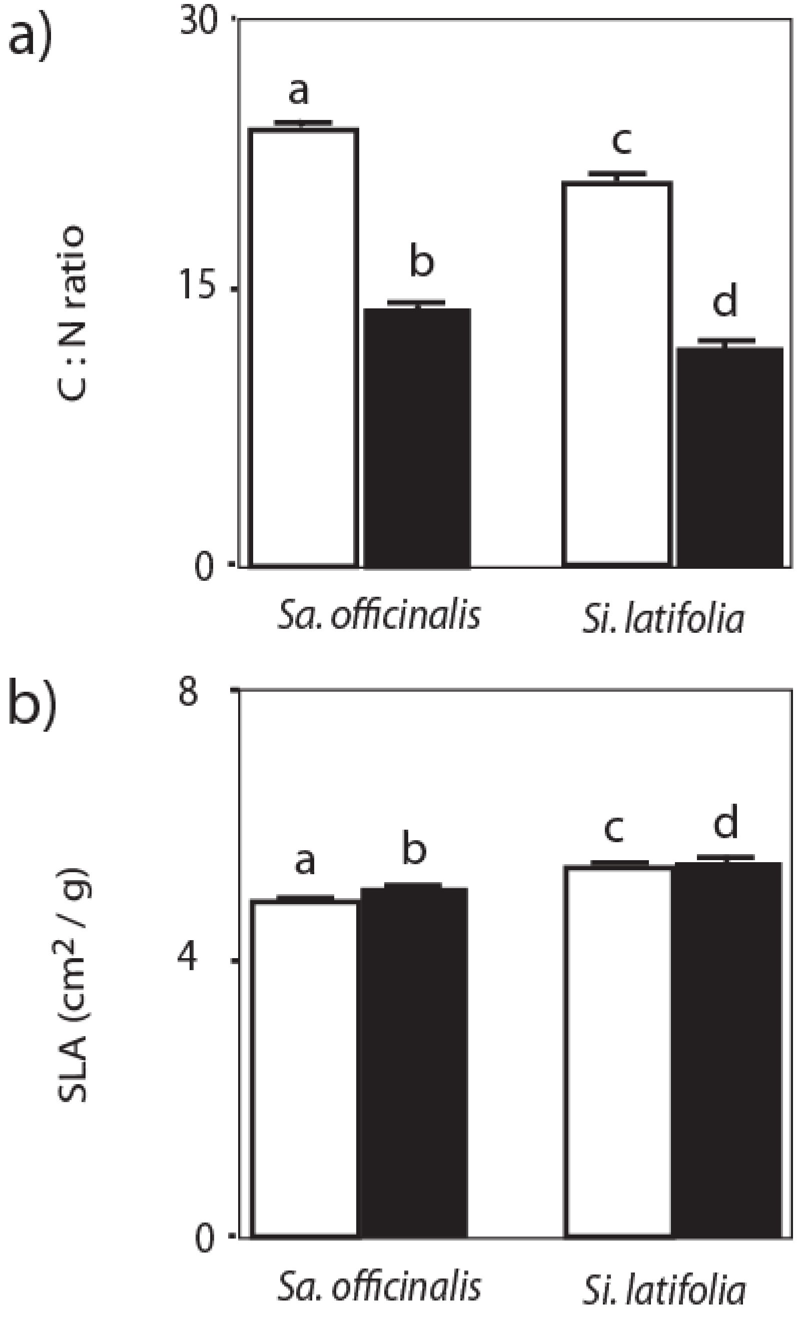
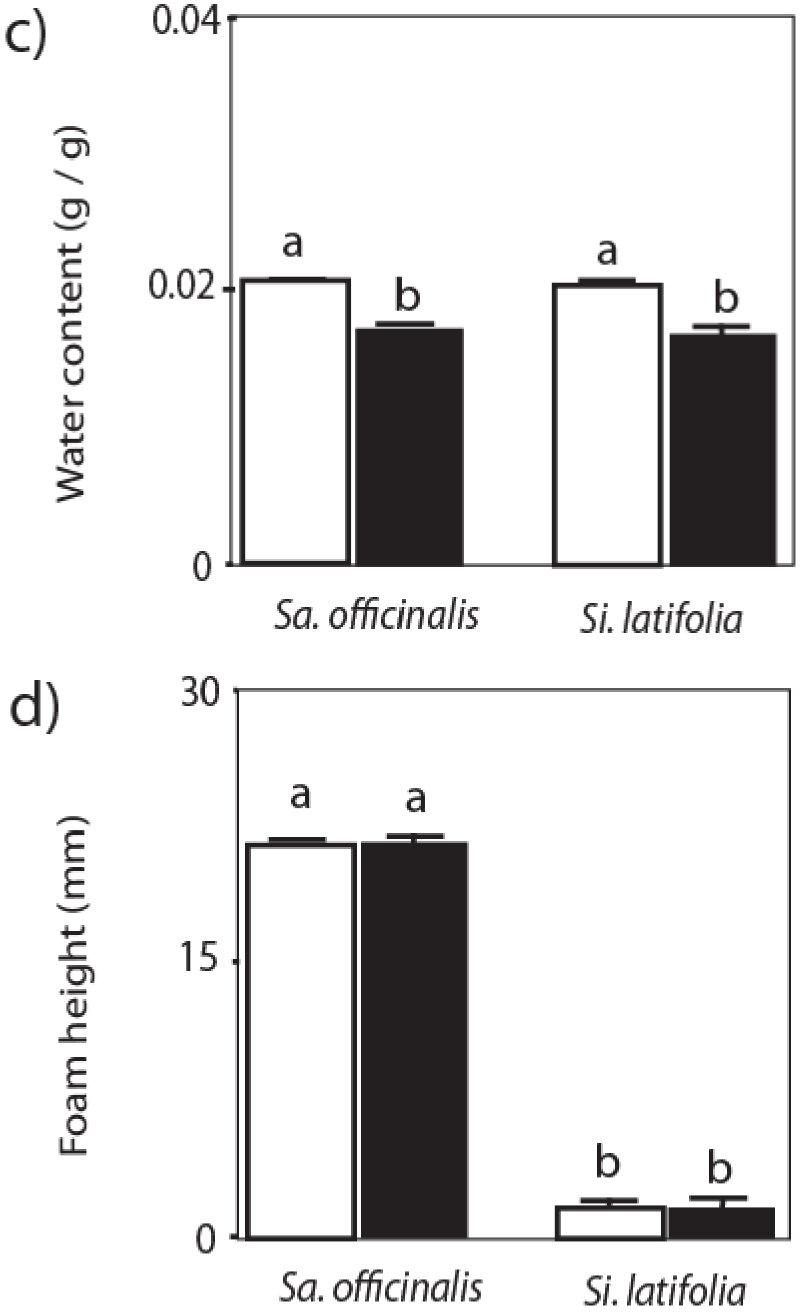


Figure 2.1: Oviposition preference (a), larval survival (b), and feeding efficiency (c) on *Si. latifolia* and *Sa. officinalis.* Open bars indicate plants in the low nitrogen treatment and closed bars indicate plants in the high nitrogen treatment. Error bars represent standard errors. Numbers above bars indicate samples size. See Table 2.1 for the significance of host species and nitrogen treatment effects.



a)
30
15
0
C : N ratio
a
b
c
d
Sa. officinalis
Si. latifolia
b)
8
4
0
SLA (cm^2 / g)
a
b
c
d
Sa. officinalis
Si. latifolia


c)
0.04
0.02
0
Water content (g / g)
a
b
a
b
Sa. officinalis
Si. latifolia
d)
30
15
0
Foam height (mm)
a
a
b
b
Sa. officinalis
Si. latifolia

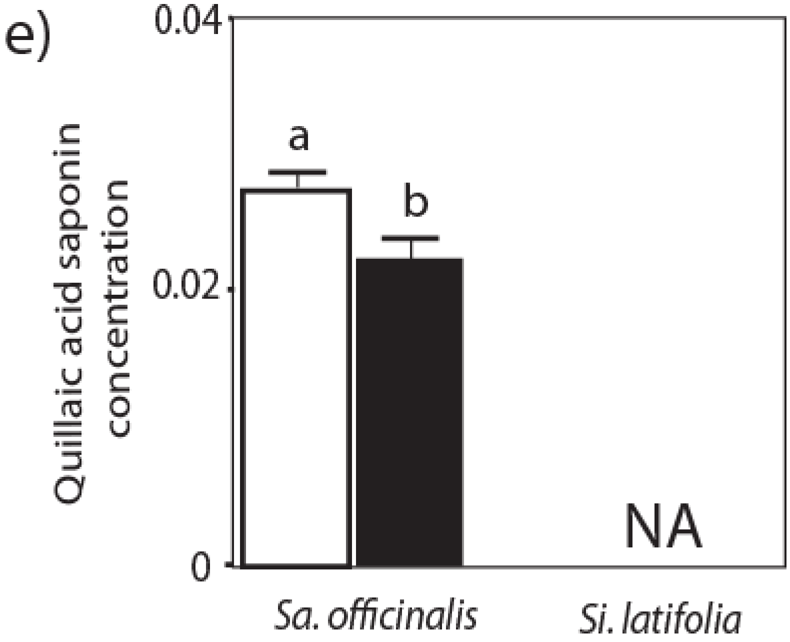


Figure 2.2: Plant trait variations between host species and nitrogen treatments. Open and closed bars indicate low and high nitrogen treatments, respectively. C: N ratio (a), specific leaf area (SLA:b), leaf water content (c), and total saponin concentration (foam height: d) were measured for both host species, and quillaic acid saponin concentration (e) was measured for *Sa. officinalis*. Error bars indicate standard errors (n = 38 in *Sa. officinalis* in both low and high nitrogen treatments, and n = 38 and 29 in *Si. latifolia* for low and high nitrogen treatments). Different letters indicate significant contrasts at $P <$ 0.05.

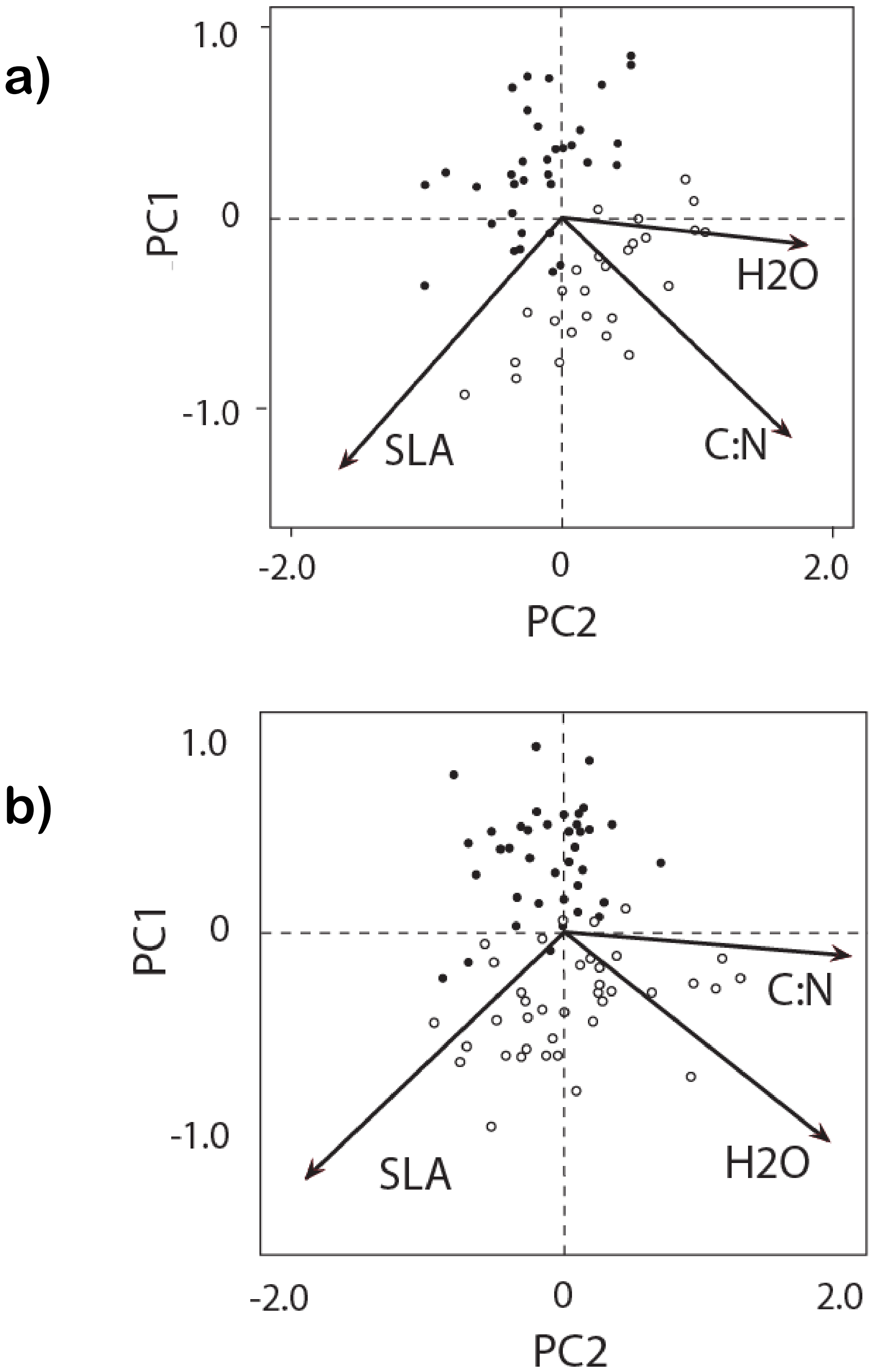


Figure 2.3: PCA plots for *Si. latifolia* (a) and *Sa. officinalis* (b) showing plants in high nitrogen treatment (black dots: n = 29 in *Si. latifolia* and 39 in *Sa. officinalis*) and low nitrogen treatment (open circles: n = 38 in *Si. latifolia* and 39 in *Sa. officinalis*). Plant traits are projected onto the plots as arrows.

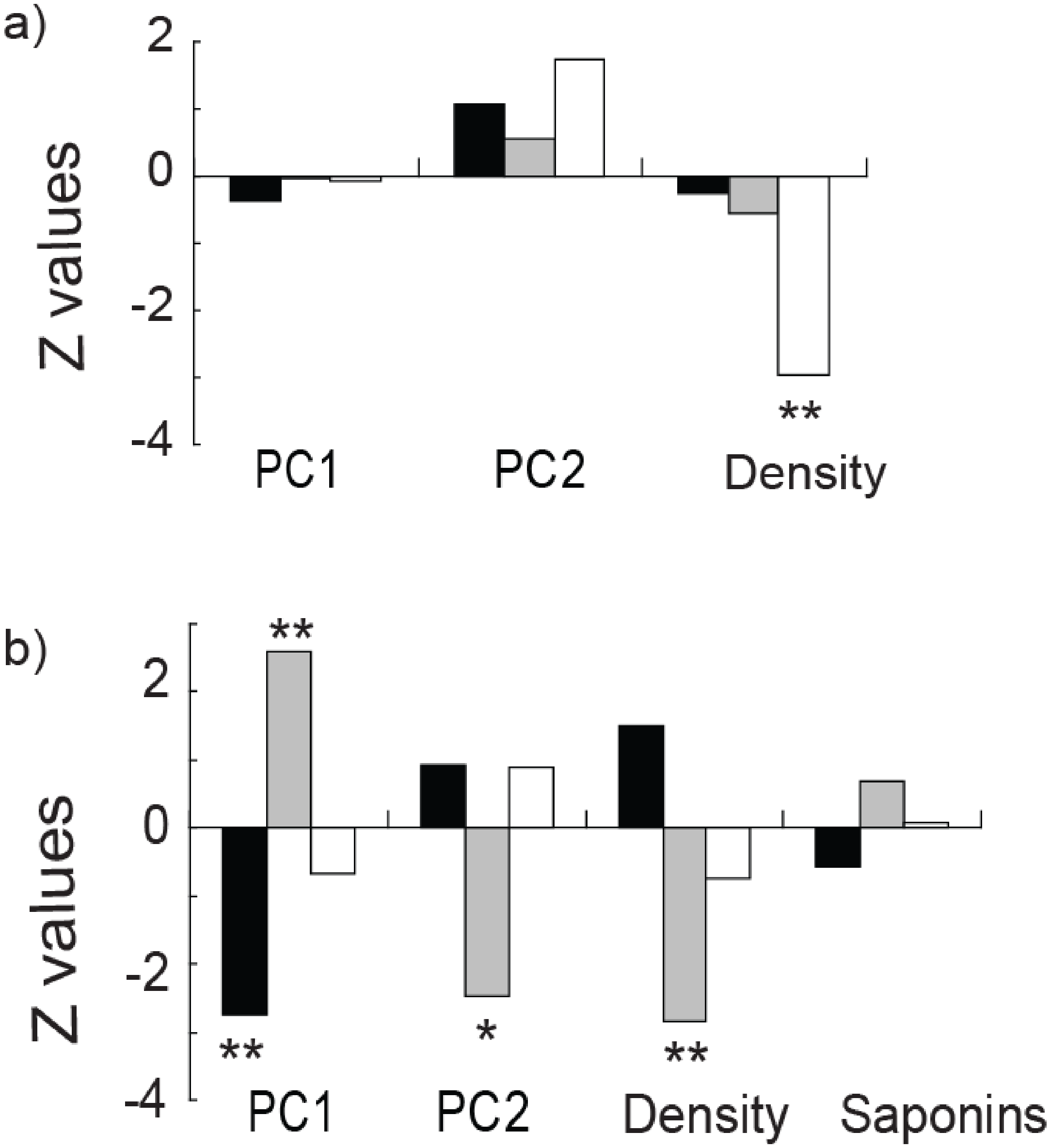


Figure 2.4: Effect size of predictors (PC1, PC2, larvae density and quillaic acid saponin concentration (in *Sa. officinalis*)) in multiple regression analyses. For *Si. latifolia* (a) and *Sa. officinalis* (b), Z values for oviposition preference (closed bars), larval survival (gray bars), and feeding efficiency (open bars) are plotted.

Table 2.1: Results of inter-specific comparisons by ANOVA. Effects of host plant species (Species), nitrogen treatment (N treatment) and larval density on oviposition frequency, larval survival and feeding efficiency.

		Estimate	Std. Error	Z	P
Oviposition					
	Species	**1.11**	**0.10**	**11.23**	**<0.0001**
	N treatment	**0.27**	**0.12**	**2.21**	**0.027**
	density	0.05	0.05	1.01	0.31
Survival					
	Species	**0.75**	**0.21**	**3.61**	**0.0003**
	N treatment	**-0.50**	**0.18**	**-2.85**	**0.004**
	density	**-0.37**	**0.12**	**-3.24**	**0.001**
Efficiency					
	Species	0.00029	0.002	0.15	0.88
	N treatment	0.0042	0.002	1.92	0.06
	density	**-0.0039**	**0.001**	**-4.05**	**0.0002**

Table 2.2: Effects of host plant species and nitrogen treatment on leaf quality—C: N ratios, specific leaf area (SLA), water content, total saponins, and quillaic acid saponins (measured only for *Sa. officinalis*).

plant trait	species		nitrogen		species x nitrogen	
	t	*P*	*t*	*P*	*t*	*P*
C:N ratios	-4.83	**<0.0001**	-16.9	**<0.0001**	0.69	0.49
SLA	13.47	**<0.0001**	4.79	**<0.0001**	-1.40	0.16
water content	-0.58	0.56	-6.08	**<0.0001**	0.094	0.93
total saponins	-17.79	**<0.0001**	0.061	0.951	-0.019	0.99
Qa saponins	-	-	-2.13	**0.041**	-	-

Table 2.3: PCA scores of plant traits in *Si. latifolia* and *Sa. officinalis* for the first and second principal components (PC1 and PC2).

	Si. latifolia		*Sa. officinalis*	
	PC1*sl*	PC2*sl*	PC1*so*	PC2*so*
CN	1.69	-1.13	2.08	-0.127
water	1.81	-0.128	1.91	-1.05
SLA	-1.65	-1.30	-1.84	-1.23

Table 2.4: Results of intra-specific comparisons in *Si. latifolia*. Effects of plant traits (PC1 and PC2) and larval density on oviposition preference, survival and efficiency.

		Estimate	Std.Error	Z	P
Oviposition	PC1	-0.056	0.16	-0.35	0.72
	PC2	0.15	0.14	1.08	0.28
	density	-0.014	0.054	-0.27	0.79
Survival	PC1	-0.013	0.36	-0.038	0.97
	PC2	0.21	0.39	0.54	0.59
	density	-0.088	0.16	-0.55	0.59
Efficiency	PC1	-0.0003	0.004	-0.081	0.94
	PC2	0.007	0.004	1.75	0.093
	density	**-0.005**	**0.0016**	**-2.95**	**0.0073**

Table 2.5: Results of intra-specific comparisons in *Sa. officinalis*. Effects of plant traits (PC1 and PC2), larval density, and quillaic acid saponin concentration on oviposition preference, survival and efficiency.

		Estimate	Std.Error	Z	P
Oviposition	**PC1**	**-0.58**	**0.21**	**-2.74**	**0.0061**
	PC2	0.16	0.17	0.92	0.36
	density	0.19	0.13	1.51	0.13
	saponins	-5.87	10.1	-0.58	0.56
Survival	**PC1**	**0.66**	**0.25**	**2.59**	**0.0096**
	PC2	**-0.75**	**0.30**	**-2.47**	**0.014**
	density	**-0.60**	**0.21**	**-2.86**	**0.0042**
	saponins	7.92	11.5	0.69	0.49
Efficiency	PC1	-0.0016	0.0026	-0.66	0.51
	PC2	0.0028	0.0031	0.89	0.38
	density	-0.0012	0.0016	-0.75	0.46
	saponins	0.0086	0.12	0.071	0.94

Chapter 3

Spatial and temporal heterogeneity in host plant availability influences the pattern of host plant use in the leafminer *Amauromyza flavifrons*

ABSTRACT

Spatial and temporal heterogeneity in resource availability is expected to influence the pattern of host plant use in herbivorous insects because selection favors female behaviors that maximize host searching efficiency. In this study, I investigated whether the characteristics of host plant patches (i.e. size, density and seasonal availability of resources) could influence the host use pattern of the leafminer *Amauromyza flavifrons* between its host plant species, *Saponaria officinalis* and *Silene latifolia.*

Although *Sa. officinalis* is an inferior host to *Si. latifolia*, the density of *A. flavifrons* was consistently higher in patches of *Sa. officinalis* compared to *Si. latifolia*. This pattern may be explained by the resource concentration hypothesis that females can find dense and persistent patches of *Sa. officinalis* more easily and stay longer to oviposit than on the sparse and ephemeral patches of *Si. latifolia*.

INTRODUCTION

The availability of suitable host plants for herbivorous insects often varies spatially and temporally across the landscape, and ovipositing females may modify their host range to maximize their fitness gain (Mayhew 1997). In herbivorous insects, females generally exhibit ranked preference for host plant species according to the plant quality for offspring performance (Jaenike 1978). However, herbivores are often found feeding on low-quality hosts, if preferred hosts are rare and females are limited in host searching time (Levins & MacArthur 1969, Mayhew 1997, West & Cunningham 2002). This is because the cost of searching outweighs the loss of fitness from accepting the low-quality host (Levins & MacArthur 1969). For example, females of *Pieris oleraceae* from areas heavily invaded by garlic mustard were found to readily use the abundant host, on which larvae survived poorly compared to their native host (Keeler & Chew 2008).

In spatially heterogeneous environments where host species grow in distinct patches, host searching efficiency may be influenced by how apparent patches are to the searching females (Root 1973, Kareiva 1983). Previous studies showed higher colonization rates by insects when patch size and/or plant density per patch were greater (Hamback & Englund 2005, Heisswolf et al 2009). This resource concentration hypothesis (Root 1973) also predicts that females should stay longer in larger and denser patches to oviposit, resulting in accumulation of herbivore populations. Although previous studies testing the hypothesis investigated the colonization of a single plant species by monophagous herbivores, patch characteristics could vary among host plant species, and influence the choice between host plant species in generalist insects.

Similarly, temporal variation in host abundance should influence oviposition behavior, such that host species that are more ephemeral should be used less than persistent hosts. For example, in the checkerspot butterfly, *Euphydryas chalcedona*, females frequently used a nutritionally poor host in the field because a superior host was ephemeral (Williams 1983). The pattern of host plant use by herbivores, therefore, may be strongly influenced by the spatial and temporal variation in the relative abundance of host plant species (Mayhew 1997).

In this study, I investigated how the resource availability and seasonal persistence of the host plant species influence the pattern of host plant use in *A. flavifrons* *Amauromyza flavifrons* (Diptera: Agromyzidae). *A. flavifrons* is oligophagous, feeding on multiple host plants within the family Caryophyllaceae (Spencer 1990). In the study area in southeast Michigan, *A. flavifrons* commonly feed on two host species, *Silene latifolia* and *Saponaria officinalis.* In a common garden experiment where both hosts are equally abundant, females prefer to oviposit on *Si. latifolia* to *Sa. officinalis* because *Si. latifolia* is a higher quality host for larval development (see Chapter 2).

Spatial variation in host plant availability is expected to influence the host use of *A. flavifrons* in the field because *Si. latifolia* and *Sa. officinalis* commonly grow in separate patches across the landscape. Because *A. flavifrons* has 3-4 generations a year (A. Uesugi, personal observations), the temporal pattern of host plant availability is also likely to influence host plant use. I predicted that host use would be enhanced for the host species that forms larger, denser and temporally stable patches.

METHODS

Field surveys

Field surveys were conducted from 2006-2008 in Washtenaw Co. Michigan in six and eight patches of *Sa. officinalis* and *Si. latifolia*, respectively. The number of patches that were visited differed between years because some patches disappeared and some were added over the study years. All patches used in this study had only one of the two host species growing. Patches of *Sa. officinalis* were found along road sides, while *Si. latifolia* grew predominantly in the edges of old fields and agricultural fields. Patches were separated at least by 1 km (Fig. 3.1).

In *Sa. officinalis* patches and small *Si. latifolia* patches, the patch size was estimated by directly measuring the length and width of the area covered by the plant species. Direct measurement was not possible in large patches of *Si. latifolia* that extended over agricultural fields, thus the area was estimated using aerial photos (available from www.Google Earth.com). In each patch, shoot density was estimated in early June 2007. Because *Sa. officinalis* patches were much denser than *Si. latifolia* patches, shoot density was estimated using 10 replicated quadrats of 1 m^2 in *Sa. officinalis* patches, and two replicates of 2 m x 5 m transects in *Si. latifolia* patches. The density was calculated as the number of shoots per square meter.

To monitor temporal change in leaf availability, study patches were visited at the beginning of each month from June to August 2007. These visits corresponded approximately to the peak mining activity of each leafminer generation. In each patch, 20 shoots of host plants were randomly selected and the number of healthy, non-senescent leaves was counted. The mean number of leaves per shoot was calculated for the subsequent analysis.

The density of leafminers was estimated per patch per generation from 2006-2008. All patches were visited within three days at the peak of mining activity during each generation. The density was estimated as the number of mines found during 30 minutes of observation time. Along a transect line, I randomly selected a nearest shoot every 50 cm, carefully checked all leaves on the shoot, and collected leaves that contained mines. The time required for checking one leaf was assumed to be consistent among host plant patches and visits within a patch over time because censuses were conducted by a single observer. Mines of all sizes were collected, including ones that larvae had exited. However, empty mines that had turned brown were excluded because they are likely to be old mines made by individuals from previous generations, which would result in overestimation of the density in later seasons.

Data Analyses

Patch sizes and shoot densities were compared between *Sa. officinalis* and *Si. latifolia* patches using the Mann-Whitney test. The non-parametric test was used because these patch characteristics were not normally distributed. Seasonal changes in leaf availability and leafminer density were examined using a Linear Mixed Model. Because not all patches were visited every year, I modeled patch as subject group. Host plant species and the number of generations were entered as fixed factors and year as a random factor.

RESULTS

Patch sizes were marginally greater for *Si. latifolia* than for *Sa. officinalis* ($U = 11$, $P = 0.059$), but *Si. latifolia* patches were variable in size (Fig. 3.2a). Shoot density within a patch was greater in *Sa. officinalis* than in *Si. latifolia* patches ($U = 0$, $P < 0.0001$, Fig.3.2b). The number of leaves per shoot was greater in *Sa. officinalis* than in *Si. latifolia* across three leafminer generations (species effect: $F_{1,13.5} = 131.5$, $P < 0.0001$, Fig. 3.3). No significant effect of generation was detected when the two host species were considered together (generation effect: $F_{1,18.5} = 0.69$, $P = 0.51$). However, the pattern of leaf availability differed between the host plant species. In *Sa. officinalis*, the leaf number increased in July but decreased in August, whereas in *Si. latifolia* the leaf number dropped in July and August. In *Si. latifolia* patches found in agricultural fields, plants themselves disappeared in later generations because the host plant was harvested along with the crop plants. *Si. latifolia* patches in old fields persisted, but most leaves senesced by the beginning of the July generation. This phenological differences between host species resulted in significant host species x generation interactions (species x generation: $F_{1,19.6} = 48.1$, $P < 0.0001$).

The density of *A. flavifrons* fluctuated over time and space (Fig. 3.4). Density was consistently higher in *Sa. officinalis* than in *Si. latifolia* patches throughout the generations (species effect: $F_{1,44} = 137.4$, $P < 0.0001$). The density also changed among generations (generation effect: $F_{1,44} = 3.8$, $P = 0.032$). The density in *Sa. officinalis* increased with generations, but *A. flavifrons* disappeared from *Si. latifolia* patches in July and August generations, resulting in significant species x generation interactions (species x generation: $F_{1,44} = 21.9$, $P < 0.0001$).

DISCUSSION

The density of *A. flavifrons* in natural patches was consistently higher in *Sa. officinalis* than *Si. latifolia* patches. This host use pattern did not agree with the pattern of oviposition preference for *Si. latifolia* over *Sa. officinalis* observed in the common garden experiment (see Chapter 2). Instead, the pattern may be explained by the resource concentration hypothesis (Root 1973), suggesting that females can easily find and stay longer in larger and denser patches. *Sa. officinalis* patches were considerably denser than *Si. latifolia* patches, possibly because *Sa. officinalis* spreads primarily through vegetative growth, whereas *Si. latifolia* spreads mostly by seeds.

Heisswolf et al (2009) found that the density of a leaf beetle *Cassida canaliculata* in patchy environments increased with patch size and host plant density, but the effect of plant density was much stronger. Similarly in *A. flavifrons*, plant density predicted leafminer density better than the patch size, since leafminer density was lower in *Si. latifolia* patches, which tended to be larger than *Sa. officinalis* patches. Visual and chemical cues from the plants may be more concentrated in denser patches and could aid females during host search (Bukovinszky et al 2005).

Moreover, the associational resistance hypothesis (Root 1973) suggests that the presence of non-host species within a patch would make host searching by females more difficult as a result of visual or olfactory interference (Hamback et al 2000, Sholes 2008). While *Sa. officinalis* formed monospecific patches, *Si. latifolia* often grew interspersed among non-host species. The present study could not separate the effect of plant density and the presence of non-host species because dense patches of *Sa. officinalis* necessarily contained fewer non-host species. Nonetheless, these patch characteristics suggest that females could locate *Sa. officinalis* plants more efficiently than *Si. latifolia* plants.

The temporal pattern of resource availability also seems to explain why *A. flavifrons* uses *Sa. officinalis* more frequently than *Si. latifolia* under natural conditions. Seasonal changes in leaf availability indicated that *Sa. officinalis* plants continue to produce new leaves throughout the generations of *A. flavifrons*, and that the host is seasonally predictable. Leafminer density increases in *Sa. officinalis* patches because the population can build up over generations.

In contrast, *Si. latifolia* is an ephemeral host, available to *A. flavifrons* only during the first generation of the year in June. Because *Si. latifolia* senesces in early summer, the first generation of leafminers developing in *Si. latifolia* patches must disperse to *Sa. officinalis* patches in the following generations to find host plants. Leafminer populations also do not persist and overwinter in *Si. latifolia* patches, thus these patches must be newly colonized every spring. Thus, even though *Si. latifolia* is a preferred host, *A. flavifrons* may be limited to use it due to costs associated with dispersal among host patches (Schtickzelle and Baguette 2003, Zera & Mole 1994,Yoder et al 2004).

Because *Si. latifolia* is available only during the first generation of the year, this host species may invoke less selection pressure on the behavior of females than does *Sa. officinalis* (Futuyma 1976). Thus, for the preference for *Si. latifolia* to be maintained in the population, the benefit of using the host must be substantial. Although leafminer larvae survive plant defense better on *Si. latifolia* than on *Sa. officinalis*, the survival difference (~20 % difference) does not seem sufficient to counteract the cost of host searching. The following chapter will examine how ecological interactions involving the third trophic levels might contribute to the shaping of oviposition preference in *A. flavifrons*.

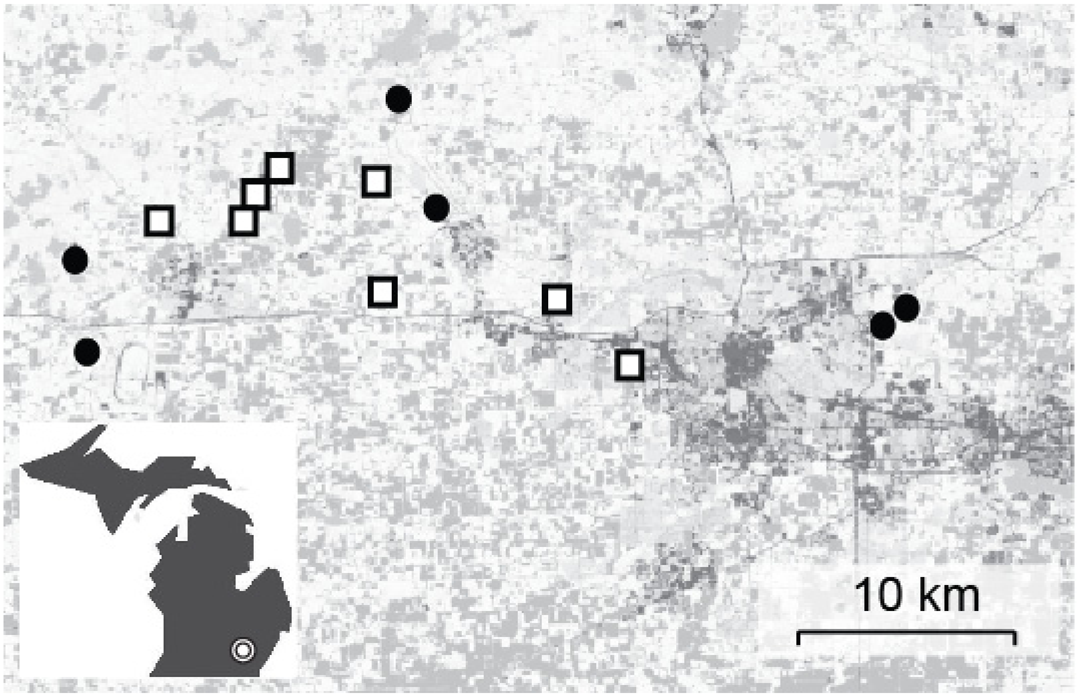


Figure 3.1: Locations of *Si. latifolia* (open squares) and *Sa. officinalis* (black dots) patches surveyed. The study was carried out in Washtenaw Co., Michigan.

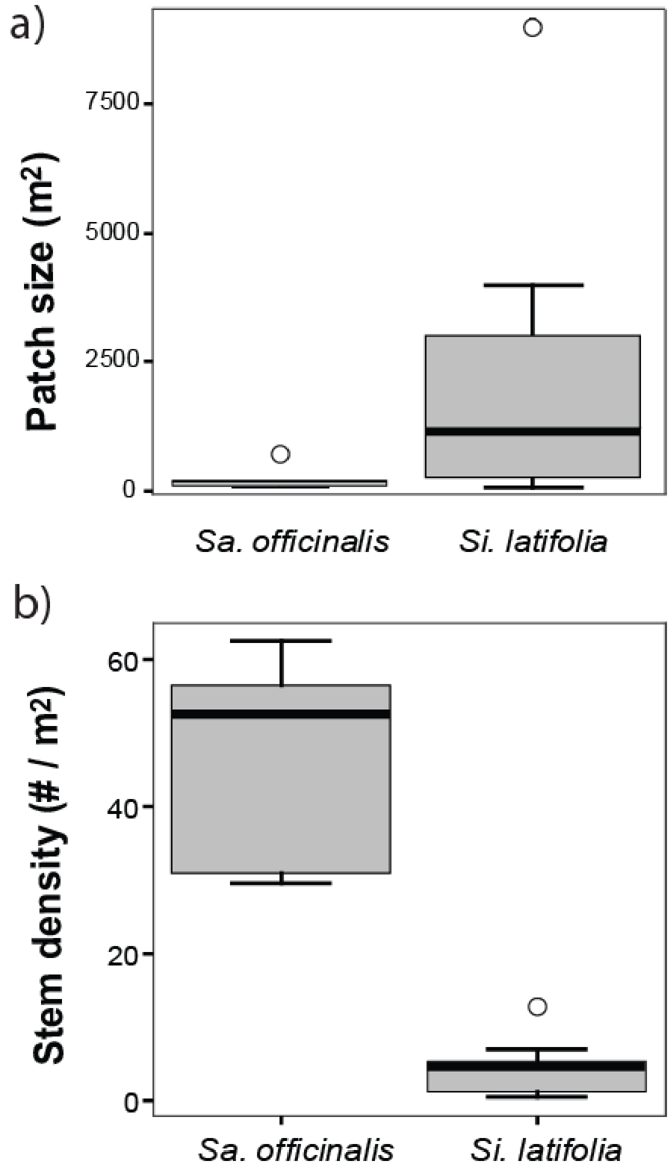


Figure 3.2: Box plots of patch size (a) and stem density (b) variation in *Sa. officinalis* and *Si. latifolia*. Lines inside the box indicate means, and circles show outliers. Species are marginally different in patch sizes (*n* = 15, *U* = 11, *P* = 0.059), and significantly different in stem densities (*n* = 15, *U* = 0, *P* < 0.0001).

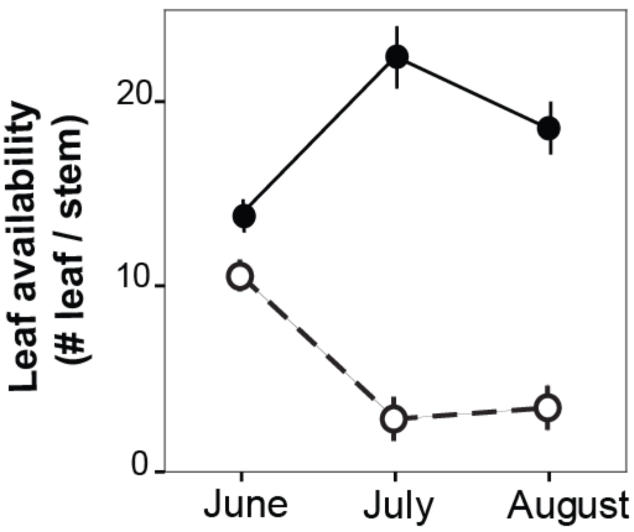


Figure 3.3: The seasonal change in mean (±SE) leaf abundance in *Sa. officinalis* patches (black dots: *n* = 6) and in *Si. latifolia* patches (open circles: *n* = 9).

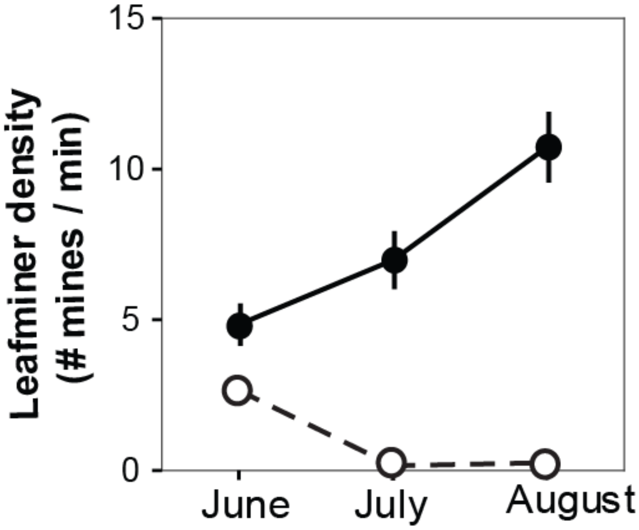


Figure 3.4: The seasonal change in mean (±SE) leafminer density in *Sa. officinalis* patches (black dots: *n* = 6) and in *Si. latifolia* patches (open circles: *n* = 9).

Chapter 4

Spatial heterogeneity in parasitism increases the impact of enemy-free space for the leafminer *Amauromyza flavifrons*

ABSTRACT

Host plant choice by herbivorous insects may be influenced by the presence of enemy-free space (EFS), a host plant on which herbivores may escape from their natural enemies. Variation in mortality from natural enemies is often attributed to differences in plant chemical, nutritional, or morphological traits between host plant species. However, when host species grow in distinct patches, spatial variation in abundance and composition of the enemies may further modify the benefit of EFS.

This study investigated the relative importance of plant and patch characteristics in providing EFS for a leafminer *Amauromyza flavifrons*. The degree of parasitism was compared between a seasonally persistent host plant, *Saponaria officinalis*, and a seasonally ephemeral host, *Silene latifolia,* when both host species were interspersed in a common garden, and when they were growing separately in monospecific patches as occurs in nature.

Overall mortality due to parasitism did not strongly differ between host species in the common garden, although mortality at larval stages was higher on *Sa. officinalis*

than on *Si latifolia*. This suggests that variation in plant traits alone does not create EFS for *A. flavifrons*. In contrast, overall parasitism was substantially lower in naturally-occurring patches of *Si. latifolia* than *Sa. officinalis*, and was independent of leafminer density, indicating that host plant specific habitat characteristics are responsible for providing EFS in *Si. latifolia* patches. This benefit of EFS may partly explain why *A. flavifrons* continues to feed on *Si. latifolia* even though it is an ephemeral and unpredictable resource.

INTRODUCTION

For insect herbivores that encounter multiple host species across the landscape, oviposition choice by females plays an important role in determining the performance of their offspring (Jaenike 1990). Although host choice may be influenced primarily by host quality as food (Futuyma and Moreno 1988), accumulating evidence suggests that herbivores may preferentially feed on host plant species that reduce mortality from natural enemies, or providing "enemy free space" (EFS: Price et al 1980, Bernays and Graham 1988, Berdegue et al 1996).

Mortality from natural enemies may differ between host plant species due to plant chemical, nutritional, or morphological traits that are specific to host species (Hunter 2003). For example, herbivores may find EFS on more toxic hosts where they obtain chemical compounds that can be sequestered for defense (Denno et al 1990, Bjorkman et al 1997, Nieminen et al 2003, Singer et al 2004). Structural refuges, such as large fruit or a protective calyx where enemies cannot reach, or leaf trichomes that hinder movement of enemies, can also provide herbivores with EFS (Feder 1995, Freese 1995, Gruenhagen and Perring 2001, Mira and Bernays 2002, Oppenheim and Gould 2002, Koller et al 2007, Obermaier et al 2008). When parasitoids use host plant specific olfactory cues to search for prey, herbivores may find EFS on plants that lack the chemical cues (Brown 1995, Gratton 2001). Finally, herbivores may escape from natural enemies by feeding on poor quality plants because enemies preferentially attack vigorous prey reared on high quality plants (Benrey and Denno1997, Teder and Tammaru 2002, Ode 2006, Koller et al 2007).

When host species grow in spatially distinct patches, mortality from natural enemies could also differ due to spatial variation in abundance and composition of the enemies among host patches (Yamaga and Ohgushi 1999, Ohsaki and Sato 1994, Murphy 2004, Craig et al 2007, Wiklund and Friberg 2008). Spatial heterogeneity in enemy load may arise from density-dependent colonization of patches by natural enemies, such that highly mobile enemies aggregate in patches where herbivore density is high in order to increase their foraging efficiency (Umbanhowar et al 2003, Vanbergen et al 2007, Pareja et al 2008, Tentelier et al 2008). Such density-dependent foraging behavior of enemies provides spatial refuges for herbivores that feed in low density patches, regardless of plant species identity.

In contrast, enemies may respond differently to the habitat characteristics where plant species grow, generating density-independent heterogeneity in enemy abundance. For example, certain host plant patches may be unsuitable for natural enemies (Yamaga and Ohgushi 1999, Murphy 2004, Craig et al 2007, Wiklund and Friberg 2008). A herbivorous lady beetle, *Epilachna pustulosa,* suffered much lower predation when feeding on blue cohosh than on alternative hosts because blue cohosh grows in dark understory forests, which predators tend to avoid (Yamaga and Ohgushi 1999). Alternatively, enemy populations may not build up in certain plant patches because host plants are ephemeral and herbivore populations do not persist over time (Ohsaki and Sato 1999). The butterfly *Pieris rapae*, for example, is known to avoid parasitism by colonizing ephemeral patches, but suffer parasitism in permanent patches (Ohsaki and Sato 1994). Such spatial heterogeneity in enemy load is likely to have important implications for EFS and host plant choices by herbivorous insects, but previous studies rarely address these habitat effects.

In this study, I investigate the relative importance of the habitat effect in creating EFS for a leafminer, *Amauromyza flavifrons* (Agromyzidae, Diptera). EFS may be especially relevant for *A. flavifrons* because the larvae are heavily attacked by multiple species of parasitoids (Scheffer 1995), including idiobiont ectoparasitoids, which terminate their prey growth and feed externally, and koinobiont endoparasitoids, which allow the host to continue developing and feed within the host body (Askew and Shaw 1986). *A. flavifrons* feed on multiple species of plants in the family Caryophyllaceae, but in this study area (southeastern Michigan) they feed mainly on two host species, *Saponaria officinalis* and *Silene latifolia* (Caryophyllaceae). Habitat effects could be potentially important in this system because these host plants generally grow in separate patches, although they are occasionally found growing intermixed. *Sa. officinalis* is a common and persistent host that are present throughout the summer and for all leafminer generations (3-4 generations per year). In contrast, *Si. latifolia* is seasonally ephemeral and available only during the first generation of each year in June, after which it senesces.

To test for the relative contribution of plant and habitat effects in providing EFS, I compared parasitism on *A. flavifrons* at two spatial contexts: when both plant species are intermixed in a common garden, and when they grow in separate patches as occurs in nature. Discrepancy in parasitism on *Sa. officinalis* and *Si. latifolia* in the common garden represent the benefit of EFS acquired through differences in plant traits alone, whereas parasitism differences between *Sa. officinalis* and *Si. latifolia* patches at a larger spatial scale show the EFS resulting from the combination of plant traits and habitat characteristics. Thus, if the degree of EFS is similar in the common garden experiment and the among-patch comparisons, it will imply that variation in plant traits is responsible for EFS. The absence of EFS in the common garden, in turn, will suggest that variation in habitat characteristics provides *A. flavifrons* with EFS across the landscape. To understand the mechanisms generating the spatial heterogeneity of parasitism among natural patches, I further tested whether parasitism is dependent or independent of the density of *A. flavifrons*.

METHODS

Common Garden Experiment

The effect of plant traits alone on EFS was examined in a common garden experiment at Matthaei Botanical Gardens (MBG: University of Michigan) in July 2007. Mortality from parasitoids was estimated by contrasting leafminer mortality in the presence ("exposed treatment") and absence ("caged treatment") of parasitoids. This method was preferred to a commonly used method of estimating parasitism from the number of parasitoids emerged because larval mortality, which is often assumed to be caused by plant defense chemicals, can also result from adult parasitoids consuming *A. flavifrons* larvae for egg production (Heimpel and Collier 1996). Thus, the exposed and caged treatments allowed us to identify which categories of leafminer mortality are caused by parasitoids.

Plants used in the experiment were grown in individual pots in the greenhouse at MBG and fertilized with two levels of nitrogen: 5 ml of 1.17 mol and 0.009 mol ammonium nitrate solution weekly. Six potted plants of the same species and nitrogen treatment were placed in a plastic cage where eight mated females were released for oviposition for 6 hrs. To assure oviposition, new females fed with 30 % honey water were used for each oviposition trial. Plants containing *A. flavifrons* eggs were kept in the greenhouse under mesh cover for four days before they were exposed in the field. This was done so that larger experimental larvae could be distinguished from larvae subsequently oviposited by the wild population of *A. flavifrons* (Scheffer 1995).

To avoid competition among larvae, a single larva per leaf was allowed to develop and the rest were killed by a needle. Most eggs hatched by the fifth day, and plants were placed in an open field at MBG under shade cloth to shield them from direct sunlight. Half of the plants were bagged with fine mesh (caged treatment) and the other half were exposed to the natural level of parasitoids (exposed treatment). A total of 40 *Sa. officinalis* (16 caged, 24 exposed) and 30 *Si. latifolia* (13 caged, 17 exposed) plants were placed in the field in a completely randomized design. Larval development lasted approximately for two weeks. Each leaf containing a leafminer at the last stage of development was individually collected in a Petri dish. Pupae were reared at a room temperature in the laboratory, and emerging insects were recorded. To assess the mortality at larval stages, all leaves were checked for mines under a dissecting scope.

Field survey of natural patches

To compare parasitism between the two host species in naturally occurring patches, field surveys of *Sa. officinalis* and *Si. latifolia* patches were conducted in June 2006, 2007 and 2008 in Washtenaw Co., Michigan in five to six patches per host plant species. This comparison was only relevant in the first generation of the year because *A. flavifrons* disappeared from the *Si. latifolia* patches as the host became unavailable by the second and third generations. Patches of *Sa. officinalis* were found along road sides, while *Si. latifolia* grew predominantly in the edge of large agricultural fields. All patches were separated at least by 1 km. Each patch was visited for 30 minutes and all leaves containing mines of *A. flavifrons* were collected.

The leaves were brought back to the laboratory and were examined under a dissecting scope to determine the mortality at the larval stage. Leaves containing living larvae were placed in a 0.5 ml microcentrifuge tubes and reared at a room temperature until adult eclosion. In 2006 field survey, I included larvae collected at earlier developmental stages, which is likely to underestimate the rate of endoparasitism because these larvae were not exposed to the parasitoid community during their entire larval development. For 2007 and 2008 survey, only larvae collected at the final instar stage were included in the calculation of leafminer fates.

Leaf C: N ratio analysis

To compare the quality of plants in the field and the common garden experiment, I measured leaf C: N ratios. In the common garden, all un-mined leaves from each plant were collected, whereas in the field, 50 leaves from various stems were collected per patch. All leaves were dried in a 50 ˚C oven, and the powdered material was analyzed using a Perkins Elmer CNO analyzer.

Data Analysis

In both host plant species, the C: N ratios of leaves collected from the natural patches were similar to that of common garden plants in the high nitrogen treatment ($t < 1.2$, $P >$ 0.2 for both species), but significantly differed from plants in the low nitrogen treatment ($t > 8.2$, $P <0.0001$ for both species: Fig. 4.1). Because the high nitrogen treatment better represents the plant condition that *A. flavifrons* and parasitoids encounter in the field, the following analyses were conducted using only data from the high nitrogen treatment in the common garden experiment.

In both the common garden experiment and the field survey, the fate of each larva was categorized as follows: 1) *larval mortality* when *A. flavifrons* larva is found dead inside its mine, 2) *ectoparasitism* when parasitoid larva develops on the dead leafminer larva, 3) *endoparasitism* when an adult parasitoid ecloses from *A. flavifrons* pupa, 4) *pupal mortality* when no insect ecloses from the pupa, and 5) *leafminer survival* when an adult leafmining fly ecloses from the pupa. *Larval mortality* and *ectoparasitism* were calculated in proportions to the number of total larvae initiated. *Pupal mortality* and *endoparasitism* were calculated in proportion to the number of total larvae that survived to pupation. All proportion data were arcsine-square root transformed. In the common garden experiment, each category of mortality was calculated for each potted plant. *Larval* and *pupal* mortality were tested for host plant species and caging treatments using two-way ANOVA, whereas *ecto*- and *endoparasitism* were tested only for host plant species with t-test, since no parasitism was observed in the caged treatment. In the field survey, mortality categories were calculated by patch by year, and compared between host plant species and survey years using Linear Mixed Model with patch as a random effect. The random effect was included because some patches, but not all, were visited multiple years.

Parasitism risk within naturally-occurring patches may be dependent or independent of the density of *A. flavifrons*. I tested the predictions by fitting Mixed-effects logistic regression model with binomial errors and logit link functions to the parasitism data (Vanbergen et al 2007). Explanatory variables fitted as fixed effects were leafminer density and host plant species. Patch was fitted as a random effect to account for the multiple visits in some patches. Leafminer density was estimated from the number of mines collected during the 30 minutes of survey period and is expressed as number of mines per minute (see Chapter 2 for details). All analyses were done with

SPSS (SPSS Inc, Chicago) except for the test of density-dependence, which was conducted using Stata 10.0 (StataCorp LP, College Station).

RESULTS

Common Garden Experiment

In caged treatment, *larval mortality* in the absence of parasitoids accounted for less than 10% of total mortality and did not differ between host plant species (Table 4.1, Fig. 4.2a). This indicates that background larval mortality, likely caused by plant defense chemicals, is equivalent in both host species. In contrast, in the exposed treatment, the *larval mortality* was substantially greater on *Sa. officinalis* than on *Si. latifolia* (32% and 1% of total mortality, respectively). The significant cage-by-host plant interaction for *larval mortality* suggests that parasitoids preferentially kill leafminer larvae on *Sa. officinalis*. Similarly, *ectoparasitism* was evident only on *Sa. officinalis* in the exposed treatment, although it accounted for only 5% of total mortality (Fig. 4.2b). *Endoparasitism* was observed on both host plant species, but the magnitude did not differ significantly between the hosts (Table 4.1, Fig. 4.2c), suggesting that endoparasitoids select their prey irrespective of host plant species. Five morpho-species of endoparasitoids were recovered, including two *Opius spp.* (Family Braconidae), one *Chrysocharis sp*. (Eulophidae), and two *Halicoptera spp*. (Family Ptelomalidae). Finally, *Pupal mortality* was greater on *Sa. officinalis* than on *Si. latifolia* in both caged and exposed treatments (Fig.4.2d). The absence of the cage effect and cage-by-host plant interactions indicates that *pupal mortality* is not caused by parasitism, and that the host plant difference in pupal mortality is due to variation in plant nutritional or chemical quality alone. Because *pupal mortality* was not caused by parasitism, I excluded *pupal mortality* when estimating the total mortality caused by parasitoids (*total parasitism*). *Total parasitism* did not differ between host plant species (Fig. 4.2e).

Field survey

Larval mortality (Fig. 4.3a) and *endoparasitism* (Fig. 4.3c) showed significant host plant species effects, whereas *ectoparasitism* (Fig. 4.3b) and *pupal mortality* (Fig. 4.3d) did not (Table 4.2). A significant year effect was observed for *endoparasitism*, *ectoparasitism* and *pupal mortality*, but host plant-by-year interactions were not significant, indicating that the differences between host plant species are consistent over three years (Table 4.2). *Total parasitism* was calculated for each patch by excluding *pupal mortality*, assuming that background mortalities in the natural patches are equivalent with that in the common garden. *Total parasitism*, the combination of *endoparasitism* and *larval mortality*, was substantially higher in *Sa. officinalis* patches (30.6 %) than in *Si. latifolia* patches (8.0 %; Fig. 4.3e).

Mortality at larval stages (*larval mortality* and *ectoparasitism*: Fig. 4.4a) and *endoparasitism* (Fig. 4.4b) did not depend on *A. flavifrons* density within a patch, but depended on the host plant species identity (Table 4.3). On average across the three census years, mortality increased in *Sa. officinalis* compared to *Si. latifolia* patches by 65.2 % at larval stages and 84.8 % by endoparasitoids. Together, *total parasitism* increased by 75.2% in *Sa. officinalis* patches (Table 4.3).

DISCUSSION

Enemy-free space (EFS) was found in patches of the ephemeral host, *Si. latifolia*. Field observations showed that total mortality due to parasitoids was 75 % lower in *Si. latifolia* patches than in *Sa. officinalis* patches, and the pattern was consistent over three years. In contrast, total mortality from parasitoids between the host plant species did not differ in the common garden experiment where the habitat effect was removed. This suggests that differences in plant characteristics alone cannot account for the EFS observed in natural patches. These results support the hypothesis that spatial heterogeneity of parasitoid abundance, rather than plant traits, is largely responsible for generating EFS across the landscape.

Although the parasitoid community as a whole did not respond differently to the host plant species in the common garden, the examination of each mortality category suggests that some parasitoids preferentially attack leafminers based on plant traits. For example, *larval mortality*, which is frequently caused by female parasitoids feeding on leafminer larvae (Heimpel and Collier 1996), was substantially higher on *Sa. officinalis* than on *Si. latifolia* plants both in the common garden and in natural patches. *Ectoparasitism* was also higher on *Sa. officinalis* in the common garden, and may suggest that larval mortality was mostly caused by the female adults of these ectoparasitoids (Jervis et al 2008). One possible explanation for this female preference may be that leaves of *Sa. officinalis* are smooth without trichomes. In contrast, leaves of *Si. latifolia* are covered with trichomes, which have been shown to slow down parasitoid movements and thus negatively affect the overall searching efficiency (Gruenhagen and Perring 2001, Carrillo et al 2008). Trichomes may be especially problematic for these ectoparasitoids of *A. flavifrons* because they are smaller than endoparasitoids (A. Uesugi, personal observation). The level of *endoparasitism* did not differ between the host species in the common garden, suggesting that plant traits may not strongly influence the foraging activities of endoparasitoids. In fact, all endoparasitoid species were reared from both host plant species. Unlike the common garden results, the field observation showed consistently higher *endoparasitism* in *Sa. officinalis* patches than *Si. latifolia* patches. This discrepancy in *endoparasitism* at the two spatial scales may suggest that the abundance of foraging endoparasitoids differ between the host patches.

Mortality due to parasitism in naturally-occurring patches was not related to leafminer density. Other studies have demonstrated that foraging parasitoids may aggregate and spend longer time searching in patches with higher prey density to increase their foraging efficiency, resulting in density-dependent parasitism risk (Umbanhowar et al 2003, Vanbergen et al 2007, Pareja et al 2008, Tentelier et al 2008). But the lack of density-dependence in this study is not surprising because such density-dependent foraging requires parasitoids to move freely across plant patches (Taylor 1993). In fact, studies that use spatial scales less than 1 ha often show density-dependent parasitism (Ray and Hastings 1996, Vanbergen et al 2007, Pareja et al 2008), whereas studies conducted at larger spatial scales generally show no pattern of density-dependent parasitism (Esch et al 2005). Distances among the natural patches in this study were much greater than that of previous studies, and were most likely beyond the range of the typical dispersal abilities of parasitoids (Elzinga et al 2006).

The variation in parasitism among naturally-occurring patches was predicted by plant species identity. This suggests that foraging parasitoids aggregate in *Sa. officinalis* patches, creating density-independent spatial heterogeneity in parasitism risk. Previous studies have suggested that enemy abundance might depend on the environmental conditions where host plants grow (Yamaga and Ohgushi 1999, Murphy 2004). Murphy (2004) demonstrated that a host shift by the Alaskan swallowtail butterfly, *Papillio machaon*, was driven by EFS on a novel host that grows in higher elevation where predacious ants were scarce. However, such variation in environmental conditions is unlikely to exist between *Si. latifolia* and *Sa. officinalis* patches, because both hosts grow in similar habitats, such as on the edge of open fields or roadsides.

A more likely explanation for the spatial heterogeneity in enemy load is that parasitoid populations cannot build up in *Si. latifolia* patches because the plant is ephemeral and senesces by the midsummer. As the plant resource diminishes, *A. flavifrons* and parasitoid populations must leave the patch or go extinct, and these patches must be newly colonized each spring. In contrast, *A. flavifrons* populations in *Sa. officinalis* patches persist throughout the season, allowing parasitoid populations to accumulate and overwinter in the patch. Thus, the insect populations are already present in *Sa. officinalis* patches in the following spring, and may serve as the source populations that disperse to *Si. latifolia* patches. Colonization of *Si. latifolia* patches by parasitoids may be particularly limited, because they can only colonize patches already occupied by *A. flavifrons* (Holt 2002). Although some specialist parasitoids are known to disperse as far as their prey (van Nouhuys and Hanski 2002, Esch et al 2005, Elzinga et al 2006), other parasitoids have limited dispersal ranges compared to their hosts (Ohsaki and Sato 1994).

Colonization of *Si. latifolia* patches may be further constrained by their lack of visibility to the foraging parasitoids. In contrast to *Sa. officinalis* that forms dense, monospecific patches, *Si. latifolia* plants often grow interspersed with non-host plants, such as alfalfa and barley in agricultural fields (see Chapter 2). A positive relationship between colonization probability and plant density or patch size has been demonstrated elsewhere (Elzinga et al 2007), potentially because plants in larger and denser patches emit more detectable volatile cues that parasitoids use to locate their prey (Pareja et al

2008). Similarly, Bukovinszky et al (2007) showed that parasitoids' tendency to arrive a patch decreased with the presence of non-host plants within a patch. Thus, the low plant density and complexity of *Si. latifolia* patches may contribute to the low probability of colonization by parasitoids in this study. This limitation in parasitoid colonization, in turn, may create spatial refuge for *A. flavifrons* that successfully disperse to *Si. latifolia* patches.

EFS in *Si. latifolia* patches may partly explain why *A. flavifrons* continues to use this ephemeral host, despite the theoretical expectation that herbivores should ignore rare and unstable hosts in order to increase the efficiency of locating suitable hosts (Bernays 1998; West and Cunningham 2002). Increased diet breadth can be favored when the quality of the rare host is higher so that the benefit balances out the cost of host searching (West and Cunningham 2002). For example, the European grapevine moth (*Lobesia botrana*) does not specialize on grape, which is an abundant and predictable resource, but includes rare alternative hosts in their diet because larvae grow better on them (Thiery and Moreau 2005). My study is similar to Theiry and Moreau's (2005) in that the plant quality of the ephemeral host, *Si. latifolia*, is higher than that of *Sa. officinalis,* favoring the inclusion of the ephemeral host into leafminer diet.

But this study emphasizes that EFS can also increase the benefit of using the ephemeral host, and may have a significant impact on the host plant choice of *A. flavifrons*, because parasitoids impose substantial mortality. Furthermore, the presence of EFS on the ephemeral host suggests that the conditions that favor polyphagy may be broader than predicted by the models based on plant-herbivore interactions alone. For example, plant-herbivore interactions suggest that the ephemeral hosts are included only when they are higher in quality than the common hosts (West and Cunningham 2002). In contrast, EFS can be potentially obtained on any ephemeral hosts regardless of their plant quality, and may expand the parameter space where ephemeral hosts are incorporated into herbivore diet. In addition, the plant phenology is consistent over years, and provides reliable EFS on the ephemeral host each year. This is in contrast to EFS based on plant trait effects, which often fluctuate in direction and strength over time (Heard et al 2006). Theoretical models of diet breadth evolution in herbivorous insects have rarely taken a tri-trophic perspective, but Singer and Stireman (2005) suggest that diet breadth expansion or initial host shifts to a novel plant is theoretically more plausible when EFS is considered. Therefore, the presence of EFS on rare plants may explain why some herbivorous insects maintain polyphagous habits in spite of costs associated with host searching.

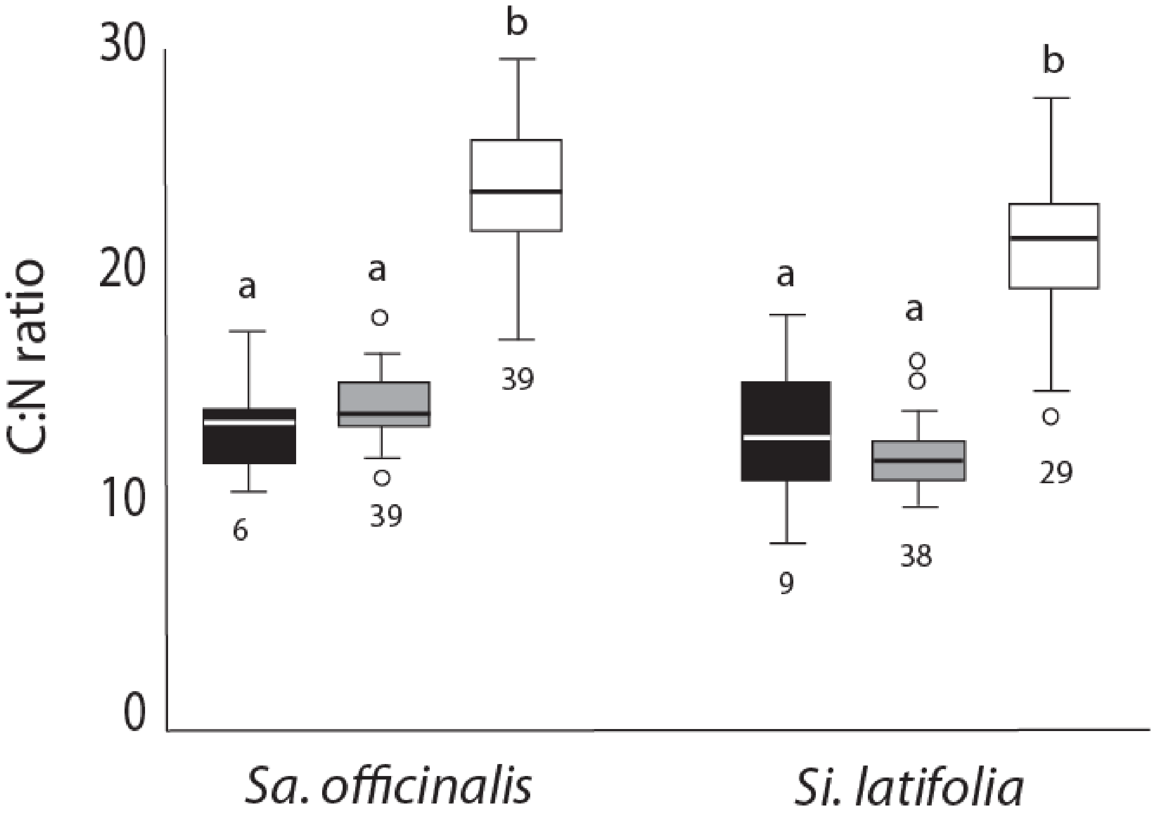
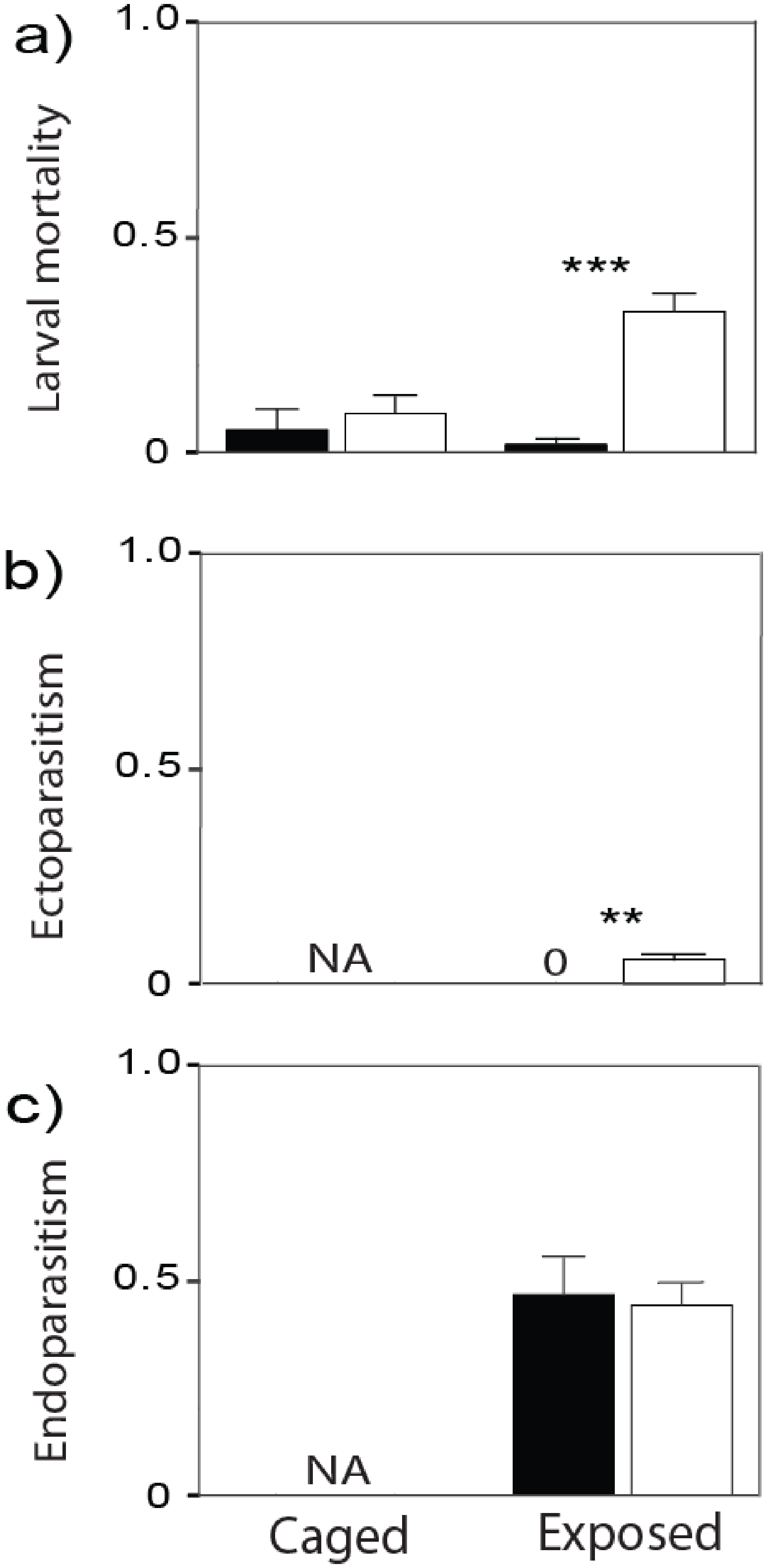


Figure 4.1: Leaf C:N ratios of *Sa. officinalis* and *Si. latifolia* plants collected in natural patches (closed boxes) and plants used in the common garden experiment grown under high (gray boxes) and low (open boxes) nitrogen treatments. Different letters indicate significant differences within host species at $P < 0.05$, and numbers below indicate sample size.


a)
1.0
0.5
0
Larval mortality
***
b)
1.0
0.5
0
Ectoparasitism
NA
0
**
c)
1.0
0.5
0
Endoparasitism
NA
Caged
Exposed

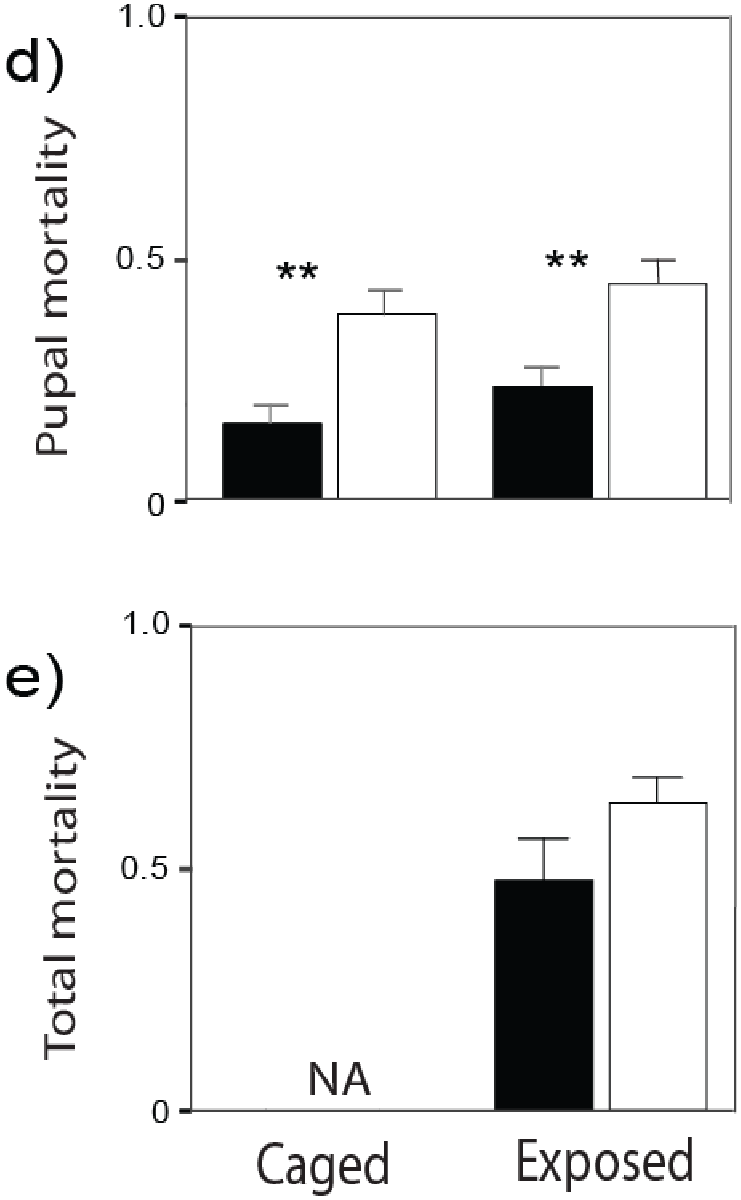
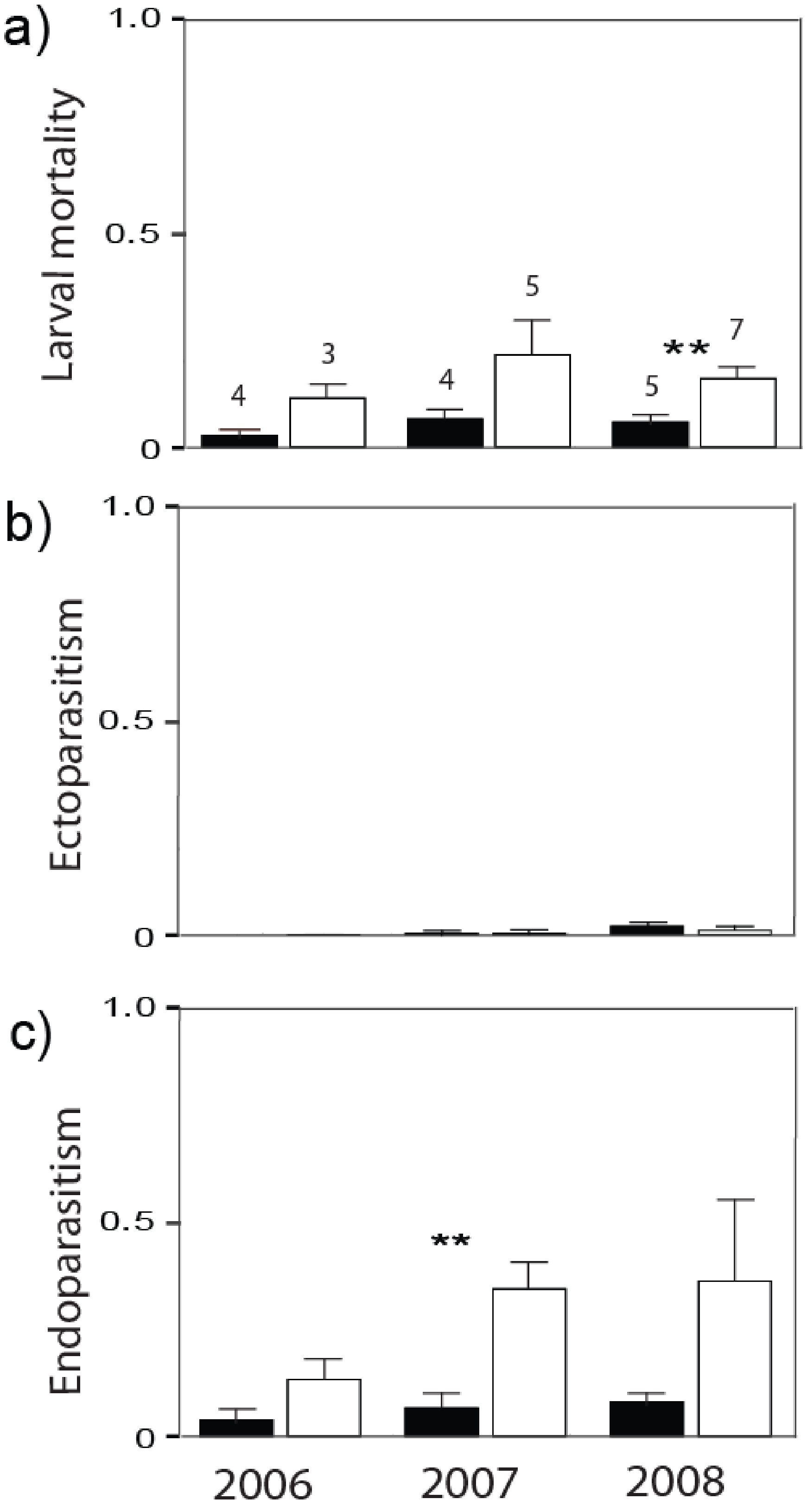


Figure 4.2: Mean proportion (± SE) of larval mortality (a), ectoparasitism (b), endoparasitism (c), pupal mortality (d), and total parasitism (e) in the common garden experiment under caged and exposed treatments. Closed bars indicate *Si. latifolia* (caged: *n* = 12, exposed: *n* = 17) and open bars indicate *Sa. officinalis* (caged: *n* = 16, exposed: *n* = 23). Asterisks indicate differences between host plant species within caging treatments (*P < 0.05, **P < 0.01, ***P < 0.0001).



a)
b)
c)
Larval mortality
Ectoparasitism
Endoparasitism
1.0
0.5
0
4
3
4
5
5
7
**
**
2006
2007
2008

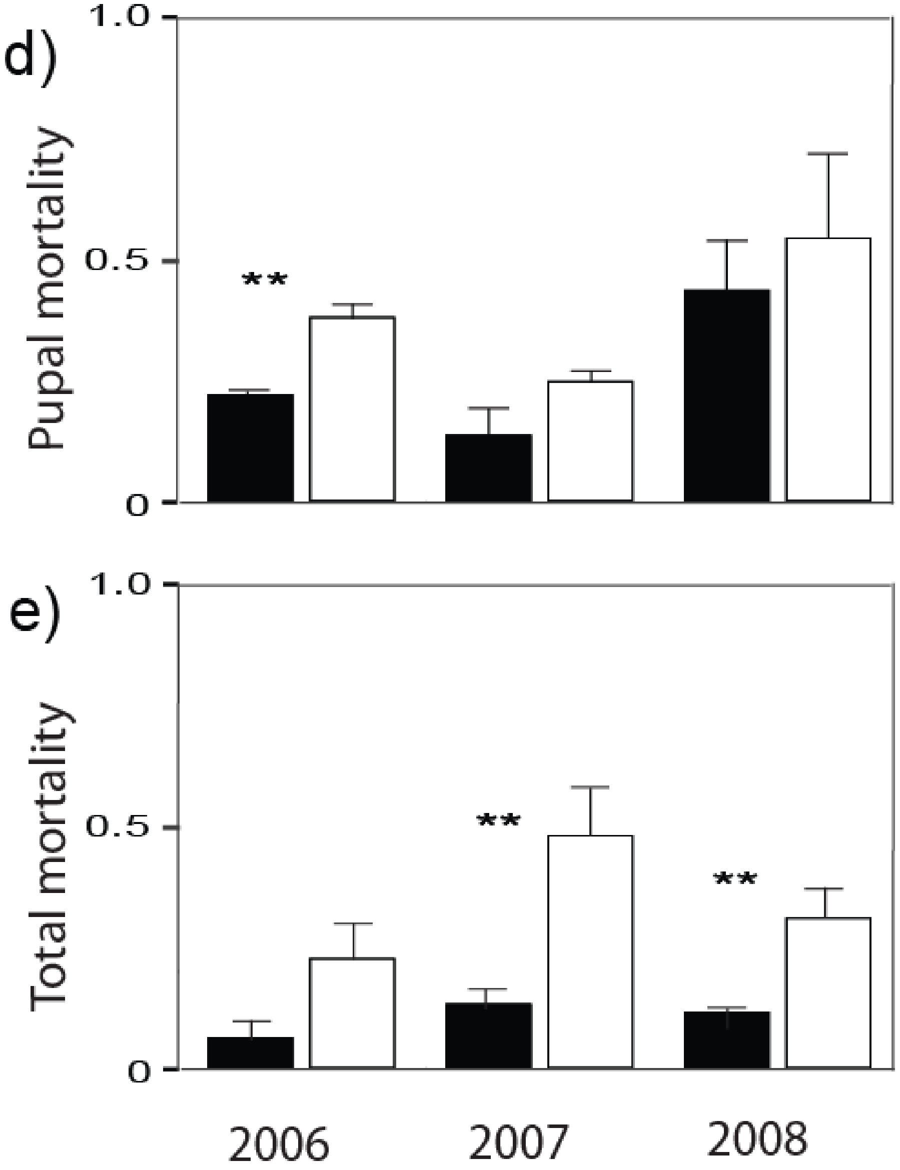


Figure 4.3: Mean proportion (± SE) of larval mortality (a), ectoparasitism (b), endoparasitism (c), pupal mortality (d), and total parasitism (e) between *Si. latifolia* (closed bars) and *Sa. officinalis* (open bars) patches in the field between year 2006 to 2008. Asterisks indicate significance between host patches for each year (*P<0.05, **P<0.01), and numbers above bars in (a) indicate sample size.

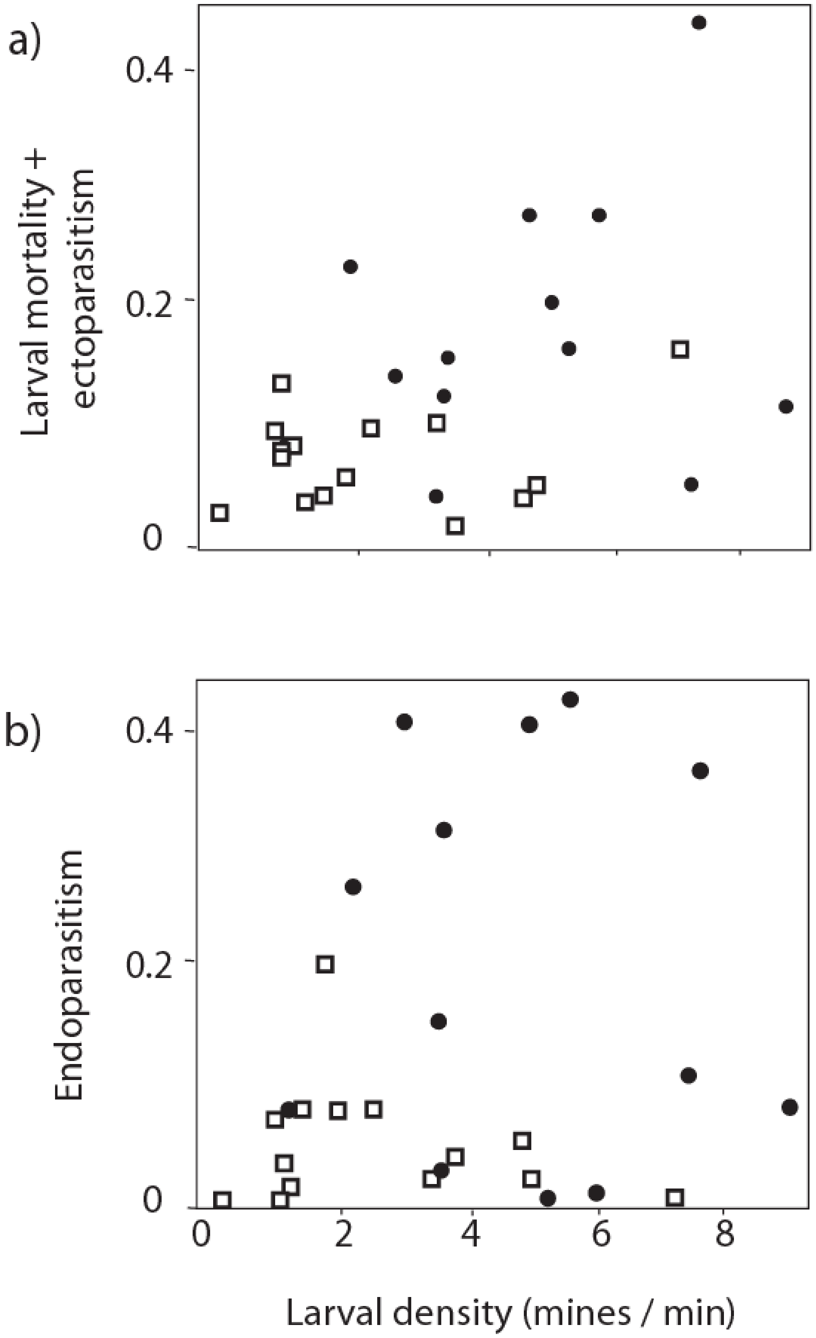


Figure 4.4: Relationship between leafminer density and mortality at the larval stages (a: larval mortality and ectoparasitism) and endoparasitism (b). Black dots indicate *Sa. officinalis* patches (*n* = 13), and open squares indicate *Si. latifolia* patches (*n* = 15). Data from three years were pooled, and patch was considered as a random effect.

Table 4.1: Effects of host plant species and caging treatment on leafminer mortality in the common garden experiment. *Larval mortality* and *pupal mortality* were tested with two-way ANOVA, and *ectoparasitism*, *endoparasitism*, and *total parasitism* were tested with t-test.

	Larval mortality		**Ecto-parasitism**		**Pupal mortality**		**Endo-parasitism**		**Total parasitism**	
	F	*P*	*t*	*P*	*F*	*P*	*t*	*P*	*t*	*P*
Host	18.6	**<0.0001**	-4.22	**0.006**	15.4	**<0.0001**	0.003	0.99	-1.8	0.082
Cage	3.9	0.055	**-**	**-**	2.6	0.11	**-**	**-**	-	-
Host x Cage	6.7	**0.012**	-	-	0.18	0.67	-	-	-	-

Table 4.2: Effects of host plant species and survey years on leafminer mortality in the field surveys of naturally-occurring patches. Each mortality category was tested with Linear-Mixed Model with patch as a random factor.

	Larval mortality		**Ecto-parasitism**		**Pupal mortality**		**Endo-parasitism**		**Total parasitism**	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Host	17.7	**<0.0001**	0.06	0.8	2.3	0.14	23.7	**<0.0001**	32.1	**<0.0001**
Year	1.9	0.17	5.6	**0.01**	4.4	**0.025**	4.2	**0.03**	4.4	**0.025**
Host x Year	0.07	0.9	0.7	0.5	0.04	0.9	0.6	0.6	0.9	0.4

Table 4.3: Results of Mixed-effect logistic regression analysis testing for the density-dependence of parasitism in natural patches. Fixed effects tested here are *A. flavifrons* density (Density) and host plant species (Species). Patch was modeled as a random effect.

Mortality category	fixed effect	Odds Ratio	95 % CI	P
Larval mortality and ectparasitism	Density	0.94	0.86 - 1.03	0.19
	Species	0.35	0.21 - 0.59	**<0.0001**
Endoparasitism	Density	1.04	0.93 - 1.16	0.48
	Species	0.15	0.05 - 0.44	**0.001**
Total parasitism	Density	0.95	0.89 - 1.02	0.16
	Species	0.21	0.12 - 0.36	**<0.0001**

Chapter 5

Natural enemies mediate life-history traits in the leafminer *Amauromyza flavifrons*: Selection for shorter development time by parasitoids

ABSTRACT

Natural enemies are expected to impose strong selection on prey life history traits that facilitate enemy avoidance. Selection on life history traits by parasitism in *A. flavifrons Amauromyza flavifrons* was investigated by estimating selection gradients on four traits—development time, adult body size, mine size and host plant quality—under natural field conditions.

Larval development time was under direct selection, supporting the *slow-growth high-mortality (SGHM) hypothesis,* which predicts that prolonged development time increases the probability of being attacked by parasitoids. There was no indirect selection on adult body size and mine size because these traits were not correlated with development time. The results indicate that the mechanism of enemy avoidance in *A. flavifrons* is to grow faster and thus narrow the window of vulnerability to parasitoid attack. Such non-random parasitism may play a significant role in shaping the evolution of life history traits of *A. flavifrons*.

INTRODUCTION

Herbivorous insects often suffer substantial mortality from natural enemies (Hawkins et al 1997) and are expected to be under strong selection pressure to evade enemy attacks (Kraaijeveld and Godfray 2003). Herbivores may adapt by increasing their defenses, for example, by thickening gall walls (e.g. goldenrod gall flies, *Eurosta solidaginis*; Abrahamson et al 1989; Weis et al 1992), by increasing their ability to sequester plant defense chemicals (pipevine swallowtail, *Battus philenor*; Fordyce and Nice 2008), or by enhancing encapsulation of parasitoid eggs and larvae (e.g. *Drosophila melanogaster*; Kraiijeveld et al 1998; Fellowes and Godfray 2000). In herbivorous insects with limited direct defense mechanisms, predation and parasitism may impose selection on life history traits that increase enemy avoidance. However, few studies have examined the role of natural enemies as selective agents on such life history traits in herbivores (Kraaijeveld and Godfray 2003; Teder and Tammaru 2001).

Interactions between leafminers and parasitoids represent a great opportunity to examine selection on life history traits by natural enemies because leafminers are particularly susceptible to attacks from parasitic wasps (Hawkins et al 1997). Previous studies of parasitoid behavior suggest that parasitism potentially acts on multiple leafminer life-history traits (Hunter 2003), which leads to four hypotheses about how leafminers might adapt to decrease parasitoid-induced mortality. The *slow-growth high-mortality (SGHM) hypothesis* (Clancy and Price 1987; Benrey and Denno 1997; Williams 1999) suggests that prolonged development time will increase the risk of attack, thereby favoring individuals with shorter development times. The *prey size hypothesis* predicts that parasitoids preferentially attack larger prey because the size of the host influences the performance of parasitoid offspring (Wang and Messing 2004; Kraaijeveld and Godfray 2003: hypothesis title is mine). This prey size preference by parasitoids would result in selection for smaller leafminer body size. The *appearance hypothesis* suggests that parasitoids selectively attack leafminers that make larger mines because they are more apparent and easier to detect (Stiling et al 1999; Cornelissen and Stiling 2006: hypothesis title is mine). Parasitoid searching behavior based on visual cues (Ayabe and Ueno 2004) or chemical cues associated with mine size (Finidori-Logli et al 1996) may favor individuals that make smaller mines. The selection on mine size, in turn, may select for increased food assimilation efficiency in leafminers because more efficient individuals require less feeding (Stiling et al 1999). Finally, parasitism may have nothing to do with these life history traits but may depend on the quality of the plants upon which herbivores feed. According to the *plant quality hypothesis*, parasitoids may be attracted to high quality plants because herbivores that feed on these plants are likely to be better quality prey (Hunter and Price 1992; Mira and Bernays 2002; Walker et al 2008; hypothesis title is mine). Host plant choice by parasitoids may, in turn, indirectly influence oviposition preference of leafminers (Price et al 1980).

Despite a wealth of studies of parasitoid behavior, most lack the ability to distinguish among these hypotheses because they do not simultaneously test associations between parasitoid-induced mortality and multiple life history traits of the prey. For example, Stiling et al (1999) observed an increase in parasitism of leafminers that made larger mines, providing support for the *appearance hypothesis*. However, increased parasitism could also be a result of prolonged development time or larger body size if these traits are correlated with mine size; neither of these traits was measured in the study. Even in studies that do measure multiple traits, identifying traits that directly influence parasitism rates is often difficult if these traits are correlated with each other (Clancy and

Price 1987; Auerbach and Alberts 1992; Cornellisen and Stiling 2006). Here, I investigate enemy-induced selection on the life history traits of a leafmining fly *Amauromyza flavifrons* Meigen *(*Agromyzidae; Diptera). The four hypotheses (above) were tested simultaneously to identify traits subject to direct and indirect selection from natural communities of parasitoids. The mode and strength of selection was also compared between two host plant species because overall parasitism rates differed between them.

METHODS

Study system

Amauromyza flavifrons Meigen is a leafmining herbivore that feeds on multiple species of plants within the family Caryophyllaceae (Spencer 1990). In southeastern Michigan, USA, it is commonly found on two plant species, *Silene latifolia* and *Saponaria officinalis*. *A. flavifrons* is multivoltine having three to four generations per year. Adults emerge in early June and females lay eggs under the epidermis layer of a host plant leaf. Eggs hatch within 3-4 days and larvae mine for 7-12 days until they exit leaves to pupate in the soil. During its larval development, *A. flavifrons* may be heavily attacked by multiple species of parasitoids in the families Braconidae, Pteromalidae, and Eulophidae (Scheffer 1995). Early instars are subject to mortality by adult female parasitoids that feed on this larval stage (Jervis and Kidd 1986; Heimpel and Collier 1996). Late instars may be heavily parasitized, with parasitism rate reaching as high as 70% (Scheffer 1995). Despite high parasitism levels, larvae seem to lack direct defensive mechanisms; no occurrence of encapsulation of parasitoid eggs or larvae has been observed in the study area (A. Uesugi, personal observations). Its high risk of parasitism coupled with restricted movement during the larval period facilitates accurate measurement of each leafminer's fate and relevant life history traits, and makes this system ideal for examining selection on life history traits by parasitoids.

Field Experiment

A field experiment was conducted in July 2007 at Matthaei Botanical Gardens (MBG) in Dixboro, Michigan, to estimate the direction and strength of selection imposed by parasitism. Larvae of *A. flavifrons* were reared either on caged plants without parasitoids to provide "before selection" treatment, or on exposed plants with natural levels of parasitoids to provide an "after selection" treatment. Plants used in the experiment were grown individually in pots in the greenhouse under two nitrogen treatments: a low nitrogen treatment received 5 ml of 0.009 mol ammonium nitrate weekly, and a high nitrogen treatment received 5 ml of 1.17 mol ammonium nitrate. Nitrogen treatments were used to test the *plant quality hypothesis*, as well as to artificially create variation in leafminer life history traits, since leaf nitrogen levels are known to influence larval development time, body size, and mine size in other herbivorous insects (Stiling et al 1999). All *Sa. officinalis* plants originated from vegetative growth from one natural patch and *Si. latifolia* plants were grown from seeds collected from another patch. Plants had been growing for two months prior to the experiment, and some *Si. latifolia* plants started flowering during the experiment.

Females of the leafmining fly were collected from one *Sa. officinalis* patch at MBG in the previous generation. Six potted plants of the same species and nitrogen treatment were placed in a plastic cage where eight 3-4 day old mated females were released for oviposition for 6 hrs. To maximize oviposition, new females fed with 30 % honey water were used for each oviposition trial. Plants containing *A. flavifrons* eggs were kept in the greenhouse under mesh cover for four days before they were exposed in the field. This was done so that larger experimental larvae could be distinguished from non-experimental larvae subsequently oviposited by the wild population of *A. flavifrons* (Scheffer 1995). To avoid competition among larvae, a single larva per leaf was allowed to develop and the rest were killed by a needle. Half of the plants were bagged with fine mesh (caged treatment) and the other half were not (exposed treatment). A total of 83 *Sa. officinalis* (16 high nitrogen/caged, 17 low nitrogen/caged, 24 high nitrogen/exposed, 26 low nitrogen/exposed) and 69 *Si. latifolia* (13 high nitrogen /caged, 17 low nitrogen /caged, 17 high nitrogen /exposed, 22 low nitrogen /exposed) plants were placed in an open field under shade cloth at MBG in completely randomized design. The shade cloth shielded plants from direct sunlight and created relatively homogeneous environments between caged and exposed treatments. Ambient temperature increased in the caged treatment only slightly (mean difference = 0.3 ˚C, t=2.2, P=0.35), and no differences in plant quality, such as leaf C:N ratio, water content, and thickness, were observed ($P > 0.2$ for all). Thus, differences in life history traits between surviving leafminer in the two treatments were assumed to be the result of selection by parasitism on parasitoid-exposed plants.

All leaves were checked daily for leafminer development. Each leaf containing a large pre-pupal larva was individually collected in a Petri dish before it emerged from the leaf to pupate in the soil. Most larvae collected in this way pupated in their Petri dish within a day of collection, but ones that did not were excluded from the analysis because they were not exposed to the field conditions during their entire larval period. Larval development time was determined as days from oviposition to pupation. Leaves containing leafminer larvae were brought to the laboratory, and photographed immediately to estimate mine size at the time of leaf collection. Adobe Photoshop software was used to calculate mine area which served as the proxy of mine size. Insects were reared to adults at room temperature (25˚C), and the body size of eclosed adult flies was estimated by measurements of thorax length, calculated with ImagePro program (Media Cybernetics Inc.), and using the margin of the pronotum and scutelum as landmarks (Partridge and Fowler 1993).

The nutritional quality of each potted plant used in the experiment was estimated by measuring leaf C: N ratio. All un-mined leaves from each plant were collected, dried in a 50˚C oven, and analyzed using a Perkins Elmer CNO analyzer. Because leaves at different ages may vary in nitrogen levels (Ikonen 2002), this method does not capture the variation in C: N ratio experienced by individual larvae. However, most larvae were found on leaves of similar ages (i.e. mature leaves with no sign of senescence) which are assumed to have similar C: N ratios.

Data analysis

Because the proportion of larvae parasitized differed significantly between host plant species (Pearson's X^2= 19.9, P<0.0001) but did not differ between leafminer sexes (Pearson's X^2<0.1, P>0.2 on both host species), the following analyses were conducted separately for host species with both leafminer sexes combined. Qualitatively similar results were obtained when the sexes were analyzed separately.

Selection gradients were estimated in multiple logistic regression analysis to identify traits under direct selection due to parasitism (Lande and Arnold 1983; Janzen and Stern 1998). Since the fitness measure used here is dichotomous (survived or dead), logistic regression was preferred over linear regression analysis (Janzen and Stern 1998).

The analysis was conducted with development time, body size, mine size and host plant quality (as estimated by C: N ratio) as predictors. To standardize the selection gradient so that it could be applied directly to micro-evolutionary equations, the average selection gradient, $\beta_{avggrad,}$ was calculated by multiplying coefficients with a conversion factor and σ, which is the standard deviation of the trait in the caged treatment (Janzen and Stern 1998). To compare the magnitude of selection gradients between host plant species, separate analyses were conducted using a dataset with both host plant species combined. The multiple logistic regression analysis was conducted as above, but including species-by-trait interaction terms.

The strength of total selection, including both direct and indirect selection, on each focal trait was estimated as a selection differential, $s = (\bar{z}' - \bar{z})$, where $\bar{z}'$ and $\bar{z}$ are the trait means in exposed and caged treatments, respectively (Lande and Arnold 1983). Selection differentials were standardized by dividing by σ, so that values could be compared between host plant species and traits under investigation (Lande and Arnold 1983). The statistical significance of selection differentials was explored by a Linear Mixed Model with caging treatment as a fixed factor, and potted plant as a random factor. The random factor was added because larvae from one plant share the same environment, and thus are not independent. To compare the magnitude of selection differentials for each focal trait between host plant species, separate analyses were conducted using a dataset with both host species combined. The Linear Mixed Model was applied as above, but species-by-cage treatment interactions were additionally included.

Phenotypic correlations among development time, adult body size and mine size were examined using data from caged treatment to exclude the potential effect of parasitism on trait correlations. Each pair of life history traits was tested using a multiple linear regression model by controlling for leaf C: N ratio and leafminer sex, which are likely to influence trait values. When environmental variation is minimized, phenotypic correlations are shown to be reliable estimation of genetic correlations (Roff 2000). In addition, the effect of C: N ratio on the life history traits of *A. flavifrons* was assessed using regression analysis. All analyses were conducted in SPSS (SPSS Inc, Chicago).

RESULTS

Mortality due to parasitism was greater on *Sa. officinalis* (84.7 %) than on *Si. latifolia* (62.9 %) (Pearson's $X^2 = 45.0$, $P<0.0001$). On *Sa. officinalis*, 268 out of 491 larvae survived to adulthood in the caged treatment, whereas 63 out of 754 survived in the exposed treatment. On *Si. latifolia*, 171 out of 251 larvae survived in the caged treatment, and 93 out of 368 survived in the exposed treatment.

A multiple logistic regression analysis revealed that, on both host plant species, development time was the only significant predictor of parasitism rate (Table 5.1). The average selection gradient ($\beta_{avggrad}$) was negative on both hosts (Fig. 5.1), indicating that parasitism rates increased with development time. The magnitude of the direct selection was slightly greater on *Si. latifolia* than on *Sa. officinalis*, but the difference was not significant (Table 5.2). Selection gradients for body size, mine size and host plant quality were not significant on either host plant species (Table 5.1 & 5.2).

Selection differentials were significantly negative for development time on both host plant species (Table 5.3, Fig. 5.2a), indicating a total selection favoring shorter development time. On *Sa. officinalis*, the mean development time decreased by 0.38 days in the exposed compared to caged treatments, and on *Si. latifolia*, the mean development time decreased by 0.35 days. Standardized selection differentials (s') indicated that the intensity of total selection was slightly greater on *Sa. officinalis* than on *Si. latifolia* (Table 5.3), but the difference was not significant (Table 5.4). Selection differentials for adult body size, mine size and host plant quality were not significant (Table 5.3, Fig. 5.2b,c,d).

Phenotypic correlations between development time, adult body size and mine size were not significant on either host species (Table 5.5). On *Si. latifolia*, increased leaf nitrogen resulted in longer development time, larger adult size, and smaller mine size ($F > 14$, $P < 0.0001$ for all). On *Sa. officinalis*, in contrast, increased nitrogen levels decreased development time ($F = 20$, $P < 0.0001$), but did not affect adult body size or mine size ($F < 0.18$, $P > 0.4$).

DISCUSSION

Parasitism causes substantial mortality in *A. flavifrons*, predicting that selection by parasitoids should be an important force shaping the evolution of life history traits in this leafminer. The selection gradient analysis indicates that parasitism directly selects against individuals with long larval development times. While overall parasitism was greater on *Sa. officinalis* than on *Si. latifolia* plants, the intensity of the direct selection did not differ on the two hosts. Thus, *A. flavifrons* seems to experience similar selection pressures from parasitoids acting on development time regardless of host plant environment. The results support the *slow-growth high-mortality (SGHM) hypothesis,* which suggests that prolonged development time increases the probability of being attacked by parasitoids (Benrey and Denno 1997). The *SGHM hypothesis* has been tested elsewhere, but previous studies have found only equivocal support for the hypothesis (reviewed in Williams 1999). Contrasting outcomes in previous studies may be partly explained by differences in methodology. In this study, *A. flavifrons* were exposed to parasitoids during their entire larval development period. In contrast, other studies have exposed herbivores to natural enemies only during a part of their larval development period (Medina et al 2005; Benrey and Denno 1997; Lill and Marquis 2001; Loader and Damman 1991). Assuming that fast growing individuals have a smaller window of vulnerability to enemy attack, these studies used larvae developing at different rates, and exposed them to enemies for the same length of time. However, the assumption could be violated if the actual window of vulnerability is longer than the time exposed during experiments (Williams 1999; Fordyce and Nice 2008). Thus, selection for shorter development may be more prevalent than reported previously if the relationship between mortality risk and exposure time to enemies is accurately assessed.

The *SGHM hypothesis* was supported by separate analyses for each host plant species, but did not hold when comparisons were made between the host plant species. Larvae develop faster on *Sa. officinalis* than on *Si. latifolia* (Fig. 5.2a), but suffer higher overall parasitism on *Sa. officinalis*. This pattern of parasitism may reflect a behavioral preference of parasitoids for *Sa. officinalis*, rather than their responses to the development time of *A. flavifrons per se* (Benrey and Denno 1997). Parasitoids may be more attracted to *Sa. officinalis* because it is a more abundant and predictable resource than *Si. latifolia* (see Chapter 4). Parasitoids may also attack *A. flavifrons* more efficiently on *Sa. officinalis* because it lacks leaf trichomes that hinder their movements (Carrillo et al 2008; trichomes are present in *Si. latifolia*). Such interspecific variation in attractiveness to parasitoids may explain why the *SGHM hypothesis* is rarely supported in studies that employ among-host comparisons to infer the relationship between development time and parasitism rate (e.g. Medina et al 2005; Preszler and Boecklen 1994; Benrey and Denno

1997). Moreover, comparisons within a host species are more relevant in this system where host plant species grow in distinct patches. Since parasitoid mobility among host patches is likely to be limited (see Chapter 4), prey selection within each host patch should be relatively independent.

The *prey-size hypothesis* was not supported in this study, as selection gradients on adult body size were not significant. This result differs from a study by Kraaijeveld and Godfray (2003), which reported that parasitoids selected against larger pupae in *Drosophila melanogaster*. Differences in the results might be explained by the types of parasitoids used in the two experiments. Pupal parasitoids are likely to prefer larger pupae because pupal size at the time of attack directly determines the maximum size of parasitoid offspring. A similar pattern is expected from other idiobiont parasitoids, which terminate prey growth and their larvae usually feed externally (Askew and Shaw 1986). Consequently, studies involving idiobiont parasitoids should generally support the *prey-size hypothesis* rather than the *SGHM hypothesis* (Clancy and Price 1987; Kraaijeveld and Godfray 2003; Wang and Messing 2004). In contrast, koinobiont parasitoids allow their insect hosts to continue developing while their larvae feed within the host's body (Askew and Shaw 1986). Koinobionts may be less choosy because the prey size at the time of attack does not necessarily reflect its final size (Benrey and Denno 1997). Similarly, choosing larger prey is not important in predators because they can compensate for poor prey quality by increasing the number of prey consumed (Williams 1999). In this study, *A. flavifrons* was attacked by both idiobiont and koinobiont parasitoids, but 89 % of parasitism was caused by koinobiont species. Consequently, I found no support for the *prey-size hypothesis*. Because the outcome of selection is likely to vary depending on the composition of natural enemies, caution is required when inferring the mode of selection in nature using laboratory experiments where herbivores are exposed to only a subset of enemies that occur in nature.

The *appearance hypothesis* was also rejected, as parasitism risk was not associated with leafminer mine size. The lack of support suggests that the parasitoids did not use visual or chemical cues to locate their prey, as predicted by the hypothesis (Ayabe and Ueno 2004). Similarly, feeding activities, calculated as leaf area mined per day, did not predict parasitism risk (data not shown). High feeding activities are often associated with increased predation risk in free-ranging caterpillars (Gotthard 2000). However, natural enemies may not be able to detect such variation in the feeding activities of leafminers that feed in concealed environments.

Leaf C: N ratios did not predict parasitism risk, contrary to expectations of the *plant-quality hypothesis*, which predicts that parasitoids will preferentially attack leafminers on high quality plants. There was no direct effect of leaf C: N ratios on parasitism, possibly because parasitoids were incapable of detecting the plant quality variation, or because C: N ratio was unrelated to the quality of leafminers as hosts. *A. flavifrons* adult size increased with leaf nitrogen levels on *Si. latifolia*, but no such correlation was found on *Sa. officinalis* (Table 1). This hypothesis also assumes that parasitoids prefer larger prey, which was also not supported in this study. Nevertheless, because nitrogen level was strongly correlated with leafminer development time, plant quality may indirectly influence the parasitism risk of *A. flavifrons* (Hunter 2003). However, the mechanisms underlying any effects of plant quality on development time are not clear at present—development time was positively correlated with plant C: N ratio on *Sa. officinalis* and negatively correlated with C: N ratio on *Si. latifolia* (Table 1). These data suggests a more complex relationship between plant quality and development time in *A. flavifrons*.

Life history traits are often correlated with one another such that direct selection acting on one trait can indirectly select for other traits (Lande and Arnold 1983). However, direct selection on larval development time did not result in significant selection differentials for other traits measured here. The absence of a positive association between development time and adult body size was particularly surprising because the resource allocation model predicts that increases in body size, which is positively correlated with fecundity in insects, should come at the cost of a longer feeding period during larval stages (Roff 1996).

A slightly negative phenotypic correlation observed in this study may, instead, suggest that there is environmental or genetic variation in growth rates within populations, such that fast growing individuals attain larger body sizes in shorter periods of time than do slow growing individuals (Berner and Blackenhorn 2007). The present study partially accounts for environmental variations in growth rates by statistically controlling for nutrient levels experienced by developing larvae (i.e. leaf C: N ratio), though there are surely other, unmeasured sources of environmental variation in growth rate. Genetic variation in growth rates might be maintained in *A. flavifrons* populations if growth rate itself represents a tradeoff with other life history characters (Chippindale et al 1997; Sevenster and Van Alphen 1993; Gotthard 2000). The genetic variation might also be maintained at mutation-selection balance with the accumulation of deleterious mutations depressing growth rates (Charlesworth 1990; Houle 1991). In either case, parasitism that selects for shorter development time may potentially act on variation in growth rates and remove slow-growing individuals that end up smaller from the population.

Overall, selection pressure on development time suggests that the mechanism of enemy avoidance in *A. flavifrons* is to grow faster, thereby narrowing the window of vulnerability to parasitism. In herbivorous insects that lack direct defense mechanisms, this avoidance mechanism may be a common adaptation, particularly in herbivores that are attacked by a suite of generalist enemies that do not selectively attack prey based on its quality (Williams 1999). Quantitative host-parasitoid food webs often show that most parasitoids that attack leafminers are generalists (Lewis et al 2002, Hirao & Murakami 2008). Whether herbivore population will respond to the selection from enemies will depend on the presence of additive genetic variation for this trait. Recent studies show that there exists ample additive genetic variation and high evolvability (ability to respond to selection; Houle 1992) for development time in many herbivorous insects, including potato beetles (Boman et al 2008), and a cactophilic fly, *Drosophila buzzatii* (Cortese et al 2002). Thus, herbivore populations exposed to intense parasitism or predation may potentially evolve shorter larval development period than populations that escape natural enemies.

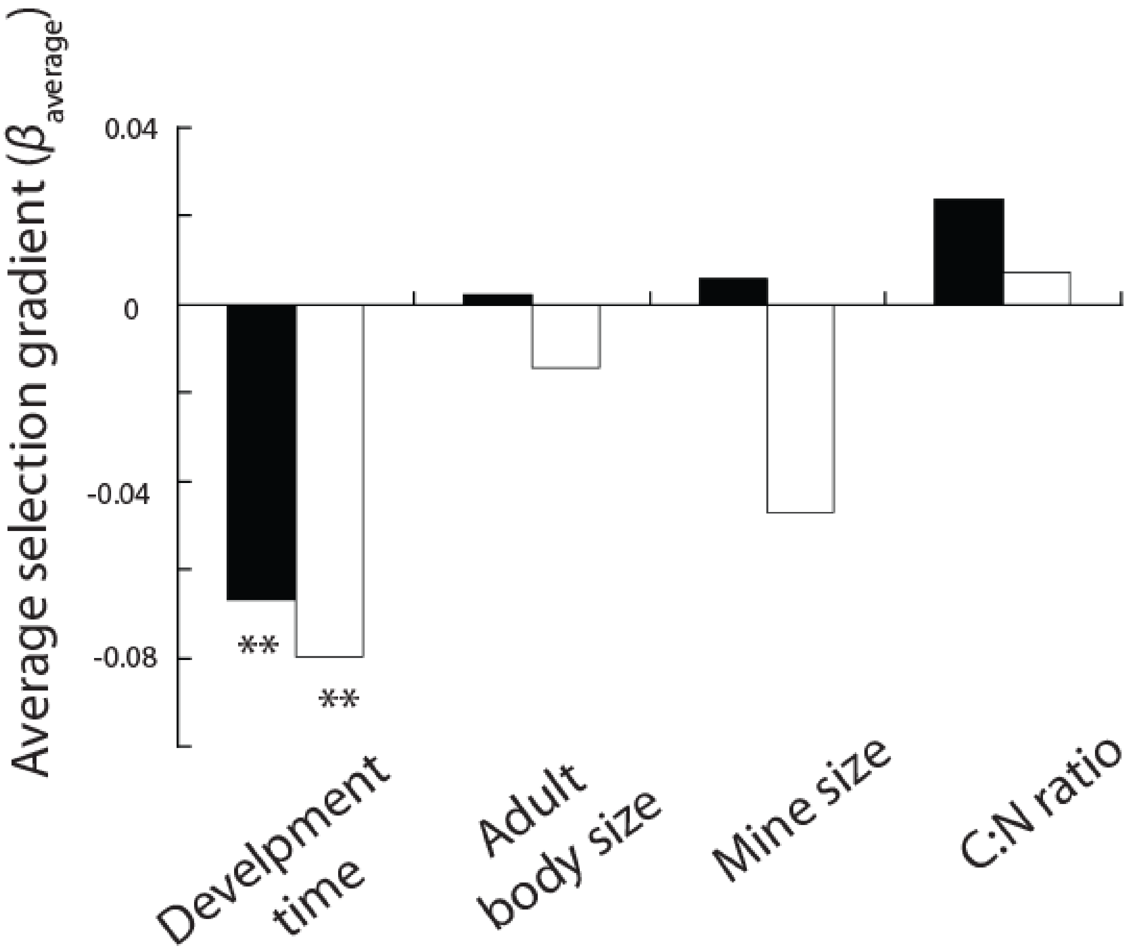
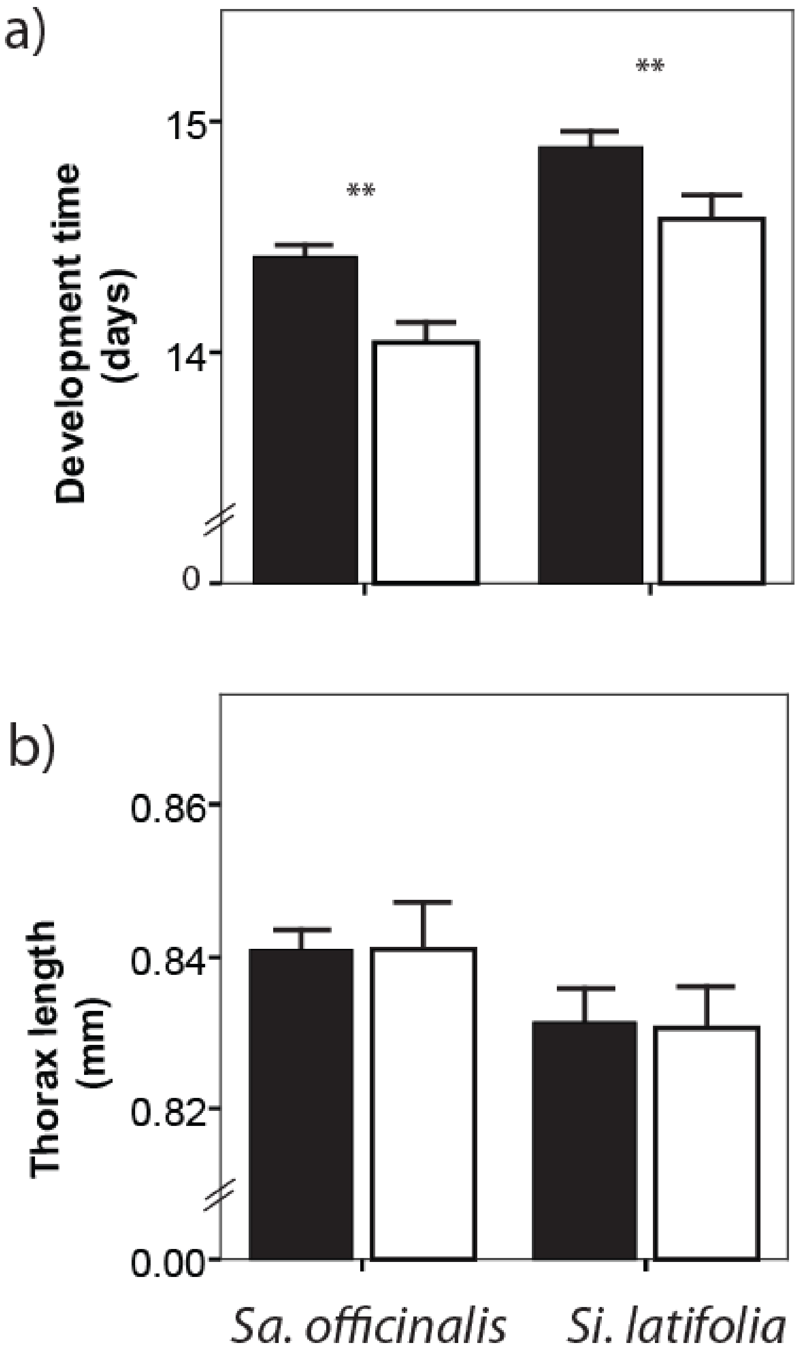


Figure 5.1: Standardized selection gradients ($\beta_{avggrad}$) for development time, adult body size, mine size and host plant quality (estimated as leaf C: N ratio) of *A. flavifrons* on *Sa. offcinalis* (closed bars: $n = 78$) and *Si. latifolia* (open bars: $n = 67$). Asterisks represent significance at $P < 0.01$.


a)
Development time (days)
15
14
0
**
**
b)
Thorax length (mm)
0.86
0.84
0.82
0.00
Sa. officinalis
Si. latifolia

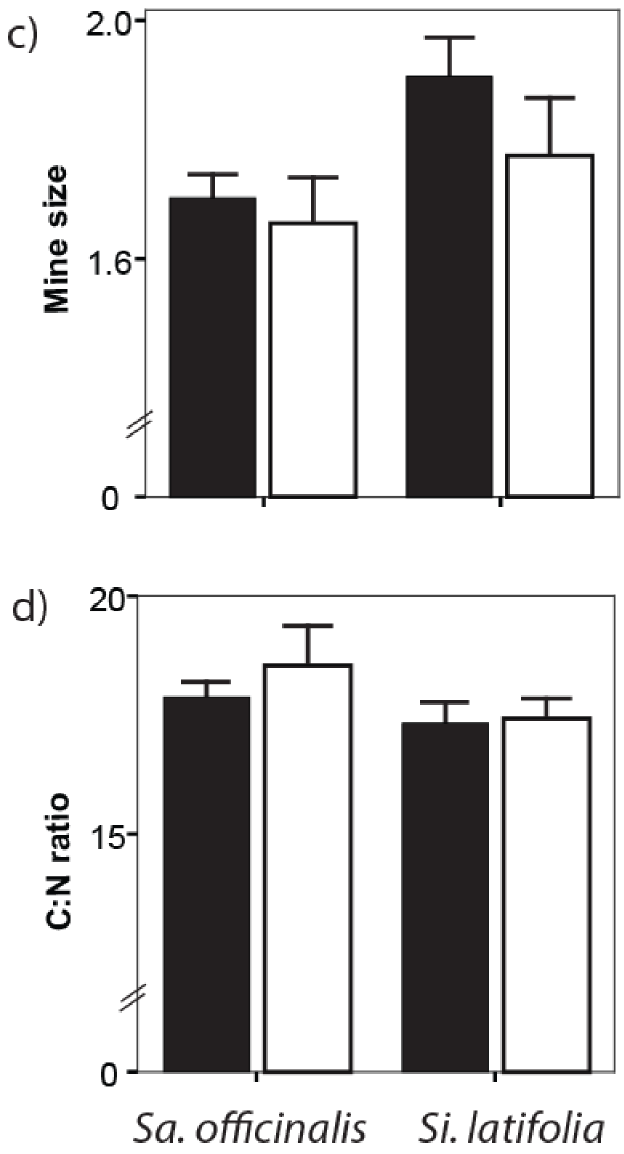


Figure 5.2: Development time (a), body size (b: measured as thorax length), mine size (c), and leaf C:N ratio (d) of larvae survived to adult on *Sa. officinalis* (*n* = 78) and *Si. latifolia* (*n* = 67) in caged (closed bars) and exposed (open bars) treatments. Error bars indicate standard error. Asterisks represent significance at $P < 0.01$ between caged and exposed treatments within each host species.

Table 5.1: Standardized selection gradients ($\beta_{avggrad}$) for the four focal traits analyzed separately for *Si. latifolia* and *Sa. officinalis*.

host	trait	$\beta_{avggrad,}$	Z	P
Si. latifolia	Development time	**-0.080**	**-2.21**	**0.027**
	Body size	-0.014	-0.06	0.95
	Mine size	-0.047	-1.72	0.084
	Nitrogen level	0.007	-1.25	0.21
Sa. officinalis	Development time	**-0.067**	**-3.07**	**0.002**
	Body size	0.002	-0.16	0.87
	Mine size	0.006	-0.23	0.81
	Nitrogen level	0.024	0.39	0.69

Table 5.2: Results of analysis showing differences in selection gradients between host plant species, as indicated by host species x trait interactions. The analysis was conducted with both host species combined.

	Estimate	Std. error	Z	P
Development time	0.16	0.14	1.18	0.23
Body size	-1.53	2.23	-0.69	0.49
Mine size	-0.16	0.16	-1.03	0.30
Nitrogen level	-0.027	0.022	-1.21	0.23

Table 5.3: Selection differentials (s') for the four focal traits analyzed separately for *Si. latifolia* and *Sa. officinalis*.

host	trait	s	s'	F	P
Si. latifolia	**Development time**	**-0.35**	**-0.37**	**6.59**	**0.011**
	Body size	-0.001	-0.07	0.014	0.905
	Mine size	-0.13	-0.16	1.31	0.253
	C/N ratio	0.11	0.02	0.027	0.87
Sa. officinalis	**Development time**	**-0.38**	**-0.49**	**11.88**	**0.001**
	Body size	0.001	0.04	0	0.98
	Mine size	-0.04	-0.07	0.25	0.618
	C/N ratio	0.67	0.13	0.72	0.40

Table 5.4: Results of analyses showing differences in selection differentials between host plant species, as indicated by host species x caging treatment interactions. The analysis was conducted with both host species combined.

	DF	*F*	*P*
Development time	591	0.13	0.72
Body size	588	0.01	0.92
Mine size	564	0.37	0.55
CN	580	0.29	0.59

Table 5.5: Pair-wise phenotypic correlations between life history traits of *A. flavifrons*, and the relationship between the life history traits and C: N ratios. Values in the upper half of the table indicate standardized correlation coefficients when *A. flavifrons* developed on *Sa. officinalis*, and the lower half when they developed on *Si. latifolia*. ***P<0.001

	Dev. time	Body size	Mine size	C:N ratio
Dev. time		-0.085	-0.04	0.26***
Body size	-0.02		0.03	-0.045
Mine size	0.03	0.01		0.026
C:N ratio	-0.29***	-0.36***	0.39***	

Chapter 6

Conclusion

The aim of this dissertation was to investigate multiple ecological processes influencing the evolution of diet breadth in herbivorous insects. Using a tritrophic system involving host plants (*Silene latifolia* and *Saponaria officinalis*), *A. flavifrons* (*Amauromyza flavifrons*), and parasitoids *A. flavifrons*, I tested three major hypotheses, including preference-performance, plant availability and enemy-free space hypotheses, which together explained the pattern of host plant use in *A. flavifrons*.

The common garden experiment showed that females preferentially oviposited on *Si. latifolia* over *Sa. officinalis*. Consistent with the preference-performance hypothesis (PPH), larval survival was greater on *Si. latifolia* than on *Sa. officinalis* in the absence of natural enemies. Increased larval mortality on *Sa. officinalis* could possibly be due to both constitutive and induced defense chemicals specific to the host species (Chapter 2).

The mortality on *Sa. officinalis* was even greater in the presence of natural enemies, supporting the enemy-free space (EFS) hypothesis. While host species were not intrinsically different in the attractiveness to the parasitoids, parasitism rates were significantly lower in the natural patches of *Si. latifolia* compared to *Sa. officinalis*. One potential explanation for this difference is that parasitoid abundance was lower in *Si. latifolia* patches because parasitoid populations cannot accumulate due to their ephemeral nature (Chapter 4). Parasitism on *A. flavifrons* did not occur randomly, but selected against individuals with longer larval development time (Chapter 5). Thus, parasitism is likely to play a significant role in shaping the evolution of oviposition preference as well as life history traits of *A. flavifrons*.

Although leafminer larvae suffer higher mortality on *Sa. officinalis* compared to *Si. latifolia*, natural patches of *Sa. officinalis* supported higher leafminer density than *Si. latifolia* patches. The resource concentration hypothesis may explain this pattern: patches of *Sa. officinalis* were denser and seasonally more stable than *Si. latifolia* patches, suggesting that ovipositing females may locate *Sa. officinalis* patches more efficiently than *Si. latifolia* patches (Chapter 3).

Overall, *A. flavifrons* population as a whole continues to use both *Si. latifolia* and *Sa. officinalis* due to a tradeoff in fitness functions: *Sa. officinalis* promotes host searching efficiency, whereas *Si. latifolia* enhances offspring performance. Although more available, the *A. flavifrons* population in southeast Michigan does not specialize on *Sa. officinalis* because when given choice, females prefer *Si. latifolia* over *Sa. officinalis*. And the oviposition preference seems to be maintained due to higher offspring survival on *Si. latifolia*. In natural patches, the risk of mortality inflicted by plant adverse effect was 1.23 fold higher on *Sa. officinalis* than on *Si. latifolia*. The risk of parasitism was even greater: larvae in *Sa. officinalis* patches were 2.26 fold higher risk of parasitism than larvae in *Si. latifolia*. This suggests that natural enemies might impose stronger selection on oviposition preference than plant chemical defense, further supporting the EFS hypothesis.

Si. latifolia patches provide *A. flavifrons* with EFS because the patches are ephemeral and the enemy population cannot accumulate over time. Thus, as *Si. latifolia* becomes more persistent and available across the landscape, EFS is expected to disappear, whereas the strength of EFS increases as the host becomes less available. This interaction between plant availability and EFS might explain why many insect herbivores include rare and ephemeral hosts in their diet (Theiry and Moreau 2005). The extent to which such an interaction broadens the condition of polyphagy can be examined by a modeling approach based on West and Cunningham (2002), but including the third trophic level into consideration.

Due to higher larval survival, females should colonize *Si. latifolia* patches whenever the plants are available to take advantage of the higher offspring fitness. In the absence of costs associated with dispersal, one might expect *A. flavifrons* to exclusively use *Si. latifolia* patches in early spring. However, the density of *A. flavifrons* was consistently lower in natural patches of *Si. latifolia* compared to *Sa. officinalis*, suggesting that the cost of dispersal might be substantial. Given the benefit of using *Si. latifolia*, how costly can dispersal be in order to maintain the preference for the host? Future studies will examine the evolution of dispersal in a temporary and spatially heterogeneous environment (McPeek & Holt 1992, Ronce 2008) with *A. flavifrons* as a model system.

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