INFLUENCES OF PEA MORPHOLOGY AND INTERACTING FACTORS ON PEA
APHIDS (ACYRTHOSIPHON PISUM)

A thesis presented to
the faculty of
the College of Arts and Sciences of Ohio University

In partial fulfillment
of the requirements for the degree
Master of Science

Natalie L. Buchman
August 2008
This thesis titled

INFLUENCES OF PEA MORPHOLOGY AND INTERACTING FACTORS ON PEA
APHIDS (ACYRTHOSIPHON PISUM)

by

NATALIE L. BUCHMAN

has been approved for
the Department of Biological Sciences
and the College of Arts and Sciences by

________________________________________
Kim M. Cuddington
Assistant Professor of Biological Sciences

________________________________________
Benjamin M. Ogles
Dean, College of Arts and Sciences
Abstract

BUCHMAN, NATALIE L., M.S., August 2008, Biological Sciences

INFLUENCES OF PEA MORPHOLOGY AND INTERACTING FACTORS ON PEA APHIDS (*ACYRTHOSIPHON PISUM*) (90 pp.)

Director of Thesis: Kim M. Cuddington

I demonstrate that pea plant (*Pisum sativum* L.) architecture only affects the reproduction rate of pea aphids (*Acyrthosiphon pisum* (Harris)) under extreme and variable environmental conditions, and that plant shape does not interact with other mechanisms which control aphid reproduction. Experiments do indicate that aphid reproduction is positively density-dependent. I found that reproduction was decreased on a pea morphology with no tendrils and many small leaves but only when warmer temperatures were experienced in a greenhouse in April. I speculate that environmental conditions similar to my findings may be the explanation for previously reported effects. In addition, plant morphology and prey distribution did not have an effect on the consumption of pea aphids by lady beetles. However, there was a significant relationship between the plant surface area and predation rate.

Approved: _____________________________________________________________

Kim M. Cuddington

Assistant Professor of Biological Sciences
Acknowledgments

I would like to thank Dr. Kim Cuddington for all of her guidance throughout my project and also for comments on my thesis, Dr. Kelly Johnson, Dr. Willem Roosenburg and Janet Duerr for all their helpful suggestions, all my undergraduate research assistants for helping with experiments and laboratory maintenance. This research was funded by the National Science Foundation (DEB 0516031).
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Chapter 1: Introduction and Literature Review

While several authors have investigated the effects of plant morphology, aphid density, environmental conditions or plant nutritional status on herbivore success, few have explored potential interactions among these factors. The main objective of my thesis research was to determine what mechanisms interact with plant morphology to affect insect herbivores and their predators. Using a pea aphid (*Acyrthosiphon pisum* (Harris)) and pea plant (*Pisum sativum* L.) system, I examined the effect of plant architecture, aphid density, environmental variation and plant nutrient availability on aphid reproduction. I also explored the vulnerability of pea aphids to predators such as multicolored Asian lady beetles (*Harmonia axyridis*) on different plant morphologies. In this chapter, I review the literature on these topics. In chapter 2, I describe experiments that were designed to examine the effect of plant architecture on aphid reproduction and the potential interactions between plant architecture, aphid density, environmental conditions and plant nutrients. Finally in chapter 3, I describe experiments to investigate the effect of plant morphology on the interaction between pea aphids and multicolored Asian lady beetles.

**Literature Review**

Investigations of the effects of plant architecture mainly focus on plant-herbivore-predator interactions and how they shape herbivore population dynamics. However, there is a large literature on the effects of plant nutrients on herbivorous insects. In the following, I will review the impacts of plant architecture, and related factors, on herbivorous insects. There are few authors who have investigated the effect of plant...
architecture on herbivorous insects. Even fewer authors have investigated the interactions of plant architecture with other factors such as environmental conditions and plant nutrients.

Throughout the thesis, I will use the terms spatial structure or architecture to refer to the size and design of a plant. Lawton’s (1986) concept of plant architecture includes size, growth form, seasonal development, persistence and variety of above-ground parts. The connectivity of these plant parts is another important aspect of architecture (Andow and Prokrym 1990). Architectural characteristics such as stem and leaf dimension, branching angles, surface complexity and canopy spacing might influence insect herbivores and their predators. In this thesis, I focus on plant size, as estimated by surface area, and architectural features of plants. Fractal dimension estimates have been demonstrated to be an effective way to characterize plant architecture (Morse et al. 1985, Gunnarsson 1992, Gee and Warwich 1994). The fractal dimension gives an indication of how completely an object appears to fill space.

I focus on pea plants and pea aphids: the study system used for my thesis project. First, I will provide a brief overview of the effects of plant architecture on insect herbivore performance. Second, I will examine the effect of environmental conditions, with an emphasis on temperature, on insect herbivore reproduction. Next, I will summarize the effects of plant nutrients on insect herbivore reproduction, and examine the interactions between plant architecture and nutrients. Finally, I will review the effect of plant architecture on predator-prey interactions. I conclude with a discussion of contradictory results found by different researchers working in the pea plant-pea aphid
system and I suggest that these divergent findings are due to different methodologies, possible variation in plant nutrients and the various environmental conditions in the different studies.

**Effects of plant architecture on insect herbivores**

Reported effects of plant architecture on herbivorous insects include changes in abundance, fecundity, population growth rate and spatial distribution. Interpretation of the reported findings are complicated by the fact that some studies report effects of architectural differences between host plants of different species, while other studies report effects of architectural differences of host plants of the same species. Thus, reported effects of architecture pertaining to different plant species may be due to other factors which differ between species such as plant nutrition. However, authors who have focused on the same species of plant have also found effects of different architectures on herbivorous insects. While I will describe some of the findings regarding different host plant species, I focus on the reports on peas.

Differences in plant architecture between different species of plants have an effect on insect population abundance. In a study on different species of British Umbelliferae that differed architecturally (finely, divided leaves or broad, undivided leaves), it was found that leaf-mining agromizid flies were more abundant on the species with broad, undivided leaves (Fowler and Lawton 1982). Cartier (1963) found greater pea aphid abundance on short pea plants as compared to tall pea plants. Cartier (1963) speculated that the decreased spacing between internodes and more clumped leaves on shorter plants provided more protection from weather, predators and other adverse conditions. Other
authors who investigated the effect of architecture on pea aphid performance found a
differed significantly between red clover, alfalfa, and broad bean, but found smaller
differences across five pea genotypes that differed architecturally (Sandstrom and
Pettersson 1994). These authors found highest aphid performance (survival, pre-
reproductive period, weight, fecundity and population growth rate) on peas, followed by
broad bean, alfalfa and red clover, respectively.

Fecundity, population growth rate and longevity of herbivores may be affected by
differences in architecture between plants of the same species. Sandstrom and Pettersson
(1994) found a reduction in fecundity and population growth rate of pea aphids on
leafless genotypes of peas as compared to other genotypes, and Karieva and Sahakian
(1990) found that on near-isogenic lines of peas there was slightly reduced population
growth of aphids on leafless plants compared to normal plants in cage and field
experiments. In a laboratory study on six morphologically different cultivars of peas, two
cultivars had significantly decreased fecundity, population growth rate and longevity of
aphids compared to four the other cultivars (Soroka and MacKay 1991). These
performance parameters were most reduced on a semi-leafless cultivar of peas (normal
stipules but with leaflets reduced to tendrils). In a field study, Soroka and MacKay
(1990) also examined the effects of different cultivars of field peas with differing
morphology on the abundance of pea aphids. They found that there was a significantly
lower density of aphids on a semi-leafless cultivar as compared to the other cultivars.
The authors suggested that this cultivar provided less preferred area for the aphids to
colonize and feed, and that the openness of the canopy increased aphid exposure to
adverse weather conditions and natural enemies. Pea aphids prefer to feed on stem terminals and abaxial surfaces of leaves (Lowe 1971, Muller 1984), so a semi-leafless architecture would provide less of these preferred areas. In contrast, Legrand and Barbosa (2000) reported no significant effect of plant architecture on reproductive rates or population growth rate of pea aphids, but did report some impact on longevity. In conclusion, some studies have found that plant architecture plays an important role in affecting herbivores (Karieva and Sahakian 1990, Soroka and MacKay 1991) while others have not (Legrand and Barbosa 2000). It is possible that these contrasting results arose because the researchers used pea varieties that differ in nutrient availability or that the different environmental conditions in the various studies interacted with plant morphology. For example, Legrand and Barbosa (2000) conducted a laboratory study and found no effect of architecture on pea aphid performance in near-isogenic peas, while Soroka and MacKay (1990, 1991) found a significant effect of architecture between different cultivars in field and laboratory studies.

**Effects of temperature on insect herbivores**

Development, reproduction, survival and behavior of insects are influenced by temperature (Dixon 1973, Bursell 1974), as are population dynamics and seasonal occurrence (Campbell et al. 1974, Logan et al. 1976, Schowalter 2000). Aphids, like all organisms, have an optimal temperature for development rate and reproduction, and any deviation from this optimum causes development time and reproduction to be reduced (Dixon 1973). When insects are reared at their upper temperature threshold, they develop more slowly compared to when they are reared in a favorable environment (Sharpe and
DeMichele 1977). In pea aphids it has been reported that population growth and survival are negatively affected by temperatures above 25°C (Kenten 1955, Barlow 1962, Campbell and Mackauer 1975). Dunbar et al. (2007) have shown that at high temperatures (35°C), symbiotic gut bacteria are killed, which causes aphids to become sterile due to the lack of essential nutrients for reproduction.

Ambient temperature can also interact with host plant characteristics to determine development, fecundity and mortality (Morgan et al. 2001). The structure of a plant could cause increased or decreased exposure to the effects of air temperature. For example, a plant with small leaves could allow greater exposure of the aphids to extreme temperatures that may adversely affect performance parameters of the herbivores. Soroka and MacKay (1990) found a reduction in aphid density on a semi-leafless variety compared to leaved cultivars, and speculated that this effect was due to increased exposure to adverse weather conditions.

**Effect of nutrient availability on herbivores**

The nutrient levels in a plant can vary greatly and are affected by soil fertilization, irrigation and light (Bernays and Chapman 1994). Many studies have focused on the importance of plant nutrients and/or secondary compounds and how they affect feeding behavior and performance of insect herbivores (Montlter 1991). Plant nutrients determine if an insect will accept and colonize a host, and once a plant is colonized determine insect growth, development and fecundity (e.g., Facknath and Lalljee 2005).

In general, phloem contains low levels of nitrogen and a high ratio of carbon to nitrogen. Therefore sap-feeders are nitrogen-limited (Auclair 1963), and the
concentration of nitrogen is thought to be a key factor in individual growth rates, fecundity, survival and population growth (Auclair et al. 1957). For example, early studies have found that *Myzus persicae*, the green peach aphid, detects and responds to various amino acids in artificial diets (Mittler 1967). In a study on pea aphids (*A. pisum*), ingestion rates increased in diets that contained added amino acids compared to diets without them (Srivastava and Auclair 1974).

Nitrogen fertilization in laboratory and field conditions has been used to determine the effect of nitrogen availability on plants and how it affects sap-sucking insects (Montliler 1991). Sudderth et al. (2005) were interested in determining if the population growth of * Macrosiphum euphorbiae*, the potato aphid, is affected by nitrogen fertilization in the presence of elevated levels of CO₂ on two host plants (*Solanum dulcamara* and *Amaranthus viridis*). In both plant species, enriched nitrogen caused an increase in aphid survival. The aphids also grew to a larger size, developed more rapidly and achieved higher densities. Nevo and Coll (2001) investigated the effect of nitrogen fertilization on the color, size, fertility and population growth rate of *Aphis gossypii*, the cotton aphid, on cotton plants. These researchers found that density, population growth rate, size and darker coloration were increased with nitrogen fertilization. In field experiments, it was also found that *A. gosypii* reached higher densities on high nitrogen fertilized cotton plants compared to low nitrogen fertilized plants (Cisneros and Godfrey 2001). Honek (1991) examined the effect of nitrogen fertilization of winter wheat and winter barley on the abundance of two cereal aphids, *Metopolophium dirhodum* (Walker) and *Sitobion avenae* (F.). The abundance of *M. dirhodum* increased with increased
nitrogen dose, while nitrogen had no effect on *S. avenae*. This researcher suggested that nitrogen supply alters within plant resource allocation, which caused an increase in the abundance of the leaf-feeding aphid, but produced no effect on the ear-feeding aphid. In conclusion, these studies suggest that nitrogen fertilization can have positive effects on herbivore performance.

Another focus regarding plant nutritional impacts on herbivorous insects has been on the effect of different ratios of nutrients (Jansson and Ekbom 2002). A deficiency of one nutrient, such as potassium, can lead to increased amount of another nutrient, such as nitrogen, and could have a positive affect on aphid performance (van Emden 1966). High concentrations of carbohydrates can have a negative effect on herbivores, because they can dilute other nutrients (Bartlet et al. 1990). An investigation of the role of nitrogen and potassium fertilization on the reproductive rates of two species of aphids (*Brevicoryne brassicae* and *Myzus persicae*) on Brussel sprouts found that an increase in nitrogen and decrease in potassium caused an increase in soluble nitrogen in the plants. This change in nutrients increased fecundity and the reproduction rate of *M. persicae*, but *B. brassae* showed a lower response to these treatments (van Emden 1966). Anwar et al. (1998) also investigated the effect of fertilizer application of four varieties of *Brassica* on aphid density. They found that the application of nitrogen fertilizer increased aphid density whereas the application of both nitrogen and phosphorus suppressed the attack of aphids. Both studies suggest that application of the appropriate nutrient ratios can be used to manage aphid density. Huberty and Denno (2006) investigated the effect of different soil levels of nitrogen and potassium on the performance (survival, growth,
development time and population density) of two phloem feeding planthoppers
(*Prokelisia dolus* and *Prokelisia marginata*) on *Spartina*. Plants that were given nitrogen
enrichment had a positive impact on both planthopper species as compared to nitrogen
deficient plants. All of these studies suggest that herbivores are directly affected by the
nutritional changes in plants caused by fertilization.

A very few researchers have examined on how different cultivars may differ in
nutrients which affect herbivorous insects. Bentz and Townsend (2001) investigated the
role of leaf nutritional content of two red maple clones, a Freeman maple cultivar, two
elm cultivars and an American elm clonal selection on the feeding injury and
performance of *Empoasca fabae* (Harris), the potato leafhopper (Hemiptera:
Cicadellidae). They found that there was a positive linear relationship between the
number of eggs laid and the foliar nitrogen and phosphorus content. Auclair et al. (1957)
examined the relative resistance of several varieties of peas to pea aphid infestation.
They found that varieties that are more susceptible to pea aphid infestation had a higher
concentration of free and total amino acids than the resistant ones. The authors suggest
that resistant varieties with lower concentrations of amino acids cause a reduction in the
growth and reproduction of aphids which would lead to a decrease in infestation.

There are several ways that plant nitrogen is measured. Total nitrogen is a
measure which includes soluble and insoluble, free amino acids and nitrogen in nucleic
acids. Amino nitrogen includes polypeptides and proteins, nonprotein amino acids, free
amino acids, while a measure of soluble nitrogen includes free amino acids, amides,
nitrates, ammonium and small peptides. The quantity of free amino acids is also used to
estimate plant nitrogen. When investing the effect of nitrogen on aphid performance researchers have used a variety of measures ranging from total nitrogen to free amino acids in phloem sap. However, it has been shown that percent total nitrogen, water-soluble nitrogen and amino nitrogen are linearly related (Maltais 1951).

**Effects of plant architecture and nutrient availability on insect herbivores**

This literature review indicates that plant architecture and nutrients both can have an impact on insect herbivores (see above). Many studies have focused on these effects singly, but only one study, to my knowledge, reported the effects of both architecture and nutrients on insect herbivores. Researchers that have addressed this interaction find that morphology and nutritional factors may not interact to alter the success of sap-sucking insects. Sandstrom and Petterson (1994) investigated the performance of 5 pea aphid clones on 5 pea genotypes. The authors found that total amino acid concentrations were not significantly different between the pea genotypes. Therefore, the amino acid composition of the pea genotypes could not explain the differences in the performance of the pea aphid genotypes. On leafless pea genotypes, fecundity and population growth rate of aphids were reduced. This study indicates that plant architecture could be influencing the performance of the pea aphid clones independent of nutrient availability. Since there has been only one study on this topic, it is unclear if this lack of interaction is generally true for pea cultivars, or whether unmeasured differences in nutrient availability have driven previous reported findings about affects of pea architecture.

**Density dependence and plant architecture**

Plant architecture can affect density-dependent processes which regulate insect
herbivore populations. To some extent architecture determines the surface area of a plant which, in turn, can alter the *per capita* availability of resources for insects. Increasing insect density on a plant that has a reduced surface area could deplete resources, causing individuals on that plant to have reduced survival and reproduction. In addition, the movement rates of herbivores could be altered by plant architecture: some plants are more easy or difficult to travel on. Structures which cause reduced movements could increase crowding.

Historically, there has been controversy about whether density-dependent processes affect insect populations (Nicholson 1954, Andrewartha and Birch 1954, Turchin 1999). It is now generally accepted that density-dependent processes play a role in population regulation (Turchin 1999, Brook and Bradshaw 2006). A density-dependent factor is any factor in which its adverse effect or beneficial influence increases or decreases as the population increases in density (Price 1984). Both time series analyses and density manipulation experiments have demonstrated that density-dependent processes affect insect herbivores (Hassell et al 1989; Woiwood and Hanski 1992, Underwood and Rausher 2000; Agrawal et al. 2004, Miller 2007).

Factors such as the plant architecture, nutrients and secondary compounds could alter density-dependent processes in insect populations by affecting the availability of resources (Underwood and Rausher 2000, Agrawal 2004, Agrawal et al. 2004, Rhainds and Messing 2005, Miller 2007). Underwood and Rausher (2000) conducted density manipulation experiments on *Epilachna varivestis*, the Mexican bean beetles, on four different soybean genotypes. These authors found that beetle population growth rate was
different among the plant genotypes. They suggested that this effect was due to differences in host plant quality. Other investigators have directly measured or characterized plant features that might alter density-dependent processes. Rhainds and Messing (2005) found that *Aphis gossypii* (Glover) are regulated by density-dependent processes on taro, *Colocasia esculenta*. On larger leaves, high densities of aphids were found, suggesting that surface area availability can influence colonization and reproduction. Agrawal (2004) found negative density dependence of the specialist aphid, *Aphis neni*, on 17 of the 18 species of milkweed investigated. The maximum population growth rate was well predicted by the variation in plant secondary compounds, trichomes, leaf carbon and nitrogen concentrations and seed mass, but the strength of density dependence was unrelated to nitrogen levels and secondary compounds. He suggested that in aphid populations, plant characters may determine the growth rate and slope of density dependence. Miller (2007) also found that the strength and shape of density dependence of the cactus bug *Narnia pullidicornis* varied with the proportion of meristems of the host plants (*Opuntia imbircata*). This author found higher cactus bug densities on host plants that had a high proportion of meristems allocated to reproduction compared to host plants with lower proportion of meristems, an important plant quality trait. These studies suggest that the sign and strength of density dependence and long-term herbivore dynamics are strongly influenced by their host plant traits such as architecture and trichome (fine outgrowth or appendage on plants) density.

**Plant architecture effects on predation rate**

Plant architecture also can play a very important role in predator-prey
interactions, and so alter populations of herbivorous insects. Plant architecture can influence predation rates by modifying the foraging behavior of predators (Carter et al 1984, Grevstad and Klepetka 1992), and altering the availability of refugia for prey (Hawkins et al 1993), thus indirectly modifying herbivore population density.

In a study on lacewings on different architectures of perennial grasses, it was found that the differing architectures modify the ability of the predators to access the prey species (Clark and Messina 1998). It has been suggested that the size of the predator may affect access to prey (Kauffman and LaRoche 1994). Messina et al. (1997) investigated the effectiveness of lacewing larvae and lady beetles at reducing *Diuraphis noxia*, wheat aphid populations, on two range grass species, crested wheat grass *Agropyron desertorum* and Indian ricegrass, *Oryzopsis hymenoides*. Crested wheat grasses provide aphids with potential refuges due to tightly rolled leaves. The authors found that both predators were less effective on crested wheat grass compared to the Indian ricegrass. The larger predator, lady beetles, had a greater reduction in predation on crested wheat grass compared to the smaller lacewing predators. The authors suggested that the smaller lacewing larvae were better at accessing aphids in the rolled leaves.

Predator movement is sometimes altered by plant architecture, and this alteration may affect prey consumption rates. Grevstad and Klepetka (1992) investigated the effects of four architecturally different, but closely related, crucifers on the predation efficiency of 5 species of lady beetles on *Brassica* oleracea, cabbage aphids. Plant morphology had a significant effect on overall predation rate, predator mobility, falling frequency and prey accessibility. One of the cultivars had a 78% higher predation rate
compared to another cultivar, because predators could not grip the surface of the leaves. Legrand and Barbosa (2003) examined the effects of plant architecture in near-isogenic peas on the predation efficiency of adult lady beetles consuming pea aphids. The authors saw a decrease in foraging efficiency on the pea that had many tiny leaves compared to the normal compound leaf morph. On the tiny leaved morph, the lady beetles spent a greater amount of time searching for aphids than on the normal morph.

Smaller scale features may also affect predator-prey interactions. Predators have increased predation efficiency on peas with reduced wax blooms (White and Eigenbrode 2000, Rutledge et al. 2003, Chang et al. 2006). Karieva and Sahakian (1990) found that coccinellid beetles controlled aphid population growth better on leafless peas than on normal pea plants. These authors suggested that the slippery leaves of the normal morph caused increased falling frequency of the beetles, which caused a decrease in predation efficiency on the normal morph. Carter et al. (1984) investigated the role of plant architecture on searching efficiency of coccinellid larvae on pea and bean plants. These authors found that the predation rate of the larvae was significantly lower on the pea plants compared to the bean plants. This effect of architecture was due to the increased tendency of the predatory larvae to fall off the smooth pea leaves. Coll and Ridgeway (1995) found that the predation rate of minute pirate bugs on thrips differed greatly between tomatoes, peppers and beans. Minute pirate bugs consumed more prey on bean plants compared to tomatoes. These authors suggest that leaf trichomes on the tomato plants interfere with predator searching ability. All of these studies indicate that predator foraging efficiency is strongly influenced by plant architecture.
Conclusions

My review of the literature reveals that while several authors have examined the effects of plant morphology, environmental conditions or plant nutritional status on herbivore success, very few have examined an interaction between these factors. The interaction between predation success, plant morphology and herbivorous insects has been more thoroughly investigated. In some cases, there are divergent results reported. For example, Karieva and Sahakian (1990) and Soroka and MacKay (1990, 1991) found an effect on plant morphology on pea aphid performance while Legrand and Barbosa (2000) did not. I suspect that some of these differences occur because of different methodologies employed, different environmental conditions or differences in plant nutrition. Soroka and MacKay (1990, 1991) used different cultivars of peas which could differ in nutritional content. In addition, these authors conducted a field study where the aphids could be experiencing different environmental conditions on the different morphologies. However, Legrand and Barbosa (2000) used near-isogenic lines of peas in a single cultivar which could minimize differences in nutritional content between the morphologies. Moreover, this study was conducted under laboratory conditions. The aim of my thesis project was to understand more completely the possible interactions of plant architecture with other mechanisms that alter herbivore populations and so explain the different effects reported in the literature.

Abstract

The effects of and interactions between plant architecture, aphid density, environmental conditions and nutrient availability on the reproduction of pea aphids (Acyrthosiphon pisum (Harris)) were investigated using four near-isogenic peas (Pisum sativum) that differ in morphology. Manipulations of aphid density (1, 5 and 10 adults per plant) were used to examine the effects of plant morphology on crowding and consequently, reproduction. It was hypothesized that the greatest crowding and lowest reproduction would be found on leafless peas at high aphid densities. The possible interaction between pea morphology and the effects of temperature and environmental exposure on pea aphid reproduction were investigated by conducting experiments in greenhouse. Finally, peas were fertilized with nitrogen at different levels to determine if differences between nutrient availability of the four different pea morphologies might drive any observed differences in aphid reproduction. Pea morphology did not alter pea aphid reproduction or crowding, as measured by mean nearest neighbor distance. Reproduction did increase with increasing adult density indicating positive density dependence. Pea morphology did have an affect on aphid reproduction under greenhouse conditions were temperature were more extreme and variable. The pea aphids on a pea morph with many small leaves had a significant reduction in the number of offspring compared to a pea type with large leaves and no tendrils. This effect was possibly due to
increased exposure to high temperature and direct sunlight on the small-leaved plant. While plants responded to fertilization treatments, this did not have an impact on aphid reproduction. I conclude that plant morphology only influenced aphid reproduction when environmental conditions were extreme, nutrient availability did not affect aphid reproduction, and there was some impact of initial adult aphid density on reproduction.

**Introduction**

Many studies have focused on how herbivore density (e.g. Underwood and Rausher 2000, Agrawal 2004), environmental conditions (e.g. Dixon 1973) and the phytochemistry of plants (e.g. van Emden 1966, Bentz and Townsend 2001) affect herbivore performance, but the physical properties of plants can also affect insect herbivore growth, reproduction and survival (Karieva and Sahakian 1990, Soroka and MacKay 1990). My main objective was to investigate how plant architecture, aphid density, environmental conditions and available nutrients of pea plants (*Pisum sativum*) affect the reproduction of pea aphids (*Acyrthosiphon pisum*). Researchers working with this system have reported contradictory findings. While some authors report strong effects of pea morphology on aphid reproduction (Soroka and MacKay 1990), others find little or no impact (Legrand and Barbosa 2000). This variation in results is possibly due to differences in methodologies, or to uninvestigated interactions between morphology and environmental conditions or plant nutrients. I aimed to find a clearer answer by simultaneously investigating several possible mechanisms.

Plant architecture is the size and design of a plant. The two major determinates of plant morphology are the spread of plant tissue through different positions in space, and
the variety of plant structures (Lawton 1986, Cloyd and Sadof 2000). Stem and leaf dimension, branching angles, surface complexity and canopy spacing are all important architecture characteristics of a plant that might influence insect herbivores. For example, plant architecture partly determines the surface area that is available to an insect herbivore, which in turn determines the availability of resources.

Some studies have found that fecundity, population growth rate and longevity of herbivores are affected by plant architecture. In laboratory studies on different cultivars of field peas, Soroka and MacKay (1991) found that aphids had reduced performance on a semi-leafless cultivar than on leaved varieties. Sandstrom and Pettersson (1994) also found a significant reduction in fecundity and population growth rate of aphids on leafless cultivars of peas as compared to other varieties. However, Karieva and Sahakian (1990) found that there was only a slight insignificant reduction in population growth of aphids on leafless plants compared to normal plants in cage and field experiments. Moreover, Legrand and Barbosa (2000) reported no significant effect of plant architecture on reproductive rates or population growth of pea aphids, but did report some impact on longevity.

Plant surface area can be altered by plant architecture: it is possible that reported effects of morphology are actually due to differences in the density of aphids driven by differences in surface area on different plants, and consequently, the strength of any density-dependent regulation of aphid growth parameters. Moreover, it is possible that the effects of morphology on the surface area of peas differed with the different cultivars used in these various studies. In many different species of aphids, population growth is
strongly affected by density (Underwood and Rausher 2000, Agrawal 2004, Rhainds and Messing 2005). For example, density-dependent processes regulate the population growth of *Aphis gossypii*, the melon aphid, on taro (Rhainds and Messing 2005). Higher densities of aphids were found on larger leaves, suggesting that surface area availability can influence colonization and reproduction.

None of the authors working with the pea plant-pea aphid system measured the surface area of the plants used. The different experimental methods used in the different studies also produced widely varying aphid densities. For example, Legrand and Barbosa (2000) removed aphid offspring every 48 hours, while Soroka and MacKay (1990) allowed aphids to reproduce on plants until death and did not remove offspring. Consequently, the results of Legrand and Barbosa (2000) may reflect a lack of density-dependent effects, while those of Soroka and MacKay (1990) may be related to a significant negative density-dependent effect of plant architecture.

Architecture can also alter the movement rates of herbivores. Structure which causes reduced movements could increase crowding, and therefore alter the effective per capita access to resources. Increased aggregation on the plants can increase intraspecific competition which may increase mortality, lower reproductive success and increase the rate of dispersal to a less dense location (Dixon 1973, Day 1986; Klindmann and Dixon 1996).

More obviously, plant architecture can determine how much shelter is provided by the plant against adverse weather conditions. Aphid performance (Dixon 1973, Bursell 1974), population dynamics and seasonal occurrence of insects are affected by
temperature (Campbell et al. 1974, Logan et al. 1976, Schowalter 2000). The effects of ambient temperature can also interact with host plant characteristics to affect development, fecundity and mortality (Morgan et al. 2001). Host plant characteristics could provide more or less protection from extreme temperature, precipitation, variation in humidity and sunlight. For example, it has been suggested that semi-leafless morphologies of peas can increase pea aphid exposure to these environmental conditions (Soroka and MacKay 1990) due to the openness of the canopy. A comparison of the field study by Soroka and MacKay (1990) and the lab study by Legrand and Barbosa (2000) is complicated by this potential interaction.

Finally, one of the most important plant characteristics for feeding behavior and performance of insect herbivores is the amount of plant nutrients and/or secondary compounds. There is a positive correlation between the amount of nitrogen in plants and the fecundity, reproduction rates and population growth of herbivorous insects (van Emden 1966, Bentz and Townsend 2001, Sudderth et al. 2005). The nitrogen content in phloem is low compared to other plant parts, so aphids must concentrate nitrogen from their diets for growth and reproduction (Auclair et al. 1957). Nitrogen fertilization has been used to determine the effect of nitrogen availability on plants and sap-sucking insects (Montller 1991). Nevo and Coll (2001) found a positive correlation between nitrogen fertilization and population growth rate in the melon aphid, *Aphis gossypii* on cotton plants. This effect can also be seen when aphids are fed on artificial diets. In a study on pea aphids, ingestion rates increased in diets that contained added amino acids compared to diets without them (Srivastava and Auclair 1974).
This relationship between nutrition and fecundity suggests that studies which have reported significant effects of plant architecture on aphid reproduction may have confounded differences in plant nutrition with differences in morphology. Nutrition may vary between plants of different morphologies because photosynthetic energy gained is determined by plant structure. For example, a leafless plant has a reduced area to collect energy compared to a broad leaf morph. It also seems likely that some structures are more energetically costly to construct. Even in studies in which nearly isogenic peas were used (Karieva and Sahakian 1990; Legrand and Barbosa 2000), it is possible that plants with different morphologies differed in nutrient status. As a result, it is unclear whether any observed correlations between plant architecture and herbivore success due to an indirect impact through changes in plant nutrition.

The motivation for this investigation was to determine the mechanisms that interact with plant morphology to alter aphid reproduction. By simultaneously examining effects of architecture, aphid density, environmental conditions and plant nutrients on a single cultivar of near-isogenic peas, I hope to determine the explanation for the contrasting results in the literature. I tested three hypotheses. First I expected reproductive rates to be reduced on leafless mutants at high aphid densities, as reported by Karieva and Sahakian (1990) and others, but that reproduction rates would be unaffected by pea morphology at low aphid densities, as suggested by Legrand and Barbosa (2000). This hypothesis is based on the idea that one impact of morphology is to modify the density-dependent regulation of population growth. Next, I tested the hypothesis that plant morphology may alter aphid reproduction rates only under harsh
environmental conditions, which could explain the discrepancy between field and laboratory studies (e.g. Soroka and MacKay 1990, Legrand and Barbosa 2000). Finally, I tested the novel hypothesis that alteration in aphid reproduction is due to plant morphology, rather than because of indirect effects of plant nutrition.

**Material and Methods**

**Plant material**

The pea plant (*Pisum sativum*) is ideally suited to study the effects of plant architecture because pea morphology is modified by simple, naturally occurring mutations. I used a near-isogenic line of peas that has reduced stipules (*st*), which was obtained from the USDA-ARS Western Regional Plant Introduction Station, Pullman, Washington. The morphologies of peas are altered by mutations in the genes *af* (*afila*) and *tl* (*acacia*). “Normal” (*AfAfTlTl* genotype) has normal, compound leaves with 1 to 2 pairs of leaflets and a number of tendrils. “Tendril” or *af* (*afafTlTl*) has leaflets replaced by tendrils. “Leaflet” or *tl* (*AfAftltl*) tendrils are converted into leaflets. “Parsley” (*afaftltl*) has an interaction between the *af* and *tl* mutations. It has tiny leaflets and branching petioles (Figure 2.1).

**Aphids**

Phloem-sucking species are ideal for examining effects of plant structure on insect herbivores, because these herbivores have little impact on the structure during feeding. For this study, I used pea aphids (*Acyrthosiphon pisum*) from a laboratory population that was founded with individuals purchased from Carolina Biological Supply. Pea aphids are a common pest species on peas. At 20-25°C, aphids mature after
growing 7-9 days and molting four times (Campbell and MacKauer 1975). Pea aphids are parthogenetic and bear live young. An adult produces young one day after maturing and produces 5-15 young per day for approximately 7 days. An adult aphid is about 0.37±0.02 cm long and the widest portion of the body is 0.159±0.017 cm. The high reproductive rate of this species under laboratory conditions is an advantage for experimental study.

**Plant surface area and fractal dimension**

I quantified plant architecture by measuring the surface area and fractal dimension of the four pea morphologies were measured. The fractal dimension characterizes the architecture or morphology of a plant (Morse et al. 1985, Gunnarsson 1992, Gee and Warwich 1994), by giving an indication of how completely an object appears to fill space. It provides a metric for the complexity and irregularity of the given object (Corbit and Garbary 1995). For example, we expect a lower fractal dimension for morphologies that have a linear and branching structure, and a high fractal dimension for those plants with more leaf tissue and less branching.

I produced two-dimensional (2D) projections of the plants by scanning them on an Epson Perfection 3490 photo scanner, and converted the image into binary format. I calculated the fractal dimension from the binary file using the box-counting dimension routine in the Frac-Lac 2004 plug-in for the NIH distributed software package Image-J (http://rsb.info.nih.gov/ij/). The box-counting dimension has been well established as a measure of plant architecture (Gunnarsson 1992, Gee and Warwich 1994). The use of 2D projections of 3D structure is based on the projections theorem (Marstrand 1954), which
states that the fractal dimension \((D_f)\) in \(n-1\) dimensional space is equal to \(D_f - 1\), where \(n\) is the Euclidean dimension. Experimental studies also have shown that the fractal dimension of 2D projections of root systems and plant structure is correlated with the 3D estimates (Nielson et al. 1997, Walk et al. 2004).

I calculated the surface area by analyzing a scan of all the plant tissue, where the tissue was cut into pieces of approximately the same size. Cut pieces were analyzed because uncut plant tissue overlaps on the scanner causing an underestimate of the surface area. Preliminary analysis indicated that tendril plants have a surface area about half that of the other morphologies for all experiments. As a result, I used 2 tendril plants compared to one of the other morphologies for all experiments. The fractal dimension was calculated by analyzing individual cut stem pieces. Scanning the entire uncut plant gives an overestimate of fractal dimension because of plant tissue overlapping on the scanner. On the other hand, scanning the entire plant cut into pieces gives an underestimate because of the increased number of edges included in the estimation.

**Experiment 1: Architecture and density effects on pea aphid reproduction**

Plants were grown in a Conviron growth chamber (Winnipeg, Manitoba, Canada) (16L:8D photoperiod; light level 481.2 μmol; 18:15°C temperature regime) until they were approximately 30 days old (approximately 15.24-20-32 cm tall). I reared pea aphids in the laboratory on fava beans \((Viva faba)\) and peas \((Pisum sativum\) cultivar “Little Marvel”) in mesh rearing containers (Bugdorm II, BioQuip products, Rancho Dominguez, CA). All experimental plants were growth in Fafard 52 mix in 5” deep x 4” wide pots. The temperature in the laboratory ranged from 18-24°C with a photoperiod of
16:8 (L:D). At the end of the growth period, plants were placed in experimental enclosures, which consisted of a circular pot with a piece of circular Styrofoam that had a square pot embedded in it. This pot was used to hold a potted plant. A piece of circular acrylic tubing topped with fine mesh (Anti-Viral insect screen, International Greenhouse Company) enclosed each experimental plant to prevent the escape of the experimental organisms.

In order to determine if pea morphology has a significant impact on reproductive rates through either density-independent or density-dependent effects, I simulated the effects of increased crowding by placing either 1, 5 or 10 adult aphids that were 7 days old (±8 hours) on the peas. The enclosures were placed under lights (16L:8D photoperiod) in the laboratory. These adults were allowed to reproduce for 72 hrs. At this time, the number of offspring and their spatial distribution on the peas were recorded. A total of 25 replicates for each morph were conducted.

To measure the spatial distribution of the aphid nymphs, I took digital photos of a cluster of aphids in a 2x2 cm² sampling area. The aphids within the sampling region were marked with a dot in Adobe Photoshop. The x,y coordinate of each point was calculated using the particle counter in ImageJ. A SAS macro created by Moser (1987) was used to calculate the mean nearest neighbor distances. All statistical analysis was completed in SAS (SAS 2007).

**Experiment 2: Architecture and environmental effects on pea aphid reproduction**

To determine if plant morphology alters exposure to experimental conditions, two sets of greenhouse experiments were conducted. The conditions in the greenhouse were
more variable than those in the laboratory, and therefore aphids experienced higher and lower temperatures. In addition, the pea aphids were subjected to direct sunlight and varying humidity levels. One set of greenhouse experiments was conducted from February 17-20, 2008 with temperatures ranging from 4.4-21.6°C. The second set of experiments was conducted from April 21-24, 2008 with temperature ranging from 11.7-30°C. In both sets of experiments, for ~30 days 6 plants of each of three pea morphs (normal, leaflet and parsley) were grown on a greenhouse bench that had capillary matting and drip lines to supply water (N.B.: There was insufficient seed to conduct experiments on tendril plants). Each of the plants was enclosed with clear acrylic tubing that was topped with anti-viral mesh. Five adult aphids aged 7 days (±8 hrs) were placed on each morph. The adults were allowed to reproduce for 72 hrs on each morph, and then the offspring were counted.

**Experiment 3: Architecture and fertilization effects on aphid reproduction**

I examined aphid reproduction on the 4 pea morphs crossed with a fertilization treatment, to determine if there is an interaction between nutrient availability and plant architecture that may affect pea aphid reproduction. Plants received either no fertilization (control), low (0.22 g/1.1 L per pot) or high (0.88g/1.1 L per pot) levels of ammonium sulphate applied to the soil as a granular form during initial planting. Only nitrogen fertilizer was used because it has been shown that fertilization of peas with phosphorus and potassium is not particularly effective (McKenzie et al. 2001a and b). The plants were grown in a pH Environmental brand growth chamber (16L:8D photoperiod with 18°C:15°C temperature regime) until they were ~30 days old. Adult aphids aged 7 days
(±8 hrs) were placed on each pea morph in the experimental enclosure previously described. Aphids were allowed to reproduce for 72 hrs on each morph and then the number of offspring produced was determined. A total of 8 replicates for each morph were conducted.

To determine plant response to the fertilizer treatments, after the aphids were removed, the experimental plants were then dried for 16 to 20 hrs in a dehydrator. Percent nitrogen was determined by combustion analysis and the total concentration of major elements was determined by acid digestion followed by Inductively Coupled Plasma (ICP) Emission Spectrometry (analysis completed by the STAR lab at the Ohio State University).

**Results**

**Experiment 1: Architecture and density effects on pea aphid reproduction**

*Surface area and fractal dimension*

The surface area of the pea plants was not significantly different between any of the experimental treatments (2-way ANOVA F=1.31; df=3,288; P=0.2168; Table 2.1), which suggests that the area available to the aphids was similar across all four pea architectures.

The fractal dimension of the pea morphologies was significantly different (2-way ANOVA F=97.23; df=3,288; P<0.0001; Table 2.2). A Tukey’s comparison of means test indicated that all four morphologies are significantly different from each other. There was no interaction between the surface area (F=0.60; df=6,288; P=0.7298) or the fractal dimension (F=0.78; df=5,288; P=0.5831) and density treatment.
Pea aphid reproduction

Plant morphology had no significant effect on the per capita reproduction of pea aphids (F=0.30; df=3,288; P=0.8241; Figure 2.2). These results are consistent with those of Legrand and Barbosa (2000) but were generated using a different experimental protocol. There was a significant difference in the number of offspring per adult between the different initial adult densities (F=9.53; df=2,288; P<0.0001). A Tukey’s comparison of means indicated that there was a positive effect of density on the reproduction of pea aphids. There was no significant interaction between morphology and initial adult density (F=0.69; P=0.6594). Levene’s test for homogeneity of variance was significant (F=2.20; df=11,288; P=0.0147), but Welch’s weighted ANOVA indicated that there was a significant effect on mean reproductive rate when the unequal variances were accounted for (F=2.21; df=11,113; P=0.0186). Table 2.3 reports the mean total number of aphids for each of the adult density treatments.

Mean nearest neighbor distance

The tendril morph with an initial adult density of 5 individuals had the highest mean nearest neighbor distance ($\bar{x} = 0.00036\pm 0.00013$ cm), while leaflet with an initial adult density of 1 individual had the lowest nearest neighbor distance ($\bar{x} = 0.00018\pm 0.00021$ cm). However a 2-way ANOVA indicates that plant morphology and initial adult density had no significant effect on the mean nearest neighbor distances (F=0.93; df=7,32; P=0.4974). Therefore plant morphology did not alter aphid crowding.
Experiment 2: Architecture and environmental effects on pea aphid reproduction

Analysis of the February greenhouse data indicated that there was no significant difference in reproduction between the morphologies (ANOVA F=0.7681; df=2,15; P=0.7681; Figure 2.3a). Analysis of the April greenhouse data indicated that there was a significant difference in aphid reproduction between the morphologies (ANOVA F=5.50; df=2,15; P=0.0161; Figure 2.3b). Tukey’s comparison of means indicated that pea aphids on the leaflet morph had a significantly higher reproduction rate compared to pea aphids on the parsley morph.

Experiment 3: Architecture and percent nitrogen effects on pea aphid reproduction

Chemical analysis

Analysis of the untransformed and rank-transformed data indicated that there was a significant effect of fertilization on the percent nitrogen in the pea plants (2-way ANOVA on raw data F=4.89; df=11,72; P<0.0001; 2-way ANOVA on rank transformed data; F=4.51; df=11,72; P<0.0001; Figure 2.4a). The lowest percent nitrogen was in the unfertilized control followed by the 0.22g fertilization treatment and the 0.88g fertilization treatment. The unfertilized and 0.22g nitrogen fertilization treatment did not significantly differ in percent nitrogen between the morphs. However, the leaflet had significantly higher percent nitrogen compared to tendril peas in the high fertilization treatment. Plant morphology or fertilization treatment had no affect on phosphorus content (F=0.88; df=11,47; P=0.5651; Figure 2.4b). The potassium content was significantly different between the morphologies (F=6.36; df=3,47; P=0.0010; Figure 2.4c) but not between the fertilization treatments (F=0.74; df=2,47; P=0.4836). Tukey’s
comparison of means indicated that the tendril morph had significantly higher phosphorus content than leaflet and normal morph. Refer to appendix A for micronutrient analysis.

Surface area and fractal dimension

I was unable to maintain similar surface area across the different morphologies in the fertilization experiment (2-way ANOVA F=3.96; df=3,84; P=0.0108; Figure 2.5a). A Tukey’s comparison of means indicated that leaflet and normal plants were significantly larger than 2 tendril plants. The leaflet morph was also significantly larger than parsley. However, plant surface area did not vary systematically between fertilization treatments (F=0.04; P=0.9623). The fractal dimension of the different peas followed the same pattern as previously observed in Experiment 1 (F=3.47; P=0.0197; df=3,84; Figure 2.5b). Leaflet had the highest fractal dimension followed by normal, parsley and tendril, respectively. However, Tukey’s analysis of the means indicated that there was only a significant difference between the leaflet and tendril morphologies. There was no effect of the fertilization on fractal dimension (F=2.33; df=2,84; P=0.1038).

Aphid Reproduction

Plant morphology and nitrogen fertilization had no significant affect on aphid reproduction (2-way ANOVA, F=1.11; df=11,84; P=0.3611; Figure 2.6). In addition, multiple linear regression indicated that there was no linear relationship between aphid reproduction and percent nitrogen, surface area or fractal dimension (F=0.31; df=3,52; P=0.8179; R²=0.0176; Figure 2.7a,b and c). The peas responded to the fertilization treatment but this did not alter the reproduction of aphids.
Discussion

I conclude that plant morphology only affects pea aphid reproduction when environmental conditions are harsh. Aphid density does have an effect on reproduction: increasing the initial adult density caused an increase in pea aphid reproduction across all pea morphologies, but density and aphid crowding did not interact with plant architecture. Finally, the pea plants responded to nitrogen treatments, but this had no effect on the reproduction of pea aphids. I suggest that previously reported effects could have resulted from interactions between morphology and environmental conditions similar to my findings, or from negative density dependence not investigated in this study.

Plant morphology had no effect on pea aphid reproduction when surface area is controlled (Figure 2.2). These results are consistent with those of Legrand and Barbosa (2000) who also used near-isogenic peas and found no effect of plant morphology on several aphid performance parameters. However these results are inconsistent with several studies which have reported differences in aphid reproduction on different plant morphologies. Karieva and Sahakian (1990) found that aphid population growth was slightly reduced on a leafless pea compared to a normal pea when the density of aphids is high. Soroka and MacKay (1991) and Sandstrom and Petterson (1994) reported that pea aphid fecundity and longevity was significantly reduced on a semi-leafless cultivar.

In my study, increasing the number of adult aphids caused increased reproduction or positive density dependence. Agrawal et al. (2004) also found positive density dependence in a study on milkweed aphids on 18 species of milkweed in the field.
However these authors did not identify the possible causes. I speculate that this effect may have resulted from increased resource flow due to increased aphid feeding.

I found that plant morphology alters aphid reproduction under greenhouse conditions. In the February greenhouse experiments there was no effect of plant morphology on aphid reproduction, but in the April greenhouse experiment there was a significant effect of morphology on aphid reproduction. The aphids on the leaflet morph had significantly higher reproduction compared to the parsley morph (Figure 2.3b). These experiments suggest that the leaflet morph could be providing some protection against high temperatures and direct sunlight, possibly preventing desiccation or sterilization that occurred on the parsley morph. Soroka and MacKay (1990) also suggested that the semi-leafless cultivar provided more exposure to adverse weather conditions due to the openness of the canopy.

Plant structure and percent nitrogen did not have an effect on pea aphid reproduction. The pea plants did respond to the different fertilization treatments, and the different morphologies responded in different ways. The highest fertilization treatment (0.88g N) had the highest percent nitrogen in all of the morphologies compared to the control and the 0.22g N treatment. In the fertilization treatments, the leaflet morph had the highest percent nitrogen followed by normal, parsley and tendril, respectively (Figure 2.5a), although percent nitrogen did not differ between morphs in the control and 0.22g nitrogen treatments. However pea aphids did not respond to changes in plant nitrogen: there was no relationship between reproduction and the percent nitrogen. Phosphorus did not differ between morphologies or fertilization treatments. Potassium was significant
different between the morphologies, the tendril morph had significantly higher potassium then normal and leaflet morphs. However pea aphids did not respond to changes in plant potassium: there was no relationship between reproduction and plant potassium content. These results are inconsistent with other studies that found a positive affect of nitrogen on herbivore growth, development and reproduction (van Emden 1966, Jansson and Ekbom 2002, Sudderth et al 2005), but those studies were not conducted on nitrogen-fixing plants such as peas. It may be that the aphids are not significantly nitrogen-limited on these types of plants and thus there is no impact of nitrogen on reproduction. In support of this hypothesis, I note that the nitrogen content of my plants was a little higher than the nitrogen content found in a pea variety that is susceptible to aphid infestation with a mean total percent nitrogen of 3.128 (Maltais 1951). Similarly, Sandstrom and Petterson (1994) found that amino acid composition did not differ between five pea genotypes and aphid reproduction was not determined by nitrogen levels. Since the results of my study are consistent with those of Legrand and Barbosa (2001) who used an isogenic line of peas, I also suspect that the use of isogenic lines of peas makes it much more likely that nitrogen levels are similar across different morphologies where there is no fertilization.

It is possible that negative density-dependence could have an impact on aphid reproduction that interacts with plant shape, where aphid densities are higher then those investigated here. Such negative density dependence could possibly explain the reported reductions in pea aphid reproduction on semi-leafless cultivars. Since tendril plants (semi-leafless) were observed to have the smallest surface area in my study, they are most likely to produce negative density-dependent controls of reproduction at higher
aphid densities than those that I investigated. It is possible that the effects observed by other authors were driven by negative density-dependence that differed between morphs, but which was not observed in my study because of lower aphid densities. However, the mean nearest neighbor distances measured in this study were not significantly different between the morphologies. Therefore it seems unlikely that the different pea morphologies will have an impact on aphid crowding at higher densities. If surface area is controlled, I predict that negative density dependence will not interact with pea morphology even at high aphid densities.

Finally, my April greenhouse experiment suggests that pea morphology does alter exposure to extreme conditions and therefore alters aphid reproduction. Soroka and MacKay (1990) also suggested a similar explanation for alteration in aphid reproduction across morphologies under field conditions. I conclude that either or both of these mechanisms of environmental exposure which varies with plant morphology and negative-density dependence which depends on plant surface area could explain effects reported by Soroka and MacKay (1990) and Karieva and Sahakian (1990) but that it is unlikely that differences in aphid crowding or plant nutrients between morphologies drive these effects.
Table 2.1: Mean surface area (cm$^2$) (±1 SE) of the four different pea morphologies with three different initial adult densities.

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Adult Density</th>
<th>Surface Area (cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1</td>
<td>233.92±22.71</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>267.41±21.66</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>237.50±18.04</td>
</tr>
<tr>
<td>Leaflet</td>
<td>1</td>
<td>253.40±25.24</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>236.61±16.41</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>221.89±18.32</td>
</tr>
<tr>
<td>Parsley</td>
<td>1</td>
<td>234.12±20.73</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>202.10±23.48</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>171.51±21.10</td>
</tr>
<tr>
<td>Tendril</td>
<td>1</td>
<td>233.93±24.66</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>215.42±27.39</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>214.92±22.86</td>
</tr>
</tbody>
</table>
Table 2.2: Mean fractal dimension (±1 SE) of the four different pea morphologies.

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Adult Density</th>
<th>Fractal Dimension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1</td>
<td>1.5423±0.0135</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.5411±0.0142</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.5356±0.0134</td>
</tr>
<tr>
<td>Leaflet</td>
<td>1</td>
<td>1.5994±0.0072</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.6001±0.0086</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.5994±0.0083</td>
</tr>
<tr>
<td>Parsley</td>
<td>1</td>
<td>1.4903±0.0010</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.4841±0.0134</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.4700±0.0148</td>
</tr>
<tr>
<td>Tendril</td>
<td>1</td>
<td>1.4610±0.0149</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.4353±0.0133</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.4115±0.0147</td>
</tr>
</tbody>
</table>
Table 2.3: Mean total number of offspring (±1 SE) of 3 different initial adult densities of the four pea morphologies.

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Adult Density</th>
<th>Total number of Offspring</th>
<th>Offspring per Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1</td>
<td>22±1.19</td>
<td>22±1.19</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>114±4.98</td>
<td>23±0.10</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>250±5.40</td>
<td>25±0.57</td>
</tr>
<tr>
<td>Leaflet</td>
<td>1</td>
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<td>23±1.06</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>124±2.50</td>
<td>25±0.70</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>240±8.26</td>
<td>24±0.82</td>
</tr>
<tr>
<td>Parsley</td>
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<td>22±1.00</td>
</tr>
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<td>122±4.45</td>
<td>24±0.88</td>
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<tr>
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<td>10</td>
<td>251±7.02</td>
<td>25±0.70</td>
</tr>
<tr>
<td>Tendril</td>
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<td>22±1.13</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>115±4.27</td>
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<tr>
<td></td>
<td>10</td>
<td>248±5.51</td>
<td>24±0.27</td>
</tr>
</tbody>
</table>
Figure 2.1. Examples of binary scanned images of cut stem pieces of the different morphologies of the near-isogenic peas.
Figure 2.2. Mean number of offspring per adult (±1 SE) for the three different initial number of adults and four pea morphologies. Means that share the same letter are not significantly different according to Tukey’s comparison of means.
Figure 2.3a and b. Mean number of offspring per adult (±1 SE) for each of the three pea morphologies in February (a) and April (b) greenhouse experiments. Means that share the same letter are not significantly different according to Tukey’s comparison of means.
Figure 2.4a, b and c. Mean percent nitrogen, phosphorus (μg/g) and potassium (μg/g) (±1 SE) of the four pea morphologies and three fertilization treatments. See text for information on significant differences.
Figure 2.5a and b. Mean surface area and fractal dimension (±1 SE) of the four pea morphologies and three fertilization treatments. See text for information on significant differences.
Figure 2.6. Mean number of offspring per adult (±1 SE) on the four pea morphologies and fertilization treatments. Reproduction is not significantly different between fertilization treatments or the four pea morphologies.
Figure 2.7a, b and c. Scatter plots of percent nitrogen, surface area and fractal dimension of peas versus number of offspring per adult pea aphid.
Chapter 3: Effect of Plant Architecture and Prey Distribution on Predation Rates

Abstract

Pea plant architecture and prey distribution were manipulated to determine the effects on the consumption of pea aphids (*Acyrthosiphon pisum* (Harris)) by multicolored Asian lady beetles (*Harmonia axyridis*). Total consumption on four near-isogenic peas that differ in morphology and two categories of aphid distribution (uniform and naturally aggregated) were examined over a 24 hour period. Plant morphology and prey distribution did not have an effect on the consumption of pea aphids by lady beetles. However, there was a significant relationship between plant surface area and predation rate. I conclude that plant surface area is more important than plant architecture in altering predator consumption rates in this system.

Introduction

Plant architecture can play an important role in predator-prey interactions. It has been shown that plant architecture can alter the availability of refugia for prey (Hawkins et al 1993, Clark and Messina 1998). Aggregation of insects may be influenced by plant shape and can have an impact on a predator’s ability to find prey (Turchin and Karieva 1989, Bommarco et al. 2007). Plant architecture is also known to influence predation rates by modifying the foraging behavior of predators (Carter et al 1984, Grevstad and Klepetka 1992). My main objective was to investigate how plant architecture of pea plants (*Pisum sativum*) and distribution of pea aphids (*Acyrthosiphon pisum*) affect the predation of the multicolored Asian lady beetle (*Harmonia axyridis*).
Plant architecture is the size and design of a plant. It has three important components: plant size or surface area, variation in plant parts, and connectivity of plant parts (Andow and Prokrym 1990). Stem and leaf dimension, branching angles, surface complexity and canopy spacing are all important architectural characteristics of a plant that might influence predation success. Plant morphology also determines surface area. An increase in the surface area of a plant alters prey density, which in turn, determines predation rate.

Plant architecture can create a refuge for some prey. Lawton (1983) suggested that if a plant is more architecturally complex, it provides more refugia for prey. These refugia can be absolute, where prey is completely concealed or partial where the predator has reduced access to prey. Clark and Messina (1998) found that lacewing larvae captured more aphids on Indian ricegrass compared to crested wheat grass. These authors suggested that the rolled leaves of crested wheat grass provided a refuge from the lacewings, while the Indian ricegrass had leaves that were too narrow to provide protected areas. In another investigation, Grevstad and Klepetka (1992) found that plant architecture of four different cultivars of cabbage had a significant influence on the predation rate of 5 species of coccinellids on cabbage aphids (*Brevicoryne brassicae*). Plant architecture could create an absolute refuge for prey because indentations on a ruffled leaf cultivar completely excluded large bodied predators. These authors also found that plant architecture also created a partial refuge for prey. Predators were less able to access prey on the middle of the undersides of leaves due to their inability to grip to the leaf surface.
Most authors that have investigated the effect of plant morphology on predation have not included the effects of prey distribution. In some cases, it is unclear whether plant structure simply alters the distribution of prey, thus altering a predator’s success, or whether plant structure directly affects predation by modifying prey refugia or altering predator search patterns. For example, two studies which report different effects of pea morphology on lady beetle predation rates have examined quite different densities and distributions of aphids on pea plants (Karieva and Sahakian 1990, Legrand and Barbosa 2003).

Prey distribution can have large impacts on predation rates. It has been suggested that aggregation of prey reduces predation success for predators that use a random search pattern, due to time spent searching in areas with low prey density (Turchin and Karieva 1989). Bommarco et al. (2007) found that prey (*Rhopalosiphum padi*) suppression by *Pterostichus cupreus* was greatest when animals were distributed evenly on spring cereals in a lattice simulation model. However, a study investigating the effect that consumption increased in areas of clumped prey. *A. confusus* increased turning frequency after prey consumption which concentrated foraging in close proximity to prey. These predators used an area-restricted search that is also characteristic of multicolored Asian lady beetles (Evans 1976).

Both small and large scale characteristics of plants can cause changes in the movement patterns of insect predators (Crist et al. 1992, Ferran and Deconchat 1992) and in so doing, alter predation rates. Legrand and Barbosa (2003) found that search time of coccinellids increased on a pea morph that had tiny leaflets. Predators spent a substantial
amount of time maneuvering over the tiny leaflets. Grevstad and Klepetka (1992) found that prey search time depended on plant architecture and also lady beetle species. Mathematical and simulation models suggest that predation success should decrease on plants that have low fractal dimension (Cuddington and Yodzis 2000, 2002). For example, a plant with a more branching architecture may take longer to search than a plant with a more compact structure with less branching and broader leaves.

Small scale architectural features, such as surface texture, can also influence predation. Investigators have found that a slippery plant surface can cause the predator to fall off of the plant more frequently, which can alter predation success (Carter et al. 1984, Karieva and Sahakian 1990). Surface structures such as trichomes can also alter movement of predators. The larvae of the predator *Propylea quatuordecimpunctata* (L.) had reduced movement on hairy potato leaves compared to glabrous bean leaves (Banks 1957). The felt-like hair of *Pelargonium* and tobacco caused alterations in movement of predatory *Chrysopu carnea* Stephens larvae (Arzet 1973). On a cultivar of cabbage that had a slippery surface but more complex architecture (more edges and less flat surfaces) coccinellids traveled over the plant more easily and fell less frequently (Grevstad and Klepetka 1992). Thus, plant architecture may ameliorate some of the negative effects of plant surface texture.

Although at least two studies report effects of pea architecture on lady beetle foraging, the effects reported differ. Legrand and Barbosa (2003) found lowest predation on a pea morph with tiny leaves, while Karieva and Sahakian (1990) found lowest predation on a normal type pea morph. It seems likely that these different reported
effects of pea architecture on predation may be due to either differences in prey
distribution or the surface texture of peas used in the experiments. Legrand and Barbosa
(2003) and Kareiva and Sahakian (1990) employed different methodologies which caused
very different distributions and densities of prey. Legrand and Barbosa (2003) placed
one aphid on the top leaf of pea plants and four aphids on the lower six leaves for a total
of 25 aphids per plant. The authors suggest that the greater architectural complexity of
the parsley morph increased predator search times and so decreased predation rates of
_Coccinella septempunctata_. However, the authors also report that this pea morph had the
largest surface area. Therefore I expect lower predation success on this large plant over a
fixed time period without any effects additional of architecture. In constrast, Karieva and
Sahakian (1990) placed adult aphids on different pea genotypes, a normal and leafless
variety, and allowed them to reproduce until the desired densities were reached, so that an
aggregated distribution was created. In this study, they found that predation by 2 lady
beetle species (_Coccinella septempunctata_ and _Hippodamia variegata_) was reduced on
normal morph compared to a leafless morph. These authors suggested that coccinellids
fall more frequently on the normal morph due to slippery surface texture of the leaves. It
is possible that the cultivars used by Karieva and Sahakian (1990) had greater surface
wax than the cultivar used by Legrand and Barbosa (2003). It is also possible that the
methods used by Karieva and Sahakian (1990) produced an aggregated distribution of
aphids which altered the impact of plant architecture, as compared to the uniform
distribution of aphids produced by the methods employed by Legrand and Barbosa
(2003). Finally, we should expect that the leafless variety of peas used by Karieva and
Sahakian (1990) to have a lower surface area than the normal type peas. Therefore it is also possible that there was a lower density of aphids on the normal morph, which produced lower predation rates.

The main objective of this investigation is to determine if plant morphology and prey distribution interact to determine predation rates. I compared pea aphid consumption by the multicolored Asian lady beetle on four different pea morphologies with two distribution patterns (uniform and naturally aggregated from reproduction). I hypothesized that consumption should be reduced on the leafless morph with aggregated prey distribution. The leafless morph has a branching architecture that could cause a decrease in predator search efficiency. If so, it will be more difficult for predators to find prey on this morph, especially when prey have an aggregated distribution, even if the effects of the surface area of the plants are taken into account.

**Materials and Methods**

**Plant material**

A near-isogenic line of peas (*Pisum sativum*) that has reduced stipules (st) was obtained from the USDA-ARS Western Regional Plant Introduction Station, Pullman, Washington. The morphologies of peas are altered by mutations in the genes *af* (afila) and *tl* (acacia). “Normal” (AfAfTtTt genotype) has normal, compound leaf types with 1 to 2 pairs of leaflets and number of tendrils. “Tendril” or *af* (afAfTtTt) has leaflets converted into tendrils. “Leaflet” or *tl* (AfAfttTt) tendrils are replaced by leaflets. “Parsley” (afafttt) has an interaction between the *af* and *tl* mutations. It has highly branching petioles and tiny leaflets (Figure 2.1). By using a line of genetically similar
peas, I attempted to minimize possible differences in phytochemistry and surface wax between the different morphologies. The four pea morphs were grown in a Conviron growth chamber (16L:8D photoperiod with 18°C:15°C temperature regime) until they were approximately 30 days old. All experimental plants were growth in Fafard 52 mix in 5” deep x 4” wide square pots.

**Insects**

Pea aphids (*Acyrthosiphon pisum*) from a laboratory population founded with individuals orginally purchased from Carolina Biological Supply Company were used. Pea aphids are a common phloem-sucking pest of peas. At 20-25°C, aphids molt four times before reaching adulthood in 7-9 days (Campbell and MacKauer 1975). Pea aphids are parthogenetic and bear live young. An adult produces 5-15 young a day for approximately 7 days.

The multicolored Asian lady beetle (*Harmonia axyridis*) was the predator species. *H. axyridis* is a well known predator of aphids and has been extensively studied and used for biological control (Koch 2003). It is a Palearctic polyphagous species that originated in the Far East (Lanzoni et al. 2004). The lady beetle life cycle proceeds from egg, through four instars, to pupal and finally adult stage (Hodek 1973) in 14-20 days (LaMana and Miller 1998). Individuals live 30-90 days (He et al. 1994). Reported average aphid consumption by an adult ranges from 15-65 aphids per day (He et al. 1994).

Figure 3.1 illustrates the relative sizes of a tendril to an aphid and multicolored Asian lady beetle. A tendril is approximately 0.068±0.0092 mm in diameter. An aphid is
0.37±0.020 cm long and 0.159±0.017 cm wide at the abdomen. Lady beetles are 0.63±0.02 cm long and 0.505±0.02 cm wide at the largest body portion.

**Plant surface area and fractal dimension**

To characterize plant architecture, surface area and fractal dimension were measured at the end of all experiments. Fractal dimension is a well known measure of plant architecture (Morse et al 1985, Gunnarsson 1992, Gee and Warwick 1994) and gives an indication of the space filling properties of an object. For example, a plant that has more compact, broad leaves would have a higher fractal dimension compared to a branching small leaf type.

To quantify surface area and fractal dimension, 2D projections were created by scanning the plants on an Epson Perfection photo scanner. The resulting image was converted into binary format and the surface area estimated and fractal dimension estimated using the box-counting dimension routine in the Frac-Lac 2004 plug-in for the NIH distributed software package Image-J (http://rsb.info.nih.gov/ij/). Other investigators have used the box-counting estimate of the fractal dimension as a measure of plant architecture (Moore et al. 1985, Gunnarsson 1992, Gee and Warwick 1994). Marstrand (1954) suggested that 2D projections of these 3D structures can be used to approximate the true fractal dimension. Other authors that have investigated root systems and plant structures have successfully used 2D projections for these 3D structures (Nielson et al. 1997, Walk et al. 2004).

To calculate surface area, the entire plant was cut into pieces to prevent an underestimate of surface area due to tissue overlap. Preliminary analysis indicated that
tendril plants have a surface area about half that of the other morphologies. As a result, I used 2 tendril plants compared to one plant of the other morphologies. Individual cut stem pieces were used to calculate fractal dimension. Scanning the entire uncut plant gives an overestimate of fractal dimension because of plant tissue overlapping on the scanner. However an underestimate occurs when scanning the entire cut plant pieces because of the increased number of edges included in the estimation.

**Predation experiments**

The effects of two different aphid distributions were examined on each of the pea morphologies. A total of 25, 4th instar (non-reproducing) aphids, in either a uniform or aggregated distribution, were added to the 30 day old plants. To produce a uniform distribution of aphids, a 2D grid with 25, 2.8x2.8 cm squares was placed behind the experimental plant and one aphid was added to the area defined by each grid square. Aggregated distributions were produced by allowing adult aphids to reproduce on the peas. The resulting offspring were counted and aphids were either removed or added to give a total of 25.

Experiments were conducted in enclosures which consisted of a circular pot that holds a piece of circular Styrofoam that has a square pot embedded in it. Enclosures were placed under lights in the laboratory with a 16L:8D photoperiod. Predators that had been starved for 24 hrs were placed in the experimental enclosures and allowed to forage for 24 hours. At the end of this period, the predator was removed and the number of aphids remaining was counted. A total of 25 replicates for each morph and distribution were conducted. The number of aphids consumed for each pea morph and spatial
distribution were then compared. All statistical analyses were completed in SAS (SAS 1990).

To quantify the spatial distribution of the aphid nymphs for the aggregated distribution, macro digital photos were taken of a cluster of aphids in a 2x2 cm² sampling area. The aphids within the sampling region were marked with a dot in Adobe Photoshop. The x,y coordinate of each point was calculated using the particle counter in ImageJ. A SAS macro created by Moser (1987) was used to calculate the mean nearest neighbor distances.

Results

Surface area and fractal dimension of experimental peas

Normality tests (Shapiro-Wilk) indicated that the distributions of surface areas for normal, parsley and tendril plants with an aggregated aphid arrangement were not normal. A square root transformation was used to normalize all data. Transformed surface area was significantly different between the pea morphologies in spite of the effort to control this factor (2-way ANOVA F=3.15; df=3,192; P=0.0262), but there was no significant difference in surface area of plants between the different aphid distribution treatments (F=0.76; df=1,192; P=0.3846; Figure 3.2a). A Tukey’s comparison of means indicated that the leaflet morph had a significantly higher surface area than the tendril morph. As expected, analysis by 2-way ANOVA indicated that the fractal dimension was significantly different between the pea morphologies (F=48.09; df=3,192; P<0.0001), indicating that the plants did differ architecturally. There was no unexpected difference in fractal dimension of plants used in either aggregated or uniform aphid
distribution treatments (F=1.61; df=1,192; P=0.2055). The tendril morph had the lowest fractal dimension followed by parsley, normal and leaflet morphologies. A Tukey’s comparison of means indicated that leaflet and normal were not significantly different from each other; but differed from tendril and parsley, which also grouped together (Figure 3.2b).

**Nearest neighbor distances between morphologies and prey distributions**

When aphids were aggregated, the mean nearest neighbor distance was not significantly different between the pea morphologies at the beginning of experiments (ANOVA, F=0.82; df=3,36; P=0.4934). The parsley morph had the largest mean nearest neighbor distance ($\bar{x} = 0.00037\pm0.00015$ cm) followed by tendril ($\bar{x} = 0.00030\pm0.000034$ cm), normal ($\bar{x} = 0.00025\pm0.000024$ cm) and leaflet ($\bar{x} = 0.00022\pm0.000023$ cm), respectively. Therefore plant shape did not alter the aggregation patterns of reproducing aphids, and predators experienced a similar distribution of prey on all morphologies. The nearest neighbor distance of the uniform distribution was approximately 2.8 cm.

**Predation rates on different pea morphologies**

Normality tests (Shapiro-Wilk) indicated that the distributions of consumption of aphids for leaflet and parsley plants with an aggregated aphid distribution, and parsley plants with a uniform aphid arrangement were not normal. A square root transformation ($\sqrt{x+1}$) was used to normalize the consumption data, but this transformation did not normalize the data for the parsley morph with a uniform distribution. The number of aphids consumed was not significantly different between the morphologies or prey distributions as indicated by a 2-way ANOVA on the transformed data (F=0.67;
Analysis of rank transformed consumption data also indicated that there was no significant difference in aphid consumption between the morphologies or distributions ($F=0.746; \text{df}=7,192; \text{P}=0.633$). In addition, a non-parametric comparison of different consumption categories was also performed. The predation data was divided into three even categories of consumption: 0-7, 8-14 and 15-21 aphids. For each of the morphologies, the proportion of replicates in each of the three consumption categories was compared using a row x column, G-test of independence (Sokal and Rohlf 1981). This test determines if the frequency in each consumption category (column) is independent of the pea morphologies (rows). Analysis of the data for a uniform distribution of aphids indicated that the frequency of consumption in the categories was dependent on morphology ($G=14.08; \text{p}=0.0288$). Examination of the contingency table indicates that the parsley morph had more low consumption trials compared to the other morphologies, a lower frequency of intermediate consumption replicates, and more high consumption trials (Table 3.1). The tendril morph had no replicates in the high consumption category. An analysis of aggregated prey distribution analysis indicated that the frequency of consumption in the categories was independent of morphology ($G=7.2690; \text{p}=0.2967$; Table 3.2).

Finally, analysis indicated that one aspect of plant shape, its surface area, did significantly alter predation rates. Spearman correlation analysis on untransformed data indicated that there is a negative correlation between consumption and surface area ($\rho=-0.27700; \text{P}<0.0001$; Figure 3.5).
Discussion

Unlike previous work, which has identified plant morphology as a control of predation rates, I found that pea plant architecture and aphid distribution did not significantly affect the predation rate of lady beetles. Previous studies indicate that plant architecture is important in determining the ability of the predator to: locate the feeding sites of prey (Fleschner 1950, Banks 1957, Dixon 1959, Evans 1976), access prey species (Clark and Messina 1998) and maneuver over the plant (Grevstad and Klepetka 1992). In particular, my results are inconsistent with those of Legrand and Barbosa (2003) and Karieva and Sahakian (1990) who used the same pea plant-pea aphid system which similar replication.

Legrand and Barbosa (2003) and Karieva and Sahakian (1990) both found a significant affect of plant architecture on predation rate of seven-spot lady beetles, *Coccinella septempunctata*. Legrand and Barbosa (2003) found a reduction in aphid consumption on the pea morphology that had tiny leaflets (parsley) compared to the normal morph. The lady beetles spent more time searching these tiny leaflets, and explored a smaller proportion of the plant. However, Karieva and Sahakia (1990) found that 2 lady beetles species (*Coccinella septempunctata* and *Hippodamia variegate*) controlled aphid population growth to a greater extent on a leafless morphology compared to a normal pea morph. In that study, lady beetles were reported to fall off of the slippery leaves of the normal morphology more frequently than on the leafless morph. Therefore, it is also possible that the surface texture of the near-isogenic lines used by these authors differed from the near-isogenic lines that were used in my investigation and
also by Legrand and Barbosa (2003). In contrast, I found low consumption rates by *H. axyridis* on all four morphologies. However, these low consumption rates did not reflect a lack of lady beetle appetite since multicolored Asian lady beetles consumed almost all aphids in a Petri plate environment (Cuddington and Buchman, unpublished).

In this investigation, I also found that pea aphid distribution did not significantly affect the number of aphids consumed by multicolored Asian lady beetles. Non-parametric analysis did suggest that there may be more lady beetles with low consumption on parsley and tendril plants with uniform distribution, but parametric analysis on transformed data indicated there was no significant difference in mean consumption rates. I conclude that the results reported by Legrand and Barbosa (2003) and Karieva and Sahakian (1990) did not differ because the methodologies employed caused very different distributions of prey. The methods used by Legrand and Barbosa (2003) produced a uniform distribution of prey, while the experiments of Karieva and Sahakian (1990) produced an aggregated distribution of prey.

The best predictor for consumption rates in this study was plant surface area. There was a significant negative relationship between surface area and predation rate. This result is not unexpected because surface area determines prey density, which in turn, determines the rate at which a predator encounters prey. A predator searching for a given number of aphids on a large plant is likely to find fewer of the prey in a fixed period of time than the same predator searching for the same number of aphids on a small plant. Karieva and Sahakian (1990) did not measure the surface area of the two pea morphologies used in their investigation. However, it is possible that their results could
be confounded by differences in total plant surface area. My results suggest that normal morph plants have a larger surface area than leafless plants, and therefore for the same number of aphids, we should expect predators to have greater consumption rates on leafless morphs as reported by these authors. On the other hand, Legrand and Barbosa (2003) measured surface area, but it is difficult to determine how these estimates were obtained. Moreover, these authors report that the parsley morph had a much larger surface area then the normal or leaflet pea morphologies. The larger surface area reported for parsley should lead us to expect the lowest consumption rate on this morphology, as reported by these authors. However, Legrand and Barbosa’s (2003) surface area estimates differ dramatically from the surface area estimates in my study (parsley surface area $\mu$=807.7 cm$^2$ Legrand and Barbosa 2003; Buchman thesis parsley surface area $\mu$=236.28±20.87 cm2). The reported surface areas for normal and leaflet morphs are more similar (surface area normal $\mu$=137.8 cm$^2$ and leaflet $\mu$=194.7 cm$^2$ Legrand and Barbosa 2003; Buchman thesis surface area normal $\mu$=254.80 cm$^2$ (±18.49 S.E.) and leaflet $\mu$=288.00 cm$^2$ (±15.87 S.E.)). However, if the reported surface area is correct, it could explain the reported results more simply than differences in plant architecture. With its large surface area, the parsley plant would have a reduced aphid density compared to the other pea morphs. I should note, however, that Legrand and Barbosa (2003) conducted an additional experiment in which the surface area of the parsley morph was reduced by removing leaves and the behavior of lady beetles was observed over a 15 minute period, as the predator moved over a plant with no aphids.
The authors report that this study indicates that predator search times were still longer on parsley plants compared to the other morphs.

In conclusion, I found that pea plant morphology and pea aphid distribution does not significantly affect predation by Asian multi-colored lady beetles. There is a significant relationship between surface area and prey consumption. Surprisingly, some previous studies on the effects of plant architecture on predation rate have failed to account for this very important factor. Authors that used a similar system either did not measure surface area (Karieva and Sahakian 1990) or measured surface area, but did not consider the importance of this factor to their results (Legrand and Barbosa 2003). Both of these authors found reduced consumption on a pea morph that probably had the largest surface area. Because prey density determines predation rates, this seems the best explanation for my results. Although it is not frequently measured, I conclude that surface area may be one of the most important plant characteristics influencing predator-prey interactions.
### Table 3.1

Frequency of low (0-7), medium (8-14) and high (15-21) pea aphid consumption by lady beetles where aphids were uniformly distributed on different pea morphologies. There were a total of 25 trials in each category.

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>56</td>
<td>36</td>
<td>8</td>
</tr>
<tr>
<td>Leaflet</td>
<td>52</td>
<td>40</td>
<td>8</td>
</tr>
<tr>
<td>Parsley</td>
<td>68</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>Tendril</td>
<td>52</td>
<td>48</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 3.2

Frequency of low (0-7), medium (8-14) and high (15-21) pea aphid consumption by lady beetles where aphids are in an aggregated distribution on different pea morphologies. There were a total of 25 trials in each category.

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>64</td>
<td>28</td>
<td>8</td>
</tr>
<tr>
<td>Leaflet</td>
<td>72</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Parsley</td>
<td>72</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Tendril</td>
<td>52</td>
<td>32</td>
<td>16</td>
</tr>
</tbody>
</table>
Figure 3.1. Photograph illustrating the relative size of a tendril morphology pea plant to a pea aphid and a multicolored Asian lady beetle.
Figure 3.2a and b. Mean surface area (cm$^2$) and fractal dimension (±1 SE) for each of the four pea morphologies used in the predation experiments with either aggregated or uniform aphid distributions. Means that share the same letter are not significantly different.
Figure 3.3. Mean number of aphids consumed (±1 SE) by lady beetles on the four pea morphologies with either aggregated or uniform aphid distributions. Aphid consumption is not significantly different between morphologies or aphid distributions.
Figure 3.4. Scatterplot of number of aphids consumed versus surface area (cm$^2$). Correlation analysis indicated that there is a significant linear relationship between number of aphids consumed by $H. axyridis$ and surface area of the pea plants.
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Appendix A: Micronutrient Data Analysis

Table A1: Mean content of micronutrient (μg/g) (±1 SE) for the normal morph and nitrogen fertilization treatments described in chapter 2.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.22gN</th>
<th>0.88gN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>14261.78±999.25</td>
<td>13589.90±540.22</td>
<td>12557.12±1161.86</td>
</tr>
<tr>
<td>Mg</td>
<td>7232.62±239.10</td>
<td>7219.94±348.90</td>
<td>6370.68±599.73</td>
</tr>
<tr>
<td>Al</td>
<td>1.85±2.19</td>
<td>2.10±1.34</td>
<td>1.60±0.81</td>
</tr>
<tr>
<td>B</td>
<td>26.74±2.06</td>
<td>25.80±1.87</td>
<td>28.08±2.46</td>
</tr>
<tr>
<td>Cu</td>
<td>9.45±0.79</td>
<td>8.27±1.20</td>
<td>9.00±1.07</td>
</tr>
<tr>
<td>Fe</td>
<td>75.33±6.08</td>
<td>80.17±6.60</td>
<td>87.00±4.65</td>
</tr>
<tr>
<td>Mn</td>
<td>85.38±20.05</td>
<td>96.08±28.20</td>
<td>110.42±22.17</td>
</tr>
<tr>
<td>Mo</td>
<td>12.65±4.45</td>
<td>4.52±1.48</td>
<td>5.10±2.26</td>
</tr>
<tr>
<td>Na</td>
<td>2347.86±295.72</td>
<td>1996.43±201.44</td>
<td>2156.73±316.47</td>
</tr>
<tr>
<td>Zn</td>
<td>145.18±18.21</td>
<td>134.80±8.07</td>
<td>128.33±11.96</td>
</tr>
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</table>

Table A2: Mean content of micronutrient (μg/g) (±1 SE) for the leaflet morph and nitrogen fertilization treatment described in chapter 2.

<table>
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<th>Control</th>
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<th>0.88gN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>13272.74±800.76</td>
<td>11902.62±1416.52</td>
<td>13648.42±977.18</td>
</tr>
<tr>
<td>Mg</td>
<td>7292.10±500.21</td>
<td>6033.26±541.61</td>
<td>6227.18±289.07</td>
</tr>
<tr>
<td>Al</td>
<td>1.12±0.53</td>
<td>2.53±1.01</td>
<td>1.14±0.45</td>
</tr>
<tr>
<td>B</td>
<td>29.38±2.70</td>
<td>26.19±2.17</td>
<td>29.61±2.20</td>
</tr>
<tr>
<td>Cu</td>
<td>9.55±1.05</td>
<td>9.29±0.89</td>
<td>10.82±0.72</td>
</tr>
<tr>
<td>Fe</td>
<td>79.52±5.01</td>
<td>83.00±2.77</td>
<td>100.77±9.79</td>
</tr>
<tr>
<td>Mn</td>
<td>104.11±26.60</td>
<td>98.60±22.05</td>
<td>113.34±20.64</td>
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<tr>
<td>Mo</td>
<td>10.17±2.23</td>
<td>13.86±5.38</td>
<td>4.26±1.69</td>
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<tr>
<td>Na</td>
<td>2098.32±131.55</td>
<td>1250.38±205.18</td>
<td>2194.79±149.19</td>
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<tr>
<td>Zn</td>
<td>145.46±8.47</td>
<td>149.54±7.27</td>
<td>115.25±6.61</td>
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</table>
Table A3: Mean content of micronutrient (μg/g) (±1 SE) for the parsley morph and nitrogen fertilization treatment described in chapter 2.

<table>
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</tr>
</thead>
<tbody>
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<td>Ca</td>
<td>11720.98±875.06</td>
<td>10308.20±601.82</td>
<td>10455.22±648.51</td>
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<tr>
<td>Mg</td>
<td>5529.16±390.50</td>
<td>5190.42±408.62</td>
<td>4939.76±335.41</td>
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<tr>
<td>Al</td>
<td>3.28±1.47</td>
<td>2.33±0.57</td>
<td>1.24±0.53</td>
</tr>
<tr>
<td>B</td>
<td>26.31±1.42</td>
<td>28.57±1.42</td>
<td>31.44±1.48</td>
</tr>
<tr>
<td>Cu</td>
<td>9.11±0.53</td>
<td>8.41±0.88</td>
<td>9.42±0.59</td>
</tr>
<tr>
<td>Fe</td>
<td>74.76±7.56</td>
<td>79.61±6.18</td>
<td>80.88±5.82</td>
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<tr>
<td>Mn</td>
<td>77.17±19.0</td>
<td>92.44±12.23</td>
<td>71.38±14.17</td>
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<tr>
<td>Mo</td>
<td>11.15±3.69</td>
<td>5.24±0.98</td>
<td>6.97±3.20</td>
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<tr>
<td>Na</td>
<td>3096.19±392.40</td>
<td>2422.46±198.15</td>
<td>3022.38±506.00</td>
</tr>
<tr>
<td>Zn</td>
<td>123.28±16.46</td>
<td>98.53±5.38</td>
<td>100.82±6.62</td>
</tr>
</tbody>
</table>

Table A4: Mean content of micronutrient (μg/g) (±1 SE) for the tendril morph and nitrogen fertilization treatment described in chapter 2.

<table>
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<th>Control</th>
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<th>0.88gN</th>
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<tbody>
<tr>
<td>Ca</td>
<td>8718.45±452.91</td>
<td>9951.86±987.89</td>
<td>8913.48±525.54</td>
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<td>Mg</td>
<td>4828.58±265.37</td>
<td>5544.76±470.19</td>
<td>4950.12±130.04</td>
</tr>
<tr>
<td>Al</td>
<td>1.43±0.47</td>
<td>2.29±0.54</td>
<td>1.98±0.70</td>
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<tr>
<td>B</td>
<td>27.55±1.62</td>
<td>23.74±1.47</td>
<td>34.99±2.75</td>
</tr>
<tr>
<td>Cu</td>
<td>7.88±0.62</td>
<td>6.80±0.19</td>
<td>8.90±0.81</td>
</tr>
<tr>
<td>Fe</td>
<td>78.58±2.09</td>
<td>105.77±11.95</td>
<td>141.38±12.35</td>
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<td>Mn</td>
<td>81.76±13.76</td>
<td>71.04±13.66</td>
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<tr>
<td>Mo</td>
<td>7.65±1.93</td>
<td>8.90±5.68</td>
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<tr>
<td>Na</td>
<td>2482.21±130.16</td>
<td>2556.03±233.88</td>
<td>3445.68±270.57</td>
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<tr>
<td>Zn</td>
<td>99.85±7.98</td>
<td>118.40±16.16</td>
<td>121.72±13.52</td>
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