The effects of plant gross morphology on the foraging efficiencies of generalist predators

by

Paula Reynolds

A thesis
presented to the University of Waterloo
in fulfillment of the
thesis requirement for the degree of
Master of Science
in
Biology

Waterloo, Ontario, Canada, 2011

AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Plant shape affects the ability of insect predators to locate prey by altering search paths or by providing partial refugia for prey. Changes in predator foraging efficiency can have significant consequences for population dynamic of both predators and prey. Yet, the relationship between plants and insect predators is not well understood despite its relevance to agriculture and biological control. The effect of plant gross morphology on predator foraging success was tested using multicoloured Asian ladybeetles, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), and green lacewing larvae, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), preying on pea aphids*, Acyrthosiphon pisum* Harris (Hemiptera: Aphididae). These predators differed in body size and therefore might be expected to have different responses to a given plant morphology. Experiments were conducted using four different pea plant morphologies (*Pisum sativum* L.) that differed in fractal dimension, but which were controlled for surface area. The consumption rate of each predator on each pea morph was determined by measuring the number of aphids consumed in a 48 hour foraging period at 3 prey densities. I also tracked predator search paths using 2D time-lapse photography to determine if the two predators search plants differently.

I found that both predators were more successful at capturing prey on plants with a higher leaf edge to leaf area ratio (lower fractal dimension). Plants with more edges were easier for predators to grip, thus increasing their mobility and manoeuvrability. Also, plants with more edges and fewer leaf surfaces had fewer locations where aphids could hide. As a result, predators are more effective at locating and capturing prey on these morphologies.

Acknowledgements

I would like to thank my supervisor, Kim Cuddington, for her guidance and wisdom throughout this project. I would also like to thank my advisory committee, Roland Hall and Stephen Murphy for providing helpful comments on this thesis. Many thanks to Lynn Hoyles for her horticultural expertise, for keeping the growth chambers running and for always making me laugh. Thank you to my lab mates, Bradley Vermunt and Stephanie Sobek for their feedback on early drafts of this thesis and for their positive support and encouragement. I am grateful for all of the assistance I received from the undergraduate students who worked in the lab maintaining the insect populations and helping with experiments. Finally, thank you to my family and friends who always have faith in me. Without their support I would not have made it to the end of this project.

Table of Contents

List of Figures

List of Tables

Chapter 1: Introduction and Literature Review

1.1 Introduction

Predation rates are dependent on prey density (Solomon, 1949; Holling, 1959). When few prey are present, predation rates are low, but these rates increase as prey density increases. Therefore, for a given number of prey individuals, I expect that predators will have lower foraging rates when these prey are spread over a large area (low prey density), as compared to when the same number of prey are concentrated over a small area (high prey density). However, prey density is not the only factor which dictates predator foraging success. In tritrophic systems consisting of plants, herbivores and predators, previous studies have shown that aspects of plant structure, such as branching and connectivity influence predator-prey interactions by reducing predator mobility and prey encounter rates (Kareiva and Sahakian, 1990; Grevstad and Klepetka, 1992; Clark and Messina, 1998a, 1998b; Legrand and Barbosa, 2003). Therefore, predictions about the efficiency of insect predators drawn from data collected in spatially simple lab environments, without spatial structure, may be incorrect.

In general, authors report that as habitat structure becomes more heterogeneous, predator foraging efficiency decreases (Kaiser, 1983; Andow and Prokrym, 1990; Lukianchuk and Smith, 1997; Cloyd and Sadof, 2000; Grez and Villagran, 2000; Gingras and Boivin, 2002; Hoddle, 2003; Legrand and Barbosa, 2003; Gingras *et al.,* 2008). For example, Legrand and Barbosa (2003) suggest that increased branching of pea plants decreases the foraging efficiency of 7-spot ladybugs. To test the hypothesis that plant morphology affects insect predator foraging behaviour, I measured the predation rates of two

predators, the green lacewing *Chrysoperla carnea* Stephens, and the multicoloured Asian ladybeetle, *Harmonia axyridis* Pallas, feeding on pea aphids, *Acyrthosiphon pisum* Harris. I compared predator consumption rates on aphids placed on leaf tissue in Petri plates and on whole plants. I also compared consumption rates on pea plants (*Pisum sativum* L.) that differed in gross morphology. In all cases, I ensured that the foraging arenas were the same volume, and that the surface area of the plant tissue was not significantly different. By keeping prey density constant I was able to isolate the effects of plant morphology on predation rates. I also tested the effects of plant morphology on predator searching behaviour by measuring and comparing predator movement trajectories.

In this chapter, I will review the literature about the effects of habitat structure on predator foraging behaviour. In chapter 2, I will discuss experiments designed to test the effects of gross morphology on predator foraging success. In chapters 3, I will describe the potential mechanisms that account for varying consumption rates on plants with different gross morphologies.

1.2 Literature Review

1.2.1 Overview

There is a large body of literature that describes the foraging behaviour of insect predators and how they respond to changes in prey density. However, the interactions between insect predators and prey often include a third trophic level ó plants. Prey species are often herbivorous; therefore, predators must search within plant canopies to locate prey. However, the interactions between plants and predators are not well understood. Throughout

2

this thesis I will use the term gross morphology. Studies have shown that several factors affect a predator's ability to search for prey on plants. Plant morphological characteristics such as leaf texture, branching, and structures that provide refugia for prey, all influence predator foraging success. Although plant-predator relationships have been previously studied there are several aspects of this relationship that have been overlooked. In particular, the effects of whole plant or gross morphology are not well understood because many studies have used different plant species, which can influence predator behaviour through phytochemical and nutritional differences. Also, in many studies plant surface areas were not controlled. Therefore, prey densities may have differed, which has a strong effect on predator consumption rates. Second, it is not clear if the effects of plant structure are similar for all predators. The literature suggests that as body size changes a predator's experience with its environment may also change. Therefore, certain morphological characteristics may not affect predator forging success in the same manner.

In this review I will summarize effects of habitat structure on insect predator foraging behaviour. I will also give a detailed description of the effects of small-scale plant structure i.e. surface texture, refugia and morphology on predator foraging efficiency. I will discuss the various search strategies that predators use to navigate in heterogeneous environments. Finally, I will review literature on the size-grain hypothesis, which suggests that body size may affect environmental experience.

1.2.2 Predator Foraging and Habitat Structure

Predator consumption rates depend on prey densities. At low density, consumption rates are low, and increase in proportion with prey density. In other words, in an area of a given size, a predator is more likely to encounter and consume prey if there is a greater number of individuals present than if there are very few, as demonstrated by Holling's work on functional responses of predators (1959, 1961, 1966). However, the size of a predator's searching environment is not the only factor that may affect its relative success. The structure of the environment also has a strong influence because it can slow predator movements, which reduces foraging efficiency. For example, in experimental plots of the same size, but with different habitat structures beetle movement was reduced in plots with greater structural heterogeneity (Weins *et al*., 1997; Grez and Villagran, 2000). However, it is challenging to accurately measure and quantify the effects of habitat structure on predator foraging success. Field experiments have the advantage of incorporating realism to the study system, but they can be labour-intensive and difficult to run. On the other hand, laboratory experiments may simplify interactions between plants and predators, but they may be useful for observing predator foraging behaviours.

While several laboratory studies have demonstrated that habitat structure affects insect predator foraging success, their applicability to real-world ecosystems is limited. When quantifying predator foraging capacity, many authors agree that measuring predator consumption rates in small experimental arenas leads to an overestimate of predator foraging rates (Kiritani and Dempster 1973; Grant and Shepard 1984; Luck et al. 1988). This may be particularly true when prey densities are low. If a predator is confined to a small enclosure it

will repeatedly search the same area and is more likely to locate prey. However, in the natural environment, if prey density is low a predator will most likely abandon the area and emigrate to a more suitable one (Luck *et al.*, 1988). However, Latham and Mills (2009) contest that laboratory observations may actually underestimate predator daily per capita consumption and the methods of measuring consumption rates in the field may be the reason. They measured both biomass killed and biomass consumed by *Harmonia axyridis* and *Chrysoperla nigricornis* and found higher consumption in the field and cages than in laboratory arenas. Whichever case is true, it is clear that measuring predator foraging rates in simple environments may lead to inaccurate conclusions about their capacity to consume prey or control pest populations in more natural conditions. Yet, these methods are often used for assessing biological control agents (e.g., Cabral *et al.*, 2007; Oliveira *et al.*, 2007).

Nonetheless, using simple environments to measure parameters, such as the attack rate and handling time of predators, can provide valuable information about how predators respond to different prey species. They can also be used to determine at what density a predator's functional response will be overwhelmed (Oaten and Murdoch, 1975). This can be a valuable first step in evaluating the predatory potential of biological control agents.

Making reliable large-scale predictions about predator-prey interactions from simple laboratory experiments is difficult, and carrying out large-scale field experiments is often labour-intensive and impractical. Therefore, incorporating habitat structure, such as plant morphology, into laboratory experiments is necessary to improve our understanding of predator mobility and foraging efficiency and to ensure accuracy for predictions about real-world settings.

1.2.3 Effects of Plant Morphology on Predator Foraging

Plants produce a variety of morphological features to protect themselves from herbivory, such as surface features like pubescence, spines, or waxes (Price, 1980). While these features may directly impede herbivore attack, they also affect the predators that feed on these herbivorous insects. In some instances, plant morphology can impede predator foraging success by decreasing mobility. However, Marquis and Whelan (1996) suggest that there may be a subtle and unrecognized relationship between plants and predators, whereby plant morphology improves the predator's ability to move through plant canopies and capture herbivorous prey.

I use the term plant morphology to refer to a variety of plant characteristics from large to very fine-scale features. Gross morphology describes the size, surface area and branching of a whole plant. Plant morphology can also refer to small-scale features such as leaf surface textures (i.e. trichomes and waxes). There is a large body of literature indicating that all these aspects of plant morphology affect predator foraging success. In the following sections I will discuss the plant morphological features that have been well studied with respect to their effects on predator foraging behaviour. These features include, leaf surface textures, plant structures that serve as prey refugia, and gross morphology.

1.2.3.1 Leaf Surface Texture

Leaf surface textures, such as hairiness or slipperiness can affect insect predators. Trichomes are hair-like projections of the plant epidermis that protect plants from herbivores (Levin, 1973). The effects of trichome density on natural enemy foraging have been examined on a variety of different plant and insect species. Intuitively, one would suspect

that as trichome density increases an insect's rate of movement would decrease. Some experimental studies have confirmed this relationship. For example, the predatory mite, *Phytoseiulus persimilis*, captured the least prey on chrysanthemums with high trichome density compared to those with intermediate and low trichome density (Stavrinides and Skirvin, 2003). Increased trichome density on cotton reduced the ability of the parasitoid, *Trichogramma pretiosum*, and the predator, *Chrysopa rufibralis*, to attack cotton bollworm eggs (Treacy *et al*., 1985). *Podisus nigrispinus*, foraging on tomatoes, sweet pepper and eggplant captured the fewest prey on tomatoes due to glandular trichomes (DeClercq *et al*., 2000). Similarly, *Picromerus bidens* captured fewer prey on tomatoes than sweet pepper and eggplant due to longer handling times on tomatoes (Mahdian *et al*., 2006). As well, Coll and Ridgway (1995) observed that *Orius insidiosus* captured fewer Western flower thrips on tomato plants than beans and sweet pepper because the leaf surface of tomatoes were dense with trichomes.

Not all studies on the relationship between trichomes and predator foraging have found an inverse relationship between trichome density and prey capture. Styrsky *et al*. (2006) found that trichomes did not inhibit the predatory abilities of fire ants on lepidopterans. In fact, more prey were consumed on pubescent isolines than glaborous ones. Romero *et al*. (2008) determined that there was a mutualism between the predatory spiders, *Peucetia flava*, *P. Rubrolineata* and glandular trichomes. In times when prey are scarce, spiders can feed on dead carcasses trapped in glandular trichomes. As a result, spiders living on plants with trichomes had a larger body mass and were more effective at defending plants from herbivore attack.

Waxy leaf surfaces can also affect the mobility and foraging of predators. Many plants produce leaf waxes to reduce water loss and to deter herbivores. Predator foraging efficiency may be greatly decreased on waxy surfaces because predators slip from leaves frequently or engage in other activities, such as grooming, rather than searching for prey (Eigenbrode *et al.*, 1996).

On pea plants (*Pisum sativum*) with different wax blooms, *Hippodmia convergens*, *Orius insidiosus* and larval *Chrysoperla carnea* control pest populations more effectively on non-wax cabbage than a waxy variety because their tarsae cannot grip the smooth surface of the waxy cabbage (Chang *et al.*, 2006). The same effect was reported by Eigenbrode *et al.* (1996) who tested *Chrysoperla carnea, Hippodamia convergens,* and *Orius insidiosus* foraging on waxy and non-wax cabbage. All predators were more effective at capturing diamondback moth (*Plutella xylostella*) on cabbage with a non-wax surface rather than a waxy one. Brachonid wasps, *Diaeratiella rapae*, attack more hosts on a reduced wax variety of cauliflower, and spent less time engaged in grooming (Gentry and Barbosa, 2006).

The interpretation of these results can be confounded by the fact that herbivores respond differently to wax blooms on their host plant. Since waxes are used by plants as a defense from herbivores, it is possible that herbivores may respond differently to plants with normal versus reduced wax blooms. For example, canola, *Brassica napus*, with reduced epicuticular wax, was found to reduce aphid numbers, whereas, it increased the number of flea beetles (Eigenbrode *et al.*, 2000). Pea aphids, *Acyrthosiphon pisum*, were less numerous on peas with reduced wax, while pea leaf weevils, *Sitona lineatus*, caused more damage on peas with reduced wax than normal wax (White and Eigenbrode, 2000). To eliminate this

potentially confounding factor, Rutledge *et al.* (2003) used near isolines of pea that did not affect the growth and fecundity of aphids. They showed that increased predation was a result of reduced wax, rather than a variable response of aphids to the different wax blooms.

Although it seems quite apparent that leaf waxes decrease the efficiency of natural enemies, this area of research has primarily focused on plants that differ obviously in wax bloom. Insect attachment on plants with amorphous waxes differing in composition rather than bloom has not been examined, despite the fact that these types of wax are prevalent in nature (Walton, 1990).

1.2.3.2 Prey Refugia

Many morphological features of plants can be used by herbivores for protection from predators. These features are known as refugia. Refugia can be either partial or complete. Complete refugia are plant structures which fully conceal herbivores. Galls are an example of complete refugia. They are abnormal plant growths that form when insects are feeding within plant tissue (Weis and Kapelinski, 1994). Herbivores that feed within complete refugia are often difficult or impossible for natural enemies to reach, thus they greatly decrease foraging efficiency. Some natural enemies, such as parasitoids have developed adaptations to reach prey in these locations. For example, the parasitoid *Itoplectis conquisitor* can successfully parasitize pupae of the European pine shoot moth, *Rhyacionia buoliana*, feeding within complete refugia by driving its ovipositor through the plant tissue to reach the host (Ball and Dahlsten, 1973).

Partial refugia include structures that do not conceal prey but prevent easy access by predators. Partial refugia affected the ability of coccinellids and chrysopids to capture

Russian wheat aphids, *Diuraphis noxia*, on crested wheatgrass, *Agropyron desertorum*, in comparison with Indian ricegrass, *Oryzopsis hymenoides* (Kauffman and LaRoche, 1994; Clark and Messina, 1998a, 1998b). Wheatgrass has flat, broad leaves while ricegrass has slender, rolled leaves. As a consequence, predators were more successful at capturing aphids on ricegrass than wheatgrass since prey fed in exposed locations. Predation and parasitism of the boll weevil, *Anthonomus grandis*, increased on cotton expressing the õfrego bractö trait (rolled-up floral bud bracts) in comparison to normal cotton plants. Rather than seeking refuge in the rolled bracts, this trait caused weevils to engage in movement from plant to plant thus exposing them to natural enemies. Prey attack was much lower on normal cotton since weevils remained in the flower buds (Mitchell *et al*., 1973). However, partial refugia are not necessarily associated with a plant structure that offers concealment. Grevstad and Klepetka (1992) observed that several species of coccinellids searching for aphids on crucifers could not reach aphids that fed on the middle of the undersides of the leaves because it may be impossible for them to grip these surfaces.

1.2.3.3 Gross Morphology

Gross morphology refers to whole plant shape, particularly the surface areas and branching of plant parts. Gross morphology can range from simple and linear to highly complex and branched. In general, researchers report that as a plant becomes more branched, predator efficiency decreases (Andow and Prokrym, 1990; Lukianchuk and Smith, 1997; Cloyd and Sadof, 2000; Gingras and Boivin, 2002; Legrand and Barbosa, 2003; Gingras *et al.,* 2008).

A useful analogy for the effect of increased branching of plants can be drawn from various experiments in artificial environments. Researchers report that as the number of possible paths increases, natural enemy foraging success decreases. Kaiser (1983) compared foraging rates of predatory mites in flat arenas with varying numbers of borders. Arenas with a greater number of borders caused predators to have the lowest predation rates because discontinuous paths slowed predator searching behaviour. Similarly, Hoddle (2003) experimented with arenas with increasing numbers of vertices. He found that each additional vertex led to a decrease in prey capture. In three-dimensional environments the same pattern has been observed. By using paper structures that differed only in the number of branches, Andow and Prokrym (1990) were able to eliminate influences of chemicals, surface textures, and size on the searching behaviour of the parasitoid *Trichogramma nubilale*. Highly branched structures differed from simple ones in that they were cut to have more finger-like projections, thus introducing more edges along which parasitoids could search. The results showed that on simple paper structures *T. nubilale* was able to locate hosts almost two and a half times faster than on complex ones. The same results were observed for *Trichogramma evanescens* searching for hosts on artificial structures that were of simple, intermediate and complex architecture. The artificial plant structures in the latter experiment differed only in the density of connections. Parasitoids attacked twice as many hosts on simple structures (Gingras and Boivin, 2002).

Studying the effects of architecture on predator foraging using real plants is challenging due to confounding factors such as plant phytochemicals, differing nutritional quality of plants and differing surface features. The use of near isogenic plants is an

effective way to study the effects of plant architecture since it eliminates these issues. Near-isolines of the same species differ in only one trait (Bottrell *et al.*, 1998). Legrand and Barbosa (2003) used pea near-isolines to study coccinellid searching abilities on plants with divergent architectures. Previously, they had found that these pea near-isolines did not alter aphid fecundity and intrinsic rate of increase, and the differing leaf morphology did not influence the within-plant distribution of aphids (Legrand and Barbosa, 2000). They showed that the predator, *Coccinella septempunctata*, was less successful at capturing prey on plants that had more junctions, a high leaf edge to leaf surface ratio and greater surface area. Ladybugs searched predominantly leaf edges; therefore on more highly branched peas with more edges, predators took longer to encounter prey. Search paths overlapped more frequently on complex plants resulting in poor efficiency. In contrast, Kareiva and Sahakian (1990) found that coccinellids were more effective at capturing prey on leafless pea morphs that leafy ones. Ladybugs fell from tendril morph plants less frequently, apparently because they could grasp the tendrils and manoeuvre on these leafless plants more easily. As a result, predators controlled aphid populations more effectively on the leafless morphology. Although these two sets of authors did not compare exactly the same range of pea morphologies, their results do represent an interesting contrast. In one case the authors conclude that highly branched plants decrease predator consumption, and in another case, the authors find increase consumption on highly branched plants. One possible explanation for these differing results is the effect of surface area. It is known that prey density affects predator consumption rates. Therefore, if differences in morphology lead to differences in

12

surface area, we should expect different consumption rates irrespective of any effect of morphology on predator movement rates.

Within a canopy of branches, the amount of connectivity between branches is also important. The parasitoid, *Trichogramma turkestanica*, encountered more eggs on simple plants because this architecture favoured linear walking at high velocities. Increasing connectivity increased the number of possible paths and directions that can be taken (Gingras *et al.,* 2008). Similarly, *Trichogramma minutum,* attacked more eggs when searching on continuous surfaces than divided surfaces, because edges redirected their search path such that previously searched areas were re-examined (Lukianchuk and Smith, 1997). Kareiva and Perry (1989) created a higher degree of plant connectivity by attaching leaves of adjacent peas together. *Hippodamia convergens* captured more prey in these altered canopies because they could travel among plants more easily than if adjacent leaves were not overlapping. Adding linear connectivity changed the trajectory of the predators and increased their efficiency. It should be noted that insects search behaviour is not completely random. Insect predators employ a variety of strategies to navigate through plant canopies to locate prey.

1.2.4 Insect Foraging Behaviour

Predators who feed on herbivores are faced with the challenge of travelling among plants to locate prey. Insects have developed a number of strategies to deal with the challenges of plant architecture. The searching behaviour of coccinellids and chrysopids has been particularly well studied since these generalist predators are conspicuous in a variety of habitats and have potential as biological control agents (Bond, 1980; Norlund and Morrison, 1990; Dixon, 2000; Koch 2003).

A common search mechanism used by entomophagous insects is a combination of area-concentrated (intensive) searching and extensive searching (Carter and Dixon, 1982; Bond, 1980). This mechanism is seen mostly in predators such as coccinellids, syrphids and chrysopids that feed on sedentary insects such as aphids (Krebs, 1973; Curio 1976; Bond, 1980). It is generally used by predatory insects whose resources are distributed in patches or clumps (Nakamuta, 1985). Intensive search is initiated when a prey item is encountered. If prey is captured, there is a period of handling time while the predator consumes the prey item. Afterwards, intensive searching continues since the predator may be aware it is within a prey patch. This period of searching is characterized by frequent turning and a reduction in speed (Carter and Dixon, 1982). If a period of time passes and no further prey is found the predator switches to extensive searching whereby it decreases turning and increases speed in an effort to locate the next patch of prey (Bond, 1980; Carter and Dixon, 1982; Nakamuta, 1985).

Understanding the sensory capabilities of these predators is crucial for predicting their capacity to recognize and to capture prey. There are several cues that insects rely on to guide them between patches. These cues can be visual, chemical, tactile (Bell, 1990) and, in some instances, learning can play a role in determining how a predator finds patches (Ferran and Dixon, 1993). For example, many insects depend on the surface features of plants to direct their searching. They will follow leaf edges or veins since these features help them to orient their search and lead them to prey (e.g. coccinellids Bell, 1990). This technique is effective since aphids are often concentrated in these areas (Ferran and Dixon, 1993). Although their sense of sight is not highly developed, coccinellids have demonstrated the

14

ability to visually recognize prey. Nakamuta (1985) found that *Coccinella septempunctata* was capable of visually deciphering prey from non-prey like objects. *Harmonia axyridis* can use both visual and olfactory cues to detect its prey. Obata (1986) found that *H. axyridis*, when presented with the choice between entering opaque bags filled with aphid-infested leaves and clean leaves, the ladybugs chose the bags with aphids. In another trial, when ladybugs could only see leaves but could not detect smell, they entered bags filled with leaves rather than control bags which were empty (Obata, 1986). Bahlai *et al*. (2008) tested the ability of *H. axyridis* to discern between the visual appearance and odour of apple and buckthorn leaves, since these ladybugs are often seen feeding on aphids on buckthorn. During visual bioassays, ladybugs chose to move towards silhouettes of leaves over blank spaces. Ladybugs did not choose buckthorn leaves over apple leaves unless olfactory cues were also included.

Conversely, lacewings do not appear to respond to visual or chemical cues. They search primarily by moving their head from side to side until their mandibles contact a prey item. Prey contact is the only cue that initiates intensive search, which means that their searching efforts are intensified following the successful attack of a prey item (Bond, 1980; Norlund and Morrison, 1990).

Internal information, gained from learning or conditioning, can also impact the way a predator searches (Ferran and Dixon, 1993). Predators may learn from previous experience, which improves their searching efficiency, while conditioning can influence the search mechanism or the type of prey captured (Bell, 1990). Conditioning has been observed in coccinellids and it affects their switch from extensive to intensive search as well as the type

of prey they seek. For example, *H. axyridis* raised on either pea aphids, *Acyrthosiphon pisum*, or *Ephestia kuehniella* eggs developed a preference for the food on which they were reared. When other food was available they consumed it but it would not cause them to switch from extensive to intensive searching. *H. axyridis* continued to search extensively until their preferred prey was encountered (Ettifouri and Ferran, 1993).

1.2.5 Predator Body Size

Based on what is reported in the literature, plant morphology can influence predator foraging success in a variety of ways. It is often argued that opposing results may be related to the body size of the predator (Vohland, 1996; Yang, 2000; Lucas *et al*., 2004). As body size changes, the way in which an individual experiences its environment also changes (With, 1994). The size-grain hypothesis states that as body size decreases, the environment experienced by terrestrial walking organisms becomes less planar and more rugose, that is, it has more contours or wrinkles (Kaspari and Weiser, 1999). With respect to morphological features such as trichomes, waxes, refugia and gross morphology there is evidence that body size is influential (eg. Lucas *et al*., 2004)

There is an allometric relationship between leg and body length for walking insects (Teuscher *et al*., 2009). Therefore, as body size increases, leg length increases in proportion. A longer leg implies a longer stride length which may be directly correlated with movement ability (With, 1994). A greater stride length also allows individuals to interact with the environment at a different scale of habitat structure, which may help lower complexity (With, 1994). For example, when a large insect is travelling across a surface with dense trichomes it will step over these obstacles because it has long enough legs to do so. A small insect, on the

other hand, may be able to travel between the spaces of the trichomes, and its mobility will also be relatively unimpeded. Medium-sized individuals, therefore, may be the most likely to be impeded by dense trichomes since they cannot step over or fit between trichomes.

It is difficult to conclude if a particular size is preferential for mobility on surfaces with trichomes. Some authors have suggested that small body size is preferable on pubescent plants (Obrycki and Tauber, 1984; Yang, 2000). Treacy *et al*. (1985) found that third instar lacewing larvae, *Chrysopa rufilabris,* were less affected by trichome presence than smaller second instars. In this case the smaller predator did not have an advantage in the pubescent environment. Conversely, Lucas *et al*. (2004) measured the efficiency of the ladybugs, *Coleomegilla maculata* and *Delphastus catalinae*, preying on greenhouse whitefly and observed that *C. maculata* were impeded by trichomes while smaller *D. catalinae* individuals were not.

With respect to smooth leaf surface covered with waxes, there is evidence that indicates body mass affect an insect's attachment to these slippery surfaces. Gorb *et al*. (2001, 2002) reported that as insect body mass increases, friction and adhesion decrease. In a centrifugal force tester they found that more acceleration was required to detach small insects than large ones because the relationship of mass-to-friction is higher in small insects. Since body mass generally increases with body size, it is possible that larger, heavier insects would slip from waxy surfaces more frequently than small insects. However, the effect of body size and mass has not been examined in relation to attachment to leaf wax.

Although studies of plant architecture and foraging capacity generally conclude that branching and prey capture are inversely related, very few studies have examined the effect

of body size on foraging in environments of varying complexity. Bearing in mind that body size and environmental experience are linked (With, 1994), and body size is correlated with stride length (Teuscher *et al.*, 2009), in environments with varying levels of branching and connectivity, one might suspect that natural enemies with longer stride lengths would have an advantage since they could step from branch to branch with ease. Their ability to span between obstacles could enable them to search a particular patch faster or a whole plant in less time than it would take a small predator to search the same plant. If this is true, large insects would be more efficient than small ones on plants with greater architectural complexity.

1.2.6 Conclusions

Upon reviewing the literature there is a strong indication that the relationship between plants and predators is not well understood. Predator foraging efficiency is strongly influenced by habitat structure: specifically, plant morphological features such as shape, size, surface textures and prey refugia. Studies have shown that these features affect predator foraging success by slowing predator movement, decreasing prey encounters, or decreasing manoeuvrability. There are two aspects of the plant-predator relationship that I believe need to be further investigated. First, the effects of gross plant morphology are not well understood. For example, Legrand and Barbosa (2003) reported that *Coccinella septempunctata* foraging on highly branched pea morphs were less successful at capturing aphids than on normal morphs. In contrast, Kareiva and Sahakian (1990) reported that ladybugs were more successful at controlling aphid populations on highly branched leafless peas. Plant surface area was overlooked in these studies and therefore it is difficult to

conclude what effect gross morphology had on ladybugs. Future studies should be careful to control plant surface area since it effects prey density, which is the most influential factor on predator consumption.

Second, while it is apparent that plant morphology has an impact on predator mobility, it is not clear what this effect is, and how it relates to predator body shape and size. Several studies indicate that body size and environmental experience are directly linked.   I could find no studies which compared the success rate of predators of differing size foraging in complex environments. Hence, it remains unclear if particular plant morphological features affect smaller predators in the same manner. A better understanding of predator and plant relationships is invaluable for understanding population dynamics of predators and prey, and is also important if predators are going to be used in a biological control context. The aim of this project is to study the effects of gross plant morphology on the foraging success of insect predators.

Chapter 2: Effects of plant gross morphology on predator foraging success

2.1 Overview

The effects of habitat structure on the consumption rates of two generalist predators: the multicoloured Asian ladybeetle, *Harmonia axyridis* Pallas, and the green lacewing, *Chrysoperla carnea* Stephens, feeding on pea aphids¸ *Acyrthosiphon pisum* Harris, were measured. Predator consumption was first compared in homogeneous environments (Petri dishes) and heterogeneous environments (whole plants). Consumption rates were also compared on four peas (*Pisum sativum* L.) with similar surface areas but different gross morphologies. I found that habitat structure had a significant effect on the predatorsø abilities to find and consume prey. Predators consumed significantly more aphids in Petri dishes than on whole plants of the same size (P<0.0001), which suggests that habitat structure has a strong influence on predator foraging behaviour. Furthermore, I found that small-scale differences in plant gross morphology also had a significant effect on the consumption rates of both ladybugs (P=0.015) and lacewings (P<0.0001). Both predators were more successful at capturing aphids on morphologies that were highly branched. I speculated that predators move more easily over highly branched plants because there are more edges to grasp.

2.2 Introduction

Previous studies have shown that spatial structure can influence predator-prey interactions by impeding predator mobility (Kareiva and Sahakian, 1990; Grevstad and Klepetka, 1992; Clark and Messina, 1998a, 1998b; Legrand and Barbosa, 2003). For

example, morphological characteristics, such as leaf waxes, can make surfaces too slippery for predators to walk across (Eigenbrode *et al.* 1996; Chang *et al.*, 2006; Gentry and Barbosa, 2006) while hairy surfaces with trichomes can be cumbersome to traverse (Treacy *et al.*, 1985; Coll and Ridgway, 1995; DeClercq *et al.*, 2000; Stavrinides and Skirvin, 2003; Mahdian *et al.*, 2006). In addition, some plant structures may provide partial or full refugia for prey, thus reducing predator attack (Mitchell *et al.*, 1973; Kauffman and LaRoche, 1994; Clark and Messina, 1998a, 1998b).

The overall shape and connectivity of plant parts (i.e. its gross morphology) can also affect a predator's foraging success (Andow and Prokrym, 1990; Lukianchuk and Smith, 1997; Gingras and Boivin, 2002; Gingras *et al.,* 2008). For example, Cloyd and Sadof (2000) found that the height, number of leaves, leaf surface area, and number of branches of variegated coleus, *Solenostemon scutellarioides*, was negatively correlated with the searching efficiency of *Leptomastix dactylopii*.

It is clear that leaf surface textures and gross morphology can alter predator mobility and yet predator efficiency is often measured in simple lab environments such as Petri dishes (Norlund and Morrison, 1990; Lee and Kang, 2004; Provost *et al.,* 2006; Cabral *et al.*, 2007; Oliveira *et al.* 2007). These studies may lead to inaccurate predictions about how predators behave in natural environments. For example, O'Neil (1989) compared the functional responses of the spined soldier bug, *Podisus maculiventris*, measured under laboratory and field conditions. In Petri dishes, *P. maculiventris* consumed an average of 4.4 Mexican bean beetles per day, and its ability to consume more prey was associated with limitations of

handling time. In field experiments, *P. maculiventris* consumed only 0.42 bean beetles per day because its ability to locate prey was reduced by the structure of the plant canopy.

In addition, it is unknown if different predators will be affected by morphological characteristics in the same manner. As body size changes, the way in which an individual experiences its environment also changes (With, 1994). The size-grain hypothesis states that as body size decreases, the environment experienced by terrestrial walking organisms becomes less planar and more rugose, that is, it has more contours or wrinkles (Kaspari and Weiser, 1999; Farji-Brener *et al*., 2004). As well, there is an allometric relationship between leg and body length for walking insects (Teuscher *et al*., 2009). Therefore, as body size increases, leg length increases in proportion. A longer leg implies a longer stride length which may be directly correlated with movement (With, 1994). A greater stride length also allows individuals to interact with the environment at a different scale of habitat structure, which in some cases may help lower complexity (With, 1994). For example, a large insect travelling across a surface with dense trichomes will step over these obstacles because it has long enough legs to do so, while a smaller predator will have less mobility since it cannot step over these structures.

While we can determine the scale-dependency of plant effects on insect predators by comparing the response of animals of different size to aspects of plant morphology, it is more difficult to isolate the effects of gross plant morphology. A comparison of different plant species confounds effects of gross morphology, surface textures and phytochemistry. For example, Mahadian *et al*. (2007) measured the functional response of predatory shield bugs (*Picromerus bidens*) on tomato, sweet pepper and eggplant. They observed that predators

were less efficient on tomatoes and concluded that plant morphology and chemistry had an influence on predator foraging behaviour. However, because these plants differed in more than one trait, it was not possible for them to draw specific conclusions about the effects of plant morphology.

Plants that are the same species but which have different shapes may allow us to isolate the effect of gross morphology, if the different varieties are sufficiently similar. Near-isolines are plants of the same species that differ in only one trait (Bottrell *et al.*, 1998). However, this approach has yielded contradictory results. Using near-isolines of pea that differed only in gross morphology, Legrand and Barbosa (2003) found that *Coccinella septempunctata* was less successful at capturing prey on pea morphs that had more junctions, and a high leaf edge to leaf surface ratio. In contrast, Kareiva and Sahakian (1990) found that *Coccinella septempunctata* and *Hippodamia variegata* were more effective at capturing prey on pea morphs which had more junctions and a high leaf edge to surface area ratio than leafy ones.

A possible explanation for these differing findings is that in both studies, pea plant surface area was not controlled. Differences in gross morphology could cause differences in the mean plant size. In fact, Legrand and Barbosa (2003) report that plants with more edges also had larger surface area. A plant with the larger surface area but the same number of aphids would have fewer aphids per unit area. Since predator consumption increases with prey density below a saturation density associated with handling time, it is possible that a larger surface area would decrease predator consumption.

This study has three objectives. I determined the effects of gross morphology on the foraging rates of the multicoloured Asian ladybeetle, *Harmonia axyridi*s, and the green lacewing, *Chrysoperla carnea*, by comparing the number of prey consumed on simple Petri dish environments versus whole plants. I hypothesized that the more heterogeneous plant structure would reduce consumption rates, even when the same surface area was available to search. Second, I investigated whether more subtle differences in gross morphology, in particular *Pisum sativum* L. near-isolines with different shapes, might alter predation successes when surface area was controlled. Finally, I compared the consumption rates of the two predators on these pea plants to determine if body size influences the effects of gross plant morphology.

2.3 Materials and Methods

2.3.1 Study System

I used near-isoline morphs of the pea cultivar õFrogelö with reduced stipules as a host plant, (USDA-ARS Western Regional Plant Introduction Station, Pullman, WA). Near-isolines reduce the confounding effects that plant phytochemicals and nutrients may have on either aphids or predators (Legrand and Barbosa, 2000). Furthermore, previous research indicated that these pea near-isolines did not affect aphid fecundity and reproduction, and that the differing leaf morphology did not influence the within-plant distribution of aphids (Legrand and Barbosa, 2000; Buchman and Cuddington, 2009).

The architecture of the near-isolines is caused by differences in 2 alleles, *af* (afila) and *tl* (acacia), at 2 loci (Wehner and Gritton, 1981). The õnormalö (AfAFTlTl) morph

consisted of several pairs of leaflets with an odd number of tendrils. The õleafletö morph (AfAftltl) had additional leaves in the place of tendrils making this morph dense with foliage. The tendril morph (afafTlTl) had only tendrils and no leaves. Lastly, the combination of both *af* and *tl* alleles creates the õparsleyö morph (afaftltl) which consists of highly branched petioles and tiny leaflets (Figure 2.1).

To quantify the differences in architecture I measured the fractal dimension of each morph. Fractal dimension describes the space-filling properties of an object and has commonly been used as a metric for describing plant morphologies (Morse *et al*., 1985; Gunnarsson, 1992; Gee and Warwich, 1994). As fractal dimension increases, the space-filling properties of the object increase. Therefore, I expected a higher fractal dimension for plants with more leaf tissue and a low fractal dimension for those that had more branching and less tissue.

Peas were potted in 12cm deep x 10cm wide pots with Pro-Mix ˵BRKø soil and watered daily. Plants were kept in growth chambers (model GC-20; Bio Chambers, Winnipeg, MB) on 16L: 8D photoperiod with a 14 °C-18 °C temperature regime for approximately 30 days. The light intensity of the growth chambers was approximately 450 mol m^{-2} s^{-1}. Maintaining constant conditions for growth helped to control the size of the peas so they had approximately equal surface areas. Since the tendril morph is leafless and has a low surface area, two seeds were planted in each pot to ensure the surface area would be similar to the leafed morphs.

Aphids were obtained from laboratory populations that were originally supplied by Carolina Biological Supply (Burlington, NC). Populations were maintained on a mixture of

õLittle Marvelö garden peas (*Pisum sativum*) and broad bean, (*Vicia faba*). Adult multicoloured Asian ladybeetles (*Harmonia axyridis*) were reared on a diet of *Ephestia kuehniella* eggs, pea aphids (*Acyrthosiphon pisum*), organic apples and sugar water. Larvae were fed *Ephestia kuehniella* eggs and pea aphids daily. Aphids and ladybugs were housed in mesh enclosures (Bugdorm II; BioQuip Products, Rancho Dominguez, CA). Green lacewing larvae (*Chrysoperla carnea*) were supplied by Plant Products (Brampton, ON). They were reared in individual Petri dishes, to avoid cannibalism, and fed *Ephestia kuehniella* eggs and pea aphids daily. All insects were kept on a 16L: 8D photoperiod, between 18 and 24°C.

2.3.2 Experiment 1: Effects of habitat structure on consumption rates

Prey consumption by *H. axyridis* and *C. carnea* was measured in Petri dishes and on whole plants. Petri dishes, 10 cm in diameter, were filled with semi-soft agar. Leaf tissue from pea plants was cut and placed on the agar surface until the Petri dish was covered. Twenty-five pea aphid nymphs (*Acyrthosiphon pisum*) were added to the Petri dishes with a fine paintbrush. Nymphs were placed in an aggregated distribution and allowed to settle for one hour. Whole plants of approximately the same surface area were inoculated with 3 adult aphids, which were allowed to reproduce for 72 hours. Afterwards, the 3 adults were removed and nymphs were counted to ensure only 25 individuals were present on each plant. Removing adults ensured that there would be no further aphid reproduction during the experimental period. To measure the spatial distribution of aphids, photographs of aphid clusters were taken in a 2 x 2cm sampling area (Figure 2.2). Using Photoshop, aphids in the sampling area were marked with a dot. The x, y coordinates of each aphid were then

measured using the particle counter in ImageJ and a SAS macro created by Moser (1987) was used to calculate two-dimensional nearest neighbour distances which acted as a surrogate for three-dimensional distances on whole plants. Petri dishes and whole plants were placed in behavioural enclosures consisting of a 10 cm pot with a Styrofoam insert, enclosed by a 20 x 30 cm acrylic cylinder with an Anti-virus mesh screen[TM] top (Figure 2.3).

Predators were starved for 24 hours and then allowed to forage in Petri dishes and on whole plants for 48 hours (16L: 8D cycle). Following the 48 hour foraging period, predators were removed and the remaining aphids were counted. Each treatment was replicated 15 times with a naïve predator. Following each trial, Petri dishes and whole plants were scanned using a CanoScan 5600F photo scanner. Using ImageJ, scans were converted into binary images and a standard box count was done using the FracLac_2.5 Release 1d plugin. The number of pixels in scanned images was used to calculate whole plant and Petri dish surface areas.

2.3.3 Experiment 2: Effects of gross morphology on consumption rates

To determine the effect of plant gross morphology on predator consumption rates, I measured the number of pea aphid nymphs consumed by *H. axyridis* and *C. carnea* on the four pea morphs (normal, leaflet, parsley, tendril) at 3 different approximate aphid densities. As previously described, adult aphids were allowed to reproduce on 30 day old pea plants reared in growth chamber conditions. After 72 hours, the number of aphid nymphs was adjusted by addition or removal to be exactly 10, 25 or 50 animals in an aggregated pattern. Experiments were conducted in the same enclosures described in experiment 1 (Figure 2.3), and consumption rates were measured using the same protocol. Exact aphid densities were

27

determined by measuring the plant surface area following the trail, and those plants with very large or small surface areas were not used. Each treatment was replicated 15-20 times.

2.4 Results

2.4.1 Experiment 1: Effects of habitat structure on consumption rates

Student's t-tests (SAS Institute, 2010) were used to compare the surface areas of Petri dishes and whole plants, as well as, mean nearest neighbour distances on Petri dishes and whole plants. All surface area and nearest neighbour data passed the Shapiro-Wilk test for normality and the Levene's test for homogeneity of variances. The surface areas of Petri dishes and whole plants did not differ for ladybug or lacewing experiments (Student's t-test, t =0.42; 28 df; P=0.679 and t =1.10; 28 df; P=0.281), and mean nearest neighbour distance of aphids on Petri dishes and whole plants did not differ (Student's t-test t=1.20; 20 df; P=0.244 and t=0.56; 20 df; P=0.581). Therefore, predators experienced the same prey density and aggregation in the two treatments.

Petri dish consumption data was non-normal according to the Shapiro-Wilk test; however, a data transformation was not appropriate because consumption was nearly 100% in many cases. Petri dish and whole plant consumption data were compared using a Mann-Whitney U test. Consumption on Petri dishes and whole plants differed significantly for both ladybugs and lacewings (Mann-Whitney U, U=23; df=1; P<0.0001 and U=18; df=1; P<0.0001; Figure 2.4). On Petri dishes, predators consumed 80-100% of all aphids present, while on whole plants they consumed approximately 30%.

2.4.2 Experiment 2: Effects of gross morphology on consumption rates

A one-way ANOVA was used to compare surface areas and fractal dimensions of each of the four pea morphs to ensure that plants were the same size, but different in fractal dimension. The surface areas of peas used for ladybug experiments did not differ significantly (one-way ANOVA $F_{3,239} = 0.13$; P=0.943). The average surface area of pea plants was about 188 cm^2 (Table 2.1), giving aphid density treatments of 0.05, 0.13 and 0.27 individuals/cm^2. In addition, the mean nearest neighbour distances of aphids did not differ on each pea morph (one-way ANOVA $F_{3,43} = 1.66$; P=0.190). Therefore, ladybugs experienced the same prey density and aggregation across the 4 different pea morphologies. However, as expected, there was a significant difference in the fractal dimensions of each pea morph (one-way ANOVA, $F_{3,28} = 175.05$; P<0.0001; Table 2.1). Tukey's comparison of means indicated that the leaflet morphology had the highest fractal dimension, followed by normal, parsley and tendril, indicating that the tendril morphology had the most branching and linear form.

Ladybug consumption data failed normality tests, and therefore was transformed using a square-root transformation, which is recommended for count data. A two-way analysis of variance on square-root transformed ($\sqrt{x + 0.5}$) data showed a significant effect of plant morphology on ladybug consumption ($F_{3, 239} = 3.56$; P=0.015). Tukey's comparison of means indicated that ladybugs consumed significantly more aphids on tendril plants compared to normal plants (Figure 2.5 a). There was also a significant effect of aphid density on consumption rates ($F_{2,239} = 71.62$; P<0.0001), but no interaction between morphology and density ($F_{6,239} = 0.85$; P=0.535).

The surface areas of pea morphs used for lacewing predation experiments did not differ significantly (Table 2.1), however their variances did. I used a Welch's one-way ANOVA for each aphid number to test that surface areas were comparable (10 aphids: F=2.05, P=0.127; 25 aphids: F=0.83, P=0.487; 50 aphids: F=0.10, P=0.962). In addition, the mean nearest neighbour distances of aphids did not differ on each pea morph (one-way ANOVA $F_{3,39}$=1.56; P=0.172). The fractal dimensions of pea morphs was significantly different (one-way ANOVA, $F_{3,30}$=73.49; P<0.0001; Table 2.1), with the lowest fractal dimension found for tendril morphs. A two-way ANOVA on square-root transformed ($\sqrt{x+0.5}$) data indicated that there was a significant effect of morphology on lacewing consumption rates ($F_{3,179}$=12.14; P<0.0001). Similarly to ladybug consumption data, Tukey's comparison of means indicated that the highest average consumption was on the tendril morph. As well, tendril and parsley had significantly different consumption rates than leaflet and normal (Figure 2.5 b). There was also a significant effect of aphid density on consumption rates ($F_{2,179}$=84.48; P<0.0001), but no interaction between morphology and density ($F_{6,239}$=1.77; P=0.108).

To determine if one predator was more efficient than the other I used a two-way ANOVA on predator type and consumption at one prey density (25 aphid nymphs). I found no significant interaction between predator type and morphology (Two-way ANOVA, $F_{3,119}$=0.61; P=0.592), indicating that the different sized predators had similar predation success on the differing plant morphology.

2.5 Discussion

I found that the foraging success of *Harmonia axyridis* and *Chrysoperla carnea* is greatly affected by gross plant morphology. Predator consumption rates were significantly higher on Petri dishes than on whole plants of the same surface area. As well, on pea near-isolines that did not differ significantly in surface area, both predators consumed significantly more aphids on tendril morphs than on leafier plants.

My results contradict the hypothesis that on highly branched, discontinuous structures predator foraging efficiency decreases (Andow and Prokrym, 1990; Lukianchuk and Smith, 1997; Cloyd and Sadof, 2000; Gingras and Boivin, 2002; Legrand and Barbosa, 2003; Gingras *et al.,* 2008). I agree that foraging behaviour is influenced by plant gross morphology; however, my results show that both *H. axyridis* and *C. carnea* were more successful at capturing prey on morphologies with more edges and branches. More specifically, my results contradict those reported by Legrand and Barbosa (2003) who indicate that the seven-spot ladybug, *Coccinella septempunctata*, captured the fewest pea aphids on parsley pea morphs when they compared normal, leaflet and parsley morphologies of the same pea near-isolines. I found that on the parsley morph the ladybug, *H. axyridis*, tended to consume more aphids than on leaflet and normal morphs.

Kareiva and Sahakian (1990) report similar results to mine when they measured aphid suppression on tendril and normal pea varietals. They observed that the ladybugs, *Coccinella septempunctata* and *Hippodamia variegata*, captured more pea aphids on tendril peas than normal ones. They attributed this difference to ladybugs falling off the normal morph because they could not grip leaves as effectively as they could tendrils. In contrast, Legrand

31

and Barbosa hypothesized that ladybugs were less successful on parsley morphs because these plants had many edges and junctions which caused predators to retrace their footsteps creating an inefficient search strategy.

There are two possible explanations for these contradictory results. First, Legrand and Barbosa used the seven-spot ladybug (*C. septempunctata*) while I used the multicoloured Asian ladybug (*H. axyridis*). Therefore, I cannot rule out the possibility that these predators have different searching strategies or consumption capacities which could account for the differences observed. However, I note that Kareiva and Sahakian (1990) also used the seven-spot ladybug and found a different result. Secondly, it is unclear if Legrand and Barbosa (2003) controlled the surface areas of peas in their study. Their methods state that they grew pea plants until they had nine leaves (in the case of the parsley morph, I assume the term õleafö refers to each cluster of tiny leaflets). They reported that the mean leaf areas of normal, leaflet and parsley morphs were 137.8, 194.7 and 807.0 cm^2. Therefore, it would appear that the overall surface areas of the normal and leaflet morphs were significantly smaller than those of the parsley morph. The large difference in surface area may have contributed to the lower consumption rates on parsley morphs since aphid density was lower on this morph. Predator consumption decreases at low prey densities (Holling, 1959). Furthermore, I observed a negative correlation between consumption and surface area (Figure 2.6); a relationship which has been reported by other authors as well (Ables *et al.* 1980; Casas 1991; Maini *et al.* 1991; Stamp and Bowers 1993; Wang *et al*. 1997; Cloyd and Sadof, 2000).

Prey inaccessibility also affects predator consumption rates (Mitchell *et al*., 1973; Grevstad and Klepetka, 1992; Kauffman and LaRoche, 1994; Clark and Messina, 1998a, 1998b). Morphs with large leaves (i.e. normal and leaflet) may provide partial refugia for prey. Pea aphids most often congregate on the undersides of leaves to feed, and this area may be inaccessible for *H. axyridis* and *C. carnea* if they require leaf and tendril edges to manoeuvre on the plant, as suggested by Kareiva and Sahakian (1990). Similarly, adaxial leaf surfaces of the crucifer, *Brassica oleracea caulorapa*, provided partial refugia for cabbage aphids because ladybugs could not cling to leaf undersides (Grevstad and Klepetka, 1992). I found that lacewings and ladybugs consumed significantly more aphids on tendril morphs than on leaflet and normal morphs. It is likely that prey inaccessibility is one of the reasons for this difference. Therefore, predators are more likely to control aphid populations on this type of morphology since manoeuvrability and prey access are increased.

There was no clear indication that body size had a strong influence on the predatorsø experiences with their spatial environment. I expected that the larger bodied *H. axyridis* might be less affected by the branching structure of parsley and tendril morphs, since it has a larger stride length which might allow it to span branching structures when walking. However, there was no indication that this predator had an advantage over smaller *C. carnea* larvae. In fact, I suspect that the larger body size of *H. axyridis* may have decreased its manoeuvrability and increased its propensity to fall off of plants.

Moreover, it is surprising that mean consumption rates for these two predators were so similar on whole plants. Predators with smaller body sizes generally have smaller guts and therefore become satiated more quickly. As well, Latham and Mills (2009) found that in

field, cage and lab tests *H. axyridis* always consumed more than the lacewing, *Chrysoperla nigricornis*. In Petri dishes, with 25 aphids, I observed that 75% of the time ladybugs consumed all aphids present, while lacewing larvae consumed all 25 aphids only about 35% of the time. However, I observed that on whole plants lacewing larvae consumed comparable quantities of prey. In fact, on tendril morphs at high prey density (50 aphids) lacewings consumed slightly more aphids on average than ladybugs (Figure 2.5). Differences in prey encounter rates may account for this difference. The smaller body size and vermiform shape of lacewing larvae may enable them to disturb prey patches less frequently. Aphids can detect plant vibrations caused by an approaching predator. This triggers the defensive response to drop from plants (Losey and Denno, 1998). Ladybugs or large-bodied predators are easier for prey to detect and avoid by dropping. Francke *et al*. (2008) found that aphid dropping behaviour reduced consumption by *H. axyridis* by 40%. Small-bodied predators, such as, a lacewing larvae, can approach prey patches without disturbing them, and therefore, may encounter more prey than ladybugs. This difference may be more pronounced if there was a larger difference between predator body sizes.

The results of this study clearly demonstrate that plant morphology has a strong influence on predator foraging success, and even subtle differences in plant gross morphology change predator consumption rates. Highly branched morphologies have a significant effect on predator foraging success, and therefore it is possible that on a larger scale these effects would significantly affect prey populations. In order to extrapolate these results to biological control recommendations, factors such as microclimate and community interactions would have to be observed more closely.

It would also be valuable to identify the underlying search mechanisms that explain why predators are more successful on certain plant morphologies than others. My observations lead me to believe that partial prey refugia and edge effects are the two main factors that dictate foraging success for these predators. Therefore, I tested these hypotheses by observing predator movement on the four pea near-isolines to see if movement patterns correspond with consumption rates. By understanding predator foraging behaviour in greater detail it will be possible to make more accurate predictions about how they will respond to prey populations in real-world ecosystems.

Figure 2.1 Binary images of leaves of pea near-isolines (*Pisum sativum* L.). From left to right: leaflet, normal, parsley, tendril.

Table 2.1 Mean surface areas and fractal dimensions of pea morphs.

Predator	Morphology	Surface Area (cm^2)	Fractal Dimension
Ladybug	Leaflet	184.6 ± 48.9	1.63 ± 0.01
	Normal	181.0 ± 37.6	1.54 ± 0.02
	Parsley	186.1 ± 47.6	1.49 ± 0.03
	Tendril	184.7 ± 52.7	1.40 ± 0.02
Lacewing	Leaflet	192.8 ± 28.4	1.63 ± 0.02
	Normal	188.6 ± 35.1	1.55 ± 0.04
	Parsley	195.3 ± 34.1	1.44 ± 0.04
	Tendril	195.3 ± 21.1	1.37 ± 0.04
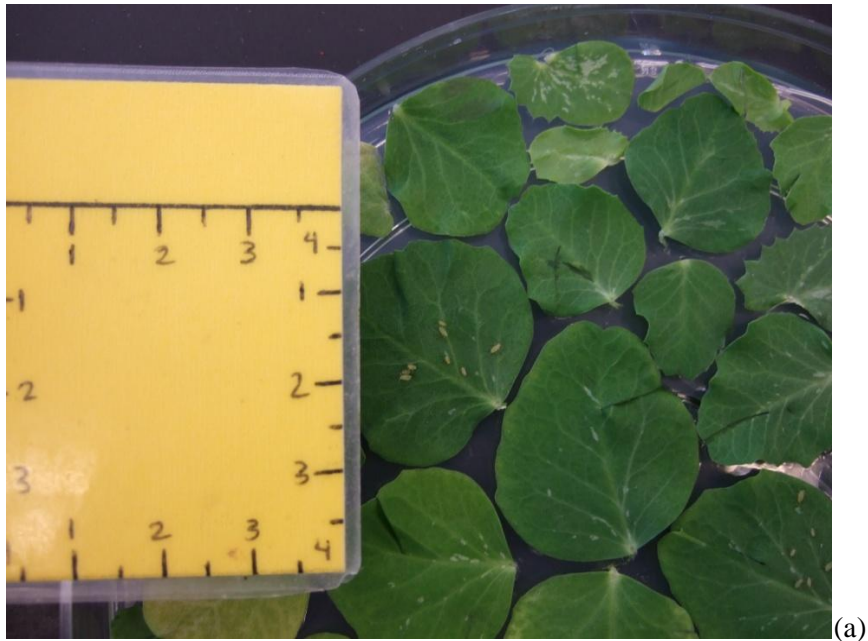
(a)
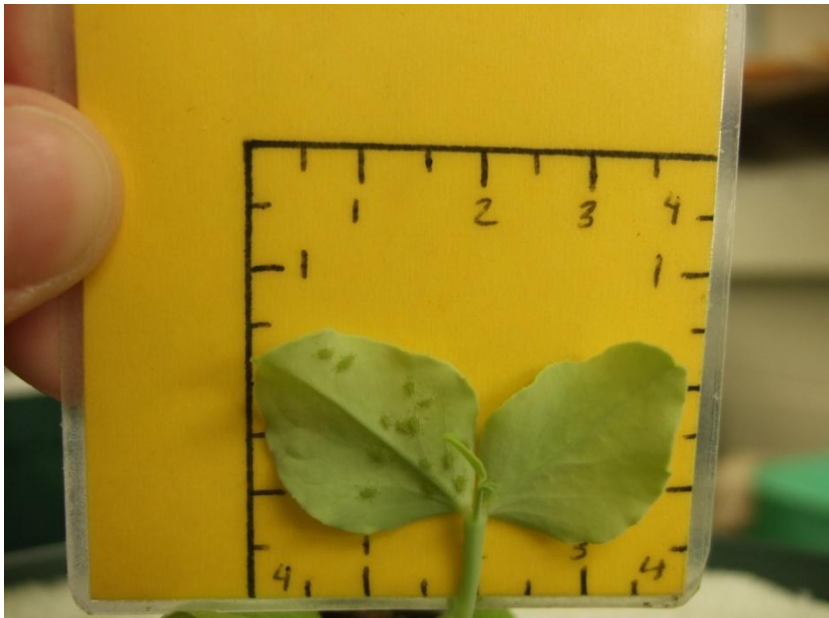
(b)

Figure 2.2 Photograph illustrating method used to calculate 2-dimensional nearest neighbour distances on (a) Petri dishes and (b) whole plants.

Figure 2.3 Behavioural enclosures used for Petri dish and whole plant foraging experiments.
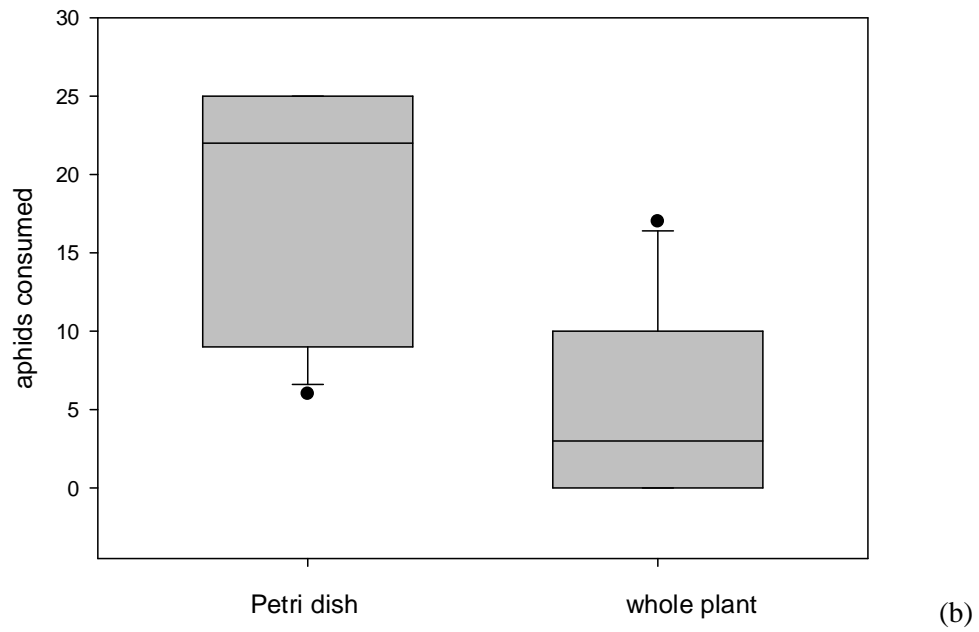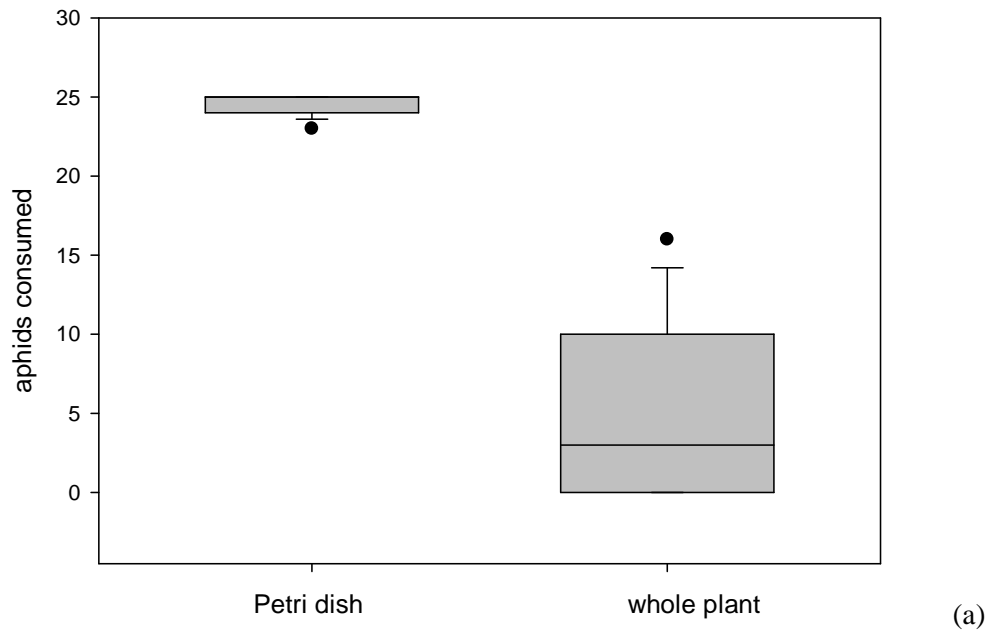
Figure 2.4 Aphid consumption by (a) *H. axyridis* and (b) *C. carnea* when foraging in Petri dishes and on whole plants with 25 aphids for 48 hours.
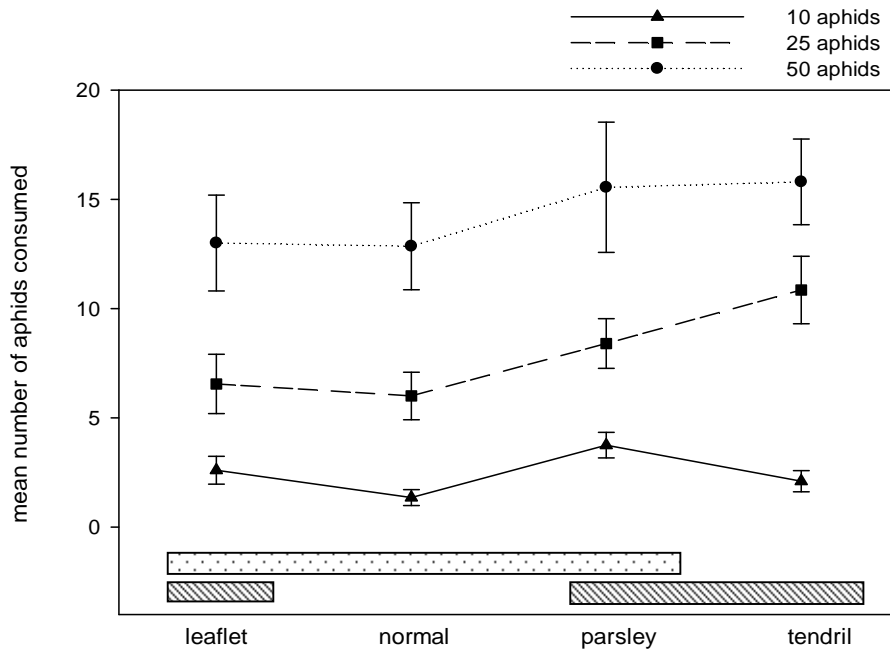
(a)
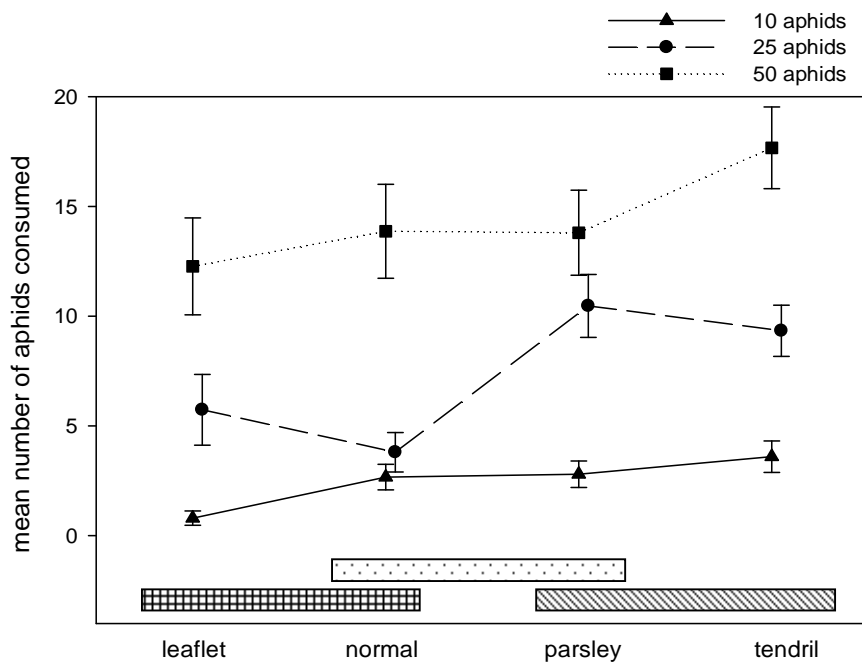
(b)

Figure 2.5 Mean aphid consumption by (a) *H. axyridis* and (b) *C. carnea* at low (10), medium (25), and high (50) aphid densities. Untransformed data ±1 S.E is shown in graphs. Bars with the same pattern show morphologies that are not significantly different according to Tukey's comparison of means.

Figure 2.6 Scatter plot of aphids consumed by *H. axyridis* versus plant surface area.

Chapter 3: Effects of plant gross morphology on predator searching behaviour

3.1 Overview

I previously concluded that the consumption rates of *Harmonia axyridis* Pallas and *Chrysoperla carnea* Stephens feeding on pea aphids (*Acyrthosiphon pisum* Ha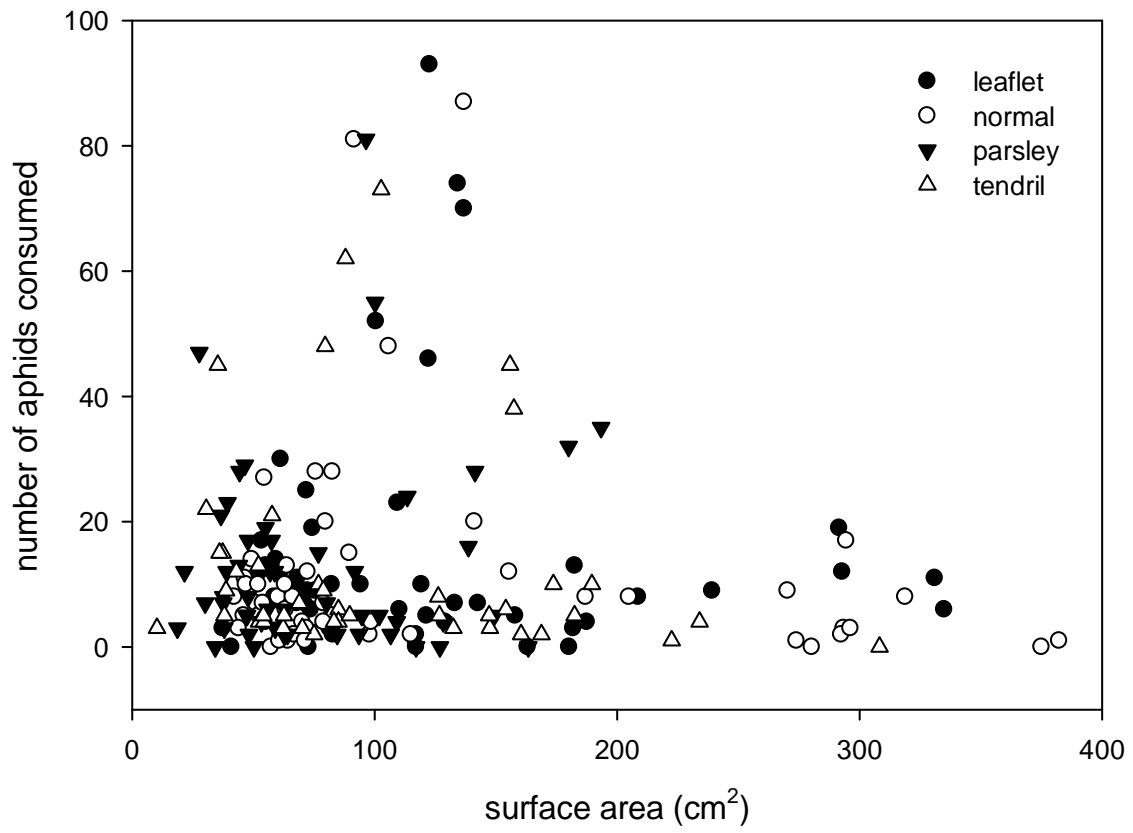rris) were altered by the different morphologies of a set of four peas (*Pisum sativum* L.) that differed only in two alleles at two loci. The searching behaviour of these two predators was analyzed on the same pea near-isolines to determine what mechanisms were responsible for these results. It has been previously suggested that the search paths of predatory insects may be altered by the branching of plant morphology. Using time-lapse photography I recorded the search paths of these two predators on the four pea near-isolines in order to test this hypothesis. I digitized two-dimensional representations of predator search paths and then measured total path length, percentage of plant covered, and path tortuosity. The search paths of *H. axyridis* were significantly longer on more highly branched morphologies (P=0.002), while the search paths of *C. carnea* did not differ. Previous observations of predator foraging behaviour led me to hypothesize that consumption rates were affected by predators falling off plants and by prey residing on the undersides of leaves where predators could not access them. To test these two hypotheses I measured the drop rates of *H. axyridis* on leafed versus leafless morphs, and found that ladybugs fell off leafed morphs more frequently than leafless ones. To test the effect of partial prey refugia on searching behaviour I used Petri dishes layered with leaf tissue. I placed Petri dishes at one of three orientations

(right-side up, upside down, and sideways) and allowed ladybugs to forage for prey. Ladybugs consumed significantly fewer prey on plates that were sideways and upside down (P<0.0001) which suggests that they cannot grip surfaces that are not oriented upward. Therefore, it is unlikely they can capture prey located on the undersides of leaves.

3.2 Introduction

The branching architecture of an individual plant is often considered a classic example of a dendritic network (Thompson, 1917). Dendritic networks are those that are made up of hierarchical branches, and habitats with this particular spatial arrangement have specific implications for the species that live within them. Because dendritic habitats are made up of a series of branches, individuals foraging in this type of environment will have restricted movement (Grant *et al*., 2007). Unless an individual's body size and stride length allows it to step across branches it will be confined to following specific search paths which may or may not lead it to prey.

There are several factors that affect prey encounter in habitats with branching structures. Some of these factors include: branch connectivity (Kareiva and Perry 1989; Randlkofer *et al*., 2010), attachment and manoeuvrability through branches (Kareiva and Sahakian, 1990), and prey inaccessibility due to partial refugia (Clark and Messina 1998a, 1998b). For example, Kareiva and Perry (1989) found that on bean plants with a large amount of leaf overlap, *Hippodamia convergens* had longer and straighter trajectories than on beans with less overlap, which they hypothesized would translate into more efficient searching. Conversely, Randlkofer *et al*. (2010) observed that connectivity prevented the

44

parasitoid, *Oomyzus galerucivorus*, from reaching upper areas of vegetation because they engaged in backwards movement or used connection points as resting places. This prevented them from continuing to search upward where most hosts were located.

Furthermore, the number of branches can significantly change a predator's foraging rate (Andow and Prokrym, 1990; Lukianchuk and Smith, 1997; Cloyd and Sadof, 2002; Gingras and Boivin, 2002; Legrand and Barbosa, 2003; Gingras *et al.,* 2008). In general, these studies have concluded that an increasing degree of plant structural complexity leads to a decrease in foraging efficiency (Randlkofer *et al.*, 2010). For example, Gingras and Boivin (2002) found that on plants with complex architecture, parasitoids attacked fewer hosts than on those structures that were simple and linear. On pea plants, Legrand and Barbosa (2003) found that ladybugs had decreased searching efficiency on highly branched plants because they retraced their steps and engaged in less õnew area searchö. Conversely, Kareiva and Sahakian (1990) observed that ladybugs were more successful at capturing prey on branched, leafless peas than leafy ones because they could manoeuvre on branched morphologies without falling off of plants.

A predator's ability to attach to plant structures and manoeuvre over heterogeneous surfaces will also influence its movement rates. Leaves with waxes can cause predators to slip (Eigenbrode *et al.*, 1996), while those with trichomes can be difficult to walk over with ease (Coll and Ridgway, 1995). Plants that do not have adequate edges for insect tarsae to grip may cause them to fall (Kareiva and Sahakian, 1990) or it may prevent them from searching particular portions of plants, where prey may be located, because they do not have adequate foot holds.

Plant structures that preclude predator search can act as partial refugia for prey. The presence of partial refugia can greatly affect predator foraging success because certain patches of prey will not be accessible. For example, Kauffman and LaRoche (1994) tested the ability of five aphidophagous coccinellids to exploit curled wheat leaves while foraging for Russian wheat aphids. They found that predator foraging success was affected by curled leaves because several of the ladybug species could not enter leaf curls where many aphids were feeding. This leaf structure served as partial refugia for prey, and only those predators that had body sizes smaller than the diameter of leaf curls could reach aphids feeding in these locations.

My objective was to observe the searching behaviours of predators on peas that differed in morphology to see if search strategies differed and if any observed differences in predator behaviour corresponded to the different consumption rates reported in the previous chapter. First, I measured the total path lengths, percentages of plants searched, and path tortuosity of *Harmonia axyridis* and *Chrysoperla carnea* on pea near-isolines that differed only in gross morphology to see if predator search paths differed on plants with different branching patterns. Based on the higher consumption rates on branched morphologies I previously observed, I hypothesized that search paths would be longer on these morphs, and that predators would search these morphs more thoroughly than leafy morphologies.

I also conducted two experiments to explore the mechanisms responsible for differences in consumption rates previously observed. I measured *H. axyridis* drop rates on leafless versus leafed morphologies to determine if there was a difference in attachment and manoeuvrability on each morph. I hypothesized that on leafless morphs, ladybugs would fall

off plants less frequently since they could grip tendrils more easily than leaves. I also hypothesized that the requirement for predators to grip tendrils and the edges of leaves might produce partial refugia in the center of large leaves. I tested this hypothesis by measuring predator consumption on Petri dishes with different spatial orientations (right-side up, upside down and sideways). I predicted that ladybugs would be unsuccessful at capturing prey on Petri dishes that were oriented downwards since they may not be able to grip flat surface with no edges while upside-down.

3.3 Materials and Methods

3.3.1 Study System

Near-isoline morphs of the pea cultivar õFrogelö with reduced stipules were used as a host plant, (USDA-ARS Western Regional Plant Introduction Station, Pullman, WA). Using isolines reduces the likelihood that plants will affect predator behaviour because they differ in only one trait (Bottrell *et al*., 1998). Previous research indicated that these particular pea isolines did not affect aphid fecundity and reproduction, and that the differing leaf morphology did not influence the within-plant distribution of aphids (Legrand and Barbosa, 2000; Buchman and Cuddington, 2009).

The morphology of the near-isolines is caused by differences in 2 alleles, *af* (afila) and *tl* (acacia), at 2 loci (Wehner and Gritton, 1981). The õnormalö (AfAFTlTl) morph consisted of several pairs of leaflets with an odd number of tendrils. The õleafletö morph (AfAftltl) had additional leaves in the place of tendrils. The combination of both *af* and *tl*

alleles creates the õparsleyö morph (afaftltl) which consists of highly branched petioles and tiny leaflets. The tendril morph (afafTlTl) had only tendrils and no leaves.

To characterize the gross morphology of these four plant morphs I used fractal dimension, and I avoided the use of the term õcomplexityö, which has commonly been used in other studies. The term õcomplexityö is quite vague, and plant structures that some researchers have identified as õcomplexö may not be perceived that way by a foraging insect. Fractal dimension describes plant morphology based on its space-filling properties. A low fractal dimension indicates a structure that is linear and branched. Highly branched morphologies would correspond to those that other authors have labeled as õcomplexö. As fractal dimension increases, plant structure becomes less linear and more space-filling. Leafy morphologies may be considered less complex since there are fewer junctions and branches, therefore, predators may have fewer search paths to choose from. However, leafy morphologies may not be simpler for predators to search because leaves may provide partial refugia for prey (Grevstad and Klepetka, 1992) or they may be too slippery for predators to walk across (Kareiva and Sahakian, 1990; Eigenbrode *et al*. 1996; Chang *et al*., 2006; Gentry and Barbosa, 2006).

Peas were potted in 12 cm deep x 10 cm wide pots with Pro-Mix ӠBRKø soil and watered daily. Plants were kept in growth chambers (model GC-20; Bio Chambers, Winnipeg, MB) on 16L: 8D photoperiod with a 14 °C-18 °C temperature regime for approximately 30 days. The light intensity of the growth chambers was approximately 450 mol m^{-2} s^{-1}. Maintaining constant conditions helped control the size and surface area of

peas so they were similar. Because tendril morphs are leafless, I planted two seeds per pot to ensure the surface areas of this morph would be similar to the leafed morphs.

Aphids were obtained from laboratory populations that were originally supplied by Carolina Biological Supply (Burlington, NC). Populations were maintained on a mixture of õLittle Marvelö garden peas (*Pisum sativum*) and broad bean, (*Vicia faba*). Adult multicoloured Asian lady beetles (*Harmonia axyridis*) were reared on a diet of *Ephestia kuehniella* eggs, pea aphids (*Acyrthosiphon pisum*), organic apples and sugar water. Larvae were fed *Ephestia kuehniella* eggs and pea aphids daily. Aphids and ladybugs were housed in mesh enclosures (Bugdorm II; BioQuip Products, Rancho Dominguez, CA). Green lacewing larvae (*Chrysoperla carnea*) were supplied by Plant Products (Brampton, ON). They were reared in individual Petri dishes to avoid cannibalism, and fed *Ephestia kuehniella* eggs and pea aphids daily. All insects were kept on a 16L:8D photoperiod, between 18 and 24 ^{o}C.

3.3.2 Experiment 1: Effects of gross morphology on predator movement

The searching behaviour of *H. axyridis* and *C. carnea* was recorded on each of the four pea near-isolines. Pea plants were removed from growth chambers after 30 days and inoculated with three adult pea aphids. Aphids were allowed to reproduce for 72 hours, and then removed. Aphid nymphs were counted and additions or removals were done until each plant had 25 nymphs in an aggregated distribution. Using plants infested with aphids helped ensure predators would engage in natural searching behaviour rather than wandering off plants. Predators were starved for 24 hours prior to experiments which increased their motivation to search for prey. Plants were placed in enclosures consisting of 20 cm pots with

Styrofoam inserts containing 12 x 10 cm square pots embedded in the Styrofoam. Pea plants were placed in the square pots and enclosed by a 20 x 30 cm acrylic cylinder with an Anti-virus mesh screen[TM] top. One predator was placed in each enclosure and its movements were recorded for ten minutes.

To record predator movements, digital cameras (Hp Photosmart 618) were clamped on retort stands and placed in front of behavioural enclosures (Figure 3.1). Digital cameras were set to take time-lapse photographs every 15 seconds for 40 frames while predators foraged on plants. If a predator wandered off the plant during the ten minute observation period, the trial was not used.

Photographs were uploaded to a computer and stacks of images were analyzed using the Manual Tracking plugin of ImageJ. Using this plugin I recorded the x,y coordinates of each movement the predator made. By tracing the predator's two-dimensional trajectory I was also able to provide an index of the total path length. I also measured an index of the total percentage of the plant searched by measuring the size, in pixels, of the plant and of the 2D trajectory and dividing these two values.

Finally, I measured path tortuosity by calculating the fractal dimension of 2D trajectories. Several authors have shown that the fractal dimension of an animal's path can be used as an index for the tortuosity of its movement (Katz and George, 1985; Dicke and Burrough, 1988; Casas and Aluja, 1997; Etzenhouser *et al*., 1998; Doerr and Doerr, 2004; Loureiro *et al*., 2007). Measuring path tortuosity is one method for characterizing insect movements through dendritic habitats. When the fractal dimension of an animal's search path is close to 1, its movement is relatively linear. As fractal dimension increases towards

2, the path becomes more convoluted. A search path with a high fractal dimension indicates that the animal is back-tracking or constrained in a certain area. Therefore, search behaviour may be inefficient. It may also indicate that the animal is engaged in an õarea restricted searchö meaning that the predator has located a patch of prey and is focusing on searching in an area of high prey density. This searching technique is used by many entomophagous insects that search for prey that aggregate in patches (Krebs, 1973; Curio, 1976; Bond, 1980; Carter and Dixon, 1982).

I tested each predator on each plant morph a total of 10 times and compared the total path length, percentage of plant covered and path tortuosity to determine if there was a difference in the efficiency with which each plant morph was searched for prey. The effect of plant morphology on each metric was compared using a one-way analysis of variance.

3.3.3 Experiment 2: Effects of gross morphology on *H. axyridis* drop rates

I measured how frequently *H. axyridis* fell off of plants while foraging. Methods for this experiment were taken from Kareiva and Sahakian (1990) so that the two studies could be compared. I compared only leaflet and tendril morphs for this experiment because these two morphs have the greatest difference in edge to surface area ratios. An individual predator was added to either a tendril or leaflet plant with a paintbrush and observed for two minutes. If a predator fell off the plant during the two minute observation period it was counted as a õdropö and the trial was ended. Each experiment was replicated 60 times with a naïve predator. Drop rates were compared using a G-test of independence.

3.3.4 Experiment 3: Effects of partial refugia on *H. axyridis* foraging success

To test the ability of *H. axyridis* to grip leaf undersides I measured aphid consumption on Petri dishes at different orientations. Ten centimeter Petri dishes were filled with agar and whole pea leaves pressed into the agar surface (Figure 3.2). Leaves were inoculated with 25 aphid nymphs in a uniform distribution. Dishes were placed in behavioural enclosures described in experiment 1, except Styrofoam inserts had additional supports to hold Petri dishes in one of three orientations: right-side up, upside down, and sideways. After being starved for 24 hours, one ladybug was added to each enclosure and allowed to forage for 24 hours. At the end of the foraging period the remaining aphids were counted. Each treatment was replicated 19 times. A Kruskal-Wallis test was used to compare median consumption at each orientation.

3.4 Results

3.4.1 Experiment 1: Effects of gross morphology on predator movement

Two-dimensional path lengths and the percentages of plants covered by the search path violated homoscedasticity according to a Levene's test, so a log transformation was applied. Plant gross morphology had a significant effect on ladybug foraging behaviour. There was a significant difference in path lengths (one-way ANOVA, $F_{3,39}=6.24$; $P=0.002$; Figure 3.3). Total path lengths were significantly longer on parsley than on normal and leaflet morphs (Table 3.1). In addition, the total percentage of plants covered by the search path differed between morphologies (one-way ANOVA, $F_{3,39}=6.60$; $P=0.001$; Figure 3.4). Ladybugs searched a significantly larger total percentage of parsley and tendril morphs than

leaflet (Table 3.1). I should note that the index of proportions of plants searched, given by the 2-dimensional approximation of the true path, are low and are of course underestimates of the true proportion of plants searched because measurements were taken from 2D images. Finally, there was no difference in ladybug path tortuosity on each of the morphs (one-way ANOVA, $F_{3,39}$=0.71; P=0.552; Figure 3.5).

Lacewing searching behaviour was not affected by plant gross morphology. There was no significant difference in total path lengths ($F_{3,39}$=0.42; P=0.739; Figure 3.3) and percentages of plants searched ($F_{3,39}$=0.57; P=0.636; Figure 3.4) on each of the four morphs. Also, there was no difference in path tortuosity ($F_{3,39}$=0.97; P=0.416; Figure 3.5).

3.4.2 Experiment 2: Effects of gross morphology on *H. axyridis* drop rates

The edge to surface area ratio of pea plants had an effect on the ability of ladybugs to attach to peas while walking on them.   *H. axyridis* fell off plant with fewer edges (leaflet morphs) more frequently than those with more edges (tendril morphs), (G-test of independence, G= 75.8; df =1; P<0.001).

3.4.3 Experiment 3: Effects of partial refugia on *H. axyridis* foraging success

There was a significant difference in ladybugsøability to capture prey on Petri dishes with different orientations. Consumption data was non-normal according to a Shapiro-Wilk test. Applying a data transformation did not improve normality so a non-parametric Kruskal-Wallis test was used. Ladybugs captured a significantly greater number of aphids on Petri dishes that faced upwards (Kruskal-Wallis, H=37; df=2; P<0.001; Figure 3.6). On plates that

were upside down and sideways, ladybugs captured, on average, 12% of aphids present while on plates that were right-side up they consumed about 80% of aphids.

3.5 Discussion

Plant gross morphology appears to have an effect on *H. axyridis* mobility but not *C. carnea*. These results demonstrate that increasing plant branching (or õcomplexityö as it is called by many authors e.g. Andow and Prokrym, 1990) does not always decrease predator movement or foraging success. While foraging on pea near-isolines, I observed that *Harmonia axyridis* searched branched morphologies (parsley and tendril) more thoroughly than compact leafy ones (leaflet and normal). This result contradicts Legrand and Barbosaøs report (2003) about *Coccinella septempunctata* foraging on similar isogenic peas. The authors found that ladybugs were inefficient when searching on parsley morphs because they would retrace their search paths, and spent more time searching tiny leaflets rather than advancing to new areas of the plant. I found that *Harmonia axyridis* had the longest search paths on parsley morphs, and consequently they searched a greater total percentage of these plants. The consumption rates reported in the previous chapter also reflect the movement behaviour of this predator. I observed that ladybugs consumed significantly more aphids on parsley and tendril morphs than on leaflet and normal.

There are several possible explanations for this difference in the observed behaviour of very similar predators. First, I suspect that my results differ from those of Legrand and Barbosa (2003) because they observed the behaviour of predators foraging on pairs of leaves, while I observed predators moving on whole plants. Legrand and Barbosa found that 7-spot

ladybugs spent more time searching clusters of leaflets on parsley morphs than whole leaves of the normal and leaflet morphs. It should be noted that I observed that multicoloured Asian ladybugs can only search the perimeter of whole leaves and cannot thoroughly search the undersides of leaves where aphids are commonly found. Therefore, these ladybugs walk around the edges of leaves quickly and then advance to a new area of the plant. On the parsley morph, ladybugs may spend a greater amount of time searching individual leaflets before they advance to a new search area, but in doing so they are more likely to encounter aphids.

The total path lengths and percentages of plants searched by lacewings did not differ on peas with different morphologies. It appears that plant gross morphology does not affect their mobility. However, lacewings consumed a greater number of aphids on tendril and parsley morphs than leaflet and normal (see chapter 2), despite the fact that they searched the same percentage of each morph. Since path lengths did not differ, but consumption rates did, lacewings likely had a higher encounter rate with aphids on tendril and parsley morphs. I speculate that on normal and leaflet morphs lacewings encountered fewer aphids because they were hidden on the undersides of leaves.

The likelihood that a predator will fall off of plants also affects its foraging efficiency. Predators that fall will spend much of their time finding their way back to the plant, and may only search the bottom portion of the plant thoroughly. I measured the frequency at which ladybugs dropped off leaflet and tendril pea morphs. Ladybugs fell off leaflet plants 33 times and off tendril plants 23 times out of 60 trials each. This behavior likely contributes to their lower consumption rates on normal and leaflet morphs, as reported

in chapter 2. My result is similar to what Kareiva and Sahakian (1990) report about the

ladybugs, *Coccinella septempunctata* and *Hippodamia variegata* foraging on leafless peas

and normal peas. They found that ladybugs fell off normal peas more frequently than on

leafless peas and concluded that this was because predators could grip tendrils of leafless

peas more effectively than whole leaves of normal peas.

The presence of prey refugia plays a significant role in predator foraging success

(Mitchell *et al*., 1973; Grevstad and Klepetka, 1992; Kauffman and LaRoche, 1994; Clark

and Messina, 1998a, 1998b). In this system, I hypothesized that there may be a partial

refugia on the underside of large leaves, because of the need for larger bodied predators to

grip leaf edges in order to remain on the plant. I tested this hypothesis by measuring

consumption rates of differently oriented Petri dishes layered with leaf tissue. I found that on

plates which were upside-down and sideways, ladybugs consumed very few aphids compared

to plates oriented upwards.

In other systems, prey inaccessibility directly influenced predator foraging success.

Clark and Messina (1998a, 1998b) observed that lacewing larvae and ladybugs captured

fewer aphids on grasses with rolled leaves where aphids could not be reached. Similarly,

ladybugs could not capture prey located on leaf undersides of crucifers (Grevstad and

Klepetka, 1992). Because ladybug tarsae cannot grip leaf surfaces, I observed that they kept

at least two tarsae on leaf edges at all times to prevent them from falling. Therefore, not all

aphids on leaf undersides were inaccessible; only those that were in the centre of the leaves.

Ladybugs could not grip Petri dishes that were upside-down, and as a result they consumed

few aphids. In fact, I suspect that they only consumed those that had fallen off the Petri

dishes. Frazer and McGregor (1994) observed similar behaviour when they examined the foraging behaviour of seven ladybug species foraging on artificial leaf and stem models. They found that ladybugs searched on the upper edges of artificial leaf disks, but ladybugs seldom walked on the central or undersides of disks.

In this particular system, the greater number of edges on parsley and tendril pea morphs appears to be the most influential morphological feature on predator search behaviour. Having more edges reduced the likelihood that *H. axyridis* fell off plants. It also reduced the amount of partial refugia that aphids could use to escape predation. To confirm this result, the drop rates and effects of prey refugia on *C. carnea* must be measured since they were only measured for *H. axyridis*.

When studying predator-prey interactions to better understand population dynamics for either ecological or practical purposes, the relationship between plants and predators has often been overlooked. My results on predator searching behaviour, in combination with the results on consumption rates from the previous chapter, indicate that plant gross morphology has an effect on insect predator foraging efficiency. This study emphasizes that plant-predator interactions should not be neglected when evaluating predator-prey dynamics. Instead, this system should be examined as a tritrophic one since each trophic level has an effect on the others.

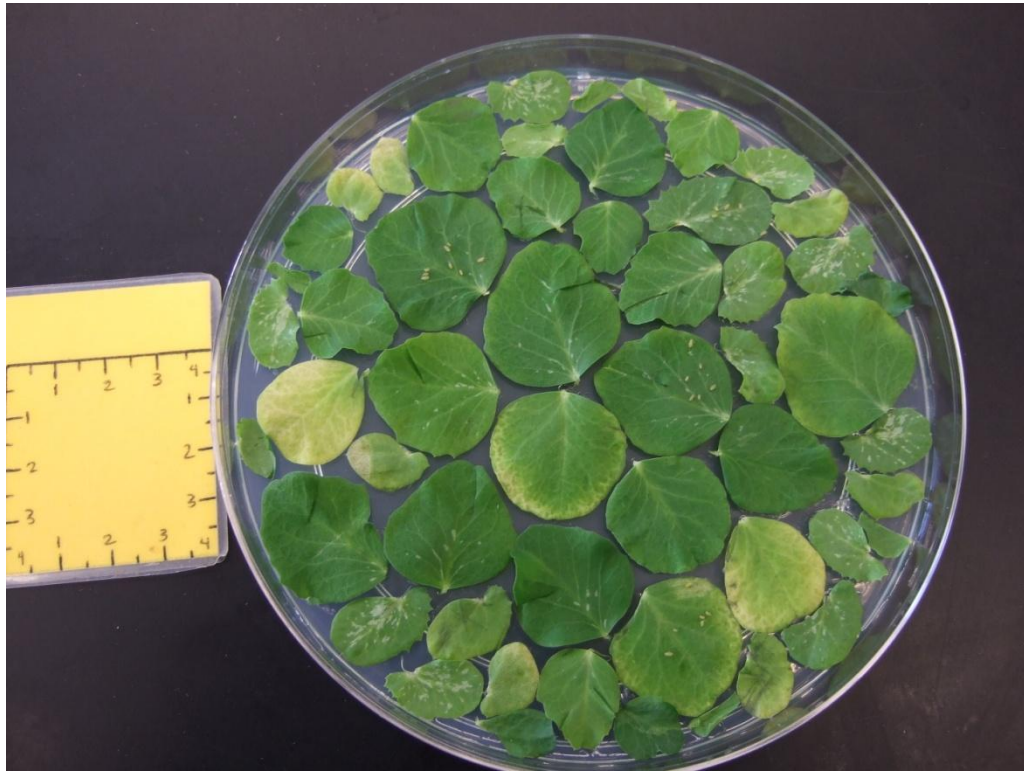**Figure 3.1** Experimental setup used to record predator movement.

Figure 3.2 Photograph of Petri dish filled with pea leaves used for experiments on partial refugia

Table 3.1 Summary of predator trajectories on the four pea near-isolines.

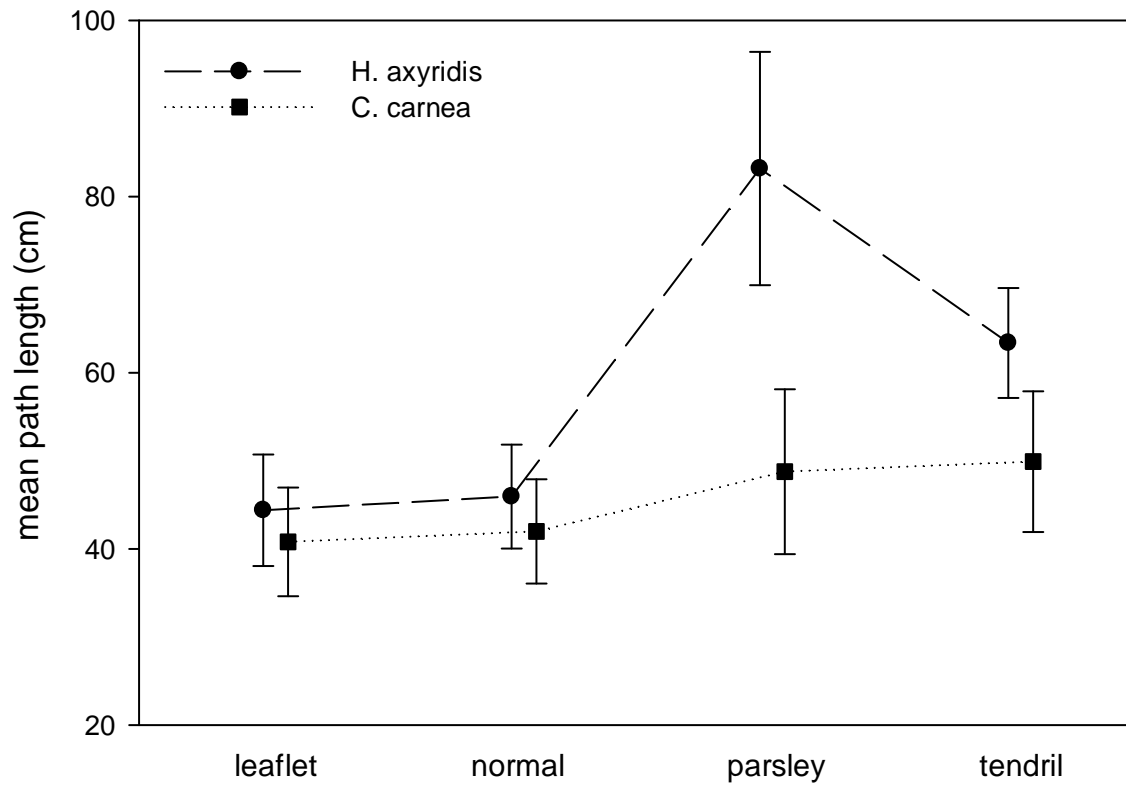| Predator	Morph	Mean path length (cm)	Mean % of plant searched	Path tortuosity
H. axyridis	Leaflet	44.38	2.92	1.22
	Normal	45.93	5.03	1.26
	Parsley	83.17	10.49	1.28
	Tendril	63.37	6.06	1.24
C. carnea	Leaflet	40.79	4.20	1.27
	Normal	41.98	4.88	1.24
	Parsley	48.76	6.78	1.18
	Tendril	49.90	5.21	1.24

Figure 3.3 Untransformed mean total path length of *H. axyridis* and *C.carnea* (±1 S.E.) on four pea near-isolines. *H. axyridis* had a significantly longer path length on the parsley morph.
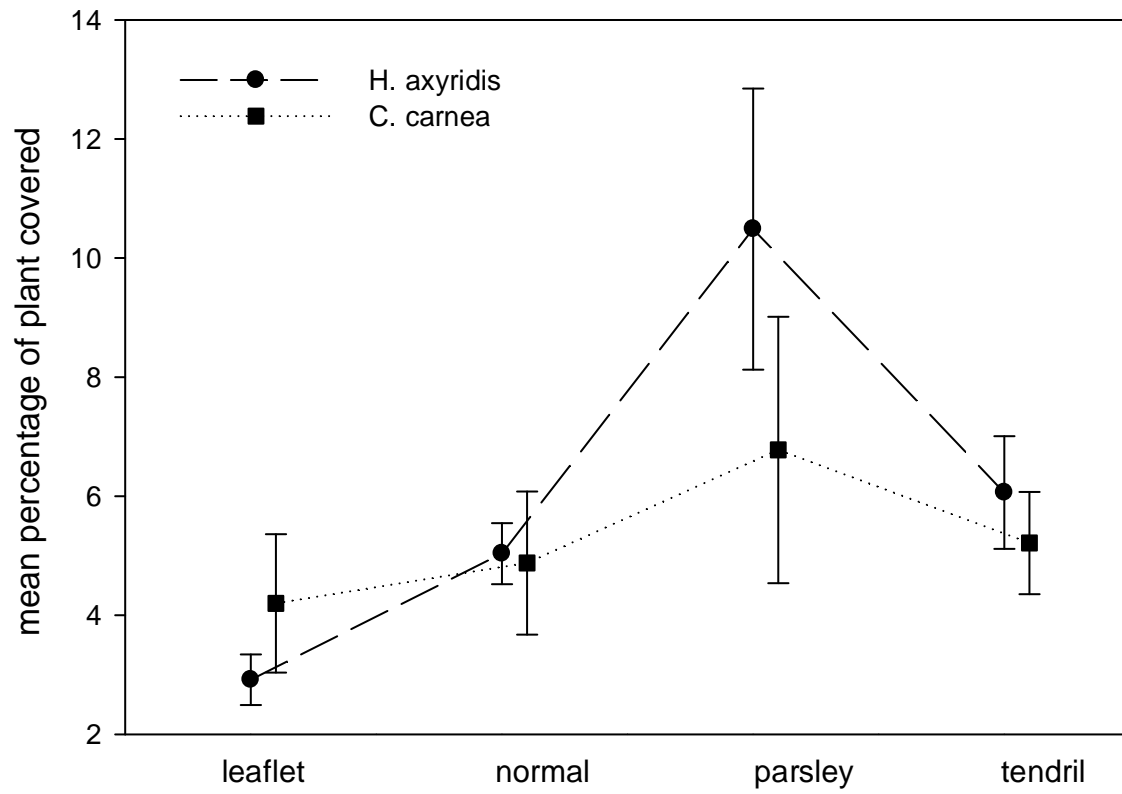
Figure 3.4 Untransformed mean percentage of plant searched by *H. axyridis* and *C.carnea* (±1 S.E.) on four pea near-isolines.
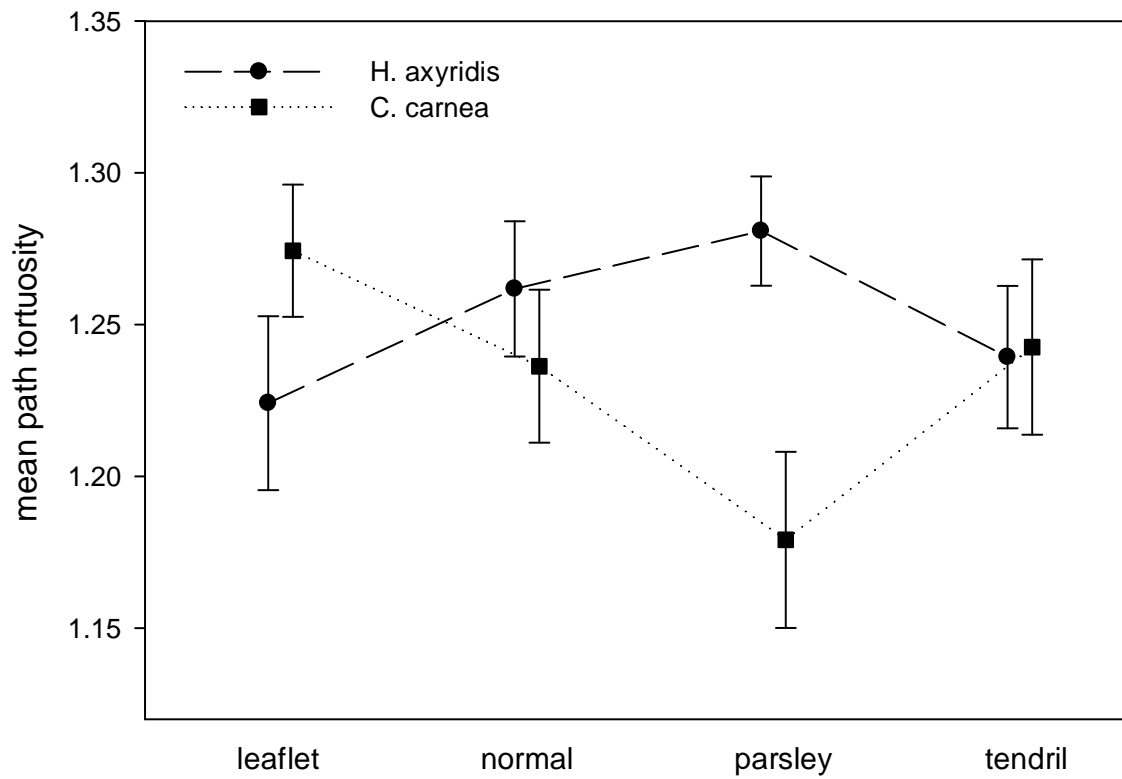
Figure 3.5 Mean tortuosity of *H. axyridis* and *C.carnea* search paths (±1 S.E.) on four pea near-isolines.
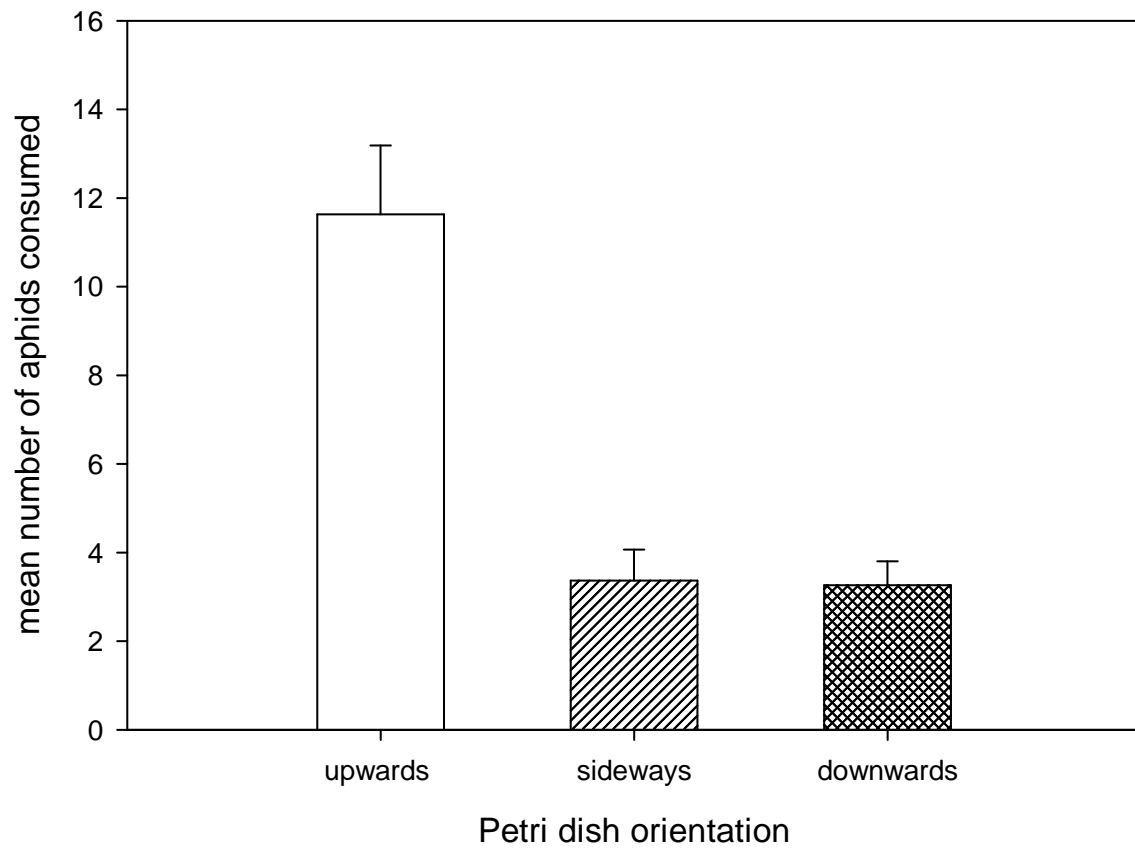
Figure 3.6 Mean consumption of aphids by *H. axyridis* (±1 S.E.) on Petri dishes with different orientations. Aphid capture was significantly higher (P<0.0001) on Petri dishes that faced upwards.

Bibliography

Ables, J. R., D. W. McCommas, S. L. Jones, and R. K. Morrison. 1980. Effect of cotton plant size, host egg location, and location of parasite release on parasitism by Trichogramma pretiosum. Southwestern Entomololgist 5:261-264.

Andow, D.A., and D.R. Prokrym. 1990. Plant structural complexity and host-finding by a parasitoid. Oecologia 82:162-165.

Bahlai, C.A., J.A. Welsman, E.C. Macleod, A.W. Schaafsma, R.H. Hallett, and M.K. Sears. 2008. Roles of visual and olfactory cues from agricultural hedgerows in the orientation behaviour of multicoloured Asian lady beetle (Coleoptera: Coccinellidae) Environmental Entomology 37:973-979.

Ball, J. C., and D.L. Dahlsten.1973. Hymenopterous parasites of Ips paraconfusus (Coleoptera: Scolytidae) larvae and their contribution to mortality. I. Influence of host tree and tree diameter on parasitization. Canadian Entomologist 105:1453-1464.

Bell, W.J. 1990. Searching behavior patterns in insects. Annual Review Entomology 35:447-467.

Bond, A.B. 1980. Optimal foraging in a uniform habitat: the search mechanism of the green lacewing. Animal Behaviour 28:10-19.

Bottrell, D.G., Barbosa, P., and F. Gould. 1998. Manipulating natural enemies by plant variety selection and modification: A realistic strategy? Annual Review Entomology 43:347-367.

Buchman N., and K. Cuddington. 2009. Inłuences of pea morphology and interacting factors on pea aphid (Homoptera: Aphididae) reproduction. Environmental Entomology 38:962-970.

Cabral, S., A.O. Soares, and P. Garcia. 2009. Predation by Coccinella undecimpunctata L. (Coleoptera: Coccinellidae) on Myzus persicae Sulzer (Homoptera: Aphididae): effect of prey density. Biological Control 50:25-29.

Carter, M.C., and A.F.G. Dixon. 1982. Habitat quality and the foraging behaviour of coccinellid larvae (Coccinella septempunctata). Journal of Animal Ecology 51:865-878.

Casas, J. 1991. Density dependent parasitism and plant architecture. Redia 74:217-222.

Casas J. and M. Aluja. 1996. The geometry of search movements of insects in plant canopies. Behavioural Ecology 8:37-45.

Chang, G.C., Neufeld, J., Eigenbrode, S.D., and G.C. Chang. 2006. Leaf surface wax and plant morphology of peas influence insect density. Entomologia Experimentalis et Applicata 119:197-205.

Clark, T.L., and F.J. Messina. 1998a. Plant architecture and the foraging success of ladybird beetles attacking the Russian wheat aphid. Entomologia Experimentalis et Applicata 86:153-161.

Clark, T.L., and F.J. Messina. 1998b. Foraging behavior of lacewing larvae (Neuroptera: Chrysopidae) on plants with divergent architectures. Journal of Insect Behavior 11:303-317.

Cloyd, R.A., and C.S. Sadof. 2000. Effects of plant architecture on the attack rate of Leptomastix dactylopii (Hymenoptera: Encyrtidae), a parasitoid of the Citrus Mealybug (Homoptera: Pseudococcidae). Environmental Entomology 29:535-541.

Coll, M., and R.L. Ridgway. 1995. Functional and numerical responses of Orius insidiosus (Heteroptera: Anthocoridae) to its prey in different vegetable crops. Annals of the Entomological Society of America 88:732-738.

Curio, E. 1976. The ethology of predation. Springer-Verlag, Berlin, Germany.

De Clercq, P., Mogaghegh, J., and L. Tirry. 2000. Effect of host plant on the functional response of the predator Podisus nigrispinus (Heteroptera: Pentatomidae). Biological Control 18:65-70.

Dicke, M. and P.A. Burrough. 1988. Using fractal dimensions for characterizing tortuosity of animal trails. Physiological Entomology 13:393-398.

Dixon AFG. 2000. Insect Predator-Prey Dynamics: Ladybird beetles and Biological Control. Cambridge University Press. Cambridge, United Kingdom.

Doerr V.A.J., and E.D. Doerr. 2004. Fractal analysis can explain individual variation in dispersal search paths. Ecology 85:1428-1438.

Eigenbrode, S.D., Castagnola, T., Roux, M.B., and L. Steljes. 1996. Mobility of three generalist predators is greater on cabbage with glossy leaf wax than on cabbage with a wax bloom. Entomologia Experimentalis et Applicata 81:335-343.

Eigenbrode S.D., Kabalo N.N., and C.E. Rutledge. 2000. Potential of reduced-wax bloom oilseed Brassica for insect pest resistance. Journal of Agricultural Entomology 17:53-63.

Ettifouri, M., and A. Ferran. 1993. Influence of larval rearing diet on the intensive searching behaviour of Harmonia axyridis [Coccinellidae] larvae. Entomophaga 38:51-59.

Etzenhouser, M.J., M. Keith Owens, D.E. Spalinger, and S. Blake Murden. 1998. Foraging behaviour of browsing ruminants in a heterogeneous landscape. Landscape Ecology 13:55-64.

Farji-Brener, A.G., G. Barrantes, and A. Ruggeiro. 2004. Environmental rugosity, body size and access to food: a test of the size-grain hypothesis in tropical litter ants. Oikos 104:165-171.

Ferran, A., and A.F.G. Dixon. 1993. Foraging behaviour of ladybird larvae (Coleoptera: Coccinellidae). European Journal of Entomology 90:383-402.

Francke, D.L., J.P. Harmon, C.T. Harvey, and A.R. Ives. 2008. Pea aphid dropping behavior diminishes foraging efficiency of a predatory ladybeetle. Entomologia Experimentalis et Applicata 127:118-124.

Frazer, B. D., and R. R. McGregor. 1994. Searching behaviour of adult female coccinellidae (Coleoptera) on stem and leaf models. Canadian Entomologist 126:389ó399.

Gee, J.M., and R.M. Warwich 1994. Metazoan community structure in relation to the fractal dimension of marine macroalgae. Marine Ecology Progress Series 103:141-150.

Gentry, G.L., and P. Barbosa. 2006. Effects of leaf epicuticular wax on the movement, foraging behavior, and attack efficacy of Diaeretiella rapae. Entomologia Experimentalis et Applicata 121:115ó122.

Gingras, D., and G. Boivin. 2002. Effect of plant structure, host density and foraging duration on host finding by Trichogramma evanescens (Hymenoptera: Trichogrammatidae). Environmental Entomology 31:1153-1157.

Gingras, D., Dutilleul, P., and G. Boivin. 2008. Effect of plant structure on searching strategy and searching efficiency of Trichogramma turkestanica. Journal of Insect Science 8:1-9.

Gorb, S., Gorb, E., and V. Kastner. 2001. Scale effects of the attachment pads and friction forces in syrphid flies (Diptera, Syrphidae). Journal of Experimental Biology 204:1421-1431.

Gorb, S.N., Beutel, R.G., Gorb, E.V., Jiao, Y., Kastner, V., Niederegger, S., Popov, V.L., Scherge, M., Schwarz, U., and W. Votsch. 2001. Structural Design and Biomechanics of Friction-Based Releasable Attachment Devices in Insects. Integrative and Comparative Biology 42:1127-1139.

Grant, J. F., and M. Shepard. 1984. Techniques for evaluating predators for control of insect pests. Journal of Agricultural Entomology 2:99-116.

Grant, E.H.C., W.H. Lowe, and W.F. Fagan. 2007. Living in the branches: population dynamics and ecological processes in dendritic networks. Ecology Letters 10:165-175.

Grevstad, F.S., and B.W. Klepetka. 1992. The influence of plant architecture on the foraging efficiencies of a suite of ladybird beetles feeding on aphids. Oecologia 92:399-404.

Grez, A.A., and P. Villagran. 2000. Effects of structural heterogeneity of a laboratory arena on the movement patterns of adult Eriopis connexa and Hippodamia variegate (Coleoptera: Coccinellidae). European Journal of Entomology 97:563-566.

Gunnarsson, B. 1992. Fractal dimension of plants and body size distribution in spider. Functional Ecology 6:636-631.

Hoddle, M.S. 2003. The effect of prey species and environmental complexity on the functional response of Franklinothrips orizabensis: a test of the fractal foraging model. Ecological Entomology 28:309-318.

Holling, C.S. 1959. Some characteristics of simple types of predation and parasitism. The Canadian Entomologist 91:385-398.

Holling, C.S. 1961. Principles of insect predation. Annual Review of Entomology 6:163-182.

Holling, C.S. 1966. The functional response of invertebrate predators to prey density. Memoirs of the Entomological Society of Canada 48:5-86.

Kaiser, H. 1983. Small scale spatial heterogeneity influences predation success in an unexpected way: model experiments on the functional response of predatory mites (Acarina). Oecologia 56:249-256.

Kareiva, P., and R. Perry. 1989. Leaf overlap and the ability of ladybird beetles to search among plants. Ecological Entomology 14:127-129.

Kareiva, P. and R. Sahakian. 1990. Tritrophic effects of a simple architectural mutation in pea-plants. Nature 345:433-434.

Kaspari, M., and M.D. Weiser. 1999. The size-grain hypothesis and interspecific scaling in ants. Functional Ecology 13:530-538.

Katz, M.J. and E.B. George. 1985. Fractals and the analysis of growth paths. Bulletin of Mathematical Biology 2:273-286.

Kauffman, W.C., and S. L. LaRoche. 1994. Searchingactivities by coccinellids on rolled wheat leaves infested by the Russian wheat aphid. Biological Control 4:290-297.

Kiritani, K., and J.P. Dempster. 1973. Different approaches to the quantitative evaluation of natural enemies. Journal of Applied Ecology 10:323-330.

Krebs, J.R. 1973. Behavioral aspects of predation. Pages 73-111 *in* P.P.G. Bateson and P.H. Klopfer, editors, Perspectives in Ethology. Plenum Press, New York, New York, USA.

Koch, R.L., 2003. The multicolored Asian lady beetle, Harmonia axyridis: A review of its biology, uses in biological control, and non-target impacts. Journal of Insect Science 32:1-15.

Latham, D.R., and N.J. Mills. 2009. Quantifying insect predation: a comparison of three methods for estimating daily per capita consumption of two aphidophagous predators. Environmental Entomology 38:1117-1125.

Lee J.H., and T.J. Kang. 2004. Functional response of Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae) to Aphis gossypii Glover (Homoptera: Aphididae) in the Laboratory. Biological Control 31:306-310.

Legrand., A and Barbosa P. 2000. Pea aphid (Homoptera: Aphididae) fecundity, rate of increase, and within-plant distribution unaffected by plant morphology. Environmental Entomology 29:987-993.

Legrand, A., and P. Barbosa. 2003. Plant morphological complexity impacts foraging efficiency of adult Coccinella septempunctata L. (Coleoptera: Coccinellidae). Environmental Entomology 32:1219-1226.

Levin, D.A. 1973. The role of trichomes in plant defense. The Quarterly Review of Biology 48:3-15.

Losey, J.E., and R.F. Denno. 1998. The escape response of pea aphids to foliar-foraging predators: factors affecting dropping behaviour. Ecological Entomology 23:53-61.

Loureiro, F., L.M. Rosalino, D.W. Macdonald, and M. Santos-Reis. 2007. Path tortuosity of Eurasian badgers (Meles meles) in a heterogeneous Mediterranean landscape. Ecological Research 22:837-844.

Lucas, E., Labreque, C., and D. Coderre. 2004. Delphastus catalinae and Coleomegilla maculata lengi (Coleoptera: Coccinellidae) as biological control agents of the greenhouse whitefly, Trialeurodes vaporariorum (Homoptera: Aleyrodidae). Pest Management Science 60:1073-1078.

Luck, R.F., B.M. Shepard, and P.E. Kenmore. 1988. Experimental methods for evaluating arthropod natural enemies. Annual Review of Entomology 33:367-391.

Lukianchuk, J.L., and S.M. Smith. 1997. Inł uence of plant structural complexity on the foraging success of Trichogramma minutum: a comparison of search on artiŁcial and foliage models. Entomologia Experimentalis et Applicata 84:221-228.

Mahdian, K., Tirry, L., and P. DeClercq. 2007. Functional response of Picromerus bidens: effects of host plant. Journal of Applied Entomology 131:160-164.

Maini, S., G. Burgio, and M. Carrieri. 1991. Trichogramma maidis host-searching in corn vs. pepper. Fourth European Workshop on Insect Parasitoids, Redia 74:121-127.

Marquis, R.J., and C. Whelan. 1996. Plant morphology and recruitment of the third trophic level: subtle and little-recognized defenses? Oikos 75:330-334.

Mitchell, H.C., Cross, W.H., McGovern, W.L., and E.M. Dawson. 1973. Behavior of the boll weevil on frego bract cotton. Journal of Economic Entomology 66:677-680.

Morse, D.R., J.H. Lawton, and M.M. Dodson. 1985. Fractal dimension of vegetation and the distribution of arthropod body lengths. Nature 314:731-733.

Moser, E.B. 1987. The analysis of mapped spatial point patterns. Pages 1141-1145 *in* Proceedings of the SAS Users Group International Conference, Dallas, Texas, USA, SAS Institute, Inc.

Nakamuta, K. 1985. Mechanisms of the switchover from extensive to area-concentrated search behaviour of the ladybird beetle, Coccinella spetempunctata bruckii. Journal of Insect Physiology 31:849-856.

Norlund, D.D., and R.K. Morrison. 1990. Handling time, prey preference, and functional response of Chrysoperla rufilabris in the laboratory. Entomologia Experimentalis er Applicata 57:237-242.

Oaten, A., and W.W. Murdoch. 1975. Functional response and stability in predator-prey systems. The American Naturalist 109:289-298.

Obata, S. 1986. Mechanisms of prey finding in the aphidophagous ladybird beetle, Harmonia axyridis [Coleoptera: Coccinellidae]. Entomophaga 31:303-311.

Obrycki, J.J., and M.J. Tauber. 1984. Natural enemy activity on glandular pubescent potato plants in the greenhouse: an unreliable predictor of effects in the Łeld. Environmental Entomology 13:679ó683.

Oliveira, H., A. Janssen, A. Pallini, M. Venzon, M. Fadini, and V. Duarte. 2007. A phytoseiid predator from the tropics as potential biological control agent for the spider mite Tetranychus urticae Koch (Acari: Tetranychidae). Biological Control 42:105-109.

O∉Neil, R.J. 1989. Comparison of laboratory and field measurements of the functional response of Podisus maculiventris (Heteroptera: Pentatomidae). Journal of the Kansas Entomological Society 62:148-155.

Price, P.W., C.E. Bouton, P. Gross, B.A. McPheron, J.M. Thompson, and A.E. Weis. 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivore and natural enemies. Annual Review of Ecology and Systematics 11:41-65.

Provost, C., E. Lucas, D. Coderre, and G. Chouinard. 2006. Prey selection by the lady beetle Harmonia axyridis: the inłuence of prey mobility and prey species. Journal of Insect Behavior 19:265-277.

Randlkofer, B., E. Obermaier, J. Casas, and T. Meiners. Connectivity counts: disentangling effects of vegetation structure elements on the searching movement of a parasitoid. Ecological Entomology 35:446-455.

Romero G.Q., and W.W. Benson. 2005. Biotic interactions of mites, plants and leaf domatia. Current Opinion in Plant Biology 8:436-440.

Rutledge, C.E., A.P. Robinson, and S.D. Eigenbrode. 2003. Effects of a simple plant morphological mutation on the arthropod community and the impacts of predators on a principal insect herbivore. Oecologia 135:39-50.

SAS Institute. 2010 SAS/STAT software: changes and enhancements through release 9.2. SAS Institute, Cary, N.C.

Solomon, M.E. 1949. The natural control of animal populations. The Journal of Animal Ecology 18:1-35.

Stamp, N.E., and M.D. Bowers. 1993. Presence of predatory wasps and stinkbugs alters foraging behavior of and non-cryptic caterpillars on plantain (Plantago lanceolata). Oecologia 95:376-384.

Stavrinides, M.C., and D.J. Skirvin. 2003. The effect of chrysanthemum leaf trichome density and prey spatial distribution on predation of Tetranychus urticae (Acari: Tetranychidae) by Phytoseiulus persimilis (Acari: Phytoseiidae). Bulletin of Entomological Research 93:343-350.

Styrsky, J.D., Kaplan, I., and M.D. Eubanks. 2006. Plant trichomes indirectly enhance tritrophic interactions involving a generalist predator, the red imported fire ant. Biological Control 36:375-384.

Teuscher, M., Brandle, M., Traxel, V., and R. Branbl. 2009. Allometry between leg and body length of insects: lack of support for the size-grain hypothesis. Ecological Entomology 34: 718-724.

Thompson, D.W. 1917. On Growth and Form. Cambridge University Press, Cambridge, United Kingdom.

Treacy, M.F., Zummo, G.R., and J.H. Benedict. 1985. Interactions of host-plant resistance in cotton with predators and parasites. Agriculture, Ecosystems and Environment 13:151-157.

Vohland K. 1996. The influence of plant structure on searching behaviour in the ladybird, Scymnus nigrinus (Coleoptera: Coccinellidae). European Journal of Entomology 93:151-160.

Walton, T.J. 1990. Waxes, cutin and suberin. Pages 105-158 *in* P. M. Dey and J.B. Harborne, editors, Methods in plant biochemistry. Academic Press Inc., San Diego, California.

Wang, B., D.N. Ferro, and D.W. Hosmer. 1997. Importance of plant size, distribution of egg masses, and weather conditions on egg parasitism of the European corn borer, Ostrinia nubilalis by Trichogramma ostriniae in sweet corn. Entomologia Experimentalis et Applicata 83:337-345.

Wehner, T.C., and E.T.Gritton. 1981. Horticultural evaluation of eight foliage types of peas near-isogenic for the genes *af, tl* and *st.* Journal of the American Society for Horticultural Science 106:272-278.

Weins, J.A., R.L. Schooley, and D. Weeks Jr. 1997. Patchy landscapes and animal movements: do beetles percolate? Oikos 78:257-264.

Weis, A.E., and A. Kapelinski. 1994. Variable selection on Eurosta's gall size. II. A path analysis of the ecological factors behind selection. Evolution 48:734-745.

White, C., and D. Eigenbrode. 2000. Effects of surface wax variation in Pisum sativum on herbivorous and entomophagous insects in the field. Environmental Entomology 29: 773-780.

With, K.A. 1994. Ontogenetic shifts in how grasshoppers interact with landscape structure: an analysis of movement patterns. Functional Ecology 8:477-485.

Yang, L.H. 2000. Effects of body size and plant structure on the movement ability of a predaceous stinkbug, *Podisus maculiventris* (Heteroptera: Pentatomidae). Oecologia 125:85-90.