

**CLOSE-RANGE PREY LOCATION, RECOGNITION AND  
FORAGING DECISIONS BY THE GENERALIST  
PREDATOR, *DICYPHUS HESPERUS* KNIGHT  
(HETEROPTERA: MIRIDAE)**

by

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MASTER OF SCIENCE

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## ABSTRACT

I investigated sensory modalities and cues used at close range by the generalist predator *Dicyphus hesperus* Knight (Heteroptera: Miridae) in location and recognition of prey. Vision was important for orientation, and olfaction and gustation for recognition of prey. Putative sense organs on the rostral tip and tarsi may be involved in gustation. Neither prey colour, nor shape impacted preference, which follows the idea of “limited attention” and its implications for generalists. Similarly, different prey elutions (by polarity/prey type) added to artificial prey elicited equivalent levels of response. A clumped distribution of artificial prey (without chemical cues) negatively impacted predator response. I also examined a tradeoff between prey defenses and nutritive value; both were proportional to prey size. Large aphid prey were preferred over small prey, regardless of defenses, but the bias toward large aphids was significantly greater when prey were not defended, which is consistent with optimal foraging theory.

**Keywords:** cues; foraging; *Dicyphus hesperus*; Miridae; predator; prey

## **DEDICATION**

To my mother, Maria

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# CHAPTER 1

## INTRODUCTION

### 1.1 Benefits of biological control

Biological control can be defined as “the use of an organism *by man* to reduce the population density of another organism” (*cf.* natural control) (Bale *et al.*, 2008). This method is gaining popularity for use in agricultural systems against insect pests as we learn more about the perils of chemical pesticides. Chemical pesticides may pose a threat to human health and the environment, bioaccumulate through food chains and reduce biodiversity (Bale *et al.*, 2008). In addition, over-reliance on chemical control can lead to pest resistance and the eradication of local natural enemy populations, leaving the environment open to re-invasion by pests (Bale *et al.*, 2008; DeBach & Rosen, 1991). Conversely, no clear cases exist in which a pest species has evolved resistance to a natural enemy (Bale *et al.*, 2008). However, some clones of pest insects are known to exhibit resistance to their parasitoids (Henter & Via, 1995). In such case, coevolutionary dynamics between host and natural enemy must prevent the evolution of outright resistance. The interest in alternatives to chemicals is also increasing because many chemicals are being banned from use, on environmental grounds, without suitable substitutions (Bale *et al.*, 2008). Development time is similar for chemical pesticides and biological control products (~10 years), but development costs are much higher for chemical pesticides because of the rigorous studies that must be performed to

demonstrate their accordance with stern ecotoxicological regulations (Bale *et al.*, 2008). This provides an immediate opportunity for biological control in agriculture.

Classical biological control, which involves the introduction of natural enemies of foreign pests to establish populations for the reduction of pest numbers, is fraught with controversy over the potential for non-target effects (Messing *et al.*, 2006). The concern has resulted from several unwise decisions to introduce exotic generalist predators, which then decimated populations of native fauna (see Hajek, 2004). This is why contemporary classical biological control often involves the introduction of specialist natural enemies. Not only is there a benefit from reduced non-target effects, but specialists are considered more efficient foragers of target prey (Hassell and May, 1986). This is because they have evolved to seek out specific signals related to these prey/hosts, and there are no alternate prey/hosts to reduce the desired functional response. Classical biological control, using specialists, has been successfully applied on a number of perennial crops, but the ephemeral nature of annual crops makes difficult the establishment of natural enemies (Bale *et al.*, 2008). In such cases, augmentative biological control, the periodic release of natural enemies on a crop, is a superior method (e.g. Obrycki *et al.*, 1997).

Both specialist and generalist natural enemies have been used successfully in augmentative control against a number of pests of both field and greenhouse crops (Bale *et al.*, 2008; Symondson *et al.*, 2002). This method provides the opportunity to employ native generalist predators to control exotic pests. In so doing, non-target effects are eliminated. As well, because they may subsist on alternative prey, generalist predators may be introduced to the crop prior to pest emergence, allowing for their establishment and increase in numbers early in the season using alternative food sources (Symondson *et*

*al.*, 2002). This may help to delay or prevent the rapid growth phase of pests that gives rise to a large infestation (Landis and van der Werf, 1997; Symondson *et al.*, 2002). However, because specialists often have a higher searching efficiency and do not typically consume alternative prey, they may more quickly suppress pest populations (Symondson *et al.*, 2002). Thus, they may be more useful for controlling pests after an outbreak than generalists, where alternative prey are available for generalists. On the other hand, specialists are more likely to become locally extinct when pest populations become reduced, while generalists may locally extinguish pest populations and still persist by consuming alternative prey, providing insurance against a reinvasion (Symondson *et al.*, 2002). As a result, fewer introductions of generalist natural enemies at the beginning of the season (seasonal inoculation) may be required to achieve similar results to those obtained from multiple releases of specialists. As well, such persistent generalists may help to prevent the establishment of exotic pests, where specialist natural enemies do not exist.

## **1.2 Predatory Heteroptera as Biocontrol agents**

Many of the predatory Heteroptera are generalist predators that also feed on plants ('true omnivores,' sensu Coll & Guershon, 2002). This additional layer of diet complexity may enable these predators to persist for extended periods in the absence of any arthropod prey whatsoever (Eubanks and Styrsky, 2005). This makes them good candidates for use in species-poor agroecosystems, such as those found in greenhouses, where prey can at times be scarce. However, the role of omnivores on the stability of such constrained food webs remains equivocal; it is thought to be determined by the

degree of interaction between different trophic levels and likely varies from one situation to another.

### 1.2.1 *Dicyphus hesperus* Knight, 1943 (Heteroptera: Miridae)

Greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae), is a serious and economically important pest of tomatoes in Canadian greenhouses (Alma, 2005), and the over-reliance on chemical pesticides has selected for resistant populations of this pest (Elhag and Horn, 1984). Greenhouse whiteflies are effectively controlled on both covered and open field tomato crops in Spain, by the two mirid predators, *Macrolophus caliginosus* Wagner and *Dicyphus tamaninii* Wagner (Dicyphini: Bryocorinae) (Lucas & Alomar, 2002b). Reluctance by North American regulatory agencies to licence either of these mirids for release in greenhouses prompted a search for a domestic equivalent. This was carried out by a research team headed by Dr. Dave Gillespie at Agriculture and Agri-Food Canada. Studies demonstrated promise in *Dicyphus hesperus* Knight (Heteroptera: Miridae: Bryocorinae), a generalist zoophytophagous predator that feeds on a variety of small soft-bodied arthropods and arthropod eggs (McGregor *et al.*, 1999). This native predator is now being used in Canadian greenhouses where it has been effective against greenhouse whiteflies and has even helped to reduce populations of two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) (McGregor *et al.*, 1999). Its potential use as a biological control agent for other greenhouse crops has also been demonstrated (Sanchez *et al.*, 2004; Shipp and Wang, 2006), which has stimulated interest by greenhouse growers as far away as the Netherlands (Hatherly *et al.*, 2007).

*Dicyphus hesperus* ranges from northern British Columbia to California and Mexico on the west coast of North America (Cassis, 1986). In the laboratory, it has been observed to feed on moth eggs, all stages of whitefly, spider mites, thrips, and aphids (Gillespie, pers. comm.). When Knight (1941) first described *D. hesperus*, it was found on whiteflower leafcup *Polymnia canadensis* L. (Compositae) but was later discovered on a variety of other host plants. Certain members of the Lamiaceae, Scrophulariaceae and Solanaceae serve as good host plants for this species (Sanchez *et al.*, 2004). Females oviposit over about 4 weeks into plant stems and leaf veins. After approximately 2 weeks, nymphs hatch and then develop through four instars, over about 5 weeks, prior to reaching the adult stage. The exact development time, fecundity and longevity all vary with diet, and longevity of adult females is inversely proportional to egg production (Sanchez *et al.*, 2004). At the northern extent of its range, *D. hesperus* exhibits 2 generations in a season, whereas warmer regions of British Columbia might facilitate 3 generations (Dave Gillespie, pers. comm.). Adults overwinter in reproductive diapause in response to decreased daylength (Gillespie & Quiring., 2005). The threshold daylength for diapause induction varies by population, according to latitude, with northern populations entering diapause sooner (Gillespie & Quiring., 2005). In nature, the southernmost populations may not even enter diapause. In central California, females appear to be in only a mild diapause during winter and reproductive individuals have been found in January near Bakersfield, California (Dave Gillespie, pers. comm.).

Extra-oral digestion is ubiquitous amongst the predatory Heteroptera (see Cohen, 1998). As water is continuously lost in this process, through evaporation and hydrolytic reactions, it is assumed a critical resource for *D. hesperus* (Gillespie & McGregor, 2000).

Sinia *et al.* (2004) found that hydrated individuals ate more prey in post treatments than dehydrated individuals, and that starved, hydrated individuals spent less time plant feeding than hydrated individuals given prey, demonstrating their need for a supplemental water source. Water can be obtained from plants, and the main function of plant feeding appears to be for its acquisition (Gillespie & McGregor, 2000). However, in omnivorous heteropterans, a plant-supplemented diet generally enhances fitness (Coll & Guershon 2002). *Dicyphus hesperus* nymphs reared on both prey and plant material develop at a faster rate than individuals given access to fresh water and prey but deprived of plant material, indicating a possible complementary role of plant-derived nutrients for nymphal development (Gillespie & McGregor 2000, Sanchez *et al.*, 2004).

*Dicyphus hesperus* cannot complete nymphal development when provided with only tomato leaves (Gillespie & McGregor 2000, McGregor *et al.* 1999, Sanchez *et al.*, 2004). However, on a plant diet of mullein (*Verbascum thapsus* L. [Scrophulariaceae]), pepper (*Capsicum annum* L. [Solanaceae]), or catnip (*Nepeta cataria* L. [Lamiaceae]) alone, some nymphs were able to complete development (Sanchez *et al.*, 2004). Development time and mortality were much lower on mullein, than on the other two. In fact, the introduction of mullein into tomato greenhouse crops has been shown to facilitate the early establishment of *D. hesperus* and to sustain individuals when prey is scarce (Sanchez *et al.*, 2003).

### **1.2.2 Plant feeding is a cause for concern**

While plant feeding by *D. hesperus* brings the benefit of longevity, there is a drawback: This predator may cause feeding lesions on tomato fruits (Gillespie *et al.*, in prep.; McGregor *et al.*, 2000; Shipp & Wang, 2006). In fact, many of the predatory

Heteroptera are known to cause similar feeding damage (Lucas & Alomar, 2002a; Schuh & Slater, 1995; Wheeler, 2000). The damage caused by *D. hesperus* is only apparent, however, in times of prey scarcity (when predator populations are high) (Gillespie *et al.*, in prep.). This may be a reflection of the relative profitabilities of leaves and fruit. Without prey, adult females provided tomato fruit lived longer than females provided leaf tissue (Gillespie *et al.*, in prep.). However, because prey are the most profitable food resource, fruit feeding can likely be avoided if the appropriate measures are taken. Behaviour manipulation is gaining popularity as a way to make natural enemies more efficacious (Roitberg, 2007), but it requires detailed knowledge of the sensorial capacities and foraging behaviours of the organism in question. In the case of *D. hesperus*, this knowledge is very limited.

### **1.3 Research focus**

As fruit feeding results from prey scarcity, knowledge of how prey are perceived and the cues that are used by *D. hesperus* in prey foraging may be useful in devising strategies to reduce its occurrence. Exploitation of behavioural responses to prey-related cues by manipulating the greenhouse environment may provide a means to this end. In addition, the efficacy of this predator for the control of whiteflies may be enhanced. An understanding of other factors that impact foraging decisions would further improve the efficacy of this approach. In this light, my research objectives were as follows:

- (1) To determine the importance of vision, olfaction and gustation in close range orientation to and in recognition/acceptance of prey by *Dicyphus hesperus*. In short trials I employed an extract of macerated prey (to capture food chemicals)

and artificial prey items (APIs) of two sizes, in various combinations, to analyze the effects of the different prey-related cues.

- (2) To determine whether foraging decisions by *Dicyphus hesperus* differ according to specific visual and/or chemical, prey-related cues. Artificial prey were used to determine the effect of prey colour, shape and arrangement. The importance of different groups of prey chemicals extracted from the external surfaces of novel and rearing prey were also assessed. Groups of compounds varied by their solubility in solvents of different polarities: methanol (polar), dichloromethane (intermediate) and hexane (non-polar). Extracts were applied to artificial prey for bioassays.
- (3) To examine foraging decisions by *D. hesperus* in response to a tradeoff between prey-size-related defensive capabilities and energetic value. To analyse this I used pea aphids, *Acyrtosiphon pisum* (Harris) (Homoptera: Aphididae), because early and late instars show a great disparity in size (energetic value), and larger instars are much better defended (Chau & Mackauer, 1997). To isolate the effects of prey defenses and energy content on prey choice, pea aphids were either immobilized or defenses were left unabated.

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## **CHAPTER 2**

# **WHAT'S THE SENSE? INVESTIGATION INTO THE SENSORY MODALITIES USED, AT CLOSE RANGE, IN PREY LOCATION AND RECOGNITION BY A ZOOPHYTOPHAGOUS MIRID.**

### **2.1 Abstract**

The predacious Miridae have been generally understudied, despite their importance as generalist predators in agroecosystems. *Dicyphus hesperus* Knight is one such predator, for which much knowledge is yet to be gained with regard to its foraging behaviour and sensory modalities used in foraging. In this study, I investigated the importance of vision, olfaction and gustation in close range orientation to and in recognition/acceptance of prey using a macerated prey extract and artificial prey items. Electron micrographs of putative sensory structures reinforce behavioural observations. The data suggest that vision, olfaction and contact chemoreception are all important in foraging for prey, and that the rostral tip and possibly the tarsi are involved in contact chemoreception.

### **2.2 Introduction**

Mirids represent nearly one third of the described species within the Heteroptera, with at least one third of those estimated to exhibit predatory habits, and of those, a number are important predators in agroecosystems (Wheeler, 2000). Yet, compared to other predacious Heteroptera, relatively little research has been performed on predatory mirids.

*Dicyphus hesperus* Knight (Heteroptera: Miridae) is a generalist zoophytophagous (i.e. mostly carnivorous but also herbivorous) predator that feeds on a variety of small soft-bodied arthropods (McGregor, 1999), and has been used to control whitefly and spider mite populations on greenhouse tomato crops (McGregor *et al.*, 1999; Gillespie & McGregor, 2000; Sanchez *et al.*, 2003). The value of zoophytophagous heteropterans as biological control agents lies in their ability to colonize crops before pest infestation and survive on plant resources in times of prey scarcity (Wiedenmann & Wilson, 1996; Wheeler, 2000); however, plant feeding may translate to fruit feeding on crop plants and the subsequent formation of lesions on fruit, which is of major concern to growers (e.g. Alomar & Albajes, 1996; McGregor *et al.*, 2000; also see Schuh & Slater, 1995). Strategies need to be formulated to reduce the incidence of fruit feeding by these predators. There is increasing interest in using behavioural manipulation to make natural enemies more efficacious (Roitberg, 2007). However, a better understanding of the sensory modalities (i.e. vision, contact/volatile chemical perception, etc.) used by predacious mirids in foraging for food is necessary to make this possible.

The foraging process for insects searching for patchily distributed prey or hosts can be broken down into a series of behavioural steps (Hassell & Southwood, 1978; Vinson, 1976; Douthett, 1959). Hassell & Southwood (1978) identified habitat (a collection of patches), patch (spatial subunit of the foraging area in which aggregations of food items occur) and food item as the distinct levels at which foraging insects search. Throughout this process, insects integrate cues from more than one sensory modality en route to finding their resource (Bell, 1990).

It has been demonstrated that *D. hesperus* females are attracted to the volatiles from certain herbivore-infested plants (McGregor & Gillespie, 2004). Many arthropod predators and parasitoids foraging for herbivores are known to exploit these herbivore-induced plant volatiles (reviewed by Vet & Dicke, 1992). These operate at the level of patch and/or habitat location because herbivore-derived odours are usually difficult to detect over long distances, with the exception of pheromones (see reviews: Vet & Dicke, 1992; Dicke & van Loon, 2000; also see Kessler & Baldwin, 2001). In addition, vision is coupled with plant odour detection by many insects when orienting to plants (e.g. Blackmer & Cañas, 2005; Hattingh & Samways, 1995; Rojas & Wyatt, 1999; Todd *et al.*, 1990; Wäckers, 1994; Wäckers & Lewis, 1994; 1999).

At close range (within patch), there is less similarity among carnivores regarding the sensory modalities they use in search of prey/hosts, and varies depending on their sensorial capacities; however, it appears that herbivore-derived cues become increasingly important with greater proximity (Vet & Dicke, 1992). Furthermore, very few behavioural studies have investigated the sensory mechanisms involved in foraging for food by Hemiptera (Backus, 1988; Freund & Olmstead, 2000; Ventura *et al.*, 2000). To the best of my knowledge, none has focussed on within-patch modalities used by mirid predators, which is the focus of the current study.

*Dicyphus hesperus* has large, well-developed eyes, suggesting the importance of the visual sensory modality to this species. But, because *D. hesperus* females are more active and find and consume prey at a higher rate at night (i.e. when it is dark) than day (VanLaerhoven *et al.*, 2003), one might assume that vision plays a minor role, if any, in prey foraging. Other carnivores may use visual, olfactory, contact or vibratory modalities

in search of prey/hosts at close range (Ferran *et al.*, 1997; Freund & Olmstead, 2000; Hassell & Southwood, 1978; Lambin *et al.*, 1996; McIntyre & Vaughn, 1997; Vinson, 1976; Wäckers *et al.*, 1998). Many heteropteran predators rely mainly on their olfactory and chemotactile capacities (Ambrose *et al.*, 1983; Freund & Olmstead, 2000; Rani & Wakamura, 1993). However, some predacious Reduviidae (Heteroptera) (e.g. Ables, 1978; Ambrose, 1991; Edwards, 1966; Parker, 1965, 1969) and Pentatomidae (Awan *et al.*, 1989) are known to utilize vision, and some nabid (Freund & Olmstead, 2000) and pentatomid (e.g. Pfannenstiel *et al.*, 1995) predators use vibrational stimuli. Foraging animals may employ such sensory modalities hierarchically (e.g. in rats, Maaswinkel & Whishaw, 1999) or they can interact, additively or synergistically (e.g. in a hymenopterous parasitoid, Fischer *et al.*, 2001; in wild hawkmoths, Raguso & Willis, 2005). As well, their importance may vary according to the stage of prey location (i.e. orientation vs. recognition/acceptance).

In this chapter, I investigated the importance of vision, olfaction and contact in close range orientation to and in recognition/acceptance of prey by *Dicyphus hesperus*. In short trials I employed a macerated prey extract and artificial prey items (APIs) of two sizes, in various combinations, to analyze the effects of the different prey-related cues. Maceration of prey was necessary to liberate, from cells and into extracts, compounds known to be phagostimulants for many insects, such as amino acids, sugars and inorganic salts (Chapman 2003; 2004).

## 2.3 Materials and Methods

### 2.3.1 Insect collection and rearing

Insects were obtained as third or fourth instar nymphs from the Pacific Agricultural Research Centre in Agassiz. There they were reared on whole tobacco plants *Nicotiana tabacum* L. (Solanaceae) and provided eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) as prey. Daylength was 16h and rearing temperatures ranged from ~23-26 °C. This colony was established from collections made in 1999 near Woody, California, USA, at 35° 43' N, 116° 49' W, at an elevation of ~300 m on white stem hedgenettle, *Stachys albens* Gray (Lamiaceae).

The third and fourth instar *D. hesperus* were removed from the colony and reared to adults, on the lab bench, in 3.4 L (320 mm high x 215 mm wide x 80 mm deep) plastic containers on fresh tobacco leaves with *E. kuehniella* eggs as prey, under L16:D8 and at  $20 \pm 3$  °C. To obtain uniform age classes, adults were removed at three day intervals, and placed into 600 ml (140 mm square x 55 mm deep) plastic containers and aged an additional 5 days, under the same conditions. Both types of containers had mesh-covered holes in the lids for ventilation. This yielded cohorts of 5-8 day-old adult females.

Because females that are maturing eggs should have an increased requirement for food resources, relative to other adult individuals (adult diet strongly impacts fecundity by female *D. hesperus*, Sanchez *et al.*, 2004), I expected them to be the most motivated to feed; hunger often increases intensive or area-restricted search (e.g. ladybirds: Carter & Dixon, 1982; e.g. darkling beetles: McIntyre & Wiens, 1999). Therefore, males and females were reared together, to allow for mating, but only females were used in experiments.

### 2.3.2 Sensory structures

A scanning electron microscope examination of *D. hesperus* proboscis, tarsi and antennae, was performed. I looked for potential chemoreceptors on the tip of the labium, as plant host-acceptance in the Heteroptera, rather stereotypically, begins with a surface exploration phase where the insect walks on the plant surface, repeatedly tapping or dabbing it with the tip of its rostrum (see Backus, 1988). I have also observed this in *D. hesperus*, and additionally, I have seen this insect dab the surface of prey with its proboscis prior to insertion of its stylets and feeding. I hypothesized that such touches were used for prey recognition and assessment, through chemoreception. Because tarsi are also known to play a role in chemoreception for many insects (see Chapman & Bernays, 1989; Chapman, 1995; 2003; Dethier, 1976), I examined these structures for potential chemoreceptors, as well. I also looked for potential sensilla on the antennae, as they are the primary olfactory structures of insects and sometimes serve to detect compounds via contact (e.g. *Periplaneta americana* L. [Blattodea: Blattidae], Ruth, 1976; *Triatoma infestans* Klug [Heteroptera: Reduviidae], Diehl *et al.*, 2003).

### 2.3.3 Starvation Pretreatment

Under L16:D8 and at  $20 \pm 3$  °C., 5-8 day-old adult females were held, without plant and prey material, in clean, clear-lidded plastic cups (Solo Cup Corp, Urbana, Ill., #P200) for three days ( $72 \pm 4$ h). Holes were cut in the centre bottom of cups, through which 10 mm diameter, sterile cotton dental rolls were passed into a distilled water reservoir for hydration. Water was held in 280 mm x 552 mm x 62 mm deep, black, watertight, plastic plant propagation trays. The plastic cups were placed on perforated (for passage of dental wicks) plant carry trays (complementary to propagation trays) that

were propped up above the water on four 57 mm green plastic plant pots, positioned at the corners of each tray.

#### **2.3.4 Prey extract**

The eggs of *E. kuehniella* were used to produce a prey extract solution for bioassays. In order to release cellular contents known to be phagostimulants for many insects such as sugars, amino acids and inorganic salts (Chapman, 2003; 2004), *E. kuehniella* eggs were macerated (2.5g) using a porcelain mortar and pestle and 10 ml methanol (99.8 %) was added. The resulting slurry was then filtered through a Buchner funnel with filter paper, and 50 ml methanol, in 10 ml aliquots, was poured over the slurry to wash any residue through the funnel. The filtrate was then concentrated, using a vacuum rotary evaporator and reconstituted with methanol to 10 ml.

#### **2.3.5 Close range chemoreception**

The purpose of this experiment was to determine if, at close range, *D. hesperus* responds to prey volatiles and/or recognizes chemicals indicative of prey on a surface in the absence of a visual or mechanotactile stimulus. The response of individuals to either the *E. kuehniella* egg solution or the solvent control (methanol only), was examined.

Experiments were conducted under dim, diffused fluorescent light ( $0.33 \mu\text{Watt}/\text{cm}^2$ ) at  $21 \pm 2 \text{ }^\circ\text{C}$ . Arenas were constructed from 60 mm diameter by 15 mm high plastic Petri dishes, with a piece of 55 mm filter paper as a substrate. Fifty  $\mu\text{l}$  of treatment or control solution were pipetted into the centre of each arena onto the filter paper substrate. In order to keep track of their location, the circumference of treatment

spots was delineated on the top of each arena using a permanent, fine-point, black marker.

Charcoal-filtered air was pumped through arenas at 0.1 L/min to prevent the habituation of individuals to any volatiles that might have been emitted from treatment solutions. Air entered through two holes opposite each other on the top edge of arenas, and out through two opposing exit holes, also on the top edge, and equidistant from neighbouring entrance holes. Air was carried into and out from arenas in 4.3/6.4 mm i.d./o.d. low density polyethylene (LDPE) tubing with 13mm polytetrafluoroethylene (PTFE) plumbing tape wrapped around the ends leading into arenas to create an airtight seal at each tubing-arena interface. A gasket was also created between the two halves of the petri dishes using a #8 rubber band, which was placed around the outside rim of dish bottoms and wrapped tightly with PTFE plumbing tape to mask rubber volatiles. Additionally, arena walls were coated with PTFE-based Fluon<sup>®</sup> AD-1 (Asahi Glass Fluoropolymers Americas, Inc., Bayonne, NJ), to prevent insects from climbing them.

Individuals were introduced into arenas through the air entrance hole nearest the observer five minutes after test solution was added to the filter paper substrate (to allow for evaporation of the solvent), and subsequently, behaviours were observed for 30 min. I hypothesized that if individuals perceived prey compounds on the surface, they would exhibit arrestment behaviour or slowed movement (orthokinesis; indicative of intensive search) and would dab the substrate with their proboscises to further examine the substrate. Therefore, I measured the time individuals spent in contact with treatment solutions, if they did so for a minimum of 3 s. I decided 3 s represented a sufficient duration of exposure to treatment spots for the assessment of surface compounds and was

approximately the amount of time required for an individual to traverse the width of a treatment spot at a moderate pace. I further noted the proportion of these females that demonstrated prolonged proboscis extension ( $\geq 5$  s) and dabbed the substrate, within the treatment spot area. A total of 16 responders were obtained for each treatment. The total time these individuals spent within the treatment spot area and the latency to first response were both analyzed by means of t-tests. I also compared the proportions of positive responders between treatments.

### 2.3.6 Importance of vision

This experiment was conducted to assess the importance of visual cues to *D. hesperus* with and without chemical cues for orientation to and recognition of prey at close range. Artificial prey items (APIs) were employed in this investigation, so that visual cues could be analysed independently of chemical cues. These were approximately the size of real prey that *D. hesperus* typically encounters in the greenhouse and in nature. APIs consisted of colourless glass beads (Halcraft USA Inc., Mt. Vernon, NY, USA, “rock candy,” #83612) of two different diameter classes: 1 mm (mean  $\pm$  SE =  $1.03 \pm 0.014$  mm;  $n = 30$ ) or 1.5 mm (mean  $\pm$  SE =  $1.63 \pm 0.018$  mm;  $n = 30$ ). Glass was chosen as it is relatively odourless and inert. These beads were either washed with methanol solvent, or washed with methanol and then coated with the *E. kuehniella* egg extract, resulting in four treatment combinations (2x2 factorial): 1 mm + solvent alone; 1 mm + prey extract; 1.5 mm + solvent alone; and 1.5 mm + prey extract.

Trials were run under the same conditions as in the previous experiment, except under greater light intensity ( $1.05 \mu\text{Watt}/\text{cm}^2$ ). The arena consisted of a glass cylinder (140 mm o.d., 2.5 mm wall) inserted into a 150 mm Petri dish lid onto which a clean 144

mm diameter disc of black Art Street<sup>®</sup> heavyweight construction paper (Riverside Paper Co, Appleton, WI, USA) was placed as substrate. On the construction paper, 14 APIs were placed symmetrically (16 mm centre to centre) in a ring, equidistant from the arena's centre (36 mm). Trials were filmed from above (~20 cm) using a Panasonic PV GS400 DV camcorder and later analysed. Construction paper and APIs were changed after each replicate.

As substrate/food contrast has been shown to be important for resource detection by other insects (e.g. Conlon & Bell, 1991; Harmon *et al.*, 1998), I sought to maximize the contrast between substrate and artificial prey. As well, I wanted to avoid any complexities associated with colour and substrate chemistry. Therefore, I used black construction paper as substrate (rather than a leaf) and colourless (rather than an arbitrary colour) glass beads as artificial prey.

Just prior to trials, individuals were placed for at least five minutes inside the chambers used to release them into the arena; 3 cm sections of 6.4/9.5 mm i.d./o.d. PVC tubing capped at one end with a polyethylene lid. The other end was closed off by inserting it into a 2 mL glass vial. The function of this was to reduce insect escape behaviour by allowing them to acclimatize to release tubes. After this acclimation period, the 3 cm section of PVC tubing (with insect) was pulled out from the glass vial and inserted into a complementary hole at the bottom of the arena at its centre. Individuals were allowed to enter the arena of their own accord; previous observations showed they are negatively geotropic and positively phototactic.

By means of a distribution chi-square test, the proportion of individuals that made contact with an API along their path was compared by treatment combination to an

expected proportion,  $P_{null}$ , corresponding to the probability of contacting an API at random. The proportions of positive responses by treatment combination were then analysed via a loglinear analysis and further compared in a pairwise fashion using the `pairwise.prop.test` command with Holm adjustment (to minimize family-wise error rate), in the statistical software application, R (ver. 2.4.1). As well, the proportion of those making contact with an API that, in addition, exhibited feeding behaviour (extended their proboscises and attempted to probe/manipulate the API for a minimum of 5 s) was analysed using a chi-square test across treatments. Trials were terminated once individuals either passed between adjacent APIs or touched an API, en route to the edge of the arena. Those individuals that took flight before reaching the ring of APIs and landed somewhere on the opposite side, were excluded from the analysis. The number of replicates obtained for the 1 mm, 1.5 mm, 1mm + prey extract, and 1.5 mm + prey extract treatments were 30, 31, 32 and 32 respectively.

Reactive distance, the minimum distance at which prey are perceived (Holling, 1966; Le Ralec *et al.*, 2005; Roitberg, 1985; Wajnberg & Colazza, 1998), was also recorded. Le Ralec *et al.* (2005) estimated this (as per Pak *et al.*, 1991; and Bruins *et al.*, 1994) for three hymenopterous parasitoids by measuring the distance at which parasitoids initiated a turn toward a host and after which made a more or less straight path to that host. Here I adopt the same protocol with some slight modifications. *Dicyphus hesperus* females sometimes initiate body rotation while moving forward and walk in an arc toward APIs. Therefore, I estimated reactive distance here as the distance from an API at which body rotation toward the API was initiated, and either where thereafter a more or less straight line was followed en route to an API, or where subsequently body rotation

continued in the same direction along the walking path, until API contact. For consistency (at the expense of some data points), I also added a criterion that, from the reactive point, body rotation toward an API be a minimum of 10 °. For a given instance, this was measured as the angle (with the anterior of the head at the vertex) subtended by the point of contact on the API and a point at an arbitrary distance ahead, but in-line with an individual's mid-line and walking path, prior to rotation.

Reactive distances and angles were calculated by measuring the distance between the API contacted and the nearest point on the head, at the eye region, of the approaching insect, just prior to the observed body rotation. For this, video frames of interest were exported as jpegs (30 fps resolution) and analysed using image analysis software: ImageJ 1.37v (National Institutes of Health, USA). In addition, latency (s) from entry into the arena to API contact and rate of approach (mm/s) from reactive distance to API contact were calculated. All of the above were analysed in separate 2-way ANOVAs with bead size and coating treatment as factors.

### 2.3.7 Null model

The expected proportion,  $P_{null}$ , is equal to the proportion of space, at 36 mm from the arena's centre, where an individual would be in contact with an API. This is a function of insect width,  $i$ , number of beads,  $n$ , average bead diameter,  $b$ , and circumference delineated by APIs (radius 36 mm),  $c$ .

$$P_{null} = \frac{i + nb}{c}$$

The body width of *D. hesperus* is 0.98-1.12mm (Kelton, 1980). However, legs extend out from the body, thereby increasing the effective insect width. Observation suggests

the foretarsi are used to contact objects for investigation and that forelegs extend laterally somewhere between 2/3 and 3/4 the width of the body when walking. Based on this, I made a conservative estimate of effective width occupied by insects at 2.7 mm, for the null model. From this, I should expect that by chance alone (assuming random movement), the probability of individual *D. hesperus* making contact with APIs of 1.03 mm and 1.63 mm would be 0.233 and 0.271, respectively.

These probabilities are in contrast to a theoretical probability calculated for a similar experiment, with *Harmonia axyridis*, by Lambin *et al.* (1996), in which the authors only considered the space occupied by artificial prey and the distance between them, and did not incorporate the width of individuals; adult *H. axyridis* are approximately 1/4 as wide (4.0 – 6.6 mm) (reviewed by Koch, 2003) as was the distance between artificial prey (ca. 2 cm). This approach, therefore, greatly underestimates the true probability of contacting a prey at random.

## **2.4 Results**

### **2.4.1 Sensory structures**

The rostral tip of *D. hesperus* (Figure 2.1A) looks similar to that of the tarnished plant bug, *Lygus lineolaris* Palisot (Heteroptera: Miridae) (Ave *et al.*, 1978; Hatfield and Frazier, 1980). There appear to be two sensory fields with eleven putative peg sensilla on each, two long potential sensilla trichoidea just proximal to these fields and ventrally, and a ring of six other hairs, more proximally, that may be sensilla trichoidea. However, without a histological examination, it cannot be confirmed that the structures described are, in fact, sensilla. The peg sensilla of *L. lineolaris* are morphologically similar to the

corresponding putative sensilla of *D. hesperus*, except those of *D. hesperus* are variable in size and shape within each field; although, roughly bilaterally symmetrical between sensory fields. They vary from broad and short to long and thin, and many have lobed tips. A single large pore was apparent at the tips of some of the putative peg sensilla, which indicates they may have a gustatory function (Figure 2.1B).

Relative to more proximal segments, the terminal antennal segment had the highest concentration of potential sensilla (Figure 2.1C). I was able to distinguish at least four types. Those that were presumed sensilla trichoidea were the most dominant, followed by those that appeared to be grooved peg sensilla, sensilla campaniformia (in shallow pits) and sensilla chaetica (Figure 2.1D). Some of the campaniform structures may be associated in some way with grooved pegs and/or trichoid structures, due to their proximity. Some of the putative s. campaniformia even appear to be attached to the bases of trichoid structures. No large terminal pores were apparent on any of these structures, but small lateral pores could be seen on many (especially apparent near their bases), including grooved pegs (Figure 2.1D, E).

Projecting from the distal surface of the pretarsis (unguitractor plate) is a pair of grooved setiform parempodia (Figure 2.1F). These may have a mechanoreceptive function as suggested by Schuh & Slater (1995). As well, there is a pair of short pointed pegs ventrally and a pair of short putative trichoid sensilla more dorsally on the distal ridge of the last tarsal segment (Figure 2.1F). Proximally, from the distal tip of the tarsus, begins a more or less uniform distribution of long, thick, grooved trichoid or chaeticoid hairs that extend the length of the tarsi.

### 2.4.2 Close range chemoreception

In the absence of an obvious visual or mechanotactile stimulus, *D. hesperus* responded to prey extract. First, there appeared to be an arrestment effect: over the 30 min observation period, responders in the prey extract treatment spent significantly more time within the substrate area exposed to solution than did those in the solvent control group (Figure 2.2;  $t_{30} = 4.26$ ,  $p = 0.0002$ ). Second, significantly more positive responses (prolonged proboscis extension in treatment spot area) occurred in the prey extract treatment (Fig. 2.3; Fisher's Exact Test, 2-tail,  $p < 0.0001$ ). I also observed an increase in antennal waving frequency in most insects when very near or on prey extract. However, there was no evidence to suggest that *D. hesperus* uses prey volatiles at close range for orientation (i.e. chemotaxis); the latency of first response was not significantly different between treatments (Fig. 2.4;  $t_{30} = 1.10$ ,  $p = 0.2809$ ).

### 2.4.3 Importance of vision

More individuals than expected from all but the 1.5 mm API without prey extract treatment contacted APIs when compared to null probabilities for respective treatments (Figure 2.5; 1 mm API:  $\chi^2(1) = 11.9672$ ,  $p = 0.0005$ ; 1.5 mm API:  $\chi^2(1) = 3.4536$ ,  $p = 0.0631$ ; 1 mm API + prey extract:  $\chi^2(1) = 36.9885$ ,  $p < 0.0001$ ; 1.5 mm API + prey extract:  $\chi^2(1) = 42.1715$ ,  $p < 0.0001$ ). Comparing proportions of positive responders across treatments, the loglinear analysis produced a final model that retained the API coating treatment x API contact interaction as the main effect. The corresponding likelihood ratio for this model was  $\chi^2(4) = 1.140$ ,  $p = 0.888$ . This indicated that coating treatment alone significantly affected API contact,  $\chi^2(1) = 9.867$ ,  $p = 0.0017$ . Odds ratios indicate that individuals were 1.60 times more likely to contact prey-extract-coated APIs

than those without. In addition, pairwise comparisons revealed only one significant difference amongst treatments; a higher proportion of individuals from the 1.5 mm API + prey extract treatment touched an API than did those from the 1.5 mm API without prey extract treatment (Figure 2.5;  $p = 0.044$ ).

Contact by individuals with APIs was usually initiated with the foretarsi, and in some cases, the proboscis was subsequently extended to APIs, followed by attempts at probing/feeding. The latter behaviour was only observed on prey extract coated APIs (Fig. 2.6; Fisher's exact test, 2-tail,  $p < 0.0001$ ) and no significant difference was detected in this regard between the two API sizes ( $\chi^2(1) = 0.004$ ,  $p = 0.9495$ ).

Reactive distance (Table 2.1) was greater in response to 1.5 mm APIs, mean  $\pm$  S.E. =  $8.12 \pm 0.56$  mm, than to 1 mm APIs, mean  $\pm$  S.E. =  $4.94 \pm 0.53$  mm (ANOVA,  $F_{1,56} = 18.3344$ ,  $p < 0.0001$ ), but coating treatment had no effect (ANOVA,  $F_{1,56} = 0.2752$ ,  $p = 0.6019$ ), nor was there a significant interaction term (ANOVA,  $F_{1,56} = 0.3524$ ,  $p = 0.5551$ ).

As in the previous experiment, I noticed an increase in antennal waving when insects were near to prey extract (coated APIs) and this was accompanied by a slowed and sometimes punctuated approach to APIs treated with prey extract: approach rate (Table 2.2) from the reactive point was significantly lower in response to prey extract coated APIs, at  $4.71 \pm 0.47$  mm/s (mean  $\pm$  S.E.) than to others at  $7.06 \pm 0.60$  mm/s (mean  $\pm$  S.E.) (ANOVA,  $F_{1,56} = 8.5625$ ,  $p = 0.0049$ ), but there was no effect of API size (ANOVA,  $F_{1,56} = 1.2806$ ,  $p = 0.2626$ ), nor was there a significant interaction term (ANOVA,  $F_{1,56} = 2.0910$ ,  $p = 0.1537$ ). However, pair wise comparisons among treatments (Tukey HSD,  $\alpha = 0.05$ ) revealed that the approach rate to 1 mm APIs

without prey extract was significantly different than that to both prey extract coated APIs (mean  $\pm$  S.E. for 1 mm solvent alone; 1 mm + prey extract; and 1.5 mm + prey extract, respectively =  $8.00 \pm 3.65$  mm;  $4.58 \pm 2.41$  mm;  $4.82 \pm 2.60$  mm). In contrast, the approach rate in the 1.5 mm solvent alone treatment (mean  $\pm$  S.E. =  $5.95 \pm 3.14$  mm) was not significantly different than that observed in any of the other treatment combinations. Therefore, the difference detected between the approach rates for prey extract coated and uncoated treatments is largely attributable to the 1 mm solvent alone treatment.

No significant differences were detected in API touch latency (API size, ANOVA,  $F_{1,56} = 0.3142$ ,  $p = 0.5769$ ; Coating treatment,  $F_{1,56} = 0.1468$ ,  $p = 0.7028$ ; Interaction,  $F_{1,56} = 0.5076$ ,  $p = 0.4785$ ) and reactive angle (API size,  $F_{1,56} = 0.4812$ ,  $p = 0.4907$ ; Coating treatment,  $F_{1,56} = 1.6297$ ,  $p = 0.2069$ ; Interaction,  $F_{1,56} = 1.1898$ ,  $p = 0.2800$ ). However, the mean reactive angle in response to 1 mm prey extract coated APIs was considerably larger than the others:  $60.195^\circ$  versus  $47.822^\circ$ ,  $46.534^\circ$ , and  $43.781^\circ$  (1.5 mm prey extract coated, 1.5 mm no extract, and 1 mm no extract, respectively).

## 2.5 Discussion

Vision, olfaction and gustatory perception all appear to be used in the location and recognition of prey by *D. hesperus*. As the cues provided by a particular food item may determine the importance of a given sensory modality in its detection, the broad sensorial capacity of this true omnivore (sensu Coll & Guershon, 2002) may facilitate the use of a wide range of food items.

SEMs revealed putative sensilla with a variety of morphologies. Although these structures are similar to receptors of known function in other arthropods, their precise

function cannot be determined without more detailed microscopy, histology and electrophysiological examination. Many of the putative peg sensilla on the tip of the rostrum may be gustatory in nature, as they possess a single terminal pore, and they may also be mechanoreceptive or olfactory (i.e. dual function) (Ryan, 2002). Other potential sensilla on the rostrum, if not gustatory, may be olfactory, mechano-, thermo- or hygrosensitive. As for the putative antennal sensilla, the variety of forms observed could be a reflection of equally variable function. Small pores are apparent on the side walls of some (including some trichoid hairs and grooved pegs), which infers an olfactory function, if they are indeed sensilla. The putative grooved peg sensilla of the antennae closely resemble sensilla of *Triatoma infestans* Klug (Heteroptera: Reduviidae) (Diehl *et al.*, 2003); however, some *T. infestans* sensilla have a large terminal pore, whereas none could be discerned on those of *D. hesperus*. Therefore, if sensilla, they likely do not serve a gustatory function. Due to their proximity, some of these grooved pegs appeared to be associated with putative s. campaniformia. It is difficult, however, to discern how they might interact without more detailed information. Some trichoid structures were also associated with putative s. campaniformia, but, whereas the aforementioned grooved pegs and putative s. campaniformia were close in proximity, these hairs looked to be connected in such a way as for the mechanoreception of weak stimuli (perhaps, vibrations on the substrate, or sound). Such trichoid hairs may act as fulcrums when contacted, generating a greater amount of force on the putative s. campaniformia at their bases than the force of contact; thus amplifying the signal. The parempodia of the pretarsus could serve a mechanosensory function, as suggested by Schuh and Slater (1995). As well, based on behavioural observations and analogous structures in other fluid feeding insects

(see reviews: Bernays & Simpson, 1982; Chapman, 1995; Chapman, 2003, and references therein), I propose a gustatory function for at least one of the pairs of short pointed sensilla on the terminal ridge of the tarsi.

In the absence of obvious visual and mechanotactile stimuli, female *D. hesperus* were capable of recognizing surface chemicals indicative of prey. Individuals responded to prey-extract-coated filter paper by waving antennae, and extending the proboscis. They also frequently dabbed at the prey extract spot with the proboscis, as if ingesting surface fluids or searching for food particles. Some species of Heteroptera, while exhibiting this dabbing behaviour, are known to secrete saliva, and then re-ingest it to taste the substrate (Miles, 1958). As well, individuals given filter paper treated with prey extract spent significantly longer within the treatment solution exposed area than did those given filter paper treated with solvent alone. However, I did not gather any evidence to suggest that volatiles, alone, are sufficient to direct the location of prey; no difference was detected between the latency to investigation of prey extract solution and solvent alone. If *D. hesperus* was capable of detecting a chemical gradient of prey chemicals, increasing in concentration toward the prey extract source, and if these predators use such information at close range to locate prey, I predicted that prey extract spots would be located more quickly than the solvent control spots.

Despite the fact that *D. hesperus* feeds on prey to a greater degree at night (VanLaerhoven *et al.*, 2003), visual detection of artificial prey items was very apparent. APIs were contacted by a much greater proportion of individuals than expected assuming random movement, and the majority of these individuals demonstrated close range body rotation to APIs. As well, the distance at which this response to APIs occurred, reactive

distance, was largely a function of API size (the reactive distance from 1.5 mm APIs was significantly greater than that from 1 mm APIs), coating treatment had no effect and there was no interaction term. This implies that visual perception of prey is of primary importance in orientation behaviour at close range by *D. hesperus*, at least under well lit conditions. Given that experiments were conducted under full light, the importance of vision for prey location under lower light intensities cannot be discerned; future work is necessary (see Conclusion). It is possible, though, that the eyes of *D. hesperus* are adapted for orientation in the dark. Alternatively, light levels may determine the importance of different sensory modalities for orientation to prey.

The proportion of individuals that contacted APIs coated with prey extract solution was significantly greater than that which contacted APIs washed with solvent, alone. This suggests that prey volatiles are important to *D. hesperus* in prey recognition and that visual and olfactory cues interact either additively or synergistically to elicit a response. For many foragers, using a combination of cues is more reliable than using any one cue by itself and success in finding a resource is improved. Orientation by male white-spotted longicorn beetles (*Anoplophora malasiaca* Thomson (Coleoptera: Cerambycidae)) to black glass rods (beetle models) was greatly enhanced when the rods were coated with female elytra extract (Fukaya *et al.*, 2004). Host location, by the pupal parasitoid *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae) was better when both vibrational and visual cues were available than when one or the other was excluded (Fischer *et al.*, 2001). Single sensory stimuli failed to elicit feeding responses to natural or artificial flowers by *Manduca sexta* L. (Lepidoptera: Sphingidae), but when plant

chemical and visual cues were present simultaneously, feeding occurred on both natural and artificial flowers (Raguso & Willis, 2005).

Although response to APIs by *D. hesperus* was enhanced when chemical cues were provided, their impact appeared to be non-directional; prey extract did not affect the latency to contact of APIs, nor did it affect reactive distance. Volatiles had their effect near the reactive point, as there was a significantly slower and often noticeably punctuated approach to prey extract treated APIs, relative to solvent treated APIs, from the reactive point. Similarly, cornicle secretions from the pea aphid, *Acyrtosiphon pisum* Harris, elicited oviposition behaviour by the parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae) at close range, but did not appear to be involved in parasitoid orientation (Battaglia *et al.*, 2000). Antennal waving by *D. hesperus* individuals was also often observed just prior to contact with prey extract treated APIs. Insects often pause and move their sensory appendages to gather information from the environment (see Bell, 1990). This short range effect could indicate that active volatiles from the prey extract were only perceived at a high enough concentration to elicit a response when individuals were close to the reactive point, or that when searching for prey, these insects only pay attention to prey volatiles upon visual detection of a prospective prey item. Either way, this appears to be an example of cross-channel potentiation (see Bell, 1990). In the case of *D. hesperus*, the addition of an olfactory cue appears to increase responsiveness to a visual one.

There may also be a positive synergistic effect between model prey (bead) size and prey volatiles: a higher proportion of individuals contacted 1.5 mm APIs coated with prey extract than 1.5 mm APIs without, whereas all other pair-wise comparisons were not

significant. Furthermore, the proportion of individuals contacting 1.5 mm APIs without prey extract was not significantly different than expected if movement were random (although only marginally n.s.), whereas all other proportions were significantly higher (highly significant) than expected. As well, multiple comparisons of approach rate from the reactive point to APIs demonstrated that insects moved significantly faster toward the smaller 1 mm APIs without prey extract than toward both sizes with prey extract, but that insect velocity toward the larger 1.5 mm APIs without prey extract was not significantly different than that toward any of the others.

For *D. hesperus*, there may be a greater perceived risk or danger associated with larger potential prey that are only visually detectable. Generalist predators encounter a variety of prospective prey in their environment and without additional sensory inputs, size may be the only measure of risk. In accordance, larger prey are often associated with increased handling time or effort, and a higher probability of failure in prey capture (Paine, 1976; Pastorok, 1981). As well, predators could potentially be subject to a greater threat of injury when handling larger prey. Oystercatchers, for example, choose smaller prey than expected from a short term, rate maximization perspective, apparently to minimize bill damage (Rutten *et al.*, 2006). This may serve to maximize food intake over the long term (Rutten *et al.*, 2006). An additional level of danger could arise where what is perceived as potential prey is actually a predator or dangerous non-prey. In this case, a larger organism might make for a more formidable opponent, thereby increasing the probability of injury or death. However, when visual inputs are reinforced with prey volatiles, there is a greater degree of certainty that what is detected visually is suitable as prey. Therefore, large APIs coated with prey extract may be perceived as less risky by

predators than large APIs alone, where the potential payoff from a prey item of this size may outweigh the risk. This could explain the disparity between these treatments in the proportion of individuals investigating APIs. As the prey extract was created from *E. kuehniella* eggs, the primary food source in rearing, there may have also been an effect of previous experience with these suitable prey and their associated volatiles. The difference observed between the small API treatments (although not significant) may be explained by the same mechanism: uncertainty presents risk of some form and that it is proportional to prey size.

The blend of volatiles arising from the prey extract would not mirror that emitted from intact *E. kuehniella* eggs, however. As eggs were macerated for the production of the extract (exposing additional compounds), the resulting blend is likely more close to that which would be emitted from already-damaged prey. Encountering and consuming injured prey is not an unusual event for *D. hesperus* (personal observation) and therefore it is not unrealistic to assume that food items might release those volatiles presented by the prey extract. It may be that damaged prey are more attractive. The Brazilian harvestman, *Iporangaia pustulosa* Mello-Leitão (Opiliones: Gonyleptidae), spent significantly more time on screened boxes containing cut up prey than on control boxes containing no prey, but there was no significant difference between the time spent on control boxes and those containing live, intact prey (Willemart & Chelini, 2007). This may be explained simply by a greater concentration of volatiles emanating from injured prey, or perhaps, injured prey are preferred (less risky). As well, the blend of prey extract volatiles does not represent the entire suite that would be emitted from a whole prey item;

only the subset captured in methanol. It appears, though, that at least some compounds involved in prey location were present.

Several insects from each treatment combination contacted APIs, but feeding behaviour was only observed on prey extract-coated APIs. This suggests that a visual cue may attract an individual, but a gustatory signal upon contact with prey is required to initiate a feeding response. A number of insects are known to contact food with their maxillary or labial palps to obtain chemical information regarding its suitability for consumption (see reviews: Bernays & Simpson, 1982; Chapman, 1995; 2003; Chapman & Bernays, 1989; and see Dethier, 1976). Accordingly, *D. hesperus* and all heteropterans examined thus far have been found to possess contact chemoreceptors on the tip of the rostrum (Backus, 1988); the first of the mouthpart structures to come into contact with food before feeding.

When not in use, the heteropteran proboscis is bent back from the head posteriorly and against the ventral surface of the body. In order to bring their mouthparts into contact with food, heteropterans and other fluid feeding insects must extend their proboscises (Backus, 1988; Chapman, 1995; Dethier, 1976). Therefore, demonstration of this behaviour often indicates that these insects, through some sensory modality, have recognized a potential food source (Dethier, 1976). Proboscis extension by *D. hesperus* was only apparent in response to APIs coated with prey extract and after tarsal contact. Minnich (1921) discovered that proboscis extension could be elicited in two nymphalid butterfly species (fluid feeders) by chemical stimulation of the tarsi. He later went on to demonstrate this behaviour in flies (Minnich, 1926; 1929), and Grabowski and Dethier (1954) eventually, with blow flies, showed clearly that the gustatory structures of the tarsi

were setae found on the distal surface of the ultimate segment. Analogous tarsal structures have since been identified in several fluid feeders and apparently serve the same function (see reviews: Bernays & Simpson, 1982; Chapman, 1995; 2003, and references therein). This leads me to believe that the setae found on the distal surface of the last tarsal segment of *D. hesperus* are gustatory in nature. Sensory hairs have been found distally on the last tarsomere of other Heteroptera as well (e.g. Reduviidae, Weirauch, 2005; and see Backus, 1988), but no direct behavioural or electrophysiological examinations have been performed to determine their function (Backus, 1988). Perhaps, very general chemical cues are perceived by the tarsi on contact, and more precise information is gathered by the rostral sensilla when a potential food source is detected. Restricting examination of objects with the proboscis until a chemical stimulus is perceived on the tarsal sensilla could enhance the efficiency of searching for prey by reducing the extent of assessment of unsuitable prey or non-food items.

It is apparent that vision provides important directional information involved in prey location at close range for *D. hesperus*, and prey volatiles at close range seem to enhance responsiveness to potential prey. Ultimately, volatile perception at very close range and/or chemotactile perception are involved in the final stages of prey recognition leading to a feeding response. Vision, olfaction and gustatory sense were all found to be important for the granivorous bug, *Coreus marginatus* L. (Heteroptera: Coreidae), in selecting seeds (Pekár & Hrušková, 2006) and vision and olfaction were found to be important in prey location for the predaceous pentatomid, *Oechalia schellebergii* Guérin-Méneville (Heteroptera) (Awan *et al.*, 1989). In conclusion, the use of all of the above prey location modalities in concert, likely maximizes the efficiency by which *D.*

*hesperus* finds suitable prey items. However, it appears that chemotactile perception alone may be enough to stimulate a feeding response in *D. hesperus*.

There may be some practical uses for the knowledge gained in this experiment, which could potentially be extended to other systems involving mirid predators similar to *D. hesperus*. At least one potential application arises from the propensity for *D. hesperus* to investigate small spheres as prospective food: encapsulated liquid food or small viscous food spray droplets could be applied to the surface of plants for use as a diet supplement when prey are scarce. This may have the effect of discouraging tomato fruit feeding demonstrated by *D. hesperus* (McGregor *et al.*, 2000) and other close relatives such as *Dicyphus tamaninii* Wagner (Alomar & Albajes, 1996), while sustaining the populations of these natural enemies in times of prey scarcity.

## 2.6 References

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**Figure 2.1 Putative sensory structures of *D. hesperus*. A. Apical view of rostrum; note the variety of forms of peg sensilla. B. Zoomed-in view of putative rostral sensilla; note the terminal pore (tp) on putative peg sensillum. C. Last antennal segment, displaying the variety and arrangement of putative sensilla. *ch*, s. chaetica; *gp*, grooved peg; *tr*, s. trichoidea. D. Closer view of some antennal sensilla; note the attachment of the campaniform structure (*ca*) to the trichoid one in the lower middle portion of the micrograph, the close proximity of another campaniform structure with a grooved peg below that, and the small pores in the side walls of many putative sensilla (especially on the trichoid ones at the top left). E. Close-up of a grooved peg; note no terminal pore and small pores near its base. F. Pretarsus and terminal portion of the tarsus; note the parempodia (*pe*), and the small pointed pegs (*spp*), positioned dorsally on the terminal ridge of the tarsus, and the more ventrally positioned small trichoid structures (*str*). *cl*, tarsal claw; *pv*, pulvillus; *up*, unguitactor plate.**

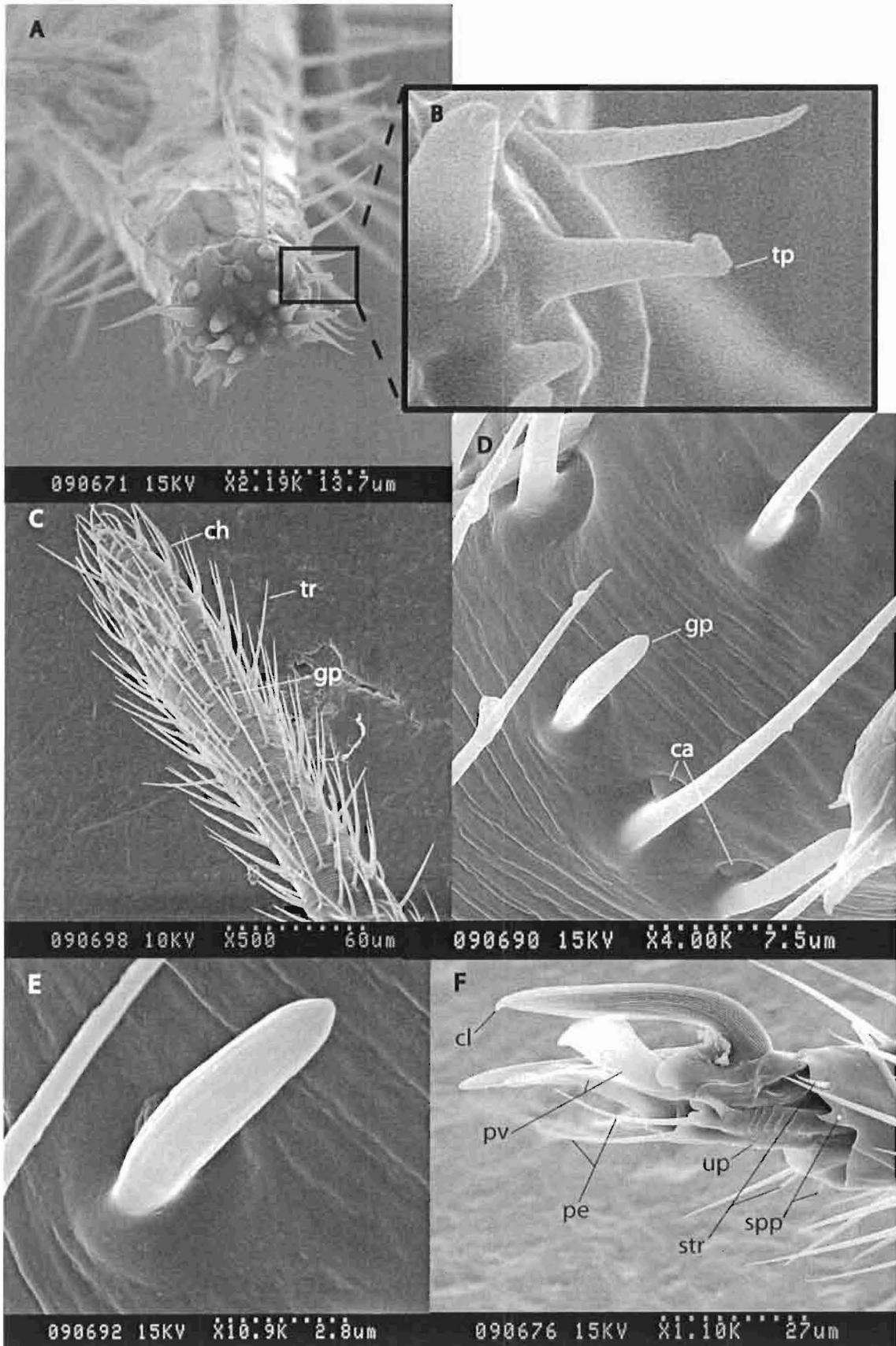


Figure 2.2 The total time (s) spent within the bounds of treatment solution (prey extract or solvent control) spots on the substrate, by *D. hesperus* that had a minimum of 3s continuous contact with said areas. Observations were made over a 30 min observation period. For each treatment, whiskers mark the 10<sup>th</sup> and the 90<sup>th</sup> percentiles, top and bottom ends of boxes indicate the 25<sup>th</sup> and 75<sup>th</sup> percentiles, horizontal solid lines through the boxes denote the median and horizontal dashed lines the mean.

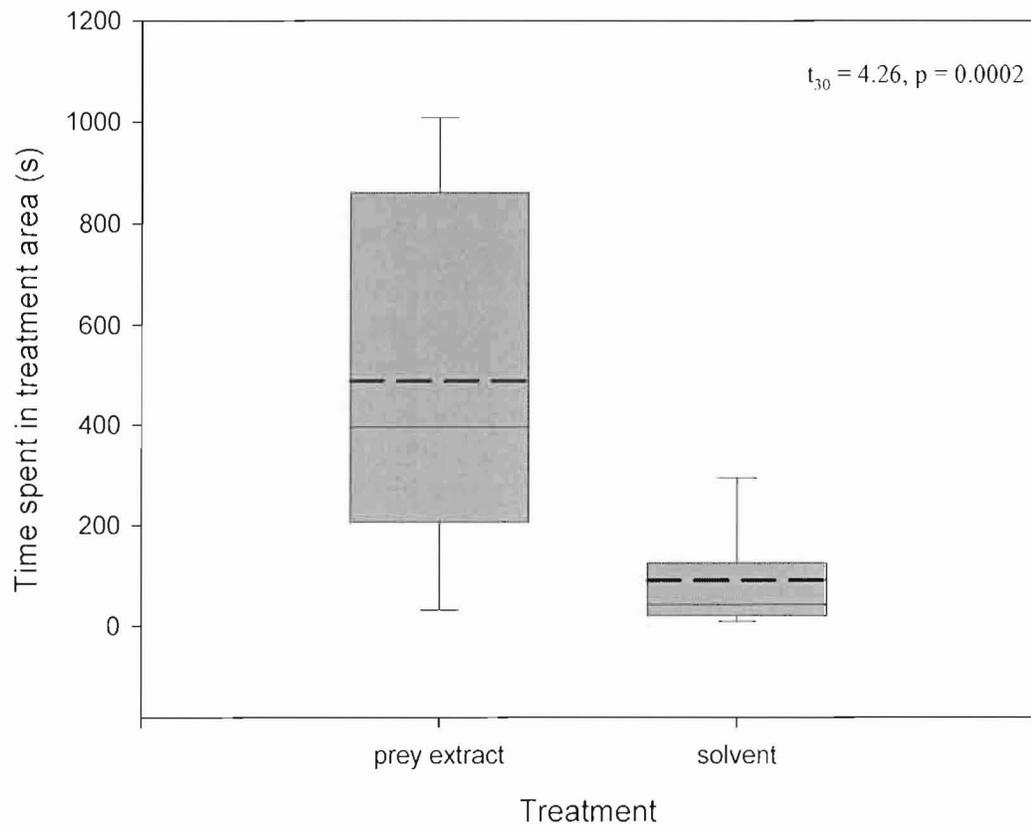


Figure 2.3 The proportion of *D. hesperus* females remaining within the bounds of treatment solution (prey extract or solvent control) spots for a minimum of 3 s that demonstrated prolonged proboscis extension (>5 s) and dabbed the substrate (positive response) versus the proportion of those who spent the minimum 3 s within treatment spots and did not (negative response).

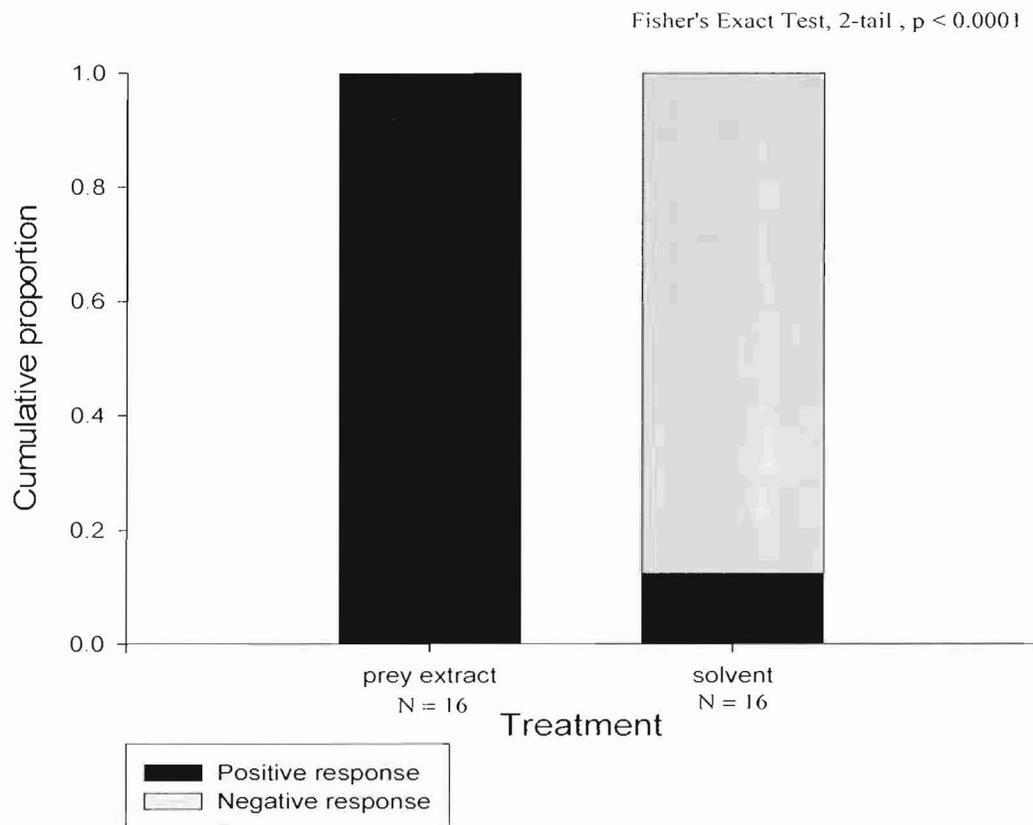


Figure 2.4 Latency (s) to event of minimum duration of contact (3s) with treatment solution spots by *D. hesperus*. For each treatment, whiskers mark the 10<sup>th</sup> and the 90<sup>th</sup> percentiles, top and bottom ends of boxes indicate the 25<sup>th</sup> and 75<sup>th</sup> percentiles, horizontal solid lines through the boxes denote the median and horizontal dashed lines the mean.

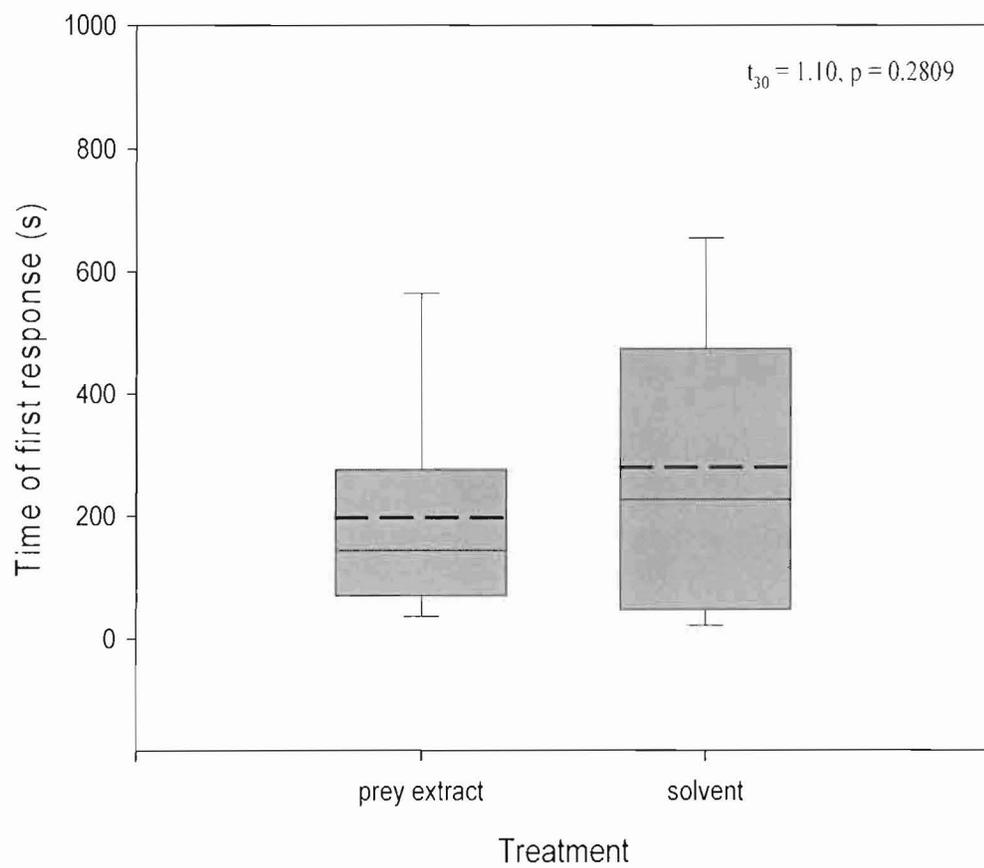


Figure 2.5 The proportions of *D. hesperus* making contact with artificial prey items (APIs) for each treatment combination. Asterisks denote whether proportions were significantly different than expected from a theoretical probability of random search. Multiple comparisons were made; a difference is indicated by no overlap in the letters above the bars between treatments.

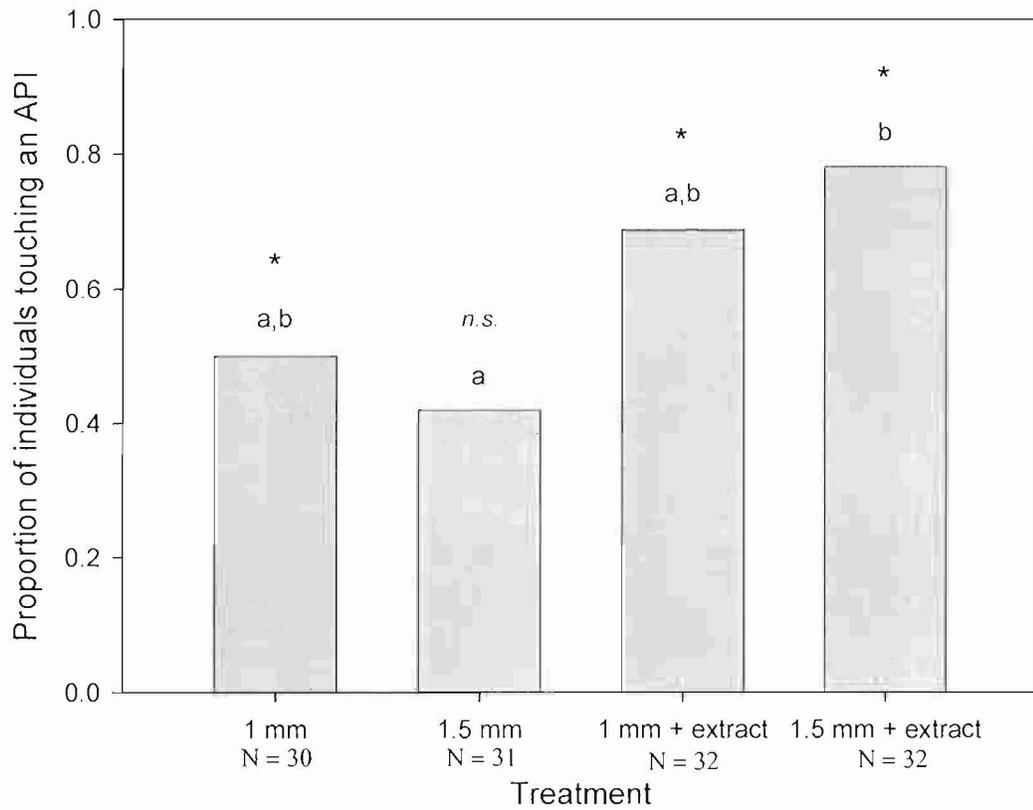
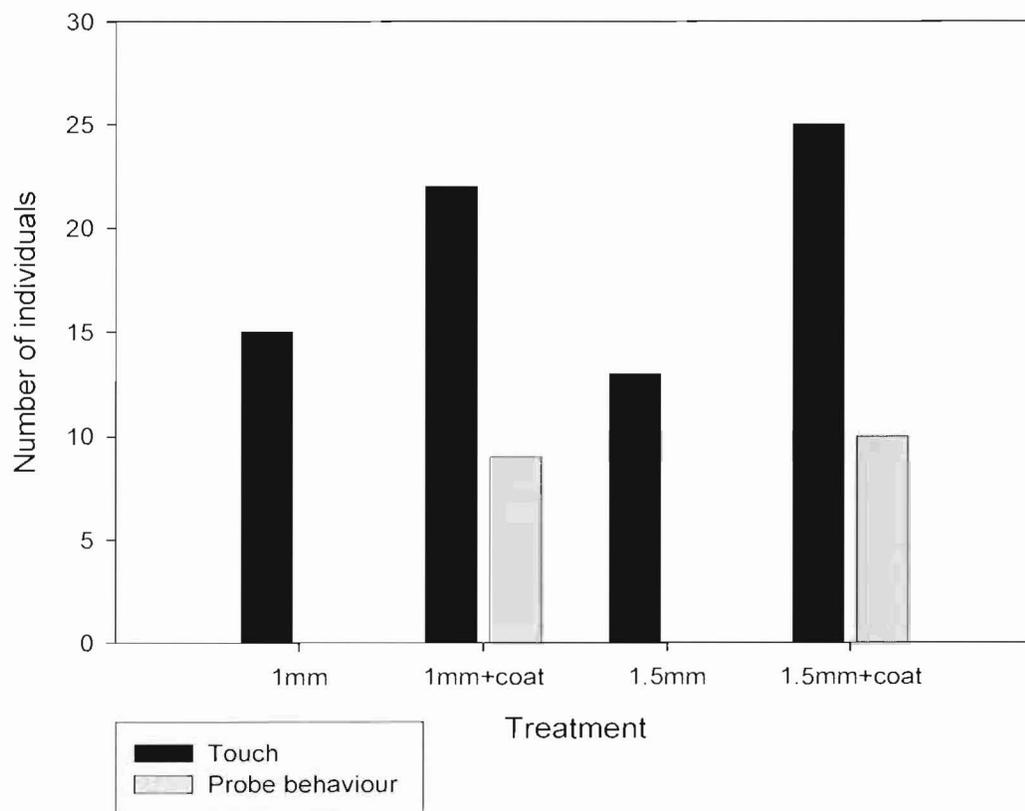


Figure 2.6 Number of *D. hesperus* exhibiting probing behaviour after contact with an API (artificial prey item). This behaviour was only observed on prey extract coated APIs (Fisher's exact test, 2-tail,  $p < 0.0001$ ) and no significant difference was detected in this regard between the two API sizes ( $\chi^2(1) = 0.004$ ,  $p = 0.9495$ ).



**Table 2.1 Analysis of variance table for the reactive distance (distance from which prey are perceived in mm) by *D. hesperus* in response to artificial prey items (APIs). Factors are size (1 or 1.5mm) and coating treatment (prey extract or solvent alone).**

<i>Source</i>	<i>DF</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>P</i>
API size	1	157.67	157.67	18.334	<0.0001
Coating	1	2.366	2.366	0.2752	0.6019
API size*Coating	1	3.031	3.031	0.3524	0.5551
Error	56	490.812	8.6		
<b>Total</b>	<b>59</b>	<b>653.879</b>			

**Table 2.2 Analysis of variance table for the rate of approach (mm/s) by *D. hesperus* from the point of reaction to an artificial prey item (API). Factors are size (1 or 1.5mm) and coating treatment (prey extract or solvent alone).**

<i>Source</i>	<i>DF</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>P</i>
API size	1	157.67	157.67	18.334	<0.0001
Coating	1	2.366	2.366	0.2752	0.6019
API size*Coating	1	3.031	3.031	0.3524	0.5551
Error	56	490.812	8.6		
<b>Total</b>	<b>59</b>	<b>653.879</b>			

## **CHAPTER 3**

# **ARE SPECIFIC CUES USED BY A GENERALIST PREDATOR?**

### **3.1 Abstract**

Insects, as with all animals, have a limited ability to pay attention to cues in their environment. Whereas, specialist carnivores may feasibly concentrate on a few reliable, specific cues from their hosts/prey, extreme generalists cannot. Extreme generalists, by definition, feed on a broader range of organisms, which may vary greatly in their physical or chemical characteristics and host plant associations. The result is an equally variable array of available prey-related cues. Therefore, it appears to be adaptive instead for generalists to focus innately on cues that are common to all or a range of their available prey/hosts. However, a previously rewarding experience with prey may cause predators to respond to specific cues related to that experience. *Dicyphus hesperus* is a broadly generalist predator of small arthropods. In the current study, I examined whether this predator responds preferentially to a range of specific prey-related visual cues, and whether body washes of different polarity, from both rearing and novel prey, differentially elicit a gustatory response. Neither prey colour nor shape impacted preference. Similarly, all body washes elicited equivalent levels of response. The latter result indicates that this predator is able to detect a broad range of prey-related compounds, but that these cues may be conserved among prey; considering the non-existence of a difference in response to novel and rearing prey. These results are consistent with the theory of limited attention.

### 3.2 Introduction

The cues an organism responds to in its environment and its sensitivity to these cues should be a reflection of their importance to the organism, given its current state. In order to reduce 'background noise', sensory mechanisms can decrease inputs, or neural mechanisms can prevent irrelevant cues from controlling behaviour (see Bernays & Wcislo, 1994 and references therein). Attention may then be focused on the relevant stimuli, using the appropriate receptive fields ('selective attention': see Dukas, 2004). For example, the wolf spider, *Schizocosa ocreata* (Hentz), switches from using both visual and vibratory cues in patch residence time decisions to responding primarily to visual cues when hungry (Persons, 1999).

When foraging for food, specialists need only respond to very specific cues that are characteristic of prey in their restricted range (Bernays & Wcislo, 1994; Steidle & van Loon, 2003; Vet & Dicke, 1992). On the other hand, generalists feed on a broader range of organisms, which may vary greatly in their physical or chemical characteristics, and hence in the cues they provide to their predators. Due to cognitive constraints ('limited attention,' see Dukas, 2004), it is not possible for broad generalists to pay attention to all specific combinations of cues that are characteristic of each type of potential prey. As a consequence, Vet & Dicke (1992) proposed, in their seminal paper on infochemical use by natural enemies, that it might be more adaptive for extremely generalist carnivores (generalist for both prey/host and plant association) to perform random search, and to not use infochemicals at all. They reasoned that, as they are less selective, generalists have more prey available to them and thus use relatively less time searching than specialists. A follow up paper by Steidle & van Loon (2003), which compared data from about 140

research papers on 95 species of parasitoids and predators, contradicts this suggestion. These data indicate that all carnivores, including extreme generalists, use infochemicals innately but that generalists more often use general cues (those present in different, non-related host/prey complexes) than specialists. As an extension of this, generalist predators should also respond to general cues of other modalities, so long as they are reliable indicators of prey identity and if predators are well-equipped to detect them (Vet and Dicke, 1992). Therefore, specific cues such as prey colour and shape should not factor into foraging decisions by naïve generalist insects, as they are not universally indicative of prey suitability; however, if particularly relevant, such cues can be learned (see Steidle and van Loon, 2003; see also review by Dukas, 2008). Unfortunately, very little work has been published that explores the innateness of responses to visual cues by broadly generalist carnivores. Interestingly, however, in one study, one of three generalist ladybird predators, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), discriminated between differently-coloured pea aphid, *Acyrtosiphon pisum* (Homoptera: Aphididae), morphs (Harmon *et al.*, 1998). The preference for red over green morphs was maintained regardless of background contrast. Conversely, there have been a number of studies on naïve parasitoids, with narrow host ranges, that illustrate their preference for a particular host colour (Michaud & Mackauer, 1994; Battaglia *et al.*, 1995; Battaglia *et al.*, 2000; Libbrecht *et al.*, 2007) or shape (Battaglia *et al.*, 1995).

Prey-related visual cues may be amplified by dense aggregations (patches) formed by many phloem-feeding herbivores (e.g. whiteflies: Byrne & Bellows, 1991; aphids and coccids: Borges *et al.*, 2006) and thus enhance their detection by both generalist and specialist predators. Because aggregation is typical of such herbivores and because

predators and parasitoids do not typically form large aggregations on leaves, the close association of multiple organisms might be a reliable indicator of their identity as prey.

Chemotactile cues or close range volatiles from the prey/host are very important for their recognition by natural enemies (see Fellowes *et al.*, 2005). As above, those with a restricted prey/host range typically use specific cues (Anton & Gnatzy, 1998; Benedet *et al.*, 1999; 2002; Battaglia *et al.*, 2000; 2002; Le Ralec *et al.*, 2005; Muratori *et al.*, 2006) more so than generalists, whereas generalists likely rely more on general cues. Short-range volatile or contact chemicals from prey that may provide specific information to carnivores include cuticular hydrocarbons (Battaglia *et al.*, 2000; Muratori *et al.*, 2006), proteins or polypeptides (Benedet *et al.*, 1999) and honeydew (Ide *et al.*, 2007). Ubiquitous nutrient compounds, such as sugars and amino acids, generally serve as phagostimulants for most insects (Chapman, 1995; 2003).

*Dicyphus hesperus* Knight (Heteroptera: Miridae) is a generalist carnivore that feeds on a variety of small, soft-bodied arthropods. At close range, prey-related visual cues direct orientation to prey by this predator (at least under full light), and prey-related volatiles and chemotactile cues are involved in prey recognition/acceptance (Chapter 1). In the current study, I investigated whether prey colour, shape, or prey aggregation affect foraging decisions by *D. hesperus*, and whether body washes of different polarity, from both rearing and novel prey, differentially elicit a gustatory response. If specific cues are important for prey recognition, I would expect predators to discriminate among the different treatment levels, but considering the constraints of limited attention on these generalists, I predict that no preference will be shown for specific cues that do not provide useful information about prey profitability. However, predators may learn to

respond to specific cues from being reared on particular prey, if such prey are profitable, and thereby exhibit a stronger response when these cues are present (associative learning). As well, repeated exposure to chemical cues from rearing prey may increase responsiveness in a way not due to conditioning (sensitization), or conversely, reduce responsiveness (habituation).

### **3.3 Materials and Methods**

#### **3.3.1 Insect collection and rearing**

Insects were obtained as third or fourth instar nymphs from the Pacific Agricultural Research Centre in Agassiz. There they were reared on whole tobacco plants *Nicotiana tabacum* L. (Solanaceae) and provided eggs of *Ephesia kuehniella* (Lepidoptera: Pyralidae) as prey. Daylength was 16h and rearing temperatures ranged from ~23-26 °C. This colony was established from collections made in 1999 near Woody, California, USA, at 35° 43' N, 116° 49' W, at an elevation of ~300 m on white stem hedgenettle, *Stachys albens* Gray (Lamiaceae).

The third and fourth instar *D. hesperus* were removed from the colony and reared to adults, on the lab bench, in 3.4 L (320 mm high x 215 mm wide x 80 mm deep) plastic containers on fresh tobacco leaves with *E. kuehniella* eggs as prey, under L16:D8 and at  $20 \pm 3$  °C. To obtain uniform age classes, adults were removed at three day intervals, and placed into 600 mL (140 mm square x 55 mm deep) plastic containers and aged an additional 5 days, under the same conditions. Both types of containers had mesh-covered holes in the lids for ventilation. This yielded cohorts of 5-8 day-old adult females. Because females that are maturing eggs should have an increased requirement for food resources, relative to other adult individuals (adult diet strongly impacts fecundity by

female *D. hesperus*, Sanchez *et al.*, 2004), I expected them to be the most motivated to feed; hunger often increases intensive or area-restricted search (e.g. ladybirds: Carter & Dixon, 1982; e.g. darkling beetles: McIntyre & Wiens, 1999). Therefore, males and females were reared together, to allow for mating, but only females were used in experiments.

Pea aphids, *Acyrtosiphon pisum* Harris (Homoptera: Aphididae), were maintained on *Vicia faba* L. (cv. Broad Windsor) (Fabaceae), at  $22 \pm 2.0$  °C, 50–60% r.h., L16:D8, in 27 cm wide x 36 cm deep x 42 cm high acrylic glass cages. Plants were replaced as necessary to maintain the colony.

### **3.3.2 Starvation pre-treatment**

Under L16:D8 and at  $20 \pm 3$  °C., 5-8 day-old adult females were held, without plant and prey material, in clean, clear-lidded plastic cups (Solo Cup Corp, Urbana, Ill., #P200) for three days ( $72 \pm 4$ h). Holes were cut in the centre bottom of cups, through which 10 mm diameter, sterile cotton dental rolls were passed into a distilled water reservoir for hydration. Water was held in 280 mm x 552 mm x 62 mm deep, black, watertight, plastic plant propagation trays. The plastic cups were placed on perforated (for passage of dental wicks) plant carry trays (complementary to propagation trays) that were propped up above the water on four 57 mm green plastic plant pots, positioned at the corners of each tray.

### **3.3.3 Physical characteristics and spacing of prey**

Three experiments were performed to determine the influence of shape, colour and clumping of prey on the predatory behaviour of *D. hesperus*. All experiments were

conducted under diffused fluorescent light ( $1.32 \mu\text{Watt}/\text{cm}^2$ ) at  $21 \pm 2 \text{ }^\circ\text{C}$  and took place in arenas comprised of 150 mm x 14 mm Petri dish lids with a 144 mm diameter disc of black Art Street<sup>®</sup> heavyweight construction paper (Riverside Paper Co, Appleton, WI, USA) as substrate. A 3/8" hole was cut through the centre bottom of each arena and into a piece of standard 38 mm x 184 mm lumber, positioned directly beneath, for the placement of 2 mL vials, which were used for the release of insects. Prior to trials, individuals were placed in the vials at the centre of arenas for 5 min. to acclimatize. Arenas were covered with 150 mm x 150 mm squares of acrylic glass to prevent insects from flying away upon their entrance.

I previously demonstrated that artificial prey of approximately 1 mm were significantly more attractive to *D. hesperus* than expected, based on a null model related to space occupied by prey. Therefore, the artificial prey items (APIs) used in the three experiments consisted of colourless glass beads/marbles (Halcraft USA Inc., Mt. Vernon, NY, USA, "rock candy," #83612), approximately 1 mm (mean  $\pm$  SE =  $1.03 \pm 0.014$  mm;  $n = 30$ ) in size and/or irregularly shaped salt clusters of about the same size. It should be noted that prey extracts were not used here as they were in the previous experiment, because no strong distance effect of odour was observed; odours only impacted the decision to contact APIs at very close proximity and did not affect reactive distance. Therefore, vision was of primary importance for prey location. Salt clusters were obtained by partially crushing non-iodized, pure canning salt (NaCl) and passing the resulting mixture of particle sizes through 1 mm and 0.75 mm pore diameter soil sieves. Those particles that remained on the 0.75 mm sieve after vigorous sifting were used as artificial prey. Different coloured APIs were generated from salt treated with Club House

food colour preparation (McCormick Canada<sup>®</sup>, London, Ontario). First, a saturated canning salt solution (at boiling) was made and 50 mL was transferred to each of three 100 mL beakers. Fifteen drops of green food colouring was added to one, fifteen of blue to another and twenty of yellow to the last one. After stirring with glass rods, and cooling to room temperature, solutions were poured into 150 mm Petri dishes and placed in a drying oven at 60 °C. Mixtures were stirred occasionally, breaking up large clusters of precipitate, until the majority of water had evaporated. Once the salt clusters (opaque aggregations of salt crystals and food colouring) were completely dry, they were crushed and separated as above. Reflectance spectra of the resulting APIs were obtained using a CARY-17 spectrophotometer (On-Line Instrument Systems Inc.), with magnesium oxide and charcoal as the white and black references, respectively (Figure 3.1). All three spectra show a high degree of reflectance in the dark to far red region (> 650 nm). However, previous studies with *D. hesperus* have been conducted under red light to simulate darkness (e.g. VanLaerhoven *et al.*, in prep.), and these predators do not appear to be responsive to visual stimuli under such conditions (Sherah VanLaerhoven, pers. comm.). As well, the majority of insects are not sensitive to light from the red region. Therefore, differently coloured APIs should appear dissimilar, as spectra differ outside of the red region.

The distribution of APIs in the arena for the investigation of the influence of prey shape followed the same template as in the first chapter; fourteen were placed symmetrically (16 mm centre to centre) in a ring, equidistant from the arena's centre (36 mm). In this configuration, spherical glass beads that had been coated with saturated (at room temperature) canning salt solution and dried and the irregularly shaped salt clusters

(both colourless) were placed alternately around the ring. The other two experiments required a slight modification to the design. To test the effects of colour, fifteen APIs were placed with the same equal spacing between neighbours (16 mm centre to centre) and in a ring, but these were 38 mm from the arena's centre. Coloured APIs (five of each) were arranged in a cyclical alternating pattern around the ring to generate an unbiased, uniform distribution. The experiment for the effect of prey clumping involved two treatments of API spacing, in a similar ring design, one with five clusters of three, closely spaced glass bead APIs (4 mm from the centre of either outside bead to centre of the middle bead), and the other with fifteen symmetrically spaced APIs, as in the colour experiment (16 mm centre to centre). As well, APIs in both treatments were 38 mm from the arenas centre. Therefore, the expected probability of contacting an API in either case was the same, as the only variable manipulated was spacing and spacing in both cases exceeded predator width.

In the three experiments, the number of predators that contacted APIs en route to the edge of the arena was counted. An instance of no contact was scored if an individual passed through the ring of APIs without touching one. Those individuals that took flight before reaching the ring of APIs and landed somewhere on the opposite side, were excluded from the analysis. The number of contacts with APIs between the prey shape classes, between colours and between prey spacing treatments were compared via a series of chi square analyses.

#### **3.3.4 Prey chemical cues**

The objective of this experiment was to determine if compounds from the external surfaces of prey elicit a gustatory response by *D. hesperus*, and if so, whether certain

classes of these compounds, grouped by their polarity, are stronger phagostimulants than others. Three body wash extracts were prepared for each of two prey species: pea aphids, *Acyrtosiphon pisum* (Harris) (Homoptera: Aphididae), and Mediterranean flour moth, *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae), eggs. The three solvents were methanol, dichloromethane and hexane (polar, moderately polar and non-polar respectively). Washes of aphids were prepared by placing them directly into 7 mL vials on dry ice that contained 1.5 mL of solvent, and then after one minute, removing vials from the dry ice and allowing them to stand at room temperature for 10 minutes. Subsequently, the liquid was removed from each vial and filtered through glass wool into another 7 mL vial. Solutions were then diluted to 200 aphid equivalents/mL, taking into consideration the total number of aphids used in each wash and the volume of fluid collected. Similarly, moth egg washes were made by placing 1.233 g (~50,000 individuals) of eggs into 7 mL vials, containing 1.5 mL of solvent, and allowing them to stand for 10 minutes at room temperature. As above, liquid was removed and filtered into 7 mL vials, but solutions were instead filled to 1 mL to generate solutions of approximately 50,000 egg equivalents/mL.

Trials were performed under diffused fluorescent light ( $1.32 \mu\text{Watt}/\text{cm}^2$ ) at  $21 \pm 2$  °C in arenas constructed from 60 mm diameter by 15 mm high plastic Petri dishes, with a piece of 55mm filter paper as a substrate. Arena walls were coated with Fluon<sup>®</sup> AD-1 (Asahi Glass Fluoropolymers Americas, Inc., Bayonne, NJ) to prevent insects from climbing them. At one end of each arena were placed five of the colourless glass APIs. Twenty-five  $\mu\text{l}$  (five aphid equivalents or 1,250 egg equivalents) of a given prey extract, or solvent alone was then pipetted over the group of APIs. Predators were subsequently

introduced from above through a hole at the arena's centre in a 2 mL vial. These vials were inserted to the arena's floor so as to hold predators in place for the 5 minutes preceding the commencement of trials, allowing them to acclimatize.

Trials lasted 15 minutes and contacts with APIs were observed. The latency to API contact, whether individuals extended their proboscises to APIs and whether they subsequently exhibited feeding behaviour (extended their proboscises and attempted to probe/manipulate the API for a minimum of 5 s) were noted. There were 23 replicates for the dichloromethane control, 24 for the aphid methanol extract, and 25 for each of the other treatments. All count data were analyzed individually by response variable, using a loglinear approach to account for the effects of prey type and solvent.

## **3.4 Results**

### **3.4.1 Physical characteristics and spacing of prey**

The likelihood of *D. hesperus* contacting APIs was neither affected by their shape nor their colour ( $\chi^2(1) = 0.667$ ,  $p = 0.414$ ,  $\chi^2(2) = 0.20$ ,  $p = 0.905$ ; shape and colour, respectively) (Table 3.1). This behaviour was affected by the distribution of prey, however ( $\chi^2(1) = 7.71$ ,  $p = 0.006$ ). Odds ratios indicate that the predators were 4 times more likely to contact the broadly spaced APIs than clumped APIs. Data from these experiments are summarized in Table 3.1.

### **3.4.2 Prey chemical cues**

The number of individuals that contacted APIs was not affected by the chemical cues on them (Table 3.2). The loglinear analysis for proportions of positive responders produced a final model that only retained response as the main effect, and thus no effect

of solvent or prey type (including solvent control). The corresponding likelihood ratio for this model was  $\chi^2(16) = 5.67$ ,  $p = 0.991$ . The total number of contacts on APIs per individual was not impacted by solvent ( $F_{2,213} = 0.041$ ,  $p = 0.960$ ), or by prey type ( $F_{2,213} = 1.843$ ,  $p = 0.161$ ), nor was there an interaction term ( $F_{4,213} = 0.779$ ,  $p = 0.540$ ).

The loglinear analysis of incidences of individuals that contacted APIs with their proboscises generated a final model that retained the response x prey treatment interaction as the main effect ( $\chi^2(2) = 36.91$ ,  $p < 0.001$ ). The likelihood ratio for this model was  $\chi^2(12) = 3.08$ ,  $p = 0.995$ . As no difference was detected between solvent treatments, data were condensed across solvents and a pairwise comparison was performed. The analysis demonstrated significantly greater proportions of positive responses to wash treatments of either prey type versus solvent alone ( $p < 0.001$  for both), but no difference between the prey wash treatments ( $p = 0.660$ ).

No individuals exhibited feeding behaviour when APIs were coated with solvent alone. A modified Fisher's exact test (2x3), with data consolidated across solvent treatments, indicated that feeding behaviour was significantly impacted by prey treatment ( $p < 0.001$ ). In order to satisfy the assumption of no expected values below 1, the loglinear analysis on feeding events was performed without solvent controls. The final model produced by the analysis had only response as the main effect, with a likelihood ratio of  $\chi^2(10) = 6.06$ ,  $p = 0.811$ , indicating no significant effect of treatment factors and no interaction term. This implied that the effect detected by the Fisher's exact test was attributable to a greater response by individuals to prey washes over solvent controls. Despite no detection of an effect by treatment in the loglinear analysis, the proportion of positive responses to both hexane aphid and dichloromethane moth egg washes was

approximately half that of each of the others. This non-detection of an interaction term between solvent and prey type is likely a result of the small number of individuals that exhibited feeding behaviour.

### **3.5 Discussion**

As predicted, there was no innate preference by *D. hesperus* for particular colour or shape of potential prey. Similarly, the generalist pentatomid predator *Eocanthecona furcellata* Wolff did not discriminate between prey manipulated into different shapes (Rani & Wakamura, 1993). Such cues provide specific information about prey, but they likely do not provide a general inference about the nutritional composition or defenses of prey. Hence, theory predicts that while searching for conspicuous prey types, it is best to divide attention among all the available types, so long as they are equal in abundance, energy content and handling time (Dukas & Ellner, 1993). This prediction holds true for the current study, as all potential prey were conspicuous and equally abundant. Energy content and handling time obviously did not factor in as potential prey were novel. As such, the predator could not know their values. As well, model prey were approximately equal in size, which provided the only information about their potential energy content and defensive capabilities.

The innate use of specific cues may not be adaptive for a generalist, but many have demonstrated the ability to learn specific visual cues associated with profitable food sources, so that they may later be used to detect and recognize similar potential food (Dukas, 2008). For example, *Diachasmimorpha longicaudata* Ashmead (Hymenoptera: Braconidae), a generalist fruit fly larval parasitoid, demonstrated a preference for fruit models of a given colour after they were exposed to larvae associated with fruit models

of the same colour. However, naïve individuals showed no colour preference (Segura *et al.*, 2007). Therefore, it is possible that *D. hesperus* can learn to discriminate visual cues associated with profitable prey.

In this study, it was assumed that *D. hesperus* was capable of discerning the chromatic differences of coloured APIs. Very few studies have been performed to determine the specifics of chromatic vision in related Heteroptera; however, I have found no evidence to suggest that the spectral sensitivity of their eyes is at all constrained. For instance, another cimicomorph species, *Triatoma infestans* Klug (Reduviidae) exhibited a photonegative response (it is nocturnal) to a broad range of wavelengths: from UV at  $\lambda = 357$  nm to dark orange at  $\lambda = 621$  nm, and even to dark red at  $\lambda > 665$  nm at relatively high intensities (Reisenman and Lazzari, 2006). However, the response to dark red may have simply been a response to heat with the greater light intensity.

Unlike the other visual cues, prey clumping had a strong effect on the probability that *D. hesperus* would contact prey. Clumped APIs were contacted significantly less frequently than evenly spaced ones. This result is the opposite of what would be expected for a predator that typically feeds on densely aggregated prey, such as whiteflies and aphids. There are two (not mutually exclusive) hypotheses that may provide an explanation for this pattern: (i) *D. hesperus* saw each group of potential prey and showed less interest because of potential difficulties attacking individuals in close proximity; and (ii) predators could not resolve individual prey of a bunch, which made them appear as one large organism. Neither hypothesis was tested directly, but neither can be discounted.

Consider the first hypothesis. Animals often group together as a means to defend themselves. Buffaloes, for example, huddle around young and with rumps facing inward. In this way, more vulnerable members and body parts are shielded by their neighbours (Begon *et al.*, 1996). Such behaviour may make prey less attractive. Neighbours may also otherwise interfere. In the case of *D. hesperus*, perhaps defensive behaviours, such as kicking (as done by aphids, see Gross, 1993), by closely approximated neighbours could interfere with the processing of a prey item and even damage its mouthparts if stylets were inserted into prey at the time. If this is true, then how can one account for the fact that *D. hesperus* consumes prey such as whiteflies and aphids, which are usually densely clumped on a leaf or stem? Perhaps, as suggested in Chapter 1 for large potential prey, the perception of risk is enhanced by uncertainty. Objects that might represent potential prey, but that cannot be detected chemically, may only be worth investigating if the available cues suggest that risk is very low. While all objects/organisms that are completely unidentifiable remotely may present some risk, perceived risk may be proportional to the number of these potentially dangerous objects/organisms in close proximity. Predatory species of ants, for example, often forage in groups and efficiently recruit in groups to subdue their prey (Štyrský & Eubanks, 2007). As well, many are known to tend honeydew-producing hemipterans, and often become more aggressive, attacking insects that they might otherwise ignore, including other predators, to protect their resource (Štyrský & Eubanks, 2007). This makes ants particularly relevant, as such hemipterans are typical prey for *D. hesperus*. In fact, *D. hesperus* has been shown to avoid tomato leaves occupied by the cornfield ant, *Lasius* sp. (Carolyn Duckham, pers. comm.). The low response rate to clumped APIs could also be due to the poor resolving

power of insect visual systems; upon entrance into the arena, APIs within each prey cluster may have been perceived by predators as contiguous parts of a large, and hence, potentially dangerous organism (hypothesis (ii)). The acuity of an insect's vision is severely limited by a high degree of diffraction-related blur that is inversely proportional to the aperture of ommatidial lenses, and is also constrained by the angular width of receptors and the interommatidial angle ( $\Delta\phi$ ) (see Land, 1997; Land & Nilsson, 2002). In addition, Horridge (2005) insists that spatial resolution in insects varies according to the specific cue and that "the performance of each cue depends on the density of sampling points and the number collaborating." For example, Giurfa *et al.* (1996) found that free-flying honeybee (*Apis mellifera*) foragers were able to detect colored targets if their visual angle was larger than  $5^\circ$  (a visual field covered by seven ommatidia), while the drone honeybee is capable of tracking models of queen bees that subtend a visual angle of only  $0.32^\circ$  (Vallet & Coles, 1993). This difference is attributable to a specialized region of high acuity on the drone's eye (Vallet & Coles, 1993). Many predators, including mantids, robber flies, dragonflies and notonectids, are also known to exhibit such specializations for detecting prey (Land, 1997; Land & Nilsson, 2002; Horridge, 2005). Visual acuity is also proportional to insect size (Land & Nilsson, 2002). In light of so much variation, to determine the visual acuity of *D. hesperus* for this task, a combination of anatomical and electrophysiological measurements, and an examination of behavioural responses to objects occupying specific visual angles would be necessary.

All six body wash extracts elicited much stronger probing and feeding responses by predators than did solvents alone, but no differences were detected amongst washes. The proportion of predators that extended their proboscises to APIs was significantly

greater in response to those treated with prey washes than those treated with solvent alone. As well, a significantly greater proportion of individuals exposed to prey wash treatments attempted to feed on APIs than did those exposed to the solvent control treatments; not one feeding attempt for the latter. However, the proportion of individuals that contacted APIs with their tarsi was not significantly different amongst treatments. Therefore, it is apparent that the predator is capable of using a number of different chemical cues from both novel and rearing prey at close range. This ability likely facilitates recognition of the variety of food sources used by *D. hesperus*.

Non-polar body surface compounds appear to be more important than polar ones for the recognition of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) prey by the generalist heteropteran predator *Eocanthecona furcellata* (Wolff) (Pentatomidae) (Rani & Wakamura, 1993). However, these predators were not naïve to *S. litura* prey. Therefore, the cues used, potentially non-polar hydrocarbons, may have been associatively learned. Non-polar hydrocarbons on the cuticle of insects are highly variable and species-specific (Howard & Blomquist, 2005). Therefore, such compounds should be reliable indicators of prey/host identity and learning to recognize those from a rewarding host/prey species could have strong fitness implications. Cuticular hydrocarbons are known to be used as cues to determine not only species but also to recognize gender and nestmates/kin, and to communicate dominance, fertility and even the tasks being carried out at a given time by individuals in ant colonies (Howard & Blomquist, 2005).

*Dicyphus hesperus* may not have responded more strongly to extracts from *its* rearing prey because the compounds contained in the extracts may have only provided very general cues; the outer surface of insect eggs is composed almost entirely of

polysaccharides, and is generally lacking non-polar hydrocarbons (Trougakos & Margaritis, 2002). Interestingly however, the eggs of two ladybird species that co-occur in nature, *Adalia bipunctata* and *Coccinella septempunctata*, are anomalously coated with hexane soluble hydrocarbons that prevent intraguild predation by each on the other (Hemptinne *et al.*, 2000). These compounds appear to flag the poisonous nature of the alkaloid-containing eggs to their heterospecifics. In addition, the primarily alkane signal coming from the eggs of *A. bipunctata* significantly reduces cannibalism (Hemptinne *et al.*, 2000). This further supports the importance of cuticular hydrocarbons in insect recognition. Whereas, cuticular hydrocarbons are extremely variable in structure at the atomic level (see Howard & Blomquist, 2005), polysaccharides are comprised of repeating chains of common sugar residues. As well, those that comprise the exochorion (outer egg layer) form a rather fibrous covering (Trougakos & Margaritis, 2002), implying low solubility, and therefore would likely not have been extracted in the washes prepared for this experiment. More general phagostimulants, such as sugars and amino acids, from eggs that may have been crushed prior to, or during the preparation of washes were likely important. As well, cues resulting from contaminants, such as the scales and frass of adult moths (from the facility where the eggs were produced) may also have been relied upon. Alternatively, the non effect of rearing prey on feeding response could also be explained by a lack of foraging success over the long (72 h) pre-trial starvation period. This may have caused predators to ignore/forget specific cues associated with the previously profitable rearing prey and adopt a more general search strategy. Another possibility is predators became habituated to certain chemical stimuli from the moth eggs. Repeated, long-term exposure to a stimulus often leads to a reduction in responsiveness

(Barnard, 2004), and moth eggs were provisioned in large numbers to these predators, throughout the rearing process.

*Dicyphus hesperus* uses cues in a way that is consistent with its generalist feeding habits. As predicted, this predator did not demonstrate an innate preference for potential prey of different shapes or colour. Theory states that it is more adaptive for extreme generalists to pursue all conspicuous prey, assuming all other factors are the same (Dukas & Ellner, 1993). However, a clumped prey distribution, uncoupled from chemical cues, negatively impacted response of *D. hesperus*. This may reflect the way *D. hesperus* assesses risk in the environment. Finally, *D. hesperus* appears to use a broad range of chemical cues from the external surfaces of prey in close range prey foraging.

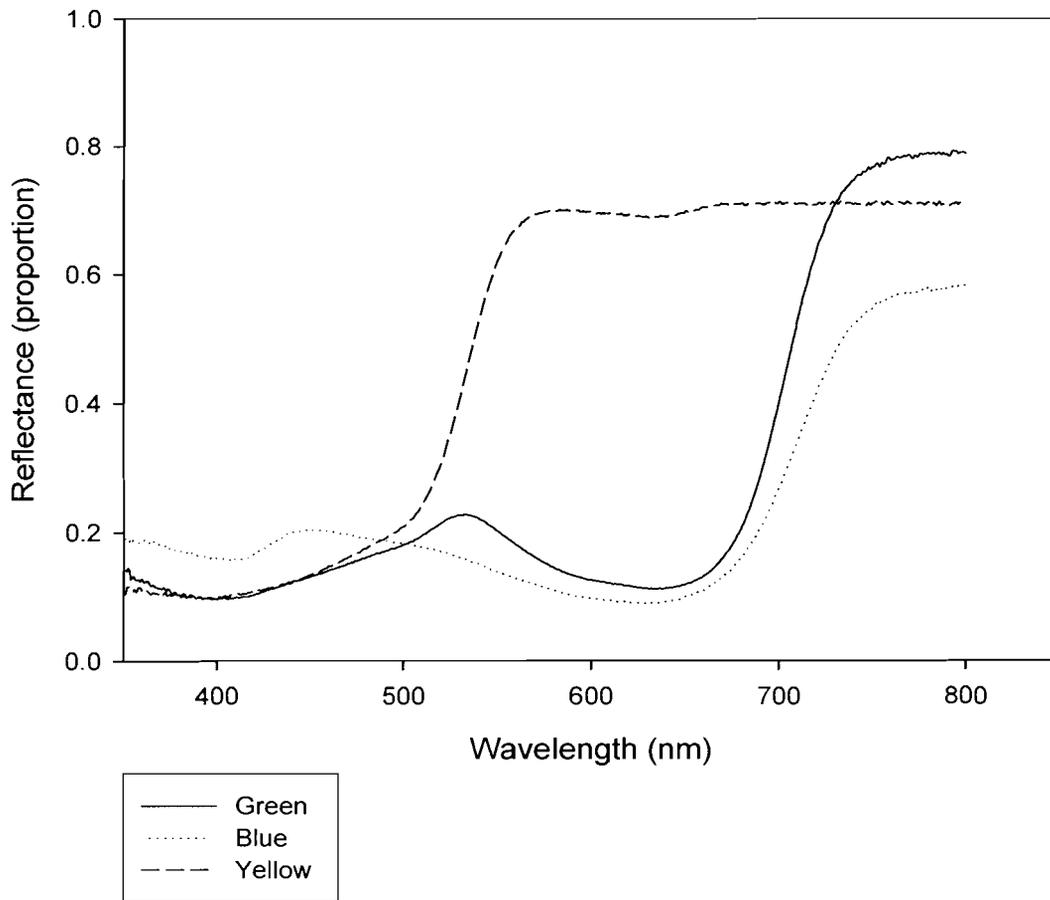
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Figure 3.1 Reflectance spectra for coloured artificial prey items (APIs), using MgO and charcoal as white and black references.



**Table 3.1** Number of *D. hesperus* making contact with different artificial prey types (level), according to the variable tested. Asterisks (\*) indicate where N corresponds to tests with only one treatment but with multiple levels.

Variable	Level	Number of contacts (Total)	N
Shape	Irregular	15	56*
	Sphere	12	
Colour	Blue	14	70*
	Green	14	
	Yellow	12	
Clumpedness	Evenly spaced	12	25
	Clumped	3	25

**Table 3.2** Count data for the number of *D. hesperus* that exhibited a given behaviour (contact with tarsus, with proboscis, or prolonged ( $\geq 5$  s) contact/probing, by proboscis) in response to artificial prey items (APIs) treated with various solutions. The shaded area indicates values that are significantly different from the rest, but not amongst themselves.

Prey	Treatment		Response Totals		
	Solvent	N	Tarsus	Proboscis	Feed
Aphid	Dichloromethane	25	19	18	9
	Hexanes	25	19	15	4
	Methanol	24	16	14	8
<i>Ephestia</i>	Dichloromethane	25	18	13	4
	Hexanes	25	22	17	8
	Methanol	25	18	14	9
Control	Dichloromethane	23	19	5	0
	Hexanes	25	18	5	0
	Methanol	25	21	4	0

## CHAPTER 4

# MANAGING A TRADEOFF: BIGGER IS BETTER DEFENDED

### 4.1 Abstract

The energetic value of prey is usually proportional to their size, but larger prey may also have better defensive capabilities. Therefore, on the one hand, choosing large prey minimizes the number of prey that need to be captured, but on the other, the effort required in each case may be disproportionately large. According to optimal diet theory, predators should manage this tradeoff by choosing prey that provide the greatest amount of resources per unit of time invested. In this study, I examined whether the generalist heteropteran predator, *Dicyphus hesperus* Knight, selects prey based on size and how prey size-related defenses impact prey choice. Extra-oral digestion (EOD) enables the predatory Heteroptera to handle a range of prey sizes (from relatively small to those even larger than themselves), as they are not constrained by gape size. This was exploited in the current study, using first (small) and fourth (large) instar pea aphids, *Acyrtosiphon pisum* Harris as prey. Fourth instars from the source colony were on average 18 times larger, by volume, than first instars and they are much better defended. To isolate the effect of prey defenses, prey were either defended or immobilized in different treatments. Large aphid prey were preferred over small aphid prey, regardless of defenses, but the bias toward large aphids was significantly greater when prey were not defended. These results are consistent with optimal diet theory.

## 4.2 Introduction

While searching for food, generalist predators may encounter a multitude of potential prey that may confer different amounts of nutrition as well as different levels of cost to the forager. Classic optimal foraging theory states that predators should select prey so as to maximize their net rate of energy return, or profitability, which is calculated as the ratio of the net energetic gain from prey to the time spent searching for, pursuing, subduing and consuming prey (Stephens & Krebs, 1986). All else being equal, energetic value is proportional to prey size, and as such, Charnov's optimal diet theory (1976) suggests that larger prey should be preferred when available. However, while prey of increasing size may contain more nutritional resources, they may also have better defensive capabilities, which in turn may increase their handling time (Chau & Mackauer, 1997; 2001; Gross, 1993; Pastorok, 1981). This presents a potential tradeoff between the energetic value of prey and the time needed by predators to pursue, subdue and consume them. If this time is disproportionately greater for larger prey than for smaller prey (so as to make them less profitable from a rate maximization standpoint), then smaller prey should be preferred.

Whereas the range of size and type of prey of chewing predators is often limited by gape size (Paine, 1976), organisms that consume prey by means of extra-oral digestion (EOD) are not constrained by gape, and generally are able to handle relatively larger prey than similarly sized chewing predators (Cohen, 1995; 1998; see Hespenheide, 1973). Cohen and Tang (1997) have shown that some reduviids can consume prey that are up to four times their own body weight via EOD. Consequently, individuals with this capability may be sated by nutrients from a single, large prey item, and could maximize

their rate of energetic return by eliminating the need to search for, pursue and subdue additional prey to achieve this state (Cohen, 1998). As well, unhindered by indigestible and potentially damaging cuticular structures, such predators are able to consume otherwise unprofitable or inaccessible, nutrient-rich food (see Cohen, 1995 and references therein).

EOD by the predatory Heteroptera, often called “lacerate and flush feeding,” involves the injection of hydrolytic enzymes from salivary glands into prey and the laceration of tissues beneath the cuticle with the stylet bundle (Cohen, 1990; 1995; 1998; Miles, 1972). The stylet bundle is remarkably flexible and capable of moving throughout the body of prey, even penetrating the narrowest of spaces, including the legs and antennae (Cobben, 1978; Cohen, 1995). The resulting slurry of digestive enzymes, small particles, dissolved nutrients and lipid micelles is then ingested by suction through a food canal that is formed between the interlocking maxillary stylets (Cobben, 1978; Cohen, 1990; 1995). Injection of enzymes/laceration of tissues and ingestion occur cyclically throughout a feeding bout (Cohen, 1995). This mode of consuming prey has been shown to yield an ingestion efficiency of more than 94% of prey nutrients, while the inert cuticle remains intact (Cohen, 1984).

The recovery of digestive enzymes in this process is important, but once ingested, they cannot be reused; they remain in the gut for the duration of digestion (Cohen, 1995; 1998). Heteropteran predators are accordingly called “nonrefluxers,” as opposed to “refluxers,” which can cyclically pump digestive enzymes into prey from the gut and re-ingest them. Nutrient acquisition in these predators is therefore limited by the amount of enzyme they can produce in a given amount of time (Cohen, 1995; 1998). As such, they

may be better served to select larger prey so as to maximize the amount of material digested per unit of enzyme. Presumably, not all enzymes are resequenced after a cycle of saliva injection and uptake of digested compounds. In turn, those enzymes left behind are free to digest the internal contents of prey as long as they remain within its cuticle and there is substrate. Hence, as the amount of substrate is proportional to prey size, reaction rates would likely be higher and substrate availability to enzymes would remain high longer, during a feeding bout on larger prey.

*Dicyphus hesperus* (Knight) (Heteroptera: Miridae) is a generalist predator that feeds by EOD on a range of small soft-bodied arthropods, of various sizes. Data presented in Chapter 1 suggest that the tendency for this predator to investigate prey is negatively affected by prey size when uncoupled with chemical cues. However, it is not clear how prey size impacts foraging decisions when real prey are used. The present study examines how *D. hesperus* manages a tradeoff between prey-size-related defensive capabilities and energetic value. Two different size classes (first and fourth instars) of pea aphids, *Acyrtosiphon pisum* (Harris) (Homoptera: Aphididae) served as prey. These prey were chosen specifically because, early and late instar pea aphids show a great disparity in size (energetic value); fourth instar pea aphids, from the source colony, were on average 18 times larger, by volume, than first instars (Nigel May, pers. comm.) and were visibly larger than the predators. As well, larger instars are much better defended (Chau & Mackauer, 1997). Such defenses, which are characteristic of many aphids, include dropping from the host plant or walking away, and kicking, striking with wings or antennae, or jerking movements, often with the hind legs and abdomen lifted off the substrate (Chau & Mackauer, 1997; and see Gross, 1993 and references therein).

Handling time by the parasitoid *Aphidius ervi* (Hymenoptera: Braconidae) on pea aphids is proportional to instar (size) (Lee Henry, pers. comm.). As well, Chau and Mackauer (1997) showed that first instar pea aphids were the least likely of all instars to exhibit any form of anti-parasitoid behaviour, when provoked, whereas, fourth instars were the most likely to respond by dropping from the host plant (see Roitberg & Myers, 1978). To isolate the effect of prey defenses for the current study, prey were either defended or immobilized in different treatments. I predict prey defenses will negatively impact prey choice by *D. hesperus* and that larger prey will be more attractive when defensive capabilities have been removed.

### **4.3 Materials and Methods**

#### **4.3.1 Insect collection and rearing**

Insects were obtained as third or fourth instar nymphs from the Pacific Agricultural Research Centre in Agassiz. There they were reared on whole tobacco plants *Nicotiana tabacum* L. (Solanaceae) and provided eggs of *Ephestia kuehniella* (Lepidoptera: Pyralidae) as prey. Daylength was 16h and rearing temperatures ranged from ~23-26 °C. This colony was established from collections made in 1999 near Woody, California, USA, at 35° 43' N, 116° 49' W, at an elevation of ~300 m on white stem hedgenettle, *Stachys albens* Gray (Lamiaceae).

The third and fourth instar *D. hesperus* were removed from the colony and reared to adults, on the lab bench, in 3.4 L (320 mm high x 215 mm wide x 80 mm deep) plastic containers on fresh tobacco leaves with *E. kuehniella* eggs as prey, under L16:D8 and at 20 ± 3 °C. To obtain uniform age classes, adults were removed at three day intervals, and placed into 600 ml (140 mm square x 55 mm deep) plastic containers and aged an

additional 5 days, under the same conditions. Both types of containers had mesh-covered holes in the lids for ventilation. This yielded cohorts of 5-8 day-old adult females.

Because females that are maturing eggs should have an increased requirement for food resources, relative to other adult individuals (adult diet strongly impacts fecundity by female *D. hesperus*, Sanchez *et al.*, 2004), I expected them to be the most motivated to feed; hunger often increases intensive or area-restricted search (e.g. ladybirds: Carter & Dixon, 1982; e.g. darkling beetles: McIntyre & Wiens, 1999). Therefore, males and females were reared together, to allow for mating, but only females were used in experiments.

#### **4.3.2 Starvation pre-treatment**

To ensure individuals were motivated to feed, 5-8 day-old adult females were held, without plant and prey material, in clean, clear-lidded plastic cups (Solo Cup Corp, Urbana, Ill., #P200) for three days ( $72 \pm 4$ h), under L16:D8 and at  $20 \pm 3$  °C. Holes were cut in the centre bottom of cups, through which 10 mm diameter, sterile cotton dental rolls were passed into a distilled water reservoir for hydration. Water was held in 280 mm x 552 mm x 62 mm deep, black, watertight, plastic plant propagation trays. The plastic cups were placed on perforated (for passage of dental wicks) plant carry trays (complementary to propagation trays) that were propped up above the water on four 57 mm green plastic plant pots, positioned at the corners of each tray.

#### **4.3.3 Investigation of meal size**

The objective of this experiment was to determine if starved *D. hesperus* females were able to obtain a larger meal from fourth instar pea aphids or if first instars were

sufficiently large to satiate them. Experiments were conducted under diffused fluorescent light ( $1.32 \mu\text{Watt}/\text{cm}^2$ ) at  $21 \pm 2 \text{ }^\circ\text{C}$ . Each predator was given access to a single, pre-weighed first or fourth instar pea aphid, inside a lidded 0.5 dram vial. Aphids were immobilized, to prevent defensive behaviours, by adhering their appendages and ventral body surface to one side of a small piece (approximately the area of the aphid footprint) of double sided masking tape (Intertape<sup>®</sup> Brand, Intertape Polymer Group, Sarasota, FL, USA). A piece (the same width and approximately twice the length of the tape piece) of aluminum foil was stuck to the other side of the tape to enable manipulation with forceps. To facilitate this process and prevent injury, aphids were anaesthetized with  $\text{CO}_2$  beforehand. As well, just prior to trials, predators were provided distilled water to ensure hydration. They were placed in 0.5 dram vials and closed in with a section of moistened cotton ( $\sim 2.5 \text{ cm}$  long x  $10 \text{ mm}$  diameter) cut from a dental roll, for 30 min to 1 hr.

All trials and events were timed. A feeding event was counted if a predator inserted its stylets into and maintained contact with prey, proboscis remaining extended, for a minimum of 10 minutes. As preliminary observations indicated that feeding events could last well over an hour, this was deemed to be a reasonable estimate of the absolute minimum duration a starved individual would spend feeding on an acceptable prey item. Most individuals that fed took short ‘breaks’ (from seconds to minutes) between feeding bouts, but they were allowed to continue feeding so long as ten minutes had not elapsed since the last bout. When ten minutes was exceeded, *D. hesperus* individuals were assumed satiated and were removed from vials to prevent further feeding. Multiple replicates were run concurrently and the initiation of feeding and feeding duration varied, which led to disparities in trial completion times. Therefore, in order to reduce variation

due to evaporative loss over time, weighing of all aphids for the calculation of meal size was performed at the same time: twelve hours from the commencement of trials. Some individuals did not attempt to feed on the aphid provided to them. These rejected aphids were weighed at the same time as the others to calculate the average change in mass over the experimental period (for each instar class) due to evaporative loss. The meal size for each predator, measured to the nearest  $\mu\text{g}$  (on a Cahn Microbalance, Cahn Instruments, el Cerritos, CA), was therefore calculated as the difference between pre- and post-trial mass minus the average decrease in mass of unmolested aphids. Data were obtained for 24 first and 23 fourth instar prey that had been fed upon and 17 of each that had not. Meal sizes and feeding rates (meal size divided by feeding duration) between instars were compared using T-tests. Both meal size and feeding rate were plotted (by instar) against aphid weights and feeding duration in multiple regression analyses.

#### **4.3.4 Attacks on prey of different sizes and defensive capabilities**

The purpose of this experiment was to determine the importance of prey size and defenses for foraging decisions by *D. hesperus*. Trials were performed under the same lab conditions as in the previous experiment. Predators were provided, simultaneously, with four small and four large prey in two treatments: defended or immobile. Defended prey were unaltered and free to move. In preliminary trials,  $\text{CO}_2$  was used to immobilize aphids, but they became animated before the trials had concluded. Therefore, immobile prey in this experiment were freshly killed prey; placed in the freezer for 2 hr at  $-10^\circ\text{C}$  and thawed for  $\frac{1}{2}$  hr at room temperature. Prey and predators were placed into arenas constructed from 60 mm diameter by 15 mm high plastic Petri dishes, with a piece of 55mm filter paper as a substrate. Arena walls were coated with Fluon<sup>®</sup> AD-1 (Asahi

Glass Fluoropolymers Americas, Inc., Bayonne, NJ) to prevent insects from climbing them. Defended prey were introduced into arenas indiscriminately and allowed to disperse freely, whereas immobile prey were haphazardly dropped into arenas from a distance of about 15 cm. The latter were also manipulated into an upright and lifelike posture. Predators were subsequently introduced from above through a hole at the arena's centre in a 0.5 dram vial. These vials were inserted to the arena's floor so as to hold predators in place for the 5 minutes preceding the commencement of trials, allowing them to acclimatize.

Vials were then removed and trials were run for 30 min to observe the number of attacks and feeding events for each prey size class, in both treatments. Attacks were classified as events when predators initiated contact with prey (i.e. contacted prey with an extended proboscis or antenna(e), or did so with tarsi after movement toward prey), and feeding events as those where a predator inserted its stylets into prey for a minimum of 10 s. T-tests were performed to compare the average number of attacks and feeding events by predators on defended versus immobile prey. As well, the proportion of attacks on large versus small prey was calculated for each individual and arcsin square root transformed. The averages of these transformed proportions, for defended versus immobile prey, were compared by way of a T-test.

## **4.4 Results**

### **4.4.1 Investigation of meal size**

Individuals obtained a significantly larger meal from fourth instar (mean  $\pm$  SE:  $0.565 \pm 0.223$  mg) than from first instar (mean  $\pm$  SE:  $0.148 \pm 0.062$  mg) pea aphids ( $t_{45} = p < 0.001$ ). In addition, multiple regressions revealed that aphid mass and feeding

duration both had a significant positive effect on meal size for predators feeding on first instars (Table 4.1a), but only feeding duration contributed significantly to predicting the outcome for those feeding on fourths (Table 4.1b). As well, the proportion of material remaining (mass) after a feeding event relative to the prefeeding mass was significantly greater after a fourth instar meal ( $0.757 \pm 0.021$ ; mean  $\pm$  SE) than a first instar meal ( $0.266 \pm 0.033$ ; mean  $\pm$  SE) ( $t_{45} = 12.151$ ;  $p < 0.001$ ).

A greater feeding rate was exhibited by individuals that were given fourth instar prey than those given first instar prey (mean  $\pm$  SE, first and fourth instars respectively:  $3.87 \pm 1.72$   $\mu\text{g}/\text{min}$ ;  $4.74 \pm 1.43$   $\mu\text{g}/\text{min}$ ); however, this difference was not quite significant ( $t_{45} = 1.887$ ,  $p = 0.066$ ), although statistical power was slightly less than desirable ( $1-\beta = 0.744$ ). Aphid mass, however, had a positive relationship and feeding duration had a negative relationship with feeding rate on first instars; both accounted for a significant amount of variation in the multiple regression model (Table 4.2a). Conversely, only feeding duration contributed significantly to the prediction of feeding rate on fourth instars (Table 4.2b).

#### **4.4.2 Attacks on prey of different sizes and defensive capabilities**

The average number of prey attacks by individual *D. hesperus* was not significantly different between treatments (mean  $\pm$  SE, immobile and defended treatments respectively:  $6.81 \pm 1.27$ ;  $9.04 \pm 1.17$ ) ( $T_{48} = 1.29$ ,  $p = 0.204$ ). The proportion of attacks on the larger fourth instars was greater than the null proportion of 0.5 for each of the two treatments (Figure 4.1; immobile:  $T_{25} = 6.85$ ,  $p < 0.001$ ; defended:  $T_{23} = 4.23$ ,  $p < 0.001$ ). However, predators given immobile prey attacked a significantly higher

proportion of fourth instars over first instars than did those provided defended prey (Figure 4.1;  $T_{48} = 4.56$ ,  $p < 0.001$ ).

The proportion of predators that exhibited feeding behaviour (success) was significantly greater in the immobile prey treatment than in the defended prey treatment (Fisher's exact test,  $p < 0.001$ ). As well, no individuals succeeded in feeding on defended fourth instar prey. Kicking behaviour by aphids appeared to repel predators. Conversely, of the feeding events on immobile prey, the average proportion on fourth instars was greater than half ( $0.680 \pm 0.096$ , SE); although not significantly greater than half ( $T_{19} = 1.78$ ,  $p = 0.0918$ ). However, as the p-value was relatively small and the statistical power was low ( $1-\beta = 0.392$ ), the interpretation remains equivocal.

## 4.5 Discussion

Prey size and defensive capabilities strongly influenced prey selection and foraging success by *D. hesperus*. As predicted, larger fourth instar pea aphids provided a significantly larger meal to *D. hesperus* than smaller first instars. As well, there was a significant increasing trend in meal size with the mass of first instar prey, whereas no such trend was found with fourth instar prey mass. This seems to indicate that fourth instars were above the minimum size necessary for satiation of predators and is further supported by the significantly higher proportion of prey material that was leftover (mass) after feeding events on fourth instars relative to first instars. On the other hand, fourth instars also displayed greater defensive capabilities than firsts when mobile. Accordingly, the predators adjusted prey selection behaviour in a way that appears to follow the predictions of optimal diet theory (Charnov, 1976). The proportion of directed attacks by the predator on the larger fourth instars was significantly lower when prey

were defended than when immobile. This illustrates an adjustment to the predicted tradeoff between the energetic content of larger prey and their enhanced defensive capabilities. In addition, in the absence of defenses, fourth instars should have been preferred to a greater extent than firsts, as was the case. However, fourth instar aphids were attacked more frequently than firsts, regardless of treatment, even though no predator was successful in consuming mobile fourth instars. Kicking behaviour by fourth instars was sufficient to stifle an attack. Therefore, if individuals behaved according to optimal diet theory, even mobile fourth instar aphids must have been perceived as more profitable to *D. hesperus* than first instars.

I contend that the observed differences in attack rates between treatments were a result of behavioural adjustments in the foraging behaviours of the predator in response to prey defenses and not simply evasion by prey. This is because aphids did not appear to avoid *D. hesperus* individuals at all in the trials (some in fact climbed on the predators), only exhibiting defensive behaviours when attacked. If it is assumed, then, that mobile aphids moved about arenas randomly, and that predators did not behave differently between treatments, one should have observed the opposite of what occurred. Larger aphids move more quickly, and hence cover more space over time than smaller aphids, so there should have been a greater proportion of interactions with larger aphids when mobile than when immobile (see Werner & Anholt, 1993). Even if it were the case that aphid movement was opposite to the direction of predators, the constrained space afforded by the arena, and a far greater maximum velocity of movement by predators than by prey (personal observation), should have nullified its effect on the potential for the predator to make contact with any particular aphid.

In two separate choice experiments, *D. hesperus* was found to encounter immobile prey (whitefly pupae) more frequently than mobile prey (two-spotted spider mites) and to encounter less mobile prey (two-spotted spider mites) more frequently than more mobile prey (predatory mites (intraguild prey)) (Brommit, 2007). The author suggested that it was the mobility of prey that enabled avoidance of predators, and that selection of prey was passive. However, both results may be misleading, and mobility, per se, may not have been that important. With regard to the former result, whitefly pupae are larger than the mobile spider mites, and as observed in the current study, *D. hesperus* shows a preference for larger prey. Further, the author observed that, “spider mites are not that fast and... *D. hesperus*, upon encountering spider mites, would often be the one to walk away, perhaps in search of higher quality whitefly.” This seems to indicate that these prey were not really avoiding predators by moving, and that predators were making a choice. In fact, I have observed several instances where *D. hesperus* has moved into close proximity with potential prey, paused for some duration, as if to assess the prey, and moved on without an interaction. Regarding the result concerning differentially mobile prey, the more mobile predatory mites often left the leaf substrate during trials, whereas the less mobile spider mites remained. This difference in behaviour may have made spider mites more vulnerable to attack. However, this appears to be a case of reduced ability to detect predatory mites or accessibility, rather than mobility, per se. Had the intraguild prey remained on leaves and moved at random, different results may have been obtained. To ensure accessibility to all potential prey in the current study, both aphids and predators were constrained to the substrate by adding Fluon<sup>®</sup> to the walls of arenas.

Aside from the possibility of satiation when consuming fourth instar prey, there appears to be a positive relationship between prey size and feeding rate by these predators, potentially making large prey doubly profitable. A greater volume of material already in fluid form in larger prey (easily removed, little or no preparation/digestion required) could account for this difference, or it may be that other ingestible material is more easily accessed in larger prey (fewer tight spaces). Cook and Cockrell (1978) found that EOD feeding rate by waterboatmen (*Notonecta glauca*), measured by the amount of dry weight consumed at fixed time intervals, was proportional to prey (*Culex molestus* larvae) size. Cohen and Tang (1997) also observed an increase in the amount of material consumed from larger prey, by two reduviids, but at the same time there was a disproportionately greater increase in handling time (decreased profitability). However, in this experiment, handling time not only included the time spent consuming prey but also the time taken by predators to pursue and subdue prey. Therefore, it is difficult to compare these results to those of the present study and to those of the Cook and Cockrell study. Cohen and Tang contend that the great expenditure of energy and materials associated with using large mobile prey necessitates spending extra time to extract appropriate nutrients and recover enzymes before abandoning such prey. Sequestration of prey contents by these means is time intensive and the recovery of enzymes is imperative. As such, functional response kinetics involving this type of predator may be more strongly impacted by size, rather than density of prey (Cohen, 1998; Cohen and Tang 1997). However, *D. hesperus* has been shown to follow type II and type III functional responses to prey much smaller than themselves (Brommit, 2007). Further, Cohen (1998) contends that EOD-using predators are predisposed to a nutrient

acquisition strategy involving the consumption of small numbers of large prey, as opposed to, the consumption of large numbers of small prey. Accordingly, *D. hesperus* became satiated on whitefly pupae faster than on mite prey, which are less than half the size (Brommit, 2007). Cohen and Tang (1997) coined the term “major investors” to describe such predators, because of the expensive investments in digestive enzymes (and venoms, where applicable), and increased injury risk in using large prey. The consumption of fewer, large prey translates to less time spent searching for, pursuing and subduing prey.

Irrespective of instar, the amount of food material removed from prey by *D. hesperus* was greater, the longer the feeding bout. The amount consumed per unit time, however, was inversely proportional to feeding duration, suggesting that the rate of food consumption declined toward the end of the feeding bout. Similarly, the rate of feeding by the waterboatmen diminished over time for all prey size classes, as extraction became more difficult (Cook and Cockrell, 1978). However, individuals that were fed in multiple short bouts, each time with a fresh prey item, consistently obtained meals of a similar size. This suggested the effect was prey-related. Therefore, apparent diminishing returns in both the Cook & Cockrell (1978) and current study could be explained, as above, by reduced digestibility of or access to ingestibles. However, it is also conceivable that for a non-refluxer (see Cohen, 1995) feeding on sufficiently large prey, or for a certain duration, the availability of digestive enzymes becomes rate-limiting. Cook and Cockrell (1978) used prey that were considerably smaller than the predators. Therefore the non-detection of a predator-related effect in the waterboatman experiment could have been an

artefact of small prey size or short overall feeding duration by control individuals (< 36min).

Although predators in this study appeared to respond to the tradeoff between defenses and energetic value in the direction predicted by optimal diet theory, it is likely too simplistic a theory to fully describe the motivations behind diet choice. Specifically, the theory ignores the need of organisms to balance their intake of macronutrients (see Raubenheimer & Simpson, 1999). The most blatant examples of this need are seen in true omnivores and in some herbivores where there are often drastic differences in the chemical makeup of different foods (Raubenheimer & Simpson, 1997; 2003). However, macronutrient levels of insects can also be highly variable, despite the oft-held view that “meat is meat” and insect prey vary only by energetic content (Fagan *et al.*, 2002; Mayntz *et al.*, 2005). Mayntz *et al.* (2005) demonstrated that invertebrate predators can forage selectively, choosing arthropod prey based on protein and lipid content, according to specific needs. It is not enough, though, for an organism to simply achieve certain nutritional thresholds; it is imperative to establish a balance of nutrients (geometrical approach to nutrition, Raubenheimer *et al.*, 1999). In fact, an overabundance of a given macronutrient can have fitness consequences (Raubenheimer *et al.*, 2005; Raubenheimer and Simpson, 1997). For example, Raubenheimer *et al.* (2005) demonstrated that excess carbohydrate consumption by *Spodoptera littoralis* caterpillars caused increased mortality. Therefore, when confined to foods with a surplus of accessible carbohydrate relative to protein, some insects regulate nutrient intake so as to obtain a moderate excess of carbohydrates and a deficit of protein/essential amino acids (Raubenheimer & Simpson, 1997).

EOD-users have been observed to leave a considerable amount of uningested prey biomass after a feeding bout, and this does not always appear to be related to gut capacity (Cohen, 1989; 1995; Cohen & Tang, 1997). There is much debate over the explanation for abandoning prey prematurely (see Cohen, 1995; 1998; and Cook & Cockrell, 1978). A common explanation is that the rate of energetic return during a feeding bout diminishes over time, and at some point (when the average rate of energy harvest is reached, for example), the cost of allocating time to this activity to the exclusion of others is prohibitive (Cook & Cockrell, 1978). However, perhaps this partial consumption of prey by *D. hesperus* and other EOD-users serves as a means to regulate their intake of nutrients. This idea stems from the finding by Cohen (1995) that hemipterans extract certain nutrients at different times within a feeding bout; proteins and glycogen are generally removed early and lipids later. Therefore, a meal, obtained by filling the gut on prey too large to be wholly consumed, or by terminating a feeding event short of satiation, would be relatively higher in the proportion of protein and carbohydrates to fat, than that obtained by entirely consuming smaller prey.

Protein consumption during the adult stage is essential for egg production by most dipterans (see Wheeler, 1996 and references therein) and fecundity is greatly impacted by the availability of particular amino acids in the adult diet of many other insects (e.g. Chang, 2004; Ferkovich & Shapiro, 2005; Mevi-Schutz & Erhardt, 2005). Certain prey-derived nutrients are also required in the adult diet of *D. hesperus* females for egg maturation (Sanchez *et al.*, 2004). In a study by Sanchez *et al.* (2004), adult *D. hesperus* females fed on a plant diet alone showed greatly reduced fecundity (no eggs were laid on most plants) as compared to those fed both plants and prey. Female longevity was also

shorter without prey on most plants. However, females lived three times longer on mullein (*Verbascum thapsis*, Scrophulariaceae) without prey than on mullein supplemented with *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs. Therefore, certain plants are at least suitable sources of energy, but they do not contain sufficient resources to facilitate oogenesis. As the non-reproductive tissues of plants are inherently low in protein, it is likely that this is one of the components that is lacking. In support of this notion, egg production by another heteropteran, *Orius insidiosus* (Say), was significantly greater when fed artificial diet supplemented with a protein fraction isolated from *Ephestia kuehniella* eggs than when fed any one of a variety of artificial diets alone (Ferkovich & Shapiro, 2005). Therefore, if larger prey (by virtue of the order of digestion) represent a meal that is more protein rich, they may more closely match the nutritional requirements of a female *D. hesperus* maturing eggs. This added benefit might help to explain why in this study, females pursued defended fourth instars to a greater extent than firsts, despite their apparent inability to capture them. To obtain the same composition of nutrients from first instar pea aphids as that which would be acquired from a single meal (to satiation) from a fourth instar, would require several meals (smaller than what could potentially be consumed) from an equal number of aphids.

The lack of success by *D. hesperus* in capturing defended larger prey is in contrast to the ability of the reduviids studied by Cohen & Tang (1997); handling prey four times their size. Predacious reduviids typically secrete strong zootoxic venoms in their saliva that help to immobilize prey (Edwards, 1961; Haridass & Ananthkrishnan, 1981). Such venoms are commonly used by predators that consume prey larger than

themselves (Hespenheide, 1973; Sabelis, 1992). Conversely, no venoms have been reported in the few studies performed on saliva composition of mirids (Agusti & Cohen, 2000; Boyd, 2003; Boyd *et al.*, 2002; Cohen & Wheeler, 1998). Nor has hyaluronidase, a “spreading factor” for the venoms of many Arthropoda, been found in these studies. Hyaluronidase attacks the extracellular matrix, essentially macerating tissues and enabling the spread of venoms as well as other digestive enzymes (Cohen, 1995; 1998; Edwards, 1961; Miles, 1972). A lack of these substances in the salivary secretions of *D. hesperus* could help to explain the difference observed between the predators. *Geocoris punctipes* (Say) (Geocoridae) is also known to produce strong venoms that quickly debilitate prey, enabling it to consume prey without having to grasp them with its legs (Cohen, 1989). However, predation by *G. punctipes* on tobacco budworm (*Heliothis virescens*) larvae also appears to be limited by prey-size-related defenses (Chiravathanapong & Pitre, 1980; Lopez, *et al.*, 1976). Although size-limited, *G. punctipes*, in a later experiment (Cohen, 2000), still consumed tobacco budworm prey that were larger, on average, than themselves (by mass, ca. one-fourth larger than feral predators and ca. one third larger than domesticated predators).

A lack of previous experience with aphid prey may also have had an effect on success rate by *D. hesperus*. Being reared exclusively on small, sessile prey (*E. kuehniella* eggs), and hence being naïve to the defenses of large mobile prey, may have made them ill prepared to handle large defended pea aphids. Vanlaerhoven *et al.* (2006) also pointed to a possible effect of rearing on foraging behaviour by *D. hesperus*. Patch retention time was greater on patches with the rearing host (*E. kuehniella* eggs) than on patches with greenhouse whitefly pupae (*Trialeurodes vaporariorum* Westwood

(Homoptera: Aleyrodidae)) or adult two-spotted spider mites (*Tetryanchnus urticae* Koch (Acari: Tetranychidae)). Another heteropteran, *Orius majusculus* Reuter (Anthocoridae), was unsuccessful, during short trials, in preying upon fourth instar pea aphids, when reared exclusively on moth eggs. Conversely, those predators reared on fourth instar pea aphids as nymphs had a 55% success rate. This difference was attributed to dissimilar methods of attack: Whereas naïve predators tended to contact aphids more laterally, and were thus repelled by aphid kicking, aphid-reared individuals tended to elicit direct attacks toward the head or posterior abdomen of aphids (where prey defenses were inadequate). However, some previously naïve predators were successful in attacking aphids when given several days (Henaut *et al.*, 2000). Perhaps the same could have been expected for *D. hesperus*. On the other hand, the *in vitro* rearing on immobile prey of other heteropteran predators, does not appear to affect their ability to handle mobile prey. *Geocoris punctipes*, taken from a colony reared on artificial diet for over 6 years, showed no significant differences relative to feral counterparts in handling time, amount of prey extracted or consumption rate, when feeding on tobacco budworm larvae, or pea aphids (Cohen, 2000). As well, Hagler & Cohen (1991), using the same colony, demonstrated that *in vitro*-reared and field-derived populations of *G. punctipes* shared almost identical patterns of prey selection. In another study, individuals from a two-year-old colony of *Deraeocoris nebulosus* (Miridae) reared on *E. kuehniella* eggs were still effective in reducing populations of aphids (Boyd & Alverson, 2003).

As with other members of the predatory Heteroptera, *Dicyphus hesperus* demonstrated a predilection for prey larger than itself over smaller prey (a “major investor”), but there was a negative effect of defenses, proportional to prey size, on prey

selection. Further research is necessary to determine whether the predators' lack of success in subduing large, defended prey is an artefact of rearing, or whether these predators are in fact incapable of utilizing fourth instar pea aphids as prey. If the former is true, there might be negative implications of certain *in vitro* rearing schemes on the use of these or similar predators in pest management programs, involving the control of large mobile prey. As well, although predators obtained larger meals from the larger fourth instar prey, a quantitative analysis of the nutrients obtained from the respective prey classes would be useful in determining the true benefit to consuming larger prey, and in determining whether prey size selection plays a role in maintaining nutritional balance.

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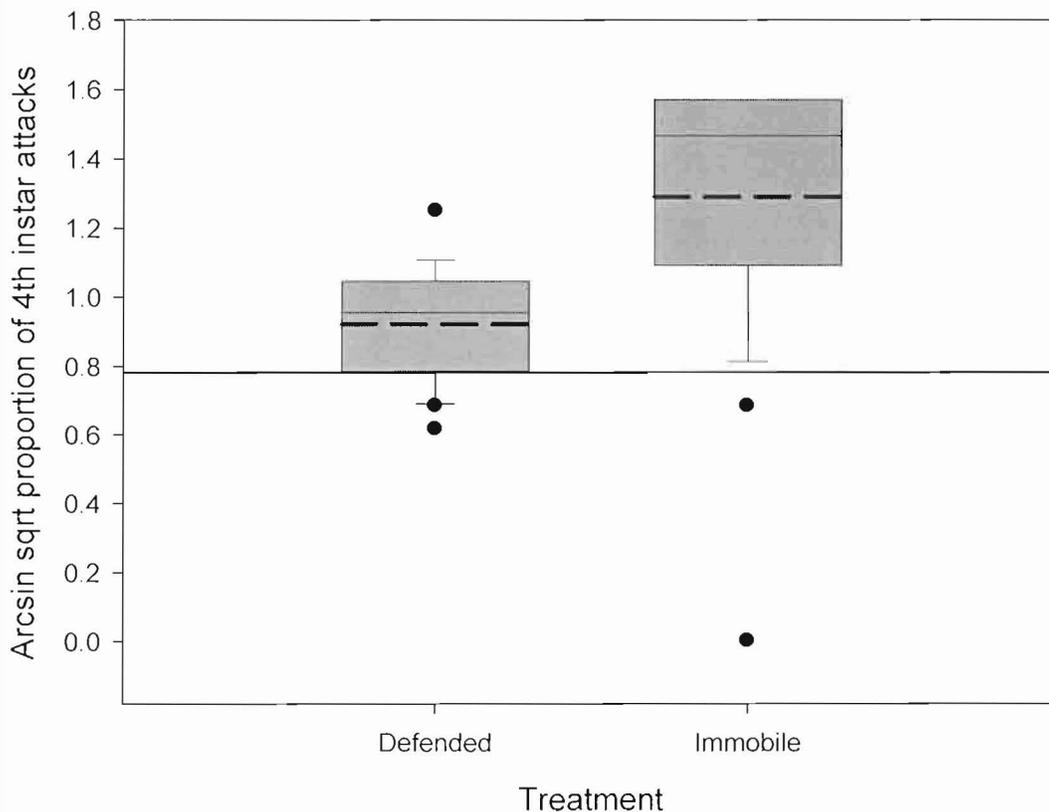
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Figure 4.1 Arcsine square root transformed proportions of fourth instar aphid attacks by *D. hesperus*. Observations were made over a 30 min observation period. For each treatment, dots signify outliers, whiskers mark the 10<sup>th</sup> and the 90<sup>th</sup> percentiles, top and bottom ends of boxes indicate the 25<sup>th</sup> and 75<sup>th</sup> percentiles, horizontal solid lines through the boxes denote the median and horizontal dashed lines the mean. The solid horizontal line through the centre of the graph denotes no preference (proportion of 0.5). Average arcsin sqrt proportions for both treatments were significantly greater than that of no preference (immobile:  $T_{23} = 6.85$ ,  $p < 0.001$ ; defended:  $T_{23} = 4.23$ ,  $p < 0.001$ ) and that for the immobile treatment was significantly greater than that for the defended treatment ( $T_{48} = 4.56$ ,  $p < 0.001$ ).



**Table 4.1 Multiple regression tables for the prediction of meal size obtained by *D. hesperus* from first (a) and fourth (b) instar pea aphids.**

a) First instars

	<i>B</i>	<i>SE B</i>	$\beta$	<i>p</i>
Step 1				
Constant	0.018	0.029		
Aphid mass	0.632	0.133	.712	< 0.001
Step 2				
Constant	- 0.100	0.028		
Aphid mass	0.534	0.125	.602	< 0.001
Feeding duration	0.001	< 0.001	.356	0.020

Note  $R^2 = .507$  for Step 1;  $\Delta R^2 = .115$  for step 2 ( $ps = 0.020$ ).

b) Fourth instars

	<i>B</i>	<i>SE B</i>	$\beta$	<i>p</i>
Step 1				
Constant	0.247	0.079		
Feeding duration	0.002	0.001	.699	< 0.001
Step 2				
Constant	- 0.100	0.126		
Feeding duration	0.002	0.001	.668	< 0.001
Aphid mass	0.065	0.045	.225	0.157

Note  $R^2 = .489$  for Step 1;  $\Delta R^2 = .050$  for step 2 ( $ps = 0.157$ ).

**Table 4.2 Multiple regression tables for the prediction of feeding rate on first (a) and fourth (b) instar pea aphids by *D. hesperus*.**

a) First instars

	<i>B</i>	<i>SE B</i>	$\beta$	<i>p</i>
Step 1				
Constant	0.006	0.001		
Feeding duration	- 4.3x10 <sup>-5</sup>	< 0.001	- .485	0.016
Step 2				
Constant	0.003	0.001		
Feeding duration	- 6.0x10 <sup>-5</sup>	< 0.001	- .671	< 0.001
Aphid mass	0.015	0.004	.603	0.001

Note  $R^2 = .235$  for Step 1;  $\Delta R^2 = .329$  for step 2 ( $ps = 0.001$ ).

b) Fourth instars

	<i>B</i>	<i>SE B</i>	$\beta$	<i>p</i>
Step 1				
Constant	0.006	0.001		
Feeding duration	- 1.3x10 <sup>-5</sup>	< 0.001	- .568	0.005
Step 2				
Constant	- 0.006	0.001		
Feeding duration	- 1.3x10 <sup>-5</sup>	< 0.001	- .567	< 0.006
Aphid mass	- 2.5x10 <sup>-5</sup>	< 0.001	- .013	0.944

Note  $R^2 = .323$  for Step 1;  $\Delta R^2 < .001$  for step 2 ( $ps = 0.944$ ).

## CHAPTER 5 CONCLUSION

### 5.1 What have we learned about *Dicyphus hesperus*?

Orientation to prey at close range by *Dicyphus hesperus* Knight is strongly directed by visual cues (at least under moderate light). Prey colour and shape do not appear to factor into foraging decisions, but prey size is important. Larger prey were more attractive to this predator, regardless of their defensive capabilities. Cohen and Tang (1997) suggest that many of the predatory Heteroptera are likewise 'major investors,' organisms that make expensive investments in digestive enzymes (and venoms, where applicable), and face increased risk of injury by using large prey. The obvious benefit to the predator is a reduction in the number of prey that predators need to pursue and subdue, which could result in the concomitant reduction of time spent in these activities overall, thereby maximizing their net rate of energy return. This tendency may have negative implications for its use in the control of smaller pests (e.g. spider mites), however, when relatively larger prey are available. On the other hand, such behaviour indicates the potential to use this predator against pests larger than itself, despite current practice to use it for controlling relatively smaller pests. However, when prey are chemically cryptic, size may have the opposite effect on prey choice by this predator, possibly due to an association of risk with the unknown, proportional to size. Similarly, closely grouped prey that are only visually perceptible were much less attractive than those spaced further apart; even though the prey they typically consume are often found in dense aggregations. As such, volatiles at close range must provide important

information for prey recognition. Further, contact with prey-related surface chemicals elicited a gustatory response by *D. hesperus* (extension of the proboscis), indicating the importance of contact chemoreception in prey recognition. However, this predator responded in this manner equally to multiple extracts of surface compounds from prey.

### **5.1.1 Potential Applications**

Food supplements are provided to natural enemies when naturally occurring foods are in limited supply. When prey are scarce or absent, providing *D. hesperus* with a food source that is superior to tomato fruit, should reduce fruit feeding. As mullein has been shown to support growth, reproduction and the longevity of these predators (Sanchez *et al.*, 2004), its presence in the greenhouse does, in fact, significantly reduce the number of feeding lesions on tomato fruit (Gillespie *et al.*, in prep.). Mullein may thus be a more profitable food source than tomato fruit. Currently, greenhouse growers in BC use mullein as banker plants (refugia for predators), which are provisioned with *Ephestia kuehniella* Zeller eggs to allow *D. hesperus* to persist when pest densities are low. However, the problem with this practice is these extra plants take up growing space and resources in the greenhouse, including water, fertilizer and labour, and they are susceptible to rots if overwatered (Dave Gillespie, pers. comm.) As well, the predators tend to remain on mullein plants if prey are added to them (thereby reducing the predators' activity on tomato), and *E. kuehniella* eggs are expensive (Gillespie, pers. comm.).

Artificial diets as food supplements may provide a cheaper and potentially better alternative to using mullein plants augmented with moth eggs. The major benefit of an artificial diet is that one can fine tune nutrient composition to elicit the desired effect.

For example, artificial diet, complemented with a protein fraction that was isolated from *Ephestia kuehniella* eggs, enhanced egg production by another heteropteran, *Orius insidiosus* Say (Ferkovich & Shapiro, 2005). In terms of *D. hesperus*, we know that females live longer, but produce fewer offspring on a diet of mullein alone than on a diet of mullein plus prey. Therefore, certain nutrients contribute differentially to the longevity and fecundity of this predator and manipulation of their ratios in food supplements could potentially help to control their population dynamics in the greenhouse.

Eliminate™ is a protein-rich commercial food spray supplement that was originally formulated for parasitoids of the southern pine beetle. These parasitoids are synovigenic, meaning that female wasps emerge with a limited number of nutrient-rich eggs, and to produce more eggs require a protein-rich diet (Quicke 1997). Preliminary research by Gillespie (pers. comm.) has shown promise for the use of Eliminate™ with *D. hesperus*. Direct application to tomato plants significantly reduced fruit feeding. As well, females were able to produce eggs without prey, and nymphs developed at a rate similar to that on mullein. However, the number of nymphs counted after several weeks was much lower than could be expected on a diet that includes prey. Therefore, some adjustments to the food spray formulation should be made if a large numerical response is required. In addition, over-supplementation may have a negative impact on predation of pests. Cage experiments demonstrated a reduction in prey feeding in the presence of Eliminate™ (Gillespie, pers. comm.). The major concern with Eliminate™, however, is that because it is a very sticky substance, if it were to get onto fruit it would impact the quality of the product, not unlike aphid honeydew (Dave Gillespie, pers. comm.).

The reliance on vision for prey location by *D. hesperus* and its responsiveness to spherical and irregularly-shaped artificial prey indicates how one might provision food supplements to enhance the efficacy of this predator and reduce fruit feeding. Food sprays, applied as a fine mist, to produce small prey-sized droplets, may maximize their efficacy. Microencapsulated artificial diets, which can produce comparable rearing yields to prey diets (e.g. Ferkovich *et al.*, 2007), could also be applied onto plants with a spray nozzle. To force the food capsules to fix onto plants, an adhesive could be added to the spray mixture. This technique is currently being used to apply microencapsulated pheromone in some mating disruption programs. Therefore, the necessary equipment is already available.

Work by Gillespie *et al.* (in prep.) suggests that fruit feeding may be a function of predator position on plants, as there does not appear to be a preference by *D. hesperus* for either leaf or fruit in the absence of prey and fruit feeding typically does not occur when prey are abundant. Whiteflies are typically found on the underside of leaves and predators may thus be retained there and plant feed in the same location. However, when prey are not available, predators may search for prey more extensively over plants, resulting in more encounters with tomato fruits. As a consequence, opportunistic herbivory on fruits could increase. In this vein, strategic application of food supplements may help to control predator location on plants, thereby reducing fruit feeding. Focusing applications on leaves may also help to keep predators in position to attack pests should they invade. As well, satiated *D. hesperus* are less likely to leave tomato plants, when disturbed, than starved individuals (VanLaerhoven *et al.*, in prep.).

## 5.2 Suggestions for future work

There are several unanswered questions that have arisen as a result of the current work. For example, although vision is important for prey location by *D. hesperus* in the day, it is uncertain whether the visual modality is used for the same purpose at night, when the majority of prey feeding occurs (VanLaerhoven *et al.*, 2003). It is possible that the location of prey patches in the daytime eliminates the need to see prey at night. Their typical herbivore prey generally form dense aggregations, which should make prey patches profitable for long durations. As such, predators could remain within patches or close by for easy access to prey at night. Conversely, the visual capacity of this predator may be such that prey can be located under low light, such as moonlight. Predatory heteropterans are known to employ networks of ommatidia in a way that dramatically increases light capture for a given point in space, while maintaining a decent level of resolution (Fischer *et al.*, 2000; Nilsson & Ro, 1994). Further, in a review on dim-light adapted vision, Warrant (2004) concluded that “nocturnal habitats are just as rich in visual details as diurnal habitats are, and nocturnal animals have evolved visual systems capable of exploiting them.” For example, at night, under very low light, *Megalopta genalis* (Hymenoptera: Halictidae) are able to locate nests (while flying) in small openings at the ends of hollow sticks, with eyes that have almost 30 times greater sensitivity to light than the eyes of diurnal worker honeybees (Warrant *et al.*, 2004). To test for the effect of light availability on the visual acuity of *D. hesperus*, prey foraging bioassays could be performed under variable light intensities. As well, a physiological examination of photoreceptor sensitivity to various intensities of light would be beneficial.

Another interesting direction for research would be to determine if quantitative differences exist between the nutrient compositions of meals from small versus large prey. Cohen (1995) found that there is an order to the digestion of macromolecules inside prey by extra-oral digesting heteropterans, where proteins and carbohydrates are digested first and lipids last. As such, prey larger than can be wholly consumed may provide more protein and carbohydrate rich meals than from otherwise identical, smaller prey. If meals are more protein rich, individuals may maximize fecundity by choosing larger prey, which could help to account for why *D. hesperus* females tended to do so in the case of pea aphids, despite not having any success in capturing and consuming them. To be adaptive, however, females must be able to handle larger prey and obtain a greater rate of return by choosing them. The lack of success on such aphids may be explained by inexperience with mobile prey. Thus an examination of the effect of rearing conditions on the ability of this predator to utilize different prey should be carried out. This may have implications for the efficacy of predators produced for biological control in mass-rearing systems. To answer the former question, a chemical analysis of meals obtained from large versus small prey would be necessary. An examination of the success on different sizes of mobile prey by *D. hesperus* reared on mobile prey would also help to sort out the fitness consequences associated with prey size.

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## **APPENDIX: DYNAMIC STATE VARIABLE MODEL**

I have constructed the framework for a dynamic state variable patch foraging model for *D. hesperus* in a tomato greenhouse, in the style of Clark and Mangel (2000). These theoretical models describe optimal behaviour for organisms given their current state. The objective of this model is to generate predictions about conditions under which tomato fruit feeding is the optimal decision for *Dicyphus hesperus*, based on an individual's internal state as well as the conditions in the patch itself.

Fitness,  $F$ , is a function of the number of eggs,  $e$ , produced by the end of an individual's life,  $T$ . Also included in my definition of fitness are several states: the number of eggs produced,  $e$ ; energy reserves,  $x_1$ ; additional nutrients for egg production (namely protein),  $x_2$ ; number of prey in patch,  $v$ ; hydration,  $h$ ; and current time,  $t$ . Thus fitness is described as  $F(e, x_1, x_2, v, h, t, T)$ .

Individuals in the model must maintain the internal states  $x_1$ ,  $x_2$  and  $h$  above critical levels or they die. There are direct costs incurred to each of these states at every time step. However these costs vary with the activity. Certain activities are also associated with state gains. Eggs are matured at a constant rate, as long as  $x_1$ ,  $x_2$  and  $h$  are above their respective threshold levels, and these states are decremented, accordingly, as  $e$  increases in value. Therefore, to maximize fitness accrued by the end of time, optimal decisions are those which maximize the probability of maintaining  $x_1$ ,  $x_2$  and  $h$  above this

reproductive threshold. This model assumes that reserves allocated to egg production cannot be reallocated to somatic condition.

The initial decision is whether to stay in the current patch. A patch is represented by a tomato plant with a certain number of whitefly prey (victims),  $v$ , ranging from zero to  $v_{max}$ . If an individual leaves the current patch in search of a new patch it expends energy (flying), over and above basal metabolic demands, thereby reducing  $x_1$ ,  $x_2$ , and  $h$  per event (i.e.  $x_1'$ ,  $x_2'$  and  $h'$ ). A departing individual will survive with a certain probability,  $s_1^d$ , which is related to the distance flown,  $d$ . Because prey are patchily distributed, plants adjacent to prey-infested plants have a greater probability of being infested than plants further away. Similarly, as compared to some baseline probability of infestation, plants adjacent to uninfested plants have a greater probability of being uninfested than those further away. Therefore, in this model, individuals encounter a patch with a given number of prey,  $v_i$ , with the probability  $\lambda_i$ , as a function  $d$ , and  $v$  at the previous patch. The subscript,  $i$ , indicates the class for a given number of prey. The 'Max' term is for the determination of the dispersal distance that maximizes expected future fitness.

\* In the following dynamic equations, for this and/or other decisions,  $\alpha$  is metabolic cost,  $\beta$  is nutrient gain,  $\gamma$  is hydration cost,  $\eta$  is hydration gain. Subscripts, other than  $i$ , denote the activity.

**Leave**

$$\text{Max } [s_1^d \left( \sum_{i=0}^{V_{max}} \lambda_i(d) * F(e, x_1', x_2', v_i, h', t + t_i, T) \right)];$$

$$x_1' = x_1 - \alpha_{1s} * d$$

$$x_2' = x_2 - \alpha_{2s} * d$$

$$h' = h - \gamma_s * d$$

$$t_1 = d * t_{constant}$$

To determine whether an individual leaves or not, the expected future fitness from leaving is compared to that of the best option if staying. If an individual is to stay, it has the option of whether to rest, plant feed, search for prey locally (intensive search) or engage in a more extensive search for prey, over the plant.

Resting provides the organism with the lowest reduction in  $x_1$ ,  $x_2$ , and  $h$  per time step and the greatest chance of survival  $s_r$ . However, making a more risky decision may have a bigger payoff.

***Rest***

$$s_r F(e, x_1'', x_2'', v, h'', t + 1, T);$$

$$x_1'' = x_1 - \alpha_{1rest}$$

$$x_2'' = x_2 - \alpha_{2rest}$$

$$h'' = h - \gamma_{rest}$$

Plant feeding leads to an overall increase in  $h$  and has an associated survival probability,  $s_{pf}$ . This activity is the only means by which an individual can increase  $h$ . The overall level of  $x_1$  and  $x_2$  decrease as a result of this activity because we know that *D. hesperus* cannot reproduce on a diet of tomato leaves alone, and without any other items in the diet, individuals have a short life span (Sanchez *et al.*, 2004). However,  $x_1$  decreases less as some energy is procured in the process.

***Plant Feed***

$$s_{pf} F(e, x_1''', x_2''', v, h''', t + 1, T);$$

$$x_1''' = x_1 - \alpha_{1pfeed} + \beta_{1pfeed}$$

$$x_2''' = x_2 - \alpha_{2pfeed} + \beta_{2pfeed}$$

$$h''' = h - \gamma_{pfeed} + \eta_{pfeed}$$

Because prey feeding by *D. hesperus* depletes water stores,  $h$  decreases accordingly and thus  $h$  is required to be above a certain level to prey feed. Therefore, if  $h$  falls below a certain level, the exclusion variable  $H(h)$  is assigned a value of 0, preventing a search response for prey; it is otherwise 1. If an individual searches intensively for prey, it will spend time,  $t_{is}$ , survive with a probability of,  $s_{is}$ , and encounter prey at a rate,  $\kappa_{is}(v)$ . The probability prey are not found is thus  $1 - \kappa_{is}(v)$ . As these probabilities are a function of prey number,  $v$ , the encounter rate decreases as prey are consumed. This is a key component of marginal value theorem (Charnov, 1976). All individuals accept prey when encountered. Prey feeding provides the greatest benefit to  $x_1$  and  $x_2$ , but it is the most risky activity, which is reflected in the associated survivorship term,  $s_v$ .

### ***Intensive Prey Search***

$s_{is}[$

#### ***Don't Find Prey***

$$(1 - \kappa_{is}(v)) * F(e, x_1''''', x_2''''', v, h''''', t + t_{is}, T) +$$

#### ***Find Prey***

$$H(h) * \kappa_{is}(v) * s_v * F(e, x_1''''', x_2''''', v', h''''', t + t_{is} + t_{vf}, T);$$

$$x_1'''''' = x_1 - \alpha_{1is}$$

$$x_2'''''' = x_2 - \alpha_{2is}$$

$$h'''''' = h - \gamma_{is}$$

$$x_1'''''''' = x_1 - \alpha_{1is} - \alpha_{1vfeed} + \beta_{1vfeed}$$

$$x_2'''''''' = x_2 - \alpha_{2is} - \alpha_{2vfeed} + \beta_{2vfeed}$$

$$h'''''''' = h - \gamma_{is} - \gamma_{vfeed}$$

$$v' = v - 1$$

Work by Gillespie *et al.* (manuscript) suggests that fruit feeding may be a function of predator position on plants, as there does not appear to be a preference by *D.*

*hesperus* for either leaf or fruit in the absence of prey and fruit feeding typically does not occur when prey are abundant. Predators may thus be retained in prey patches (typically on the underside of leaves) and plant feed in the same location. When prey are scarce, predators may search for prey more extensively over plants, resulting in more encounters with tomato fruits. As a consequence, opportunistic herbivory on fruits could increase. The ‘extensive search’ decision represents an attempt to capture this behaviour.

The extensive search decision is accompanied by a very similar set of outcomes as intensive search. The major differences are a greater amount of time spent searching,  $t_{es}$ , a reduced probability of survival,  $s_{es}$ , and the probability of ending up on tomato fruit,  $\tau$ , after searching. Because more time and energy are expended in this type of search, there are greater costs associated with  $x_1$ ,  $x_2$ , and  $h$ . As well, because of the increase in area searched, the probability of finding prey,  $\kappa_{es}(v)$ , is greater than  $\kappa_{is}(v)$ . The probability of finding prey and tomato fruit at the end of a search bout are mutually exclusive, as prey do not colonize tomato fruits. If a fruit is found, individuals must decide whether to feed on fruit or not. The decision is again based on maximizing potential future fitness gains.

***Extensive Prey Search***

$s_{es}[$

***Don't Find Prey or Tomato Fruit***

$$(1 - \kappa_{es}(v) - \tau_t) * F(e, x_1^{''''''}, x_2^{''''''}, v, h^{''''''}, t + t_{es}, T) +$$

***Find Prey***

$$H(h) * \lambda_{es}(v) * s_v * F(e, x_1^{''''''}, x_2^{''''''}, v', h^{''''''}, t + t_{es} + 1, T) +$$

**Find Tomato Fruit**

Max [

**Don't Eat Fruit**

$$\tau_t * F(e, x_1^{t+1}, x_2^{t+1}, v, h^{t+1}, t + t_{es}, T);$$

**Eat Fruit**

$$\tau_t * s_v * F(e, x_1^{t+1}, x_2^{t+1}, v, h^{t+1}, t + t_{es} + 1, T);$$

]

$$x_1^{t+1} = x_1 - \alpha_{1es}$$

$$x_2^{t+1} = x_2 - \alpha_{2es}$$

$$h^{t+1} = h - \gamma_{es}$$

$$x_1^{t+1} = x_1 - \alpha_{1es} - \alpha_{1vfeed} + \beta_{1vfeed}$$

$$x_2^{t+1} = x_2 - \alpha_{2es} - \alpha_{2vfeed} + \beta_{2vfeed}$$

$$h^{t+1} = h - \gamma_{es} - \gamma_{vfeed}$$

$$v^t = v - 1$$

$$x_1^{t+1} = x_1 - \alpha_{1es}$$

$$x_2^{t+1} = x_2 - \alpha_{2es}$$

$$h^{t+1} = h - \gamma_{es}$$

$$x_1^{t+1} = x_1 - \alpha_{1es} - \alpha_{1vfeed} + \beta_{1tfeed}$$

$$x_2^{t+1} = x_2 - \alpha_{2es} - \alpha_{2vfeed} + \beta_{2tfeed}$$

$$h^{t+1} = h - \gamma_{es} - \gamma_{tfeed} + \eta_{tfeed}$$

The model framework, in full, is as follows:

$$F(e, x_1, x_2, v, h, t, T) =$$

Max [

*Leave*

$$\text{Max } [s_l^d * (\sum_{i=0}^{K_{\max}^d} \lambda_i(d) * F(e, x_1^i, x_2^i, v_i, h^i, t + t_{ps}, T)];$$

*Stay*

Max [

*Rest*

$$s_r F(e, x_1^{''}, x_2^{''}, v, h^{''}, t + 1, T);$$

*Plant Feed*

$$s_{pf} F(e, x_1^{'''}, x_2^{'''}, v, h^{'''}, t + 1, T);$$

*Intensive Prey Search*

$s_{is}[$

*Don't Find Prey*

$$(1 - \kappa_{is}(v)) * F(e, x_1^{''''}, x_2^{''''}, v, h^{''''}, t + t_{is}, T) +$$

*Find Prey*

$$H(h) * \kappa_{is}(v) * s_v * F(e, x_1^{'''''}, x_2^{'''''}, v', h^{'''''}, t + t_{is} + 1, T);$$

*Extensive Prey Search*

$s_{es}[$

*Don't Find Prey or Tomato Fruit*

$$(1 - \kappa_{es}(v) - \tau_t) * F(e, x_1^{''''''}, x_2^{''''''}, v, h^{''''''}, t + t_{es}, T) +$$

*Find Prey*

$$H(h) * \kappa_{es}(v) * s_v * F(e, x_1^{'''''''}, x_2^{'''''''}, v', h^{'''''''}, t + t_{es} + 1, T) +$$

*Find Tomato Fruit*

Max [

*Don't Eat Fruit*

$$\tau * F(e, x_1^{''''''''}, x_2^{''''''''}, v, h^{''''''''}, t + t_{es}, T);$$

*Eat Fruit*

$$\tau * s_v * F(e, x_1^{'''''''''}, x_2^{'''''''''}, v, h^{'''''''''}, t + t_{es} + 1, T);$$

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