2015

An experimental analysis of the effects of plant and prey quality on the life history and behaviour of Dicyphus hesperus Knight (Hemiptera: Miridae), an omnivorous biological control agent

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An experimental analysis of the effects of plant and prey quality on the life history and behaviour of *Dicyphus hesperus* Knight (Hemiptera: Miridae), an omnivorous biological control agent

By

Meghan Ann Vankosky

A Dissertation
Submitted to the Faculty of Graduate Studies
through the Department of Biological Sciences
in Partial Fulfillment of the Requirements for
the Degree of Doctor of Philosophy
at the University of Windsor

Windsor, Ontario, Canada

2015

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Declaration of Co-Authorship / Previous Publication

I. Co-Authorship Declaration

I hereby declare that this thesis incorporates material that is result of joint research, as follows: The general introduction (Chapter 1) and general conclusion (Chapter 7) were authored solely by me. The data chapters (Chapters 2 through 6) are co-authored by Dr. Sherah VanLaerhoven and myself. In all cases (Chapters 2 to 6) I developed the key ideas and experimental designs, and I analyzed and interpreted the data and prepared the manuscripts. Dr. Sherah VanLaerhoven contributed to the experimental design, manuscript editing, and provided funding and logistical support for the data collected and described in all five chapters.

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Thesis Chapter	Publication title/full citation	Publication status*
Chapter 5	Are omnivores picky eaters? The prey preference of *Dicyphus hesperus,* an omnivorous biological control agent of greenhouse whitefly (*Trialeurodes vaporariorum*).	In review by *Biological Control*
Chapter 6	Plant and prey quality interact to influence the foraging behaviour of an omnivorous insect, *Dicyphus hesperus*	In revision for *Animal Behaviour*

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Abstract

Dicyphus hesperus Knight (Hemiptera: Miridae) is an omnivorous natural enemy of greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) that can be used in biological control programs. Omnivorous natural enemies consume both plant and prey food and offer certain advantages and disadvantages to biological control; therefore, understanding these species prior to their use is important. Much is currently known about *D. hesperus*. However, variation in the quality of plant and/or prey resources for consumers is common in agroecosystems. The impacts of varying within-species resource quality on *D. hesperus* have not been investigated. The objective of my dissertation was to investigate the effect of varying plant and prey quality on the life history and behaviour of *D. hesperus*. To approach this question, I used nitrogen (N) fertilizer to manipulate the quality of tomato plants, *Solanum lycopersicum* L. (Solanales: Solanaceae). Prey reared on high and low N tomato plants were offered in feeding trials to represent natural variation in prey quality. I observed how these factors, independently or simultaneously, affected oviposition preference (Chapter 2); development and survival of *D. hesperus* nymphs (Chapter 3); olfactory response (Chapter 4); prey preference and consumption rate (Chapter 5); and the activity budget (Chapter 6) of *D. hesperus*. Based on optimal oviposition theory, optimal foraging theory, and the plant vigor and plant stress hypotheses, I predicted that high N tomato plants, and whitefly prey reared on those plants, would be most preferred. I also predicted that the behaviour of *D. hesperus* would vary in response to the quality of the resources available. As expected, both factors influenced the life history and behaviour of *D. hesperus*. For example, high N tomatoes were preferred for oviposition and prey reared on high N plants were preferred for consumption. Foraging behaviour of *D. hesperus* adults also varied in response to varying levels of plant N when prey from high and low N tomatoes were provided. My results can be used to inform the development of biological control programs using omnivores, and *D. hesperus* in particular. My research highlights the importance of considering within-species variation in quality when making pest management decisions.

Dedication

To my parents, Gary and Marion,

My siblings, Mallary and Gavin,

and

Grannie Annie.

Thank you for everything.

In Loving Memory of

Macey Vankosky (August 31, 1992 – January 12, 2011)

and

Dr. Lloyd Dosdall (July 21, 1952 – June 12 2014).

Acknowledgements

I have a rather long list of people to acknowledge. Without these people, my thesis might not have happened and I might not be the same person I am today.

I am grateful to Dr. Sherah VanLaerhoven for the opportunity to study entomology as a member of her lab at the University of Windsor, and for her unwavering support, encouragement, and mentorship. It has been an honour to work with Sherah. In particular, Sherah gave me the freedom to approach this project as I have, and her faith in my abilities has meant more than I can say.

For their guidance, support, and patience, I thank my committee members, Dr. Ken Drouillard, Dr. Dennis Higgs, and Dr. Oliver Love. I would also like to thank Dr. Lynda Corkum for her contribution to my thesis committee prior to her retirement and for her friendship. I also extend my gratitude to Dr. Bill Snyder for agreeing to serve as my external examiner and for travelling here from Washington State University to do so.

I thank the faculty and staff in the Department of Biological Sciences for their various contributions to my degree. Everyone here is friendly, approachable, and helpful. I am thankful for their advice, assistance, conversations, and friendship. Without them I would have been in big trouble.

I am extremely grateful to my lab-mates, Vincenzo Pacheco and Krystal Hans. The three of us have spent nearly every day together since 2011. They listened to me vent about anything and everything and took care of the *Dicyphus* colony when I was away, and trusted me with the blow fly colonies when they were away. Without them, this experience would not have been nearly so much fun, or educational, or quite so entertaining. I'd also like to thank Lida Nguyen-Dang for being the best undergrad thesis student in the history of undergrad thesis students.

I acknowledge all of the agencies that supported my research, including NSERC, the Ontario Graduate Scholarship, the Government of Ontario Early Researcher Award, the Canada Foundation for Innovation, the Ontario Innovation Trust, and the University of Windsor. In addition, I would like to thank all of the letter writers who nominated me for Departmental Awards, including the Dr. Joseph E.J. Habowsky Graduate Teaching Award and the Graduate Excellence Award.

I need to take several lines to thank many very important people who I think of as my "Windsor Family." First, I am grateful beyond words for the friendship of Holly Hennin and her family, Todd, Lynn, Angela and Ben, who welcomed me into their home for the holidays and on other special and not-so-special occasions. No one should spend the holidays alone, so at those times when I was not able to travel home to Alberta, it was heartwarming to know that I had a place to go and a family to celebrate with.

Second, my friendships with Christine Madliger and Chris Harris have saved my sanity in the past several months. Here's to drinking rum and discussing the joys of grad school at Ruthven, to our shopping trips (sans Chris, because that would be asking way too much), to dinners, to walks with Dar and Timber, and to everything else we have shared. Thank you so much.

The third, and largest part of my "Windsor Family" includes all of the denizens of Partington Palace, past and present. At the risk of leaving anyone out, and because the list is huge, I won't try to name names. Thank you for all of the incredible parties and other good times we shared. It meant a lot to me the first time I was invited over for a sangria night, and every time since. It has been a pleasure to share in your triumphs and successes, to celebrate your marriages, and the births of your babies. I'm glad that I could be there for you when times were tough, and I'm glad that you were all there for me during the rougher patches as well. I have made many great friends during my time here. When I left Edmonton, I never expected to find myself part of such a tight-knit, supportive, and welcoming group again so soon, but you proved me wrong and I am thankful for that.

There are no words to express my thanks to my family for their support during my seemingly never-ending educational journey. It has been hard to be so far away from home for so long, but I have always known that my parents, Gary and Marion; my siblings, Gavin, Macey, and Mallary; my Grannie Annie; and everyone else had my back. For their love, strength, and understanding I will be forever thankful. To my Dad, thank you for your sense of humor about my education, and for always answering with "30" whenever you were asked what I'd be when I was finished my degree.

Taking on a PhD is a significant time commitment. Since I started in September 2010, a lot has changed, and I have changed as well. Sometimes the events that shape us

are terrible – the deaths of my sister and my MSc co-supervisor come to mind – while others are positive – friendships, marriages, and births. I would like to finish by thanking everyone who has had an impact on my life, good or bad, happy or sad, since September 2010. Thanks to you, I am where I am today – I am the person that I am today. For that I am grateful.

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Biological control: History and definitions

The first recognized and documented example of modern biological control was pioneered by Charles Valentine Riley and Albert Koebele, who identified and introduced the vedalia beetle, *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae), to citrus orchards on the west coast of the United States to control the cottony cushion scale, *Icerya purchase* Maskell (Hemiptera: Monophlebidae) (Caltagrione and Doutt 1989). This early success story paved the way for similar pest management programs until the discovery that certain chemical products developed during World War I and World War II could be applied to crop plants to kill insect pests with great efficacy (Casida and Quistad 1998; Perdikis *et al.* 2008). The popularity of biological control has only begun to recover to pre-War levels in the past several decades, as widespread insecticide resistance and other negative consequences of insecticide use have been identified (Casida and Quistad 1998; Kogan 1998; Perdikis *et al.* 2008). Biological control provides an important alternative to chemical insecticides; it has certain characteristics that are appealing to producers and consumers (Casida and Quistad 1998; Buitenhuis *et al.* 2014).

Biological control takes advantage of naturally occurring predator-prey or parasitoid-host relationships to manage populations of herbivores that reach pest status in agroecosytems (DeBach 1964; Bale *et al.* 2008). Predators and parasitoids are natural enemies of herbivores (DeBach 1964). Pathogens, such as fungi or bacteria can also be classified as natural enemies (Lacey *et al.* 2001). Biological control tactics can be classified as one of several types depending on the natural enemy used, the method of application, and whether or not the natural enemy is endemic or introduced to the habitat where biological control is used.

Classical biological control (also referred to as introduction biological control; Hopper 2003) utilizes introduced natural enemies from the region of origin of the pest (Caltagrione 1981; Eilenberg *et al.* 2001; Hopper 2003). These programs aim to establish long-term management of the pest by introducing a natural enemy that is able to persist in the pest's new habitat (Caltagrione 1981; Eilenberg *et al.* 2001). The vedalia beetle, for

example, was released into citrus orchards in 1888 and 1889, and the beetle continues to suppress cottony-cushion scale 100 years later (Caltagrione and Doutt 1989). Classical biological control programs have also been used to control weeds. For example, *Galerucella calmariensis* (L.) (Coleoptera: Chrysomelidae) is an extremely successful biological control agent of purple loosestrife, *Lythrum salicaria* L. (Myrtales: Lythraceae), an invasive weed of wetlands in the United States and Canada (Blossey *et al.* 2001).

Augmentation biological control is characterized by the release of natural enemies of the target pest that are reared or collected *en masse* in order to augment naturally occurring populations (Hopper 2003; van Lenteren and Bueno 2003). This type of biological control can be subdivided into inoculation and inundation biological control (van Lenteren and Bueno 2003). Inundation biological control aims for immediate pest suppression but lasts for a limited time due to the life history of the natural enemy, the population dynamics of the pest, and/or climatic factors that prevent the natural enemy from persisting in the environment (Eilenberg *et al.* 2001; van Lenteren and Bueno 2003). Inoculation biological control can result in season-long pest suppression, as well as immediate pest suppression (Eilenberg *et al.* 2001; van Lenteren and Bueno 2003). One example of an important natural enemy used in augmentation biological control programs is *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae), a parasitoid that is released into greenhouses to control *Trialeurodes* sp. Cockerell (Hemiptera: Aleyrodidae) and *Bemisia* sp. Altus Lacy Quaintance & Baker (Hemiptera: Aleyrodidae) of whitefly (van Lenteren *et al.* 1996). Another example is ladybeetles (Coleoptera: Coccinellidae) that are used to control aphids in greenhouses (Obrycki and Kring 1998). Entomopathogens can also be used in inoculation biological control programs (Lacey *et al.* 2001).

Finally, conservation biological control focuses on increasing populations of natural enemies and improving their efficacy against herbivorous pests using conservation strategies in agroecosystems (Landis *et al.* 2000; Eilenberg *et al.* 2001). Beetle-banks, for example, provide an undisturbed habitat within the boundary of the agroecosystem where important predaceous beetles (Coleoptera) and other natural enemies of insect pests can take refuge from agricultural practices and find alternative sources of food when pest densities are low (Landis *et al.* 2000). Other important

conservation practices include the provision of shelterbelts or corridors into the interior of the agroecosystem and the use of conservation tillage to avoid killing natural enemies that overwinter in the soil (Dent 1995; Van Driesche and Bellows 1996; Eilenberg *et al.* 2001). Landis *et al.* (2000) provide a thorough review of habitat manipulation methods that can be utilized as part of a conservation biological control program.

What makes a good biological control agent?

A number of authors (Smith 1935; Hagen *et al.* 1976; van Lenteren and Woets 1988; Bale *et al.* 2008) have compiled lists of characteristics required for natural enemies to be effective biological control agents. These characteristics pertain to the biology and ecology of the natural enemy and include: i) ability to disperse to locations of pest infestation; ii) rates of population increase that are on par or exceed the rate of population increase of the pest; iii) ability to identify and locate areas of high pest density; iv) a narrow range of prey or host targets; v) the ability to survive during prey or host scarcity; and vi) multivoltinism or increased longevity of the growth stage that has the greatest impact on the pest population (Smith 1935; Hagen *et al.* 1976; van Lenteren and Woets 1988; Bale *et al.* 2008). Natural enemies that are highly host-specific allow agroecosystem managers to avoid negative impacts of biological control programs on non-target species (Bale *et al.* 2008). There are some additional characteristics that host-specific natural enemies need to possess: their lifecycles should be highly synchronized with their host and in the case of parasitoids, they should have a method of marking or identifying already parasitized hosts (Bale *et al.* 2008).

Highly host-specific natural enemies have been historically preferred for use in biological control programs (Symondson *et al.* 2002). Therefore, the vast majority of well-documented and oft-released natural enemies have been parasitoids (Symondson *et al.* 2002), with a few important exceptions, such as *R. cardinalis*. Highly host-specific natural enemies tend to be most effective in perennial agroecosystems, such as orchards (Symondson *et al.* 2002). For this, and other reasons, less of our research effort has been placed on developing biological control programs in ephemeral or annual crop systems (Symondson *et al.* 2002), although there are some notable successes (see Wiedenmann and Smith 1997 for examples). In ephemeral agroecosystems, the characteristics that

define an effective biological control agent are slightly different. Host synchrony and high rates of reproduction are less important in ephemeral agroecosystems, while persistence remains important (Wiedenmann and Smith 1997). Polyphagy by natural enemies is also important because both the plant and arthropod community structure change seasonally (Wiedenmann and Smith 1997). In annual agroecosystems, omnivory is probably an advantageous characteristic, as by definition, omnivores can utilize both plant and arthropod food resources to survive when their preferred prey is absent (Coll and Guershon 2002). Therefore, in annual cropping systems, generalist predators that have a diet comprised of multiple species, or multiple taxa might be more effective than specialists, at least in some cases. Contrary to long-held opinion, generalist predators, either alone or as part of a community of predators, are actually quite effective biological control agents; for example, in a review of pertinent literature, Symondson *et al.* (2002) found that more than 75% of biological control programs using generalist predators were successful.

Regardless of whether specialist or generalist natural enemies are most suitable for a specific biological control program, it is essential to have a thorough understanding of both the natural enemy and the target pest prior to initiating biological control programs. Determining if natural enemies possess the necessary characteristics to be successful, and under which conditions those characteristics are most advantageous, should be the focus of our research efforts prior to natural enemy release. Bale *et al*. (2008) acknowledge that thorough investigation of natural enemies prior to their release in a biological control program is time consuming, but the amount of time needed to gain enough knowledge to develop an efficient and effective biological control program is generally on par with the time needed to develop and test novel insecticide products. Without careful study, biological control programs can fail to reduce pest populations or can have serious unforeseen ecological or environmental consequences (Bale *et al.* 2008).

Ecological theory and biological control

Smith (1935) clearly stated that the factors that would contribute most to the success of biological control were biotic factors, especially those that impact the population of the natural enemy. Although some abiotic factors are undoubtedly

important, such as climatic factors that might influence the establishment of natural enemies (Bale *et al.* 2008), most of the characteristics of an effective natural enemy are directly or indirectly related to population growth. In the case of generalist predators, the factors that influence predation efficiency should also be considered. Ecological theories relevant to population growth and reproduction have been studied extensively, providing a broad knowledge base that can be used as the foundation for studying the life history and behaviour of natural enemies of important agricultural pests. These theories should also be useful in developing predictive models for biological control programs and perhaps provide insight as to why biological control programs have failed in the past.

Optimal Foraging Theory

Optimal foraging theory predicts the foraging decisions made by optimal predators in fine-grained habitats where the predator has complete knowledge of the prey available (Emlen 1966; Krebs 1977; Pyke *et al.* 1977; Stephens and Krebs 1986). Following optimal foraging theory, the decisions of an optimal predator should maximize energy intake while minimizing energy expenditure (Emlen 1966; MacArthur and Pianka 1966; Stephens and Krebs 1986). In general, optimal foraging theory predicts that prey items that provide the greatest amount of energy with the least amount of energy expenditure should be selected first and that no other types of prey should be included in the diet so long as those prey are available (Emlen 1966; MacArthur and Pianka 1966; Krebs 1977). Following this logic, so long as the costs associated with searching for and handling prey items are equal, the quality of a prey item can be defined based upon the nutritional value or energy gained from its consumption, such that high quality prey provide greater benefits. Using natural or artificial foraging arenas, a variety of prey species, of varying quality, can be offered to predators to determine prey preference. For example, shore crabs, *Carcinus maenas* (L.) (Decapoda: Portunidae) provided with prey *ad libitum* selected prey of the optimal size to minimize energy expenditure and maximize energy intake (Elner and Hughes 1978). An optimal predator should have a diet that consists of only one prey type, unless that type of prey becomes exhausted, the foraging costs associated with locating or handling that prey type increase, or prey of greater nutritional value are encountered (Emlen 1966; MacArthur and Pianka 1966; Krebs 1977; Stephens

and Krebs 1986). In biological control research, optimal foraging theory can be used to determine the diet breadth of different species of natural enemy. The diet breadth of a natural enemy can be important in evaluating its fit as a biological control agent in different types of agroecosystems, against different species of pests. In short, information regarding the prey preferences of natural enemies is important when designing biological control programs.

Optimal foraging theory can also be used to predict the patch residence time of a predator or parasitoid. According to Emlen (1966) a prey patch should be abandoned when the quality of the patch has declined to be equal to that of other patches. Patch quality changes over time usually as result of 'resource depression,' which is the direct result of predators removing prey items from the habitat without prey items being replenished (Charnov *et al.* 1976). The amount of time a natural enemy is willing to remain in a given patch depends on aspects of both the prey and the predator (Charnov *et al.* 1976). The giving up time of a natural enemy in a patch might be an accurate indicator of its efficiency. Wiedenmann and Smith (1997) recommend that effective natural enemies in ephemeral agroecosystems should have superior searching ability allowing them to locate prey that are present in low density, increasing their patch residence time. Studying the patch residence time and efficiency of prey removal during that time could provide valuable insight regarding the success of biological control.

Finally, the feeding behaviour of a predator might change in order to optimize its rate of food intake in response to the quality of the available resources. Slansky and Feeny (1977) were the first to observe and document compensatory feeding behaviour while studying the development of *Pieris rapae* L. (Lepidoptera: Pieridae) provided with plant material that varied in nitrogen (N) concentration. In their experiments, caterpillars consumed greater amounts of plant material with low N concentration than plant material with high N concentration, but the overall growth rate of individuals across all diets was equal (Slansky and Feeny 1977). By increasing the rate of intake of poor quality food, organisms can maintain an optimal rate of nutrient uptake and subsequently, maintain their rate of development (Slansky and Feeny 1977). Natural enemies used in biological control programs should also be able to compensate for low quality prey by consuming more of it. This type of behaviour would be beneficial in biological control programs, as

it could increase the rate of pest mortality. It is even possible that certain agroecosystem management practices could be used to reduce the quality of prey food intentionally in order to increase the rate of prey consumption. These management practices might include exposing prey to increased risks of predation or disease, or limiting fertilizer inputs to host plants.

Optimal Oviposition Theory

Jaenike (1978) originally predicted that insect herbivores should deposit their eggs on the host plants that would optimize offspring development. This idea is also known as the preference – performance hypothesis, where female preference for oviposition sites optimizes offspring performance (Thompson 1988). Insect species that do not practice parental care and have larval stages with limited dispersal abilities are expected to follow optimal oviposition theory, as the offspring of those females are limited to the food source where the eggs are deposited, and the fitness of the female is dependent on the survival of her offspring (Jaenike 1978; Thompson 1988). A number of insect herbivores have been found to follow the predictions of optimal oviposition theory, most of those are specialists or oligophagous (Gripenberg *et al.* 2010). Examples include many Lepidoptera, such as *Polygonia c-album* (L.) (Lepidoptera: Nymphalidae) (Nylin and Janz 1993), but also members of other orders such as *Delia radicum* (L.) (Diptera: Anthomyiidae) (Kostal and Finch 1994; Hopkins *et al.* 1996). A few predators have also been identified to choose oviposition sites based on the quality and availability of future food sources, such as some ladybeetles (Coleoptera: Coccinellidae) and hoverflies (Diptera: Syrphidae) (Hemptinne *et al.* 1993).

Although the selection of oviposition sites by female insects does not always follow the predictions of optimal oviposition theory, especially if the best host for the female is not the best host for her offspring (Videla *et al.* 2012), this is an important aspect of natural enemy behaviour to examine prior to their use in biological control programs. Optimal oviposition theory provides a good framework for the study of oviposition choices made by female insects and the subsequent development of their offspring. For example, multiple potential oviposition and larval hosts can be provided simultaneously or alone, to first gauge the preference of the female, as well as the

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conditions under which eggs are deposited, and second, determine the rate of development of the offspring using those resources. The experimental design and statistical analysis of choice and no-choice experiments is well documented in the literature (Quade 1979; Roa 1992; Manly 1993; Lockwood 1998; Scheirs *et al.* 2000; Larrinaga 2010), making these tests easy to perform and analyze in the laboratory. Importantly, studying insect reproduction under the framework of optimal oviposition theory can inform us of the conditions required to maximize the reproduction and success of the natural enemy in natural settings.

Plant Vigor and Plant Stress Hypotheses

The plant vigor (Price 1991) and plant stress hypotheses (White 1984) were proposed to explain how pest outbreaks are related to characteristics of the host plant. The plant vigor hypothesis predicts that animal populations gain more nutritional and energetic benefits from consuming plants that have high concentrations of nutrients and that are growing vigorously (Price 1991). Vigorous plants should be large and healthy, able to compensate for herbivory, and have invested very little in defenses, both physical and chemical (Coley *et al*. 1985). As a result of these qualities, Price (1991) predicted that animals feeding on vigorous plants would be able to convert plant material to energy and growth more efficiently, resulting in faster population growth. Contrary to the plant vigor hypothesis, the plant stress hypothesis predicts that insects feeding on stressed plants will grow and develop faster, resulting in more rapid population growth (White 1984). White (1984) proposed that stressed plants have more 'free' nutrients in their tissues, which are more readily available for conversion into energy and growth by the herbivore. 'Free' nutrients result when plants mobilize nutrients stored in senescing or damaged plant tissues and transport them to healthy tissues or storage organs to increase the chances of plant survival (Hill 1980; Buchanan-Wollaston 1997). White (1984) based his hypothesis on his observation that insect populations feeding on drought or nutrient stressed plants tended to reach outbreak densities more often than those feeding on unstressed plants.

Many herbivores have been investigated to determine if their population dynamics can be predicted by either the plant vigor or plant stress hypothesis, with mixed results. Many insect herbivores follow the predictions of the plant vigor hypothesis (see Prada *et*

al. 1995; Inbar *et al.* 2001; Dhileepan 2004), whereas others, such as leafcutter ants, *Atta colombica* Guérin-Méneville (Hymenoptera: Formicidae), follow the predictions of the plant stress hypothesis (Meyer *et al.* 2006). The number of herbivore species that conform to the plant vigor hypothesis outweighs those that conform to the plant stress hypothesis (Cornelissen *et al.* 2008). Interestingly, feeding guild (sap-sucker, chewer, etc.) and the preferred age of vegetation for consumption (i.e. senescence or flush feeder) can predict whether or not an herbivore responds positively to vigorous or stressed plants (Cornelissen *et al.* 2008; White 2009). Because of the lack of overwhelming support for one hypothesis or the other, the two have come to be considered as the extreme and opposite ends of a continuum, with the response of herbivores to plant health falling anywhere between the two (Price 1991; White 2009).

The plant vigor and plant stress hypotheses can be used to provide important insight to both pest and natural enemy population dynamics and behaviour that can be used to improve upon biological control programs. For example, it is important to know if a pest exploits healthy or stressed plants, and if the signals released by the host plant differ with its status. Plants release herbivore induced plant volatiles (HIPV) when attacked (Vet and Dicke 1992; Dicke *et al.* 2003); it is possible that the quality and identity of the chemical cue released by stressed and healthy plants could differ, which would alter the ability of natural enemies to locate their target pest. The status of the host plant could also impact the stability of the pest population. For example, populations of *Bemisia argentifolii* Bellows and Perring (Hemiptera: Aleyrodidae) are known to increase as the nitrogen concentration of its host plant increases (Bi *et al.* 2003). If pest population dynamics are unstable, or fluctuate significantly, then it is important to ensure that the natural enemy selected for biological control can respond appropriately.

Finally, the quality of the host plant might have direct or indirect effects on the health of the natural enemy. For example, pests feeding on stressed plants might accumulate higher levels of allelochemicals than their counterparts that consume vigorous plants, which have fewer defenses (Coley *et al.*1985). Predators that consume large numbers of pests with high concentrations of sequestered allelochemicals might exhibit lethal or sub-lethal effects as a result (Malcolm 1990). It is also logical to predict that natural enemies that consume some plant material, such as omnivores or parasitoids

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that utilize pollen resources can be directly affected by the status of the host plant, in negative or positive ways. This prediction needs to be investigated to prevent failure or other unexpected outcomes of biological control programs.

The study system

   The use of omnivores in biological control programs is fairly recent, gaining attention in the mid-1990s, as illustrated by the publication of *Zoophytophagous Heteroptera: Implications for Life History and Integrated Pest Management* by Alomar and Wiedenmann (1996). Omnivores are defined as organisms that gain energy by consuming foods from multiple trophic levels (Pimm and Lawton 1978; Coll and Guershon 2002). Zoophytopagous predators are omnivores that are able to feed on both plant and prey material simultaneously during a single life stage (Coll and Guershon 2002) and are referred to as omnivores herein. Many omnivorous Hemiptera of the suborder Heteroptera have been investigated for use in biological control programs, including *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), *Orius albidipennis* (Reuters) (Hemiptera: Anthocoridae), *Orius tristicolor* (White) (Hemiptera: Anthocoridae), *Nesidiocoris tenius* (Reuter) (Hemiptera: Miridae), *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae), *Dicyphus tamaninii* Wagner (Hemiptera: Miridae), and *Dicyphus hesperus* Knight (Hemiptera: Miridae), (Alomar and Albajes 1996; McGregor *et al.* 1999; VanLaerhoven *et al.* 2000; Perdikis *et al.* 2008; Sobhy *et al.* 2010; Castañé *et al.* 2011). Most of these species are considered to be generalist predators, as they consume a number of prey species (Riechert and Lockley 1984).

   Omnivores have several characteristics that make them good biological control agents. Because many omnivores are generalists, it is common to find that omnivorous natural enemies colonize habitats before the pest does (Naranjo and Gibson 1996), or at the same time (Gabarra *et al.* 2004). In addition, by consuming a mixed diet omnivores are more likely to persist when prey is rare or absent in a habitat compared to their predator and parasitoid counterparts (Bugg *et al.* 1987; Naranjo and Gibson 1996; Settle *et al.* 1996; Wiedenmann *et al.* 1996; Lalonde *et al.* 1999; Coll and Guershon 2002). Both of these characteristics are very important, as persistence and early colonization result in the natural enemy being present during the early stages of pest population

growth. Therefore, biological control with omnivores does not often suffer from the lag-time that is associated with biological control using predators and parasitoids that colonize habitats after the pest does (Van Driesche and Bellows 1996; Kogan *et al.* 1999). Moreover, it is during this period of latent pest population growth that natural enemies may have the greatest impact on the pest (Wiedenmann and Smith 1997).

One challenge associated with the use of omnivores in biological control programs is that plant feeding by the omnivore can result in plant damage and yield loss (Alomar and Albajes 1996; McGregor *et al.* 2000; Shipp and Wang 2006; Sanchez and Lacasa 2008; Arnó *et al.* 2010). For example, both *D. tamaninii* and *N. tenius* will feed upon the fruit of tomato (*Solanum lycopersicum* L., Solanales: Solanaceae) plants when its prey is absent or scarce, resulting in unsalable fruit (Alomar and Albajes 1996; Sanchez and Lacasa 2008). Therefore, there are economic thresholds for these omnivores in tomato crops (Alomar and Albajes 1996; Sanchez and Lacasa 2008). Theoretically, there are other times at which plant feeding might be more beneficial to the omnivore than prey feeding. This might occur if the plant material is nutrient rich or if plant feeding is associated with very low search and handling costs. To ensure that biological control programs that utilize omnivores are effective it is important to identify the conditions in which omnivores may choose to plant feed rather than prey feed.

Dicyphus hesperus is a generalist omnivore that is believed to have potential in biological control programs. McGregor *et al.* (1999) identified *D. hesperus* as an effective natural enemy of greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) and two-spotted spider mites, *Tetranychus utricae* Koch (Acari: Tetranychidae) on tomato plants. Because *D. hesperus* oriented to whitefly-infested sentinel tomato plants and completed development on those plants, they suggested that it could be used as a biological control agent in tomato crops (McGregor *et al.* 1999). Work to follow up on this conclusion revealed that *D. hesperus* prefers to feed from tomato leaves rather than tomato fruits when prey is present or absent, suggesting that yield losses due to its plant feeding would be minimal (McGregor *et al.* 2000), although damage via fruit feeding has been observed (Shipp and Wang 2006).

Since 1999, we have learned a lot about this omnivore in terms of its interactions with other biological control agents, its biology and ecology, and its host plant and prey

preferences. Multiple studies have investigated the relationship between *D. hesperus* and *E. formosa,* as both are natural enemies of whitefly. In choice tests, *D. hesperus* exhibited no preferences for whitefly nymphs that had been parasitized by *E. formosa* compared to unparasitized nymphs (McGregor and Gillespie 2005), however, Bennett *et al.* (2009) observed that *D. hesperus* do have an overall negative impact on *E. formosa* populations. The relationship between the two natural enemies is described as asymmetrical, as *E. formosa* has no negative impact on *D. hesperus* (Bennett *et al.* 2009). Some work has also been done to determine the susceptibility of *D. hesperus* to certain fungal pathogens used in greenhouses to control whitefly, such as *Beauveria bassiana* (Vuilleman) (Hypocreales: Clavicipitaceae) and *Paecilomyces fumosoroseus* (Wize) (Eurotiales: Trichocomaceae) (Labbe *et al.* 2006; Alma *et al.* 2007; Alma *et al.* 2010). In habitats where *B. bassiana* was applied, *D. hesperus* was careful to avoid preying upon infected whitefly nymphs, especially at later stages of infection (Labbe *et al.* 2006). In early studies, Alma *et al.* (2007) observed that *D. hesperus* was not susceptible to infection by *P. fumosoroseus*; however, in later trials *D. hesperus* died as a result of *P. fumosoroseus* infection (Alma *et al.* 2010). Overall, *D. hesperus* does have the potential to be incorporated into biological control programs with other natural enemies or pathogens, so long as studies have been conducted to assess their compatibility beforehand.

The effects of temperature and day-length on the life history of *D. hesperus* have also been studied. The proportion of daylight hours in each day over time has an effect on the reproductive abilities of *D. hesperus*; reproductive diapause occurs if the number of daylight hours decreases below 13 or 14 h, depending on the population of *D. hesperus* (Gillespie and Quiring 2005). For their work, and that of Gillepsie *et al.* (2004), populations of *D. hesperus* originated from British Columbia, Canada, or California, USA. The BC and CA populations did not differ statistically or biologically in terms of their response to temperature during development (Gillespie *et al.* 2004). For example, the threshold temperatures for male development were 8.01 ± 0.21°C and 8.4 ± 0.14°C for the BC and CA population, respectively (Gillespie *et al.* 2004). For females, developmental thresholds were 7.8 ± 0.22°C and 7.9 ± 0.21°C for the BC and CA populations respectively (Gillespie *et al.* 2004). In general, as the rearing temperature increased, the time required for nymph development decreased for temperatures below

35°C (Gillespie *et al.* 2004). When females are held at 23°C, five to seven days are required for reproductive system development after adult emergence before egg laying begins (Gillespie and Quiring 2005).

Certain aspects of *D. hesperus* behaviour are also related to temperatures and light regimes. Specifically, VanLaerhoven *et al.* (2003) observed that *D. hesperus* are more active at night, and consume prey at a greater rate during the night than during the day. Temperature also has an effect on the activity budget of *D. hesperus*, as observed by Sparkes (2012). Sparkes (2012) determined the effects of temperature on the foraging behaviour and activity budget of *D. hesperus* when the mean daily temperature was held at 23°C, but temperatures fluctuated over the course of the day with either a high or low amplitude to simulate temperature variation due to climate change. Temperature regime had a significant effect on the activity budget of *D. hesperus*; when temperatures reached greater daily highs, insects were much less active during daylight hours than when temperatures were maintained at 23°C (Sparkes 2012).

Because *D. hesperus* is a generalist, it has been important to identify its preferred species of host plant and prey. In choice experiments where adults were allowed to select their host plant, mullein (*Verbascum thapsus* L., Lamiales: Scrophulariaceae) and tobacco, (*Nicotiana tabacum* L., Solanales: Solanaceae) were preferred, pepper (*Capsicum annuum* L. Solanales: Solanaceae) and corn (*Zea mays* L., Poales: Poaceae) were among those that were not preferred, and preference for tomato was intermediate (Sanchez *et al*. 2004). The grouping of preferred and non-preferred hosts observed by Sanchez *et al.* (2004) differed when prey was provided. Using a patch residence time model, VanLaerhoven *et al.* (2006) also tested host plant preference. Their results were similar to those of Sanchez *et al.* (2004), as insects remained on mullein and tomato plants significantly longer than on chrysanthemum, *Chrysanthemum coronarium* L. (Asterales: Asteraceae), and pepper plants (VanLaerhoven *et al.* 2006). Ma *et al.* (2009) observed similar results, in which *D. hesperus* were more likely to leave tomato and pepper plants compared to mullein plants. Mullein and tomato are also among the preferred oviposition hosts of *D. hesperus*, but again, preference depends on the presence or absence of prey (Sanchez *et al*. 2004).

The studies described above examined host plant preference by manipulating the species of host plant available to *D. hesperus*, but the quality of those host plants was not manipulated. In monocultures, *D. hesperus* are limited to only one species of host plant, and the quality (or suitability) of those plants might vary over spatial and temporal scales. The impacts of plant quality on host plant selection by *D. hesperus* have not been investigated previously; however, it is likely that plants of a single species that vary in quality will be more or less attractive to *D. hesperus*, as plant quality is known to impact insect development, as well as that of other animals (Mattson 1980).

Variation in nutritional content, allelochemical concentration, and of physical or morphological plant characteristics can all affect the quality of a host plant for insect consumption (Mattson 1980; Coley *et al.* 1985). Coley *et al.* (1985) propose that the overall quality of a host plant is dependent on the resources available in the environment in which it grows. In general, good quality host plants are those with excess nutrients available for growth, the potential for rapid growth, low levels of defensive compounds, and no structural defenses (Coley *et al.* 1998). In contrast, low quality host plants are those growing in nutrient deficient conditions that grow slowly and invest highly in both chemical and structural defenses (Coley *et al.* 1985). Therefore, it is not just the caloric or nutritional value of a plant that determines its suitability; quality is the sum of all of the plant characteristics that affect the insect's ability to utilize that plant.

Nitrogen (N) applied in fertilizer can be used to easily manipulate plant quality. The effects of N-fertilizer on tomato plants are well documented in the literature. For example, Jauset *et al.* (1998) treated tomato plants with 84, 140, and 308 ppm N in solution and found that in addition to differences in percent N between treatments, N-fertilizer also affected leaf water content and plant biomass. Increased leaf N content and water content are both considered indicators of high quality food sources (Scriber and Slansky 1981). Plant biomass is associated with vigorous growth, providing excess plant material for consumption, generally with fewer defenses (Coley *et al.* 1985). It has also been observed that tomatoes receiving low levels of N have twice the concentration of phenoloics as those that received high levels of N (Stout *et al.* 1998); therefore, tomato plants that receive higher levels of N-fertilizer have lower levels of toxins. Finally, tomato plants that received high levels of N-fertilizer had thinner leaf cuticle, making

those leaves easier to pierce and consume (Jauset *et al.* 2000). To elucidate the effects of plant quality on *D. hesperus* host plant selection, I manipulated the quality of tomato plants using nitrogen (N) fertilizer, to obtain high quality (vigorous, high leaf N, high water content, low toxin content, thin cuticle) and low quality (nutrient stressed, low leaf N, low water content, high toxin content, thick cuticle) plants, following the methods used by other authors studying the plant vigor and plant stress hypotheses (Prada *et al.* 1995; Jauset *et al.* 1998; Jauset *et al.* 2000; Inbar *et al.* 2001; Dhileepan 2004; Meyer *et al.* 2006). Using these plants, I tested the preference of *D. hesperus* for high and low quality tomato plants as oviposition hosts (Chapter 2). Following the predictions of optimal oviposition theory, I expected that female *D. hesperus* would lay the most eggs on high quality tomato plants and the fewest on low quality tomato plants. I expected to observe this result in both choice tests, where tomato plants of varying quality were provided simultaneously, and in no-choice tests, when only one tomato plant of a single quality was provided.

As optimal oviposition theory predicts that female choice and offspring performance should be positively correlated (Jaenike 1978; Thompson 1988), it is important that investigators determine the influence of the host plant that the female selects for oviposition on the performance of her offspring. A lot is known about how different species of host plant and prey affect the development of *D. hesperus* nymphs. For example, in studies conducted using excised tomato leaflets, nymphs provided with prey and a source of water, or prey and plant material, have much higher rates of survival than nymphs provided with only prey (Gillespie and McGregor 2000). The development of nymphs fed a diet of plant and prey material is also faster than nymphs provided with prey and a source of freshwater, suggesting that *D. hesperus* consumes nutrients from its host plant when extracting water for prey feeding (Gillespie and McGregor 2000). McGregor *et al.* (1999) observed that nymphs fed only tomato plant material did not survive past the first instar. In comparison, other species of host plant do support development of *D. hesperus* nymphs to the adult stage in the absence of prey, although at very low rates (Sanchez *et al.* 2004). For example, when reared on mullein 32.4% of nymphs survived to the adult stage, and on pepper 2.2% of nymphs reached the adult stage (Sanchez *et al.* 2004).

Overall, the development and survival of *D. hesperus* nymphs depends on the combination of plant and prey species available for consumption (McGregor *et al.* 1999; Gillespie and McGregor 2000; Sanchez *et al.* 2004). However, none of the studies described above investigated the impacts of varying plant quality, of a single plant species, on the development and survival of *D. hesperus* nymphs. Using tomato plants of varying quality, manipulated as described in Chapter 2, I evaluated the effects of plant quality on the development time and survival of *D. hesperus* nymphs (Chapter 3). In one experiment, small amounts of prey were provided to each individual nymph during the early stages of development but not the later stages to mimic habitats where prey becomes scarce, as often observed in ephemeral agroecosystems (Wiedenmann and Smith 1997). In another experiment, no prey food was provided at any stage of development. In both of these experiments, plant quality was manipulated but prey quality was not. Because female preference and offspring performance should be positively correlated (Jaenike 1978; Thompson 1988), I expected that *D. hesperus* nymphs would perform best on tomato plants of the quality preferred by ovipositing females in Chapter 2.

The species and availability of prey also affects the development time, survival, and patch residence time of *D. hesperus*. When prey were available, for example, Sanchez *et al.* (2004) observed that tomato and mullein were the most suitable host plants for nymphal development and survival. Certain species of prey also appear to be more preferred by *D. hesperus*, as females abandoned patches without prey significantly faster than patches with prey available and females were more likely to remain in patches with two-spotted spider mites than those with whiteflies as time passed (VanLaerhoven *et al.* 2006).

The quality of an herbivore is closely tied to the quality of the plant food it consumes (Mattson 1980; Bentz and Larew 1992; Blua and Toscano 1994; Blackmer and Byrne 1999; Lill and Marquis 2001; Crafts-Brandner 2002; Ode 2006). As a result, if plant quality in a monoculture varies, then so will the quality of the herbivore as prey for a predator or omnivore. Similar to plant quality, the value of prey to the predator or omnivore is the sum of its nutritional value, caloric content, toxins, and defenses. All of these can be influenced by the quality of the host plant (Ode 2006; Correa *et al.* 2014). Variation in prey quality across time and space due to variation in plant quality may have

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significant impacts on both the life history and behaviour of natural enemies but this has not been investigated for many species, including *D. hesperus*.

To determine if host plant quality affected the life history and behaviour of *D. hesperus* via its prey food, I manipulated the rearing host quality of whitefly nymphs by isolating adult whitefly on tomato plants from the high N and low N-fertilizer treatments. Plant N is known to affect whitefly size (of both adults and nymphs), egg mortality, rate of population increase, fecundity, development time, and host plant preference (Bentz and Larew 1992; Jauset *et al.* 1998; Blackmer and Byrne 1999; Jauset *et al.* 2000; Crafts-Brandner 2002; Bi *et al.* 2003). Importantly, whitefly reared on high N host plants are known to have increased levels of free amino acids (Crafts-Brander 2002). In addition, herbivores feeding on low N tomato plants are exposed to higher levels of phenolic compounds (plant defensive compounds; Stout *et al.* 1998), which are among the toxic compounds known to decrease herbivore (prey) quality (Kaplan and Thaler 2010). Therefore, whitefly reared on high N host plants should have been exposed to lower levels of toxins and should have greater concentrations of amino acids than whitefly nymphs reared on low N plants, making whitefly reared on high N plants better prey for predators. For ease of nomenclature, I refer to whitefly reared on high N tomatoes as high quality prey; low quality prey was reared on low N tomato plants.

Whitefly prey from high and low N tomato plants were used in four experiments. In the first experiment, developing *D. hesperus* nymphs were reared on high and low quality prey in the laboratory (Chapter 3). For this experiment, I predicted that nymphs would develop faster and have greater survival rates when feeding on high quality prey than on low quality prey. In the second experiment, I investigated the effects of plant and prey quality on the patch residence time of *D. hesperus* adults in a greenhouse experiment, with the expectation that the patch residence time of adults would be greatest when both high quality prey and plant food was available (Chapter 3). Third, I used experimental designs similar to those used in tests of optimal foraging theory to determine if prey quality affected 1) the rate at which *D. hesperus* consumed whitefly nymphs, and 2) the preference of *D. hesperus* for whitefly nymphs (Chapter 5). Based on Slansky and Feeny's (1977) observation of compensatory feeding by insects in response to low quality foods, I predicted that *D. hesperus* would consume greater numbers of

whitefly reared on low quality tomato plants compared to whitefly reared on high quality tomato plants. I also predicted that *D. hesperus* would demonstrate a preference for high quality prey in choice experiments, based on the predictions of optimal foraging theory (Stephens and Krebs 1986). Finally, I used focal observations in a laboratory study to determine the combined effects of variation in prey and plant quality on the foraging behaviour of *D. hesperus* (Chapter 6). In this experiment, I expected that the proportion of the activity budget that *D. hesperus* devoted to plant feeding would be dependent on the quality of the prey, where the incidence of plant feeding would decrease as prey quality increased.

In addition to the preferences of *D. hesperus* for different species of prey and host plants, the distribution of *D. hesperus* in tomato crops and the cues that might affect its ability to locate its host plants and prey have also been investigated. Specifically, Sanchez *et al.* (2002) sampled populations of *D. hesperus* on tomato plants and found that the distribution of adult *D. hesperus* among plants was aggregated, similar to the distribution of their whitefly prey. McGregor *et al.* (1999) observed that adults oriented to whitefly infested host plants and McGregor and Gillespie (2004) found that female *D. hesperus* preferred odors from whitefly-infested tomato plants in assays using a y-tube olfactometer. Their results also indicated that female *D. hesperus* had no preference for the odors of mite-infested plants, but were attracted to odors from pepper leaves infested with *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (McGregor and Gillespie 2004). However, the importance of olfactory cues is probably more important over greater distances, as Hazard (2008) observed that visual cues were important for host location at close range. The distribution and host location abilities of *D. hesperus* need to be well understood to ensure that this natural enemy comes into contact with the target pest in the habitats were it is released. We might assume that the distribution of *D. hesperus* in a monoculture would be easy to predict, however, if plant quality varies, the quality of the cues that *D. hesperus* use to locate their hosts might also vary and their distribution may be different than expected based on the results of Sanchez *et al.* (2002) and McGregor and Gillespie (2004). To address this possibility, I used a y-tube olfactometer to investigate the response of *D. hesperus* to tomato plants of varying quality, with and without whitefly infestation (Chapter 4). For this experiment, I predicted that plant odors

from high quality host plants would be most attractive to *D. hesperus*, and that the presence of whitefly nymphs and previous foraging experience on tomato plants would increase the responsiveness of *D. hesperus* to odors in the olfactometer.

Research objective

The overall objective of the research described in my dissertation was to determine the effect of variation in plant and prey quality on *D. hesperus*. To accomplish this objective, life history and behavioural traits important to the success of *D. hesperus* as a biological control agent were chosen for investigation and experiments were designed to test those traits using the foundation provided by key ecological theories as a research model. By addressing the effects of plant and prey quality on the behaviour and life history of *D. hesperus*, biological control programs using this natural enemy can be made more effective. In addition, this information should be useful in designing biological control programs using other species of omnivores, or to improve upon existing programs.

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Chapter 2. Does host plant quality affect the oviposition decisions of an omnivore?

Introduction

Reproduction is necessary in order for individuals to pass on their genetic information to subsequent generations. Thus, we assume that the primary goal of all individuals is to maximize their fitness (Krebs and Davies 1993). Key aspects of animal behaviour have been modeled on this basic assumption, including feeding behaviour (MacArthur and Pianka 1966; Stephens and Krebs 1986) and reproductive behaviour (Jaenike 1978). Decisions regarding reproduction encompass numerous tradeoffs such as the number of offspring to produce, when to begin reproducing, and providing offspring with parental care, among others (Stearns 1992). Parental care allows adults to nurture and protect their offspring, increasing their ultimate reproductive fitness (Krebs and Davies 1993; Tallamy 1999; Wong *et al.* 2013). However, there are costs associated with parental care (Krebs and Davies 1993). Many insect species simply deposit their eggs in presumably suitable habitats and leave the resultant offspring to fend for themselves (Jaenike 1978; Tallamy 1999).

Where parental care is not practiced and offspring dispersal capability is limited, optimal oviposition theory predicts that females should attempt to maximize their fitness by selecting oviposition hosts that will result in the highest offspring performance and survival (Jaenike 1978). Optimal oviposition theory is also known as the preference-performance hypothesis because it is tested by evaluating i) female preference for oviposition sites, and ii) the performance of offspring at those oviposition sites (Thompson 1988). For some herbivorous insects there is a strong correlation between female preference and offspring performance on different species of host plants. Examples include *Papillo machaon* L. (Lepidoptera: Papilionidae) (Wiklund 1981), *Euphydryas editha* (Boisduval) (Lepidoptera: Nymphalidae) (Ng 1988; Singer *et al.* 1988), *Polygonia c-album* (L.) (Lepidoptera: Nymphalidae) (Nylin and Janz 1993), and *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) (Coll 1996). Optimal oviposition behaviour has also been observed for predators, such as *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), which was found to avoid laying eggs near declining aphid (Hemiptera: Aphididae) colonies (Hemptinne *et al.* 1993). For many herbivorous insects,

however, no strong correlations have been found (Thompson 1988; Jaenike 1990; Mayhew 1997).

There are a number of hypotheses that might explain the lack of strong positive correlation between preference and performance. A simple explanation is that different measures of offspring performance correlate with female preference better than others, such that for a single insect species, one measure might correlate strongly with preference while another does not (Thompson 1988; Nylin *et al.* 1996). Nylin *et al.* (1996) suggest that in order to fully test optimal oviposition theory for any given insect species, the most appropriate measure of offspring performance is their fitness, however, to measure the fitness of the offspring, each individual would have to be followed for its entire life cycle. This would be extremely difficult and time consuming, especially for long-lived species. Thomson (1988) reviewed several other possible explanations for the lack of correlation between preference and performance. First, using the time hypothesis, he suggests that if female preference for a specific host is relatively new, sufficient time may not have passed for the offspring to adjust, resulting in reduced performance (Thompson 1988). Oviposition preference by females might also be affected by changing abundance of host plants in the plant community, such that females express a preference for an inferior larval host simply because it is common in the community (patch dynamics hypothesis; Thompson 1988). Females might also express a preference for host plants where the risk of predation or parasitization is lower, even though that host plant is not optimal for offspring development (enemy free space hypothesis; Thompson 1988). Finally, it has recently been suggested that many insects are simply bad mothers that select host plants that maximize their performance and fecundity at the expense of their offspring (Scheirs *et al.* 2000; Mayhew 2001). This has been observed for a the grass miner, *Chromatomyia nigra* (Meigen) (Diptera: Agromyzidae) (Schiers *et al.* 2000).

Host plant quality varies in the field and may also contribute to the weakness of correlations between adult preference and offspring performance, as Nylin *et al.* (1996) suggest. For example, Leather (1985) observed that *Panolis flammea* Dennis and Schiffermüeller (Lepidoptera: Noctuidae) preferred to oviposit on *Pinus contorta* Douglas (Pinales: Pinaceae) from specific geographic regions with different climatic conditions. Secondary metabolites of host plants also vary in the field. Therefore,

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Wheeler and Ordung (2005) investigated the effects of terpenoids on the preference of *Melaleuca quinquenervia* (Cav.) S.T. Blake (Myrtales: Myrtaceae) by *Boreioglycaspis melaleucae* Moore (Hemiptera: Psyllidae). They found that the terpenoid profile did affect oviposition preference, where plants with a profile primarily consisting of viridiflorol were preferred relative to those whose profile consisted of *E*-nerolidol (Wheeler and Ordung 2005). Plant nitrogen (N) levels also tend to vary greatly within plant species, both temporally and spatially (Mattson 1980). Although Nadel *et al.* (2008) found no effect of N on the oviposition preferences of *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), effects of N on oviposition preferences have been observed for *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) (Minkenberg and Ottenheim 1990). In the absence of natural enemies, N is believed to affect the oviposition preference of *Hydrellia valida* Loew (Diptera: Ephydridae) on saltmarsh cord grass, *Spartina alterniflora* Loisel. (Poales: Poaceae) (Stiling *et al.* 1992). Nitrogen does increase the rate of development, size, fecundity, and survival rate of insects feeding on plant material (Mattson 1980; Hunt *et al.* 1992; Jauset *et al*. 1998; Brodbeck *et al.* 2001), although the effects tend to be species specific. Therefore, it is likely that within-species N variation impacts the oviposition behaviour of a wide range of herbivorous species.

Omnivores obtain nutrients and energy from multiple trophic levels (Pimm and Lawton 1978; Coll and Guershon 2002), and may be classified as facultative, opportunistic or obligate according to diet (Coll and Guershon 2002). Omnivores that consume plant and animal materials are common in the Insecta across at least 12 orders (Coll and Guershon 2002). Due to their wealth of diversity, omnivores are gaining favour as biological control agents (Lalonde *et al.* 1999 and references therein, Lundgren 2009; Ågren *et al.* 2012), and are often assessed as potential biological control agents. For example, Messelink *et al.* (2014) and Pérez-Hedo (2015), recently evaluated a number of mirid omnivores as biological control agents of aphids (Hemiptera: Aphididae). One of the primary benefits of omnivores in biological control programs is their ability to persist in the absence of prey, which stabilizes the ecosystem (Lalonde *et al.* 1999). However, some groups of omnivores, including members of the Miridae such as *Dicyphus tamaninii* Wagner (Hemiptera: Miridae) and *Nesidiocoris tenius* (Reuter) (Hemiptera: Miridae) are known to cause economic levels of damage to their host plants (Alomar and

Albajes 1996; McGregor *et al.* 2000; Sanchez and Lacasa 2008; Castañé *et al.* 2011). To prevent significant levels of omnivore damage to host plants, or plant structures of agricultural and economic importance, the behaviour of omnivores needs to be well understood before their release into agroecosystems. Studying the oviposition preference of omnivores in monocultures where plant quality varies might provide important insight regarding their behaviour that can be used to inform the decisions of agroecosystem managers.

The cues driving oviposition choices of omnivores are not as well understood as those of herbivores and predators. Their selection of sites for oviposition may depend on host plant quality, prey quality, or a combination of both. Some work has been done to identify preferred host plant species of omnivores (Coll 1996; Coll and Guershon 2002; Groenteman *et al.* 2006; Lundgren and Fergen 2006; Seagraves and Lundgren 2006). However, few studies address the fact that plant suitability for oviposition likely varies between plants of the same species. The goal of this research was to identify the effects of N fertilization on the oviposition decisions of a potential biological control agent and omnivore, *Dicyphus hesperus* Knight (Hemiptera: Miridae), using choice and no-choice tests. For both tests, female omnivores were expected to prefer plants fertilized with high rates of N-fertilizer (≥ 200 ppm N) relative to plants fertilized with low rates of N-fertilizer (< 200 ppm N) as oviposition hosts. In choice tests, female omnivores were expected to prefer high N (≥ 200 ppm N) plants as feeding hosts, as indicated by the number of females found on the plants at the end of the oviposition period.

Materials and Methods

Insect and plant rearing

Dicyphus hesperus is a good model species for testing the effects of within-species variation in plant quality on the oviposition decisions of omnivorous insects. *Dicyphus hesperus* is easily reared in the laboratory (McGregor *et al.* 1999), is an obligate zoophytophagous omnivore (Gillespie and McGregor 2000), and has a Canada-wide distribution (Maw *et al.* 2000). *Dicyphus hesperus* can be used as a biological control agent of whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) in

greenhouses (McGregor *et al.* 1999), making it a practical species for modeling omnivore behaviour.

A colony of *D. hesperus* is maintained at the University of Windsor, Windsor, Ontario, Canada. The colony was originally established in Agassiz, British Columbia with insects collected from *Stachys albens* A. Gray (Lamiales: Lamiaceae) (white stem hedge nettle) in California, USA (35º42'15"N, 118º50'00"W, approximately 500 m elevation) (McGregor and Gillespie 2004). Insects from this colony were subsequently transferred to the University of Windsor (Sparkes 2012). Conditions in the rearing laboratory are maintained at 20 ± 5°C with a light:dark photoperiod of 16:8 h and 50 ± 10% relative humidity provided by a humidifier (Nortec Humidity Inc., Ogdensburg, New York, USA).

To rear *D. hesperus*, adult insects are held on one of their host plants, *Nicotiana tabacum* L. (Solanales: Solanaceae), grown in a greenhouse at the University of Windsor (McGregor *et al.* 1999; Sparkes 2012). Rearing cages are modified 61 cm^3 collapsible cages with mesh sides and top (BioQuip Products, Rancho Dominguez, California, USA). Each cage holds two to three *N. tabacum* plants with four to eight large leaves. After seven to 10 days, adults are removed from the oviposition plants using an aspirator and the insect-free plants are placed inside a second rearing cage to allow nymphs to hatch and develop isolated from adult *D. hesperus*, as like many predaceous Hemiptera, *D. hesperus* are opportunistic cannibals (Carayon 1961; Groenteman *et al.* 2006; Laycock *et al.* 2006). *Dicyphus hesperus* have four nymphal instars and the development period from oviposition to adult eclosion ranges from approximately 15 to 30 d at temperatures ranging from 19 to 27°C (Gillespie *et al.* 2004). Females begin to deposit eggs after five to seven days (Gillespie and Quiring 2005). In the rearing room, four colony cages are maintained: one oviposition cage, and three developmental cages, resulting in a new cohort of adult insects emerging approximately once per week. Throughout development, *D. hesperus* are provided with preserved *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (Beneficial Insectary Inc., Guelph, Ontario, Canada), eggs as prey *ad libitum* (Sparkes 2012).

Tomato plants, *Solanum lycopersicum* L. (Solanales: Solanaceae) were used as the experimental host plant. The development and reproduction of *D. hesperus* has

largely been evaluated on tomato plants (McGregor *et al.* 1999; McGregor *et al.* 2000; Gillespie and McGregor 2000; Sanchez *et al.* 2004), as tomatoes are an important greenhouse crop in Canada, worth $334 million in exports in 2005 (Agriculture and Agri-Food Canada 2006) and $496 million in 2011 (Statistics Canada 2012). Results have indicated that tomato is a suitable host for *D. hesperus* as long as prey are available; without prey no nymphs survive (McGregor *et al.* 1999; Sanchez *et al.* 2004). Patio hybrid tomato seeds (Stokes Seeds Canada, Thorold, Ontario, Canada) were sown in BM6 potting soil containing 78% peat moss plus perlite (Berger, Saint-Modeste, Quebec, Canada) in 8 x 8 x 9 cm square black pots, to a depth of two cm. In the greenhouse, seedlings sprouted under natural light and temperature regimes. At the four-leaf stage (BBCH stage 14; Feller *et al.* 1995), seedlings were transplanted into 8-inch (~ 20 cm) green plant pots, filled to 2.5 cm from the top with the same potting medium. Fertilization of the seedlings to manipulate tomato quality began the day following transplantation. Seedlings were randomly assigned to one of four fertilizer treatments: 50, 100, 200 and 400 ppm N. Each plant within each treatment received 100 ml (after Jauset *et al.* 1998) of the appropriate fertilizer solution daily for seven days, and every second day thereafter. Alberta Agriculture and Rural Development (2009), recommends fertilization at a rate of 200 ppm N for greenhouse tomatoes, which is roughly equivalent to 87 kg N ha^{-1} (Chen *et al.* 2008). Fertilizer solutions were mixed using 6-11-31 Hydroponic Fertilizer and 15.5-0-0 calcium nitrate (Plant-Prod® Canada, Brampton, ON, Canada) as described in Table 2.1. All plants were watered as needed to prevent wilting.

Approximately 24 d after the initiation of fertilization, plants were used in oviposition experiments. Prior to use, each plant was measured and the height, stem diameter, and BBCH growth stage (following Feller *et al.* 1995) was recorded. At the end of the choice experiment, the aboveground biomass of each plant was collected, dried in brown paper bags and measured using a Precisa BJ 100M balance (Precisa Ltd., Tongwell, Milton Keynes, UK).

No-choice and choice preference trials
In both no-choice and choice trials, female *D. hesperus* were allowed to oviposit for 72 h, after which adults were removed from the cages. In the choice trials, the number of adults

collected from each of the four plants in the cage was recorded and the plants were isolated. After seven days, each plant was inspected daily for emerging nymphs, which were collected immediately following the first observation of nymphs on at least one plant. Nymphs were collected three times over six days using a fine haired paintbrush, counted, and preserved in 70% ethanol. On the final collection date, plants were destroyed; each leaf, branch, and the main stem was carefully inspected for remaining nymphs. Throughout oviposition trials, all plants were fertilized and watered as described above.

In no-choice trials, individual plants were enclosed using a mesh bag tied closed around the top edge of the pot. Five adult female *D. hesperus*, at least seven days old, were collected from the oviposition cage in the rearing colony and transferred to each plant pot inside a plastic vial. The vial was placed against the main stem of the plant, with the cap off.

In choice trials, four tomato plants (with labeled pots), one from each fertilizer treatment, were placed inside a 55 x 55 x 60 cm mesh-walled cage. Plants were placed in the four corners of the cage so that the plants did not touch. The arrangement of the four plants was pseudo-randomized, such that no four cages in a block had the same arrangement. Female *D. hesperus*, at least seven days old, were collected into vials and released into each cage by placing the opened vial on the floor in the center of the cage, equidistant from all plants. Five female *D. hesperus* per plant were released into each cage (20 females per cage).

Egg mortality

As female *D. hesperus* insert eggs into plant tissues during oviposition (Gillespie *et al.* 2004), the number of emerging nymphs was used as a proxy measurement of oviposition in this experiment. To ensure that nymphal emergence was an accurate proxy for oviposition, egg mortality on plants from each of the four N-fertilizer treatments was determined in the laboratory. Branches from tomato plants were collected from plants that had been fertilized for approximately 24 d and the stems were inserted through a hole in the lid into a 60 ml Solo® cups (Lake Forest, Illinois, USA) filled with water. At 16:00 h, female *D. hesperus* were collected from the oviposition cage of the colony and placed

upon the tomato branches, with two or three females per branch. Branches were under constant surveillance by an observer between 16:00 and 21:00 h. The observer recorded the time of first oviposition, all oviposition events, and all attempts at oviposition. At the end of the observation period, females were removed from the tomato branches and each branch was isolated. Seven to 10 d later, branches were inspected for nymphs, with each branch inspected three times. Nymphs were counted and preserved in 70% ethanol. Oviposition events were observed on four branches of 50, 200, and 400 ppm N-fertilized tomato plants and on three branches of 100 ppm N-fertilized tomato plants.

Statistical analysis

Plant height, stem diameter, dry biomass, and fruit yield were analyzed using one-way analysis of variance (ANOVA) in SAS (SAS Institute 2009) to determine if fertilizer treatment had an effect on physical aspects of plant quality. Data for plant growth stage was not normal or homoscedastic, and could not be transformed to meet those assumptions. Therefore, plant stage was analyzed using the Kruskal-Wallis test for non-parametric ANOVA; significant Kruskal-Wallis results were followed up with paired Wilcoxon tests to compare N-fertilizer treatments (SAS Institute 2009). For this analysis, α was adjusted using the Bonferroni correction to account for multiple comparisons of means (SAS Institute 2009). Dry biomass and fruit yield were transformed using the natural log (LN) transformation to meet the assumptions of ANOVA (SAS Institute 2009); raw data are presented in the results. Where significant N-fertilizer effects were observed for plant height, stem diameter, dry biomass, and fruit yield, means were compared using the LSMEANS statement with the PDIFF and ADJUST=BON functions specified in the model (SAS Institute 2009). The PDIFF statement returns results to compare all possible pairs of treatments and the ADJUST=BON statement returns Bonferroni adjusted *p*-values that can be compared to α = 0.05 (SAS Institute 2009). The relationship between N-fertilizer treatment and dry plant biomass was tested using linear regression analysis in SAS (SAS Institute 2009). Relationships between host size and offspring number have been observed for several species, including *Eoreuma loftinia* (Dyar) (Lepidoptera: Crambidae) (Reay-Jones *et al.* 2007) and *Otiorhynchus sulcatus* F. (Coleoptera: Curculionidae) (Clark *et al.* 2011). Therefore, biomass may need to be

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incorporated into the analysis as a covariate. Biomass was tested as a potential covariate using the general linear model in SAS (SAS Institute 2009).

No-choice trials were analyzed by comparing the mean number of nymphs collected per plant in each treatment using one-way ANOVA in PROC MIXED with replicate as a random effect (SAS Institute Inc. 2009). Where significant fertilizer treatment effects were observed, means were compared using the PDIFF function with the ADJUST=BON statement as described above (SAS Institute 2009).

Choice tests should not be analyzed using univariate statistics because choice experiments involve the acceptance of a given host with the simultaneous rejection of an alternative host, which violates the assumption of treatment independence required for parametric testing (Roa 1992; Manly 1993; Larrinaga 2010). Several alternative methods for the analysis of choice experiments have been proposed, including the use of multivariate analysis of variance (MANOVA) (Roa 1992; Manly 1993; Lockwood 1998), repeated measures models (Larrinaga 2010), and non-parametric approaches including the Quade Test (Quade 1979; Conover 1999) and Friedman's Test (Friedman 1937; Conover 1999). For example, Scheirs *et al.* (2000) tested oviposition host preference of *Chromatomyia nigra* (Meigen) (Diptera: Agromyzidae) using the Quade Test (Quade 1979; Conover 1999) and presented preference in order of ranks.

For the purposes of this experiment, biomass was first tested as a potential covariate affecting plant preference as described above but was not significant (see Results below). Therefore, MANOVA was used to determine if plant quality affected host plant preference of *D. hesperus* based upon adult location at the end of 72 h and oviposition host preference based on the number of nymphs collected per plant using the repeated measures model in SPSS to calculate within-treatment effect statistics (Lockwood 1998, IBM SPSS Statistics 21.0 2012), where $\alpha = 0.05$. Where MANOVA results were significant, treatment means were separated using pairwise comparisons, which were controlled for error using the Bonferroni correction of α (IBM SPSS Statistics 21.0 2012).

The assumption that egg mortality was equal on plants from each N-fertilizer treatment was tested using chi-square analysis. Using the number of oviposition events recorded and the number of nymphs collected, the percentage of hatched nymphs was

calculated and the mean success rate for each N-fertilizer treatment was calculated and compared with chi-square analysis using the PROC FREQ procedure in SAS (SAS Institute 2009). For this analysis, $\alpha = 0.05$.

Results

Tomato plants that received 200 and 400 ppm N-fertilizer were at a more advanced growth stage at the start of the choice experiments than tomato plants that received 50 and 100 ppm N-fertilizer ($\chi^2 = 22.16$, df $= 3$, $p < 0.0001$; Table 2.2). Similarly, N-fertilizer treatment affected stem diameter ($F_{3,92} = 8.50$, $p < 0.0001$), plant height ($F_{3,92} = 11.89$, $p < 0.0001$), dry biomass ($F_{3,92} = 173.09$, $p < 0.0001$), and fruit yield ($F_{3,11} = 18.56$, $p < 0.0001$), such that plants that received more N-fertilizer were larger and had greater yields (Table 2.2). Regression analysis showed that N-fertilizer treatment had a significant linear effect on plant biomass ($F_{1,94} = 318.73$, $p < 0.0001$, $R^2 = 0.7722$), where biomass increased as fertilizer N concentration increased. Biomass differed between all N-fertilizer treatments (Table 2.2). Although not quantified, tomato plants that received 200 and 400 ppm N-fertilizer had dark green foliage and plants that received 50 and 100 ppm N-fertilizer had yellow-green coloured leaves.

In no-choice trials, N-fertilizer had a marginally significant effect on the number of nymphs per plant ($F_{3, 57} = 2.86$, $P = 0.0449$). Plants treated with 200 ppm N-fertilizer had a mean ($\pm$ SE) of 24.0 ± 3.0 per plant, which was significantly more than plants treated with 100 ppm N-fertilizer (15.5 ± 2.0 nymphs per plant). Plants receiving 50 and 400 ppm N fertilizer had means of 17.9 ± 2.1 and 21.0 ± 2.8 nymphs per plant, respectively, but these treatments were not different from the others.

Although dry plant biomass increased linearly as the rate of N-fertilizer increased, dry plant biomass did not have an effect on the mean number of *D. hesperus* nymphs per plant ($F_{1,94} = 0.00$, $p = 0.98$); therefore, biomass was not treated as a covariate in the model and MANOVA was used determine the effect of N-fertilizer on the oviposition preferences of female *D. hesperus*. The location of adult *D. hesperus* was affected by N-fertilizer (Hotelling's Trace $= 30.559$, df $= 3, 21$, $P < 0.0001$). After 72 h, the most adult females were found on plants receiving 200 ppm N-fertilizer and the fewest were found on plants receiving 50 ppm N-fertilizer (Figure 2.1). The number of *D. hesperus* nymphs

collected per plant was also affected by N-fertilizer treatment (Hotelling's Trace = 10.624, df = 3, 21, $P < 0.0001$). Again, the most nymphs were found on plants fertilized with 200 and 400 ppm N-fertilizer, and the fewest were found on plants fertilized with 50 ppm N-fertilizer (Figure 2.2).

During the five hours in which oviposition events were observed to test for differences in egg mortality across the N-fertilizer treatments, female *D. hesperus* only attempted to oviposit on the stems or large leaf veins of tomato plants. Oviposition events were only observed on four of eight 50 ppm N-fertilized tomato branches and on three of five 100 ppm N-fertilized tomato branches. The mean time to the first oviposition event was 100 ± 49 min, 197 ± 41 min, 82 ± 9 min, and 94 ± 41 min for females on 50, 100, 200, and 400 ppm N-fertilized tomato branches, respectively. Oviposition events were observed on all 200 and 400 ppm N tomato branches used in this experiment. Egg mortality was less than 20% on plants from all fertilizer treatments: 83.33, 100, 81.25, and 81.67% of eggs hatched on 50, 100, 200, and 400 ppm N-fertilized tomato branches, respectively. Chi-square results indicated no differences in egg mortality between the four levels of plant quality ($\chi^2 = 2.8092$, df = 3, $p = 0.4220$).

Discussion

Plant quality, as manipulated by N-fertilizer, had a significant effect on the physical characteristics of the tomato plants used for these experiment. The stage of growth, height, stem diameter, dry biomass, and fruit yield were all affected by fertilizer treatment. Other physical indicators of plant quality were also affected, but not quantified. Plant colour, for example, varied with fertilizer treatment, with low N-fertilized plants having yellow-green leaves and high N-fertilized plants having dark green leaves. The effects of N-fertilizer on plant N and protein content have been well documented for tomato (Jauset *et al.* 1998) and other plants (Mattson 1980; Bi *et al.* 2003; Wang *et al.* 2006; Athar *et al.* 2011); therefore, plant N content was not assessed. Although plant biomass and yield varied significantly between the N-fertilizer treatments, plant biomass was not a significant predictor of oviposition preference. Because all females observed while investigating egg mortality deposited eggs into the stems and large leaf veins, it is possible that the biomass or the area the stems and leaf veins might

be predictors of oviposition preference. Unfortunately, only whole plant biomass was measured in this experiment. The diameters of plant stems were measured, and did vary significantly with N-fertilizer inputs; however, measurement of stem diameter is not informative enough to reach solid conclusions regarding the effect of stem or leaf vein availability on oviposition preference.

As predicted, tomato plant quality did have an effect on the preference of *D. hesperus* for oviposition hosts. In both the choice and no-choice tests, the most nymphs were collected from 200 ppm N-fertilized plants and the difference in nymph numbers between 200 and 400 ppm N-fertilized tomato plants was not statistically significant. This observation supports our initial prediction that high N-fertilized plants are preferred for oviposition by *D. hesperus* females. We also observed that females laid eggs on 50 and 100 ppm N-fertilized tomato branches 50 and 40% of the time, respectively, compared to 100% of the time on 200 and 400 ppm N-fertilized tomato branches, which lends support to this conclusion. Nitrogen is vital for insect development and is a limiting resource (Mattson 1980). Therefore, it makes sense that more nymphs would be found on plants with greater potential N reserves, in this case, plants receiving 200 and 400 ppm N-fertilizer. Similar effects of N-fertilizer on oviposition have been observed for herbivores, including several species of whitefly (Bentz *et al.* 1995; Jauset *et al.* 1998; Ortega-Arenas *et al.* 2006), but information regarding the within-species host preferences of omnivores are lacking. Most tests of host plant preferences of omnivores have investigated differences in preference between different host plant species (Coll 1996; Coll and Guershon 2002; Lundgren and Fergen 2006; Seagraves and Lundgren 2006). For example, ovipositing *D. hesperus* preferred mullein (*Verbascum thaspus* L., Laminales: Scrophulariaceae), *Stachys albotomentosa* (Laminales: Laminaceae), and tobacco relative to broad bean (*Vicia sativa* L., Fabales: Fabaceae), pepper (*Capsicum annuum* L. Solanales: Solanaceae), and corn (*Zea mays* L., Poales: Poaceae) (Sanchez *et al.* 2004). When prey items were available on host plants, *S. albotomentosa*, tobacco, and tomato were the most preferred oviposition hosts (Sanchez *et al.* 2004). We found one other study where the effects of N on omnivore preferences were investigated. In this case, *Orius albidipennis* Reuter (Hemiptera: Anthocoridae) did not exhibit a preference for plants with different levels of leaf N, but females did exhibit an increased propensity to

guard their oviposition sites on high N plants (Groenteman *et al.* 2006). Other work has examined the effects of genetic differences (Lundgren *et al.* 2009), induced plant defenses (Agrawal *et al.* 1999; Agrawal and Klein 2000), and specific plant parts (Eubanks and Denno 1999) on host plant choice, oviposition, survival, and fitness of omnivores.

In choice tests, more adult female *D. hesperus* were found on 200 and 400 ppm N-fertilized plants after 72 h. Recent literature has suggested that there is a fundamental conflict between adult females and their offspring, sometimes referred to as 'optimal bad motherhood' (Mayhew 2001), where females lay eggs on hosts that are more beneficial to their own performance rather than that of their offspring (Scheirs *et al.* 2000; Mayhew 2001; Videla *et al.* 2012). Because female *D. hesperus* appear to prefer high N-fertilized plants for feeding and for oviposition, it will be most parsimonious if nymph performance correlates to female preference, as predicted by Jaenike (1978). The performance of *D. hesperus* nymphs on tomato plants of varying quality is described in Chapter 3.

Interestingly, although female *D. hesperus* exhibited a preference for high N-fertilized tomato plants for oviposition, a substantial number of nymphs were collected from tomato plants fertilized with 50 ppm N (no-choice: 17.9 ± 2.1; choice: 7.9 ± 1.1). There are several potential explanations for this observation. If *D. hesperus* are time limited dispersers, then laying eggs on low quality plants may represent a tradeoff between current opportunity and lost opportunity if search time for a better quality plant is excessive (Levins and MacArthur 1969; Mayhew 1997). However, *D. hesperus* adults may live for upwards of 28 days (Gillespie and Quiring 2005), which suggests that these insects are more likely to be egg limited, or constrained by egg production. If this is the case, then *D. hesperus* should be more selective and avoid suboptimal host plants, unless time becomes short and an egg absolutely must be deposited, as predicted by Jaenike (1978) and Mayhew (1997).

Selection of low N-fertilized tomato plants might be an artifact of the experimental design; only a brief window of the potential oviposition period of *D. hesperus* was observed. McNamara and Houston (1986) suggest that the entire lifetime of an insect must be observed to fully understand its oviposition preferences, as each oviposition decision impacts the potential fitness of a female. Studies of this nature,

however informative, are not always feasible. In the literature, oviposition periods as short as 4 h have been reported (Videla *et al.* 2012); compared to such short-term trials, an oviposition period of 72 h should accurately represent the oviposition preference of *D. hesperus*. It is more likely that density dependent factors affect the oviposition preference of *D. hesperus*. Fretwell and Lucas (1970) predict that competitors of equivalent abilities should eventually be distributed evenly across the landscape, such that each individual has equal access to resources. *Dicyphus hesperus* are reared in high density conditions (M.A. Vankosky, *personal observation*), and relatively low numbers of *D. hesperus* adults were collected on low N-fertilized plants at the end of the three day oviposition period. Therefore, low N-fertilized plants might be good alternative oviposition hosts, as there is less competition between adults and potentially less competition between nymphs on these plants. Moreover, by considering density dependence in a model, a broader range of resources may become acceptable. This could also apply to low N-fertilized plants, which are certainly not the best hosts, but provide an acceptable alternative when densities are too great on high N-fertilized plants. It is also important to note that *D. hesperus* are opportunistic cannibals (Laycock *et al.* 2006), so low N-fertilized plants might also represent enemy free space, which is believed to affect oviposition behaviour (Price *et al.* 1980; Thompson 1988).

Within a biological control framework, the results of the experiments described here should be encouraging for scientists and agriculturalists interested in employing omnivores as biological control agents. When provided with plants of the same species, but of varying quality, the oviposition behaviour of *D. hesperus,* and potentially other omnivores, is predictable. When planning and executing biological control programs, a thorough understanding of the behaviour of the biological control agent is key to success (Bale *et al.* 2008). Our results add to the present knowledge regarding the behaviour of *D. hesperus*, and provides a framework with which to study the behaviour of other omnivores with biological control potential.

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Table 2.1. The amounts of 6-11-31 hydroponic fertilizer and 15.5-0-0 calcium nitrate (CaNO$_3$) required for 20 L solutions of each fertilizer treatment.

Fertilizer Treatment	6-11-31 (g)	CaNO$_3$ (g)	Water (L)
50 ppm N	5.75	4.25	20.0
100 ppm N	11.5	8.50	20.0
200 ppm N	23.0	17.0	20.0
400 ppm N	46.0	34.0	20.0

Table 2.2. Treatment means (± SE) for measurements of physical plant characteristics. For each characteristic, means with the same letters are not significantly different.

Characteristic	Fertilizer Treatment			
	50 ppm N	**100 ppm N**	**200 ppm N**	**400 ppm N**
Growth stage*	56 ± 1**a**	57 ± 1**a**	61 ± 1**b**	61 ± 1**b**
Stem diameter (mm)	8.96 ± 0.16**ab**	9.63 ± 0.21**ab**	10.44 ± 0.25**b**	9.91 ± 0.22**b**
Height (cm)	29.89 ± 0.60**a**	29.50 ± 0.58**a**	33.34 ± 0.77**b**	33.73 ± 0.62**b**
Dry biomass (g)	14.52 ± 0.47**a**	20.36 ± 0.63**b**	31.50 ± 1.02**c**	41.05 ± 1.59**d**
Fruit yield (g)	145.72 ± 24.16**a**	401.98 ± 38.36**b**	539.88 ± 44.45**b**	791.32 ± 174.22**b**

*Growth stage was recorded to the nearest whole growth stage using BBCH guidelines for tomato plants (Feller *et al.* 1995)
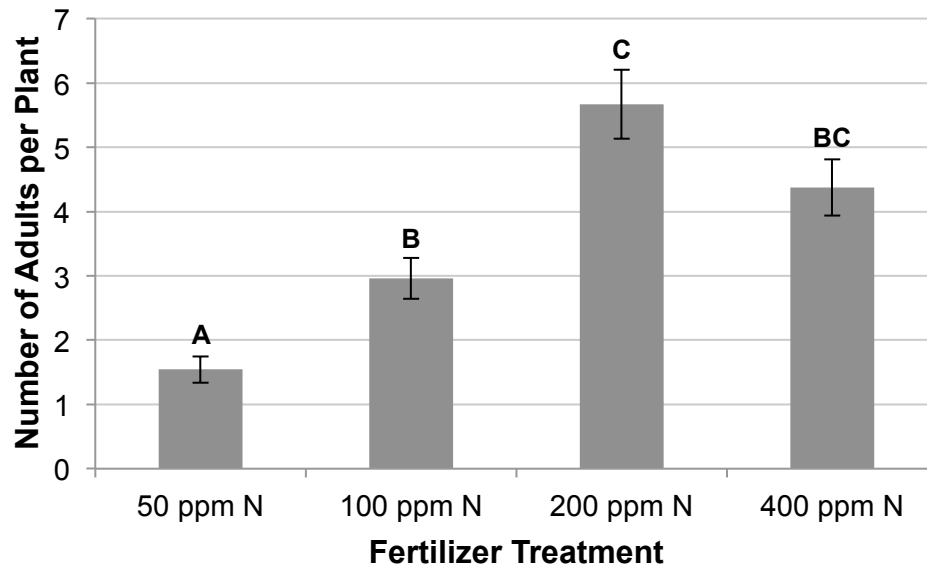
Figure 2.1. Mean (± SE) number of adult *Dicyphus hesperus* per plant in choice experiments after 72 h for each level of tomato plant quality. Plant fertilizer treatment had a significant effect on adult distribution (Hotelling's Trace = 30.559, df = 3, 21, $p < 0.0001$). Means with the same letters are not significantly different (Bonferroni adjusted $p > 0.05$).
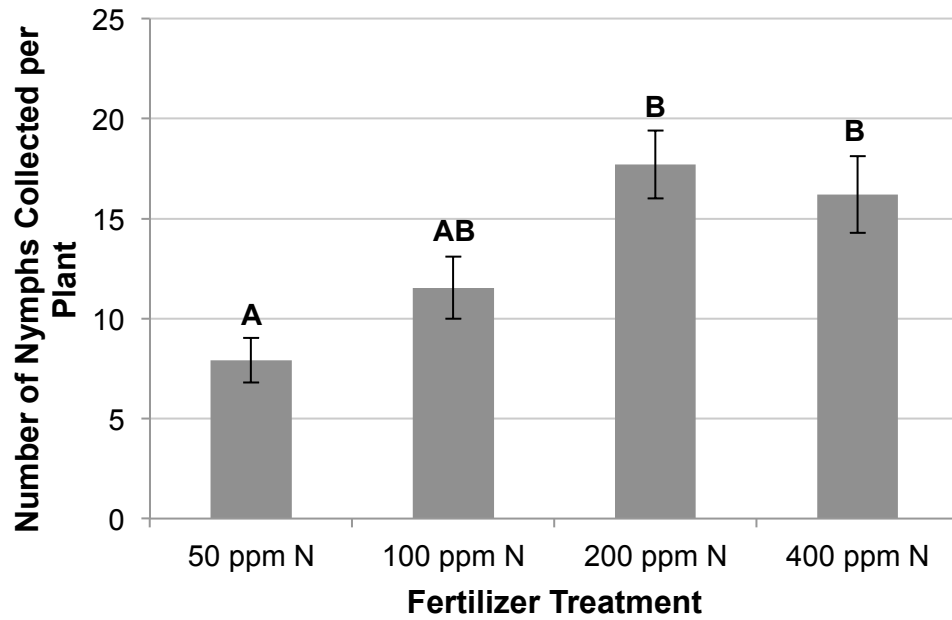
Figure 2.2. Mean (± SE) number of *Dicyphus hesperus* nymphs collected per plant in choice experiments for each level of tomato plant quality. Plant fertilizer treatment had a significant effect on the number of nymphs (Hotelling's Trace = 10.624, df = 3, 21, $P <$ 0.0001). Means with the same letters are not significantly different (Bonferroni adjusted *p* > 0.05).

Chapter 3. The effect of plant and prey quality on the development, survival, and patch residence time of an omnivorous biological control agent, *Dicyphus hesperus.*

Introduction

Knowledge regarding the development time and survival of juvenile insects has many practical applications, in a number of fields. The field of forensic entomology uses temperature data and the developmental rate of blow fly maggots (Diptera: Calliphoridae) to estimate the post-mortem interval (PMI) used in legal investigations (Tomberlin *et al*. 2011). The field of agricultural entomology relies heavily upon a thorough understanding the life cycle and development of crop pests. Farmers can use this type of information to disrupt the linkage between pest and plant phenology by altering planting dates (Teetes 1981). A thorough understanding of pest development can also be used to make decisions regarding the best time to apply insecticides or other pest management options, following the economic injury level concept (Higley and Pedigo 1993). In other cases, knowledge regarding the most vulnerable stage of pest development is important to achieve effective levels of pest management. For example, larvae of *Sitona lineatus* (L.) (Coleoptera: Curculionidae) are protected by root nodules, so properly timing the application of control measures to target the adult or egg stage is vital to reduce their populations (Ester and Jeuring 1992; Steene *et al.* 1999).

The efficacy of biological control is also highly dependent on a thorough understanding of the factors that affect the survival and development of both the target pest and the natural enemy used as a biological control agent. Natural enemy release or pathogen application should be timed to target vulnerable pest stages. For example, *Bacillus thuriengensis* δ-endotoxins target mosquito larvae (Diptera: Culicidae), thus the toxins need to be applied where and when larvae are present (Lacey 2007). Some of the most difficult pests to manage are multivoltine, that is, they undergo multiple generations in a single growing season (Gullan and Cranston 2005). The greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae), is one such example. Biological control agents to target these pests should be long-lived, multivoltine, and persistent (Smith 1935; Hagen *et al.* 1976; van Lenteren and Woets 1988; Bale *et al.* 2008).

To meet this need, interest in the use of omnivores as biological control agents is increasing. Omnivores consume foods from multiple trophic levels (Pimm and Lawton 1978; Coll and Guershon 2002); therefore, omnivores used in biological control consume plants and their herbivores. Although there are cons associated with omnivores, including yield loss if certain plant structures are targeted for plant feeding (McGregor *et al.* 2000; Castañé *et al.* 2011), there are a number of important benefits associated with the use of omnivores in biological control programs. One key benefit comes from the ability of omnivores to survive in habitats where prey is absent because plant material can replace prey food in the diet (Bugg *et al.* 1987; Naranjo and Gibson 1996; Settle *et al.* 1996; Wiedenmann *et al.* 1996; Coll and Guershon 2002). Persistence of omnivores within agroecosystems ensures that natural enemies of the target pest are present prior to pest colonization, eliminating the lag-time in pest suppression that is associated with delayed arrival of natural enemies to a newly infested habitat (Van Driesche and Bellows 1996; Kogan *et al.* 1999). The ability of omnivores to persist can also help to reduce the economic and logistic costs associated with biological control. Specifically, strategies for the release of natural enemies are based largely on costs associated with rearing and release (Grevstad 1999). Knowing that the natural enemy being released has multiple strategies to ensure its own survival reduces some of the economic risk associated with biological control.

The ability of omnivores to persist in habitats where one food type is absent is dependent on a number of factors. First, there are different 'degrees' of omnivory, ranging from obligate omnivores that require both plant and prey for successful development, reproduction or survival, to opportunistic omnivores that only consume alternative foods when the opportunity to do so presents itself (Coll and Guershon 2002). When one type of food is absent, obligate omnivores are less likely to persist than omnivores that have more flexible diet requirements. Second, the survival of omnivores on a single food type depends on the species of plant or prey available for consumption. For example, when *Dicyphus hesperus* Knight (Hemiptera: Miridae) nymphs were reared in the absence of prey, 34% of nymphs reared on mullein (*Verbascum thaspus* L., Lamiales: Scrophulariaceae) survived to the adult stage, compared to only 4.6% of nymphs reared on catnip (*Nepeta cataria* L., Lamiales: Lamiaceae), and 2.2% of nymphs

reared on sweet pepper (*Capsicum annuum* L., Solanales: Solanaceae) (Sanchez *et al.* 2004). Third, persistence of omnivores may depend on the developmental stage of the omnivore when one of its food sources becomes scarce or absent. For example, first instar nymphs of some predaceous Hemiptera may be limited to feeding exclusively on plant material, at least for the first few hours or days after hatching (Parker 1981; Coll and Guershon 2002; Groenteman *et al.* 2006). A shortage of prey during this time would not have a significant impact on development and survival. However, if the shortage persists, then negative impacts on development and survival may be observed. Finally, the quality of the food available for feeding when one type of food is absent may impact the survival and persistence of omnivores. Nitrogen, for example, affects the survival and development of juvenile insects (Mattson 1980; Hunt *et al.* 1992; Jauset *et al.* 1998; Brodbeck *et al.* 2001).

The tendency of a biological control agent to remain in the habitat where it was released is also an important consideration in designing effective biological control programs. Residence time within a habitat patch is generally modeled as an optimal behaviour, such that an animal will only remain in the patch so long as it gains some fitness benefit from doing so (Krebs and Davies 1993). Following this theory, as resources become scarce or the quality of a resource is diminished the likelihood of leaving the patch should increase (Charnov 1976; Charnov *et al.* 1976). Both prey and plant resources available in a patch should influence the patch residence time decisions of omnivores. VanLaerhoven *et al.* (2006) investigated the patch residence time of *D. hesperus* in habitats that varied in terms of both the species of host plant and prey available and found that both resources were important predictors of patch residence time. Therefore, it is reasonable to predict that in a monoculture cropping system, the quality of the plant resource and prey resource will also interact to influence the patch retention time of an omnivore.

The objective of the present study was to determine the effects of plant and prey quality on the development, survival, and patch residence time of *D. hesperus*, an omnivore and biological control agent of greenhouse whitefly, *T. vaporariorum* (McGregor *et al.* 1999). Female *D. hesperus* do exhibit an oviposition preference for plants that receive greater concentrations of nitrogen in inorganic fertilizer solutions

(Chapter 2). Therefore, development and survival data collected from the present experiments can be used to further investigate if *D. hesperus* follow the predictions of optimal oviposition theory (Jaenike 1978), which has been tested for many herbivores (Thompson 1988; Gripenberg *et al.* 2010), and for some predators (Lundgren and Fergen 2006), but has not been widely tested for omnivores. This information can also be used to predict how plant vigor (Price 1991) and plant stress (White 1984) might impact populations of *D. hesperus* in greenhouses, and improve our knowledge regarding the persistence and residence time of *D. hesperus* in tomato crops when prey and plant quality vary.

To meet our objectives, three experiments were designed in which developing *D. hesperus* nymphs had access to plant and prey foods of varying quality in different conditions. In the first experiment (*Effect of prey quality*) *D. hesperus* nymphs were reared individually in laboratory conditions to determine the effect of a) prey quality, and b) plant availability on development time and survival. For all experiments, a survivor was defined as any nymph that reached the adult stage. In the second experiment (*Effect of plant quality – no prey*) *D. hesperus* nymphs were reared on whole tomato plants that varied in quality to determine the effect of plant nitrogen variation on nymph development and survival. The third experiment (*Effect of plant quality – with prey*) was similar to the second experiment, with the exception that small amounts of prey were provided during the early stages of development. In ephemeral agroecosystems, natural enemy interactions with their prey are often interrupted, resulting from removal of host plants at harvest, or the rapid decline and recovery of pest populations during the growing season (Wiedenmann and Smith 1997). The ability of a natural enemy to persist in an ephemeral habitat may be negatively affected as a result of these factors (Wiedenmann and Smith 1997). Therefore, the goal of the third experiment was to investigate how varying plant quality might influence the persistence of *D. hesperus* when its prey become absent during the course of development. In all of these experiments, the accumulated degree-days required for development and the survival of each insect included in the experiment was measured. Using this information, we tested three predictions for each of the three experiments. First, we predicted that *D. hesperus* nymphs fed high quality plant or prey material would develop faster than nymphs fed low

quality plant or prey material. Rate of juvenile development is important, as juvenile insects are considered to be at greater risk of predation or exposure to disease than adults (Clancy and Price 1987; Lill and Marquis 2001). When feeding on low quality host plants, or host plants with many chemical defenses, the length of juvenile development and juvenile mortality increase (Lindroth and Bloomer 1991). Therefore, quicker development on high quality prey is considered to be beneficial, even if faster growth is associated with smaller adult body size and lower fecundity (Awmack and Leather 2002). Second, we predicted that the survival rate of *D. hesperus* nymphs would be higher when nymphs were fed high quality plant or prey material. Finally, we predicted that the probability of *D. hesperus* nymph survival over time, estimated using survivor functions, would vary when the quality of plant and prey food varied.

We also conducted a fourth experiment to determine the effects of plant and prey quality (and the potential interaction of both factors) on the patch residence time of *D. hesperus* adults. For this experiment, we predicted that both plant and prey quality would influence the patch residence time of *D. hesperus* adults, such that adults would be most likely to remain on high quality host plants with high quality prey for longer periods of time compared to low quality host plants with low quality prey.

Materials and Methods

Insects and plants

All insects used in the experiments described herein originated from a rearing colony of *D. hesperus* maintained at the University of Windsor as described in Chapter 2. Insects in the colony are held on *Nicotiana tabacum* L. (Solanales: Solanaceae) plants and fed flour moth eggs (*Ephestia kuehniella* Zeller, Lepidoptera: Pyralidae) (Beneficial Insectary Inc., Guelph, Ontario, Canada) (McGregor *et al.* 1999; Sparkes 2012; Chapter 2). For all development time experiments, first instar *D. hesperus* nymphs were collected from *N. tabacum* plants in the rearing colony within 24 h of nymph emergence (following McGregor *et al.* 1999; Gillespie and McGregor 2000; Gillespie *et al.* 2004), using a fine-bristled paintbrush. To ensure that all nymphs were less than 24 h old when experiments were initiated, all emerged nymphs were cleaned from the *N. tabacum* plant the day before nymphs were collected for the experiment. Adult female *D. hesperus*, seven to ten

days old, were used in the patch residence time experiment. These females were collected from the rearing colony using an aspirator and isolated in 60 ml plastic cups (Solo Cup Company, Lake Forest, USA) with only a source of water for 48 h. The purpose of the isolation period was to standardize the level of hunger between individuals prior to release, as hunger levels influence certain behaviours of *D. hesperus* including prey consumption rate (Gillespie *et al.* 2012).

'Patio Hybrid' tomato plants, *Solanum lycopersicum* L. (Solanales: Solanaceae) were used as the host plant in all experiments. All tomato plants were grown from seed in the greenhouse at the University of Windsor, using seeds from Stokes Seeds Canada (Thorold, Ontario, Canada). Details regarding plant propagation are available in Chapter 2 and are only briefly summarized here. To manipulate plant quality, transplanted tomato seedlings were haphazardly assigned to one of four nitrogen (N)-fertilizer treatments: 50, 100, 200, or 400 ppm N. Fertilizer solutions were mixed using 6-11-31 Hydroponic Fertilizer and 15.5-0-0 calcium nitrate (Plant-Prod® Canada, Brampton, ON, Canada). Fertilizer was applied daily for seven days following transplantation, and every second day thereafter until the plants were destroyed. Each seedling received 100 ml of the appropriate solution at each fertilizer application (after Jauset *et al.* 1998).

Trialeurodes vaporariorum nymphs were used as prey in all experiments where prey quality was manipulated. 'High' quality prey items were defined as whitefly nymphs reared on 400 ppm N-fertilized tomato plants and 'low' quality prey were those reared on 50 ppm N-fertilized tomato plants. This method of manipulating prey quality was selected because diet affects both the physiological and morphological characterisics of developing herbivores in natural settings where plant nutrient levels, especially N levels, vary across time and space (Mattson 1980). Importantly, *T. vaporariorum* and other species of whitefly are known to respond positively to host plants that are N-rich in terms of a number of life history traits, including developmental rate (Bentz and Larew 1992; Blua and Toscano 1994; Jauset *et al.* 1998; Blackmer and Byrne 1999; Bi *et al.* 2003). Whiteflies were reared in 55 x 55 x 60 cm cages constructed using metal dowels and white mesh. The cages, containing either 50 or 400 pmm N-fertilized tomato plants, were placed in the greenhouse and *T. vaporariorum* nymphs were collected by hand from *N. tabacum* plants in the same greenhouse to initiate colonies in each cage (see Chapter 5).

Effect of prey quality

One hundred and twenty first instar nymphs of *D. hesperus* were collected from the rearing colony and haphazardly assigned to one of six treatment groups (n = 20) in a full factorial design with three levels of prey quality (high, low, and no prey), with or without 200 ppm N-fertilized tomato leaflets present (Table 3.1). This level of fertilization is roughly equal to the recommended rate of fertilization for greenhouse tomatoes (AARD 2009). Tomato leaflets were harvested from plants that had been fertilized for at least 24 d. The stem of each leaflet was inserted into a ½ dram glass vial (Fisher Scientific Co., Pittsburgh, USA) that was sealed with parafilm (Pechinay Plastic Packaging, Chicago, USA), and filled with water using a syringe. Nymphs provided with tomato leaflets were held in 250 ml glass Mason jars (Bernardin Ldt., Richmond Hill, Ontario, Canada), as the jars were large enough to accommodate the tomato leaflet and the ½-dram vial. Nymphs without leaflets were difficult to find in the jars and as a result nymphs were often killed accidentally during handling. Therefore, those nymphs were reared inside 60 ml plastic cups that were lined with filter paper and held a small piece of wet cotton to maintain internal humidity. All *D. hesperus* nymphs in treatment groups with prey were fed three whitefly nymphs daily. All whitefly nymphs were of the late fourth instar, which are characterized by the appearance of red eyes (Byrne and Bellows 1991). Whitefly nymphs were collected using a fine-bristled paintbrush from either of the high or low quality whitefly rearing colonies to avoid significant damage to the prey. *Dicyphus hesperus* nymphs were observed daily, and the date of each molt, of death, or of adult emergence was recorded (after McGregor *et al.* 1999; Gillespie and McGregor 2000; Gillespie *et al.* 2004). Nymphs that survived were those that reached the adult stage.

Effect of plant quality – no prey

Dicyphus hesperus nymphs, less than 24 h old were placed inside clip-cages on whole tomato plants in the greenhouse. Clip-cages were handmade using 5.5 x 5.5 cm cardboard squares covered with white felt and a 50 mm diameter Petri dish. Small holes were drilled along the edges of the Petri dishes and covered with white fabric to allow air circulation. Leaves of whole tomato plants were pressed between the felt and the Petri dish and cages were secured in place with elastic bands. Three clip cages, holding one nymph each, were

placed on each plant and 12 plants from each of the four N-fertilizer treatments were used in the experiment. Nymphs were observed daily and the date upon which each *D. hesperus* nymph molted, emerged as an adult, or died was recorded (following McGregor *et al.* 1999; Gillespie and McGregor 2000; Gillespie *et al.* 2004).

Effect of plant quality – with prey

Dicyphus hesperus nymphs were reared inside clip-cages on whole plants as described above, with the exception that nymphs were provided with prey food on Days 1, 4, 7, and 10 of the experiment. *Ephestia kuehniella* eggs were provided as prey. On days that nymphs were fed, five eggs were placed onto the surface of the tomato leaf inside the clip-cage using a paintbrush. Data was collected and recorded following McGregor *et al.* (1999), Gillespie and McGregor (2000) and Gillespie *et al.* (2004), as described above.

Patch residence time

The effect of prey quality and plant quality, manipulated as described above, on the patch residence time of female *D. hesperus* was tested in cages in the greenhouse using a full factorial experimental design with three levels of whitefly prey quality (no prey, high, and low) and four levels of plant quality (50, 100, 200, and 400 ppm N-fertilized). Each cage (55 x 55 x 60 cm) contained three tomato plants from the same N-fertilizer treatment that had been fertilized for at least 24 d and had six to 10 true leaves but were not flowering. Inside each cage, the three plants were placed in a diagonal line from the back left-hand corner to the front right-hand corner, such that the leaves of neighbouring plants did not touch (VanLaerhoven *et al.* 2006). On the day that each replicate was initiated, 50 to 60 whitefly nymphs of either high or low quality were placed on a lower leaf of the central tomato plant in the cage. No prey was added to control cages. One female that had been isolated for 48 h, as described above, was transferred onto the leaf with the prey patch using forceps. All replicates were initiated before noon and checked daily at approximately the same time (after VanLaerhoven *et al.* 2006), based on the diurnal changes in activity levels *D. hesperus* (VanLaerhoven *et al.* 2003). Every second day the patches of whitefly prey were replenished to prevent patch depletion (after VanLaerhoven

et al. 2006). The date on which each trial was initiated and the date on which the insect was first observed off of the central plant was recorded for analysis.

Accumulated degree-day calculations

Accumulated degree-days (ADD) were used to standardize the time required for development based on daily temperatures, as the developmental rate of insects is temperature dependent (Gullan and Cranston 2005). Temperature was recorded hourly using Smartbutton data loggers (ARC Systems Inc., Surrey, British Columbia, Canada) for the duration of all development time (and patch residence time) experiments. Using this information, the daily degree-day (°d) contribution to *D. hesperus* development was calculated using: $°d = [(T_{max} + T_{min})/2] - T_{base}$, where T_{max} and T_{min} are the daily maximum and minimum temperatures and T_{base} is the developmental threshold temperature for the species in question (from McMaster and Wilhelm 1997). Developmental threshold temperatures used for calculations were 8.01°C for males and 7.8°C for females (Gillespie *et al.* 2004). The total (or accumulated) °d required for development of each *D. hesperus* nymph was calculated by adding the °d values for each day of its developmental period using thermal summation (Gullan and Cranston 2005). Accumulated °d were calculated for the entire developmental period (first instar to adult emergence), and for each of the four instars. Development of a specific instar was defined as the period between the first day that a given instar was observed until the day before the exoskeleton from the molt to the subsequent instar was found. This definition avoided overlap between instars and prevented overestimation of ADD. For nymphs that reached adulthood, ADD was calculated using gender specific threshold values. If nymphs died before reaching adulthood, gender average °d-values were used for thermal summation.

Statistical analysis

Three predictions were tested for all three of the development time experiments. First, analysis of variance (ANOVA) was used to determine if there were effects of plant and prey treatments on the time required for development of *D. hesperus* nymphs (for each instar and for development to adulthood). For these analyses, the GLM procedure was used (SAS Institute 2009), where $\alpha = 0.05$. Type III sums of squares were reported (SAS

Institute 2009), as the different treatment groups had unequal sample size (n) at the later stages of development due to death of the nymphs. Where plant or prey quality effects were observed, differences between levels of plant and prey quality were explored using the PDIFF function with the ADJUST=BON statement to return Bonferroni corrected *post hoc* results that were compared to $\alpha = 0.05$ (SAS Institute 2009). Prior to ANOVA analysis, the normality of the data for each treatment group (Shapiro-Wilks test) and equality of variance (Levene's test) were assessed (SAS Institute 2009). If the data failed to meet the assumptions after transformation, then the Kruskal-Wallis non-parametric equivalent of ANOVA was performed using the NPAR1WAY procedure (SAS Institute 2009). For any transformed data, results are summarized using back-transformed values.

To test our prediction that nymph survival would be greatest when nymphs were fed high quality plant or prey material, the proportion (or number) of *D. hesperus* nymphs that survived to the adult stage and the proportion of nymphs that died were calculated. Some nymphs reared on whole plants escaped their clip-cages; these lost nymphs were excluded from the calculation, as their fate could not be determined. In the laboratory study, no nymphs were lost so the counts of survivors and non-survivors could be compared. The proportions (or number) of survivors were compared between treatments using chi-square analysis in SAS using PROC FREQ (SAS Institute 2009). Significant results were further investigated using the subdividing procedure (Zar 2010). The CATMOD procedure, which allows for the analysis of a two-factor experiment with count data (SAS Institute 2009) was used to determine the effect of prey quality, plant availability, and the interaction therein, on the survival of nymphs reared in the laboratory (*Effect of prey quality* experiment). For all chi-square analyses, *p*-values were compared to $\alpha = 0.05$.

To determine the effects of plant and prey quality on the probability of nymph survival over time, the survivor functions of the nymphs were generated and the slopes of the functions were compared using the LIFETEST procedure (SAS Institute 2009). This analysis uses information regarding the time of death and/or survival of all individuals in a population to describe the probability of survival of individuals in the population sampled (SAS Institute 2009). This analysis is similar to the Cox Proportional Hazard model (Cox and Oakes 1984). For our analysis, non-parametric estimates of the survivor

function were calculated for each treatment group in each experiment using the Kaplan-Meier method, and the resultant survivor functions of each treatment group were compared using a log-rank chi-square test (SAS Institute 2009). Where significant differences in the survivor functions for different treatments were observed ($p < \alpha = 0.05$), differences between the survivor functions were identified using the ADJUST=SIDAK command in the STRATA line of the model (SAS Institute 2009). This command performs all possible paired comparisons and returns Šidák adjusted p-values that can be compared to $\alpha = 0.05$ (SAS Institute 2009). For the laboratory experiment (*Effect of prey quality* experiment) the DIFF=CONTROL('TREATMENT') command was used to specify Treatment 2 (no prey, plant) treatment as the control and perform all possible comparisons between Treatment 2 and the other treatments (SAS Institute 2009).

To determine the effects of plant and prey quality on the probability of *D. hesperus* females remaining in a patch over time, the LIFETEST procedure (SAS Institute 2009) was used as described above. The time spent on the central plant (patch) was compared between treatments in terms of the number of days that each insect spent in the patch. In addition, two-factor ANOVA was used to determine if plant quality, prey quality, or the interaction therein, had an effect on patch residence time (SAS Institute 2009). A power analysis was also conducted for this experiment using Equation 11.23 described by Zar (2010), which calculates ϕ using the number of treatment groups (k=12 for this experiment), and the mean square error and mean square group values from the ANOVA analysis. Once ϕ is known, it is used to estimate the power of the analysis based on the numerator and denominator degrees of freedom (11 and 228, respectively, for this experiment) (Zar 2010).

Results

Effect of prey quality

Nymphs in Treatment 1 (no prey, no plant) survived for 3.05 ± 0.25 d (mean ± SE). None of the nymphs in this treatment survived to the second instar. Nymph mortality was also high in Treatment 2 (no prey, plant); nymphs that died lived for 12.00 ± 1.97 d. Due to high mortality in Treatment 1, Treatments 1 and 2 (both with no prey provided) were not

included in the ANOVA analysis. Some of the Treatments did not meet the assumptions of parametric ANOVA so the raw data was transformed using the square root transformation. The quality of whitefly prey did not affect the ADD required for development of any of the four instars or development from the first instar to the adult stage, and no significant interaction effects of prey quality and plant availability were observed (Table 3.2). The availability of plant material did effect the development of *D. hesperus* nymphs (Table 3.2), except for development of second instar nymphs (Table 3.2). When plant material was available, nymphs developed more quickly than when plant material was not available (Table 3.3).

The number of nymphs that survived to emerge as adults in Treatments 2, 3, 4, 5, and 6 was different ($\chi^2 = 15.9655$, df $= 4$, $p = 0.0031$). The chi-square test was subdivided to determine which treatments had more nymphs emerge than the others; the fewest nymphs reached the adult stage in Treatment 5 (low quality prey, no plant; Table 3.1). Contingency table analysis was used to determine the effects of plant availability and prey quality on survivorship. Because there was no survival in Treatment 1, both Treatments 1 and 2 needed to be removed to keep this analysis balanced. Prey quality ($\chi^2 = 5.05$, df $= 2$, $p = 0.0246$) and plant availability ($\chi^2 = 65.17$, df $= 1$, $p < 0.0001$) affected the number of nymphs that survived to adulthood. There was also an interaction between the two factors ($\chi^2 = 5.21$, df $= 1$, $p = 0.0225$). When plant material was available during development, nymph survivorship was equal regardless of prey quality. In the absence of plant material, survivorship was greater when nymphs were provided with high quality prey (Figure 3.1).

The probability of nymph survival over time differed between the six treatments (log-rank $\chi^2 = 179.2145$, df $= 5$, $p < 0.0001$). Nymphs in Treatment 1 (no prey, no plant) survived for the least amount of time, with the probability of survival dropping to zero before nymphs accumulated 100 °d (Figure 3.2); the survival function of this treatment was significantly different from those of the other five treatments (Šidák adjusted $p < 0.0001$ for all paired comparisons; Figure 3.2). To further analyze the probability of nymph survival, Treatment 2 (no prey, plant) was specified as the 'control' and compared to the other treatments. The survival function of the nymphs in Treatment 2 was marginally different from that of nymphs in Treatment 3 (high prey, no plant; $\chi^2 =$

7.4391, df = 1, p = 0.0315), Treatment 4 (high prey, plant; χ^2 = 6.7357, df = 1, Šidák adjusted p = 0.0464), and Treatment 6 (low prey, plant; χ^2 = 6.9869, df = 1, Šidák adjusted p = 0.0404), but there was no difference between Treatment 2 and Treatment 5 (low prey, no plant; χ^2 = 0.5599, df = 1, Šidák adjusted p = 0.4543) (Figure 3.2).

Effect of plant quality – no prey

Plant quality did not affect the development of first instar nymphs ($F_{3,42}$ = 2.49, p = 0.0732), second instar nymphs (χ^2 = 1.6653, df = 3, p = 0.6447), or fourth instar nymphs ($F_{3,6}$ = 0.33, p = 0.8026) when nymphs were reared in clip-cages on whole plants with no prey provided (Table 3.4). Plant quality did affect the ADD required for third instar development ($F_{3,21}$ = 4.40, p = 0.0150; Table 3.4). Finally, plant quality did not affect nymph development from the first instar to the adult stage ($F_{3,6}$ = 0.56, p = 0.6605; Table 3.4).

In this experiment, 144 *D. hesperus* nymphs were reared on whole tomato plants without prey. Of those nymphs, 115 died, 18 were lost, and 12 nymphs survived to the adult stage. Plant quality did not affect the proportion of nymphs that reached the adult stage (χ^2 = 7.0206, df = 3, p = 0.0712), nor did plant quality affect the probability of nymph survival over time (log-rank χ^2 = 1.3784, df = 3, p = 0.7106; Figure 3.3).

Effect of plant quality – with prey

When nymphs were provided with *E. kuehniella* eggs on Days 1, 4, 7, and 10, plant quality had no effect on the development of first instar ($F_{3,44}$ = 0.10, p = 0.9593), second instar ($F_{3,44}$ = 1.13, p = 0.3465), third instar ($F_{3,44}$ = 0.73, p = 0.5375), or fourth instar ($F_{3,19}$ = 1.33, p = 0.2945) *D. hesperus* nymphs when all nymphs were included in the analysis (Table 3.4). The total development time of these nymphs was not affected by plant quality ($F_{3,19}$ = 2.24, p = 0.1166). Only one nymph died before Day 10; the remainder of the nymphs that did not reach adulthood survived for two to 18 days after feeding ceased. When only nymphs that survived to the adult stage were included in the analysis, plant quality did not affect development time of first instar ($F_{3,19}$ = 0.10, p = 0.9578), second instar ($F_{3,19}$ = 0.46, p = 0.7157), or fourth instar ($F_{3,19}$ = 1.33, p = 0.2945) nymphs. The total development time of surviving nymphs was not affected by plant

quality ($F_{3,19} = 2.24$, $p = 0.1166$). Plant quality did affect the development time of the third instar nymphs that emerged as adults ($F_{3, 19} = 6.28$, $p = 0.0038$), such that nymphs reared on 50 and 400 ppm N-fertilized tomato plants developed fastest (Table 3.4). Most nymphs had reached the third instar when feeding ceased.

Of the 144 nymphs included in this experiment, six were lost and 33 survived to adulthood when fed *E. kuehniella* eggs on Days 1, 4, 7, and 10. The proportion of nymphs that survived to the adult stage was not different between the four levels of plant quality ($\chi^2 = 6.8864$, df = 3, $p = 0.0756$). In the first 200 ADD after feeding ceased, 79.67% of the nymphs died and 20.33% lived to the adult stage. Of the 33 nymphs that reached the adult stage, 25 molted in the first 200 ADD after feeding ceased; these individuals accounted for 88, 80, 44, and 91% of the total number of adults that emerged from nymphs reared on 50, 100, 200, and 400 ppm N-fertilized plants, respectively. Percent adult emergence was not equal across the four levels of plant quality ($\chi^2 = 18.0420$, df = 3, $p = 0.0004$), with the lowest percentage emerging from 200 ppm N-fertilized tomato plants ($\chi^2 = 17.8922$, df = 1, $p < 0.001$). Conversely, of the nymphs that reached the adult stage, 13, 20, 56, and 9% of the total number of adults that emerged from nymphs reared on 50, 100, 200, and 400 ppm N-fertilized tomato plants did so after accumulating more than 200 ADD. These percentages were not equal ($\chi^2 = 56.5306$, df = 3, $p < 0.001$); a significantly greater percentage of adults emerged when nymphs were reared on 200 ppm N-fertilized tomato plants ($\chi^2 = 54.00$, df = 1, $p < 0.001$).

The probability of nymph survival over time was not affected by plant quality over the course of the entire experiment (log-rank $\chi^2 = 5.3949$, df = 3, $p = 0.1451$). When survivor functions were calculated from Day 10, when feeding ceased, until the last nymph molted or died, plant quality did not affect the probability of nymph survival over time (log-rank $\chi^2 = 4.6380$, df = 3, $p = 0.2003$; Figure 3.4).

Patch residence time
Across the 12 treatments in the experiment, the shortest mean (± SE) residence time was 3.75 ± 0.73 d and the longest mean residence time was 8.60 ± 1.38 d. The mean (± SE) patch residence times for all treatments are given in Table 3.5. The probability of adult *D. hesperus* remaining in a given habitat or patch over time was not affected by prey quality

(log-rank χ^2 = 4.0507, df = 2, p = 0.1320; Figure 3.5), or plant quality (log-rank χ^2 = 1.0778, df = 3, p = 0.7824; Figure 3.6). The number of days that adult *D. hesperus* remained in a given habitat patch was not affected by plant or prey quality, as there were no differences in residence time between the 12 treatments included in this experiment ($F_{11,228}$ = 0.98, p = 0.4690). Subsequently, there was no effect of prey quality ($F_{2,228}$ = 2.06, p = 0.1321), or plant quality ($F_{3,228}$ = 0.39, p = 0.7452) and there was no interaction of these factors ($F_{6,228}$ = 0.91, p = 0.4907). The mean (± SE) patch residence time of all 240 female *D. hesperus* included in the experiment was 6.08 ± 0.35 d. This experiment had k=12 treatment groups, with 11 numerator and 228 denominator degrees of freedom. For the ANOVA, ϕ =0.9459, based on this value, the power of this analysis was approximately 0.55.

Discussion

Predictions regarding the amount of time that a biological control agent will remain in a habitat patch may be helpful in predicting the success of a biological control program. VanLaerhoven *et al.* (2006) observed that both the species of prey and plant available affected the patch residence time of *D. hesperus*. In their study, females remained on tomato plants for 4.4 ± 0.29 d and remained in patches of whitefly prey for 3.3 ± 0.18 d (VanLaerhoven *et al.* 2006). The 240 female *D. hesperus* included in our study remained on the tomato plant where they were released for 6.08 ± 0.35 d, almost two days longer than observed by VanLaerhoven *et al.* (2006). In the current experiment, the patch residence time of *D. hesperus* was not affected by plant quality or prey quality, however, the power of the analysis to detect differences between the 12 treatment groups in this experiment was only 0.55. To increase the power of this analysis, the simplest approach would be to increase the sample size, with the expectation that in doing so the variation within the treatment groups would be reduced and the variation among treatment groups would increase (Zar 2010). An alternative approach would be to decrease the number of treatment groups (k), as power decreases as k increases (Zar 2010). Increasing the sample size of the present experiment would be difficult due to logistical constraints including time and space, and the number of tomato plants and insects that would be required. In this experiment, the variation in patch residence time

between individual insects within each treatment was greater than expected. This should be considered in future attempts to assess the patch residence time of *D. hesperus* when prey and plant quality vary.

The development of third instar *D. hesperus* nymphs was influenced by plant quality when nymphs were reared on whole tomato plants in the greenhouse, both when no prey was provided and when nymphs were provided with *E. kuehniella* eggs until Day 10. In both experiments, development time was shortest on plants of the highest (400 ppm N-fertilized) and lowest quality (50 ppm N-fertilized), and longest on plants of intermediate quality (100 and 200 ppm N-fertilized). These results both support and contradict the results of previous studies regarding omnivore development on host plants of varying N nutrition. For example, Groenteman *et al.* (2006) reported that *Orius albidipennis* (Reuter) (Hemiptera: Anthocoridae) nymphs completed first instar development approximately 18 days earlier when nymphs were reared on high N cotton plants (*Gossypium hirsutum* L., Malvales: Malvaceae), relative to low N cotton plants. Following their results, one would expect that *D. hesperus* nymphs reared on low N plants would require more time to develop, however, in our experiments, this was not observed. Increased rates of development on both high and low N-fertilized tomato plants provides support for both the plant vigor hypothesis (Price 1991) and the plant stress hypothesis (White 1984), suggesting that nymphs of this omnivore benefit from feeding on both N-stressed plants and plants with surplus N. Therefore, both hypotheses may be used to predict the development and potentially the population growth of *D. hesperus*.

In the laboratory experiment where prey quality effects on development were tested, we observed that development was faster when tomato leaves were present relative to when tomato leaves were absent for all instars except the second instar. Gillespie and McGregor (2000) observed a similar result in experiments where nymphs were provided with *E. kuehniella* eggs and either tomato leaves or water. In their study, nymphs developed through the first, third, and fourth instars approximately one day faster when tomato leaves were available relative to when tomato leaves were absent, depending on the gender of the nymph, but no effect on second instar development was observed (Gillespie and McGregor 2000). This is an interesting result that appears to be consistently observed for nymphs of this species. The feeding habits of juvenile insects

may aid in explaining our observations. Many juvenile insectsincrease their rate of food consumption as their developmental stage increases, with the penultimate and final instars consuming significantly more units of food than early instars (Raman *et al.* 1994; Schoonhoven *et al.* 2005). Contrary to this observation, some predatory nymphs have been observed to consume significantly more prey at earlier instars than at later instars. For example, nymphs of the omnivorous thrips, *Frankliniella schultzei* Trybom (Thysanoptera: Thripidae) consume more prey during the course of second instar development than at any other developmental stage (Milne and Walter 1997). The voracious appetite of second instar nymphs for prey food may be the result of the inability of first instar nymphs to consume prey (Parker 1981; Coll and Guershon 2002; Groenteman *et al.* 2006). Ability to utilize prey and plant hosts is limited by the physical ability of predators and herbivores, and switching between diet types during development is commonly observed as a result (Coll and Guershon 2002). If first instar *D. hesperus* nymphs are limited to plant feeding, then the absence of a plant effect on development of the second instar might be explained by an increase in prey feeding at that stage. To our knowledge, the ability of first instar *D. hesperus* to consume prey has not been investigated. However, in the experiment described by Gillespie and McGregor (2000), nymphs had *ad libitum* access to prey during development in all treatment groups, and the second instar was the only instar unaffected by tomato availability, lending some support to this theory. In our experiment, only three prey items were provided each day and none of the prey was removed. Therefore, plant availability may not have had an effect on the development of second instar *D. hesperus* nymphs because those individuals had extra prey available for consumption compared to third and fourth instar nymphs. An observational study to determine the prey and plant feeding abilities of *D. hesperus* at each instar would improve our understanding of its development in the presence and absence of plant material.

Although tomato plant quality did have an effect on the development of *D. hesperus* nymphs, no difference in the survivorship of nymphs between the four levels of plant quality was observed when nymphs were reared on whole tomato plants without prey. In this experiment, survival ranged from 3.1 to 14.3% when nymphs were reared on whole tomato plants of varying quality. Similarly, the survival of *D. hesperus* nymphs to

the adult stage was not affected by plant quality when nymphs were reared on whole tomato plants and provided with prey until Day 10. In this experiment, survivorship ranged from 14.3 to 33.3% when nymphs were reared on tomato plants of varying quality. Based on previous work, where authors recorded no survival of *D. hesperus* nymphs beyond the fourth instar when reared on tomato plants without prey (Sanchez *et al.* 2004), we expected that none of the nymphs would survive to the adult stage when prey was absent. The survivorship of *D. hesperus* nymphs on tomato plants without prey in our experiment was greater than the survivorship of *D. hesperus* nymphs reared on catnip and pepper without prey, but not greater than the survivorship of nymphs reared on mullein without prey, as reported by Sanchez *et al.* (2004).

The greenhouse where our tomato plants were grown and where our experiments were conducted is infested with *T. vaporariorum*. Therefore, although tomato leaves were inspected with the naked eye and clip-cages were only installed on tomato leaves without whitefly nymphs, some prey residues may have been present and subsequently utilized by *D. hesperus* nymphs in all experiments. However, the clip-cages remained on the same tomato leaf for the duration of the development of each nymph, except in rare cases where the tomato leaf senesced and the clip-cage needed to be moved. If prey was present on the leaves inside the clip-cages, it would have been utilized early in development, following that, nymphs would have only had access to plant material for feeding. As such, both of the experiments conducted on whole plants should adequately represent the development and survival of *D. hesperus* nymphs with limited prey early in development. Taken together, these two experiments provide support for the prediction that *D. hesperus* is able to persist in habitats without prey, which is generally predicted for omnivores (Bugg *et al.* 1987; Naranjo and Gibson 1996; Settle *et al.* 1996; Wiedenmann *et al.* 1996; Coll and Guershon 2002). Our results also suggest that development of *D. hesperus* nymphs is possible on tomato plants when prey is limited. Some authors predict that development of omnivores in the absence of prey may limit the reproductive ability of emerging adults (Coll and Guershon 2002), as prey shortages during the adult stage do (De Clercq and Degheele 1992). We did not assess the reproductive capacity of the individuals that molted to the adult stage in our experiments; this would be an interesting avenue of inquiry for future work on this system.

The probability of nymph survival, and chances of adult emergence on tomato plants in this experiment was approximately equal for all levels of plant quality. However, when *D. hesperus* nymphs were fed until Day 10, an interesting effect of plant quality was observed after Day 10. Specifically, in the first 200 ADD after Day 10, the vast majority of nymphs either died or molted to the adult stage. During this period, significantly fewer nymphs than expected emerged as adults when reared on 200 ppm N-fertilized tomato plants. The nymphs reared on 200 ppm N-fertilized tomato plants that did not emerge as adults before accumulating 200 °d required upwards of 400 °d before adult emergence, but also enjoyed a higher rate of adult emergence after accumulating more than 200 °d compared to nymphs from the other levels of plant quality. This observation is important in light of the fact that the recommended fertilizer rate for tomatoes grown in greenhouses is approximately 200 ppm N (AARD 2009). *Dicyphus hesperus* nymphs reared on 200 ppm N-fertilized tomatoes may require more time to develop, but are also more likely to emerge as adults after an extended juvenile stage, in the absence of prey.

In the laboratory, the probability of nymph survival was affected by the combination of plant and prey material available for consumption. For example, nymphs had similar chances of survival when provided with only prey reared on high N tomato plants and when provided with plant and prey material simultaneously. When only tomato leaves were available for consumption, the survival function of those nymphs was similar to that of nymphs fed only prey reared on low quality tomato plants. In previous work with *D. hesperus*, McGregor *et al.* (1999) observed that nymphs reared on excised tomato leaves did not survive to the second instar in the absence of prey. In our experiment in the laboratory, nearly 50% of the nymphs reared on excised tomato leaves survived to the adult stage without consuming prey. Both our experiment and that of McGregor *et al.* (1999) were conducted under controlled laboratory conditions, with mean daily temperatures that differed by no more than 1 or 2°C. One difference between our study and that of McGregor *et al.* (1999) was the variety of tomato plant used; we used 'Patio Hybrid' tomatoes, while McGregor *et al.* (1999) used 'Variety Trust' tomatoes. Differences in plant handling or tomato plant variety may have contributed to the difference in results between these two studies. When whitefly prey was available, the

survivorship of nymphs in this experiment was very similar to that of *D. hesperus* nymphs fed *E. kuehniella* eggs as in the experiment described by McGregor *et al.* (1999). Similar to McGregor *et al.* (1999), but contrary to our results, Sanchez *et al.* (2004) also observed poor survivorship of *D. hesperus* nymphs reared on tomato plants in the absence of prey. Sanchez *et al.* (2004) also controlled the temperature and photoperiod under which *D. hesperus* nymphs were held, however, in their experiment, nymphs were held on whole tomato plants, rather than on excised tomato leaves, as in our experiment and that of McGregor *et al.* (1999). Induced responses of whole tomato plants to feeding by *D. hesperus* may explain the differences between our results and those of Sanchez *et al.* (2004), as Agrawal *et al.* (1999) observed that induced responses in cotton due to herbivory reduced plant feeding by omnivores, even in the absence of prey.

Based on the oviposition preferences of female *D. hesperus* for whole tomato plants grown under different N-fertilizer regimes (Chapter 2), and the predictions of optimal oviposition theory (Jaenike 1978; Thompson 1988), we expected that *D. hesperus* nymphs would develop fastest, and enjoy significantly greater survivorship on 200 and 400 ppm N-fertilized tomato plants. Although we did observe that high quality tomato plants increased the rate of development of *D. hesperus* nymphs, low quality plants also increased their rate of development, yet low quality plants were the least preferred oviposition hosts (Chapter 2). Therefore, female *D. hesperus* preference for high quality plants follows the predictions of optimal oviposition theory and their lack of preference for low quality plants is contradictory to optimal oviposition theory. It has commonly been observed in the literature regarding oviposition theory that some aspects of offspring performance correlate well to female preference while others do not (Thompson 1988; Mayhew 1997). This is a key criticism of tests of optimal oviposition theory. It would be helpful to investigate other metrics of offspring fitness not measured in the current study to see how those correlate to female preference for oviposition hosts. For example, the fecundity of emerging females or longevity of the adult stage of the offspring should be investigated.

The results of these experiments provide important insight to the development of omnivores when plant and prey hosts vary in quality. In greenhouses where *D. hesperus* are employed as biological control agents of whitefly, the success of the biological

control program might be improved by altering the N status of tomato plants, or by supplementing developing nymphs with whitefly prey reared on high quality plants. Our development time experiments on whole plants also support the prediction that omnivores can persist and develop if prey is scarce or absent (Bugg *et al*. 1987; Naranjo and Gibson 1996; Settle *et al.* 1996; Wiedenmann *et al.* 1996; Coll and Guershon 2002), which is important in natural settings where prey population dynamics may be cyclic or unpredictable. It is our hope that this work can provide a strong background for further investigation of the behaviour and development of omnivores, especially those with biological control applications.

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Table 3.1. The six treatment groups the factorial experiment to determine the effect of prey quality on development and survival of *Dicyphus hesperus* nymphs in the *Effect of prey quality* experiment. The number of nymphs that survived to the adult stage is given for each treatment; adult emergence in Treatments 2, 3, 4, 5, and 6 was compared using a chi-square test. Results indicated that significantly fewer nymphs reached the adult stage in Treatment 5 than in the other four treatments ($\chi^2 = 6.635$, df $= 1$, p < 0.01).

Treatment	Prey Quality	Plant Availability	Number of Adults Emerged
1	None	No	0
2	None	Yes	8
3	High	No	10
4	High	Yes	18
5	Low	No	3
6	Low	Yes	19

Table 3.2. Summary of ANOVA results to determine the effect of prey quality, plant availability, and their interaction on the development time (accumulated degree days, ADD) of each of the four instars of *Dicyphus hesperus* nymphs, and of complete nymph development. Nymphs were reared in the laboratory with prey quality and plant availability manipulated (*Effect of prey quality* experiment). For all analyses, $\alpha = 0.05$. Significant effects are given in bold font.

Developmental stage	Effect	F-value (df)	*p*-value
First Instar	Prey Quality	3.33 (1,74)	0.0719
	Plant Availability	**17.71 (1,74)**	**<0.0001**
	Prey*Plant	0.91 (1,74)	0.3419
Second Instar	Prey Quality	0.08 (1,69)	0.7760
	Plant Availability	3.27 (1,69)	0.0748
	Prey*Plant	1.17 (1,69)	0.2833
Third Instar	Prey Quality	1.84 (1,67)	0.1800
	Plant Availability	**43.59 (1,67)**	**<0.0001**
	Prey*Plant	0.23 (1,67)	0.6314
Fourth Instar	Prey Quality	1.43 (1,46)	0.2377
	Plant Availability	**27.85 (1,46)**	**<0.0001**
	Prey*Plant	2.11 (1,46)	0.1530
First to Adult	Prey Quality	2.20 (1,46)	0.1448
	Plant Availability	**119.43 (1,46)**	**<0.0001**
	Prey*Plant	2.82 (1,46)	0.1000

Table 3.3. The effect of plant availability on the mean (± SE) accumulated degree days (ADD) required for development of *Dicyphus hesperus* for each of four instars and for complete nymph development from first instar to the adult stage. In each row of the table, means with the same letter were not significantly different ($p > 0.05$).

Developmental Stage	ADD with Plant	ADD without Plant
First Instar	65.91 ± 1.57 **a**	78.52 ± 2.83 **b**
Second Instar	48.69 ± 1.41 **a**	53.43 ± 2.17 **a**
Third Instar	53.09 ± 2.21 **a**	74.15 ± 2.16 **b**
Fourth Instar	92.53 ± 2.27 **a**	118.74 ± 3.73 **b**
First Instar to Adult	259.24 ± 1.92 **a**	310.37 ± 5.23 **b**

Table 3.4. The mean (± SE) accumulated degree days (ADD) required for the development of first, second, third, and fourth instar *Dicyphus hesperus* nymphs, as well as for the full developmental period of nymphs that reached the adult stage for each level of plant quality, as indicated by the rate of N-fertilizer application. Where plant quality affected development, the instar stage is given in bold font. Within a row, means with the same letters are not significantly different ($p > 0.05$).

Developmental Stage	Plant Quality, Manipulated by Rate of N-Fertilizer Application			
	50 ppm N	100 ppm N	200 ppm N	400 ppm N
Effect of plant quality – no prey				
First Instar	129.19 ± 15.18	97.88 ± 14.51	84.97 ± 5.12	102.28 ± 9.27
Second Instar	142.59 ± 30.22	96.22 ± 8.54	106.26 ± 10.24	158.99 ± 36.83
Third Instar	113.48 ± 7.22**ab**	163.58 ± 15.42**b**	143.40 ± 13.72**ab**	105.10 ± 13.50**a**
Fourth Instar	192.28 ± 7.92	251.24*	214.91 ± 36.68	190.13 ± 33.72
First to Adult	460.53 ± 13.17	626.0*	464.87 ± 53.48	476.90 ± 72.85
Effect of plant quality – with prey				
First Instar	76.69 ± 3.65	77.78 ± 4.11	77.98 ± 3.46	75.30 ± 4.27
Second Instar	63.95 ± 2.10	59.80 ± 2.60	62.94 ± 2.25	59.07 ± 1.91
Third Instar	71.19 ± 1.52	75.36 ± 3.18	74.86 ± 1.92	72.44 ± 1.55
Fourth Instar	110.21 ± 12.30	149.83 ± 40.52	177.06 ± 34.47	119.72 ± 11.78
First to Adult	300.31 ± 4.84	289.77 ± 7.34	311.80 ± 10.48	301.58 ± 10.39
Effect of plant quality – with prey, survivors only				
First Instar	73.51 ± 7.85	77.29 ± 5.76	73.58 ± 3.59	71.69 ± 6.84
Second Instar	58.01 ± 2.58	56.50 ± 5.93	64.76 ± 5.26	59.01 ± 5.06
Third Instar	64.96 ± 2.68**a**	76.48 ± 5.38**ab**	81.39 ± 2.71**b**	66.28 ± 3.15**a**
Fourth Instar	110.21 ± 12.30	149.83 ± 40.52	177.06 ± 34.47	119.72 ± 11.78
First to Adult	300.31 ± 4.84	289.77 ± 7.34	311.80 ± 10.48	301.58 ± 10.39

*Only one individual completed development of marked growth stages when nymphs were reared on 100 ppm N-fertilized whole plants.

Table 3.5. The minimum, maximum, and mean (± SE) patch residence time (d) of adult *Dicyphus hesperus* females placed in habitat patches consisting of different quality tomato plant hosts and provided with prey of varying quality. Control habitats had no prey provided.

Prey Quality	Plant Quality	n	Minimum Time (d)	Maximum Time (d)	Residence Time (d)
Control	50 ppm N	20	1	21	6.15 ± 1.05
	100 ppm N	20	1	37	5.95 ± 1.77
	200 ppm N	20	1	13	3.75 ± 0.73
	400 ppm N	20	1	16	6.20 ± 1.03
High Quality	50 ppm N	20	1	18	5.85 ± 1.23
	100 ppm N	20	2	19	8.60 ± 1.38
	200 ppm N	19	1	25	6.42 ± 1.32
	400 ppm N	20	1	22	7.45 ± 1.27
Low Quality	50 ppm N	20	1	20	5.95 ± 0.99
	100 ppm N	20	1	22	4.85 ± 1.05
	200 ppm N	21	1	19	6.29 ± 1.08
	400 ppm N	20	1	28	5.50 ± 1.36
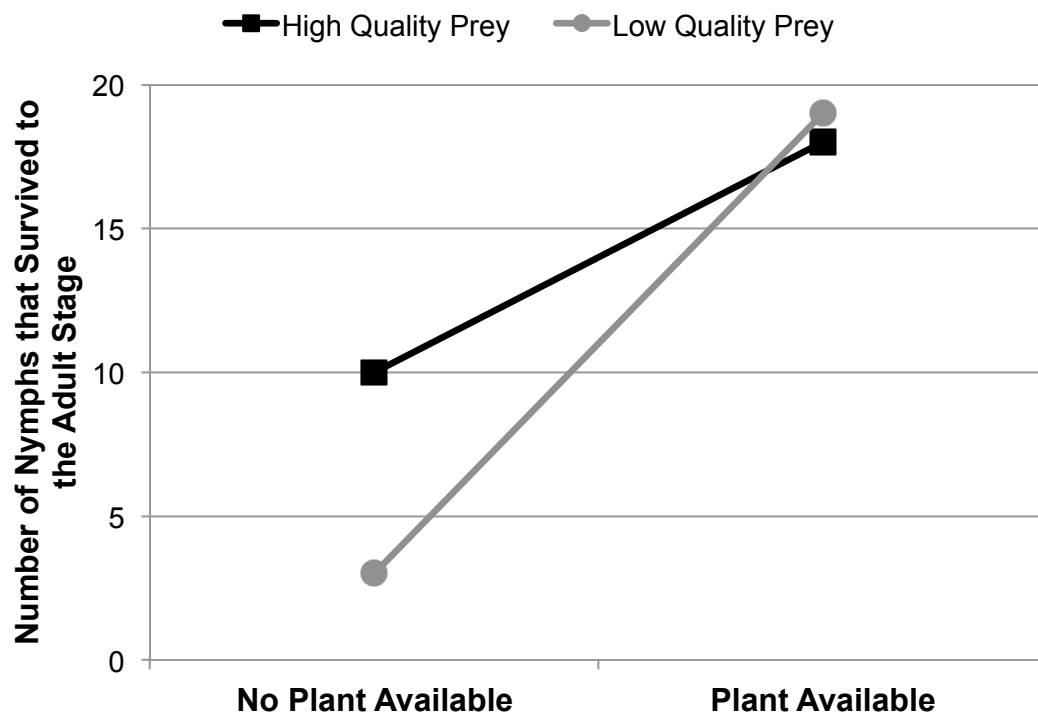
Figure 3.1. The interaction between prey quality and plant availability on the survival of *Dicyphus hesperus* nymphs reared from the first instar in the laboratory in the *Effect of prey quality* experiment. When no plant material was available to developing nymphs, more nymphs survived if nymphs were fed high quality whitefly prey.
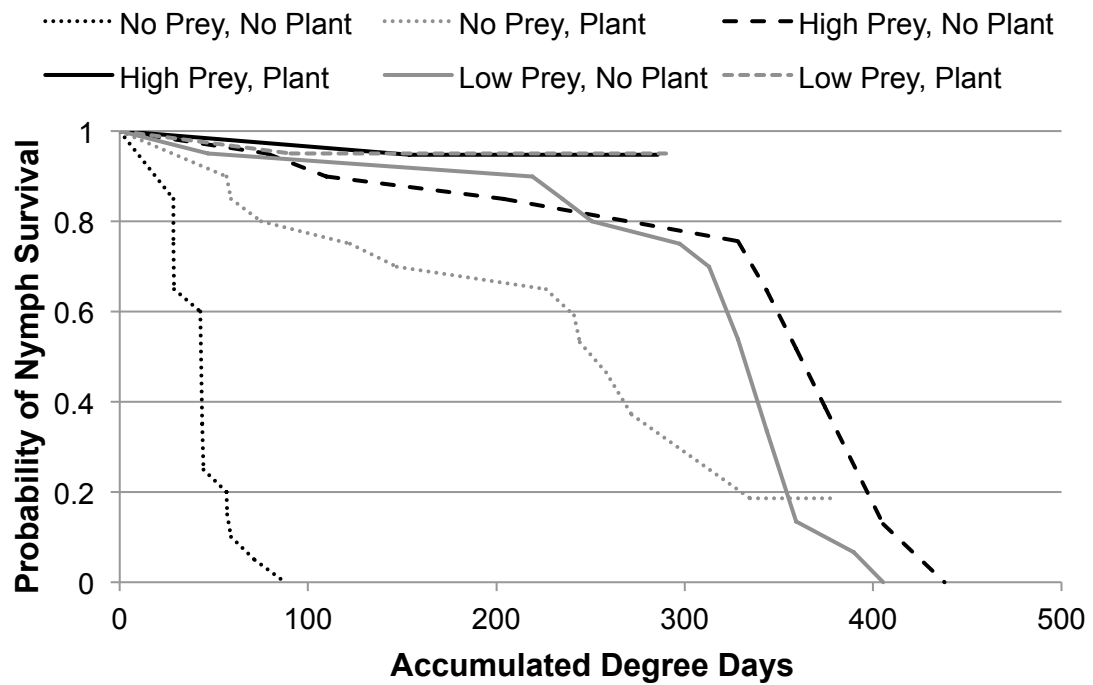
Figure 3.2. The survivor functions describing the probability of the survival of *Dicyphus hesperus* nymphs over time when nymphs were reared in conditions where plant availability and prey quality was manipulated in the laboratory (*Effect of prey quality* experiment). For treatments where the line representing the survivor function does not reach the *x*-axis, the probability of nymphs dying in that treatment was never zero at any time during the experiment.
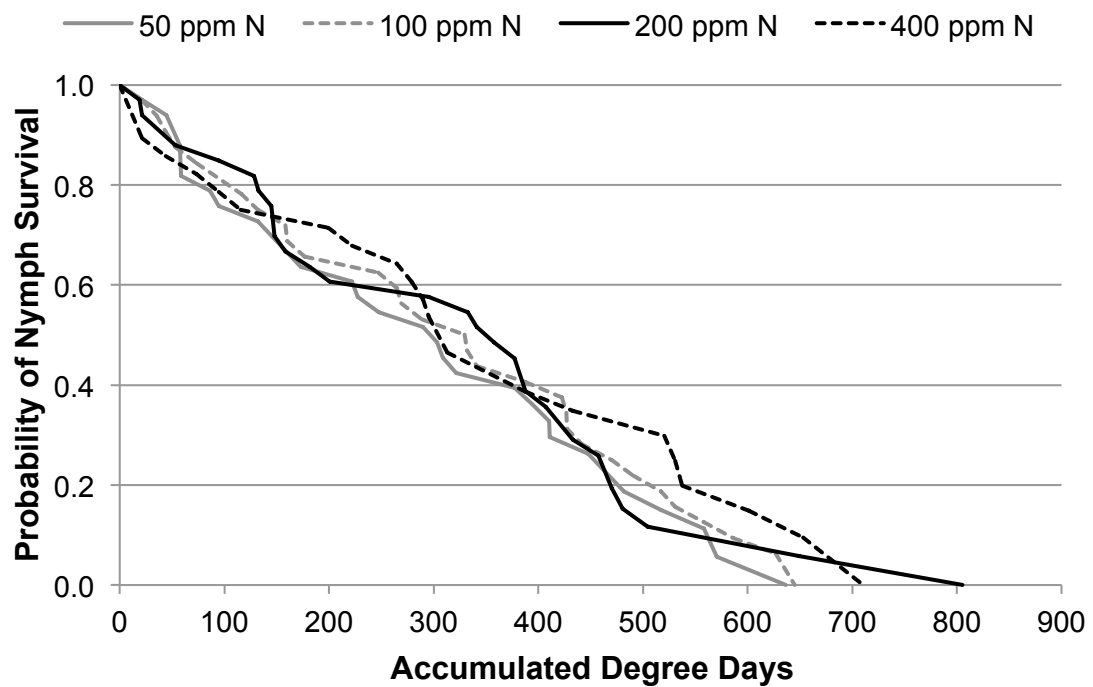
Figure 3.3. The survivor functions describing the probability of the survival of *Dicyphus hesperus* nymphs over time when nymphs were reared in clip-cages on whole tomato plants in the greenhouse, where plant quality was manipulated with N-fertilizer and no prey was provided (*Effect of plant quality – no prey* experiment).
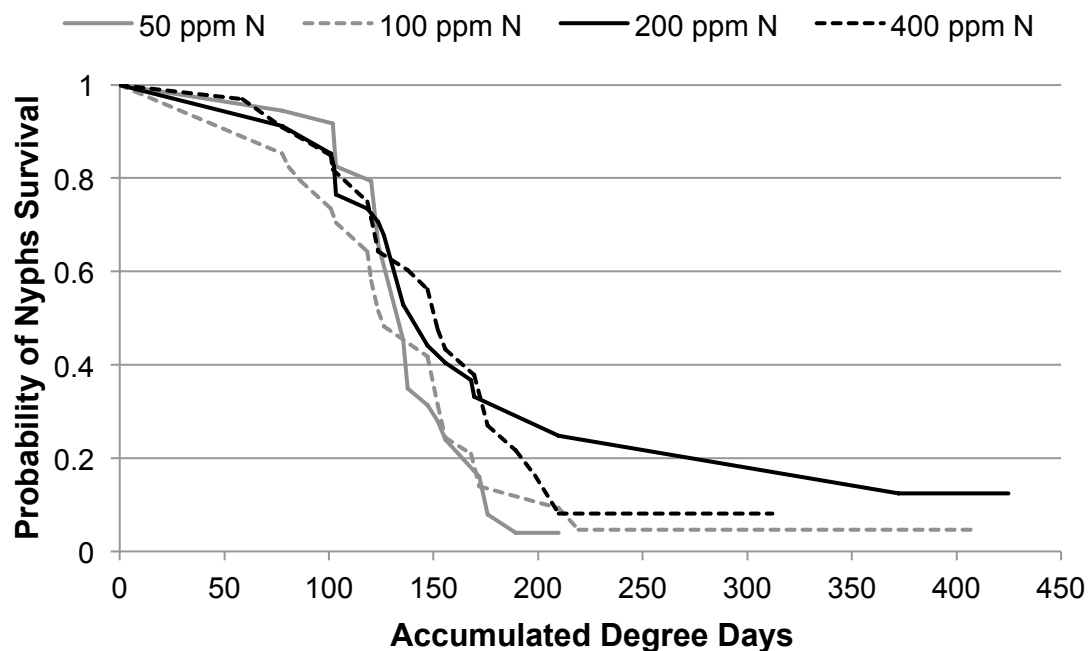
Figure 3.4. The survivor functions describing the probability of survival of *Dicyphus hesperus* nymphs reared on 50, 100, 200, and 400 ppm N-fertilized tomato plants when nymphs were provided with prey food on Days 1, 4, 7, and 10. All nymphs were held inside clip-cages on whole plants in the greenhouse. The survivor functions were determined beginning on Day 10, the last day on which prey were provided. For plant quality treatments where the line representing the survivor function does not reach the x-axis, the probability of nymphs dying in that treatment was never zero at any time in the experiment.
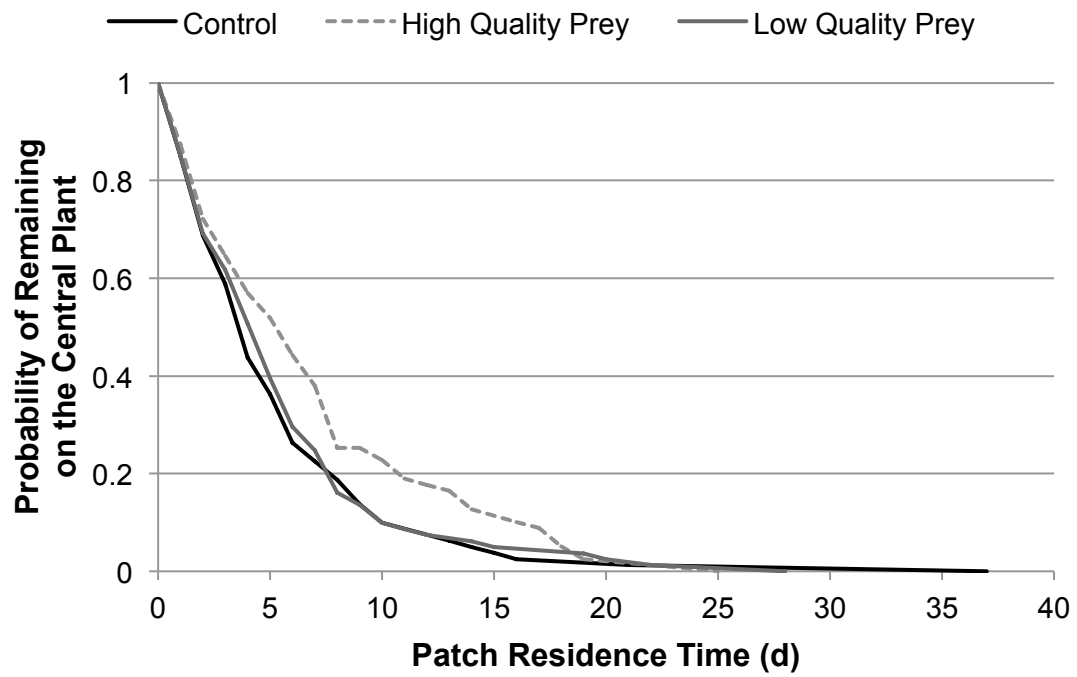
Figure 3.5. The survivor functions describing the probability of adult female *Dicyphus hesperus* remaining on the central plant upon which adults were released in patch residence time experiments when prey quality was manipulated.
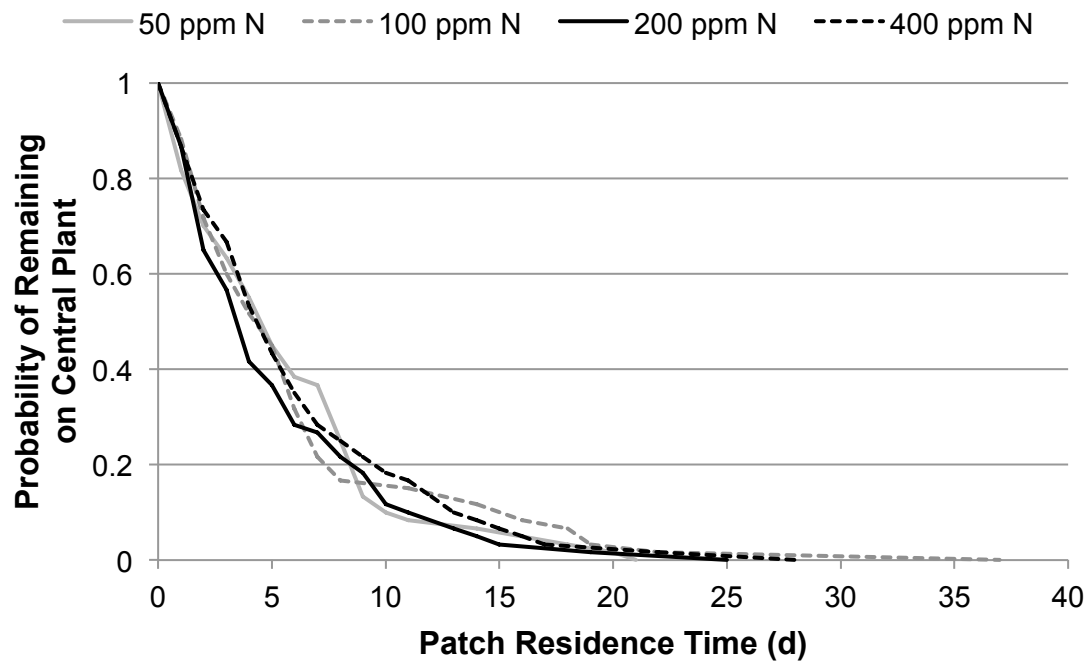
Figure 3.6. The survivor functions describing the probability of adult female *Dicyphus hesperus* remaining on the central plant upon which adults were released in patch residence time experiments when plant quality was manipulated.

Chapter 4. Through the Y-tube: the effects of plant quality, whitefly infestation, and foraging experience on the olfactory response of *Dicyphus hesperus*

Introduction

 Dicyphus hesperus Knight (Hemiptera: Miridae) is a generalist omnivore that was identified as an effective biological control agent of greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae), on tomato plants, *Solanum lycopersicum* L. (Solanales: Solanaceae), by McGregor *et al.* in 1999. Since then, our knowledge of this insect has grown, with information now available regarding its development (McGregor *et al.* 1999; Gillespie and McGregor 2000), diapause (Gillespie and Quiring 2005), and host plant and prey preferences (Sanchez *et al.* 2004; VanLaerhoven *et al.* 2006). Understanding the basic biology of omnivores such as *D. hesperus* is important, as there are both significant potential benefits and drawbacks to their use as biological control agents. For example, omnivores are better able to persist in a specific habitat when their prey are scarce or absent compared to predators or parasitoids (Bugg *et al.* 1987; Wiedenmann *et al.* 1996; Coll and Guershon 2002). However, omnivores might also cause economic injuries to their host plant due to excessive plant feeding or feeding on harvestable plant structures, as *Dicyphus tamaninii* Wagner (Hemiptera: Miridae) and *Nesidicoris tenius* (Reuter) (Hemiptera: Miridae) are known to do (Alomar and Albajes 1996; Sanchez and Lacasa 2008; Castañé *et al.* 2011). Omnivores (and generalists) also pose a challenge to biological control in that they may not feed readily upon the target pest if alternate prey are available (Harmon and Andow 2004; Koss and Snyder 2005), or they may be reluctant to leave a preferred host plant in order to find prey on an alternative host plant (VanLaerhoven *et al.* 2006; Frank 2010).

 Manipulating certain characteristics of agroecosystems may allow producers to improve the efficacy of biological control using omnivores. Manipulating fertilizer inputs, for example, may indirectly affect the efficacy of omnivores, especially if altered fertilizer regimes increase the omnivore's population density or if fertilizer inputs have an impact on the olfactory cues emitted by the host plant. It is of particular interest to determine if varying levels of nitrogen (N)-fertilizer inputs increase or decrease the attractiveness of plants to omnivorous natural enemies searching for host plants and prey.

Insects use a variety of host plant and prey cues when foraging and searching for hosts. A generalized pattern of search has been described for herbivores (Bernays and Chapman 1994; Schoonhoven *et al.* 2005) in which olfactory and visual cues play key roles when insects are out of contact with the host (Bernays and Chapman 1994; Schoonhoven *et al.* 2005). Once the insect has contacted a potential host, olfactory, gustatory, and tactile cues are used to determine host suitability (Bernays and Chapman 1994; Schoonhoven *et al.* 2005). Unlike pheromone-tracking insects that follow concentration gradients, herbivores rely on visual cues at a distance, as plants do not generally emit odors in a concentration gradient over distances greater than several centimeters (Bernays and Chapman 1994). During the process of host searching, the physiological state of the insect (i.e. age, hunger, and egg load) can affect the suitability of encountered hosts (Singer 1971; Jaenike 1990). Predators and parasitoids are also known to use olfactory cues to locate their prey using herbivore-induced plant volatiles (HIPV), which allow for an indirect line of communication between plants and the natural enemies that target their herbivores (Vet and Dicke 1992; Dicke *et al.* 2003).

There is evidence in the literature that suggests that *D. hesperus* use HIPV to locate their prey. For example, in greenhouse-scale releases of *D. hesperus*, insects oriented towards whitefly-infested tomato plants (McGregor *et al.* 1999). In this experiment, *D. hesperus* did not orient towards tomato plants infested with two-spotted spider mites, *Tetranychus urticae* Koch (Acari: Tetranychidae), which suggests that this omnivore can discriminate between HIPV resulting from infestation by different pest species (McGregor *et al.* 1999). In addition, results from assays using a y-tube olfactometer showed that female *D. hesperus* were more attracted to a combination of whitefly and tomato odors than to tomato odors alone (McGregor and Gillespie 2004).

The effect of plant quality on the olfactory response of female *D. hesperus* has not been studied, to date. However, if plant nutrition plays a role in the ability of *D. hesperus* to locate its hosts in greenhouses or other agroecosystems where monocultures are grown, differences in plant quality may impact the efficacy of *D. hesperus* as a biological control agent. Therefore, our objective was to investigate the influence of host plant quality, as manipulated by nitrogen (N)-fertilizer inputs, on the preference and response of *D. hesperus* to olfactory cues in the laboratory. Four experiments were performed using a y-

tube olfactometer in order to test insect preference for tomato foliage with and without whitefly infestation, as well as the effect of previous experience foraging on the host plant and prey on the response of *D. hesperus*. In these experiments, *D. hesperus* exhibited a 'preference' by selecting one odor or the other by walking down one arm of the y-tube olfactometer. A 'response' was defined as the selection of an odor, regardless of which odor was selected. Using data from the four experiments, we tested four primary predictions. First, we expected that *D. hesperus* would prefer the plant and prey odors over the control in the olfactometer, and that as plant N increased, the attractiveness of the odors would also increase. Second, we expected that previous experience foraging on host plant and prey material would increase the responsiveness of *D. hesperus* to odors. Third, we expected that more *D. hesperus* would respond to odors in the olfactometer as plant quality increased. Finally, we expected that insects exposed to test odors similar to those previously experienced would respond to odors in the olfactometer more often than those without previous experience and that the rate of response would increase with increasing plant quality.

Materials and Methods

Plant propagation and insect rearing

Details regarding plant propagation for these experiments are described in Chapter 2 and are summarized below. 'Patio Hybrid' tomato (*Solanum lycopersicum* L., Solanales: Solanaceae) seeds were grown in small pots filled with BM6 potting soil (78% peat moss, Berger, Saint-Modeste, Quebec, Canada). Plants were grown in a greenhouse at the University of Windsor, Ontario, Canada under natural light and temperature regimes. Seedlings with four true leaves were transplanted into 20 cm pots and then assigned to one of four N-fertilizer treatment groups (50, 100, 200 or 400 ppm N) using a haphazard method. Fertilizer treatments were prepared by mixing 6-11-31 hydroponic fertilizer and 15.5-0-0 calcium nitrate (Plant-Prod® Canada, Brampton, ON, Canada) as described in Chapter 2. Plants began receiving fertilizer the day after transplantation; N-fertilizer was applied daily for seven days and on alternating days thereafter until the plants were discarded. All plants received 100 mL of the appropriate fertilizer solution at each application (following Jauset *et al.* 1998). Water was provided as required in addition to

the fertilizer to prevent wilting. Tomato leaves and stems for the olfactometer experiments were collected from plants that had been receiving fertilizer for at least 24 d.

Dicyphus hesperus were reared from insects originally collected off of *Stachys albens* A. Gray (Lamiales: Lamiaceae) plants in California, USA (35º42'15"N, 118º50'00"W) (McGregor and Gillespie 2004; Sparkes 2012). The colony is maintained at $20 \pm 5°C$, with a 16:8 h light:dark photoperiod, and $50 \pm 10\%$ humidity, provided by a humidifier (Nortec Humidity Inc., Ogdensburg, New York, USA) (Chapter 2). In the colony, *D. hesperus* obtain energy and nutrients from *Nicotiana tabacum* L. (Solanales: Solanaceae) plants and frozen *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs (Sparkes 2012).

Adult female *D. hesperus* used in olfactometer experiments were collected from the rearing colony seven to 14 days post-adult eclosion. Naïve insects (Table 4.1) were placed individually into 60 mL plastic cups (Solo Cup Company, Lake Forest, Illinois, USA) and held without food for 72 h before being placed into the olfactometer. Each cup held a small piece of moistened cotton to provide a water source. Cups were opened after 48 h to re-wet the cotton if needed. To gain experience foraging on tomato plants prior to the experiments (Table 4.1), females were released into a 61 cm^3 cage (BioQuip Products, Rancho Dominguez, California, USA) holding a 200 ppm N-fertilized tomato plant with *E. kuehniella* eggs for prey (modified from Blackmer *et al.* 2004). Females remained in this cage for at least 24 h and were then transferred to 60 mL plastic cups as described above. To gain experience foraging on tomato plants and whitefly prey (Table 4.1), female *D. hesperus* were held in a similar cage for at least 24 h with both food types available (modified from Blackmer *et al.* 2004), before being isolated in 60 mL plastic cups.

The olfactometer apparatus

The olfactometer apparatus used for this experiment was modeled after those described by McGregor and Gillespie (2004), and Tansey *et al.* (2010). The olfactometer was assembled inside a fume hood such that the insect intake was near the sash and the odor sources were against the inside wall and below the line of sight of the insects inside the y-tube. Components are described following the path of airflow through the apparatus: air

flowed through 76 cm of 0.64 cm Tygon® tubing (Saint-Gobain, Courbevoie, France) from the lab-air source to an airflow meter (Analytical Research Systems [ARS], Gainesville, Florida), followed by a carbon filter (ARS, Gainesville, Florida) located 30 cm from the airflow meter. Air was forced through room temperature water inside a sealed 1 L wide-mouth Ball jar (Ball Corporation, Broomfield, Colorado), 20 cm from the carbon filter to humidify the air before it reached the insect. The air was split by a y-shaped plastic splitter 25 cm from the humidifier, and then flowed 30 cm into 125 mL Pyrex flasks (Corning Incorporated Life Sciences, Tewksbury, Massachusetts) holding the odor sources and stoppered with #5 black rubber stoppers. Air leaving the flasks travelled 30 cm to reach the external odor adapters (OLFM-XO-2425M, ARS, Gainesville, Florida) and finally, the body of the y-tube (OLFM-YT-2425F, ARS, Gainesville, Florida). Airflow in the olfactometer was maintained at approximately 0.3 ± 0.05 L min^{-1}. Evaporating dry ice was used as a smoke test to examine the pattern of airflow through the arms and body of the y-tube (after Blackmer *et al.* 2004). There was a region of turbulence in the inner zone of the intersection of the arms and body of the y-tube where air sources mixed. Along the outer zones of the intersection, airflow was laminar with no apparent mixing of odor sources.

Three to six tomato leaflets with the stems attached were used as the odor source in the olfactometer. All plant materials were harvested from the top half of tomato plants. To keep tomato foliage fresh, water was provided in a half-dram glass vial (12 x 35 mm, Fisher Scientific Co. Pittsburgh, Pennsylvania) sealed around the stems with parafilm (Pechinay Plastic Packaging, Chicago, Illinois). For all trials, one odor source was provided. The second flask was a blank control holding a half-dram glass vial, filled with water and sealed with parafilm with a small hole to mimic any parafilm gaps around the tomato stems in the test flask.

Following each replicate of each experiment, the y-tube was cleaned with warm soapy water (Sparkleen 1, Fisher Scientific Co. Pittsburgh, Pennsylvania), rinsed with 70% ethanol and dried by hand. When the location of the odor was changed then all components of the apparatus from the flasks to the y-tube were cleaned as described. Five to 10 replicates were run before the odor location was changed.

Olfactometer experiments

Four experiments were performed in which insect response to each level of plant quality (50, 100, 200, and 400 ppm N) was determined with varying prey availability using insects with differing degrees of previous experience foraging on tomato plants and whitefly prey. The conditions tested in each experiment are given in Table 4.1. Each individual *D. hesperus* female placed in the olfactometer was considered a replicate. For each level of plant quality in each experiment, replicates were performed until 40 insects had responded to the odors presented. A response was defined as movement of the insect in the y-tube 5 cm past the junction of the two arms. At the beginning of each replicate, insects were gently transferred into the y-tube by hand and given 20 minutes to choose either the left or right arm. The replicate was terminated as soon as a choice was made or if the insect did not make a choice before the end of the 20 min period. Insects that did not make a choice were recorded as non-responders. The response or non-response of all insect replicates was recorded, as was the direction the insect moved in the y-tube and the odor selected by responding insects. Replicates were run between 11:00 and 19:00. The temperature inside the fume hood where the experiments were performed was monitored using a Smartbutton data logger (ARC Systems Inc., Surrey, British Columbia, Canada). The mean (± SE) daily temperature in the fume hood between 11:00 and 19:00 on the days the experiments were conducted was 21.5 ± 0.2°C.

Only one olfactometer apparatus could be running at any given time. Therefore, multiple days of testing were required in order to observe 40 responses. The dates on which replicates were performed for each level of plant condition for each experiment are given in Table 4.1. Insofar as it was possible, insects from a single generation of the rearing colony were used for testing insect response to each level of plant quality in each experiment. However, the availability of plant material, especially of plant material infested with whitefly, was not always consistent because the whitefly population in the greenhouse crashed frequently. As a result, insects of multiple generations were required for testing for some experiments. For all experiments, female *D. hesperus* were handled as consistently as possible to reduce variation between experiments.

Statistical analysis

Olfactometer experiments are commonly analyzed using chi-square to test the null hypothesis of no difference in choice between the arms of the olfactometer (McGregor and Gillespie 2004; Blackmer *et al.* 2004; Moayeri *et al.* 2006; Zhong *et al.* 2011; and others). Details are given below regarding the specific analyses used to test each prediction.

Prediction 1. The preference of *D. hesperus* for the odor versus the control was tested by comparing the number of insects that selected the odor in the olfactometer to those that selected the control using PROC FREQ (SAS Institute 2009) for each level of plant quality in each of the four experiments. Only insects that responded in the olfactometer were included in this analysis (following McGregor and Gillepsie 2004; Moayeri *et al.* 2006; Zhong *et al.* 2011). Because four comparisons were made for each experiment, *p*-values were compared to a Bonferroni-adjusted α-value of 0.0125 to control for error associated with performing multiple comparisons using data from a single experiment.

A second chi-square analysis was performed to compare the number of insects that selected the odor in the olfactometer (rather than the control) between the four levels of plant quality within each experiment to determine if increasing plant quality also increased attractiveness of the odors. For this analysis, the null hypothesis of equal preference for plant odors across the four levels of plant quality was tested using the "TESTP = (0.25 0.25 0.25 0.25)" command added to the TABLES statement of the FREQ procedure (SAS Institute 2009). Each of the four experiments was analyzed independently and *p*-values were compared to α = 0.05 to determine statistical significance. If the null hypothesis was rejected for any of the experiments, differences in the numbers of *D. hesperus* that selected the odor were determined by subdividing the chi-square analysis (Zar 2010).

Prediction 2. To test the effect of previous experience on *D. hesperus* responsiveness, first the responses of naïve *D. hesperus* (Exp. 1) and *D. hesperus* with experience foraging on tomato (Exp. 2) were compared. In both of these experiments, no prey was present in the olfactometer. A second analysis compared the responses of *D. hesperus* with experience foraging on tomato (Exp. 3) to those with experience foraging

on tomato + whitefly (Exp. 4). In both Exp. 3 and Exp. 4, odors from whitefly-infested tomato leaves were used in the olfactometer. For each pair of experiments, a contingency table was used to determine the effect of prior experience on the response of *D. hesperus* to the odors provided using the FREQ procedure (SAS Institute 2009). For both analyses, $\alpha = 0.05$.

Prediction 3. For each experiment, replicates were performed until 40 insects had selected either the control or the plant/prey odor offered in the olfactometer, for each level of plant quality, in order to test Prediction 1. Therefore, the number of responses to the odors was equal across all four levels of plant quality, due to the nature of the experimental design. However, the response of insects to odors in the olfactometer, regardless of the odor selected, was of interest and could be compared using the number of insects that did not respond in the olfactometer, as these numbers were different across the four levels of plant quality in each of the four experiments. For each of the four experiments (Table 4.1), chi-square analysis was used to determine if equal numbers of insects did not respond in the olfactometer using the FREQ procedure with "TESTP = (0.25 0.25 0.25 0.25)" specified in the TABLES statement (SAS Institute 2009). For each experiment, $\alpha = 0.05$. If the null hypothesis of equal numbers of responses between the four levels of plant quality was rejected for any of the experiments, the chi-square analysis was further subdivided following the method described by Zar (2010) to determine which levels of plant quality elicited different numbers of responses than expected.

Prediction 4. The percentages of female *D. hesperus* that did and did not respond in the olfactometer were calculated for each level of plant quality for all four experiments. For example, in Experiment 1, 40 females responded to 400 ppm N tomato leaves and 28 did not, with a total of 68 replicates, therefore, 58.82% *D. hesperus* females responded to the odor while 41.18% did not. The percentage values were used to determine the effects of previous foraging experience, plant quality, and the interaction of the two factors on the response of *D. hesperus* in the olfactometer using analysis of variance and weighted least squares estimates for categorical data (PROC CATMOD with *freq* and *prob* specified in the MODEL statement; SAS Institute 2009). For

comparison, the four experiments were paired as described in Prediction 2. Resultant *p*-values were compared to α = 0.05 to determine significance.

Results

General observations

The time required for insects to respond to odors was extremely variable, as some females selected an odor in two minutes or less, and others required upwards of 18 to 20 minutes to make a choice. Insects that responded quickly tended to walk along the outer walls of the olfactometer, where airflow was more laminar. Some insects sat on the olfactometer walls in the turbulent zone, where odor sources mixed; some of these females selected an odor, while others did not. Occasionally, females would attempt to oviposit in the y-tube. Oviposition attempts were observed in all areas of the olfactometer. This behaviour was observed in all four experiments.

Prediction 1

Dicyphus hesperus females selected the test odor as often as the control odor in the olfactometer at all levels of plant quality, in all four experiments (Table 4.2). Tomato plant quality, as manipulated by N-fertilizer concentration (50, 100, 200 or 400 ppm N), did not affect the preference of *D. hesperus* for plant odors in the olfactometer (Exp. 1: χ^2 = 1.000, df = 3, *p* = 0.8013, N = 72; Exp. 2: χ^2 = 1.5057, df = 3, *p* = 0.6809, N = 87; Exp. 3: χ^2 = 1,7595, df = 3, *p* = 0.6238, N = 79; Exp. 4: χ^2 = 0.7429, df = 3, *p* = 0.8631, N = 70).

Prediction 2

The response of insects with previous experience on tomato (Exp. 2) differed from the response of naïve insects (Exp. 1) (χ^2 = 6.8453, df = 1, *p* = 0.0089). Naïve insects responded to odors less often than expected (Figure 4.1a), whereas insects with previous experience foraging on tomato plants responded to plant odors more often than expected. The response of insects in Exp. 3 (previous experience foraging on tomato) was also compared to that of insects in Exp. 4 (previous experience foraging on tomato+whitefly). There were no difference between these two experiments in the expected versus observed

numbers of insects that responded in the olfactometer when whitefly infested tomato foliage was provided ($\chi^2 = 0.0645$, df = 1, $p = 0.7995$; Figure 4.1b).

Prediction 3

The number of *D. hesperus* females that did not respond in the olfactometer was equal for all levels of plant quality in Exp. 1 ($\chi^2 = 4.5333$, df = 3, $p = 0.2093$), Exp. 2 ($\chi^2 = 4.5946$, df = 3, $p = 0.2040$), and Exp. 3 ($\chi^2 = 3.5238$, df = 3, $p = 0.3177$, N = 84). In Exp. 4, where *D. hesperus* had previous experience foraging on whitefly infested tomato, the number of insects that did not respond in the oflactometer was different between the four levels of plant quality ($\chi^2 = 12.7000$, df = 3, $p = 0.0053$, N = 80). Of the 80 *D. hesperus* females in this experiment, 20, 13, 33, and 14 did not respond when the olfactometer held whitefly infested foliage harvested from 50, 100, 200, and 400 ppm N-fertilized tomato plants, respectively. The most females did not respond in the olfactometer when 200 ppm N-fertilized whitefly-infested tomato foliage was provided, compared to when whitefly-infested tomato foliage from the other levels of plant quality was provided ($\chi^2 = 11.2667$, df = 1, $p = 0.0008$).

Prediction 4

The percentages of female *D. hesperus* that did and did not respond to tomato plant odors in the olfactometer when females did and did not have experience foraging on tomato plants (Exp. 1 vs. Exp. 2) were not affected by plant quality ($\chi^2 = 4.54$, df = 3, $p = 0.2087$), nor was there a significant interaction effect of plant quality and previous foraging experience ($\chi^2 = 4.25$, df = 3, $p = 0.2361$). The percentage of responses was affected by the previous foraging experience of *D. hesperus* ($\chi^2 = 11.24$, df = 1, $p = 0.0008$), such that a greater percentage of females with experience foraging on tomato responded in the olfactometer than females that were naïve to tomato plants.

The percentages of *D. hesperus* that did and did not respond to odors from whitefly-infested tomato plants were also compared to determine if plant quality or foraging experience influenced the response of the insects (Exp. 3 vs. Exp. 4). Neither plant quality ($\chi^2 = 5.81$, df = 3, $p = 0.1213$), nor previous foraging experience ($\chi^2 = 0.32$, df = 1, $p = 0.5715$) had an effect on the percentage of responses and non-responses in the

olfactometer. There was an interaction effect of plant quality and previous experience on the response percentages ($\chi^2 = 10.00$, df = 3, $p = 0.0186$). The interaction effect observed was driven by the difference in response of females between Exp. 3, where plant quality had no effect on response ($\chi^2 = 2.285$, df = 3, $p = 0.5153$), and Exp. 4, where plant quality did affect the response of females in the olfactometer ($\chi^2 = 8.3308$, df = 3, $p = 0.0396$). In Exp. 4, a significantly greater percentage of females did not respond in the olfactometer in the presence of 200 ppm N-fertilized whitefly-infested tomato plants than the percentage of females that did not respond in the presence of whitefly-infested tomato foliage from the other levels of plant quality ($\chi^2 = 6.9441$, df = 1, $p = 0.0084$; Figure 4.2).

Discussion

Host plant location by herbivorous insects is a complicated process that requires olfactory, visual, gustatory, and tactile cues (Bernays and Chapman 1994; Schoonhoven *et al.* 2005). Predators of herbivores are known to use HIPV to locate their prey (Vet and Dicke 1992; Dicke *et al.* 2003). Because omnivores obtain nutrients from their host plants and prey, omnivores likely use a combination of visual, olfactory, gustatory, and tactile cues associated with the plant in addition to HIPV to locate host plants. Olfactometer studies aim to decrease the complexity of a natural environment by providing only olfactory cues, although visual cues can be easily incorporated into the apparatus (Blackmer and Canas 2005). Breaking down a complex sensory environment into its components in this manner can help researchers determine if particular cues are important during host plant location. The objective of the current study was to determine if *D. hesperus* can use host plant odors and HIPV to distinguish between plants of different nitrogen nutrition, and how previous experience with host plants and prey influences olfactory response.

As predicted, previous experience of female *D. hesperus* affected their response in the olfactometer. Females were more responsive if they had previous experience with tomato, relative to naïve insects. Interestingly, previous experience with whitefly-infested tomato did not increase the responsiveness of *D. hesperus* to HIPV relative to those individuals with experience on tomato alone. Previous experience appears to have species-specific effects on the olfactory response of insects in olfactometer studies. For

example, contrary to our results, Blackmer *et al.* (2004) did not observe a positive effect of previous experience on the response of *Lygus hesperus* (Knight) (Hemiptera: Miridae) to odors in a y-tube olfactometer. In our study and that of Blackmer *et al.* (2004), insects were allowed the same amount of time to gain experience with the host plants tested in the olfactometer. Based on our results, we suggest that producers releasing *D. hesperus* to manage greenhouse whitefly populations on tomato plants consider holding adults on tomato plants prior to their release, as this may shorten the time needed for insects to colonize tomato plants.

No preference of *D. hesperus* females for the odor in the olfactometer, relative to the control, was observed at any level of plant quality in any of the four experiments. This was unexpected, as McGregor and Gillespie (2004) did observe a preference for whitefly-infested tomato plants relative to control odors in y-tube olfactometer trials. There were some differences in olfactometer design between these two studies that may have contributed to this difference in results. Specifically, our olfactometer had larger holding chambers for the odors, controlled the airflow before the air was split, and used a slightly higher rate of airflow than the olfactometer apparatus described by McGregor and Gillespie (2004). The choice of the insect may have also been affected by turbulence near the junction of the arms of the y-tube with the body of the olfactometer. Turbulence at olfactometer intersections affects the ability of insects to distinguish between odors, and therefore, with their ability to make an informed choice (Vet *et al.* 1983). Therefore, it is possible that insects only made accurate choices if they walked along the outer walls of the olfactometer, where airflow was laminar. As we only recorded the final choice (or lack thereof) of the insect, and not path taken inside the olfactometer, our study cannot be used to conclude that turbulence at the y-tube intersection affected the decisions of *D. hesperus*. However, as turbulence is an issue associated with olfactometer studies (Vet *et al.* 1983), the possibility that turbulence affected the behaviour of *D. hesperus* cannot be discounted.

In addition to not observing a preference of *D. hesperus* females for the odor relative to the control, we also observed no difference in the number of insects that did not respond to plant odors of varying quality in three of the four experiments. In Exp. 4, females that had previous experience foraging on whitefly-infested tomato were least

responsive to HIPV from 200 ppm N-fertilized tomato plants. This observation also contributed to an interaction of plant quality and previous foraging experience when the percentages of insects that did and did not respond to HIPV from whitefly-infested tomato plants were compared. Based on our previous observations in which females preferred high quality tomato plants for oviposition in choice tests (Chapter 2), we expected that the response of insects to high quality tomato plants in the olfactometer would be similar. However, this was not the case, and the difference in response to odors from plants of high quality is difficult to explain.

One possible explanation for our results in these olfactometer experiments is that detection of plant odors elicits a response by *D. hesperus* that the olfactometer was not designed to measure. Based on the results of McGregor and Gillespie (2004), we expected *D. hesperus* to walk upwind towards the odor source. However, Hazard (2008) observed that detection of prey odors caused *D. hesperus* females to stop walking and to begin probing the area where the odor was detected. In his test arenas, Hazard (2008) observed that prey odors were not used for orientation to prey at close range, but that prey odors did result in the arrestment of broad scale searching behaviours (i.e. walking). Rather, females relied on visual cues to locate prey after arrestment (Hazard 2008). In prey choice experiments, it was observed that *D. hesperus* seem to rely heavily upon their visual ability to locate prey, as pale yellow whitefly nymphs were not consumed when placed on filter paper after six hours, but were readily consumed in half of that time if placed on tomato leaves (M.A. Vankosky, *personal observation*). Based on these observations, we suspect that female *D. hesperus* in the olfactometer responded to HIPV and tomato odors in a similar manner to that described by Hazard (2008): detection of the odors resulted in arrestment of walking behaviours and insects initiated local search behaviours that would normally involve gustatory and visual cues. However, both of those types of cues were absent in our olfactometer apparatus, and could not be measured.

The effects of the physiological state of insects on behaviour is another important factor to consider, as the condition of an insect can significantly affect its behaviour and host preference during host plant selection (Jaenike 1990). When insects are in poor condition or have been deprived of oviposition substrates for long periods of time, they

become less choosy, as their priority is focused their own survival and fitness, rather than that of the their offspring (Jaenike 1990). In the present study, females had been caged with males and were probably gravid (VanLaerhoven *et al.* 2006). They had also been held for 72 h without food or an oviposition substrate. In this situation, even the faintest attractive stimuli may have elicited close-range searching behaviours similar to those observed by Hazard (2008), and unfortunately, those behaviours were not measured in the present study. Interestingly, in some trials, females that did not respond by selecting either arm of the olfactometer did attempt to oviposit in the olfactometer, providing some support for this explanation.

As *D. hesperus* are known to discriminate between tomato plants of differing nitrogen content for oviposition in choice experiments (Chapter 2), it is important to understand the basis for this discrimination. Therefore, further investigation into the olfactory response of *D. hesperus* to tomato plant and HIPV odors emitted from whitefly-infested tomato plants is required to fully understand the behaviour of this biological control agent. It would be illuminating to incorporate other plant or prey-related cues into the design of the olfactometer. Blackmer and Canas (2005) describe one such modification to an olfactometer used to investigate the effect of visual cues on the response of *L. hesperus* to odors from one of its host plants, following poor responses of *L. hesperus* to odors alone (Blackmer *et al.* 2004). In the modified olfactometer, visual cues were attractive to *L. hesperus* without odors present and males were only attracted to odors in the presence of visual cues (Blackmer and Canas 2005). Like *L. hesperus*, we suspect that *D. hesperus* are likely to be more responsive when visual and olfactory cues are offered simultaneously rather than alone.

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Table 4.1. Description of the four experiments and treatment groups within each experiment conduced using the olfactometer. Experiments differed in terms of prey availability and previous foraging experience of *Dicyphus hesperus* females. The dates that replicates were conducted on are provided.

	Plant Quality	Prey	Previous Experience	Dates Performed
Exp. 1	50 ppm N	None	Naïve	25, 27-29 June 01-05 July 2012
	100 ppm N	None	Naïve	17-22 June 2012
	200 ppm N	None	Naïve	11,13-16 June 2012
	400 ppm N	None	Naïve	03-08, 10 June 2012
Exp. 2	50 ppm N	None	Tomato	08, 09, 11-14 May 2013
	100 ppm N	None	Tomato	02-04, 06-08 February 2013
	200 ppm N	None	Tomato	09-11 Feb 2013 14-15 May 2013
	400 ppm N	None	Tomato	21, 23, 29, 30 January 2013 01 February 2013
Exp. 3	50 ppm N	Whitefly	Tomato	18 May 2013 13, 24-25 June 2013
	100 ppm N	Whitefly	Tomato	21-22 May 2013 14, 16 June 2013
	200 ppm N	Whitefly	Tomato	19 May 2013 17-20 June 2013
	400 ppm N	Whitefly	Tomato	16-17 May 2013 20-21, 23, 26, 28 June 2013 16 July 2013
Exp. 4	50 ppm N	Whitefly	Tomato and Whitefly	26 May 2013 02, 17 July 2013
	100 ppm N	Whitefly	Tomato and Whitefly	23, 27 May 2013 05, 15 July 2013
	200 ppm N	Whitefly	Tomato and Whitefly	25 May 2013 30 June 2013 01, 03-05 July 2013
	400 ppm N	Whitefly	Tomato and Whitefly	24 May 2013 07-09, 16 July 2013

Table 4.2. Chi-square test results to compare the selection of the odor and control arms of the olfactometer by female *Dicyphus hesperus*. All *p*-values were calculated with one degree of freedom. For each Experiment, *p*-values were compared to α = 0.025 (Bonferroni adjusted for four comparisons within each Experiment).

		Number of Responses			
	Fertilizer Treatment	**Odor**	**Control**	χ^2	***p*-value**
Exp. 1	50 ppm N	18	22	0.40	0.5271
	100 ppm N	18	22	0.40	0.5271
	200 ppm N	21	19	0.10	0.7518
	400 ppm N	15	25	2.50	0.1138
Exp. 2	50 ppm N	25	15	2.50	0.1138
	100 ppm N	18	22	0.40	0.5271
	200 ppm N	20	20	0.00	1.0000
	400 ppm N	24	16	1.60	0.2059
Exp. 3	50 ppm N	23	17	0.90	0.3428
	100 ppm N	20	20	0.00	1.0000
	200 ppm N	21	19	0.10	0.7518
	400 ppm N	15	25	2.50	0.1138
Exp. 4	50 ppm N	20	20	0.00	1.0000
	100 ppm N	15	25	2.50	0.1138
	200 ppm N	17	23	0.90	0.3428
	400 ppm N	18	22	0.40	0.5271

A

B
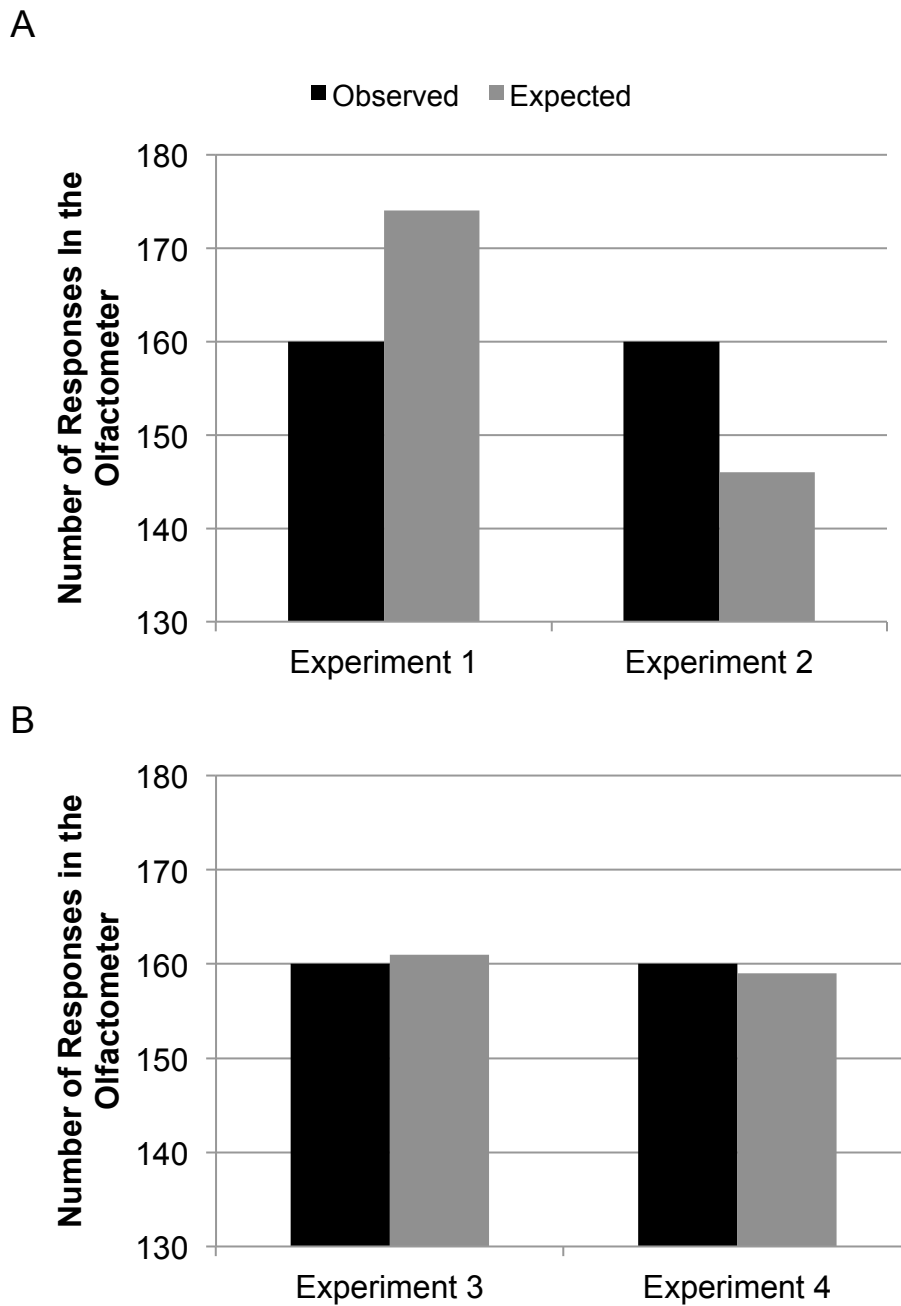
Figure 4.1. The number of observed and expected responses of *Dicyphus hesperus* to odors offered in the olfactometer. In panel A, the number of responses of *D. hesperus* was affected by foraging experience ($\chi^2 = 6.8453$, df $= 1$, $p = 0.0089$). In panel B, foraging experience had no effect on response ($\chi^2 = 0.0645$, df $= 1$, $p = 0.7995$).
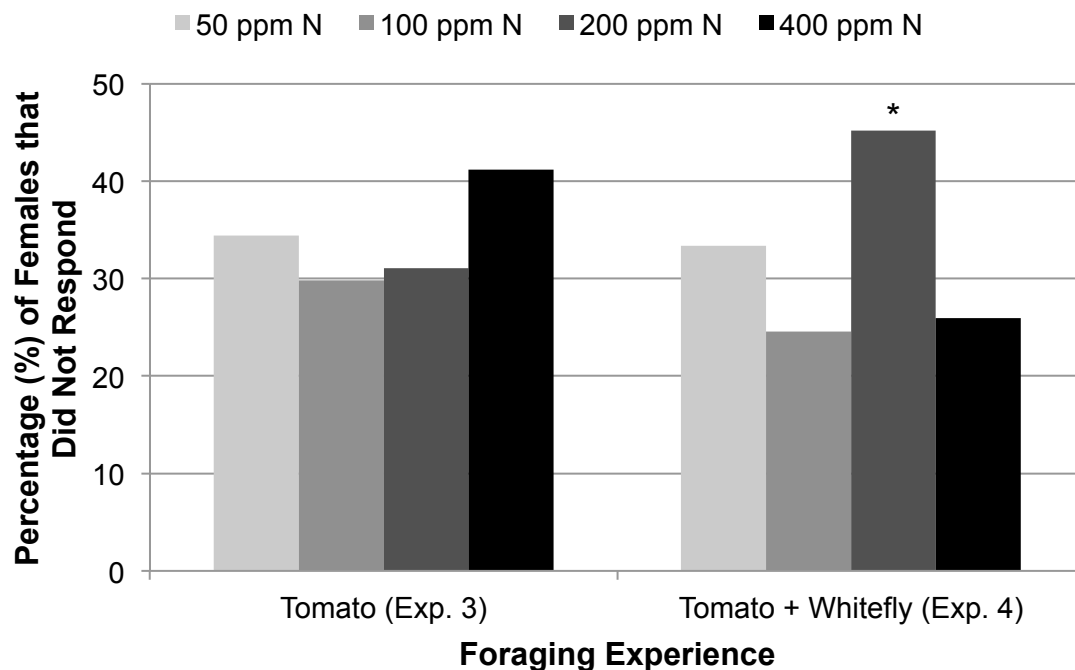
Figure 4.2. The interaction between plant quality and previous experience on the percentage of *Dicyphus hesperus* females that did not respond in the olfactometer in Exp. 3 and Exp. 4, where the previous foraging experience of the females differed. Equal percentages of females did not respond in the olfactometer for all four levels of plant quality in Exp. 3. When *D. hesperus* had experience foraging on whitefly-infested tomato plants (Exp. 4), a greater percentage of females did not respond in the olfactometer when 200 ppm N-fertilized whitefly infested tomato plant material was present compared when plant material from the other three levels of plant quality was present (χ^2 = 6.9441, df = 1, p = 0.0084), as indicated by the asterisk.

Chapter 5. Are omnivores picky eaters? The prey preference of *Dicyphus hesperus*, an omnivorous biological control agent of greenhouse whitefly (*Trialeurodes vaporariorum*)

Introduction

There are a number of advantages associated with using omnivores, especially zoophytophagous predators, as biological control agents. For example, diet mixing improves the stability of omnivore population dynamics (Singer and Bernays 2003). Plant feeding enables these omnivores to survive on plant hosts during times of prey scarcity (Bugg *et al.* 1987; Naranjo and Gibson 1996; Wiedenmann *et al.* 1996; Coll and Guershon 2002), and can facilitate the establishment of the omnivore simultaneously with, or prior to pest establishment (Gabarra *et al.* 2004; Castañé *et al.* 2011). Plant feeding by some omnivores is also known to induce plant defence responses, with negative consequences for pest species (Pérez-Hedo *et al.* 2015). These traits can reduce the need for multiple releases of biological control agents and enable omnivores to maintain pest populations in the latent growth phase, below their economic threshold (Wiedenmann and Smith 1997). Despite these advantages, there are also some inherent difficulties associated with omnivores used as biological control agents. Firstly, plant feeding can damage the crop the omnivore is supposed to protect (Shipp and Wang 2006; Calvo *et al.* 2009; Arnó *et al.* 2010; Castañé *et al.* 2011). *Nesidiocoris tenius* (Reuter) (Hemiptera: Miridae) plant feeding, for example, results in necrotic rings and the abortion of tomato, *Solanum lycopersicum* L. (Solanales: Solanaceae), flowers (Sanchez 2008; Sanchez and Lacasa 2008; Castañé *et al.* 2011), resulting in economic losses that warrant the use of economic thresholds for this omnivore (Sanchez and Lacasa 2008; Calvo *et al.* 2009). *Dicyphus tamaninii* Wagner (Hemiptera: Miridae) also requires the use of thresholds and monitoring to prevent tomato yield losses when *D. tamaninii* is used to manage greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) (Alomar and Albajes 1996). Other issues include cannibalism (Laycock *et al.* 2006) and low reproductive rates compared to prey species (Carayon 1961). Importantly, the feeding behaviour of omnivores can be difficult to predict and has been described as "ambiguous" by Alomar and Albajes (1996). These disadvantages need to be

overcome in order to ensure the success of biological control programs that utilize omnivores. Therefore, it is important to determine the diet breadth, prey preference, and potential consumption rate of the target pest by an omnivore before initiating large-scale biological control programs.

Following ecological theory, predators are generally expected to exhibit a Type II functional response, such that the consumption rate of the predator increases linearly as prey density increases until the predator becomes satiated (Holling 1959; Holling 1966), and select their prey following the predictions of optimal foraging theory (Emlen 1966; Krebs 1977; Pyke *et al.* 1977; Stephens and Krebs 1986). Because of physical limitations, an important assumption of optimal foraging theory is sequential encounter and handling of prey items (Stephens and Krebs 1986). As a result, predators are faced with prey handling decisions when multiple prey items are available simultaneously. These decisions are generally based on economics, where prey items are rejected if the costs (search, handling, predation risk) outweigh the benefits (energy, nutrients) (Krebs and Davies 1993). Assuming that a predator is able to evaluate potential prey items and assess potential costs and benefits before attacking, we might predict that an optimal predator should discriminate between two prey items of the same species if the two items differ in terms of energetic or nutritional gain or associated costs. As zoophytophagous omnivores act as both herbivores and predators, their feeding behaviour is less predictable than that of pure predators (Alomar and Albajes 1996), and is poorly understood (Singer and Bernays 2003; Gillespie *et al.* 2012). This poses a challenge to the development of biological control programs using omnivores.

A number of zoophyotphagous Hemiptera have been investigated for their potential as biological control agents in the past 20 years. Some examples include *Macrolophus caliginosus* Wagner (Hemiptera: Miridae), *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), *D. tamaninii*, *N. tenius*, *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae), and *Dicyphus hesperus* Knight (Hemiptera: Miridae) (Alomar and Albajes 1996; Wiedenmann *et al.* 1996; Barnadas *et al.* 1998; McGregor *et al.* 1999; Sanchez and Lacasa 2008; Castañé *et al.* 2011; Messelink *et al.* 2014). In Canada, a significant body of work has focused on the use of *D. hesperus* for biological control of the greenhouse whitefly and the two-spotted spider mite, *Tetranychus urticae* Koch

(Acari: Tetranychidae) (McGregor *et al.* 1999). *Dicyphus hesperus* has been recorded across Canada and in several northwestern States (Kelton 1980; Henry and Wheeler 1988) and is easily reared in laboratory conditions on one of its host plants and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs for mass release into the field or greenhouse (McGregor *et al.* 1999; Gillespie and McGregor 2000). Several preferred host plant and prey species of *D. hesperus* have been identified. Host plants include *Verbascum thapsus* L. (Lamiales: Scrophulariaceae), and members of the Solanaceae such as *S. lycopersicum* (tomato), *Capsicum annuum* L. (pepper), and *Nicotiana tabacum* L. (tobacco) (Gillespie and McGregor 2000; Sanchez *et al.* 2004; VanLaerhoven *et al.* 2006). Acceptable species of prey include *T. vaporariorum*, *T. urticae*, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripdae), and eggs of *E. kuehniella* (McGregor *et al.* 1999; Shipp and Wang 2006; VanLaerhoven *et al.* 2006). The functional response of *D. hesperus* is dependent on prey species; for example individuals preying on *T. vaporariorum* exhibit the Type II functional response and individuals preying on *Phytoseiulus persimilis* Athias-Henriot (Acarina: Phytoseiidae) exhibit the Type III functional response (Brommit 2007). The intraguild interactions of *D. hesperus* with *Encarsia formosa* Gahan (Hymenotpera: Aphelinidae), an important parasitoid of greenhouse whitefly (van Lenteren *et al.* 1996; Hoddle *et al.* 1998), have also been investigated (McGregor and Gillespie 2005; Labbe *et al.* 2006; Brommit *et al.*, in review). Specifically, although *D. hesperus* exhibits no preference for feeding on *E. formosa* parasitized whitefly, *D. hesperus* has negative effects on *E. formosa* populations (Bennett *et al.* 2009).

Although a great deal is known about the preferences of *D. hesperus* for different species of prey, the response of *D. hesperus* to prey of a single species that vary in quality or suitability has not been addressed. Prey might vary in terms of palatability, ease of handling, nutritional quality, or energetic value. Variation in plant quality is known to impact the life history, fitness, and nitrogen content of herbivores (Bentz and Larew 1992; Blua and Toscano 1994; Jauset *et al.* 1998; Blackmer and Byrne 1999; Crafts-Brandner 2002; Bi *et al.* 2003), despite a number of adaptations that herbivores may use handle variation in the nutritional value of their host plants (Mattson 1980), such as compensatory feeding (Slansky and Feeny 1977). As a result, foraging predators are

likely to encounter prey of varying quality in natural settings. Following the predictions of optimal foraging theory, high quality prey, which feed on high quality host plants, should be preferred over low quality prey that consume low quality host plants (Stephens and Krebs 1986); however, the behaviour of *D. hesperus* might not conform to this prediction. Rather, *D. hesperus* might have the ability to compensate for low quality prey by consuming prey at a greater rate, or by plant feeding. To better undersand the predatory behaviour of *D. hesperus*, we manipulated prey quality, but not plant quality, 1) in no-choice arenas to determine if the consumption rate of prey items by *D. hesperus* changes as prey quality changes, and 2) in choice arenas to determine if *D. hesperus* discriminates between prey items of the same species when prey quality differs. For all experiments, greenhouse whitefly nymphs were used as prey. *Trialeurodes vaporariorum* is ubiquitous (van Lenteren *et al.* 1996), and poses a significant threat to plant productivity worldwide (Byrne and Bellows 1991; Pappas *et al.* 2013). It is also known to be resistant to many insecticide formulations (van Lenteren *et al.* 1996; Gorman *et al.* 2002; Bi and Toscano 2007; Pappas *et al.* 2013). For the first experiment, we tested the prediction that *D. hesperus* would consume more low quality whitefly nymphs than high quality whitefly nymphs in a given period of time. In the second experiment, we tested the prediction that *D. hesperus* would prefer to consume high quality whitefly prey over low quality whitefly prey.

Materials and Methods

Plant propagation

All experiments were conducted on 'Patio Hybrid' tomato plants, grown at the University of Windsor, Ontario, Canada. Tomatoes were grown from seed (Stokes Seeds Canada, Thorold, Ontario, Canada) in BM6 potting soil (Berger, Saint-Modeste, Quebec, Canada). Tomato seedlings were transplanted into 8-inch (~ 20 cm) green pots at the four-leaf stage. After transplantation, seedlings were randomly assigned to one of three fertilizer regimes: 1) 'low quality' seedlings receiving 50 ppm nitrogen (N)-fertilizer, 2) seedlings receiving 200 ppm N-fertilizer, and 3) 'high quality' seedlings receiving 400 ppm N-fertilizer. In their work to test the response of greenhouse whitefly to plant nitrogen nutrition, Jauset *et al.* (1998) fertilized tomato plants with 84, 140, and 308 ppm N in

solution and observed that increasing N concentration in the fertilizer subsequently increased plant nitrogen level, leaf numbers, and leaf water content. The nitrogen treatments selected for this study were based on the recommended N rate for tomatoes of 200 ppm N (AARD 2009). High N inputs resulted in significantly greater aboveground plant biomass and yield than low N inputs (Chapter 2). Solutions of N-fertilizer consisted of 5.75 g, 23.0 g, and 46 g of 6-11-31 Hydroponic Fertilizer and 4.25 g, 17.0 g, and 34.0 g of 15.5-0-0 calcium nitrate (Plant-Prod® Canada, Brampton, ON, Canada) dissolved in 20 L freshwater, for 50, 200 and 400 ppm N-fertilizer solutions, respectively. Seedlings received 100 ml of the appropriate fertilizer solution daily for seven days and every second day thereafter (following Jauset *et al.* 1998). Plants were watered as needed.

Insect rearing

All *D. hesperus* used in experimental trials were obtained from a colony maintained at the University of Windsor. The colony originated from insects collected from *Stachys albens* A. Gray (Lamiales: Lamiaceae) plants in California, USA (35º42'15"N, 118º50'00"W) (VanLaerhoven *et al.* 2003; McGregor and Gillespie 2004). All developmental stages of *D. hesperus* are held on *N. tabacum* plants and *E. kuehniella* eggs (Beneficial Insectary Inc., Guelph, Ontario, Canada) are provided *ad libitum* to provide protein (McGregor and Gillespie 2004). As adult *D. hesperus* are known to cannibalize nymphs (Laycock *et al.* 2006), adults and nymphs are separated in the colony. New generation nymphs emerge approximately two weeks following oviposition, depending on temperature, and undergo four instars before molting to the adult stage, approximately 20 days after hatching (Gillespie *et al.* 2004). The colony is maintained in a rearing room with a 16:8 h light: dark photoperiod at 20 ± 5°C and 50 ± 10% humidity (Nortec Humidity Inc., Ogdensburg, New York, USA).

   Trialeurodes vaporariorum are pests in the greenhouse at the University of Windsor, and often reach outbreak densities (M.A. Vankosky, *personal observation*). Therefore, whitefly nymphs were readily available for experiments. To expose whitefly nymphs to host plants varying in N nutrition, two whitefly colonies were established in separate 55 x 55 x 60 cm white mesh cages in the greenhouse under ambient conditions with adults collected from *N. tabacum* using an aspirator (also see Chapter 3). One

colony was provided with 50 ppm N-fertilized tomato plants as feeding and development hosts, and the other with 400 ppm N-fertilized tomato plants. Previous research has demonstrated that plants with high levels of N are preferred feeding and oviposition hosts of whitefly (Bentz and Larew 1992; Bi *et al.* 2003) and that feeding upon high N plants increases survival rates and decreases development time (Bentz and Larew 1992; Blua and Toscano 1994; Jauset *et al.* 1998; Blackmer and Byrne 1999). There is also evidence that feeding on high N host plants, fertilized with approximately 1300 ppm N $Ca(NO_3)_2$, twice weekly increases the concentration of free amino acids in the tissues of whitefly relative to whitefly feeding on plants with an N deficit (Crafts-Brandner 2002). For these experiments, high quality prey were those reared on 400 ppm N fertilized tomato plants, and 'quality' refers to any differences, nutritional or physiological, that result from whitefly development on high and low N host plants.

All whitefly used in these experiments were fourth instar nymphs, to reduce variation between individual prey items or the ability of *D. hesperus* to feed on nymphs due to morphological differences between developmental stages. Fourth instar nymphs were easily identified by the presence of 'red eyes' (Byrne and Bellows 1991). Fourth instar nymphs are also non-feeding (Byrne and Bellows 1991), which ensured that prey quality would not change during the experiment. Whitefly nymphs were collected by hand from plants inside the whitefly colony cages using a fine-bristled paintbrush to prevent damage to the nymphs during collection and transfer. For both high and low quality whitefly nymphs, four samples of ten fourth instar nymphs were randomly collected from the leaves of different tomato plants in the colonies and their length and width were measured at 4x magnification using a Meiji dissecting microscope (Meiji Techno America, San Jose, California, USA). Lengths and widths were reported in mm and compared between low and high quality treatments with analysis of variance (ANOVA) using PROC MIXED (SAS Institute 2009).

Rate of prey consumption

Adult female *D. hesperus*, seven to 10 days old, were collected from the rearing colony for use in experiments. These insects were isolated from conspecifics and held in 60 ml Solo® cups (Lake Forest, Illinois, USA) for 48 h. Each cup contained a small piece of wet

dental cotton (Richmond Dental, Charlotte, North Carolina, USA) to prevent dehydration of the insect. Insects were treated as such to standardize hunger levels among subjects, as hunger is known to affect the number of prey that adult *D. hesperus* consume in a given time (Gillespie *et al.* 2012).

On the day of the experiment, test arenas were set up in 50 mm Petri dishes. Each arena contained a piece of Whatman® Grade 1 qualitative filter paper (Maidstone, Kent, UK) moistened with fresh water to prevent desiccation, and 20 whitefly nymphs placed on a leaflet clipped from a 200 ppm N-fertilized tomato plant to facilitate *D. hesperus* foraging. In pilot trials for these experiments, *D. hesperus* females foraging on filter paper rarely consumed any prey after 6 h, but readily consumed prey placed on tomato leaves in 3 h (M.A. Vankosky, *personal observation*). Tomato leaflets were prey-free prior to placement inside the arena. Whitefly prey was placed in a patch on the leaflet, to mimic natural prey distribution. Prepared test arenas were placed on a countertop in the room housing the *D. hesperus* colony, thus the insects did not experience any changes in temperature, humidity, or photoperiod at any time during the experiment.

After the test arenas were prepared, a single female was placed inside each arena, away from the tomato leaflet and the arenas were closed and visually isolated from nearby arenas by placing a 15mm tall metal ring from a 250 ml Mason jar (Bernardin Ldt., Richmond Hill, Ontario, Canada) around each arena. After 3 h, females were removed from the arena with an aspirator. The number of whitefly nymphs consumed in each arena was determined using a dissecting microscope at 2.5x magnification. Whitefly nymphs that had been consumed by *D. hesperus* appeared flattened and the appearance of the red eyes was altered or the eyes were missing completely relative to unconsumed nymphs (M.A. Vankosky, *personal observation*). The experiment was repeated 40 times for both levels of prey quality between 03 January and 26 January 2014, during daylight hours. Experiments were initiated between 09:30 and 11:15 h; this timing was coordinated with the timing of the 48 h prey-deprivation period. As all female *D. hesperus* were allowed to feed for 3 h, the consumption rates of high and low quality prey could be compared following Peterson and Renaud (1989) using one-way analysis of variance (ANOVA) (SAS Institute 2009). For statistical testing $\alpha = 0.05$.

Prey choice

Female *D. hesperus* were collected and deprived of prey for 48 h and test arenas were set up as described above, with the exception that patches of high and low quality prey were provided simultaneously. Both prey patches in each arena contained 15 whitefly nymphs. Patches were located near the tip or the stem of the leaflet to enable the human observer to discriminate between prey quality patches. Location of the low and high quality prey patches on the leaflets was alternated every four replicates; the experiment was repeated 74 times (n = 73 as one arena was contaminated with a second omnivore). All replicates of the experiment were conducted between 03 and 10 February 2014 and on 02 and 05 March 2014, during daylight hours, with replicates initiated between 08:45 and 15:00 h (timing was coordinated with the 48 h prey-deprivation period). After the arenas were prepared, a single female was placed into each arena, away from the leaflet. As described above, the arenas were closed, visually isolated from other arenas, and females were allowed to feed for 3 h, after which the females were removed and the number of consumed prey of both levels of quality was determined.

Two factors may have impacted prey choice in this experiment: the quality of the prey and the location of the prey patches on the tomato leaflets. A two-factor ANOVA (SAS Institute 2009) was used to test for location and interaction effects. ANOVA results indicated that location of prey patches did not have a significant effect on the number of prey of each quality that was consumed and that there was no significant interaction effect (see Results). Therefore, patch location was not included in further analyses.

Feeding choice or preference experiments violate the assumption of independence between treatments necessary for parametric statistics; as such, univariate ANOVA should not be used (Roa 1992, Manly 1993, Larrinaga 2010). Alternative statistical methods include multivariate ANOVA (MANOVA), chi-square analysis, Chesson's α_i analysis, and a number of other options (Chesson 1983; Peterson and Renaud 1989; Roa 1992; Manly 1993; Lockwood 1998; Prince *et al.* 2004; Larrinaga 2010). Chesson's α_i is advantageous as it can be calculated for a variety of different experimental conditions, including when prey is depleted during the feeding period (Chesson 1983), as in our experiment. If only two types of food are compared, as in our experiment, the α_i -values can be compared using a *t*-test (Chesson 1983). Due to the advantages listed, and the

inappropriateness of parametric univariate tests, Chesson's α_i was used for analysis of feeding preference. Values of α_i were calculated for each trial for both high and low quality whitefly prey using the equation for Case 2, in which food depletion occurs:

$\alpha_i = \frac{\ln(n_i - r_i/n_i)}{\sum_{j=1}^{m} \ln(n_j - r_j/n_j)}$, where α_i is the preference of the predator for food type i, n_i is the

amount of prey of type i available, r_i is the amount of food type i eaten and m is the number of food types offered (from Chesson 1983). As α_i increases, the proportion of that food type in the diet increases, with values of 1.0 indicating a pure diet (Chesson 1983). Mean α_i, variance (s^2), standard deviation (s), and standard error were calculated for both high and low quality prey. For each quality of prey, the descriptive statistics were used to

calculate t_s using: $t_s = \frac{\bar{\alpha}_i - 0.5}{\sqrt{s^2/k}}$, where k is the number of trials ($k = 73$ for this experiment)

(from Chesson 1983). To test the null hypothesis of no preference, the resultant t_s-values were compared to t_{crit} −values of 1.993, 2.646, 2.896, and 3.431 respectively, for $\alpha = 0.05$, 0.01, 0.005 and 0.001 with 72 degrees of freedom (values from Zar 2010) to estimate the p-value for the test. This analysis was used by Schmidt *et al.* (2012) to determine the feeding preferences of wolf spiders (*Pardosa milvina* (Hentz) Araneae: Lycosidae). To test for differences in the absolute number of whitefly nymphs of each quality consumed during the 3 h period, we used MANOVA (IBM SPSS Statistics 2012) to generate Hotelling's T^2 statistics (Schmidt *et al.* 2012). For this analysis $\alpha = 0.05$.

Results

No statistical difference in the mean (± SE) length of whitefly nymphs reared on low quality (0.767 ± 0.015 mm) and high quality (0.741 ± 0.006 mm) tomato plants was observed (ANOVA: $F_{1, 6} = 2.76$, $p = 0.1478$). Similarly, no significant difference was observed between the widths of nymphs reared on low (0.538 ± 0.015 mm) and high (0.504 ± 0.007 mm) quality tomato plants (ANOVA: $F_{1, 6} = 4.44$, $p = 0.0796$).

In the 3 h feeding period for the no-choice trials, female *D. hesperus* consumed a minimum of three and a maximum of 16 low quality whitefly nymphs. When provided with high quality prey, a minimum of one and a maximum of 11 whitefly nymphs were consumed. The number of high and low quality prey consumed by *D. hesperus* females

differed (ANOVA: $F_{1, 78} = 9.51$, $p = 0.0028$), as more low quality prey was consumed than high quality prey (Figure 5.1).

In choice arenas, *D. hesperus* females consumed a minimum of zero and a maximum of 13 prey in 3 h. Several females consumed no low quality prey, resulting in six individuals with a diet comprised of exclusively high quality whitefly nymphs. Only one individual of the 73 included in the analysis consumed only low quality prey. Location of the prey patches did not affect the number of prey of each type consumed (ANOVA: $F_{1, 113} = 0.59$, $p = 0.4446$), and there was no interaction between location and prey quality (ANOVA: $F_{1, 113} = 0.01$, $p = 0.9073$). Therefore, location was not incorporated into calculations for Chesson's α_i –values. Mean ($\pm$ SE) α_i –values indicated that the diet of *D. hesperus* was dominated by high quality prey; high quality prey accounted for $62.97 \pm 0.03\%$ of the diet, which was significantly more than 50% of the diet ($t_s = 4.27$, df $= 72$, $p < 0.001$). Low quality prey accounted for $37.63 \pm 0.03\%$ of the diet, which was significantly less than 50% of the prey consumed by female *D. hesperus* ($t_s = -4.27$, df $= 72$, $p < 0.001$). In terms of absolute consumption during choice trials, female *D. hesperus* consumed significantly more high quality than low quality whitefly nymphs (MANOVA: $F_{1, 72} = 10.9$, $p = 0.001$; Figure 5.2).

Discussion

In no-choice trials the rate of prey consumption by *D. hesperus* females was affected by prey quality, with females consuming more low quality nymphs, reared on low N tomato plants, than high quality nymphs, reared on high N tomato plants. Because there was no significant difference in the size of whitefly nymphs reared on high and low N tomato plants, the difference in rate of prey consumption cannot be attributed to prey size, but to differences in prey quality. This result suggests that *D. hesperus* adjust their rate of prey consumption to compensate for low nutrient or energy gains associated with whitefly nymphs reared on low N tomato plants. Because N is considered limiting in terrestrial ecosystems (Mattson 1980), there are a number of physiological or behavioural adaptations that consumers may utilize to increase N intake (Mattson 1980). A simple compensatory behaviour is to increase the rate of food consumption (Slansky and Feeny 1977; Mattson 1980; Slansky and Wheeler 1992).

Slansky and Feeny (1977) were among the first to document increased rates of food consumption for herbivores on low quality resources. In their observations of fifth instar *Pieris rapae* L. (Lepidoptera: Pieridae) larvae feeding on plants across a range of N concentrations larvae consumed more low N than high N plant material but larvae feeding on both high and low N diets had equal growth rates (Slansky and Feeny 1977). A number of other herbivores have demonstrated similar compensatory feeding behaviour when challenged with diets deficient in N and/or phosphorus (P). Examples include *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) (Slansky and Wheeler 1989), *Samea multiplicalis* (Guenée) (Lepidoptera: Crambidae) (Wheeler and Halpern 1999), *Daphnia magna* Straus (Cladocera: Daphniidae) (Plath and Boersma 2001), and *Prokelisia dolus* Wilson (Hemiptera: Delphacidae) (Huberty and Denno 2006). Natural fluctuation in nutrient levels have also been associated with increased feeding rates of herbivorous insects. For example, Oishi *et al.* (2006) observed that larvae of the oak silk moth, *Antheraea yamamai* (Guérin-Méneville) (Lepidoptera: Saturniidae) in no-choice conditions consumed more *Quercus acutissima* Carruthers (Fagales: Fagaceae) leaf tissue from leaves of the lower crown (low N) than from the upper crown (high N).

Predators are also known to increase their rate of consumption to compensate for low quality prey. For example, when the predatory ladybeetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), was offered *Aphis nerii* B de F. (Hemiptera: Aphididae) with high and low N content in no-choice arenas, more low N than high N aphids were consumed (Couture *et al.* 2010). For this experiment, *A. nerii* were reared on tropical milkweed, *Asclepias curassavica* L. (Gentianales: Apocynaceae), treated with two levels of N fertilizer to manipulate prey quality (Couture *et al.* 2010). Similar compensatory feeding rates were observed for *H. axyridis* feeding on cereal aphids when aphids were reared on low N wheat plants (*Triticum aestivum* L., Poales: Poaceae) relative to aphids reared on wheat receiving high N inputs (Aqueel and Leather 2012). Finally, Khanamani *et al.* (2014) observed that the predator *Typhlodromas bagdasarjani* Wainstein & Arutunjan (Acari: Phystoseiidae) increased its rate of consumption when fed two-spotted spider mites reared on mite-resistant eggplant, *Solanum melongena* L. (Solanales: Solanaceae) relative to mites reared on susceptible eggplants.

As the above examples demonstrate, compensatory feeding behaviour is common among both herbivores and predators. In searching the *Web of Science* for compensatory feeding literature with regard to prey quality, we found only one reference to omnivores. In that study, Visanuvimol and Bertram (2011) examined the effect of P on the life history of crickets, *Acheta domesticus* L. (Orthoptera: Gryllidae), and found that crickets did not compensate for low P levels via increased consumption rates (Visanuvimol and Bertram 2011). This is contrary our observations; however, there were a number of differences in the design of these two experiments. Importantly, Visanuvimol and Bertram's (2011) experiment modified P in an artificial diet, whereas in our study N levels in the rearing hosts of whitefly nymphs were manipulated to affect the quality of actual prey items. These two studies are difficult to compare, but taken together, they highlight the need for further investigation regarding the effect of nutrient deficiency on the feeding rate of omnivores. Gillespie *et al.* (2012) found that *D. hesperus* consumed different amounts of prey after being held on different species of host plant. This result supports our conclusion that *D. hesperus* engages in compensatory feeding behaviours, and suggests that this type of behaviour can be used to respond to prey availability and quality across a range of conditions.

In choice arenas, *D. hesperus* females exhibited a significant preference for whitefly nymphs reared on high N tomato plants when foraging. Optimal foraging theory suggests that animals should make informed decisions when foraging so as to maximize energy or nutritional intake (Stephens and Krebs 1986). Our results suggest that *D. hesperus*, a generalist zoophytophagous omnivore, makes foraging decisions based on the quality of available prey when plant quality is consistent. However, our observations do not fully agree with the predictions of optimal foraging theory. For example, the model predicts that the predator ranks different types of food and those of lower rank should not be included in the diet unless foods of higher rank are absent, or the costs associated with handling low quality foods is negligible (Krebs 1977; Stephens and Krebs 1986). In other words, a predator should consume a pure diet, and if the diet is mixed, then high quality prey should first be depleted. This was not the case for *D. hesperus*, as only 11% of insects consumed a pure diet, and one individual consumed only low quality prey. Moreover, none of the insects that consumed a mixed diet had completely depleted their

supply of high quality prey. It is possible that *D. hesperus* had incomplete knowledge of the prey offered, which is reasonable, as none had previous exposure to whitefly nymphs as prey. Alternatively, females might have exhibited a starvation effect, as insects had been deprived of both plant and prey food for the 48 h preceding the feeding trials. Starvation may have resulted in consumption of the first prey encountered, regardless of quality. It would be interesting to test the effects of previous diet on prey choice by *D. hesperus*. In a recent experiment, Schmidt *et al.* (2012) found that wolf spiders provided a diet of high quality fruit flies prior to experimentation preferred high quality fruit flies in choice tests, while spiders fed low quality fruit flies did not exhibit a preference. Very few other researchers have investigated the effects of intraspecific prey quality on predator choice, although many have compared predator preferences for two or more different prey species (Hazzard and Ferro 1991; Eubanks and Denno 2000; Reitz *et al.* 2006; Ferrer *et al.* 2008). Overall, the results of this experiment are important as they indicate that *D. hesperus* females do discriminate between prey items of the same species that vary in quality, although not perfectly, as predicted by optimal foraging theory.

Previous work with *D. hesperus* has focused on its preference for and performance on different species of prey and host plants (McGregor *et al.* 1999; Gillespie and McGregor 2000; Sanchez *et al.* 2004; Shipp and Wang 2006; VanLaerhoven *et al.* 2006), allowing biological control planners to predict the best habitats to employ *D. hesperus* in, and to predict the predation potential of *D. hesperus* against different pest species. This makes sense, as the composition of the plant and prey community utilized by *D. hesperus* is a primary determinant of its diet (Gillespie *et al.* 2012). Our results indicate that *D. hesperus* foraging decisions are dependent on prey quality when only a single species of prey is available. However, our experiments do not address the long-term impacts of consuming a low quality diet on *D. hesperus*, or the effects of both host plant and prey quality variation on foraging decisions. These questions must be addressed to achieve a fuller understanding of *D. hesperus* and hopefully, zoophytophagous predators in general.

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Figure 5.1. The mean (± SE) number of prey consumed by *Dicyphus hesperus* females in 3 h when provided with whitefly nymphs reared on low N tomato plants ('low quality prey') or whitefly nymphs reared on high N tomato plants ('high quality prey') in no-choice arenas. Significantly more low quality nymphs were consumed than high quality nymphs (ANOVA: $F_{1,78} = 9.51$, $p = 0.0028$).
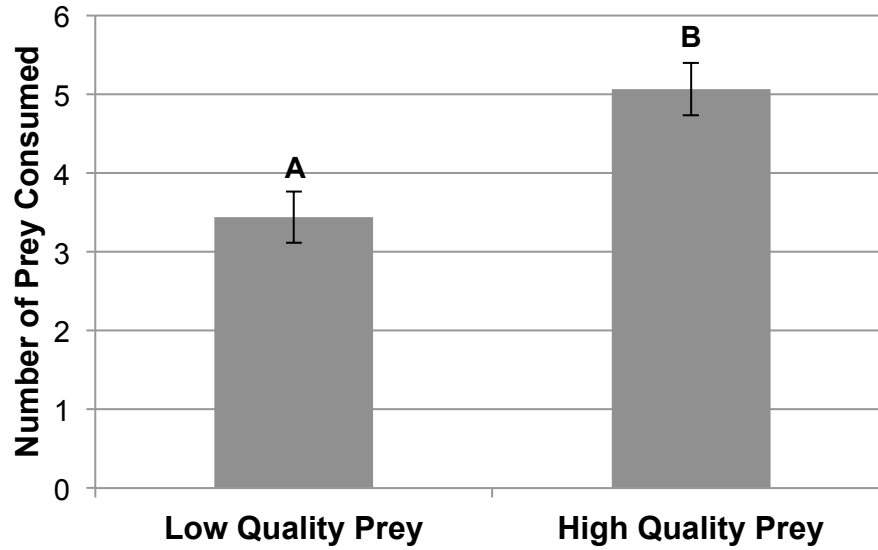
Figure 5.2. The mean (± SE) number of prey consumed in 3 h when starved *Dicyphus hesperus* females were allowed to choose between patches of whitefly nymphs reared on low N tomato plants ('low quality prey') and whitefly nymphs reared on high N tomato plants ('high quality prey') for feeding. Significantly more high quality prey items were consumed than low quality prey items (Hotelling's T^2: $F_{1, 72} = 10.9$, $p = 0.001$).

Chapter 6. Plant and prey quality interact to influence the foraging behaviour of an omnivorous insect, *Dicyphus hesperus*

Introduction

      Early ecosystem models led Pimm and Lawton (1978) to conclude that omnivores should be rare in ecosystems; however, omnivores are more common in ecosystems than originally thought. In the Insecta, omnivores occur in approximately 40 families (Coll and Guershon 2002). Due to the prevalence of omnivores, understanding the role of omnivores in ecosystems has been the focus of a significant body of research, including a special feature in *Ecology* (Agrawal 2003). Because omnivores consume a mixed diet, obtaining energy and nutrients from multiple trophic levels (Coll and Guershon 2002; Agrawal 2003), the feeding behaviour of omnivores is often unpredictable (Coll 1996; Agrawal *et al.* 1999; Singer and Bernays 2003). This is because the foraging behaviour of omnivores does not necessarily fit well with traditional foraging models, such as optimal foraging theory (Stephens and Krebs 1986), or the functional response model (Holling 1959; 1966). However, there are situations in which it might be important to predict the behaviour of an omnivore with some level of precision. For example, omnivores might be important biological control agents in agroecosystems (Alomar and Wiedenmann 1996; McGregor *et al.* 1999), but only if they do not significantly damage crop plants as some omnivores are known to do (Alomar and Albajes 1996; Agrawal *et al.* 1999; McGregor *et al.* 2000).

      A more thorough understanding of when and why omnivores consume a mixed diet might improve our ability to predict the feeding behaviour of omnivores. However, there are a number of potential factors that might contribute to the consumption of a mixed diet of plant and animal tissues, including but not limited to: prey availability, the risks associated with specific prey or plant hosts, toxin dilution, and a lack of essential nutrients or minerals in the primary diet (Freeland and Janzen 1974; Westoby 1978; Rapport 1980; Bjorndal 1991; Hailey *et al.* 1998; Singer and Bernays 2003). It is unlikely that the feeding behaviour of omnivores is driven by only one of these factors. In addition, the factors that affect the feeding behaviour of individuals of different species, or of the same species, might vary considerably. Within any one species of omnivore,

feeding behaviour likely varies depending on the habitat, life history stage, and the current condition of each individual. Because of the diversity of possible hypotheses and the potential for multiple interacting factors, it is a challenge to understand the feeding behaviour of omnivores from a theoretical standpoint.

Direct observation of omnivores may help researchers to identify the primary factors that influence feeding decisions so that testable hypotheses can be developed. For example, Rosenheim *et al*. (2004) used focal observations of the omnivore *Lygus hesperus* (Knight) (Hemiptera: Miridae) in the field and in the laboratory to determine if it was causing yield losses in important crops in California. Focal observations of feeding and foraging behaviour have been utilized to identify the factors that affect the preference of *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) for two thrips species, *Frankliniella occidentalis* (Pergande) and *F. bispinosa* (Morgan) (Thysanoptera: Thripidae), that differ in size and mobility (Reitz *et al.* 2006). Similarly, VanLaerhoven *et al.* (2000) used focal observations to compare the search time of *Orius tristicolor* (White) (Hemiptera: Anthocoridae) for their prey on beans (*Phaseolus vulgaris* L., Fabales: Fabaceae) damaged artificially or by herbivores, in the presence and absence of prey. Focal observations were also used to determine if the foraging behaviour of the assassin bug, *Zelus renardii* Kolenati (Hemiptera: Reduviidae), changed with its developmental stage (Cisneros and Rosenheim 1998). Although focal observations must be applied on a case-by-case basis, they yield knowledge specific to the species of interest. This information can be directly applied to situations where omnivores are important, such as in ecosystems where omnivores can be used as biological control agents, or to future hypothesis testing.

Dicyphus hesperus Knight (Hemiptera: Miridae) is an omnivorous generalist (McGregor *et al.* 1999; Wheeler 2001), endemic to North America (Kelton 1980; Henry and Wheeler 1988). *Dicyphus hesperus* was first tested in Canada for its potential to control small-bodied crop pests such as greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) on tomato plants (*Solanum lycopersicum* L., Solanales: Solanaceae) with favorable results (McGregor *et al.* 1999). However, excessive plant feeding by *D. hesperus* on tomato leaves may have negative effects on the plant, or *D. hesperus* may quit prey feeding in favour of plant feeding. To implement

effective biological control programs using *D. hesperus* or other omnivores, we need to understand the factors that induce plant and prey feeding and how those factors might interact and influence the activity budget of the omnivore.

Using focal observations of *D. hesperus* foraging behaviour in the laboratory, we tested three hypotheses. First, we tested the null hypothesis of no difference in the proportion of time that *D. hesperus* females dedicated to different foraging behaviours (H_{01}) to determine if the behaviour of this insect was more similar to that of a predator or a herbivore. Second, we tested the null hypothesis of no effect of plant or prey quality on the activity budget of foraging *D. hesperus* females (H_{02}). This information may be used to better predict the foraging behaviour of omnivores in natural conditions. Finally, we tested the null hypothesis that prey handling time and prey consumption rate would not be affected by prey or plant quality (H_{03}). Overall, our objective for this study was twofold: to improve our understanding of *D. hesperus* foraging to improve its efficacy as a biological control agent, and to improve upon our understanding of the foraging behaviours of omnivores in general.

Materials and Methods

Insect and plant rearing

A colony of *D. hesperus*, originating from *Stachys albens* A. Gray (Lamiales: Lamiaceae) plants in California (Sparkes 2012) is maintained at the University of Windsor for experimental purposes. Insects are held on *Nicotiana tabacum* L. (Solanales: Solanaceae) plants, which serve as a food plant and oviposition medium (McGregor *et al.* 1999; Gillespie and McGregor 2000). To provide protein and otherwise supplement nutrients extracted from the host plant, frozen *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs are provided in excess (McGregor *et al.* 1999; Gillespie and McGregor 2000). More specific colony rearing details can be found in VanLaerhoven *et al.* (2003), McGregor and Gillespie (2004), Sparkes (2012) and Chapter 2. For this experiment, female *D. hesperus*, seven to 14 days old were randomly selected from the rearing colony and held individually in 60 ml Solo® cups (Lake Forest, Illinois, USA) provisioned with moistened dental cotton (Richmond Dental, Charlotte, North Carolina, USA), to provide a source of water. Females were held under these conditions for 48 h prior to behavioural

observations to standardize hunger levels between individual insects, to reduce variation that might occur during the experiment due to differences feeding rates resulting from differing hunger levels, as have been observed by Gillespie *et al.* (2012) for *D. hesperus*. In pilot trials, we observed that females held in isolation with only water for 72 h resulted in constant bouts of plant feeding lasting greater than 1 h. Isolation for 24 h with only water resulted in erratic behaviour when females were placed into experimental arenas. Insects held in isolation for 48 h with only water for consumption were calm when placed into experimental arenas and readily consumed both plant and prey material.

All experiments were performed using tomato, *S. lycopersicum*, as the host plant. 'Patio Hybrid' tomato seeds (Stokes Seeds Canada, Thorold, Ontario, Canada) were sown into potting soil (78% peat plus 22% perlite; Berger, Saint-Modeste, Quebec, Canada). Following emergence of the third and fourth leaves, tomato seedlings were transplanted into green pots filled with the same potting medium and haphazardly assigned to one of four nitrogen (N)-fertilizer treatments that varied in N concentration: 50, 100, 200 and 400 ppm N (Chapter 2). Fertilizer treatments were obtained by dissolving the appropriate masses (Table 6.1) of Hydroponic Fertilizer (6-11-31) and calcium nitrate (15.5-0-0; Plant-Prod® Canada, Brampton, ON, Canada) in 20 L freshwater. Each seedling received 100 ml of their assigned N-fertilizer solution (after Jauset *et al.* 1998), delivered daily for the first seven days, followed by every second day until the plants were no longer needed. Seedlings were watered as needed to prevent wilting. All plants were grown in a greenhouse with a natural photoperiod (no manipulation of photoperiod with artificial lighting). Monthly temperatures in the greenhouse in 2013 are provided in Table 6.2.

Experimental design and focal observations

The effect of plant and prey quality on the foraging behaviour of *D. hesperus* was tested using a two-factor experimental design, testing two levels of prey quality (high and low) and four levels of plant quality (50, 100, 200, and 400 ppm N). Arenas for observing insect foraging behaviour consisted of 50 mm Petri dishes, each containing a small tomato leaflet (approximately 1 to 2 cm long). Ten freshly collected *T. vaporariorum* nymphs were placed on the tomato leaflet using a fine-bristle paintbrush. Low quality whitefly nymphs were collected from 50 ppm N-fertilized tomato plants and high quality

nymphs were collected from 400 ppm N-fertilized tomato plants (see Chapters 3 and 5 for whitefly colony rearing details). All prey offered to an individual insect were of the same quality. Whitefly nymphs used as prey in this experiment were of the late third instar or fourth instar, when the bodies of the nymphs are a pale yellow colour and the nymphs have red eyes (Byrne and Bellows 1991). At this developmental stage, nymphs are non-feeding (Byrne and Bellows 1991), so we expect that no change in prey quality should occur during behavioural observations.

Immediately after an arena had been prepared, a single female *D. hesperus* that had been deprived of both prey and plant food for 48 h, was placed into the arena with plant and prey material. Each *D. hesperus* female was observed for 60 minutes, beginning five minutes after the insect was placed in the arena, or upon the first signs of feeding behaviour (described below). If a female was in the process of feeding (on plant or prey) at the conclusion of the 60-minute observation period, recordings continued until the end of the feeding bout. Methodologies used to record the foraging behaviour of *D. hesperus* were modified from work by Cisneros and Rosenheim (1998), Rosenheim *et al*. (2004), and Reitz *et al.* (2006). Behavioural observations were performed using a dissecting microscope (Meiji Techno America, San Jose, California, USA) at 2.5x magnification with a 10x ocular lens, such that the entire insect could be observed, including the insertion of the mouthparts or ovipositor into plant or prey material. During the 60-minute observation period, the behaviour of the insect was recorded in an Excel spreadsheet (Microsoft Corporation, Microsoft® Excel®: Mac 2011, v. 14.4.2) for every 20 s interval. If the insect's behaviour changed inside an interval, both behaviours were recorded if the switch occurred approximately halfway through the 20 s interval. Otherwise, the most dominant behaviour during each 20 s interval was recorded. Behaviours were defined as follows (values in parentheses were used to record behaviours): 1) resting (r); 2) walking (w), where the insect moved without contact between the mouthparts and plant or prey; 3) grooming (g); 4) plant probing (t1), where the stylets were in contact with plant material; 5) plant feeding (t2), where the stylets were inserted into the plant for more than 5 s; 6) prey probing (y1), where the stylets were in contact with prey; 7) prey feeding (y2), where the stylets were inserted into a whitefly nymph for more than 5 s; and 8) oviposition (o) (after Rosenheim *et al.* 2004).

During prey feeding bouts, the stylets could be seen moving inside the cuticle of the whitefly nymphs. The number of prey items consumed was recorded, as was the amount of time spent feeding on each individual prey item. The foraging behaviour of ten insects was observed for all eight of the plant quality by prey quality treatments (n = 80 insects).

Calculations and statistical analysis

After all observations were complete, the total time each of the 80 insects spent on each of the eight focal behaviours was calculated, followed by the proportion of total time spent on each behaviour. The proportion of the activity budget that was comprised of each of the behaviours was calculated using the total time that each insect was observed. Four behaviours, resting, walking, grooming, and oviposition, were rare compared to the other behaviours. Because our focus was on the foraging behaviours of *D. hesperus* we pooled those four behaviours together (referred to as 'other' behaviours) (after Reitz *et al.* 2006).

To determine if the foraging activity budget of *D. hesperus* females was balanced (H_{01}), the proportions of time spent on each behaviour were compared for all of the insects observed (n = 80). Several insects only engaged in one or two of the five behaviours included in the analysis. To determine if any of these insects could be considered statistical outliers, the Dixon's test for outliers was conducted, following the methods of Dixon and Massey (1951). Critical values for the test for outliers were obtained from Johnson and Leone (1994). Unfortunately, the datasets did not meet the assumptions of parametric analysis of variance (ANOVA), before or after transformations were applied (SAS Institute 2009). Therefore, the Kruskal-Wallis non-parametric ANOVA equivalent (SAS Institute 2009) was used to determine if the activity budget of *D. hesperus* was balanced. All analyses were performed using the NPAR1WAY procedure with the 'Wilcoxon' command in the PROC statement (SAS Institute 2009). Results were compared to $\alpha = 0.05$ to determine statistical significance. Significant Kruskal-Wallis test results were followed by ten Wilcoxon-Mann-Whitney paired comparison tests to identify which behaviours occupied a statistically different proportion of the activity budget (SAS Institute 2009). Results of the paired comparison tests were adjusted for multiple means comparisons using the Bonferroni correction (SAS Institute

2009), hence, Wilcoxon-Mann-Whitney results were compared to $\alpha = 0.005$ to determine statistical significance.

The effects of plant quality, prey quality, and the interaction of the two factors on the activity budget of female *D. hesperus* (H_{02}) were determined using a two-factor multivariate analysis of variance (MANOVA). The analysis was performed using the PROC GLM procedure with the 'MANOVA' statement (SAS Institute 2009). For MANOVA, *p*-values were compared to $\alpha = 0.05$ to determine significance. The use of MANOVA for activity budget analyses is common in the literature (see Cisneros and Rosenheim 1998; Reitz *et al.* 2006), as it allows for the analysis of multiple dependent variables simultaneously without losing predictive power (Foster *et al.* 2006). To further explore significant two-way MANOVA results, one-way MANOVA and univariate ANOVA analyses were used (Foster *et al.* 2006; SAS Institute 2009). One-way MANOVA analyses were used to further explore significant interactions, in a similar procedure to the 'SLICE' function used to explore interactions in two-factor ANOVA analyses (SAS Institute 2009). One-way MANOVA results were compared to $\alpha = 0.05$ to determine significance. Error associated with multiple comparisons using ANOVA was controlled using the Bonferroni adjustment (SAS Institute 2009).

Total prey handling time was calculated (total handling time = total prey probing + total prey feeding) and divided by the number of prey consumed by the insect to calculate the mean per prey handling time for each insect. To determine if plant quality or prey quality affected the number of prey consumed and the mean per prey handling time (H_{03}), two-way ANOVA analyses were conducted. Analysis was performed using the GLM procedure (SAS Institute 2009). For significant models, comparisons of treatment means were conducted using the PDIFF function with Bonferroni adjustment of α to avoid Type I Error (SAS Institute 2009).

Results

Of the five behaviours included in the analysis, prey feeding, prey probing, plant feeding, plant probing, and 'other' behaviours, six of 80 insects did not engage in 'other' behaviours (resting, walking, oviposition, and grooming). All females probed the tomato leaflet; however, three of the 80 observed females did not engage in plant feeding for

more than 5 s. Three of 80 females did not consume any prey; of those individuals, two did not attempt to prey feed during the observation period. One of these two females spent time plant probing, plant feeding, and on 'other' behaviours. The third female that did not prey feed spent 2.78% of the time probing the tomato leaflet, and the remainder of the time (97.22%) feeding from the tomato leaflet. This individual was determined to be a statistical outlier (Dixon's test statistic = 0.5885, n=80, $p \ll 0.01$) and was removed from the dataset for the statistical analyses regarding the total activity budget.

The activity budget of female *D. hesperus* was not balanced (Kruskal-Wallis test, $\chi^2 = 185.517$, df = 4, $p < 0.0001$). On average, females spent significantly more time feeding on prey than on any other behaviour and devoted the least amount of time to 'other' behaviours (Figure 6.1).

The interaction between plant quality and prey quality was significant (MANOVA: Wilk's $\lambda = 0.6620$, $F_{15, 185.36} = 1.99$, $p = 0.0179$), indicating that the foraging behaviour of *D. hesperus* depends on both the quality of the host plant and the quality of the prey that are encountered while foraging. Examples of the interactions observed as prey and plant quality varied are given for plant feeding and plant probing in Figure 6.2. Neither plant quality (MANOVA: Wilk's $\lambda = 0.7588$, $F_{15, 185.36} = 1.30$, $p = 0.2058$), nor prey quality (MANOVA: Wilk's $\lambda = 0.9203$, $F_{5, 67} = 1.16$, $p = 0.3380$), had a significant effect on the activity budget of *D. hesperus* females.

To statistically determine which behaviours were affected by the interaction of plant and prey quality, the dataset was further analyzed using two one-way MANOVA tests. In the first one-way MANOVA, the dataset was 'sliced' in order to determine: a) if *D. hesperus* were fed low quality prey, did the activity budget vary with plant quality, and b) if *D. hesperus* were fed high quality prey, did the activity budget vary with plant quality? When *D. hesperus* were provided with high quality prey, plant quality did not have an effect on the activity budget of *D. hesperus* (MANOVA: Wilk's $\lambda = 0.6238$, $F_{12, 84.96} = 1.38$, $p = 0.1903$). Therefore, when high quality prey was available, insects spent approximately the same proportion of their time on all behaviours in the activity budget, regardless of plant quality. When low quality prey was offered, plant quality did affect the activity budget of *D. hesperus* (Wilk's $\lambda = 0.4432$, $F_{15, 88.739} = 2.03$, $p = 0.0216$). Plant quality had a marginally significant effect on the mean time spent plant probing

(ANOVA: $F_{3, 36} = 3.02$, $p = 0.0424$; Figure 6.3) and plant feeding (ANOVA: $F_{3, 36} = 3.02$, $p = 0.0422$; Figure 6.4).

In the second one-way MANOVA, the data was 'sliced' to determine how prey quality affected the activity budget for each level of plant quality included in the experiment. Prey quality did not have an effect on the activity budget when *D. hesperus* were foraging on 50 ppm N-fertilized tomato leaflets (MANOVA: Wilk's $\lambda = 0.5617$, $F_{4, 15} = 2.93$, $p = 0.0567$), 100 ppm N-fertilized tomato leaflets (MANOVA: Wilk's $\lambda = 0.5279$, $F_{5, 14} = 2.50$, $p = 0.0806$), or 400 ppm N-fertilized tomato leaflets (MANOVA: Wilk's $\lambda = 0.9033$, $F_{5, 13} = 0.28$, $p = 0.9169$). Prey quality had a significant effect on the activity budget of *D. hesperus* when females were observed foraging on 200 ppm N-fertilized tomato leaflets (MANOVA: Wilk's $\lambda = 0.4678$, $F_{4, 15} = 4.27$, $p = 0.0167$). This effect was subsequently explored using one-way ANOVA. The results of this test indicated that *D. hesperus* spent significantly less of their time (ANOVA: $F_{1, 18} = 10.92$, $p = 0.0039$) probing the plant when high quality prey was available (mean ± SE: 12.71 ± 1.19%) than when low quality prey was available (20.09 ± 1.88%). None of the other behaviours included in the activity budget (prey feeding, prey probing, plant feeding, or 'other') were affected by prey quality when female insects were foraging on 200 ppm N-fertilized tomato leaflets (ANOVA: $p > 0.05$).

Prey quality did not affect the number of whitefly prey consumed (ANOVA: $F_{1,72} = 0.05$, $p = 0.8194$), as female *D. hesperus* consumed 3.58 ± 0.20 high quality whitefly nymphs relative to 3.50 ± 0.27 low quality whitefly nymphs. Plant quality did not affect the number of prey consumed during the observation period (ANOVA: $F_{3,72} = 1.70$, $p = 0.1744$; Table 6.3) and there was no significant interaction between prey quality and plant quality on the number of prey consumed (ANOVA: $F_{3,72} = 1.64$, $p = 0.1879$). Mean prey handling time was not affected by prey quality (ANOVA: $F_{1,72} = 0.01$, $p = 0.9393$), as females spent 555 ± 29 s handling high quality prey and 551 ± 35 s handling low quality prey. There was no significant effect of plant quality on mean prey handling time (ANOVA: $F_{3,72} = 1.32$, $p = 0.2735$; Table 6.3), and no significant interaction between plant quality and prey quality on prey handling time (ANOVA: $F_{3,72} = 0.36$, $p = 0.7813$).

Discussion

In foraging arenas, female *D. hesperus* exhibited a number of behaviours. Four of the behaviours were relatively rare, and were grouped together for the analysis. Behaviours associated with food consumption (plant and prey probing, plant and prey feeding) were much more prevalent in the activity budget. Females spent significantly more time prey feeding (approximately 40% of the activity budget), than on any other behaviour. The activity budgets of other generalist predators and omnivores have been determined in the laboratory and the field using methods similar to those used here (Cisneros and Rosenheim 1998; Rosenheim *et al.* 2004; Reitz *et al.* 2006). For example, *L. hesperus* has a wide diet breadth (Wheeler 2001), with some populations considered important predators of crop pests (Hagler and Naranjo 1994; Rosenheim *et al.* 2004), and other populations considered important crop pests themselves (Rosenheim *et al.* 2004). Rosenheim *et al.* (2004) observed a population of *L. hesperus* from California and found that their activity budget did not include any predatory behaviours. In fact, the activity budget of the observed *L. hesperus* nymphs and adults was dominated by resting, followed by walking and plant feeding (Rosenheim *et al.* 2004). Their results are contrary to our results for *D. hesperus*. The activity budget of nymphs and adults of the assassin bug, *Z. renardii*, were observed by Cisneros and Rosenheim (1998), who found that *Z. renardii* spent more than 20% of the observation period resting, more than 30% walking, and less than 15% of the observation period feeding. Compared to *Z. renardii* and the California population of *L. hesperus*, the activity budget of *D. hesperus* suggests that it occupies more of its time with food consumption. This might imply that *D. hesperus* is also a more efficient predator than *L. hesperus* or *Z. renardii*. However, the setting in which the observations were made should also be considered. For example, *Z. renardii* were observed in the field (Cisneros and Rosenheim 1998), whereas *D. hesperus* was observed in the laboratory. The activity budget of *D. hesperus* females might be strikingly different in field conditions, and this deserves investigation in the future.

Analysis of the activity budget as a whole revealed that the foraging behaviour of *D. hesperus* females was affected by an interaction of both plant and prey quality. When high quality prey were provided, the prevalence of behaviours in the activity budget was unaffected by plant quality. With low quality prey, plant quality did affect the proportion

of time females spent probing and feeding on plant material. Females spent a greater proportion of their time probing plant material when plant quality was high (23% of the activity budget at 400 ppm N) compared to when plant quality was low (16 and 18% of the activity budget on 100 and 50 ppm N plants, respectively). It is possible that females were able to derive more energy or essential nutrients (such as N, see Mattson 1980) from high quality plant material than from low quality whitefly nymphs. Alternatively, optimal foraging models predict that if low quality prey items require significantly less energy to locate and handle than high quality prey, they will be accepted in the diet, especially if they are highly prevalent (Stephens and Krebs 1986; Krebs and Davies 1993). In the test arenas used for this study, the tomato leaflet was more likely to be encountered than the whitefly prey. Therefore, it is possible that females spent more time probing the plant material as it was more common, and hence, more likely to yield a greater energy return than the less common whitefly prey. However, with low quality prey, females spent a greater proportion of the time plant feeding on low quality plants (26% of the activity budget on 50 ppm N plant material) than on high quality plants (14% of the activity budget on 200 ppm N plant material), which is contrary to our predictions. Many herbivores and predators are known to compensate for low quality foodstuffs by increasing their rate of food consumption (Slansky and Feeny 1977; Mattson 1980; Slansky and Wheeler 1992). Recently, compensatory behaviour was observed for *D. hesperus* feeding on low quality whitefly prey in no-choice arenas (Chapter 5). Therefore, although insects spent more time probing at high quality plant material, increased rates of feeding on low quality plant material in the presence of low quality prey probably represents a compensatory mechanism.

Effects of prey quality on the activity budget were only observed when females were placed in arenas with 200 ppm N-fertilized tomato leaflets. In these arenas, *D. hesperus* spent significantly more time probing the plant material in the presence of low quality prey (20% of the activity budget), than in the presence of high quality prey (12% of the activity budget). Previous work with *D. hesperus* has found that foraging females exhibit a preference for high quality prey in choice tests (Chapter 5); therefore, it is logical that insects would spend less time probing for plant food when high quality prey is available. This result also fits with the predictions of optimal foraging theory, as

discussed above. Overall, the activity budget of *D. hesperus* is complex. It consists of a number of important behaviours that are affected by prey quality and plant quality in different ways, depending on the combination of food materials available. Because *D. hesperus* are omnivores, and can survive on some host plants in the absence of prey (Sanchez *et al.* 2004), it is reasonable to conclude that characteristics of both the plant host and prey host affect their activity budget.

In addition to comparing the prevalence of *D. hesperus* foraging behaviours and the effects of plant and prey quality on the overall activity budget, the data collected from the behavioural observations was also used to test the impacts of plant and prey quality on prey handling time and rate of prey consumption. Total prey consumption is often used as a measure of predation capacity (Ibrahim and Rahman 1997; Calixto *et al.* 2013). No effect of prey or plant quality on the time spent handling each prey item was observed, as the mean handling time was in the range of 9 min across all prey and plant treatment combinations. Similarly, we observed no effects on the mean number of prey items consumed across the treatment combinations. This result was unexpected, as previous work with *D. hesperus* has demonstrated that foraging females consume more low than high quality whitefly nymphs in the same amount of time (Chapter 5); however, the time insects were allowed to forage differed by two hours between these two experiments. When taken together, these results suggest that over short periods of time, plant and prey quality cannot be manipulated to increase the predation capacity of *D. hesperus*. However, prey handling and prey consumption rate may change over time, as insects become satiated (following the predictions of the Type II functional response; Holling 1959, 1966), or prey becomes more difficult to locate due to patch depletion via exploitation depression (Charnov 1976; Charnov *et al.* 1976). Predator satiation is often observed in feeding trials. The number of prey required to become satiated depends on the predator, and on the developmental stage of the predator. For example, Cabral *et al.* (2009) observed that adult *Coccinella unidecimpunctata* L. (Coleoptera: Coccinellidae) became satiated after consuming 90 aphids, compared to 130 aphids required for larval satiation. The time to satiation may also vary depending on a number of factors, including the condition of the predator at the start of a feeding bout. After 24 h starvation, for example, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) consumed 31 aphids

in 85 min to reach satiation, relative to 14 aphids consumed in 35 min after 6 h of starvation (Kumar *et al.* 2002). The observation period used for the present study was probably not long enough to observe effects such as satiation or exploitation depression on prey handling time or rate of prey consumption. Future work with this insect should include longer periods of observation than those used here, although there are some important logistical considerations that would have to be taken into account, such as the need for direct observation by an observer using a microscope rather than using video technology due to the small size of the omnivore and its prey.

Chubaty *et al.* (2014) recently modeled the evolution of omnivory using a simulation in which the foraging environment varied in terms of quality and prey and plant availability. Their model indicated that herbivores, omnivores, and predators can coexist in the same habitat, and that the classification of an organism as a predator, omnivore, or herbivore was dependent on the relative availability of plant and prey materials (Chubaty *et al.* 2014). However, they also observed that when prey quality increased, so did the benefits of feeding on prey (Chubaty *et al.* 2014). We observed a similar effect of plant availability on foraging behaviour, and a similar effect of prey quality, where the availability of high quality prey decreased the amount of time spent probing 200 ppm N plant material relative to the availability of low quality prey.

Overall, our results suggest that over relatively short periods of time (~ 1 h) of observation, prey and plant quality do affect the activity budget of foraging *D. hesperus* females; however, the same factors do not affect the rate of prey consumption by this omnivore. This is valuable insight into the activity budget of *D. hesperus*. The use of focal observations also provides the basis upon which to compare the behaviour of this omnivore to other omnivorous insects. Within the framework of biological control, our results indicate that the activity budget of *D. hesperus* is dependent on the interaction of plant and prey quality; using this information to manipulate *D. hesperus* is not straightforward. In terms of omnivore behaviour, our results indicate that aspects of omnivore behaviour can be predicted using rules that generally apply to herbivores, such as compensatory feeding behaviour (Slansky and Feeny 1977), whereas other aspects can be predicted using rules that generally apply to predators, such as optimal foraging theory (Stephens and Krebs 1986). The use of focal observations of omnivore behaviour is a

powerful, if time consuming, method by which to test the predictions of models in order to unite theory and reality and to identify areas of future study.

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Table 6.1. The masses of 6-11-31 hydroponic fertilizer and 15.5-0-0 calcium nitrate ($CaNO_3$) required for 20 L solutions of each N-fertilizer treatment. Pre-weighed chemicals were mixed with 20 L freshwater and solutions were held in plastic jugs for storage.

Fertilizer Treatment	6-11-31 (g)	CaNO₃ (g)	Water (L)
50 ppm N	5.75	4.25	20
100 ppm N	11.5	8.50	20
200 ppm N	23.0	17.0	20
400 ppm N	46.0	34.0	20

Table 6.2. Monthly maximum, minimum, and mean temperatures in the greenhouse where tomato plants were grown in 2013 and 2014 for use in focal observations of *Dicyphus hesperus* feeding behavior.

Month	Minimum Temperature (°C)	Maximum Temperature (°C)	Mean Temperature (°C)
January	11.5	36.0	25.08
February	12.5	43.0	25.63
March*	12.5	54.5	26.68
April*	7.5	57.0	26.48
May	11.0	46.5	27.90
June	18.5	41.5	28.26
July	14.0	40.5	25.88
August	13.0	44.0	26.90
September	11.0	44.5	23.28
October	10.0	39.5	24.05
November	15.5	34.0	22.98
December	17.0	26.0	21.90

*Missing for 2013, information provided is from 2014.

Table 6.3. The mean (± SE) number of prey consumed by female *Dicyphus hesperus* and the mean (± SE) time spent prey handling in test arenas where insects foraged for prey on 50, 100, 200, and 400 ppm N-fertilized tomato leaflets.

Plant Quality	Number of Prey Consumed	Prey Handling Time (s)
50 ppm N	3.4 ± 0.4	521 ± 50
100 ppm N	3.6 ± 0.3	629 ± 49
200 ppm N	4.1 ± 0.3	515 ± 35
400 ppm N	3.1 ± 0.3	547 ± 44
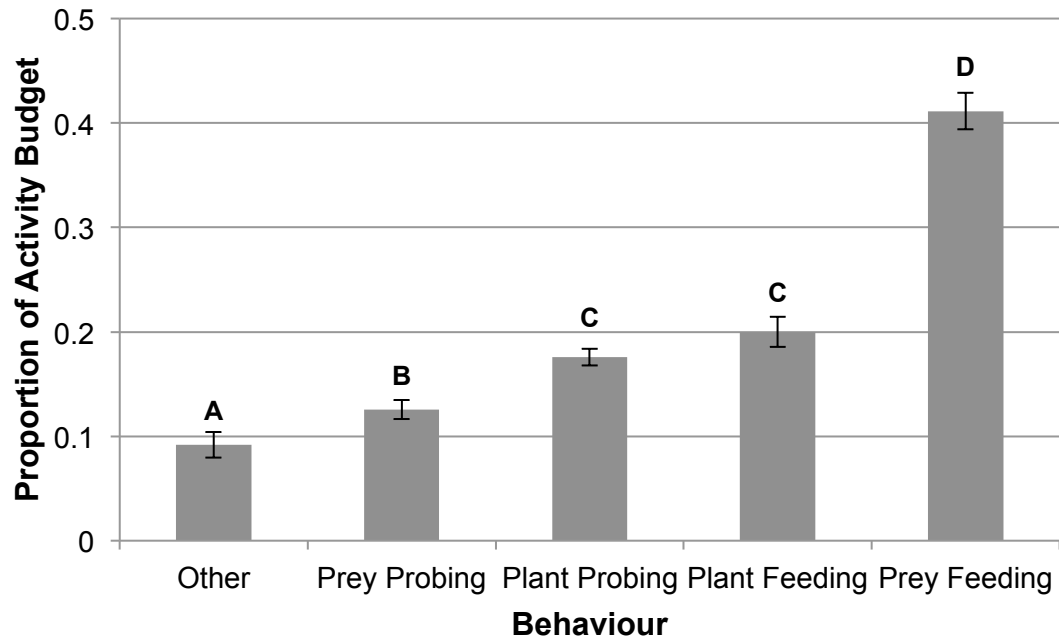
Figure 6.1. The difference (Kruskal-Wallis test, $\chi^2 = 185. 517$, df $= 4$, $p < 0.0001$) in the mean ($\pm$ SE) proportion of the activity budget of *Dicyphus hesperus* devoted to foraging and 'other' behaviours across all plant and prey quality treatment combinations. Means with the same letter are not significantly different (Wilcoxon-Mann-Whitney test: $p > 0.005$).

A: Proportion of Activity Budget Spent Plant Feeding
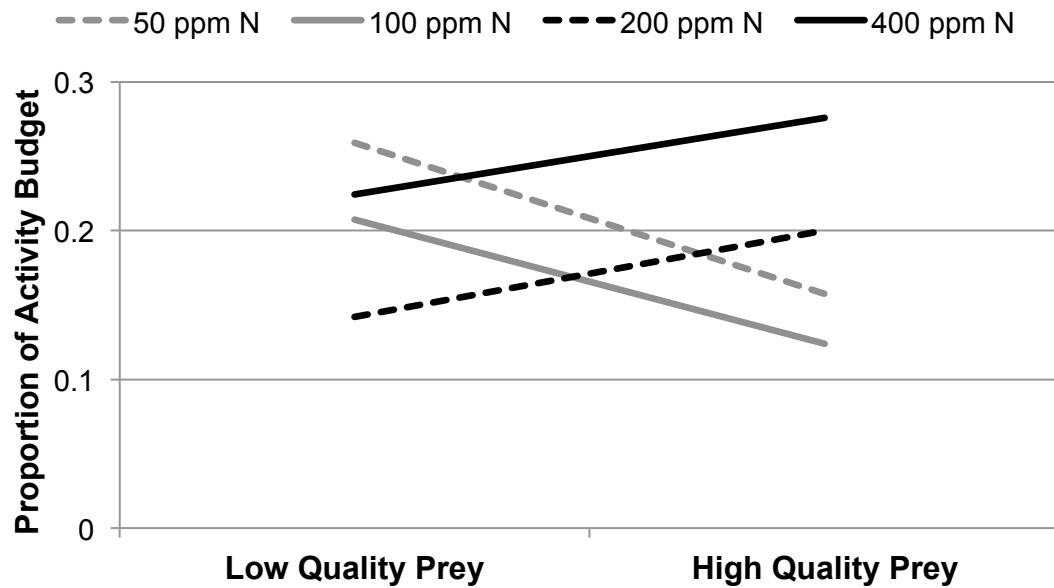
B: Proportion of Activity Budget Spent Plant Probing
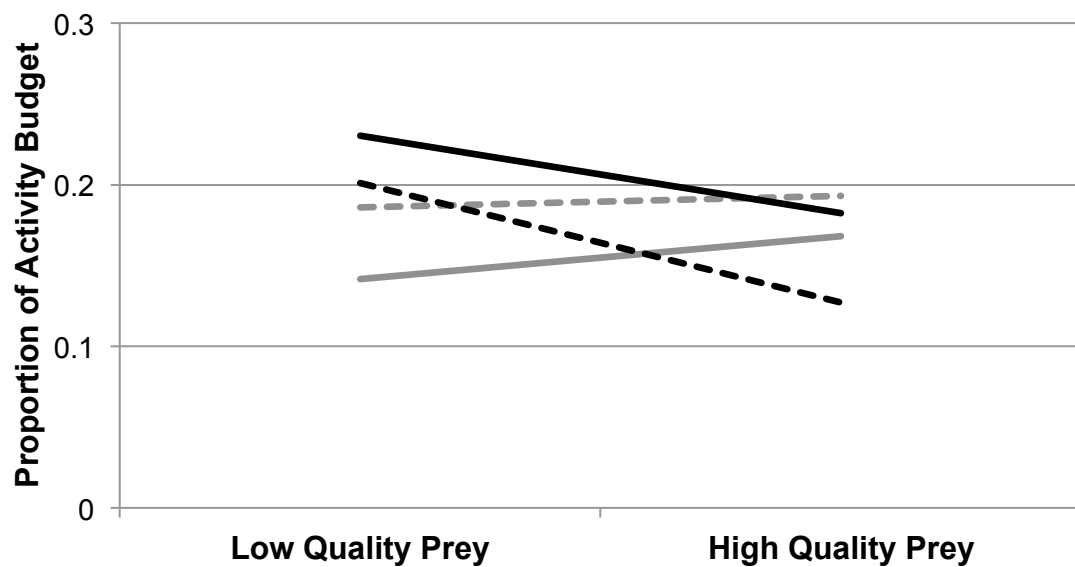
Figure 6.2. The effect of the interaction between whitefly prey quality and tomato plant quality on the mean proportion of the activity budget spent plant feeding (A) and plant probing (B) by foraging *Dicyphus hesperus* females that were observed for 1 h. Standard error (SE) bars are not shown.
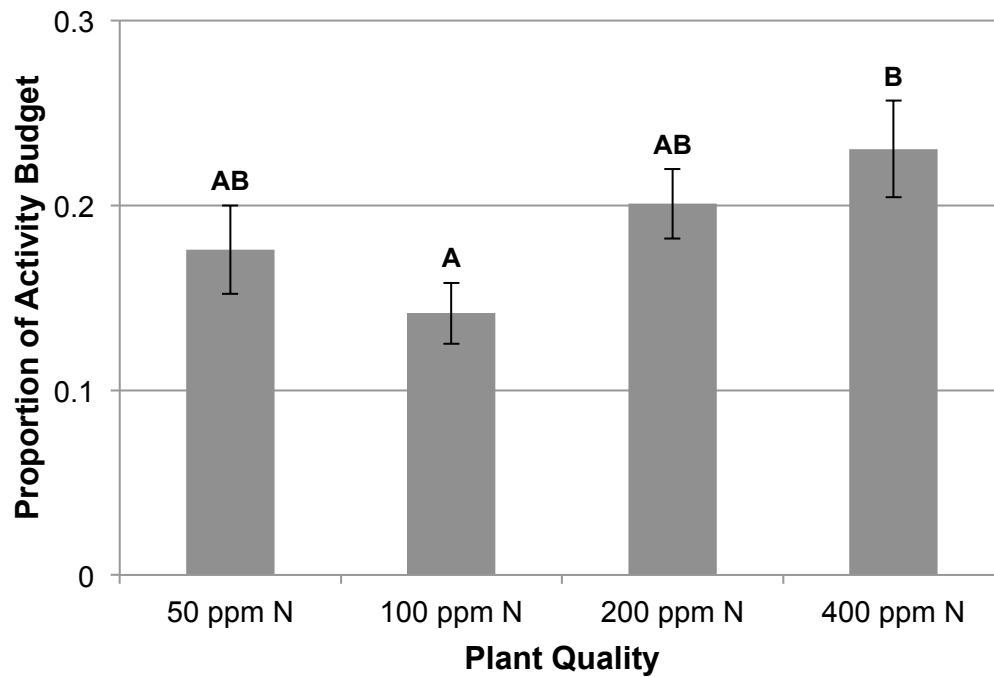
Figure 6.3. The mean (± SE) proportion of the activity budget spent plant probing when female *Dicyphus hesperus* were provided with low quality whitefly nymphs as prey in foraging arenas. Means with the same letter(s) are not significantly different ($p > 0.05$).
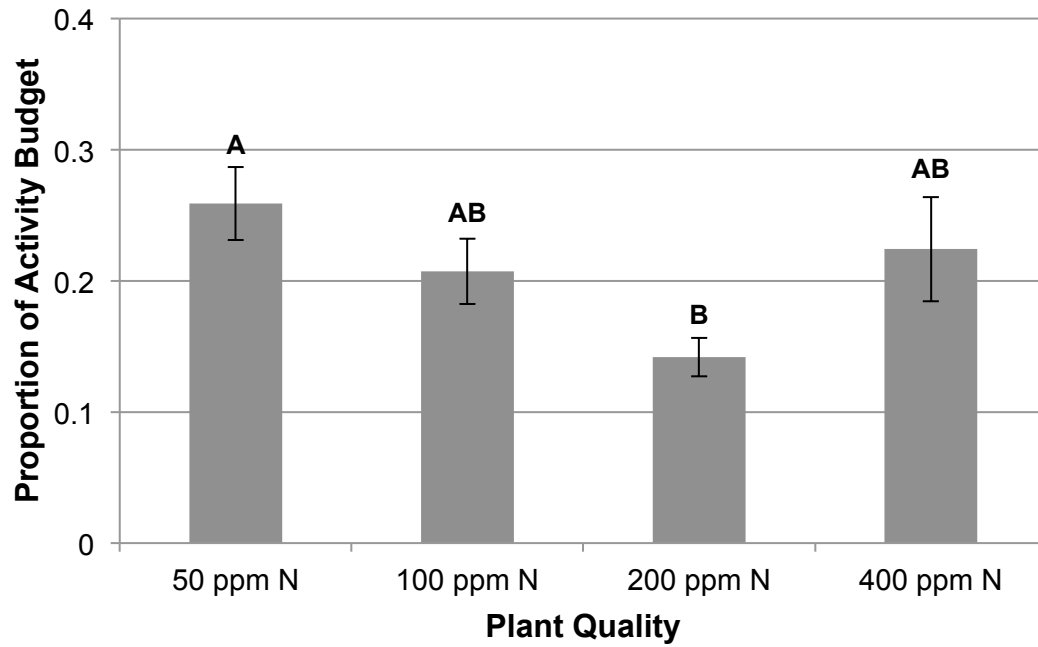
Figure 6.4. The mean (± SE) proportion of the activity budget spent plant feeding when female *Dicyphus hesperus* were provided with low quality whitefly nymphs as prey in foraging arenas. Means with the same letter(s) are not significantly different ($p > 0.05$).

Chapter 7. Plant and prey quality affect the life history and behaviour of the omnivore *Dicyphus hesperus*: implications for biological control programs and suggestions for the future

Research overview

Biological control programs are an important tool for the management of pests that can help to ensure the sustainability of food production in the present and the future (Bale *et al.* 2008). The success of biological control programs for pest management in agroecosystems requires a thorough understanding of the pest and the natural enemy (Bale *et al.* 2008). Therefore, all aspects of the behaviour and life history of the natural enemy should be investigated as part of the process of designing and implementing a biological control program. For some natural enemies, this task is easier than for others. Omnivores, for example, represent a unique challenge in the design of biological control, as unlike their predator and parasitoid counterparts, their life history and behaviour are closely tied to plants as well as the targeted insect pest. To ensure that omnivores behave as expected requires a close look at the effects of plant availability and quality on the omnivorous natural enemy. This is in addition to understanding the interactions of the omnivore with the target pest and non-target species that occupy the same habitat.

All types of natural enemies (predator, parasitoid, omnivore, or pathogen) must possess certain characteristics in order to be effective biological control agents. Consider a hypothetical predator to be used in a biological control program. If the predator's population is relatively small and its rate of population increase is very low compared to the pest population, then the chances of the predator having a significant impact on the pest are also low. Similarly, if a female parasitoid to be used in biological control produces only five eggs in her lifetime, then a single female will only remove five individuals from the pest population, and that is only if she is 100% successful in locating a host and depositing an egg within it. From these examples it is clear that reproductive capacity is an important characteristic of a natural enemy that must be considered when assessing novel natural enemies for biological control programs (Smith 1935; Hagen *et al.* 1976; van Lenteren and Woets 1988). Other important characteristics include the ability to disperse, longevity of the stage that directly affects the pest, and persistence in

the habitat over long periods of time, even when prey populations are low (Smith 1935; Hagen *et al.* 1976; van Lenteren and Woets 1988).

As the characteristics that define effective natural enemies are generally biotic (Smith 1935), the use of ecological theory as a basis for biological control research is logical. Certain ecological theories have been tested extensively by studying the biology and behaviour of a number of animal species, including insects. For example, optimal foraging theory has been tested since its definition over 50 years ago and it is the topic of several books (for example: Stephens and Krebs 1986; Krebs and Davies 1993). Because of the wealth of information and examples available, these theories provide an invaluable guideline that can be used as the basis of research projects in a variety of systems. Ecological theories that apply to the study of natural enemies for biological control include optimal oviposition theory, optimal foraging theory, and the plant vigor/plant stress hypotheses (Chapter 1). I used these three theories as the foundation upon which to design the experiments and define and test the predictions of my dissertation research regarding an important omnivorous natural enemy of greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae).

Greenhouse whitefly is a cosmopolitan pest of many important agricultural plants that is known for having developed resistance to all insecticides applied against it (Byrne and Bellows 1991; Pappas *et al.* 2013). As a result, the management of *T. vaporariorum* in greenhouses requires creative solutions. Biological control is an important option and is widely practiced, utilizing a number of predators, parasitoids, and pathogens (van Lenteren *et al.* 1996). Because of the economic importance of greenhouse crops (tomatoes accounted for $496 million in exports in 2011; Statistics Canada 2012), there is a constant push to improve the biological control programs that are currently employed and to identify novel biological control agents of greenhouse whitefly. *Dicyphus hesperus* Knight (Hemiptera: Miridae) was identified as a biological control agent of greenhouse whitefly on tomato plants as a result of a research initiative to identify generalist predators with biological control potential in greenhouse ecosystems (McGregor *et al.* 1999).

Although a great deal of information has been gathered regarding the life history and behaviour of *D. hesperus* for its use in biological control programs for greenhouse

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whitefly and other small insects, some gaps exist. For example, the preferred host plant and prey species of *D. hesperus* have been identified, as well as its performance when reared on those species (Sanchez *et al.* 2004; VanLaerhoven *et al.* 2006). However, within-species differences in plant quality exist. For example, the nitrogen status of plants is known to vary within and between plants in all ecosystems (Mattson 1980). Importantly, plant quality affects prey quality (Mattson 1980; Minkenberg and Ottenheim 1990; Crafts-Brandner 2002). Within-species differences in the nitrogen status of the host plant of *D. hesperus* might have an effect on its biology and behaviour, but this has not been investigated. Furthermore, because *D. hesperus* can obtain energy via plant and prey feeding, the nutritional status of both its plant and prey hosts might play an important role in determining its success as a biological control agent. This is an important knowledge gap to fill. Therefore, the overall objective of my dissertation research was to investigate the effects of varying tomato plant and whitefly quality on aspects of the life history and behaviour of *D. hesperus*, in order to add to our knowledge regarding this important biological control agent and of omnivores in general.

Key findings and their relevance to the application of *D. hesperus* in biological control programs

Reproduction and survival

In biological control programs, effective natural enemies need to reproduce efficiently and persist when the target pest is absent or scarce (Smith 1935; Hagen *et al.* 1976; van Lenteren and Woets 1988; Bale *et al.* 2008). Using the framework of optimal foraging theory (Jaenike 1978; also preference-performance hypothesis; Thompson 1988), I investigated the effect of tomato plant quality on host selection by reproductive females (Chapter 2). I also investigated the effect of plant and prey quality on the development and survival of *D. hesperus* nymphs (Chapter 3), with the expectation that nymphs would develop fastest and have the greatest survival rate on the preferred host plants for oviposition. These experiments also allowed for the investigation of the development and survival of *D. hesperus* with respect to the predictions of the plant vigor/plant stress hypotheses.

Tomato plants of different quality, as manipulated by N-fertilizer inputs, did have different numbers of *D. hesperus* nymphs emerge after females were allowed 3 d to oviposit (Chapter 2), indicating female preference for oviposition hosts. High quality plants had the most nymphs (200 and 400 ppm N), whereas low quality tomato plants had the fewest nymphs (50 and 100 ppm N) (Chapter 2). Based on the preference of *D. hesperus* females, I expected that nymph development would be fastest on high quality tomato plants. This was not the case, as third instar nymphs developed fastest on both the extremely high and low quality plants when equal numbers of prey were provided across all levels of plant quality (Chapter 3). No other nymphal stage was affected by plant quality in terms of development time. This result agrees with the predictions of both the plant vigor and plant stress hypotheses. This result is also interesting from a practical perspective, as female *D. hesperus* did lay eggs on low quality tomato plants in both choice and no-choice tests. It is important that nymphs develop quickly on low quality plants. Although low quality plants may represent a poor choice by the female, it might also be an adaptive choice that gives either the female or her offspring some advantage (Mayhew 2001). The quick development of nymphs on low quality plants also suggests that *D. hesperus* have different mechanisms for dealing with sub-par plant hosts, such as compensatory feeding on low quality host plants (Slansky and Feeny 1977; Mattson 1980), as I observed for *D. hesperus* feeding on whitefly prey reared on low N tomato plants (Chapter 5). Therefore, although *D. hesperus* populations might persist and increase in density faster on plants with high levels of N, populations should be able to persist on plants with low levels of N as well. Both of these possibilities contribute to the suitability of *D. hesperus* as a biological control agent.

I also expected that *D. hesperus* nymphs would have greater rates of survival on high quality tomato plants compared to low quality tomato plants based on the oviposition preferences of *D. hesperus* females (Chapter 2), but this was not the case (Chapter 3). For some individual nymphs reared on high quality tomato plants there was an increased chance of molting to the adult stage after a prolonged period of development (Chapter 3). I also observed that *D. hesperus* nymphs were able to survive to the adult stage on diets that consisted of only tomato plants, with no prey provided (Chapter 3). This is contrary to observations reported by McGregor *et al.* (1999) and Sanchez *et al.*

(2004), in which no nymphs survived to the adult stage on pure diets of tomato leaves. The tomato leaves used in these experiments had no evidence of whitefly infestation prior to the start of the experiments. However, it is possible that whitefly eggs or early-instar nymphs that were not visible to the naked eye were present on the tomato leaves, and were not removed. If whitefly residues of this nature were present, I expect that they would have been present on all of the tomato leaves where *D. hesperus* nymphs were isolated, as the greenhouse facilities at the University of Windsor are infested with whitefly and they are difficult to exclude from whole tomato plants. If whitefly eggs or early-instar nymphs were present they would have been utilized early in development. As no further prey were provided, this situation would be analogous to a natural habitat in which prey populations crash or are exhausted. Therefore, these results provide further evidence that *D. hesperus* populations can persist in habitats were prey resources are scarce or absent; this is an important characteristic of omnivores (Bugg *et al.* 1987; Naranjo and Gibson 1996; Settle *et al.* 1996; Wiedenmann *et al.* 1996; Coll and Guershon 2002) that contributes to their value as biological control agents.

Host plant and prey detection
Effective natural enemies have to be able to disperse to affected habitats and locate prey within that habitat (Smith 1935; Hagen *et al.* 1976; van Lenteren and Woets 1988; Bale *et al.* 2008). To determine if the quality of tomato plants, with or without whitefly infestation affected the response of *D. hesperus* to tomato plant odors, y-tube olfactometer trials were conducted (Chapter 4). I expected that the preference of female *D. hesperus* for high quality tomato plants in oviposition trials (Chapter 2) would be reflected in the olfactometer trials, as olfactory cues are one possible method that *D. hesperus* females can use to distinguish between host plants of varying quality. However, plant quality did not influence *D. hesperus* preference for odors in the olfactometer (Chapter 4). In fact, no preference for tomato plants odors, with or without whitefly infestation was observed (Chapter 4). These results are somewhat confusing, as preferences for tomato odors have been observed in previous work, both in y-tube olfactometer studies (McGregor and Gillespie 2004) and in greenhouse-scale releases (McGregor *et al.* 1999).

There are several possible explanations for the differences between my results and those of other authors. Differences in the designs of olfactometers are one possible explanation, as the McGregor and Gillespie's (2004) olfactometer had two air sources compared to only one in the olfactometer that I used. Another possible explanation is that turbulence in the olfactometer made it difficult for *D. hesperus* to determine the source of the plant odor, as discussed in Chapter 4. The distance between the odor source and the insect was also relatively short, and this might have had an effect on the searching behaviour of *D. hesperus* females. At shorter distances, other sensory modalities might be more important for host location than others. For example, Hazard (2008) observed that olfactory cues arrested *D. hesperus* movements and prey location after arrestment was achieved using gustatory and visual cues. It would be helpful to incorporate different sensory cues into the olfactometer and complete olfactometer trials with different distances between the insect and odor source to gain more insight into the relative importance of different cues under different conditions.

The results of the olfactometer trials I conducted were also used to determine if the quality of tomato plants, with and without whitefly infestation, affected the response of *D. hesperus* in the olfactometer. For these analyses, a response was defined as entry into either arm of the y-tube olfactometer regardless of the location of the odors, whereas insects that did not respond did not enter either arm of the y-tube (Chapter 4; McGregor and Gillespie 2004; Moayeri *et al.* 2006). Results of these analyses indicated that the previous foraging experience of *D. hesperus* had an effect on their response in the olfactometer, such that naïve insects were least responsive (Chapter 4). This result suggests that insects reared *en masse* for release into greenhouses as part of a biological control program would be more responsive to odors from tomato plants if they have previously foraged on tomato plants. Although tomato plants are not often used for rearing *D. hesperus* colonies, it would be relatively simple to transfer individuals to tomato plants for several days before mass release using the same handling techniques used for colony maintenance.

Foraging behaviour and prey consumption

There are several different aspects of foraging behaviour and prey consumption that can be considered when evaluating natural enemies for biological control programs. To date, the ability and propensity of *D. hesperus* to utilize prey of a single species that varies in quality has not been investigated, nor have the foraging decisions of *D. hesperus* when presented with plant and prey material of a single species that vary in quality. To fill this knowledge gap I used optimal foraging theory research as a guideline to determine if prey quality affected *D. hesperus* preference for whitefly prey using choice tests (Chapter 5). In these experiments, the quality of the rearing host determined the quality of the prey, hence whitefly reared on 400 ppm N-fertilized tomato plants were designated as high quality prey. In choice tests, *D. hesperus* exhibited a preference for high quality whitefly nymphs relative to low quality nymphs during a 3 h foraging bout (Chapter 5). This result was expected, as optimal foraging theory predicts that high quality prey should be preferred (Krebs 1977; Stephens and Krebs 1986), however, the diet of *D. hesperus* was not pure, which contradicts other predictions of optimal foraging theory (Stephens and Krebs 1986). I also compared the response of *D. hesperus* to low and high quality prey in no-choice tests (Chapter 5). In this experiment, I observed that *D. hesperus* consumed more low quality than high quality prey (Chapter 5). This is likely an example of a compensatory response by *D. hesperus* to low quality prey. Many insect herbivores and predators are known to compensate for low quality foods by increasing their rate of food intake (Slansky and Feeny 1977; Wheeler and Halpern 1999; Oishi *et al*. 2006); this is one of the first studies to report compensatory prey feeding by an omnivore.

Because prey quality varies naturally due to variation in plant quality (Mattson 1980; Minkenberg and Ottenheim 1990; Crafts-Brandner 2002), this information can be used in future biological control programs that use *D. hesperus*. Based on my results, improving plant quality might increase the attractiveness of a habitat to *D. hesperus* via the presence of preferred high quality prey. Reducing the rate of fertilizer application to tomato crops might also increase whitefly mortality via *D. hesperus* predation, as *D. hesperus* can increase their rate of prey intake in response to prey quality. Unfortunately, manipulating tomato plant quality in an effort to improve biological control of whitefly is not as simple as it sounds, for a number of reasons. For example, reducing the rate of N

fertilization would likely reduce tomato plant yield. Increasing the rate of N fertilization would likely contribute more to the growth of the pest population than to *D. hesperus* populations, this is contrary to our goal of pest suppression. Understanding the impact of prey quality on the preferences of *D. hesperus* could be used to improve *D. hesperus* sampling protocols. The distribution of *D. hepserus* between plants is aggregated, like the distribution of its prey (Sanchez *et al.* 2002). Preference for high quality prey might result in greater aggregation of *D. hesperus* on high quality plants, which could result in an over- or underestimation of the *D. hesperus* population if only high or low quality plants are sampled.

To examine the relationship of plant and prey quality on the foraging behaviour of *D. hesperus* I used focal observations, similar to those described by several authors (see Cisneros and Rosenheim 1998; VanLaerhoven *et al.* 2000; Rosenheim *et al.* 2004; Reitz *et al.* 2006) to observe the behaviour of *D. hesperus*. In 1 h foraging periods, *D. hesperus* spent more time prey feeding than on any other activity (Chapter 6), regardless of the quality of plant and prey resources available. This was quite different from the behaviour of other predators that were observed in a similar manner which were found to spend more time resting or walking than feeding (Cisneros and Rosenheim 1998; Rosenheim *et al.* 2004), and might indicate that *D. hesperus* are more efficient than other predators. I also observed an interaction of plant and prey quality on the time *D. hesperus* dedicated to plant and prey feeding (Chapter 6). For example, when low quality prey was provided, *D. hesperus* females spent a greater amount of time probing the plant material (Chapter 6). Because increased plant probing and plant feeding can result in yield losses to the plant (McGregor *et al.* 2000; Shipp and Wang 2006; Sanchez and Lacasa 2008; Castañé *et al.* 2011), it would be best to ensure that high quality prey is present in habitats where *D. hesperus* is used as a biological control agent.

Suggestions for future research

The life history and behaviour of *D. hesperus* is affected by plant and prey quality. Therefore, future research on this omnivore should incorporate manipulations of plant and prey quality when investigating other aspects of its life history and behaviour. For example, one aspect of *D. hesperus* behaviour that my research did not address was

their functional response to prey. Functional response models can be used to understand the interaction of a predator with its prey in a variety of conditions, including when prey density and predator satiation vary (Holling 1959; 1966). Some work has been done to determine how different prey species, including greenhouse whitefly nymphs, and *E. formosa*-parasitized whitefly nymphs affect the functional response of *D. hesperus* (Brommit *et al.* in prep). Incorporating the effects of plant quality and prey quality into functional response models for *D. hesperus* would increase the realism of functional response tests and further our understanding of the foraging behaviour of this whitefly predator. This information could also inform biological control planners of the habitat conditions in which *D. hesperus* is most suited for use.

The incorporation of plant quality does add to the complexity of the research questions that we can ask, but natural ecosystems, including agroecosystems, are complex. It is a disservice to biological control research to simplify agroecosystems too much. That being said, one important shortcoming of the research conducted for my dissertation was that it was all performed in simplified test arenas or exclusion cages, although whole tomato plants were used as often as possible. Small-scale research of this nature is important, but small-scale research is best treated as a pilot study. Before the results of my research are applied to actual biological control programs, they should be validated on a larger scale, such as in greenhouse trials. For example, the effects of plant quality on dispersal, oviposition preference, and the development and survival of nymphs could be determined in greenhouse compartments by releasing *D. hesperus* into tomato plants receiving different rates of N-fertilizer. Focal observations of *D. hesperus* foraging behaviour could be made in the same greenhouses to validate the information collected in the laboratory study described in Chapter 6.

Another aspect of my research that might be considered both a pro and a con is that the majority of the data was collected by observing individual insects. Oviposition preference (Chapter 2) was tested using cages that held multiple insects as experimental units. In all other cases, individual *D. hesperus* were treated as experimental units. The use of individuals in experiments such as those described in Chapters 3 through 6 does allow us to consider variation between individual insects, which can provide a great deal of insight. Unfortunately, the use of individual insects as replicates does not account for

interactions between individuals. As a result, potential effects of plant quality on competition between individuals were not assessed. Plant quality might have interesting effects on the relationships between individuals that could help or hinder biological control programs, thus, the effects of plant quality on competition should be a focus of future research on this omnivore.

   Dicyphus hesperus has significant potential as a biological control agent in tomato greenhouses (McGregor *et al.* 1999), and probably in other agroecosystems where whiteflies are pests. The results presented in my dissertation provide important insight into the interactions of *D. hesperus* with its host plant and prey resources when both vary in quality. This information can be used to refine future biological control programs using *D. hesperus* and to shape future *D. hesperus* research efforts. In addition, this research can be used to inform future research on the life history and behaviour of other omnivores with potential in biological control programs. Omnivores have the potential to do a lot of good in agroecosystems, but could also have negative impacts on the agroecosystem such as significant yield losses (Coll and Guershon 2002; Shipp and Wang 2006; Sanchez and Lacasa 2008; Castañé *et al.* 2011). It is very important to have a thorough understanding of the life history and behaviour of omnivores that are employed as natural enemies in biological control programs in order to amplify their contributions and minimize their potential for negative impacts.

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