INFLUENCE OF PLANT ARCHITECTURE ON TRITROPHIC INTERACTIONS BETWEEN WINTER CANOLA (*BRASSICAE NAPUS*), APHIDS (HEMIPTERA: APHIDIDAE) AND *HIPPODAMIA CONVERGENS* (COLEOPTERA: COCCINELLIDAE).

by

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Abstract

Winter canola production in the south-central US is commonly threatened by a complex of aphid species that can cause up to 70% in yield loss. Aphid species vary in their life-history traits, performance (sequestration/excretion of secondary compounds; glucosinolates), vertical distribution within the plant, and temporal dynamics across the growing season. Colonizing behavior of these aphids may be affected by intrinsic characteristics of the host plant (bottom-up effects), such as nutritional value, secondary compounds, or plant architecture. Understanding bottom-up effects may enable the evaluation of plant-level interactions that are influencing predator-prey dynamics. The goal of my research project is to understand aphid population dynamics in different canola plant structures, assess whether aphid quality (sequestration/ excretion of glucosinolates) is influenced by feeding location on the canola plant, and if so, assess the impact on the existing predator communities, specifically the development and fitness of immature and adult *Hippodamia convergens*. A combination of filed and greenhouse experiments provided novel contributions that will help shape our understanding of key factors regulating aphid population growth in canola fields, which will lead to more judicious use of insecticides and better sampling strategies.

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Dedication

I dedicate this work to my grandfather who inspires me to be a better person every day.

Chapter 1 - INFLUENCE OF PLANT ARCHITECTURE ON TROPHIC INTERACTIONS BETWEEN WINTER CANOLA (*Brassica napus*), APHIDS (HOMOPTERA: APHIDIDAE) AND *Hippodamia convergens* (COLEOPTERA: COCCINELLIDAE)

INTRODUCTION

In the US alone, ecosystem services provided by beneficial insects (pollinators and natural enemies) are estimated at $57 billion dollars annually (Losey and Vaughan 2006); $4.5 billion of these are attributed to natural enemies, predominantly generalist predators in different agricultural settings (Landis et al. 2008). Ecosystem services vary with landscape complexity (landscape fragmentation and number of species inhabiting the agroecosystem) and intensity of agricultural practices (Dale et al. 2007). Simplification of land uses such as, herbicide tolerant varieties, increasing dependence on agrochemicals, and greater demands for food and biofuel production are reducing biodiversity of beneficial organisms in agroecosystems (Landis et al. 2000). For instance, in the North Central US, increasing corn acreage for biofuel production reduced the natural biological control services for the soybean aphid (*Aphis glycines*) by an estimated 24% (Landis et al. 2008). In contrast, Lu et al. (2012) and Wolfenbarger et al. (2008) demonstrated that widespread adoption of genetically modified cotton that produces the insecticidal toxin of *Bacillus thuringiensis* increased abundance of generalist predators over the past decade; increase abundance of natural enemies are primarily due to decrease in overall insecticide use. Therefore, newly introduced crops or changes in management practices in already existing agroecosystems might provide alternative sources or sinks for pests and/or beneficial arthropods in different agroecosystems.

Insects generally discern between high-quality or low-quality habitats using environmental cues (e.g., olfactory cues, flower or alternative food availability, etc.) preferring, in most cases, environments with the highest quality resources (Jauker et al. 2009). High resource environments usually offer reduced competition and preferred host plants. Clear distinctions between a source or sink resource depends on the level or types of ecosystem services provided by existing landscapes. Source type environments are able to sustain and increase survival and reproduction of existing beneficial organisms, such as pollinators and predators, which then spill into existing crop landscapes. Sink type environments, on the other hand, are often referred to as "ecological traps", meaning that they cannot sustain or increase survival and reproduction of beneficial insects and pests (Dwernychuk and Boag 1972).

Newly introduced crops, such as winter canola (*Brassica napus*) to the Southern Great Plains, make spatial and temporal changes in agroecosystems that lead arthropods to make maladaptive habitat choices when assessing these novel environments (Lu et al. 2012, Wolfenbarger et al. 2008, Landis et al. 2008). Newly introduced canola might serve as a source or a sink resource for pest and beneficial organisms in a well-established agroecosystem. Thus, this review will focus on understanding the consequences associated with adding canola to the landscape by concentrating on interactions occuring at the plant-level and how these canola-herbivore interactions may influence predator-prey dynamics in the system.

WINTER CANOLA-WINTER WHEAT SYSTEM

Winter canola belongs to the Brassicae family of plants and is a profitable first generation biofuel that yields up to 1,793 kg/ha and producing approximately 40% oil when crushed (Tickell 2000, Smith et al. 2007, Dansby 2008, Peeper and Boyles 2008, Peeper et al. 2009). After introduction of winter hardy varieties in 2001, winter canola was proposed as a primary

rotation crop with winter wheat (*Triticum aestivum*), which is the most important crop to the South Central US (Peeper et al. 2009, Franke et al. 2009). Crop rotation between canola and wheat is beneficial for weed management, soil fertility, and disease management, since canola and wheat have no diseases in common. Due to these economic and agronomic advantages, canola acreage has increased from zero in 2004 to > 81,000 hectares (200,000 acres) in 2012 in the South Central US alone, with an estimated 70% increase from 2011 to 2012 in the continental US (US Canola Association website; USDA November 2012 Crop Production Report). Rapid establishment of canola in the region may significantly reduce or enhance natural biological control services in winter wheat given that newly established canola provides alternative niches, novel prey items, and its management programs differ significantly from those in wheat.

Wheat/canola management

Winter wheat production in the South Central US is highly sustainable; less than 16% of the winter wheat fields are treated with insecticides annually (Giles, Hein and Perirs 2008, Giles and Walker 2009). Many winter wheat producers rely on already established natural enemies communities for pest suppression. For example, in 2006, scouting for weeds, insects, and diseases accounted 86, 57, and 56%, of the winter wheat acres in Kansas alone, respectively, while only 3% of the winter wheat acreages surveyed were treated mainly with insecticides (USDA, Kansas Chemical usage 2007). In contrast, since the introduction of winter canola to the South Central US, a complex of different aphid species threatens production annually. The combined feeding effect of these aphid species can cause up to 70% in yield loss if aphid populations are left unmanaged (Giles at al 2006, Giles et al. 2011). Presently, approximately 90% of the canola fields are treated with broad-spectrum insecticides annually, typically

synthetic pyrethroids (Franke et al. 2009, Knodel 2011). Insecticides are applied mainly during canola flowering, which is peak attraction time for pollinators and other natural enemies dispersing from nearby habitats and using canola as a resource (Baggen et al. 1999).

Despite the rapid increase in canola acreage and corresponding increases in insecticide usage, data is lacking with regards to the potential landscape-level impacts of adding canola to an existing, wheat-dominated, agricultural system, and its influence on the associated arthropods or their ecosystem services. Source/sink relationships are important to understand in order to manage new crops in a sustainable manner (Raghu et al. 2011). Both winter crops are attacked by unique aphid complexes that are host specific (Table 1). Aphid species found colonizing canola might serve as suitable prey for the natural enemies present in these new wheat-canola landscapes, and thus, providing new resources for natural enemies and parasitoids (i.e., sources) or sinks within the landscapes (i.e., higher preference for canola where insecticide applications are more frequently used). Aphids attacking canola mitigate secondary compounds produced by canola differently and which may have fitness consequences to biological control organisms using aphids as a food resource (i.e., sinks). The consequences associated with adding canola to the landscape are not limited to landscape-level impacts on ecosystem services and such effects may extend to the plant level.

CANOLA APHID COMPLEX

Aphids attacking canola include *Lipaphis erysimi* (turnip aphid), *Myzus persicae*, (green peach aphid), and *Brevicoryne brassicae* (cabbage aphid) (Franke et al. 2009, Berlandier et al. 2010, Royer and Giles 2008-10) (Table 1). Feeding damage by aphids results in seedling death, curling, yellowing, stunting, deformation of developing flower heads, flower abortion, reduced pod set, and/or disease transmission (Ahuja et al. 2009, Franke et al. 2009, Hopkins et al. 2009,

Royer and Giles 2008-10, Berlandier et al. 2010, Palumbo, 2012). Severe pest infestations can result in losses of up to 70% of the canola crop, especially when aphids form dense colonies on different structures of canola plants (Giles et al 2011).

Biology

Aphid populations attacking canola are composed of apterous females that rapidly produce viviparous young through parthenogenesis (Hughes 1963). Conversely, populations can remain asexual under certain conditions and reproduce as apterous adults until environmental conditions are ideal for development. Under cold climatic conditions, sexual winged forms (males and females) of aphids are produced, mate, and lay their eggs in aerial parts of alternative host plants. Aphids overwinter as eggs on winter Brassicae crops or weeds. During early spring wingless aphids are hatched giving raise to "fundatrix" mothers that produce the first generation of parthenogenic nymphs. Winged aphid migrants are then primarily dispersed by wind currents (Hughes 1963). Temperature, feeding location within the plant, and host quality are crucial factors affecting aphid development and influence both number of nymphs produced and longevity at all life stages (Sidhu and Singh 1964, Costamanga et al. 2013). All life stages of aphids feed on canola plants, and all life stages of the plants are susceptible to their attack.

Phenology

Occurrence of these aphid species varies across the canola growing season. In the Southern Great Plains, the turnip aphid is an early season pest that attacks canola during seedling establishment in the fall months (Royer and Giles 2008-2010) (Fig. 1B). Turnip aphid colonies are found colonizing whole canola seedlings and in most cases their phenology does not overlap with other aphid species in the field (Berlandier et al. 2010, Hopkins et al. 2009). The green peach aphid (Fig.1A) and the cabbage aphid (Fig. 1C) attack canola mainly from the early flower

to early pod stages during late spring (April to March) and occur simultaneously in canola production fields (Boyles et al. 2007, Royer and Giles 2008). Cabbage aphid colonies are mainly found colonizing the top flowering canopy, while green peach aphid are predominately found colonizing bottom structures of the canola plant, which is mainly comprised of mature, fully expanded leaves (Merritt 1996, Hopkins, Ekbom and Henkow 1998, Hopkins et al. 2009, Berlandier et al. 2010). Other arthropod species are known to be more abundant in particular plant structures and feeding location has been reported to influence both fitness, and natural enemy recruitment rates (Idris and Roff 2002, Pekar 2005, Smallengange et al. 2007, Berberet et al. 2009, Fernandes et al. 2011, Grigollo et al. 2013). Conversely, natural enemy feeding habits can have disproportionate effects on aphid population growth based on where the natural enemies are feeding. Costamagna et al. (2013) reported that while bottom-up forces affect distribution and fitness (size and population growth) of soybean aphids (*Aphis glycines*) in soybean (*Glycine max*), top-down control by lady beetles is the dominant force suppressing aphid population growth. Predation was therefore higher on upper rather than lower soybean nodes, which resulted in non-consumptive reduction of aphid population growth because most of the surviving aphids were located on lower plant nodes, where rates of increase were reduced (Costamagna et al. 2013). The reason for the differences in vertical distributions for cabbage and green peach aphids in canola is not known. Occurrence in different plant structures may be influenced or regulated by predation, aphid feeding preferences, or simply by competition between the two aphid species.

Host range

Not only do aphids attacking canola vary in vertical distribution across the canola plant, but they also vary in their host range and quality towards natural enemies. Turnip and cabbage

aphids are Brassica specialists, attacking various cultivated and wild Brassicae species; these species mitigate glucosinolates (secondary compounds of Brassicae) by sequestration (Hopkins et al. 2009). Conversely, green peach aphid is a polyphagous species that attacks plants in over 40 families; mitigating glucosinolates through excretion of toxic constituents (Weber 1985, Pratt et al. 2008, Palumbo 2012) (Table 1). Hosts used by the green peach aphid include ornamental plants, vegetables as well as weed plants (Palumbo 2012).

Interactions between plant chemistry and aphid biology may result in differential occurrence of aphid species across the canola plant. Reproductive versus somatic (vegetative) tissues in the plant might exhibit significant differences in levels of both nutrients and defensive secondary metabolites, and variation in the quality of these tissues might influence aphid demography (Brown et al. 2003b, Lambton and Hassall 2005, Smallengange et al. 2007). Hence, specific plant structures have the potential of being more toxic than other feeding sites for non-specialists pests, such as the green peach aphid and better feeding sites for specialist herbivores such as cabbage aphid (Hopkins et al. 2009, Pratt et al. 2008). Previous studies have reported a gradient of toxicity (quality) to predators where cabbage aphid is more toxic to natural enemies than green peach aphid (Cole 1997a, Cole 1997b, Francis et al. 2000, Francis et al. 2001, Pratt et al. 2008, Kos et al. 2011a, Kos et al. 2011b, Kos et al. 2012). Therefore, defensive chemistry of the plant (glucosinolates) might not only play an import role in aphid population dynamics and within plant distribution, but also have indirect repercussions to natural enemies in the system.

GLUCOSINOLATES MITIGATION BY CANOLA APHIDS

Glucosinolates are nitrogen-sulfur secondary compounds derived from amino acids characteristic to all Brassica plants and are generally toxic to arthropod pests and beneficial organisms (Cole 1997a, Cole 1997b, Kazana et al. 2007, Ratzka et al. 2002, Hopkins et al. 2009,

Ahuja et al. 2009). Glucosinolates are stored in plant vacuoles where they have little biological activity. Upon hydrolysis through tissue damage (herbivore feeding or other exogenous causes) , glucosinolates come into contact with the myrosinase enzyme and are transformed into bioactive defense products (Pratt et al. 2008, Hopkins et al. 2009, Gutbrodt et al. 2012). When glucosinolates come into contact with the catalytic enzyme (myrosinase) they yield aliphatic (isothiocyonates), aromatic, or indolic (nitriles) glucosinolates (Reichelt et al. 2002, Kos et al. 2011a, Kos et al. 2011b, Kos et al. 2012) (Fig. 2). More specifically, aliphatic glucosinolates are derived from methionine and aromatic glucosinolates are derived from phenylalanine or tyrosin while indolic glucosinolates are derived from the amino acid tryptophan. Additional variations in each group are achieved by chain elongation, oxidation, or hydroxylation of side chain (Hopkins 2009). Glucosinolate profiles among Brassicae plants vary between species and cultivars (Hopkins 2009). To date, 120 glucosinolates have been described belonging to three structural groups (Mewis et al. 2006).

Glucosinolates provide olfactory and chemoreceptor cues, which are characteristic signatures for all Brassicae plants (Cole. 1997a, Cole. 1997b, Hopkins et al. 2009). Although glucosinolates are toxic to most herbivore species, some specialist herbivores have evolved to mitigate and use these compounds for host recognition and defense. The diamondback moth (*Plutella xylostella*) uses non-volatile glucosinolates as ovipositing cues (Sun et al. 2009), while specific glucosinolates deter oviposition of *Pieris rapae* (De Vos et al. 2008). Aphid colonies have greater growth rates when feeding on plants that express greater glucosinolate concentrations while the parasitoid *Diaeretiella rapae* prefers feeding on Brassicae specialist aphids and recognize this species by their glucosinolate volatile emissions (Blande et al. 2008, Newton et al. 2009). Specialist herbivores counteract and take advantage of glucosinolate

toxicity in different manners. For example, some lepidopterans have a nitrile specifier protein, which diverts glucosinolate hydrolysis toward less toxic compounds (Wittstock et al. 2004), whereas *Plutella xylostella* possesses a glucosinolate sulfatase (protein) that desulfates glucosinolates (Ratzka 2002); other insects such as turnip and cabbage aphids sequester these toxic compounds (Müller et al. 2001, Bridges et al 2002). Being that glucosinolates hydrolyze through tissue damage by herbivore feeding (Fig. 2), feeding by specialists or generalist herbivores changes glucosinolates profiles of the plant. For instance, Hopkins et al (2009) reported that feeding by cabbage aphid (specialist) makes aliphatic glucosinolates increase and indolic glucosinolates stay the same within the plant after a 7 d feeding period, while feeding by green peach aphid (generalist) makes both aliphatic and indolic glucosinolates increase in the same time frame.

Glucosinolate sequestration

Sequestering glucosinolates is possible for the specialists cabbage aphid and turnip aphid because both species have evolved a specific myrosinase pathway, independent from that of the plant, which enables them to mimic the host plant defense mechanism (Kazana et al. 2007, Pratt et al. 2008) (Table 1). For cabbage aphid and turnip aphid, the myrosinase enzyme is stored in compartmentalized crystalline microbodies located in the sarcoplasm of the non-flight muscle (Kazana et al. 2007). These species acquire the myrosinase enzyme early in their development and take up initial glucosinolates from the maternal haemocoel (Kazana et al. 2007). In later instars, these same aphids then acquire glucosinolates directly from their host plant. Upon predator attack, the enzyme is released from the sarcoplasm of the non-flight muscle, hydrolyzing the glucosinolates, thus making the insects toxic to predators (Pratt et al. 2008).

Indolic glucosinolates are the most abundant in the plant tissues, but aliphatic glucosinolates are more abundant in the adult cabbage aphid. Evidence to date suggests that adult cabbage aphids are selectively sequestering non-toxic forms of aliphatic glucosinolates and avoid indolic glucosinolates. Such compounds can negatively affect performance since they may become bioactive independently of myrosinase activity (Kos et al. 2011b). Consequently, selective sequestration by cabbage aphid may also explain why adults are more toxic to natural enemies than nymphs (Kos et al. 2011b). Being that glucosinolates are also part of the aphid defense mechanism, these secondary compounds act as feeding stimulants for the specialist turnip aphid and cabbage aphid. Additionally, differential expression of glucosinolates in different host plants can directly influence turnip aphid or cabbage aphid abilities to deter predators because glucosinolate chemical profiles vary not only within the plant but also between cultivars and species (Gabrys et al 1997, Cole. 1997a, Van Dam and Domen 2008, Van Dam et al 2008, Guigo et al. 2010, Kabouw et al 2010, Kramer et al. 2011).

Glucosinolate excretion

The green peach aphid alternatively mitigates glucosinolates by excretion through honeydew (Pratt et al. 2008); however, the direct mechanism for the excretion pathway is not well understood (Pratt et al. 2008, Hopkins et al. 2009, Kos et al. 2011a,b). Green peach aphid is a generalist species and nutrients (free amino acids) other than glucosinolates are important feeding stimulants (Cole 1997a). Although green peach aphid excretes most non-toxic glucosinolates acquired through the phloem, some indole breakdown products have been observed in green peach aphid. These compounds may actually negatively affect non-specialist aphid (green peach aphid) performance and fitness (Kim et al. 2008). Glucosinolate concentrations observed in cabbage aphid are about 10 times higher than those found in green

peach aphid, thus providing evidence of sequestration and excretion occurring in cabbage aphid and green peach aphid, respectively (Bridges at al 2002, Winde and Wittstock 2011). Breakdown products sequestered by adult cabbage aphid are mostly aliphatic glucosinolates (isothiocyonates), while glucosinolates reported in green peach aphid are primarily indolic glucosinolates (nitriles) (Bridges at al. 2002, Winde and Wittstock 2011).

Selective sequestration rather than excretion by the specialist cabbage aphid make this species more toxic to natural enemies, but within plant distribution of colonies might also influence aphid toxicity, given that plant architecture influences the resource base available to herbivorous insects (Chrispeels et al. 1999, Lengrand and Barbosa 2000). The optimal defense hypothesis predicts: 1) higher concentrations of defense compounds (glucosinolates in the case of *Brassicae* species) are expected in younger, more valuable tissues (flowers) and 2) that generalist herbivores will avoid strongly protected, glucosinolate-rich tissues due to potential toxic effect (Gutbrodt et al. 2012). In soybeans, greater reproductive rates have been reported when aphids where restricted to top (reproductive) compared to bottom (vegetative) canopy plant structures (Costamagna et al. 2013). In addition, newer plant tissues support higher numbers and healthier soybean aphids than older plant tissues (McCornack, et al. 2008, Costamagna et al. 2013). Hence, specific plant structures have the potential of being more or less toxic than other feeding sites for specialists and non-specialist pests and this might influence pest demography and natural enemy fitness (Hopkins et al. 2009, Pratt et al. 2008).

CANOLA-APHID-PREDATOR INTERACTIONS

Unlike wheat aphids, aphids attacking canola are exposed to glucosinolates and therefore may negatively affect natural enemy fitness and performance (Table 1). Additionally, if predation rates are higher on upper rather than lower canola canopy parts, as observed by

Costamagna et al. (2013), natural enemies such as lady beetles are more likely to be exposed to more toxic cabbage aphid colonies. Thus, management of canola with significant pesticide applications, coupled with different ecological sinks within the plant created by varying degrees of aphid quality (glucosinolate sequestration/excretion) may create more detrimental sink resources in the canola-wheat system. The impact of within-plant distribution of aphids and their interactions on predatory natural enemy communities in established canola-wheat systems is unknown.

Coccinellids

In a landscape study performed from 2011 to 2013, lady beetles (Coleoptera: Coccinellidae) were the second most abundant generalist natural enemy found in wheat-canola landscapes, and therefore selected as study species for this thesis (Cibils-Stewart and McCornack, unpublished data). There are approximately 6,000 species of lady beetles worldwide belonging to 370 genera; 482 of these species are found in North America, belonging to 61 different genera (Vandenberg 2002, Escalona and Slipin'ski 2011). Most lady beetles are aphidophagous predators of honeydew-producing insects in the Order Hemiptera (sub-order Sternorrhyncha), though some are pytopaghous or mycophagous (Giorgi et al. 2009). Even among predaceous groups, alternative foods such as pollen, sap, or nectar are used when prey numbers are low, and sometimes as a supplementary requirement (Lundgren 2009a).

Coccinellids are important natural enemies, both as adults and immature stages, of many insect pest species (Obrycki and Kring 1998, Zhu and Park 2005, Emden and Harrington 2007). For instance, since coccinellid densities were incorporated into *Aphis gossipii* economic thresholds in cotton, insecticide treatments were reduced by half, saving producers an average of $ 3.6 (US) per hectare or $9.00 (US) per acre (Wratten and Powell 1991, Schmidt et al. 2004,

Obrycki et al. 2009). Additionally, Chambers at al. in (1983) reported Coccinellids as effective natural enemies of wheat aphids (Table 1). Presently, ecosystem services within and around wheat fields could be affected by the addition of canola to the landscape given that prey quality/availability as well as plant diversity on the landscape affects distribution and abundance (Landis et al. 2000, Lavandero et al. 2006). Several studies demonstrate that value of intercropping Brassicae crops with wheat to enhance populations of arthropod predators as well as increase wheat returns (Tingey and Lamont 1988, Vandermeer 1995, Altieri and Nicholls, 1999, Parajulee and Slosser 1999, Sarker et al. 2007, Ali and Wahla 2009, Khan et al. 2009). This is possibly due to the availability of alternative foods such as nectar, pollen and prey (Patt et al. 1997, Tylianakis et al. 2004, Lundgren 2009, Seagraves et al. 2010); however, the direct impact of canola aphids on development and fitness of predators has not been reported, specifically. Coccinellids are predators both as adults and larvae. Consequently prey consumption increases as prey density increases (type II functional response). In many cases this guild of predators feed on aphids and occupies the same habitats and niches as their prey (Hodek 1973). This makes lady beetles a meaningful candidate to study the impacts of lanscape changes on beneficial arthropod movements in agroecosystems.

Adult lady beetles have high dispersal (Gardiner et al 2009) and searching capacities (Evans 2003) that enable them to locate isolated patches of ephemeral and unpredictable prey, such as aphids (Hodek 1973, Evans 2003, Hagler and Naranjo 2004). These predatory species deploy a combination of visual and olfactory cues to search for suitable prey patches (Hajek 2004, Seagraves 2009). Larvae, on the other hand, have reduced dispersal capacity, reduced eyesight and chemosensory mouthparts. Adult females therefore determine larval developmental patches by selecting suitable prey patches to oviposite eggs (Evans 2003). Lady beetle larvae are

subject to prey populations selected by their mothers at a local scale (within-field level) due to their limited dispersal capacity (Evans 2003). Ovipositing adults carefully select the oviposition sites for offspring and clutch size is often directly correlated with suitability (Williams and Flaxman 2012) and availability (Evans 2003) of prey diet. Period of food scarcity can be mitigated by dispersal of adults, and alternative food items (i.e., nectar and pollen) (Hodek 1973). Cannibalism in larvae is common to mitigate food scarcity periods (Hodek 1973). Consequently, novel prey, offered by the addition of canola to wheat-pastures systems (Table 1), might directly influence biology and fitness of immature (size and developmental duration) and adult (reproductive output and size) lady beetles, independently.

Coccinellid biology

Lady beetles have a holometabolous life cycle with 4 stages of development (egg, larvae, pupae, and adult). In general, 5-20% of their preimaginal developmental time is spent as eggs, 55-65% as larvae, and 20-25% as pupae (Honeˇk and Kocourek 1988, Dixon 2000). Female lady beetles are usually larger than males, and adult body size in general is an indirect measure of fitness; bigger females are more likely to oviposit a greater number of eggs, while bigger males have an increased chance of mating (Dixon 2000, Tsaganou et al. 2004, Phoofolo et al. 2009, Kajita and Evans 2010, Hodek et al. 2012). To avoid prey scarcity periods caused by the "bust and boom" nature of aphid populations, some lady beetles lay trophic eggs, which are non-hatching and serve as food for the newly hatched larva. Consequently, egg cannibalism enables faster development and higher survival under low prey conditions (Roy et al. 2007). For instance, *Harmonia axyridis* females produced 56% more infertile eggs in low-food patches compared to high-food patches (Perry and Roitberg 2005). Conversely, presence and quality of prey has a direct effect on retention and oviposition of aphidophagous lady beetles within a

habitat (Seagraves 2009). Eggs are laid singly or in clusters, depending on the species (Brown et al. 2003a). Lady beetle fecundity can be defined as the number of eggs laid per female, while fertility is the number of viable progeny per female. Hence, fecundity, fertility and egg retention can be affected by diet quality and the addition of new prey items to the system (Evans 2003, Williams and Flaxman 2012) (Table 1).

After four larval instars, larvae discontinue feeding and use an anal pad (cremaster) to attach to a substrate, which aids in transition to the pupal stage. Duration of the larval period is species specific, but is both temperature and resource (food availability and quality) dependent for all lady beetle species (Hodek et al. 2012). Hodek et al. (2012) demonstrated that body mass and duration of each larval stage differs according to prey type, and concluded that it was probably correlated to quality of prey. Risk of cannibalism and intraguild predation among immatures might increase with differing degrees of prey quality within the canola system (Table 1). In most cases, lady beetles pupate in the vegetation where larvae developed. The adult beetle emerges from the pupal skin through a slit at the front of the dorsal surface. Adults are able to adjust their developmental rate and adult weight in response to food abundance as a density dependent responce.

Hippodamia convergens

The convergent lady beetle (*H. convergens)* is a widely distributed lady beetle, occurring in temperate as well as tropical regions and this species is native to the Western US (Vargas et al. 2012a). When aphid population decline in the late summer, this species migrates to higher elevations in the foothills of the Sierra Nevada Mountains canyons where they aggregate and overwinter, feeding on pollen and nectar (Hajek 2004). During the early spring, adults mate and are carried by wind currents to the central valley where they establish, feed and reproduce (Hajek

2004). Michaud and Qureshi (2006) reported that *H. convergens* is bivoltine in the High Plains with an estivation period during summers (reproductive diapause) and hibernation periods during the winter. The length of these periods is variable between years (Vargas et al. 2012b).

The convergent lady beetle is the most abundant predatory lady beetle in mixed species aggregations of Coccinellids in Kansas during spring, where wheat and canola systems are prevalent (Nielson and Currie 1959, Dogan et al. 1996, Nechols and Harvey 1998, Michaud and Qureshi 2006, Cibils-Stewart and McCornack, unpublished data). Like most coccinellids, convergent lady beetles undergo four larval instars before they pupate and become adults. Convergent lady beetles are oligophagous predators that accept a wide range of food, but this species requires aphids for proper development and reproduction (Hodek and Honeˇk 1996). They complete their preimaginal development (egg to pupa) within 14 d (Omkar and Pervez 2002, Phoofolo et al. 2009). Adult lady beetles can live for an extended period of up to three months (females usually living longer than males), having reached their reproductive capacity at one month. Variation in adult body size is largely determined by resources allocated to the final instar. It is in this instar that lady beetles consume 60-80% of the total aphids (Lee and Kang 2004, Berner and Blackenhorn 2007, Phoofolo et al. 2008, Phoofolo et al. 2009). Fourth instars must reach a minimal weight threshold prior to successfully pupating (Phoofolo et al. 2008, Phoofolo et al. 2009). Given that convergent lady beetles are the most abundant lady beetles in the canola-wheat systems, this species was selected to evaluate whether within-plant distribution of aphids affects aphid quality (sequestration/excretion) and if so, how this might influence tritrophic interactions.

Tritrophic interactions

A complex of aphid species that either sequester (cabbage aphid) or excrete (green peach aphid) glucosinolates can be found in winter canola (Table 1). Aphid species that sequester glucosinolates (turnip aphid and cabbage aphid) are more toxic to natural enemies, such as predatory coccinellids, than those that excrete these compounds (green peach aphid) (Kazana et al. 2007, Pratt et al. 2008, Kos et al. 2011ab). Toxicity of sequestering or excreting aphids varies with plant cultivar, being that glucosinolates are differentially expressed among different brassica species (Francis et al. 2000, Cole 1997a;b, Francis et al. 2001, Pratt et al. 2008, Hopkins et al. 2009, Kos et al. 2011ab). Additionally, toxicity levels and/or quality of these aphid species might be directly affected by colony feeding location within the plant, since biochemical contents differ among plant structures. Varying prey quality created by these interactions may directly influence mortality, development, growth rates and fecundity of populations of natural enemies inhabiting these canola-dominated habitats (Giles at al. 2002, Tsaganou et al. 2004, Giles et al. 2005).

PROJECT GOALS AND OBJECTIVES

A better understanding of the canola agroecosystem, specifically, and the interactions with the surrounding highly-managed landscapes, in general, is required to improve management programs and preserve beneficial insects in the Southern Great Plains (Raghu et al. 2011). Winter wheat crops are capable of sustaining high populations of natural enemies like the convergent lady beetle, green lacewings (*Chrysoperla* sp.) and parasitoids. To date, these natural enemies have provided sufficient control for cereal aphids (Fig 1) in general, which is evident in the limited use of insecticides to manage aphids in wheat grown in this region (Brewer and Elliott 2004). Understanding how the addition of canola is affecting these natural enemy

communities, the biology of the aphid pests in the system, and how predators are using the canola and associated landscapes is crucial to the successful deployment of new biofuel crops like canola into existing production systems.

By evaluating interactions happening at the canola plant level, we can model trophic interactions occurring between species (pests and beneficial organisms). Ultimately, this will shape our understanding of how these interactions affect the dynamics and structure of communities between newly introduced crops and its established surroundings (Guigo and Corff 2010). The three objectives of my thesis were to:

1) Evaluate if aphid (turnip, cabbage and green peach aphid) (Table 1) feeding location (reproductive vs. somatic tissues) in canola influences aphid demographics (measured by comparing λ of different aphid species) or aphid quality (measured by glucosinolates within aphids);
2) Evaluate how within-plant distribution impacts specialist cabbage aphid demography by developing a stage-structured matrix model for aphid populations restricted to either the reproductive or the vegetative plant tissues;
3) Evaluate whether previous feeding location of specialist cabbage aphid and generalist green peach aphid prey in canola impacts immature convergent lady beetle fitness (pupal weights), consumption rates (number of aphids eaten) and developmental rates (measured as duration of each larval stage).

Understanding the vertical distribution of insect pests on the host plant and its possible interactions with other trophic levels is important to develop effective IPM programs that will save time, cut costs involved with pest monitoring, and deepen our understanding of tritrophic interactions (Berberet et al. 2009, Fernandes et al. 2011, Grigolli et al. 2013).

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FIGURES AND TABLES

Figure 1.1.Brassica aphids; A) *Myzus persicae*, (green peach aphid, GPA), B) *Lipaphis erysimi* (turnip aphid, TA), and C) *Brevicoryne brassicae* (cabbage aphid, CA)

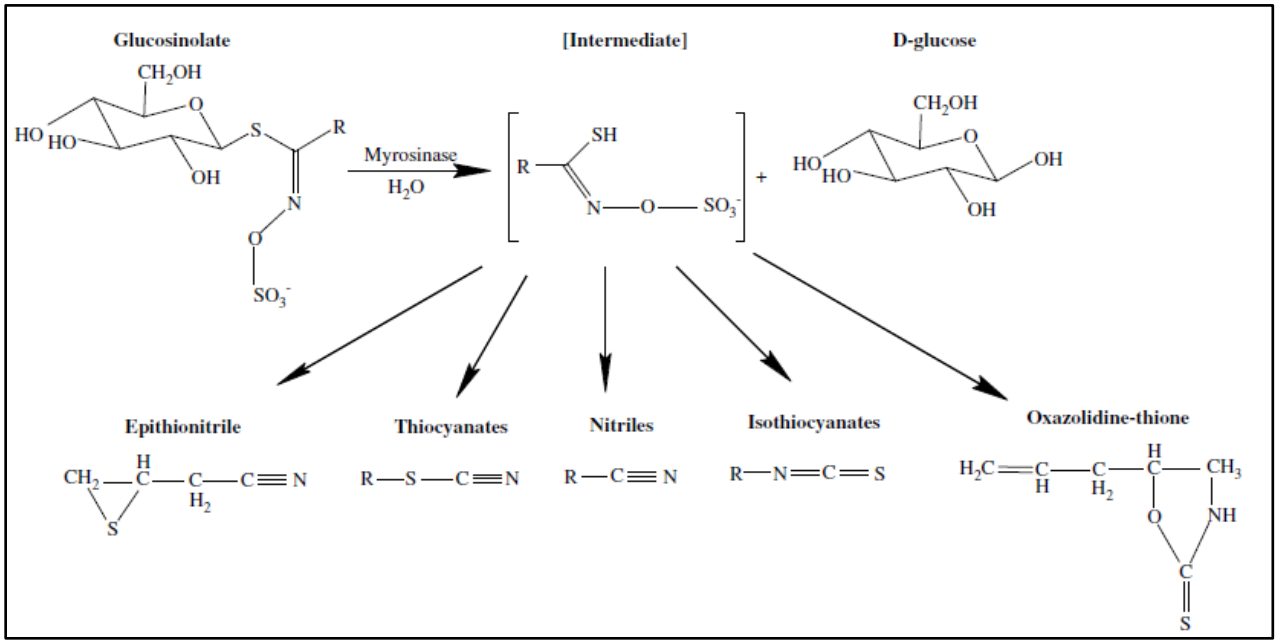

Figure 1.2. Glucosinolates hydrolysis products (reproduced from Vig et al. 2009).

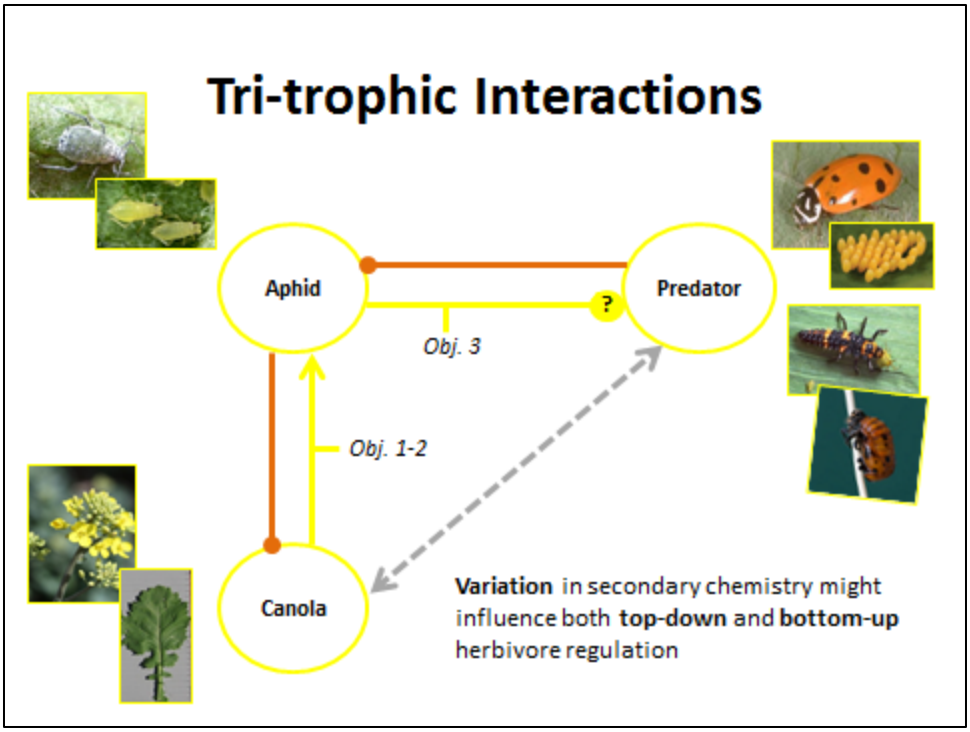

Figure 1.3. The objective of this thesis were to: 1) evaluate if aphid (CA, GPA & TA) feeding location (reproductive vs. somatic tissues) in canola influences aphid demographics (measured by comparing λ of different aphid species) or aphid quality (measured by GLS within aphids); 2) evaluate how within-plant distribution impacts cabbage aphid demography on different canola plant structures by developing a stage-structured matrix model for populations restricted to either the reproductive or the vegetative plant tissues; and 3) evaluate if previous prey (GPA & CA) feeding location in canola impacts immature convergent lady beetle fitness (pupal weights), consumption rates (number of aphids eaten) and developmental rates (measured as duration of each larval stage).

Table 1.1. Characteristics of aphids attacking winter canola and winter wheat in the Southern Great Plains. Wheat aphids include: *Diuraphis noxia* (Russian wheat aphid) and *Schizophis graminum* (Green-bug); canola aphids include: *Lipaphis erysimi* (turnip aphid), *Myzus persicae*, (green peach aphid), and *Brevicoryne brassicae* (cabbage aphid).

	Canola Aphids			**Cereal Aphids**	
	Lipaphis erysimi	***Myzus persicae***	***Brevicoryne brassicae***	***Duraphis noxia***	***Shizophis graminum***
Appearance	Olive-green with a wax	Yellowish-green	Grayish-green with wax	Pale green with small cornicles	Pale green with a green stripe down the middle of their body
Phenology	Early season pest	Throughout the year	Late season pest	Cereal growing season	Cereal growing season
Location preference	Seedling stage	Vegetative	Reproductive	Rolled leaves on upper parts of plants	Throughout the cereal plant
Host range	Brassicae specialist	Generalist	Brassicae specialist	Cereal crop specialist	70 graminaceous species
Glucosinolate	Sequester	Excrete	Sequester	Not exposed	Not exposed

Chapter 2 - FEEDING LOCATION AFFECTS POPULATION GROWTH RATES AND GLUCOSINOLATE CONTENT IN APHIDS (HEMIPTERA: APHIDIDAE) ON WINTER CANOLA (*Brassica napus*).

ABSTRACT

Winter canola production in the South Central US is threatened by a complex of aphid species, including *Lipaphis erysimi, Myzus persicae,* and *Brevicoryne brassicae*. These aphid species vary in their biology, performance, vertical distribution within the canola plant and temporal dynamics across the growing season. Colonizing behavior of these aphids may be affected by intrinsic characteristics of the host plant (bottom-up effects), such as nutritional value or toxic secondary compounds. Understanding these bottom-up effects is a critical first step in understanding potential interactions with aphid predators and parasitoids, which provide valuable ecosystem services. Therefore, the goal of this research was to evaluate how feeding location impacts aphid growth rates and glucosinolate levels in the absence of aphid predation. In field and greenhouse trials (2011-2013), plants were infested and aphids were caged onto single plants in two locations: reproductive tissue or vegetative tissue. Populations were left undisturbed for a three-week period. Results indicate that aphid growth rates were significantly greater ($P < 0.0001$) when aphid feeding was restricted to reproductive tissues, regardless of aphid species. Additionally, glucosinolate profiles significantly differed among aphid species ($P < 0.0001$) and across tissue organs previously attacked by *M. persicae* or *B. brassicae* ($P < 0.0001$). These results suggest a need for sampling plans that account for unequal aphid growth rates based on aphid distributions within the plant. In addition, the ability of predators to reduce aphid

populations below economic levels may depend on how aphid quality affects predator consumption and developmental rates.

INTRODUCTION

In the South Central US, winter canola (*Brassica napus*) production has increased from zero in 2004 to over approximately 81,000 hectares (200,000 acres) in 2012 (Peeper et al. 2009, Franke et al. 2009, U.S Canola Association 2013, USDA 2012). Since its introduction, canola yields have been threatened by a complex of aphid species including *Lipaphis erysimi* (turnip aphid or TA), *Myzus persicae*, (green peach aphid or GPA), and *Brevicoryne brassicae* (cabbage aphid or CA) (Franke et al. 2009, Berlandier et al. 2010, Royer and Giles 2010). These three species of aphids form dense colonies on the canola plant that result in wilting, flower abortion, reduced oil-seed content and reduced pod set, all of which can result in yield reductions of up to 70% if populations are left unmanaged (Berlandier et al. 2010). Seasonality of aphid species occurring in canola production fields varies throughout the life cycle of the canola plant. While the turnip aphid is an early season pest that attacks canola seedlings during late fall, mixed infestations of green peach and cabbage aphids are common in flowering canola fields during early spring (Royer and Giles 2010). Additionally, occurrence of cabbage and green peach aphid also varies among plant structures. Green peach aphid colonies are predominantly observed colonizing the lower leaves of the canopy (hereafter referred to as vegetative tissue), whereas cabbage aphid colonies are mainly observed on flowering racemes in the upper canopy (hereafter referred to as reproductive tissue) of canola plants (Merritt 1996, Hopkins et al. 1998, 2009, Berlandier et al. 2010).

Occurrence of aphids on different plant structures might be a result of interactions between top-down effects (i.e., natural enemy forces), bottom-up effects (i.e., secondary

compounds and/or nutrition of plants), or competition among aphid species (Wilson et al. 1982, Hacker and Bertness et al. 1995, Costamagna et al. 2013). The primary forces governing canola aphid population growth rates on canola needs further investigation. Feeding on different plant structures is known to influence the fitness of herbivores in other systems. For example, the population growth rate for apterous aphids was greater when feeding on the lower plant strata of chilli plants (*Capsicum annum*) compared to the upper plant strata (Idris and Roff 2002). Conversely, *Aphis gossypii* (Fernandes et al. 2011) and *Anthonomus grandis* (Grigollo et al. 2013) in cotton (*Gossypium hirsutum*) had greater fecundity when feeding in the upper plant strata compared to lower plant strata. Selective feeding on plant structures can indirectly impact natural enemies, which is often mediated by secondary compounds found in plants. For example, *Pieris brassicae* caterpillars preferred feeding on glucosinolate-rich flowers of black mustard (*Brassicae nigra*) compared to glucosinolate-poor leaves (Smallengange et al. 2007), which increased fecundity. Soler et al. in 2005 reported herbivores displayed feeding preference for flower tissue containing higher levels of glucosinolates, which is used as an effective biochemical defense strategy against natural enemies. Costamagna et al. (2011 and 2013) demonstrated that the disproportionate effects of feeding by generalist predators caused higher number of soybean aphids (*Aphid glycines*) in the lower canopy of soybeans (*Glycine max*) even though aphid fecundity is lower compared to soybean aphids feeding in the upper canopy. The effects of aphid feeding location on canola aphid population growth rates is not known, additionally, differences in concentration levels of secondary compounds like glucosinolates by developing aphid populations has not been studied.

Bottom-up forces might contribute to the distribution of herbivores within the plant canopy; consequently, these same factors might affect suitability of aphids for predators.

Glucosinolates are nitrogen-based, sulfur-containing compounds that are characteristic to brassica plants (Kos et al 2011ab), generally serving as a species-specific plant chemical defense against herbivores (Cole 1997ab, Borek et al. 1998, Wittstock and Halkier 2002, Ratzka et al. 2002, Kazana et al. 2007, Hopkins et al. 2009, Ahuja et al. 2009). In other brassica plants, glucosinolate concentrations are typically higher in reproductive tissues compared to more mature vegetative tissues (Merritt 1996, Smallengange et al. 2007). Toxicity to herbivores results from the breakdown of glucosinolates into other compounds, which are classified as aliphatic (isothiocyonates), aromatic, or indolic (nitriles) (Kos et al. 2011ab,. 2012). While glucosinolates are toxic to most generalist herbivore species, specialist herbivores that utilize brassica crops have evolved different mechanisms to counteract their toxicity (Pratt et al. 2008, Kim et al. 2008, Hopkins et al. 2009, Gutbrodt et al. 2012). For instance, specialist herbivores like cabbage and turnip aphid sequester glucosinolates as a defense against predators (Weber 1985, Awmack and Leather 2002, Kazana et al. 2007, Pratt et al. 2008, Hopkins et al. 2009). Alternatively, the generalist herbivore green peach aphid mitigates toxicity of intact glucosinolates through honeydew excretion (Pratt et al. 2008). Although green peach aphid excretes most aliphatic glucosinolates, some indole breakdown products have been reported to occur in green peach aphids and are known to negatively affect aphid reproduction (Kim et al. 2008). Therefore, glucosinolate content in canola may be influencing the composition, abundance and vertical distribution of the attacking aphid complex since this complex is made up of both generalists and specialist herbivores.

As the acreage of planted canola increases, so will use of insecticides to manage canola aphid outbreaks; however, the impact of these landscape-level impacts on existing ecosystems in a historically wheat-dominated landscape is not known (Brewer et al. 2004, Franke et al. 2009,

Knodel 2011). In addition to potential non-target effects from insecticide use, canola may serve as a potential source or sink for natural enemies. For example, novel prey items offered by the canola landscape may disrupt existing ecosystem services by either providing new sources for natural enemies or sinks that reduce services from existing systems. It is imperative to focus on source-sink relationships occurring at the canola plant-level to understand these broader landscape-level effects. For this study, we wanted to determine if feeding location on canola had any effects on aphid population growth rate and their quality as a food source for aphid predators based on levels of defensive compounds. Therefore, the three objectives of this research were to: 1) determine if feeding location (reproductive versus vegetative tissues) influences growth rates of turnip, cabbage and green peach aphid in winter canola, 2) determine if glucosinolate composition in specialist (cabbage aphid) and generalist (green peach aphid) aphids is affected by feeding locations, and 3) determine if glucosinolate composition varies between reproductive and vegetative tissues of an infested canola plant. Based on previous literature, we predict that the reproductive tissue will have higher levels of glucosinolates than the vegetative tissue, and higher levels of glucosinolates would yield higher growth rates for brassica specialist aphids**.** We also predict that glucosinolate concentrations will be greater in the specialist aphid (cabbage aphid) compared to the generalist aphid (green peach aphid) due to differences in ability to mitigate deleterious effects of glucosinolates encountered during feeding (Kazana et al. 2007). If varying levels of glucosinolates exist between plant structures (Smallengange et al. 2007), we could then hypothesize that feeding location will have a stronger positive effects on growth rates of the specialist cabbage aphid that sequester rather than excrete compounds.

MATERIALS AND METHODS

Aphids and plant material

Aphids (turnip, cabbage and green peach aphids) used for this experiment were obtained from laboratory-reared colonies maintained at Kansas State University (Manhattan, KS). Adult turnip aphids were collected from infested canola seedlings at Ashland Bottoms Research Farm in Riley County near Manhattan, KS (N 39.63590, W -96. 38425) during late fall in 2011. Adult cabbage and green peach aphids were collected from winter canola fields in Barber County near Kiowa, KS (N 36.998414, W -98.456797) during early spring in 2011. Aphids were transported to the laboratory in coolers and transferred to potted, vernalized canola seedlings. Prior to aphid establishment in the laboratory, winter canola (variety Riley) was seeded in a soil mix that contained all required minerals and nutrients (proprietary soil blend provided by M. Stamm, canola breeder, Kansas State University and Oklahoma State University) and maintained in the greenhouse at 22ºC ± 3 under natural light during daylight hours and supplemented with artificial lights at night to ensure a 16:8 hr (light:dark) photoperiod. Canola plants were then artificially vernalized for a two month period with a 12:12 hr (light:dark) photoperiod at a constant 4ºC to induce reproductive maturity (Murphy and Scarth 1994*).* Vernalized plants were then used for aphid colonies and greenhouse experiments. Greenhouse plants were watered daily. Aphid colonies were maintained at 22ºC ± 2, 60-70% RH, and a 16:8 hr (light:dark) photoperiod (Kos et al. 2011a) in growth chambers (Percival, model AR22L; Perry, IA). Voucher specimens (*Brevicoryne brassicae* nymphs and adults) were deposited in the Department of Entomology Museum (voucher number = 228).

Aphid population growth rates

The impact of feeding location on population growth rates of aphids attacking canola was evaluated under predator-free conditions in both greenhouse and field studies using mesh enclosure cages. A field study was replicated in three trials at the Ashland Bottoms Research Farm using small canola fields (18.3 x 30.5 m): two parallel trials in 2011 (4 April to 13 May and 27 April to 26 May) and one trial in 2013 (21 April to 13 May). A complementary greenhouse study was replicated in two trials under greenhouse conditions from 27 October to 17 November 2011 and 11 October to 2 November 2012. All trials were conducted on plants at the reproductive stage (Canola Council of Canada, 2013). We evaluated the main effects of aphid species (cabbage or green peach aphid) at predetermined locations on the plant (reproductive and vegetative tissues). In the field trials, all treatments were arranged in a completely randomized design. A similar design was used for the greenhouse study with the exception of species as a main effect, where turnip aphid was added to the greenhouse study. Since turnip aphids are rarely found in canola during flowering, they were added to greenhouse trials to determine if the flowering stage of plant development is a poor resource for this aphid species.

In the 2011 and 2013 field trials, a total of 20, 36 and 60 plants, respectively, were infested with either cabbage or green peach aphid. An experimental unit consisted of a single canola plant, which were spaced 0.2 m apart along an edge row of the canola field fields used in the trials (18.3 x 30.5 m). In the 2011 and 2012 greenhouse trials, a total of 90 and 15 vernalized canola plants, respectively, were infested with either, turnip, cabbage or green peach aphid. In the greenhouse (6 × 7.3 m), canola plants were arranged in a CRD, with plants spaced 0.4 m apart; where plant location was re-randomized weekly to account for variation in greenhouse conditions (light, temperature, air movement).

Aphid movement was restricted to either vegetative or reproductive tissues using mesh cages. Cages enclosed either a single leaf in the lower canopy (vegetative tissue) or the flowering raceme in the upper canopy (reproductive tissue); the cage design was adapted from Soper et al. (2013). Both cage types were deployed on the same plant (experimental unit) to enable direct comparisons between feeding location and thus reducing between-plant variability (e.g., size, nutritional composition, etc). The base of each enclosure cage was secured using 15 cm zip-ties (Gardner Bender; Butler, WI) at the node between the leaf and the plant stem in the lower canopy or below the last flower of the flowering raceme. To allow free-movement of aphids within the cage, cylindrical supports were used inside the mesh sleeves to keep mesh from draping across flowers or leaves. Supports were made of 14-gauge, galvanized steel wire rope (Impex Systems Group Inc., Miami, FL).

When 30% of flowers opened on the main raceme (Canola Council of Canada, 2013), vegetative and reproductive enclosure cages were infested with five, newly-reproductive, apterous adult aphids of the same species. Aphids were transported to greenhouse or field trials using 2 ml Eppendorf vials (Fisher Scientific Inc.; Waltham, MA). Experimental plants were infested using a fine camel hair paintbrush and aphids were deposited into cages directly on the leaf or flower tissue. Aphid populations remained in cages for approximately 21 d. At the end of this period, cages and aphids were removed by excising the secondary stem and placing plant material and cages in 3.78 L plastic bags (Great Value; Wal-Mart, Manhattan, KS). Bags were immediately placed in the freezer to stop aphids from reproducing. Freezing also allowed for effective counting of static aphids, which were visually counted and numbers were recorded using a hand counter.

For two experimental plants in all trials, temperature (ºC) was recorded inside both cage types and on adjacent open vegetation of the same plant at 1 hr intervals using data loggers (HOBO Pendant®; Onset; Pocasset, MA). Loggers were secured to the different plant locations using 15 cm zip-ties (Gardner Bender, Butler, WI) and deployed at the time aphids were infested on cages for the duration of the experiment. Temperature data was used to determine if any cage effects existed that would influence population growth. Additionally, temperature data was used to determine degree-day accumulation for aphids, which was calculated using procedures outlined by Pruess (1983) and Wagner et al. (1984). Specifically, cumulative degree-days at all locations were calculated as:

$$Degree - day\ (base\ X) = \sum \left(\frac{HT+LT}{2}\right) - X \quad (1)$$

where HT is the daily high temperature mean, LT is the daily low temperature mean, and X is the base developmental threshold temperature. If subtracting the base results in a negative number, the degree-day calculation equals zero. Degree-day accumulations are determined by summing the degree-day calculations over time. Different developmental thresholds were used because these thresholds are often species-specific. For all CDD, we used X = 5.8 ºC for cabbage aphid (Diaber 1970) and X = 4.0 ºC for green peach and turnip aphids (Weed 1927).

Glucosinolates profiles

Upon terminating the field trial in 2013, glucosinolate content was quantified independently for both aphid and plant samples from 40 randomly selected plants infested with either cabbage or green peach aphids. For each plant, fifteen apterous adults were removed from either feeding location using a fine camel hair paintbrush (Chaplin-Kramer et al. 2011) and placed directly in a 2 ml Eppendorff vials (Fisher Scientific Inc.; Waltham, MA) that contained 500 µl of 90% methanol. From the same cages, approximately 150 mg of either vegetative or

reproductive plant tissue were excised using a razor blade and placed directly in 2 ml Eppendorff vials (Fisher Scientific Inc.; Waltham, MA) that contained 750 µl of 90% methanol. A total of 160 samples were extracted for glucosinolates; 80 aphid samples and the paired 80 plant samples. Aphids and plant samples were taken between 12:00 and 14:00 to minimize any potential impact of natural fluctuations in glucosinolate levels (Johnson et al. 2012) on 13 May 2013 and stored at -20°C until glucosinolate concentrations were analyzed.

All aphid and plant tissue samples were analyzed for total aliphatic and indolic glucosinolate concentrations. Additionally, all dectable glucosinolate compounds within the sample were identified and extracted and quantifiedusing extraction protocols as descrbied in Kliebenstein et al. (2001a). The identified glucosinolates that were absolutely quantified in the aphid and plant samples included: 3-hydroxy-pent-4-enyl, But-3-enyl, Pent-4-enyl, 4-methoxy-indol-3-ylmethyl, N-methoxy-indol-3-ylmethyl, and Indole-3-ylmethyl. There were two additional unknown indole glucosinolates that were relatively quantified do to the lack of purified standards to compare with. Glucosinolate concentrations were identified by comparing retention times and UV absorption spectra to purified standards (Fisher Scientific Inc.; Walthham, MA) per methods described by Reichelt et al. (2002). Retention and UV absorption was measured via high-performance liquid chromatography (HPLC) with diode-array detection (DAD) using an Agilent 1100 system (Agilent 1100 HPLC Pump Hewlett, Packard HP, Bordentown, NJ) (Kliebenstein et al. 2001b; Reichelt et al. 2002). Absorption was measured at 229 nm and converted to micromoles per gram using response factors determined from purified standards (Reichelt et al. 2002). Values are reported in nmol/15 apterous adult aphids and nmol/150 mg of plant tissue.

Statistical analysis

Aphid population growth rates. Data pertaining to aphid population growth rates for field and greenhouse trials were analyzed using a mixed model analysis of variance (ANOVA) (PROC MIXED, SAS 2009). Assumptions of normality for growth rate data were tested according to the Shapiro-Wilk test statistic (PROC UNIVARIATE, SAS 2009). The ANOVA models were evaluated for the main effects of feeding location, aphid species, and an interaction between location and species. The *LS MEANS* statement using an adjusted Tukey method was used to make treatment comparisons at $\alpha = 0.05$. Growth rate data was not significantly different between trials within greenhouse ($F = 2.59$; $df = 1, 172$; $P < 0.85$) and field ($F = 3.95$; $df = 1, 186$; $P < 0.92$) studies. Thus, data were combined and trial was added as a random variable in the model. Additionally, mean maximum and minimum temperatures recorded inside both cage types and on an adjacent open area of the canopy were analyzed for the main effect of location for field and greenhouse trials with ANOVA (*PROC MIXED,* SAS Institute 2009). Mean differences were compared using the least-squares mean difference (LSD) from an adjusted Tukey Kramer method for multiple comparisons with the level of significance set at $\alpha = 0.05$. Cumulative-degree days were calculated for data loggers place inside both cage types and on an adjacent open area of the canopy using daily maximum and minimum temperature means for greenhouse and field studies, using equation (1).

Glucosinolate profiles. All data pertaining to total indolic and total aliphatic concentrations as well as eight select glucosinolate concentrations detected in samples from the 2013 field trials were analyzed with an ANOVA using the *PROC MIXED* in SAS (Institute 2009). Assumptions of normality for data were tested according to the Shapiro-Wilk test statistic (PROC UNIVARIATE, SAS Institute 2009). For all aphid and plant samples, ANOVA models

were used to test main effects of feeding location, aphid species and the two-way interaction between feeding location and aphid species. Mean differences were compared using the least-squares mean difference (LSD) from an adjusted Tukey Kramer method for multiple comparisons with the level of significance set at $\alpha = 0.05$.

RESUTLS

Aphid population growth rates

Field Study. There was a significant main effect of feeding location (F = 68.8; df = 1, 172; $P < 0.0001$), where growth rates were higher for aphids feeding on reproductive tissues compared to vegetative tissues. The main effect of aphid species was also significant (F = 4.6; df = 1,172; P = 0.033), where growth rates were overall higher on the reproductive versus vegetative tissues. Despite significant main effects, there was a significant interaction between feeding location and aphid species (F = 2.59; df = 1, 172; $P < 0.05$), where the cabbage aphid had significantly higher population growth rates than the green peach aphid when both were restricted to reproductive tissues (Fig. 1A, Appendix. Table 2.4).

Under field conditions, there were no significant differences between maximum (F = 0.17; df = 2, 17; P = 0.84) or minimum (F = 0.01, df = 2, 17; P = 0.99) mean daily temperatures observed at any of the locations tested, including caged (reproductive and vegetative) and open locations (Fig. 2). Although the caged locations within the plants were 0.3°C (vegetative cage) to 0.7°C (reproductive cage) warmer than the open locations, degree-day estimates for caged and open location populations indicate that there were no differences between the possible number of aphid generations across cage treatments. Estimates included five generations of cabbage aphid (152 degree-day generation time) and six generations of green peach aphid (152.2 degree-day

generation time). Therefore, degree-day estimates suggest that there were no cage effects influencing aphid growth (Table 1).

Greenhouse Study. There was a significant main effect by feeding location (F = 68.8; df = 1, 186; $P < 0.0001$) where growth rates were higher for aphids feeding from reproductive tissues compared to vegetative tissues. Although there was a significant main effect by aphid species (F = 8.52 df = 1, 186; P = 0.0003) as well, there was also a significant interaction between feeding location and aphid species (F = 9.83; df = 1, 186; $P < 0.0001$). Similar to the field study, the specialist cabbage aphid had significantly higher population growth rates than generalist green peach aphid when both were restricted to reproductive tissues (Fig. 1A, Appendix. Table 2.4).

Contrary to temperatures observed in the field, there were significant differences between caged and open locations on experiment plants for maximum (F = 35.4; df = 2, 11; $P < 0.002$) and minimum (F = 4.98; df = 2, 11; P= 0.0077) daily temperatures observed during the studies (Fig. 2). Although the caged locations within the plants were 4.3°C (vegetative) and 4.2°C (reproductive) warmer than the opened locations, the degree-day estimates for caged and open locations indicate that no differences existed between the number of aphid generations across cage treatments, showing that there were no cage effects influencing aphid growth (Table 1). Specific estimates included six generations for cabbage aphid (152 degree-day generation time) and eight generations for green peach aphid (152 degree-day generation time).

Glucosinolate profiles

Aphid samples. The main effect of aphid species was significant (F = 14.88; df = 1, 76; P = 0.0002), where cabbage aphid had greater amounts of aliphatic glucosinolates than green peach aphid (Table 2; Appendix Table 2.5); these effects were additive between feeding locations

observed (F = 14.11; df = 1, 76; P = 0.0003). Significant interactions were observed between feeding location and aphid species for total aliphatic concentrations (F = 14.11; df = 1, 76; P =0.0003). Cabbage aphids restricted to feeding on reproductive plant parts had greater concentrations of aliphatic glucosinolates compared to the other species by location combinations (Table 2; Appendix Table 2.5). For indolic compounds, significant differences among aphid species (F = 34.36; df = 1, 76; P < 0.0001) were found; where indolic concentrations were 14-fold higher for cabbage aphid (0.028 nmol/15 aphids ± 0.001) than for green peach aphid (0.002 nmol/15 aphids ± 0.001SEM). These effects were additive between feeding locations observed (F = 7.51; df = 1, 76; P = 0.0076). Additionally, a significant interaction between feeding location and aphid species was observed where cabbage aphid feeding on reproductive tissues had higher concentration of total indolic compounds than the other treatment combinations tested (F = 8.22; df = 1, 76; P = 0.0054) (Table 2; Appendix Table 2.5).

For concentrations of select aliphatic glucosinolates, there was a significant interaction between feeding location and species, where cabbage aphid samples feeding on reproductive tissues had significantly higher concentrations of 3-hydroxy-pent-4-enyl (F = 5.75; df = 1, 76; P = 0.018), but-3-enyl (F = 11.78; df = 1, 76; P = 0.001), and pent-4-enyl (F = 14.37; df = 1, 76; P = 0.0003) than all other treatments (Fig. 3A; Table 3; Appendix Table 2.7). A similar pattern was observed for select indolic glucosinolate concentrations. Specifically, cabbage aphid restricted to feeding on reproductive tissues had significantly higher concentrations of UnknIndole2 (F = 10.29; df = 1, 76; P = 0.002) and 4-methoxy-indol-3-ylmethyl (F = 6.51; df = 1, 76; P < 0.012) than all other treatments tested (Fig. 3A; Table 3; Appendix Table 2.7). Furthermore, the cabbage aphid had significantly higher concentrations of Indole-3-ylmethyl (F = 27.73; df = 1,

76; $P < 0.0001$) compared to the other treatment combinations (CA-R, GPA-R, and GPA-V). Concentrations of UnkIndole1 ($F = 13.64$; $df = 1, 76$; $P = 0.004$) and N-methoxy-indol-3-ylmethyl ($F = 29.25$; $df = 1, 76$; $P < 0.0001$) were significantly higher in cabbage aphid than in green peach aphid, regardless of feeding location (Fig. 3A; Table 3; Appendix Table 2.7).

Plant samples. For aliphatic glucosinolate content, there was a significant main effect of tissue type ($F = 86.61$; $df = 1, 76$; $P < 0.0001$), where concentrations in reproductive tissue were higher than those observed in vegetative tissues, regardless of the aphid species that fed at those locations (Table 2; Appendix Table 2.6). We observed a similar effect of tissue type on indolic glucosinolate content ($F = 12.72$; $df = 1, 76$; $P = 0.0006$), where concentrations were significantly higher in reproductive tissue compared to vegetative tissue (Table 2; Appendix Table 2.6). There were no significant interactions for either indolic content ($F = 3.49$; $df = 1, 76$; $P = 0.65$) or for total aliphatic compounds tested ($F = 2.48$; $df = 1, 76$; $P < 0.12$).

Only reproductive plant tissues had detectable levels of aliphatic glucosinolates for select compounds tested (Fig. 3B; Table 3). Of these, But-3-enyl ($F = 70.49$; $df = 1, 76$; $P < 0.0001$) and Pent-4-enyl ($F = 63.38$; $df = 1, 76$; $P < 0.0001$) were significantly higher on reproductive tissue exposed to cabbage aphid compared to reproductive tissue exposed to green peach aphid. Concentrations of 3-hydroxy-pent-4-enyl ($F = 51.1$; $df = 1, 76$; $P < 0.0001$) were not significantly different between reproductive tissues exposed to either species (Fig. 3B; Table 3; Appendix Table 2.8). For select indolic compounds, only reproductive tissue had measurable concentrations of Indole-3-ylmnethyl ($F = 46.76$; $df = 1, 76$; $P < 0.0001$) when compared to the other treatment combinations. Only vegetative tissue had measurable concentrations of 4-methoxy-indol-3-ylmethyl ($F = 29.6$; $df = 1, 76$; $P < 0.0001$), where vegetative tissues exposed to cabbage aphid had higher concentrations than when exposed to green peach aphid ($F = 8.86$;

df = 1, 76; *P* = 0.0039) (Fig. 3B; Table 3; Appendix Table 2.8). Concentrations of UnkIndole1, UnknIndole2, and N-methoxy-indol-3-ylmethyl were not detected in tissue samples (Fig. 3B; Table 3; Appendix Table 2.8).

DISCUSSION

In field and greenhouse studies, specialist (turnip and cabbage aphid) and generalist aphids (green peach aphid) were restricted to reproductive and vegetative tissue of the canola plant to test for the effects of feeding location on population growth rates and glucosinolate content in aphids. Results indicate that all aphid (turnip, cabbage and green peach aphid) population growth rates were equal when restricted to feeding on vegetative plant parts, yet specialist aphids (turnip and cabbage aphid) had significantly higher growth rates than generalist aphids (green peach aphid) when restricted to reproductive tissues (Fig. 1). While higher population growth rates for other brassica pests (*Pieris brassicae)* have been reported in reproductive versus vegetative tissue of *Brassicae nigra* (Smallegange et al. 2007), to our knowledge this is the first study showing feeding location effects on aphid population growth rates in canola.

Furthermore, concentrations of aliphatic and indolic glucosinolate compounds were significantly higher in reproductive versus vegetative tissue (Table 2). Similar to other study findings (Weber 1985, Pratt et al. 2008, Hopkins et al. 2009, Kazana et al. 2007), we found that the generalist green peach aphid had lower glucosinolate concentrations than specialist cabbage aphid. These differences were likely a result of cabbage aphid sequestering glucosinolates rather than excreting them as the generalists do, which also coincided with the higher levels of glucosinolates in the reproductive tissues. Sequestration of glucosinolates, especially of aliphatic compounds, is reported elsewhere to directly influence cabbage aphid toxicity to predators (Kos

et al. 2011b). In a separate, no-choice laboratory bioassay, *Hippodamia convergens* larvae were restricted to diets of specialist (cabbage aphid) and generalist (green peach aphid) aphids that were reared on different canola structures to evaluate prey suitability (see Chapter 4). In this study, *H. convergens* larvae were capable of surviving exclusively on a diet of canola-reared aphids. However, cabbage aphid feeding location within a plant, more specifically cabbage aphid restricted to reproductive tissues, negatively affected larval consumption and developmental rates (see Chapter 4).

Higher population growth rates in reproductive versus vegetative tissues, regardless of aphid species, in our studies suggests that reproductive tissue of canola might be functioning as a 'nutritional source' for aphids. McCornack et al. (2008) and Costamagna et al. (2013) reported lower performance for aphids in soybean and differences in growth rates were mainly attributed to a combination of top-down and bottom-up factors governing aphid population growth. However, under field conditions cabbage aphid colonies are observed predominantly in the reproductive tissues of the canola plant, wheras green peach aphid colonies are observed in the lower plant canopy (Merritt 1996, Hopkins et al.1998, Hopkins et al. 2009, Berlandier et al. 2010). Hacker and Bertness (1995) reported that marsh aphids (*Uroleucon ambrosiae*) were more abundant on hosts that enabled them to avoid predation, even though the poor hosts slowed their growth rates. Avoidance of predation may be the case for canola, where the green peach aphid colonies might colonize lower plant parts in the field to avoid predation even though population growth rates are compromised.

Glucosinolate concentrations measured from plant tissues did not vary when exposed to feeding by specialist cabbage aphid or generalist green peach aphid species. Although not significantly different, aliphatic glucosinolate content was 29% higher in reproductive tissues

exposed to feeding by cabbage aphid compared to tissues fed on by green peach aphid (Table 2). Our results corroborate findings by Mewis et al (2006), which reported that feeding by cabbage aphid (specialist) increases aliphatic glucosinolates in *Arabidopsis thaliana*. Additionally, the feeding mode, duration, and species-specific salivary secretions have been demonstrated to play an important role in secondary compound expression (Kessler and Baldwin 2002). Cole (1997) compared feeding modes of cabbage and green peach aphid in different brassica plants, and even though performance of cabbage and green peach aphid were not different among brassica hosts, the green peach aphid probed significantly less and ingested significantly more xylem than cabbage aphid. However, once initial phloem contact was established, cabbage aphids spend significantly less time in the phloem salivation phase (Cole 1997). Feeding duration was not measured in our studies, but prolonged xylem feeding and reduced probing by green peach aphid observed by Cole (1994) might explain the differences in glucosinolates concentrations between reproductive tissues exposed to feeding from either aphid species (Table 2). More intensive feeding duration studies using electrical penetration graph *(*EPG) could help explain differences between specialist and generalist aphids in canola to determine if the specialist and generalist are stimulating different glucosinolate production in canola indeed varies according to aphid species feeding and feeding location.

Glucosinolate concentrations were lower in generalist versus specialist aphids in our study, which is supported in the literature (Weber 1985, Pratt et al. 2008, Hopkins et al. 2009, Kazana et al. 2007). In our study, acquisition of aliphatic and indolic glucosinolates by the specialist cabbage aphid was directly influenced by feeding location (Table 2). A further study including the baseline glucosinolate levels in aphids prior to the experiments would help confirm these findings. Presumably glucosinolate levels will be null without feeding. Selective

sequestration of aliphatic versus indolic compounds by cabbage aphid has been reported by Kos et al (2011b). Specialist cabbage aphids selectively sequester aliphatic compounds because formation of hydrolysis toxic compounds by indole glucosinolates can negatively affect cabbage aphid performance (Kos et al. 2011b). Additionally, greater sequestration of aliphatic compounds directly influences cabbage aphid toxicity to natural enemies without adversely affecting growth rates and development (Kos et al. 2011b).

We further explored correlations between glucosinolate concentrations observed in plant tissue with levels detected in the aphids feeding on the tissue at the locations tested using simple linear regression (PROC REG, SAS institute 2009). Linear model revealed that the composition of compounds within aphids was not related to compounds in plants (reproductive: $F = 0.12$; $df = 1, 39$; $P = 0.7226$; vegetative: $F = 2.56$; $df = 1, 39$; $P = 0.1196$). Last, we used multiple regression analysis (*PROC REG*, SAS institute 2009) to determine if relationships existed between specific compounds and aphid growth rates. The analysis revealed that the concentration of Pent-4-enyl (select aliphatic measured) had a significant positive effect on cabbage aphid growth rates on reproductive tissue ($F = 4.64$; $df = 1, 19$; $P = 0.0449$). Despite the effect, Pent-4-enyl only explained 20% of the variation in growth rate for cabbage aphid restricted to reproductive tissue. Absorption peaks for unknown compounds were also detected by the HPLC analysis, which cannot be identified without mass spectrometry (Appendix Figure 4).

Overall, our studies show that aphid feeding location directly influenced demography of aphids in canola. In the absence of migration events, top-down forces and herbivore competition, growth rates were higher for all aphid species (turnip, cabbage and green peach aphid) that fed on glucosinolate-rich, reproductive tissues. In prior field observations, specialist cabbage aphid

colonies have been predominantly found in flowering canopy while generalist green peach aphid colonies were observed on vegetative tissues. Green peach aphid colonies might be shifting to vegetative tissues to avoid predation although feeding on vegetative tissue might compromise population growth, which is observed in other systems (Hacker and Bertness 1995, Costamanga et al. 2013). Generalist green peach aphid is less protected against predators than specialist cabbage aphids. Recall, this species sequesters rather than excretes glucosinolates, which may further explain the occurrence of specialist herbivores in glucosinolate-rich plant parts and generalists on low-glucosinolate vegetative tissue. Since cabbage aphids had higher levels of glucosinolates when restricted to reproductive tissue, we conclude that, in canola, feeding location may indirectly affect predator performance due to suitability of aphids colonizing different canola plant structures (see Chapter 4). Future studies that involve adult natural enemy oviposition preference may provide a further understanding of the landscape-level impact of adding canola to an already existing landscape. Understanding how feeding location of herbivores affects natural enemy development, and the sources-sinks created by aphids at the plant level will help us understand the impacts of adding canola acres into the landscape, and the potential influence on surrounding crops. Future studies regarding oviposition preference of natural enemies in canola as well as choice studies involving aphids that are restricted to different canola plants are required to understand broader landscapes effects of adding canola to already existing landscapes with other crop covers.

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FIGURES AND TABLES

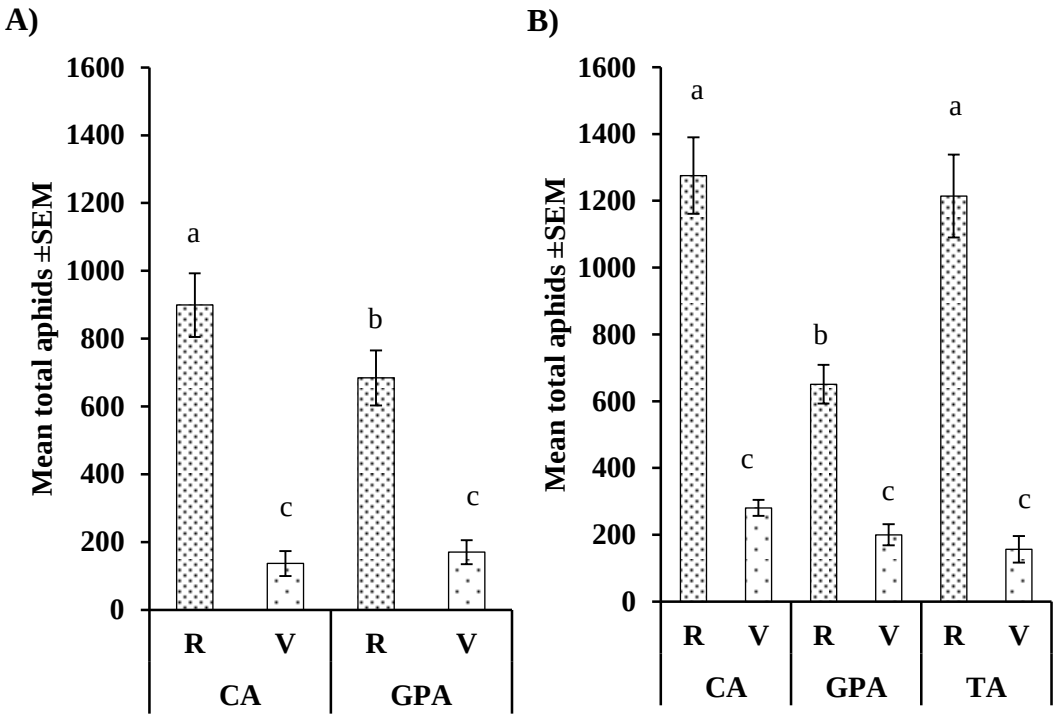

Figure 2.1. Mean ± SEM population growth rates for *Brevicoryne brassicae* (cabbage aphid: CA), *Myzus persicae*, (green peach aphid: GPA), and *Lipaphis erysimi* (turnip aphid: TA) restricted to either reproductive (R) and vegetative (V) tissues of canola (*Brassicae napus*) in field (A) and greenhouse (B) study during 2011-2013. Different letters above bars indicate a significant difference between treatments using an adjusted Tukey method ($P < 0.05$).

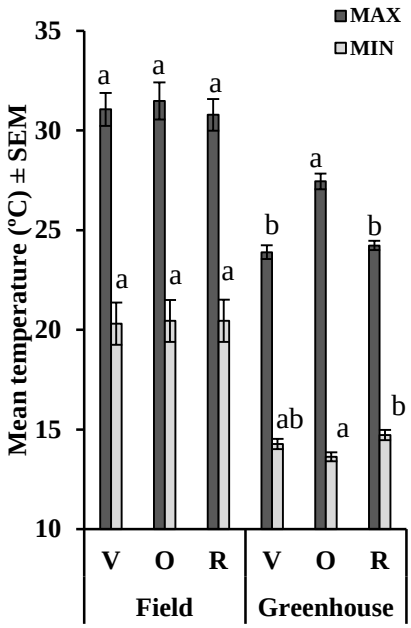

Figure 2.2. Mean ± SEM maximum and minimum temperatures (°C) recorded inside vegetative (V) and reproductive (R) cages or on adjacent open (O) locations of experimental canola (*Brassicae napus*) plants in field and greenhouse studies. Different letters above bars indicate a significance difference between treatments at $P < 0.05$ using Fisher's Protected LSD.

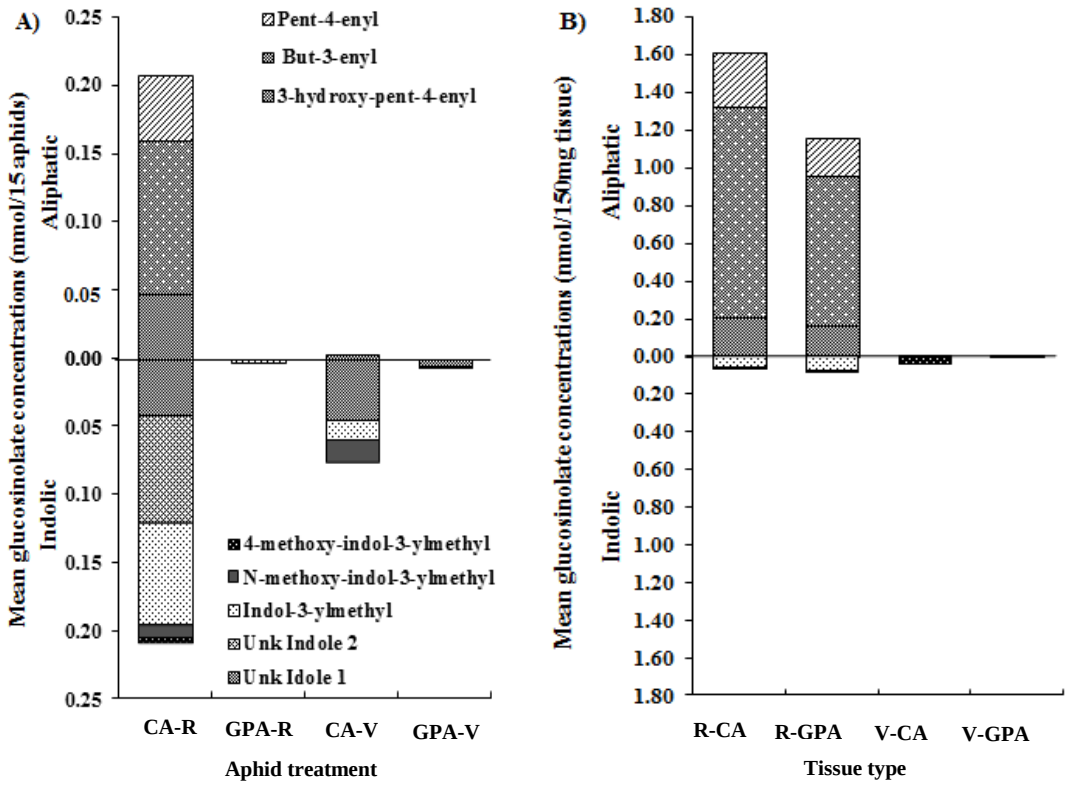

Figure 2.3. Select glucosinolate mean concentrations for A) cabbage (CA) or green peach (GPA) aphids that were previously feeding on vegetative (V) or reproductive (R) tissues of the canola plant and B) for fresh canola plant tissue from V or R structures that were previously feed upon by CA or GPA, for 21 d. Field studies were conducted at the Ashland Bottoms Research Farm near Manhattan, Kansas in 2013. Each bar represents the mean of 20 independent samples. Aliphatic and indolic glucosinolates are shown above and below the x-axis, respectively.

Table 2.1. Degree-day accumulation for CA, GPA, and TA calculated from inside cages (V = vegetative and R = reproductive structures) or outside cages (O) for greenhouse (GH) and field (F) studies. Lower developmental thresholds used were 5.8 ºC (CA) and 4.0 ºC (GPA and TA) to compute values for cumulative degree days.

		DD	
EXP	**LOCATION**	**CA**	**GPA/TA**
F	**V**	715.6	780.4
	O	725.7	790.5
	R	713.4	778.2
GH	**V**	982.8	1116.0
	O	1046.4	1174.2
	R	1011.9	1145.2

Table 2.2. Mean concentrations of aliphatic and indolic content (nmol) measured in the 2013 field study in cabbage (CA) or green peach (GPA) aphids (light grey columns) that had fed on vegetative (V) or reproductive (R) canola tissues for 21 d; and for plant tissue from V or R exposed to feeding by aphids (dark grey columns). Letters that are different within rows of the light or the dark grey columns indicate a significant difference between treatments ($P < 0.05$) using Fisher's Protected LSD.

	Concentrations (nmol/15 aphids) ±SEM				**Concentrations (nmol/150 mg tissue) ±SEM**			
	R		**V**		**R**		**V**	
	CA	**GPA**	**CA**	**GPA**	**CA**	**GPA**	**CA**	**GPA**
Total indolic	0.209 ± 0.042 a	0.003 ± 0.002 b	0.077 ± 0.021 b	0.006 ± 0.005 b	0.066 ± 0.011 a	0.086 ± 0.021 a	0.044 ±0.007 b	0.015 ± 0.008 b
Total aliphtaic	0.206 ± 0.054 a	0.000 ± 0.000 b	0.003 ±0.003 b	0.000 ±0.000 b	1.608 ± 0.175 a	1.15± 0.24 a	0.000 ± 0.000 b	0.007 ± 0.007 b

Table 2.3. Mean concentrations of select glucosinolates measured in 2013 field study in cabbage (CA) or green peach (GPA) aphids (light grey columns) that had fed on vegetative (V) or reproductive (R) canola tissues for 21 d; and for plant tissue from V or R exposed to feeding by aphids (dark grey columns). Letters that are different within rows of the light or the dark grey columns indicate a significant difference between treatments ($P < 0.05$) using Fisher's Protected LSD.

		Concentration (nmol/15 aphids) ±SEM				Concentration (nmol/150 mg tissue) ±SEM			
		R		V		R		V	
	Glucosinolate	CA	GPA	CA	GPA	CA	GPA	CA	GPA
Aliphatic	3-hydroxy-pent-4-enyl	0.047 ±0.019 a	0.000 ±0.000 b	0.000 ±0.000 b	0.000 ±0.000 b	0.204 ±0.033 a	0.159 ±0.037 a	0.000 ±0.000)b	0.000 ±0.000 b
	But-3-enyl	0.112 ±0.032 a	0.000 ±0.000) b	0.003 ±0.003 b	0.000 ±0.000 b	1.114 ±0.127 a	0.794 ±0.188 b	0.000 ±0.000 c	0.007 ±0.007 c
	Pent-4-enyl	0.047 ±0.012 a	0.000 ±0.000 b	0.000 ±0.000 b	0.000 ±0.000 b	0.289 ±0.043 a	0.197 ±0.043 b	0.000 ±0.000 c	0.000 ±0.000 c
Indolic	Unk Idole 1	0.042 ±0.015 a	0.000 ±0.000 b	0.045 ±0.016 a	0.005 ±0.005 b	0.000 ±0.000 a	0.000 ±0.00 a	0.000 ±0.000 a	0.000 ±0.000 a
	Unk Indole 2	0.078 ±0.025 a	0.000 ±0.000 b	0.000 ±0.000 b	0.000 ±0.000 b	0.002 ±0.002 a	0.006 ±0.005 a	0.000 ±0.000 a	0.000 ±0.000 a
	Indol-3-ylmethyl	0.076 ±0.011 a	0.003 ±0.002 b	0.014 ±0.003 a	0.001 ±0.001 b	0.062 ±0.009 a	0.079 ±0.001 a	0.001 ±0.001 b	0.002 ±0.002 b
	N-methoxy-indol-3-ylmethyl	0.010 ±0.003 b	0.000 ±0.000 c	0.018 ±0.007 a	0.000 ±0.000 c	0.000 ±0.000 a	0.000 ±0.000 a	0.000 ±0.000 a	0.000 ±0.000 a
	4-methoxy-indol-3-ylmethyl	0.003 ±0.001 a	0.000 ±0.000 b	0.000 ±0.000 b	0.000 ±0.000 b	0.002 ±0.002 b	0.000 ±0.000 b	0.043 ±0.007 a	0.013 ±0.006 b

APPENDIX

Unknown compounds. Unknown compound 4.6 had lower expression in plant tissue than it did in aphid samples, while compound 5.1 had higher expression in plant than it did on aphid samples (Fig. 4). Feeding location does not seem to influence unknown chemical acquisition for green peach aphid, but seems to have a direct effect on cabbage aphid unknown chemical acquisition. More specifically, unknown chemical 5.1 is higher for cabbage aphid restricted to reproductive tissue, while unknown chemical 4.6 is higher for cabbage aphid restricted to feeding on vegetative plant tissues of the canola plant (Fig. 4). These compounds might be essential compounds for aphid nutrition although for our study they showed weak relationships with our samples; further analysis of these compounds are needed to determine if there were any differences in the unknown compounds.

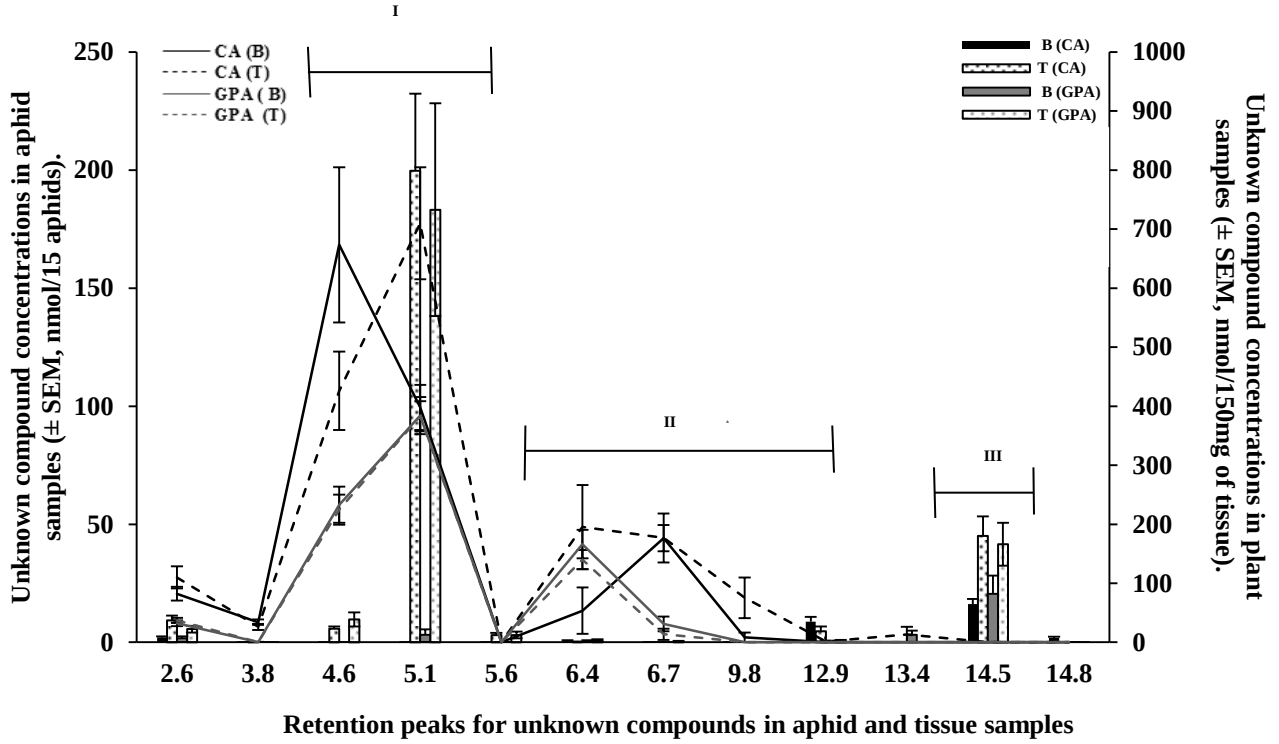

Figure 2.4. Mean absorbance (mAu) for unknown compounds found in cabbage (CA) or green peach (GPA) aphids that were previously feeding on vegetative (V) or reproductive (R) tissues of the canola plant (lines, primary axis) and for fresh canola plant tissue from V or R structures that were previously fed upon by CA or GPA (bars, secondary axis), for a three week period. Section (I) in the graph represents unknown compounds that were expressed in both aphid and plant tissue samples, section (II) represents unknown compounds only expressed in aphid samples, and section (III) represents unknown compounds only expressed in plant tissue samples. Numbers on the x-axis represent retention times where the compound peaked for the HPLC analysis.

Table 2.4. Results from test of fixed effects (*PROC MIXED*) of population growth rates of different aphid species (*Brevicoryne brassicae* and *Myzus persicae*) in two feeding locations (reproductive = R, and vegetative = V) under field and greenhouse conditions.

Factor	df	*F*	*P*
Field			
species	**1,172**	**4.6**	**0.033**
feeding location	**1,172**	**68.8**	**< 0.0001**
species*feeding location	**1,172**	**2.59**	**0.05**
Greenhouse			
species	**2,186**	**8.52**	**0.0003**
feeding location	**1,186**	**68.8**	**< 0.0001**
species*feeding location	**2,186**	**9.83**	**< 0.0001**

Population growth rates were measured after a three week period

Table 2.5. Results from test of fixed effects (*PROC MIXED*) of feeding location (reproductive = R, and vegetative = V) on aliphatic and indolic glucosinolates uptake by different aphid species (*Brevicoryne brassicae* and *Myzus persicae*).

Factor	df	*F*	*P*
Aliphatic in aphids			
species	**1,76**	**14.88**	**0.0002**
feeding location	**1,76**	**14.11**	**0.0003**
species*feeding location	**1,76**	**14.11**	**0.0003**
Indolic in aphids			
species	**1,76**	**34.36**	**<0.0001**
feeding location	**1,76**	**7.51**	**0.0076**
species*feeding location	**1,76**	**8.22**	**0.0054**

Glucosinolates in aphids were measured after a three week period using an HPLC analysis.

Table 2.6. Results from test of fixed effects (*PROC MIXED*) of tissue type (reproductive = R, and vegetative = V) on aliphatic and indolic glucosinolates concentrations when attacked by different aphid species (*Brevicoryne brassicae* and *Myzus persicae*).

Factor	df	*F*	*P*
Aliphatic in tissues			
species	**1,76**	**2.34**	**0.13**
tissue type	**1,76**	**86.61**	**< 0.0001**
species*feeding location	**1,76**	**2.48**	**0.12**
Indolic in tissues			
species	**1,76**	**0.11**	**0.74**
tissue type	**1,76**	**12.72**	**0.0006**
species*feeding location	**1,76**	**3.49**	**0.65**

Glucosinolates in plant tissue were measured after a three week period using an HPLC analysis.

Table 2.7. Results from test of fixed effects (*PROC MIXED*) of feeding location (reproductive = R, and vegetative = V) on eight select glucosinolates uptake by different aphid species (*Brevicoryne brassicae* and *Myzus persicae*).

Factor	df	*F*	*P*
Aliphatic in aphids			
3-hydroxy-pent-4-enyl			
species	**1,76**	**5.75**	**0.0189**
feeding location	**1,76**	**5.75**	**0.0189**
species*feeding location	**1,76**	**5.75**	**0.0189**
but-3-enyl			
species	**1,76**	**12.88**	**0.006**
feeding location	**1,76**	**11.78**	**0.001**
species*feeding location	**1,76**	**11.78**	**0.001**
pent-4-enyl			
species	**1,76**	**14.37**	**0.0003**
feeding location	**1,76**	**14.37**	**0.0003**
species*feeding location	**1,76**	**14.37**	**0.0003**
Indolics in aphids			
UnkIndole1			
species	**1,76**	**13.64**	**0.004**
feeding location	**1,76**	**0.15**	**0.701**
species*feeding location	**1,76**	**0**	**0.946**
UnknIndole2			
species	**1,76**	**10.29**	**0.002**
feeding location	**1,76**	**10.29**	**0.002**
species*feeding location	**1,76**	**10.29**	**0.002**
Indole-3-ylmethyl			
species	**1,76**	**59.24**	**< 0.0001**
feeding location	**1,76**	**32.67**	**< 0.0001**
species*feeding location	**1,76**	**27.73**	**< 0.0001**
N-methoxy-indol-3-ylmethyl			
species	**1,76**	**29.25**	**< 0.0001**
feeding location	**1,76**	**2.57**	**0.1132**
species*feeding location	**1,76**	**1.97**	**0.1649**
4-methoxy-indol-3-ylmethyl			
species	**1,76**	**3.32**	**0.0723**
feeding location	**1,76**	**3.32**	**0.0723**
species*feeding location	**1,76**	**6.51**	**0.0127**

Glucosinolates in aphids were measured after a three week period using an HPLC analysis.

Table 2.8. Results from test of fixed effects (*PROC MIXED*) of tissue type (reproductive = R, and vegetative = V) on eight select glucosinolate concentrations when attacked by different aphid species (*Brevicoryne brassicae* and *Myzus persicae*).

Factor	df	*F*	*P*
Aliphatic in plants			
3-hydroxy-pent-4-enyl			
species	**1,76**	**0.82**	**0.329**
tissue type	**1,76**	**51.1**	**< 0.0001**
species*feeding location	**1,76**	**0.82**	**0.3292**
but-3-enyl			
species	**1,76**	**1.9**	**0.1719**
tissue type	**1,76**	**70.49**	**<0.0001**
species*feeding location	**1,76**	**2.06**	**0.155**
pent-4-enyl			
species	**1,76**	**2.27**	**0.136**
tissue type	**1,76**	**63.38**	**< 0.0001**
species*feeding location	**1,76**	**2.27**	**0.136**
Indolics in plants			
UnkIndole1	**not detected**		
UnknIndole2			
species	**1,76**	**0.54**	**0.46**
feeding location	**1,76**	**2.74**	**0.102**
species*feeding location	**1,76**	**0.54**	**0.46**
Indole-3-ylmethyl			
species	**1,76**	**0.89**	**0.3495**
tissue type	**1,76**	**46.76**	**< 0.0001**
species*feeding location	**1,76**	**0.63**	**0.43**
N-methoxy-indol-3-ylmethyl			
species	**1,76**	**1**	**0.3205**
feeding location	**1,76**	**1**	**0.3205**
species*feeding location	**1,76**	**1**	**0.3205**
4-methoxy-indol-3-ylmethyl			
species	**1,76**	**11.48**	**0.0011**
tissue type	**1,76**	**29.6**	**< 0.0001**
species*feeding location	**1,76**	**8.86**	**0.0039**

Glucosinolates in plant tissue were measured after a three week period using an HPLC analysis.

Chapter 3 - FEEDING LOCATION AFFECTS DEMOGRAPHY OF CABBAGE APHID (*Brevicoryne brassicae*) ON WINTER CANOLA (*Brassica napus*).

ABSTRACT

The cabbage aphid (*Brevicoryne brassicae*) is a perennial pest that specializes on plants of the Brassicaceae family, attacking canola mainly before and during flowering. Under field conditions, cabbage aphids typically colonize the upper flowering canopy. Dynamics of aphids in the flowering canopy might be due to effects of plant quality (bottom-up) or effects of predation (top-down) forces. Understanding plant-insect interactions is important for the management of aphids in canola. The goal of our study was to evaluate how within-plant distribution impacts cabbage aphid demography. A stage-structured matrix model was constructed for aphids restricted to reproductive or vegetative plant tissues of canola. We found that feeding location significantly affects cabbage aphid demographics; the finite rate of increase (λ) for cabbage aphids was higher (λ = 1.24) for aphids restricted to reproductive tissues compared to aphid restricted to vegetative tissues (λ = 1.17). Aphids with higher λ exhibited shorter generation times (T = 12.6 d) and higher reproductive rates (R_0 = 16.2). Prospective analyses showed that there was a nymph-skewed stable distribution, and elasticity values revealed that λ is most sensitive to future changes in stasis (adults staying in the adult stage) and mortality of adults. Retrospective analyses indicated that contributions from growth of nymphal stage 2-3, 3-4, and 4 to adult accounted for nearly all of the variation in λ between the treatments, but adult fecundity (0.05) was driving population dynamics. Monitoring programs should target adults and penultimate instars colonizing reproductive tissues of canola plants in the field.

INTRODUCTION

Winter canola (*Brassica napus*) is a profitable biofuel crop that has increased in acreage in South Central US since the introduction of winter hardy varieties. New varieties have allowed growers to rotate canola with winter wheat (*Triticum aestivum*) (Dansby 2008, Ash 2012), the most abundant crop of this region (Peeper et al. 2009, Franke et al. 2009). Since its introduction, winter canola has been attacked by a complex of aphid species including *Lipaphis erysimi* (turnip aphid), *Myzus persicae* (green peach aphid), and *Brevicoryne brassicae* (cabbage aphid), (Franke et al. 2009, Berlandier et al. 2010, Royer & Giles 2010). If aphids form dense, losses of up to 70% in yield have been reported when aphid infestations are left untreated (Royer and Giles 2010, Berlandier et al. 2010, Giles et al. 2011). After the adoption of treated seed for the management of early season turnip aphids, cabbage aphids have become the most damaging species for winter canola in mixed species infestations (Royer and Giles 2010).

Cabbage aphids are herbivorous perennial pests restricted to Brassicaceae, and attack canola mainly during early flowering and pod development (Boyles et al. 2007, Berlandier et al. 2010, Royer and Giles 2010). Cabbage aphids develop through four non-reproductive, nymphal instars before reaching physiological maturity and starting parthenogenic reproduction (Hughes 1963). Cabbage aphid populations developing on brassica crops like canola are primarily composed of apterous (wingless) females that rapidly produce viviparous young with telescopic generations (Hughes 1963, Kindlmann and Dixon 1989). When growing cabbage aphid populations reach high densities, alates (winged morphs) are produced and disperse to new host plants or move longer distances. As temperatures cool in late summer to early fall, sexual winged forms (males and females) of aphids are produced, mate, and lay their eggs in apical parts of the host plant, which can be one of several cruciferous crops (Boyles et al. 2007). Conversely,

populations can remain asexual in the southern part of the US and reproduce as apterous adults until environmental conditions are ideal for development. For populations under sexual reproduction, eggs hatch the following spring giving raise to fundatrix mothers that disperse and produce the first generation of parthenogenic nymphs on new hosts (Hughes 1963). In both asexual and sexual reproduction, the fate of newly deposited apterous nymphs is subject to deposition sites selected by their fundatrix mothers at a local scale (within-field level). Little is known about the consequence of deposition site selection by reproductive females. Canola plants also produce secondary compounds as defense mechanisms against herbivores. Additionally, as a consequence of being a brassica specialist, cabbage aphids are capable of mitigating exposure to allelochemicals (glucosinolates; Ahuja et al. 2009) produced by cruciferous plants by sequestration in body tissue (Chew 1975, Slemens and Mitchell-Olds 1996, Ratzka et al. 2002, Kazana et al. 2007, Pratt et al. 2008). Allelochemical composition varies among different cultivated and uncultivated brassica species. Thus, toxicity toward natural enemies, fecundity, age at maturity, and survival of cabbage aphid populations is directly affected by host plant species (Merritt 1996, Francis et al. 2000, Chaplin-Kramer et al. 2011, Kos et al. 2011a, 2011b, 2012). In canola, little is known regarding the effect of top-down and bottom-up forces on cabbage aphid populations during critical growth stages where management is most effective (early flowering).

In the field, cabbage aphid colonies are predominantly observed colonizing the top flowering canopy of the canola plant, specifically the reproductive tissues (Merritt 1996, Hopkins et al. 1998, 2009, Peeper and Boyles 2008, Berlandier et al. 2010). Interestingly, occurrence of herbivores in different plant structures is a phenomenon not unique to aphids attacking canola. Feeding location has been reported to influence both fitness as well as natural

enemy recruitment for herbivorous insects/arthropods in other crop/cultivated pants systems including, chili (Idris and Roff 2002), cotton (Fernandes et al. 2011, Grigollo and Souza 2013), alfalfa (Berberet et al. 2009), sunflowers (Pekar 2005), and other brassica crops, like *Brassicae nigra* (Smallengange et al. 2007). Reproductive structures of the canola plant might serve as better food resources for cabbage aphid and therefore influence aphid demographics (Petersen et al. 2002, Brown et al. 2003, Smallegange et al. 2007, Malik et al. 2010). Conversely, natural enemy feeding habits can also have disproportionate effects on aphid population growth based on where the natural enemies are feeding, which has been documented for soybean aphid in soybean (Costamagna et al. 2013). Therefore, studying interactions between top-down predation, bottom-up forces, location preference, or simply competition between the aphid species found colonizing the same plant structures may explain the primary forces responsible for regulating vertical distributions observed in the field (Idris and Roff 2002, Pekar 2005, Berberet et al. 2009, Fernandes et al. 2011). Demographic models based on sensitivity analyses as life-table response experiments (LTRE) can help quantify the effects of feeding location on vital rates (reproduction, growth, and survival) and contributions to population growth rate or λ (Caswell1996, 2011). Changes in the vital rates for individuals can be vital to our understanding of populations (Mills et al. 1999). Consequently, demographic models have been widely used in ecotoxicology (e.g., *Acyrthosiphon pisum* in Dauer et al. 2012) as well modeling invasive species (e.g., *Jacobaea vulgaris* in Hamda et al. 2012).

Therefore, the objective of this study was to use sensitivity analysis and a retrospective life table response experiment (LTRE) approach to determine the extent to which feeding location affects aphid vital rates (survival, growth, and reproduction). The LTRE method allowed us to decompose the effects of each stage-specific vital rate and their contributions to λ.

In the first experiment, aphid populations were experimentally restricted to reproductive or somatic (vegetative) parts of the plants and population growth rates (λ) were estimated for aphids on different locations on the plant. In a second experiment, individual aphids were restricted to reproductive or somatic (vegetative) parts of the plants to determine the effect of feeding location on the vital rates of individual cabbage aphids, which were modeled with a stage-structured matrix population model. A prospective analysis was used to test how potential changes in vital rates (i.e, reproduction, growth, and survival) influenced the finite rate of population growth (λ) of the cabbage aphid in each treatment location (reproductive vs. vegetative). Lastly, we used a retrospective LTRE to decompose the contributions of different vital rates to variation in λ among the experimental treatments. Demographic population analyses are useful to predict pest outbreaks and dynamics of colonization in crops (Kasap and Alten 2006). Understanding aphid demography will also assist in the formulation of more reliable and sustainable management programs for winter canola in the South Central region of the US.

MATERIALS AND METHODS

Aphids and plant materials

Cabbage aphids used for all experiments were obtained from laboratory-reared colonies maintained at Kansas State University in the Department of Entomology (Manhattan, KS). In April 2011, adult cabbage aphids were collected from winter canola fields in Barber County Kansas and transported to the laboratory in coolers where they were provided with young potted canola seedlings as a food source. Using modified procedures from Kos et al. (2011a), colonies were maintained at 22ºC ± 2, 60-70% relative humidity, and a 16:18 hr (light:dark) photoperiod. Canola (variety Riley) was seeded in a special soil mix that contained all required minerals and nutrients (M. Stamm, canola breeder, Kansas State University and Oklahoma State University)

and maintained in the greenhouse with a 16:8 hr (light:dark) photoperiod. Canola plants were then artificially vernalized in a growth chamber for approx. 2 months at 12:12 hr (light:dark) photoperiod and constant 4ºC to induce reproductive maturity (Murphy and Scarth 1994*)*. Vernalized plants were watered daily and used to maintain aphid colonies. To maintain colony vigor, apterous aphids were transferred to non-infested, vernalized canola plants every other week. Transferring of aphids to new plants consisted of excising an aphid-infested leaf and placing it atop of new leaves from vernalized canola plants to allow independent aphid movement to fresh plant tissue. Voucher specimens (*Brevicoryne brassicae* nymphs and adults) were deposited in the Department of Entomology Museum (voucher number = 228).

Colony dynamics

To assess the impacts of feeding location on aphid population growth rates in the absence of predation, field and greenhouse experiments were conducted using two types of mesh enclosure cages adapted from Soper et al. (2013). This experiment was conducted in a production canola field at Ashland Bottoms Research Farm near Manhattan, KS from 27 April to 18 May 2011 and repeated under controlled greenhouse conditions at Kansa State University (Manhattan, KS) from 25 October to 15 November 2011. Specifically, apterous cabbage aphids were restricted to either the upper flowering canopy or a single leaf in the lower canola canopy using enclosure cages. Both cage types were placed on the same plant to enable direct comparison of plant location on aphid population growth, thus reducing between-plant variability (e.g., size, nutritional composition). Canola plants were arranged in a completely randomized design and equal numbers of aphids were assigned to either reproductive or vegetative tissues of each canola at the start of the experiment. Each cage consisted of white, no-see-um mesh (Quest Outfitters, Sarasota, FL) with zippered tops (23 cm diameter and 71 cm long). Zippers provided

access to either the flowering raceme or the vegetative leaf after a cage was secured to a plant structure. The base of each enclosure cage was secured to the canola plant using 15-cm plastic cable-ties (Gardner Bender, Butler, WI), which were located below the last flower of the flowering raceme or the node between the leaf and the plant stem depending on enclosure cage type. To allow free-movement of aphids within the cage, we added cylindrical supports made of 14-gauge, galvanized steel wire (Impex Systems Group, Inc. Miami, FL). Supports kept the mesh from resting on the flowers or the leaves or disrupting growing aphid populations. In companion experiments both under field and greenhouse conditions across multiple years using the same cage design, we demonstrated that there was no cage effect in our experimental cage treatments; cumulative degree day was the same for aphids in enclosed to reproductive and vegetative plant parts (see chapter 2 for results).

Each enclosure cage was infested with five, newly reproductive, apterous adult cabbage aphids that were transported in a 2-ml Eppendorff vial (Fisher Scientific Inc., Waltham, MA) to greenhouse or field experiments. Aphids were transferred to the experimental plants with fine, camel hair paintbrushes and deposited directly on the canola flowers or leaves accordingly. Aphid populations remained on caged sections of the plant for a 3-week period. At the end of the study, cages and aphids were removed by excising the base of each canola plant and placing all plant material and attached cages in 3.78-L (1 gallon) size plastic bags that were then placed in a freezer. Freezing stopped aphids from reproducing and allowed for effective counting of aphid populations at the end of the experiment. Plant structures within enclosure cages were removed and aphid numbers within cages were visually counted in the laboratory, numbers were recorded using a hand counter. For the greenhouse trial, aphids were also categorized into different groups

based on visible morphological structures, which included apterous nymphs, alatoid nymphs and adults, or alatoid adults.

The finite rate of population change was calculated for a 21-d period (λ_{21}) as a ratio of population densities at the start (N_0.) and end (N_{21}) of the field or greenhouse trials, where: $\lambda_{21} = N_{21}/N_0$. To compare results between population and individual aphid experiments, we then calculated the daily rate of change (λ_d) as: $\lambda_d = (N_{21}/N_0)^{1/2}$. After calculating the finite rate of population growth for each location (aphids populations restricted to flowering or reproductive tissue), a one-way analysis of variance or ANOVA (PROC ANOVA, SAS 2009) was used to compare aphid populations restricted to either the top (reproductive) or bottom (vegetative) plant structures in both trials (greenhouse and field); means were separated using the Tukey method at $\alpha = 0.05$. Last, the structure of populations (apterous nymphs, alatoid nymphs and adults, and alatoid adults) from the greenhouse trial were compared between locations (flower and leaf) using a one-way ANOVA (PROC ANOVA, SAS 2009). The LS MEANS statement was used to make pair-wise comparisons between plant locations and means were then separated using the Tukey method ($\alpha = 0.05$).

Individual aphid demographics

Growth, survival, and reproduction of cabbage aphids was recorded using single aphid cages to obtain vital rates of aphids feeding on apical reproductive tissues or basal vegetative tissues of the canola plant under greenhouse conditions at Kansas State University from 23 September to 28 October 2012. In this experiment, individual aphids were randomly assigned to one of three different plant locations on the sample canola plant: 1) flowering structure (reproductive tissue) and the 2) top and 3) bottom surfaces of a single leaf (somatic or vegetative tissues) from the mid-canopy of the same plant. Enclosure cages (Converters Inc., Huntingdon

Valley, PA) were adapted from Nagaraj et al. (2005) and consisted of a 0.5 cm thick foam rectangle (outside rectangle dimensions = 6.2 × 3.6 cm, inside rectangle dimensions =5.1 × 2.5 cm) with adhesive glue on the cage top and bottom. For cages placed on leaves, cage adhesive was used to secure no-seeum mesh one side of the cage, which kept aphids from escaping, allowed for adequate ventilation, and facilitated aphid counting; remaining adhesive side was stuck to the leave on the leave treatments to contain the aphids. For aphids restricted to flowers, two cages were stuck together with a single flower stem between the two cages; no-seeum mesh was used on the outside of the cages to restrict aphids to reproductive structures. Aphids were not exposed to the sticky sides to reduce incidental mortality.

Each treatment was infested with two, apterous adult cabbage aphids (first generation or G_1) per individual cage (n = 10 per treatment). On the first day nymphs were produced, approximately 48 hrs after infestation, G_1 adults were removed and a cohort of five nymphs (second generation or G_2) was left in each cage until they reached their penultimate instar. Following Chaplin-Kramer et al. (2011), we left nymphs together until they reached their penultimate stage (4th instar) to reduce the likelihood of nymphs escaping individual cages. Only G_2 nymphs were used to estimate demographic attributes for aphid populations in this experiment. Once G_2 aphids reached their penultimate instar they were placed in individual cages and responses were tracked for each aphid (n = 50 per treatment). Nymphal development (instar changes), adult fecundity, and survival of each individual G_2 aphid was recorded daily for the entire lifespan of each aphid. Development was determined by counting the number of aphid exuvia (exoskeleton molts), which were removed daily. In addition, number of nymphs at each stage was recorded daily and allowed us to correlate body size with number of exuvia to calculate proportion of nymphs at a given instar or age. Once all G_2 aphids reached reproductive

stages, the number of G_3 nymphs produced by each G_2 was recorded and newly deposited G_3 nymphs were removed daily to determine fecundity and reproductive rates (Chaplin-Kramer et al. 2011). Therefore, 150 individual cabbage aphids (50 aphids per treatment) restricted to three locations within the canola plant were monitored through their complete life cycle.

The life cycle of individual cabbage aphids was categorized into six different stages: four nymphal stages followed by reproductive and post-reproductive adults. Total stage duration (d) for each nymphal stage as well as total duration of the pre-reproductive, reproductive, and post-reproductive periods (d) was calculated; a two-way ANOVA (PROC MIXED, SAS 2009) was performed using location (flowers, leaf top and leaf bottom) and either instar (total stage duration) or aphid stage (total duration) as main effects. The LS MEANS statement was used to make pair-wise comparisons among treatments and means were then separated for multiple comparisons using the Tukey method at $\alpha = 0.05$. In this study, treatment location was a fixed effect, and individual canola plants were random components of the mixed model, since we wanted to determine if feeding location influenced aphid demography. Mean daily nymph production was also calculated and compared among treatments using a two-way ANOVA (PROC MIXED, SAS 2009). Means were then separated for multiple comparisons using the Tukey method ($\alpha = 0.05$).

In addition to differences in life stage duration, we compared transition matrix parameter estimates for aphids at all feeding locations. The cabbage aphid life cycle was generalized into a 6×6 stage-classified transition matrix with a daily time-step (Fig. 1). Apterous aphid adults produced only female clones by parthenogenesis, and fecundity rates (F) were calculated as the mean number of female offspring produced by each adult female per day. Growth rates (G) were calculated as the product of daily survival (a) and the probability of transitioning to the next

stage (g). Stasis was the product of daily survival (a) and the probability of remaining in that stage class (s). We parameterized a 6 × 6 stage-classified matrix for each treatment; aphids restricted to flowers, leaf tops and leaf bottoms.

Prospective analysis

We used a prospective analysis to identify demographic parameters that would be predicted to have a large effect on future changes in λ (Caswell 1996, 2011). Matrix elements were calculated for each treatment in separate matrices using a deterministic matrix population model approach (Caswell 2011). For each matrix, we calculated the finite population growth rate (**λ**), stable age distribution (**w**), reproductive value of each stage class (**v**), sensitivities ($\mathbf{s_{i,j}}$) and elasticities ($\mathbf{e_{i,j}}$). Elasticities measure the effect of a proportional change in a matrix element on λ and enable direct comparisons of demographic rates between survival (bounded between 0 and 1) and fecundity (bounded between 0 and infinity) among the treatments. Our matrix elements were products of different demographic rates and we calculated lower-level elasticities for all vital rates that comprise the proposed matrix elements.

Retrospective analysis

A life-table response experiment (LTRE) model based on fixed effects and a single classification design was used to calculate contributions of different lower-level vital rates to variation in λ among different treatments (Caswell 1996, 2011). Treatments used for the retrospective analysis included aphids restricted to the flowering canopy and a mean matrix for aphids restricted to upper or lower parts of the leaves. Leaf treatments were combined because responses and rates were similar among aphids restricted to the bottom and top leaves of the same canola plant (see results section). The formula for the fixed-effect LTRE design was: (equations 10.2 and 10.3 of Caswell 2001):

$$\Delta\lambda = \lambda^m - \lambda^r \approx \sum_{i,j} (a_{ij}^m - a_{ij}^r) \frac{\partial \lambda}{\partial a_{ij}}\bigg|_{\mathbf{A}^+} \quad \text{where} \quad \mathbf{A}^+ = \frac{(\mathbf{A}^m + \mathbf{A}^r)}{2} \tag{1}$$

Here, *m* = aphids restricted to apical reproductive tissue and *r* = mean aphids restricted to basal vegetative tissue of the experimental canola plants. All prospective and retrospective analyses were performed in R (version 2.13.0) using the popbio package (Stubben and Milligan 2007).

RESULTS

Colony dynamics

Daily rate of population growth (λ) for cabbage aphids was 1.28 and 1.17 for populations restricted to the reproductive structures and 1.16 and 1.11 for populations restricted to somatic (vegetative) structures in greenhouse (F = 11.14; df = 1,59; $P < 0.001$) and field (F = 8.85; df = 1,35; $P < 0.05$) trials, respectively. Mean total number of aphids recorded after 21 d was 1,104 and 1,090 for populations restricted to the reproductive structures and 185 and 348 for populations restricted to somatic (vegetative) structures in greenhouse and field trials, respectively. Flower structures supported 5.9 and 3.1 fold more aphids than lower leaves of the canola plants after a 3-wk period in greenhouse and field trials, respectively.

For the greenhouse trial, cabbage aphid populations restricted to feeding on the reproductive parts of the canola plant after a 3-wk period consisted of 72% (785.7 6 ± 74.07 SEM) apterous nymphs and adults, 12% (116.8 ± 13.82 SEM) alatoid nymphs, and 16% (201.6 ± 34.18 SEM) alates; whereas populations restricted to feeding on the vegetative parts of the same plant consisted of 80% (162.1 ± 23.29 SEM) apterous nymphs and adults, 5% (8.5 ± 1.56 SEM) alatoid nymphs, and 15% (28.1 ± 5.22 SEM) alates, respectively. Although overall population densities were significantly lower (F = 11.14; df = 1,59; $P < 0.001$) when aphids were restricted to vegetative tissue compared to reproductive tissues, proportion of apterous nymphs and adults

(F = 0.15; df = 1,58; P = 0.70) and alates (F = 0.46; df = 1,58; P = 0.49) within these populations did not significantly differed between feeding locations. Proportion of alatoid nymphs, on the other hand, was significantly higher (F = 16.83; df = 1,58; P < 0.0001) when populations were restricted to reproductive versus the vegetative tissues.

Individual aphid demographics

Only 25, 36, and 27 of the 50 aphids survived to adulthood on the flower, leaf top and leaf bottom treatments, respectively. Daily nymph counts from surviving aphids were used for the fecundity analysis. Developmental times were significantly faster (F = 19.86; df = 2,75; P < 0.0001) for cabbage aphids restricted to flowers compared to aphids developing on top or bottom of leaves. Significant interactions (F = 6.63; df = 6,300; P < 0.0001) between plant location and duration of nymphal stage were also observed (Fig. 2). Shorter duration of the post-reproductive, pre-reproductive and then reproductive stages were observed for all the treatments regardless of feeding location (F = 2.96; df =4, 225; P = 0.02) (Fig. 3). Stage duration of aphids across all plant locations were 7.6 to 9.2 d for pre-reproductive, 19.8 to 21.7 d for reproductive, and 3.9 to 8.1 d for post-reproductive periods. Aphids restricted to flowers had a significantly shorter post-reproductive duration (F = 2.96; df = 4, 225; P = 0.02) than aphids on leaves (Fig. 3). Hence, mean (± SEM) overall life cycle duration was shorter for aphids restricted to flowers (31.3 ± 3.08 d) than those exposed to upper (38.6 ±5.88 d) or lower parts of the leaves (37.1 ± 6.50 d) (F = 9.98; df = 4,225; P < 0.001).

Mean daily fecundity was also significantly greater for aphids restricted to reproductive canola structures compared to aphids on either top or bottom vegetative tissues (F = 19.86; df= 2, 75; P < 0.001). Mean daily fecundity values (± SEM) were 3.3 ± 0.1 aphids per female per day for aphids restricted to flowers, but 2.0 ± 0.1 and 2.2 ±0.1 for aphids restricted to top or bottom

leaf parts, respectively. Vital rates of survival, stasis, and mean daily fecundity were calculated for each treatment location pooling across aphid vital rates within a treatment (Table 1). These values were used for the prospective analysis. Cabbage aphids restricted to reproductive structures of the plant had higher demographics performance than aphids restricted to somatic plant tissues (Table 2).

Population growth rate (λ) was higher for aphids restricted to the reproductive parts of canola plant ($\lambda = 1.25$) compared to aphids restricted to the vegetative tissues ($\lambda = 1.17$) (Table 2). Damping ratios (ρ) and time of convergence (t_{20}), measured in d, showed that populations restricted to flowers converge faster to the stable-age distribution (5.7 d) than aphids restricted to vegetative structures (t_{20} = 9.3-10.4 d) (Table 2). Stable age distribution (**w**) revealed that young nymphs are the most abundant stage class among all populations (> 0.30), and reproductive values (**v**, or mean number of offspring theoretically produced by post-transients) were highest for reproductive adults (> 9), followed by the last instar stage (fourth instars) (< 5); these trends were consistent across treatments (Fig. 4). Elasticity values indicated that λ is most sensitive to changes in stasis (s_5) and survival of adults (a_5).

Results from the retrospective analysis using the mean between the two leaf treatments compared to the matrix of individual aphids restricted to reproductive tissue showed that the sum of all elements in the contribution matrix $\sum c = 0.074$ was an good approximation to the expected treatment effects (0.0739) ($\Delta\lambda$: $\lambda^m - \lambda^r = 0.074$). Positive contributions of improved adult fecundity (F_5: 0.05), growth from nymphal stage 2-3 (g_2: 0.04), 3-4 (g_3: 0.02), and 4 to adult (g_4: 0.01), and survival of nymphal stage 3 (a3: 0.005) accounted for nearly all of the variation in λ between the aphid populations restricted to canola flowers versus leaves on the same plant. These five demographic rates accounted for ~74% of the effects of feeding location on fecundity,

growth, and survival of the cabbage aphids (Fig. 6). There were negative contributions for stasis of early nymphal stages on the variation in λ, where only stasis of nymphal stage 2 was higher for aphids on leaves (s_2 = -0.025).

DISCUSSION

When mixed-age aphid populations or same-aged aphid adults were restricted to different regions of canola plants, the intrinsic rate of growth (λ) was significantly higher for aphids on reproductive tissues. The demographic performance of individual cabbage aphids on canola flowers exhibited shorter generation times (*T*), higher net reproductive rates and higher fecundities than aphids on somatic plant tissues. Similar trends in intrinsic growth rates and fecundity estimates have been reported for aphids in soybean and differences in growth rates were mainly attributed to a combination of top-down and bottom-up factors governing aphid population growth (McCornack et al. 2008, Costamaga et al. 2013). In canola, plant-herbivore interactions might be more complex since they involve allelochemicals (glucosinolates) that act synergistically or antagonistically on both herbivores and natural enemies in the agricultural system.

It is well documented that different plant tissues (reproductive versus somatic) of varying host plants can provide different resources to herbivorous insects (Smallegange et al. 2007, Malik et al. 2010). For example, Smallegange et al. (2007) reported that flowers of *Brassica nigra* (black mustard) contain levels of glucosinolates five times higher than those of leaves, and Malik et al. (2010) reported similar differences in wild radish (*Raphanus sativus*). Differences in resource allocation might differentially affect herbivore growth rates and development. For instance, nitrogen availability is directly correlated to growth and development of most aphid species, since aphids acquire essential nitrogen from amino acids that are translocated through

the phloem of host plants (Walter and DiFonzo 2007, Winde and Wittstock 2011, Winder et al. 2012). Amino acids are actively transported through the host plant; therefore, availability and concentrations can differ not only across different plant organs but also according to plant phenology. In the case of oilseed rape (*Brassicae napus*), Malagoli et al. (2005) demonstrated that essential nitrogen resources available for herbivores change throughout its life cycle. During stem elongation, leaves of oilseed rape act as sinks of nitrogen, whereas during early flowering stages nitrogen pools are shared among plant organs including leaves (30%), stems (35%), and flowers (15%). Conversely, 66% of the nitrogen within leaves is mobilized or re-distributed to the reproductive tissues during early pod formation (Augustinussen 1987, Malagoli et al. 2005). The authors concluded that nitrogen initially allocated to winter leaves is allocated to spring leaves and subsequently to pods at the end of the growing season (Augustinussen 1987, Malagoli et al. 2005). Differences in nitrogen between reproductive and somatic plant parts may possibly be correlated to differences in glucosinolate concentrations. The nitrogen component of glucosinolates might be contributing positively to the faster and more fecund development of the specialist cabbage aphid in glucosinolate rich flower tissues (see Chapter 2). Future research should investigate the major nutrient components in canola plants that promote faster developmental times and higher rates of fecundity among aphids.

Three possible mechanisms can explain the increased demographic performance of aphids on canola flowers than leaves. First, glucosinolates are feeding stimulants to cabbage aphids and other specialist herbivores. Agrawal and Kurashige (2003) reported that high concentrations of glucosinolates within host plants can also negatively affect demography of specialist herbivores such as the white cabbage butterfly, *Pieris rapae*. In our study, cabbage aphids had a shorter generation time (*T*) and higher fecundity when restricted to reproductive

parts of the canola plant. Tradeoffs between nitrogen and glucosinolate concentrations in different canola plant structures may explain demographic differences we observed (see chapter 2); however, we did not measure changes in plant constituents over the duration of our studies. Higher nitrogen concentrations in reproductive versus somatic tissue (Augustinussen 1987, Malagoli et al. 2005) due to amino acid translocation, which may explain higher intrinsic rate of growth (λ) and higher fecundity on reproductive structures. Second, higher concentrations of glucosinolates, specifically isothiocyonates, and aliphatic-hydrolyzed glucosinolates in the reproductive tissues may explain the shorter generation times (*T*) displayed by experimental aphids. Isothiocyonates are known to have negative effects on herbivore life history traits (Bridges et al. 2002, Agrawal and Kurashige 1999, Agrawal et al. 2003, Smallegange et al. 2007, Malik et al. 2010, Winde and Wittstock 2011). Lastly, inherent differences in life histories between aphid species colonizing canola. Fitness consequences resulting from shorter generations can be a result of feeding in glucosinolate rich tissues of the plant, but specialization for glucosinolate sequestration may counteract fitness costs by increasing female fecundity (Agrawal and Kurashige 2003). Shelton (2005) reported that spatial variation in defenses within or among plant tissues could slow the evolution of resistance to herbivores by creating uneven selective pressure on herbivores and their natural enemies. If glucosinolates are being differentially sequestered according to feeding locations select aphid species, then feeding location will affect aphid toxicity. Therefore, variation in toxicity levels will indirectly affect demographic parameters of natural enemies such as survival and fitness (Francis et al. 2000, 2001, Brown et al. 2003, Pratt et al. 2008, Hopkins et al. 2009, Kos et al. 2011a, 2011b). Quantification of glucosinolates within different plant tissues and herbivores needs further investigation (see Chapter 2).

In a broader context, our findings may have implications on beneficial organisms in the canola system. Lady beetles (Coleoptera: Coccinellidae) are one of the most important natural enemies of aphids in several agricultural crops. Coccinellids disperse by flight and often land in the top of a soybean canopy and then proceed to search for their prey within the plant (Cibils et al. *personal observation*). Coccinellids are therefore more likely to land on the reproductive parts of the canola where they will encounter aphid populations with higher fitness, however, prey might be more toxic due to glucosinolate sequestration and exert a fitness cost to lady beetle feeding (See Chapter 4). Third trophic level interactions need to be further studied since they will further develop our understanding of agro-toxicology and its interactions in tritrophic agricultural systems.

Our prospective analysis showed that there was a nymph skewed stable distribution, which is common in growing populations (Taylor 1979). Reproductive values were higher for adults and the penultimate nymphal stage since only adult aphids are reproductive. Elasticity values revealed that λ is more sensitive to future changes in stasis and survival (a) of adults. Even with a nymph-skewed stable stage distribution, nymphal stages have the highest mortality rates within populations; therefore, adults and penultimate instars are more important in shaping cabbage aphid populations. Chemical management should target adult aphids and older nymphal stages, but most populations have overlapping telescoping generations and stage distribution would be difficult to target in chemically-based management programs. Controversially understanding demographic composition of aphid populations under field conditions can provide more effective biological control programs where natural enemy releases target nymphal aphid stages that have the greatest impacts on λ. Retrospective analysis indicated that contributions from nymphal growth stages 2 to 3, 3 to 4, and 4 to adult (Fig. 6) accounted for nearly all of the

variation in λ between the feeding locations. Prolonged early nymphal stage is likely detrimental for population growth since earlier aphid stages are less toxic (smaller body size) and should be preferred by predators. The observation that stasis in early nymph development results in negative contributions to λ may be explained by higher mortality among early nymphal stages in cabbage aphids. Higher mortality in early developmental stages is common in arthropod populations due to a higher degree of predation. To our knowledge these are novel contributions to further understand factors affecting aphid demographic parameters. We recommend the combination of sensitivity and LTRE methods to further the understanding of how within-plant interactions can directly affect aphid demographics.

Understanding canola-aphid-predator interactions has important implications for pest management. Canola producers currently manage aphid outbreaks by applying pesticides during the canola blooming period. Current monitoring programs do not record aphid distributions within the canola canopy or potential effects of glucosinolate sequestration on natural enemy communities. Understanding the interactions between secondary compounds, feeding location and its relation to allelochemical sequestration by aphids and potential impacts on the natural enemy community might facilitate best management practices that reduce pesticide usage and enhance natural control in winter canola fields. Understanding aphid demography in canola, and interactions with natural enemies might improve monitoring and management programs, and help to understand effects of allelochemicals at other trophic levels, such as effects of prey feeding location on natural enemy communities. Our results indicate that feeding location influences demography of aphids directly, different plant structures are being either sources or sinks to cabbage aphids. Source-sink relationships within the plant are directly affecting demography of aphids within the canopy and therefore creating additional source-sinks within

the canopy for third tropic levels. Varying degrees of allelochemicals acquisition by the cabbage aphids in different plant structures might be directly influenced by feeding location, which is a new contribution to agro-toxicology and eco-toxicology (see Chapter 2).

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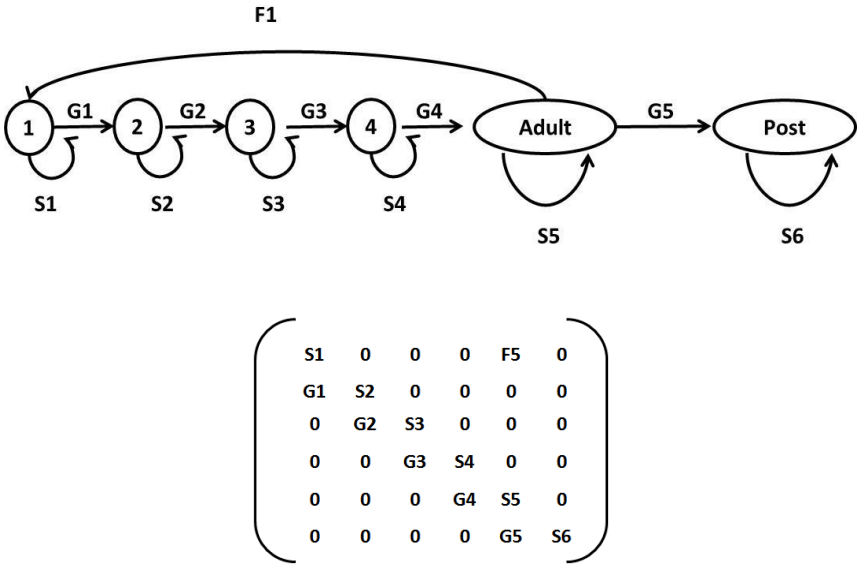
FIGURES AND TABLES



$$\begin{pmatrix} S1 & 0 & 0 & 0 & F5 & 0 \\ G1 & S2 & 0 & 0 & 0 & 0 \\ 0 & G2 & S3 & 0 & 0 & 0 \\ 0 & 0 & G3 & S4 & 0 & 0 \\ 0 & 0 & 0 & G4 & S5 & 0 \\ 0 & 0 & 0 & 0 & G5 & S6 \end{pmatrix}$$

Figure 3.1. The life cycle and corresponding stage-classified transition matrix for female cabbage aphids (*Brevicoryne brassicae*). Stage 1 = first instar, stage 2 = second instar, stage 3 = third instar, stage 4 = fourth instar, adults = reproductive females, and post = post-reproductive adults. Arrows indicated transitions, where G = growth (i.e., aphid survives from one stage to the next), S = stasis (i.e., aphid survives and stay in the same stage) and F = fecundity (number of nymphs per reproductive female).

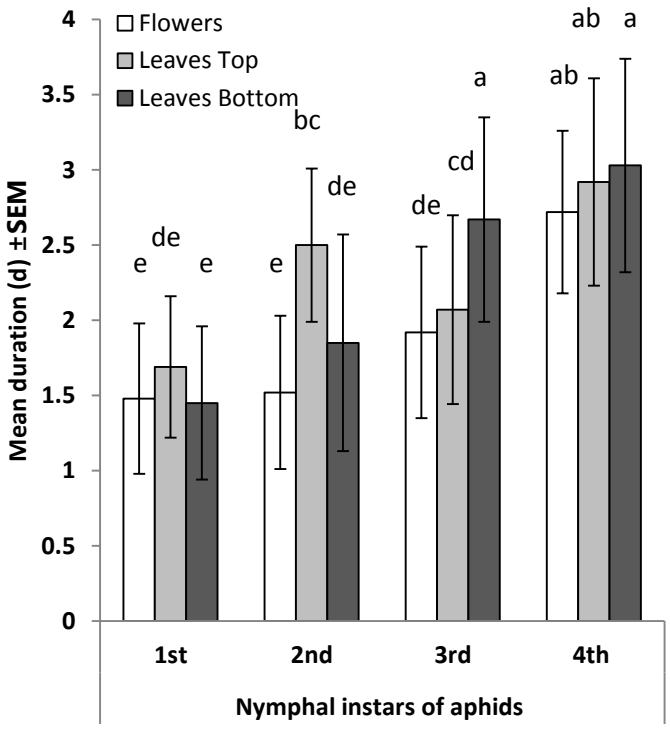


Figure 3.2. Nymphal stage duration (d ± SEM) cabbage aphid populations restricted to different locations within a canola plant (flowers, tops and bottoms of leaves) for the greenhouse trial in 2012. Different letters indicate significance between treatments at $P < 0.05$.

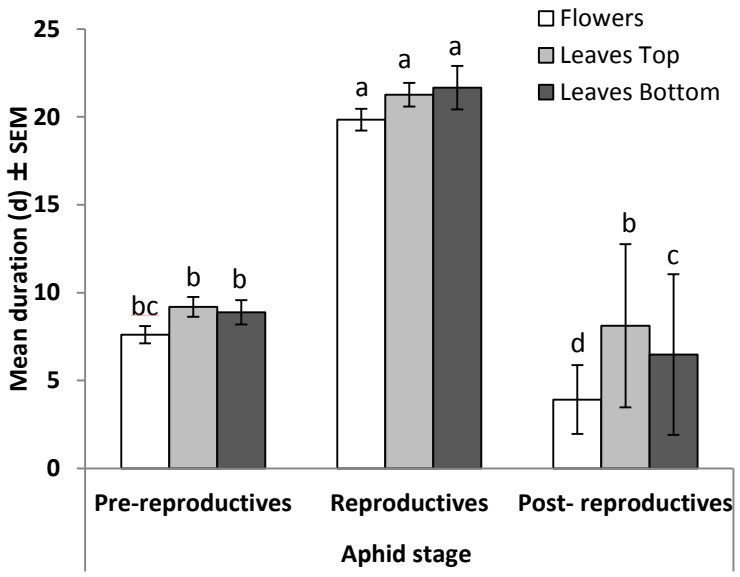


Figure 3.3 Duration (d ± SEM) for pre-reproductive, reproductive and post-reproductive periods for cabbage aphid populations restricted to three locations within a canola plant (flowers, leaves bottom and leaves tops) for the 2012 greenhouse trial. Different letters indicate significance between treatments at $P < 0.05$.

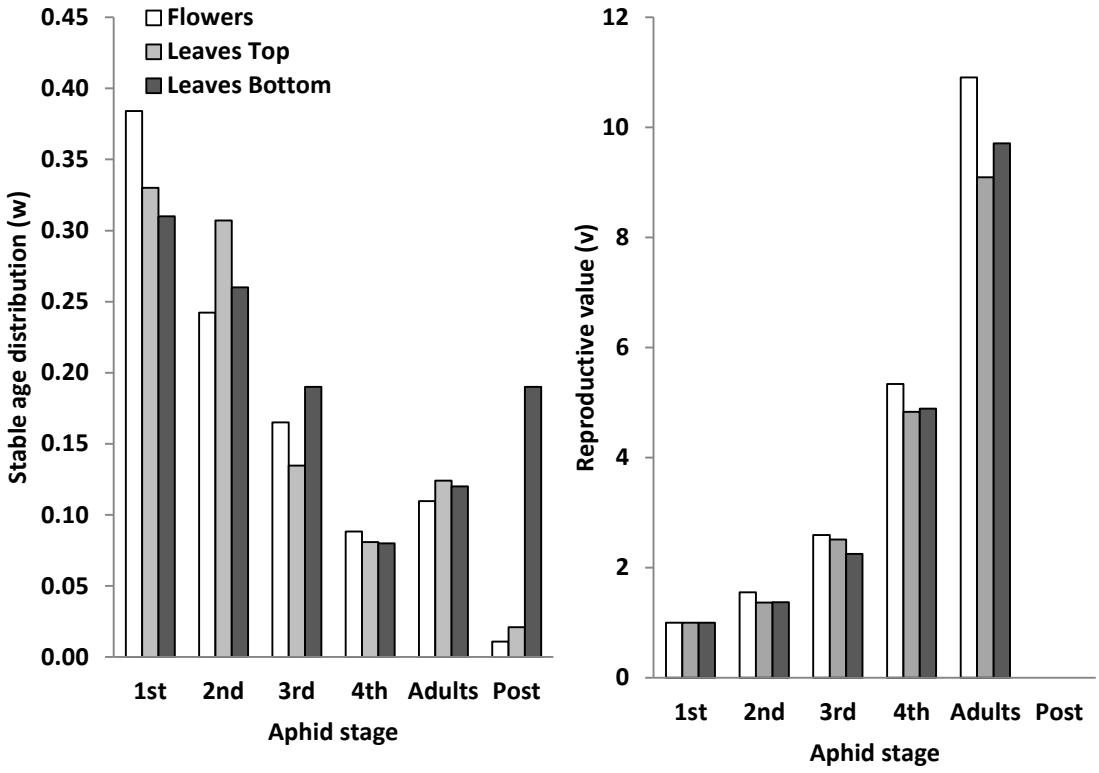


Figure 3.4. Stable stage distribution and reproductive value for experimental populations of cabbage aphids restricted to three different canola plant structures (flower, leaves top, and leave bottom) in a greenhouse study conducted in 2012.

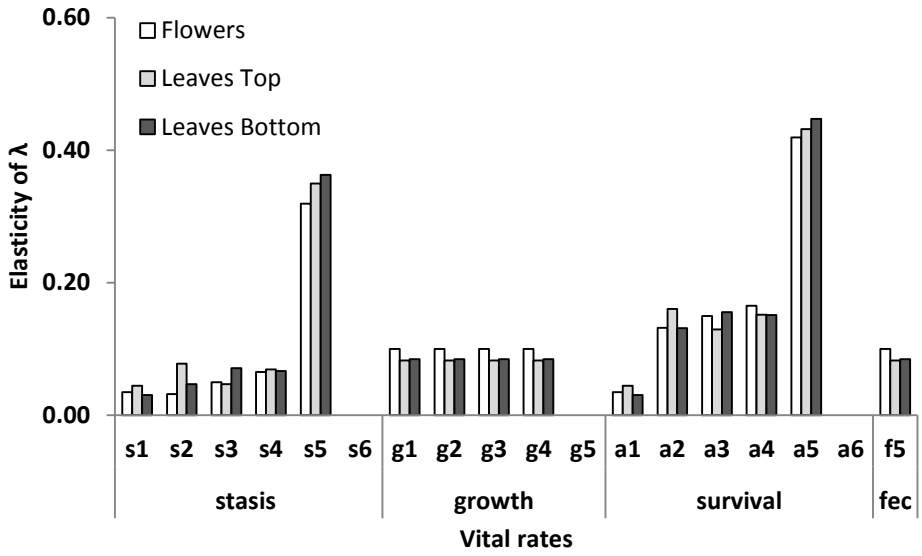


Figure 3.5. Elasticity values for lower-level vital rates for three populations of CA restricted to different locations (flowers, leaf tops and bottoms) within the canola plant canopy.

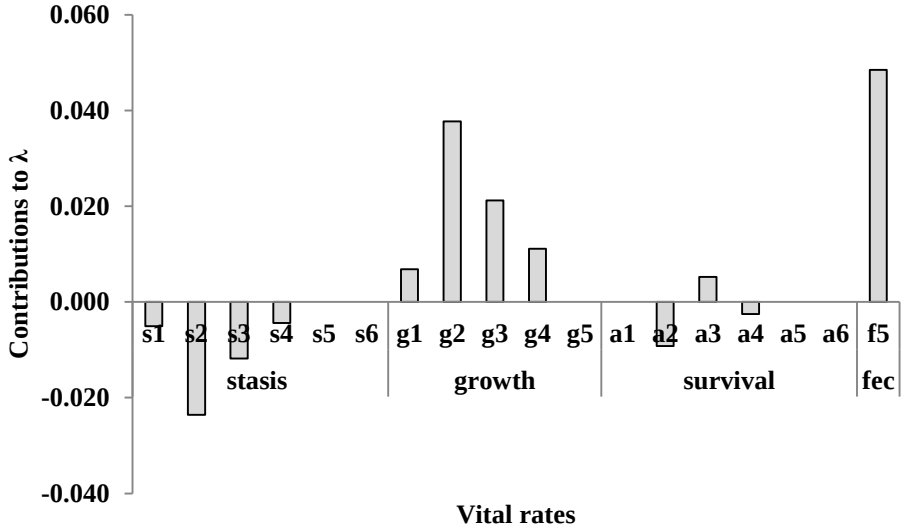


Figure 3.6. Contributions of lower- level vital rates to variation in the finite rate of population change (λ) between cabbage aphid populations restricted to flowers versus vegetative tissues of canola plants.

Table 3.1. Vital rates for individual cabbage aphids feeding on canola, which include: growth (G), stasis (S), and fecundity (F; females produced by females per d) rates for nymph (S_{1-4}), adults (S_5), and post-reproductive (S_6)) cabbage aphids restricted to three different structures of the canola plant (Cage location), in 2012.

	Cage location		
Vital rates	**Flowers**	**Leaf Top**	**Leaf bottom**
n	50	50	50
S1	0.32	0.41	0.31
S2	0.3	0.57	0.42
S3	0.41	0.43	0.53
S4	0.49	0.53	0.52
S5	0.95	0.95	0.95
S6	0.74	0.88	0.85
G1	0.6	0.56	0.63
G2	0.57	0.33	0.46
G3	0.41	0.39	0.29
G4	0.37	0.34	0.33
G5	0.05	0.05	0.05
F5	3.25	2.04	2.15

Table 3.2. Growth rates (λ) and asymptotic properties for cabbage aphids restricted to three different canola plant structures (flowers, leaves top and leave bottom), 2012. Results obtained from the prospective matrix analysis.

	Cage location		
Parameters	**Flowers**	**Leaves Top**	**Leaves Bottom**
λ	1.248	1.174	1.171
ρ	1.68	1.33	1.37
t_{20}	5.73	10.38	9.34
R_0	16.15	9.05	9.46
T(d)	12.56	13.71	14.21

Where λ = finite rate of population change, ρ = damping ratios, t_{20} = days to model convergence, R_0 = net reproductive rate, T = Generation time in days.

Chapter 4 - FEEDING LOCATION OF APHIDS (HEMIPTERA: APHIDIDAE) ON CANOLA AFFECTS DEVELOPMENT AND CONSUMPTION RATES OF IMMATURE *Hippodamia convergens* (COLEOPTERA: COCCINELLIDAE).

ABSTRACT

Green peach aphid *(Myzus persicae*, GPA) and cabbage aphid *(Brevicoryne brassicae*, CA) exhibit different feeding preferences for canola (*Brassica napus*) plant structures when observed under field conditions. These plant structures exhibit differences in allelochemical compositions. Depending on the degree to which aphid sequester (CA) or excrete (GPA) these chemicals we hypothesize that aphid feeding location on canola might directly affect prey suitability for predators such as coccinellids. A no-choice bioassay was conducted to evaluate the effect of aphid feeding location within canola on their suitability as prey for larval stages of *Hippodamia convergens*. Newly hatched *H. convergens* were fed either CA or GPA *ad libitum* that were previously restricted to reproductive or vegetative canola structures. In addition, we evaluated predator response on two *ad libitum* control diets: the soybean aphid (*Aphis glycines*, SA) as a non-canola aphid and a non-aphid diet, eggs of the Mediterranean flour moth, *Ephestia kuniella*. Results showed that feeding location of canola aphids had no effect on overall survival of immature convergent lady beetles, but significantly affected developmental rates ($P < 0.0001$). Specifically, *H. convergens* larvae that fed on canola aphids reared on vegetative plant parts had significantly faster developmental rates than larvae restricted to a diet of aphids reared on canola reproductive structures. Daily consumption rates were greatest for lady beetle larvae feeding on soybean aphid followed by green peach aphid and cabbage aphid, respectively ($P < 0.0001$). However, aphid location only affected *H. convergens* consumption rates when feeding on

cabbage aphid. Overall, trade-offs between developmental rate, consumption, and body weight were observed among treatments. Our data also suggests that *H. convergens* is capable of surviving exclusively on a diet of canola aphids and feeding location within the plant directly affects their developmental rate.

INTRODUCTION

Prior to 2004, commercial production of winter canola (*Brassica napus*) was non-existent in Southern Great Plains of the USA. In less than a decade, production in this region continues to increase with planted acreage now exceeding 80,937 ha (200,000 acres) (Peeper et al. 2009, Franke et al. 2009, U.S Canola Association; USDA 2012). Rapid adoption is driven primarily by agronomic and economic benefits provided to producers who rotate canola with winter wheat (*Triticum aestivum*), which has been a staple crop in this region for decades (Peterson and Westfall 1994, Blackshaw et al. 2001, Giles et al. 2008, Giles and Walker 2009, Peeper et al. 2009, Franke et al. 2009). Winter canola is also a profitable, first-generation biofuel crop (Tickell 2000, Peeper et al. 2009). Spatial and temporal changes in the landscape due to the expansion of novel crops such as winter canola might cause arthropod communities to make maladaptive habitat choices when choosing these new prey patches over existing prey patches (Lu et al. 2012, Wolfenbarger et al. 2008, Landis et al. 2008).

In this new canola-wheat rotaion, each cropping system is attacked by unique pest complexes that are host specific. As a result, aphid species found colonizing canola may or may not serve as suitable alternate prey for natural enemies that once inhabited a predominantly wheat or grassland landscape (Franke et al. 2009, Knodel 2011). Consequently, introducing new prey items into a relatively stable cropping system like wheat may alter existing ecosystem services by either providing new sources of predators and parasitoids or sinks (pulling services

away from wheat systems); thus, reducing beneficial insects within these changing landscapes. With respect to population reductions, canola landscapes may be classified as "sinks" since 90% of the canola produced in the Southern Great Plains is treated with broad-spectrum insecticides (i.e,. synthetic pyrethroids) annually (Franke et al. 2009). In comparison, less than 16% of the winter wheat fields are treated with insecticides annually (Giles et al. 2008, Giles and Walker 2009). Moreover, aphids attacking canola interact with secondary compounds (allelochemicals) produced by the canola plant and chemical defenses may have direct fitness consequences for biological control organisms that prefer these aphids as a resource, thus acting as biological sinks (Cole 1997ab, Francis et al. 2000, 2001, Pratt et al. 2008, Kos et al. 2011a, 2011b). Despite the continued increase in canola acreage and corresponding increases in insecticide usage, we lack understanding of potential landscape-level impacts of adding canola to a historically wheat-dominated landscape (Brewer et al. 2004, Franke et al. 2009, Knodel 2011). In addition to studying the broader landscape-level effects, it is also imperative to focus on plant-level interactions that govern predator-prey dynamics at a local sacle.

Foliar insecticide applications in canola coincide with the flowering stage, which is peak attraction time for pollinators and natural enemies dispersing from nearby habitats and using canola as a resource (Baggen et al. 1999). Aphid species attacking canola predominantly during flowering include a generalist, the green peach aphid (*Myzus persicae*) and a specialist, the cabbage aphid (*Brevicoryne brassicae*) (Franke et al. 2009, Berlandier et al. 2010, Royer and Giles 2008). The green peach aphid is a polyphagous pest that can utilize over 400 plant families. Green peach aphids mitigate allelochemicals (glucosinolates) produced by canola through excretion (Weber 1985, Pratt et al. 2008, Hopkins et al. 2009). Cabbage aphids, on the other hand, are a perennial pest restricted to plants in the Brassicaceae family, which includes winter

canola (Hughes 1963). Cabbage aphids are specialist species and evolved mechanisms to sequester glucosinolates using an aphid-endogenous enzyme that mitigates chemical toxicity (Weber 1985, Kazana et al. 2007, Pratt et al. 2008, Hopkins et al. 2009). Under field conditions, these two species have different vertical distributions within the canola canopy. Green peach aphids occur on the lower leaves (vegetative tissue) of the plant while cabbage aphid colonies primarily colonize the flowering racemes (reproductive tissue). Differential occurrence of herbivores in different plant structures is a phenomenon not unique to canola aphids. Reasons for these differences may be the result of top-down (predator preference) or bottom-up forces (plant quality or nutrient composition), competition between aphid species (space, resources), or a combination of factors (Merritt 1996, Idris and Roff 2002, Smallegange et al. 2007, Pekar 2005, Berberet et al. 2009, Fernandes et al. 2011). For natural enemies using canola aphids as a resource, prey/host selection can directly affect fitness which has been observed in other production systems (Idris and Roff 2002, Pekar 2005, Berberet et al. 2009, Fernandes et al. 2011). Bottom-up forces, such as variation in host quality (i.e., allelochemicals) influence prey/host toxicity (fatty acid content or toxicity through sequestration) and thus have direct repercussions on mortality, development, growth rates, fecundity and recruitment of natural enemies in other brassica species (Gabrys et al 1997, Cole. 1997a, Giles at al. 2002, Brown et al. 2003b, Giles et al. 2005, Lambton and Hassall 2005, Smallengange et al. 2007, Van Dam et al 2008a, 2008b, Guigo et al. 2010, Kabouw et al 2010, Kramer et al. 2011). To our knowledge, these plant-level interactions between aphids occupying different structures and natural enemies that encounter such prey patches have not been studied for canola specifically (see Chapters 2 and 3).

A complex of natural enemies is observed on winter wheat in the Southern Great Plains and their impacts on cereal aphid suppression are well documented (Chambers 1986, Michels et al. 2001). Lady beetles (Coccinellidae), green lacewings (*Chrysopera* spp), damsel bugs (*Nabis* spp), syrphid flies (*Syrphidae* spp), and spiders (Araneae), among others species are abundant in these wheat-dominated landscapes (Chambers 1986, Michels et al. 2001). Lady beetles specifically play a critical role in cereal aphid suppression (Kring et al. 1985, Michels and Behle, 1991, Obrycki and Krig 1998, Michels at al. 2001, Phoofolo et al. 2007, Obrycki et al. 2009) and also co-occur with canola flowering. Among all coccinellid species observed in this system during the spring when canola and wheat aphids are more abundant, *Hippodamia convergens* (convergent lady beetle) is the most abundant Coccinellidae species in the South Central Region of the US (Nielson et al. 1959, Dogan et al. 1996, Nechols and Harvey 1998, Michels et al. 2001, Michaud and Qureshi 2006). Similar to other predatory lady beetles, *H. convergens* are effective predators both as adults and larvae. Furthermore, *H. convergens* adults are capable of using alternative foods such as pollen, sap, or nectar when prey numbers are low (Hodek 1973, Lundgren 2011); these alternative resources become readily abundant during early spring with the addition of flowering canola in the landscape. Adult lady beetles in general have a high dispersal capacity that enables them to use available resources in wheat and surrounding landscapes easily (Hodek 1973, Hajek 2004, Seagraves 2009). Conversely, immature lady beetles are restricted to prey patches that were maternally selected by ovipositing females at a local scale (within-field level) due to their limited dispersal capacity (Evans 2003). Hence, in the context of source-sink relationships, recruitment can play an integral part in determining the impact of adding new resources to predator-prey system. In addition, gravid females can determine larval fitness through searching and oviposition decisions (Evans 2003). Due to their

limited dispersal, novel prey offered by the addition of canola to the landscapes may directly influence biology and fitness of immature (size and developmental duration) lady beetles more directly if adult females use canola fields as ovipositional sites.

To our knowledge, the suitability of canola aphids as a diet for immature *H. convergens* and associated impacts on its life history traits have not been evaluated. Although different prey species can be accepted by predatory lady beetles in these new canola lanscapes (Kalushkov and Hodek 2001); suitability studies can determine if newly introduced aphid species are essential or alternative prey for lady beetles (Hodek and Honek 1996). While essential prey ensures survival and fecundity of predators, alternative prey only provides nutrients necessary for survival (Hodek and Honek 1996, Kalushkov and Hodek 2001). The objectives of this study were to quantify if feeding location (reproductive versus vegetative tissues) of prey in canola affects immature convergent lady beetle survival, development, and consumption rates in a non-choice bioassay. By evaluating source-sink dynamics at the plant level, we can further understand trophic interactions occurring between species (both pest and beneficial) at the landscape-level. More specifically, such interactions may influence community dynamics and structure for newly introduced crops into established agroecosystems (Guigo and Corff 2010).

MATERIALS AND METHODS

Aphids and plants

Canola aphids (green peach and cabbage aphids) used in feeding assays were collected from natural field populations and maintained under laboratory conditions at Kansas State University (Manhattan, KS). In April 2011, adults from both species were collected from winter canola fields in Barber County Kansas (N 36.998414, W -98.456797) and transported to the laboratory in coolers where they were provided with young, potted canola seedlings as a food

source. Aphid colonies were maintained in growth chambers (Bio-Temp Scientific Inc., BT-1-49 WC, Bradenton, FL) at 22 ± 2 ºC, 60-70% RH, with a 16:8 hr (light:dark) photoperiod (Kos et al. 2011a). Canola (variety Riley) was seeded in a special soil mix that contained sulfur, ammonium nitrate, micronutrients, peat moss, perlite and two types of slow release fertilizers (proprietary soil blend, M. Stamm, canola breeder, Kansas State University and Oklahoma State University). Plants were maintained in the greenhouse at 22 ± 3ºC under natural light during daylight hours and supplemented with artificial lights at night; photoperiod was kept at 16:8 hr (light:dark). Canola plants were artificially vernalized in a growth chamber for approx. 2 months at 12:12 hr (light:dark) and constant 4ºC to induce reproductive maturity (Murphy and Scarth 1994). Vernalized plants were then used for all experiments.

Soybean aphid (*Aphid glycine)* colonies (biotype 1) were also maintained at 22 ± 2ºC, 60-70% RH, 16:18hr (light:dark) photoperiod in a growth chamber (Bio-Temp Scientific Inc., BT-1-49 WC, Bradenton, FL). Adult soybean aphids were obtained from a source colony maintained at Iowa State University (Ames, Iowa). Soybean aphid colonies were reared on susceptible soybean plants (variety A1026832, Asgrow®, St Louis, MO) that were grown in horticultural flats (5 × 10 cells) (Deepots, Hummert International). Flats were placed in plastic storage tubs (587.7 × 42.9 × 16.2 cm, L×W×H) (Sterilite storage box, Leominister, MA) filled with water; tubs allowed pots to receive water *ad libitum*. Once soybean plants were in vegetative stages 3 to 6 (V3-V6) (Fehr et al. 1971), soybean aphids were transferred to plants. Two flats of soybeans were planted in the greenhouse bi-weekly to adequately maintain soybean aphid colonies. Aphids were transferred to new plants using the same techniques and timing as for the canola aphid colonies. Voucher specimens (*Brevicoryne brassicae* nymphs and adults) were deposited in the Department of Entomology Museum (voucher number = 228).

Lady beetles

Adult *H. convergens* were collected from commercial corn fields in Washington County Kansas in August 2012 (N 39.999561, W -97.344964). Adult beetles were collected (n = 300) using a hand-held aspirator and brought to Kansas State University (Manhattan, KS) in coolers. Adults were maintained at 22 ± 2ºC, 60-70% RH, 16:8 hr (light:dark) photoperiod in a growth chamber (Percival, AR22L, Perry, IA). *H. convergens* adults were provided with honey and a supplementary, artificial diet mix that consisted of a mixture pollen substitute, tropical fish flakes, cichlid pellets, sun-dried *Gammarus pulexm* and *Ephestia kuehniella* eggs *ad libitum* (see Lundgren et al. 2011 for complete diet composition); *E. kuehniella* eggs were obtained from a commercial supplier (Beneficial Insectary Inc., Redding, CA). Additionally, soybean aphids from laboratory colonies described above were included as part of the daily *H. convergens* diet regime to avoid reproductive diapause (Vargas et al. 2012). *H. convergens* adults were kept in commercial rearing cages (61 × 61 × 61cm) (BugDorms, BioQuip inc., CA, USA), which were cleaned weekly.

All adults were left in cages for 2 wk to facilitate multiple matings and increase chances of viable egg production. Adults were sexed using size, morphological characters on the last abdominal segment, and overall coloration (Kova'r 1996). Once sexed, females were placed in individual plastic containers (4.5 × 4.5 × 1.5 cm) and provided with soybean aphids *ad libitum*, a water-saturated cotton gauze, a droplet of honey (0.3 × 0.2 cm diameter) and approx. 0.5 mg of non-aphid diet mix daily. Individual containers were checked daily for egg production. Once eggs were deposited, females were moved to new containers and eggs remained undisturbed in the same container until hatching. Newly hatched larvae were placed into individual cells (128 per tray) of a clear bioassay tray (BAC128, Bio-Serv®, Frenchtown, NJ). Larvae were restricted to the same cells using clear, 16-cell adhesive lids (BACV16, Bio-Serv®, Frenchtown, NJ).

Larvae were provided with *E. kuehniella* eggs *ad libitum* and a water-saturated cotton gauze throughout their larval development. After rearing one generation of *H. convergens* in the laboratory from field-collected adults, newly hatched larvae from this first generation (i.e., second generation larvae) were used for all no-choice bioassay trials. To avoid maternal effects, larvae of multiple mothers were randomly selected. Voucher specimens (*Brevicoryne brassicae* nymphs and adults) were deposited in the Department of Entomology Museum (voucher number = 228).Colonies were maintained for future studies; field collected female and male adults were added regularly to avoid consanguinity or inbreeding effects (Soares et al. 2001).

No-choice bioassay

We tested effects of various prey diets on the life history attributes of immature *H. convergens* using a no-choice feeding assay. Different aphid diets were produced by restricting cabbage or green peach aphids on either reproductive (flowering racemes) or vegetative (mid-canopy leaves) canola plant structures using enclosure cages. Adult aphids (5 per cage) were placed on plant structures 3 wk prior to initiating the experiment. Cages used for this experiment were adopted from Soper et al. (2012) (see Chapter 2-3 for cage design details). Canola plants used to produce aphid treatments were arranged in a completely randomized design in the greenhouse at 22 ± 3ºC, under natural light supplemented by artificial lights; plants were kept in a 16:8 hr (light:dark) photoperiod.

Second generation *H. convergens* larvae were individually placed into bioassay tray cells within 8 hr of hatching; larvae were placed in similar trays and lids used for rearing the first generation of larvae. Only newly hatched lady beetles that were still feeding on their egg case were selected for this experiment to further control for hatching time. Larvae were arranged in a completely randomized design and assigned to one of six diets, which included: green peach

aphids from reproductive (GPA-R) or vegetative (GPA-V) tissues, cabbage aphid from reproductive (CA-R) or vegetative (CA-V) tissues, an aphid control (*Aphis glycines* [SA]), and non-aphid diet (*E. kuniella* eggs). *H. convergens* larvae were fed the same diet for the duration of their larval development. Aphids were collected daily from infested plants as needed for the feeding assays and provided to larvae *ad libitum*, which was 10 aphids per d (unpublished data, X.C.S.). Aphids were placed within cells with a fragment of leaf material from their host plants to reduce mortality caused by handling. For the *E. kuniella* diet, eggs (approx. 0.12 g) were added to cells as needed daily to ensure *ad libitum* supply. All larvae were reared in a growth chamber (Percival, AR22L, Perry, IA) at 22 ± 2ºC, 60-70% RH, 16:8 hr (light:dark) photoperiod. Overall, a total of 168 newly hatched *H. convergens* larvae from 50 adult females were used for this feeding assay. The experiment was repeated at three different times (blocks): block 1 had 4 larvae per treatment; blocks 2 and 3 each had 12 larvae per treatment.

The main effect of aphid feeding location (reproductive versus vegetative structures) on *H. convergens* mortality (survival to adult emergence), development, and consumption rates were determined through daily observations. For all aphid diets, consumptive effects were further categorized into total number aphids consumed or partially consumed as well as number of unconsumed aphids. Consumption values were not recorded for *H. convergens* reared on *E. kuniella* eggs as these larvae were fed eggs *ad libitum*. Larval development (instar change) was determined by presence/absence of exuvia in the bioassay cells. Exoskeletons, remaining aphids, and plant material were removed from all cells and replaced with new diet daily; new bioassay trays were changed weekly to reduce infection and mold creation. After larvae completed development, pupal mass were recorded (10^{-4} mg, Denver Instrument, Pinnacle Analytical Scale,

Denver, CO) and compared among diet treatments. Once individual pupal mass were recorded, pupae were placed back into the bioassay trays and time to adult emergence was recorded.

Statistical analysis

All data pertaining to survival to adulthood, development, consumption, and pupal mass of immature lady beetles in our study were analyzed using SAS for Windows (SAS Institute 2009). An analysis of variance (*PROC MIXED*) procedure was performed with diet as a fixed effect and block as a random effect for all parameters. The *LS MEANS* statement using an adjusted Tukey method was used to make treatment comparisons ($\alpha = 0.05$).

RESULTS

Mortality did not differ among prey treatments ($F = 1.52$; $df = 3, 21$; $P = 0.23$). In our experiment, 30.1% of all *H. convergens* (n = 65) larvae observed did not reach pupation. A small portion of these larvae (38 total or 17.6 %) escaped the bioassay trays whereas the remaining 12.5% (n = 27) were found dead. Hence, aphid diets reared on pre-determined feeding locations of a canola plant did not affect larval survival.

Aphid diet significantly affected larval developmental times ($F = 37.83$; $df = 5, 95$; $P < 0.0001$). Mean overall preimaginal development was significantly longer for lady beetle larvae reared on the prey control diet (SA), whereas larvae that fed strictly on canola aphids-plant location treatments (i.e., GPA-R, CA-R, CA-V, and GPA-V) were intermediate to soybean aphid and non-aphid diet. Thus, larvae reared on non-aphid diet had the shortest overall larval developmental time, which was 12.9 ± 0.4 d (Fig. 1A). We further explored the effects of diet on developmental durations within each instar (Fig. 1B). Larvae reared on soybean aphid had the longest developmental times during the first three instars compared to the other treatments (first: $F = 3.98$; $df = 5, 95$; $P = 0.0025$, second: $F = 3.15$; $df = 5, 95$; $P = 0.0113$, and third: $F = 12.36$;

df= 5, 95; *P* < 0.0001); but spent the least amount of time as fourth instars (*F*= 9.86; *df*= 5, 95; *P* < 0.0001). In contrast, larvae reared on green peach aphid had the shortest developmental times in the first three instars, compared to other diets, and fourth instar development was longer. However, the development of fourth instar *H. convergence* was shorter if green peach aphids were reared on vegetative versus reproductive tissues. Larvae reared on cabbage aphids had longer development in the first three instars compared to those reared on green peach aphids, and it was comparatively shorter in the fourth instar. In general, development on cabbage aphids was progressively longer as larvae increase in stage. Also, there was no difference in development of any *H. convergence* instar when reared on cabbage aphids from reproductive tissue or vegetative tissue (Fig. 1B). Meanwhile, lady beetles on the SA diet spent 24.0% of their preimaginal time as fourth instars; immatures on the GPA-R diet spent 55.9% of their preimaginal development as fourth instars.

Aphid consumption rates varied significantly across the diets tested (*F* = 48.69; *df* = 4, 85; *P* < 0.0001); recall, the *E. kuniella* egg diet was excluded from consumption rate calculations as total number of eggs consumed was not recorded. Mean number of aphids consumed per d was significantly greater for immature lady beetles exposed to the prey control diet (SA), followed by GPA treatment regardless of aphid previous location in the canola plant, and CA diet treatments, with CA-R diet corresponding to lower consumption overall (Fig. 2A). Lady beetles in the SA control diet consumed 31.4 % more aphids daily than those exposed to the CA-T. Consequently, larval lady beetles left significantly more partially consumed (*F* = 73.45, *df*= 4, 85; *P* < 0.0001, Fig. 2B) and unconsumed aphids (*F* = 3.85; *df* = 4, 85; *P* = 0.0064, Fig. 2C) in the CA-R, CA-V, GPA-R, GPA-V and SA respectively.

Lastly, we compared consumption rates for larvae within each developmental stage (Fig. 3). Those that fed on soybean aphid ate significantly more prey as larval development proceeded (first: $F = 12.72$; $df = 4, 85$; $P < 0.0001$, second: $F = 7.99$; $df = 4, 85$; $P < 0.0001$, and third: $F = 4.94$; $df = 4, 85$; $P = 0.0013$). However, no significant differences in prey consumption were observed among most of the diets once larvae reached the fourth instar with the exception of cabbage aphids reared on vegetative tissues ($F = 3.05$; $df = 4, 85$; $P = 0.0213$). For this diet, larvae ate 12% fewer larvae than any of the other canola aphid diets.

In addition to rates of development, pupal masses were obtained and compared among diets tested; pupal mass was used as a measure of adult fitness. Significantly higher mass were observed for lady beetles that consumed soybean aphid, followed by *E. kuniella* eggs, and last canola-reared aphid diets regardless of species (GPA, CA) and aphid feeding location (V, R) ($F= 48.67$, $df = 5, 94$; $P < 0.0001$) (Fig. 4). Pupal weights were about 40% smaller when *H. convergens* larvae were reared on canola aphid diets compared to the control diets (soybean aphid and *E. kuniella* eggs) (Fig 4).

Overall development (from egg hatch to adult ecolsion) was longer for larvae exposed to soybean aphid or canola-reared aphids restricted to feeding on reproductive parts of the canola plant (CA-R and GPA-R) ($F = 29.54$; $df= 5, 94$; $P < 0.0001$). In addition, previous feeding location by CA had no significant effects on overall development, while larvae feeding on green peach aphids that previously fed on vegetative plant parts (GPA-V) displayed shorter overall developments than those restricted to the GPA-R diet; larvae feeding on *E. kuniella* eggs displayed the shortest overall developmental times ($F = 29.54$; $df= 5, 94$; $P < 0.0001$) (Fig. 5).

DISCUSSION

In this study, immature convergent lady beetles were used as a model organism to test the effect of feeding location on suitability of cabbage and green peach aphid prey offered in newly introduced winter canola landscapes. Although feeding location of aphids in canola had no direct effects on pupal mass or survival, aphid feeding location within the canola plant significantly affected development and consumption rates of *H. convergens* larvae. Moreover, trade-offs between developmental rates, consumption rates, and fitness (measured indirectly as pupal weights) for immature *H. convergens* were observed across the different diets tested. Immature lady beetles reared on aphids from control diets had developmental times similar to those reared on canola aphids; however, pupal weights and consumption rates were greater for larvae in the aphid control diets. Longer developmental times observed for *H. convergens* feeding on the aphid-control diet is likely a biological trade-off with body size (Phoofolo et al. 2007).

It is well documented that lady beetle pupal masses can be used to estimate adult reproductive fitness (Hodek and Honeˇk 1996, Dixon 2000, Phoofolo et al. 2008, Kajita et al. 2010). Pupal weight reflects adult size which directly influences fecundity, longevity and mating success (Phoofolo et al. 2008). For this study, immature *H. convergens* that were restricted to a diet of canola aphids, regardless of feeding location (vegetative or reproductive tissues), attained pupal weights that were 39.9% smaller than larvae reared on either of the control diets (soybean aphid or *E. kuniella* eggs) tested (Fig. 4). In addition, overall differences in pupal weights observed in this study are similar to the differences reported between cereal aphid diets used in Phoofolo et al. (2007). For our study, the findings suggest that aphids reared on canola are an inferior diet for *H. convergence* compared to other species tested because of the smaller pupae size.

Aphids that feed on canola have evolved different mechanisms to process allelochemicals (in particular glucosinolates) produced by these plants. Cabbage aphids sequester toxic compounds and green peach aphids excrete these deleterious chemicals. Because numerous studies using other brassica species have documented the negative effects of glucosinolates on natural enemy fitness and population development (Blackman 1967, Gabrys et al. 1997, Cole 1997a, Van Dam et al. 2008a, Van Dam et al. 2008b, Guigo et al. 2010, Kabouw et al. 2010, Kramer et al. 2011), we hypothesized that *H. convergence* would be adversely affected by feeding on canola aphids. We also predicted that a greater negative effect on fitness would be observed when lady beetle larvae fed on cabbage aphids because they retain the allelochemicals within their bodies. Finally, although direct measures of diet quality were not quantified in this study, previous research has shown that there are distinct differences in glucosinolate concentrations for aphids reared on upper (reproductive) versus lower (vegetative) canola plant structures and that these differences are species-specific (see Chapter 2). Therefore, we conclude that differences in larval development observed in this study are due to toxic compounds experienced by *H. convergens* larvae during feeding and these differences are related to aphid species, plant location, or their interactions. However, other possible explanations include differences in aphid size, nutritional quality, phenotypic plasticity, or digestibility among species. Future no-choice studies should involve providing lady beetles lower predetermined amounts (g) of aphids regardless of species to control for intake rates and differences in aphid size across species (Jessie 2013); other studies report differences in consumption rates that are related to prey size for other lady beetles species (Elliott et al. 1994, Tsaganou et al. 2004). Nutritional quality, such as proportion of fat and/or protein of aphids from different plant

structures should also be measured since it can directly influence predator performance (Giles et al. 2002).

Even though feeding location of aphids in canola did not influence pupal mass, consumption rates were directly affected by the previous feeding location, but only for specialist cabbage aphids on canola ($P < 0.0001$). Specifically, lady beetles restricted to feeding on cabbage aphids form reproductive parts of the canola plant consumed 21% fewer aphids than those lady beetles restricted to feeding on cabbage aphids from vegetative canola parts. We speculate that the reduced feeding by *H. convergens* larvae may be related, at least in part, to higher exposure of allelochemicals due to higher concentrations in reproductive parts of the canola plant and ability of cabbage aphids to sequester these defensive compounds. Differences in nutritional quality of cabbage aphids between reproductive and vegetative plant parts may also affected larval consumption rates, but we did not evaluate the effect of nutritional quality in this experiment. Feeding *ad libitum* assumes that predators like *H. convergens* have an unlimited amount of food within a defined area. In an unlimited food environment, we still observed differences in consumption rates between the species tested. However, to account for differences in nutritional quality between these species, future studies that restrict the daily number of aphids available to *H. convergens* larvae could be used to distinguish deleterious effects of diet from nutritional inadequacies (Jessie 2013). For example, Phoofolo et al. (2007) performed such a study to test for differences in nutritional quality of cereal aphids on *H. convergens* development. Suitability could be measured only when prey items were provided to immature lady beetles in suboptimal levels rather than *ad libitum* diets. Future studies including daily voracity and consumption rates for each larval instar should be included. Such calculations account for duration of the feeding period as well as daily weight changes for each larvae stage (Soares et al.

2001) parameters that were not included in this study. When looking at consumption rates across individual larval stages (Fig 3), the degree of acceptance of specific diets by developing *H. convergens* larvae varied across treatments. Early instars consumed significantly fewer cabbage aphids but significantly more soybean aphids followed by green peach aphids (Fig 3).

Additionally, we formally explored correlations between ordinal instar stage (explanatory variable) and consumption rates (response variable or number of aphids consumed per stage) for each aphid diet using simple linear regression (*PROC REG*, SAS 2009) (Fig. 3). Not surprisingly, there was a positive relationship between larval instar and number of aphids consumed; as larvae changed from one instar to the next, the number of aphids consumed within each diet also increased (Table 1). However, the slopes or rate at which they consumed aphids within each successive instar was not equal between the diets tested. Larvae feeding strictly on cabbage aphids restricted to the vegetative parts of the plant had the highest coefficient of determination (0.73). Lady beetle larvae that consumed green peach aphids reared on vegetative leaves or soybean aphid had the lowest coefficient of determination (0.65) (Table 1). Variation in the coefficient of determination can be explained by the positive relationship between instar duration and total number of aphids consumed within an instar for each diet tested (Table 1). Slopes for regressions vary across treatments from 1.72 for lady beetles on the soybean aphid (SA) diet to 2.60 for larvae reared on the CA-R diet; differences in slopes might explain different degrees of prey acceptance by lady beetles in study (Table 1). The intercept of the regression represents the number of aphids consumed when experiment started, approx. 8 hours after egg hatch. Negative intercepts may therefore represent conditioning of immature lady beetles to diets that are more detrimental or toxic such as cabbage aphid over diets that are less toxic like green peach aphid.

Time required by *H. convergens* larvae to complete development was also affected by the aphid diets we tested. Lady beetles restricted to canola aphids reared on reproductive plant parts had developmental rates similar to larvae restricted to the aphid control diet (soybean aphid). Previous feeding location of specialist cabbage aphid did not significantly influence lady beetle development; however, there was a trend for longer development, which is consistent with developmental differences observed when reared on green peach aphids. Specifically, lady beetles that fed on green peach aphids confined to vegetative plant structures developed 17% faster than larvae restricted to feeding on green peach aphids confined to reproductive plant structures. Plant specific growth is consistent with reproductive plant parts where higher allelochemicals concentrations than vegetative plant parts were reported (see Chapter 2). Trade-offs between developmental duration and consumption explain life history responses displayed by larvae in our study. Phoofolo et al. (2008; 2009) showed that developing larvae need to acquire a minimal weight threshold to discontinue feeding and growth prior to pupating. In our study, larvae exposed to canola aphids (green peach aphid and cabbage aphid) and to the non-aphid diet (*E. kuniella* eggs) spent significantly more time in each successive instar (Fig. 1B). Conversely, larvae that fed only on soybean aphids displayed a similar trend during the first three instars but developmental time was reduced during the last larval stage (Fig. 1B). In addition, *H. convergens* larvae that fed on soybean aphids *ad libitum* developed faster than larvae fed a non-aphid diet or canola aphids regardless of species or previous prey location. As reported in other studies (Tasaganou et al. 2004, Cabral et al. 2006, Phofolo et al. 2008) the length of the pupal stage was not significantly different across treatments. Cabral et al. (2006) stated that a lack of effect is most likely due to metabolic rates and the effects on pupal development.

Interactions between aphid diet, developmental time and consumption for *H. convergens* larvae reared on predetermined diets affected the pupal weights, which will indirectly affect overall fitness as adults. Longer developmental times observed for the lady beetles reared on soybean aphid is likely a biological trade-off with body mass (Phoofolo et al. 2007). Lady beetles restricted to feed on soybean aphids *ad libitum* continued to feed in their latest instar stage to acquire higher pupal mass, while lady beetles in the other canola aphid diets discontinued feeding and pupated once they acquired the minimal mass threshold (Fig 4). Conversely, canola aphids (cabbage aphid or green peach aphid) were a poor quality diet compared to soybean aphid; larvae spent less time as fourth instars and produced smaller pupae as a result. Lady beetles on the non-aphid diet displayed intermediate developmental time as well as intermediate pupal size, which suggest that *E. kuniella* eggs were nutritionally better than canola aphid diets because pupae were significantly larger but took less time to reach the pupal stages, although reproductive success of lady beetles in this diet was not measured and might be compromised since convergent lady beetles require aphids in their diet to avoid reproductive diapause (Vargas et al. 2012). Variation in adult body size of lady beetles is largely determined by food resources allocated to the final instar, when 60-80% of the aphids are consumed (Lee and Kang 2004, Berner and Blackenhorn 2007, Phoofolo et al. 2008, 2009). The direct correlation between canola aphid toxicity and direct effects on larval development requires further investigation.

Overall, our data suggests *H. convergens* larvae are capable of surviving exclusively on a diet of canola aphids although feeding location within a plant directly affects their development rate. These trade-offs are important to understand, especially in the context of adding new crops to an agricultural landscape and understanding the implications of such actions on existing

natural enemy communities. Future studies should examine the direct effects of different canola aphid diets from different plant parts influence on *H. convergens* adults. Diet can greatly affect reproductive output of adults and level of control these ecosystem services exert on pest populations. Additionally, choice-studies that reflect more field situations (i.e., mixed species diet) would also further our understanding of the canola system and its interaction with existing natural enemy communities. Comprehensive behavioral studies that analyze coccinellid feeding behavior when restricted to different prey will enable us to better understand movement among and between crops, ultimately shaping our understanding of coccinellid biology. A better understanding of behavioral ecology will enable the inclusion of coccinellid predation into economic thresholds for aphid management in wheat-canola systems.

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FIGURES AND TABLES

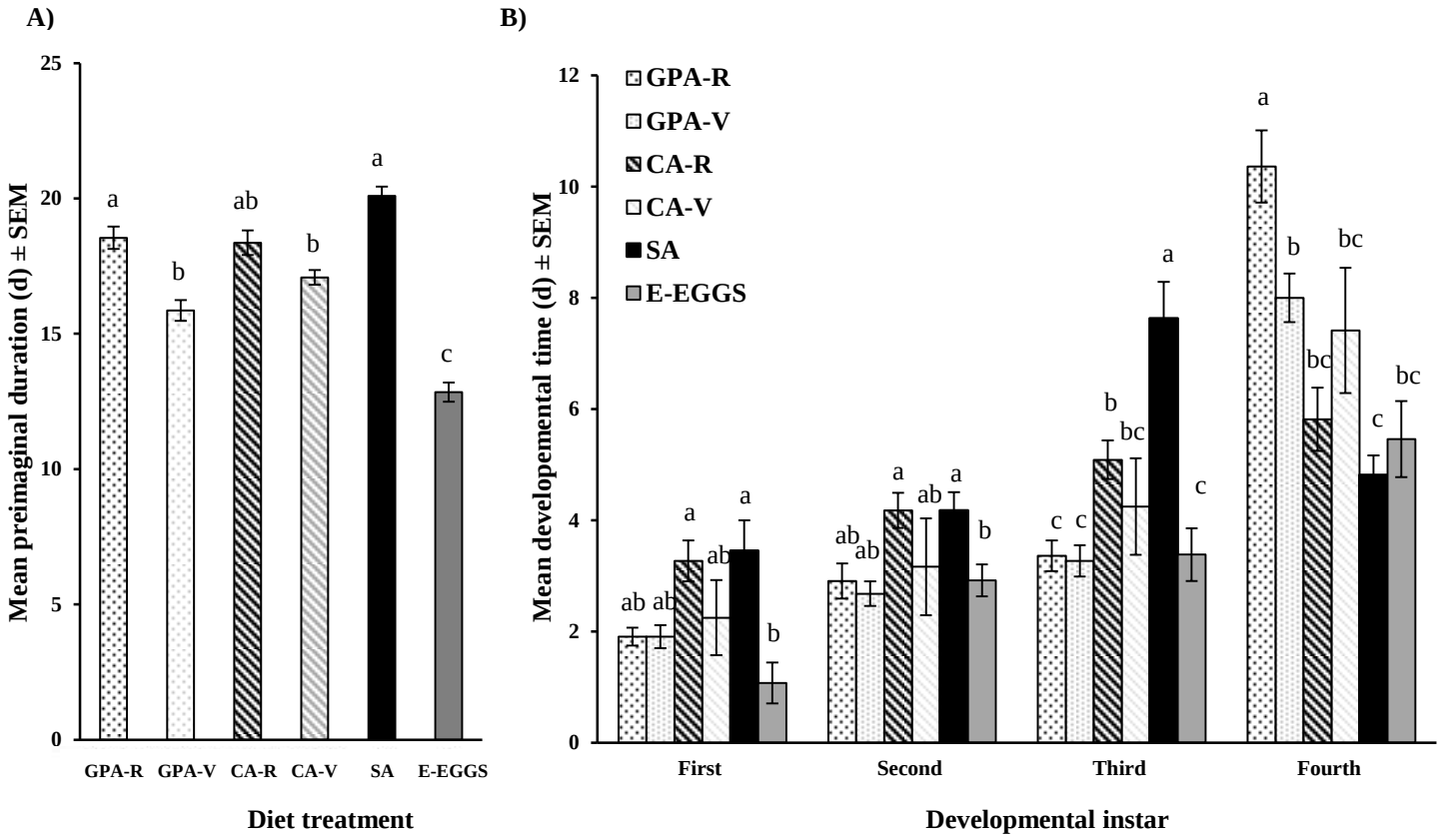

Figure 4.1. No-choice bioassay assessing effects of different aphid diets on *Hippodamia convergens* A) mean preimaginal developmental time (d) ± SEM and B) mean duration of specific stages (d) ± SEM. Diet treatments included: *Myzus persicae* (GPA) and *Brevicoryne brassicae* (CA) previously confined to reproductive (GPA-R and CA-R) or vegetative (GPA-V and CA-V) structures of canola plants, an aphid control (*Aphis glycines*, SA), and a non-aphid diet (*Ephestia kuniella* eggs). Bars with the same letters are not significantly different ($P < 0.05$).

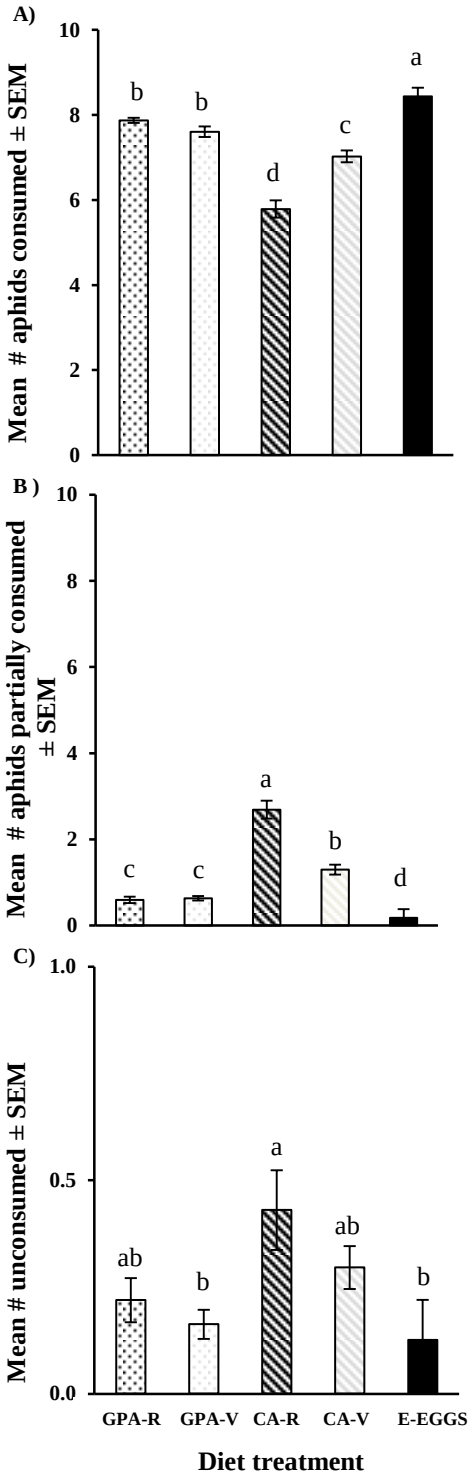
A)
Mean # aphids consumed ± SEM
10
8
6
4
2
0
b
b
d
c
a
B)
Mean # aphids partially consumed ± SEM
10
8
6
4
2
0
c
c
a
b
d
C)
Mean # unconsumed ± SEM
1.0
0.5
0.0
ab
b
a
ab
b
GPA-R
GPA-V
CA-R
CA-V
E-EGGS
Diet treatment

Figure 4.2. A) Consumption rates ± SEM for *H. convergens* larvae restricted to different aphid diets: *Myzus persicae* (GPA) and *Brevicoryne brassicae* (CA) previously confined to reproductive (GPA-R and CA-R) or vegetative (GPA-V and CA-V) structures of canola plants, and an aphid control (*Aphis glycines*, SA). Consumption was categorized as mean number of aphids (10 initial aphids) that were A) fully consumed, B) partially consumed, C) and unconsumed. Bars with the same letters are not significantly different ($P < 0.05$).

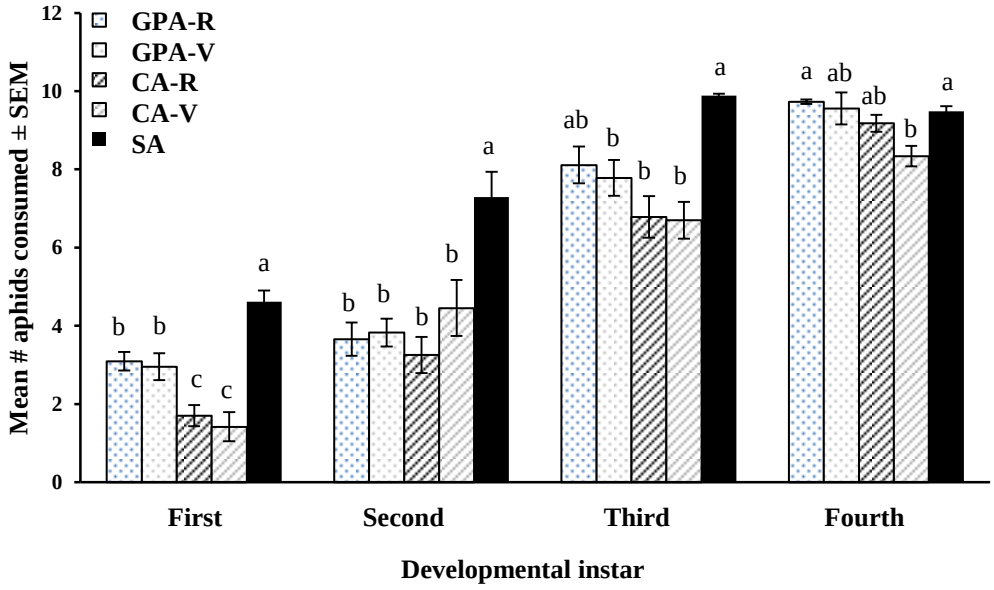

Figure 4.3. Mean number of aphids consumed ± SEM within each *H. convergens* larval stage for aphid diets tested: *Myzus persicae* (GPA) and *Brevicoryne brassicae* (CA) previously confined to reproductive (GPA-R and CA-R) or vegetative (GPA-V and CA-V) structures of canola plants, and an aphid control (*Aphis glycines*, SA). Bars with the same letters are not significantly different ($P < 0.05$).

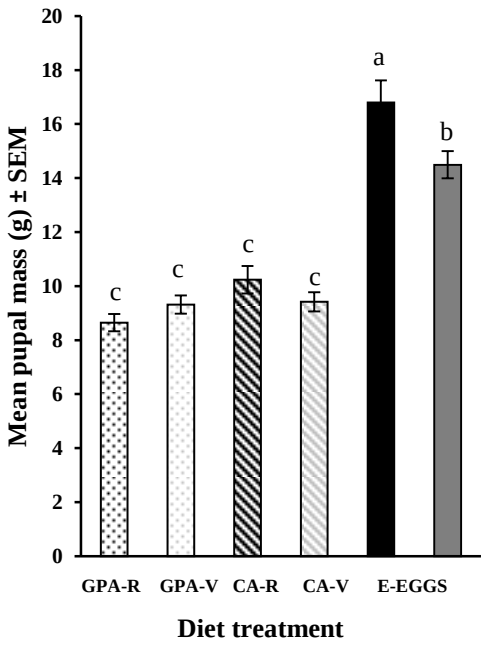

Figure 4.4. Mean pupal weights ± SEM for *H. convergens* larvae restricted to six different diets. Diet treatments included: *Myzus persicae* (GPA) and *Brevicoryne brassicae* (CA) previously confined to reproductive (GPA-R and CA-R) or vegetative (GPA-V and CA-V) structures of canola plants, an aphid control (*Aphis glycines*, SA), and a non-aphid diet (*Ephestia kuniella* eggs). Bars with the same letters are not significantly different ($P < 0.05$).

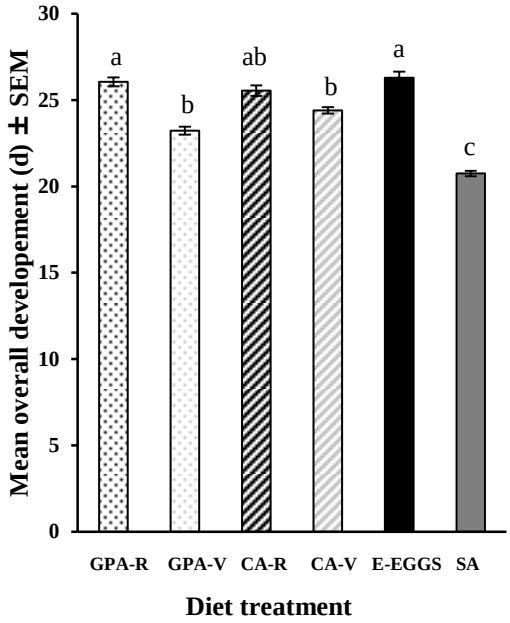

Figure 4.5. Mean overall development time (d) ± SEM for lady beetles in in six diet treatments, *Myzus persicae* (GPA), *Brevicoryne brassicae* (CA) previously feeding on (GPA-R, CA-R) or vegetative (GPA-V, CA-V) canola plant structures and two controls: SA (*Aphis glycines*, prey control) and *Ephestia kuniella* eggs (non-aphid diet). Bars with the same letters are not significantly different ($P < 0.05$).

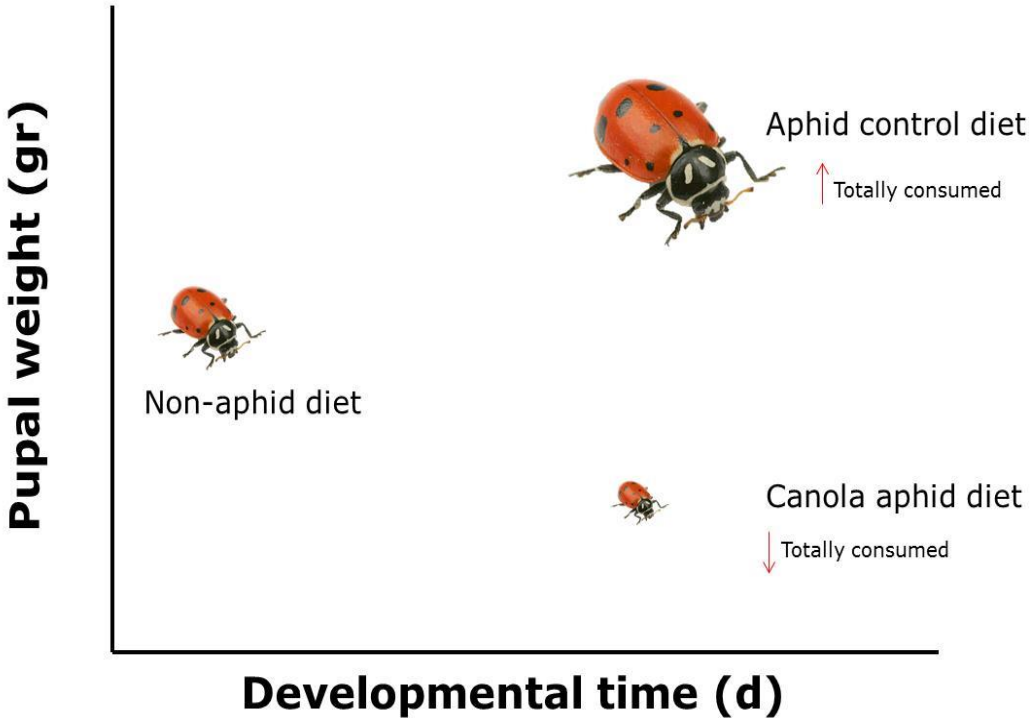

Figure 4.6. Hypothetical relationship between larval developmental times (d) and pupal mass (g) for *Hippodamia convergens* feeding on canola aphids and control diets.

Chapter 5 - SUMMARY AND CONCLUSIONS

Despite the continued rise in canola (*Brassica napus*) acreage and corresponding increases in insecticide usage throughout the South Central US, the potential impacts of adding canola into a historically wheat-dominated landscape are unknown (Brewer et al. 2004, Franke et al. 2009). To understand the broader landscape-level effects associated with adding new crops to established landscapes, it is imperative to first focus on plant-level interactions. Under field conditions, turnip aphid (*Lipaphis erysimi*), green peach aphid (*Myzus persicae*) and cabbage aphid (*Brevicoryne brassicae*), are known to exhibit different feeding preferences for canola plant structures. Feeding preferences might directly affect aphid demography and/or allelochemical (glucosinolates) composition, being that glucosinolate profiles might vary within the plant and affect aphid quality through sequestration (turnip and cabbage aphid) or excretion (green peach aphid). As a result of within plant variation, different degrees of sources or sinks may exist within a canola plant and indirectly impact natural enemies that use canola as a resource. Therefore, the goal of this thesis was to determine tritrophic interactions occurring between canola, aphids and a key predator in this system, *Hippodamia convergens*. Specifically, we were interested in whether feeding location of aphids within the canola plant canopy directly affects aphid demographics and aphid quality (allelochemicals composition) under predator-free conditions. In addition, we sought to quantify the indirect implications of prey feeding location on life history response of immature *H. convergens* in no-choice bioassays.

Chapter 2 outlined an experiment evaluating population growth rates and glucosinolate compositions of aphids restricted to specific locations within a canola plant. Specifically, two specialists, turnip and cabbage aphid, and a generalist, green peach aphid, were restricted to feeding on vegetative vs. reproductive tissues of the canola plant under predator-free conditions.

Turnip, cabbage, and green peach aphid population growth rates were equal when restricted to feeding on vegetative plant parts, yet specialist turnip and cabbage aphids displayed significantly higher growth rates than generalist green peach aphid when restricted to reproductive tissues. Furthermore, glucosinolate composition was significantly higher in reproductive structures versus vegetative structures regardless of previous exposure to any of the aphids used in this study. Higher glucosinolate concentrations in reproductive tissue directly affect glucosinolate profiles of the specialist cabbage aphid. Overall, this study suggests that when restricted to vegetative tissues with lower glucosinolate levels, aphid population growth rates are similar across all three species. However, when aphid populations were restricted to feeding on glucosinolate-rich reproductive tissues, specialist cabbage aphid not only outperformed (higher growth rates) generalists green peach aphid, but also had greater concentrations of glucosinolates and thus, added protection towards predators. Previous research shows that glucosinolate acquisition varies between generalist and specialist aphids (Weber 1985, Pratt et al. 2008, Hopkins et al. 2009, Kazana et al. 2007). In our study, the levels of aliphatic and indolic glucosinolates in the specialist cabbage aphid were higher on reproductive structures. Reproductive parts of the canola plant are therefore serving as a source of glucosinolate for specialist cabbage aphid, since higher degrees of glucosinolate acquisition by specialists increases their toxicity towards generalist natural enemies (Kos et al. 2011).

In Chapter 3, we used sensitivity and LTRE demographic analyses to model the elasticity and contributions to variation in rates of increase (λ) of individual specialist cabbage aphids restricted to different canola structures. These studies were used to determine the extent feeding location of individual aphids in canola affects demographic parameters such as fecundity and longevity. Results indicate that finite rates of increase (λ) were higher ($P < 0.05$) when aphids

were restricted to reproductive tissues compared to vegetative tissues, further confirming the findings in Chapter 2. Additionally, cabbage aphids restricted to reproductive tissue exhibited shorter generation times and higher reproductive rates than those restricted to vegetative tissues. Prospective analyses showed that there was a nymph-skewed stable stage distribution where elasticity values revealed that λ is most sensitive to future changes in stasis (adults staying in the adult stage) and mortality of adults for all feeding locations. Overall, retrospective analyses indicated that contributions from growth of nymphal stage 2 to 3, 3 to 4, and 4 to adult accounted for nearly all of the variation in λ between the treatments, but adult fecundity was driving population dynamics. Hence, when restricted to glucosinolate rich reproductive tissue (Chapter 2) in the absence of predation, specialist cabbage aphids not only had greater added protection from predators, but also higher fecundity rates and shorter generation times than cabbage aphid restricted to vegetative tissues. Adult longevity is compromised when cabbage aphids are restricted to reproductive tissues, which is likely due to the higher concentration of indolic glucosinolates in these plant structures (Chapter 2). Consequently, indolic glucosinolates can negatively affect specialist aphid performance (Kos et al. 2011). These demographic studies are novel contributions to our understanding of how within-plant interactions can directly affect cabbage aphid demographics.

Last, in a no-choice laboratory experiment (Chapter 4), *H. convergens* life history responses were evaluated based on the prey suitability of a specialist cabbage aphid and a generalist green peach aphid that previously fed on different canola plant structures. Results indicate that feeding location by canola aphids had no effect on overall *H. convergens* survival and pupal weights; however, aphid feeding location affected developmental rates ($P < 0.0001$) and overall consumption by developing larvae ($P < 0.0001$). Specifically, *H. convergens* larvae

that fed on green peach aphids reared on vegetative tissue had faster developmental rates than larvae restricted to a diet of aphids reared on canola reproductive tissues. Additionally, daily consumption rates were higher for larvae feeding on aphid control diets, followed by green peach aphid and then cabbage aphid ($P < 0.0001$). Aphid feeding location only affected *H. convergens* consumption rates when feeding on the specialist cabbage aphid; consumption was lower for larvae feeding on cabbage aphid that were previously restricted to the reproductive parts of the canola plant. When predators fed soybean aphids were compared to those that were fed canola aphids, developmental times on soybean aphids were similar to or slower than canola aphids fed on reproductive and vegetative plant parts, respectively. However, pupal weights were higher for lady beetles on soybean aphid diet. Overall, our data suggests that *H. convergens* larvae are capable of surviving exclusively on a diet of canola-reared aphids, although adult fitness (based on body weight) is compromised. Interestingly, aphid-feeding location within the canola plant did not affect pupal mass but directly affected larval consumption and developmental rates. Tradeoffs between body weight, developmental rate and total consumption within various diet treatments were observed in this study. Our findings have potential important implications regarding the introduction of canola in the landscape. Specifically, immature lady beetles are restricted to prey patches that were maternally selected by ovipositing females at a local scale (within-field level) due to their limited dispersal capacity (Evans 2003). In the context of source-sink relationships, recruitment can play an integral part in determining the impact of adding new resources to predator-prey system. In other words, gravid females can determine larval fitness through searching decisions and oviposition decisions (Evans 2003). Our study suggests that at a landscape-scale novel prey offered by canola can influence adult fitness by negatively affecting adult size, whereas at a plant-scale reproductive canola plants support greater amounts of

specialist aphids that are less suitable to immature lady beetles (Chapter 2) and directly influence consumption and development. Our project may be the first study that has evaluated how feeding location of aphid prey might impact existing natural enemy communities using canola as a resource.

Current monitoring programs in canola in the south central US do not take into account aphid distributions within the canola canopy, current natural enemy communities within the landscape or the potential effects of glucosinolates on those natural enemy communities. This thesis provides novel contributions that further shape our understanding of how within-plant interactions can affect aphid populations and natural enemy communities in newly introduced canola systems. Futures studies regarding the effects of feeding location on aphid populations in the presence of predation and aphid competition are required. Additionally, intraguild predation, fecundity trials, diet quality, ovipositional behavior studies, and choice studies for lady beetles and other predators in the canola system need further investigation to fully understand the effects of allelochemicals on the third trophic level. The study of such plant-insect interactions will ultimately enable us to develop best management practices that may conserve natural controls within winter canola fields in the South Central US.

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