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Spatial and Temporal Dynamics of Predator-Prey Interactions in Winter Wheat

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SPATIAL AND TEMPORAL DYNAMICS OF PREDATOR-PREY INTERACTIONS IN WINTER WHEAT

DISSERTATION

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Agriculture, Food, and Environment at the University of Kentucky

By
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2015

ABSTRACT OF DISSERTATION

SPATIAL AND TEMPORAL DYNAMICS OF PREDATOR-PREY INTERACTIONS IN WINTER WHEAT

Aphids (Hemiptera: Aphididae) are pests of multiple cropping systems, primarily due to the viruses they vector and direct crop damage that is exacerbated by their rapid population growth. In Kentucky, grain aphids (*Rhopalosiphum padi* and *Sitobion avenae*) cause significant yield loss to winter wheat as vectors of Barley Yellow Dwarf virus (BYDV), prompting the routine application of insecticides. Coupled with growing human populations and decreasing arable land, it is increasingly evident that biological control services provided by natural enemies represent a viable long-term management option.

Aphids are preyed upon by a diverse array of predators that can be exploited in conservation biological control. I designed a field experiment to monitor dispersal into and out of wheat fields, and how these movements were affected by the surrounding habitat. Analysis revealed there are significant movements of *R. padi* into the wheat in the fall, and *S. avenae* in spring, and that these movements are slowed down by forested edges. Natural, field-bordering weed strips were used as a conservation biological technique to enhance predator populations. Results showed that while weed strips did not affect the yield of the crop, aphid abundance, or BYDV incidence, it did significantly increase the abundance of natural enemies. Dominant predators included Coccinellidae, Anthocoridae, Chrysopidae larvae, and Braconidae.

Using molecular gut-content analysis, I screened multiple species of predators and found strong trophic linkages between aphids and *Orius insidiosus* and multiple species of coccinellids, namely *Coccinella septempunctata* and *Coleomegilla maculata*. In aphidophagous systems, intraguild predation (IGP) can interfere with the biological control potential so I also screened coccinellids for IGP using newly designed primers. To identify intraguild prey DNA in coccinellids, I designed species-specific primers for *C. maculata* and *C. septempunctata* to use in PCR-based molecular gut-content analysis. Results revealed high frequencies of IGP between coccinellids that are significantly higher in weed strip plots. However, I observed no detectable impact on aphid predation during these

increased times of IGP, suggesting it does not interfere with biological control of aphids in this system. I discuss the role of weed strips in winter wheat as part of an integrative pest management strategy.

Key words: aphids, generalist predator, winter wheat, biological control

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SPATIAL AND TEMPORAL DYNAMICS OF PREDATOR-PREY INTERACTIONS IN WINTER WHEAT

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Chapter 1: Introduction

Agricultural intensification over the last century, while leading to high yields and increased food production, has also contributed to a range of negative ecological consequences, including losses in biodiversity, pollution, and erosion (Matson et al. 1997, Foley et al. 2005, Robertson et al. 2014). To maximize the ecosystem services provided by agriculture, we must understand how to sustainably increase crop yields in agroecosystems (Power 2010). This requires knowledge of how the ecological processes interact in these highly complex agroecosystems (Robertson et al. 2014). Conservation biological control aims to increase one particular ecosystem service, pest regulation, by promoting the impact of natural enemies in the system (Debach and Rosen 1991, Fiedler et al. 2008). This can be done through habitat manipulation, which provides resources such as pollen, nectar (Eubanks and Denno 2000), physical refugia, or alternative prey and hosts (Landis et al. 2005). In addition to providing pest management services, habitat manipulations can also provide other services such as biodiversity conservation, waste water treatment, and weed suppression (Fiedler et al. 2008). On-farm management can have substantial impacts on both the landscape- (Tscharntke et al. 2007a) and the farmscape-level (Collins et al. 2002), promoting invertebrate biodiversity and pest suppression. This dissertation will focus on habitat management at the farmscape-level.

Field margins can be used for promoting natural enemy abundance, particularly in cereal crops (Holland et al. 2008, Dong et al. 2012, Birkhofer et al. 2014). These areas of non-crop habitat can provide more vegetative diversity that will in turn be home to a more diverse group of generalist predators that can aid in pest suppression (Marshall and

Moonen 2002, Costamagna and Landis 2011). Generalist predators are good biological control agents because they can help reduce pest populations and thus the damage caused by herbivores in agroecosystems (Oelbermann and Scheu 2009). This is due, in part, to their ability to be the first colonizers of these highly disturbed environments, and survive on alternative prey (Murdoch et al. 1985, Chiverton 1987, Settle et al. 1996, Landis and Van der Werf 1997). In cereal crops, there exists a diverse group of natural enemies, both epigeal and aerial, such as Anthocoridae (Hemiptera), Carabidae (Coleoptera), Chrysopidae (Neuroptera), Coccinellidae (Coleoptera), Linyphiidae (Araneae) and Staphylinidae (Coleoptera) (Harwood and Obrycki 2005), therefore giving us the opportunity to enhance these predators already in the system.

In the field, direct observation of predator feeding events are difficult to observe due to their size and infrequency, and determining prey remains in the guts of predators is not always accurate, especially when soft-bodied prey are consumed or when the predators are liquid feeders. Therefore, a variety of molecular tools are helpful in identifying the food web of a system, such as enzyme electrophoresis, polyclonal and monoclonal antibodies, and polymerase chain reaction (PCR) to detect prey DNA (Symondson 2002, Sheppard and Harwood 2005). PCR-based molecular gut-content analysis is now a widely used tool for elucidating food webs in agroecosystems (e.g. Lundgren et al. 2014, Raso et al. 2014, Schmidt et al. 2014, Wallinger et al. 2014) and provides invaluable information about predator diets, intraguild predation, and cannibalism (Furlong 2015). Utilizing both field and molecular techniques maximizes our chances of teasing apart the ecological interactions in the system, and allows us to integrate the information into pest management recommendations (Chen et al. 2000).

This project will use molecular techniques, specifically PCR and enzyme-linked immunosorbent assay (ELISA), on arthropods in winter wheat. This data will give insight into the winter wheat-aphid-predator food web, as well as examine the spatial and temporal movement of an aphid-vectored virus, thus allowing us to make more accurate pest management recommendations.

In Kentucky, winter wheat is a valuable crop bringing in over $200 million annually (KYSGGA, 2013) and is an integral part of the state's unique crop rotation system. Winter wheat is double-cropped with corn and soybean, which results in higher yields and reduced pest problems. Most of Kentucky farmers practice conservation tillage, leaving at least 30% of the crop residue on the soil when planting (Holland 2004). This process has ecological and economic benefits, such as enhanced erosion control, nutrient cycling, and pest management, while still maintaining high yields (Halvorson et al. 2006, De Vita et al. 2007, Yau et al. 2010). Nonetheless, aphids are a major pest in cereals, mainly due to the staggering numbers they can reach in a season and their role as vectors of plant viruses (Blackman and Eastop 2007).

Two of the most agriculturally important aphid species occur in Kentucky winter wheat, the bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae), and the English grain aphid, *Sitobion avenae* (F.) (Hemiptera: Aphididae) (Rochow 1961, Blackman and Eastop 2007). Aphids have evolved host-alternating behaviors that allow them to better exploit plants (Dixon 1971), and will migrate between primary and secondary hosts throughout the year to complete reproduction (Dean 1974). While in many parts of the world *R. padi* is heteroecious migrating between a primary, woody host, and a secondary, herbaceous host (Dixon 1971, Dean 1974) it has no woody host in

Kentucky and will feed on Gramineae. *Sitobion avenae* is monoecious, spending the entire year on Gramineae (Leather 1993), such as cereals and pasture grasses. In areas with Mediterranean climates, such as Kentucky, both species of aphids are anholocyclic on winter wheat, so that only asexual female clones are produced (Blackman and Eastop 2007).

Rhopalosiphum padi and *S. avenae* are important vectors of Barley Yellow Dwarf virus (BYDV), causing substantial yield loss worldwide and resulting in routine, and sometimes unnecessary, insecticide applications (Irwin and Thresh 1990, Pike 1990). BYDV was first described as an infection in small grains in California in the 1950's (D'Arcy and Burnett 1995), however it was not realized until later that there are several strains of the virus and it was most likely a combination of these strains that comprised this initially described infection (Irwin and Thresh 1990, Halbert and Voegtlin 1995). Rochow (1970) characterized five strains of the virus based on its main vector, and they included MAV (*S. avenae*), RPV (*R. padi*), RMV (*Rhopalosiphum maidis* (Fitch)), SGV (*Schizaphis graminum* (Rondani)), and PAV (*R. padi* and *S. avenae*). Each strain of the virus is specifically vectored by particular aphid species (Irwin and Thresh 1990, Halbert and Voegtlin 1995); it relies entirely on aphids for its movement into crop fields and subsequent development of, and development on, plants. Luteoviruses, like BYDV, are transmitted by aphids in a circulative, nonpropagative way, so that once the virus infects its host, it moves through the aphid requiring recognition and transportation, but the virus does not infect or replicate in the aphids (Gray and Gildow 2003).

The circulative manner in which the virus is transported through the aphid from the gut to the hemocoel requires that these parts recognize the specific aphid vector and

allow transmission (Irwin and Thresh 1990). The aphid possesses luteovirus receptors on cells from the salivary glands to the gut, however the selectivity of these receptors most likely varies with the location and the strain (Gray and Gildow 2003). The processes of BYDV transmission are divided into four stages; virus transmission from the phloem of the host plant into the aphid, recognition and acquisition of the virus into the aphid gut, movement into the hemocoel, and finally transmission of the virus from the salivary gland of the aphid into a host plant. Once an aphid has acquired the virus, it is infected with BYDV for the remainder of its life and has the potential to infect healthy plants (Gildow et al. 2004).

Routine insecticide use is commonplace in Kentucky for control of aphids and BYDV, although widespread use is not sustainable when coupled with the aphids' rapid generation time and unique life cycle (Bass et al. 2014). Recently, pyrethroid resistance was found for the first time in *S. avenae* in the United Kingdom (Foster et al. 2014) indicating an urgent need to reduce the chemical inputs in cereal crops globally and investigate more sustainable options. Therefore, the objective of this dissertation is to explore conservation biological possibilities in winter wheat in western Kentucky. Specifically, I will examine the role of semi-natural habitats on the dispersal patterns of aphids and BYDV. Additionally, I will look at the effect of natural, field-bordering weed strips on aphids and their natural enemies. Lastly, using molecular gut-content analysis, we will study the aphid food web in relation to the weed strips in an effort to make biological control recommendations in this crop.

Chapter 2 : Semi-natural habitats in the farmscape affect immigration of cereal aphids

2.1 Abstract

Non-crop habitats in agriculture are important in promoting natural enemy abundance and diversity, and thus aid in pest suppression. Agricultural management can alter trophic interactions between predators and their prey, and landscapes with higher proportions of semi-natural habitats may experience lower pest pressure. Therefore, I looked at grain aphid dispersal around the edges of winter wheat fields in western Kentucky with fields consisting of various types of edges: road, forest, grass, water, or winter wheat. Aphids were sampled throughout the growing season (November – June) using aerial sticky traps, and a subset were screened for Barley Yellow Dwarf virus (BYDV) using enzyme-linked immunosorbent assay (ELISA). *Rhopalosiphum padi* was the predominant aphid species moving into the fields in the fall, and *Sitobion avenae* in the spring, although in significantly lower numbers. Fields bordered by forests had lower dispersal rates by both aphid species throughout the year. Winter wheat fields adjacent to other winter wheat fields had the highest rate of *S. avenae* movement, while grass edges had the highest rates of *R. padi* movement. I did not detect any edge effect on BYDV incidence in aphids. I propose that these differences in dispersal patterns are due to the seasonal differences in these aphid species, as well as natural enemy presence, and discuss this interaction in the context of biological control.

2.2 Introduction

Semi-natural habitats can promote natural pest suppression in agricultural systems (Tscharntke et al. 2007a) by contributing to the diversity and abundance of natural enemies which move into crops and provide biological control (Alhmedi et al. 2009, 2011). However, the global expansion of croplands, and specifically monocultures (Meehan et al. 2011), has led to losses in biodiversity (Foley et al. 2005). Agricultural intensification threatens these natural services, such as predation, pollination, or parasitism that are enhanced by diverse crop landscapes (Kremen et al. 2002, Krewenka et al. 2011). Within agroecosystems, conservation biological control provides a valuable opportunity to mitigate environmental degradation through habitat modification and enhanced diversification of the environment for the purpose of pest suppression (Debach and Rosen 1991, Landis et al. 2000, Gurr et al. 2004). As a result, the local habitat management scheme determines the abundance and diversity of biological control agents (Koh and Holland 2015) and it is becoming increasingly evident that the community of natural enemies in an agroecosystem is an important part of achieving this goal of pest suppression (Crowder et al. 2010)

In cereals, aphids (Hemiptera: Aphididae) are global pests as vectors of 28% of the world's known plant viruses (Hogenhout et al. 2008) and vector Barley Yellow Dwarf virus (BYDV), causing approximately 17% yield loss in non-outbreak years (Plumb 2002). In Kentucky winter wheat, the most damaging and important vectors are the bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae) and the English grain aphid, *Sitobion avenae* (F.) (Hemiptera: Aphididae) (Rochow 1961, 1969). Cereal aphid

populations fluctuate seasonally, forming a ‘multispecies complex’ (Vickerman and Wratten 1979, Brabec et al. 2014) of different aphid species on a crop. Aphids have evolved various types of life cycles such as host-alternating that allows them to better exploit plants (Dixon 1971). While in many parts of the world *R. padi* is heteroecious migrating between a primary, woody host, and a secondary, herbaceous host (Dixon 1971, Dean 1974) it has no woody host in Kentucky and will feed on Gramineae. *Sitobion avenae* is monoecious, spending the entire year on Gramineae (Leather 1993), such as cereals and pasture grasses. In areas with Mediterranean climates, such as Kentucky, both species of aphids are anholocyclic on winter wheat, so that only asexual female clones are produced (Blackman and Eastop 2007). In addition to parthenogenesis, aphids also have short generation times and telescoping generations (Kindlmann and Dixon 1989), traits which contribute to their complex lifestyle and ability to reach large populations quickly. Pyrethroid insecticides are routinely used to control for aphid pests, however coupled with widespread use and recent resistance shown in Europe, these practices are no longer sustainable (Bass et al. 2014). Additionally, such intensively managed crops and simple landscapes may lead to higher populations of aphids (Birkhofer et al. 2008, Diel et al. 2013), therefore agricultural management is important in controlling these pests.

Fortunately, a diverse group of natural enemies preys on aphids in cereals (Harwood and Obrycki 2005) and plays an important role in suppressing aphid populations (Schmidt et al. 2003). These include foliar-foraging Coccinellidae adults and larvae, Syrphidae larvae, Chrysopidae larvae (Wratten and Powell 1991, Schmidt et al. 2003), ground-dwelling spiders (Araneae), ground beetles (Carabidae), and rove beetles

(Staphylinidae), as well as a variety of parasitoids (Symondson et al. 2002, Schmidt et al. 2003). The local landscape composition heavily influences the abundances of these predators and parasitoids, such as hoverflies, ladybeetles, and carabids, which are all increased by semi-natural habitats (Tscharntke et al. 2005, Roume et al. 2011, Alignier et al. 2014). Additionally, reduced management intensity and increased vegetation complexity help to conserve web-building spiders, which contribute significantly to aphid biological control (Nyffler and Sunderland 2003, Diel et al. 2013). The movement of these mobile predators between crop and non-crop habitats during their lifetime can aid in pest suppression (Wratten et al. 2003, Werling and Gratton 2010), however ecosystem services can be influenced at multiple scales depending on the mobility of the predators (Tscharntke et al. 2005, Werling and Gratton 2010). There is also evidence that more complex agroecosystems increase both natural enemy populations and pest populations but only sometimes resulting in less damage by pests (Van Emden 1990, Marino and Landis 1996, Thies et al. 2005) so understanding these dispersal processes is important to maximizing biological control potential.

Given these management issues with cereal aphids, this project sought to study the dispersal patterns of aphids relating to local landscape (hereafter referred to as farmscape) characteristics. I predict that semi-natural habitats, such as forests, will slow aphid dispersal, while adjacent crops such as winter wheat and fescue will increase *R. padi* and *S. avenae* movement.

2.3 Methods

2.3.1 Field Site

Aphid movement was monitored in conventionally managed winter wheat fields during the 2012-2013 field season at the University of Kentucky Research and Education Center (UK-REC) in Princeton, Kentucky, USA (GPS coordinates 37.1 N, 87.9 W). Soft red winter wheat (*Triticum aestivum* L.) (Pembroke variety, 2012, Clements Ag Supply, Springfield, Kentucky, USA) was planted in October 2012 in accordance with standard agronomic practices for the region using a John Deere 1590 Planter (Deere & Company, Moline, Illinois, USA) (planting rate: 3.15 seed/m² in 0.191 m rows). Nitrogen was applied twice, at 18.14 kg/acre on the first application (February 2013) and 36.29 kg/acre on the second application (March 2013). Aphid monitoring commenced two weeks after planting at winter wheat emergence (Feekes scale 1-2) and continued throughout the growing season, until two weeks prior to harvest on 21 June 2013. Four fields were selected (Fig. 2.1), each containing eight or ten individual aphid traps, dependent on field size (trap description below). All fields were at least 1 km apart to avoid spatial autocorrelation between replicates.

2.3.1.1 Aphid Sampling

Aerial aphid traps (Fig. 2.2) were placed around each field and designed to intercept some flying and wind-dispersed aphids. Metal fence posts (1.2 m tall) were fixed in the ground to support a PVC pipe (diameter: 0.03m, height: 2 m) on which removable, double-sided sticky traps were placed (0.3m x 0.3m). Vertical positioning of

the traps was selected to intercept aphids given their movement typically occurs between 0 and 3 m (e.g. Johnson 1957; Taylor 1974). Traps were made with aluminum insect screening (0.3 m x 0.3 m; mesh size: 1 cm x 1 cm) (Phifer Incorporated, Tuscaloosa, Alabama, USA), pulled taught and sprayed with Tangle-Trap© Sticky Coating spray (The Tanglefoot Company, Grand Rapids, Michigan, USA). Each trap was placed inside the field, 1 m from the edge and approximately 50 m from adjacent traps. These were collected weekly between November 6 and December 19, and again from March 4 through June 4. During the winter (Jan 3, Jan 18, Jan 31) traps were collected every two weeks and no sampling was undertaken in February due to adverse weather conditions. Traps were left *in situ* for the duration of the sample period (7 or 14 days) after which they were removed and transferred to the freezer for subsequent counting and virus analysis (described below). Reduced sampling was undertaken during the winter because both aphid activity and virus incidence are significantly lower within the region (K.A. Kowles, pers. obs.).

2.3.2 Virus Incidence

A random subset of alate aphids intercepted by the traps was removed, identified, and individually placed in 1.5 µL microcentrifuge tubes. Only aphids from the outside of traps were used (N=5 per trap per sample period) unless aphids were too scarce during that time. Triple-antibody sandwich Enzyme-linked Immunosorbent Assay (ELISA) was used to screen for the presence or absence of BYDV using Barley Yellow Dwarf virus-PAV kits (Agdia Incorporated, Elkhart, Indiana, USA). Humid boxes were made to create environmental conditions conducive to ELISA. Each humid box consisted of a plastic

Tupperware® box (L 20.3 cm x W 15.9 cm x H 9.6 cm) (Tupperware Corporation, Orlando, Florida, USA) with a wet paper towel; all incubation steps were conducted in the humid box. Between each step, all liquid in the microtiter plate was ejected and the plate was washed with Phosphate Buffered Saline solution with Tween ® (polysorbate 20 sorbitan monolaurate (PBST, ACC0011, Agdia) to remove excess material without disrupting the antigen/antibody binding process. Individual 96-well microtiter plates were coated with anti-BYDV-PAV capture antibody (CAB 27500, Agdia) and placed inside the humid box; following this step, plates were washed three times, and for all subsequent steps plates were washed eight times. The aphids were diluted with general extraction buffer (ACC 00111, Agdia) and homogenized using sterilized pellet pestles (Kimble-Chase Kontes™, Rockwood, Tennessee, USA); 100 µL of each homogenized sample was added to two wells on the microtiter plate. In addition, positive (LPC 27500, Agdia) and negative (LNC 27500, Agdia) controls were added to each plate. The sample was incubated overnight at room temperature, after which 100 µL of detection antibody (SRA27500, Agdia) and enzyme conjugate (ECA 27500, Agdia) were added. After two h incubation, the plate was washed and 100 µL of purinenucleosidephosphorylase (PNP) buffer and PNP tablets (ACC0011, Agdia) were added. This final step was conducted in the humid box and placed in the dark for 1 h to allow the color reaction to develop. Finally, the absorbance was read at 405 nm using a Thermo Labsystems Multiskan Plus © spectrophotometer (Fisher Scientific Company LLC, Pittsburgh, Pennsylvania, USA). The average of the two readings were taken for each sample; a sample was considered positive for BYDV if it was greater than three standard deviations above the average of

the negative controls (after Frey et al. 1998).

2.3.2 Statistical Analysis

Number of aphids collected per 24 h was calculated by dividing the number caught on each trap by the duration of the trapping period in days. I used a repeated measures multivariate analysis of variance (PROC GLM in SAS 9.3) with a Poisson distribution to examine the effect of edge type on species and aphid number captured by the traps. Regression analysis was conducted with mean temperatures for the sample period and each species of aphids moving in and out of the fields. To examine the seasonal effects of BYDV, aphids were grouped by month for analysis and virus incidence was measured by the proportion of aphids testing positive for BYDV and arcsine square root transformed.

2.4 Results

2.4.1 Seasonal Dynamics

A total of 5,629 aphids were intercepted across all traps over the growing season, with significantly more aphids immigrating into (N = 3,172) versus emigrating out of (N = 2,432) ($F_{1,1220} = 25.97$, $P < 0.0001$) winter wheat fields. There were significantly more total *R. padi* (N = 4,494) than total *S. avenae* (N = 1,135) ($F_{1,1220} = 239.36$, $P < 0.0001$).

There was significant temporal variation in the movement of both species of aphids ($F_{3,2520} = 453.7$, $P < 0.0001$); *R. padi* immigration ($F_{6,2533} = 379.37$, $P < 0.0001$) and emigration ($F_{6,2533} = 325.03$, $P < 0.0001$) rates peaked in November and December (Fig. 2.3a, b) and *S. avenae* immigration ($F_{6,2533} = 121.42$, $P < 0.0001$) and emigration ($F_{6,2533} = 199.99$, $P < 0.0001$) rates peaked in April and May (Fig. 2.4a, b).

Temperature data was collected using Kentucky Mesonet monitoring stations, with temperatures ranging from a low of 7.1 °C in February to a high of 25.3 °C in June. The mean number of *R. padi* moving into or out of fields was not significantly related to the temperature over the growing season (immigrating: $F_{1,16} = 1.06$, $P = 0.372$, $R^2 = 0.004$; emigrating: $F_{1,16} = 1.87$, $P = 0.191$, $R^2 = 0.049$). Conversely, *S. avenae* captured on traps was significantly correlated with temperature, with increased movement in the spring as the temperatures rose (immigrating: $F_{1,16} = 11.55$, $P = 0.004$, $R^2 = 0.383$; emigrating: $F_{1,16} = 16.13$, $P = 0.001$, $R^2 = 0.471$).

BYDV infection in the total aphid population ranged from 5% in March 2013 to 21% in January, but infection rates did not differ significantly between *R. padi* and *S. avenae* ($F_{1,536} = 2.24$, $P = 0.135$). There were no effects of edge type on viral rate ($F_{4,536} =$

0.54, P = 0.708), but there was significant temporal variation ($F_{6,536}$ = 2.84, P = 0.01) (Fig. 2.5) with BYDV incidence peaking in January for *S. avenae* and *R. padi*.

2.4.2 Edge Effects

There were five different edge types (Fig. 2.1) and those had a significant effect on both aphid species' movement ($F_{4,2523}$ = 23.94, P < 0.0001) with significant effects of date ($F_{17,2523}$ = 250.24, P < 0.0001) and field ($F_{3,2522}$ = 39.14, P < 0.0001), as well as interactions with date and field ($F_{9,7569}$ = 6.02, P < 0.0001) and date and edge ($F_{12,7569}$ = 17.87, P < 0.0001). Forested edges had the lowest rates of movement for *R. padi* moving into and out of the fields, while edges bordered with grasses had the highest rates. Conversely, fields bordered by winter wheat had the highest rates of *S. avenae* movement on the outside of fields, and those bordered by grass had the lowest rates.

2.5 Discussion

This study showed that cereal aphids, *Rhopalosiphum padi* and *Sitobion avenae*, colonize winter wheat at distinct times over the season and this movement is significantly affected by the local farmscape. Using aerial aphid traps, I found lower rates of aphid movement in fields bordered by forests compared to those bordered by winter wheat, which had the highest rates of aphid movement. Additionally, winter wheat fields bordered by grasses affected *R. padi* and *S. avenae* differently; it increased *R. padi* movement while decreasing *S. avenae* movement.

My results on BYDV incidence in Kentucky winter wheat are consistent with other North American studies on aphid vectors indicating rates in non-outbreak years between 0 - 17% (Halbert and Voegtlin 1995, Plumb 2002). Infection rates of aphids in the 2012-2013 growing season in western Kentucky ranged from 5-21% (Fig. 2.5). *Rhopalosiphum. padi* is widely considered the most important BYDV vector because of its numbers and vectoring ability (Halbert and Pike 1985), but I did not detect any difference in BYDV incidence between *R. padi* and *S. avenae*, although there were significantly more *R. padi* overall, which had populations peaking in the fall. While I did not detect any edge effects on virulence, the significantly lower dispersal rates of *R. padi* along forested edges is still crucial for BYDV management. The greatest yield losses are from winter wheat infected at early growth stages and this primary infection is determined by the number of migrating aphids, the proportion infected, and the length of time the crop is susceptible (Tatchell et al. 1988). Therefore, if we can reduce one of these variables, it can make a substantial impact on yield loss caused by BYDV.

This study found no measurable relationship between *R. padi* movement and temperature, however there was a significant relationship with *S. avenae*. The lack of a consistent pattern between the two species is not surprising; significant correlations have been found between aphids and temperature, but interactions between aphid species, host plants and natural enemies complicate these relationships (Dewar and Carter 1984). In the case of *R. padi*, this particular species has a very strong 'migratory urge' and will initiate take off even in adverse weather conditions (Walters and Dixon 1983).

Edge had a significant effect on the dispersal rates of aphids, and it differed between species. While winter wheat fields bordered with forests had the lowest rates of aphid movement of both aphid species studied, the highest rates for *R. padi* were fields bordered with grasses and for *S. avenae* fields adjacent to other winter wheat fields. These differences may be due to the host-alternating behavior of these aphids; *R. padi* may be moving from drying summer grasses and moving into the winter wheat, which accounts for the high dispersal rate, while *S. avenae* is moving between winter wheat fields. Forests, on the other hand, may act as a physical barrier to dispersal, especially since aphids are not good fliers (Dixon 1985). My results agree with those of Alignier et al. 2014 who found wooded areas were negatively correlated with aphid populations, and positively correlated with increased aphid predators. While I did not measure natural enemy movement in this study, they may have contributed to the lower rate of aphids on forested edges, specifically coccinellids (Gardiner et al. 2009b, Woltz and Landis 2014). The area between forests and agricultural crops are highly traveled by arthropods, and as a result are highly affected by edges (Fahrig 2003). Mobile predators can move through multiple crops within their lifetime (Wratten et al. 2003), therefore edge effects can

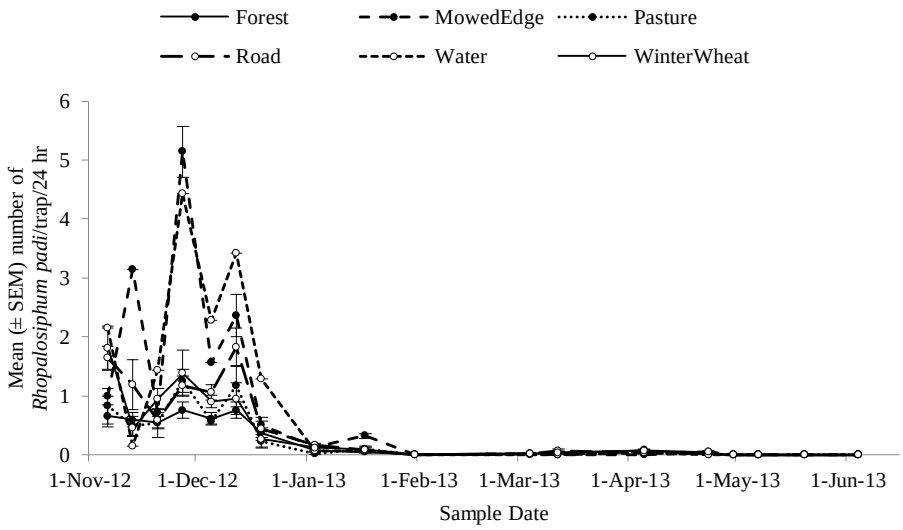
benefit the crop through aiding in the dispersal of predatory arthropods (Roume et al. 2011).

Complex landscapes, those with a higher percentage of wooded areas and hedgerows, have significantly higher rates of parasitism on pest insects (Marino and Landis 1996), but in some cases also higher rates of aphid colonization (Thies et al. 2005). This may be due to the increased number of trees available for host-alternating aphids, which is true in Europe but not here in Kentucky (Roschewitz et al. 2005). Therefore, these unique aspects of each cropping systems must be studied on an individual level, especially since the spatial arrangement of crops and their adjacent border habitats play an important role in the population dynamics of pest species (Kennedy and Storer 2000, Fievet et al. 2007). This study shows that semi-natural habitats can help reduce aphid dispersal into winter wheat fields through a variety of mechanisms that most likely involve the local landscape and natural enemies, and further studies of these mechanisms could help to maximize the biological control potential.

Figure 2.1 Images of the four winter wheat fields used during the 2012-2013 season. Field 1 (a) is 9.42 acres and has two edges with roads and two with grass. Field 2 (b) is 13.5 acres with two edges of forest, one of winter wheat and one of grass. Field 3 (c) is 6.1 acres with winter wheat on two edges, a road on one and a small pond on the last. Field 4 (d) is 11.6 acres and has two edges of forest, one with road and one with grass. Image data: Google 2014.

Figure 2.2. Aerial aphid trap designed to catch aphids dispersing in and out of winter wheat fields. Traps were made of PVC and aluminum mesh, and sprayed with Tanglefoot©. Traps were left up *in situ* for 7 or 14 days, and aphids counted and screened for Barley Yellow Dwarf virus (BYDV).

a.



b.

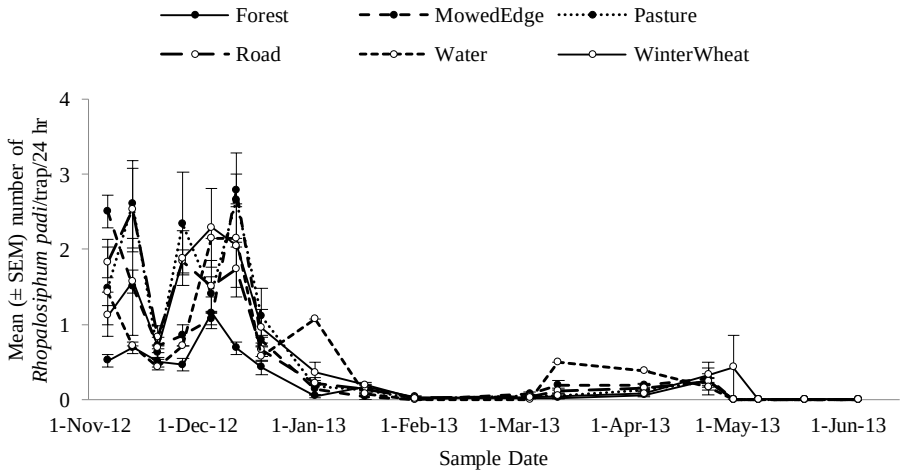


Figure 2.3. a. *Rhopalosiphum padi* (mean ± SEM/24 h) captured on the inside of the field bordered by different edge types (forest, grass, road, water, winter wheat) over the 2012-2013 growing season in winter wheat. b. *R. padi* (mean ± SEM/24 h) captured on the outside of the field bordered by different edge types (forest, grass, road, water, winter wheat) over the 2012-2013 growing season in winter wheat.

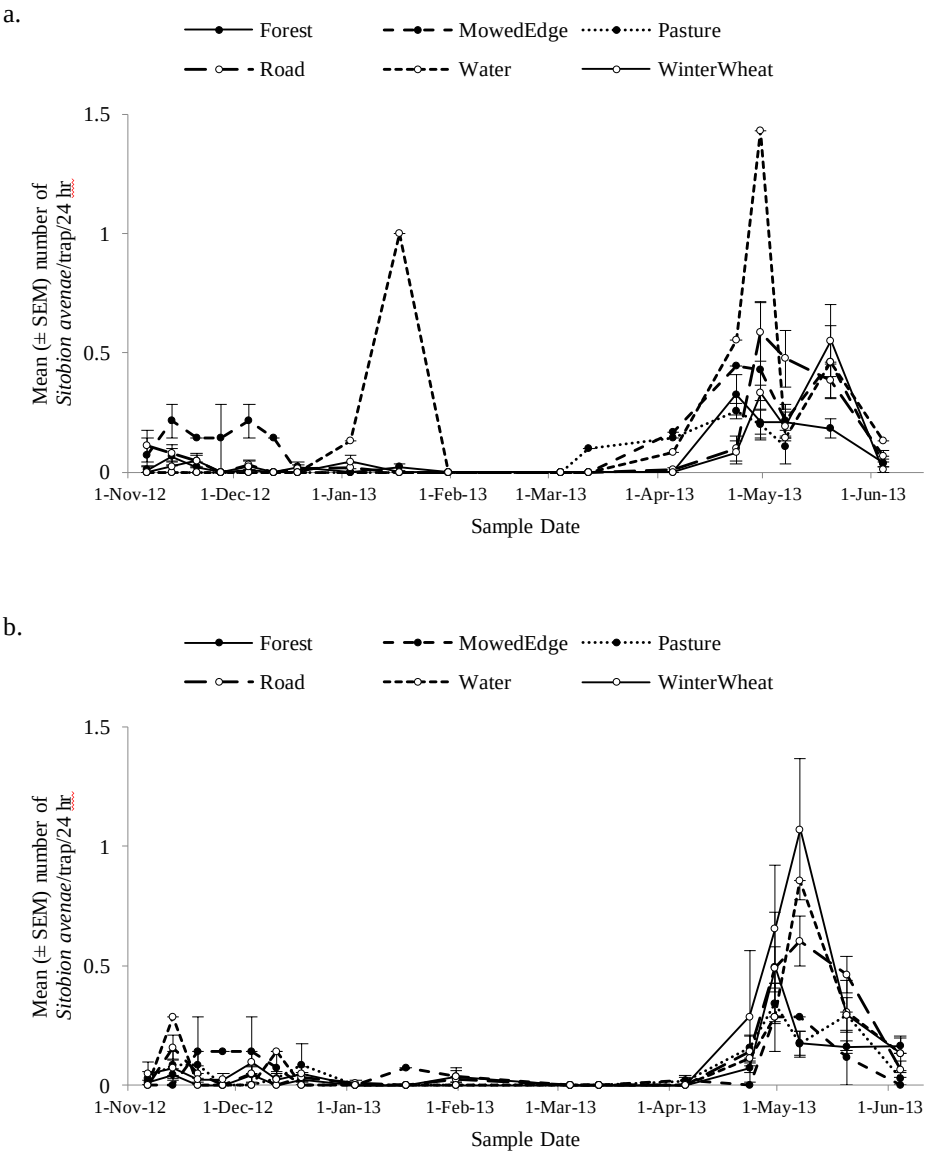


Figure 2.4. a. *Sitobion avenae* (mean ± SEM/24 h) captured on the inside of the field bordered by different edge types (forest, grass, road, water, winter wheat) over the 2012-2013 growing season in winter wheat. b. *S. avenae* (mean ± SEM/24 h) captured on the outside of the field bordered by different edge types (forest, grass, road, water, winter wheat) over the 2012-2013 growing season in winter wheat.

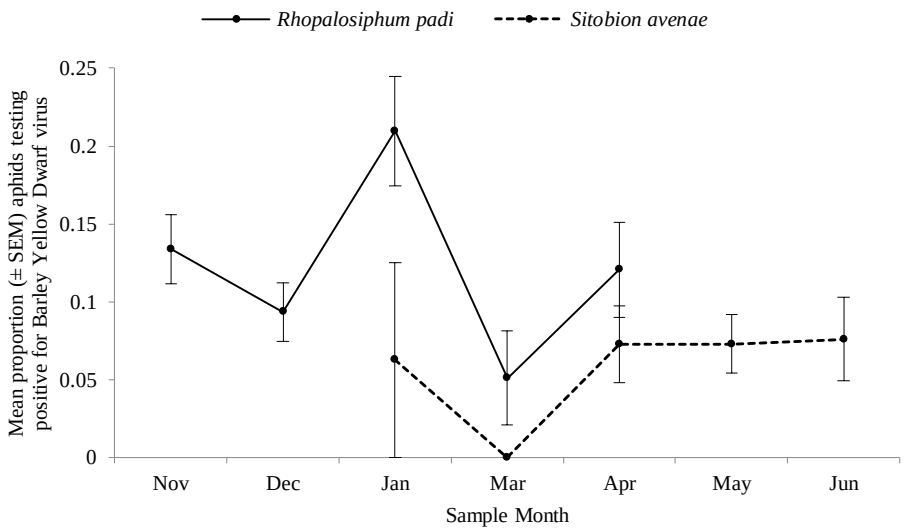


Figure 2.5. Mean (± SEM) proportion of aphids, *Rhopalosiphum padi* and *Sitobion avenae*, testing positive for Barley Yellow Dwarf virus (BYDV).

Chapter 3 : Field-bordering weed strips enhance aphidophagous predators in winter wheat

3.1 Abstract

Natural enemies in agroecosystems provide valuable ecosystem services through pest regulation, and their populations can be enhanced through conservation biological control and habitat manipulations. Field margins in cereal crops have been studied extensively for aphid control, in an attempt to reduce yield loss by these plant virus vectors. In Kentucky winter wheat, aphids vector Barley Yellow Dwarf virus (BYDV) and cause substantial yield loss. Therefore, I set out to test the effects of natural, field-bordering weed strips on natural enemy and pest populations in wheat, as well as BYDV incidence in pests and plants. The experiment was conducted over two growing seasons in 2010-2011 and 2011-2012 in replicated fields, using natural weeds. Aphid populations or BYDV incidence were not different between treatments, but fields with weed strips had significantly higher natural enemy populations, specifically Anthocoridae, Braconidae, Coccinellidae, and Chrysopidae. These results suggest that aphids and their natural enemies respond differently to habitat manipulations, and increased natural enemy abundance does not directly lead to increased pest control.

3.2 Introduction

Agricultural biodiversity can lead to increased pest suppression by natural enemies (e.g. Altieri 1999, Gurr et al. 2003). However, intensification of agricultural practices has led to losses in biodiversity (Foley et al. 2005, Bianchi et al. 2006) that can negatively impact natural pest control. Natural pest control has environmental benefits such as reduced chemical inputs and landscape conservation (Bianchi et al. 2006). Consequently, conservation biological control, the manipulation of the environment to enhance natural enemies for pest suppression, is crucial as we seek to combat these losses (Debach and Rosen 1991). These naturally occurring enemies provide a valuable ecosystem service by contributing to pest suppression (Losey and Vaughan 2006) and we can further increase their role through habitat manipulation (Landis et al. 2000, Gurr et al. 2004) by providing alternative resources such as nectar and pollen (Eubanks and Denno 2000), refugia, and alternative prey or hosts (Landis et al. 2005).

The landscape surrounding agricultural fields can directly influence the abundance and diversity of natural enemies in the crop (Schmidt and Tscharntke 2005, Tscharntke et al. 2005, Alignier et al. 2014). There is evidence that crop pests are controlled by natural enemies that live in the crop year-round, or migrate between the crop and non-crop areas (Holland et al. 2012) as they utilize resources in both habitats (Rusch et al. 2010). Therefore, the type of non-crop habitat can influence the natural enemy population and biological control services provided at both the landscape level (Tscharntke et al. 2007b), as many natural enemies can disperse long distances, and local level (Collins et al. 2002, Meek et al. 2002, Sarthou et al. 2014). Specifically, field

margins can provide an increased level of vegetative diversity that can be home to a more abundant (Holland et al. 2008, Dong et al. 2012), diverse assemblage of predators (Marshall and Moonen 2002, Birkhofer et al. 2014) which can then provide top-down control of insect pests (Costamagna and Landis 2011). Farm management programs that promote the use of unsown field margins can successfully contribute to conservation biological control (Holland et al. 2008, Birkhofer et al. 2014).

Generalist predators (Symondson et al. 2002) can reduce pest populations in agroecosystems, in part due to their ability to survive in highly disturbed environments and on alternative prey (Murdoch et al. 1985, Chiverton 1987, Landis and Van der Werf 1997). In cereal crops, there exists a diverse group of natural enemies, both epigeal and aerial, that routinely feed on pest aphids such as Anthocoridae (Hemiptera), Carabidae (Coleoptera), Chrysopidae (Neuroptera), Coccinellidae (Coleoptera), Linyphiidae (Araneae) and Staphylinidae (Coleoptera) (Harwood and Obrycki 2005). The most effective biological control may be achieved by a diversity of natural enemy guilds so that pests are attacked throughout their lifetime (Holland et al. 2008). The composition of these guilds is determined by a variety of factors, such as the management of the crop and the type and proportion of crop and non-crop habitats (Thies and Tscharntke 1999).

In grain crops, cereal aphids (Hemiptera: Aphididae) vector Barley Yellow Dwarf virus (BYDV) in a circulative, persistent manner. Once the virus is acquired, an aphid will be infected for life (Irwin and Thresh 1990). While over twenty species of aphids are capable of vectoring BYDV (Halbert and Voegtlin 1995), two of the most crucial species are the bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae) and the English grain aphid, *Sitobion avenae* (F.) (Hemiptera: Aphididae) (Rochow 1969, Plumb

2002). Aphids are capable of exponential growth (Blackman and Eastop 2007) which results in large, within-field aggregations with a strong but ephemeral spatial pattern (Winder et al. 1999, 2001, 2005). Given the spatial heterogeneity of aphids within a crop and its relationship to economic loss, understanding this spatial pattern could improve biological control possibilities (Winder et al. 1999). Additionally, the spread of aphid-vectored viruses and natural enemies are correlated through complex multi-trophic interactions (Garzon et al. 2015). Predation may help slow the spread of these viruses (Moore et al. 2010) so manipulations that focus on predator enhancement may contribute to overall biological control.

Two years of extensive field work were conducted in Kentucky winter wheat to evaluate the effectiveness of natural, field-bordering weed strips that can be used as a no-input, no-cost habitat manipulation to increase natural enemy abundance. I focused on foliar natural enemies, which constitute the key naturally occurring predators and parasitoids of cereal aphids (Ramsden et al. 2015), and epigeal spiders in the family Linyphiidae which capture cereal aphids in their webs and prey on them (Sunderland et al. 1986, Harwood et al. 2001b). I also wanted to examine the spatio-temporal relationship between aphid vectors and BYDV, and how virus incidence may be affected by natural field boundaries. My hypothesis is that weed strips will help increase the abundance of aphid natural enemies, which will consequently have a larger pest suppression effect and will contribute to the overall yield of the wheat.

3.3 Materials and Methods

3.3.1 Field Site

Fields of soft red winter wheat, *Triticum aestivum* L. (Pembroke variety, 2010, Clements Ag Supply, Springfield, Kentucky, USA), were grown during the 2010-2011 and 2011-2012 seasons at the University of Kentucky Research and Education Center (UK-REC) in Princeton, Kentucky, USA (GPS coordinates 37.1 N, 87.9 W). Four fields (125 m x 55 m) were planted each year, on October 13, 2010 and October 18, 2011, in accordance with standard agronomic practices for the region (planting rate: 3.15 seed/m^2 in 0.191 m rows) using a John Deere 1590 Planter (Deere & Company, Moline, Illinois, USA). No insecticides, herbicides, or fungicides were applied to any of the fields. Nitrogen was applied twice, at 18.14 kg/acre on the first application (February 21, 2011 and February 20, 2012) and 36.29 kg/acre on the second application (March 7, 2011 and March 5, 2012).

3.3.1.1 Wheat Harvesting

At the end of the growing season, wheat fields were harvested on June 20, 2011 and June 8, 2012 using a Wintersteiger combine with a 1.52 m header (Wintersteiger AG, Ried, Austria). In 2011, due to an equipment malfunction, half of the fields were harvested with a John Deer 4425 combine with a 4.57 m header (Deere & Company, Moline, Illinois, USA). Grain was weighed using a Parker weigh cart (Model 1500R, Parker Industries, Jefferson, Iowa, USA) and a Avery Weigh-Tronix scale (Avery Weigh-Tronix LLC, Fairmont, Minnesota, USA).

3.3.1.2 Weed Strips

To assess the effects of bordering weed strips on natural enemies and pest control, each field was divided into two treatments, a control plot (unmanipulated) and a weed strip plot. The treatments were separated by a 15 m winter wheat buffer in the center of the field. Weed strips were created by leaving a 3 m strip of uncultivated soil around the field edge to allow for natural weed growth. Each field was further surrounded by a winter wheat buffer zone to avoid edge effects.

3.3.2 Sampling Effort

Within each field, a grid system was established, creating 32 equally-sized subplots measuring 13.75 m x 13.75 m. Each subplot was sampled approximately every two weeks during the spring (March-June) for aphids and predators. Ten figure-eight sweeps were conducted in each plot, and samples were transferred into whirl-pack bags filled with alcohol and returned to the laboratory for subsequent identification. In parallel, foliar predators were hand-collected from the field and stored individually in 1.5µL microcentrifuge tubes containing 95% ethanol and transferred to a -20°C freezer until DNA extraction (see Chapter 4). Ground-dwelling spiders were collected from sheet webs using an aspirator and similarly prepared for DNA extraction.

3.3.2.1 Sticky Traps

Mini-sticky traps were used to monitor spider prey availability and quantify aphid falling rates. Traps (7.5 cm^2, 1.5 cm x 5 cm, 2 mm thick) consisted of plastic painted with

brown acrylic paint to minimize any visual stimulus on the ground (after Harwood et al. 2001, 2003). Each trap was covered with an acetate sheet coated with Tangle-Trap© Sticky Coating spray (The Tanglefoot Company, Grand Rapids, Michigan, USA). Traps were placed at random within each plot and left *in situ* for 24 h, before the acetate sheet was removed and returned to the laboratory for identification.

3.3.2.2 BYDV Sampling

Aphids and plants were also collected during each sample period, from each plot within all fields, to screen for Barley Yellow Dwarf virus. Ten individual aphids were hand collected using an aspirator from each plot, stored individually in 1.5µL microcentrifuge tubes and kept at -20°C until screening. Ten plant samples were hand collected and stored in sample bags at -20°C until screening. Enzyme-linked immunosorbent assay (ELISA) methods were used to assess BYDV incidence in plants and aphids as described in Chapter 2.

3.3.3 Statistical Analysis

During the 2010-2011 field season, major flooding in one of the fields during spring 2011 affected the abundance and subsequent distribution of arthropods and this field was therefore excluded from statistical analyses. Additionally, during the 2011-2012 field season an equipment malfunction in the planter reduced sampling replication to three fields. Therefore, for each field season, three fields were used in the analyses and are henceforth categorized as Fields A-C (2011) and Fields D-F (2012). Prior to yield analysis, grain weights were corrected for moisture and converted to bushels/acre. An

analysis of variance (ANOVA) was used to examine the effect of treatment on winter wheat yield.

To examine the effect of treatment (weed strips) on aphid abundance, counts of aphids from sweep samples were used and analyzed with a repeated measures mixed model analysis of variance (PROC MIXED in SAS 9.3) assuming a Poisson distribution with field as a random effect. The effect of treatment on natural enemy abundance was analyzed using a repeated measures multivariate analysis of variance (PROC GLM) assuming a Poisson distribution. Virus incidence was measured by the proportion of aphids or plants testing positive for BYDV and square root arcsine transformed before any analyses. Plants were grouped by month to examine the seasonal effects of the virus. A repeated measured mixed model assuming a binary distribution was used to analyze the effect of treatment on virus incidence in plants and aphids.

3.3.3.1 Spatial Analysis

Spatial Analysis by Distance IndicEs (SADIE) (Perry and Hewitt 1991, Perry 1995, Perry et al. 1999) was used to examine the spatial and temporal patterns of aphid and natural enemy populations in the field (SADIEshell version 1.22). Analyses were conducted for each field and each sampling date. SADIE employs a grid system and count data to quantify the distance, D, needed for the organisms to reach either a uniform or an aggregated distribution. Every location was assigned a cluster index, with a positive patch index of v_i for counts that were above the mean and a negative gap index of v_j for counts below the mean; an index > 1.5 indicated a patch and an index < -1.5 indicated a gap. The entire sample was also given an index of aggregation, I_a, to indicate significant

aggregation (> 1), a random distribution (= 1) or a regular sample (< 1). After approximately 20,000 randomizations for each test, a probability was generated for a formal test of randomness, P_a. I then interpolated the indices to create two-dimensional contour maps and visualize the patches and gaps with Surfer mapping software version 9.11.947 (Golden Software Inc., Golden, Colorado, USA).

3.4 Results

3.4.1 Pest and Natural Enemy Abundance

Approximately 100,000 aphids were collected over the two years and were comprised of two species, *R. padi* and *S. avenae*. Significantly ($F_{1, 238} = 7.46$, $P = 0.0068$) more aphids were collected in 2011 (75,626) compared to 2012 (21,411). Ground-based aphid availability was measured using sticky traps, and was significantly higher in 2011 (Fig. 3.1a) than 2012 (Fig. 3.1b) ($F_{1,915} = 4.66$, $P = 0.0003$).

A total of 11,541 foliar aphid natural enemies were captured in sweep nets in 2011 and 2012, representing four major families including Coccinellidae, Anthocoridae, Chrysopidae and Nabidae. Over half of these (51.1%) were Coccinellidae (Coleoptera) composed of five species: *Coccinella septempunctata* L., *Coleomegilla maculata* DeGeer, *Harmonia axyridis* (Pallas), *Hippodamia convergens* Guérin-Méneville, and *Cycloneda munda* (Say) (results discussed in detail in Chapter 5). The next most abundant predator was *Orius insidiosus* (Say) (Anthocoridae: Hemiptera) which made up 19.8% of the predators (results discussed in Chapter 4). Aphid parasitoids were also present (Braconidae: Hymenoptera). Green lacewing larvae (Chrysopidae: Neuroptera) were composed of two species, *Chrysopa oculata* Say and *Chrysoperla plorabunda* (Fitch) (tentative). Damsel bugs (Nabidae: Hemiptera) were also present each year, but in extremely low numbers. Additionally, approximately 1,000 epigeal spiders in the family Linyphiidae were hand collected.

3.4.2 Barley Yellow Dwarf Virus Incidence

3.4.2.1 Plants

In 2011 2,626 plant samples were screened for BYDV with 4.72% testing positive (Fig. 3.2). There was no difference between treatments ($F_{1,253} = 0.72$, $P = 0.396$) on plant infection rate, but there was a field effect ($F_{2,253} = 7.31$, $P = 0.003$). Time of year had a significant effect on the infection rate in plants ($F_{4,253} = 8.4$, $P < 0.0001$) with plants collected in the May having the highest rate of infection. Since there was no detectable difference in infection rates between treatments, plants collected in 2012 were not screened for BYDV.

3.4.2.2 Aphids

In 2011, 4,635 total aphids were screened for BYDV with 12.2% testing positive (Fig. 3.3a). In 2012 1,522 total aphids were screened with 19.6% testing positive (Fig. 3.3b). There was no difference between treatments on aphid infection rates in 2011 ($F_{1,397} = 2.88$, $P = 0.09$) but there was a significant field effect ($F_{2,30} = 3.98$, $P = 0.03$). In 2012 there was no difference between treatments ($F_{1,265} = 1.16$, $P = 0.283$) but there was a significant date effect ($F_{4,265} = 12.97$, $P < 0.0001$). Significantly lower infection rates were detected in aphids early and late in the season (Fig. 3.3b). There were no significant differences in infection rates between the two aphid species ($F_{1,3749} = 0.51$, $P = 0.477$).

3.4.3 Weed Strips

During the 2010-2011 growing season, weeds were abundant in all four fields. Dominant species included common ragweed (Asterales: Asteraceae) (*Ambrosia artemisiifolia* L.), giant ragweed (Asterales: Asteraceae) (*Ambrosia trifida* L.), Johnson

grass (Poales: Poaceae) (*Sorghum halepense* (L.)), and horseweed (Asteracles: Asteraceae) (*Conyza canadensis* (L.) Cronquist). In the 2011-2012 season, weeds were much less abundant and the dominant weed species was horseweed, *C. canadensis*.

3.4.3.1 Effect of Weed Strips on Aphid Abundance

There was no effect of treatment on aphid abundance in 2011 (Fig. 3.4a) ($F_{1,418}$ = 0.14, P = 0.701) but there was a significant effect of date ($F_{4,418}$ = 94.7, P < 0.0001). Conversely, in 2012 there was a significant effect of treatment on aphid abundance in 2012 (Fig. 3.4b) ($F_{1,485}$ = 15.08, P = 0.0001). There were also significant effects of date ($F_{5,485}$ = 288.83, P < 0.0001), field ($F_{2,30}$ = 27.2, P < 0.0001), and an interaction of date and treatment ($F_{5,485}$ = 3.04, P = 0.01). There were significantly more aphids in weed strip plots than in control plots in 2012 (Fig. 3.4b) (t_{485} =26.77, P < 0.0001). In each year, aphid populations peaked on one sample date (2011: 25-May, 2012: 26-April) where abundances were significantly higher than all the other time points. Also, there was no difference in the number of aphids captured in sticky traps in weed strip plots or control plots in 2011 ($F_{3,44}$ = 1.19, P = 0.326) or 2012 ($F_{3,24}$ = 1.21, P = 0.327).

3.4.3.2 Effect of Weed Strips on Natural Enemies

In 2011, natural enemy abundance differed significantly between weed strip fields and control fields ($F_{6,2837}$ = 6.35, P < 0.0001), with effects of date ($F_{2,2842}$ = 4.38, P < 0.0001) and field ($F_{4,2842}$ = 2261.23, P < 0.0001). Specifically, coccinellids and green lacewings had significantly higher abundances in weed strip plots. In 2012, fields with weed strips had significantly higher natural enemy abundances ($F_{6,3244}$ = 68.09, P <

0.0001) with effects of date ($F_{5,3249} = 878.29$, $P < 0.0001$) and field ($F_{2,3249} = 349.15$, $P < 0.0001$). Coccinellid adults (Fig. 3.5a, e) and larvae (Fig. 3.5b, f) (described in detail in Chapter 5), green lacewings (Fig. 3.5c, g), insidious flower bugs (described in detail in Chapter 4), and parasitoids (Fig. 3.5 d, h) were significantly higher in weed strip plots compared to control plots.

3.4.3.3 Effect of Weed Strips on Wheat Yield

There was no effect of the weed strips on yield in 2011 ($F_{1,132} = 2.01$, $P = 0.159$) but there was a significant effect of field ($F_{2,132} = 42.14$, $P < 0.0001$). In 2012, there was a significant effect of weed strips on yield ($F_{1,204} = 67.1$, $P < 0.0001$) with control plots having higher yields. There were also significant effects of field ($F_{2,204} = 292.94$, $P < 0.0001$) and interaction of field and treatment ($F_{2,204} = 58.87$, $P < 0.0001$). When I analyzed the relationship between yield and distance to field edge, there was no relationship in either weed strip plots (2011: $F_{1,50} = 1.23$, $P = .273$, 2012: $F_{1,96} = 0.17$, $P = 0.684$) or control plots (2011: $F_{1,79} = 0.49$, $P = 0.485$, 2012: $F_{1,105} = 0.21$, $P = 0.65$).

3.4.3.4 Spatial Dynamics of Aphids and BYDV

When the spatial structure in control and weed strip fields was compared, considerable field-to-field variation was observed (Table 3.1, 3.2). In 2011, control and weed strip fields showed random distribution, and significant patches and gaps, whereas during 2012 only control fields showed any significant spatial pattern, in the form of aggregated patches.

I used ELISA to screen plants and aphids for BYDV and then examined the spatial relationship within the field. Infection rates were low in the fall and winter, and were grouped by month for spatial analysis. Contour maps show the spread of BYDV from the fall (Fig. 3.7a) to the spring (Fig. 3.7b) from the edge of the field to the center. Early in the season, there were no patches of infected plants but later in the season two out of the three fields had significant aggregations of virulence (Table 3.3). Conversely, there was no evidence of patches or gaps of virulent aphids in wheat fields in either 2011 (Table 3.4) or 2012 (Table 3.5).

3.5 Discussion

This study showed that field-bordering weed strips significantly increase aphid natural enemies in winter wheat. Fields with weed strips supported higher populations of coccinellid adults and larvae, green lacewing larvae, and aphid parasitoids in the subfamily Aphidiinae. Aphid populations did not differ significantly between treatment and control plots, nor was a reduction in BYDV infection rates in plants or aphids seen between treatments. Additionally, there was no measurable difference in yields between plots with and without weed strips. My study aligns with the increasing body of evidence that habitat manipulations in agroecosystems can aid in natural enemy enhancement (e.g. Gurr et al. 2004), although a direct reduction in injury to the crop by the pest has rarely been seen (Bianchi et al. 2006). Nonetheless, studies like mine are important because it measures the success of habitat manipulations with natural enemy abundance, pest populations, and crop yield. There may be reluctance on the behalf of farmers to adopt conservation biological control tactics as a part of an integrated pest management system due to the lack of studies showing their direct impact on crop yield (Gurr et al. 2000), so conducting experiments such as this will more fully evaluate the benefits of sustainable agriculture.

Increased landscape complexity can increase parasitoid activity and diversity (Zhao et al. 2014). Weed strip fields did have significantly higher populations of aphid parasitoids, but this did not lead to lower populations of aphids or lower BYDV infection rates. This may be due to the biology of the aphid vector; once an aphid is parasitized, it must remain alive for some time as a host, which may give them enough time to vector

BYDV to healthy plants (Smyrnioudis et al. 2001). Due to the dynamics of this pathogen, only a small number of aphids are required for an outbreak of BYDV (Power 1991) so this time may be crucial. My spatial analysis (Fig. 3.7) showed that, even in the cold winter months, aphids are capable of transmitting BYDV and causing significant within-field infection. While I found increased natural enemy populations in weed strip fields, it did not result in lower transmission of BYDV. A recent study found that disease reduction in cereals may not be the result of predation by natural enemies on aphid vectors, but rather their presence alters the behavior of vectors, resulting in lower transmission (Long and Finke 2015).

Higher proportions of semi-natural habitats will enhance biological control of pests, but time and spatial scales must be taken into account, as well as the species of natural enemies present (Alignier et al. 2014). In this study, weed strips increased some species of predators, while others remained unaffected. Natural enemies respond differently to habitat manipulations, as well as the plants within them (Frank et al. 2009), and management of the individual species requires knowledge of their habitat preferences (Thomas and Marshall 1999). Information from conservation biological control studies such as mine can be incorporated into integrated pest management programs. Additionally, the use of spatial information can aid in the localized, within-field control of pests (Thomson and Hoffman 2013).

The climate conditions in the two field seasons differed drastically, which resulted in aphid populations appearing in the wheat fields almost a month earlier. Alternations of high and low years of aphid populations can be the result of changes in climate (Dixon 1985) or cyclical pressure from natural enemies, such as coccinellids (Hodek and Honek

1996). Climate had a significant effect on the epigeal spider populations. Very few linyphiid spiders were collected in spring 2012 due to the drought; these web-building, sit-and-wait predators (Sunderland et al. 1986) were most likely driven elsewhere due to lack of resources. Therefore, no spiders were screened for prey DNA in this study. However, examining both epigeal and foliar spider predators in this winter wheat system would give valuable insight into the aphid food web. Control for BYDV is accomplished using preventative insecticides, which are sometimes unnecessary given that climate, along with other biotic factors, are mainly responsible for its virus spread (Pike 1990, Smyrnioudis et al. 2001). Continued use of chemical control for aphid viruses can result in insecticide resistance or negative impacts on beneficial insects (Irwin and Thresh 1990). Therefore, large field studies that examine the specific mechanisms responsible for predator enhancement are increasingly important. The weed strips in winter wheat significantly increased the abundance of natural enemies in two field seasons, despite drastic differences in climate and the amounts of weeds each year. If we are to create successful integrative pest management programs, it is important to tease apart the interaction between field margins, natural enemies and pest suppression.

Table 3.1. Index of aggregation, I_a, and mean cluster index, v_i for patches and v_j for gaps, with associated probability, P_x, from randomization test of SADIE, for aphids counted in weed strip and control plots of winter wheat in the 2011 spring season. Bold font denotes where a significant measurable spatial pattern can be detected.

2011 Aphids						
18-Apr-11	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
A (trt)	0.951	0.563	0.991	0.464	0.653	0.181
B (trt)	1.152	0.165	1.079	0.246	1.259	0.057
C (trt)	0.954	0.589	0.947	0.618	1.016	0.389
A (con)	1.411	**0.012**	1.5	**0.004**	1.34	**0.024**
B (con)	1.276	0.056	1.266	0.061	1.016	0.084
C (con)	0.887	0.733	0.914	0.677	0.888	0.793

29-Apr-11	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
A (trt)	0.901	0.698	1.04	0.330	0.876	0.804
B (trt)	0.965	0.522	0.852	0.844	0.96	0.543
C (trt)	1.03	0.368	0.994	0.435	0.977	0.482
A (con)	1.171	0.138	1.056	0.297	1.058	0.296
B (con)	1.411	**0.018**	1.421	**0.012**	1.357	**0.025**
C (con)	1.16	0.17	1.244	0.073	1.073	0.260

10-May-11	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
A (trt)	0.819	0.871	0.844	0.898	0.812	0.920
B (trt)	1.21	0.12	1.12	0.204	1.241	0.077
C (trt)	1.25	0.084	1.191	0.121	1.124	0.209
A (con)	1.337	0.038	1.411	0.012	1.324	0.028
B (con)	1.602	**0.001**	1.619	**0.000**	1.4	**0.013**
C (con)	1.198	0.141	1.122	0.224	1.13	0.035

25-May-11	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
A (trt)	1.193	0.127	1.045	0.3374	1.185	0.122
B (trt)	1.515	**0.001**	1.396	**0.011**	1.452	**0.004**
C (trt)	1.124	0.197	1.239	0.073	1.199	0.103
A (con)	1.199	0.123	1.155	0.168	1.207	0.108
B (con)	1.493	**0.002**	1.345	**0.019**	1.23	0.068
C (con)	0.902	0.668	0.867	0.777	0.968	0.528

Table 3.2. Index of aggregation, I_a, and mean cluster index, v_i for patches and v_j for gaps, with associated probability, P_x, from randomization test of SADIE, for aphids counted in weed strip and control plots of winter wheat in the 2012 spring season. Bold font denotes where a significant measurable spatial pattern can be detected.

2012 Aphids						
14-Mar-12	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
D (trt)	1.323	**0.037**	1.284	0.047	1.309	**0.036**
E (trt)	1.28	0.06	1.243	0.067	1.195	0.113
F (trt)	0.901	0.675	0.829	0.893	1.071	0.267
D (con)	1.095	0.26	1.062	0.311	1.017	0.385
E (con)	1.302	0.052	1.124	0.196	1.143	0.135
F (con)	1.404	**0.021**	1.29	0.056	1.173	0.129
28-Mar-12	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
D (trt)	1.092	0.243	1.111	0.214	1.092	0.229
E (trt)	1.056	0.315	1.142	0.184	1.044	0.314
F (trt)	1.314	**0.048**	1.18	0.129	1.146	0.157
D (con)	1.36	**0.031**	1.548	**0.001**	1.326	**0.032**
E (con)	1.01	0.409	1.008	0.410	1.025	0.364
F (con)	1.194	0.131	1.219	0.085	1.184	0.108
13-Apr-12	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
D (trt)	1.392	**0.02**	1.357	**0.022**	1.304	**0.043**
E (trt)	1.082	0.265	1.03	0.351	0.976	0.495
F (trt)	1.013	0.411	1.013	0.395	0.994	0.452
D (con)	1.15	0.181	1.195	0.102	1.066	0.279
E (con)	0.855	0.809	0.861	0.827	1.081	0.241
F (con)	0.922	0.629	0.935	0.625	0.989	0.453
24-Apr-12	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
D (trt)	1.292	0.066	1.43	**0.012**	1.416	**0.016**
E (trt)	0.952	0.554	1	0.443	1.044	0.317
F (trt)	1.596	**0.001**	1.566	**0.002**	1.525	**0.004**
D (con)	1.603	**0.002**	1.502	**0.006**	1.41	**0.016**
E (con)	0.902	0.685	0.86	0.819	0.899	0.734
F (con)	1.317	**0.039**	1.471	**0.005**	1.241	0.069
8-May-12	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
D (trt)	1.378	**0.024**	1.298	**0.044**	1.127	0.171
E (trt)	n/a	n/a	n/a	n/a	n/a	n/a
F (trt)	0.931	0.612	0.947	0.573	1.062	0.274
D (con)	0.987	0.468	1.084	0.246	0.925	0.630
E (con)	n/a	n/a	n/a	n/a	n/a	n/a
F (con)	1.199	0.116	1.21	0.098	1.07	0.274

Table 3.3. Index of aggregation, I_a, and mean cluster index, v_i for patches and v_j for gaps, with associated probability, P_x, from randomization test of SADIE, for plants testing positive for Barley Yellow Dwarf virus (BYDV) as detected by enzyme-linked immunosorbent assay (ELISA) in the 2011 spring season. Bold font denotes where a significant measurable spatial pattern can be detected; those labeled as 'n/a' did not have high enough infection rates to conduct spatial analysis.

2011 Barley Yellow Dwarf virus-positive plants						
Field A	Index of Aggregation		Cluster of gaps		Cluster of patches	
Date	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
Nov	0.982	0.429	-0.976	0.441	1.017	0.362
Jan	1.153	0.200	-1.151	0.206	1.265	0.116
Mar	n/a	n/a	n/a	n/a	n/a	n/a
9-Apr	n/a	n/a	n/a	n/a	n/a	n/a
29-Apr	0.989	0.497	-0.997	0.406	0.993	0.907
8-May	0.923	0.572	-0.921	0.571	0.929	0.549
24-May	0.999	0.450	-1.003	0.438	0.912	0.758
Field B	Index of Aggregation		Cluster of gaps		Cluster of patches	
Date	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
Jan	1.328	0.079	-1.321	0.072	1.210	0.121
Mar	1.004	0.423	-1.015	0.394	1.096	0.271
9-Apr	0.876	0.718	-0.896	0.628	0.787	0.927
29-Apr	0.936	0.527	-0.910	0.581	1.101	0.276
8-May	1.528	**0.024**	-1.461	**0.031**	1.346	0.059
24-May	n/a	n/a	n/a	n/a	n/a	n/a
Field C	Index of Aggregation		Cluster of gaps		Cluster of patches	
Date	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
9-Apr	n/a	n/a	n/a	n/a	n/a	n/a
29-Apr	0.924	0.571	-0.912	0.600	0.959	0.476
8-May	1.586	**0.015**	-1.587	**0.014**	1.574	**0.016**
24-May	1.560	**0.020**	-1.566	**0.020**	1.530	**0.022**

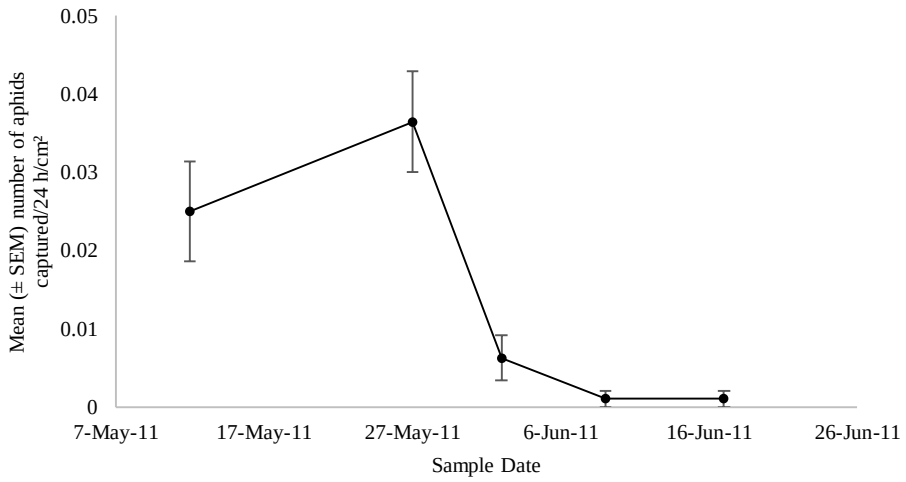
Table 3.4. Index of aggregation, I_a, and mean cluster index, v_i for patches and v_j for gaps, with associated probability, P_x, from randomization test of SADIE, for aphids testing positive for Barley Yellow Dwarf virus as detected by enzyme-linked immunosorbent assay in the 2011 spring season. Bold font denotes where a significant measurable spatial pattern can be detected.

2011 Barley Yellow Dwarf virus-positive aphids						
10-Apr-11	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
A	1.506	**0.030**	-1.507	**0.025**	1.435	**0.034**
B	1.122	0.224	-1.070	0.291	1.113	0.225
C	0.826	0.818	-0.871	0.718	0.906	0.623
29-Apr-11	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
A	1.603	**0.021**	-1.622	**0.021**	1.875	**0.006**
B	0.913	0.603	-0.859	0.753	0.892	0.658
C	1.018	0.358	-1.015	0.374	0.988	0.421
10-May-11	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
A	1.053	0.311	-1.046	0.322	1.207	0.134
B	1.074	0.269	-1.181	0.159	1.054	0.288
C	0.956	0.481	-0.948	0.488	0.914	0.591
18-May-11	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
A	1.580	**0.021**	-1.612	**0.016**	1.178	**0.016**
B	1.256	0.118	-1.306	0.087	1.145	0.191
C	0.798	0.880	-0.797	0.891	0.823	0.888
27-May-11	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
A	1.303	0.099	-1.329	0.088	1.280	0.095
B	0.748	0.956	-0.774	0.939	0.832	0.854
C	n/a	n/a	n/a	n/a	n/a	n/a

Table 3.5. Index of aggregation, I_a, and mean cluster index, v_i for patches and v_j for gaps, with associated probability, P_x, from randomization test of SADIE, for aphids testing positive for Barley Yellow Dwarf virus as detected by enzyme-linked immunosorbent assay in the 2012 spring season. Bold font denotes where a significant measurable spatial pattern can be detected.

2012 Barley Yellow Dwarf virus-positive aphids						
30-Mar-12	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
D	1.419	**0.049**	-1.479	**0.030**	1.519	**0.025**
E	n/a	n/a	n/a	n/a	n/a	n/a
F	2.312	**0.0002**	-2.254	**0**	2.348	**0**
12-Apr-12	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
D	0.892	0.66	-0.928	0.560	0.858	0.769
E	0.947	0.5054	-0.983	0.420	0.955	0.493
F	n/a	n/a	n/a	n/a	n/a	n/a
26-Apr-12	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
D	1.036	0.322	-1.038	0.312	1.007	0.367
E	1.346	0.069	-1.348	0.062	1.331	0.059
F	0.925	0.5671	-0.925	0.568	0.879	0.724
8-May-12	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
D	0.786	0.9026	-0.786	0.898	0.829	0.835
E	n/a	n/a	n/a	n/a	n/a	n/a
F	1.444	**0.044**	-1.447	**0.045**	1.47	**0.033**

a. 2011



b. 2012

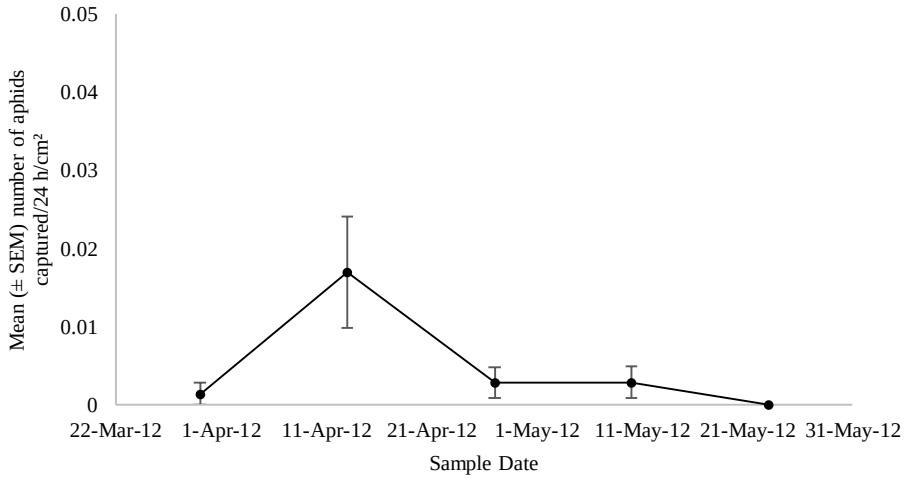


Figure 3.1. Mean (± SEM) number of potential aphid prey captured on mini-sticky traps in a. 2011 and b. 2012. Ground-based traps represent aphids caught over 24 hr, per cm².

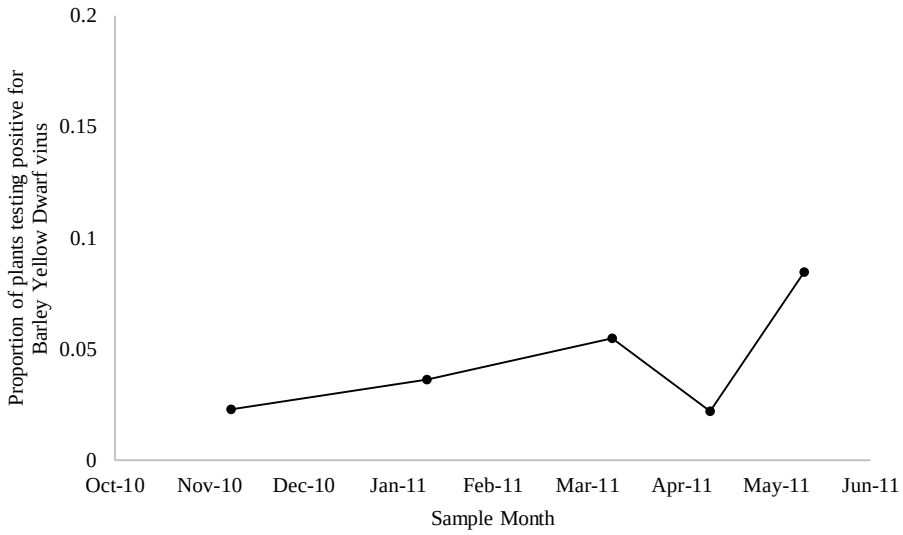
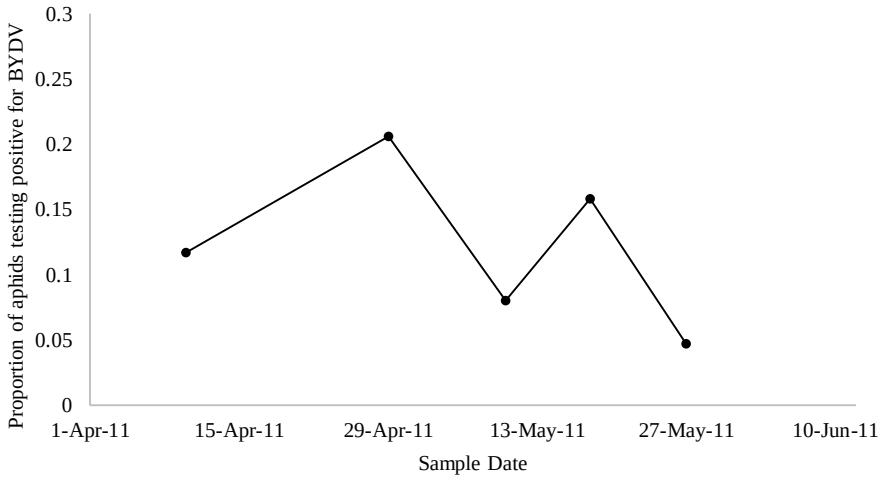


Figure 3.2. Barley Yellow Dwarf virus detected in plants using ELISA over the growing season.

a. 2011



b. 2012

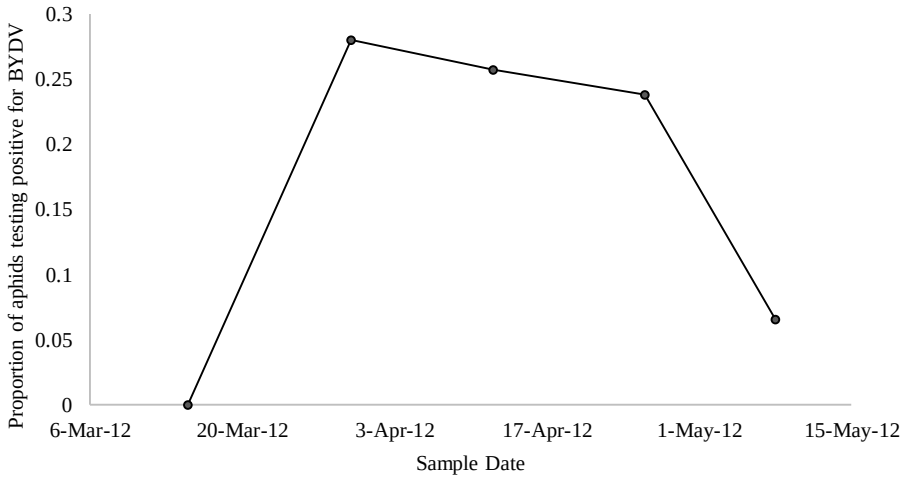


Figure 3.3. BYDV in aphids detected using ELISA in a. 2011 and b. 2012

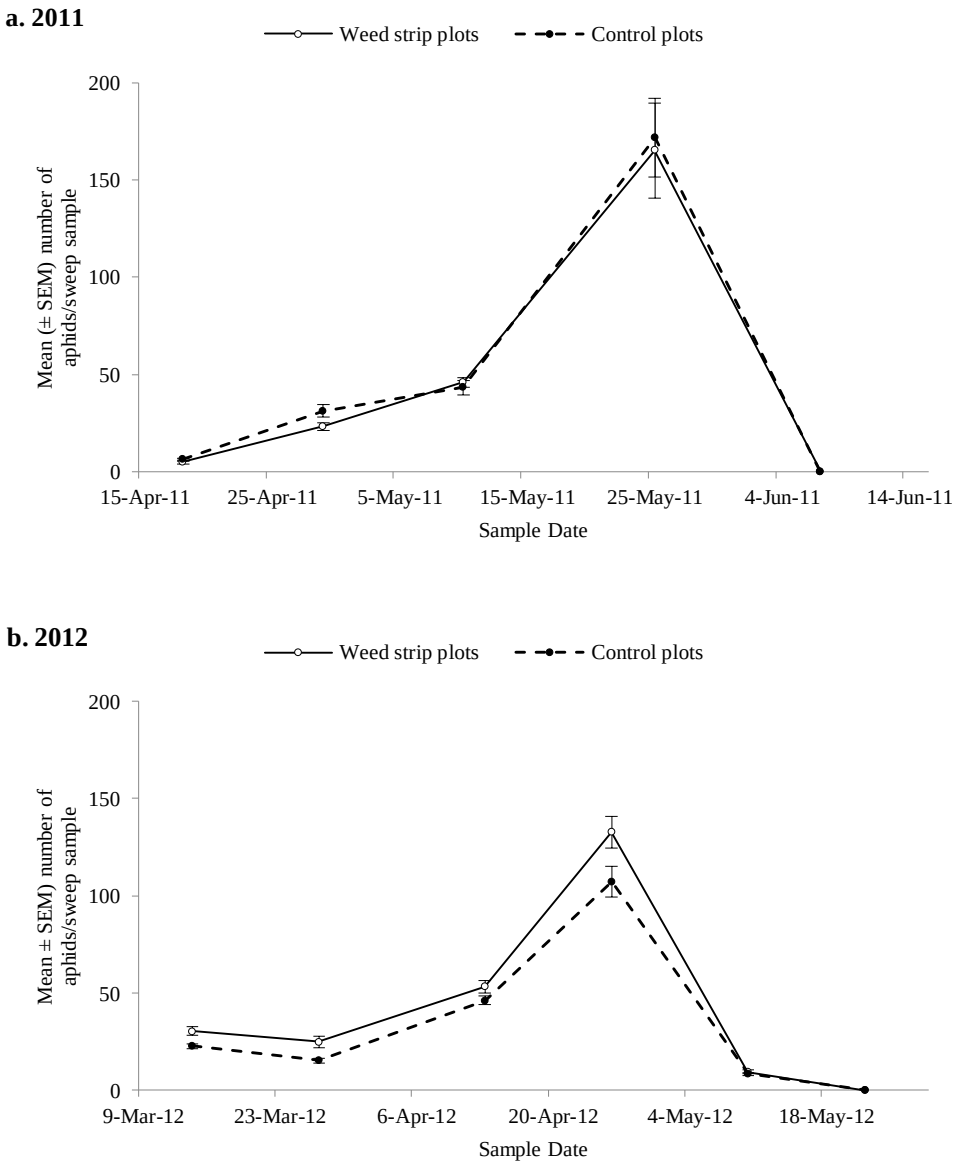
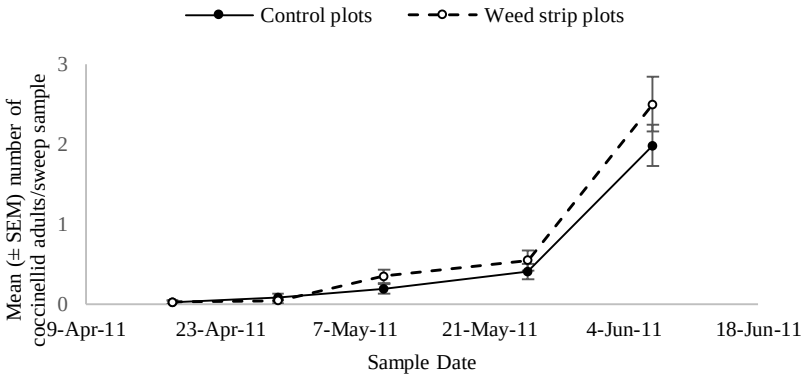
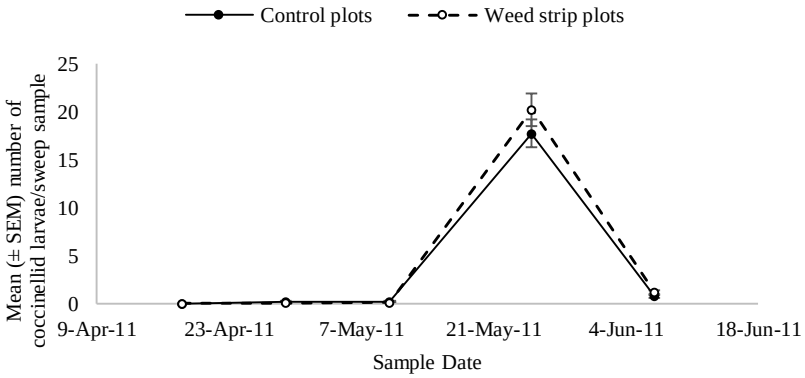


Figure 3.4. Mean (± SEM) number of aphids in weed strip and control plots per sweep sample, consisting of ten figure-eight sweeps, in winter wheat in a. 2011 and b. 2012

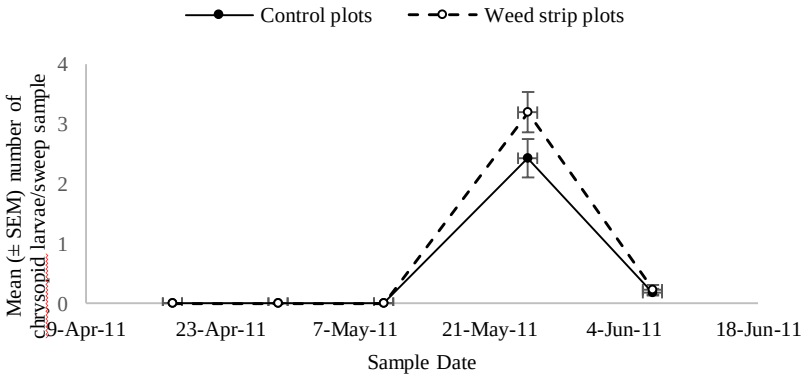
a. 2011 Coccinellid adults



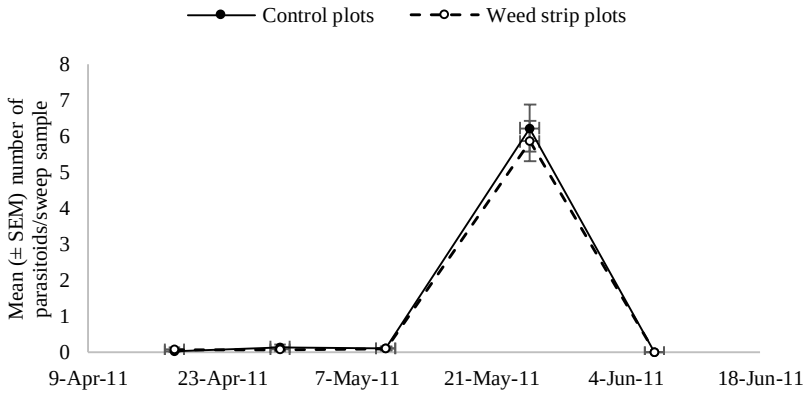
b. 2011 Coccinellid larvae



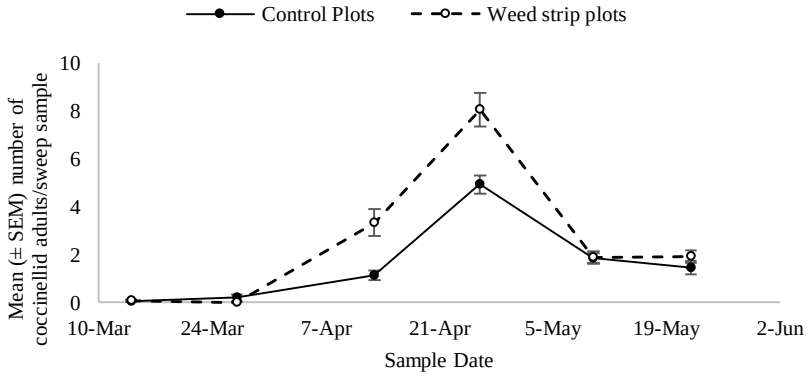
c. 2011 Chrysopid larvae



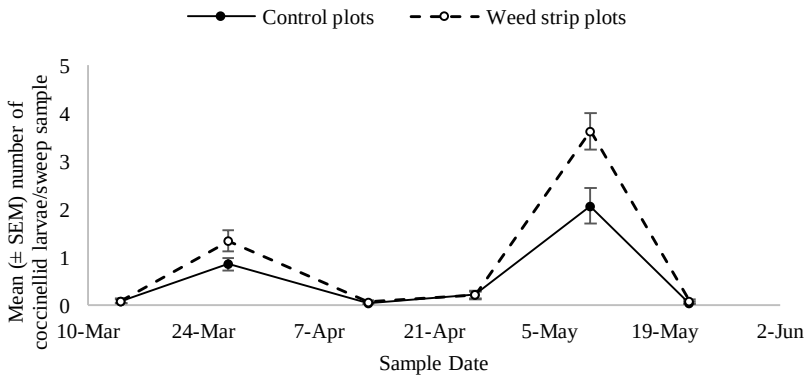
d. 2011 Parasitoids



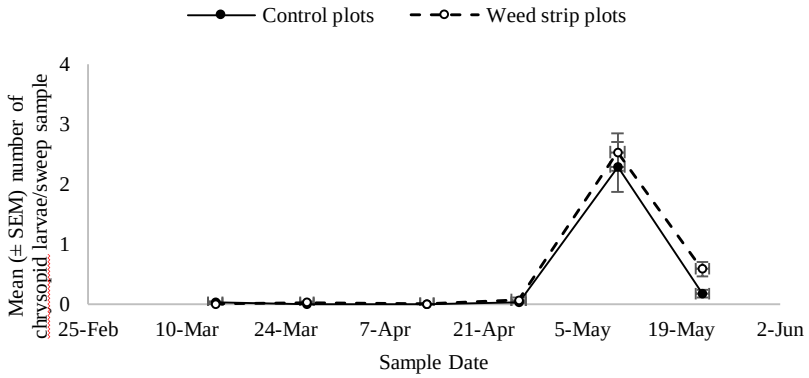
e. 2012 Coccinellid adults



f. 2012 Coccinellid larvae



g. 2012 Chrysopid larvae



h. 2012 Parasitoids

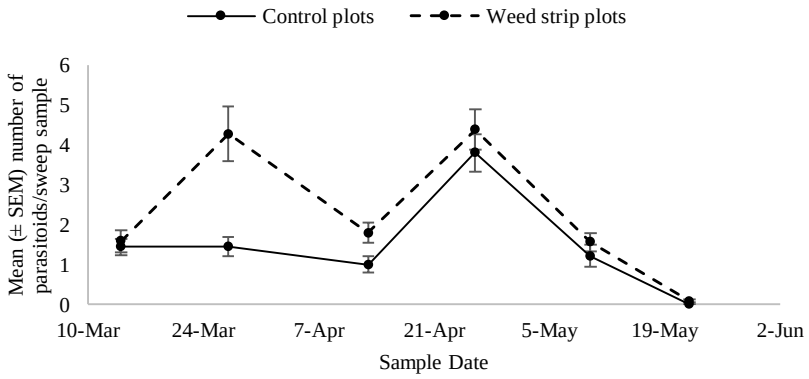


Figure 3.5. Mean (± SEM) number of most abundant natural enemies in weed strip plots and control plots per sweep sample, consisting of ten figure-eight sweeps, in winter wheat in 2011: a. coccinellid adults, b. coccinellid larvae, c. chrysopid larvae, d. parasitoids and in 2012: e. coccinellid adults, f. coccinellid larvae, g. chrysopid larvae, h. parasitoids

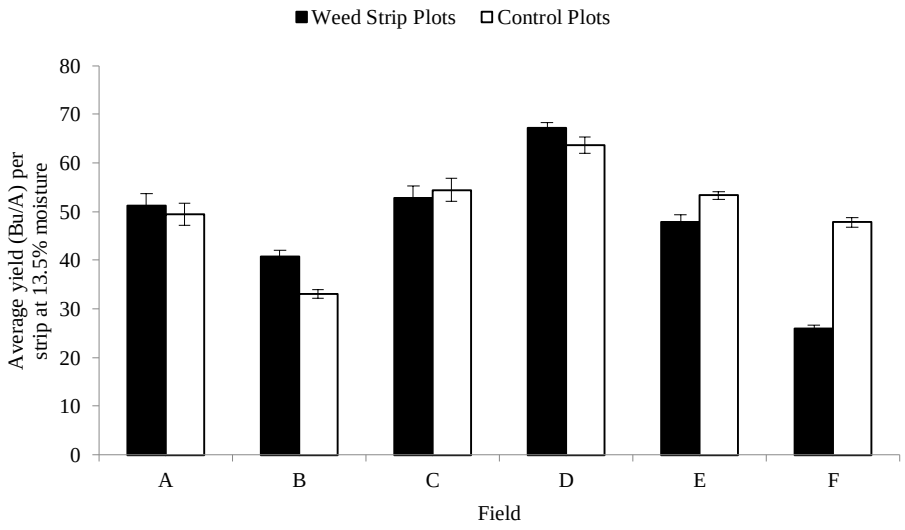


Figure 3.6. End of season wheat yields in control plots and weed strip plots in 2011 (Fields A, B, C) and 2012 (Fields D, E, F) averaged by strip in Bu/acre.

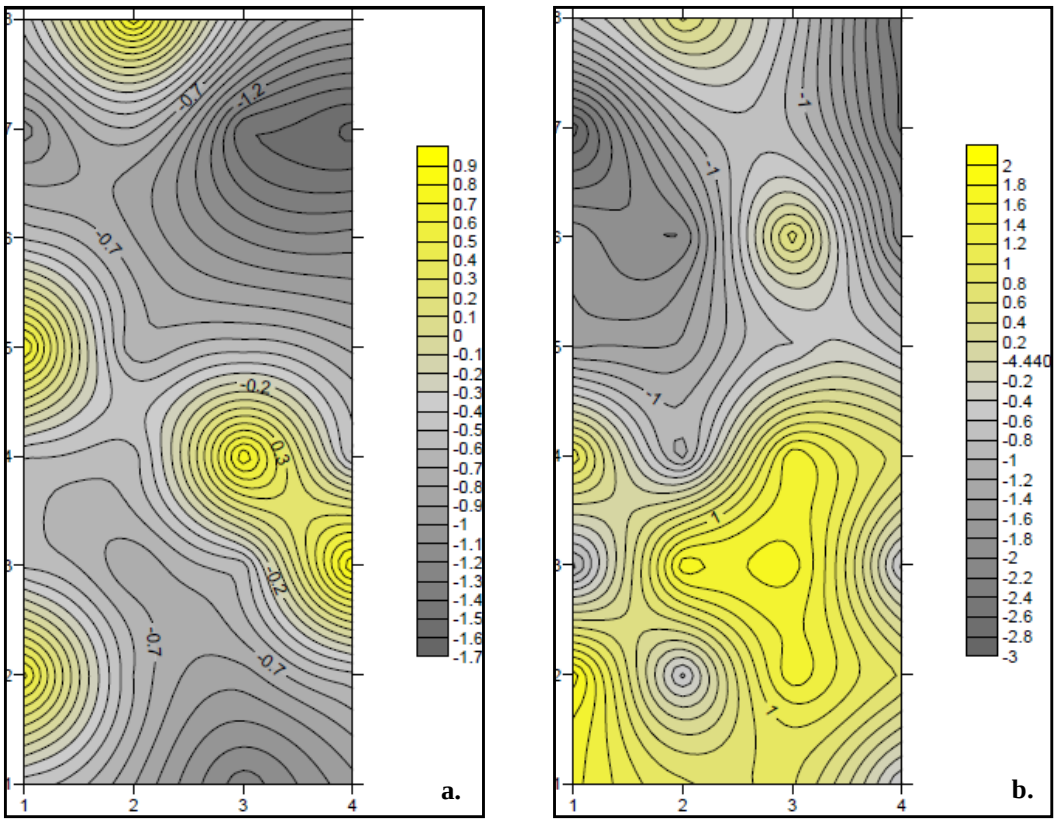


Figure 3.7. Contour maps of Barley Yellow Dwarf virus in winter wheat fields in (a) November and (b) January in Field B. The key with negative values indicates gaps and positive values indicates a patch. A unit that belongs to a patch is indicated by $v_i > 1$ whereas a gap is indicated by neighboring unit with values of $v_j<-1$. Values of $v < -1.5$ indicate significantly larger gaps, and values $v > 1.5$ indicate significantly larger patches. The horizontal and vertical axes represent the coordinate system used for sampling, with each sample unit measuring 188m².

Chapter 4 : Spatial and temporal synchrony between a generalist predator and pest aphid in winter wheat facilitates high predation rates

4.1 Abstract

Understanding the biological control value of a natural enemy requires knowledge of the unique spatial and temporal dynamics between predators and their prey, and this can give insight into how to potentially manipulate these dynamics for maximum pest suppression. Grain aphids are major pests in cereals and cause substantial yield loss as vectors of Barley Yellow Dwarf virus (BYDV). Within these agroecosystems, generalist predators can reach high densities and have the potential to suppress aphid populations, particularly through conservation biological control whereby populations of natural enemies are enhanced. I conducted a two-year field study in winter wheat to examine the temporal and spatial relationship between a generalist predator, *Orius insidiosus* (Hemiptera: Anthocoridae), and a pest aphid, *Sitobion avenae* (Hemiptera: Aphididae), to test the hypothesis that natural, field-bordering weed strips increase the abundance of predators. Furthermore, using molecular gut-content analysis, I identified the strength of trophic connectedness between these predators and their aphid prey. Both *O. insidiosus* and *S. avenae* had very low populations early in the spring, peaking in density during wheat flowering and subsequently declining rapidly as the plant senesces. Although treatments did not increase natural enemy abundance, populations of predators and prey showed strong spatial structure with significantly clumped distributions that were positively associated. Molecular analysis revealed that 36% of *O. insidiosus* contained

detectable DNA of *S. avenae*. These results suggest that despite the generalist feeding habits of *O. insidiosus*, these aphids constitute an important component of their diet, and the spatiotemporal association between the two imply it could serve as an important natural enemy of aphids in winter wheat agroecosystems.

4.2 Introduction

Agroecosystems are a complex series of interactions webs between biotic and abiotic variables (Welch and Harwood 2014), all influencing the temporal and spatial dynamics of predator and prey populations (Campos-Herrera et al. 2013). These interaction webs are further influenced by modification of the environment or on-farm management practices that enhance existing natural enemy populations to aid in pest suppression (Debach and Rosen 1991). Modification of the environment through conservation biological control has been widely adopted as a successful strategy for pest control by enhancing predator populations to exert top-down control of insect pests (Costamagna and Landis 2011, Dong et al. 2012, Holland et al. 2012). Successful implementation of such approaches requires a fundamental understanding of the effect of different habitat types on the abundance and diversity of natural enemies (Landis et al. 2000, Gurr et al. 2004). This is often accomplished through the provisioning of alternative resources such as nectar or pollen (Eubanks and Denno 2000a), physical refugia and alternative prey or hosts (Landis et al. 2005).

In cereal agroecosystems, a diverse complex of natural enemies persist and they can be exploited for conservation biological control, including many epigeal and aerial predators that feed on soft-bodied aphids (Harwood and Obrycki 2005). In cereals, aphids (Hemiptera: Aphididae) are global pests primarily because they vector 28% of the world's known plant viruses (Hogenhout et al. 2008) including vector Barley Yellow Dwarf virus (BYDV), which causes up to 17% yield loss worldwide (Plumb 1983). BYDV is vectored by specific aphid species (Irwin and Thresh 1990), and relies entirely

on aphids for its transfer to, and insertion into, plants. Twenty-five species of aphids are reported to vector BYDV (Halbert and Voegtlin 1995), although one of the most crucial species for virus transmission is the English grain aphid, *Sitobion avenae* (F.) (Rochow 1969, Plumb 2002). This aphid is responsible for the secondary infection of BYDV in the spring, routinely causing substantial yield loss (Irwin and Thresh 1990, Plumb 2002). The unique biology of aphids, including parthenogenesis, exponential growth, and alate and apterous morphs (Blackman and Eastop 2007), allows their populations to form large aggregations with strong spatial patterns in the field that are ephemeral in space and time (Winder et al. 1999, 2001, 2005a). This spatial pattern is typically correlated to yield loss (Chapin et al. 2001) and the interactions between aphids and their natural enemies (Harwood et al. 2001, Winder et al. 2005b, Rahman et al. 2010). Deciphering these, and other, interactions within an agroecosystem can assist with developing sampling strategies for pests and to characterize the importance of a particular predator for biological control and use in IPM programs (Cantrell and Cosner 1991, Holland 2004).

One such predator is the insidious flower bug, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae). Abundant in agroecosystems throughout the US Midwest (Rutledge and O'Neil 2005) and, as a foliar-foraging predator (Schmidt et al. 2008), it is a valuable control agent against a variety of aphid species (Landis and Van der Werf 1997, Obrycki and Kring 1998, Fox et al. 2004, Harwood et al. 2007, 2009). In the context of biological control of *S. avenae*, which infests the heads of winter wheat, it is likely that a foliar-foraging predator, such as *O. insidiosus*, will play a greater role in pest suppression (Holland et al. 2008, 2012). These predators are highly mobile (Montserrat et al. 2004), actively search for food using plant and prey cues (Cantelo and Jacobson 1979, Lattin

1999, Arab et al. 2007), and conspecific cues with sex and trail pheromones (Aldrich et al. 2007). Additionally, they supplement their diet with pollen in many crops (Coll and Ridgway 1995, Coll and Guershon 2002) and typically show distinct seasonal variation in populations in the spring, peaking during crop anthesis (Dicke and Jarvis 1962, Isenhour and Marston 1981). Consequently, *O. insidiosus* does not colonize crops early in the season before pest numbers increase (Veres et al. 2012), but will become more abundant later in the season through reproduction and immigration into the field (Isenhour and Yeargan 1981). Although the spatial and temporal associations between predator and prey are important in the foraging behavior of a predator and its ultimate decision to feed on a pest (Cantrell and Cosner 1999), significant spatiotemporal patterns are not always indicative of a strong trophic linkage (Winder et al. 2001). It is therefore important to decipher the strength of trophic connection in relation to predator and prey spatiotemporal associations.

Two years of extensive field research were conducted in Kentucky winter wheat to examine the spatiotemporal association between *O. insidiosus* and *S. avenae* to correlate population densities to trophic connectivity. The primary goal was to evaluate the effectiveness of natural field boundaries (weed strips) that can be used as a no-input, low-cost form of habitat manipulation to increase predator abundance. My hypothesis was that the increased vegetative diversity that characterizes weed strips will provide supplemental resources to the predator thereby increasing populations throughout the year. Additionally, the spatial pattern of *O. insidiosus* and *S. avenae* was monitored using a grid-based sampling method to test the hypothesis that populations of predator and prey will show significant levels of aggregation in space and time. Finally, predation will be

assessed using molecular gut-content analysis and correlated to population density to test the prediction that increased rates of predation will occur where populations are positively associated with each other.

4.3 Materials and Methods

4.3.1 Field Sampling

Field sampling methods were similar to those described in Chapter 3.

4.3.2 Molecular Detection of Predation

Total DNA was extracted from crushed whole body specimens of *O. insidiosus* using QIAGEN DNeasy Tissue Kits (QIAGEN Inc., Chatsworth, California, USA) following the manufacturer's animal tissue protocol. Primers for *S. avenae* (Hemiptera: Aphididae) (EgaCOllF2: AGATGAAATTAAATGTCCCA and EgaCOllR: AGTTTTTATTGTCTACTTCAATTAAA), which produce a 159 base pair amplicon, were used (after Chen et al. 2000). To test for specificity of these primers, they were screened for cross-reactivity against 180 non-target arthropod species (listed in Chapman et al. 2013). PCR reactions were 50 µL each and consisted of 1 x Takara Buffer (Takara Bio Inc., Shiga, Japan), 0.2 mM of each dNTP, 0.2µM of each forward and reverse primer, 1.25 U Takara *Ex Taq*™ and template DNA (1 µL of total DNA). PCR reactions were carried out in Bio-Rad PTC-200 and C1000 thermocyclers (Bio-Rad Laboratories, Hercules, California, USA). The PCR cycling protocols were 94°C for 1 minute followed by 35 cycles of 94°C for 30 seconds, 56°C for 30 seconds and 72°C for 45 seconds. Amplification success was determined using electrophoresis with 10 µL of PCR product in 1.5% SeaKem agarose (Lonza, Rockland, Maine, USA) stained with GelRed (0.1 mg/µL; Biotium Inc., Hayward, California, USA).

4.3.3 Statistical Analysis

To analyze the effect of weed strips on predator and pest abundance, I used a repeated measures multivariate analysis of variance (PROC GLM in SAS 9.3) assuming a Poisson distribution and field set as a random effect, and the effect of treatment on predation was similarly analyzed assuming a binary distribution. Each year was analyzed separately to enable the temporal synchrony between the predator and prey within a field season to be assessed.

4.3.3.1 Predation Index and Spatial Analysis

Spatial Analysis by Distance IndicEs (SADIE) methods were used as described in Chapter 2.

SADIE Association Analysis (described in detail in Winder et al. 2001, Perry and Dixon 2002) was used (N_AShell version 1.0) to examine the spatial relationship between predators and prey by measuring the degree of local clustering at each sample location. An overall index of association (X) was produced, with a positive association signified for $X > 0$ ($P < 0.025$) and a negative association signified for $X < 0$ ($P > 0.975$). If two populations are aggregated in the same area, they will be considered as locally associated; if one population has a positive patch and the other a negative gap, they will be considered locally disassociated. Contour maps of predators and prey from Surfer mapping software were then overlaid to produce a visualization of the interacting populations. I also used association analysis to examine the local relationship of prey availability and proportion of predators testing positive for aphid DNA. For each of the 32 sampled locations, a predation index was calculated and integerized based on the

proportion of predators testing positive, which was square root arcsine transformed prior to analysis. A positive, local association in this analysis indicates that aggregations of aphids and predation events on aphids are occurring in the same areas.

4.4 Results

4.4.1 Seasonal Abundance

Approximately 100,000 aphids were collected over the two years. Significantly ($F_{1, 238} = 7.46$, $P = 0.0068$) more aphids were collected in 2011(75,626) compared to 2012 (21,411). A subset of approximately 4,500 aphids in the first year and 1,500 in the second year were identified to species and this data used to extrapolate species abundances in each field. The remainder of aphids were counted and categorized as Aphididae. There were 1,977 *Orius insidiosus* collected in sweep nets in 2011 and 313 in 2012, with year having a significant effect on the number of collected ($F_{1,398} = 186.13$, $P < 0.0001$). An additional 523 and 89 were hand collected in 2011 and 2012, respectively, for molecular gut-content analysis. Aphids and *O. insidiosus* showed strong seasonal variability with populations peaking in late May in 2011 (Fig. 4.1a-c) and subsequently crashing. In 2012, a similar pattern was observed, with both pest and predator peaking in late April (Fig. 4.1 d-f) and subsequently declining rapidly.

4.4.2 Weed Strips

During the 2010-2011 growing season, weeds were abundant in all four fields. Dominant species included common ragweed (Asterales: Asteraceae) (*Ambrosia artemisiifolia* L.), giant ragweed (Asterales: Asteraceae) (*Ambrosia trifida* L.), Johnson grass (Poales: Poaceae) (*Sorghum halepense* (L.)), and horseweed (Asteracles: Asteraceae) (*Conyza canadensis* (L). Cronquist). There was no significant effect of weed strips on the abundance of aphids or *O. insidious* during this year (Fig. 4.2a) ($F_{2,1416} =$

0.37, P = 0.688) but there was a significant effect of date ($F_{4,1417}$ = 294.55, P < 0.0001), field ($F_{2,1417}$ = 6.86, P = 0.001), and interaction between date and field ($F_{2,1417}$ = 5.90, P = 0.003).

In the 2011-2012 season, weeds were much less abundant, and the dominant weed species was horseweed, *C. canadensis*. Aphid and *O. insidiosus* populations were significant higher in weed strip plots than control plots (Fig. 4.2b) ($F_{1,1078}$ = 17.52, P < 0.0001). There were also significant effects of date ($F_{5,1079}$ = 583.8, P < 0.0001) field ($F_{2,1079}$ = 55.46, P < 0.0001) as well as interactions between date and field ($F_{2,1079}$ = 52.79, P < 0.0001) and date and treatment ($F_{1,1079}$ = 33.80, P < 0.0001).

4.4.3 Spatial Distribution

Aphid counts were sufficiently high for spatial analysis on four sample dates in 2011 and five dates in 2012. On a whole-field level, aphids (Fig. 4.3a) only showed a significant spatial pattern in the form of patches and gaps in the 2012 field season (Table 4.1d). The abundance of *O. insidiosus* was sufficiently high to complete the spatial analysis on 25 May 2011 and 26 Apr 2012. On a field level, *O. insidiosus* populations showed significant spatial structure in both years (Table 4.1a ,c), with significant patches and gaps of predators occurring on these dates (Fig. 4.3b). When the spatial structure in control and weed strip fields was compared, considerable field to field variation was observed (Table 4.2). In 2011, control and weed strip fields showed random distribution, and significant patches and gaps, whereas during 2012 only control fields showed any significant spatial pattern, in the form aggregated patches. SADIE association analysis was conducted between *O. insidiosus* and aphids for each sample date in 2011 and 2012.

All three fields in both years showed significant positive spatial association (Fig. 4.4a, b, Table 4.3), indicating the same aggregation type, patch or gap, with the two populations.

4.4.4 Predation

There were 523 field-caught *O. insidiosus* from the 2011 field season screened for *S. avenae*, and 214 (41 %) tested positive (Fig. 4.5a). In contrast, only 89 *O. insidiosus* were collected in 2012 and only nine (10 %) screened positive for S. *avenae* DNA (Fig. 4.5b). In both years, there was no significant difference in predation by *O. insidiosus* caught in treatment fields compared to control fields ($P > 0.05$ on all sample dates).

4.4.4.1 Predation Index

The distribution of predation was analyzed by month (May 2011 and April 2012), to coincide with peak abundance of *O. insidiosus*. In 2012, there were extremely low sample sizes due to drought so only one field (Field D) was used in the analysis. Field C in 2011 (Table 4.4a) revealed significant gaps and patches of *O. insidiosus* testing positive for *S. avenae*, but this pattern was not seen in other fields in 2011 or in 2012 (Table 4.4b). However, when predation events were locally correlated with aphid populations, we found positive relationships in each field (Table 4.5). Field B in 2011 showed the only significant association between aggregations of aphids and patches of *O. insidious* testing positive for aphid DNA ($P = 0.014$), but Fields C and D ($P = 0.056$ and $P = 0.071$, respectively) showed strong spatial associations.

4.5 Discussion

This study revealed a strong temporal and spatial relationship between the insidious flower bug, *Orius insidiosus,* and the English grain aphid, *Sitobion avenae*, leading to high levels of aphid predation by the generalist predator. Previous field research has shown *O. insidiosus* to be a prominent aphid predator, but my research uniquely examined the spatial and temporal association between the two and its implication for biological control in winter wheat. Both predator and prey showed a similar temporal pattern in the spring, with aphids appearing first and slowly increasing until their exponential growth phase peaked in late May (Fig. 4.1). *Orius insidiosus* appeared slightly later, but peaked during the same time as aphid populations, coinciding with wheat pollination, and subsequently declining rapidly during plant senescence, in a similar trend to the occurrence of this predator during corn anthesis (Dicke and Jarvis 1962) and soybean flowering (Isenhour and Marston 1981). Temporal synchrony (Welch and Harwood 2014) and spatial dynamics (Cantrell and Cosner 1999) are important components of a natural enemy's success in regulating pest populations. Furthermore, since *S. avenae* infests the heads of wheat, and *O. insidiosus* is a foliar-foraging predator (Schmidt et al. 2008), timing is crucial for mediating their interactions.

In addition to temporal changes in *O. insidiosus* populations, my data also revealed strong spatial patterns between predator and prey populations. The significant, positive, local association between *O. insidiosus* and aphids indicated both positive-positive (areas with both patches of *O. insidiosus* and aphids) and negative-negative association (areas with relatively few *O. insidiosus* and aphids). These results imply that

following colonization of the crop by *O. insidiosus*, these predators create local aggregations to areas where aphids are most abundant. Additionally, using SADIE analysis, I was able to find positive local associations between *O. insidiosus* testing positive for *S. avenae* and aggregations of aphids within the field. This indicates that where there are high density clusters of aphids, there are high levels of detectable *S. avenae* predation by *O. insidiosus*, and where they are low density gaps of aphids, I found lower levels of detectable aphid predation. The capability of a predator to react to a patchily distributed prey, such as aphids, is crucial in determining their likely success in biological control. Other successful biological control agents have been shown to similarly aggregate towards their prey in crops, such as coccinellids, which move towards areas of high aphid density (Agarwala and Bardhanroy 1999, Rahman et al. 2010). Furthermore, generalist predators *Pterostichus melanarious* and *P. madidus* aggregate towards cereal aphids in winter wheat while the aphid population is increasing (Winder et al. 2005b) and *P. melanarious* aggregates to areas of high slug density (Bohan et al. 2000). It was also found that linyphiid spiders were able to locate areas of high prey resources, such as aphids and Thysanoptera, for web-building and high prey interception (Harwood et al. 2013).

Orius insidiosus was screened for the presence of *S. avenae* DNA because this species was dominant during the sampling effort, representing 98.5 % of the total aphids identified. A strong trophic linkage was revealed, with 36 % of these predators screening positive for prey DNA in their gut. In a soybean agroecosystem in a neighboring state (Indiana), this same predator was reported as having detectable soybean aphid (*Aphis glycines*) DNA in 32% of individuals screened (Harwood et al. 2007), a very similar rate

to that observed here. In other agroecosystems, generalist predators have been reported as preying of aphids at a much lower rate (e.g., Harwood et al. 2004; Opatovsky et al. 2012; Chapman et al. 2013; Winder et al. 2013) and these low predation rates could be attributed to the low nutritional quality of aphids to many predators (Bilde and Toft 1994, Toft 1995, Jorgensen and Toft 1997). However, even though they are nutritionally poor to some predators, due to their highly ephemeral and aggregative nature (Winder et al. 1999), they are abundant and easily available (Eubanks and Denno 2000b). The strong temporal pulse of *O. insidiosus* in late spring leads them to aggregate to patches of aphids using chemical cues given off by their prey (Lattin 1999, Aldrich et al. 2007). This is further supported by the spatial predation analysis (Table 4.5) which revealed a positive spatial association between predation events and aphid spatial pattern in the field.

My central hypothesis was that natural, field-bordering weed strips would help enhance *O. insidiosus* that would consequently enhance suppression of aphids. However, my study did not show a significant, consistent increase in the abundance of *O. insidiosus* in weed strip plots compared to control plots with no weed strips. In the 2011 field season there was no difference in populations of predators or pests, but in stark contrast to the hypothesis, in 2012, control plots showed a higher abundance of *O. insidiosus* and aphids than weed strip plots. Other studies examining the response of *O. insidiosus* to weedy vegetation reveal mixed results. In soybeans, weedy and diverse vegetation increased their abundance (Shelton and Edwards 1983, Lundgren et al. 2008, Lundgren et al. 2009), but when grassy corridors were established (Kemp and Barrett 1989), these corridors acted as a sink for the predators, increasing abundance only in the corridors. Additionally, surrounding cotton fields, vegetative buffers increased *O. insidiosus* populations but also

pest thrips in the adjacent cotton fields (Olson and Wackers 2007). Clearly the contrasting results across systems provide clear evidence suggesting predator-, pest-, crop- and region-specific effects are likely occurring. Teasing apart the specific mechanisms driving population enhancement of predators is critical for evaluating natural enemies in biological control. While one approach of conservation management may be sufficient in a certain crop, that may not be universally the case and mechanisms of attraction to weed strips, flowering border vegetation or other refuge habitat by both predator and pest (e.g., olfactory cues, food resources, refuge from harsh conditions within the crop system), characterizing the specific role of such habitats is key when maximizing biological control services afforded by generalist predators.

In agroecosystems, generalist predators can aid in suppression of pest populations if they are present in substantial numbers early in the season when pest density is low (Ehler and Miller 1978, Settle et al. 1996). In contrast to this phenomenon, I found *O. insidiosus* to be an important late-season predator of *S. avenae*, with predator populations positively spatially associated with prey populations. Despite their generalist feeding habits, over 40 % of *O. insidious* screened positive for *S. avenae* DNA, suggesting potential for pest suppression as part of the wider natural enemy community. Late season control of aphids in winter wheat is still important in this system because of the seasonal dynamics of aphids and their role as virus vectors. Large populations of *S. avenae* are capable of producing alates. Once these mobile forms acquire BYDV they can inoculate healthy plants at considerable distance and speed. The presence of an aphid predator with high predation rates could slow their movement and the rate of spread of the virus.

Table 4.1. Index of aggregation, I_a, and mean cluster index, v_i for patches and v_j for gaps, with associated probability, P_x, from randomization test of SADIE in winter wheat fields. Data are presented for counts of (a) *Orius insidiosus* and (b) aphids sampled on 25-May-11 and (c) *O. insidiosus* and (d) aphids sampled on 26-Apr-2012. Bold font denotes where a measurable spatial pattern can be detected.

a.

25-May-11
Orius insidiosus

	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
A	1.495	**0.026**	-1.401	**0.044**	1.309	0.075
B	1.369	0.063	-1.265	0.107	1.241	0.113
C	1.505	**0.035**	-1.421	**0.048**	1.492	**0.028**

b.

25-May-11
Aphids

	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
A	1.339	0.063	1.234	0.104	1.269	0.083
B	1.228	0.133	1.135	0.192	1.161	0.157
C	0.920	0.570	0.966	0.454	0.998	0.379

c.

26-Apr-12
Orius insidiosus

	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
D	0.859	0.742	-0.857	0.779	0.868	0.752
E	2.001	**0.001**	-1.807	**0.002**	1.911	**0.001**
F	1.624	**0.016**	-1.640	**0.017**	0.661	0.065

d.

26-Apr-12
Aphids

	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
D	1.267	0.116	1.299	0.083	1.124	0.210
E	2.010	**0.001**	1.904	**0.003**	1.883	**0.002**
F	1.480	**0.035**	1.531	**0.023**	1.249	0.100

Table 4.2. Index of aggregation, I_a, and mean cluster index, v_i for patches and v_j for gaps, with associated probability, P_x, from randomization test of SADIE, for *Orius insidiosus* and counted weed strip and control (non-weed strip) plots of winter wheat in (a) 2011 and (b) 2012. Bold font denotes where a significant measurable spatial pattern can be detected.

a.

25-May-11 *Orius insidiosus*						
	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
A (trt)	1.205	0.127	-1.121	0.195	1.246	0.075
B (trt)	1.539	**0.002**	-1.467	**0.005**	1.548	**0.002**
C (trt)	1.337	**0.038**	-1.177	0.134	1.241	0.078
A (con)	0.714	1.000	-0.745	0.996	0.755	0.993
B (con)	1.612	**0.001**	-1.400	**0.013**	1.399	**0.015**
C (con)	1.172	0.147	-1.140	0.174	1.087	0.245

b.

26-Apr-12 *Orius insidiosus*						
	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
D (trt)	0.900	0.682	-0.871	0.799	0.899	0.707
E (trt)	1.056	0.325	-1.063	0.276	1.306	**0.032**
F (trt)	1.376	**0.028**	-1.174	0.134	1.191	0.114
D (con)	1.010	0.412	-1.113	0.209	1.009	0.411
E (con)	0.983	0.477	-0.997	0.435	1.121	0.195
F (con)	1.192	0.114	-1.194	0.110	1.249	0.067

Table 4.3. Summary of SADIE analyses of local spatial association between *Orius insidiosus* and aphids. Probability of P < 0.025 denotes significant positive association, and P > 0.975 denotes significant negative dissociation (after Winder et al. 2001). Bold font denotes where a significant measurable spatial pattern can be detected.

	25-May-11			26-Apr-12	
Field	**X**	**P**	Field	**X**	**P**
A	**0.798**	**<0.0001**	D	0.246	0.137
B	**0.700**	**<0.0001**	E	**0.729**	**<0.0001**
C	**0.424**	**0.011**	F	0.291	0.052

Table 4.4. Index of aggregation, I_a, and mean cluster index, v_i for patches and v_j for gaps, with associated probability, P_x, from randomization test of SADIE, for *Orius insidiosus* predation events on *Sitobion avenae* in winter wheat during the month of May in (a) 2011 and April in (b) 2012. Also shown are corresponding SADIE statistics for aphids for the month of May in (a) 2011 and April in (b) 2012. Bold font denotes where a significant measurable spatial pattern can be detected.

a.

May 2011
Orius insidiosus predation on *Sitobion avenae*

	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
A	0.82	0.821	-0.791	0.934	0.776	0.941
B	0.881	0.692	-0.868	0.737	0.93	0.567
C	1.776	**0.004**	-1.759	**0.006**	1.895	**0.009**

May 2011
Aphids

	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
A	1.331	0.065	-1.225	0.109	1.26	0.087
B	1.248	0.119	-1.123	0.212	1.085	0.243
C	0.85	0.767	-0.861	0.748	0.87	0.75

b.

April 2012
Orius insidiosus predation on *Sitobion avenae*

	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
D	0.882	0.674	-0.886	0.668	0.867	0.722

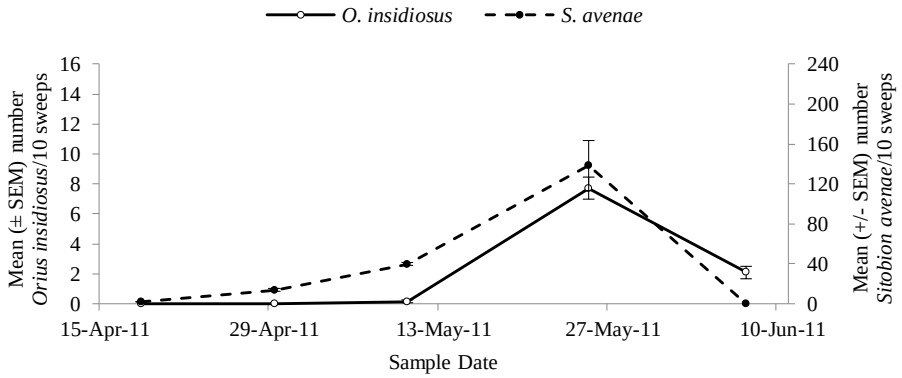
April 2012
Aphids

	Index of Aggregation		Cluster of gaps		Cluster of patches	
Field	**I_a**	**P_a**	**Mean V_j**	**P_j**	**Mean V_i**	**P_i**
D	1.345	0.079	-1.306	0.08	1.254	0.096

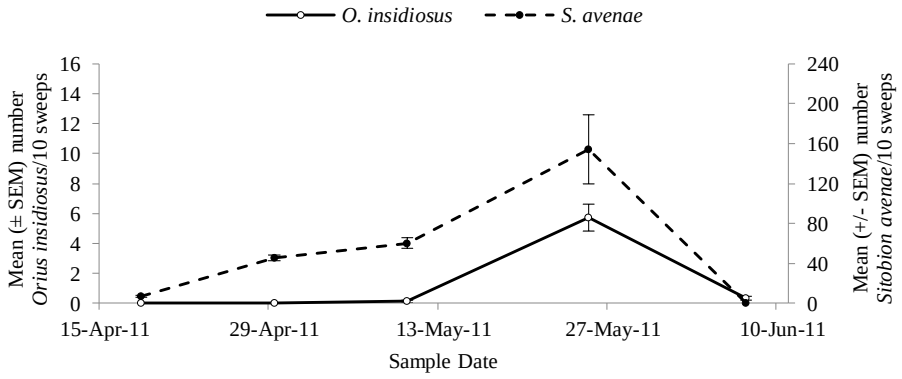
Table 4.5. Summary of SADIE analyses of local spatial association between *Orius insidiosus* predation events and aphids. Probability of $P < 0.025$ denotes significant positive association, and $P > 0.975$ denotes significant negative dissociation.

Year	Field	**X**	**P**
2011	A	0.161	0.2
2011	B	**0.436**	**0.014**
2011	C	0.332	0.056
2012	D	0.302	0.071

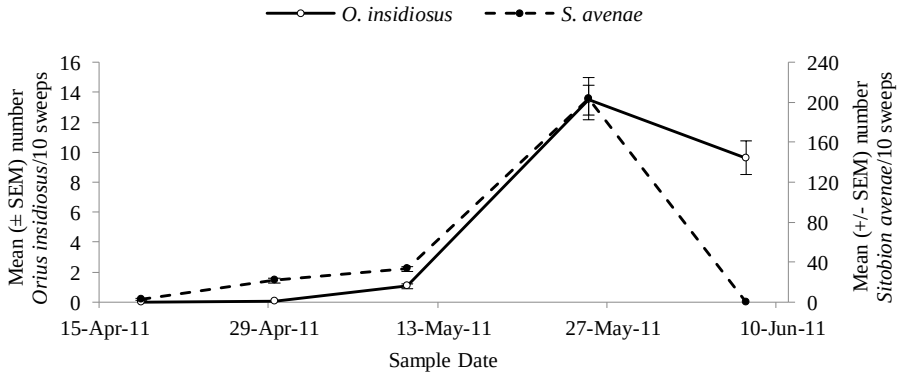
a. 2011



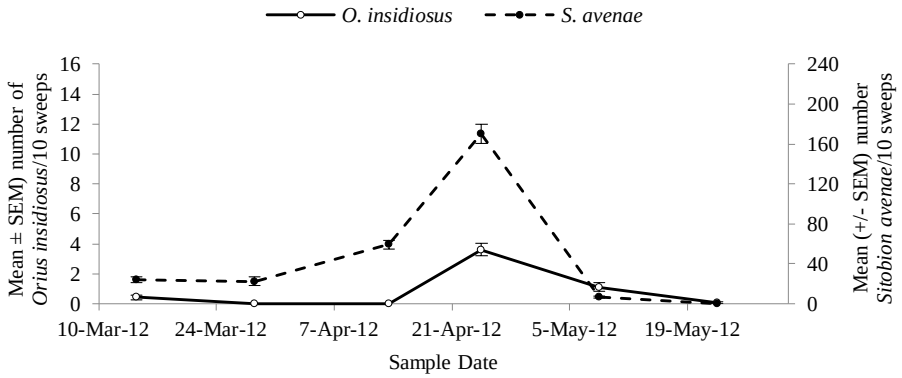
b. 2011



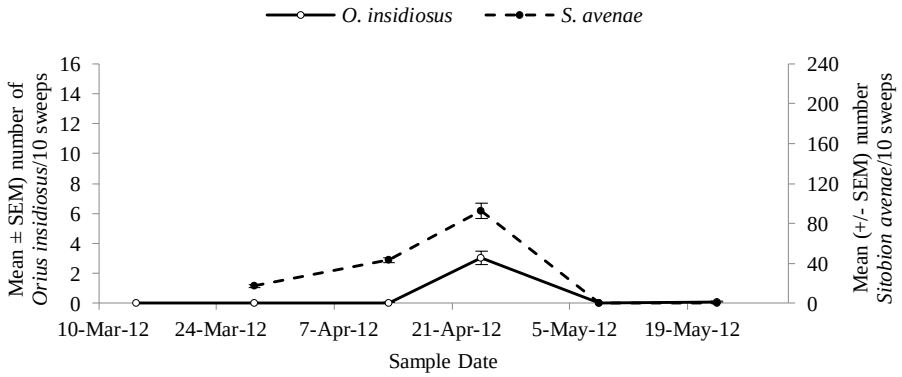
c. 2011



d. 2012



e. 2012



f. 2012

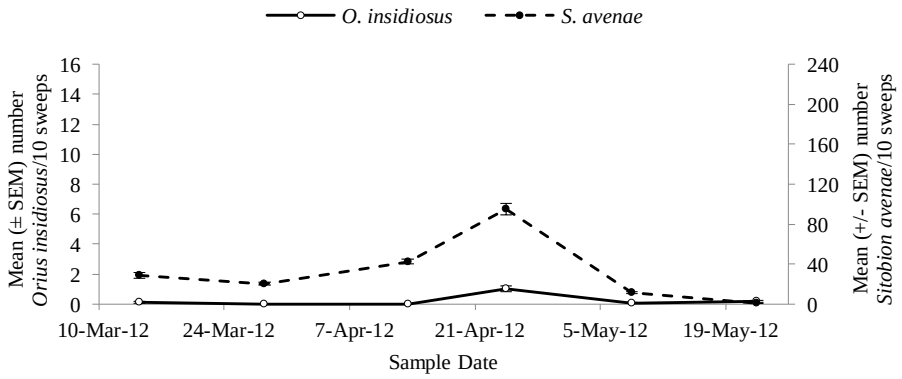
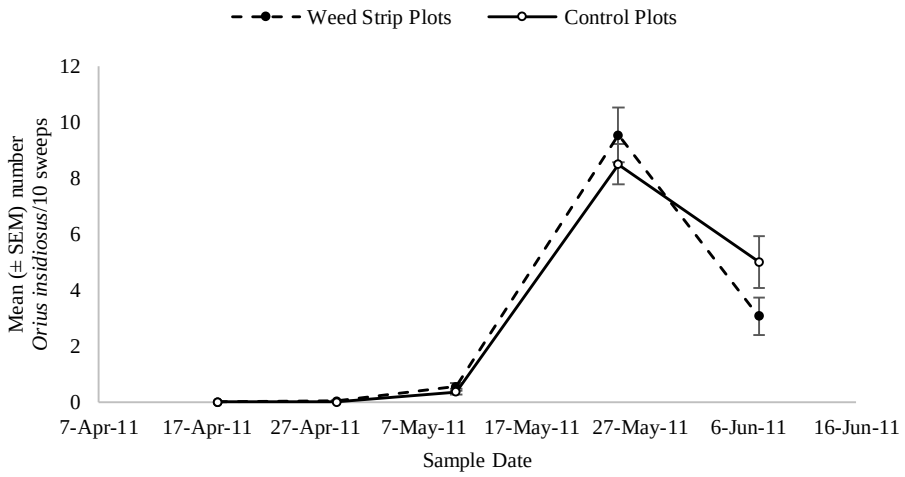


Figure 4.1. Mean number (± SEM) of *Sitobion avenae* and *Orius insidiosus* captured in ten figure-eight sweep net samples in three winter wheat fields in 2011 (a, b, c) and 2012 (d, e, f).

a. 2011



b. 2012

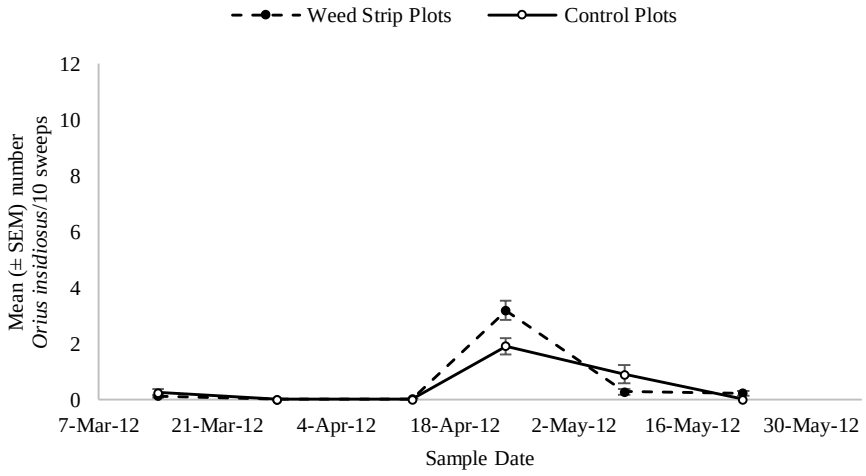


Figure 4.2. Mean number (± SEM) *Orius insidiosus* (Hemiptera: Anthocoridae) in 2011 (a) and 2012 (b) caught in ten figure-eight sweeps in winter wheat fields.

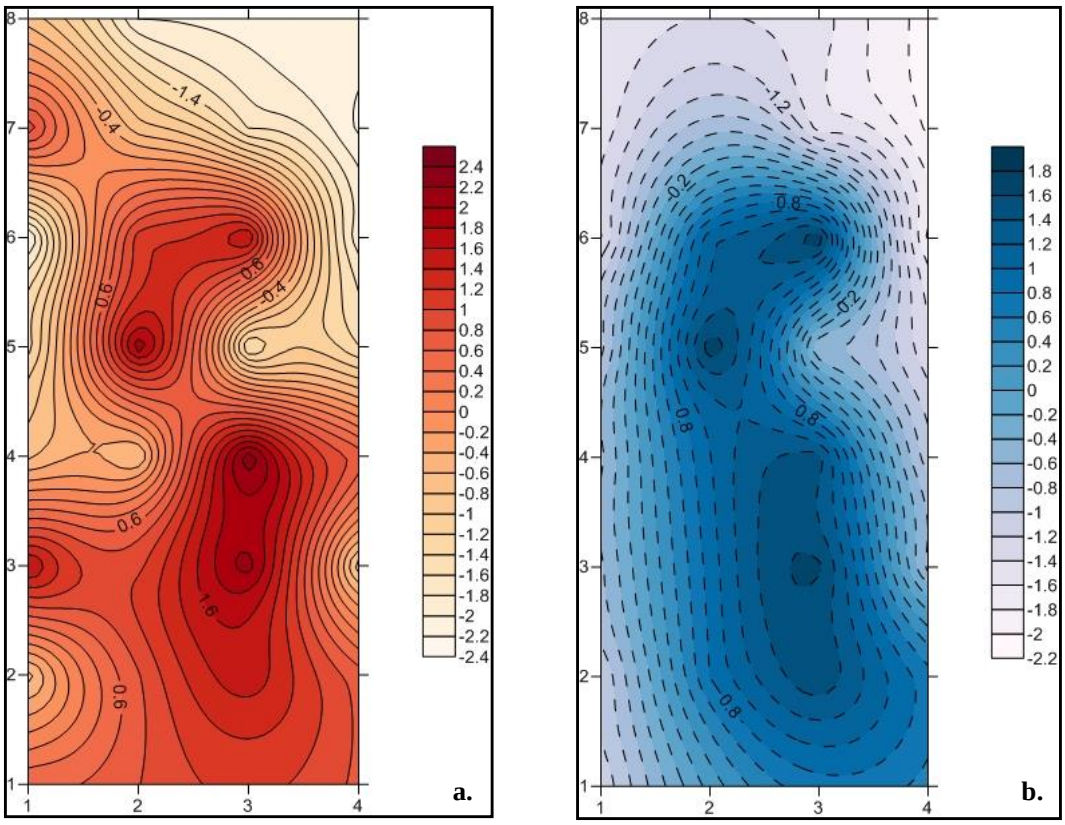


Figure 4.3. Contour maps of clustering in winter wheat fields on 25-May-2011 for (a) aphids and (b) *Orius insidiosus*. The key with negative values indicates gaps and positive values indicates a patch. A unit that belongs to a patch is indicated by $v_i > 1$ whereas a gap is indicated by neighboring unit with values of $v_j<-1$. Values of $v < -1.5$ indicate significantly larger gaps, and values $v > 1.5$ indicate significantly larger patches. The horizontal and vertical axes represent the coordinate system used for sampling, with each sample unit measuring 188m².

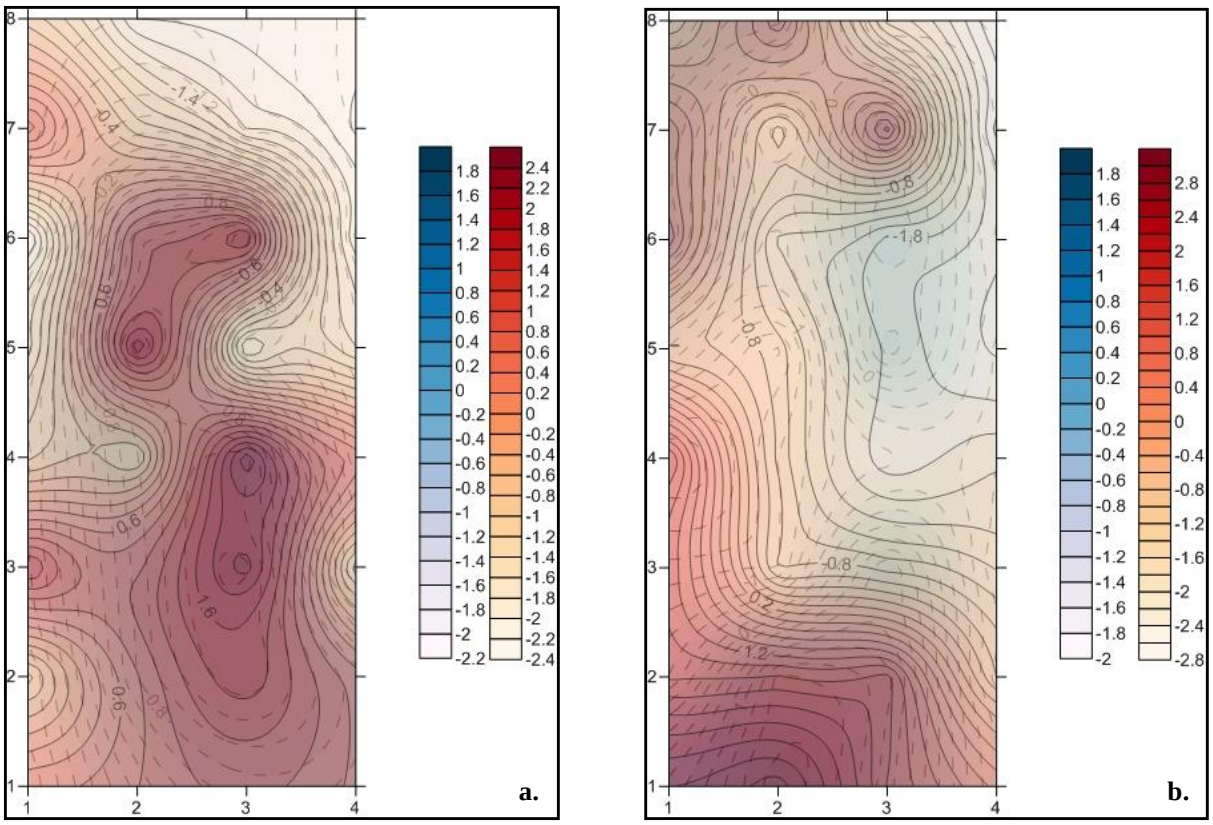
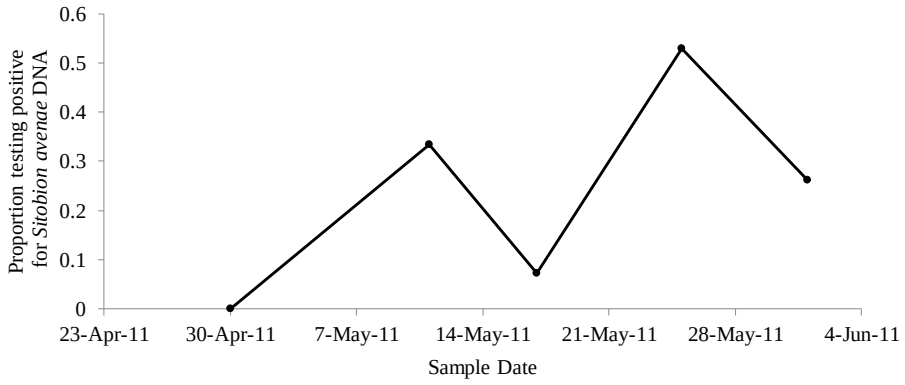


Figure 4.4. Contour maps of local spatial association showing positive association between *Orius insidiosus* and aphids in winter wheat on 25-May-2011 in (a) Field A and (b) Field C. The key with negative values indicates dissociation and positive values indicate association between *Orius insidiosus* (red) and aphid species (blue). Areas associated with small negative values show strong dissociation (light-colored areas), and areas associated with large positive values show strong association (dark-colored areas) between insidious flower bugs and aphids. The horizontal and vertical axes represent the coordinate system used for sampling, with each sample unit measuring 188m².

a. 2011



b. 2012

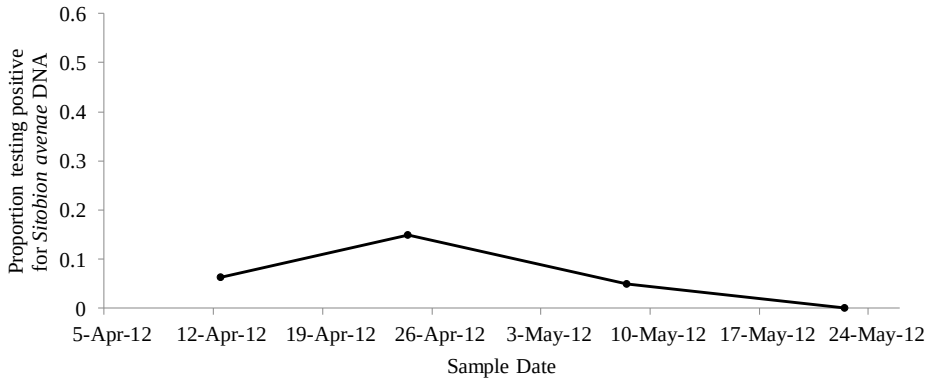


Figure 4.5. The proportion of field-caught *Orius insidiosus* testing positive for *Sitobion avenae* DNA using PCR-based molecular gut content analysis during the (a) 2011 and (b) 2012 field spring seasons.

Chapter 5 : Habitat manipulation through weed strips promote aphid predation by coccinellids in winter wheat

5.1 Abstract

Natural enemies provide valuable ecosystem services in agroecosystems in the form of pest suppression. Conservation biological control aims to enhance natural enemy populations through management of the local habitat, and understanding which natural enemies have the greatest impact on pests is essential to creating successful habitat manipulations. In this study, I focused on coccinellids, some of the most dominant aphid predators in agroecosystems, and which have been shown to respond positively to on-farm management. Specifically, I examined the biological control of grain aphids, *Rhopalosiphum padi* and *Sitobion avenae*, provided by coccinellids in Kentucky winter wheat. In a two-year field study, I examined the coccinellid community in winter wheat and its response to natural, field-bordering weed strips. I utilized molecular gut content analysis to identify the major aphid predators in this system and whether or not predation was affected by habitat manipulation. I identified five species of coccinellids in winter wheat, *Coccinella septempunctata, Coleomegilla maculata, Harmonia axyridis, Hippodamia convergens,* and *Cycloneda munda*. PCR-based molecular gut content analysis revealed that all five species of coccinellids are major aphid predators, many of which tested at very high frequencies for aphid predation (>50%). Furthermore, I found in both field seasons adult and larval coccinellid populations were higher in plots bordered by weed strips compared to control plots. This effect was species-specific,

suggesting that different coccinellid species respond differently to the non-crop habitats surrounding winter wheat. *C. maculata* and *C. septempunctata*, the two most dominant coccinellid predators in this system, both displayed higher predation frequencies with increased aphid populations. These results implicate coccinellids as valuable natural enemies of grain aphids in winter wheat, and suggest that on-farm manipulations can be used to increase predator populations as part of an integrated pest management program.

5.2 Introduction

Agroecosystems are ephemeral, frequently disturbed habitats and the regularity of harvest can interfere with pest suppression by natural enemies (Bjorkman et al. 2004). Conservation biological control, through habitat manipulation (Landis et al. 2000, Gurr et al. 2004) increases natural enemy populations through the provisioning of resources such as pollen or nectar (Eubanks and Denno 2000), physical refugia, and alternative prey or hosts (Landis et al. 2005). Habitat management for control of grain aphids in cereals has been extensively studied, including manipulations such as grassy margins (Holland et al. 2012, Birkhofer et al. 2014, Ramsden et al. 2015) or wildflower strips (Frank et al. 2009, Anjum-Zubair et al. 2010). However, while some studies show that field margins promote natural enemy populations such as coccinellids in cereals (Dong et al. 2012), others do not show an increase or benefit to biological control (Holland et al. 2008, Caballero-Lopez et al. 2012), indicating that these differences may be species- or crop-specific.

Predaceous Coccinellidae are an important part of biological control in agriculture (Hagen 1962, Hodek and Honek 1996, Obrycki and Kring 1998) and have been shown to be effective biological control agents against aphids (Hagen 1962, Kring et al. 1985, Obrycki and Kring 1998, Hodek and Michaud 2008). Numerically dominant predators in cereal fields, coccinellids also occupy all of the niches of their prey, and have a high searching capacity (Obrycki et al. 2000). In addition, individual coccinellid species vary in their prey and habitat choices as well as their life history behaviors, leading to variable impacts on aphid populations (Hodek and Honek 1996). Moreover, the landscape around

crops plays an important role in the diversity and abundance of coccinellid predators (Elliott et al. 1999, Gardiner et al. 2009a, Gardiner et al. 2009b) and this can be exploited for biological control purposes. Coccinellids hibernate in the surrounding non-crop habitats, surviving on prey in these hibernation areas before moving into crops (Honek 1989, Hodek et al. 1993, Bianchi and van der Warf 2004). Therefore, the food supply in non-crop habitats will affect coccinellids' fecundity (Ferran and Dixon 1993) and their migration into crops (Hodek et al. 1993), which will ultimately impact their biological control capabilities (Bianchi and van der Warf 2004).

A key component of successful aphid biological control is early season predation before pest populations have reached outbreak levels (Settle et al. 1996, Landis and Van der Werf 1997, Harwood et al. 2004, 2007). Early in the season when prey is at low densities, predators must be active and efficient foragers (de Roince et al. 2013), but they need not have a high predation rate because the impact of consuming small numbers of aphids will be large (Murdoch et al. 1985, de Roince et al. 2013). Pest regulation requires strong temporal synchrony between the predator and pest (Welch and Harwood 2014) and this is especially true for pest aphids which are an ephemeral resource that varies greatly in space and time (Dixon 1985, Winder et al. 1999, 2001). In the field, coccinellids begin to lay eggs when the aphid colonies reach a certain density (Honek 1980), thus establishing a temporal synchrony with the pest population. The timing of aphid infestation in the crop impacts the population dynamics of both the pest and predator. Coccinellids respond to early infestations of aphids with an increase in reproduction, and this can lead to higher levels of pest suppression, but a late aphid

infestation may cause a delay in coccinellid reproduction (Honek 1978, Bianchi and van der Warf 2004).

Numerous studies have attempted to quantify the relationship between aphids and coccinellids in cereal crops, but due to the complex nature of the temporal and spatial dynamics between them (Kindlmann and Dixon 1989) and the variation in predator density from year to year (Freier et al. 2007), results have been inconsistent. More importantly, given the push towards sustainable agriculture and value of ecosystem services, I wanted to determine the significance of coccinellids as biological control agents in Kentucky winter wheat. My goals were to identify the coccinellid community in winter wheat and the strength of the aphid-coccinellid food web using molecular gut-content analysis. Additionally, I sought to determine the effects of field-bordering weed strips on coccinellid populations. I predict that the weed strips will have a higher vegetative diversity and complexity that helps increase the coccinellid population, and this will, in turn, aid in aphid suppression.

5.3 Materials and Methods

Field sampling methods were the same as Chapter 3 with the following exceptions.

5.3.1 Feeding Trials

On May 21, 2013 approximately 300 adult *Coccinella septempunctata* were collected from winter wheat and the surrounding weeds in Lexington, Kentucky, USA at Spindletop Research Farm (GPS coordinates 38.1 N, 84.5W). In the laboratory, they were kept in individual plastic cups (0.07 m D x 0.04 m H) in an environmental chamber (24°C, photoperiod 16 h:8 h light:dark). All beetles were starved for 48 hours prior to the experiment, but given access to water via a moist cotton ball. At the beginning of the experiment, 100 *C. septempunctata* were fed a single *Rhopalosiphum padi* (Hemiptera: Aphididae). If any beetles did not feed on the aphid, they were not used for the experiment. Upon completion of feeding, 10 beetles were immediately preserved in 95% ethanol (t = 0 h) that was previously frozen to prevent regurgitation by the beetles. The remaining 90 beetles were given a "chaser" prey of *Drosophila melanogaster* to simulate natural conditions and maintained in the environmental chamber at above conditions; 10 *C. septempunctata* were removed and preserved at 95% EtOH after 1,2,4,8,12,16,24,36 and 48 hours and stored at -20°C. The same methods were applied for 100 *C. septempunctata* fed *Sitobion avenae*.

Laboratory colonies of *Coleomegilla maculata* were reared on a mixed diet of bird cherry-oat aphid, *R. padi*, and pea aphid, *Acyrthociphon pisum* (Harris), in an environmental chamber (24°C, photoperiod 16h:8h light:dark). The same procedures

were used for 200 adult *C. maculata* as described above, with the addition of 100 second instar larvae fed *R. padi* and 100 second instar larvae fed *S. avenae*.

5.3.2 Molecular Detection of Predation

Total DNA was extracted from crushed whole body specimens of all Coccinellidae using QIAGEN DNeasy Tissue Kits (QIAGEN Inc., Chatsworth, California, USA) following the manufacturer's animal tissue protocol. Primers are listed in Table 5.1 (Chen et al. 2008). To test the specificity of these primers, the primers were screened for cross-reactivity against 180 arthropod species (listed in Chapman et al. 2013). PCR reactions were 12.5 µL each and consisted of 1 x Takara Buffer (Takara Bio Inc., Shiga, Japan), 0.2 mM of each dNTP, 0.2µM of each forward and reverse primer, 1.25 U Takara *Ex Taq*™ and template DNA (1 µL of total DNA). PCR reactions were carried out in C1000 thermocyclers (Bio-Rad Laboratories, Hercules, California, USA). The PCR cycling protocols were 94° for 1 minute followed by 35 cycles of 94 ° for 30 seconds, 56 ° for 30 seconds and 72 ° for 45 seconds. Amplification success was determined using electrophoresis with 10 µL of PCR product in 1.5% SeaKem agarose (Lonza, Rockland, Maine, USA) stained with GelRed (0.1 mg/µL; Biotium Inc., Hayward, California, USA).

5.3.3 Statistical Analyses

To analyze the effect of weed strips on predator abundance and predation rates, I used a repeated measures multivariate analysis of variance (PROC GLM in SAS 9.3) assuming a Poisson and binary distributions, respectively. A Bonferroni correction was

used for multiple comparisons. The relationship between availability of prey resources and proportion of predators screening positive was correlated following square root arcsine transformation of gut-content data. A regression with forward selection at the P = 0.05 significance level was conducted.

5.4 Results

5.4.1 Predator and Pest Abundance

A total of 10,144 aphid predators were caught in sweep nets in 2011 and 2012; 58.15% of these were Coccinellidae composed of five species: *Coccinella septempunctata, Coleomegilla maculata, Harmonia axyridis, Hippodamia convergens,* and *Cycloneda munda*. Within the coccinellids, 4,254 were larvae and 1,645 were adults. In both field seasons, *C. septempunctata* and *C. maculata* were the dominant coccinellid species. There were 102,254 aphids captured in sweep nets containing the two most abundant aphid pest species, *Rhopalosiphum padi* and *Sitobion avenae* (Hemiptera: Aphididae).

5.4.1.1 Effect of weed strips

There was a significant effect of treatment of coccinellid abundance in 2011 (Fig. 5.1 a, b) ($F_{10,4723} = 15.11$, $P < 0.0001$) and 2012 (Fig. 5.1 c, d) ($F_{9,5423} = 57.73$, $P < 0.0001$). In 2011, all species of coccinellids were significantly higher in weed strip plots except that *H. convergens* was higher in control plots (Table. 5.1a). In 2012, all species of coccinellids were significantly higher in weed strips plots as well, except that *C. munda* and *H. axyridis* adults, which occurred in very small numbers, but were significantly higher in control plots (Table. 5.1b).

Date also had a significant effect on the coccinellid abundance in 2011 ($F_{4,4732} = 2652.62$, $P < 0.0001$) with larval populations peaking on 25-May-2011 and adult populations peaking later on 7-June-2011. Similarly, in 2012 date had a significant effect

($F_{5,5431}$ = 959.8, P < 0.0001) with larval populations peaking on 26-April-2012 and adult populations peaking on 12-May-2012. Year had a significant effect on coccinellid numbers, ($F_{2,2035}$ = 160.83, P < 0.0001) with significantly more larvae in 2011 ($F_{1,2036}$ = 153.82, P < 0.0001) and adults in 2012 ($F_{1,2036}$ = 167.37, P < 0.0001).

5.4.2 Molecular Analysis of Predation

A total of 1,780 coccinellids were hand-collected from winter wheat between March and June 2011 and 2012 for molecular gut-content analysis. In 2011, 511 adults and 740 larvae were screened for aphid DNA and in 2012, 431 adults and 97 larvae were screened (Table 5.3). All five species tested positive for *R. padi* and *S. avenae* DNA either as adults or larvae. *C. septempunctata* larvae displayed the highest predation frequencies on *S. avenae* (0.84) and on *R. padi* (0.30).

5.4.2.1 DNA decay rate

The rates of *R. padi* DNA decay are shown in Figure 5.3. The DNA detectability half-life was 2.5 h (r^2 = 0.881) for *C. septempunctata* adults, 5 h (r^2 = 0.948) for *C. maculata* adults and 3 h (r^2 =0.854) for *C. maculata* 2nd instar larvae fed *R. padi* in feeding trials.

5.4.2.2 Effect of weed strips

Aphid predation was significantly affected by weed strips, but this effect varied between species and lifestages. *C. septempunctata* adults and larvae showed a significant increase in predation rates on both *R. padi* and *S. avenae* in weed strip plots compared to

control plots (larvae, 2011: $F_{2, 562} = 13.8$, $P < 0.0001$, 2012: $F_{2, 38} = 1.38$, $P = 0.264$, adults: 2011: $F_{2, 401} = 3.98$, $P = 0.019$, 2012: $F_{2, 224} = 6.46$, $P = 0.002$). *H. convergens* adults had higher predation rates in control plots (2011: $F_{2, 63} = 8.88$, $P = 0.0004$, 2012: $F_{2, 104} = 2.71$, $P = 0.071$) as did *H. axyridis* larvae (2011: $F_{2, 87} = 4.19$, $P = 0.018$).

5.4.2.3 Prey availability

The proportion of *C. maculata* testing positive for *R. padi* increased as populations increased (Fig. 5.2 a) (2011, adults: arcsine proportion positive = -0.035+ 0.181 x *R. padi* abundance, $F_{1,9} = 26.88$, $P < 0.001$, $r^2 = .749$, larvae: arcsine proportion positive = -0.43 + .194 x *R. padi* abundance, $F_{2,5} = 7.73$, $P = 0.032$, $r^2 = .563$) and similarly for *S. avenae* populations (Fig. 5.2b) (2011, adults: arcsine proportion positive = 0.473+ 0.006 x *S. avenae* abundance, $F_{1,9} = 6.90$, $P = 0.028$, $r^2 = .434$, larvae: arcsine proportion positive = 3.431 + 0.002 x *S. avenae* abundance, $F_{2,5} = 16.63$, $P = 0.01$, $r^2 = 0.983$). Adult *H. convergens* consumed more *R. padi* with increasing aphid abundance in 2012 (Fig. 5.2c) (arcsine proportion positive = -0.136 + 0.190 x *R. padi* abundance, $F_{2,7} = 19.68$, $P = 0.002$, $r^2 = 0.711$).

5.4.2.4 Temporal effects

The only coccinellid whose predation frequency was affected by date was *C. maculata* larvae which showed a decreasing proportion testing positive for *S. avenae* over the season, each year (Fig. 2b) (2011: arcsine proportion positive = 3.431 - 0.656 Week number, $r^2 = 0.927$, $F_{2,5} = 17.6271$, $P < 0.0001$, 2012: arcsine proportion positive = 1.153 – 0.288 Week number, $r^2 = 0.987$, $F_{2,2} = 235.59$, $P < 0.001$).

5.5 Discussion

In this study, I examined the coccinellid community in Kentucky winter wheat and its biological control potential for pest suppression against grain aphids. Molecular gut-content analysis revealed that four species of coccinellids, *Coccinella septempunctata, Coleomegilla maculata, Harmonia axyridis,* and *Hippodamia convergens*, share a strong trophic linkage with *Rhopalosiphum padi* and *Sitobion avenae*. Aphid prey detection ranged from 0 to over 80%. In many of the species tested, more than half of the adults and larvae screened for *S. avenae* tested positive for aphid DNA. This study adds to the growing body of literature that names coccinellids as important biological control agents of aphids in agroecosystems.

C. maculata and *C. septempunctata* adults and larvae had a significantly higher proportion testing positive for aphid DNA as aphid populations increased, indicating a temporal synchrony between predator and pest. Successful pest regulation requires a predator that can adapt to a periodically changing environment (Welch and Harwood 2014), such as an aphid population that is ephemeral in space and time (Winder et al. 1999, 2001). Coccinellids begin to lay eggs when the aphid colonies reach a certain density (Honek 1980), both establishing a temporal synchrony and provisioning for their offspring (Hemptinne and Dixon 1997). As aphid populations increase, adult and larval *C. maculata* and *C. septempunctata* respond by consuming more prey items, as shown by molecular analyses. Understanding these cyclical dynamics both within a season and over the long term can help growers with integrative pest management strategies regarding natural enemies.

In addition to studying the coccinellid-aphid food web in winter wheat, I examined the effect of field-bordering weed strips on these predators, and if the habitat manipulation affected aphid predation. Over two field seasons, *C. septempunctata* and *C. maculata* had significantly higher populations in plots bordered by weed strips compared to control plots. Additionally, these were the two most abundant species collected in winter wheat. The effect of the weed strips was species- and lifestage-specific. The only two coccinellid species that were significantly higher in control plots were *C. munda* adults and *H. convergens* larvae, which appeared in the wheat in low numbers throughout the season. *C. munda* did test positive for aphid DNA in both field seasons, but the numbers collected were so low that it this species will most likely not make an impact on aphid populations through predation

While coccinellids are highly mobile generalist predators that readily move between several habitats throughout one lifetime (Honek and Hodek 1996), the surrounding landscape may affect relatively mobile and immobile predators differently (Bianchi et al. 2007, Rand and Tscharntke 2007) and even species differently (Elliott et al. 1999, Schmidt et al. 2008). Adult coccinellids emerge from hibernation in the early spring when aphid number in the field are low (Bianchi and van der Werf 2004) and move into crops to oviposit. Compared to their early instar offspring, adults are relatively more mobile. This could account for the difference we saw between the species, and adults and larvae within each species. There were large differences in the weed cover in the two field seasons; 2011 was a wet year that produced lush, full weed strips while the drought in 2012 caused only sparse weeds to fill in the strips. I saw a reduction in the number of aphids and coccinellid predators, which is consistent with a drought year

(Barton and Ives 2014). Nonetheless, the effect on *C. septempunctata* and *C. maculata* was consistent in both years regardless of the weather. This indicates that it may not be the amount, or composition of the weed strips, that influence these predatory species, but rather the placement of them. Previous research indicates that the increased vegetative diversity provided by field margins can enhance the coccinellid population (Alhmedi et al. 2009, Dong et al. 2012, Holland et al. 2012, Villegas et al. 2013) in addition to other natural enemies (Albajes et al. 2009, Birkhofer et al. 2014) but also that these types of habitat manipulations are complicated (Frank et al. 2009, Ranjha and Irmler 2014).

I used molecular gut content analysis to examine the half-life detectability in two of the major predators in the system, *C. maculata* (adults and larvae) and *C. septempunctata* (adults), feeding on *R. padi*. I found that not only did the DNA half-life detectability differ between the coccinellid species, but within a species, between life stages. This was not surprising, because even for closely related species, like predators in the family Coccinellidae, the DNA half-life is specific for each predator-prey combination, and cannot be estimated (Gagnon et al. 2011). Additionally, the level of detection is predator, prey and even life-stage specific (Greenstone et al. 2010, Ingels et al. 2013); in some cases, adult and larval ladybeetles have different rates of digestion (Ingels et al. 2013). Not surprisingly, coccinellid adults and larvae have different digestive capabilities and food requirements (Michaud and Qureshi 2005, Ingels et al. 2013). Larvae use extra-oral digestion and will sometimes regurgitate fluid back into chewed up prey before sucking it back into their mouths (Hodek and Honek 1996), so this behavior may explain the shorter DNA detectability time that we found in *C. maculata*. Relatively short DNA detection periods help to give a clearer interpretation of

field data (Sheppard and Harwood 2005), while predators with a longer DNA detectability will show a higher incidence of prey remains in their guts compared to those predators with shorter detectability intervals (Greenstone et al. 2010). Therefore, caution should be taken when interpreting field data using molecular techniques.

This study helps highlight the strong biological control potential of coccinellids against aphids in winter wheat. The habitat manipulation and predation results showed species- and lifestage-specific differences between the predators, emphasizing the need to focus on effects of biodiversity in agroecosystems. Nevertheless, other natural enemies should be investigated for full season control of grain aphids in winter wheat.

Table 5.1. Primers utilized for gut content analysis (Chen et al 2000).

Aphid Species	Primer Name	Sequence	Amplicon Size
Sitobion avenae	EgaCOllF2	AGATGAAATTAAATGTCCCA	159 bp
	EgaCOllR	AGTTTTTATTGTCTACTTCAATTAAA	
Rhopalosiphum padi	BcoaCOIIF4	TCATTCATGAACAATTCAAG	148 bp
	BcoaCOIIR2	GAATAGGTATAAATCTGTGATTAATA	

Table 5.2. Mean number (± SEM) of coccinellid larvae and adults in (a) 2011 and (2) 2012 and caught in ten figure-eight sweeps.

a.

Date	**3-Apr-11**	**3-Apr-11**	**17-Apr-11**	**17-Apr-11**	**9-May-11**	**9-May-11**	**25-May-11**	**25-May-11**	**9-Jun-11**	**9-Jun-11**
Treatment	**Control**	**Weed**	**Control**	**Weed**	**Control**	**Weed**	**Control**	**Weed**	**Control**	**Weed**
Larvae										
Coccinella septempunctata	0	0	0.229 ± 0.091	0.104 ± 0.054	0.104 ± 0.068	0.109 ± 0.046	9.556 ± 1.180	11.792 ± 1.226	0.201 ± 0.201	0.063 ± 0.046
Hippodamia convergens	0	0	0.021 ± 0.021	0	0	0	1.022 ± 0.259	0.417 ± 0.142	0	0
Coleomegilla maculata	0	0	0	0	0.042 ± 0.0.029	0.022 ± 0.022	4.6 ± 0.480	4.5 ± 0.416	0.656 ± 0.144	0.896 ± 0.191
Cycloneda munda	0	0	0	0	0	0	0.022 ± 0.022	0.167 ± 0.091	0	0.021 ± 0.021
Harmonia axyridis	0	0	0	0	0	0	1.489 ± 0.301	2.708 ± 0.536	0.063 ± 0.0462	0.188 ± 0.064
Adults										
Coccinella septempunctata	0.021 ± 0.021	0	0.021 ± 0.021	0.021 ± 0.021	0.042 ± 0.029	0	0.2 ± 0.068	0.188 ± 0.057	0.313 ± 0.073	0.604 ± 0.145
Hippodamia convergens	0	0	0	0	0	0	0.044 ± 0.031	0	.063 ± .035	0.063 ± 0.035
Coleomegilla maculata	0	0	0.042 ± 0.029	0.021 ± 0.021	0.104 ± 0.045	0.326 ± 0.083	0.111 ± 0.047	0.146 ± 0.060	1.396 ± 0.201	1.58 ± 0.240
Cycloneda munda	0	0.021 ± 0.021	0.021 ± 0.021	0	0.042 ± 0.029	0.022 ± 0.022	0.044 ± 0.031	0.146 ± 0.051	0	0
Harmonia axyridis	0	0	0	0	0	0	0	0.063 ± 0.035	0.201 ± 0.079	0.25 ± 0.091

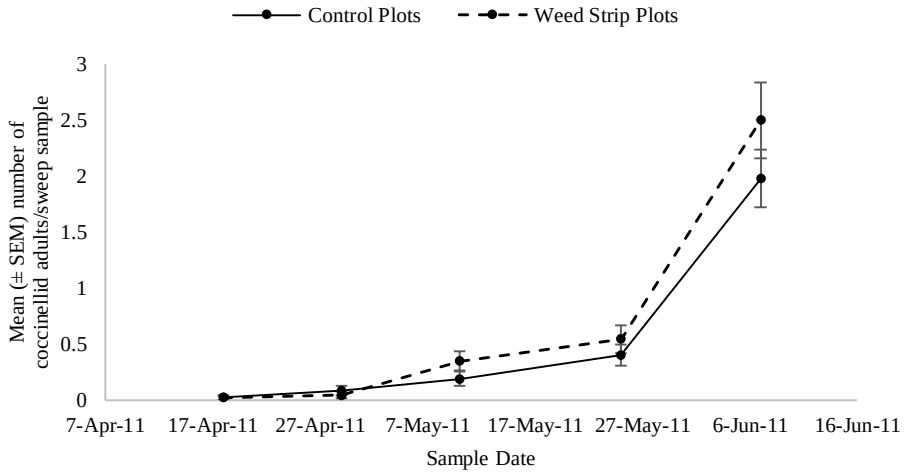
b.

Date	14-Mar-12	14-Mar-12	28-Mar-12	28-Mar-12	14-Apr-12	14-Apr-12	26-Apr-12	26-Apr-12	11-May-12	11-May-12	23-May-12	23-May-12
Treatment	**Control**	**Weed**	**Control**	**Weed**	**Control**	**Weed**	**Control**	**Weed**	**Control**	**Weed**	**Control**	**Weed**
Larvae												
Coccinella septempunctata	0.0833 ± 0.0501	0.042 ± 0.029	0.646 ± .121	1.188 ± .222	0.021 ± 0.021	0.0208 ± 0.0208	0.0201 ± 0.0201	0	0.875 ± 0.194	1.406 ± 0.161	0	0.0208 ± 0.0208
Hippodamia convergens	0	0.021 ± 0.021	0.021 ± 0.021	0.0417 ± 0.029	0	0.0208 ± 0.0208	0	0	0.031 ± 0.031	0.031 ± 0.031	0	0
Coleomegilla maculata	0	0	0.083 ± 0.040	0.104 ± 0.045	0	0.0208 ± 0.0208	0.083 ± 0.050	0.208 ± 0.089	1.063 ± 0.258	2.031 ± 0.322	0.042 ± 0.029	0.063 ± 0.035
Cycloneda munda	0	0	0	0	0	0	0	0	0	0	0	0
Harmonia axyridis	0	0	0	0	0	0	0.021 ± 0.021	0	0	0.063 ± 0.063	0	0
Adults												
Coccinella septempunctata	0	0	0	0	0.875 ± 0.180	2.896 ± 0.510	2.417 ± 0.245	3.896 ± 0.534	0.406 ± 0.109	0.406 ± 0.118	0.188 ± 0.071	0.229 ± 0.074
Hippodamia convergens	0	0	0.021 ± 0.021	0	0.208 ± 0.059	0.412 ± 0.102	2.354 ± 0.285	3.979 ± 0.331	0.844 ± 0.169	0.906 ± 0.182	0.396 ± 0.088	0.604 ± 0.118
Coleomegilla maculata	0	0.042 ± 0.042	0.021 ± 0.021	0	0	0.021 ± 0.021	0.042 ± 0.042	0.083 ± 0.065	0.563 ± 0.127	0.489 ± 0.127	0.771 ± 0.202	1.083 ± 0.190
Cycloneda munda	0	0.021 ± 0.021	0	0	0	0	0	0	0.031 ± 0.031	0.063 ± 0.043	0.104 ± 0.045	0
Harmonia axyridis	0.042 ± 0.029	0.021 ± 0.021	0	0	0.042 ± 0.029	0	0.104 ± 0.045	0.083 ± 0.040	0	0.031 ± 0.031	0	0

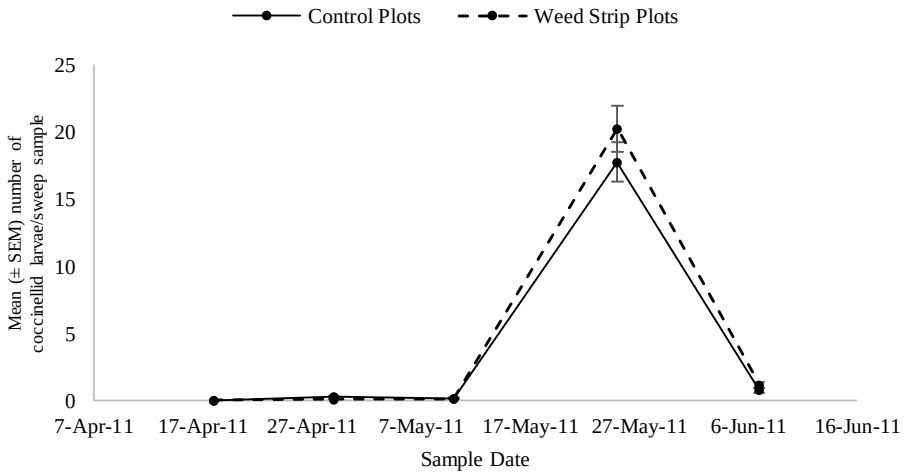
Table 5.3. Results of PCR-based molecular gut content analysis showing the proportion of each coccinellid adult and larval species in each field season collected from winter wheat testing positive for each aphid species.

Predator Species		**Proportion testing positive for prey DNA**	
2011 ADULTS	N	*Rhopalosiphum padi*	*Sitobion avenae*
Coccinella septempunctata	278	0.176	0.525
Coleomegilla maculata	115	0.078	0.296
Cycloneda munda	17	0.059	0.588
Harmonia axyridis	41	0.049	0
Hippodamia convergens	60	0.2	0.45
2011 LARVAE	N	*Rhopalosiphum padi*	*Sitobion avenae*
Coccinella septempunctata	374	0.302	0.837
Coleomegilla maculata	283	0.297	0.527
Harmonia axyridis	83	0.47	0.771
2012 ADULTS	N	*Rhopalosiphum padi*	*Sitobion avenae*
Coccinella septempunctata	231	0.169	0.45
Coleomegilla maculata	81	0	0
Cycloneda munda	2	0	0.5
Harmonia axyridis	3	0	0.667
Hippodamia convergens	114	0.088	0.149
2012 LARVAE	N	*Rhopalosiphum padi*	*Sitobion avenae*
Coccinella septempunctata	45	0.044	0.667
Coleomegilla maculata	52	0.039	0.135

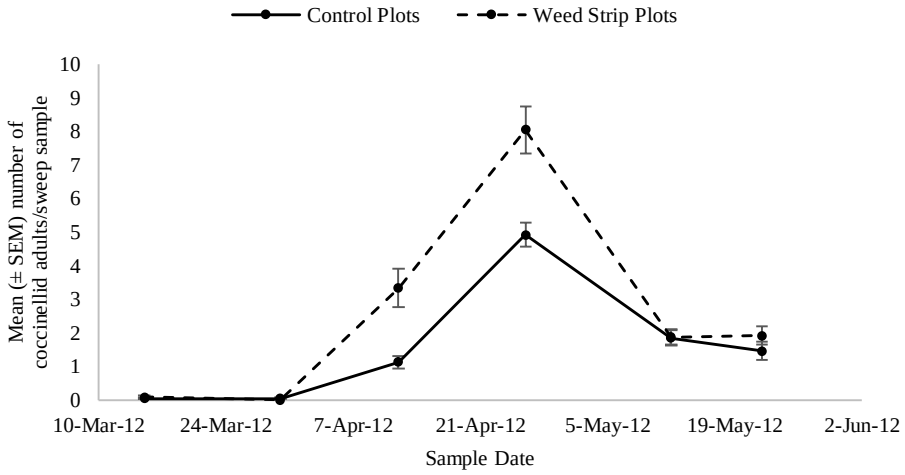
a. 2011 Adults



b. 2011 Larvae



c. 2012 Adults



d. 2012 Larvae

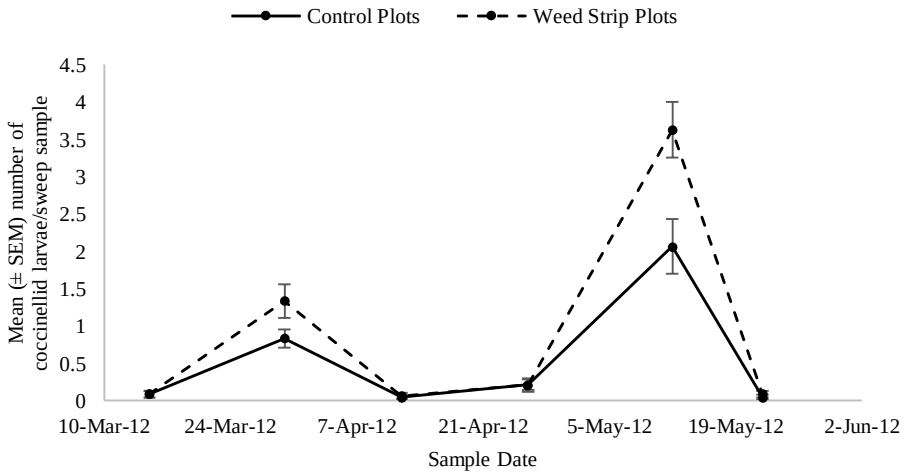
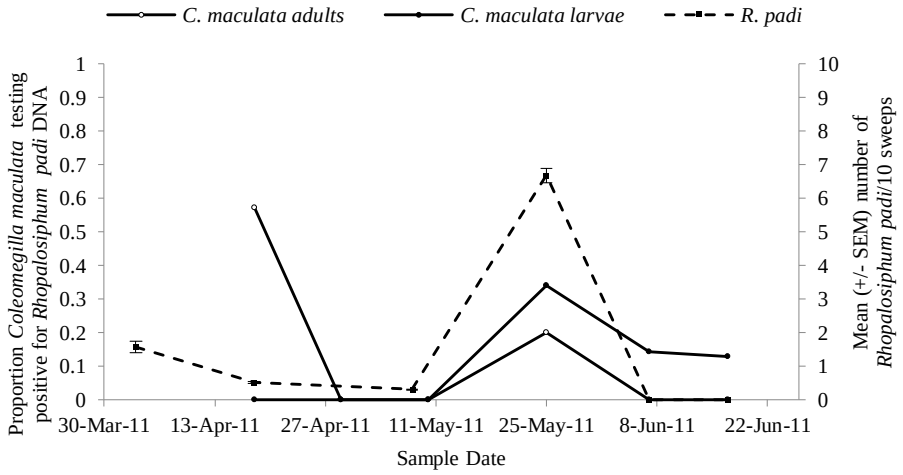
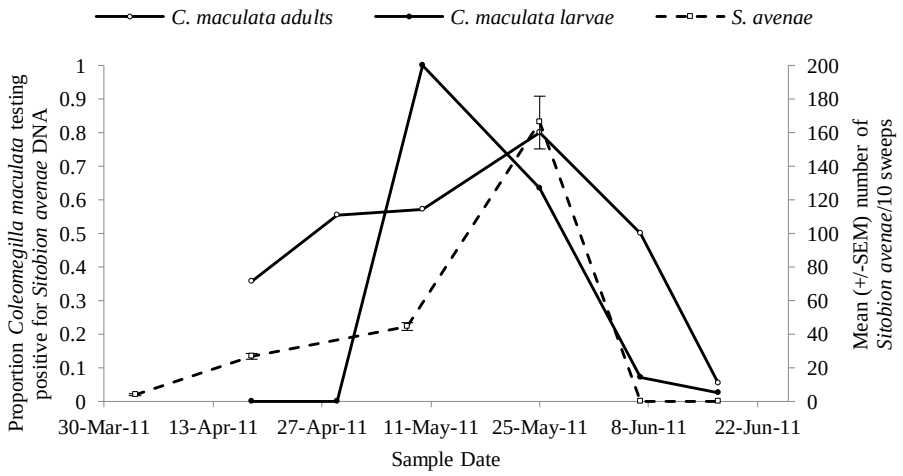


Figure 5.1. Mean number (± SEM) of coccinellid adults (all species) in (a) 2011 and (c) 2012 and coccinellid larvae (all species) in (b) 2011 and (d) 2012 caught in ten figure-eight sweeps.

a.


C. maculata adults
C. maculata larvae
R. padi
Proportion Coleomegilla maculata testing positive for Rhopalosiphum padi DNA
Mean (+/- SEM) number of Rhopalosiphum padi/10 sweeps
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10
9
8
7
6
5
4
3
2
1
0
30-Mar-11
13-Apr-11
27-Apr-11
11-May-11
25-May-11
8-Jun-11
22-Jun-11
Sample Date

b.


C. maculata adults
C. maculata larvae
S. avenae
Proportion Coleomegilla maculata testing positive for Sitobion avenae DNA
Mean (+/-SEM) number of Sitobion avenae/10 sweeps
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
200
180
160
140
120
100
80
60
40
20
0
30-Mar-11
13-Apr-11
27-Apr-11
11-May-11
25-May-11
8-Jun-11
22-Jun-11
Sample Date

c.

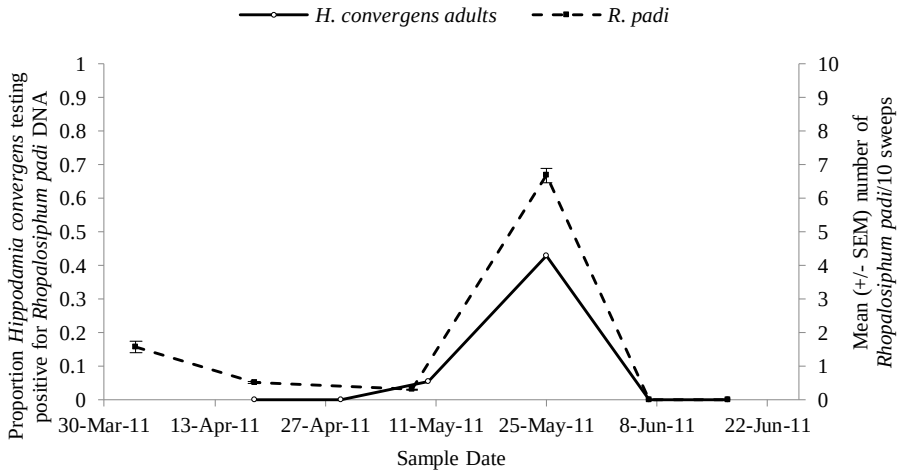
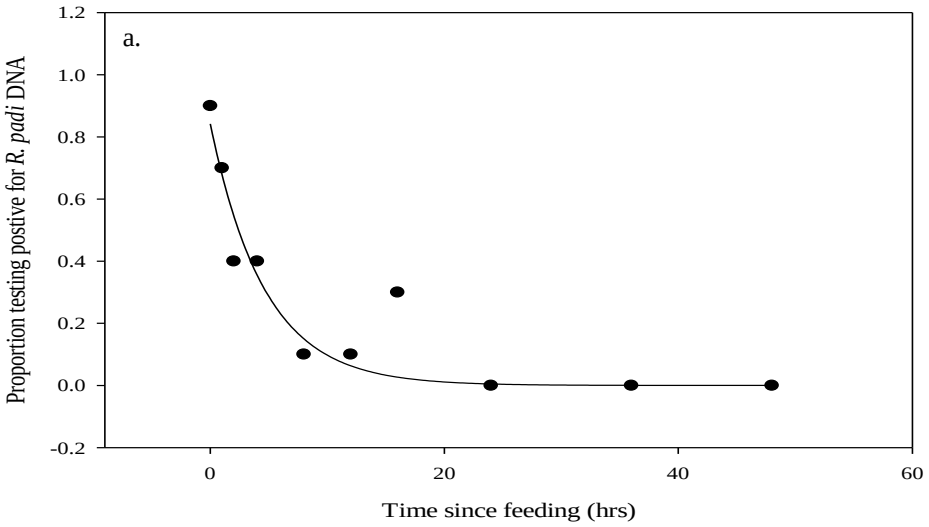
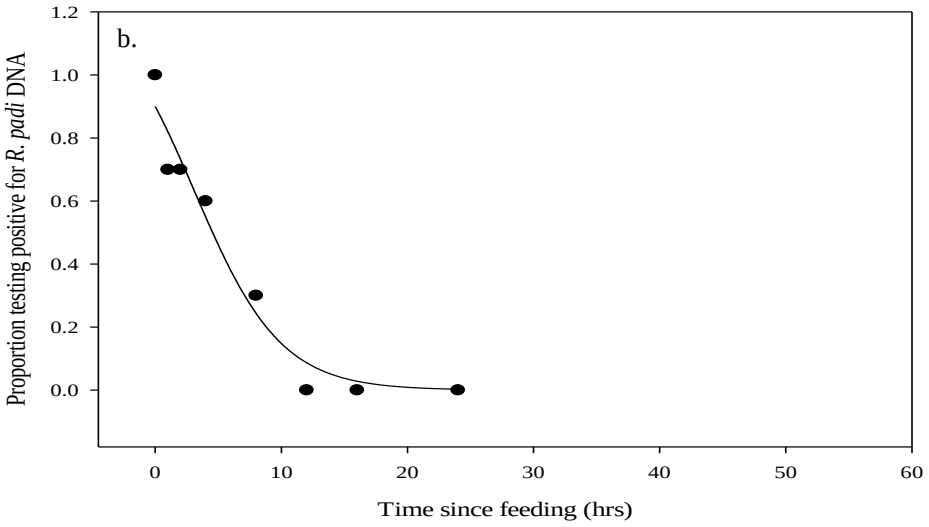


Figure 5.2. Mean (± SEM) of prey aphid captured in sweep samples on secondary axis, and proportion of coccinellid predators screening positive for aphid DNA on primary axis. a. *Coleomegilla maculata* adults and larvae testing positive for *Sitobion avenae*, with *S. avenae* populations, b. *C. maculata* adults and larvae testing positive for *Rhopalosiphum padi*, with *R. padi* populations, c, *Hippodamia convergens* adults testing positive for *R. padi*, with *R. padi* populations


a.
Proportion testing postive for R. padi DNA
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0
20
40
60
Time since feeding (hrs)


b.
Proportion testing positive for R. padi DNA
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
Time since feeding (hrs)

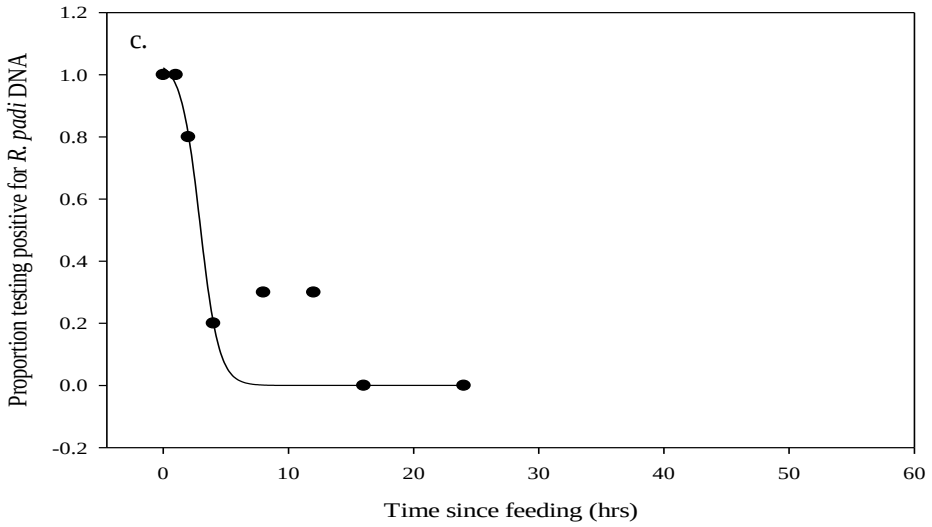


Figure 5.3. Detection of DNA of *Rhopalosiphum padi* following consumption. A. *Coccinella septempunctata* adults: detectability half-life = 2.5 h; B. *Coleomegilla maculata* adults: detectability half-life = 5 h; C. *C. maculata* larvae: detectability half-life = 3 h.

Chapter 6 : Intraguild predation in a coccinellid community: influence of habitat manipulations

6.1 Abstract

Habitat manipulations in agroecosystems provide resources to generalist predators in an effort to increase their populations for pest suppression. However, the increased abundance and diversity of natural enemies can sometimes lead to intraguild predation which may interfere with the biological control in a system. Aphidophagous systems are ideal for studying IGP due to the large population outbreaks of aphids that bring together multiple natural enemies, such as coccinellids. In this study, I examined levels of IGP in a coccinellid community in Kentucky winter wheat and whether or not it was affected by natural, field-bordering weed strips. Using molecular gut-content analysis, species-specific primers were designed to identify coccinellid intraguild prey, *Coleomegilla maculata* and *Coccinella septempunctata*, which were the two most abundant species identified in a previous study. Analysis revealed IGP by three species, *C. maculata*, *C. septempunctata*, and *Harmonia axyridis* with more than half of *C. maculata* and *H. axyridis* larvae screening positive for *C. septempunctata* DNA. Additionally, *C. maculata* collected in weed strip plots had significantly higher proportions testing positive for intraguild predation compared to those collected in control plots, with no difference in proportions testing positive for aphid DNA. These results suggest that coccinellids are effective biological control agents of pest aphids, and their populations can be successfully manipulated through on-farm management without significant interference from IGP.

6.2 Introduction

Habitat manipulations in agroecosystems have been widely adopted as successful strategies for pest control by enhancing generalist predator populations for top-down control of insect pests (Costamagna and Landis 2011, Dong et al. 2012, Holland et al. 2012). These modifications can provide alternative resources for generalist predators such as nectar or pollen (Eubanks and Denno 2000), physical refugia, and alternative prey or hosts (Landis et al. 2005). Each modification has an impact on the abundance and diversity of natural enemies in the system (Landis et al. 2000, Gurr et al. 2004), and for a predator to be an effective pest management resource, it must respond positively to population manipulations (Furlong and Zalukci 2010). Generalist predators may feed from more than one trophic level, such as conspecifics, other predators or herbivores (Polis et al. 1989) so increased numbers of natural enemies will not always lead to increased levels of pest suppression (Davey et al. 2013).

Generalist predators are often involved in intraguild predation (IGP) (Raso et al. 2014) and it has been suggested that one of the mechanisms behind the increase in natural enemies in complex environments is the reduction of IGP (Langellotto and Denno 2004). IGP is the killing and subsequent eating of species that compete for the same resources, and it is capable of significantly altering the distribution and abundance of the species involved (Polis et al. 1989). IGP and cannibalism can have significant effects on the structure of a community (Polis 1981, 1988, Polis et al. 1989). In cases where the goal is primary productivity of the plant, such as high yields in agroecosystems, IGP can

destabilize a system or interfere with pest suppression (Rosenheim et al. 1993, Holt and Polis 1997, Finke and Denno 2005). An increased abundance and diversity of predators can interfere with pest suppression (Rosenheim et al. 1993, Finke and Denno 2005), especially when there are more intraguild predators than predators (Finke and Denno 2005). IGP is widespread among biological control agents, and sometimes these predator-predator interactions can lead to the breakdown of biological control (Rosenheim et al. 1995, Vance-Chalcraft et al. 2007). Non-lethal effects are also possible with IGP, forcing some predators to limit their movements as a result of predators (e.g. Harwood and Obrycki 2005), or in areas of behavior, prey consumption or development (Noppe et al. 2012, Moreno et al. 2014). Therefore, identifying the value of each predator (Hagler and Blackmer 2013) and the most effective combination of predators (Hindayana et al. 2001) for maximum pest suppression is important.

Much work has been done concerning IGP in aphidophagous systems (reviews: Lucas 2005, Hemptinne et al. 2012). Aphidophagous guilds represent a good model system for IGP because the temporal distribution of large densities of aphid prey during brief periods of outbreaks promotes a spatial and temporal co-occurrence of natural enemies, specifically coccinellids (Winder et al. 1999, Burgio et al. 2002, Holland et al. 2004, Lucas 2005). Adult coccinellids oviposit in young, high density patches of aphids so offspring have food and time for development (Hemptinne and Dixon 1997). Therefore, multiple species of coccinellids may be aggregated in the same aphid patch, competing for the same resource (Hodek and Honek 1996). Because of this competition, the presence or absence of alternative or extraguild prey is crucial in determining in

maintaining the occurrence of IGP (Gillespie and Quiring 1992, Lucas et al. 1998). For example, IGP will decrease with increasing extraguild prey, such as aphids (Lucas et al. 1998).

Much of the previous work on IGP has conducted in the laboratory, and while this information may give insight into the outcome of intraguild interactions, they may not be indicative of what is happening on a larger field-scale (Harwood and Obrycki 2005). Studying IGP in laboratory arenas can overestimate levels of IGP by forcing predators to eat intraguild prey (Lucas et al. 2009). Interpreting laboratory results is difficult because in the field there are other factors, such as alternative prey, no spatial limitations or multiple life stages of a predator (Moreno et al. 2014). Although small arthropods engage in cryptic feeding events that are difficult to observe, molecular gut-content analysis has allowed us to identify partially digested remains or fluid-feeding insects (Sheppard and Harwood 2005). Molecular studies have revealed substantial levels of IPG in a variety of agroecosystems, such as cotton (Hagler and Blackmer 2013), soybeans (Gagnon et al. 2011) and winter wheat (Davey et al. 2013).

Theory on IGP is based on the simplest food webs and often doesn't take into account habitat structure (Janssen et al. 2007). Recently, many studies have addressed the question of multiple predator effects and habitat complexity and found that a more structured habitat can reduce negative interactions between predators and enhance pest suppression (e.g., Warfe and Barmuta 2004, Harvey and Eubanks 2005, Finke and Denno 2006), but this effect is habitat specific so more studies are needed (Griffen and Byers 2006). Field-bordering weed strips have the potential to increase natural enemy

populations and thus predator-predator interactions, which can lead to intraguild predation. In the previous chapter, I found that fields with weed strips had significantly higher populations of coccinellids, *Coccinella septempunctata* and *Coleomegilla maculata*. My hypothesis is that the increased coccinellid abundance in weed strip fields will lead to higher encounter rates and thus higher levels of IGP compared to control fields. To test this, I designed species-specific primers for the most abundant intraguild prey to screen field-collected coccinellid predators for intraguild predation in this winter wheat system.

6.3 Materials and Methods

Field sampling methods were the same as Chapter 3 with the following exceptions.

6.3.1 Molecular Detection of Predation

Total DNA was extracted from crushed whole specimens of *Coccinella septempunctata* Linnaeus. and *Coleomegilla maculata* (De Geer) the two most abundant coccinellids in the study fields, using QIAGEN DNeasy Tissue Kits (QIAGEN Inc., Chatsworth, California, USA) following the animal tissue protocol. To obtain coccinellid sequences for primer design, we employed the cytochrome *c* oxidase subunit I (COI) primers LCO-1490 (Folmer et al. 1994) and HCO-700ME (Breton et al. 2006), which produce a ~710 bp amplicon (A.K.A. the "Folmer fragment" or animal barcoding region of Hebert et al. 2003). PCR reactions (25 µL) consisted of 1x Takara buffer (Takara Bio Inc., Shiga, Japan), 0.2 mM of each dNTP, 0.2 mM of each primer, 0.625U Takara Ex Taq™ and template DNA (2 µL of total DNA). PCR reactions were carried out in Bio-Rad PTC-200 and C1000 thermal cyclers (Bio-Rad Laboratories, Hercules, California, USA). The PCR cycling protocols were 94 °C for 1 min followed by 50 cycles of 94 °C for 50 s, 40 °C for 45 s, 72 °C for 45 s and a final extension of 72 °C for 5 min. Reaction success was determined by electrophoresis of 8 µL of PCR product in 1.5% SeaKem agarose (Lonza, Rockland, Maine, USA) stained with gel red (0.1 mg/µL). Reactions that yielded significant product were purified with QIAGEN MinElute PCR purification kit according to the manufacturer's guidelines. Cycle sequencing reactions were carried out in both the forward and reverse directions using labeled dideoxynucleotides (ABI Big-

Dye Terminator mix v. 3.0; Applied Biosystems, Foster City, Califonia, USA; ABI sequencer) in an ABI 9700 thermal cycler. The separation of cycle sequencing reaction products was done by Applied Biosystems 3730XL or 3730 DNA Analyzers.

6.3.1.1 Primer Design

To design primers to test for intraguild predation by coccinellids, *C. septempunctata* and *C. maculata* sequences were aligned with coccinellid COI sequences downloaded from GenBank. MUSCLE (Edgar 2004) was used to align the sequences. The data set was searched for sites where *C. septempunctata* and *C. maculata* had bases unique to those species relative to all other sequences present. Primer names reflect the position of the 5' base in relation to the 658 bp Folmer fragment. The primers that were designed and optimized are listed in Table 6.1. The optimal PCR cycling protocol for both primer pairs with Takara reagents (as above) was 94 ℃ for 1 min followed by 45 cycles of 94 ℃ for 45 s, 62 ℃ for 45 s, 72 ℃ for 15 s. To test the specificity of these primers, the primers were screened for cross-reactivity against 180 arthropod, mollusk and nematode species (listed in Chapman et al. 2013).

All hand-collected coccinellids were screened for aphid predation described in the previous chapter. Sample sizes were only sufficiently large to screen adult and larval *Coccinella septempunctata*, *Coleomegilla maculata*, adult *Hippodamia convergens*, and larval *Harmonia axyridis* for the presence of intraguild predation with the newly designed coccinellid primers.

6.3.2 Statistical Analysis

Gut-content data were square root arcsine transformed before analyses were performed. To analyze the effect of weed strips on predator abundance and predation rates, I used a repeated measures analysis of variance (PROC GLM in SAS 9.3) assuming Poisson and binary distributions, respectively. The relationship of prey availability and the proportions of predators screening positive for intraguild predation or aphid predation was correlated and a regression (PROC REG) with forward selection at the $P = 0.05$ significance level was conducted. Since gut-content data is prey species- but not lifestage-specific, the availability of coccinellids as intraguild prey was calculated by combining both larvae and adults of each species. Furthermore, because we are concerned with biological control of both aphid pests, aphid predation in the regression model was on calculated based on predation events on *Rhopalosiphum padi* and *Sitobion avenae*, and prey aphid availability was both species combined.

6.4 Results

A description of the coccinellid and aphid species collected in the field is detailed in Chapter 5.

6.4.1 Molecular Detection of Predation

I detected both pest DNA and intraguild prey DNA in three of the coccinellid species, *C. septempunctata*, *C. maculata* and larval *H. axyridis* (Table 6.2). *C. septempunctata* adults and larvae tested positive for *C. maculata* DNA at very low levels, 5.4% and 4.3% respectively. More *C. maculata* larvae (62.5%) tested positive for *C. septempunctata* DNA than adults (2.4%). *C. septempunctata* DNA was detected more frequently (50.6%) in *H. axyridis* larvae than *C. maculata* (8.9%). Significantly more larval *C. septempunctata* ($F_{3,2967} = 33.56$, $P < 0.0001$) tested positive for *C. maculata* ($P = 0.007$), *R. padi* ($P < 0.0001$), and *S. avenae* ($P < 0.0001$) DNA compared with adults. Similarly, significantly more larval *C. maculata* ($F_{3,1328} = 39.93$, $P < 0.0001$) tested positive for *C. septempunctata* ($P < 0.0001$), *R. padi* ($P < 0.0001$), and *S. avenae* ($P = 0.022$) DNA compared to adults. Aphid predation by coccinellids is discussed is more detail in Chapter 5.

6.4.1.1 Weed Strip Effect

Treatment had a significant effect on predation by *C. maculata* (2011: $F_{3,704} = 2.62$, $P = 0.05$), *H. axyridis* (2011: $F_{4,175} = 5.38$, $P = 0.0004$), *C. septempunctata* (2012: $F_{3,1086} = 16.06$, $P < 0.0001$), and *C. maculata* (2012: $F_{3,282} = 2.61$, $P = 0.052$).

Significantly higher proportions of *C. maculata* collected in weed strips plots tested positive for *C. septempunctata* DNA compared to those found in control plots in 2011 ($P = 0.01$) and 2012 ($P = 0.022$). Significantly lower proportions of *C. septempunctata* collected in weed strip plots tested positive for *C. maculata* DNA compared to control plots 2011 ($P = 0.036$).

6.4.2 Prey Availability

The proportion of *C. maculata* larva screening positive for *C. septempunctata* DNA decreased over time in 2011 (arcsine proportion positive = 2.965 – 0.459 (week number), $r^2 = 0.651$, $F_{1,5} = 12.18$, $P = 0.018$). *C. septempunctata* adults had increasing amounts of detectable *C. maculata* DNA with increasing *C. maculata* abundance and decreasing aphid abundance in 2011 (arcsine proportion positive = 2.662 + 0.311 (*C. maculata* abundance) – 0.01 (aphid abundance), $r^2 = 0.801$, $F_{4,5} = 10.06$, $P = 0.013$) (Fig. 6.2, 6.3).

6.5 Discussion

Utilizing molecular gut-content analysis and species-specific primers, analysis of adult and larval coccinellids in Kentucky winter wheat revealed that these predators are tightly connected to grain aphids in winter wheat, and also engage in high levels of IGP. Aphid prey detection ranged from 3% to over 80% and intraguild prey detection ranged from 2% to over 60%. *C. septempunctata* was most often the intraguild prey, with frequency of detection 62.5% in *C. maculata* larvae and 50% in *H. axyridis* larvae. Additionally, there was increased intraguild prey DNA detected in *C. maculata* found in weed strip fields compared to control fields, and this pattern was not detected in any of the other coccinellid species. Overall, this study was successful in designing primers for PCR-based gut-content analysis to detect IGP and aphid predation in a winter wheat agroecosystem.

Coccinellids have been introduced intro North America for biological control of aphids and other homopteran pests (Debach and Rosen 1991, Snyder et al. 2004). *C. septempunctata* and *H. axyridis* have been introduced and successfully established in much of North America (Brown and Miller 1998) and while both feed on aphids (Hodek and Honek 1996), they do engage in high levels of IGP (Obrycki et al. 1998, Kajita et al. 2000). The competition for food and predation can have consequences on the biodiversity of aphidophagous coccinellids, resulting in the displacement of native coccinellids in agroecosystems (Colunga-Garcia and Gage 1998, Michaud 2002, Pell et al. 2008). However, data from the previous chapter revealed that *H. axyridis* is not a dominant coccinellid species in this system, but it is an aphid predator. Additionally, while more

than 50% of larval *H. axyridis* tested positive for *C. septempunctata* DNA, it is unlikely that with the numbers we collected and screened (N = 79) that there was a negative impact on the biodiversity of coccinellids. *C. septempunctata* was the most abundant species, although it was also the most common intraguild prey item, suggesting that its numbers were also not negatively impacted by IGP in this system.

In the previous chapter, it was it revealed that field-bordering weed strips enhance coccinellid populations, but this effect was species specific. I found that *C. septempunctata* that *C. maculata* had higher populations in weed strip plots than control plots, but the same pattern was not evident in other coccinellids. The increased abundance of *C. maculata* and *C. septempunctata* in weed strips, as well as the aggregative response of coccinellids (Evans and Yousseff 1992), may lead to a higher encounter rate and more predation. I found significantly more *C. maculata* that tested positive for *C. septempunctata* DNA in weed strip fields, suggesting this may have been the case. However, regardless of evidence of higher rates of IGP in weeds strip plots, there was no difference in aphid predation rates between treatments. Although coccinellids may be encountering intraguild prey at higher rates and feeding on them accordingly, aphid predation remains unaffected. In some aphidophagous systems, the presence of extraguild prey may decrease the amount of IGP but this is dependent upon the combination of predators as well as the predator mobility, size and specificity (Lucas et al. 1998). Within an agroecosystem several species of coccinellids coexist (Hodek and Honek 1996) and this equilibrium may be maintained by a variety of mechanisms including temporal separation of oviposition (Dixon 2007) or behavioral strategies (Ware and Majerus 2008). In Kentucky winter wheat, *C. septempunctata* was more numerically

dominant and appeared earlier in the season than *C. maculata*, but *C. maculata* was the dominant intraguild predator.

Molecular-gut content analysis is a valuable tool that allows us to identify trophic linkages in the field (Symondson 2002), but we are limited in our scope while studying intraguild predation for a variety of reasons. Prey detection success can be different between species (Greenstone et al. 2007, King et al. 2008, Traugott et al. 2012) and PCR-based DNA detection rates are not necessarily equal to the proportion of prey consumed in the field (Rosa et al. 2014), so detection rates should be interpreted with caution. Laboratory-based feeding experiments can be used to adjust DNA detection rates, but are only feasible when small numbers of predator-prey combinations are being assessed (Szendrei et al. 2010). Predators with longer DNA detectability times may appear to be disproportionately strong biological control agents, as they will more frequently test positive for prey DNA (Greenstone et al. 2007). The rate of DNA decay, and thus the half-life, is dependent on a variety of factors, such as temperature, the size and age of both the predators and prey, meal size (Hagler and Naranjo 1997, Chen et al. 2000, Hoogendoorn and Heimpel 2001), as well as the predator's feeding mode and digestive physiology (Greenstone et al. 2007). Results from the previous chapter show that *C. maculata* (5 h) adults have a DNA half-life twice as long as *C. septempunctata* (2.5 h) adults when consuming *R. padi* aphids. Feeding studies with all combinations of intraguild predators and prey were not possible for this study, but would provide more insight into these relationships.

One limitation of PCR-based molecular gut-content analysis is that it does not distinguish between predation, scavenging or secondary predation, and this can lead to an

overestimation of predation frequencies (Foltan et al. 2005). Some generalist predators, such as coccinellids, do engage in scavenging (Sunderland 1996) as well as cannibalism (Hodek and Honek 1996). Cannibalism, similar to IGP, can disrupt the biological control potential of a natural enemy, but studies have contrasting results on the risk of IGP compared to cannibalism (Hemptinne et al. 2000, Agarwala and Yasuda 2001). A potential method of assessing cannibalism or scavenging would be with immunomarking prey items, then detection by immunoglobulin G (IgG)-specific enzyme-linked immunosorbent assay (ELISA) in the guts of target predators (Hagler 2006, Hagler 2011, Zilnik and Hagler 2015). However, this method relies on the introduction of marked prey into an experimental area, and may limit the results. While I was able to distinguish between the species of prey eaten using the primers designed, they are not lifestage-specific. Typically, predator size ratio and mobility will determine the outcome of an IGP interaction, with smaller ones killed by larger ones and more mobile predators with an advantage over less mobile predators (Lucas et al. 1998, Hodge 1999). This is not always the case, however, especially when intraguild prey are in aggregations, such as with coccinellids (Lucas et al. 1998). Additionally, egg predation is very common (Polis et al. 1989) and without careful experimental manipulations, I was not able to definitively show this. Nonetheless, studies examining the behavior and ecology of predators and prey interacting in complex agroecosystems will aid in our understanding of IGP (Hodge 1999).

Effective biological control of aphids would require early season predators that can find aphids even when they are present at low densities (Murdoch et al. 1985, Murdoch and Briggs 1996, de Roince et al. 2013). Later in the season, due to the

exponential growth rate of aphids, successful biological control would require a specialized predator or one with a high predation rate. Therefore, a combination of both generalist and specialist predators that were separated temporally could be effective at pest suppression. In this system, I detected high frequencies of aphid DNA across multiple species of coccinellids in the spring season, in addition to substantial levels of IGP between coccinellids. When field-bordering weed strips were used as a habitat manipulation to promote natural enemy abundance, I observed an increase in coccinellid numbers. Moreover, as aphid populations increased, more coccinellids tested positive for aphid DNA (Chapter 5). Combined with results from this study, these data suggest that coccinellids are valuable biological control agents in winter wheat, and aphid pest suppression might be enhanced through field-bordering weed strips without interference from IGP.

Table 6.1. Coccinellid primers designed and optimized for molecular got content analysis.

Coccinellid Species	Primer Name	Sequence	Amplicon Size
Coccinella septempunctata	C7-345-F	TTGACTACTCCCACCTGCC	202 bp
	C7-546-R	AAGAGGTGTCTTATCAAGGTTTATG	
Coleomegilla maculata	Cmac-442-F	TCCTCTAATCTAGCTCATAATGGAT	135 bp
	Cmac-576-R	GGAGAATAGCTGTAATTAATACTGATCAG	

Table 6.2. Results of PCR-based gut-content analysis showing the proportion of each coccinellid adult and larval species testing positive for intraguild and aphid DNA.

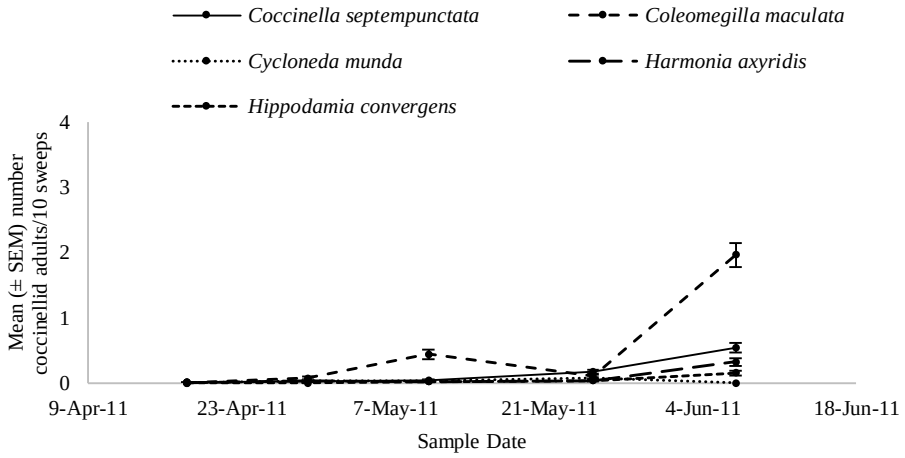
a.

Adult Species		Proportion testing positive for prey DNA			
	N	*C. maculata*	*C. septempunctata*	*R. padi*	*S. avenae*
Coccinella septempunctata	485	0.054	.	0.165	0.485
Coleomegilla maculata	126	.	0.024	0.032	0.238
Hippodamia convergens	32	.	0	0.031	0.031

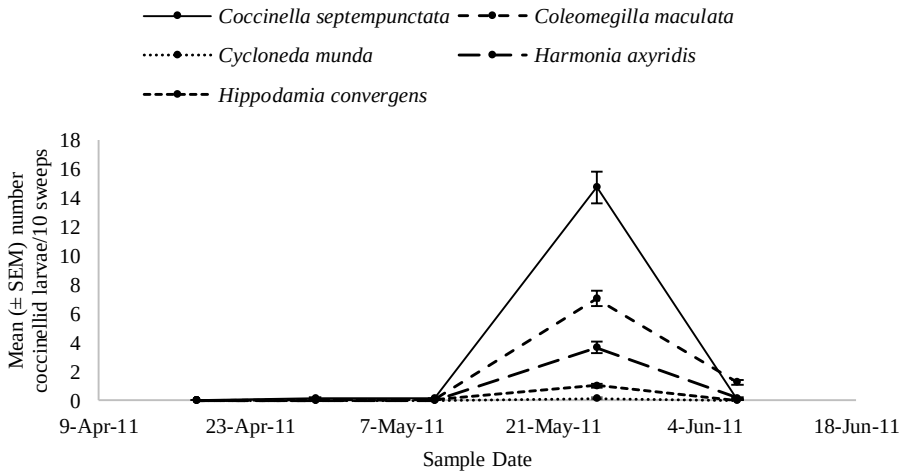
b.

Larval Species		Proportion testing positive for prey DNA			
	N	*C. maculata*	*C. septempunctata*	*R. padi*	*S. avenae*
Coccinella septempunctata	420	0.043	.	0.036	0.819
Coleomegilla maculata	336	.	0.625	0.256	0.464
Harmonia axyridis	79	0.089	0.506	0.468	0.772

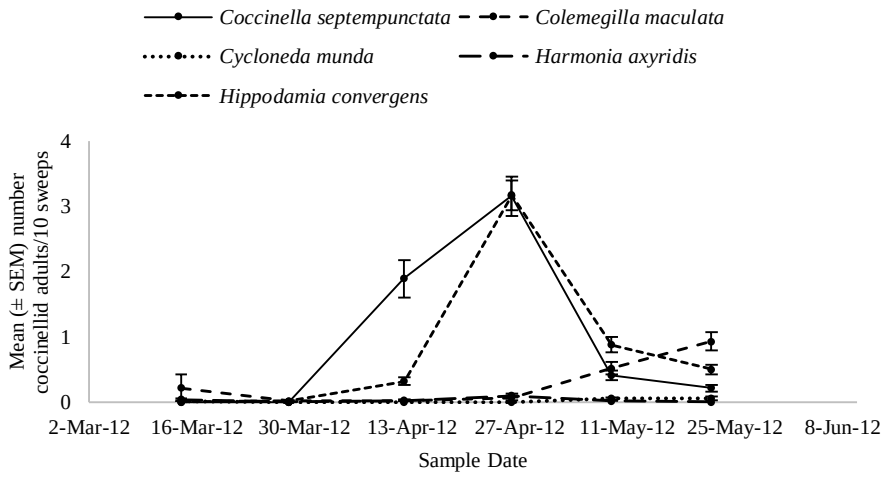
a. 2011 Adults



b. 2011 Larvae



c. 2012 Adults



d. 2012 Larvae

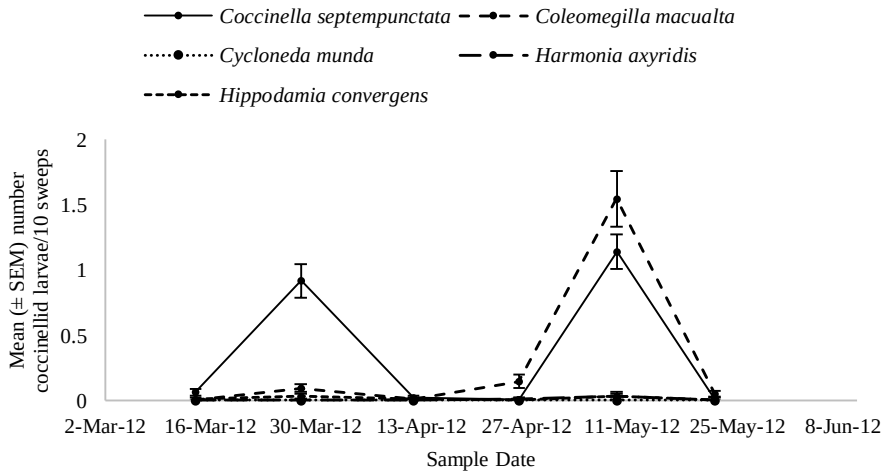
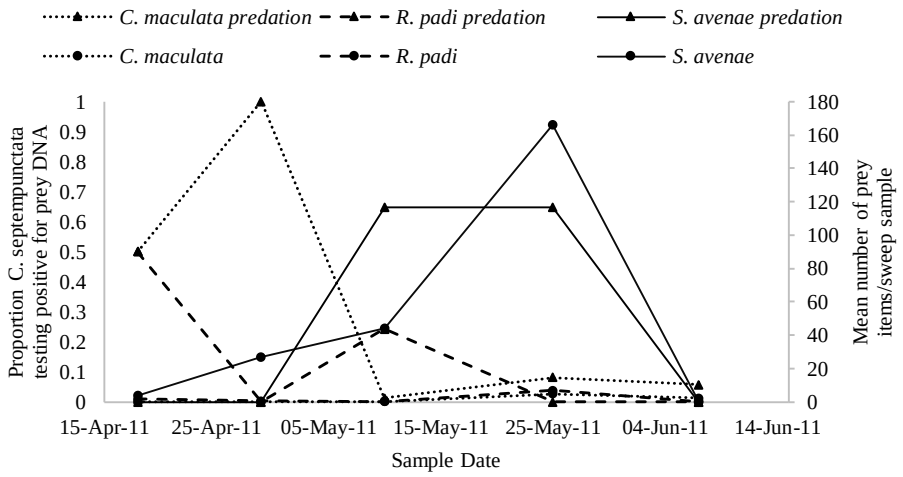
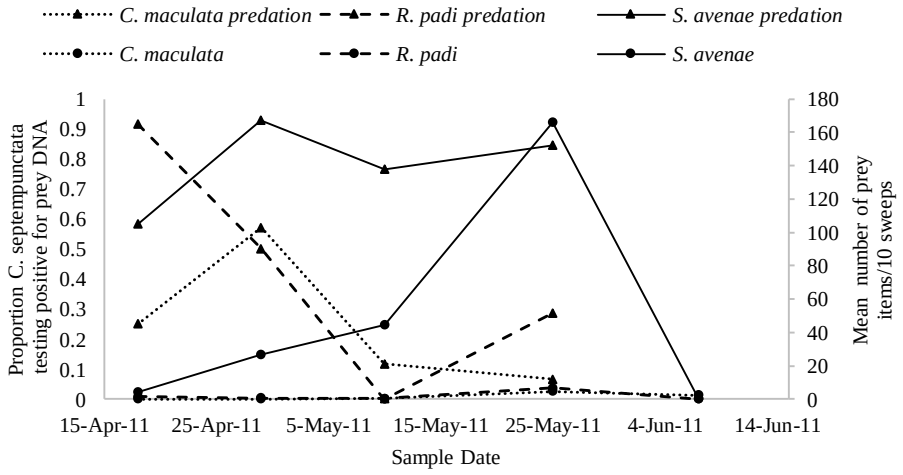


Figure 6.1. Mean number (± SEM) of coccinellid adults in (a) 2011 and (c) 2012 and coccinellid larvae (all species) in (b) 2011 and (d) 2012 caught in ten figure-eight sweeps. The five species represented are *Coccinella septempunctata*, *Coleomegilla maculata*, *Cycloneda munda*, *Harmonia axyridis*, and *Hippodamia convergens*.

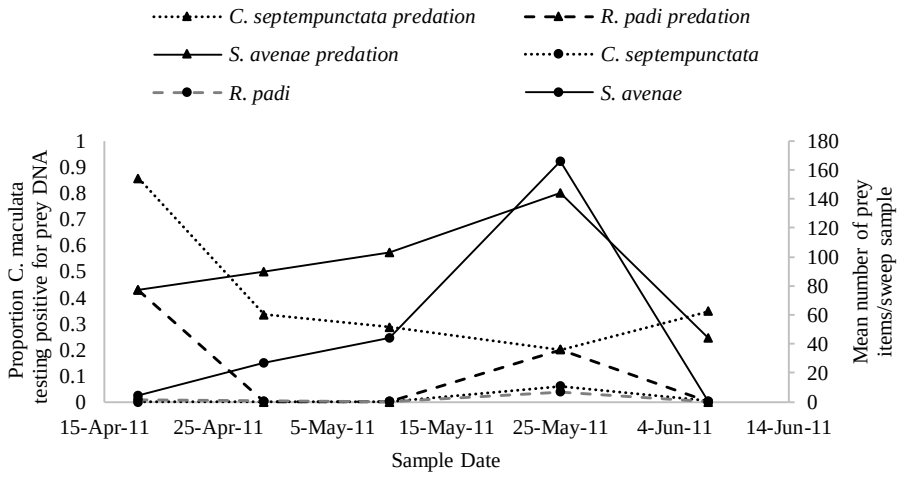
a.


C. maculata predation
R. padi predation
S. avenae predation
C. maculata
R. padi
S. avenae
Proportion C. septempunctata testing positive for prey DNA
Mean number of prey items/sweep sample
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
20
40
60
80
100
120
140
160
180
15-Apr-11
25-Apr-11
05-May-11
15-May-11
25-May-11
04-Jun-11
14-Jun-11
Sample Date

b.


C. maculata predation
R. padi predation
S. avenae predation
C. maculata
R. padi
S. avenae
Proportion C. septempunctata testing positive for prey DNA
Mean number of prey items/10 sweeps
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
20
40
60
80
100
120
140
160
180
15-Apr-11
25-Apr-11
5-May-11
15-May-11
25-May-11
4-Jun-11
14-Jun-11
Sample Date

c.



d.

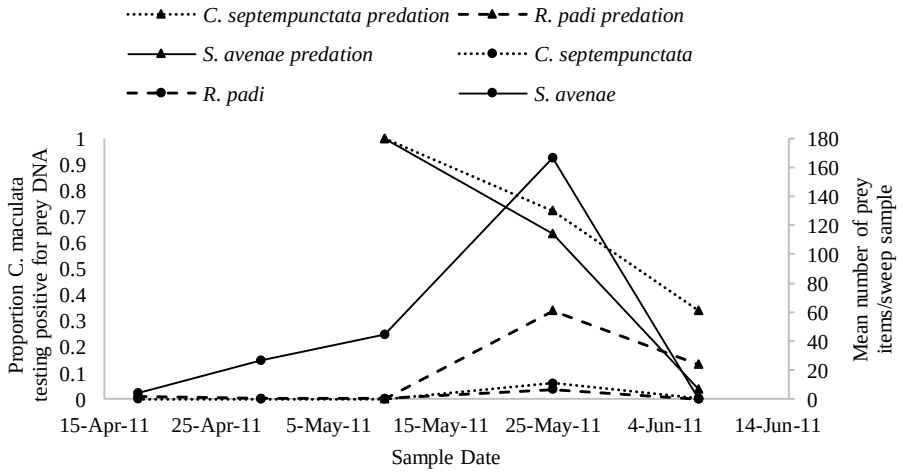
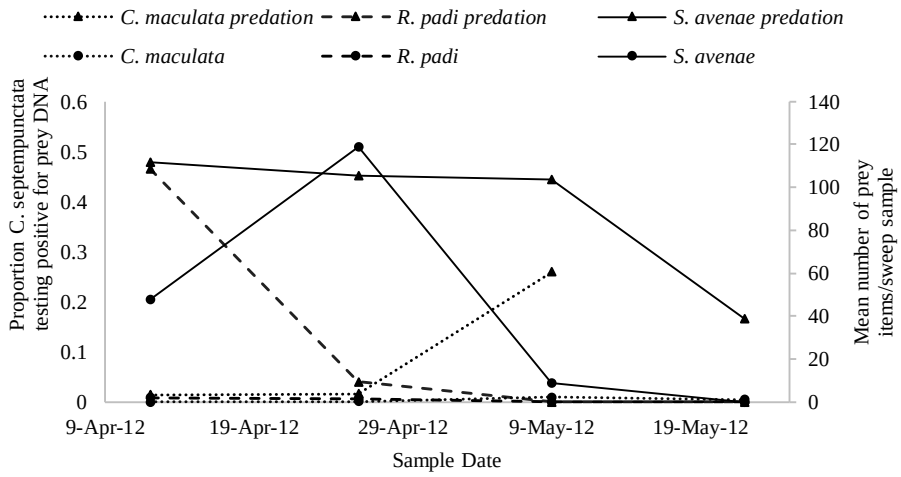
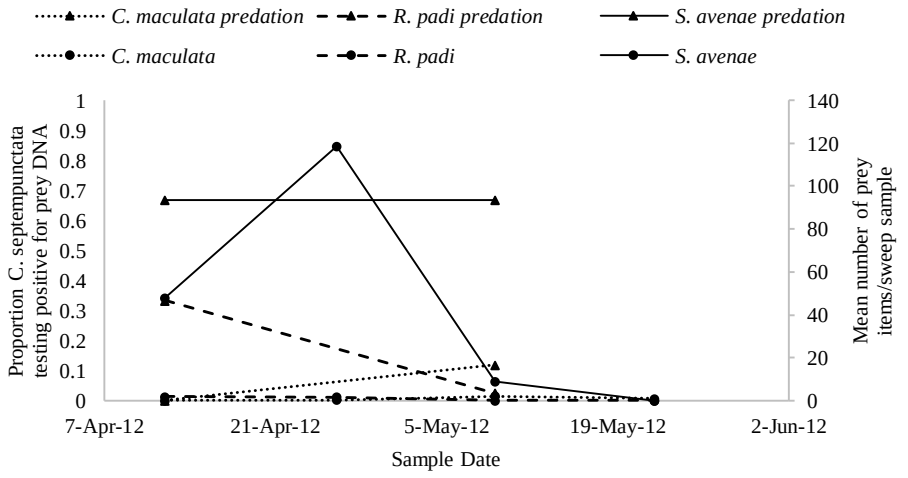


Figure 6.2. Proportion of intraguild predators testing for intraguild prey and pest aphids on primary axis, prey availability of intraguild prey and pest aphids in secondary axis in 2011. a. *Coccinella septempunctata* adults screening positive for *Coleomegilla maculata*, *Rhopalosiphum padi*, and *Sitobion avenae* b. *C. septempunctata* larvae screening positive for *C. maculata*, *R. padi*, and *S. avenae* c. *C. maculata* adults screening positive for *C. septempunctata*, *R. padi*, and *S. avenae* d. *C. maculata* larvae screening positive for *C. septempunctata*, *R. padi*, and *S. avenae*.

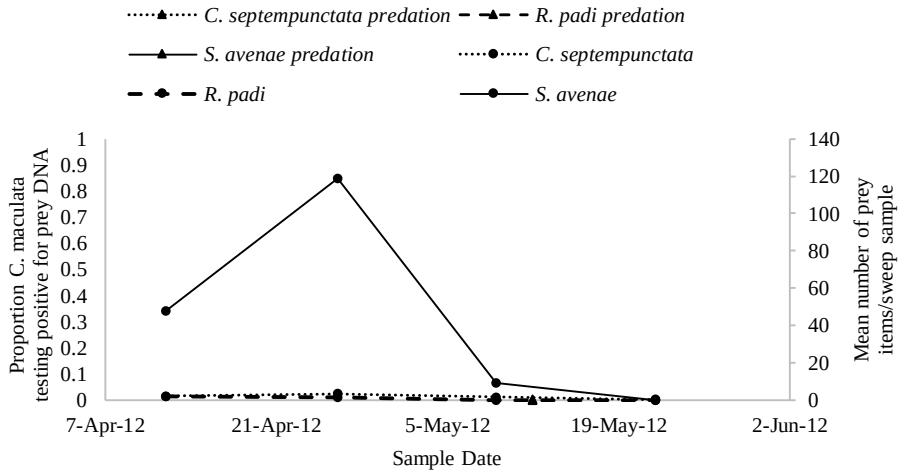
a.


C. maculata predation
R. padi predation
S. avenae predation
C. maculata
R. padi
S. avenae
Proportion C. septempunctata testing positive for prey DNA
Mean number of prey items/sweep sample
0
0.1
0.2
0.3
0.4
0.5
0.6
0
20
40
60
80
100
120
140
9-Apr-12
19-Apr-12
29-Apr-12
9-May-12
19-May-12
Sample Date

b.


C. maculata predation
R. padi predation
S. avenae predation
C. maculata
R. padi
S. avenae
Proportion C. septempunctata testing positive for prey DNA
Mean number of prey items/sweep sample
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
20
40
60
80
100
120
140
7-Apr-12
21-Apr-12
5-May-12
19-May-12
2-Jun-12
Sample Date

c.



d.

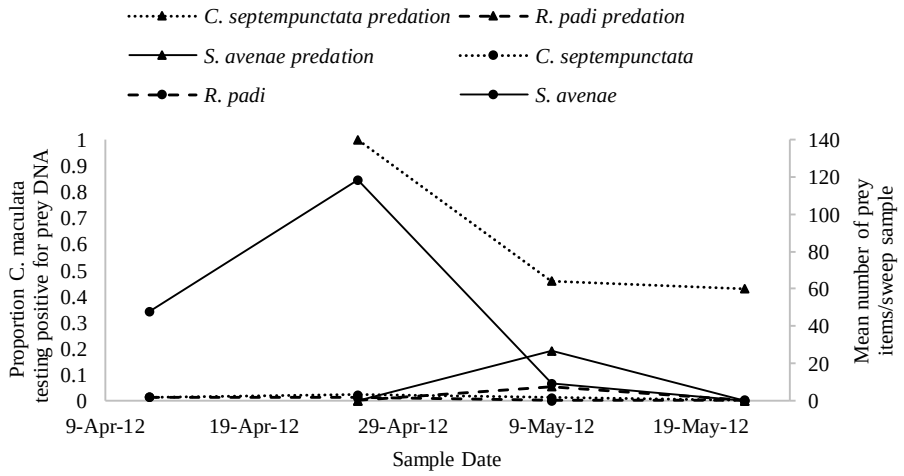


Figure 6.3. Proportion of intraguild predators testing for intraguild prey and pest aphids on primary axis, prey availability of intraguild prey and pest aphids in secondary axis in 2012. a. *Coccinella septempunctata* adults screening positive for *Coleomegilla maculata*, *Rhopalosiphum padi*, and *Sitobion avenae* b. *C. septempunctata* larvae screening positive for *C. maculata*, *R. padi*, and *S. avenae* c. *C. maculata* adults screening positive for *C. septempunctata*, *R. padi*, and *S. avenae* d. *C. maculata* larvae screening positive for *C. septempunctata*, *R. padi*, and *S. avenae*

Chapter 7 : Summary

Growers are always looking for ways to achieve higher yields and lower pest pressure, all while still making the largest profit. Many agricultural producers rely on regular pesticide applications to avoid losses from pests and diseases. Currently, the most common form of integrated pest management (IPM) is "sample, spray and pray" and does not incorporate the impact of natural enemies (Zalukci et al. 2015). However, overuse of pesticides has been harmful to the environment, detrimental to natural biological control services (Macfadyen et al. 2014) and has caused insecticide resistance in multiple species of aphids (see Bass et al. 2014, Foster et al. 2014), requiring the need for alternate pest control methods. Conservation biological control is a sustainable management technique that can help reduce chemical dependency and promote ecological benefits. This project examined the effects of natural field borders on generalist predator and pest populations, Barley Yellow Dwarf virus (BYDV) incidence and wheat yield in an effort to explore more sustainable options for winter wheat in western Kentucky. This chapter summarizes the key findings from my doctoral research.

Rhopalosiphum padi and *Sitobion avenae* are the main pest aphid species migrating into Kentucky winter wheat in the fall and spring, respectively. Wheat fields bordered with grasses will most likely experience high dispersal rates of *R. padi* as they are moving from drying, summer grasses into freshly planted wheat in the fall. Conversely, forested edges reduce the dispersal rates of *R. padi* and *S. avenae*; this could be because forests are acting as a barrier for the poor flying aphids, or a source of natural enemies preying on them (Gardiner et al. 2009b). A follow-up experiment would be

helpful in determining the edge effects on natural enemies, and aid in understanding this important interaction for biological control purposes.

In a two-year field experiment, we examined no-input weed strips as a conservation biological control possibility for winter wheat. Overall, there were no differences in yield between weed strip plots and control plots. However, there were significantly more natural enemies in weed strip plots in both years. These predators included *Coccinella septempunctata*, *Coleomegilla maculata*, *Chrysopa oculata*, *Chrysoperla plorabunda* (Fitch) (tentative), and *Orius insidiosus*, some of which showed considerable spatial and temporal association with the pest aphids. The most abundant natural enemies were coccinellids, which were significantly enhanced by the weed strips. Aphids and coccinellids have a temporal synchrony (Hemptinne and Dixon 1997), which was supported by my data. *C. maculata* and *C. septempunctata* showed spatial separation in their populations, but still remained synchronous with the pest populations.

I used PCR-based molecular gut-content analysis to identify major aphid predators in winter wheat. It was revealed that four species of coccinellids, *C. oculata*, *C. plorabunda* (Fitch) (tentative), and *O. insidiosus* all tested positive for pest aphid DNA, some at very high frequencies (>80%). Coccinellids were the most abundant and voracious aphid predators, specifically *C. maculata and C. septempunctata*. I designed species-specific primers to identify any intraguild predation (IGP) that may disrupt the biological control potential of these predators. *C. maculata* and *Harmonia axyridis* were most often the intraguild predator, and *C. septempunctata* was most often the intraguild prey. Additionally, there was an increase in detection of intraguild prey DNA in predators collected in weed strip plots. The increase in coccinellid populations may have led to a

higher encounter rate and thus more IGP, although there was no difference in aphid DNA detected. These results suggest that in this system IGP in the coccinellid community does not interfere with biological control of aphids.

The ultimate goal of molecular gut-content analysis is to assess the impact natural enemies are having on prey populations (Greenstone et al. 2007). This is so the impact of natural enemies in each study can be tailored to the specific needs of the farmer and his crop (Macfadyen et al. 2014). However, in agricultural systems it is difficult to determine the exact role of a single predator or pest (Furlong 2015), and ultimately the population suppressive effect of natural enemies (Furlong and Zalucki 2010). In fact, few studies actually show the ecological impact of predators, parasitoids, or pathogens on pest populations for a pest management program (Furlong and Zalucki 2010, Furlong 2015). The first step is to understand the relationship between the appropriate predator and pest that needs to be manipulated, but these are not always accurately defined in agroecosystems. Molecular techniques have helped advance our knowledge of predator-prey relationships (see Hagler and Blackmer 2013, Firlej et al. 2014, Moreno et al. 2014, Raso et al. 2014, Brown et al. 2015) but these methods are only qualitative. Ecological sampling methods must be conducted in tandem to quantify the effect natural enemies are having on pest populations.

This research adds to the growing body of literature on conservation biological control. There was no difference on crop yield between plots that had weed strips and control plots, suggesting this habitat manipulation may not be conducive to promoting increased yields in winter wheat. However, field-bordering weed strips did enhance natural enemy populations, especially the valuable aphid biological control agents,

coccinellids. Given the unique temporal dynamics of this crop, further work should investigate habitat manipulations specifically for the fall, and possibly epigeal arthropods, as well as parasitism rates on aphids. Additionally, in years of extreme climate, no-input weeds are not feasible, so other options should be considered. With the increasing technology of precision agriculture and molecular techniques, we can continue to explore these ecological questions with field- and laboratory-based experiments.

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Vickerman, G. P., and S. D. Wratten. 1979. Biology and pest status of cereal aphids (Hemiptera, Aphididae) in Europe – review. Bulletin of Entomological Research **69**:1-32.

Villegas, C. M., J. A. Verdugo, A. A. Grez, J. Tapia, and B. Lavandero. 2013. Movement between crops and weeds: temporal refuges for aphidophagous insects in Central Chile. Ciencia E Investigacion Agraria **40**:317-326.

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Ward, D., and Y. Lubin. 1992. Temporal and spatial segregation of web-building in a community of orb-weaving spiders. Journal of Arachnology **20**:73-87.

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Warfe, D. M., and L. A. Barmuta. 2004. Habitat structural complexity mediates the foraging success of multiple predator species. Oecologia **141**:171-178.

Welch, K. D., and J. D. Harwood. 2014. Temporal dynamics of natural enemy-pest interactions in a changing environment. Biological Control **75**:18-27.

Werling, B. P., and C. Gratton. 2010. Local and broadscale landscape structure differently impact predation of two potato pests. Ecological Applications **20**: 1114-1125.

Winder, L., C. J. Alexander, J. M. Holland, W. O. C. Symondson, J. N. Perry, and C. Woolley. 2005a. Predatory activity and spatial pattern: the response of generalist carabids to their aphid prey. Journal of Animal Ecology **74**:443-454.

Winder, L., C. J. Alexander, J. M. Holland, C. Woolley, and J. N. Perry. 2001. Modelling the dynamic spatio-temporal response of predators to transient prey patches in the field. Ecology Letters **4**:568-576.

Winder, L., G. J. K. Griffiths, J. N. Perry, C. J. Alexander, J. M. Holland, P. J. Kennedy, and A. Birt. 2005b. The role of large-scale spatially explicit and small-scale localized processes on the population dynamics of cereal aphids. Bulletin of Entomological Research **95**:579-587.

Winder, L., J. N. Perry, and J. M. Holland. 1999. The spatial and temporal distribution of the grain aphid *Sitobion avenae* in winter wheat. Entomologia Experimentalis Et Applicata **93**:277-290.

Woltz, J. M., and D. A. Landis. 2014. Coccinellid response to landscape composition and configuration. Agricultural and Forest Entomology **16**:341-349.

Wratten, S. D., M. H. Bowie, J. M. Hickman, A. M. Evans, J. R. Sedcole, and J. M. Tylianakis. 2003. Field boundaries as barriers to movement of hover flies (Diptera : Syrphidae) in cultivated land. Oecologia **134**:605-611.

Yau, S. K., M. Sidahmed, and M. Haidar. 2010. Conservation versus Conventional Tillage on Performance of Three Different Crops. Agronomy Journal **102**:269-276.

Zhao, Z. H., C. Hui, D. H. He, and F. Ge. 2013. Effects of position within wheat field and

adjacent habitats on the density and diversity of cereal aphids and their natural enemies. Biocontrol **58**: 765-776.

Zhao, Z. H., C. Hui, S. Hardev, F. Ouyang, and F. Ge. 2014. Responses of cereal aphids and their parasitic wasps to landscape complexity. Journal of Economic Entomology **107**: 630-637.

Zilnik, G., and J. R. Hagler. 2013. An immunological approach to distinguish arthropod vivphagy from necrophagy. Biocontrol **58**: 807-804.

VITA

KATELYN A. KOWLES

Curriculum Vitae

Section I: Background Information

Affiliation: Department of Entomology, University of Kentucky

Title: Graduate Research Assistant, Ph.D. Candidate

Educational Background

M.S., Biology
Eastern Illinois University, Charleston, IL
Completed: August 2009
GPA: 4.0
Thesis: *Dynamics of aggregation formation in Japanese beetles,* Popillia japonica*: characteristics of pioneers versus joiners*

B.S., Biology
University of Kentucky, Lexington, KY
Completed: December 2006

Relevant Positions

Graduate Research Assistant, Invertebrate Ecology Lab (Dr. James D. Harwood)
University of Kentucky, Department of Entomology
August 2009 – July 2015

Graduate Research Assistant, Behavioral Ecology Lab (Dr. Paul V. Switzer)
Eastern Illinois University, Department of Biological Sciences
June 2007 – August 2009

Undergraduate Independent Researcher, Center for Ecology, Evolution and Behavior (Dr. Philip Crowley)
University of Kentucky, Department of Biology
Project title: Brood size in the parasitic wasp, *Copidosoma bakeri*
January 2007 – June 2007

Awards

- American Arachnological Society Annual meeting, poster competition runner-up, June 2010

Fellowships

- University of Kentucky Graduate School Fellowship, July 2013-June 2014, $15,000
- Tracy Farmer Center for the Environment, Karri Casner Environmental Sciences Fellowship, March 2012, $1,000
- University of Kentucky Graduate School Fellowship, July 2010-June 2011, $15,000

Travel Grants

- University of Kentucky Graduate School Travel Grant (Portland, OR), November 2014, $400
- North Central Branch of the Entomological Society of America Presidential Scholarship (Des Moines, IA), March 2014, $250
- University of Kentucky Graduate School Travel Grant (London, England), August 2013, $800
- Kentucky Small Grain Growers Association (KYSGGA) Travel Grant (London, England) to visit Rothamsted Research Station, March 2013, $2,520
- University of Kentucky Graduate School Travel Grant (Knoxville, TN), November 2012, $400
- North Central Branch of the Entomological Society of America Presidential Scholarship (Lincoln, NE), March 2012, $250
- Entomological Society of America, International Congress of Entomology Travel Award (Daegu, South Korea), March 2012, $2,405
- North Central Branch of the Entomological Society of America Presidential Scholarship (Minneapolis, MN), March 2011, $250
- University of Kentucky Graduate School Travel Grant (San Diego, CA), December 2010, $400

Section II: Research

Grants Written and Received as Principal Investigator

- Kentucky Small Grains Growers Association (KYSGGA), "Tracking the source of aphid-vectored virus in winter wheat." August 2012, $8,262
  Co-Principle Investigator #1: James D. Harwood, Co-PI #1: Katelyn A. Kowles, PI#2: Douglas W. Johnson
- Kentucky Small Grains Growers Association (KYSGGA), "Tracking the source of aphid-vectored virus in winter wheat." August 2011, $8,521
  Co-Principle Investigator #1: James D. Harwood, Co-PI #1: Katelyn A. Kowles, PI#2: Douglas W. Johnson

Research Publications

Tigreros, N, R Jadhav, **KA Kowles**, BP Nathan and PV Switzer. 2010.

Physiological status of male and female *Popillia japonica* (Coleoptera: Scarabaeidae) affects mating and grouping behavior. *Environmental Entomology*, 39(2).

Kowles, KA and PV Switzer. 2012. Dynamics of aggregation formation in Japanese beetles, *Popillia japonica*. *Journal of Insect Behavior*. 25: 207-221.

Research Talks and Posters Presented

i. Invited Oral Presentations

Kowles, KA, DW Johnson and JD Harwood. 2012. Biological control in Kentucky winter wheat: Using molecular tools to assess sustainable management techniques. XXIV International Congress of Entomology. Daegu, South Korea.

Kowles, KA. 2013. Identifying predator-prey relationships in Kentucky winter wheat using spatial and molecular techniques. Ecolunch. Biology Department, University of Kentucky. Lexington, Kentucky.

Harwood, JD, **KA Kowles** and KD Welch. 2014. Spatiotemporal relationships between aphids and generalist predators. European Congress of Entomology. York, United Kingdom.

ii. Oral Presentations

Kowles, KA, DW Johnson and JD Harwood. 2010. Virus transmission in winter wheat: potential suppression by natural enemies. Entomological Society of America National Meeting, San Diego, CA.

Kowles, KA, DW Johnson and JD Harwood. 2011. Biological control in winter wheat: potential role of spiders in reducing transmission of Barley Yellow Dwarf virus. North Central Branch of the Entomological Society of America, Minneapolis, MN.

Kowles, KA, DW Johnson and JD Harwood. 2011. The effect of habitat manipulation on spider distribution and predation of viruliferous aphids. American Arachnological Society Meeting, Portland, OR.

Kowles, KA, DW Johnson and JD Harwood. 2011. Barley Yellow Dwarf virus in Kentucky winter wheat: potential suppression through habitat manipulation. Mid-South Association of Wheat Scientists Meeting, Olive Branch, MS.

Kowles, KA, DW Johnson and JD Harwood. 2011. Barley Yellow Dwarf virus in winter wheat: examining the spatial and temporal movement of its aphid vectors. Ohio Valley Entomological Association. Frankfort, KY.

Kowles, KA, DW Johnson and JD Harwood. 2011. Barley Yellow Dwarf virus in winter wheat: examining the spatial and temporal movement of its aphid vectors. Entomological Society of America National Meeting, Reno, NV.

Kowles, KA, DW Johnson and JD Harwood. 2012. Integrating natural weed strips in winter wheat production promotes biological control. Southeast Branch of the Entomological Society of America, Little Rock, AR.

Kowles, KA, DW Johnson and JD Harwood. 2012. Biological control in Kentucky winter wheat: using molecular tools to create sustainable management techniques. North Central Branch of the Entomological Society of America. Lincoln, NE.

Kowles, KA, DW Johnson and JD Harwood. 2013. Molecular and spatial analysis of an aphidophagous predator in winter wheat: implications for conservation biological control. University of Kentucky Center for Ecology, Evolution and Behavior Spring Symposium. Lexington, KY.

Kowles, KA, KJ Athey, DW Johnson and JD Harwood. 2013. Molecular and spatial analysis of an aphidophagous predator in winter wheat: implications for conservation biological control. 2nd International Symposium on the Molecular Detection of Trophic Interactions. Lexington, KY.

Kowles, KA, DW Johnson, and JD Harwood. 2013. Tracking virulent aphid population dynamics and predator-prey relationships in winter wheat.11th International Congress of Ecology. London, England.

Kowles, KA, KJ Athey, DW Johnson and JD Harwood. 2013. Sweet, destructive aphids: predation by Coccinellidae in a winter wheat agroecosystem. Ohio Valley Entomological Association Meeting, Indianapolis, IN.

Penn, HJ, **KA Kowles** and JD Harwood. 2013. Spatial relationships between ants, prey and border vegetation in a soybean agroecosystem. Ohio Valley Entomological Association Meeting, Indianapolis, IN.

Kowles, KA, KJ Athey, DW Johnson and JD Harwood. 2013. Tracking aphid predation though molecular and spatial analysis. Entomological Society of America National Meeting. Austin, TX.

Penn, HJ, **KA Kowles** and JD Harwood. 2013. Spatial relationships between ants, prey and border vegetation in a soybean agroecosystem. Entomological Society of America National Meeting. Austin, TX.

Kowles, KA, DW Johnson, and JD Harwood. 2014. Quantifying the prevalence of intraguild predation in winter wheat: impact on biological control. North Central Branch of the Entomological Society of America. Des Moines, IA.

Muncy, AD, **KA Kowles**, and JD Harwood. 2014. Virulent aphid population dynamics in Kentucky winter wheat: a land use analysis. North Central Branch of the Entomological Society of America. Des Moines, IA.

Johnson, DW, **KA Kowles** and JD Harwood. 2014. Virulent aphid movement in Kentucky winter wheat. Mid-South Association of Wheat Scientists Meeting. Madison, AL.

Muncy, AD, **KA Kowles**, and JD Harwood. 2014. Modeling the effects of border vegetation on aphid-vectored virus transmission in winter wheat. National Conference on Undergraduate Research. Lexington, KY.

Kowles, KA, HJ Penn, DW Johnson, and JD Harwood. 2014. DNA detection methods in aphid honeydew: implications for wheat biological control. Entomological Society of America National Meeting. Portland, OR.

iii. Poster Presentations

Kowles, KA and PV Switzer. 2009. Pioneering and aggregation formation in Japanese beetles, *Popillia japonica*. Eastern Illinois University Graduate Student Exposition, Charleston, IL.

Kowles, KA and PV Switzer. 2009. Dynamics of aggregation formation in Japanese beetles, *Popillia japonica*. Entomological Society of America National Meeting, Indianapolis, IN.

Kowles, KA, DW Johnson, and JD Harwood. 2010. Biological control of insects in winter wheat: potential role of spiders in reducing transmission of Barley Yellow Dwarf Virus. American Arachnological Society meeting, Greenville, NC.

Kowles, KA, DW Johnson and JD Harwood. 2011. Tritrophic linkages in Kentucky winter wheat: examining the role of spiders in an aphid-vectored virus. Graduate Student Interdisciplinary Conference, Lexington, KY.

Harwood, JD, CA Allen, KJ Johansen, **KA Kowles**, H McKenrick, JA Peterson, JM Schmidt, KD Welch and TE Whitney. 2011. Disentangling the spider's web: insights from complex terrestrial ecosystems. University of Kentucky Department of Entomology 120th Anniversary poster reception, Entomological Society of America National Meeting, Reno, NV.

Schmidt, JM, **KA Kowles**, EG Chapman and JD Harwood. 2012. Conservation biocontrol promotes natural enemies and reduces virus proliferation. Kentucky Innovation and Entrepreneurship Conference, Louisville, KY.

Kowles, KA, DW Johnson and JD Harwood. 2012. Habitat manipulation to promote sustainable management of viruliferous aphids. Entomological Society of America National Meeting, Knoxville, TN.

Kowles, KA, DW Johnson and JD Harwood. 2013. The spatial distribution of viruliferous aphids and natural enemies in Kentucky winter wheat: examining sustainable management options. Virginia Tech FREC Graduate Research Symposium. Blacksburg, VA.

Wente, RL, **KA Kowles**, JM Schmidt and JD Harwood. 2013. Intraguild predation within an aphidophagous community: interactions between Chrysopidae and Coccinellidae and their potential for biological control. Entomological Society of America National Meeting. Austin, TX.

Muncy, AD, **KA Kowles**, and JD Harwood. 2014. Understanding the effects of bordering vegetation on aphid-vectored virus transmission in winter wheat. Earth and Environmental Science Research Symposium. Lexington, KY.

Section III: Teaching

i. Teaching

General Entomology (Entomology 300), University of Kentucky
Teaching assistant, Fall 2011
Assisted with lab, designed and graded students' practical-style quizzes, mid-term and final exams. Students had insect collections and collecting field trips that I assisted with.

Agroecology (Plant and Soil Science 597), University of Kentucky
Guest Lecturer, February 2010 and February 2011
Taught portion of laboratory on biological control, focusing on conservation biological control in agriculture and highlighting my Ph.D. research.

Natural Resources and Environmental Sciences (NRES 301), University of Kentucky
Guest Lecturer, October 2013
Gave lecture on applying to and attending graduate school in the sciences, focusing on my Ph.D research.

Biological Control (Entomology 680, Biological Control), University of Kentucky
Guest Lecturer, April 2014
Gave lecture on molecular techniques for assessing predation.

ii. Mentored Students

Anna Muncy, Undergraduate Natural Resources and Environmental Sciences (NRES) and Geology Major
Winter 2013 – Present
BIO395 Project title – Modeling of the effects of border vegetation on aphid-vectored virus transmission in winter wheat

Rebecca Wente, Undergraduate Agricultural Biotechnology Major
Spring 2013 – Dec 2013
Project title: Intraguild predation within an aphidophagous community

Hannah Ali, Undergraduate Biology Major
Fall 2013
BIO395 Project title – Quantifying Barley Yellow Dwarf virus (BYDV) in a winter wheat microcosm

iii. Undergraduate Awards

Rebecca Wente, Undergraduate Research Travel Award (Austin, TX), August 2013, $415

ESA President's Prize, 1st Place.
Wente, RL, **KA Kowles**, JM Schmidt and JD Harwood. 2013. Intraguild predation within an aphidophagous community: interactions between Chrysopidae and Coccinellidae and their potential for biological control. Entomological Society of America National Meeting. Austin, TX.

Anna Muncy
NCB Student Competition, 2nd Place
Muncy, AD, **KA Kowles**, and JD Harwood. 2014. Virulent aphid population dynamics in Kentucky winter wheat: a land use analysis. North Central Branch of the Entomological Society of America. Des Moines, IA

EES Research Symposium Poster Competition, 1st Place

Muncy, AD, **KA Kowles**, and JD Harwood. 2014. Understanding the effects of bordering vegetation on aphid-vectored virus transmission in winter wheat. Earth and Environmental Science Research Symposium. Lexington, KY.

Section IV: Extension and Outreach

Extension Talks Given

Kowles, KA and JD Harwood. 2010. Using generalist predators in the management of wheat pests. Kentucky Winter Wheat Annual Meeting, Hopkinsville, KY.

Kowles, KA, DW Johnson and JD Harwood. 2012. Barley Yellow Dwarf virus in winter wheat: examining the spatial movement of its aphid vectors. University of Kentucky Winter Wheat Annual Meeting, Hopkinsville, KY.

Kowles, KA, DW Johnson and JD Harwood. 2013. Tracking the source of aphid-vectored virus in winter wheat. University of Kentucky Wheat Field Day. Princeton, KY.

Kowles, KA, DW Johnson and JD Harwood. 2014. Monitoring virulent aphid movement in Kentucky winter wheat. University of Kentucky Winter Wheat Annual Meeting, Hopkinsville, KY.

Participation in Outreach Events

- Insect Night Walk at the Arboretum, September 2014, 2015
- Science fair judge (grades 4 & 5) at Seton Catholic School, December 2013
- "BugFest" at Cincinnati Museum, June 2013
- Organism Extravaganza at Claysmill Elementary School, March 2013
- "Arbor Day" at the Lexington Arbortum, April 2012
- Russell Cave Elementary School (grades 1 & 2) Insect Presentations, February 2011, Lexington, KY
- Woodlawn Elementary School (grades 1 – 5 and families) Insect Presentations, November 2010
- Hats off Day at the Kentucky Horse Park. August 2010
- Raven Run Night Walk. July 2010, 2011
- "Bugs All Day" at the Lexington Explorium, April 2010, 2011, 2012
- Cassidy School (grade 1) Insect Presentations, April 2010, Lexington, KY
- Landsdowne Elementary School (grades 1-3) Insect Presentations, March 2010, Lexington, KY
- Science Fair judge (grades K-12) Fayette County Science Fair, February 2010, 2011, 2014

- Organized tour of Entomology Department at the University of Kentucky for 1st grade students, November 2009
- University of Kentucky College of Agriculture Roundup, October 2009
- "Trees, Trails and Creatures" at the University of Kentucky Arboretum, October 2009, 2010
- University of Kentucky Pest Control Short Course, September 2009
- Science Fair judge (grades 9-12), Charleston High School Science Fair, January 2009, Charleston, IL
- Carl Sandburg Elementary School (grade 1) Insect Presentations, March 2008, Charleston, IL

Section V: Service

Professional Societies

- Entomological Society of America
- International Organization of Biological Control
- American Arachnological Society
- Delta Epsilon Iota
- Phi Sigma
- Gamma Sigma Delta

Committees

- University of Kentucky Graduate Council member, 2010-2012
- University of Kentucky Graduate Student Congress: Department representative 2009-2010; **President**, 2010-2011
- University of Kentucky H. Garman Entomology Club: **President** 2011-2012
- Member of University of Kentucky Linnaean Games Team, 2009-2012
- University of Kentucky Entomology Extension Committee, 2009-present
- Eastern Illinois University Biological Sciences Graduate Student Association: **Vice President**, 2008-2009
- Eastern Illinois University Graduate Student Advisory Council: **Executive Board member**, 2008-2009
- Women in Science and Mathematics at Eastern Illinois University, 2007-2009

Volunteering

- Student volunteer at the Entomological Society of America Annual Meeting 2013
- Student volunteer at the North Central Branch ESA Annual Meeting 2012
- Student volunteer at the North Central Branch ESA Annual Meeting 2011
- Student volunteer at the Entomological Society of America Annual Meeting 2010
- Student volunteer at the UK Center for Ecology, Evolution and Behavior Symposium 2010
- Student volunteer at the American Mosquito Control Association Meeting 2010
- Student volunteer at the North Central Branch ESA Annual Meeting 2010

- Student volunteer at the Entomological Society of America Annual Meeting 2009

Reviewing and Editorial

Reviews for:

- Biological Control (4)
- Bulletin of Entomological Research (1)
- Arthropod-Plant Interactions (1)