

HISTORICAL, LANDSCAPE AND RESOURCE
INFLUENCES ON THE COCCINELLID
COMMUNITY IN MISSOURI

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ABSTRACT

The diversity and abundance of native lady beetle (Coccinellidae) species in North America is declining. This decline has been attributed to the introduction and establishment of exotic lady beetles, including *Coccinella septumpunctata* and *Harmonia axyridis*. This is worrisome because coccinellids, both native and exotic, provide important biological control services in many agricultural systems. To date, most of the studies documenting negative effects of exotic species on the community of native lady beetles have been done in agricultural habitats, whereas grasslands have been suggested as potential refuge habitats for native species from the pressures of exotics. The objectives of this research were 1) to document the impact of the introduction of exotic species on the community of native lady beetle species occurring in Missouri using a historical dataset (1930-present), 2) to determine whether different grassland habitats provide similar refuges for native species by comparing the community of native lady beetle species in natural and agricultural tall grass habitats, 3) to determine what factor(s) may make one grassland habitat a better refuge habitat for native coccinellid species than others, and 4) to determine if native species are being actively displaced from foraging habitats by exotic lady beetle species.

Using a dataset from museum and personal collections with eighty years of haphazardly-collected lady beetles, I documented changes in the composition of the native lady beetle community in Missouri before and after the introduction and establishment of *C. septempunctata* and *H. axyridis*. Abundances of several native species have changed throughout time, with some of these changes occurring prior to the

establishment of any exotic species in our region, suggesting that other factors such as land use change may have played a role in these shifting community demographics. From field studies, I have also found that lady beetle communities in contemporary grassland habitats vary, with the communities found in remnant and restored native tallgrass prairies more similar to each other than to the community found in agricultural tall fescue. Surprisingly, agricultural tall fescue had a greater abundance and diversity of native lady beetle species than either native prairie habitat, whereas all grassland habitats had similarly low abundances of exotic beetles. Given the well-documented displacement of native lady beetles from many agricultural habitats, these results support the importance of grassland refuge habitats for the persistence of native lady beetle species in the landscape.

Concurrent with the finding of a greater abundance and diversity of coccinellids in agricultural tall fescue habitats, there was also a greater abundance of aphids, a common food resource for many predatory insects. An abundance of aphids could promote lady beetle aggregation and reduce the vulnerability of lady beetle eggs to predation in these habitats. Aphid abundance was manipulated within grassland plots to study the impacts of prey availability on lady beetle abundance and species composition and the consumption of coccinellid eggs. There was no overall effect of aphid abundance on the abundance or composition of lady beetles. Nor was there a difference in the proportion of lady beetle eggs consumed between ambient and aphid reduction treatments.

Caged manipulations were also conducted to determine if the foraging behavior of native species was impacted by the presence of exotic lady beetles. In these experiments,

individual species of native lady beetles were allowed to forage among patches varying in the abundance of aphids either in the presence or absence of exotic species. The presence of the exotic species did not affect the foraging location of native lady beetles. The majority of beetles, both native and exotic, were found foraging within patches of high prey abundance.

The overall findings of my research suggest that there are many factors, potentially working synergistically, that have led to the decline of native coccinellids in North America.

Chapter 1

Literature review and introduction

Native lady beetle species in North America have been decreasing in abundance in recent decades (Wheeler & Hoebeke, 1995; Alyokhin & Sewell, 2004; Harmon et al., 2007; Losey et al., 2007; Gardiner et al., 2011). Declines in the abundances of several species of native coccinellids have been documented throughout North America including *Adalia bipunctata* (L.), *Brachiacantha ursina* (F.), *Chilocorus stigma* (Say), *Coccinella novemnotata* Herbst, *Coccinella transversoguttata richardsoni* Brown, *Cycloneda munda* (Say), *Hippodamia convergens* and *Hippodamia tredecimpunctata* (Say) (Wheeler & Hoebeke, 1995; Elliott et al., 1996; Colunga-Garcia & Gage, 1998; Alyokhin & Sewell, 2004; Evans, 2004; Harmon et al., 2007; Losey et al., 2007; Gardiner et al., 2009a; Gardiner et al., 2009c; Smith & Gardiner, 2013a). Because many species of lady beetles are important components of natural enemy guilds that contribute to natural biological control, decreases in the abundance of these species raises concern regarding the status of their populations as well as the causes of their decline (Harmon et al., 2007; Losey et al., 2007).

In addition to the suite of native lady beetle species in North America, several exotic species have been introduced with the goal of increased agricultural pest control (Gordon, 1985; DeBach & Rosen, 1991; Day et al., 1994; Cottrell & Yeargan, 1998; Obrycki et al., 1998a; Obrycki & Kring, 1998b; Gardiner et al., 2011). Two of these introduced exotic species, *Coccinella septempunctata* L. and *Harmonia axyridis* Pallas, have become pervasive throughout much of North America (Elliott et al., 1996; Snyder et al., 2004; Harmon et al., 2007; Losey et al., 2007).

Coccinella septempunctata was intentionally introduced into the United States several times by the U.S. Department of Agriculture (USDA) beginning in 1957, with approximately 150,000 individuals released through 1973 (Angalet et al., 1979; Gordon, 1985; Schaefer et al., 1987; Wheeler & Hoebeke, 1995). While high numbers of beetles were released, it appears that these releases failed to establish permanent populations of this potential biological control agent (Angalet et al., 1979; Schaefer et al., 1987). Despite this failure to establish, *C. septempunctata* was later detected in New Jersey as well as in Quebec, in the early 1970s (Wheeler & Hoebeke, 1995). Because of these findings, additional efforts were made to release and distribute *C. septempunctata* in both the United States and Canada (Schaefer et al., 1987). *C. septempunctata* spread rapidly throughout North America in the late 1970s (Wheeler & Hoebeke, 1995). This spread was likely due to a combination of intentional releases as well as unintentional introductions and spread occurring likely via trade routes (Day et al., 1994). By 1986, the establishment of *C. septempunctata* was documented throughout the Northeast and Midwest regions of the United States as well as five provinces in Canada (Schaefer et al., 1987) and across the country by the mid-1990s (Wheeler & Hoebeke, 1995). Beginning in the early 1990s, *C. septempunctata* was the most commonly seen species of *Coccinella* east of the Rocky Mountains (Gordon & Vandenberg, 1991) and was documented within all 48 contiguous states (Wheeler & Hoebeke, 1995). *Coccinella septempunctata* continues to be an abundant coccinellid species throughout North America, where there are concerns regarding its impacts on native coccinellid biodiversity (Harmon et al., 2007; Finlayson et al., 2008; Hesler & Kieckhefer, 2008; Evans et al., 2011a).

Similar to *C. septempunctata*, there were repeated unsuccessful attempts at introducing *H. axyridis* into North America beginning in 1916 and continuing through the 1980s (Gordon, 1985; Koch et al., 2006). Like *C. septempunctata*, it is thought that *H. axyridis* may have also arrived in North America via trade routes (Day et al., 1994). Despite being introduced before *C. septempunctata*, the first established populations of *H. axyridis* were not discovered until 1988 in southeastern Louisiana (Chapin & Brou, 1991). *Harmonia axyridis* then spread quickly throughout the continent afterwards (Brown et al., 2011b) through a combination of natural dispersal and intentional releases as a biological control agent (Heimpel & Lundgren, 2000; Koch et al., 2006). *Harmonia axyridis*, similar to *C. septempunctata*, is now commonly found throughout much of North America (Koch et al., 2006; Brown et al., 2011a).

The establishment of these two exotic coccinellids, while useful for biological control purposes, has been correlated negatively with changes in native lady beetle communities (Elliott et al., 1996; Colunga-Garcia & Gage, 1998; Obrycki, 2000; Michaud, 2002; Brown, 2003; Koch, 2003; Alyokhin & Sewell, 2004; Snyder et al., 2004; Koch et al., 2006; Harmon et al., 2007; Lucas et al., 2007; Finlayson et al., 2008; Brown et al., 2011b; Roy et al., 2012). Therefore it is possible that these exotic beetles may negatively impact native species.

Several hypotheses seek to explain how exotic coccinellids might impact native species. Displacement of native coccinellids could occur as the consequence of direct antagonistic interactions with exotic species such as predation and competition for resources (Agarwala and Dixon 1992, Cottrell and Yeargan 1998, Elliott Norman et al. 1996, Evans E W 1991, Gardiner Mary M. et al. 2011, Michaud 2002, Obrycki J.J. and

Kring 1998, Snyder et al. 2004). Predation of the eggs and larvae of native coccinellid species occurs more frequently than that of exotic eggs (Prasad & Snyder, 2004; Cottrell, 2005; Kajita et al., 2010; Smith & Gardiner, 2013a). It has been shown in laboratory studies that both native and exotic beetles will readily consume the eggs of native species such as *Coleomegilla maculata* (DeGeer), while the native species will generally avoid consumption of the eggs of exotic species (Cottrell & Yeargan, 1998; Cottrell, 2005).

Resource competition, whereby species compete for a common resource such as food or overwintering habitat, is another potential mechanism explaining the displacement of native coccinellid species by exotics in the field. Many species of coccinellids share common food resources, in particular aphids. If exotic species are more voracious consumers of aphids than native species (Bahlia, 2014), native species could be competitively excluded from a habitat when resources are scarce (Sloggett & Majerus, 2000; Evans, 2004), but coexistence may occur in areas of high aphid abundance (Evans, 1991; Ives et al., 1993; Obrycki et al., 1998a). As a result, both native and exotic coccinellids may aggregate in areas of high aphid density (Schellhorn & Andow, 2005b; Evans & Toler, 2007). A lack of this resource should then have the opposite effect, with coccinellids leaving areas of lower resources in search of prey. A long-term study by Evans (2004), found that decreases in the abundance of native lady beetles in Utah alfalfa fields were related to increased abundances of the exotic species *C. septempunctata*, which reduced the amount of aphids locally available to other predators.

Aphid abundance could also mediate the displacement of native species by exotic species by influencing the occurrence of predation on the eggs, larvae and pupae of native beetles (Obrycki et al., 1998a; Schellhorn & Andow, 1999b). As a common food

resource for many coccinellids, the presence of aphids could decrease rates of predation on immature coccinellids, in particular predation upon eggs (Cottrell, 2005). In North America, the predation of eggs of native species occurs more frequently than eggs of exotic species (*C. septempunctata* and *H. axyridis*) (Prasad & Snyder, 2004; Cottrell, 2005; Kajita et al., 2010; Smith & Gardiner, 2013b; Smith & Gardiner, 2013a). Predation also occurs between larvae of predacious Coccinellidae with exotic species often being the predator (Obrycki & Kring, 1998a; Phoofolo & Obrycki, 1998; Snyder et al., 2004). However, predation of eggs and larvae is not limited to consumption by other coccinellids. Several aphidophagous arthropods such as the green lacewing (*Chrysoperla carnea* Stephens), harvestmen (Opilionidae), and the soft-winged flower beetle [*Collops nigriceps* (Say)], are also documented predators of the eggs of the native lady beetle *Coleomegilla maculata* DeGeer (Phoofolo & Obrycki, 1998; Gardiner et al., 2011; Smith & Gardiner, 2013a).

Some coccinellid species appear to have developed mechanisms to persist in a habitat despite competition. One such mechanism is resource partitioning, whereby organisms that have similar food and/or habitat requirements use the resources in different ways. For example, species may forage in different habitats or at different times of the day, and by doing so, are capable of coexistence. Spatial partitioning has been shown between adults of the common native species *C. maculata* and adults of both common exotic species, *C. septempunctata* and *H. axyridis*. In a sweet corn habitat in New York, *C. maculata* and *H. axyridis* were observed to space themselves while foraging for aphids such that *C. maculata* occupied the lower two thirds of corn plants and *H. axyridis* could be found in the upper third, where aphids were most abundant

(Musser et al., 2006). Similar partitioning was observed in a laboratory experiment using *C. maculata*, *C. septempunctata* and *H. axyridis* foraging for pea aphids on alfalfa plants. In this study, both the exotic beetle, *H. axyridis*, and the native beetle, *C. maculata*, were displaced to the plant margins by the dominant foraging behavior of *C. septempunctata* (Cardinale et al., 2006).

While there is evidence to suggest that the ever-increasing abundance of exotic lady beetles in North America may negatively impact populations of native coccinellids, this relationship may merely be correlative. There are other factors that may have contributed to the widespread decline in native lady beetle species. Anthropogenic habitat modification, including increasing urban land area (McKinney, 2002; Szlavecz et al., 2011), fragmentation of natural habitats (Kareiva, 1987; Gibbs & Stanton, 2001) and agricultural intensification (Letourneau, 1998; Björklund et al., 1999; Iperti, 1999; Grez et al., 2013), can negatively impact native arthropod species. There has been a well-documented growth in urban/suburban populations in the United States since the early 1900s (Szlavecz et al., 2011), and this increasing population has led to an increase in urban land cover and a decrease in rural lands, which broadly include natural and semi-natural habitat as well as agricultural lands (Berry, 1978). Agricultural systems have long been known to provide nutritional resources for many species of native coccinellids at various times during the growing season (Evans, 2003; Evans, 2004; Lundgren, 2009) and the importance of natural and semi-natural habitats for resources has become more apparent in recent years (Gardiner et al., 2009a; Gardiner et al., 2009c; Gardiner et al., 2011; Schmidt et al., 2011; Diepenbrock & Finke, 2013). These anthropogenic modifications may have caused continual changes in the native coccinellid community

over time, potentially impacting individual populations well before the arrival of any exotic species.

Habitat modification and species invasion are two of the leading causes of biodiversity loss (Czech & Krausman, 1997; Wilcove et al., 1998; Kolar & Lodge, 2001; Foley et al., 2005; Sala, 2006; Ricciardi, 2007). Although their contributions to the decline of native species are often investigated independently, interactions between them are likely (Suarez et al., 1998; Stachowicz et al., 1999; Seabloom et al., 2003; Tylianakis et al., 2007; Powell et al., 2011). For example, it has been hypothesized that degraded ecosystems facilitate the displacement of native species by invasive species, with habitat disturbance shifting the competitive advantage from previously well-adapted natives to exotic invaders (Howarth, 1991; Blair, 1996; Suarez et al., 1998; Stachowicz et al., 1999; Byers, 2002; Seabloom et al., 2003; Evans, 2004; Didham et al., 2005; Hansen et al., 2005; Didham et al., 2007; Tylianakis et al., 2007; Moles et al., 2008; Powell et al., 2011). However, this relationship may also be correlative, with native species merely being more vulnerable to environmental stresses or experiencing greater barriers to recruitment than exotic species in disturbed habitats (Gurevitch & Padilla, 2004; MacDougall & Turkington, 2005; King & Tschinkel, 2008). Whichever mechanism is at work, it is clear that habitat modification and loss can disadvantage native species and promote invader success, making relatively undisturbed natural habitats important refuges for native species (Howarth, 1991; Blair, 1996; Evans, 2004). Therefore, given that landscapes are increasingly dominated by anthropogenically-modified habitats, the remaining fragments of natural habitat can serve a critical role in the conservation of native biodiversity (Norton & Miller, 2000; McNeely & Schroth, 2006).

The objectives of this research were to 1) document the impact of the introduction of exotic species on the community of native lady beetle species occurring in Missouri using a historical dataset (1930-present), 2) determine whether different grassland habitats provide similar refuges for native species by comparing the community of native lady beetle species in remnant native tallgrass prairies, restored native tallgrass prairies, and agricultural tall fescue fields, 3) determine what factor(s) may make one grassland habitat a better refuge habitat for native coccinellid species than others and 4) determine if native species are being actively displaced from foraging habitats by exotic lady beetle species.

Chapter 2

The relationship between exotic lady beetle species establishment and long-term changes in the native lady beetle community in Missouri

Introduction

In recent decades, there has been a notable decline in several native species of lady beetles that were once commonplace in North America (Wheeler & Hoebeke, 1995; Alyokhin & Sewell, 2004; Harmon et al., 2007; Losey et al., 2007; Gardiner et al., 2011). Abundances of several species of native coccinellids have been documented to be declining throughout the continent including *Adalia bipunctata* (L.), *Brachiacantha ursina* (F.), *Chilocorus stigma* (Say), *Coccinella novemnotata* Herbst, *Coccinella transversoguttata richardsoni* Brown, *Cyclonedaa munda* (Say), *Hippodamia convergens* and *Hippodamia tredecimpunctata* (Say) (Wheeler & Hoebeke, 1995; Elliott et al., 1996; Colunga-Garcia & Gage, 1998; Alyokhin & Sewell, 2004; Evans, 2004; Harmon et al., 2007; Losey et al., 2007; Gardiner et al., 2009a; Gardiner et al., 2009c; Smith & Gardiner, 2013a). Because many species of lady beetles are important components of natural enemy guilds that contribute to natural biological control, decreases in the occurrences of these species raises concern regarding the status of their populations as well as the causes for their decline (Harmon et al., 2007; Losey et al., 2007).

In addition to the suite of native lady beetle species in North America, several exotic species have been introduced with the goal of increased agricultural pest control (Gordon, 1985; DeBach & Rosen, 1991; Day et al., 1994; Cottrell & Yeargan, 1998; Obrycki et al., 1998a; Obrycki & Kring, 1998b; Gardiner et al., 2011). Two of these

introduced exotic species, *Coccinella septempunctata* L. and *Harmonia axyridis* Pallas, have become pervasive throughout much of North America (Elliott et al., 1996; Snyder et al., 2004; Harmon et al., 2007; Losey et al., 2007). The establishment of these two exotic coccinellids, while useful for biological control purposes, has been correlated negatively with changes in native lady beetle communities (Elliott et al., 1996; Colunga-Garcia & Gage, 1998; Obrycki, 2000; Michaud, 2002; Brown, 2003; Koch, 2003; Alyokhin & Sewell, 2004; Snyder et al., 2004; Koch et al., 2006; Harmon et al., 2007; Lucas et al., 2007; Finlayson et al., 2008; Brown et al., 2011b; Roy et al., 2012). Therefore it is possible that these exotic beetles may somehow be displacing the native species.

Several hypotheses seek to explain how exotics might displace natives. This displacement of native coccinellids could occur as the consequence of direct antagonistic interactions with exotic species such as predation and competition for resources (Agarwala and Dixon 1992, Cottrell and Yeargan 1998, Elliott Norman et al. 1996, Evans E W 1991, Gardiner Mary M. et al. 2011, Michaud 2002, Obrycki J.J. and Kring 1998, Snyder et al. 2004). Predation of the eggs and larvae of native coccinellid species occurs more frequently than that of exotic eggs (Prasad & Snyder, 2004; Cottrell, 2005; Kajita et al., 2010; Smith & Gardiner, 2013a). It has been shown in laboratory studies that both native and exotic beetles will readily consume the eggs of native species such as *Coleomegilla maculata* (DeGeer), while the native species will generally avoid consumption of the eggs of exotic species (Cottrell & Yeargan, 1998; Cottrell, 2005). Interspecific competitive interactions for resources can also lead to displacement of native species by exotics. Exotic species have been shown to be more voracious consumers than many native species (Bahlia, 2014) and therefore could competitively

exclude native species when resources are limited. This competitive exclusion was demonstrated in laboratory mesocosms using the native beetle *C. maculata* and two exotic species, *C. septempunctata* and *H. axyridis* (Cardinale et al., 2006). In this study, *C. septempunctata* was found to be behaviorally dominant, displacing both *C. maculata* and *H. axyridis* away from their normal foraging area in the top and center of plants outwards towards the margins of the plants.

While much attention has been paid to the ever-increasing abundance of exotic lady beetles in North America, their relationship with the declining abundance of native species may merely be correlative. There are other factors that may have contributed to the widespread decline in native lady beetle species. Anthropogenic habitat modification, including increasing urban land area (McKinney, 2002; Szlavecz et al., 2011), fragmentation of natural habitats (Kareiva, 1987; Gibbs & Stanton, 2001) and agricultural intensification (Letourneau, 1998; Björklund et al., 1999; Iperti, 1999; Grez et al., 2013), can negatively impact native arthropod species. There has been a well-documented growth in urban/suburban populations in the United States since the early 1900s (Szlavecz et al., 2011), this increasing population has led to an increase in urban land cover and a decrease in rural lands, which broadly include natural and semi-natural habitat as well as agricultural lands (Berry, 1978). Agricultural systems have long been known to provide nutritional resources for many species of native coccinellids at various times during the growing season (Evans, 2003; Evans, 2004; Lundgren, 2009) and the importance of natural and semi-natural habitats for resources has become more apparent in recent years (Gardiner et al., 2009a; Gardiner et al., 2009c; Gardiner et al., 2011; Schmidt et al., 2011; Diepenbrock & Finke, 2013). These anthropogenic modifications

may have caused continual changes in the native coccinellid community over time, potentially impacting individual populations well before the arrival of any exotic species.

The goal of this study was to examine the potential influence of exotic coccinellid invasion on the native coccinellid community in Missouri. Using museum records of native species, I compared the composition of the native community before and after the establishment of the first exotic beetle species to establish in Missouri, *C. septempunctata*. I also examined the potential for continuous change in the native community over a longer timescale to determine whether the community was already in flux prior to the arrival of the exotic, or whether there was an abrupt change in the community following the establishment of the exotic species.

Methods

Data collection

Coccinellid specimens from the Enns Entomological Museum (University of Missouri, Columbia, Missouri), personal research collections, and The Lost Ladybug Project were examined, and all Missouri specimens were identified to species (Gordon, 1985) for inclusion in the dataset. Consistent state records of coccinellids were available from the Enns Entomological Museum beginning in 1930. Personal research collections added data from the years 1994-2011. Data were also available from The Lost Ladybug Project, a citizen science program aimed at documenting the changes in lady beetle distribution across North America, for the years 2007-2011(www.lostladybug.org). For the purpose of this study, I focused on species within the tribe Coccinellinae, the tribe to which both *C. septempunctata* and *H. axyridis* belong.

Coccinella septempunctata first appears in the dataset as one individual in 1979, with a higher number of occurrences beginning in 1986, indicating establishment at this point. The establishment of this species in Missouri in 1986 coincides with the suggested time frame of *C. septempunctata* establishment in the Midwest region (Obrycki et al., 1987; Wheeler & Hoebeke, 1995). The first occurrence of *H. axyridis* in the collection occurred in 1993, with greater frequency of occurrence beginning in 2003. Because *C. septempunctata* established before *H. axyridis*, I compared the native community in the museum collection before and after the year of *C. septempunctata*'s establishment. If the species composition of the community in the years prior to establishment is different from the composition after establishment of *C. septempunctata*, then it is consistent with the idea that the establishment of this beetle influenced subsequent changes to the native coccinellid community.

Museum collections provide an excellent resource for documenting changes in biodiversity over a long time period (Suarez, 2004), with the caveat that collections are a product of multiple collectors and may suffer from collection bias from individuals seeking out species considered to be rare and potentially causing an over-representation of such species in a museum collection (Guralnick & Van Cleve, 2005). To ensure that the likelihood of detecting the presence of species did not vary based on sampling intensity over time, I examined the relationship between the number of specimens in the collection and the detected level of species richness over time (PROC GLM; SAS v. 9.3, SAS Inc., Cary, NC, USA). Data were clumped into ten-year collection periods so that the number of specimens per sample period was large enough for this analysis. A positive relationship would mean that the number of documented species in the collection

is dependent on the number of species deposited into the museum collection in a given time period, indicating a potential bias. No relationship would mean that the number of species detected was independent of the number of specimens deposited, and is therefore a good representation of the lady beetle community throughout the time course used in this study.

Comparison of native coccinellid communities before and after establishment of C. septempunctata

The species composition of the native coccinellid community was compared prior to and after the determined year of *C. septempunctata* establishment in 1986 (Obrycki et al., 1987; Wheeler & Hoebeke, 1995). Because abundance information is not reliable from museum collections (Guralnick & Van Cleve, 2005), communities were described based on species occurrence (presence/absence). Occurrence data reduce the effect of collector bias in the abundance of individual species deposited into the collections. I combined species occurrence records across five year time periods to examine patterns of species presence through time that are robust to fluctuations in species deposited into the museum collection.

I visualized differences in native lady beetle communities before and after establishment of *C. septempunctata* using NMDS (non-metric multidimensional scaling). The ordination program PC-ORD (McCune & Grace, 2002; McCune & Mefford, 2011) was used to create a Sørensen distance matrix, which quantified differences in the composition of communities among the five year time periods. MRPP (multi-response permutation procedure) was then done to determine how different these communities were before and after the establishment of exotic species. This difference is described by

A, which assesses both how dissimilar defined groups are from one another as well as how similar sample units within each group are to one another (McCune & Grace, 2002).

Examination of continuous community change

To determine if changes in the species composition of the native lady beetle community were continuous, I compared the similarity of communities that are closer in time to those that lagged further apart in time. Data were lumped into seventeen 5 year time periods, resulting in community comparisons with time lags that varied from 1 time step to 16 time steps. A dissimilarity matrix was created using Euclidean distances, which represent the amount of variation in community composition (presence/absence) across five year time periods (Collins et al., 2000). Using this matrix, I determined if communities that were closer in time were more or less similar to each other than to those that were more distant in time by examining the relationship between the Euclidean distance and the square root of the time lag between communities using linear regression (PROC REG; SAS v. 9.3). A positive slope indicates that the community is undergoing directional change, becoming more different over time. A negative slope indicates that the community is undergoing convergence, becoming more similar over time. And if the slope is not different from zero, then the community is considered to be stable through time (Collins et al., 2000). To determine whether changes in the native coccinellid community occurred in relation to exotic establishment, the sub-set of comparisons between communities that span the time period during which *C. septempunctata* became established were visualized graphically. If the residuals from these comparisons all occur above the regression line, then there is evidence for an abrupt shift in native community composition in relation to the establishment of this exotic species. If these comparisons

are distributed both above and below the regression line, then it can be concluded that the native community was continuously changing over time with no direct impact from the exotic species.

Results

Descriptive data

Recorded species richness was not dependent on the total number of specimens represented in the collection used to create our dataset (Fig. 1, $r^2 = 0.168$, $p = 0.314$). In fact, the time period with the highest total specimens, 2000-2011, yielded the least number of species, while time periods with lower numbers of total specimens had the greatest amount of species richness.

Abundances of several species varied over time (Fig. 2). For example, *A. bipunctata*, the two-spotted lady beetle, was consistently present in the museum collection prior to 1996, and has not been recorded since this time period. *C. maculata* first appeared in the collections in 1974, occurring occasionally in small numbers until the early 2000s at which time it became a more commonly collected species. *C. munda*, while present throughout the duration of our study became a more frequently observed species in the 1970s and has since been collected regularly despite being reported to be in decline. *H. tredecimpunctata* was a commonly collected beetle until 1980, after which time it was only recorded in two additional years, 1994 and 1995. Similarly, *N. venusta* was only collected once since 1947, in 2011. These trends in species abundances in our museum collection reflect only the specimens deposited, and are not an unbiased estimate

of species abundance, but provide some insight into overall changes in the native lady beetle community composition within the region.

Comparison of native coccinellid communities before and after establishment of C. septempunctata

The native coccinellid communities present before and after the establishment of *C. septempunctata* overlap only slightly, indicating an overall dissimilarity between these communities in the composition of native coccinellid species present (Fig. 3; MRPP $A = 0.116$, $p = 0.0021$, stress = 9.23). The low stress value indicates that these data give accurate depictions of each community (Clarke, 1993). These data suggest that the identity of species that make up the native lady beetle community present in Missouri prior to the establishment of *C. septempunctata* is different from the identity of species that make up the native lady beetle community after the establishment of the exotic species.

Examination of continuous community change

I found continuous long-term community change in the eighty-year dataset based upon the presence and/or absence of all of the native species known to our state. The relationship between community differences and the time lag between communities was positive (Fig. 4; $\beta_1 = 0.41$, $r^2 = 0.36$, $p < 0.0001$), which is indicative of gradual directional change as the native coccinellid community became increasingly different over time in the state of Missouri. There was no trend for community comparisons that span the establishment of the exotic species to be more dissimilar than those that do not span establishment, suggesting that this change occurred independent of the influence of exotic lady beetle establishment.

Discussion

Like other studies of North American coccinellids, I found evidence for decreases in the population size of several native coccinellid species in Missouri (Elliott et al., 1996; Alyokhin & Sewell, 2004; Evans, 2004; Harmon et al., 2007). Of the species found to have been decreasing in collection frequency in Missouri, there were declines of *A. bipunctata*, *C. novemnotata*, *H. tredecimpunctata* and *N. venusta* (Fig. 2) severe enough to suggest that these species may no longer be present within the state. Both *A. bipunctata* and *C. novemnotata* have been well-documented as declining in North America (Wheeler & Hoebeke, 1995; Elliott et al., 1996; Harmon et al., 2007). Harmon et al. (2007) describe the declines of both of these previously abundant species and the relationship between their declines and the establishment and subsequent population increase of both exotic species based on a review of available literature. In this review, it was noted that declines in *A. bipunctata* appear to be correlated with the establishment of *C. septempunctata*. It is likely that this decline was then further exacerbated by the introduction of *H. axyridis*, a species which shares *A. bipunctata*'s preference for arboreal habitats. Similar to our findings, Harmon et al. (2007) also noted that *C. novemnotata* decreased in population size prior to the establishment of either exotic species. He suggests that this might be due to a combination of the effects of decreased foraging habitat, as *C. novemnotata* is known to prefer foraging within agricultural landscapes (Gordon, 1985), and potential niche overlap with the exotic beetle of the same genus, *C. septempunctata* (Gordon, 1985), which may have led to competition and predation events between these two species (Obrycki, 2000; Harmon et al., 2007).

Concurrent with declines in the abundance of *A. bipunctata* and *C. novemnotata* the abundance of other native species appeared to remain stable or increase over time. For example, *C. maculata* first appeared in the museum collection as one individual in 1974, but then was regularly collected in the mid-1990s. While some species appear to have been negatively affected by the presence of exotic beetles which consume similar food resources, it is possible that *C. maculata* was capable of thriving due to its ability to utilize a wide variety of non-aphid food items (Lundgren & Seagraves, 2011; Choate & Lundgren, 2013) and was thereby released from the pressure of food resource competition with the exotic beetles. Surprisingly, populations of both *C. munda* and *H. convergens* were consistently collected in Missouri over the past eighty years. Both of these species have been noted to be declining broadly throughout North America (Colunga-Garcia & Gage, 1998; Turnock et al., 2003; Gardiner et al., 2009b).

While it is apparent that the establishment of *C. septempunctata* in 1986 and later *H. axyridis* coincide with a period of change within the native coccinellid community (Fig. 3), the pressures of these exotic species may not have been the only influence contributing to changes in the native community composition. My data show that the native lady beetle community in Missouri did not experience an abrupt shift around the time of the establishment of the exotic species, but has instead been undergoing gradual change for the past century (Fig. 4). One factor that is correlated with this long-term community change is land use change, as increasing amounts of land have become urbanized and less land has been used for crop production throughout the state of Missouri (Table 1, Fig. 5). Agricultural land may have historically provided important resources for lady beetles, including food (e.g., aphids, scales, mites, fungi, etc.) and

habitat requirements (e.g., overwintering sites necessary for cold weather survival) (Gordon, 1985; Nault & Kennedy, 2000; Obrycki, 2000; Fothergill, 2010). Reductions in these resources may have had severe consequences for organisms, such as lady beetles, that forage throughout the landscape for nutritional resources and require areas in which they can safely survive through lethal winter temperatures. A study on long term species trends of coccinellids with respect to land use change was recently conducted in Central Europe, in which they found a correlation between reductions in the population sizes of two native species, *C. septempunctata* and *A. bipunctata*, and a reduction in their preferred foraging grounds (Honek et al., 2013). This loss of habitat is further intensified by the somewhat recently established exotic species, *H. axyridis*, which is already displaying the ability to displace native species with overlapping niches in Europe (Roy et al., 2012).

Loss of preferred habitat and the introduction of exotic species are two major factors known to contribute to biodiversity loss (Wilcove et al., 1998). Long term studies using museum data, such as the one presented here, provide the opportunity to track changes within communities over greater time scales than are possible with manipulative experiments. This information can then be used to attempt to mediate the loss of taxonomic diversity within important guilds, such as predatory lady beetles, which provide the ecosystem service of natural pest suppression.

Table 1. Acres in agricultural production and number of farms as determined by national census data.

Census year	Total acres	Number of farms
1930	33743019	255940
1935	35054542	278454
1940	34739598	256100
1945	35278251	242934
1950	35123143	230045
1954	34195379	201614
1959	33155226	168672
1964	32691618	147315
1969	32420284	137067
1974	29801127	115711
1979	30848898	121955
1982	29266609	112447
1987	29209187	106105
1996	30100000	110000
1997	30100000	110000
1998	30100000	110000
2011	29000000	106500

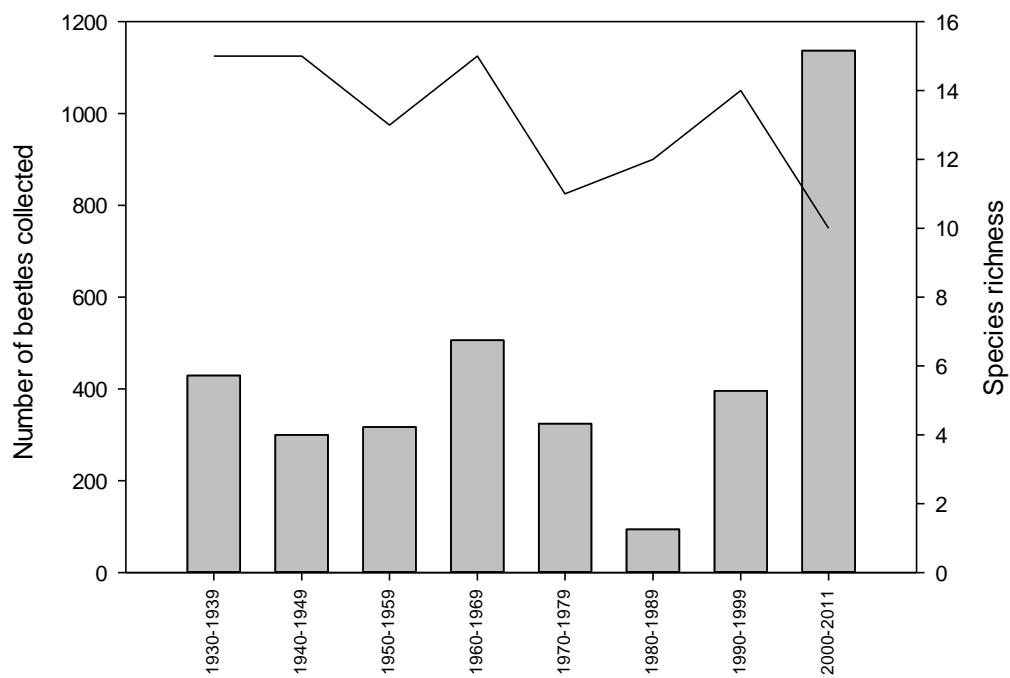
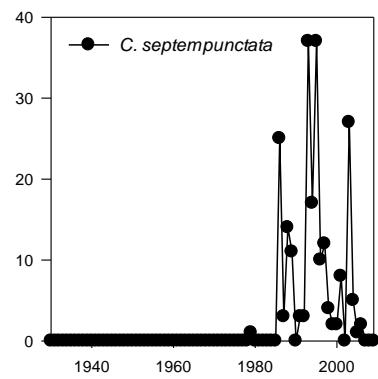
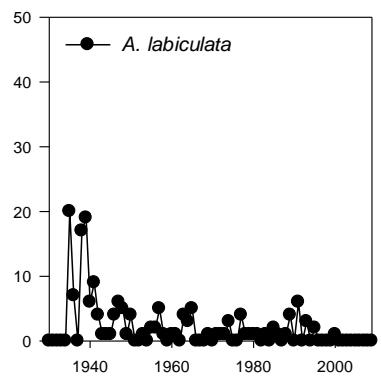
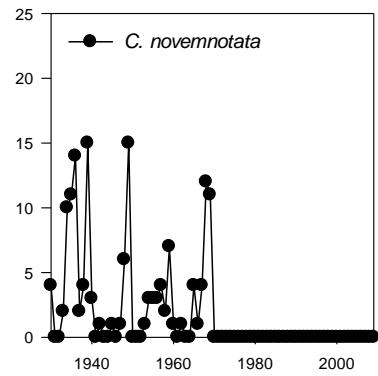
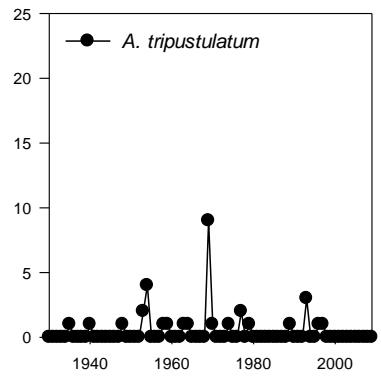
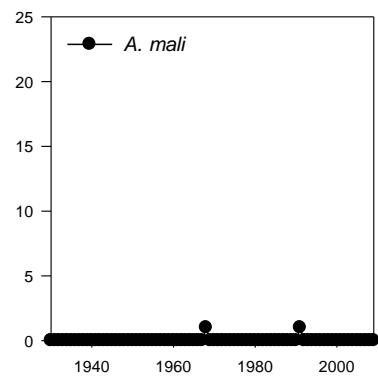
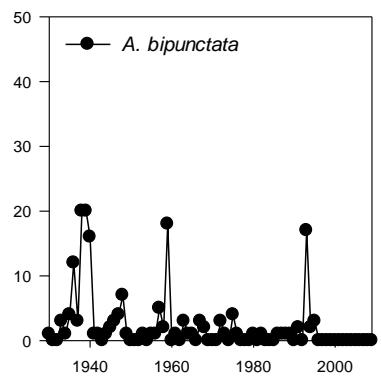
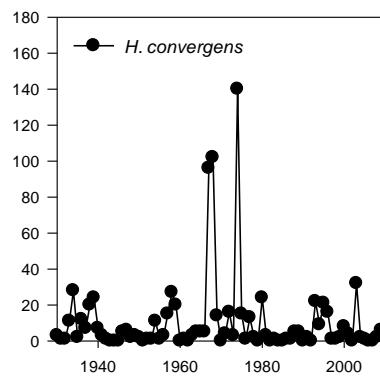
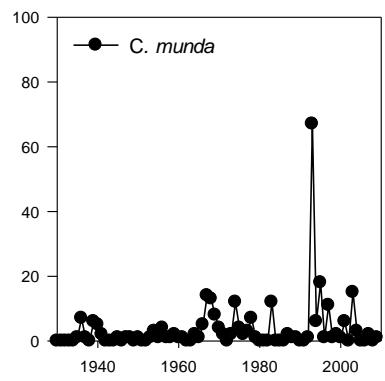
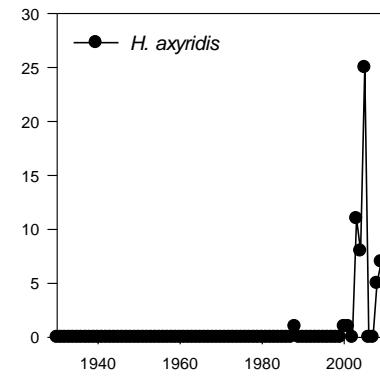
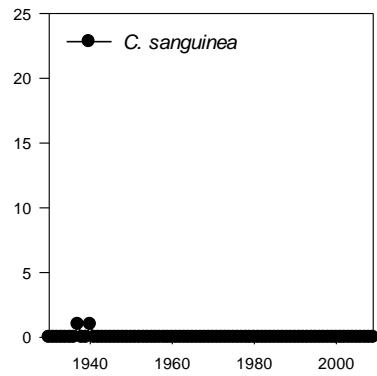
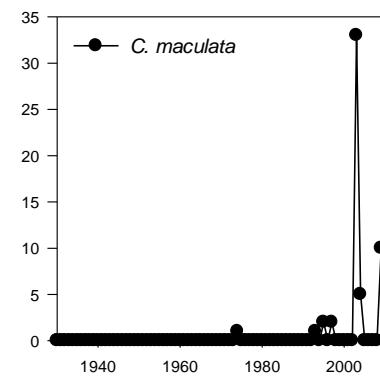
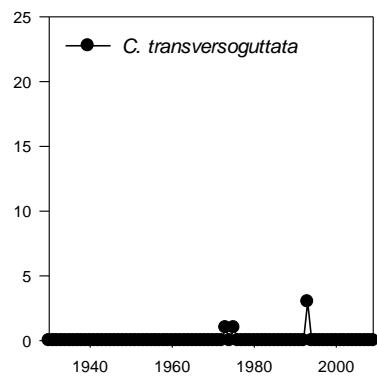
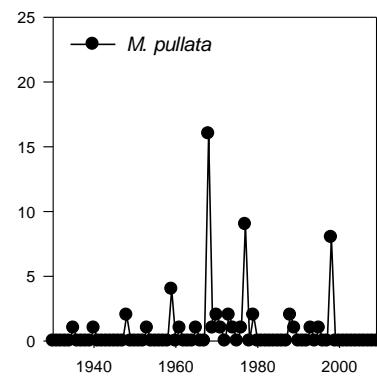
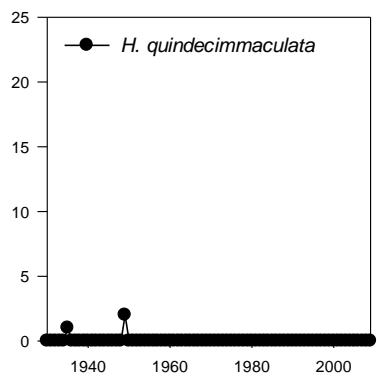
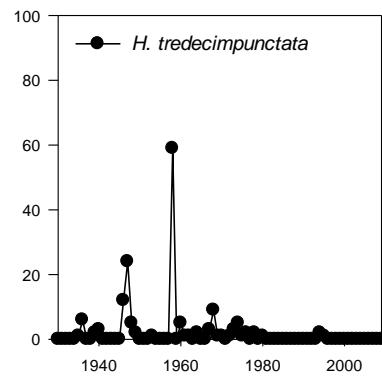
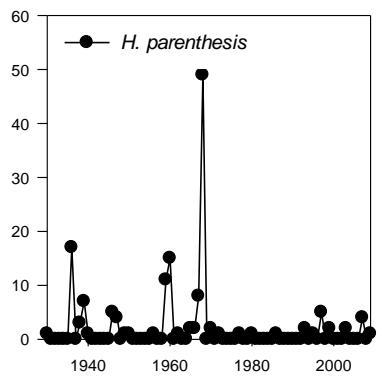
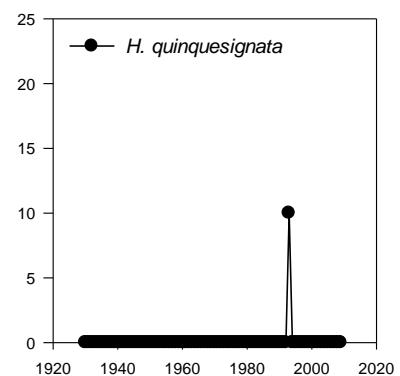
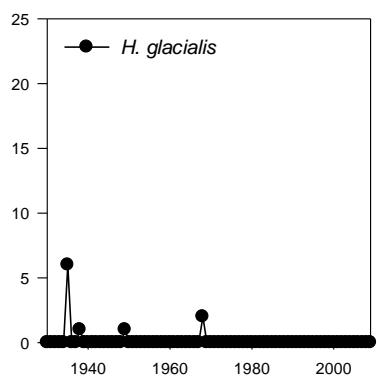


Figure 1: The number of native and exotic lady beetle specimens present in the collection (indicated by the bars) and the number of lady beetle species represented (species richness, indicated by the continuous line) in collection periods. The number of species detected was not related to the total number of beetles ($r^2 = 0.168, p = 0.314$)







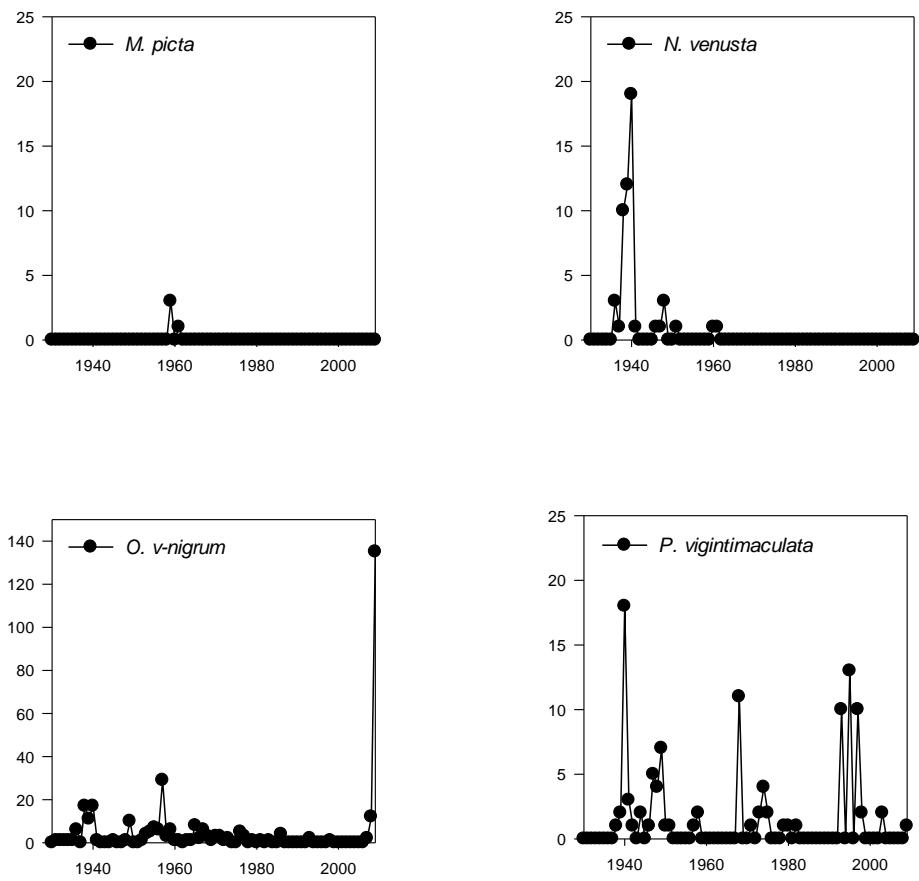


Figure 2: Number of specimens in the collection per year for each lady beetle species. Twenty species of native lady beetles and two exotic species (*Coccinella septempunctata* L. and *Harmonia axyridis* Pallas) within the tribe Coccinellinae were collected in Missouri between the years of 1930 and 2011.

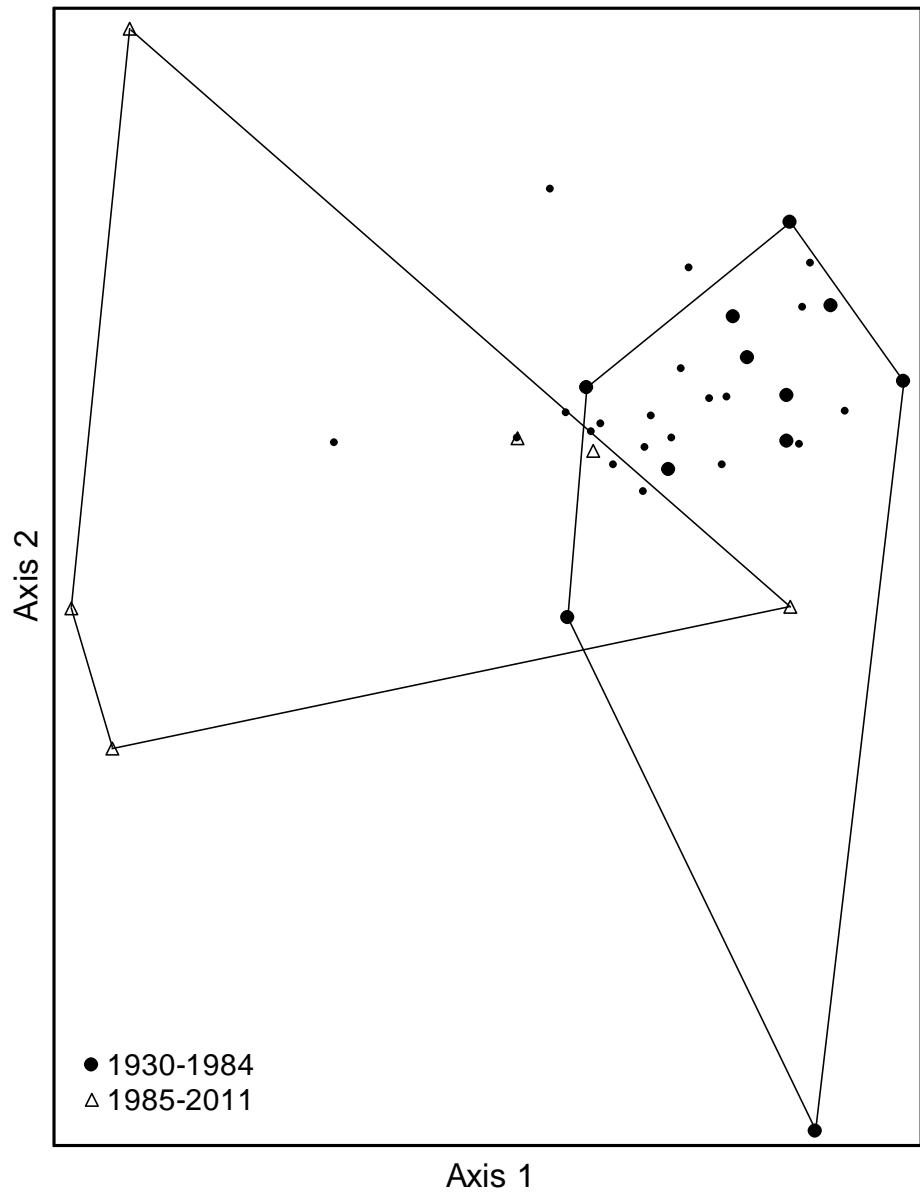


Figure 3: Non-metric multidimensional scaling representation of the native lady beetle community composition based on presence/absence data before and after the establishment of the exotic species, *Coccinella septempunctata* L., in 1986 (MRPP $A = 0.116$, $p = 0.0021$, stress = 9.23).

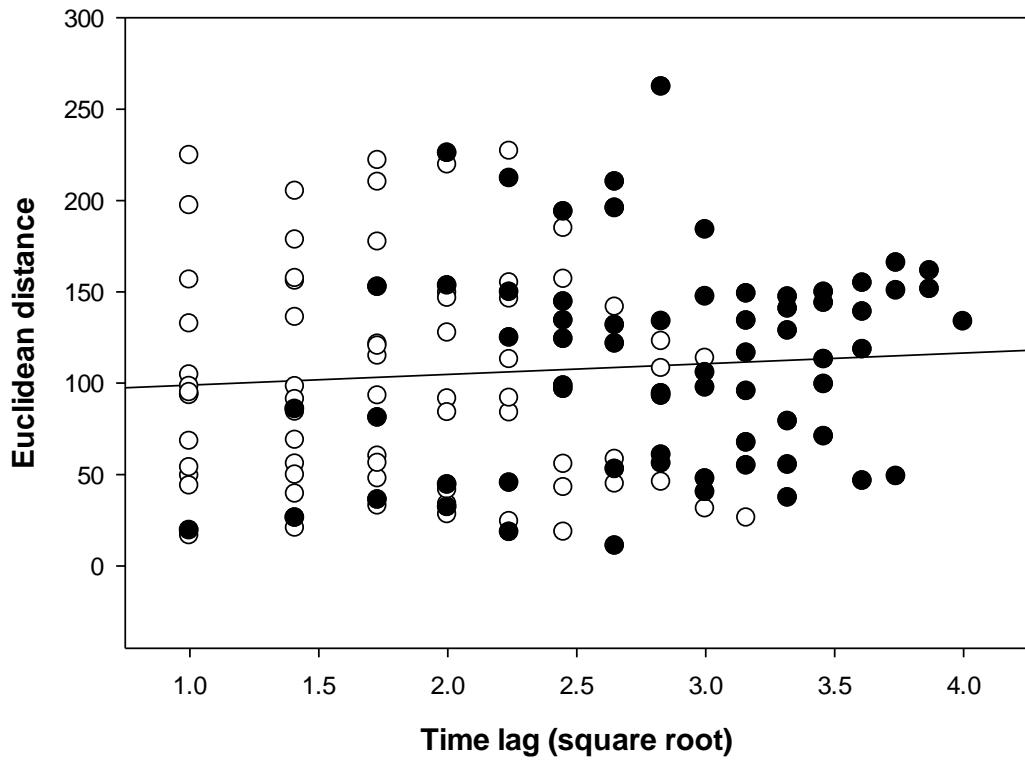


Figure 4: Dissimilarity in the species composition of native coccinellid communities with increasing time lags between communities. All comparisons between communities occurring prior to or after exotic establishment are indicated by open circles (○). Comparisons between communities occurring before and after establishment of *Coccinella septempunctata* L. are shown by black circles (●). Analysis of the amount of change between communities shows continuous directional community change through time ($\beta_1 = 0.41$, $r^2 = 0.36$, $p < 0.0001$) as the native coccinellid community became increasingly dissimilar over the course of eighty years.

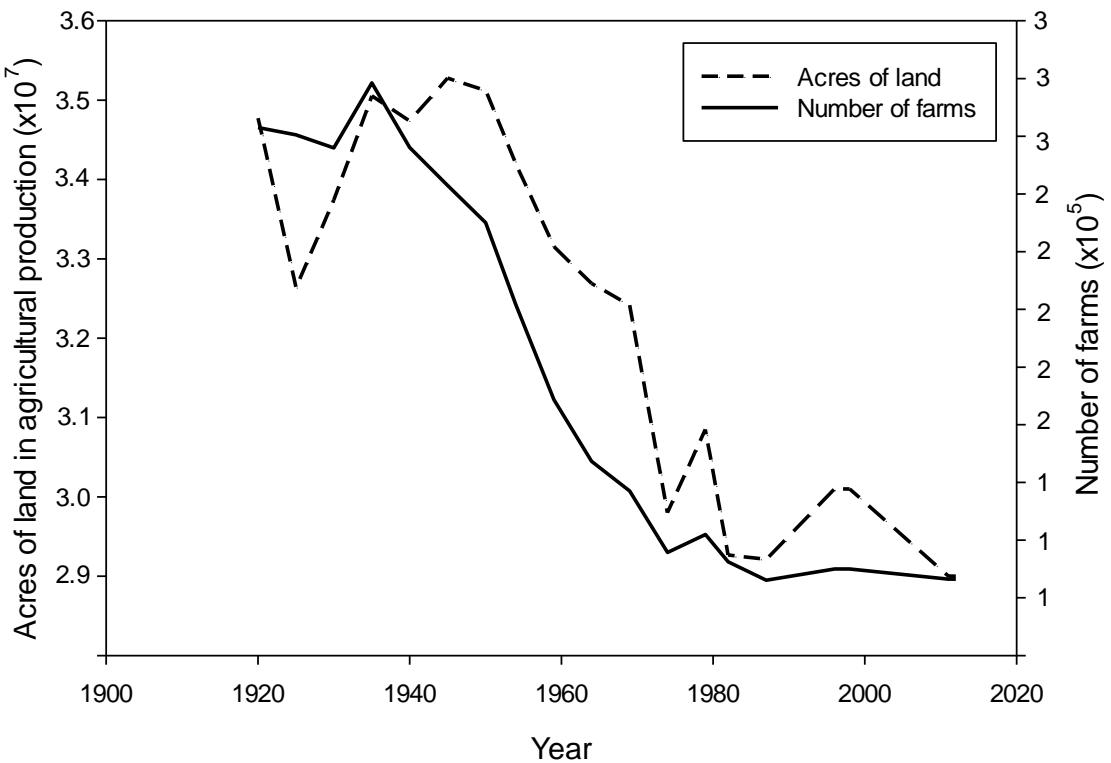


Figure 5: Decrease in total acreage of land used for agricultural crops and total number of farms in the state of Missouri from 1930-2011 ($r^2 = 0.76, p < 0.0001$; $r^2 = 0.874, p < 0.0001$ respectively).

Chapter 3

Refuge for native lady beetles (Coccinellidae) in perennial grassland habitats

Introduction

Habitat modification and species invasion are two of the leading causes of biodiversity loss (Czech & Krausman, 1997; Wilcove et al., 1998; Kolar & Lodge, 2001; Foley et al., 2005; Sala, 2006; Ricciardi, 2007). Although their contributions to the decline of native species are often investigated independently, interactions between them are likely (Suarez et al., 1998; Stachowicz et al., 1999; Seabloom et al., 2003; Tylianakis et al., 2007; Powell et al., 2011). For example, it has been hypothesized that degraded ecosystems facilitate the displacement of native species by invasive species, with habitat disturbance shifting the competitive advantage from previously well-adapted natives to exotic invaders (Howarth, 1991; Blair, 1996; Suarez et al., 1998; Stachowicz et al., 1999; Byers, 2002; Seabloom et al., 2003; Evans, 2004; Didham et al., 2005; Hansen et al., 2005; Didham et al., 2007; Tylianakis et al., 2007; Moles et al., 2008; Powell et al., 2011). However, this relationship may also be correlative, with native species merely being more vulnerable to environmental stresses or experiencing greater barriers to recruitment than exotic species in disturbed habitats (Gurevitch & Padilla, 2004; MacDougall & Turkington, 2005; King & Tschinkel, 2008). Whichever mechanism is at work, it is clear that habitat modification and loss can disadvantage native species and promote invader success, making relatively undisturbed natural habitats important refuges for native species (Howarth, 1991; Blair, 1996; Evans, 2004). Therefore, given that landscapes are increasingly dominated by anthropogenically-modified habitats, the

remaining fragments of natural habitat can serve a critical role in the conservation of native biodiversity (Norton & Miller, 2000; McNeely & Schroth, 2006).

Over the past 30 years, North American lady beetle (Coccinellidae) communities have experienced a marked decrease in the proportion of native individuals (Harmon et al., 2007). For example, *Adalia bipunctata* (L.), *Brachiacantha ursina* (F.), *Coccinella novemnotata* Herbst, *Cyclonedra munda* (Say), and *Hippodamia convergens* Guérin-Méneville are all native species in decline (Wheeler & Hoebeke, 1995; Elliott et al., 1996; Colunga-Garcia & Gage, 1998; Michaud, 2002; Alyokhin & Sewell, 2004; Losey et al., 2007; Gardiner et al., 2009c; Gardiner et al., 2011). This decline in native species is commonly attributed to the introduction and establishment of non-native coccinellids, including *Coccinella septempunctata* L. and *Harmonia axyridis* Pallas (Gordon, 1985; Obrycki & Kring, 1998b; Alyokhin & Sewell, 2004; Evans et al., 2011b); however, it is unclear whether this relationship is causal or merely correlative (Elliott et al., 1996; Evans, 2004; Harmon et al., 2007; Gardiner et al., 2011). It is also unknown to what extent, if any, the negative association between native and exotic lady beetles is mediated by habitat modification and loss (Evans, 2004; Losey et al., 2007; Gardiner et al., 2009c; Evans et al., 2011b). Most studies documenting the potential displacement of native beetles by non-natives focus on agricultural habitats (e.g. alfalfa, corn, and soybeans) (Wheeler & Hoebeke, 1995; Elliott et al., 1996; Colunga-Garcia & Gage, 1998; Michaud, 2002; Turnock et al., 2003; Alyokhin & Sewell, 2004; Evans, 2004; Snyder et al., 2004; Snyder & Evans, 2006; Harmon et al., 2007; Gardiner et al., 2009c). However, evidence suggests that natural and semi-natural habitats, such as perennial grasslands, may serve as refuges for native lady beetles by resisting the invasion of exotic species and/or providing

a consistent source of resources (Gardiner et al., 2009b; Gardiner et al., 2009c; Gardiner et al., 2011).

Common perennial grassland habitats within the mid-western region of the US that may act as refuge habitats for native lady beetle species include remnant and restored tallgrass prairies and grasses grown agriculturally as forage crops. Tallgrass prairie is a specialized ecosystem comprised largely of native grasses that was once the predominant land cover in the central portion of North America (Samson & Knopf, 1994; Samson et al., 2004). At present, only a small fraction of the original prairie remains as scattered patches of remnant prairie throughout its former range (Samson & Knopf, 1994; Samson et al., 2004), although additional tallgrass prairie habitat is being added as former agricultural fields are being actively restored. Vegetation in this habitat is dominated by warm-season bunchgrass species including *Andropogon gerardii* Vitman (big bluestem), *Panicum virgatum* L. (switchgrass), *Sorghastrum nutans* (L.) Nash (indiangrass), and *Schizachyrium scoparium* (Michx.) Nash (little bluestem) with a wide variety of flowering plants interspersed within these grasses, including *Amorpha canescens* Pursh (lead plant), *Echinacea spp.* Moench (coneflowers), and *Rudbeckia hirta* L. (black-eyed Susan) (Howe 1994, Bock and Bock 1998). Tall fescue, *Lolium arundinaceum* (Schreb.) Darbysh, is a non-native cool-season bunchgrass commonly grown as a forage crop, and is one of several agricultural products produced on land that was historically covered by tallgrass prairie (Hoveland, 2000). It was introduced into the US from Europe in the 1800's, and is currently found growing throughout the US and Canada. These three types of perennial grassland habitats fall along a continuum of modification intensity with remnant tallgrass prairies being a minimally-disturbed native habitat, restored tallgrass

prairies being a relatively more-disturbed native habitat previously used as agricultural fields, and tall fescue fields being a frequently-disturbed perennial agricultural system dominated by an exotic plant species.

The goal of this study was to evaluate the conservation value for native lady beetles of different perennial grassland habitats that vary in degree of habitat modification and disturbance. I did this by comparing the abundance, diversity, and species composition of native and exotic coccinellids in remnant tallgrass prairies, restored tallgrass prairies, and tall fescue grown as a forage crop. I hypothesized that natural grassland systems such as remnant and restored tallgrass prairies, which experience infrequent disturbances due to active management or natural causes, may provide a greater refuge for native coccinellid species than the more frequently-disturbed forage crop, where exotic grass species like tall fescue have been introduced and are harvested several times during the growing season.

Materials and Methods

Study area

Coccinellid communities were compared across five remnant tallgrass prairies, five restored tallgrass prairies, and four tall fescue fields throughout central and southwest Missouri, USA (between 38° 92' N, 91° 70'W and 37° 8'N, 94° 51'W) (Figs. 6 and 7). Sites were chosen based on the similarity of the field size ($F_{2,11} = 1.84$, $p = 0.21$; mean \pm SEM: remnant = $0.44 \pm 0.12 \text{ km}^2$, restored = $0.62 \pm 0.12 \text{ km}^2$, and tall fescue = $0.27 \pm 0.14 \text{ km}^2$), surrounding landscape, and management regime (Table 2). For statistical analyses, sites were divided into three blocks by prairie type as defined by

soil substrate, soil moisture, and geographic locality (Table 2). All three perennial grassland habitats were represented in each block, but only one block contained replicates of each habitat. All sites were historically not glaciated (Lyle, 2009) and within areas documented to have been previously dominated by tallgrass prairie (Schroeder, 1982).

In Missouri, both remnant and restored tallgrass prairie habitats are managed primarily by state agencies and/or private environmental groups, with removal of invasive plants, addition of native seed, and fire regimes employed to maintain the sites (Clubine, 2011). Tall fescue is particularly well adapted to the soil and weather conditions of this area, and millions of hectares of Missouri prairie and forest have been converted to tall fescue since 1960 (Henning et al., 1993; Missouri Department of Conservation, n.d.).

Coccinellid sampling

Coccinellid adults were collected monthly June-August 2010 and May-September 2011 using 18 x 23 cm unbaited yellow sticky cards (Stephens & Losey, 2004)(Pherocon AM, Great Lakes IPM, Vestaburg, MI, USA). Ten cards were collected per site on each sampling date. I summed the number of beetles collected on the ten cards by site across sampling dates for 2010 and 2011 separately (cumulative seasonal abundance). The ten cards were located along an 80 m transect with two cards every 20 m. Cards were attached to a 1.8 m metal fence post, with one card located at ground level and a second card at the average height of the surrounding vegetation (Fig. 8). Cards were left undisturbed in the field for one week then collected and stored in a -20° C freezer until processing. Coccinellids were later removed from the cards in the

laboratory, cleaned using HistoClear II (National Diagnostics, Atlanta, GA, USA), and identified to the lowest taxonomic unit possible, most to species (Gordon, 1976, 1985).

Comparison of lady beetle community abundance and diversity

The abundance and diversity of native and exotic coccinellid species were compared across grassland habitat types and sampling years. The main and interactive effects of habitat type (remnant tallgrass prairie, restored tallgrass prairie, or tall fescue forage crop) and time (2010 or 2011) on the cumulative seasonal abundance, species richness, or species evenness of native or exotic coccinellids were tested in separate mixed-model repeated measures analyses of variance with block and block*habitat type included as random factors (PROC MIXED, SAS v. 9.1). For each analysis, a variety of covariance structures were examined using the Bayesian Information Criterion and a compound symmetry covariance structure was determined as the best-fit model in every case. *A priori* comparisons of treatment means were done using *t*-tests with a Bonferroni correction of α values (PROC MIXED, SAS v. 9.1).

Comparison of lady beetle community species composition

Differences in coccinellid community species composition across grassland habitat types were visualized using nonmetric multidimensional scaling (NMDS) (PC-ORD, MjM Software Design, Gleneden Beach, Oregon) (Clarke, 1993; McCune & Grace, 2002). NMDS was used to create a dissimilarity matrix using the Sørensen distance coefficient (Kruskal, 1964a, b; Mather, 1976). Stress levels were obtained by fitting the dissimilarities to distances that were calculated using randomly assigned starting conditions, and a two-dimensional solution was chosen as the best representation of the dissimilarities among grassland types. Differences in coccinellid community

species composition across grassland types were tested statistically using multi-response permutation procedures (MRPP) based on the rank-transformed Sørensen distance matrix (PC-ORD, MjM Software Design, Gleneden Beach, Oregon) (Mielke, 1984; Mielke & Berry, 2001). The test statistic A and its resulting p -value from the MRPP analysis tested the null hypothesis of no difference in average within-group ranked distances.

For these multivariate analyses of community species composition, the cumulative species abundance across sampling periods and years for each site was used. Summing across time periods enabled examination of patterns of species assortment among habitats that were robust to seasonal differences in species abundances that may have occurred during the course of the study (*sensu* Wimp et al., 2010).

Comparison of vegetation across habitats

Vegetation was sampled in each field site during the month of July in 2010. Samples consisted of five randomly chosen 30.5cm^2 quadrats per location. All vegetation was removed from these quadrats (Fig. 9), brought back to the laboratory and identified to the lowest taxonomic unit possible. Samples were then dried and weighed to determine the biomass of each vegetation type represented (forbs and grasses).

The total biomass and genus richness of all plants, the biomass of forbs, grasses and thatch separately, and the generic richness of forbs and grasses separately were compared across grassland habitats. All comparisons were done using generalized linear models (PROC GLM, SAS v. 9.3). Prior to sampling, two fescue sites were mowed; these two sites were removed from biomass analysis.

Comparison of aphid abundance

The abundance of aphids in each habitat type was monitored monthly by using a combination of pan traps and the aforementioned yellow sticky cards used for coccinellid sampling. Pan traps consisted of a one liter plastic container filled with a 1:3 mixture of propylene glycol and water, suspended approximately one meter above the ground (Fig. 10). Two pan traps were used in each site and left in the field for one week per month during the 2011 season. Additionally, aphid abundance on monthly sticky cards traps was recorded.

The seasonal abundance of aphids was determined by averaging the total number of aphids collected each month in the pan traps and yellow sticky cards. These values were then compared across habitat types using generalized linear models (PROC GLM, SAS v 9.3).

Results

Comparison of lady beetle community abundance and diversity

Overall, native lady beetles were much more abundant and species rich than exotic lady beetles (Fig. 11). In two seasons of sampling, a total of 627 coccinellid adults were collected, but only 34 individuals (5.4% of the community) were exotic in origin (Table 3). Representative individuals were found from 24 native coccinellid species from 3 subfamilies (Coccinellinae, Scymninae, and Sticholotidinae) and 3 exotic coccinellid species from the subfamily Coccinellinae.

When comparing the abundance and diversity of lady beetles across different perennial grasslands, only native species responded to changes in the habitat type (main

effect of habitat type on native abundance: $F_{2,6} = 124.58$, $P < 0.0001$ and native species richness: $F_{2,6} = 5.08$, $p = 0.051$; Fig. 11). The total abundance of native beetles in tall fescue forage crops was four times greater than that found in either remnant or restored natural tallgrass prairies ($t = 14.32$, $p < 0.0001$ and $t = 14.02$, $p < 0.0001$, respectively; Fig 11a). Likewise, tall fescue also had a higher number of native species present than either prairie habitat ($t = 2.83$, $p = 0.03$ and $t = 2.76$, $p = 0.033$, respectively; Fig. 11c). However, exotic species abundance and richness remained consistently low across all grassland habitat types (main effect of habitat type on exotic abundance: $F_{2,6} = 2.66$, $p = 0.149$ and exotic species richness: $F_{2,6} = 1.16$, $p = 0.375$; Fig. 11b,d). These patterns were consistent across years, with no significant main or interactive effects found that included the factor ‘time.’ Despite the greater abundance and species richness of coccinellid communities in forage crops than prairie habitats, there was no overall difference in species evenness across habitats (fescue and native prairie: $t = -0.48$, $p = 0.64$; fescue and restored prairie: $t = -0.88$, $p = 0.39$; restored and native prairie: $t = -0.43$, $p = 0.67$).

Comparison of lady beetle community species composition

There was a significant difference in the identity of species that composed the lady beetle community across perennial grassland habitat types (MRPP $A = 0.912$, $p < 0.01$; Fig. 12). This difference arose because the coccinellid species composition in tall fescue habitats was different from that found in either of the two natural tallgrass prairie habitats (remnant vs. fescue: MRPP $A = 0.26$, $p < 0.01$; restored vs. fescue: MRPP $A = 0.28$, $p < 0.01$). Species composition did not differ between restored and remnant tallgrass prairies (MRPP $A = -0.0545$, $p = 0.80$).

To verify that community differences were not an artifact of one particularly abundant species (*Scymnus rubricaudus* Casey) or two species that were found only as singletons (*Scymnus ardelio* Horn and *Scymnus postpictus* Casey), I reanalyzed the community data in two ways: 1) in the absence of *S. rubricaudus*, and 2) in the absence of the two singletons. The results of these analyses were consistent with the full NMDS, with the composition of species in tall fescue still being significantly different than that found in either prairie habitat (Figs. 13, 14).

Comparison of vegetation across habitats

Total plant biomass did not differ amongst habitat types ($F_{2,11} = 0.22, p = 0.81$; Fig. 15a), however, the biomass of both grasses and forbs did vary by habitat (forbs: $F_{2,11} = 4.78, p = 0.032$, grasses: $F_{2,11} = 7.32, p = 0.0095$; Fig. 16), with the highest biomass of grass and lowest biomass of forbs in the fescue habitat. Prairies had greater overall generic richness than fescue ($F_{2,13} = 3.9, p = 0.047$; Fig. 15b), and this pattern was driven by the fact that native prairie had a higher forb richness than agricultural tall fescue and restored prairie was intermediate ($F_{2,13} = 4.92, p = 0.026$; Fig. 17). All habitats had a similar richness of grass genera ($F_{2,13} = 0.24, p = 0.79$; Fig. 17).

Comparison of aphid abundance across habitats

Aphid abundance was greater in fescue habitats than in either native tallgrass habitat ($F_{2,11} = 8.47, p = 0.0059$; Fig. 18). This difference was driven by the number of aphids collected on yellow sticky cards ($F_{2,11} = 8.38, p = 0.0062$) and not from those collected in pan traps ($F_{2,11} = 0.35, p = 0.7129$).

Discussion

I found evidence that both natural and agricultural perennial grasslands may act as a refuge for and source of native lady beetle diversity and abundance in the landscape. While exotic species dominate lady beetle communities in annual cropping systems (Elliott et al., 1996; Colunga-Garcia & Gage, 1998; Michaud, 2002; Alyokhin & Sewell, 2004; Evans, 2004; Snyder et al., 2004; Harmon et al., 2007; Gardiner et al., 2009c; Fothergill, 2010; Evans et al., 2011b; Gardiner et al., 2011), including those in the Midwestern region of the U.S. (Elliott et al., 1996; Colunga-Garcia & Gage, 1998; Gardiner et al., 2009c; Fothergill, 2010), they were found to be exceedingly rare in my collections across all perennial grassland types (Fig. 11b, d). In this case, more than 95% of the individuals in the lady beetle community were native in origin, including some species that are in decline, such as *Cycloneda munda*, *Hippodamia convergens*, and *Brachiacantha ursina*, with *Cycloneda munda* occurring consistently across all habitats and in both years. This finding is in stark contrast to results from row crop systems like soybeans, where exotic species can comprise as much as 90% of the community (Gardiner et al., 2009c), but is consistent with historical descriptions of lady beetle communities in the mid-western US prior to 1985, in which native individuals averaged 95% of the coccinellid assemblage (Harmon et al., 2007). The dominance of native species and rarity of exotic species in all perennial grasslands studied suggests that these habitats may provide a critical refuge for native lady beetles in an increasingly disturbed and agriculturally-dominated landscape.

Although all perennial grasslands appeared to serve as a refuge for native lady beetles, the diversity, abundance, and species composition of natives varied among the

grassland habitat types, indicating that some habitats may be of greater conservation value than others. Surprisingly, it was the habitat considered the most degraded and with the lowest plant diversity, tall fescue (Fig. 15b), in which the diversity and abundance of native lady beetles was the highest (Fig. 11a, c). The greater abundance of coccinellids in tall fescue was largely due to one species, *S. rubricaudus*, which dominated the fescue coccinellid community. This species was over ten times more abundant in the tall fescue habitat during the two year period than in either the remnant or restored tallgrass prairie habitats and at least four times more abundant than any other species overall (Table 3). Additionally, two native species, *S. ardelio* and *S. postpictus*, were found exclusively in tall fescue, although only a single individual of each species was collected. *Coleomegilla maculata* (De Geer) was nearly exclusive to the tall fescue, with twenty individuals collected within this habitat and only a single individual recorded from a restored tallgrass prairie. Species exclusive to tallgrass prairie habitats were predominantly within the subfamily Scymninae, including several in the genera *Brachiacantha*, *Hyperaspis*, and *Scymnus*. The beetles in this subfamily are some of the smallest coccinellids in North America, with *Scymnus* species noted to be less than three mm in total body length (Gordon, 1976), and are often not included in coccinellid surveys.

Although competitive displacement by exotic species is a commonly cited mechanism for native lady beetle decline (Elliott et al., 1996; Alyokhin & Sewell, 2004; Evans, 2004), it is not likely that differences in the native lady beetle communities between tall fescue and tallgrass prairies were due to competitive interactions between native and exotic coccinellids. The same three exotic species, *Coccinella septempunctata*, *Harmonia axyridis*, and the fungus-feeding *Psyllobora vigintimaculata*

Say, were found across all grassland types, and they were equally rare in all cases. Therefore, it does not appear that tall fescue promoted native species by more successfully resisting the invasion of exotic species. Alternatively, I hypothesize that the quality of refuge provided by grassland habitats may be determined by the availability of resources, such as the abundance of aphid prey. Aphids were more abundant in fescue fields (Fig. 18) and may provide a plentiful and/or high-quality food source that directly benefits native lady beetle performance or promotes the coexistence of native lady beetle species by diminishing competitive interactions for limiting resources (Losey et al., 2012). One might predict that tallgrass prairies would supply a greater abundance and diversity of prey resources for lady beetles, because prairies are more vegetationally-diverse than tall fescue fields (Siemann, 1998; Siemann et al., 1998; Haddad et al., 2001; Reich et al., 2001; Haddad et al., 2011)(Fig. 17). However, tall fescue fields also contain a variety of different host plant species in the understory, including red clover (*Trifolium pratense* L.), which supports abundant populations of the pea aphid (*Acyrthosiphon pisum*) (Harris) (L. Diepenbrock, personal observation). In addition, tall fescue is a cool-season grass that may promote a larger early-season population of aphids than the warm-season prairie grasses.

The incorporation of perennial grassland habitats, both natural and agricultural, within human-modified landscapes has the potential to benefit native lady beetles. While tall fescue fields harbored the greatest abundance and diversity of native lady beetles, suggesting the highest conservation value, native beetles were very common within prairie habitats as well. Furthermore, there were no differences among lady beetle communities in remnant and restored tallgrass prairies, suggesting that the restoration of

formerly cultivated fields to tallgrass prairie habitats can be as effective as the preservation of tallgrass prairie remnants in conserving lady beetle biodiversity. Ultimately, deciphering which grassland characteristics are responsible for promoting the persistence of native lady beetles while simultaneously preventing invasion by exotic species will be important not only for understanding lady beetle biology and community dynamics, but also for informing future land management for the conservation of these important natural enemies.

Table 2. Characteristics of sites used for comparing coccinellid communities.

Site	Habitat type	County	Coordinates	Area (Km ²)	Surrounding landscape	Land management	Type of Prairie
Friendly Prairie	Remnant tallgrass prairie	Pettis	N 38° 33.08 W 93° 17.89	0.162	Fescue, conservation reserve program	Burn	Dry-Mesic Chert
Brun's Tract	Restored tallgrass prairie	Pettis	N 38° 37.09 W 93° 28.22	0.647	Fescue/cow pasture	Burn	Dry-Mesic Chert
Busy B's Farm	Fescue	Pettis	N 38° 70.43 W 93° 42.90	0.486	Fescue/cow pasture, conservation reserve program	Mow	Dry-Mesic Chert
Prairie State Park 21C	Restored tallgrass prairie	Barton	N 37° 30.43 W 94° 33.67	1.05	Tallgrass prairie, fescue	Burn	Dry-Mesic Sandstone/Shale
Prairie State Park 22B	Restored tallgrass prairie	Barton	N 37° 31.00 W 94° 32.13	0.324	Tallgrass prairie, corn/soybean rotation	Burn	Dry-Mesic Sandstone/Shale
Prairie State Park 27A	Remnant tallgrass prairie	Barton	N 37° 30.41 W 94° 32.47	0.647	Tallgrass prairie, old field	Burn	Dry-Mesic Sandstone/Shale
Prairie State Park 22E	Remnant tallgrass prairie	Barton	N 37° 30.32 W 94° 33.04	0.324	Tallgrass prairie, corn/soybean rotation, old field	Burn	Dry-Mesic Sandstone/Shale
Farm 1	Fescue	Barton	N 37° 46.68 W 94° 40.92	0.324	Fescue/cow pasture, corn/soybean rotation	Mow	Dry-Mesic Sandstone/Shale
Farm 2	Fescue	Barton	N 37° 46.38 W 94° 40.92	0.202	Fescue, old field	Mow	Dry-Mesic Sandstone/Shale
Twenty-Five Mile Prairie Conservation Area	Remnant tallgrass prairie	Polk	N 37° 46.91 W 93° 31.76	0.451	Fescue/cow pasture, restored tallgrass prairie	Burn	Dry Limestone/Dolomite
Twenty-Five Mile Prairie Conservation Area	Restored tallgrass prairie	Polk	N 37° 46.64 W 93° 31.72	0.901	Fescue/cow pasture, remnant tallgrass prairie	Burn	Dry Limestone/Dolomite
Tucker Prairie	Remnant tallgrass prairie	Callaway	N 38° 56.96 W 91° 59.55	0.591	Corn/soybean rotation, roadway, fescue	Burn	Hardpan
Prairie Fork Conservation Area	Restored tallgrass prairie	Callaway	N 38° 54.38 W 91° 44.32	0.162	Restored tallgrass prairie, corn/soybean rotation	Burn	Hardpan
Mc Credie Research Farm	Fescue	Callaway	N 38° 94.76 W 91° 90.96	0.06	Public fishing lake, corn/soybean rotation	Burn	Hardpan

Table 3. Total number of lady beetle individuals of each species collected in five remnant tallgrass prairies, five restored tallgrass prairies, and four tall fescue fields in 2010, 2011, and summed across both years (Total).

	2010			2011			Total		
	Remnant Tallgrass	Restored Tallgrass	Fescue	Remnant Tallgrass	Restored Tallgrass	Fescue	Remnant Tallgrass	Restored Tallgrass	Fescue
Native Coccinellidae									
Subfamily Coccinellinae									
<i>Coleomegilla maculata</i>	0	1	14	0	0	6	0	1	20
<i>Cycloneda munda</i>	2	10	17	4	16	18	6	26	35
<i>Hippodamia convergens</i>	0	0	1	2	1	1	2	1	2
Subfamily Scymninae									
<i>Brachiacantha albifrons</i>	13	0	0	0	0	0	13	0	0
<i>Brachiacantha decempustulata</i>	1	0	0	0	0	0	1	0	0
<i>Brachiacantha ursina</i>	0	0	0	0	1	0	0	1	0
<i>Didion sp.</i>	0	5	4	0	0	0	0	5	4
<i>Hyperaspis fibriolata</i>	0	0	0	1	0	0	1	0	0
<i>Hyperaspis undulata</i>	0	5	2	4	5	0	4	11	2
<i>Scymnus americanus</i>	1	0	1	1	3	4	2	3	5
<i>Scymnus apicanus</i>	1	0	10	2	4	1	3	4	11
<i>Scymnus ardelio</i>	0	0	1	0	0	0	0	0	1
<i>Scymnus brullei</i>	0	0	1	0	1	1	0	1	2
<i>Scymnus compar</i>	0	0	0	1	0	0	1	0	0
<i>Scymnus iowensis</i>	0	0	0	4	2	0	4	2	0
<i>Scymnus kansanus</i>	0	0	0	2	0	0	2	0	0
<i>Scymnus lacustris</i>	1	2	1	2	4	0	3	6	1
<i>Scymnus loweii</i>	0	1	0	0	0	0	0	1	0
<i>Scymnus postpictus</i>	0	0	1	0	0	0	0	0	1
<i>Scymnus pulvinatus</i>	0	2	1	4	0	17	4	2	18
<i>Scymnus rubricaudus</i>	9	6	109	12	5	119	17	11	228
<i>Stethorus punctum</i>	0	0	11	2	1	3	2	1	14
Subfamily Sticholotidinae									
<i>Delphastus pusillus</i>	3	3	0	0	0	0	3	3	0
<i>Microweisia misella</i>	9	15	29	24	9	16	33	24	45
Exotic Coccinellidae									
Subfamily Coccinellinae									
<i>Coccinella septempunctata</i>	0	0	2	3	6	4	3	6	6
<i>Harmonia axyridis</i>	1	3	2	0	0	1	1	3	3
<i>Psyllobora vigintimaculata</i>	0	3	4	1	2	2	1	5	6

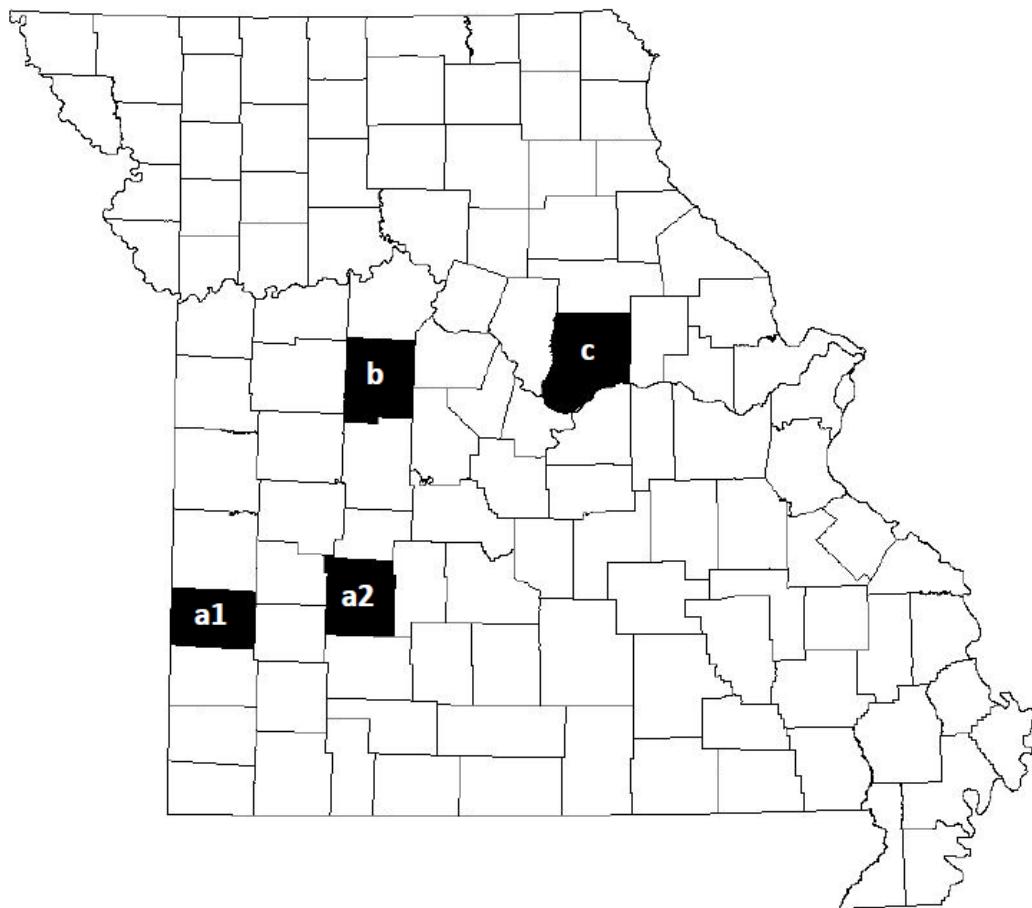


Figure 6: Map of the state of Missouri, USA showing the locations of field sites by county where lady beetles were collected. Sites in counties designated by the same lower case letters were considered part of the same block for statistical analyses. Sites in Barton (a1) and Polk (a2) counties included three remnant tallgrass prairies, three restored tallgrass prairies, and two tall fescue habitats. Pettis (b) and Callaway (c) counties each included one remnant tallgrass prairie, one restored tallgrass prairie, and one tall fescue habitat.



**Tucker Prairie
(Native)**



**Prairie Fork
Conservation Area
(Restored)**



Fescue Field

Figure 7: Images representative of the three habitat types used for this study: Tucker Prairie, a native tallgrass prairie located in Callaway County (MO), Prairie Fork Conservation Area, a prairie that has been restored from agriculture located in Callaway County (MO), and a fescue field from Barton County (MO).



Figure 8: Yellow sticky card sampling in a fescue habitat. One card is positioned just above the ground surface and the other is positioned close to the height of the plant canopy.



Figure 9: Removal of vegetation for analysis of plant genera richness and biomass.



Figure 10: Aphid pan trap set in field. Traps consisted of a 1:3 propylene glycol:water mixture suspended just above the canopy to attract aphids migrating into the site.

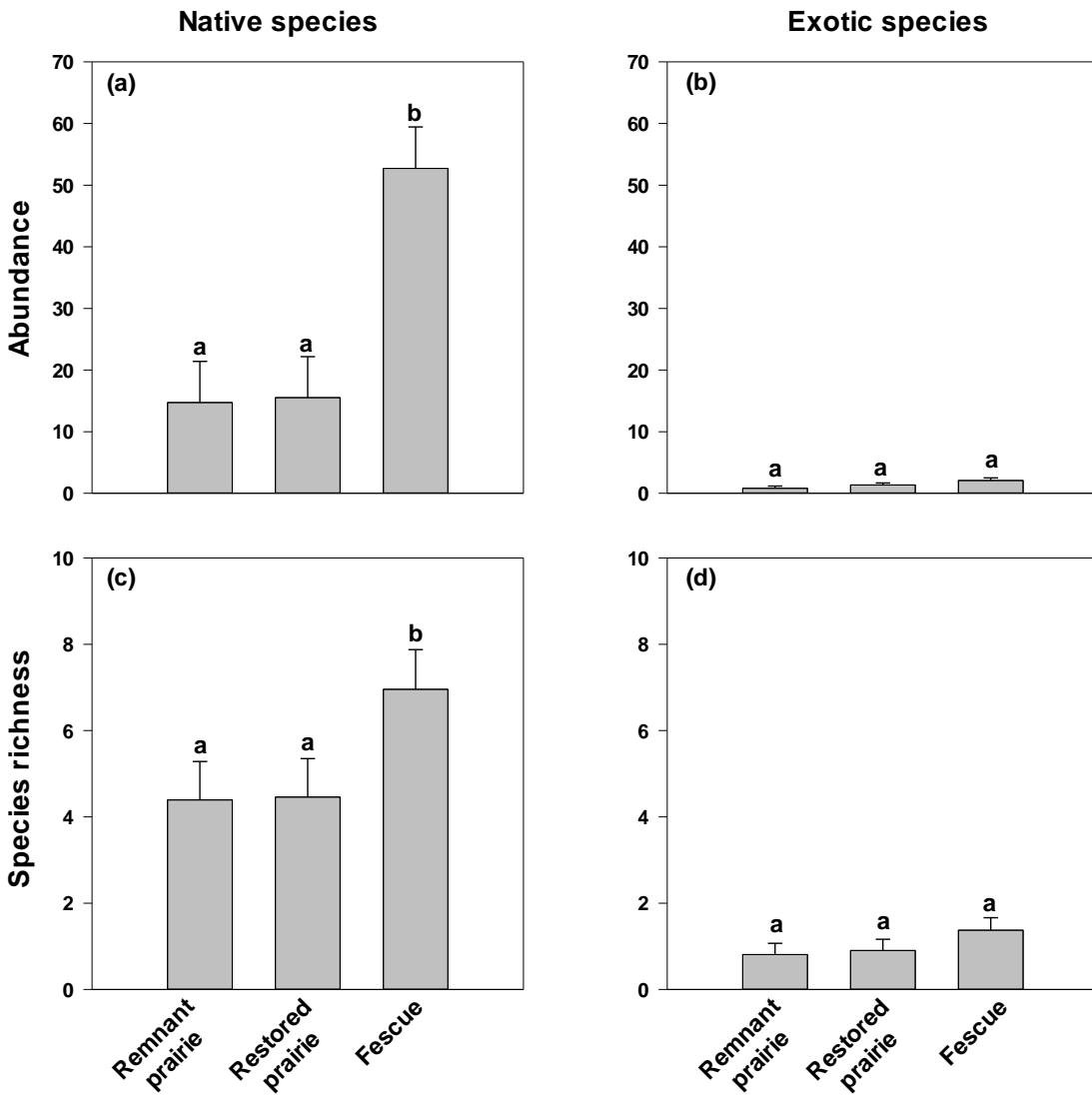


Figure 11: The cumulative seasonal abundance and species richness of native (a, c) and exotic (b, d) lady beetle species within remnant tallgrass prairies, restored tallgrass prairies, and tall fescue forage crops averaged across two years (2010 and 2011). LSmeans \pm 1 SEM are shown. LSmeans with different letters are significantly different ($p < 0.05$).

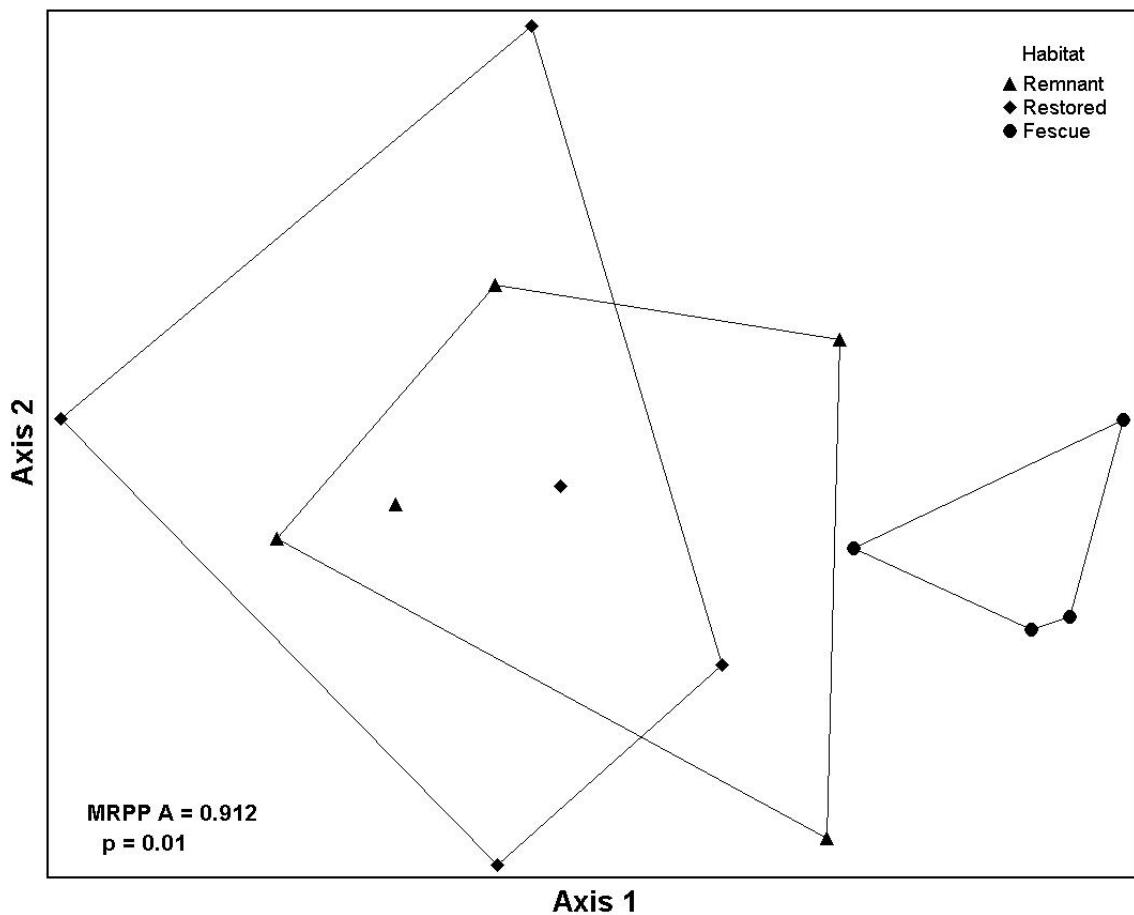


Figure 12: Two-dimensional representation of the species composition of lady beetle communities found in 5 restored tallgrass prairie habitats (\blacklozenge), 5 remnant tallgrass prairie habitats (\blacktriangle), and 4 tall fescue forage crops (\bullet) based on nonmetric multidimensional scaling (NMDS, stress value = 12.47).

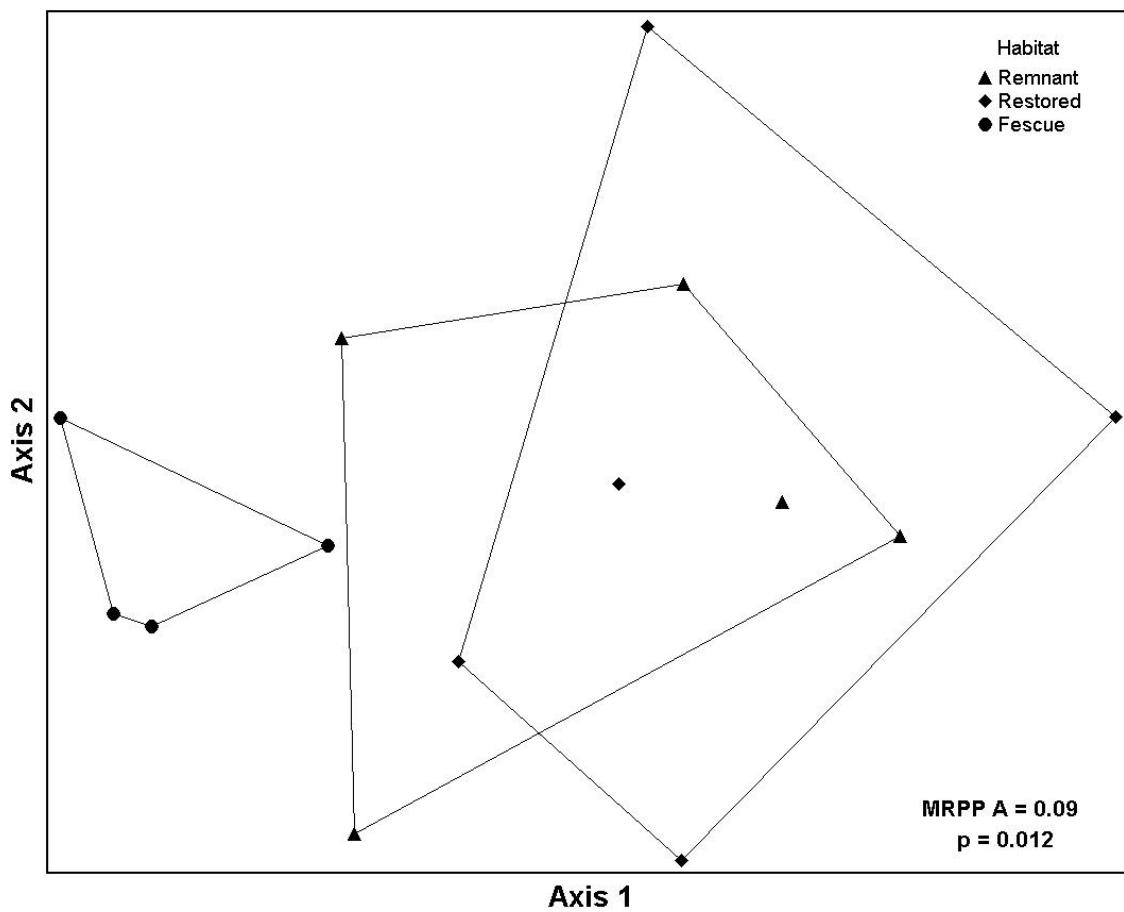


Figure 13: Comparison of coccinellid communities by habitat types in the absences of the abundant native beetle, *Scymnus rubricaudus* Casey. Data are for five restored tallgrass prairie habitats (♦), five remnant tallgrass prairie habitats (▲), and four tall fescue forage crops (●) based on nonmetric multidimensional scaling (NMDS, stress value = 12.47).

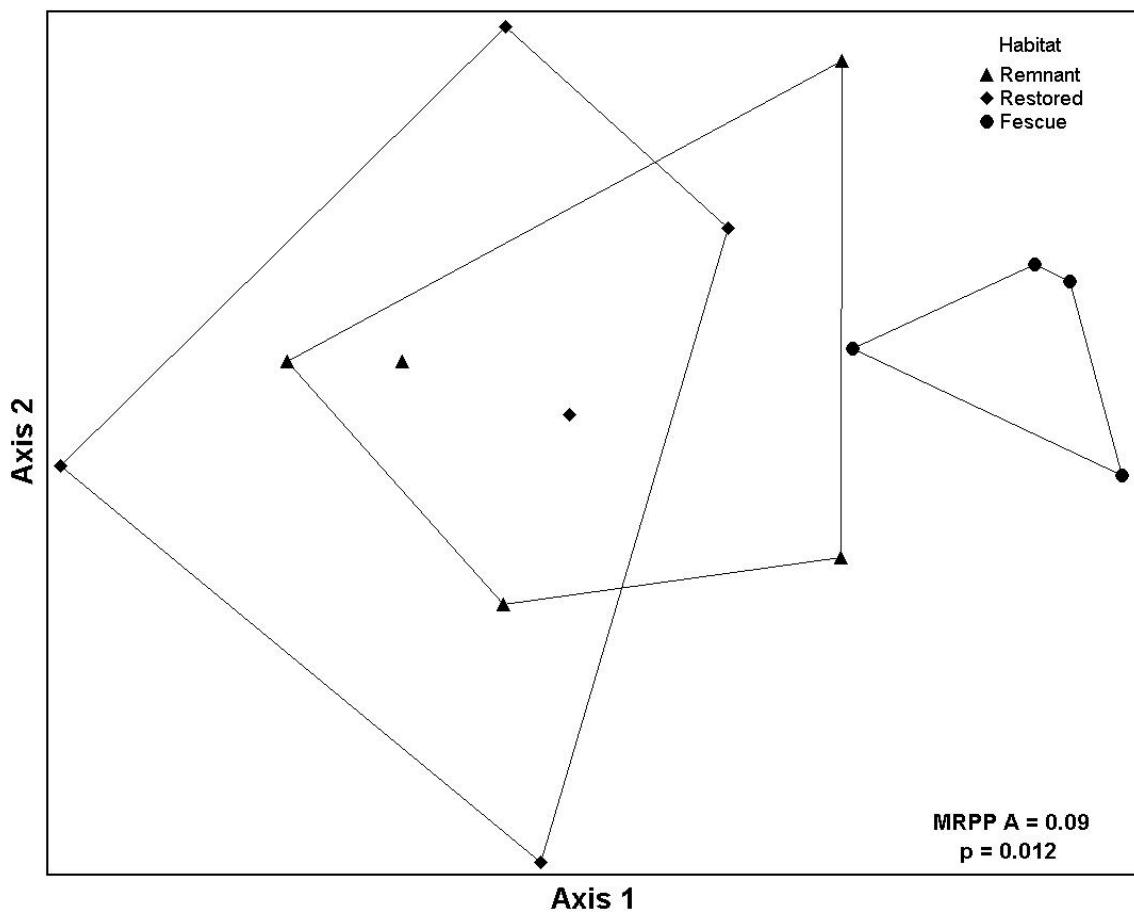


Figure 14: Comparison of coccinellid communities by habitat types in the absence of *Scymnus ardelio* Horn and *Scymnus postpictus* Casey, the two species represented by singletons in the dataset. Data are for five restored tallgrass prairie habitats (\blacklozenge), five remnant tallgrass prairie habitats (\blacktriangle), and four tall fescue forage crops (\bullet) based on nonmetric multidimensional scaling (NMDS, stress = 12.53).

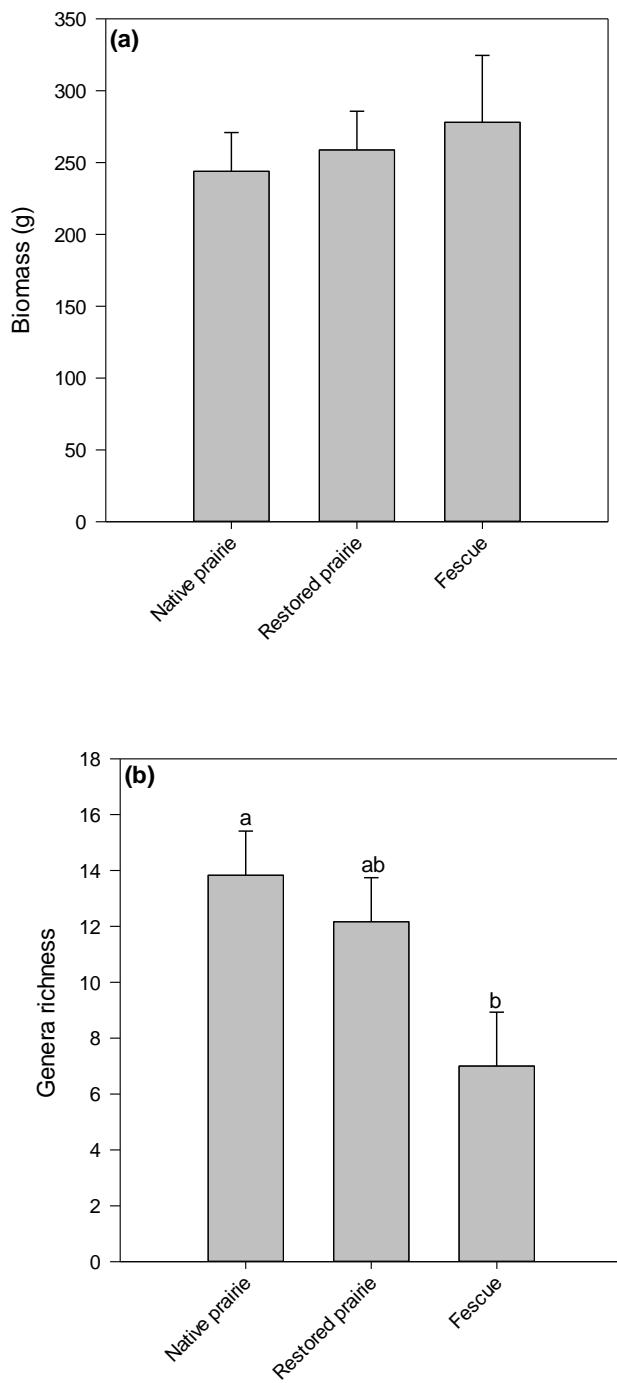


Figure 15: Total biomass (a) and generic richness (b) across habitats. Total biomass, comprised of forbs, grasses and thatch, was similar across habitats ($F_{2,11} = 0.22, p = 0.81$). Native tallgrass prairie habitats contained the greatest number of genera, agricultural tall fescue habitats the least, and restored prairie habitats were intermediate ($F_{2,13} = 3.9, p = 0.047$).

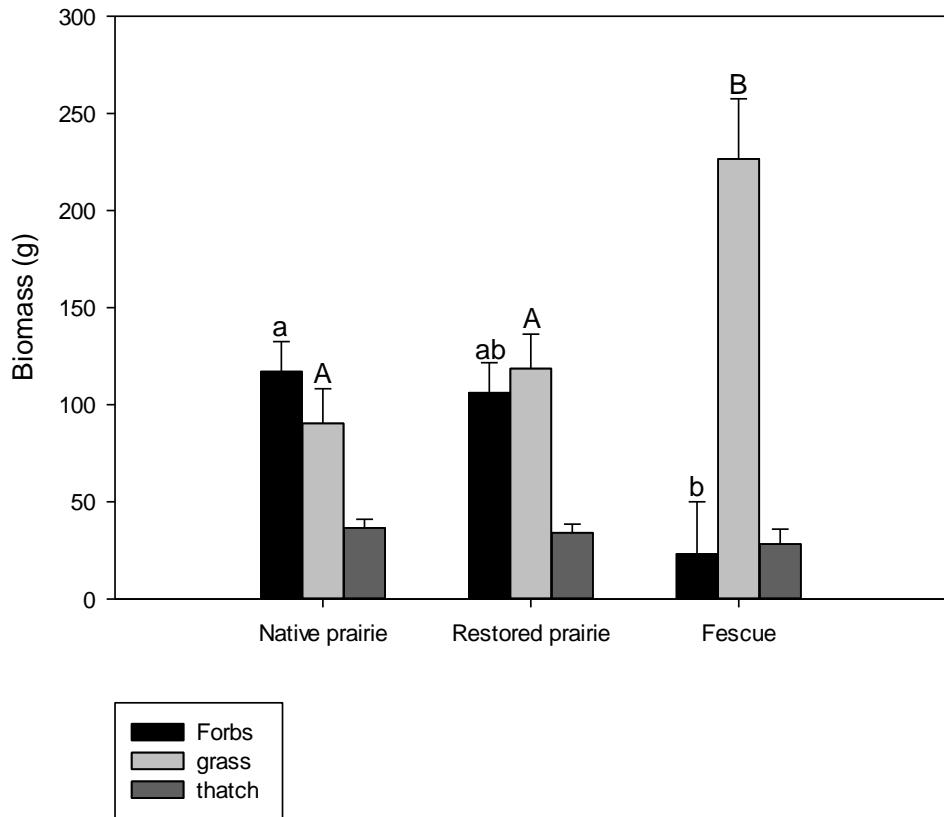


Figure 16: Biomass of forbs, grasses, and thatch across habitat types. Biomass of both forbs and grasses varied by habitat ($F_{2,11} = 4.78, p = 0.032$ and $F_{2,11} = 7.32, p = 0.0095$, respectively). Means with different letters are significantly different at the $p < 0.05$ level. Lower case letters were used to compare forb generic richness while upper case letters were used for grass generic richness.

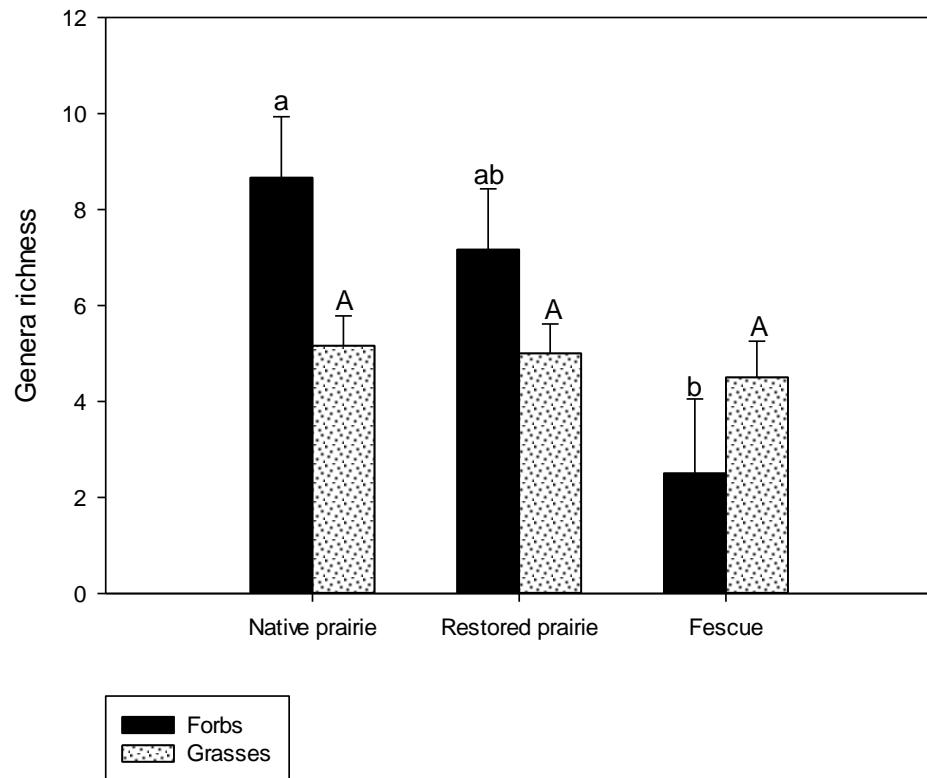


Figure 17: Generic richness of forbs and grasses across habitat types. Native prairie had a higher forb richness than agricultural tall fescue ($F_{2,13} = 4.92, p = 0.026$), with restored prairie being intermediate. All habitats had a similar richness of grasses ($F_{2,13} = 0.24, p = 0.79$). Means with different letters are significantly different at the $p < 0.05$ level. Lower case letters were used to compare forb generic richness while upper case letters were used for grass generic richness.

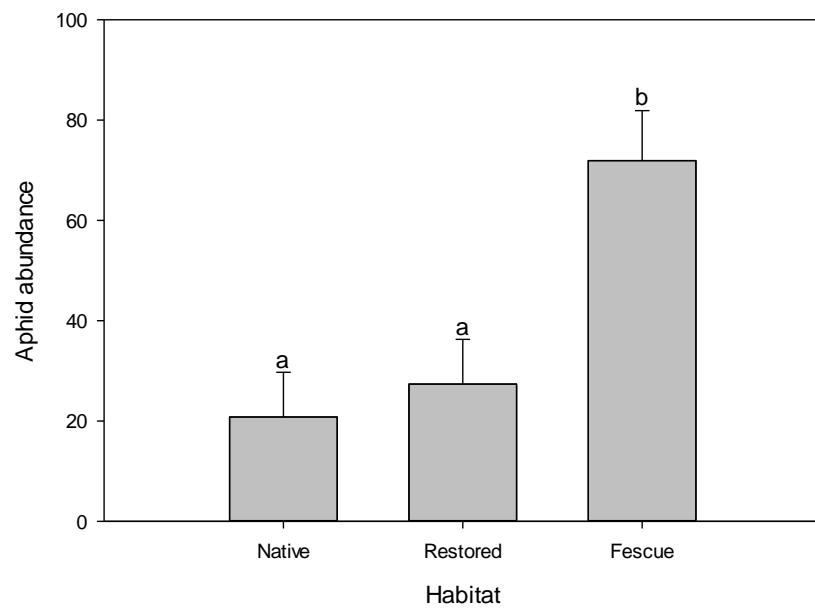


Figure 18: Seasonal aphid abundance. Seasonal aphid abundance varied by habitat, with fescue having the highest number of total aphids ($F_{2,11} = 8.47, p = 0.0059$). Means with different letters are significantly different at the $p < 0.05$ level.

Chapter 4

The influence of aphid abundance on coccinellid (Coleoptera: Coccinellidae) egg predation and adult community composition in natural and agricultural tallgrass habitats in Missouri

Introduction

Over the past few decades, lady beetle (Coleoptera: Coccinellidae) communities in North America have experienced a noticeable decrease in the abundance and species richness of native beetles (Harmon et al., 2007). *Adalia bipunctata* (L.), *Brachiacantha ursina* (F.), *Coccinella novemnotata* Herbst, *Cyclonedda munda* (Say), and *Hippodamia convergens* Guérin-Méneville are all native species considered to be in decline (Wheeler & Hoebeke, 1995; Elliott et al., 1996; Colunga-Garcia & Gage, 1998; Michaud, 2002; Alyokhin & Sewell, 2004; Losey et al., 2007; Gardiner et al., 2009c; Gardiner et al., 2011). This decline in native species is generally considered as displacement occurring in response to the introduction and establishment of exotic coccinellids, including *Coccinella septempunctata* L. and *Harmonia axyridis* Pallas (Gordon, 1985; Obrycki & Kring, 1998b; Alyokhin & Sewell, 2004; Snyder et al., 2004; Evans et al., 2011b); however, it is unclear whether this relationship is causal or correlative (Elliott et al., 1996; Evans, 2004; Harmon et al., 2007; Gardiner et al., 2011).

Resource competition, whereby species compete for a common resource such as food or overwintering habitat, is one potential mechanism explaining the displacement of native coccinellid species by exotics in the field. Many species of coccinellids share common food resources, in particular aphids. If exotic species are more voracious consumers of aphids than native species (Bahlia, 2014), native species could be

competitively excluded from a habitat when resources are scarce (Sloggett & Majerus, 2000; Evans, 2004), but coexistence may occur in areas of high aphid abundance (Evans, 1991; Ives et al., 1993; Obrycki et al., 1998a). As a result, both native and exotic coccinellids may aggregate in areas of high aphid density (Schellhorn & Andow, 2005b; Evans & Toler, 2007). A lack of this resource should then have the opposite effect, with coccinellids leaving areas of lower resources in search of prey. A long-term study by Evans (2004), found that decreases in the abundance of native lady beetles in Utah alfalfa fields were related to increased abundances of the exotic species *C. septempunctata*, which reduced the amount of aphids locally available to other predators.

Aphid abundance could also mediate the displacement of native species by exotic species by influencing the occurrence of predation on the eggs, larvae and pupae of native beetles (Obrycki et al., 1998a; Schellhorn & Andow, 1999b). As a common food resource for many coccinellids, the presence of aphids could decrease rates of predation on immature coccinellids, in particular predation upon eggs (Cottrell, 2005). In North America, the predation of eggs of native species occurs more frequently than eggs of exotic species (*C. septempunctata* and *H. axyridis*) (Prasad & Snyder, 2004; Cottrell, 2005; Kajita et al., 2010; Smith & Gardiner, 2013b; Smith & Gardiner, 2013a). Predation also occurs between larvae of predacious coccinellids with exotic species often being the predator (Obrycki & Kring, 1998a; Phoofolo & Obrycki, 1998; Snyder et al., 2004). However, it is important to note that predation of eggs and larvae is not limited to consumption by other coccinellids. Several aphidophagous arthropods such as the green lacewing (*Chrysoperla carnea* Stephens), harvestmen (Opilionidae), and the soft-winged flower beetle [*Collops nigriceps* (Say)], are also documented predators of the eggs of the

native lady beetle *Coleomegilla maculata* DeGeer (Phoofolo & Obrycki, 1998; Gardiner et al., 2011; Smith & Gardiner, 2013a).

In a previous study, it was determined that native coccinellids are abundant in both agricultural tallgrass habitats [tall fescue, *Lolium arundinaceum* (Schreb.) Darbysh] and natural tallgrass prairie habitats that are native to regions in Missouri (Diepenbrock & Finke, 2013), with exotic species present at much lower numbers. A surprising finding of this study was that the introduced agricultural grass habitats harbored both a greater abundance and diversity of native species than the natural tallgrass prairie habitats. These fescue habitats also had a greater abundance of aphids (see Fig 18, Chapter 3), which are a common food source for many species of lady beetles and may act as an attractant to these species, reducing competition as well as predation on immature stages. Therefore, I hypothesized that a high abundance of aphids would attract a greater diversity and abundance of native coccinellid species as well as reduce egg predation as compared with areas in which aphids are absent.

The goal of this study was to determine if the availability of aphids as prey items influences the lady beetles community associated with tallgrass habitats. To do this, I decreased aphid abundances in plots through aphicide application and left other plots at ambient levels of aphid abundance. I then monitored the coccinellid community present within each plot as well as predation upon sentinel coccinellid egg masses in relation to aphid abundance.

Materials and Methods

Field plot experimental design

Ten 5m x 5m plots, with a 3m buffer between plots, were established. Plots were randomly assigned as “reduced” or “ambient” aphid density treatments, with five replicates per treatment. Reduced aphid density treatments were treated with the systemic aphicide Fulfill® 50WG (Syngenta), applied at the recommended rate of 193 g/ha along with the adjuvant Induce® (Syngenta, 2.5 µl/10 gal water) via a backpack spray tank at the beginning of each five week study period. Ambient aphid density treatments were sprayed with water only. The reduced aphid density plots were visually monitored throughout the study for the presence of aphids, and aphids were manually exterminated when found. This entire field plot design was replicated across three independent sites, one located in a tall fescue field, one in a restored tallgrass prairie and one in a remnant tallgrass prairie, for a total of 30 plots. All sites are located in Callaway County, Missouri (N 38° 48' 39.7254", W 91° 55' 47.8302").

Coccinellid sampling

Coccinellid adults were collected weekly for five weeks during the 2012 and 2013 growing seasons using 18 x 23 cm unbaited yellow sticky cards (Stephens & Losey, 2004)(Pherocon AM, Great Lakes IPM, Vestaburg, MI, USA). Five cards were collected per plot per week and used as sub-samples. Within each plot, four 1.8 m metal fence posts were installed approximately 1 m diagonally inward from the corners of plots (Fig. 19B) with cards attached at the average height of the surrounding vegetation (Diepenbrock & Finke, 2013). One card was affixed to a post in the center of each plot just above ground level (Fig. 19A). All cards were left in the field for one week, then

collected and stored in a -20° C freezer until processing. Coccinellids were later removed from the cards in the laboratory, cleaned using HistoClear II (National Diagnostics, Atlanta, GA, USA), and identified to the lowest taxonomic unit possible, most to species (Gordon, 1976, 1985).

The main and interactive effects of treatment (ambient or reduced aphids) and year (2012 or 2013) on the cumulative seasonal abundance and species richness of native and exotic lady beetles were tested in separate mixed-model repeated measures analyses of variance with site included as the random block in the model (PROC MIXED, SAS v. 9.3). For each analysis, a variety of covariance structures were examined using the Bayesian Information Criterion and a compound symmetry covariance structure was determined as the best-fit model in every case. Comparisons of treatment means were done using *t*-tests with a Bonferroni correction of α values (PROC MIXED, SAS v. 9.3).

The potential influence of aphid manipulation on the native coccinellid community composition was visualized using nonmetric multidimensional scaling (NMDS) (PC-ORD, Mjm Software Design, Gleneden Beach, Oregon) (Clarke, 1993; McCune & Grace, 2002). Using the cumulative generic abundance sampled across sampling periods, NMDS was used to create a dissimilarity matrix using the Sørensen distance coefficient (Kruskal, 1964a, b; Mather, 1976). Stress levels were obtained by fitting the dissimilarities to distances that were calculated using randomly assigned starting conditions, and a two-dimensional solution was chosen as the best representation of the dissimilarities between treatments. Differences in coccinellid community composition between treatments were tested statistically using multi-response permutation procedures (MRPP) based on the rank-transformed Sørensen distance matrix

(PC-ORD, MjM Software Design, Gleneden Beach, Oregon) (Mielke, 1984; Mielke & Berry, 2001). The test statistic A and its resulting p -value from the MRPP analysis tested the null hypothesis of no difference in average within-group ranked distances.

A posteriori examinations of community differences by treatment across the three habitats were conducted using NMDS and MRPP to explain the within-treatment differences discovered in our initial NMDS analysis.

Egg predation

Coccinellid egg masses were placed into the field as sentinel prey to determine if predation is influenced by the presence of aphids and if the eggs of native lady beetle species are consumed more frequently than those of exotic species. Eggs of the species *H. convergens*, a common native species in mid-Missouri noted to be declining elsewhere in North America (Harmon et al., 2007), and *C. septempunctata*, an abundant exotic species, were collected from laboratory colonies and frozen to prevent development (Gardiner et al., 2011). Because egg masses vary greatly in number of eggs, I standardized the egg mass size at ten eggs per egg mass. To prepare the egg masses, ten individual eggs were fixed to a piece of 4 cm x 9 cm cardstock using a water soluble glue (Elmer's products, Columbus, OH) (Fig. 20A) (Gardiner et al., 2011; Werling et al., 2011), referred to hereafter as ‘egg cards.’ Four egg cards were then attached to a 25 cm x 30 cm white corrugated plastic board using binder clips and elevated approximately 25 cm off of the ground on wire H-Bracket sign holders (Fig. 20C; Hillman Group, Cincinnati, OH). The four cards consisted of one ‘open’ and one ‘caged’ egg card for each species (Fig. 2B, C). Cages were created using either the lid or the bottom of a 100 mm x 15 mm petri dish with a 4 cm diameter hole removed and covered with fine mesh

(Fig. 20B, Werling, pers. Comm.). Cages prevented predators from direct access to eggs, which provided a reference for the number of eggs that fall off for reasons other than consumption. Two plastic boards with four egg cards per board were placed in each plot and used as sub-samples. The experiment was completed once in 2012 and once again in 2013.

Data were analyzed to look for effects of aphid removal on the proportion of eggs consumed. To do this, I used a mixed-model repeated measure analysis of variance with habitat as a random factor (PROC MIXED; SAS v. 9.3, SAS Inc., Cary, NC, USA). Because the data were not normally distributed, the proportion of eggs consumed per replicate was arcsine square root transformed prior to analysis. For each analysis, a variety of covariance structures were examined using the Bayesian Information Criterion and a compound symmetry covariance structure was determined as the best-fit model in every case. *A priori* comparisons of treatment means were done using *t*-tests with a Bonferroni correction of α values (PROC MIXED, SAS v. 9.3).

Laboratory testing for non-target effects of aphicide application

I examined the potential for non-target effects of the aphicide on the adults of five species of beetles and the eggs and larvae of two species. Adult and larval beetles can come into contact with the aphicide in one of two manners: (1) direct contact or (2) consumption of treated aphids. Effects of direct contact were tested by placing one adult beetle or one beetle larva, fed to satiation, into a 100 mm x 15 mm petri dish with a 7 cm filter paper disc on the base. Either 1 ml of water or 1 ml of Fulfill® mixed to standard specifications was applied directly to the beetle and filter paper and the time to death (days) was recorded, with filter papers moistened as needed to ensure that beetles

remained hydrated. Ten replications of each treatment were performed for the adults of five species (*C. maculata*, *C. munda*, *C. septempunctata*, *H. axyridis* and *H. convergens*), and the larvae of two species (*C. septempunctata* and *H. convergens*). To determine if the aphicide reduced adult and larval longevity, I looked at the effect of treatment (aphicide or water) on the time to death for each species individually and grouped by native or exotic status using the ANOVA from generalized linear models (PROC GLM; SAS v. 9.3, SAS Inc., Cary, NC, USA).

The effect of feeding on treated aphids was examined by allowing adults of the native species *H. convergens* and the exotic species *C. septempunctata* to feed to satiation on bird cherry-oat aphids (*Rhopalosiphum padi* L.) feeding on soft red winter wheat (*Triticum aestivum* L.) that had previously been treated with either water or Fulfill[®] sprayed directly on the aphids. Upon satiation, individual beetles were transferred to 100 mm x 15 mm petri dishes with a 7 cm filter paper disc, and time to death (days) was recorded with the filter paper moistened as needed. To determine if the ingestion of treated aphids reduced beetle longevity, I examined the effect of treatment on time to death using the ANOVA from generalized linear models for each species separately (PROC GLM; SAS v. 9.3, SAS Inc., Cary, NC, USA).

Additionally, I tested for direct effects of the aphicide on the eggs of laboratory-reared *H. convergens* and *C. septempunctata*. Individual eggs were collected from rearing cages and then placed on a 7 cm filter paper disc inside a 100mm X 15mm petri dish. Treatments consisted of either 1 ml of Fulfill[®] or 1 ml of tap water. Twenty individual eggs of each species were exposed to each treatment and then checked twice daily to document hatching. Data were scored categorically as hatched or not hatched

and analyzed using a chi-square test to determine if eggs treated with the aphicide hatched at a similar frequency to those treated with water alone.

Results

Comparison of lady beetle community abundance and diversity

Aphid treatment did not impact the abundance or diversity of lady beetles, however there was a difference in the abundance of beetles by year. In 2012, there was a greater overall abundance of beetles captured than in the following year (Fig. 21A; $F_{1,69} = 39.69, p < 0.0001$), however, there was no impact on total species richness across years (Fig. 21B; $F_{1,69} = 0.18, p = 0.6746$). The greater abundance was driven entirely by native species, with native lady beetles more than twice as abundant in 2012 than in 2013 (Fig. 22A; $F_{1,69} = 42.87, p < 0.0001$). There was no difference in the species richness of either native or exotic coccinellid species across years (Fig. 22B; $F_{1,69} = 0.01, p = 0.9344$ and Fig 23B; $F_{1,69} = 0.01, p = 0.9344$ respectively). The abundance of exotic species was similarly low for both years (Fig. 23A; $F_{1,69} = 0.04, p = 0.852$); these species contributed minimally to the total lady beetle abundance and diversity for each year.

Comparison of lady beetle community generic composition

Coccinellid community composition did not vary by aphid treatment, as indicated by the almost complete overlap of communities by treatment (Fig. 24 A = -0.011, $p = 0.737$, stress = 12.352). This community comparison is based on genus presence and abundance. Identity of genera within each treatment was very similar, likely drawing from the regional pool of species.

An *a posteriori* analysis comparing the response of communities to treatments across sites showed that the coccinellid communities in the aphid reduction and ambient treatments grouped together by site, which suggests that these communities may be influenced more by the site than smaller-scale plots (Fig. 25; $A = 0.362$ and $p < 0.0001$ stress = 12.35178).

Egg predation

There was a significant interaction between year and aphid treatment on the consumption of eggs of the native species (Fig. 26A; $F_{1,54} = 11.41$, $p = 0.0014$). *Hippodamia convergens* suffered the greatest amount of predation in 2012 in plots with ambient aphid loads. There was no difference in the proportion of eggs of the exotic beetle consumed (Fig. 26B; $F_{1,54} = 0.91$, $p = 0.3437$).

Across all plots, there was a greater proportion of eggs of the native species, *H. convergens*, consumed compared to the proportion of eggs of the exotic species, *C. septempunctata*, consumed (Fig. 27; $F_{1,114} = 8.07$, $p = 0.0053$). Because predators had access to eggs of both species in close proximity, this suggests that generalist predators have a preference for eggs of the native species.

Laboratory testing for non-target effects of aphicide application

Direct exposure through surface contact with Fulfill® aphicide does not impact the longevity of any of the species tested in this study. Adults of the native lady beetles species, *C. maculata*, *C. munda*, and *H. convergens*, and the exotic species, *C. septempunctata* and *H. axyridis*, persisted for similar durations of time after exposure to either the aphicide or water (Fig. 28A; $F_{1,4} = 0.02$, $p = 0.893$). Similarly, the larvae of both the native beetle, *H. convergens* and the exotic beetle, *C. septempunctata*, survived

for a similar amount of time after exposure to either the aphicide or water (Fig. 28B; $F_{1,1} = 0.52$, $p = 0.601$). Exposure to the aphicide also did not impact the proportion of eggs that hatch as compared to water alone for either *H. convergens* or *C. septempunctata* (Table 4).

Longevity of the exotic beetle, *C. septempunctata*, was reduced after consumption of aphicide-treated aphids (Fig. 29A; $F_{1,18} = 5.21$, $p = 0.035$), while longevity of the native beetle, *H. convergens*, was not significantly impacted (Fig. 29B; $F_{1,17} = 3.07$, $p = 0.098$). These data suggest that there may be a species-specific response to the application of this chemical, however this may also be an artifact of the difference in voracity of these two species, with *C. septempunctata* known to be a more voracious predator than *H. convergens* (Bahlia, 2014), therefore likely consuming a greater quantity of treated aphids, which may in turn reduce the longevity of this species.

Discussion

In the current study, I found no effect of aphid treatment (reduced or ambient aphid load) on either the predation of sentinel egg masses or on overall native lady beetle communities. The findings of this study were contrary to my expectations, with aphid abundance perhaps not as strong a driver of native coccinellid community composition as previous data had suggested.

Surprisingly, there was no overall effect of aphid abundance on the proportion of coccinellid eggs consumed. Previous studies have shown that predation of coccinellid eggs occurs at a lower frequency in the presence of an alternate food resource such as aphids (Agarwala & Dixon, 1992; Cottrell, 2005). However, those studies were not

performed within field settings, which allow for a wide variety of generalist predators to encounter and potentially consume the eggs. While there was no overall effect of the treatment, there was a significantly greater proportion of *H. convergens* eggs consumed in the ambient aphid treatment in 2012 than in the aphid reduction treatment. In 2012, there was a greater mean ambient abundance of aphids as compared to 2013 (100.26 ± 7.638 and 83.7852 ± 7.8154 respectively; $F_{1,69} = 9.85$, $p = 0.0025$). It is possible that a variety of generalist predators were attracted to the greater abundance of aphids in 2012 while foraging in the grassland habitats. These generalist predators likely contributed to the consumption of the sentinel egg masses.

Predation on egg masses of the native coccinellid, *H. convergens*, occurred at a greater frequency than predation on the exotic species, *C. septempunctata*, regardless of aphid abundance. It is likely that the eggs of *C. septempunctata* possess greater chemical defenses than those of *H. convergens*. Chemical defenses associated with the eggs of the two common exotic species, *C. septempunctata* and *H. axyridis*, have been well-documented (Hemptinne et al., 2000; Agarwala & Yasuda, 2001; Kajita et al., 2010). Such defenses often serve as deterrents for potential egg predators, aiding in the survival of eggs of these species in field settings. To the best of my knowledge, only one study has looked at similar defenses in the native species used in our study. In this study, it was found that eggs of *H. convergens* had similar defensive chemicals to the two exotic species, although at much lower concentrations (Kajita et al., 2010). It is likely that if eggs of the native species are not as well defended as those of the exotic species, that they are a more suitable prey item for generalist predators than the chemically-defended eggs of the exotic.

There was also no difference in native coccinellid communities directly related to the aphid treatments (Fig. 24). However, when compared across years, there was an overall greater number of lady beetles collected in 2012 (100.26 ± 7.54) than in 2013 (83.79 ± 7.82) (Fig. 21). Similar to previous research conducted in tallgrass habitats (Diepenbrock & Finke, 2013), this greater overall abundance was driven by a higher abundance of native species and not exotic species (Fig. 22A, 23A). Interestingly, species richness did not vary by year (Fig. 21B, 22B, 23B).

It is important to acknowledge that the availability of aphid prey is only one of several potential factors influencing both the susceptibility of coccinellids to predation and coccinellid community composition. In a comparison of coccinellid community composition considering site as a predictor variable, it seems that the site has a stronger influence over community composition than aphid abundance (Fig. 25). Despite the commonly accepted idea that most lady beetle species are major predators of aphids, they also consume a wide variety of other food items including fungal spores, pollen, nectar, plant materials, fruits (Forbes, 1883; Triltsch, 1999; Lundgren et al., 2004; Lundgren, 2009), and other arthropods such as mites, mealybugs and larvae of other insects (Evans, 2009; Giorgi et al., 2009), which could vary by site.

This study highlights the importance of considering a wide spectrum of variables when attempting to study natural insect communities in a field setting. While most studies trying to understand the mechanisms underlying community structure occur within controlled mesocosms, it is important to put these factors into perspective and consider how they may interact at a larger field scale. For example, in the present study, year was more important than aphid manipulation with regard to both egg predation and

coccinellid community composition. Large scale factors which vary by year, such as weather, may be very important in determining fine scale interactions such as predation events that in turn can impact entire communities of organisms.

Table 4. Comparison of the proportion of coccinellid eggs hatching after exposure to aphicide (Fulfill®) versus the proportion of eggs hatching when exposed to water.

Species	Treatment		χ^2	d.f.	p
	Fulfill	Water			
<i>Coccinella septempunctata</i>	8	10	0.8	1	0.3711
<i>Hippodamia convergens</i>	13	14	0.238	1	0.6256

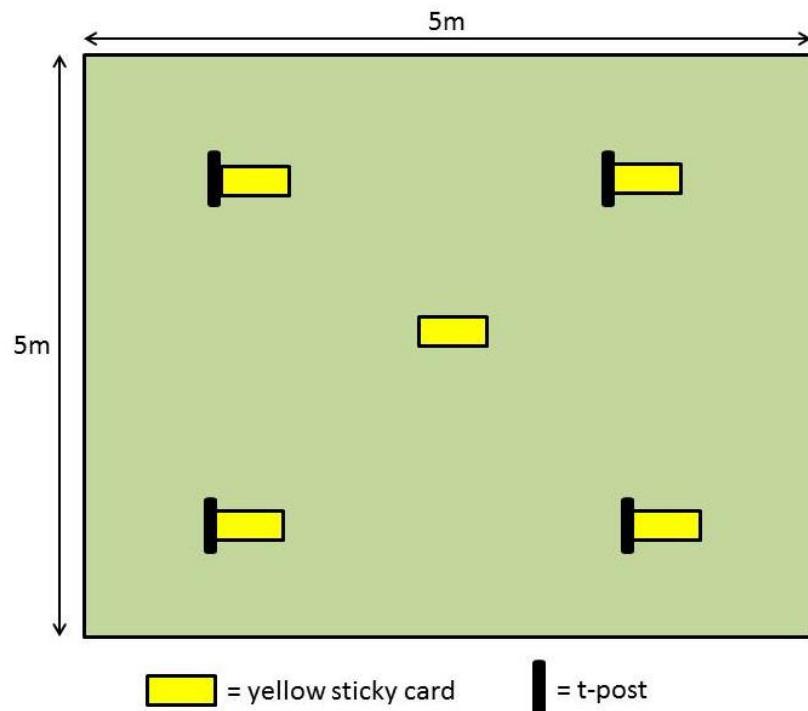


Figure 19: Experimental plot in Tucker Prairie (Callaway County, Missouri). (A) The nearest plot can be distinguished by the four 1.8 m metal posts with yellow sticky cards attached at the height of vegetation and one additional card in the center of the plot just above ground level. (B) Diagram of basic plot design. All plots were 5 x 5 m with four 1.8 m t-posts installed approximately 1 m diagonally inward from corners to which sticky cards were attached weekly at the height of the nearby vegetation. One additional card was attached to a small post in the center of each plot just above the ground

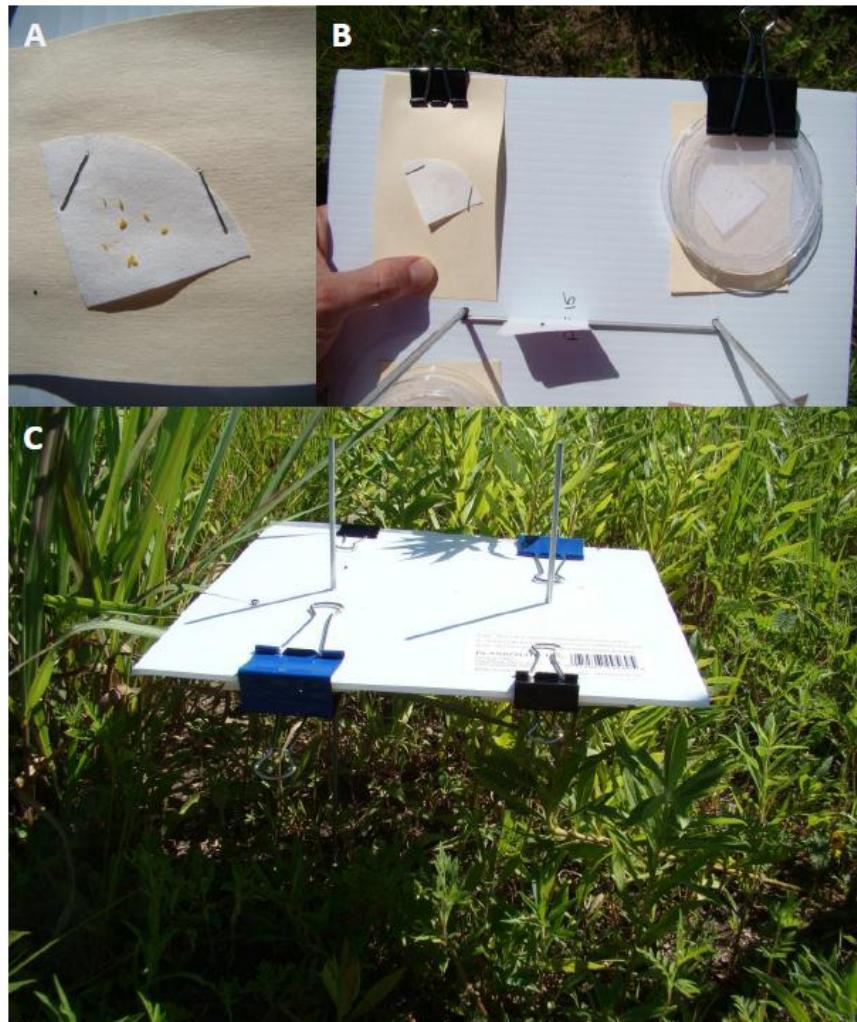


Figure 20: Egg cards for measuring predation of native and exotic coccinellid eggs. (A) Sentinel prey (eggs) before deployment in plots. (B) ‘Open’ and ‘cage’ treatments for measuring egg predation. On the left is an ‘open’ card, which is easily accessible to foraging predators, and on the right is a ‘caged’ card which is covered by a cage comprised of a petri dish with fine mesh covering a 4 cm diameter hole removed from the dish. (C) Full set up in the field comprised of two ‘open’ and two ‘caged’ egg masses from each species.

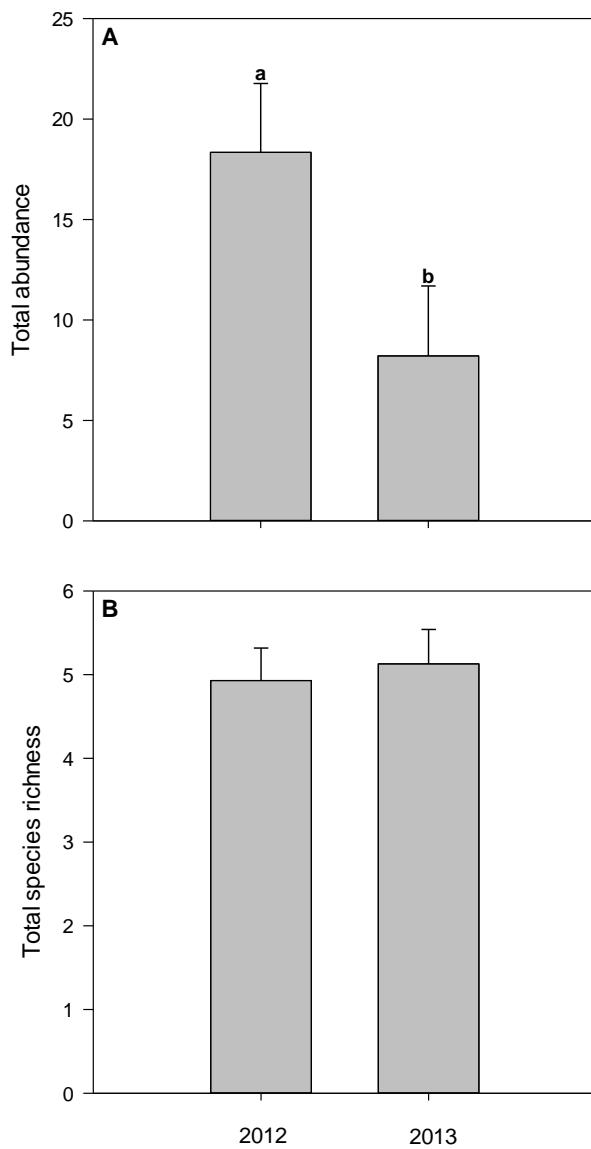


Figure 21: Comparison of overall lady beetle (A) abundance and (B) species richness by year of study. There was a greater abundance of total coccinellids recorded in 2012 than in 2013 ($F_{1,69} = 39.69, p < 0.0001$), with no difference in the total species richness in either year of our study ($F_{1,69} = 0.18, p = 0.6746$). Means with different letters are significantly different at the $p < 0.05$ level.

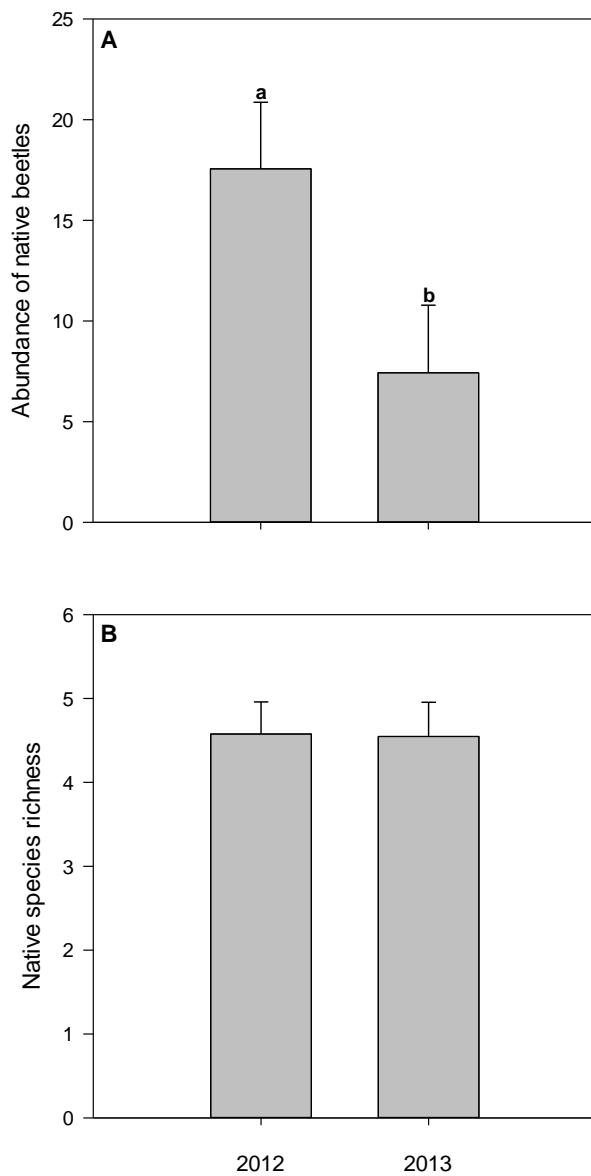


Figure 22: Comparison of native lady beetle (A) abundance and (B) species richness by year. There was a greater abundance of native beetles collected in 2012 than in 2013 ($F_{1,69} = 42.87, p < 0.0001$), however, there was no difference in the species richness for either year ($F_{1,69} = 0.01, p = 0.9344$). Means with different letters are significantly different at the $p < 0.05$ level.

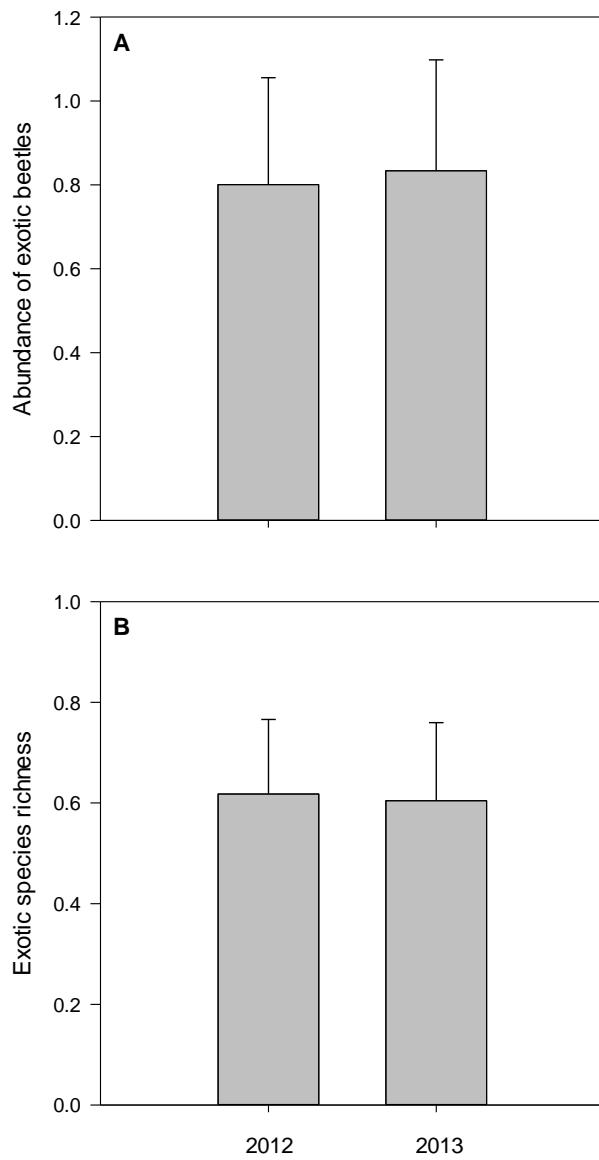


Figure 23: Comparison of exotic lady beetle (A) abundance and (B) species richness by year. There was no difference in either the abundance or species richness of exotic lady beetles collected by year of study ($F_{1,69} = 0.04, p = 0.852$; $F_{1,69} = 0.01, p = 0.9344$, respectively).

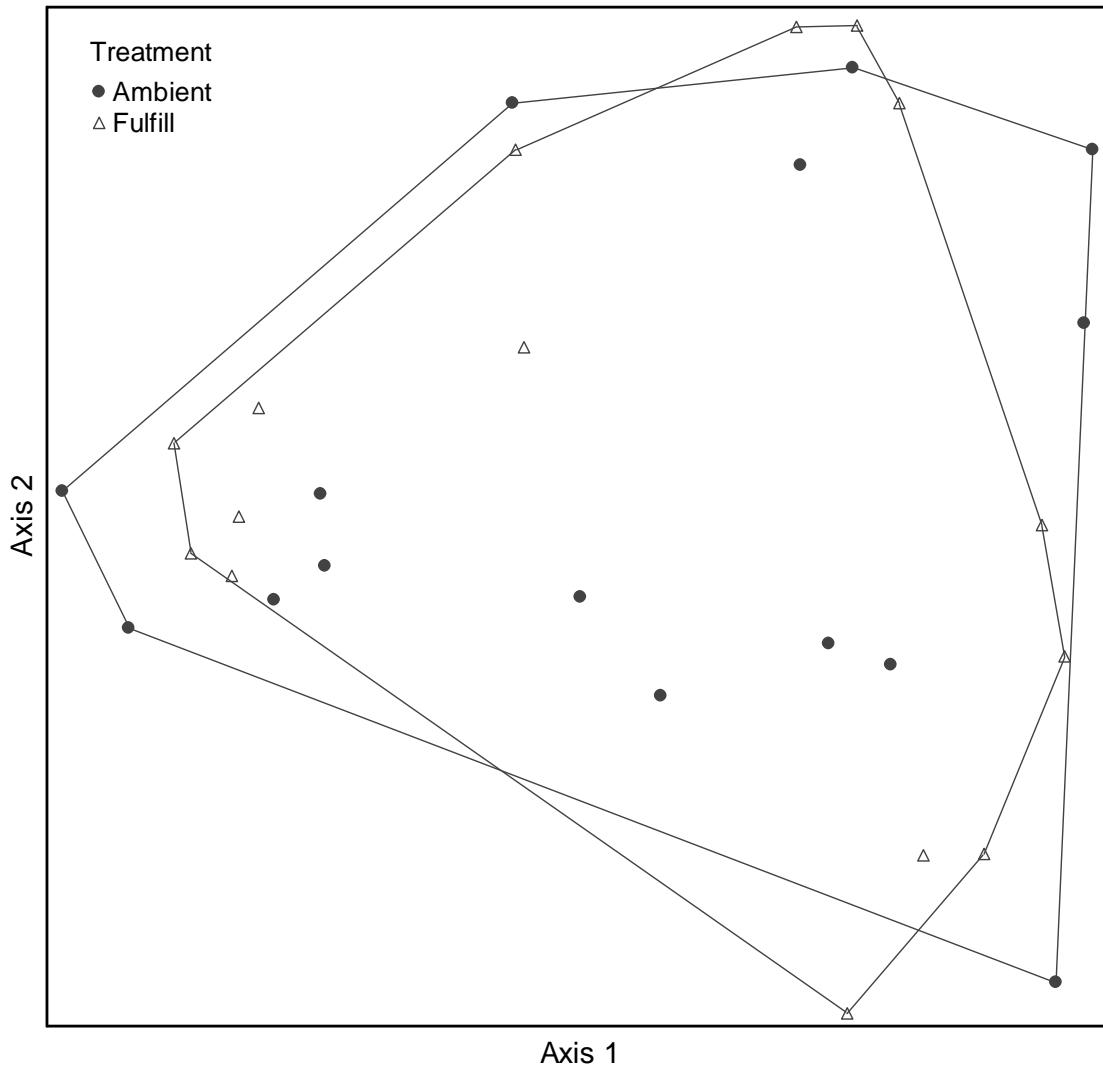


Figure 24: Comparison of native coccinellid community composition between reduced aphid (Fulfill) and ambient aphid abundance treatments. The native coccinellid community, as described by generic presence and abundance within plots, was not different between treatment groups ($A = -0.011$, $p = 0.737$, stress = 12.352).

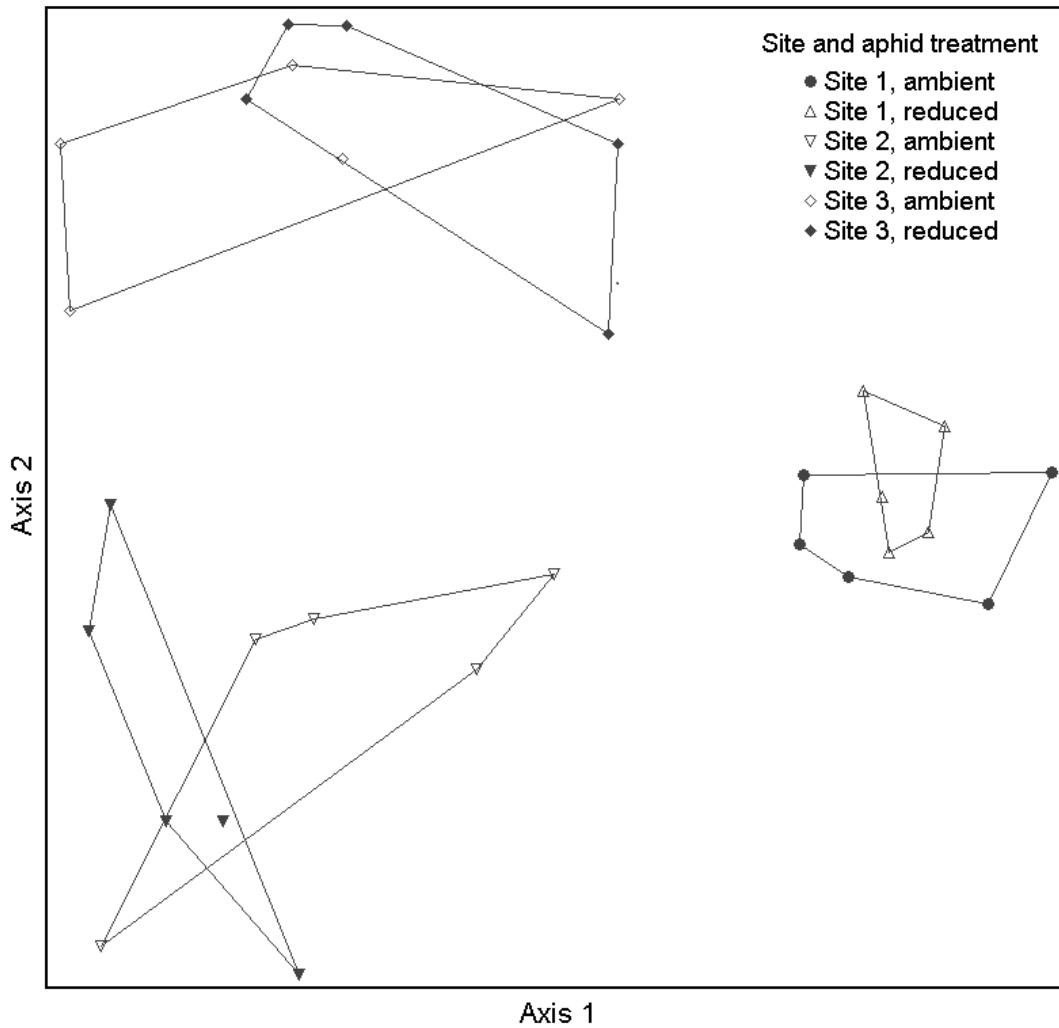


Figure 25: Comparison of native lady beetle communities between treatments across three sites in different habitat types: agricultural tall fescue, restored tallgrass prairie and remnant tallgrass prairie. An *a posteriori* analysis of the coccinellid community within each habitat suggests that site may have a stronger influence on the community composition than aphid abundance ($A = 0.362$, $p < 0.0001$, stress = 12.35178).

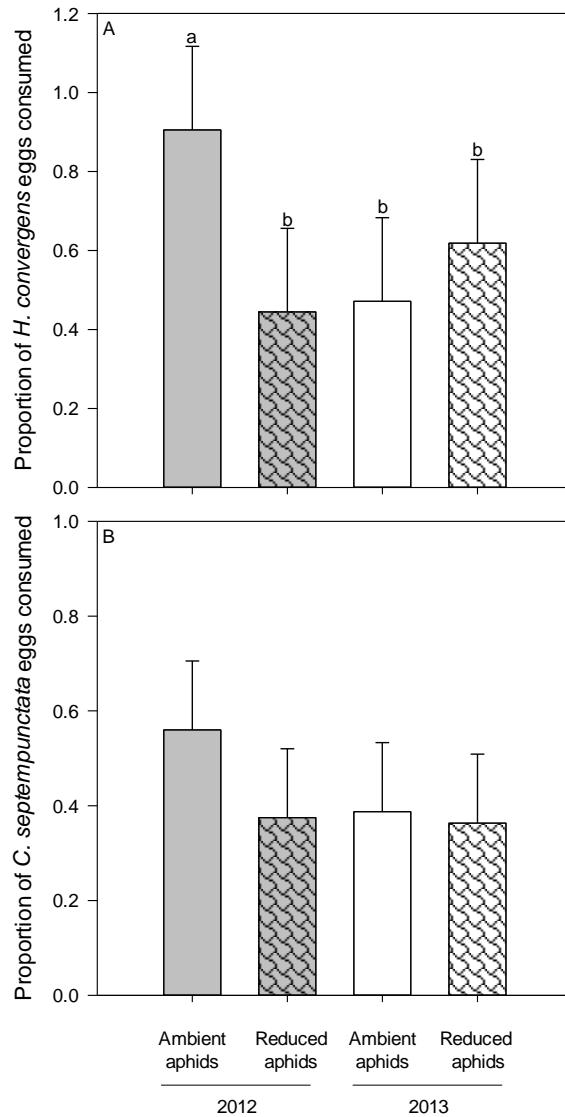


Figure 26: Proportion of coccinellid eggs consumed within reduced aphid and ambient aphid plots by year. (A) *H. convergens* eggs consumed within each treatment by year. A greater proportion of the eggs of the native coccinellid, *H. convergens*, were consumed within the ambient treatment plots during the growing season of 2012 than the reduced aphid treatment of that same year or in either treatment the following year ($F_{1,54} = 11.41$, $p = 0.0014$). (B) Proportion of *C. septempunctata* eggs consumed within each treatment by year. There was no difference in the proportion of eggs consumed of the exotic species with respect to treatments or years ($F_{1,54} = 0.91$, $p = 0.3437$). Means with different letters are significantly different at the $p < 0.05$ level, means with no associated letters are not significantly different from one another.

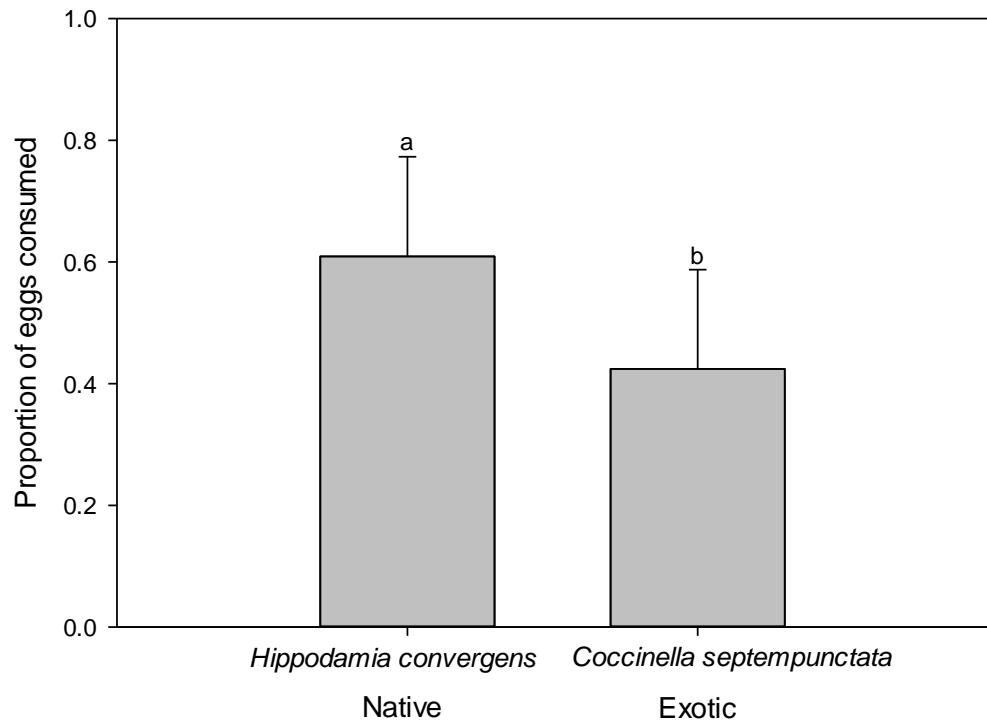


Figure 27: Comparison of the proportion of native and exotic eggs consumed across treatments and years. Overall, there was a greater proportion of eggs of the native lady beetle, *Hippodamia Convergens* Guérin-Méneville , consumed than eggs of the exotic species, *Coccinella septempunctata* L. ($F_{1,114} = 8.07$, $p = 0.0053$). Means with different letters are significantly different at the $p < 0.05$ level.

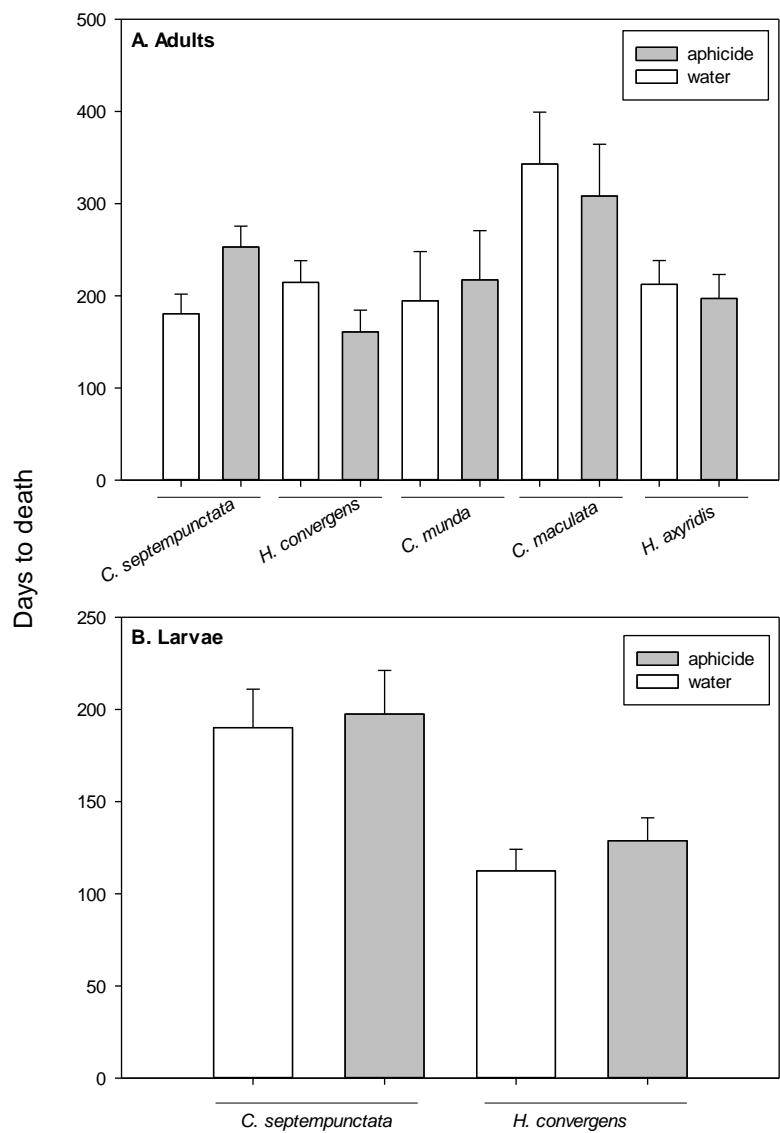


Figure 28: Effects of aphicide treatment on adult and larval mortality. There was no difference in time to death for (A) adults of the species *Coleomegilla maculata* DeGeer, *Cycloneda munda* Say, *Hippodamia convergens* Guérin-Méneville, *Coccinella septempunctata* L. and *Harmonia axyridis* Pallas when directly exposed to the aphicide as compared with water ($F_{1,4} = 0.02, p = 0.893$) or for (B) larvae of the species *H. convergens* or *C. septempunctata* ($F_{1,1} = 0.52, p = 0.601$).

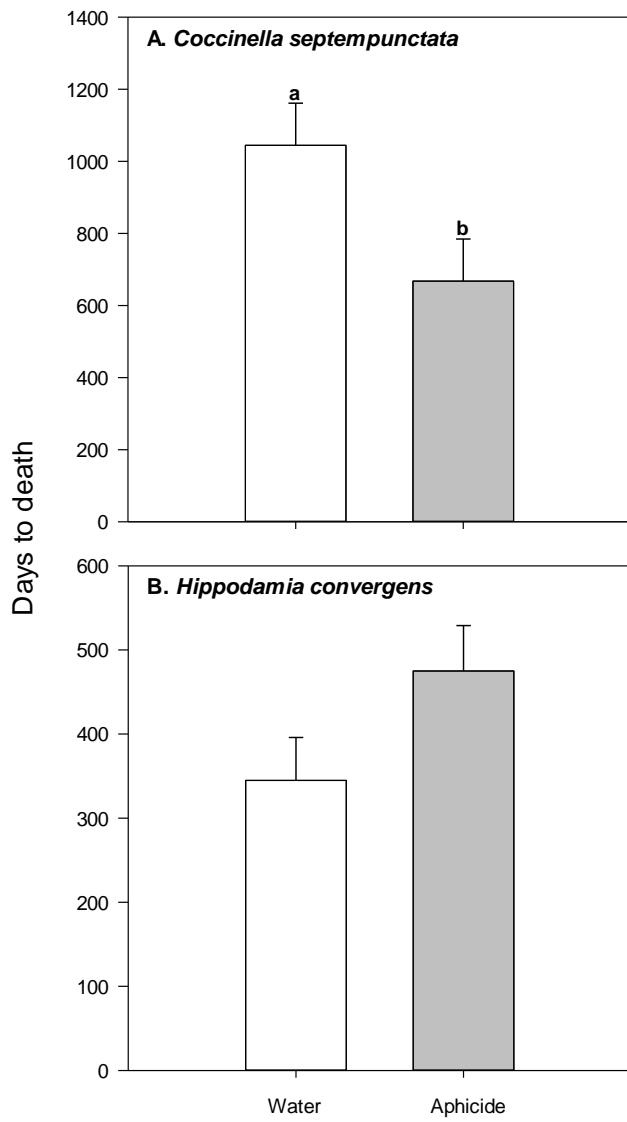


Figure 29: Effects of consuming aphicide-treated aphids on adult *Hippodamia convergens* Guérin-Méneville and *Coccinella septempunctata* L. *Coccinella septempunctata* experienced a reduction in longevity after consuming bird cherry-oat aphids that had recently been treated with an aphicide (Fig. 4A; $F_{1,18} = 5.21, p = 0.035$), while the longevity of the native species, *H. convergens*, was unaffected (Fig. 4B; $F_{1,17} = 3.07, p = 0.098$)

Chapter 5

Does the presence of the exotic coccinellid, *Coccinella septempunctata* L., influence the foraging behavior of native coccinellids species?

Introduction

Competition for shared resources can alter where and when organisms with similar resource requirements forage to meet their nutritional needs (Park, 1941; Schoener, 1974; Sutherland, 1983). This competition can determine if species are capable of coexisting within a given habitat, or if they will be competitively displaced through consumptive/exploitative competition (Armstrong & McGehee, 1980; Schoener, 1983). It has been shown that in the presence of a superior competitor, less competitive species may choose to forage in lower quality patches or on lower quality prey items to avoid competition. For example, Milinski (1982) found that three-spined stickleback fish (*Gasterosteus aculeatus* Linnaeus) that were competitively inferior opted to forage for a higher number of small prey (*Daphnia magna* Straus) when in the presence of superior competitors; however, in the absence of competition, all fish preferred to consume larger prey items (Milinski, 1982). In this study, the inferior competitor expended more energy feeding on a lower quality prey item in order to avoid direct competition with the competitively dominant fish.

In recent decades, noticeable declines in the abundance of several species of native lady beetles in North America have led to much concern regarding potential causes of their decline (Wheeler & Hoebeke, 1995; Elliott et al., 1996; Alyokhin & Sewell, 2004; Harmon et al., 2007; Losey et al., 2007). Concurrent with these declines, the

abundances of two exotic species, *Coccinella septempunctata* L. and *Harmonia axyridis* Pallas, have increased in areas where native coccinellids were previously common (Wheeler & Hoebeke, 1995; Elliott et al., 1996; Colunga-Garcia & Gage, 1998; Michaud, 2002; Alyokhin & Sewell, 2004; Harmon et al., 2007; Losey et al., 2007; Finlayson et al., 2008; Bahlia, 2014). It has been hypothesized that these introduced, exotic species have had negative impacts on native coccinellids through interspecific competition for food resources (Evans, 1991; Obrycki et al., 1998a; Evans, 2000; Michaud, 2002; Evans, 2004; Evans et al., 2011b).

Aphids are a common food resource shared by many species of predatory coccinellids. Their sugary excretion, referred to as honeydew, is an attractant for mobile predators including lady beetles (Evans & Richards, 1997). Induced outbreaks of aphids have been shown to attract both native and exotic coccinellids (Elliott et al., 1996; Evans, 2004). However, competition for this shared resource may result in displacement of native species through competitive interactions with exotic species (Alyokhin & Sewell, 2004; Evans, 2004; Snyder et al., 2004), since exotic species have been shown to be more voracious than native species in their consumption of aphid prey (Bahlia, 2014).

Several laboratory studies have explored larval competitive interactions between native and exotic coccinellid species. In one such study, larvae of the native lady beetle, *Coleomegilla maculata* (DeGeer), were paired with either conspecifics or with larvae of the exotic species, *C. septempunctata*. When provided a minimal diet of one mature apterous pea aphid (*Acyrthosiphon pison* Harris) per larva per day, interactions between conspecific pairings did not affect survival compared to unpaired individuals. However, when paired with the exotic competitor, *C. maculata* survival decreased significantly. In

these same pairings, *C. septempunctata* survival increased (Obrycki et al., 1998b). The increased survival of *C. septempunctata* in the presence of an interspecific competitor as compared to a conspecific larva or no pairing (maintained on one mature pea aphid per day) occurred because these larvae captured and consumed more prey than their native counterparts and were also observed to consume dead larvae of *C. maculata*.

Comparisons of larval interactions that were performed at higher aphid densities did not favor the survival of any one species, native or exotic, at the expense of another (Evans, 1991; Obrycki et al., 1998b), which suggests that prey abundance can mediate antagonistic interactions like competition.

Similar to other organisms that share a limiting resource, some coccinellid species appear to have developed mechanisms to persist in a habitat despite competition. One such mechanism is resource partitioning, whereby organisms that have similar food and/or habitat requirements use the resources in different ways. For example, species may forage in different habitats or at different times of the day, and by doing so, are capable of coexistence. Spatial partitioning has been shown between adults of the common native species, *C. maculata*, and adults of the common exotic species, *C. septempunctata* and *H. axyridis*. In a sweet corn habitat in New York, *C. maculata* and *H. axyridis* were observed to space themselves while foraging for aphids such that *C. maculata* occupied the lower two thirds of corn plants and *H. axyridis* could be found in the upper third, where aphids were most abundant (Musser et al., 2006). Similar partitioning was observed in a laboratory experiment using *C. maculata*, *C. septempunctata* and *H. axyridis* foraging for pea aphids on alfalfa plants. In this study, both the exotic beetle, *H. axyridis*, and the native beetle, *C. maculata*, were displaced to

the plant margins by the dominant foraging behavior of *C. septempunctata* (Cardinale et al., 2006).

The goal of this study was to determine if a common exotic coccinellid, *C. septempunctata*, influences the foraging location of adult native lady beetles. To do this, I created habitats in a greenhouse with patches of prey at different densities. I then recorded where the native lady beetles chose to forage in the presence or absence of exotic beetles. I hypothesized that in the presence of the exotic competitor *C. septempunctata*, native individuals would choose to forage in patches of low prey density to avoid contact with their potential competitor.

Methods

Laboratory-reared coccinellids

Adult coccinellids used in this experiment were obtained from laboratory colonies maintained at the Ashland Road Greenhouses at the University of Missouri. All beetles were raised in 60 x 60 x 60 cm BugDorms (MegaView Science Co., Ltd., Taichun, Taiwan) and fed a diet consisting of bird cherry-oat aphids (*Rhopalosiphum padi* L.) fed on soft red winter wheat (*Triticum aestivum* L.) and pea aphids (*Acyrtosiphon pisum* Harris) fed on fava bean (*Vicia faba* L.). All colonies were maintained at a 16:8 light-dark cycle with a temperature range of 22-25°C. Beetle colonies were originally started with hand-collected adults from alfalfa, corn, tall fescue and tallgrass prairie habitats within Boone and Callaway Counties in central Missouri.

Experimental design

The experimental design was a split-plot design with coccinellid treatments applied to the whole cage (whole plot factor) and manipulation of aphid abundance happening within cages to create prey patches (sub-plot factor). Experiments were conducted in 47.5 x 47.5 x 93 cm BugDorms (MegaView Science Co., Ltd., Taichun, Taiwan) with the same ambient conditions as described for colony rearing. Into each BugDorm, I placed three rows of plants, each row consisting of three 15.24 cm pots, with each pot containing ten two-week old wheat plants (Fig. 30). The center row of plants was maintained as the aphid-free row, and rows to the left and right were randomly assigned to either a high aphid density, in which each pot of plants in the row received 250 bird cherry-oat aphids, or a low aphid density, in which each pot of plants received 50 bird cherry-oat aphids. Aphids were allowed 24 hours to settle before beetles were introduced.

The coccinellid treatments were applied to the whole cage in a 3 x 2 factorial design with native species identity (one of three possible species present) crossed with the presence of an exotic species (*C. septempunctata* present or absent). Into each BugDorm, five adult females and one adult male of one of three native species, *H. convergens*, *C. munda*, or *C. maculata* were introduced on the center plant of the ‘no aphid’ patch. In the cages receiving exotic beetles, two adult female *C. septempunctata* were introduced on the same plant as the native coccinellids. I also included one treatment containing only *C. septempunctata* without native beetles present and one treatment with no beetles present, resulting in a total of eight experimental treatments.

The experiment was repeated over five consecutive time periods, with two complete replications of the eight treatments per time period, for a total of ten replications.

After twelve hours, I counted how many beetles were located in the high density prey patch, in the low density prey patch, in the aphid-free patch, or off the plants. These observations were made at approximately 8am when the beetles were most actively foraging (pers. obs.). This is consistent with previous studies, which found that coccinellids forage more frequently and consistently in the morning (Mack & Smilowitz, 1979; Iperti, 1999; Ximenez-Embun et al., 2014). Using these data, I then examined how species identity, aphid abundance, and the presence of exotic species affected the number of native beetles found foraging in a prey patch with block as a random factor (PROC ANOVA; SAS v 9.3).

Results

There was no influence of the presence of the exotic beetle, the species identity of the native beetles, or any interactions of these factors on where native beetles chose to forage (Table 5; Fig. 31). Aphid abundance was the only factor found to significantly influence the foraging location of native lady beetles in this study (Fig. 32; $F_{2,162} = 44.53$, $p < 0.0001$). Frequently, both native and exotic beetles were observed foraging on the same plants in the high aphid density patches, so it is possible that aphids were not limiting and there was no opportunity for competition in this study.

Discussion

I found no evidence that the presence of *C. septempunctata*, an exotic coccinellid species that has previously been shown to be a superior competitor, influenced the foraging location of any of the native species of lady beetles. A greater number of adults of all native species were found within the high aphid abundance patches than in either the low aphid or aphid-free patches (Fig. 31), regardless of the presence of the exotic beetles. This finding is consistent with the idea that in areas of high aphid abundance both native and exotic species may aggregate (Schellhorn & Andow, 2005a; Evans & Toler, 2007) and even coexist (Evans, 1991; Ives et al., 1993; Obrycki et al., 1998b).

One potential reason why this study did not show evidence of resource partitioning, while other studies have clearly documented its occurrence, may be that I tested adults. Studies focusing on larval interactions have found that individuals will spatially partition themselves so as to avoid interspecific competition, which may result in the death of the less competitive individual (Obrycki et al., 1998a). Predation events between larvae as well as predation of the vulnerable egg and pupal stages can negatively impact survival success (Takahashi, 1993; Schellhorn & Andow, 1999a; Schellhorn & Andow, 1999b; Evans, 2003). Because of this, spatial partitioning may be integral to the survival of offspring during these life stages (Hemptonne et al., 1992; Osawa, 1992; Takahashi, 1993; Sakuratani & Nakamura, 1997). However, adult coccinellids are not as vulnerable to predation as the immature stages, and therefore may be less prone to spatial partitioning.

Another reason why my results may be inconsistent with previous studies is the spatial scale of my study. Unlike other studies in which spatial resource partitioning was documented, I did not confine adult coccinellids to a single plant. Because beetles were

not forced to compete within a confined area, but rather were freely able to forage among the habitat patches and within patches with more than one plant, it is possible that direct competition between the exotic and native species did not occur in this experiment. In a field-level study of the response of two adult species of lady beetles, *H. convergens* and *C. septempunctata*, to manipulated aphid abundance on fire weed, Ives et al. (1993) found a similar response with both species responding to aphid abundance by foraging longer in patches with a higher abundance as compared to patches with lower aphid abundance. It was also noted that, while these adult beetles generally foraged longer in high abundance patches, they were also individually prone to leave a particular patch quickly (Ives et al., 1993).

Although the spatial scale used in this study was larger than a single plant, it was still much more limited than the spatial scale of other field studies that found partitioning. Coccinellids, like other mobile predatory organisms are capable of spatial, diel and seasonal partitioning at the whole-habitat scale (Elliott & Kieckhefer, 1990; Ximenez-Embun et al., 2014), which explains the coexistence of several species within the same habitat throughout the growing season. In a study by Ximenez-Embun et al. (2014), all three of these methods of partitioning were documented within the aphidophagous guild in alfalfa fields of Chile. Coccinellids constituted the majority of aphid predators in this guild. They found that coccinellids in this system were active during the daytime, while other predators were more active at night, and that certain species as well as life stages of coccinellids could be found at distinct locations within the habitat (e.g. on plants and/or on the ground). They also found seasonal partitioning within the four most commonly collected coccinellid species, with two species only collected during the spring season

and the other two collected at different seasonal frequencies throughout the study. It has also been suggested that coccinellids spatially partition their habitat use at the landscape scale. While exotic coccinellids often dominate the lady beetle communities in annual cropping systems (Elliott & Kieckhefer, 1990; Elliott et al., 1996; Alyokhin & Sewell, 2004), native species are often found foraging in natural and semi-natural perennial grasslands (Landis et al., 2000; Evans, 2004; Gardiner et al., 2009a; Gardiner et al., 2009c; Diepenbrock & Finke, 2013). It is thought that native species forage in perennial habitats to escape interspecific competition with exotic species.

Resource partitioning at the habitat or landscape scale may be important for the persistence of many native lady beetle species in North America as their exotic counterparts have become increasingly abundant in agricultural habitats. While some studies have shown such partitioning within habitats (Ives et al., 1993; Ximenez-Embun et al., 2014), the temporal and spatial scale of my study may not have permitted this sort of habitat partitioning by beetles to arise.

Table 5: Main and interactive effects of variables that may impact the number of native lady beetles foraging in a prey patch.

Effect	F value	p
Native species identity	$F_{2,162} = 0.18$	0.8376
Presence of exotic species	$F_{1,162} = 0.30$	0.5833
Native species identity* presence of exotic species	$F_{2,162} = 0.00$	0.9955
Aphid abundance	$F_{2,162} = 44.53$	< 0.0001*
Native species identity* aphid abundance	$F_{4,162} = 0.37$	0.8266
Presence of exotic species* aphid abundance	$F_{2,162} = 0.31$	0.7333
Native species identity* presence of exotic species* aphid abundance	$F_{4,162} = 1.71$	0.1493

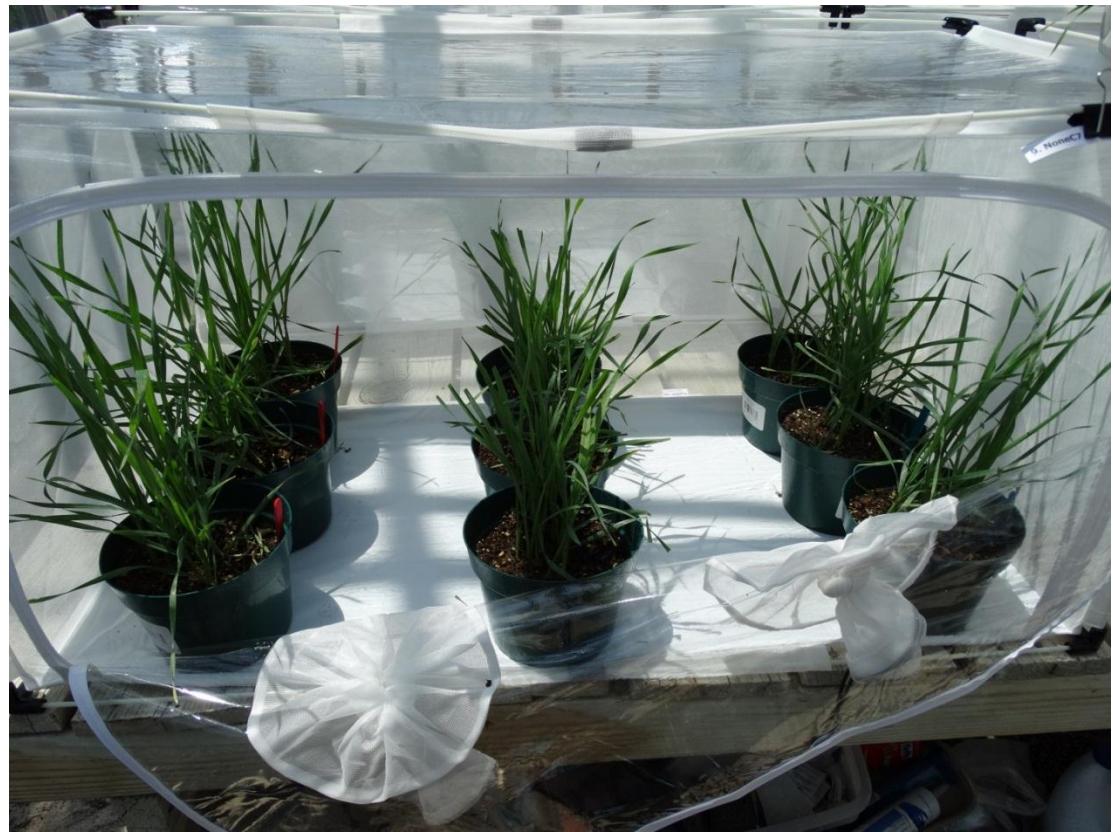


Figure 30: Greenhouse experimental mesocosms. Aphid abundance, the presence of an exotic lady beetle species, and the species identity of native lady beetles were manipulated to determine which, if any, of these factors determined where native lady beetles choose to forage.

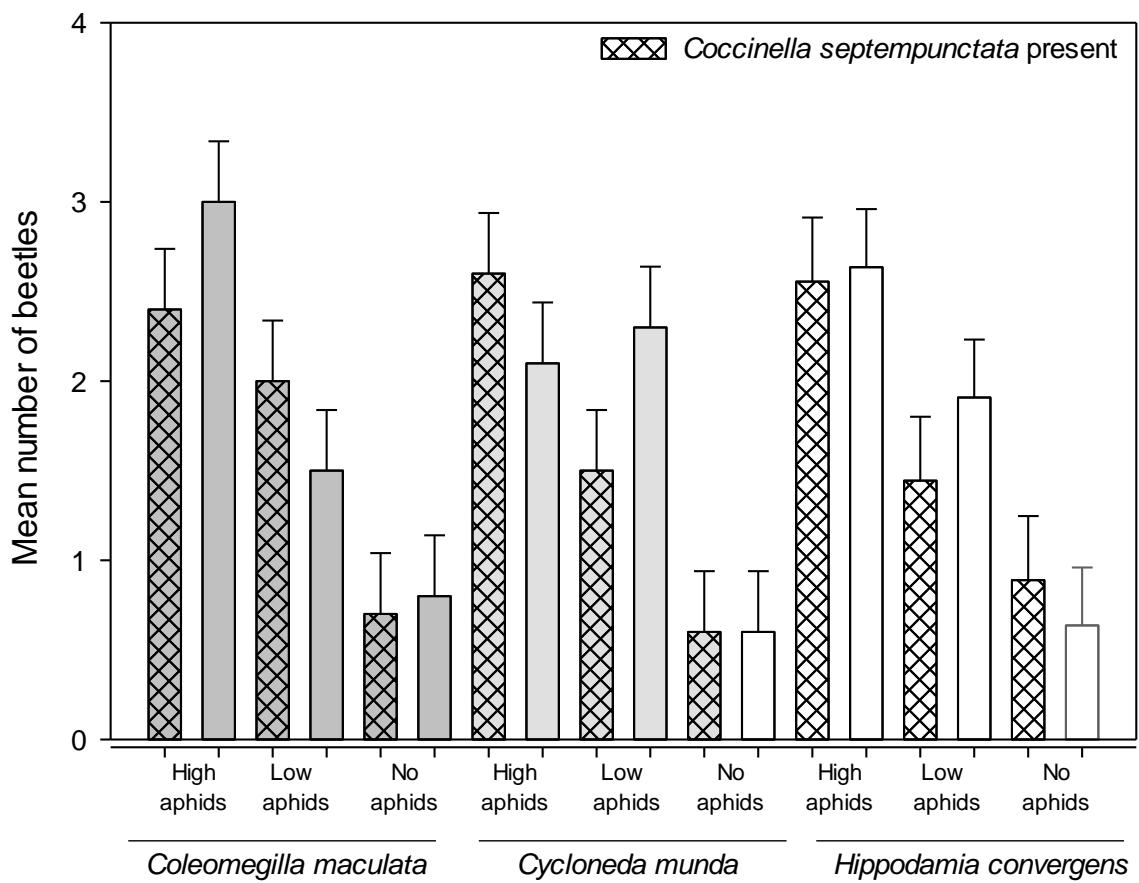


Figure 31: Mean number of lady beetles from each of three native species foraging within manipulated prey (aphid) density patches with or without the exotic species, *Coccinella Septempunctata* L., present.

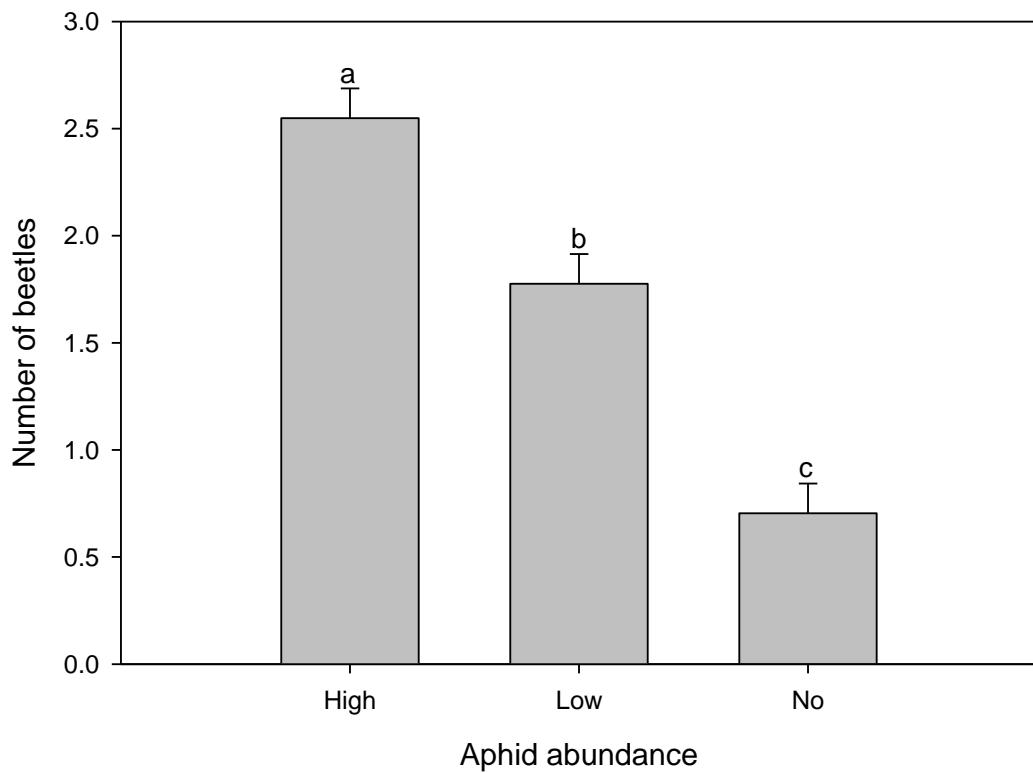


Figure 32: Mean number of lady beetles foraging within prey patches of varying aphid abundance. Aphid abundance was the only experimentally manipulated factor found to influence where native species foraged ($F_{2,162} = 44.53, p < 0.0001$). Means with different letters are significantly different at the $p < 0.05$ level.

Chapter 6

Conclusions

One of the goals of this research was to understand how the native coccinellid community has changed over the past decade within the state of Missouri. There has been much research documenting the decline in many native species throughout the continental United States and potential factors underlying these declines. To the best of my knowledge, only one other study attempts to document historical changes in coccinellid communities, however that study only encompassed 30 years of data (Bahlia, 2014), which is a much shorter time frame than this study. Because I was able to obtain 80 years of community data, my study was able to capture long-term, continuous community change occurring even prior to the arrival of any exotic species in this region, which suggests that the exotic species may not be the only driver of changes within the native lady beetle community.

My research also considered the role of perennial grassland habitat for the persistence of native lady beetles. Perennial grasslands have been correlated with increased abundances of native lady beetles in nearby crop habitats (Gardiner et al., 2009c). The focus of this research was to consider such habitats, both natural perennial grasslands and perennial agricultural grasslands, as potential refuge habitat for native coccinellids. The more disturbed perennial habitat, agricultural tall fescue, had a greater abundance and diversity of native lady beetle species than the natural tallgrass prairie habitats. However, all grasslands had very low abundances of exotic species present and significantly more native than exotic coccinellids. Because of this, it appears that all perennial grasslands may serve as refuge habitat for native lady beetles.

What is unclear though is what makes one type of grassland, particularly one that is more frequently disturbed, a better refuge habitat than any other type of grassland. As part of this study, I also looked at aphid abundance in each of the habitat types and found a higher abundance of aphids in the agricultural fescue. Because aphids are a common food resource for many species of lady beetles, it is possible that there was a numerical response of the native lady beetles into the fescue habitats containing this readily available food resource. To test this idea, I created ambient aphid abundance and aphid reduction plots within three grassland sites. Within each of these sites, I determined the coccinellid community present and looked at the amount of predation occurring on sentinel egg masses. Egg predation was included in this study because the ability of coccinellids to survive this vulnerable stage in their life cycle may be related to the availability of alternate prey items (aphids); in the presence of an abundance of alternative prey items, a greater number of eggs may survive and enable the persistence of native coccinellids locally. Contrary to expectations, I did not find an overall difference in the native coccinellid community or in the predation of eggs with respect to aphid abundance. Rather, I found a greater abundance, but not diversity, of native species in the first year of study (2012) than in the second and a difference in the proportion of native eggs consumed by aphid abundance in only the first year of study. It is likely that the greater abundance of native coccinellids in 2012 corresponded with a greater overall abundance of generalist predators in that same year that could have consumed the eggs of the native coccinellids in addition to any other available food resources.

In addition to field studies, I also used greenhouse mesocosms to look at the potential for displacement of native species by exotic species from areas of high prey (aphid) abundance. Several studies have looked at this question, either by confining larvae or adults of native and exotic species to a small area with or without prey available and have found antagonistic interactions such that the larvae of the native beetle died and/or was consumed (Obrycki et al., 1998b) or that the adults were displaced to forage in locations further from the highest density of aphids on the same plant (Ives et al., 1993). Field studies have also shown an apparent large scale displacement of native beetles with increased abundances of the highly voracious exotic species *Coccinella septempunctata* exhausting prey resources thus forcing natives to presumably forage elsewhere (Evans, 2004). In the current study, there were distinct patches of high, low and no aphid abundance where native species foraged both in the presence and absence of the exotic species *C. septempunctata*. Unlike both the confined and field scale studies, at this intermediate scale, native beetles were found foraging within the patches of high aphid density regardless of the presence of the exotic species. It is likely that in the abundance of prey, these beetles do not have to compete for resources therefore there is no displacement of the less voracious native species.

Considering the data presented here, it is clear that there is not one definitive answer for the cause of decline in the native lady beetle populations. It is likely that a combination of land use change, climatic factors and changes in the availability of resources from the constantly increasing abundance of exotic beetles have all impacted the native coccinellid communities. Understanding how these factors affect native species is important to the long term survival of native species such as lady beetles that

are part of an important community of predators that we rely on for the control of insect pests in agricultural systems.

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VITA

Lauren Marie (Hart) Diepenbrock's love of the natural world began early in life, playing in the woods near her childhood home in Palm Bay, FL. When not at school, she could be found climbing a tree or rescuing hornets from the pool. Lauren's family moved to St. Louis, Missouri in 1993, where she completed her secondary education and discovered a love for music in addition to science. She attended the University of Missouri for her undergraduate degree where she was able to participate in the band program while earning a bachelor's degree in Biology. During her college years, she discovered the joy of research working as a technician and later an undergraduate researcher in the laboratory of Dr. Donald Riddle.

After college, Lauren began her graduate studies in the biomedical sciences at Upstate Medical University in Syracuse, NY where she quickly realized that this type of research was not her true passion. She stayed in the area and earned a Master's degree of Science Education from Syracuse University where she took her first ecology course and was hooked. After completing her science education degree, Lauren returned to St. Louis, MO where she taught high school science for a few years. It was while teaching high school that she realized how much she loved ecology and sought out opportunities to return to school and continue her studies.

In 2007, Lauren joined the laboratory of Dr. Walter Tschinkel at Florida State University where she completed a Master's degree in Ecology and Evolutionary Biology and studied the natural history of trap-jaw ants. After completing her second master's degree, she moved back to Missouri once again to further her entomological education

and in 2009 joined the laboratory of Dr. Deborah Finke in the Division of Plant, Insect and Microbial Sciences. While at the University of Missouri, Lauren studied the regional lady beetle communities within the state while also being an active member of the Conservation Biology program and earning a graduate certificate in this area. While it may have taken a while to discover her true scientific passions, she is grateful for all of the opportunities provided throughout her academic life and to have finally found her scientific niche.