

THE IMPACT OF LANDSCAPE DIVERSITY AND
INVERTEBRATE COMMUNITY DYNAMICS ON
MONARCH BUTTERFLY (*DANAUS PLEXIPPUS*)
OVIPOSITION AND SURVIVAL

By

KELSEY ANN DEAL

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Dr. Kristen Baum

Thesis Adviser

Dr. Amy Frazier

Dr. Eric Rebek

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Abstract: The monarch butterfly (*Danaus plexippus*) eastern migratory population has experienced declines due to several factors including habitat loss. Monarchs rely exclusively on milkweeds as a host plant and conservation efforts are interested in increasing habitat resources by planting milkweed in towns and cities. Therefore it is important to identify factors that influence monarch use of and success (i.e., survival) in urban areas. I evaluated three monarch response variables to assess monarch fecundity and survival at multiple life stages. Monarch egg abundance, egg to 4th and 5th instar survival, and 4th and 5th instar to adult survival were compared to three categories of predictor variables: plant characteristics, invertebrate community dynamics, and landscape context. I established experimental plots at garden sites around Stillwater, Oklahoma by planting three tropical milkweed (*Asclepias curassavica*) plants at each location. Landscape characteristics for each site were quantified using the Oklahoma Ecosystem Mapping Project. Plant characteristics and invertebrate community variables such as predator species richness, aphid density, and percent herbivory were estimated by monitoring study sites during the fall (mid-August through October) of 2015 and 2016. To quantify survival rates, 4th and 5th instar monarch caterpillars were collected from study sites and reared individually in the lab. Plant height was the only variable that significantly influenced egg abundance with a positive correlation between the average maximum plant height and the total number of eggs at a site. Egg to 4th and 5th instar survival was positively influenced by predator species richness and patch richness density but negatively influenced by plant height, aphid density, and percent herbivory. Lastly, 4th and 5th instar survival was positively influenced by predator species richness and negatively influenced by aphid density. Examining how additional landscape characteristics and community interactions influence monarch oviposition and survival will provide insights into the contribution of urban areas to support the monarch population, with important implications for urban wildlife ecology and conservation.

TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION.....	1
II. METHODOLOGY.....	8
Study Sites and Experimental Plots.....	8
Landscape Context.....	9
Site Monitoring: Plant Characteristics and Invertebrate Community Dynamics...10	
Parasitism Rates.....	11
Analyses.....	11
III. RESULTS.....	14
2015 Summary.....	14
2016 Summary.....	15
Monarch Oviposition.....	15
Monarch Egg to 4th and 5th Instar Survival (Influence of Predation).....	16
Monarch 4th and 5th Instar to Adult Survival (Influence of Parasitism).....	17
IV. DISCUSSION.....	18
REFERENCES.....	23
APPENDICES.....	32

LIST OF TABLES

Table	Page
Table 1. Overview of predicted interactions between variables	32
Table 2. Reclassified land cover classes	33
Table 3. Overview of observed interactions between variables	34
Table 4. Results of linear and logistic regressions.....	35

LIST OF FIGURES

Figure	Page
Figure 1. Map of study sites in 2015 and 2016.....	36
Figure 2. Example of patch richness density analysis at three scales.....	37
Figure 3. Total number of eggs and plant height at all scales.....	38
Figure 4A. Plant height and egg to 4 th and 5 th instar survival at 100- and 200-m scales.....	39
Figure 4B. Predator species richness and egg to 4 th and 5 th instar survival at 100- and 200-m scales.....	40
Figure 4C. Aphid density and egg to 4 th and 5 th instar survival at 100- and 200-m scales.....	41
Figure 5A. Aphid density and egg to 4 th and 5 th instar survival at the 400-m scale.....	42
Figure 5B. Percent herbivory and egg to 4 th and 5 th instar survival at the 400-m scale.....	43
Figure 5C. Patch richness density and egg to 4 th and 5 th instar survival at the 400-m scale.....	44
Figure 6. Overall tachinid parasitism rates at study sites in 2016.....	45
Figure 7A. Predator species richness and 4 th and 5 th instar to adult survival at all spatial scales.....	46
Figure 7B. Aphid density and 4 th and 5 th instar to adult survival at all spatial scales.....	47

CHAPTER I

INTRODUCITON

The eastern migratory population of the monarch butterfly (*Danaus plexippus* L. [Lepidoptera: Nymphalidae]) produces several generations per year on its summer breeding grounds east of the Rocky Mountains (Malcolm et al., 1993; Flockhart et al., 2013). The final generation produced each year migrates up to 4,000 km south to reach overwintering grounds in central Mexico (Urquhart and Urquhart, 1978; Solensky, 2004). The overwintering monarchs return to the United States in the spring and produce the first generation on their spring breeding grounds in the southern U.S., which completes their annual cycle (Cockrell et al., 1993; Malcolm et al., 1993).

The monarch's migration has been identified as an endangered biological phenomenon (Brower and Malcolm, 1991; Semmens et al., 2016) due to a steady decline in monarchs observed at the overwintering grounds. Monarchs occupied 2.91 hectares of forest at the overwintering grounds in Mexico during the 2016-2017 season (World Wildlife Fund, 2017). While this estimate is an improvement from the historic low of 0.67 hectares estimated in 2013- 2014, the current population size is 50 percent smaller than the historic average population size of 6 hectares (Vidal and Rendón-Salinas, 2014). Thus, the monarch butterfly is currently being considered for listing as threatened under the Endangered Species Act (Center for Biological Diversity et al., 2014).

Habitat loss in the overwintering and breeding grounds has been identified as the primary reason for monarch decline. Forested areas in Mexico that provide shelter for overwintering monarchs have decreased in size due to illegal logging (Brower et al., 2002). Loss of breeding ground habitat has also occurred, especially in the midwestern United States where a decrease in milkweed abundance has been attributed to increased herbicide use in agricultural fields (Pleasants and Oberhauser, 2013; Pleasants, 2017). Milkweed is the only host plant fed upon by monarch larvae and is, therefore, crucial to the survival of the species. Finally, other factors such as pesticide use (Pecenka and Lundgren, 2015), extreme weather events (Calvert et al., 1984; Brower et al., 2004), predators (Oberhauser et al., 2015; De Anda and Oberhauser, 2015), and disease (Altizer and Oberhauser, 1999; Satterfield et al., 2015) have been identified as contributing to monarch decline.

Monarch conservation efforts in North America have focused on increasing milkweed availability throughout the landscape, including urban areas. For example, the Mayors' Monarch Pledge is a program promoted by the National Wildlife Federation that encourages cities to create habitat for monarchs and other pollinators (Mayors' Monarch Pledge - National Wildlife Federation). The White House Pollinator Health Task Force issued the National Strategy to Promote the Health of Honey Bees and Other Pollinators (White House, 2015), which led to the formation of the National Pollinator Garden Network. This network is composed of organizations representing a range of interests from garden clubs and the garden trade to conservation and federal agencies (Program Partners - National Wildlife Federation). Many other organizations and individuals are also focused on providing monarch habitat in urban areas.

Because of this emphasis on urban areas, it is important to identify factors that influence monarch use of and success (i.e., survival) in urban areas. Cutting and Tallamy (2015) examined the impact of urban land use by establishing study sites in native meadows (natural treatment) and managed lawns and gardens (urban treatment). They estimated immature monarch survival by

monitoring plants for eggs and larvae through the 4th instar stage. Fifth instars were excluded from their analyses because 5th instars often move away from milkweed plants to pupate and can be difficult to locate. They found that monarch survival was not significantly different between natural meadows and managed gardens. Another study focused on land use and immature monarch survival analyzed citizen science data collected at volunteer-selected sites (Nail et al., 2015). Study locations were categorized into six possible site types: garden, natural area, crop-based agricultural area, non-crop agricultural area, roadside, or other (city parks, backyards, golf courses, etc.). They found that natural and non-crop agricultural sites were correlated with higher immature monarch survival rates, but the remaining site types did not significantly affect survival in their model. Nail et al. (2015) highlighted the need for additional studies that incorporate the role of other factors such as predators, parasites, and plant characteristics on the interaction between site type and monarch survival.

Important parasites that influence monarch survival are tachinid flies (Diptera: Tachinidae). These flies are parasitoids that primarily target lepidopteran larvae. Flies lay their eggs on the host, which upon hatching burrow into the caterpillar where the fly larvae develop and ultimately kill the host. Fly larvae emerge from their hosts after three instar stages to pupate in the leaf litter and will emerge after 10-14 days (Oberhauser et al., 2007). Tachinid flies rely on nectar resources during their adult stage, with some species identified as important pollinators (Rader et al., 2011).

Previous research has focused on the diversity of tachinid flies that parasitize monarchs as well as the impact of these parasitoids on monarch populations. A total of twelve species of tachinid flies including eight different genera are documented as parasitizing monarch butterflies (Arnaud, 1978). *Lespesia archippivora* (Riley) is the most well known of these species, accounting for 75% of the 1,146 tachinid flies recently identified from citizen scientist contributed specimens (University of Minnesota Monarch Lab, 2017). The remaining flies from this study comprised an additional six species of tachinid flies including a newly described

species. Tachinid flies are usually considered generalists, although *Lespesia archippivora* densities track the monarch population, with years of high monarch densities followed by years of high parasitism (Oberhauser, 2012). Overall, tachinid flies parasitize approximately 13% of the eastern monarch population but the local rate of parasitism can vary substantially (2-90%) (Oberhauser et al., 2007).

The monarch is also parasitized by an obligate spore-forming protist (*Ophryocystis elektroscirrha*; OE). *Ophryocystis elektroscirrha* replicates inside the developing pupa, and when the adult butterfly emerges it is covered in thousands of dormant spores. Vertical transmission of OE is the most common mode of infection, with 1st instar caterpillars consuming spores deposited by infected adult females (Altizer et al., 2000). Once a caterpillar has consumed spores they will lyse in the midgut and reproduce in both the caterpillar and pupal stage of the host. Very high spore loads can lead to death before eclosion, while intermediate level spore loads cause decreased life span, decreased wingspan, decreased mass at emergence, reduced flight performance, and increased rate of weight loss for adults (Altizer and Oberhauser, 1999; Altizer, 2001; De Roode et al., 2008). Previous research has shown that approximately 8% of the eastern North American monarch population is infected by OE in a given year (Altizer et al., 2000).

The rate of OE infection also fluctuates seasonally and two main mechanisms have been identified as contributing to this pattern. Migratory culling occurs when infected individuals are removed from the population because they do not survive the long-distance migration to overwintering grounds (Bradley and Altizer, 2005). Likewise, migratory escape occurs when monarchs move out of areas of high parasite concentration (Loehle, 1995; Satterfield et al., 2015). Therefore, levels of infection at overwintering grounds are lower than levels observed on summer breeding grounds (Bartel et al., 2011). These mechanisms are also demonstrated by higher infection rates found in the non-migratory monarch population in southern Florida and the western migratory population that experiences a shorter seasonal migration than the eastern population (Altizer et al., 2000). These findings, coupled with the potential listing of the monarch

butterfly as threatened, have led to an increased interest in the influence of spatial and temporal variations on monarch-parasite interactions.

Previous research on the relationship between site characteristics and monarch-parasite dynamics has focused on land use. Andreoli (2015) compared parasitism of monarch larvae between study sites located in rangelands, hay fields, and roadsides and found that recently burned rangelands had a higher percentage of monarchs parasitized by tachinid flies compared to those in native hay fields and roadsides. However, she observed no difference in the OE parasitism rate among the three land use types. An additional study found no difference in tachinid fly and OE parasitism rates between managed prairies and roadsides (Mueller and Baum, 2014).

The first objective of this study was to evaluate if monarch use of sites for oviposition was related to site characteristics. The number of monarch eggs at a site was compared to parameters of plant characteristics, the invertebrate community, and landscape context (Table 1). Plant height was used as the plant characteristic of interest since adult butterflies may be more likely to detect taller milkweed plants. Monarchs have a 5-m short-term perception distance and a 25-m long-term perception distance (Zalucki and Kitching, 1982a). Additionally, taller plants may have more leaves and, therefore, height could be a measure of host plant quality. Thus, I expected a positive relationship between plant height and egg abundance.

Variables indicative of the invertebrate community included aphid density per plant, percent herbivory, and predator species richness. Aphid density and percent herbivory could be possible indicators of host plant quality and I expected that plants with greater aphid densities and greater percent herbivory would have lower monarch egg abundance. In contrast, predator species richness may indicate a higher-quality milkweed patch that can support a greater diversity of arthropod species, and I expected higher monarch egg abundance with higher predator species richness. Lastly, landscape context plays an important role in how female monarchs perceive the landscape. Modeling efforts have demonstrated the importance of landscape configuration on

fecundity (Zalucki et al., 2016), and therefore I expected sites with higher patch richness densities to contain more monarch eggs.

The second objective of this study was to evaluate monarch survival from egg to 4th and 5th instar in the field, which reflects mortality due to predation. I used the same parameters of interest (Table 1) as the first objective to evaluate monarch larval survival (egg to 4th and 5th instar). Plant height may influence survival because milkweed plants that are taller provide more structure that could attract predators or provide refugia for caterpillars. As for variables that represent the invertebrate community, I predicted that sites with greater predator species richness would have lower egg to late instar survival rates. Additionally, aphid density and percent herbivory both have the potential to influence predator abundance and, therefore, plants with higher aphid density and percent herbivory may have lower rates of larval survival (De Anda and Oberhauser, 2015). The landscape context may influence predator dispersal rates among habitat types. Therefore, I predicted sites with higher patch richness density would have lower monarch survival rates because landscape heterogeneity may facilitate the movement of predators.

Lastly, I evaluated monarch survival from 4th and 5th instar to adult, which reflects mortality due to parasitism by tachinid flies and OE. Plant height, aphid density, percent herbivory, predator species richness, and patch richness density were applied to this last monarch response variable (Table 1). Taller plants may attract tachinid flies to a study site because these plants are easier to detect within the landscape and, therefore, plant height could have a negative influence on 4th and 5th instar-to-adult survival. I predicted that two of the invertebrate community metrics would have a negative impact on monarch survival. Aphid density and percent herbivory could attract tachinid flies due to the increase in plant volatiles that are released as a consequence of aphid and herbivore damage to the plant. In contrast, I predicted a positive relationship with monarch survival and predator species richness because the presence of predators may influence

parasitism success by tachinids if their host (monarch caterpillar) is preyed upon by other arthropods. Finally, it is unclear what type of relationship patch richness density will have on late instar-to-adult survival because heterogeneous landscapes have the potential to benefit both parasitoid flies and monarch larvae.

CHAPTER II

METHODOLOGY

Study Sites and Experimental Plots

Twelve study sites were selected in the fall of 2015 and 28 study sites were selected in the fall of 2016 (Figure 1). Sites were identified throughout Stillwater (population size approximately 47,000), which is located in the north central part of Oklahoma, based on participant (homeowner) interest in contributing to this project and a minimum separation distance of 800 m between sites (see Landscape Context methods below). Oklahoma is an area of special interest for monarch butterflies because of its role during both spring and fall migration and breeding periods (Baum and Sharber, 2012; Batalden and Oberhauser, 2015).

An experimental plot was established at each site by planting a cluster of three tropical milkweed plants (*Asclepias curassavica* L.). This procedure followed techniques proposed by Tischendorf and Fahrig (2000) for sampling a single patch in the center of each site and scaling out from the center to compare landscape characteristics between sites. The plot of three milkweed plants functioned as the center of the area of interest for each location. Plants were established within a 5-m radius at the majority of study sites.

However, participant preferences for plant placement were also taken into consideration; therefore, in 2015 two sites were planted with milkweed plants spaced up to 10 m apart and an additional two sites had plants spaced up to 20 m apart. Likewise, in 2016 two sites were established with milkweed plants separated up to 10 m and one site separated up to 20 m. Lastly, participants had the option of using three potted plants instead of planting milkweed in their yards. Potted plants were used for the experimental plot at two study sites in 2015 and seven study sites in 2016.

Landscape Context

The spatial scale of interest was determined based on monarch and parasitoid dispersal abilities. Throughout their fall migration monarchs fly great distances with an overall pace of 32 km/day (Howard and Davis, 2015). However, monarchs move shorter distances during the breeding season and experience briefer lifespans of two to five weeks compared to migratory monarchs that can live up to nine months (Oberhauser, 2004). Breeding females have an average birth-to-death distance of 11 km, but this may vary from 1 km to 18 km based on habitat composition (Zalucki, 1983; Zalucki et al., 2016). Thus, our study sites were distributed within a landscape of 16.8 km (north to south) by 20.8 km (east to west) to address potential movement distances for ovipositing females. Previous research suggests some species of tachinids can move 100 m to 200 m, with a few individuals documented moving up to 400 m (Rader et al., 2011). Likewise, other parasitoids have been studied at scales up to 500 m based on their dispersal abilities (Bennett and Gratton, 2012). To address these diverse dispersal abilities, a nested set of three scales (radii of 100, 200, and 400 meters) was used to compare survival and parasitism rates to landscape context (Thies et al., 2003) (Figure 2).

The study sites were characterized using data from the Oklahoma Ecological Systems Mapping Project (10-m resolution), which defines 165 vegetation types across the state of Oklahoma. For the purposes of this study, the original Oklahoma Ecological Systems Mapping

Project vegetation types were reclassified into eight major land cover classes (see Table 2). Patch richness density was selected as the landscape variable of interest and this metric was estimated by dividing the number of reclassified land cover types at a study site by the area of the study site (area of 100 m scale = 31,600 m², area of 200 m scale = 126,400 m², area of 400 m scale = 502,400 m²). This initial value was divided by 10,000 and then multiplied by 100 to convert to 100 hectares (McGarigal and Marks, 1995).

Site Monitoring: Plant Characteristics and Invertebrate Community Dynamics

Sites were monitored weekly for the presence of monarchs in the fall of 2015 and 2016. The number of eggs and instars per milkweed plant were recorded. Structures (e.g., fences, brick walls, and planters) near milkweed plants were scanned for presence of monarch chrysalises. Plant height was recorded in cm by measuring the height of the tallest tropical milkweed branch above ground level and included flowers, buds, or seedpods, if present.

The invertebrate community was quantified by recording predator species richness, aphid density, and percent herbivory. The presence of potential monarch predators was recorded and separated by order or family: ants (Formicidae), lacewing larvae and eggs (Neuroptera), lady beetle adults and larvae (Coccinellidae), syrphid fly pupae and larvae (Syrphidae), spiders (Thomisidae, Salticidae, and others), and true bugs (Hemiptera). This information was used to calculate predator species richness for each site. Additionally, the number of aphids was recorded in density categories (0, 1-10, 11-100, and 101-1000 per plant) based on the guidelines outlined by the Monarch Larva Monitoring Project for citizen scientists (Monarch Larva Monitoring Project, 2016). The aphids are tended by ants, and the ants themselves are potential monarch predators (Oberhauser et al., 2015). Lastly, the percentage of leaf damage, both from herbivore consumption and disease, was assessed for each plant and placed into the following categories: 1 = 0%, 2 = <5%, 3 = 5-25%, 4 = >25% (Monarch Larva Monitoring Project, 2016). Leaves with spider mite damage were incorporated into this parameter and received a higher score for percent

herbivory.

Parasitism Rates

To quantify parasitism rates, I collected fourth and fifth instar monarch caterpillars; collecting late instars allowed time for larvae to be exposed to parasites in the field. Once collected, caterpillars were reared individually in 64-ounce plastic containers with ventilated lids to document parasitism. Tropical milkweed leaves were collected from greenhouse-grown plants, sterilized in a solution of 10% bleach and 90% water, and then rinsed thoroughly before being given to caterpillars (Altizer and Oberhauser, 1999). Caterpillars were checked at least once a day and fresh leaves were provided as needed until the caterpillars pupated. Monarch chrysalises were also collected from study sites when detected on nearby structures (e.g., fences, brick walls, and planters) and separated into rearing containers for eclosion.

Tachinid fly larvae or pupae were recorded when they were observed in the rearing container. The number of fly larvae that emerged per caterpillar was recorded. Occurrence of OE was evaluated using the tape count method once adult butterflies emerged. The tape count method involves placing a piece of clear tape on the ventral side of the butterfly's abdomen to remove some scales and examining the scales under a microscope for presence of OE spores (Altizer et al., 2000). If spores were observed the spore load or intensity of infection was estimated by examining the sample under 400x magnification and assigning a value based on the following scale: 0, no spores; 1, one spore; 2, 2-20 spores; 3, 21-100 spores; 4, 101-1000 spores; and 5, > 1000 spores (Altizer et al., 2000).

Analyses

A suite of independent variables was utilized in the analyses of factors influencing monarch oviposition, egg to 4th and 5th instar monarch survival, and 4th and 5th instar to adult monarch survival (Table 1). Maximum mean plant height was calculated by averaging the

tropical milkweed plant heights at a study site for each week and selecting the maximum mean value for the entire sampling season. The average number of flowers per milkweed plant was also estimated for each study site but was excluded from the analyses due to its high correlation with plant height.

Predator species richness was calculated based on observations of six possible types of invertebrates (ants, lacewing larvae and eggs, lady beetle adults and larvae, syrphid fly pupae and larvae, spiders, and true bugs) that are documented predators of monarch eggs and larvae (De Anda and Oberhauser, 2015). Predator species richness was summed across all weeks within a sampling season, with a maximum value of six. Maximum aphid density per plant reflects the highest possible category of aphid density observed during the sampling season (0, 1-10, 11-100, and 101-1000 per plant). Maximum percent herbivory likewise reflects the greatest level of percent herbivory observed across the span of the sampling season (1 = 0%, 2 = <5%, 3 = 5-25%, 4 = >25%). Lastly, patch richness density was calculated for each study site at each scale (radii of 100, 200, and 400 m) using the Oklahoma Ecological Systems Mapping Project reclassified land cover classes (Table 2). Urban class area or the percentage of urban land cover class at a study site was another land cover metric of interest but it was excluded from the analyses due to its high correlation with patch richness density.

The program R was used to conduct all statistical analyses. Linear regression was used to analyze the relationship between monarch egg abundance (total abundance summed across the sampling season) and plant characteristics (maximum mean plant height), the invertebrate community (predator species richness, maximum aphid density per plant, and maximum percent herbivory per plant), landscape context (patch richness density), and number of years sampled (one or two). First instar caterpillars were also included in this parameter if it was clear that they were independent from egg records based on the timing of surveys and developmental periods (i.e., they represented individuals that were not previously recorded as eggs). This analysis was done for all three scales (radii of 100, 200, and 400 m) with the corresponding patch richness

densities for each scale.

A logistic regression (stepwise method, both selection) was used to compare monarch survival (total eggs at a site and 4th and 5th instars collected) to the independent variables of interest (Table 3 and Table 4) at the three scales (radii of 100, 200, and 400 m). Similarly, the intensity of parasitism per site was compared to the explanatory variables of interest (Table 3). A logistic regression was conducted using the number of healthy monarchs reared from a study site and the number of remaining individuals (parasitized by tachinid flies or OE or died of unknown causes).

CHAPTER III

RESULTS

2015 Summary

Twelve study sites were established in 2015 and monitored for monarch larvae from August to October. Fourth and fifth instars were collected from 11 of these sites but only 8 study sites were used for analyses based on a sample size threshold of at least four caterpillars (Nail et al., 2015). During this sampling year 92 monarch larvae were collected from these 8 sites. Sixty-six emerged as healthy butterflies, 14 were parasitized by tachinid flies, 5 were infected with OE, and 7 died of other causes. The overall fly parasitism rate was 15.2% and parasitism rates per site varied from 5.6% to 25%. Fly parasitism was observed at 6 of the 8 study sites used for analyses. Parasitism by OE was observed at 3 of the 8 study sites and ranged from 9.1% to 40% at individual sites. Larvae collected during the 2015 field season had an overall OE parasitism rate of 5.4%.

2016 Summary

Twenty-eight study sites were sampled in the fall of 2016 (August through October). Eleven of the 12 study sites used in 2015 were also sampled in 2016, and an additional 17 study sites were established. However, only 16 study sites met the sample size threshold of at least 4 larvae collected throughout the field season. Therefore 16 study sites were used in the analyses of the 2016 data and these sites included 4 of the same study sites used in the 2015 analyses. A total of 267 monarch larvae were collected in 2016. Additionally, a total of eleven chrysalises were collected at 3 study sites from structures near the milkweed patch or on a milkweed plant. There were 168 healthy monarchs, 75 parasitized by tachinid flies, 6 infected with OE, and 18 that died of other causes. The overall fly parasitism rate was 28.1% and the overall OE parasitism rate was 2.2%. Fly parasitism was observed at 9 of the 16 sites used for analyses and the parasitism rate per site ranged from 10.2% to 80% (Figure 6). Three of the 16 study sites had monarchs that were infected with OE and the parasitism rates at these sites ranged from 6.1% to 28.6%.

Monarch Oviposition

A multiple linear regression was used to determine the interaction between the explanatory variables of interest and monarch egg abundance at a location (Table 4). The stepwise selection of the set of variables that best fits the dataset revealed that average maximum plant height was the only significant variable of interest (estimate= 1.00, standard error= 0.202, p-value= <0.001, adjusted R^2 = 0.553). There was a positive relationship between plant height and total number of eggs per site and this analysis indicates that for every 1-cm increase in plant height the total number of eggs per site is expected to increase by an average of 1.00 eggs (Figure 3). Patch richness density was not selected in the best-fit model and thus landscape context did not significantly influence egg abundance. The other explanatory variables (predator species richness, aphid density, percent herbivory, and number of years sampled) were also excluded from the best-fit model for the total number of eggs per site.

Monarch Egg to 4th and 5th Instar Survival (Influence of Predation)

While monarch egg abundance was only influenced by one of the six possible explanatory variables, monarch survival from egg to 4th and 5th instar was influenced by all variables except for the number of years sampled (Table 4). The model that best explained the data set for survival at the 100- and 200-m scales included average maximum plant height, predator species richness, and maximum aphid density of 101-1000 aphids per plant. The model for the analysis at the 400-m scale similarly included predator species richness and maximum aphid density (101-1000 aphids per plant) but also incorporated the two highest maximum percent herbivory categories (5-25% and >25%), and patch richness density.

Plant height had a positive effect on oviposition (see previous section) but exhibited a negative effect on monarch survival. The average maximum plant height negatively influenced survival at the 100- and 200-m scales (estimate= -0.011, standard error= 0.005, p-value= 0.039, Figure 4A). Therefore, as plant height increased by 1 cm the odds of survival decreased by 0.989.

The three variables related to the invertebrate community had varying effects on monarch survival from egg to 4th and 5th instar. Predator species richness had a significant positive effect on survival at the 100- and 200-m scales (estimate= 0.434, standard error= 0.119, p-value= <0.001, Figure 4B) but did not have a significant effect at the 400-m scale (estimate= 0.226, standard error= 0.116, p-value= 0.052). Thus, as predator species richness increased at the two smallest scales the odds of survival increased by a factor of 1.544. Conversely, both aphid density and percent herbivory had a negative effect on monarch survival. A maximum density of 101-1000 aphids per plant decreased the odds of survival by a factor of 0.365 at the 100- and 200-m scales (estimate= -1.009, standard error= 0.240, p-value= <0.001, Figure 4C) and decreased the odds of survival by 0.454 at the 400-m scale (estimate= 0.789, standard error= 0.257, p-value= 0.002, Figure 5A). Lastly, herbivory negatively influenced survival but only at the 400-m scale. The maximum percent herbivory category of >25% decreased the odds of survival by 0.486 (estimate= -0.722, standard error= 0.294, p-value= 0.014, Figure 5B). The maximum percent

herbivory category of 5-25% also had a negative impact on the odds of survival but this effect was not significant (estimate= -0.863, standard error= 0.560, p-value= 0.123).

Finally, patch richness density at the 400-m scale positively influenced egg to late instar survival. As patch richness density increased the odds of survival increased by a factor of 1.077 (estimate= 0.074, standard error= 0.027, p-value= 0.006, Figure 5C). This measure of landscape context was included in the best-fit model only at the 400-m scale and only in the context of egg to 4th and 5th instar survival.

Monarch 4th and 5th Instar to Adult Survival (Influence of Parasitism)

A logistic regression was used to compare the number of healthy adults to the number of other larvae (parasitized by OE or tachinid flies or died of unknown causes) at a site. Two of the six possible variables had a significant effect on monarch 4th and 5th instar to adult survival (Table 4). Predator species richness had a positive impact on the number of healthy adult monarchs with an increase in one predator species category recorded at a site increasing the odds of survival by 1.5 butterflies (estimate= 0.403, standard error= 0.181, p-value= 0.026, Figure 7A). This relationship was detected at the three scales of interest. Maximum aphid density was the second variable with a significant effect on monarch 4th and 5th instar to adult survival. The aphid density category of 101-1000 aphids per plant decreased the odds of the number of healthy adult monarchs by a factor of 0.406 (estimate= -0.901, standard error= 0.398, p-value= 0.023, Figure 7B). Patch richness density determined for 100 m, 200 m and 400 m did not improve the model based on stepwise method using bidirectional elimination. Thus landscape context did not significantly influence 4th and 5th instar to adult survival in this study.

CHAPTER IV

DISCUSSION

Monarch egg abundance, egg to 4th and 5th instar survival, and 4th and 5th instar to adult survival were influenced differently by plant height, predator species richness, aphid density, percent herbivory, and patch richness density (Table 3). The number of eggs at a site was positively influenced by plant height and the linear regression displayed an almost one-to-one ratio for the increase in plant height and the corresponding number of eggs at a site. This suggests adults may detect taller plants more readily and these plants may provide more resources for developing caterpillars. Possible interactions from invertebrate community level metrics or landscape level metrics did not significantly impact this model and, thus, plant characteristics may be the most important factor for monarch oviposition.

Zalucki and Kitching (1982b) also found that oviposition was higher on larger plants and plant height may be an accurate indicator of overall plant size and quality for some milkweed species. Different habitat types such as gardens and natural areas experienced significantly different oviposition rates in one study and the higher plant quality found in gardens may have contributed to this outcome (Cutting and Tallamy, 2015). Future research should focus on determining the impact of certain management practices on host plant quality and the subsequent influence on monarch oviposition.

Monarch survival from egg to 4th and 5th instar was impacted by several of the measured parameters, depending on spatial scale. Predator species richness positively impacted survival at the two smallest scales, and may be an indicator of the overall quality of the milkweed patch. This is supported by habitat suitability models created for monarch egg and larvae, as well as for monarch predators and parasitoids (McCoshum et al., 2016). Areas that are highly suitable for monarchs overlap with predicted occurrence locations for the greatest number of predators (McCoshum et al., 2016). Also, there may be interactions between the predator species themselves and/or with other prey at a site, which may further contribute to the observed patterns. Thus, monarch eggs are more likely to survive to late instars when a greater number of predator species are present.

The influence of aphid density on egg to 4th and 5th instar survival may provide more information on the impact of one of these specific predators. Previous research found it difficult to separate the interaction of ants and aphids on monarch survival because ant presence is significantly correlated with aphid density (Prysby and Oberhauser, 2004). Ants acquire food in the form of honeydew from aphids and, therefore, ants may be influenced by aphid density (Henderson and Jeanne, 1992). Several of our study sites experienced the greatest maximum aphid density of 101-1000 aphids per plant, which had a negative impact on monarch egg to 4th and 5th instar survival. Thus, higher aphid densities at a site may lead to higher ant predation on monarch eggs and larvae.

Another significant trend that emerged from analyses of the survival of eggs to 4th and 5th instars was the impact of landscape heterogeneity on monarch survival. Patch richness density was used as the metric of interest to examine the influence of landscape context on monarch oviposition and survival. However, patch richness density only had a significant effect on egg to 4th and 5th instar survival and only at the 400-m scale. This finding demonstrates the importance of scale when detecting the impact of landscape context on community interactions such as

predation (Levin, 1992). Additionally, areas along the urban-rural gradient can be viewed as providing similar benefits expected from other heterogeneous landscapes (Pereira-Peixoto et al., 2016). A higher diversity in habitat types may facilitate more diverse insect communities and reduce predation pressures on monarch eggs and caterpillars (Tschardt et al., 2012). Other studies observed landscape context as influencing host-parasite interactions (Thies et al., 2003; Bennett and Gratton, 2012), but this effect was not detected for my analyses of late instar to adult survival.

Invertebrate community level effects were the only metrics that were significant for monarch 4th and 5th instar to adult survival. Of these metrics, aphid density had a negative impact on survival and predator species richness had a positive impact on survival. Survival from late instar to adult primarily reflects parasitoid mortality in this study since we removed 4th and 5th instars from the field to study parasitism. These results suggest tachinid flies may be more likely to parasitize monarch caterpillars at locations with high densities of aphids, and less likely to parasitize monarch caterpillars at locations with more predator species. Natural enemies such as tachinid flies may be able to locate prey based on plant volatiles that are released through herbivore damage (Paré and Tumlinson, 1999). Thus tachinid flies may be able to detect milkweed plants with higher aphid densities because of the presence of increased volatiles. While higher aphid densities increased tachinid fly parasitism, tachinids were less likely to parasitize monarchs in areas with a high diversity of other predators. Monarch caterpillars parasitized by tachinid flies may also be vulnerable to predation by other invertebrates and thus parasitism rates may be influenced by the diversity of monarch predators at a site (Oberhauser et al., 2015). Future studies should estimate predator abundance in addition to diversity to further evaluate the relationship between tachinid fly parasitism rates and other invertebrate community dynamics.

These findings have important implications for monarch conservation. Additional research should evaluate whether managing for beneficial insects such as predators as well as

reducing aphid densities in garden environments could reduce monarch mortality due to tachinid flies. These two factors explained more of the survival of 4th and 5th instars to adults than variables related to plant characteristics or landscape context. Thus, it is possible that management practices affecting the invertebrate community could reduce monarch mortality due to tachinid flies in gardens and other green spaces.

Parasitoid wasps and other pupal predators may also influence monarch survival from 4th and 5th instar to adult, but would not have been observed for the 4th and 5th instars that we collected for this study since they were removed from study sites prior to pupation. *Pteromalus cassotis* (Hymenoptera: Pteromalidae) is a notable pupal parasitoid of monarchs and has been observed parasitizing up to 100% of chrysalises at a study site (Stenoien et al., 2015).

Additionally, monarch chrysalises are susceptible to other predators such as paper wasps and ants (Oberhauser et al., 2015; McCoshum et al., 2016). I collected eleven chrysalises from our study sites, but I did not observe any pupal parasitoids, although a tachinid fly did emerge from one of the field-collected chrysalises. Thus, survival rates from 4th and 5th instar to adult observed for this study may be an overestimate if pupal parasitism would have occurred. Pupal parasitism and predation may play an important role in monarch survival and future research should incorporate this mortality factor into assessing habitat suitability for monarchs (McCoshum et al., 2016).

Recent research suggests that the region including Oklahoma and Texas contributes, on average, about 11% of the monarchs inhabiting overwintering grounds (Flockhart et al., 2017). While the Midwest contributed the largest mean percentage (38%) of the six possible regions (Midwest, North-central, Northeast, Northwest, Southeast, and Southwest), the annual variability in regional contributions is high, emphasizing the need for conservation efforts that support the entire breeding range. The south central U.S. has traditionally been viewed as important for monarchs for providing nectar resources during spring and fall migration, and milkweed for spring reproduction. However, not much is known about monarchs that reproduce in the southern

region late in the year. These reproductively active monarchs are referred to as pre-migrants, fall-breeding monarchs, or late-breeding monarchs (Baum and Mueller, 2015), and the offspring produced by these monarchs are referred to as the “5th generation”. I tagged (with Monarch Watch tags) and released all individuals that emerged as healthy adult butterflies in 2015 and 2016. Two of the 66 butterflies tagged in 2015 were recovered on the overwintering grounds in Mexico, representing a 3.03% tag recovery rate. Tag recoveries have not been released for 2016 yet. These tag recoveries indicate that 5th generation monarchs successfully migrate to Mexico and contribute to the number of monarchs observed on the overwintering grounds.

The impact of predators and parasitoids will vary across the monarch’s spring, summer, and fall breeding grounds and, thus, impact conservation efforts differently (McCoshum et al., 2016). Similar assessments of factors influencing monarch survival at different stages should be conducted throughout the monarch’s breeding grounds as well as across land use types.

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APPENDICES

Table 1: The predicted interactions between explanatory variables of interest and the monarch response variables (egg abundance, egg to 4th and 5th instar survival, and 4th and 5th instar to adult survival).

Category	Variable	Predicted Effect on Egg Abundance	Predicted Effect on Survival (Egg to 4 th and 5 th Instar)	Predicted Effect on Survival (4 th and 5 th Instar to Adult)
Plant Characteristic	Plant Height	Positive	Unsure	Negative
Invertebrate Community	Predator Species Richness	Positive	Negative	Positive
	Aphid Density	Negative	Negative	Negative
	Percent Herbivory	Negative	Negative	Negative
Landscape Context	Patch Richness Density	Positive	Negative	Positive
Other	Years Sampled	None	None	Unsure

Table 2: Oklahoma Ecological Systems Mapping Project vegetation types that were prevalent within the study area and the reclassified land cover classes used in calculating patch richness density.

Vegetation Types	Reclassified Land Cover Class
<ul style="list-style-type: none"> • Urban High Intensity • Urban Low Intensity 	Urban
<ul style="list-style-type: none"> • Ruderal Deciduous Woodland • Ruderal Deciduous Shrubland and Young Woodland • Ruderal Eastern Redcedar Woodland and Shrubland • Ruderal Mixed Deciduous-Eastern Redcedar Woodland 	Ruderal
<ul style="list-style-type: none"> • Crosstimbers: Pasture/Prairie • Crosstimbers: Post Oak- Blackjack Oak Forest and Woodland • Crosstimbers: Young Post Oak- Blackjack Oak Woodland • Crosstimbers: Post Oak- Eastern Redcedar Forest and Woodland • Crosstimbers: Post Oak- Eastern Redcedar Slope Forest • Crosstimbers: Post Oak- Blackjack Oak Slope Forest • Crosstimbers: Eastern Redcedar Woodland and Shrubland 	Crosstimbers
<ul style="list-style-type: none"> • South Central Interior: Riparian Hardwood Woodland • South Central Interior: Riparian Herbaceous Wetland • South Central Interior: Riparian Barrens • South Central Interior: Bottomland Shrubland and Young Woodland • South Central Interior: Bottomland Mixed Evergreen-Hardwood Forest • South Central Interior: Bottomland Hardwood Forest • South Central Interior: Bottomland Barrens 	South Central Interior
<ul style="list-style-type: none"> • Barren • Open Water 	Other
<ul style="list-style-type: none"> • Disturbed Soil Pasture 	Disturbed Soil Pasture
<ul style="list-style-type: none"> • Row Crops 	Row Crops
<ul style="list-style-type: none"> • Central Mixedgrass: Prairie/Pasture 	Central Mixedgrass

Table 3: The observed interactions between explanatory variables of interest and the monarch response variables (egg abundance, egg to 4th and 5th instar survival, and 4th and 5th instar to adult survival). Significant effects are noted in bold font.

Category	Variable	Observed Effect on Egg Abundance	Observed Effect on Survival (Egg to 4 th and 5 th Instar)	Observed Effect on Survival (4 th and 5 th Instar to Adult)
Plant Characteristic	Plant Height	Positive (all scales)	Negative (100- and 200-m scale)	None
Invertebrate Community Dynamics	Predator Species Richness	None	Positive (100- and 200-m scales)	Positive (all scales)
	Aphid Density	None	Negative : 101-1000 per plant (all scales)	Negative : 101-1000 per plant (all scales)
	Percent Herbivory	None	Negative : >25% (400-m scale)	None
Landscape Context	Patch Richness Density	None	Positive (400-m scale)	None
Other	Years Sampled	None	None	None

Table 4: Results of step-wise model selection in R for three separate monarch response variables (egg abundance, egg to 4th and 5th instar survival, and 4th and 5th instar to adult survival). Significant p-values are noted in bold font.

Response Variable	Scale (m)	Regression	Model Parameter	Estimate	Std error	z value	p-value
Egg abundance (Total eggs per site)	100, 200, and 400	Linear	Average Maximum Plant Height	1.00	0.202	4.949	<0.001
Survival (Egg to 4 th and 5 th Instar)	100 and 200	Logistic	Predator Species Richness	0.434	0.119	3.634	<0.001
			Maximum Aphid Density (101-1000 aphids per plant)	-1.010	0.240	-4.212	<0.001
			Average Maximum Height	-0.011	0.005	-2.065	0.039
	400	Logistic	Predator Species Richness	0.226	0.116	1.945	0.051
			Maximum Aphid Density (101-1000 aphids per plant)	-0.789	0.257	-3.069	0.002
			Maximum Percent Herbivory (5-25%)	-0.863	0.560	-1.542	0.123
			Maximum Percent Herbivory (>25%)	-0.722	0.294	-2.454	0.014
			Patch Richness Density	0.074	0.027	2.724	0.006
	Survival (4 th and 5 th Instar to Adult)	100, 200, and 400	Logistic	Predator Species Richness	0.402	0.180	2.228
Maximum Aphid Density (101-1000 aphids per plant)				-0.901	0.398	-2.262	0.024

Figure 1: Study sites for 2015 and 2016 field seasons. The black squares indicate 2015 study sites and the red circles indicate 2016 study sites. Study sites marked with both a black square and a red circle were sampled both years.

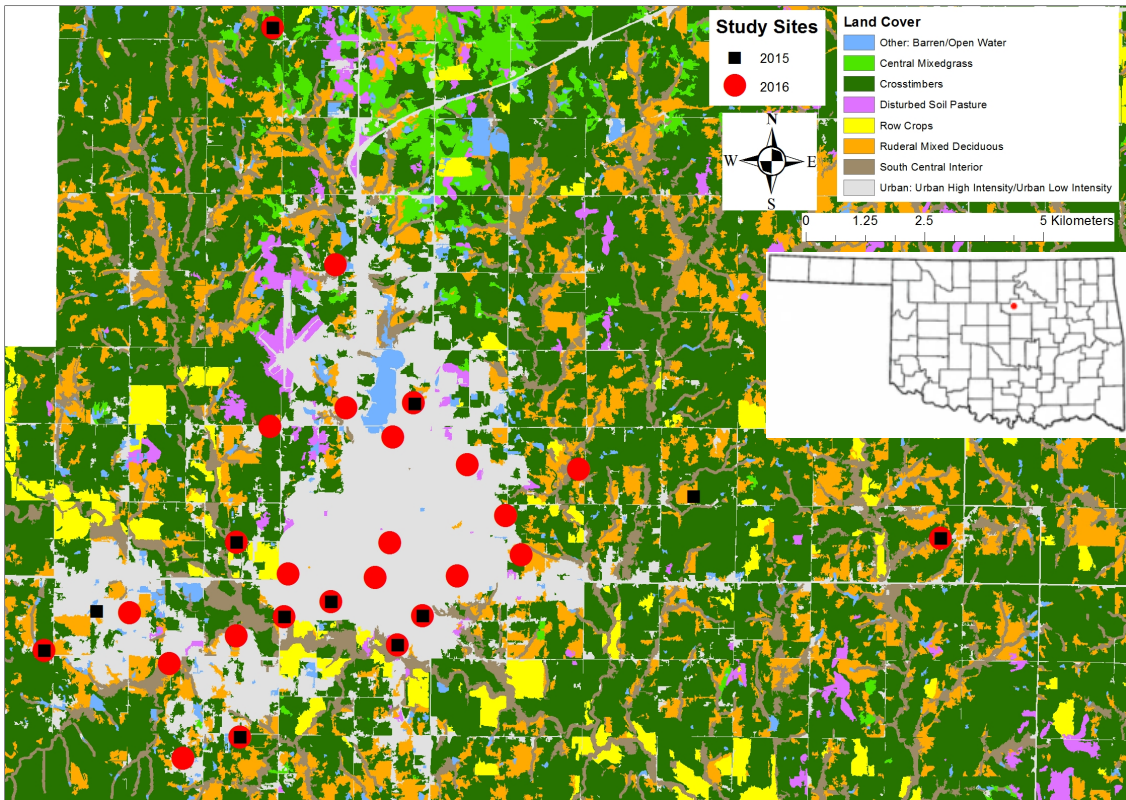


Figure 2: Example of a single study site analyzed at three radii (100, 200, and 400 meters) using the reclassified land cover classes from the Oklahoma Ecological Systems Mapping Project. The black point in the center represents the milkweed patch that was sampled during the field season.

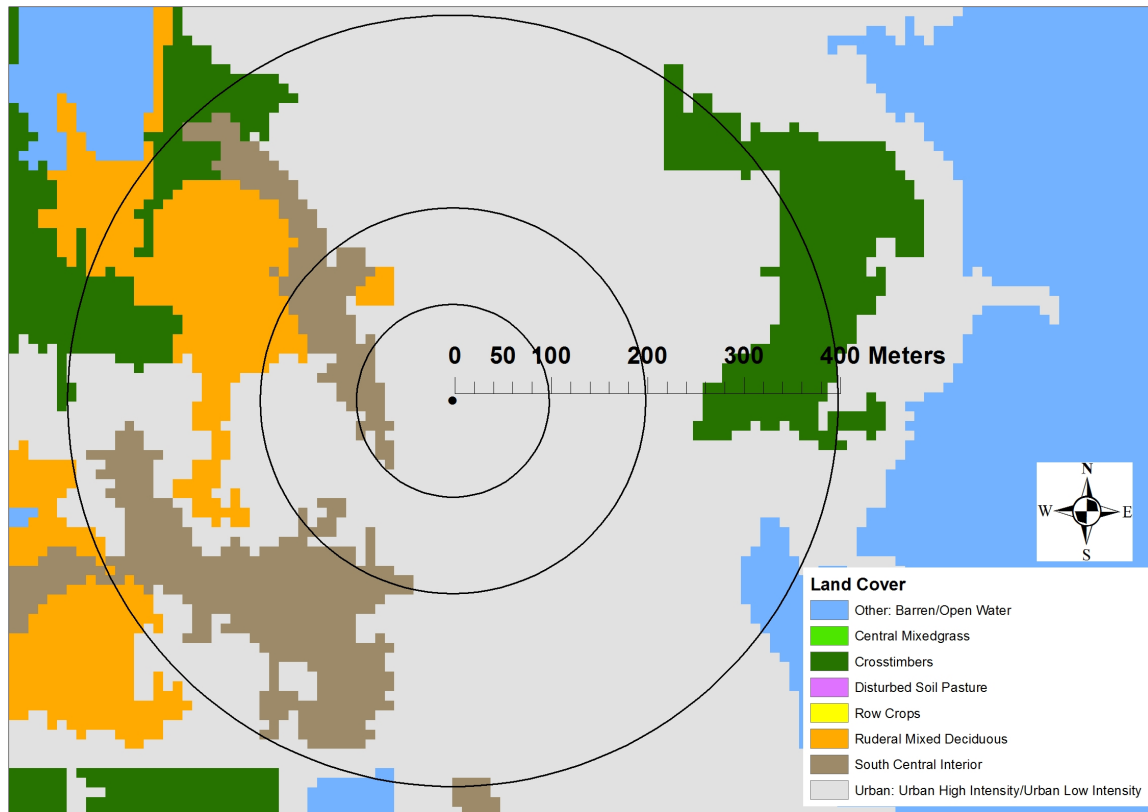


Figure 3: Linear regression comparing the total number of eggs at a site and the average maximum plant height at a site (estimate= 1.00, standard error= 0.202, p-value= <0.001, adjusted $R^2= 0.553$).

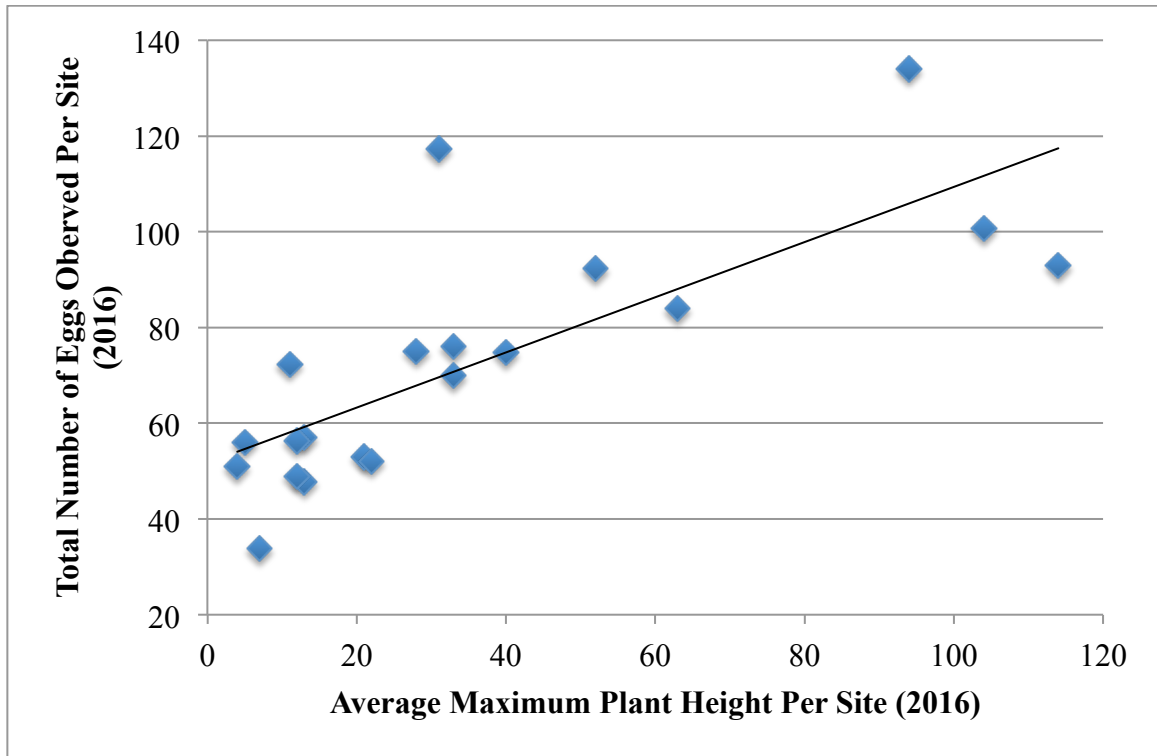


Figure 4A: Predicted values for average maximum plant height and egg to late instar survival at 100- and 200-m scales. The predicted values were derived from the logistic regression model that included predator species richness (estimate= 0.434, standard error= 0.119, p-value= <0.001), maximum aphid density of 101-1000 aphids per plant (estimate= -1.00, standard error= 0.240, p-value= <0.001), and average maximum height (estimate= -0.011, standard error= 0.005, p-value= 0.039). The best-fit line was derived using the predicted values.

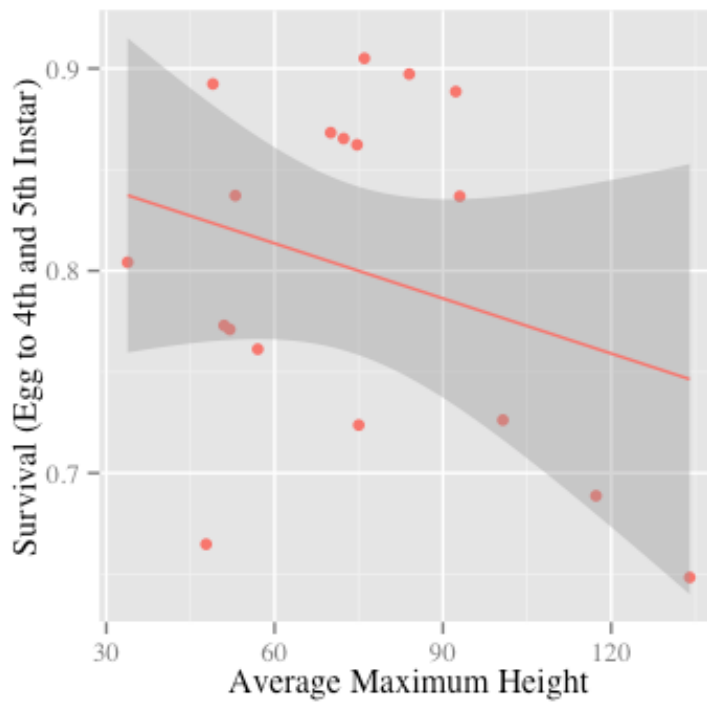


Figure 4B: Predicted values for predator species richness and egg to late instar survival at 100- and 200-m scales. The predicted values were derived from the logistic regression model that included predator species richness (estimate= 0.434, standard error= 0.119, p-value= <0.001), maximum aphid density of 101-1000 aphids per plant (estimate= -1.00, standard error= 0.240, p-value= <0.001), and average maximum height (estimate= -0.011, standard error= 0.005, p-value= 0.039). The best-fit line was derived using the predicted values.

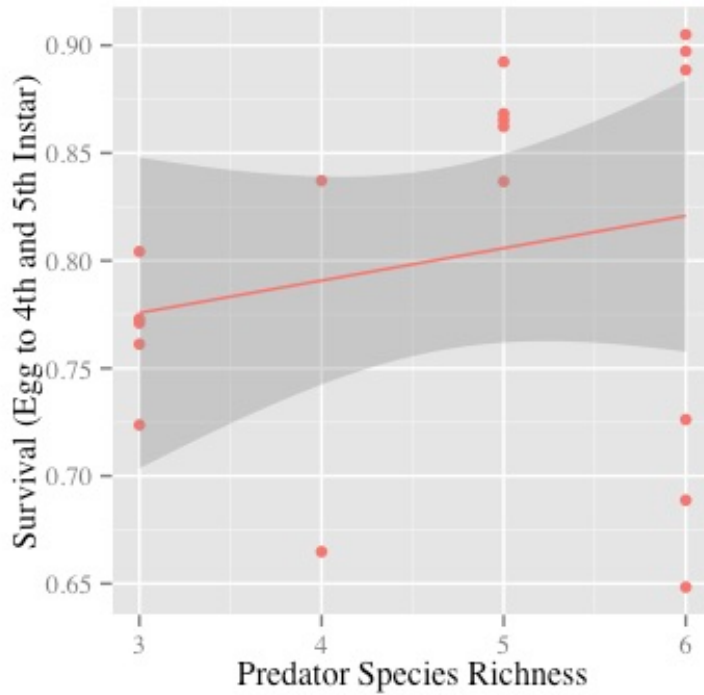


Figure 4C: Predicted values for maximum aphid density and egg to late instar survival at 100- and 200-m scales. The two highest categories for maximum aphid density are represented on the x-axis (11-100 aphids per plant and 101-1000 aphids per plant). The predicted values were derived from the logistic regression model that included predator species richness (estimate= 0.434, standard error= 0.119, p-value= <0.001), maximum aphid density of 101-1000 aphids per plant (estimate= -1.00, standard error= 0.240, p-value= <0.001), and average maximum height (estimate= -0.011, standard error= 0.005, p-value= 0.039).

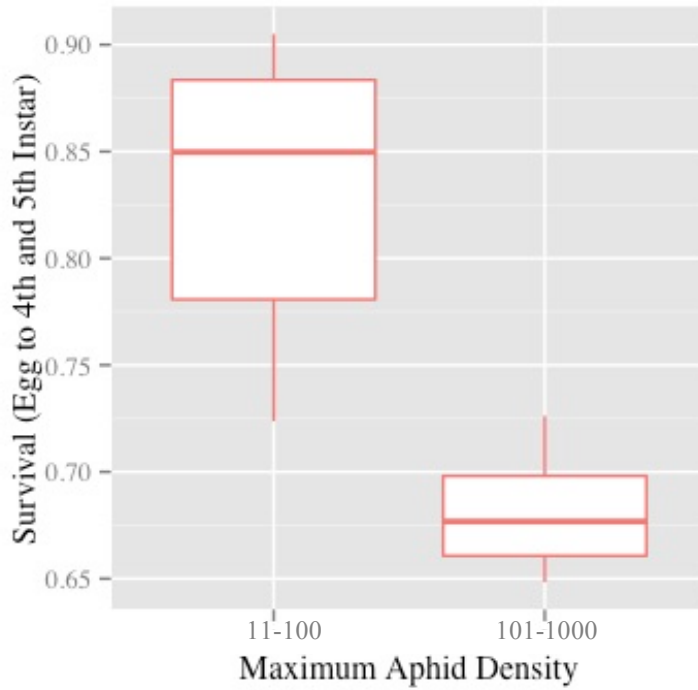


Figure 5A: Predicted values for maximum aphid density and egg to late instar survival at the 400-m scale. The two highest categories for maximum aphid density are represented on the x-axis (11-100 aphids per plant and 101-1000 aphids per plant). The predicted values were derived from the logistic regression model that included predator species richness (estimate= 0.226, standard error= 0.116, p-value= 0.051), maximum aphid density of 101-1000 aphids per plant (estimate= 0.789, standard error= 0.257, p-value= 0.002), maximum percent herbivory (estimate= -0.722, standard error= 0.294, p-value= 0.014), and patch richness density (estimate= 0.074, standard error= 0.027, p-value= 0.006).

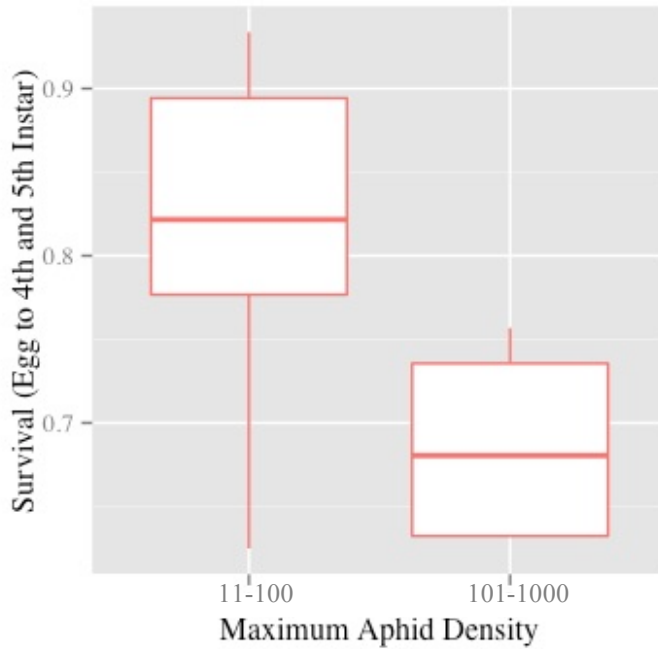


Figure 5B: Predicted values for maximum percent herbivory and egg to late instar survival at the 400-m scale. The three highest categories for maximum percent herbivory are represented on the x-axis (less than 5% leaf damage, 5-25% leaf damage, and greater than 25% leaf damage). The predicted values were derived from the logistic regression model that included predator species richness (estimate= 0.226, standard error= 0.116, p-value= 0.051), maximum aphid density of 101-1000 aphids per plant (estimate= 0.789, standard error= 0.257, p-value= 0.002), maximum percent herbivory (estimate= -0.722, standard error= 0.294, p-value= 0.014), and patch richness density (estimate= 0.074, standard error= 0.027, p-value= 0.006).

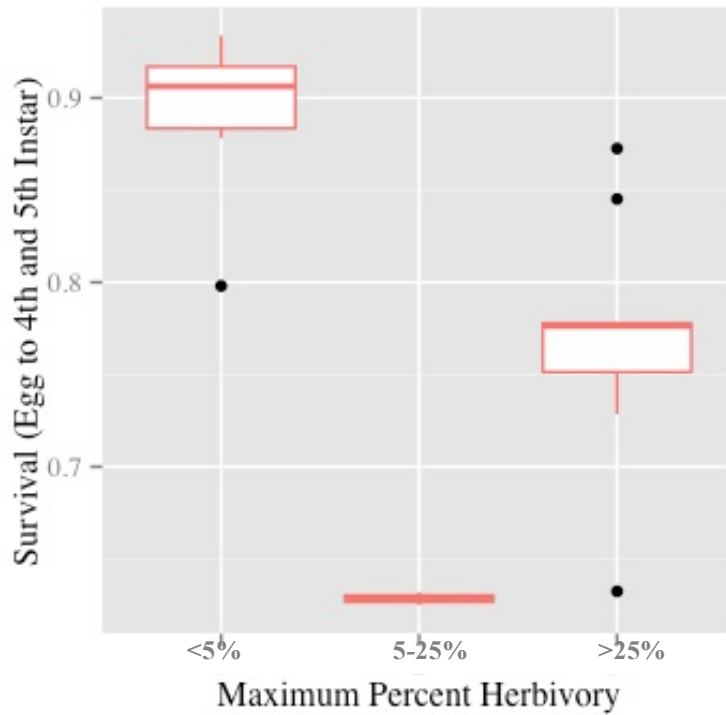


Figure 5C: Predicted values for patch richness density and egg to late instar survival at the 400-m scale. The predicted values were derived from the logistic regression model that included predator species richness (estimate= 0.226, standard error= 0.116, p-value= 0.051), maximum aphid density of 101-1000 aphids per plant (estimate= 0.789, standard error= 0.257, p-value= 0.002), maximum percent herbivory (estimate= -0.722, standard error= 0.294, p-value= 0.014), and patch richness density (estimate= 0.074, standard error= 0.027, p-value= 0.006). The best-fit line was derived using the predicted values.

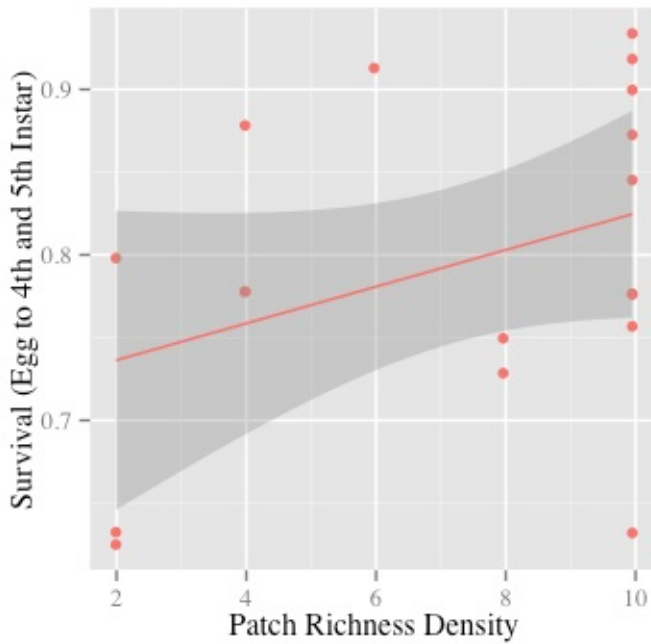


Figure 6: Overall tachinid parasitism rates at study sites in 2016 that met the minimum threshold of a sample size of four larvae collected.

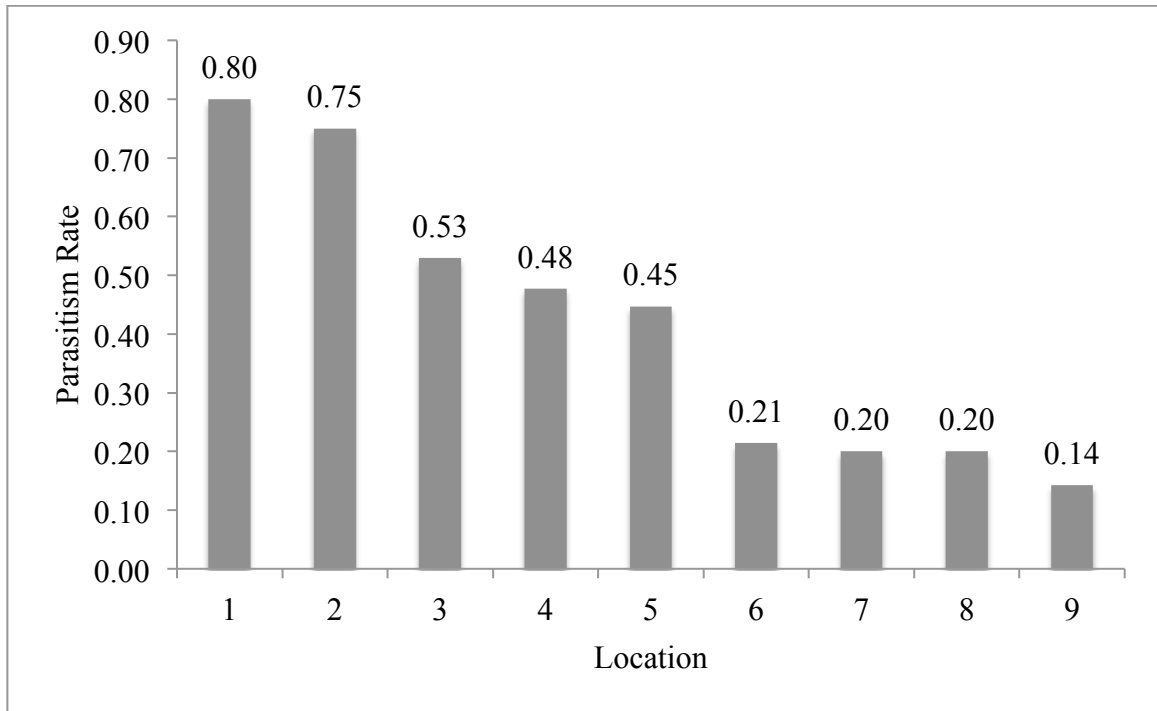


Figure 7A: Predicted values for predator species richness and 4th and 5th instar to adult survival at all spatial scales. The predicted values were derived from the logistic regression model that included predator species richness (estimate= 0.403, standard error= 0.181, p-value= 0.026) and maximum aphid density of 101-1000 aphids per plant (estimate= -0.901, standard error= 0.398, p-value= 0.024). The best-fit line was derived using the predicted values.

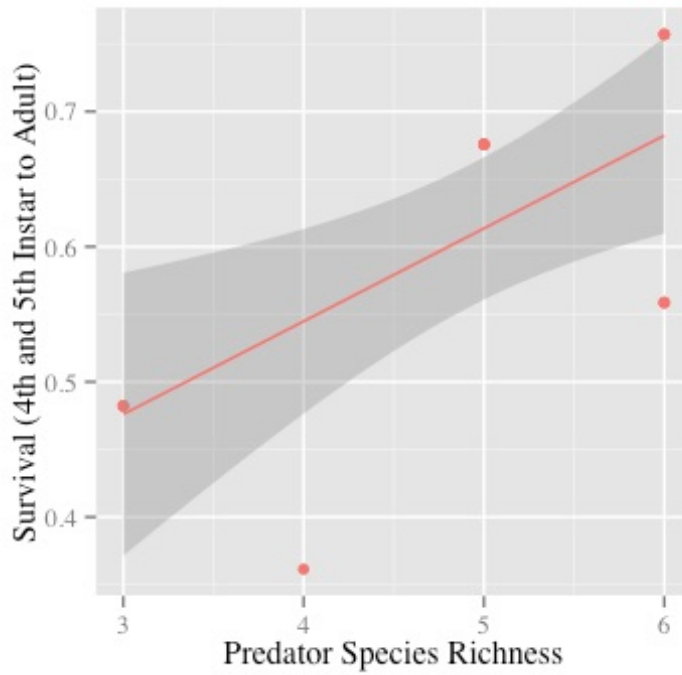
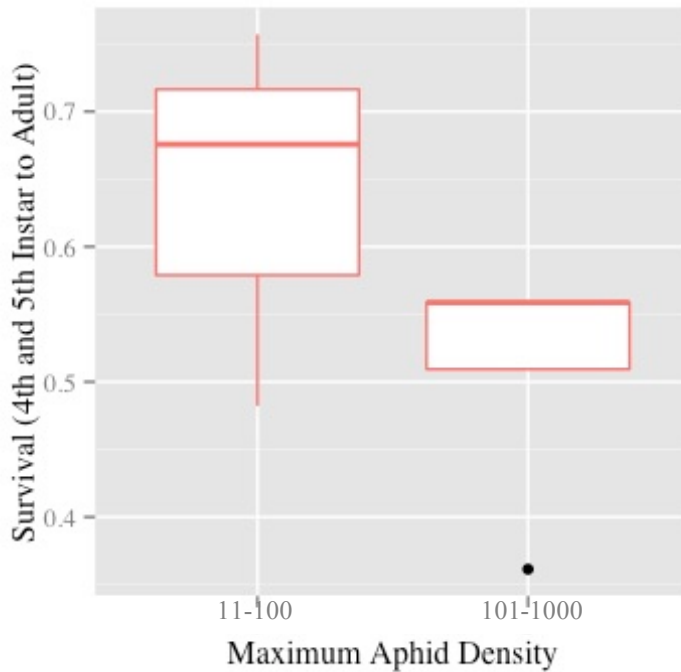


Figure 7B: Predicted values for maximum aphid density and 4th and 5th instar to adult survival at all spatial scales. The two highest categories for maximum aphid density are represented on the x-axis (11-100 aphids per plant and 101-1000 aphids per plant). The predicted values were derived from the logistic regression model that included predator species richness (estimate= 0.403, standard error= 0.181, p-value= 0.026) and maximum aphid density of 101-1000 aphids per plant (estimate= -0.901, standard error= 0.398, p-value= 0.024).



VITA

Kelsey Ann Deal

Candidate for the Degree of

Master of Science

Thesis: THE IMPACT OF LANDSCAPE DIVERSITY AND INVERTEBRATE
COMMUNITY DYNAMICS ON MONARCH BUTTERFLY (*DANAUS*
PLEXIPPUS) OVIPOSITION AND SURVIVAL

Major Field: Zoology

Biographical:

Education:

Completed the requirements for the Master of Science in Zoology at Oklahoma State University, Stillwater, Oklahoma in May, 2017.

Completed the requirements for the Bachelor of Arts in Biology at Baylor University, Waco, Texas in 2013.

Experience:

History of Mathematics Teaching Assistant

2017 Spring Semester

Patterns in Science Teaching Assistant

2016 Fall Semester

Introductory Biology Laboratory Teaching Assistant

2014 Fall Semester, 2015 Spring Semester, 2015 Fall Semester, 2016
Spring Semester

Mentoring of 3 College of Education Pre-service Science Teachers

2015 Spring Semester, 2016 Spring Semester, 2017 Spring Semester

Professional Memberships:

Zoology Graduate Student Society at Oklahoma State University (2014 – 2017)

Sigma Xi National Research Society (2015 – 2017)

Citizen Science Association (2015– 2016)