THE INTERACTING INFLUENCES OF HABITAT CONTEXT AND PREDATORS ON MONARCH BUTTERFLY (*DANAUS PLEXIPPUS* L.) OVIPOSITION AND SURVIVAL IN AGRICULTURAL LANDSCAPES

By

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ABSTRACT

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Monarch butterflies (*Danaus plexippus* L.) are among the most recognizable and beloved insect species in North America. However, concern about the long-term persistence of monarch populations has grown during recent years due to the declining overwintering aggregations of both the eastern and western population segments. One hypothesized driver of the decline of the eastern population segment is the reduction of milkweed host plants from agricultural landscapes through the use of herbicide-tolerant row cropping systems. Proponents of this hypothesis reason that with access to fewer host plants, monarchs are not able to lay their full complements of eggs during their lifetimes, resulting in lower population growth rates during the summer breeding season in the core of the breeding range. This hypothesis has motivated research efforts to determine which species of milkweed attracts the greatest oviposition rates and where these plants can be positioned to maximize egg laying by monarchs. Here I propose that monarch conservation efforts would also benefit from increased knowledge regarding the impact of predators on monarch eggs and larvae, as boosting survival during early life stages could substantially increase monarch breeding productivity. I performed several field experiments to investigate how habitat context and interactions with predators influence monarch oviposition and predation pressure. Using sentinel milkweed host plants and monarch eggs placed in various habitat treatments, I found that monarchs exhibit oviposition habitat preferences that vary between corn and grasslands depending on the year. I also showed that monarch egg survival over 72 h varies by year, but can be as low as 10% in grasslands. These experiments demonstrate that numbers of eggs observed by previous weekly surveys of various habitats reflect both the effects of oviposition preferences and predation rates and that grasslands represent relatively risky monarch egg habitats. Next, I sought to determine which predators were responsible for monarch egg mortality in grasslands by video monitoring over 150 monarch eggs. I found that a diversity of predators consumed monarch eggs, with a plurality of eggs consumed by spiders and a majority of eggs consumed during nocturnal hours. Finally, I sought to determine how the presence of ants influences monarch oviposition patterns and how aphid presence and species on host plants shapes monarch interactions between ants and monarch neonates. I measured first instar survival at 96 h on plants infested with *Myzocallis asclepiadis*, *Aphis asclepiadis*, or no aphids and with and without ants excluded and found the lowest monarch survival on *Myzocallis*-infested plants , but this effect disappeared when ants were excluded. I also found that wild monarchs laid significantly more eggs on plants with ants excluded, indicating that monarchs avoid ovipositing on plants with ants. Taken together, these studies demonstrate that reducing predation pressure on monarchs during early life stages has the potential to serve as an effective way to increase monarch productivity on limited land space in agricultural areas and provides important information regarding how habitat context and interactions with predators and other arthropods interact to influence monarch oviposition patterns and egg and early larval survival. I interpret the results of these studies in the context of monarch conservation and make recommendations for further study.

Dedicated to Michaela and Jay

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CHAPTER 1: INTRODUCTION

Importance of Agroecosystem Biodiversity

Agroecosystems represent the Earth's dominant terrestrial biome (Ellis and Ramankutty 2008) and are essential to meet growing global needs for food, energy, and ecosystem services (Tilman et al. 2002, Zhang et al. 2007, Chappell and LaValle 2009, Foley et al. 2011, Alexander et al. 2015). However, intensification and expansion of agriculture are among the leading drivers of global environmental degradation and biodiversity declines through the processes of habitat loss, landscape simplification, increased chemical inputs, and disruption of ecological processes (Tilman et al. 2001, Tscharntke et al. 2005). Beyond the moral and ethical arguments for biodiversity conservation, these trends generate an unsustainable path by undermining the ecosystem services on which agricultural production itself depends. For example, landscape simplification destabilizes natural pest control and pollination services by removing habitat requisites for source populations of beneficial arthropods (Bianchi et al. 2006, Klein et al. 2007, Rusch et al. 2016). While the impact of species loss on agroecosystem function remains largely unknown, it could involve non-linear effects and positive feedbacks, which argues for a precautionary approach regarding decisions involving biodiversity (Chapin et al. 2000). Furthermore, conservation biologists warn that currently protected natural areas are insufficient to conserve much of global biodiversity (Tscharntke et al. 2005). As more is demanded from agroecosystems, the idea that we must design multifunctional landscapes is increasingly recognized, and reconciling biodiversity conservation and agricultural production is one of the major challenges to sustainable agriculture (Tilman et al. 2001, Brussaard et al. 2010, Raudsepp-Hearne et al. 2010, Quinn 2012, Tscharntke et al. 2012, Werling et al. 2014).

Landscape Simplification Drives Agroecosystem Biodiversity Loss

Landscape simplification resulting from agricultural intensification is considered an important mechanism of agroecosystem biodiversity loss (Tscharntke et al. 2005). This process involves loss of habitat diversity and natural areas at local and landscape levels as crop fields become larger, increasingly homogenized, and spatially connected (Benton et al. 2003). Several decades of research has repeatedly highlighted this pattern, particularly in highly modified European landscapes. Stoate et al. (2001) reviewed research documenting agriculturally-driven biodiversity loss across Europe during the post-World War II period and found a consistent pattern of species loss associated with hedgerow removal, farm amalgamation, and increased chemical inputs. In their review of British agroecosystems Robinson and Sutherland (2002) largely attribute post-World War 2 declining species richness of plants, arthropods, birds, and mammals to increased chemical inputs and removal of non-crop vegetation at the local-level and loss of habitat heterogeneity at the landscape-level. Benton et al. (2003) found similar patterns across Europe and argued that multi-scale practices associated with agricultural intensity drive biodiversity loss through the common mechanism of habitat homogenization. For example, they point out that agrochemical use harms biodiversity both directly by eliminating pests and other species but also indirectly through the loss of structural habitat heterogeneity. A review by Stoate et al. (2009) updated the status of European agroecosystems to reflect dramatic agricultural policy and economic changes in the early 21st Century. They found some positive outcomes for biodiversity thanks to reduced chemical use, wetland restoration, and protection of some natural areas. However, threats to biodiversity through landscape simplification persist, stemming from increasing demand for bioenergy, trade liberalization, and changes to subsidy programs.

Although much of the literature linking agricultural intensification to biodiversity loss is based on European landscapes, these patterns are evident in other regions. Recent grassland bird declines in North America have been attributed to agricultural landscape simplification (Brennan and Kuvlesky 2005, Quinn et al. 2017). Flynn et al. (2009) reviewed studies documenting relationships between agricultural intensity and biodiversity throughout much of North and Central America (spanning Costa Rica to the northern United States) and found patterns of both species richness and trait diversity loss for mammals and birds (but not herbaceous or understory plants) associated with agricultural intensification. The effects of agricultural intensification on biodiversity in North America are best documented for arthropods, with notable declines within intensified landscapes of pollinators (Kremen et al. 2002, Grixti et al. 2009) and natural enemies (Purtauf et al. 2005, Chaplin-Kramer et al. 2011). Loss of arthropod natural enemies drives increased pesticide use, highlighting the potential for costly economic and environmental feedbacks associated with high-input agricultural practices (Meehan et al. 2011). Recently, long-term monitoring studies have brought attention to the phenomenon of global insect declines, with agricultural intensification considered a leading cause (Hallmann et al. 2017, Sánchez-Bayo and Wyckhuys 2019; but see Saunders et al. 2019a). These studies exemplify the usefulness of insects and other arthropods as indicators of environmental change and as model taxa for understanding ecological functions, associated feedbacks, and underlying drivers of biodiversity in agricultural and other ecosystems.

Stemming the Loss of Biodiversity in Agroecosystems

With the recognition of the broad trend of biodiversity loss through agricultural intensification, researchers and decision makers debate the best way to design landscapes that are simultaneously economically and ecologically sustainable. One potential for merging production agriculture with biodiversity conservation comes from realizing synergies between these related goals and designing landscapes that serve multiple functions (Brussaard et al. 2010, Raudsepp-Hearne et al. 2010). This will require a greater understanding of the function of biological diversity within agroecosystems, mechanisms leading to the loss of species, and specific practices which support both biodiversity and economic interests (Chappell and LaValle 2009). Already, progress has been made toward improving ecosystem services in some agricultural landscapes. For example, some midwestern US farmers are strategically adopting prairie plantings within crop fields to reduce costly erosion and to support native communities including pollinators and biocontrol species (Liebman et al. 2013, Schulte et al. 2017). To optimize economic and conservation goals, practitioners must determine where biodiversity conservation can be integrated into agricultural practices and clearly delineate circumstances when land must be removed from agriculture. Due to limited land space, a tradeoff exists between intensively farming smaller plots of land in order to free up more areas for conservation and farming larger areas in ways that allow biodiversity to be incorporated. The tension between these two strategies has been termed land sparing versus sharing (Green et al. 2005)

Rapidly changing agricultural technologies and practices further complicate the goal of designing multifunctional landscapes. Some of these changes have positive outcomes for agricultural biodiversity. For example, the widespread adoption of reduced and no-tillage practices in the mid-20th century promotes greater species richness of soil microbes and invertebrates (Landis et al. 2000, Derpsch et al. 2010). But other practices, particularly the use of pesticides and fertilizers, tend to reduce opportunities to incorporate biodiversity conservation in agricultural landscapes (Stoate et al. 2009). The development of genetically-modified crops can have both positive and negative outcomes for biodiversity and ecosystems (Wolfenbarger and Phifer 2000, Stoate et al. 2009, Jacobsen et al. 2013, Emani 2014). For example, *Bt* cotton is much less harmful to non-target natural enemy communities than traditional insecticides, because it is highly targeted to organisms consuming the crop (Naranjo 2006). However, genetically modified cropping systems still have the potential to indirectly harm non-target organisms (Marvier et al. 2007), and uncertainties about unintended consequences of new agricultural practices and technologies highlight the need for more research about the basic processes driving species loss in agroecosystems.

Monarch Butterfly Declines in Agricultural Landscapes

Monarch butterflies (*Danaus plexippus* L.) are among the most recent species associated with agroecosystems to suffer precipitous population declines. The decline of this abundant and widespread species in eastern North America highlights the challenge of maintaining biodiversity within agricultural landscapes, where often the primary objective is to intercept as much primary production as possible. Monarch butterflies may have benefited from breeding habitat associated with 20th century agricultural practices, which are estimated to have supported comparable amounts of milkweed to the prairies they replaced in midwestern North America and potentially more milkweed than occurred in the pre-settlement forests of eastern North America (Pleasants 2015). But shrinking overwintering aggregations of both the eastern and western North American population segments since the 1990’s indicate that monarch populations are in decline (Brower et al. 2012, Semmens et al. 2016, Schultz et al. 2017, The Xerces Society 2019). Monarchs have long been a model organism for studying ecological and evolutionary phenomena, and a substantial body of literature exists on monarch biology. Due to concerns about monarch population viability, this species increasingly serves as both a flagship and model species for agroecosystem biodiversity conservation (Guiney and Oberhauser 2009). Indeed, monarchs may be representative of a broader trend of species loss in agricultural landscapes. However, important questions still remain about the ecology of monarch butterflies. Developing science-based strategies to restore populations of this once plentiful and culturally important species holds the potential to generate attention, support, and conservation solutions for other imperiled agricultural species (Diffendorfer et al. 2014, Gustafsson et al. 2015, Agrawal 2019).

Estimating Monarch Butterfly Population Declines

Monarch Migratory Cycle

Although concern has grown recently about the viability of the eastern North American population of monarch butterflies, estimating the size of this population is made difficult by its unique and complicated migratory life history (Wilcox et al. 2019). The eastern monarch population undertakes a multigenerational annual migration between overwintering sites in central Mexico and breeding sites in the northern United States and southern Canada (Brower 1995, Urquhart and Urquhart 2008) (**Fig. 1.1**). The northward flight in spring is accomplished over two successive generations. Subsequently within the northern breeding range 2–3 generations are produced during the summer, and the entire southward migration to overwintering sites is completed by a single generation in reproductive diapause (Solensky 2004). As a consequence of the multiple biotic and abiotic environmental requisites for the species to complete this annual cycle, determining the relative influence of different variables on monarch population dynamics is very complex (Flockhart et al. 2015, Saunders, Ries, et al. 2019). Although this migratory behavior complicates the understanding of monarch biology, estimates of the population at certain stages of the migration do provide consistent annual gauges of the population size (Inamine et al. 2016, Saunders, Ries, et al. 2019).

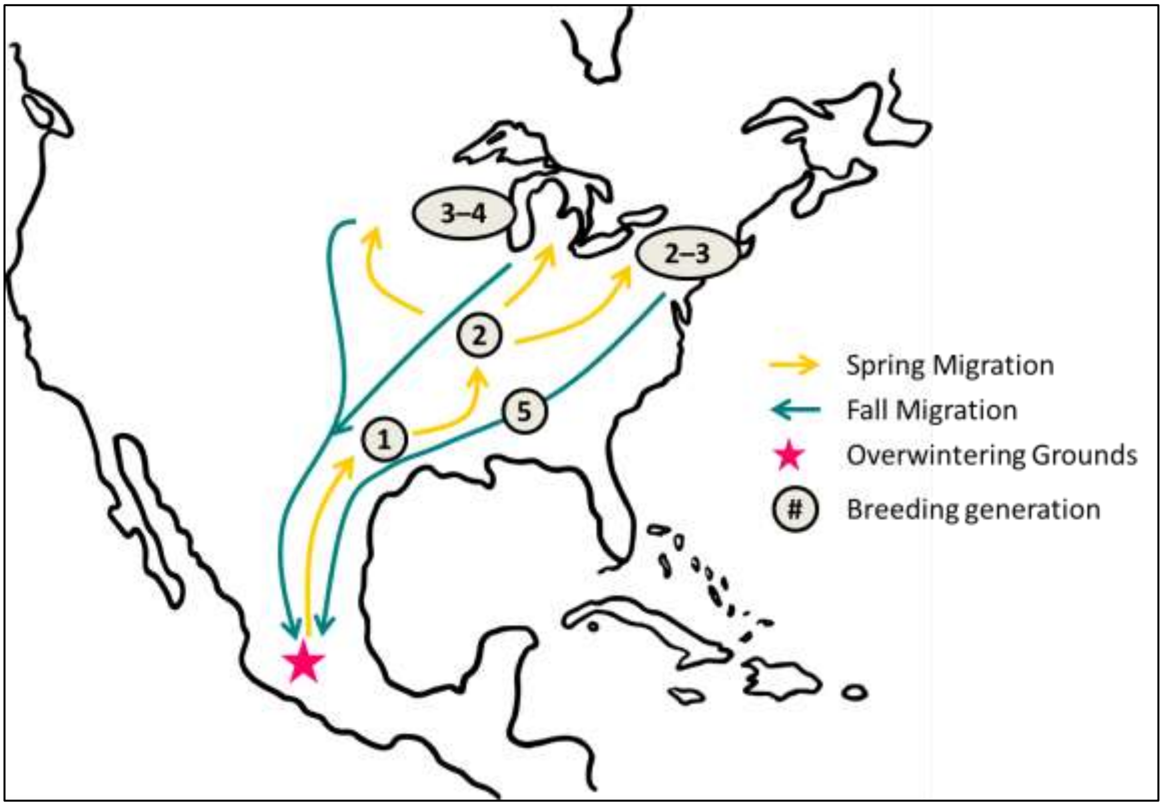

Figure 1.1. Map depicting the eastern North American monarch butterfly annual migration and seasonal breeding habitat (Adapted from Oberhauser et al. (2017)).

Overwintering Aggregation Population Estimates

Measuring the area occupied by the overwintering monarch aggregation in central Mexico generates one useful estimate of the size of the monarch population. Reliable measurements began in the winter of 1994–1995 and demonstrate a downward trend during the past two decades (**Fig. 1.2**). From 1994 to 2018 the overwintering aggregation occupied a mean area of approximately 5.67 ha, with a range of 0.67 ha (winter of 2013–2014) to 18.19 ha (winter of 1996–1997). The population fluctuates widely, but multiple studies show a significant, long-term population decline (Brower et al. 2012, Inamine et al. 2016, Saunders, Ries, et al. 2019).

And although the population has rebounded significantly following its lowest recorded size in 2013, the mean overwintering aggregation of 3.46 ha since 2004 is still below the recovery target of 6 ha (Thogmartin, López-Hoffman, et al. 2017) . Researchers hypothesize numerous non-exclusive drivers of recent monarch overwintering aggregation population declines including increasing pathogen occurrence, reduced and degraded breeding and overwintering habitat, extreme weather events, and increased mortality during migration (Solensky 2004, Belsky and Joshi 2018). Population estimates made at other stages of the annual cycle have been used to shed some light on the mechanisms driving the overwintering monarch declines.

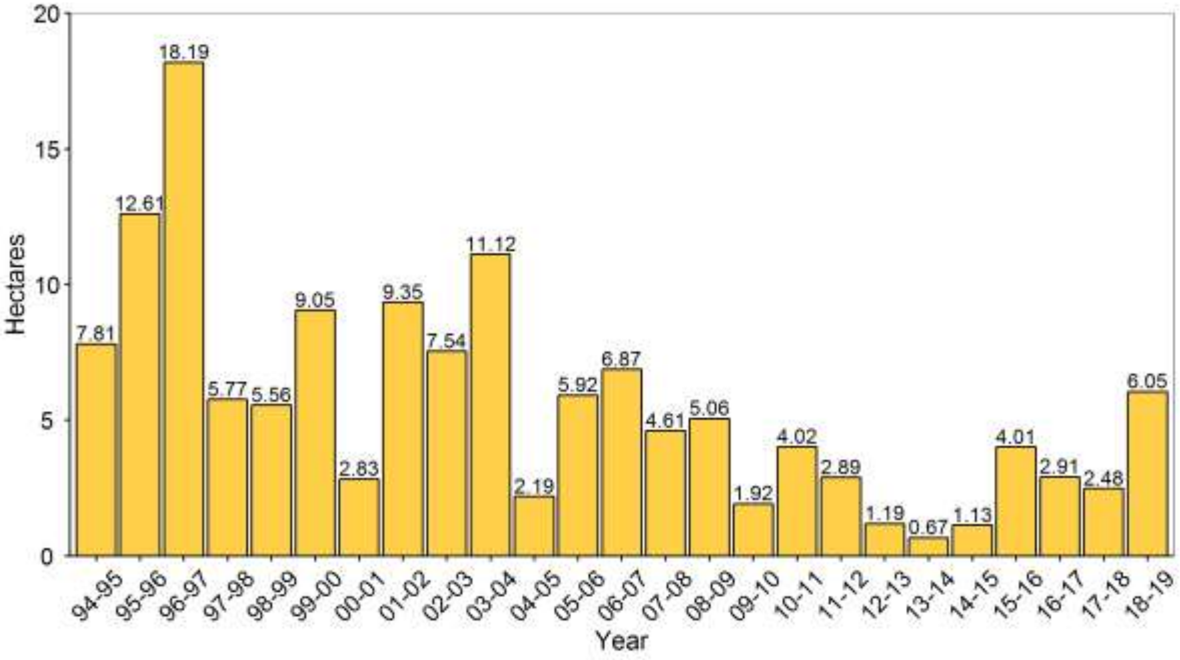

Figure 1.2. Monarch butterfly overwintering aggregation population estimates measured in hectares (Adapted from Monarch Watch Blog, 2019).

Other Monarch Population Estimates

Researchers also measure the eastern North American monarch population by counting individual monarchs at various life stages in the summer breeding season and during the fall migration. Notably, two independent monarch population counts conducted during these times

(North American Butterfly Association and Illinois Butterfly Monitoring Network counts) have *not* demonstrated declines (Ries et al. 2015). While both counts documented annual fluctuations matching those of overwintering aggregations in Mexico, neither found evidence of overall declining populations from 1993–2014. Pleasants et al. (2017), in contrast, argued that summer counts are biased to only include monarchs in non-agricultural areas, missing the declines in agricultural fields that occur via milkweed loss. Furthermore, monarch egg density estimates collected from 1997–2014 by researchers and volunteers throughout the breeding range show evidence of a decline beginning in 2007 (Stenoien et al. 2015).

Analysis of long-term fall migrating monarch counts at Cape May, New Jersey (1992–2010) and Peninsula Point, Michigan (1996–2014) also demonstrate no significant overall decline in migrating monarch numbers and no correlation between southward migrants and overwintering aggregations (Davis 2012, Badgett and Davis 2015). However, population estimates of southward migrating monarchs from 1995–2014 on Long Point Peninsula, Ontario declined by 5% annually. The decline was especially pronounced from 2004–2014. The conflicting long-term monarch population trends among these three migratory funnels could result from sampling butterflies originating in different regions. Cape May and Peninsula Point likely sample butterflies coming from largely non-agricultural regions. The Long Point site, however, is probably sampling migrants coming from the agricultural landscapes in southeastern Ontario, which presumably has been more impacted by agricultural activities including milkweed loss through herbicide use.

Boyle et al. (Boyle et al. 2019) recently calculated long-term population indices for monarchs and milkweed using over 100 years of museum and herbarium data. Museum records from across North America indicated a rise in both monarch and milkweed populations during the early 20th century, followed by a decline beginning in the mid-20th century. The authors hypothesized that agricultural disturbance benefitted *A. syriaca*, which resulted in monarch population increases. However, farm amalgamation beginning in the 1950s reduced milkweed numbers in agricultural landscapes and resulted in a monarch population decline. This study has received criticism for its sampling bias correction methods (Ries et al. 2019), further confounding the true long-term monarch population trend.

Alternative Interpretations of Population Estimates

The alternative interpretations of monarch population trends have stimulated a recent debate in the literature about the causes of overwintering monarch declines (Dyer and Forister 2016, Pleasants et al. 2016, Agrawal 2019). As a result, more sophisticated modeling efforts are needed to determine the true monarch population status and drivers (Dyer and Forister 2016). Several studies have begun to address this need. A population viability analysis based on overwintering aggregation and summer egg density count data predicted that the eastern population of monarchs have an 11–57% chance of quasi-extinction (defined as the loss of a viable monarch population when the overwintering aggregations shrink to 0.01–0.25 ha) within the next 20 years (Semmens et al. 2016). Inamine et al. (Inamine et al. 2016), however, found no correlation between summer/fall monarch counts and overwintering aggregations, arguing that monarch numbers are declining during the fall migration. Other studies which account for variables influencing year-to-year monarch population fluctuations found evidence for a correlation between summer breeding productivity and winter aggregations (Flockhart et al. 2015, Thogmartin, Wiederholt, et al. 2017, Saunders, Ries, et al. 2019).

The emerging picture points to multiple factors contributing to the decline of the eastern North American migratory monarch population (Agrawal 2019, Wilcox et al. 2019). Whatever the cause, the overwintering monarch declines are alarming and threaten the viability of the population. Concerns about these trends prompted a petition to the United States Fish and Wildlife Service in 2014 to protect the monarch under the Federal Endangered Species Act (The Center for Biological Diversity et al. 2014). The declines have also motivated research investigating monarch ecology and biology to find ways to prevent the loss of the migration phenomenon.

Hypothesized Drivers of Monarch Declines

Migration-related Declines

Although changes in monarch migratory behavior and survival are still uncertain, researchers have proposed some plausible mechanisms of monarch declines. First, increased mortality during spring and fall migrations could be caused by human disturbance to migrating butterflies. Indeed, roads are a known cause of high butterfly mortality (Mckenna et al. 2001). Availability of nectar resources fueling monarchs during their southerly migration could also impact migration success (Brower et al. 2006). Although no studies have found a direct link between migratory nectar resources and the monarch population size, Saunders et al. (2019) found the degree of landscape greenness, a surrogate for nectar availability along the migration route, was a significant predictor of overwintering colony size during the fall migration. Changing climatic conditions could also be increasing mortality pressure on migrating monarchs. Using 13 years of monarch transect counts (1996–2008) collected by volunteers throughout Ohio (northern breeding range), Zipkin et al. (2012) found that monarch summer arrival times and reproductive output in Ohio were partly explained by spring weather in Texas, the breeding region for the northward migrating overwintering generation. In Texas, springs with above average precipitation (and to some degree very dry years) and non-extreme temperatures were associated with higher abundance in Ohio. Summer climate in Ohio did not affect arrival times, but higher than average temperatures appeared to increase abundance except for the warmest areas. The authors predicted that projected drying trends for Texas springs and warming trends for Ohio could benefit monarchs, but alternatively they could also be harmed by warming Texas springs and increased extreme summer heat waves in Ohio. A more recent long-term dataset summer monarch counts showed a similar trend; wetter, cooler Texas springs resulted in more monarchs during the summer breeding season in Illinois (Saunders et al. 2018).

In contrast, there is evidence that the introduction of non-native tropical milkweed (*Asclepias curassavica* L.) in the southeastern United States could be changing the migratory dynamics of monarchs by inducing them to break reproductive diapause to breed there during the winter rather than migrate to Mexico (Batalden and Oberhauser 2015). Recent research also indicates that these non-migrating monarch populations could be at greater risk of infection and mortality from the protozoan parasite *Ophryocystis elektroscirrha* McLaughlin and Myers (Altizer and de Roode 2015, Satterfield et al. 2015). Indeed, it is hypothesized among disease ecologists that the migratory behavior evolved in part as a mechanism to avoid or cull this pathogen from the population (Bartel et al. 2011). More research is needed to assess the interactions among exotic host plants, pathogens, climate, migratory behavior, and the population dynamics of monarchs.

Overwintering Mortality

Extreme weather events affecting overwintering colonies further complicate understanding of monarch population fluctuations, as flooding and extreme cold occasionally cause high mortality. Brower et al. (2004) described several high monarch-mortality weather events from 1977–2004 and concluded that the most lethal events occurred when monarchs became simultaneously wet and cold. The authors made detailed observations of two colonies during an unprecedented cold and wet period in January 2002 and estimated that 500 million monarchs were killed across the overwintering range. Following the storm, monarchs moved into higher elevation areas. This raised concerns, because they moved into forest thinned by logging activities where monarchs are more vulnerable to weather-related mortality (Anderson and Brower 1996). A winter storm that took place in the overwintering range in March 2016 is thought to have caused significant monarch mortality (Brower et al. 2017).

Climate change could alter the frequency and level of extreme weather events in ways that might impact the long-term viability of the overwintering aggregations. Oberhauser and Peterson (2003) used ecological niche modeling combined with climate change models to argue that by 2053 increased cool, rainy periods will completely eliminate appropriate microclimate conditions for monarchs within the current overwintering range. In contrast, Flockhart et al. (2015) predicted that climate change will reduce the frequency of severe weather events in the overwintering range enough to offset mortality due to their projections of overwintering habitat loss. These contradictory interpretations highlight the need for more research assessing the long-term viability of monarchs in the face climate change and human disturbance.

Overwintering Habitat Loss

Destruction and degradation of monarch overwintering habitat is also considered a major threat to the persistence of the eastern population of monarchs. Industrial logging of the preferred monarch overwintering tree, the oyamel fir (*Abies religiosa* Kunth), has taken place since the late 1800s. Efforts to sustainably manage forests in this region began in the early 1920s. In 1986 policies began to officially protect areas of forest and have continued to strengthen and expand notably through the establishment of the Monarch Butterfly Biosphere Reserve (MBBR) in 2000 (Ramírez et al. 2015). However, logging continues to threaten overwintering monarch habitat. Ramírez et al. (2015) examined overwintering habitat loss from 1986–2012 and found that 8% of the MBBR was disturbed or lost during this period. Climatic change may also interact with habitat loss to threaten overwintering monarch aggregations. Ramírez et al. (2015) generated climate models that predicted suitable climatic conditions for oyamel firs in the monarch overwintering range will decrease by half by 2030 and be completely lost by 2090. Although overwintering habitat is crucial to the persistence of the eastern population of monarch butterflies, strong circumstantial evidence and recent modeling efforts suggest that in the nearer term, population viability is more limited more by breeding habitat loss in the northern range than by perturbations to overwintering habitat (Flockhart et al. 2015, Semmens et al. 2016).

Summer Breeding Habitat Loss

Spring and summer breeding habitat is crucial to the persistence of the eastern monarch butterfly population. The overwintering aggregations of monarch butterflies comprise adults produced mostly in breeding habitat in the northeastern and midwestern regions of the US and southern Canada (Wassenaar and Hobson 1998, Flockhart et al. 2017). Although they utilize most species in the family Asclepiadaceae (Oberhauser 2004), eastern North America’s summer breeding population relies heavily on common milkweed (*Asclepias syriaca* L.). Analysis of cardenolide compounds in overwintering monarchs showed that before glyphosate-resistant crop adoption, 92% of individuals fed on common milkweed as larvae during their previous summer (Malcolm 1993). Isotope analysis of the 1997 overwintering aggregation revealed that half of the individuals hatched in the Midwest, showing that this region contributed highly to the eastern population (Wassenaar and Hobson 1998). Traditionally common milkweed occurred naturally within grasslands and as a perennial weed in agricultural fields. However, owing to the widespread adoption of glyphosate-resistant corn and soybeans beginning in 1996, common milkweed has been reduced significantly in agricultural fields (Fernandez-Cornejo and McBride 2002, Freese and Crouch 2015). Hartzler (2010) found a 90% decrease in area occupied by common milkweed in Iowa agricultural fields from 1999–2010, and Pleasants & Oberhauser (2013) estimated a 58% overall decline of common milkweed within the midwestern United States during this same time period (Hartzler 2010, Pleasants and Oberhauser 2013). Additionally, Boyle et al. (Boyle et al. 2019) argue that North American milkweed declines significantly predate and continued after the adoption of herbicide-tolerant cropping systems, indicating that additional or alternate mechanisms are driving milkweed declines. The concurrence of common milkweed and monarch declines supports the idea that common milkweed infestations in agricultural fields were historically important to the persistence of monarchs in this region. However, specific mechanisms behind these patterns and the relative contributions of different habitats and population drivers remain unclear.

Influences on Monarch Breeding Habitat Productivity

Monarch Egg Density Patterns

The coincidence of monarch and common milkweed declines prompted several studies to assess the underlying mechanisms linking these trends. The use of common milkweed by monarchs in agricultural fields is well documented. Oberhauser et al (2001) measured monarch oviposition in different habitat types across the breeding range and found differences by location. In Minnesota/Wisconsin (pooled sites) there were greater egg densities in corn fields than nonagricultural areas and corn field edges. In Iowa there were greater egg densities in corn and soybean fields than in corn field edges. Maryland and Ontario had no significant egg density differences between agricultural and nonagricultural habitats. Because agricultural lands are so expansive in the Midwest, the researchers estimated that agricultural habitats contribute 45 and 73 times more monarchs than nonagricultural habitats in Iowa and Minnesota/Wisconsin, respectively (Oberhauser et al. 2001). To further understand the effects of common milkweed loss from agricultural landscapes on monarch production, Pleasants and Oberhauser (2013) monitored monarch eggs on common milkweed within crop and non-crop habitats in Iowa from 2000–2003 and observed an average of 3.89 times more monarch eggs per milkweed stem in crop (soybean and corn) than non-crop (natural areas, CRP land, pastures and old fields) habitats. Combining these findings with similar Monarch Larvae Monitoring Program (MLMP) egg per stem estimates from habitats throughout the Midwest and estimates of common milkweed stem per hectare from corresponding habitat types, they estimatlisted an 81% reduction in monarch egg production from the Midwest from 1999–2010 (Pleasants and Oberhauser 2013). More recent analyses incorporating contemporary MLMP egg counts revealed that overall across the northern breeding region monarch egg densities in non-crop areas have been declining from

2007–2014 (Stenoien et al. 2015). The authors speculated that the decrease of milkweed in agricultural areas during this time reached a level at which monarchs were less able to locate remaining host plant disparate patches. Pitman et al. (2018) found a similar trend in southern Ontario of more monarch eggs laid on milkweed growing in agricultural landscapes than non-agricultural landscapes and roadsides. These studies demonstrate the contribution of agricultural habitats to the monarch population and show patterns of greater monarch egg densities in midwestern agricultural than non-agricultural habits. They have also motivated further research into the ecological mechanisms underlying observed monarch egg density patterns and their implications for monarch conservation.

Pleasants and Oberhauser (2013) suggested several potential explanations for their observations of higher monarch egg densities on crop versus non-crop habitat in the Midwest. They proposed that monarchs lay more eggs per stem in agricultural fields because milkweed patches in these habitats tend to be smaller, higher in nutrients, and/or more apparent to ovipositing females against backgrounds of soybean and corn monoculture. Indeed, experiments have demonstrated monarch oviposition site selection is driven by various host plant attributes, and monarchs are highly chemoreceptive to compounds in their host plants (Haribal and Renwick 1998). Through field and flight cage experiments in Queensland, Australia, Zalucki and Kitching (1982) observed that egg-laying monarchs selected host plants that were growing singly versus those growing in patches and preferred young over mature plants and leaves. Interestingly, they also observed that monarchs of different ages preferred different host species. Similar to lab experiments by Flockhart et al. (2012) they found no evidence that monarchs avoided laying eggs on plants that already had high densities of monarch eggs or larvae. Other work, however, observed negative density-dependent oviposition in monarchs, with females laying fewer eggs on plants that already had monarch eggs present versus plants with no eggs present (Dixon et al. 1978). Oyeyele and Zalucki (1990) demonstrated that wild monarchs in Queensland preferentially oviposited on African milkweed (*Gomphocarpus fruticosus* L.) with intermediate levels of cardiac glycoside versus those with low or high levels of this compound. However, their study found no relationship between host plant nitrogen concentration and oviposition selection. Although larval development is impacted by host plant nitrogen content, with increased feeding behavior compensating for low-nitrogen food (Lavoie and Oberhauser 2004), plant nitrogen content as a driver of oviposition site selection has not been demonstrated. The literature on monarch chemical ecology and individual host plant preference is extensive, and it is clear that monarchs can react to host plant chemical cues. Monarch oviposition site selection is a function of many interacting variables, and it is plausible that milkweed growing in agricultural fields could be more attractive to egg-laying monarchs due to its patchy distribution, greater nitrogen content, tendency to be younger or have younger leaves due to human disturbance (Pleasants 2015), and greater apparency, which has been shown to affect oviposition behavior of lepidopterans (Feeny 1976, Wiklund 1984).

Monarch Egg and Larval Survival

Estimates of Immature Monarch Survival

Monarch production is not only dependent on the number of eggs laid, but it is also a function of the survival of eggs and larvae to the adult stage. Mortality during the egg stage could potentially obscure monarch production estimates based solely on egg counts if eggs die and disappear rapidly between sampling periods. Several observational studies have sought to quantify the survival rates of monarchs during egg, larva, and pupa life stages (for literature summary see **Table B1**). Early work by Borkin (1982) estimated survival at different life stages of monarchs breeding in an old field in Wisconsin. Based on a weekly observations of eggs and larvae, she estimated that 12% of monarchs survived from the egg to the pupal stage. De Anda and Oberhauser (2015) estimated lower survival rates (1.7% survival from egg to second instar) for a population monitored for two breeding seasons in Minnesota. Nail et al. (2015) analyzed monarch survival data from 18 years of volunteer-collected field observations from sites across North America and lab-reared pupae parasitism rates from the northern summer breeding range. They estimated egg to adult monarch survival from 4.3 to 5.8% for spring and summer broods, respectively. Oberhauser et al. (2001) reported survival rates of 15% or less for egg to fifth instar from sites across the northern breeding range. These high mortality rates coupled with increasing conservation concerns beg the question of which drivers of monarch mortality and survival are most important in the breeding range.

Monarchs and Natural Enemies

Most studies estimating immature monarch survival contend that predators and parasitoids (hereafter "natural enemies") are an important cause of the high mortality rates observed. Zalucki et al. (2001) showed that survival of first instar monarchs was dependent on both the cardenolide concentrations and latex defenses of host plants (which slow the growth of larvae and mire them, respectively) and mortality from natural enemies. Many natural enemies prey on monarch eggs and larvae, and several studies have quantified predation and parasitism on monarch eggs and larvae. Oberhauser et al. (2015) compiled a list of monarch natural enemies in the literature. Adult monarch predators included birds, mammals, wasps, and dragonflies, while eggs, larvae, and pupae are consumed or parasitized by a variety of arthropods including ants, lacewing larvae, stinkbugs, spiders, wasps, and tachinid flies. Borkin (1982) assumed that much of the mortality she observed in immature monarchs was due to natural enemies based on predation and parasitoid observations in the field and the emergence of tachinid flies from 11.5% lab-reared pupae collected from field sites. De Anda and Oberhauser's (2015) study of monarch survival rates in Minnesota provided strong support for natural enemies as the main driver of mortality based on the following evidence: direct observations of predation; dead larvae which had been sucked or chewed by predators; low cardenolide content in common milkweed combined with a lack of larvae mired in milkweed latex; no evidence of failure by first instars to establish at a feeding site (i.e. no spike in mortality from days four to five); and a positive correlation between mortality and presence of flowers, which were thought to attract natural enemies.

Quantifying the Impact of Natural Enemies of Monarch Survival

Other studies have sought to empirically test the impact of natural enemies on immature monarch survival (see Appendix 1 for summary of this literature). Using predator exclosures Zalucki et al. (2001) found first instar monarchs raised on common milkweed in exclosures had higher survival (92% and 72%) than on plants open to predators (24% survival). Prysby (2004) monitored monarch eggs on common milkweed with various exclosure treatments and found survival was greatest on the treatment excluding terrestrial and aerial natural enemies, intermediate on the treatment excluding terrestrial natural enemies only, and lowest on the control. Most of the mortality occurred during the egg stage. Further work at this field site revealed that survival was much lower (5%) over seven days for monarch eggs placed on milkweed plants with ants (*Formica montana* Wheeler) and aphids than plants with aphids alone or neither aphids nor ants. In one pilot study Prysby (2004) noted that eight eggs placed on a milkweed plant were consumed by *Formica montana* ants within 90 minutes. As with Borkin (1982), Prysby (2004) also lab-reared a subset of pupae from the study site from two years and found a comparable rate of tachinid fly parasitism (mean 15 and 23%, years 1999 and 2000 respectively), with higher rates at sites in agricultural than non-agricultural settings. Oberhauser et al. (2015) similarly placed lab-bred monarch larvae, prepupae (5th instar larvae that had stopped eating) and pupae in various settings and treatments to measure the effect of paper wasp (*Polistes domulina* Christ) predation and *Petromalus* spp. wasp (likely *Petromalus cassotis* Walker) parasitism. Observations of larvae and pupae in open cages revealed high predation (up to 76%) from *Polistes* wasps and high rates of parasitism (up to 59%) from *Petromalus* wasps. A second experiment revealed higher *Petromalus* parasitism rates on pupae fed common milkweed than tropical milkweed. In a third experiment they placed pupae in various settings including human structures, goldenrod, trees, and common milkweed with and without monarch frass present. They found the greatest parasitism rates on milkweed plants with frass, supporting the hypothesis that monarchs leave milkweed and pupate on other structures to avoid parasitism. Finally, Calvert (2004) monitored immature monarch survival from egg to fifth instar at two sites in Texas and found generally lower survival rates outside than inside fire ant exclosures: 0 vs. 27%; 0.5 vs. 1.6%; and 1.4 vs. 16% respectively for their three experimental periods. As with other related studies, much of this mortality occurred between eggs and first instar stages.

Habitat-dependent Natural Enemy Pressure

Clearly natural enemies have a significant impact on the survival of immature monarchs. As with immature monarch survival estimates in general, research on the variability of natural enemy mortality on monarchs reveals that this effect is habitat-dependent. The relationship between natural enemies of agricultural pests and local and landscape-level habitat characteristics is well documented, with more natural enemies typically associated with greater habitat heterogeneity (Bianchi et al. 2006). It is plausible to hypothesize that immature monarchs would experience greater mortality in agricultural landscapes with more non-agricultural land cover. However, Oberhauser et al (2015) observed greater rates of *Petromalus* parasitism on monarch pupae in agricultural than non-agricultural experimental sites. Prysby (2004) similarly noted higher tachinid fly parasitism on fourth and fifth instar larvae in cornfields and cornfield edges than non-agricultural habitats. She hypothesized that these differences could be due to several reasons including higher densities of monarch larvae attracting more tachinid flies in agricultural landscapes and greater apparency to tachinid flies of monarchs in simplified agricultural habitats. As with other studies, Zalucki and Kitching (1982) observed high rates of egg to fifth instar mortality (92–98%) for monarchs feeding on various sized patches of African milkweed at three field sites in Queensland Australia, with most of the mortality occurring during the egg stage. They found mortality was negatively correlated with patch size and hypothesized that natural enemies are attracted to larger host plant patches. Oberhauser (2012) found high immature monarch density was correlated with high tachinid fly parasitism the following year in various non-crop habitats across the Midwest (described as "…gardens, railroad right-of-ways, roadsides, abandoned fields and pastures, natural habitats, and restored prairies…"), suggesting levels of natural enemy pressure on monarchs could be complicated by predator-prey cycles. Patterns of mortality among various habitats and landscapes could also vary as a function of the age of immature monarchs. Given the interacting influences of habitat, oviposition rates, natural enemy abundance, and survival on monarch populations, more research is needed to understand contributors to the decline of monarchs. Such an understanding would aid effective conservation of monarch butterflies facing a future of increasingly simplified agricultural landscapes, which potentially support fewer natural enemies but have reduced abundance of common milkweed.

Monarch Response to Landscape Breeding Habitat Loss

Understanding the effects of monarch breeding habitat loss would be improved by information about how egg-laying behavior and survival interact and are affected by breeding habitat on the landscape level. Specifically, it is unclear how egg-laying females respond to the composition, configuration, and quality of breeding habitat on the landscape. Declining milkweed abundance on the landscape could force monarchs to lay more eggs on the few remaining milkweed plants, thereby decreasing reproductive output through negative density dependence by top-down (increased per capita natural enemy or disease pressure) and/or bottom-up (intraspecific competition) forces. Indeed, lab experiments and field observations have demonstrated negative density-dependent survival for monarch butterflies via intraspecific competition (Flockhart et al. 2012). And Prysby (2004) suggested that density-dependent predation could have caused observations of greater immature monarch predation in agricultural field edges than in natural areas. This mechanism is also consistent with observations of greater monarch egg densities on smaller patches of milkweed and in agricultural areas with fewer milkweed plants (Oberhauser et al. 2001). An alternative (but not completely mutually exclusive) explanation is that monarchs searching for egg-laying sites are unable to lay their full complement of eggs once milkweed falls below a critical density on the landscape. Indeed, Stenoien et al. (2015) suggested this as a potential reason for overall declines of monarch egg densities observed beginning in 2007, when glyphosate-resistant soybean and corn adoption reached approximately 70%. Additionally, simulation models of female monarch egg movement and egg laying demonstrate that such changes in milkweed spatial distribution on the landscape could reduce the number of eggs laid per butterfly (Grant et al. 2018). However, this explanation has never been demonstrated empirically (Flockhart et al. 2015). Increased understanding of the interplay between egg-laying behavior and top-down and bottom-up density-dependent survival of monarchs in response to host plant distribution on the landscape is recognized as important for informing monarch conservation efforts and ecological knowledge (Flockhart et al. 2015, Nail et al. 2015).

Current Breeding Habitat Conservation Measures

The growing evidence that breeding habitat loss is driving recent declines in monarch butterflies has prompted a tremendous effort to increase milkweed host plant abundance within the breeding range. Various organizations and programs have formed to promote milkweed plantings and citizen science monarch data collection efforts (summarized in (USDA 2015)). Notably, in May 2015 in response to a presidential memorandum, the Whitehouse released the "National Strategy to Promote the Health of Honey Bees and other Pollinators" in which monarch conservation was a major priority (US Pollinator Health Task Force 2015). The plan sets a goal for the recovery of the monarch overwintering aggregations to an average of 6 ha and the protection of 7 million acres of habitat for pollinators, primarily through the Federal Conservation Reserve Program, enhancement of roadside habitats, and the funding of schoolyard habitats and pollinator gardens. However, despite these efforts, knowledge about monarch breeding habitat and ecology is still lacking. Important questions remain about what landscapes and habitat types are most productive for monarchs, how monarchs interact with natural enemies in different habitat contexts, and what practices can be implemented to conserve monarchs and biodiversity as a whole in agroecosystems. Answers to these questions will not only aid in monarch conservation practices, but also would enhance knowledge of agroecosystem functioning and inform the overarching challenge of conserving biodiversity in agricultural landscapes.

Summary

Studying and conserving migratory species pose unique challenges, and monarchs are no exception (Wilcove and Wikelski 2008, Bowlin et al. 2010, Zipkin et al. 2012). Concerns about the viability of monarch butterfly populations highlights the vulnerability of even abundant agricultural-associated species to landscape simplification. The multitude of potential drivers of these declines, including climate change, increased migration mortality, and habitat loss along all steps of the annual cycle, underlines the difficulty of understanding monarch declines and potential recovery. The need to manage productive breeding habitat for monarchs in the northern breeding range is becoming increasingly clear. Although this is a challenging task, it also represents an opportunity to both increase understanding about basic ecological processes and biodiversity functions in agroecosystems and to generate attention and support for biodiversity conservation in these ever-simplified landscapes.

The following chapters address three aspects of monarch biology that will help inform monarch conservation and knowledge regarding their interactions with predators. Chapter 2 describes an experiment using sentinel milkweed plants to determine how breeding habitat type influences both attraction for monarch oviposition and predation risk for monarch eggs and larvae. Chapter 3 presents the results of a video monitoring study investigating which predator taxa consume monarch eggs in grasslands, and at what times of day this predation takes place. Chapter 4 delves deeper into specific interactions among monarch neonates, the aphids with which they cohabit milkweed host plants, and ants which are attracted by aphids and prey upon monarchs. Chapter 5 summarizes and contextualizes this new knowledge with regard to monarch conservation and breeding ecology and provides recommendations for further study in this area.

CHAPTER 2: HABITAT TYPE INFLUENCES *DANAUS PLEXIPPUS* (LEPIDOPTERA: NYMPHALIDAE) OVIPOSITION AND EGG SURVIVAL ON *ASCLEPIAS SYRIACA* (GENTIANALES: APOCYNACEAE)

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Abstract

As agricultural practices intensify, species once common in agricultural landscapes are declining in abundance. One such species is the monarch butterfly (*Danaus plexippus* L.), whose eastern North American population has decreased approximately 80% during the past 20 years. One hypothesis explaining the monarch's decline is reduced breeding habitat via loss of common milkweed (*Asclepias syriaca* L.) from agricultural landscapes in the north central United States due to adoption of herbicide tolerant row crops. Current efforts to enhance monarch breeding habitat primarily involve restoring milkweed in perennial grasslands. However, prior surveys found fewer monarch eggs on common milkweed in grassland versus crop habitats, indicating potential preference for oviposition in row crop habitats, or alternately, greater egg loss to predation in grasslands. I tested these alternative mechanisms by measuring oviposition and egg predation on potted *A. syriaca* host plants. This study revealed that habitat context influences both monarch oviposition preference and egg predation rates, and that these patterns vary by year. I found higher monarch egg predation rates during the first 24 h after exposure and that much of the predation occurs at night. Overall I documented up to 90% egg mortality over 72 h in perennial grasslands, while predation rates in corn were lower (10–30% mortality) and more consistent between years. These findings demonstrate that weekly monarch egg surveys are too infrequent to distinguish oviposition habitat preferences from losses due to egg predation and

suggest that monarch restoration efforts need to provide both attractive and safe habitats for monarch reproduction.

Introduction

Agricultural expansion and intensification are among the greatest threats to global biodiversity (Tilman et al. 2001, Tscharntke et al. 2005), and developing cropping systems that simultaneously support biological diversity and other ecosystem services remains a significant challenge (Tilman et al. 2002, Foley et al. 2011, Glamann et al. 2017). As agricultural practices intensify, even species that were historically abundant in agroecosystems can be impacted (Van Dyck et al. 2009, Stanton et al. 2018). One such case is the migratory eastern North American population of the monarch butterfly (*Danaus plexippus* L.), which has declined precipitously during the past two decades in its Mexico overwintering range (Taylor 2009, Brower et al. 2012) and by some estimates in also its summer breeding range (Stenoien et al. 2016, Pleasants et al. 2017). While the reasons for monarch declines are likely multifactorial (Thogmartin, Wiederholt, et al. 2017, Belsky and Joshi 2018), one hypothesis suggests that declining abundance of common milkweed (Asclepias syriaca L.) in the US Midwest is a key driver (Pleasants and Oberhauser 2013, Thogmartin, Wiederholt, et al. 2017); but see (Inamine et al. 2016). Common milkweed (hereafter milkweed) historically occurred in natural prairie habitats throughout the region and later as a common weed in row crops (Bhowmik and Bandeen 1976, Pleasants 2015). However, primarily due to widespread adoption of herbicide tolerant corn and soybean and associated herbicide use, milkweed has declined in agricultural crops. Recent losses of cropland milkweed in Midwestern region of the US are well documented (Hartzler 2010, Zaya et al. 2017). A synthesis of milkweed surveys by Pleasants (Pleasants 2017) estimated that nearly 40%

of Midwest milkweed disappeared between 1999–2014, which includes primarily plants lost from herbicide application in row crops but also a smaller portion lost from grasslands converted to agriculture.

In eastern North America monarch butterflies oviposit on milkweed in both natural and agricultural habitats, and studies have noted consistent trends in egg density in these habitats (Oberhauser et al. 2001, Pleasants and Oberhauser 2013, Pitman et al. 2018). Monarch egg surveys in the Midwestern US found greater numbers of monarch eggs per milkweed stem in agricultural fields than in adjacent non-agricultural grasslands in July and August (Oberhauser et al. 2001, Pleasants and Oberhauser 2013). Pitman et al. (2018) found similar patterns of more monarch eggs on milkweed in corn and soybean versus roadside and other non-agricultural habitat in southern Ontario. This pattern implies that milkweed loss from crop fields may have a disproportionately large effect on landscape-level monarch breeding productivity. Based on the numbers of eggs/stem in different habitats, Pleasants (2017) estimated that milkweed declines from Midwestern row crops resulted in a 76% reduction of monarch milkweed resources in the region. This hypothesis has served as the impetus to increase milkweed stems in the monarch breeding range (Thogmartin, López-Hoffman, et al. 2017). However, questions remain regarding the influence of habitat type and habitat configuration on monarch productivity and mechanisms underlying egg density, and conservation efforts would benefit greatly from more knowledge in these areas (Landis 2017, Grant et al. 2018).

Greater monarch egg densities on milkweed in agricultural versus non-agricultural habitats have been largely interpreted as habitat-related differences in attractiveness for oviposition preference (Pleasants and Oberhauser 2013, Pitman et al. 2018). Proposed mechanisms of increased attraction to agricultural milkweed include higher plant quality, smaller patch size, and greater apparency against a monoculture background (Pleasants 2015, Pitman et al. 2018). However, because egg surveys in these studies were conducted on a weekly basis, reported egg densities could reflect differential predation rates as well as oviposition preference (Pleasants 2015). Indeed, monarch eggs and larvae are known to experience high rates of predation in grasslands (Borkin 1982, Prysby 2004, De Anda and Oberhauser 2015, Oberhauser et al. 2015). And survival estimates based on population age structure suggest that monarchs experience lower survival in non-agricultural grasslands versus agricultural fields in the Midwest breeding region (Oberhauser et al. 2001). It is therefore plausible that the observed pattern of more eggs in corn could be due to reduced predation in addition to, or instead of, increased oviposition (**Fig. C1**). Furthermore, if predation is the primary driver of egg mortality in these systems, this could mean that grassland areas where milkweed restoration efforts are common (Thogmartin, López-Hoffman, et al. 2017) represent higher risk habitat for monarchs. The research presented here focuses on understanding the relationships among habitat type, monarch oviposition, and predation risk in agroecosystems in order to enhance the effectiveness of monarch habitat restoration and conservation efforts.

During the summers of 2016 and 2017, I conducted experiments in southwestern Michigan to quantify monarch butterfly oviposition and egg predation on potted common milkweed plants placed in corn, soybean, bare ground, and grassland habitat treatments. My goal was to determine if monarch egg densities differ, and if so, if this a function of oviposition habitat preference, differential predation, or a combination of these non-mutually exclusive causes (**Fig. C1**). I hypothesized that if oviposition was the major driver of this pattern, I would observe greater numbers of eggs laid by wild monarchs in agricultural versus grassland habitats when sampled at a high frequency (1–2 times daily) as this would limit time for predation losses.

Alternatively, if predation was the dominant cause of monarch egg densities, I would observe greater rates of predation on monarch eggs within grasslands versus agricultural habitats. I interpret the results in the context of monarch conservation efforts and recommend further work to advance knowledge about monarch breeding biology and habitat management.

Methods

Study Site Description

All experiments were conducted at the Michigan State University Kellogg Biological Station Long Term Ecological Research Site (KBS LTER) Cellulosic Biofuels Diversity Experiment (CBE) located in Hickory Corners, Michigan (42.407 N, 85.374 W). The CBE was established in 2008 and includes 12 biofuel cropping system treatments arrayed as 12 adjoining 9.1 m by 27 m plots each replicated within four randomized blocks (**Fig. 2.1**). Individual plots are immediately adjacent, and between the blocks and surrounding the entire experiment are 12.2 m wide alleyways of mowed turf grass. The experiments used four of these treatments: corn, soybean, bare ground, and prairie. Corn and soybean were grown in rotation with both crops present in each year. The bare ground treatment was formerly continuous corn, but during 2016 and 2017 treated with glyphosate to prevent plant colonization. The prairie plots were a species mixture comprising six grass and four forb species native to Michigan. A meteorological station 200 m north of the CBE measures a variety of weather variables including hourly temperature 3 m above ground and rainfall to the nearest mm (https://lter.kbs.msu.edu/datatables/13). For detailed information on agronomic practices, prairie planting seed mixes, and experimental site history see Dickson and Gross (Dickson and Gross 2015).

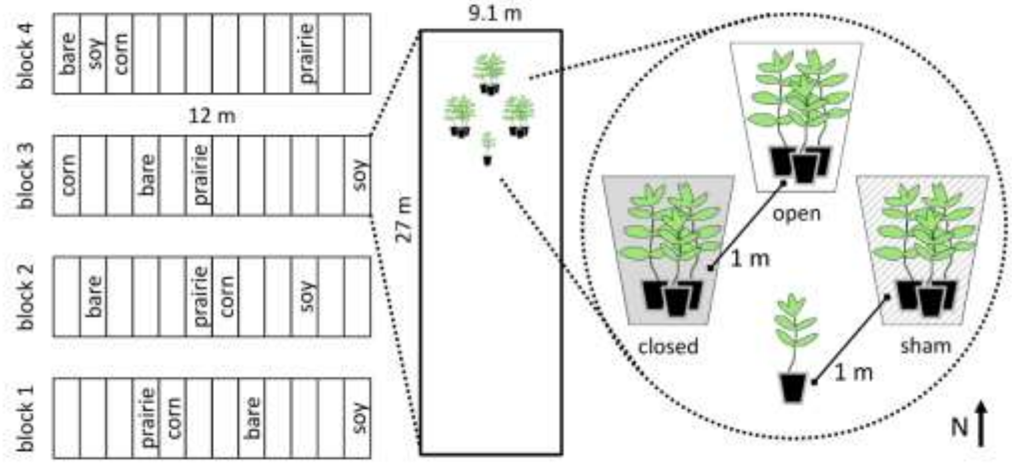

Figure 2.1. Experimental plot arrangement (left) and configuration of potted *Asclepias syriaca* (L.) patches within one experimental plot for monarch oviposition and survival experiments in 2016 and 2017. Each plant patch comprised three adjoining pots, patches were arranged in an equilateral triangle 1 m apart, and one of three exclosure treatments was randomly assigned to each patch (for survival experiments). The single plant on the south side of the triangle was included during the oviposition experiments in 2017 only.

Host Plants

None of the treatments used for the study contained naturally growing milkweed. Because plant age, nutrition, and other plant condition variables could be influenced by habitat and affect attractiveness to ovipositioning monarchs (Pleasants 2015), I used potted *A. syriaca* plants for all experiments to control for these factors. I sourced plants from native Michigan genotype seeds grown at Wildtype Nursery, Mason, Michigan. Plants were grown in three separate cohorts each year corresponding to the experimental periods. Seeds were planted in a nursery greenhouse in Fafard® 3B Mix (Sun Gro Horticulture, Agawam, MA), fertilized with Osmocote Plus 15:9:12 NPK slow release fertilizer (Everris NA, Inc., Dublin OH), and watered *ad libitum*. One week prior to deployment, each plant was transferred to a single plastic 1 L pot and acclimatized in an outdoor courtyard on the Michigan State University campus. Plants were 14–19 weeks old and approximately 50 cm tall at deployment and remained in a vegetative (i.e. pre-flowering) stage throughout the experiments. Occasionally a plant would be damaged or die in the field, in which case it was replaced using a spare plant from the same cohort.

In July and August of 2017, plants were colonized by the oleander aphid *Aphis nerii* Boyer de Fonscolombe (Hemiptera: Aphididae) while growing in the greenhouse. Although this species occurs naturally and concurrently with monarchs under field conditions in the study region, I removed them to provide consistency with previous deployments of plants. To remove aphids, every plant used in 2017 experiments was rinsed under running water and brushed with a small paint brush 3–5 days before deployment. I then submersed each individual plant in a solution of short-lived Safer® Insect Killing Soap (Woodstream Corporation, Lititz, PA) to kill any remaining aphids. On the morning of each deployment, plants were rinsed in fresh water to remove any remaining soap.

Plant Deployment

Common milkweed is a clonal species and typically grows in patches (Bhowmik and Bandeen 1976). I mimicked this patchiness by arranging potted plants in three groups of three adjoined pots, with each pot containing a single milkweed stem (hereafter each group of three pots is called a “patch”) in each treatment plot. Individual pots were 10 x 10 cm, so the plant stems within each patch were approximately 14 cm apart. The three patches were positioned 1 m apart arranged in a north-pointing equilateral triangle within each experimental plot for a total of nine plants per plot in 2016 (**Fig. 2.1**), and a total of 144 plants (nine plants x four treatments x four replicates). In 2016 the plants were randomly placed near the centroid of either the north or south half of the plot. In 2017 plants were always placed at the north centroid to accommodate an unrelated experiment. In 2017 a tenth plant was added south of the triangle such that it was 1 m from the two southern patches, for a total of 160 plants (ten plants x four treatments x four replicates). The purpose of adding this plant was to perform a parallel experiment not presented here; however, eggs laid on this plant were counted and included in oviposition checks (see 'Oviposition Experiment' section of Methods). Each pot was anchored to the ground with a 1 m bamboo stake, and the three stakes of each patch were tied at the top for added stability. Plants were hand watered as needed every 1–2 days.

Oviposition Experiment

To determine if habitat context influences monarch oviposition choice, I conducted an oviposition experiment during three separate time periods representing the early, mid and late summer breeding generations. Egg checks began the day immediately following plant deployment. To minimize the potential effects of predation on egg counts, I checked plants 1–2 times per day for eggs laid by wild monarchs (except when prevented by weather or travel logistics; see **Fig. C2**). During the 2016 field season I conducted one egg check from 10:00–12:30 and a second egg check from 13:00–15:30 on a typical day. In 2017 I performed daily egg checks from 10:00–12:30 and then from 17:00–19:30 to better sample eggs laid in the afternoon hours. Egg checks were performed by visually inspecting stems and both upper and lower surfaces of all leaves. I removed eggs as they were detected to prevent double-counting, damage to the plants by subsequent larvae, and to minimize the influence of con-specific eggs on oviposition. I also recorded each observation of an adult monarch butterfly within the study plot or immediate grassy border. Each oviposition check took approximately 2.5 h, and I standardized adult butterfly observations to number of individuals observed per h.

Oviposition Data Analysis

For each day in each plot I calculated the number of eggs laid per stem by summing all the eggs observed and dividing by the number of plants present in the plot that day. In cases where the number of plants varied within a single day (e.g. a dead plant was replaced in the morning and then checked in the afternoon), I used the mean number of plants present for the day. Because oviposition events were generally rare, I averaged all daily values of eggs per stem to generate a single mean eggs per stem per day value for each of the 16 plots within each of the two study years for analysis.

All statistical analyses were performed in R version 3.4.3 (Nasr et al. 2017). I tested the effect of the habitat treatment on numbers of eggs observed with negative binomial generalized linear mixed effects models using the glmer.nb function in the MASS package (Venables and Ripley 2002). Habitat treatment was included as a fixed effect, block as a random effect, and the number of plants present as an offset variable. I used an analysis of deviance in base R (Nasr et al. 2017) to perform a chi-square likelihood ratio test comparing the full model to a null model to determine if habitat treatment had a significant effect on oviposition. Pairwise contrasts of modeled treatment means were performed using the emmeans function with a Holm-adjustment for multiple comparisons (Lenth et al. 2018).

Survival Experiment

To determine the impact of habitat context on 72 h monarch egg survival, I performed three separate experiments (23 August 2016, 17 July 2017, and 23 August 2017; **Fig. C2**) that followed the fates of monarch eggs placed on milkweed plants. Each survival experiment immediately followed the previously described oviposition experiments using the same plants (**Fig. 2.1**). Using the same plants as the oviposition experiment ensured that plants were colonized by each habitat's local predator community, as all plants were deployed for at least eight days before the start of each survival experiment. Within each plot, the three plant patches was randomly assigned to the three exclosure treatments, with the exclosures covering the three adjoining plants: a full exclosure (hereafter "closed" treatment) intended to exclude all predators; an open treatment allowing access by all predators; and a sham treatment designed to allow entry by predators while controlling for cage effects on abiotic conditions. For the closed treatment, I removed any predators on plants before applying the exclosure.

I constructed predator exclosures using No-see-um Netting (Skeeta Inc., Bradenton, FL) supported by 142 cm tall, 46 cm diameter steel tomato cages. To prevent entry by ground predators, the bottom of the no-see-um barrier was sewed closed into a bag shape with tomato cage tines pierced through the netting into the ground. Sham treatments were identical to the full exclosures, but with the addition of three 75 cm long vertical slits in the netting to allow entry by arthropod predator and the bottom left open to ground predators. Open treatments received a tomato cage, but no netting (**Fig. C3**).

All monarch eggs used in experiments were produced by a colony housed at Michigan State University in East Lansing, Michigan. Approximately 60 h before egg deployment, a potted *A. syriaca* plant was introduced to the colony butterflies for oviposition. Approximately 12 h before the experiment, eggs were gently removed from the plant and placed onto moistened paper towels in large plastic petri dishes and stored overnight at approximately 7° C to arrest development. The following morning eggs were transported to the field in a cooler with ice packs. I attached eggs to leaves by gluing one egg per leaf to the undersides of the four uppermost fully emerged leaves where monarchs naturally lay most eggs (Zalucki and Kitching 1982a). To attach eggs, a tiny droplet of Elmer's ® Glue-All (Elmer's Products, Columbus, Ohio) was placed on the end of a fine paint brush, which was subsequently used to gently pick up and attach eggs onto plants. Laboratory feeding trials (Hermann et al. in press) and field video recording (see Chapter 3) demonstrated that a variety of arthropods (including members of Coleoptera, Lepidoptera, Hymenoptera, Hemiptera, Orthoptera, Dermaptera, Neuroptera, and Araneae) will feed on monarch eggs glued to plants using this method and that eggs hatch normally. Video surveillance also demonstrated that very few glued eggs fall off plants; of 152 eggs monitored for an average of 60 h, three or fewer fell off the plant eggs monitored for an average of 60 h only one fell off the plant (see Chapter 3). Furthermore, by day three of the 2017 survival experiments several eggs began to hatch, demonstrating that egg development was unaffected by the gluing method.

Four eggs were affixed to each plant in August 2016 and July 2017 deployments (for a total of 576 eggs per experiment), and three eggs per plant were used in August 2017 (for a total of (432 eggs). To compare survival among treatments, I checked egg survival every 2–3 hours for 24 h and again at 48 h and 72 h. At each survival check I identified and counted arthropod predators on plants in the open and closed exclosure treatments to the lowest taxonomic level possible in the field. I categorized arthropods as monarch predators if they met one or more of the following criteria: 1) the species or family is commonly considered a predatory taxon; 2) no-choice lab trials indicate that the taxon consumes monarch eggs (Hermann et al. in press); and/or 3) I directly observed predators consuming monarch eggs during experiments. Because I only recorded presence/absence of ants for August of 2016, all predator data presented from August 2016 excludes ants, while for July and August 2017 I include ant abundance data.

Survival and Predator Data Analysis

Egg survival at 72 h was assessed separately for each of the three deployments using binomial generalized linear mixed models using the glmer function in the R package lme4 (Bates et al. 2015). In all models, the individual patch was considered a random effect, and depending on the model, either exclosure treatment or habitat were considered fixed effects. To test if predators were the cause of egg mortality, I first evaluated the effect of exclosure treatment on survival response. I predicted that survival would be higher in predator exclusion treatments than in sham or fully open ones. I used an analysis of deviance in base R (Nasr et al. 2017) to perform a chi-square likelihood ratio test comparing the model that included both the fixed effect of exclosure type and the random effect of individual patch to a null model which only included the random effect of patch. I then performed post-hoc comparisons in the same manner as for the oviposition analysis (described in 'Oviposition Data Analysis' section of Methods).

After confirming that predators reduced survival of monarch eggs (see Results), I used the same approach used to determine the effect of exclosure to test the effect of habitat treatment on survival of monarch eggs in the open exclosures only. I used a binomial generalized linear mixed effects model with survival as the response variable, habitat treatment as a fixed effect predictor variable, and individual patch as a random effect, and each deployment date was assessed separately. The full model was compared with the null, which included the random effect of patch only. I performed this analysis for each of the three experimental periods. For periods in which survival was significantly influenced by habitat treatment, I proceeded to post-hoc, pairwise comparisons of survival within all four treatments using the R package emmeans (Lenth et al. 2018).

I analyzed predator abundance separately from survival data. For analyses I summed the total number of predators across all surveys repeated during the first 72 h (9, 13, and 14 surveys for August 2016, July 2017, and August 2017 experimental periods, respectively) within habitat treatment and exclosure treatment (i.e. summed across dates within each plot). Because predators were rarely found in exclosure cages (**Table 2.1**), I only performed statistical analysis using surveys from the plants in the open exclosure treatments. To determine if habitat treatment significantly affected total predator numbers, I modeled total predator numbers as a function of habitat treatment using negative binomial generalized linear models using the glmer.nb function in the R package MASS (Venables and Ripley 2002). As with the survival data, I performed this analysis for each of the three experimental periods separately. In experiments where habitat significantly influenced total predator numbers, I proceeded to post-hoc Holm-adjusted pairwise comparisons among habitats using the R package emmeans. Because ants were often the most abundant arthropods I observed and are considered important monarch predators (Calvert 2004, Prysby 2004, Mooney and Agrawal 2008), I repeated the same analysis for ants separately for the July 2017 and August 2017 experiments when ant numbers were recorded.

Table 2.1. Results of predator surveys conducted during three monarch butterfly survival experiments in the summers of 2016 and 2017. Numbers are mean predators observed per stem per survey pooled across habitat treatments within closed and open exclosure treatments. Gray cells indicate instances of predators observed in closed exclosure treatments. NA (not applicable).

		Mean Number of Predators/Stem/Survey					
Order	Family	August 2016		July 2017		August 2017	
		Closed	Open	Closed	Open	Closed	Open
Orthoptera	Acrididae	0	0	0	0.001	0	0.010
	Tettigoniidae	0	0	0	0	0	0.008
	Gryllidae (Nemobiinae)	0	0	0	0	0	0.008
	Gryllidae (Oecanthinae)	0	0	0	0	0	0.002
Dermatpera	Forficulidae	0.002	0.009	0	0.033	0	0.027
Hemiptera	Lygaeidae (sp. *Lygaeus kalmii*)	0	0.028	0	0.115	0	0.002
	Miridae	0	0	0	0	0	0.016
	Nabidae & Reduviidae	0	0.002	0	0.027	0	0.006
	Pentatomidae (nymph & adult)	0	0	0.001	0.004	0	0.008
	Anthocoridae	0	0	0	0.001	0	0.002
Neuroptera	Chrysopidae & Hemerobiidae	0	0.002	0	0	0	0
Coleoptera	Coccinellidae (larva & adult)	0.005	0.014	0	0.007	0	0.002
	Cantharidae (larva)	0	0.002	0	0	0	0
Hymenoptera	Formicidae	NA	NA	0	0.244	0	0.171
Araneae	(all families)	0	0.007	0.003	0.016	0	0.011
Opiliones	(all families)	0	0	0	0	0	0.048
Gastropoda	(all families)	0	0	0	0.004	0	0.002
	Total	0.007	0.065	0.004	0.454	0	0.322

Results

Oviposition Experiment

I observed a low but generally steady rate of adult monarch butterfly visits to the study sites (**Table 2.2**). Visitation rates ranged between 0.09–0.14 adults observed/h (i.e. one individual for every 7–11 h of observation), apart from July 2017, when I observed an average of 0.5 monarch/h (one individual for every 2 h of observation). Butterflies were typically observed flying over plots, but I also observed them nectaring within prairie plots, and on one occasion ovipositing on milkweed plants in a bare treatment.

Table 2.2. Total numbers of monarch adults and eggs observed during 2016 and 2017 monarch oviposition monitoring experiments. Adult numbers are observations noted during egg-counting surveys. Egg numbers are the total numbers of eggs observed on potted milkweed plants during egg checks.

Year	Deployment Date Range	Days checked	Adults Obs.	Adults Obs./Hour ± SEM	Total Eggs Observed	Eggs Obs./Stem/Day ± SEM
2016	June 7–24	16	6	0.088 ±0.048	1	0.0004 ±0.0004
	July 1–August 3	13	4	0.092 ±0.066	18	0.0102 ±0.0047
	August 9–23	10	6	0.140 ±0.073	149	0.0965 ±0.0293
2017	June 13–30	8	7	0.125 ±0.075	28	0.0220 ±0.0049
	July 12–16	5	16	0.520 ±0.162	29	0.0374 ±0.0284
	August 15–21	6	3	0.100 ±0.045	42	0.0448 ±0.0173

Overall monarch oviposition differed between 2016 and 2017. First examining all habitat treatments and deployments combined, in 2016 oviposition increased exponentially over the course of the breeding season, ranging between 0.0004 to 0.10 eggs/stem/d laid from June–August in contrast to 0.022 to 0.04 eggs/stem/day from June–August 2017, respectively (**Table 2.2**).

Oviposition habitat preference patterns also differed notably between the two study years. In both 2016 and 2017, habitat treatment had a significant effect on monarch oviposition (2016, $\chi^2 = 20.98$, df = 3, $p < 0.001$; 2017, $\chi^2 = 9.46$, df = 3, $p = 0.024$). In 2016, monarch butterflies laid significantly more eggs on *A. syriaca* in the corn versus the other treatments (**Fig. 2.2**). Oviposition did not differ between prairie and bare treatments, but oviposition was significantly lower in soybean versus prairie and bare treatments. In 2017, egg laying was greater in prairie versus soybean habitat treatments, but no other treatment pairs differed.

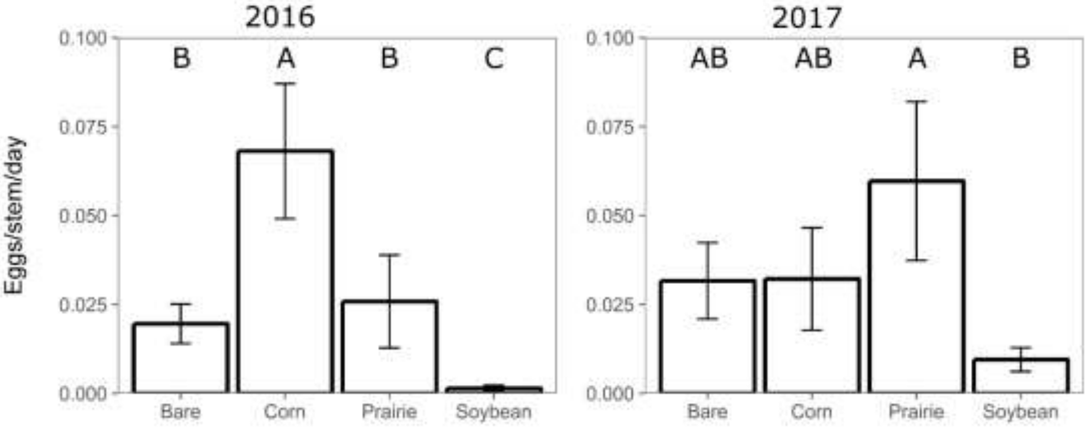

Figure 2.2. Monarch oviposition expressed as mean ± SEM eggs observed per milkweed stem per day (*n* = 4 replicates per treatment). Grouping letters represent post hoc Holm-adjusted pairwise contrast groupings of estimated marginal means from negative binomial generalized linear mixed model, $\alpha = 0.05$.

Survival Experiment

For each of the three experimental periods, survival of eggs differed among exclosure treatments (August 2016, $\chi^2 = 9.93$, df = 3, p = 0.019; July 2017, $\chi^2 = 9.40$, df = 3, p = 0.024; August 2017, $\chi^2 = 13.41$, df = 3, p = 0.004). In August of both years, survival in the closed treatment was significantly greater than in the open and sham treatments, and survival in open versus sham did not significantly differ (**Fig. 2.3A and 2.3C**). In July 2017, survival patterns were similar to the August studies, although in this period, survival in the sham did not differ statistically from that of the closed treatment (**Fig. 2.3B**). These results combined with the observations of near-complete exclusion of predators in the closed treatments (**Table 2.1**) indicate that the exclosure treatments were effective at increasing survival via reduced predation levels, and that the cage effect on survival was small. Therefore, I investigated the effect of habitat treatment on survival using only the open exclosure treatments.

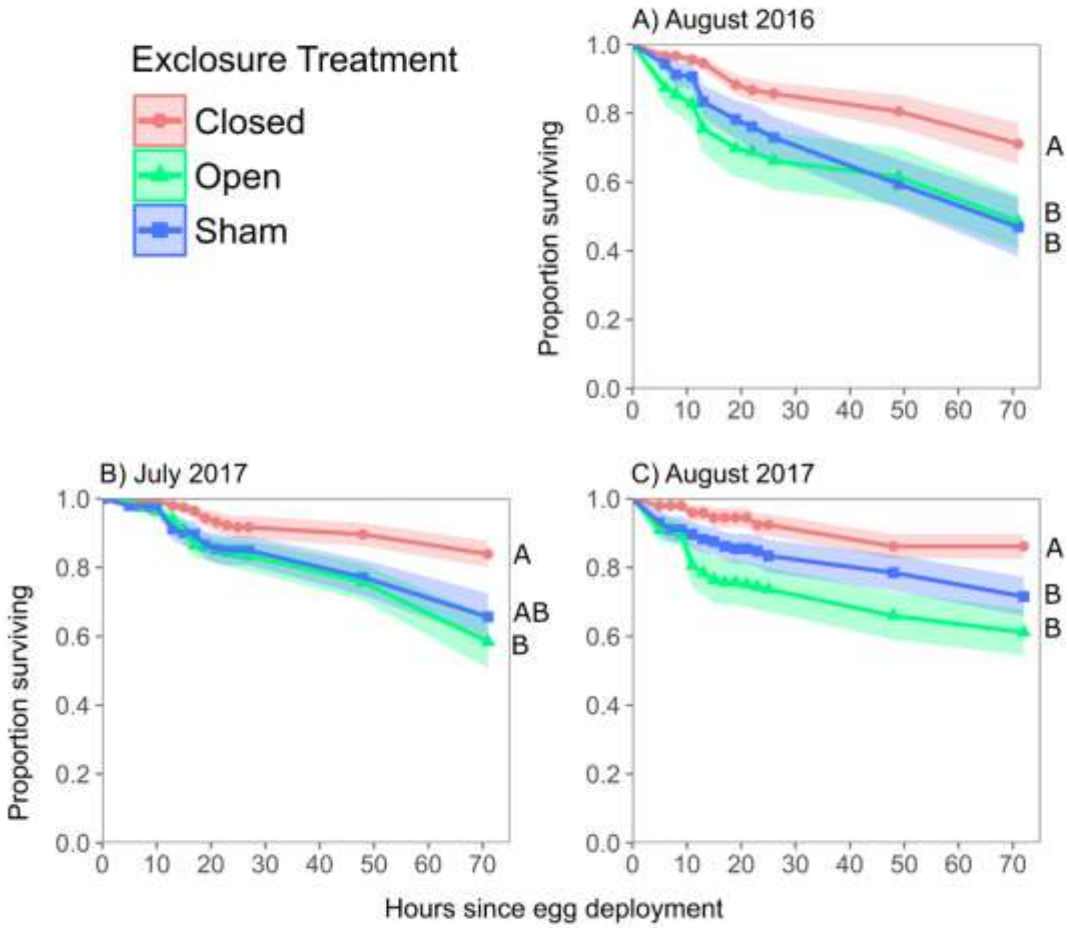

Figure 2.3. Monarch egg and first-instar survival to 72 h under three exclosure treatments for three experimental periods combined across all habitat treatments: A) August 2016; B) July 2017; and C) August 2017. Points represent arithmetic means of survival across the four experimental blocks, and colored areas are SEM. Grouping letters represent post hoc pairwise contrast groupings of estimated marginal means from binomial generalized linear mixed model, $\alpha = 0.05$.

Survival varied with habitat treatment for the August 2016 and 2017 experimental periods, but was similar across all habitats in July 2017 (August 2016, $\chi^2 = 43.98$, df = 3, $p < 0.001$; July 2017, $\chi^2 = 4.34$, df = 3, $p = 0.227$; August 2017, $\chi^2 = 15.02$, df = 3, $p = 0.002$). In August 2016 overall survival to 72 h was highest in bare soil and corn treatments, intermediate in soybean, and lowest in prairie (**Fig. 2.4A**). In August 2017, survival was highest on milkweeds in corn and lowest on those in soybean, with intermediate survival in bare and prairie treatments (**Fig. 2.4B**). Repeated egg survival checks (every 2–3 h) during the initial 24 h revealed that up to 50% of egg predation occurred during the first night following egg deployment. I reran the same survival analysis at 24 h and found the same effects of exclosure and habitat treatments on egg survival as at 72 h.

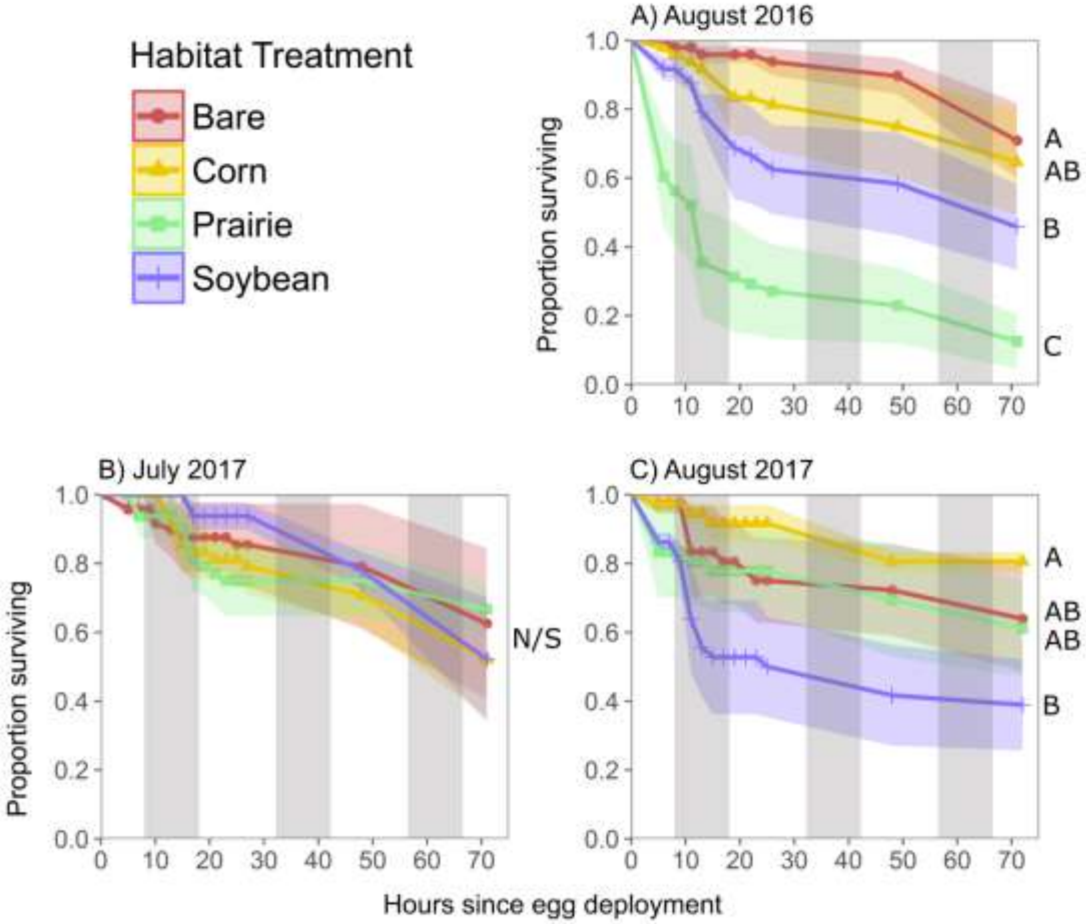

Figure 2.4. Monarch egg and first-instar survival to 72 h in four habitat treatments for three experimental periods: A) August 2016; B) July 2017; and C) August 2017. Points represent arithmetic means of survival across the four experimental blocks, and colored areas are SEM. Grouping letters represent post hoc Holm-adjusted pairwise contrast groupings of estimated marginal means from binomial generalized linear mixed model, α = 0.05. Shaded columns represent nighttime hours from 20:00 to 6:00.

Predators on Experimental Plants

Predators rarely breached exclosures, with no predators found in the exclosure cages in August 2017, and 10–100x more predators per stem in open versus closed exclosure treatments in August 2016 and July 2017 (**Table 2.1**). I observed five arthropod families feeding on sentinel monarch eggs: Coleoptera: Cantharidae (larva, likely *Chauliognathus pensylvanicus* DeGeer); Coleoptera: Coccinellidae (adult, *Harmonia axyridis* Pallas); Hemiptera: Nabidae (adult, unknown sp.); Hemiptera:Miridae (adult, unknown sp.); and Dermaptera:Forficulidae (adult, *Forficula auricularia* L.). Total predator numbers varied by habitat for the August 2017 experiment (August 2017, $\chi^2 = 8.06$, df = 3, p =0.045), but not for the August 2016 or July 2017 survival experiments (August 2016, $\chi^2 = 1.72$, df = 3, p = 0.63; July 2017, $\chi^2 = 4.45$, df = 3, p = 0.22) (**Fig. C4**). When ants were examined in isolation they exhibited a similar pattern; ant numbers varied by habitat in August 2017 ($\chi^2 = 13.72$, df = 3, p =0.003), but not in July 2017 ($\chi^2 = 5.74$, df = 3, p =0.12). In the August 2017 sampling, I observed significantly more predators on plants in the bare treatment primarily driven by ants.

Discussion

Both the differential oviposition and predation influenced egg loss hypotheses of monarch egg abundance among different habitats. I found habitat context influenced monarch oviposition patterns, with corn as the most attractive egg laying habitat in 2016 and prairie the most attractive in 2017. Eggs also experienced differential predation across habitats and over time. Egg predation was greatest in August of both years with bare and corn treatments consistently providing the safest habitats for egg survival. In combination, these findings demonstrate that weekly egg surveys of oviposition often performed in monarch population monitoring programs reflect the combined effects of oviposition preference and predation losses in different habitats.

In 2016, I observed the most eggs laid within corn plots. This pattern is consistent with observations by Pleasants and Oberhauser (2013) and Pitman et al. (2018) and supports the hypothesis that monarchs prefer *A. syriaca* growing in corn as oviposition habitat. In 2017, however, oviposition differences among treatments were less pronounced, with the most eggs laid in the prairie treatment and the only significant pairwise treatment difference between prairie and soybean. Contrary to my prediction and the findings of egg survey studies (Oberhauser et al. 2001, Pleasants and Oberhauser 2013, Pitman et al. 2018), soybean was consistently and significantly the least selected oviposition habitat in both years.

I found that predation on monarchs during the first 72 h varied strongly by habitat type, and in one instance reached 90% (prairie in 2016). However, I note that habitat effects on predation rates varied through time. In August 2016 survival was greatest in bare and corn, intermediate in soybean, and lowest in prairie. In July of 2017, survival was equivalent among all four habitat treatments. In August of 2017, I observed the greatest survival rate in corn, the lowest in soybean, and intermediate in bare and prairie. The highest monarch egg predation rates observed in the prairie treatment indicate that perennial grasslands can be relatively risky habitats for monarch eggs.

Several non-exclusive mechanisms could explain why monarchs exhibited oviposition habitat preference and experienced differential predation by habitat and why these patterns differed among years. Pleasants (2015) hypothesized that milkweed growing in corn is more attractive for oviposition due to higher nitrogen from agricultural fertilization, younger vegetation from frequent disturbance, and greater apparency against a monoculture background.

Because my study held host plant age, fertilization, and density constant while still detecting differential oviposition, I do not find support for plant quality variables as key drivers of oviposition preference. I cannot rule out the possibility that host plant apparency affected oviposition preference. However, if this were the primary driver it seems likely that plants in the bare plots would receive the most eggs, especially considering the milkweed plants were always beneath the height of the surrounding vegetation in the other three treatments, with the exception of those in the soybean and corn treatments during the month of June. I interpret this to indicate that other factors besides apparency still contribute to oviposition. Another possible explanation also put forth by Pleasants (2015) is that monarchs are attracted to the shaded milkweed plants growing under corn. Indeed, Agrawal et al. (2012) found that *A. syriaca* grown in shade had lower concentrations of defensive compounds, lower leaf toughness, and supported higher growth rates of monarch larvae than *A. syriaca* plants exposed to full sun.

Differences between the two study years in preferred oviposition habitat indicate that habitat preference may be mediated by other factors that vary from year to year. The most obvious difference between 2016 and 2017 was weather. During 2017 southwestern Michigan experienced a significant mid-summer drought, with exceptionally warm temperatures and rainfall 50% below average (https://lter.kbs.msu.edu/datatables/7). These conditions resulted in visibly water-stressed corn plants in the treatment plots compared with 2016. Monarchs seeking more humid habitats for oviposition during this dry period may have targeted prairie plots, whose vegetation stayed significantly greener and less wilted than the corn plants . Finally, it is possible that monarchs could adjust oviposition preference based on some signal of predation risk, which could vary by both habitat and year. However, in a post-hoc analysis I found no evidence for a positive correlation between oviposition preference and survival (**Fig. C5**).

Further studies into the mechanisms driving monarch oviposition habitat preference would be useful in helping to design or manage breeding habitat for monarchs.

Overall mean monarch egg density in the study was similar in 2016 and 2017 and was similar to those found in other monarch egg surveys in the region that reported eggs/milkweed stem. I found mean ranges of 0.001–0.069 (soybean and corn) and 0.003–0.022 (soybean and prairie) eggs/stem/d in 2016 and 2017, respectively. In 2000 from June–August Oberhauser et al. (2001) observed means of 0.0134 and 0.0612 egg/stem/survey in non-agricultural and corn sites, respectively, in their weekly upper Midwest surveys. In July–August of 2015 and 2016 Pitman et al. (2018) observed an overall mean of 0.1 eggs/stem/weekly survey in in southern Ontario. At 14 sites across Iowa, on *A. syriaca* Pocius et al. (2018) found approximately 0.001, 0.006, and 0.006 eggs/stem/weekly survey in June–August of 2015, 2016, and 2017, respectively. Finally, Blader (2018) found a range of 0.064–1.2 mean eggs/stem/week at four prairies in Iowa.

Patterns of oviposition across each breeding period differed between 2016 and 2017. The summer of 2016 started with very low egg numbers in early June followed by an exponential increase in oviposition in July and August (**Table 2.2**). In 2017, however, oviposition was intermediate in June and slowly increased through the summer. Low early summer oviposition could be explained by very few monarchs returning in the spring of 2016 from Mexico, as monarch numbers are correlated between sequential stages in the northerly migration (Pleasants and Oberhauser 2013, Inamine et al. 2016). Although monarch overwintering aggregations preceding the summer of 2016 were larger than in 2017 (4.01 versus 2.91 Ha), a snowstorm in March 2016 killed 30–40% of the overwintering population of monarchs in at least two of the major overwintering colonies before they departed Mexico (Brower et al. 2017), which could have resulted in the low oviposition I observed in early 2016. Monarch egg numbers, however, did not reflect the number of adult monarchs observed at the field sites, which was overall consistent between months and years with the exception of July 2017 when I saw 5-fold higher numbers. It is possible that most of these observations were male butterflies patrolling their territories, and the numbers of males observed in one small area could be more limited by the size of the study area rather than the number of individual butterflies on the landscape.

Higher egg survival in the closed exclosure treatments compared with the open and sham treatments indicated that predators were responsible for much of the mortality I observed in the survival experiment. However, eggs within full exclosures did experience some mortality (14–28% average over 72 h). This level of background mortality was likely due to a combination of predators sometimes breaching the exclosures (**Table 2.1**) and eggs occasionally falling off plants due to disturbance from observers frequently removing netting to check egg status. Low survival rates in prairie in 2016 supported my prediction that monarch eggs experience greater predation rates on milkweed in prairie versus crop habitats. However, this pattern did not hold for the following year. July 2017 was characterized by relatively moderate 72 h monarch egg survival (50–60% mean treatment survival) with no significant differences among habitat treatments. In August 2017 survival rates in all treatments matched those in August 2016 except in prairie where survival was considerably higher (10% versus 60% mean prairie survival in August 2016 versus 2017). In July, egg survival rates were moderate and not significantly different among the habitat treatments. It is plausible that one or more important grassland predator population does not reach meaningful abundance until late summer. Indeed, many generalist predators frequenting grasslands in the study area reach their highest abundances in late summer (Fiedler and Landis 2007). Nevertheless, the annual variability in August predation rate in prairie remains unexplained. Although abiotic conditions, such as the 2017 drought, may impact predation rates of monarch eggs, it seems unlikely that the abiotic conditions would affect the habitat treatments so unevenly. Perhaps more probable is that a key predator group in prairie underwent a decline in its population cycle between the two years. Generalist predator numbers can vary greatly from year to year as part of normal population cycling, or in response to alternate prey available in a given habitat (Bahlai et al. 2013). Indeed, long-term research of insect communities at the study site show large year to year variation in abundance and biocontrol service of the ladybeetle (Coleoptera: Coccinellidae) community, and at times these population cycles diverge between annual crop and perennial habitats within a given year (Bahlai et al. 2013).

Although exclosure cages provided strong evidence that predators were responsible for much of the egg mortality, I did not detect an effect of mean predator numbers per milkweed stem on monarch egg survival rate. In this experiment the bare habitat was the only treatment to have significantly more total predators and ants per milkweed stem than other treatments, and bare plots were among the consistently safer habitats for monarch eggs. Notably, ants were the most numerically abundant predators on milkweed plants when their numbers were recorded in the 2017 experiments. Although ants are considered important predators of monarch eggs and larvae (Calvert 2004, Prysby 2004, Mooney and Agrawal 2008), I never directly observed ants consuming monarch eggs. These results align with those of Pitman et al. (2018), who found that milkweed predator abundance was not a predictor of monarch egg density. I speculate that, aside from ants, many monarch egg predators could be highly mobile (e.g. Miridae and Coccinellidae) or visit plants at night and may be underrepresented in predator surveys. Furthermore, some predators could be more voracious than others, making overall predator numbers less meaningful to egg disappearance rates. More research regarding the identities, natural history, and population dynamics of monarch egg and larva grassland predators is crucial to develop schemes to increase monarch production in US agricultural landscapes.

Results of these experiments indicate that variation in monarch productivity among different habitats is a function of both oviposition habitat preference and differential survival due to predation. This has important implications for monarch population monitoring and conservation efforts. Because much of the predation I recorded occurred at night and during the first 24 h following oviposition, weekly egg surveys are not adequate to determine monarch oviposition habitat preferences in habitats with different levels of predation. This would be particularly true in years like 2016, when rapid predation between survey periods could inflate estimates of oviposition difference between grassland and crop habitats. I recommend that monarch egg surveys seeking to elucidate predictors of oviposition habitat section either be conducted more frequently or be combined with egg predation studies.

Finally, this study investigated monarch oviposition habitat preference and predation rates at a relatively fine spatial scale considering the long-distance dispersal capabilities of monarch butterflies and the much larger habitat patches occurring in agricultural landscapes. While the experimental design was useful in that it operated as a “choice test” for monarchs that could easily move among the study plots, I recommend further oviposition habitat experiments at the landscape level to complement existing observational studies and to validate recent agent based monarch movement models by Grant et al. (2018). It would also be useful to determine whether egg density is more strongly related to the number of egg laying monarchs in a given area or the number of eggs laid per individual. It is also possible that some predators could move among the small habitat patches (Blitzer et al. 2012). I expect that similar future experiments in large scale agricultural landscapes with larger habitat patches may have less predator community spillover and would show stronger predation differences among habitats.

The potential for high predation rates in grassland habitats is an important consideration for monarch conservation, as monarch breeding habitat restoration efforts are primarily proposed to increase milkweed plantings in perennial grasslands occurring on conservation lands, roadsides, and other rights-of-way (Thogmartin, López-Hoffman, et al. 2017). Developing management strategies to enhance monarch egg and larva survival in grasslands could make restoration more tenable and efficient in terms of land use. Indeed, a recent rangewide monarch population model estimated that increasing monarch survival and fecundity rates each by 2% in the northcentral breeding range would stabilize the eastern North American monarch population (Oberhauser et al. 2017). Consideration of egg and larva survival in monarch habitat management strategies could greatly increase chances of a successful monarch restoration effort. One promising possibility is the reintroduction of disturbance regimes to grasslands supporting monarch habitat. Recent work by Haan and Landis (2019a) demonstrated that late summer mowing of milkweed patches both generates new milkweed growth that is highly attractive for monarch oviposition, but also increases egg and larvae survival before predators fully recolonize plants. Recent work has also highlighted how milkweed species plays a role in influencing monarch oviposition and larval survival and performance (Pocius, Debinski, Bidne, et al. 2017, Pocius, Debinski, Pleasants, et al. 2017, Pocius et al. 2018). Monarch conservation would benefit from a better understanding of how grassland type (e.g. prairies, roadsides, old fields), milkweed species, and disturbance regimes interact to influence monarch oviposition and predation risk.

During much of the 20th century monarchs likely benefited from agricultural practices in the north central US through the creation of better *A. syriaca* growing conditions with few monarch predators. Now relegated to breeding in relatively small areas of perennial grasslands, monarchs are left with fewer host plants in more risky habitats. As conventional agricultural practices attempt to deliver ever more primary productivity from limited lands, they will inevitably result in more simplified habitats at both the field and landscape scales (Landis et al. 2000, Rusch et al. 2016). Consequently, even species like monarch butterflies that once thrived in agricultural landscapes could be at risk. Conserving global biodiversity will require intentional efforts to determine both the underlying causes of species declines and management practices which will allow for biodiversity to exist alongside productive agricultural systems.

CHAPTER 3: VIDEO SURVEILLANCE REVEALS A DIVERSE AND LARGELY NOCTURNAL COMMUNITY OF *DANAUS PLEXIPPUS* (L.) EGG PREDATORS

Abstract

The decades-long decline in overwintering populations of the eastern North American migratory monarch butterfly (*Danaus plexippus* L.) has caused concern over its long-term persistence. Losses of the monarch's primary host plant, common milkweed (*Asclepias syriaca* L.), from annual crop fields in its core summer breeding range prompted efforts to conserve monarchs by planting additional milkweed in non-agricultural perennial grasslands. However, grasslands harbor abundant and diverse communities of arthropod natural enemies, which can exert high predation pressure on monarch eggs and larvae. While diurnally active predators of monarchs are relatively well known, no studies have investigated if nocturnal predators contribute to monarch eggs and larval mortality. I utilized video surveillance cameras to monitor sentinel monarch eggs on naturally occurring milkweed in four grassland types in southern Michigan to identify predators of monarch eggs and neonates, and to determine if nocturnally-active species imposed significant predation pressure. I also observed predation on a limited number of 4th instar monarch larvae. Overall, I found at least ten arthropod taxa consumed monarch eggs and larvae, with significantly more (74%) of the predation events occurring during crepuscular or nighttime hours than during daytime. Taxa observed to attack monarch eggs included European earwigs (*Forficula auricularia* L.), tree crickets (*Oecanthus* sp.), a lacewing larva (Neuroptera), plant bugs (Miridae), small milkweed bugs (*Lygaeus kalmii* Stål), ants (Formicidae), spiders (Araneae: Salticidae and other spp.), harvestmen (Opiliones), and velvet mites (Trombidiformes: Trombidiidae). Larvae were attacked by a ground beetle (*Calleida* sp.),

jumping spider (Araneae: Salticidae), and spined soldier bug (*Podisus maculiventris* Say). These results highlight the importance nocturnal arthropod predation, and I recommend future research investigating arthropod species interacting should include observations during both night and day. I also observed a high degree of variability in predation pressure among study sites, indicating a need for more data regarding the habitat drivers of differential predation pressure on monarch eggs and the potential to reduce predation on monarchs during early life stages to achieve help restore the declining populations.

Introduction

The monarch butterfly (*Danaus plexippus* L.) has emerged as a species of conservation concern, as overwintering aggregations of both the eastern and western North American migratory populations have declined precipitously over the past three decades (Brower et al. 2012, Schultz et al. 2017, The Xerces Society 2019). It is estimated that since the 1990's the eastern migratory population has declined by approximately 80% (Brower et al. 2012, Semmens et al. 2016) and the western population has declined by over 90% (Schultz et al. 2017, The Xerces Society 2019). A significant amount of research has sought to understand the drivers of these declines. For the eastern population, multiple factors appear to contribute, including: destruction of overwintering habitat (Ramírez et al. 2015); exposure to insecticides (Oberhauser et al. 2001, Krischik et al. 2015); increased pathogen loads (Altizer and de Roode 2015); and increased mortality during migration (Ries et al. 2015, Inamine et al. 2016). However, the loss of breeding habitat in agricultural landscapes in the Midwestern US has received the most attention as a driver of monarch declines (Pleasants and Oberhauser 2013, Thogmartin, Wiederholt, et al. 2017). In this region, common milkweed (*Asclepias syriaca* L.) is the primary larval host plant

(Malcolm et al. 1993). Loss of common milkweed is primarily due to the widespread adoption of herbicide tolerant row crops, which has largely eliminated it from conventionally managed fields, representing an estimated overall 46% reduction in available stems on the landscape from 1999–2014 (Pleasants et al. 2017).

Due to the near absence of *A. syriaca* in agricultural crop fields, monarchs currently depend on host plants in non-agricultural habitats, primarily grasslands including field edges, fallow fields, and roadsides. This poses a major challenge for the monarch population recovery, as these habitats represent a relatively small portion of the former milkweed habitat in the monarch's summer breeding range. A recent analysis suggested that at least 1.3 billion milkweed stems must be added to the region to restore the monarch overwintering population to a sustainable average of 6 ha, requiring the conversion of more than half of marginal agricultural lands to grassland milkweed habitat (Thogmartin, López-Hoffman, et al. 2017).

In addition to the challenges to monarch conservation efforts imposed by limited land area, monarch eggs and larvae experience high levels of mortality by arthropod predators. Field experiments show that grasslands are riskier habitats for monarch eggs than row crops; eggs on milkweed in corn (*Zea mays* L.) and soybean (*Glycine max* L.) experience lower predation (≤ 50%) in comparison to up to 90% mortality in restored prairie habitat (Myers et al. 2019). This pattern is consistent with the findings of other research in which grasslands harbor more abundant and diverse predator communities and exhibit greater predation pressure on herbivorous arthropods than do agricultural crop fields (Werling et al. 2011, 2014). It is apparent that monarchs are not only relegated to smaller areas of breeding habitat, but that this remaining habitat potentially exposes monarch eggs and larvae to increased predation pressure.

Although monarchs naturally experience high egg and larval mortality, taking measures to increase their survival by even modest levels could be an effective conservation strategy. A population model by Oberhauser et al. (2017) indicated that increasing monarch fecundity by 4% in the Midwest breeding range would be sufficient to halt the decline of the Eastern migratory population. This could potentially be achieved by managing habitats to increase the number of monarch eggs laid and/or the overall survival of eggs to adulthood. For example, a recent study showed that grassland disturbance could be used as a tool to elicit greater monarch egg laying per milkweed stem (Haan and Landis 2019a). This practice also decreased the number of predators visiting milkweed plants for 3–4 weeks, possibly creating an opportunity for monarch eggs to escape predation during their most vulnerable life stages.

Efforts to increase monarch survival would benefit from more knowledge about the predators responsible for egg and larval mortality. A review by Oberhauser et al. (2015) compiled a list of known monarch egg and larval predators, which included 12 taxa. This work drew primarily from field observations (Smithers 1973, Borkin 1982, Zalucki and Kitching 1982b, Lynch and Martin 1993, De Anda and Oberhauser 2015) as well as a smaller number of field experiments (Calvert 2004, Prysby 2004) and laboratory studies (Koch et al. 2003, 2005, Rayor 2004, Rafter et al. 2013, Oberhauser et al. 2015). Hermann et al. (in press) generated a more comprehensive list of potential monarch egg and larva predators by performing laboratory feeding trials with a suite of arthropods collected from common milkweed. Of the 75 arthropod taxa tested, 34 consumed eggs and 30 consumed neonates, suggesting that monarch egg and larvae predators are more diverse than previously thought.

One important knowledge gap regarding monarch butterfly egg and larval predators is the potential importance of nocturnal predators and predation events. Investigations of predation on the eggs of other Lepidopteran species indicate that nocturnal predation is as significant, if not more so, than diurnal predation. A study in corn, cotton (*Gossypium* sp. L), and soybean fields in Texas found that equivalent or greater predation on sentinel eggs of *Helicoverpa zea* Boddie and *Spodoptera exigua* Hübner took place nocturnally versus diurnally, with the dominant predator groups differing by time of day (Pfannenstiel 2005). In previous studies, I observed nocturnally-active taxa resting on milkweed stems during the day and found evidence that monarch eggs suffered significant nocturnal predation (Myers et al. 2019). However, this study made few direct observations of predators and predation events, leaving unresolved the relative importance of day versus night predation levels on monarch eggs.

I used video surveillance to increase knowledge of monarch predation in grasslands. This technique has proved useful for elucidating important predators in grasslands as well as annual and perennial crops (Grieshop et al. 2012). It has an advantage over lab feeding trials in that it reveals how and when predators and prey interact in field settings. Using a simple, relatively inexpensive video camera recording system I monitored monarch eggs during the summers of 2017 and 2018 in southern Michigan. I also present the findings of a small number of video observations of fourth instar larvae tethered to *A. syriaca* plants. I sought to determine: 1) the proportion of monarch eggs consumed by predators in various grassland habitats; 2) the identity of the predators; and 3) the relative proportion of predation occurring during day versus night. Based on prior field observations, I predicted that at least half of predation events would occur during crepuscular or nocturnal time periods. I interpret my results in the context of monarch butterfly conservation in grasslands and provide suggestions for further research regarding monarch predator-prey interactions.

Methods

Study Sites

I conducted monarch video surveillance at six sites in southern Michigan. In the summer of 2017 I performed video monitoring at two sites: 1) a residential backyard garden in East Lansing, Michigan (hereafter "garden site"); and 2) a biofuels cropping experiment at the Michigan State University (MSU) Kellogg Biological Station (hereafter "KBS site") in Hickory Corners, Michigan. The biofuels experiment had 9.1 x 27 m plots of corn and 10-species prairie replicated across four blocks (Dickson and Gross 2015). The trials in 2017 tested surveillance methods and produced valuable preliminary information on predator identity and timing of predation events. Aside from final testing performed at the garden site in June, all 2018 video monitoring was performed on the MSU campus in East Lansing, Michigan at four sites representing different grassland habitats where *A. syriaca* frequently occurs in the study region (**Fig. 3.1**). Sites consisted of 1) a grassy cornfield edge (hereafter "corn edge"), 2) an old field dominated by Canada goldenrod (*Solidago canadensis* L.) and cool season grasses (hereafter "old field"), 3) a marsh edge dominated by reed canary grass (*Phalaris arundinacea* L.; hereafter "marsh edge"), and 4) an un-mowed fencerow with autumn olive (*Elaeagnus umbellata* Thunberg) and cool season grasses (hereafter "fencerow"). All four sites had an abundance of naturally growing *A. syriaca* and were actively used as breeding habitat by wild monarchs. Details regarding locations and dominant vegetation of each site are included in **Table D1**. For a detailed timeline including dates and numbers of eggs monitored for each site see **Table D2**

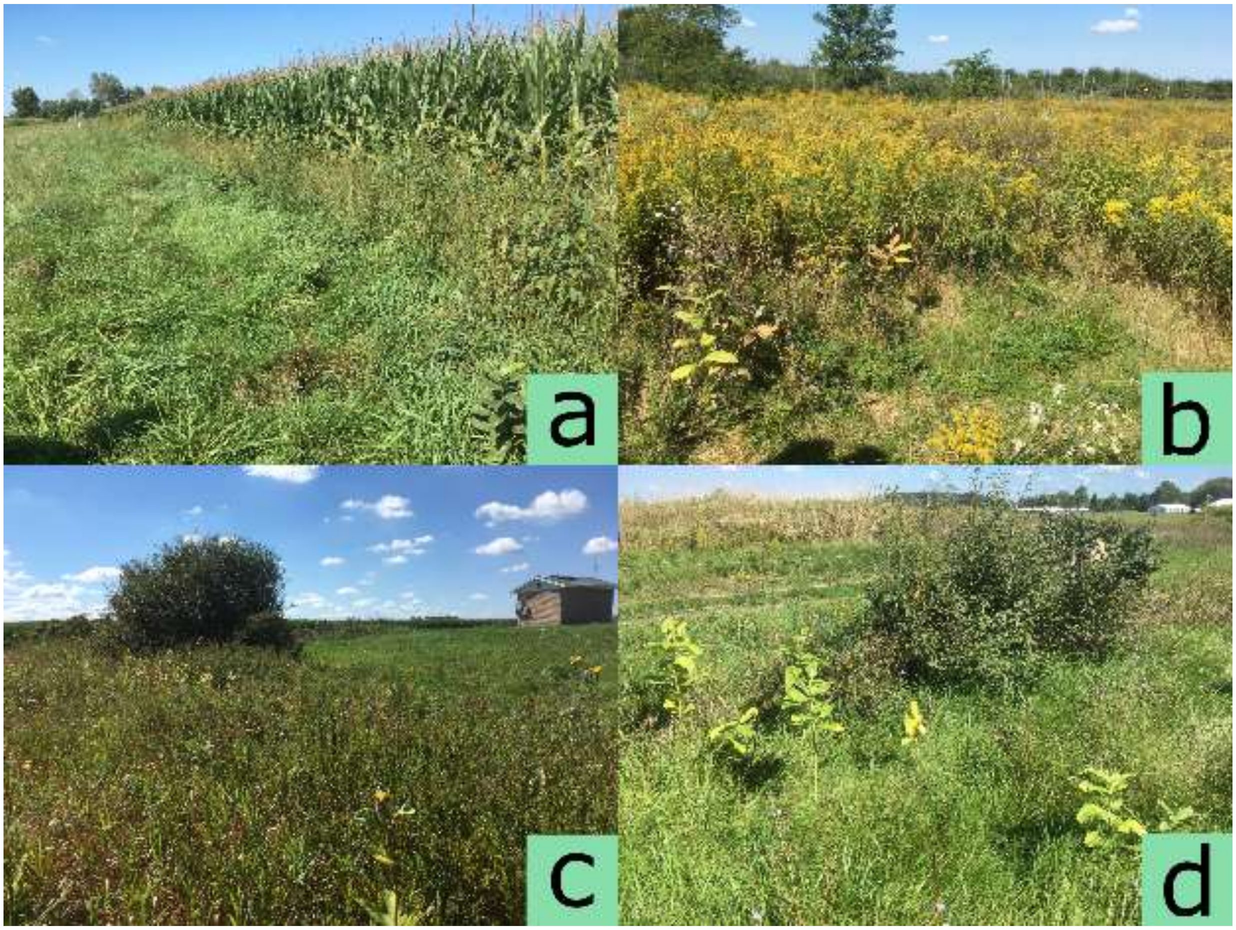

Figure 3.1. Grassland study sites used for monarch butterfly egg video surveillance on the Michigan State University campus in East Lansing, Michigan: a) corn edge; b) old field; c) marsh edge; d) fencerow.

Study Plants and Egg Placement

Monarch eggs used for these studies were produced by lab colonies originating from wild captured monarch butterflies caught locally in each of the study years. I provided captive butterflies with potted *A. syriaca* plants for oviposition and collected eggs by gently removing them by hand from the plants as needed for the study. I transported eggs to the field in plastic Petri dishes on moist paper towel. All *A. syriaca* plants used for the study were between 50 and 75 cm tall and in a vegetative (i.e. pre-flowering) stage. In 2017 at the KBS site, I monitored eggs placed on potted *A. syriaca* plants in the field. These plants were Michigan native genotypes grown from seed (Wildtype Nursery, Mason, Michigan). At all other sites I used naturally occurring *A. syriaca* stems for video monitoring.

Placement of the eggs on milkweed plants mimicked the natural oviposition sites of monarch butterflies (De Anda and Oberhauser 2015). On each plant I affixed eggs on the central underside of one of the two uppermost fully emerged leaves. In preliminary trials in 2017 I affixed 1–10 eggs per leaf spaced at least 1 cm apart. In 2018, I affixed two eggs per leaf approximately 1 cm apart. Placing eggs in this fashion allowed for observation of multiple predation events within a single camera frame. Eggs were attached by squeezing a small droplet of Elmer's ® Glue-All (Elmer's Products, Columbus, Ohio) onto the tip of a fine paintbrush, lifting an egg by dabbing it onto the glue droplet, and gently wiping it onto the leaf so that the base of the egg was glued to the leaf.

In 2017 I tested the egg gluing method by monitoring ten monarch eggs on a single leaf of an *A. syriaca* plant growing in the garden site. Four of the eggs were laid by a gravid monarch bagged over the plant, and six were affixed using this gluing method. After one night of video monitoring I found nine out of the 10 eggs were removed by predators with only one naturally laid (i.e. non-glued) egg remaining. These results demonstrated that predators would consume both eggs glued to leaves and naturally laid eggs. A prior lab study (Hermann et al. in press) used the same gluing method and confirmed that a diversity of predators readily eat eggs glued to leaves using this method. Finally, the results of the study presented below demonstrated that glued eggs rarely fall off plants in the field.

Video Camera Setup and Monitoring Procedure

I used a camera setup modeled after Grieshop et al. (2012), which included a closed circuit security camera with infrared night vision recording to a secure digital (SD) memory card through a miniature digital video recorder (DVR). The system was powered by two sealed lead acid batteries wired in parallel and housed with the DVR inside a plastic toolbox (**Fig. 3.2**). In preliminary studies in 2017 I used an outdoor security camera (Q-See model QOCDC36 Digital Peripheral Solutions Inc., Anaheim CA), FPV Mini C-DVR (Jingxinhong Model Co., LTD. Shenzhen, China), and 12 v 8 Ah sealed lead acid batteries (model ML8-12, Mighty Max Battery, Brooklyn, NY). For better video quality and battery life, in 2018 I used to a 1080P HD Security Camera (model HN-IA60E200FS, Honic Electronic Technology Co., Ltd, Zhongshan, Guangdong, China), HD Micro SD DVR (model ADIB00UMZM2IG, Coomatec Intelligence, Ltd, Shenzhen, China), and 12 v 18 Ah sealed lead acid batteries (ExpertPower Direct, Paramount, CA, USA). Both setups were designed to film continuously for 24 h on one battery charge.

Figure 3.2. Camera setup for monarch butterfly (*Danaus plexippus* L.) egg video surveillance study.

Cameras were mounted on a short section of iron rebar pounded into the ground and positioned so that all sentinel eggs were viewable in the image frame (**Fig. 3.2**). To prevent the portion of the leaf containing the sentinel eggs from moving out of focus due to wind or nyctinastic plant movement, leaves were secured to the cameras at an appropriate focal distance using a twist-tie. To prevent image overexposure I created a light diffusing shade by positioning a 0.5 x 0.5 m piece of white corrugated plasti-board mounted on a step-in fence post behind the plant such that it filled the background of the video frame. I checked the focus and position of the image frame using a portable liquid crystal display (LCD; model S801H, Shenzhen Eyoyo Tech. Co., Ltd, Los Angeles, CA).

I deployed eggs and 1–3 cameras per site on a regular weekly schedule (for details see **Table D2**), with minor adjustments to avoid rainy conditions. I revisited the cameras daily to change the batteries and check the eggs. Observations of each set of eggs continued until all eggs were gone or until the end of the week (i.e. for a range of 24–96 h of video monitoring). The same individual plants were used each week unless leaves were damaged or plants grew too tall, in which case I selected a nearby comparable plant. The process was repeated weekly from 3 July 2018 to 4 September 2018, except for the week of 23 July. At the outset of the experiment I deployed cameras at the old field and fencerow sites, later adding the marsh edge and corn edge sites. On July 30 I removed the two cameras from the fencerow site due to limited predation events occurring there and increased the number of cameras monitoring at the old field (**Table D2**). I monitored a total of 29 eggs in 2017 and 123 eggs in 2018 for a mean of 59 h per egg.

Fourth Instar Monitoring

On 18 September 2017 I performed a limited study to video monitor fourth instar monarch larvae at the KBS site. I selected four healthy fourth instar larvae from a lab colony and tethered them onto potted *A. syriaca* plants so that they stayed in the camera frame. Tethering involved loosely tying a 10 cm length of cotton sewing thread to the mid-section of the caterpillar between the legs and prolegs. I then applied a small drop of Krazy Glue® (Elmer's Products Inc., Westerville, OH) to the thread to adhere it to the dorsal side of the caterpillar. I glued the other end of the thread to the underside of an upper leaf and placed the caterpillar on the leaf. Lab trials showed that caterpillars tethered using this method actively fed and molted out of the tether seemingly unharmed. One plant/larva/camera unit was placed in each of the four prairie plots at the KBS site. I left them for 24 h, retrieving the footage and remaining caterpillars the following day.

Video Processing

To streamline processing of the over 9000 hours of video footage generated, I only reviewed instances when eggs were found missing or empty at the end of the monitoring period. I first viewed footage using the freely available VLC Media Player (VideoLan Organization) at 10x speed to locate the instance of predation and subsequently reviewed footage in real time to record predator identities, start and end times of predation events, and behaviors of interest. I could not always determine exactly when the egg disappeared, especially at night with lower quality night vision footage. In instances when multiple predators visited an egg during one night, I only considered it a predation event if I could clearly see sustained feeding behavior at the exact spot of the egg.

Statistical Analysis

All statistical analyses were performed in R version 3.4.2 (R Core Team 2017). I generated Kaplan-Meier survivorship curves to model predation rates of individual eggs at the four grassland sites in 2018 using the function survfit in the R package Survival (Lin and Zelterman 2002, Therneau 2015). Instances of predation were considered mortality events, eggs that hatched or survived to the end of the monitoring period were considered censored, and the eggs which suffered unknown or other fates (i.e. desiccated, dislodged, or on the mammal-clipped plant, see Results) were not included in the survival analysis. I contrasted survival curves among the four grasslands using the Mantel-Haenszel test with the function survdiff in the R package Survival (Lin and Zelterman 2002, Therneau 2015) . I tested for differences in the occurrence of predation between night and day using a G-test of independence (Sokal and Rohlf 1995), with a null hypotheses of equal numbers of eggs eaten during day versus night.

Results

I determined the fates of 145 of the 152 eggs total monitored. Of these, 25% were predated before hatching (**Fig. 3.3**), with predation events observed in all sites. The remaining eggs were intact at the end of the monitoring period (56%), hatched (13%), or visibly shriveled and desiccated (4%). Additionally, two eggs (1%) were on a host plant that was clipped down by a mammal, and one egg (< 1%) was dislodged from the plant by a milkweed tussock moth caterpillar (*Euchaetes egle* Dury). Of the 19 eggs that hatched while under surveillance, two on the same leaf were consumed during the first instar by a single spined soldier bug nymph (*Podisus maculiventris* Say). Of the remaining larvae, 15 exited the camera frame (and thus their fates are unknown) and two were still present at the end of the monitoring period. Fates could not be ascertained for seven eggs that went missing or were recorded as empty at the end of the video monitoring period, but for which no distinct predation event was confirmed. Egg predation rates were greatest in the old field (14/50 eggs predated) and lowest at the fencerow site (1/14 eggs predated). However, survivorship was not significantly different among the four grassland sites ($\chi^2 = 7.1$, df = 3, p = 0.07), with overall modeled survival ± standard error over 24, 48, and 96 h of 0.90 ± 0.03, 0.84 ± 0.04, and 0.75 ± 0.05, respectively.

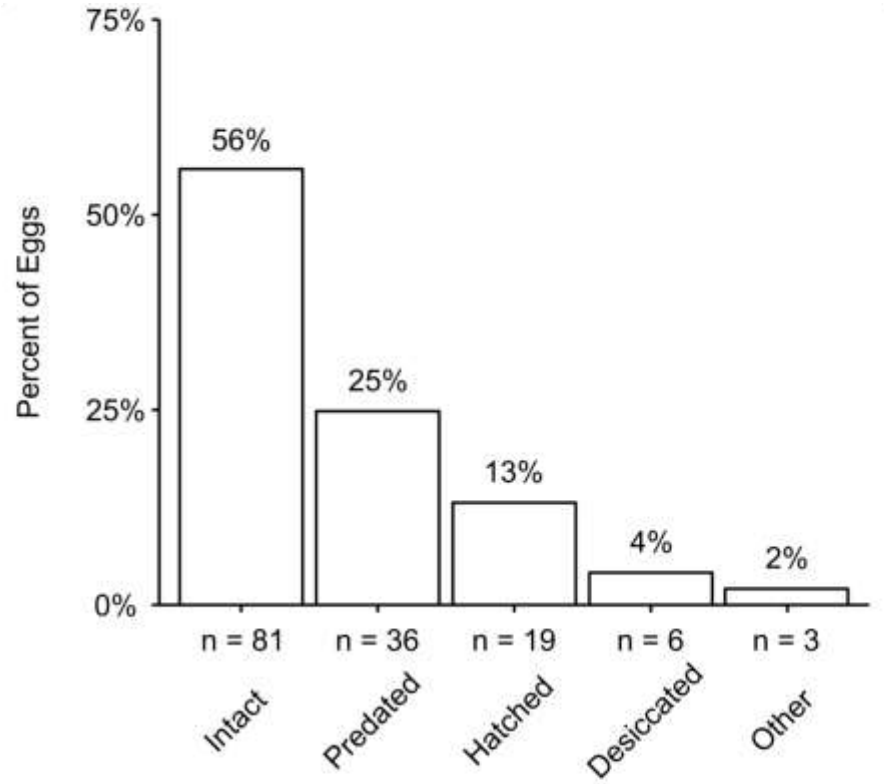

Figure 3.3. Fates of 152 monarch eggs monitored in video surveillance studies in 2017 and 2018. Eggs in the "other" category include two eggs on a plant clipped down by a mammal and one egg knocked off the plant by a milkweed tussock moth caterpillar (*Euchaetes egle* Dury). Not shown are the remaining seven eggs, whose fates could not be ascertained with certainty.

I observed a total of 10 predatory taxa from eight orders consuming monarch eggs (**Table 3.1**). Confirmed egg predators included both obligate predatory groups (i.e. orders Araneae, Opiliones, and Neuroptera, and family Trombidiidae) and omnivores (i.e. *Forficula auricularia* L., *Lygaeus kalmii* Stål, *Oecanthus* sp., and families Miridae and Formicidae). In terms of total eggs eaten, European earwigs (*F. auriculaia*) were the most voracious (10 eggs consumed over three predation events). However, spiders (Salticidae and other unidentifiable species) were responsible for the greatest number of individual egg predation events (five eggs consumed over five predation events). Despite their presence on milkweed stems during this study, only one ant species (likely *Prenolepis imparis* Say) was observed consuming a monarch egg

Table 3.1. Predatory taxa and details of predation events observed via video surveillance of 152 monarch eggs in 2017 and 2018.

Year	Order	Taxon	Locations	Number eggs eaten	Number events	Date(s)	Time	Mean time on egg (H:M:S) ± SEM
2017	Dermaptera	European earwig (*Forficula auricularia*)	KBS, Garden	9	2	6/11–9/5	22:51–23:14	0:23:00 *
	Hemiptera	Plant bug (Miridae, sp. unknown)	Garden	2	1	7/8	20:11–20:59	0:47:30 ± 0:00:30
2018	Dermaptera	European earwig (*Forficula auricularia*)	Garden	1	1	6/11	22:54–23:00	0:06:00 *
	Orthoptera	Tree cricket (*Oecanthus* sp.)	Old field	5	3	7/17–8/22	22:31–01:10	0:07:36 ± 0:02:12
	Neuroptera	Lacewing (Chrysopidae or Hemerobiidae, sp. unknown)	Old field	1	1	7/4	23:46–23:47	0:01:00 *
	Hemiptera	Plant bug (Miridae, sp. unknown)	Fencerow	1	1	6/25	unclear	unclear *
		Small milkweed bug (*Lygaeus kalmii*)	Old Field, Marsh Edge	6	3	9/4–9/6	12:55–19:52	0:09:50 ± 0:01:08
	Hymenoptera	Ant (Formicidae, likely *Prenolepis imparis*)	Corn Edge	1	1	9/6	21:31–22:27	0:56:00 *
	Araneae	Jumping spider (Salticidae, unknown sp.)	Corn Edge	1	1	8/22	10:01–10:07	0:06:00 *
		Other Spider (unknown sp.)	Old field, Marsh Edge	4	4	8/2–8/21	22:19–3:12	1:21:15 ± 0:05:27
	Opiliones	Harvestman (unknown sp.)	Garden	1	1	6/9	23:23--23:43	0:20:00 *
	Trombidiformes	Mite (unknown sp.)	Old field	2	1	8/30	11:03--14:52	1:54:00 ± 0:29:00

* SEM not included for predator taxa that ate only one egg or when exact timing of predation was indeterminable.

Two out of the four fourth instar monarch caterpillars monitored were predated. One was attacked and consumed by a jumping spider (family Salticidae). The other was attacked, killed, and partially consumed by a beetle *Calleida* sp. (family Carabidae) and subsequently fed upon by several other arthropod taxa including meadow and cone-head katydid (*Orchelimum* sp. and *Neoconocephalus* sp., respectively), a yellow jacket (*Vespula* sp.), a fly (likely family Tachinidae), a ground cricket (subfamily Nemobiinae), and a harvestman. Detailed timelines and descriptions of these events are included in **Table 3.2**. The other two tethered fourth instars were found the following day, and review of the video footage revealed no encounters with predators.

Table 3.2. Unique interactions between monarch (*Danaus plexippus* L.) and various predators observed during egg and larva video surveillance.

Year	Date	Number and life Stage	Location	Species Involved	Interaction Description
2017	9/5	2 eggs	KBS Site	Thirteen-lined ground squirrel	The upper half of the milkweed stem was found on the ground adjacent to the pot with both eggs still intact. The stem was excised by a chewing mammal, likely a thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*), which were very common at the KBS site.
	9/18	1 fourth instar	KBS site	Ground beetle Meadow katydid Yellow jacket Fly (likely Tachinid) Cone-head katydid Harvestman Ground cricket	At 16:17 the caterpillar was attacked by a ground beetle (*Calleida* sp.), and dropped from the leaf hanging by the tether. The beetle then reached the caterpillar and dragged it back to the leaf and began consuming it. At 16:42 a meadow katydid arrived, chased away the beetle and began consuming the caterpillar, shortly after which the beetle returned and fed on the caterpillar alongside the katydid until 16:57, when both abruptly departed. During the following hours the caterpillar remains were scavenged by meadow katydid(s) (*Orchelimum* or *Conocephalus* sp.) at 17:38, 3:56, and 5:15, yellow jacket(s) (*Vespula* sp.) at 17:39, a fly (likely Tachinidae) at 17:41, a cone-head katydid (*Neoconocephalus* sp.) at 20:44, a harvestman (Opiliones: family unknown) at 1:51, and a ground cricket (subfamily Nemobiinae) at 3:11.
	9/18	1 fourth instar	KBS Site	Jumping spider Meadow katydid	Caterpillar was killed and eaten by a jumping spider (Salticidae) from 14:43–15:13. The caterpillar remains were later scavenged by at least two meadow katydids from 16:52–17:22.

Table 3.2. (Cont'd).

2018	6/12	1 egg	Garden Site	Earwig Tree cricket Harvestman	The egg was inspected by potential predators four times within three hours, with the true time of predation indeterminable. Predator visits were: earwig (*Forficula auricularia*) at 23:14; tree cricket (*Oecanthus* sp.) at 23:30; harvestman at 00:40; and earwig at 1:33.
	8/9	1 first instar	Marsh Edge	Milkweed weevil	At 23:40 milkweed weevil (*Rhyssomatus lineaticollis*) walked past a first instar larva, brushing up against it. The larva dropped on a silk thread, which subsequently adhered to the weevil. The larva was then pulled along on the silk by the weevil for several cm until the silk stuck to the leaf and the larva was eventually able to climb back to the leaf and resume feeding.
	8/17	1 egg	Old field	Milkweed tussock moth larva	A milkweed tussock moth caterpillar (*Euchaetes egle*) walked across the leaf and passed over the monarch egg at 00:40, after which the egg was no longer visible, apparently having been dislodged by the caterpillar as no evidence of feeding was observed.
	8/20	1 egg	Old field	Spider Slug	After egg was consumed by a spider (Araneae: unknown sp.) with only the empty chorion remaining, a slug (likely *Deroceras reticulatum*) arrived 17:20 and consumed the chorion leaving no discernable remnant of the egg.
	8/20	1 first instar	Corn Edge	Small milkweed bug	At 17:14 the first instar dropped on a silk from lower leaf surface when a small milkweed bug (*Lygaeus kalmii*) crawled onto the upper leaf surface. At 17:30 it reappeared back on the leaf.
	9/7	2 first instars	Old field	Spined soldier bug	From 11:11–13:34 a spined soldier bug nymph (*Podisus maculiventris*) consumed both first instar larvae. The first predation event lasted 1 h 6 m and the second lasted 1 h 9 m.

Supporting my prediction, predators consumed more eggs during nighttime versus daytime hours. Of the instances for which I could confirm the time of predation (35/36 eggs predated), significantly more total eggs were consumed during night versus day (χ^2 = 6.64, df = 1, p = 0.01), with 71% of eggs consumed and 74% of predation events occurring during nocturnal/crepuscular hours between 20:00 and 6:00 (**Fig. 3.4**). For predator groups with multiple feeding observations, temporal feeding patterns were consistent; earwigs, tree crickets, and non-Salticidae spiders all fed exclusively at night, while small milkweed bugs fed during the day. Predation on first instar monarch larvae by a spined solider bug and on fourth instars by a *Calleida* sp. beetle and jumping spider occurred during the day.

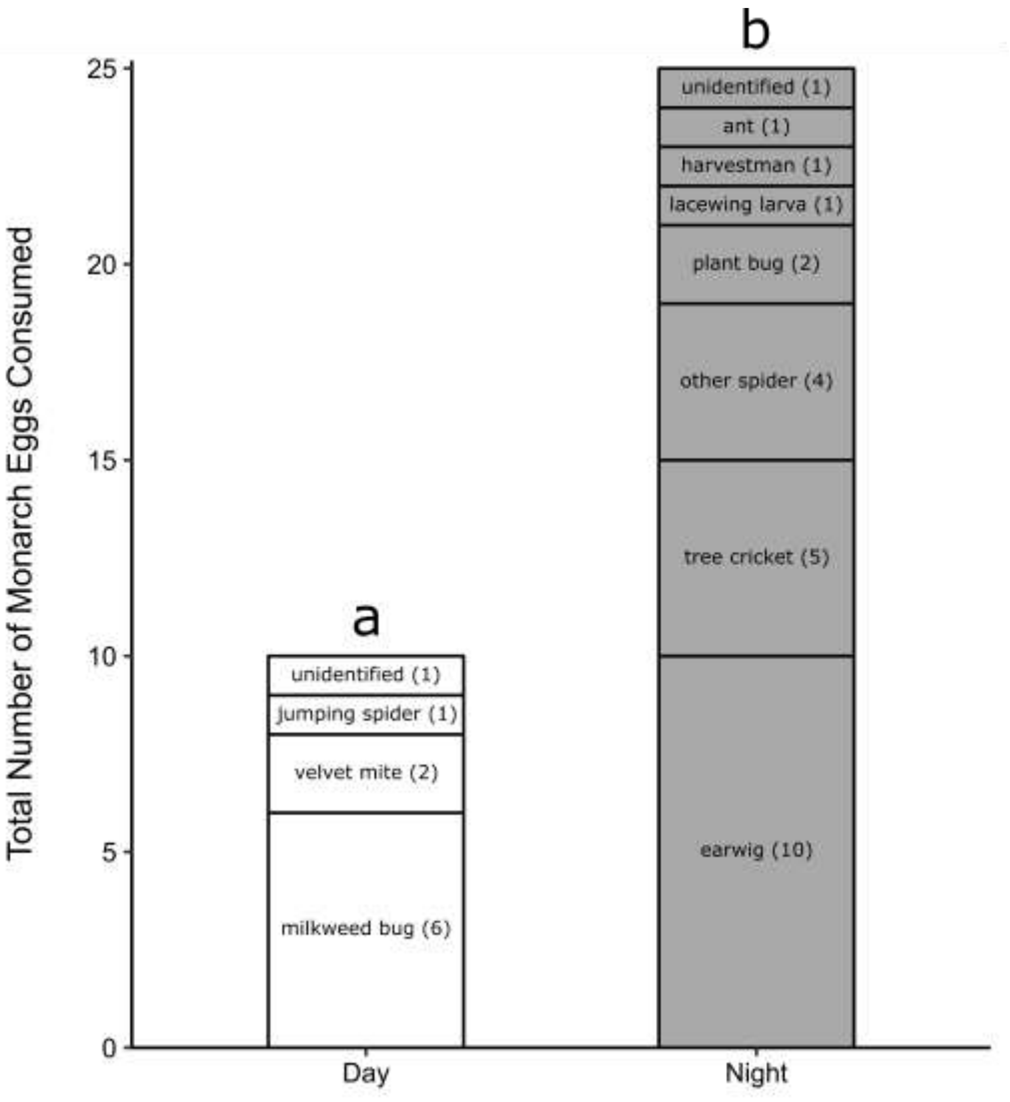

Figure 3.4. Number of monarch butterfly (*Danaus plexippus* L.) eggs (total = 35) observed through video surveillance consumed by predators during day (6:00–20:00) and night (20:00–6:00) on naturally growing common milkweed (*Asclepias syriaca* L.) in southern Michigan. Letters above bars represent a significant difference between the numbers of eggs predated during day versus night (G-test; $\chi^2 = 6.64$, df = 1, p = 0.01).

I also observed several noteworthy interactions that defy simple categorization. For example, on one occasion the internal contents of a monarch egg were consumed by a spider, and the chorion was later consumed by a slug. In another case, two monarch eggs were on a stem clipped off at its base by a mammal—likely a thirteen-lined ground squirrel (*Ictidomys tridecemlineatus* Mitchell) which were very abundant at KBS and known to clip young plant stems (Cleary and Craven 1994). Although the eggs remained on the leaf, the plant rapidly desiccated, and it is unlikely neonates could survive to find a fresh host plant. Details regarding these events along with descriptions of predation events on first and fourth instar monarch caterpillars are included in **Table 3.2**.

Discussion

I documented multiple interactions between immature monarchs and their predator community, expanding knowledge of monarch biology and predator-prey ecology. Monarch egg predators included arthropods from at least 10 species and eight orders. Spiders carried out the plurality of predation events on monarch eggs, while earwigs ate the greatest total number of eggs. Following my predictions, much of this predation (74% of predation events) took place during crepuscular or nighttime hours. These observations are consistent with other studies finding high nocturnal mortality of monarch eggs (Myers et al. 2019) and high rates of nocturnal predation on other Lepidopteran eggs (Pfannenstiel 2005). Different predator communities consumed monarch eggs at night versus during the day. Earwigs, tree crickets, non-Salticidae spiders, plant bugs, a lacewing larva, a harvestman, and an ant consumed eggs exclusively at night, while small milkweed bugs, a spined soldier bug, a velvet mite, and a jumping spider consumed eggs exclusively during the day.

I found relatively high survival of monarch eggs compared with other studies in the north central US. Overall, in this study a total of 69% of monarch eggs survived (i.e. intact or hatched at the end of monitoring) over an average of 59 h of monitoring per egg, although monarch egg predation was as high as 90% during one 24 h period (2017 Garden site). In contrast, Prysby (2004) found approximately 27% survival of eggs over 72 h in Wisconsin grasslands and De

Anda and Oberhauser (2015) found approximately 18% survival over 72 h in Minnesota restored prairies. Myers et al. (2019) found variable monarch egg survival ranging from 12.5–67% over 72 h in Michigan restored prairie sites. Another study in 2018 found similar variability across space, with 0–80% monarch egg survival to 72 h across 15 sites near Lansing, Michigan (Dr. N. Haan personal communication). Taken together, these studies indicate that monarch egg survival in grasslands is highly variable depending on the site, time of year, and potentially other variables. I found that at least 77% (n = 36) of eggs that died before hatching did so as the result of predation. Past monarch survival studies inferred that egg survival was primarily driven by predation, but relied on evidence from predator exclosures or anecdotal observations and did not directly observe and record all predation events (Zalucki and Kitching 1982b, Prysby 2004, De Anda and Oberhauser 2015, Myers et al. 2019).

Predation was more common at night than during the day in terms of both total eggs eaten and total number of predation events. The prevalence of nocturnal predation could explain why other monarch studies in the breeding range did not find strong correlations between diurnal predator numbers on milkweed and survival rates of monarch eggs and larvae (De Anda and Oberhauser 2015, Myers et al. 2019). De Anda and Oberhauser (2015) found that spiders were the only predator group correlated with monarch egg survival in Minnesota grasslands. However, they only observed jumping spiders consuming monarchs. My results suggest that they missed most spider predation events because they frequently take place nocturnally. My results showing the importance of nocturnal natural enemies mirror those of recent work demonstrating the often-overlooked occurrence of nocturnal pollination (Macgregor et al. 2015, Knop et al. 2017). These studies point to a need for more focus on nighttime observations to expand knowledge of species interactions.

It is noteworthy that I only recorded one instance of ant predation on a monarch egg, as ants have been shown to be effective monarch egg predators (Hermann et al. in press, Calvert 2004, Prysby 2004), and are often the most numerous predators observed on common milkweed in the study region (Haan and Landis 2019a, Myers et al. 2019). One explanation for the relative lack of ant predation events in this study is that ants may have been less actively foraging during the primary study period in July and August or seeking different resources. For example *Prenolepis imparis* and *Formica subsericea* (Say), both common species on *A. syriaca* at the field sites (unpublished data), are known to decrease foraging activities during mid-summer (Fellers 1989). An alternative explanation is that during this study period ants may have been focused on collecting carbohydrates (i.e. honeydew and nectar) rather than protein (i.e. insect eggs). Indeed, *P. imparis* is known collect most of its protein from freeze-killed arthropods after the first frost in the fall (Talbot 1943), so would be less likely to consume a monarch egg during the study period. This study may have under-sampled early season predation in general, and future research should determine the importance of spring and early summer predation on monarch eggs by ants and other predator groups.

I also documented several other interesting interactions among monarch eggs, larvae, and predators, which can offer the basis for further studies into monarch predator-prey interactions. For example, I documented instances where several different predators visited eggs or their remains during a single night (either multiple taxa, or multiple representatives from the same taxon). Multiple scavengers also visited fourth instar larvae after they were partially consumed. These redundant predator visits suggest that some areas have especially high predation rates, and had I been able to deploy more cameras and eggs I may have recorded an even greater diversity of predator taxa.

I also documented multiple examples of non-consumptive interactions that could ultimately influence monarch survival. These included instances of plants being clipped by vertebrates, eggs being knocked off plants due to passing arthropods, and others where larva were disturbed and dropped on the silk (**Table 3.2**). The silk-dropping behavior was observed twice, and in both instances was caused by interactions with common milkweed herbivores. These observations indicate that first instar larvae are highly sensitive to disturbance, which may affect their survival in positive or negative ways. While dropping on a silk can be effective for avoiding predation, it likely requires neonates to initiate new feeding sites, which is considered a dangerous activity due to milkweed chemical and latex defenses (Zalucki et al. 2001). Dropping from leaves could also leave larvae vulnerable to other predators. Future studies could investigate under what conditions anti-predator behaviors are most adaptive. Additionally, observers monitoring first instars should take care to avoid altering their results by eliciting silk dropping.

I suggest that conservation efforts could increase monarch population growth by enhancing survival during their vulnerable early life stages. Although there are a number of studies documenting monarch predator-prey interactions and a recent profusion of papers regarding monarch butterfly declines and conservation needs, there has been almost no emphasis on the potential to stabilize monarch populations by increasing survival rates of eggs and early instars. Immature monarch survival could be enhanced by, for example, prioritizing milkweed establishment in habitats with lower predation rates, or by managing milkweed in ways that reduce predator numbers (Haan and Landis 2019b). Identifying the most important monarch predators and learning more about the circadian and seasonal phenology of predation is an important step toward finding ways to increase monarch early life stage survival within grasslands.

Increased understanding of species interactions is crucial not only to the field of community ecology but also to conservation, agriculture, and other ecological applications. As agricultural and other land use practices continue to intensify globally, preserving biodiversity and associated ecosystem services will become increasingly challenging (Brussaard et al. 2010, Foley et al. 2011). Monarch butterflies and several high-profile pollinator species have brought national attention to declines of species that were formerly common on agricultural landscapes (Gustafsson et al. 2015). Stemming these losses while simultaneously supporting agricultural productivity and ecosystem services within agricultural landscapes will require increased understanding the ecology and natural history of these systems. Natural history knowledge not only points to ways to achieve these multiple goals, but also highlights ways in which they might be at incompatible. For example, grasslands within agricultural landscapes are managed for delivery of biocontrol services from their predator communities in addition to habitat for declining agricultural species (Schulte et al. 2017). However, my results show that predation is the main source of mortality for monarch eggs. The same could be true for other imperiled grassland arthropods, demonstrating the need for careful consideration informed by natural history knowledge in the design of landscapes for multiple ecosystem services. Such an understanding has never been more valuable, as halting biodiversity loss and protecting associated ecosystem services is ultimately necessary for the persistence of agriculture itself.

CHAPTER 4: APHIDS AND ANTS INTERACT TO INFLUENCE MONARCH BUTTERFLY (*DANAUS PLEXIPPUS* L.) OVIPOSITION AND EARLY INSTAR LARVAL SURVIVAL

Abstract

Declining populations of monarch butterflies at the Mexican overwintering grounds of the eastern North American population have motivated efforts to better understand monarch ecology. Significant attention has been paid to learning more about monarch breeding ecology with the goal of managing more attractive and productive habitat in the core of the monarch breeding range. Much of this work involves determining how milkweed host plant species and spatial distribution can maximize oviposition by monarchs. Less research, however, has focused on how interactions between monarchs and other common milkweed herbivores and predators shape monarch breeding ecology and conservation success. An increased understanding of how these interactions impact the survival of monarchs during early life stages could inform the management of more productive monarch breeding habitat. I performed a field experiment in a Michigan cool season grassland manipulating aphid species and ant access to sentinel *Asclepias syriaca* (L.) plants where I placed first instar monarchs to determine: 1) how ants influence wild monarch oviposition preferences; and 2) how aphid species and presence interact with ants to influence neonate monarch survival. I found that monarchs avoided ovipositing on plants with ants and that both species of aphids (*Myzocallis asclepiadis* and *Aphis asclepiadis*) attracted ants, but ant accessibility only resulted in lower monarch neonate survival on plants infested with *M. asclepiadis*. These results indicate that monarch breeding management should consider interactions among monarchs and other arthropods to optimize conservation efforts on limited

land space. Specifically, conservation efforts might seek to reduce ants and/or aphids on milkweed in monarch breeding habitat to increase the survival of monarch neonates.

Introduction

Migratory populations of monarch butterflies have recently experienced dramatic declines in both the eastern and western portions of their North American range. This includes shrinking overwintering aggregations (Brower et al. 2012, Semmens et al. 2016, Schultz et al. 2017, The Xerces Society 2019) and summer breeding populations (Stenoien et al. 2015, Pleasants et al. 2017; but see Inamine et al. 2016, Badgett and Davis 2015). In response to declines of the eastern population segment, a significant effort is underway to increase monarch breeding habitat within the core breeding range in the north central region of the US. These efforts seek to compensate for the milkweed host plants lost from row crop fields due to the adoption of herbicide-tolerant cropping systems, and they primarily involve planting milkweed within non-agricultural grasslands (Thogmartin, López-Hoffman, et al. 2017). Recent studies, however, indicate that egg and early instar monarchs experience more variable and at times very high mortality in grasslands compared to agricultural fields (Myers et al. 2019). This adds to the challenge of monarch population recovery, as monarchs are now consigned to significantly fewer breeding areas, and remaining habitat is likely not as productive as former agricultural habitat because of high predation pressure. However, much is still unknown about monarch-predator interactions, and more knowledge regarding how monarchs are impacted by predators in grasslands would be valuable for the creation of more productive breeding habitats on limited space.

A variety of experiments and observations revealed a diversity of monarch egg predators in grasslands (Hermann et al. in press, Oberhauser et al. 2015, Myers et al. 2019; also see Dissertation Chapter 3), however fewer studies have investigated the predators of early instar monarch caterpillars, which are known to suffer high mortality rates (Zalucki et al. 2001, De Anda and Oberhauser 2015). Additionally, monarch larval interactions with predators are complex and can be structured by interactions with other taxa including herbivores, predators, and host plants. Aphids are especially abundant on milkweed host plants and can indirectly affect monarchs in numerous ways. For example, lab feeding trials have revealed that lady beetles are less likely to consume monarch caterpillars when their preferred prey, aphids, are present (Koch et al. 2005). Furthermore, in a lab experiment in the absence of predators, monarch caterpillars grew more quickly on milkweed infested with aphids due to a defense tradeoff on the part of milkweed plants; defending against aphids reduced defense against monarchs, resulting in healthier monarch larvae (Ali and Agrawal 2014). In contrast, field studies indicate mortality of first instar larvae is generally higher on plants with aphids, because aphids attract ants which in turn feed on monarch larvae (Mooney and Agrawal 2008). A further complication is that multiple species of aphids commonly occur on milkweed, with differing life histories and relationships with ants that range from antagonistic to tightly mutualistic depending on both aphid species and host plant genotype (Mooney and Agrawal 2008, Smith et al. 2008). Although ant-aphid-milkweed interactions are well studied, little work has investigated how monarchs are impacted by these interactions or how outcomes for monarchs change with different aphid species.

Interactions with other arthropods could also impact monarch oviposition and larval survival through non-consumptive effects. Some lepidopterans avoid ovipositing on plants occupied by predatory ants, and can distinguish predatory ants from herbivores and non-predatory ants (Sendoya et al. 2009). It is plausible that the presence of ants (and aphids by attracting ants) has similar non-consumptive effects on monarchs, which demonstrate oviposition preferences based on a variety of other milkweed plant characteristics (e.g. Malcolm and Brower 1986, Pitman et al. 2018, Pocius et al. 2018). Predator avoidance behaviors could also have negative effects on early instar monarch larvae, which can drop on silk when disturbed by predators and other invertebrates (Malcolm et al. 1999; also see Dissertation Chapter 3). Although this is likely an effective predator avoidance strategy, silk dropping may require larvae to begin a new feeding site, which could be lethal due to milkweed latex and chemical defenses (Zalucki et al. 2001).

I sought to determine how the presence and species of aphids on milkweed structured survival of monarch neonates and oviposition patterns by wild monarchs. I studied two common species of milkweed aphids with different life histories and ant-associations: *Aphis asclepiadis* Fitch and *Myzocallis asclepiadis* Monell (hereafter *Aphis* and *Myzocallis*, respectively). While *Aphis* usually forms dense colonies on the tops of the uppermost leaves of milkweed plants and are actively tended by ants, *Myzocallis* typically inhabits the undersides of the lower leaves of milkweed plants in sparser colonies where ants consume aphid honeydew and prey upon the aphids themselves (Mooney and Agrawal 2008, Smith et al. 2008). Due to these differing interactions with ants, *Aphis* colonies typically grow in the presence of ants, whereas *Myzocallis* colonies decline in the presence of ants (Mooney and Agrawal 2008).

I hypothesized that the differing feeding patterns and ant-associations exhibited by *Aphis* and *Myzocallis* would lead to differing interactions between ants and monarchs utilizing the same plants. First, I hypothesized that first instar monarchs would suffer greater predation rates on plants infested with *Aphis* versus *Myzocallis*, because *Aphis* colonies would attract predatory ants to the apex of milkweed plants where first instar monarchs typically feed (De Anda and Oberhauser 2015), thus increasing the likelihood that ants would encounter and interact with monarch caterpillars. Second, I hypothesized that wild monarchs would avoid ovipositing on milkweed plants with ants present versus plants with ants absent, because their larvae would likely experience greater survival on plants without ants. I tested these hypotheses via a field experiment by measuring monarch neonate survival and adult oviposition on sentinel milkweed plants infested with the two species of aphids, with and without ants. I interpret my results in the context of monarch conservation and make recommendations for further research.

Methods

Site description

I conducted the experiment within an old field (i.e. an abandoned farm field) on the Entomology Farm on the campus of Michigan State University, East Lansing, Michigan (42.6925, -84.4915). Dominant vegetation included the graminoids *Poa* spp. and *Elytrigia repens* (L.) and forbs *Solidago canadensis* (L.), *Taraxacum officinale* (L.), and *Symphyotrichum pilosum* Wild. The field was bounded on the west by a research apple orchard and on all other sides by lanes of mowed grass. *Asclepias syriaca* occurred naturally at the site, and monarch adults and larvae were frequently observed.

Sentinel Plants

I sourced *A. syriaca* plants from Wildtype Nursery (Mason, Michigan). The nursery grew plants from seeds collected from multiple individual plants in southern Michigan. Seeds were planted in a greenhouse in a 512-cell germination tray in Fafard® Germination Mix (Sun Gro Horticulture, Agawam, MA). Once the seedlings had true leaves they were transplanted to 38-cell plug trays containing Fafard® 3B Mix (Sun Gro Horticulture, Agawam, MA), fertilized with Osmocote Plus 15:9:12 NPK 3–4 month slow release fertilizer (Everris NA, Inc., Dublin OH), and watered ad libitum.

I received plants from the nursery on August 6, 2018, 1 week prior to the start of the field experiment, and immediately potted each plant in a clear plastic 32 oz. deli cup with Suremix ™ perlite potting soil (Michigan Grower Products, Inc., Galesburg, MI). At this time, plants were colonized with *Myzocallis* and small numbers of *Aphis nerii* Boyer de Fonscolombe and *Myzus persicae* Sulzer. To reserve a set of plants with only *Myzocallis,* I selected 30 plants with only *Myzocallis* and held them in a research greenhouse on the Michigan State University campus (approximately 20–28˚ C, L:D 14.5:9.5 h). To create sets of plants with no aphids and with *Aphis asclepiadis* only, I used compressed air to gently blow all aphids off 60 of the plants and placed them in a clean reach-in Insect Rearing Chamber (Model IN034-LT-MP, Darwin Chambers, St. Louis, MO) at 25˚ C and L:D 16:8 h. To ensure I removed all aphids from the plants, I repeated this process for two additional days. Each day I switched the plants between two growth chambers, increasing the temperature to 50º C overnight on the empty chamber to kill any remaining aphids.

On August 9 I randomly split the 60 aphid-free plants between the two growth chambers, with 30 plants per chamber. I inoculated one set of these 30 plants with *Aphis* from a lab colony maintained on *A. syriaca* and originating from *Aphis* collected in East Lansing, MI. I first transferred six *Aphis* individuals to each plant by gently brushing them onto the top leaves of plants using a fine paint brush. Because aphid numbers had not increased significantly over 24 h, on August 10 I collected additional *Aphis* from common milkweed stands on the Michigan State University campus and placed 20 more individuals on each of the 30 plants to ensure a successful innoculation. I then left plants in the growth chamber for 3 d to allow aphid numbers to increase, hand watering plants as needed.

Prior to deploying the plants in the field, I recorded numbers of aphids on all the plants. Because the *Myzocallis*-infested plants had accumulated honeydew, I also gently rinsed them with water and allowed them to dry. I then transported the plants to the experiment site and selected the 25 tallest, most upright plants from each of the three treatment sets for the experiment.

Field Experiment

The experimental setup comprised 20 plots arrayed in two north-south running transects (i.e. 10 plots each). Within transects, plot centers were 4 m apart, and each plot included three treatments; one plant infested with *Aphis*; one plant infested with *Myzocallis*; and one control plant with no aphids. At 15 random plots I included an additional milkweed plant with Tanglefoot (The Scotts Company LLC, Marysville, OH) applied to the base of its stem to prevent ant access (Smiley 1986, Bishop and Bristow 2006, Jones et al. 2017). Of these 15 plants, five were randomly assigned to each of the three aphid treatments. I placed each potted plant in a socket which I created in advance using a golf cup cutter, such that the lip of the pot was level with the ground surface. Within each plot, the potted plants were placed randomly at points 0.25 m from the transect every 1 m. Points on opposite sides of the transect were staggered in a north-south orientation by 0.5 m to maximize distance among plants within a plot **(Fig. 4.1).**

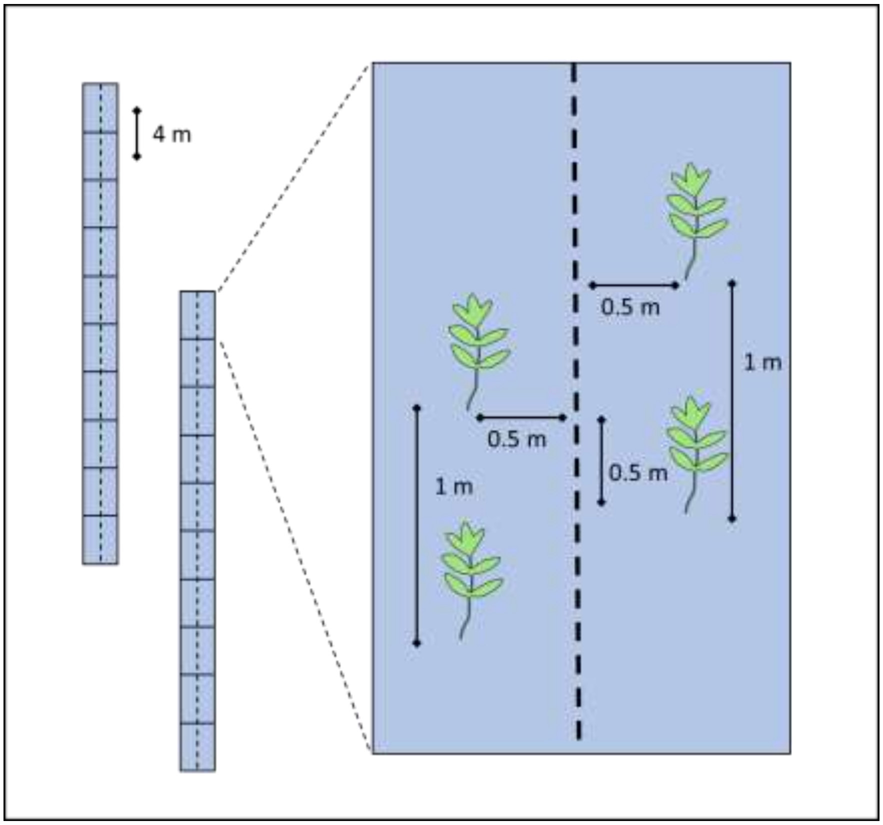

Figure 4.1. Transect and plot layout for summer 2018 aphid-ant-monarch interaction experiment showing (left) two transects with 10 plots each and (right) expanded view of a single plot. Each plot included three *A. syriaca* plants open to ants and randomly assigned to one of three aphid treatments. At 15 random plots a fourth plant was included, which had ants excluded and was randomly assigned to one of the three aphid treatments.

At 18:00 on August 13, I placed the plants in the field, and on August 14 I placed three first instar monarch butterfly larvae on each plant. Larvae were sourced from a lab colony, originating from wild monarchs collected from nearby locations in southern Michigan. I transported larvae from the lab to the field site in large Petri dishes with moist paper towel. Using a fine tipped paintbrush, I gently lifted larvae from the Petri dish and placed them on the uppermost fully emerged leaves of the plants. I recorded the number of monarch larvae, species and numbers of aphids, ants and other predators on each plant at the time of deployment (0 h) and at 24, 48, 96 h thereafter. Checking plants involved visually searching all leaves for monarch neonates and other arthropods. I also counted wild monarch eggs laid on the plants at 0, 24, 48, 96, and 144 h following caterpillar deployment. I marked the location of each monarch egg by making a small mark with a black permanent marker adjacent to each egg and writing the date the egg was found on the upper side of the leaf. On each subsequent visit I checked previously marked eggs and recorded if they were present or absent.

Statistical Analysis

All analyses were performed in R version 3.4.2 (Nasr et al. 2017), and a summary of all models used is available in Appendix **Table E1**. I assessed the effects of aphid species and ant exclusion treatment on monarch larval survival at 96 h using binomial generalized linear mixed effects models with the function glmer in the package lme4 (Bates et al. 2015). Aphid and ant exclusion treatments were considered fixed effects and individual plot was considered a random effect. I fit models with individual predictor variables and additive and interactive effects of predictor variables. I tested model fit by comparing between a null model with the random effect of plot only and performing nested comparisons among models with individual and interactive fixed effects using likelihood ratio tests (LRT) with the anova function (Nasr et al. 2017). I used the emmeans function to generate Holm-adjusted pairwise comparisons of modeled means among the treatments to determine which predictors significantly influenced survival (Lenth et al. 2018).

For all count data (aphid, ant, predator, and wild monarch egg counts) I modeled mean counts/plant/day as a function of treatment variables using generalized linear models fit with a Poisson distribution with the glm function (Nasr et al. 2017). I assessed the efficacy of the experimental treatments by modeling aphid and ant counts as functions of the categorical aphid and Tanglefoot treatment variables, respectively. I also modeled counts of wild monarch eggs laid/plant/day as a function of Tanglefoot treatment to examine the effect of ants on monarch egg laying rates. I examined non-ant predators/plant/day as a function of aphid treatments, Tanglefoot treatments, and additive and interactive effects of aphid and Tanglefoot treatments to examine how aphids and ants interacted to influence predator numbers. To determine if treatments significantly influenced response variables, models were compared with null models using the likelihood ratio tests with the anova function (Nasr et al. 2017). For models with significant predictor variables, as above, I used the emmeans function to generate Holm-adjusted pairwise comparisons of modeled means among the treatments (Lenth et al. 2018).

To confirm that wild monarch egg observations truly represented oviposition preferences and were not an artifact of egg predation differences among treatments, I performed a survival analysis on marked egg data. Using the function survfit the R package Survival (Therneau and Grambsch 2000; Therneau 2015), I generated 72 h egg survivorship curves. The oviposition data were appropriate for this type of time-to-event analysis, because individual eggs were tracked, and because of the high variability in observation time periods due to oviposition occurring throughout the study. Eggs that disappeared or were found with their contents emptied were considered predated, and eggs that were present at the end of 72 h or at the end of the study were considered censored. To determine if ant presence affected egg survival, fitted survivorship curves for plants with and without ants excluded were contrasted using the Mantel-Haenszel test with the function survdiff in the R package Survival (Therneau and Grambsch 2000; Therneau 2015).

Results

Aphid and Predator Counts

Aphid colonization and ant exclusion treatments were effective in creating the anticipated treatment effects on aphid abundance (*Aphis* LRT $\chi^2 = 2700.9$, df = 2, $p < 0.001$; *Myzocallis* LRT $\chi^2 = 28964$, df = 2, $p < 0.001$). The control plants remained virtually aphid-free throughout the experiment with mean ± SEM 0.13 ± 0.13 and 0.13 ± 0.05 *Aphis* and *Myzocallis*, respectively, observed per plant per day (**Fig. 4.1**). Aphid-treated plants also had very little cross-contamination; plants infested with *Aphis* averaged 11.6 ± 3.6 *Aphis* versus 0.2 ± 0.1 *Myzocallis* observed per plant per day ± SEM, and plants infested with *Myzocallis* averaged 101.8 ± 14.1 *Myzocallis* versus 0.45 ± 0.45 *Aphis* observed per plant per day ± SEM. Similarly, Tanglefoot effectively excluded most ants from milkweed plants, with a mean ± SEM of 0.04 ± 0.03 ants/plant/observation on plants with ants excluded and 0.37 ± 0.12 ants/plant/observation on ant accessible plants (ant LRT $\chi^2 = 31.84$, df = 1, $p < 0.001$; **Fig. 4.3**). Ant abundance correlated with aphid abundance; *Myzocallis*-infested plants had higher numbers of both aphids and ants than *Aphis*-infested plants (**Fig. 4.3**). I observed numerous and variable numbers of non-ant predators on the plants (**Table E2**), with the best fitting model of predator numbers included both the interactive and additive effects of aphid and Tanglefoot treatments as predictor variables (LRT full vs. null model $\chi^2 = 15.75$, df = 5, $p = 0.008$). However, pairwise comparisons of model mean counts of predators/plant/day did not vary significantly as with aphid or Tanglefoot treatments (**Fig. 4.4**).

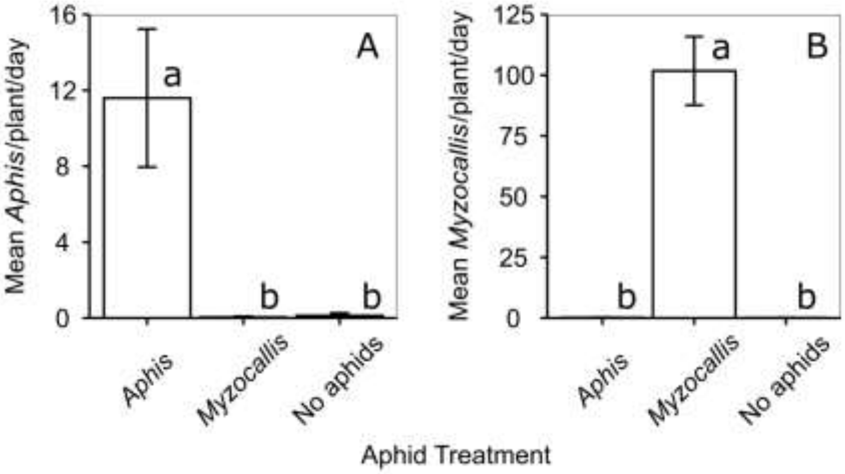

Figure 4.2. Mean ± SEM numbers of *Aphis asclepiadis* (A) and *Myzocallis asclepiadis* (B) observed per plant per day for 96 h on the three aphid treatments. Lower case grouping letters represent post-hoc Holm-adjusted pairwise contrast groupings of estimated marginal means from Poisson generalized linear mixed model, α = 0.05.

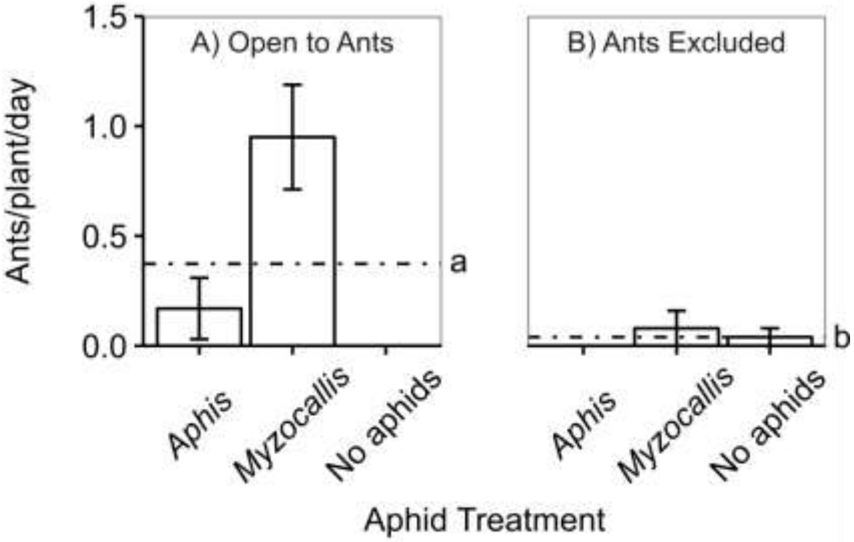

Figure 4.3. Mean counts ± SEM ants observed per plant per day for 96 h in two ant exclusion treatments and three aphid treatments. Dashed lines are overall means for ant exclusion treatments, and lower-case grouping letters represent post-hoc Holm-adjusted pairwise contrast groupings of estimated marginal means from Poisson generalized linear mixed model, α = 0.05.

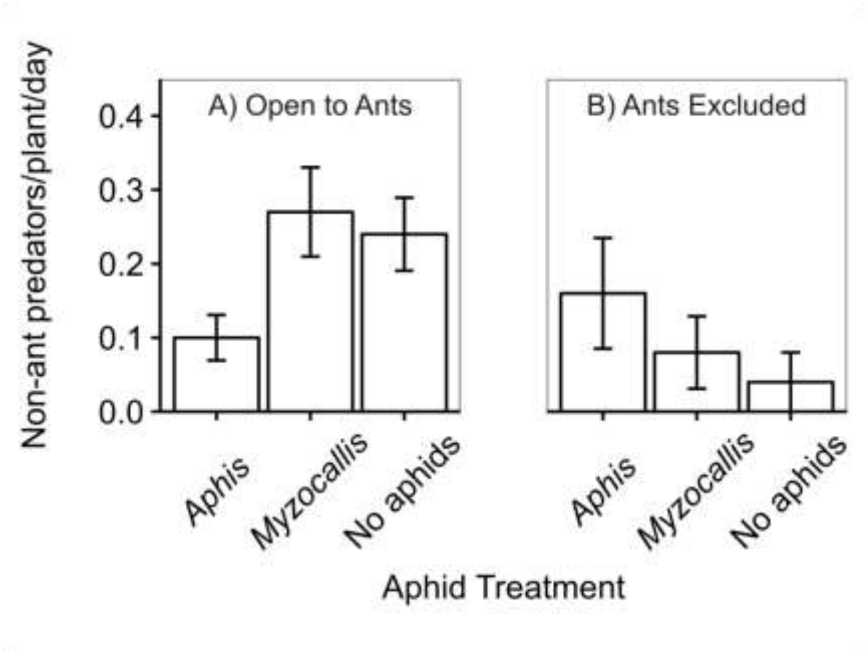

Figure 4.4. Mean numbers ± SEM of non-ant predators observed/plant/day for 96 h among the three aphid treatments and two ant exclusion treatments.

Neonate Survival

Ants and aphids interacted to influence monarch larval survival, with the best performing model including interactive and additive effects of aphid and ant treatments (null versus full interactive model LRT: $\chi^2 = 32.37$, df = 5, $p < 0.001$). However, the strength and direction of the ant-aphid-monarch interactions differed depending on the aphid species present (**Fig. 4.4**). When plants were accessible to ants, neonate survival at 96 h was lowest on plants infested with *Myzocallis*, intermediate on those infested with *Aphis*, and highest on plants with no aphids present (**Fig. 4.4A**). When ants were excluded from plants with *Myzocallis*, monarch survival increased by 9 fold from 5.0 ± 2.7% to 43 ± 16% mean ± SEM on non-Tanglefoot versus Tanglefoot plants, respectively. However, excluding ants from plants with *Aphis* did not significantly change monarch survival, with only a slight decrease from 28 ± 7.2% to 20 ± 20% on non-Tanglefoot versus Tanglefoot plants, respectively (**Fig. 4.4B**). In the model that only considered aphid treatments (i.e. averaging across ant treatments), the presence of both aphid species treatments negatively affected monarch survival to 96 h, with 38% lower survival on *Aphis*-infested plants and 69% lower survival on *Myzocallis*-infested plants versus plants with no aphids (mean percent survival ± SEM: *Aphis,* 26 ± 6.8%; *Myzocallis* 13 ± 4.8%; no aphids 42 ± 7.8%; LRT $\chi^2 = 20.6$, df = 2, $p < 0.001$). The presence of ants, however, did not independently affect monarch survival ($\chi^2 = 2.5$, df = 1, p = 0.11).

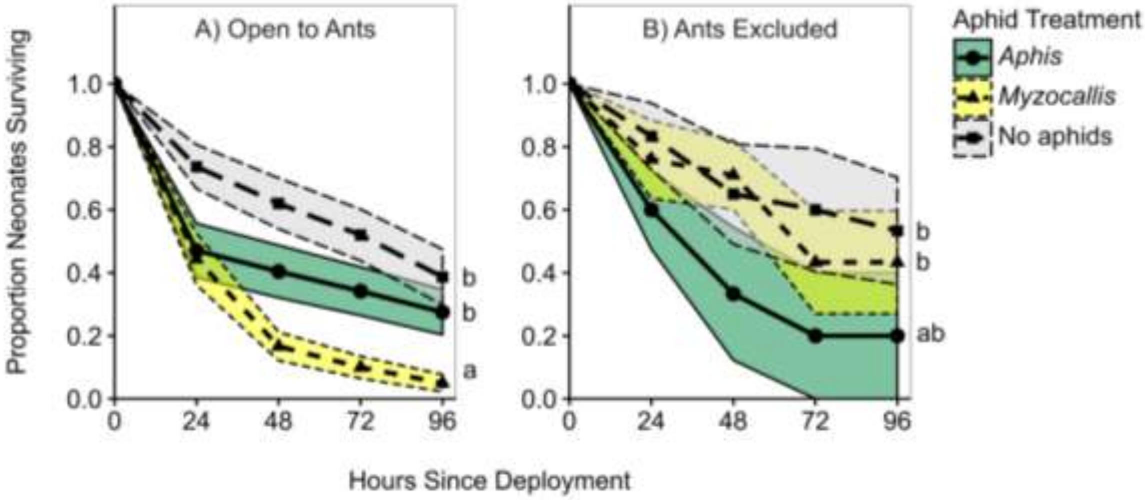

Figure 4.5. Mean ± SEM proportion of monarch larvae surviving over 96 h on three aphid and two ant exclusion treatments. Lower case letters represent post-hoc Holm-adjusted pairwise contrast groupings of estimated marginal means from binomial generalized linear mixed model, $\alpha = 0.05$.

Wild Monarch Egg Counts

I observed a total of 89 eggs laid on the 75 plants over the course of the experiment. Monarchs laid significantly (2.7-fold) more eggs on plants with ants excluded versus plants accessible to ants, with a mean ± SEM of 0.40 ± 0.09 eggs/plant/day laid on plants with ants excluded and 0.15 ± 0.47 eggs/plant/day on ant-accessible plants ($\chi^2 = 19.42$, df = 1, $p < 0.001$; **Fig. 4.6**). Moreover, the fewest eggs were found on plants with the greatest numbers of ants (see *Myzocallis* treatment **Figs. 4.3 and 4.6**). I found no significant difference in 72 h egg survival between plants with and without ants excluded ($\chi^2 = 0.5$, df = 1, p = 0.48), with modeled survival at 24, 48, and 72 h ± SE of 100 ± 100%, 54 ± 10%, and 0%, respectively, for plants with ants excluded and 98 ± 2.0%, 71 ± 7.6%, and 11 ± 5.8% for plants with ants not excluded.

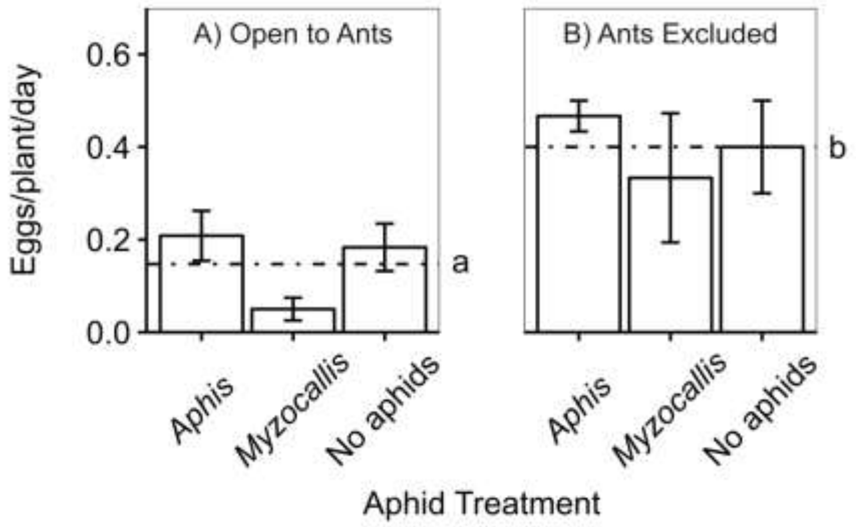

Figure 4.6. Mean counts ± SEM of monarch eggs observed per plant per day for 144 h in two ant exclusion treatments and three aphid treatments. Dashed lines are overall means for ant exclusion treatments. Grouping letters represent post-hoc Holm-adjusted pairwise contrast groupings of estimated marginal means of eggs/plant/day for each ant treatment averaged across aphid treatments from Poisson generalized linear mixed model, $\alpha = 0.05$.

Discussion

I found that both the presence and species of aphids on monarch host plants structured interactions between monarch larvae and ants. I also found that wild adult monarch butterflies adjusted their oviposition preferences based on the presence of ants, with fewer eggs laid on plants accessible to ants versus ants excluded. These findings have important implications for managing grasslands to optimize monarch breeding productivity on limited space in agricultural landscapes.

These experiments confirmed the results of a past study by Agrawal and Mooney (2008) showing milkweed plants infested with aphids attract ants which reduce monarch neonate survival. This work expanded the understanding of these interactions by demonstrating that this effect varies by aphid species. The interaction between ants and aphid species, however, ran contrary to my hypothesis; I found that relative to plants with no aphids, in the presence of *Myzocallis*, ants had a negative effect on monarch survival while on *Aphis*-infested plants, the presence of ants had no significant effect on monarch survival. There are several plausible explanations for this finding. First, *Myzocallis*-infested plants tended to have much higher numbers of aphids than *Aphis*-infested plants (**Fig. 4.2**), and this treatment also attracted more ants (**Fig. 4.3**), so it is possible that higher monarch mortality with *Myzocallis* was merely due to greater aphid and ant abundance. Second, it is possible that the ant species attracted to different aphid species (or co-varying numbers of aphids) accounted for these differences in monarch mortality. Plants infested with *Myzocallis* attracted a greater diversity of ants including *Tapinoma sessile* Say, *Crematogaster cerasi* Fitch, *Formica* sp., *Myrmica* sp., and at least one unidentified ant species, while plants infested with *Aphis* only attracted *Myrmica* and *Formica* sp. Lab feeding trials by Hermann et al. (in press) demonstrated that *C. cerasi* and *Formica vinculans* Wheeler consumed monarch neonates, while *Formica subsericea* Say and *T. sessile* did not. However, I observed one instance of monarch neonate predation by *F. subsericea* on a wild milkweed plant at the field site (Myers unpublished data). Moreover, different species of ants in the temperate region engage in different behaviors depending on time of year (Fellers 1989), and some species have feeding patterns that vary seasonally (Talbot 1943). It is possible that ant species attracted to *Myzocallis* are more predatory, while ants attracted to *Aphis* are focused on consuming honeydew. This mechanism is consistent with typical interactions between these two aphid species and ants; *Aphis* typically forms ant mutualisms while *Myzocallis* is consumed by ants. Additionally, the distribution of aphids on plants could have influenced monarch-ant encounters in a different way from what I anticipated. It is possible that the more sparsely distributed *Myzocallis* brought ants into contact with monarch larvae more frequently than *Aphis,* which occurred in aggregations. Finally, it is plausible that *Aphis*-tending ant behavior could have indirectly benefited monarchs by repelling other predators from host plants. This idea is supported by the observation that excluding ants increased other predator numbers on plants infested with *Aphis* (**Fig 4.3**). Indeed, Agrawal and Mooney (2008) also found that ants reduced other predators on milkweed, and other documented examples include Coccinellidae avoiding plant patches with aphid-tending ants (Oliver et al. 2008) and increased dispersal by spiders in the presence of ant chemical cues (Mestre et al. 2014).

As I predicted, wild monarchs oviposited more on plants with ants excluded versus plants with ants not excluded. This behavior was demonstrated for a species of Nymphalid butterfly native to Brazil, *Eunica bechina* Hewitson, which uses visual cues to avoid ovipositing on host plants with aphid-tending ants (Sendoya et al. 2009). Survivorship analysis demonstrated no significant difference in egg survival between plants with and without ants, supporting the idea that these findings were the result of butterfly oviposition behavior rather than an artifact of differential egg predation by ants during time intervals between the egg observations. This pattern is also consistent with the *E. bechina* system, which experiences very little egg predation by aphid-tending ants but suffers high larval predation. These results, however, run contrary to those of Pitman et al. (2018), who did not find predators to be a direct driver of monarch egg abundance at the milkweed patch scale. Taken together with this study’s findings, it appears that monarchs may avoid predators at the scale of individual plants rather than plant patches.

Results of these experiments demonstrate ways that aphids and ants impact monarch oviposition and survival patterns in grasslands. Further work is needed to clarify the patterns I observed and determine underlying mechanisms to both learn more about predator-prey interactions and inform monarch conservation efforts. First, the relatively low sample size of ant-excluded plants decreased statistical power such that the effect of ants on plants infested with *Aphis* was indeterminable (**Fig. 4.5**). Further field experiments could seek to resolve if *Aphis* and ants truly interact in a way that does not impact the survival of monarch neonates or if these results were driven by differences in aphid abundance, which co-varied with aphid species due to unexpectedly slow growth and establishment of *Aphis* colonies on plants after inoculation. Future experiments should improve aphid inoculation methods to create more comparable numbers of aphids per plant for different aphid species. It is also unclear from this study what specific cues (e.g. chemical or visual) caused monarchs to avoid ovipositing on ant-inhabited plants, and studies testing different predator cues could help determine mechanisms driving monarch oviposition. Non-consumptive effects could have also influenced monarch neonate survival through direct stress to monarch larvae or indirectly via predators modifying one another's behavior. Research on non-consumptive or trait-mediated effects of predators on herbivores is a growing area of study and has not been demonstrated for monarchs. Determining how non-consumptive predator interactions shape monarch neonate survival, adult monarch oviposition behavior, and the repellence of other monarch predators would be valuable to this relatively new area of study.

In addition to advancing basic questions regarding species interactions, this work also has the potential to benefit monarch conservation efforts. Increasing monarch early life stage survival in grassland breeding habitat in the north-central US has untapped potential as a conservation strategy for recovering the eastern North American monarch population. Indeed, population models indicate that increasing survival by 4% would increase the overwintering aggregations to proposed conservation targets (Oberhauser et al. 2017). Several studies show that summer disturbance to milkweed through fire (Baum and Sharber 2012) and mowing (Fischer et al. 2015, Haan and Landis 2019a, Knight et al. 2019) increases the attractiveness of milkweed plants for monarch oviposition. Haan and Landis (2019a) also demonstrate that mowing temporarily reduces aphid and predator numbers on milkweed plants, which they suggest could increase monarch survival (Haan and Landis 2019b). My work not only supports this idea, but also shows that the interaction between ants and aphids on milkweed plants is more important to monarch than merely the presence of either group itself. This suggests that either reducing ant populations (via e.g. chemical baits or ground disturbance) or reducing aphid populations (via e.g. mowing senescing milkweed plants) within grasslands could both be effective options for enhancing monarch breeding productivity. Furthermore, monarchs' avoidance of ovipositing on ant-infested plants demonstrates that ovipositing monarchs are likely to respond to predator management in ways that optimize the survival of their offspring.

Species interactions are vastly complex, with effects that are often indirect, counter-intuitive, often amplified in ways that are consequential for ecosystem function (Harvey et al. 2017). Here I show ways in which the monarch butterfly, a species of conservation concern, is impacted both directly and indirectly by other species which co-habit milkweed host plants in grasslands. Resolving these types of complex interactions not only advances basic ecological knowledge but is also important for practical concerns including protecting imperiled species, conserving biodiversity as a whole, and maintaining important ecosystem services provided by biodiversity. This knowledge is particularly important for agroecosystems, where the biodiversity declines and the resultant erosion of ecological networks and ecosystem services via agricultural intensification represents an existential threat to both irreplaceable ecological communities and human societies that depend on them (Wood et al. 2015).

CHAPTER 5: SUMMARY AND CONCLUSIONS

Shrinking monarch butterfly populations in North America have sparked a passionate discussion among ecologists and conservation biologists regarding the drivers and degree of the decline and the best course of action for recovering the species. A primary point of contention regarding the Eastern migratory population segment involves whether restoring milkweed plants in the monarch’s core breeding range in the north-central US will help to bolster numbers of monarchs overwintering in Mexico. To this end, a significant research effort has focused on determining which species of milkweed are most attractive for monarch oviposition and most supportive of larval growth (Pocius, Debinski, Pleasants, et al. 2017, Pocius et al. 2018) and how host plants can be managed in agricultural landscapes to maximize egg laying by female monarchs (Grant et al. 2018, Pitman et al. 2018, Haan and Landis 2019a, 2019b). An implicit assumption with this strategy is that high predation rates experienced by monarchs during the egg and larval stages is inevitable and can be overcome by providing ample host plants so that female monarchs can lay their full complements of eggs during their lives. A complementary approach would be to reduce predation losses. My work adds to this effort with information regarding which habitats are attractive for monarch oviposition and it also highlights the importance of considering predation in monarch breeding habitat conservation efforts.

Through multiple years of quantifying monarch oviposition on sentinel *A. syriaca* plants, I found that monarchs demonstrate oviposition preferences based on habitat context. During the 2016 season, this preference followed my predictions and the observations previous egg surveys (Pleasants and Oberhauser 2013, Pitman et al. 2018), with more eggs laid on milkweed in corn versus grassland habitat treatments. However, these patterns differed in 2017, with monarchs preferring to lay eggs in grassland habitat, possibly due to a prolonged drought preceding my observations. I also found that egg predation rates varied by habitat and year, with higher and more variable late summer predation rates in grasslands versus corn fields. Video observations revealed several taxa which depredated monarch eggs in grasslands, and showed that the majority of this predation takes place during crepuscular and nighttime hours. Although video observations only revealed one instance of egg predation by ants, later oviposition studies showed that in grasslands monarchs lay more eggs on plants with ants excluded. Because ant and other predator abundances are likely to co-vary with habitat, it is possible that year-to-year predator variation could drive the varying oviposition habitat preferences I observed, and future research should seek to distinguish these effects. Furthermore, my work shows that the weekly egg surveys conducted in other monarch oviposition studies are likely conflating oviposition preferences with differential predation rates.

The variation I observed in monarch egg mortality has important implications for modeling and predicting inter-annual monarch population fluctuations. During the past four years a flurry of publications have sought to model and predict the underlying drivers of both monarch butterfly population declines and the dramatic annual fluctuations in populations around this longer-term decline. These studies have focused primarily on climate variables including summer temperatures and rainfall along the migratory route and northern summer breeding range. My work indicates that varying predation pressure on monarch eggs and larvae could also drive the degree to which monarchs build their numbers during the spring and summer within the breeding range. Long-term studies show that generalist predator populations can vary from year to year in agroecosystems (Bahlai et al. 2013). Furthermore, predation pressure could covary with climate variables already associated with monarch population fluctuations. For example, as climate change is predicted to raise nighttime temperatures more rapidly than daytime temperatures, it is plausible that monarchs may experience greater nocturnal predation during warming nights. Indeed, experimental nighttime warming in grasslands resulted in greater predation by spiders on herbivorous grasshoppers to such a degree that the resulting trophic cascade significantly impacted the plant community (Barton and Schmitz). Further research is needed to determine the effects of climate variables and inter-annual predator population fluctuations on monarch predation rates in order to incorporate these predictors into monarch population models.

My experiments and observations opened many questions about how monarchs interact with predators and how this knowledge could be used to improve monarch conservation. First, I found high predation rates on first instar larvae, but witnessed relatively few actual instances of predation, with only *Formica subsericea* Say and *Podisus maculiventris* Say observed consuming this life stage in the field. Considering that first instars typically suffer the greatest mortality of any monarch life stage, it is important to learn more about which predators consume them. Zalucki et al. (2001) demonstrated that many first instars die from becoming mired in milkweed latex when they initiate their first feeding sites. However, I found a strong association between ant presence and early larval mortality. It is possible that monarchs are more vulnerable to milkweed latex miring if they are disturbed by ants and forced to start multiple feeding sites. Future studies involving intensive, sustained field observations of monarch neonates would be useful to determine why this life stage experiences such low survival.

This work indicates possible avenues for enhancing monarch breeding productivity within agricultural landscapes. Foremost, I found that managing interactions between monarchs, predators, and other arthropods in grassland systems could play a key role in monarch conservation efforts. Reducing predators within monarch breeding habitat could greatly increase the survival of eggs and early instar larvae. This could be achieved via multiple interventions ranging from strategic disturbance of milkweed plants (Haan and Landis 2019b) to suppressing predators through chemical means (Parr et al. 2016). Specifically reducing interactions between ants and aphids by limiting numbers of one or both of these groups on milkweed plants would likely increase the survival of monarch neonates and increase monarch oviposition. However, any activity seeking to suppress ants or other predators should be undertaken with caution and consideration of unintended ecological consequences and compatibility with other management goals. For example, ants perform many important ecosystem functions within temperate grasslands (Wills and Landis 2018), and are of benefit to some lepidopterans, particularly in the family Lycaenidae (Pierce et al. 2002). Furthermore, reducing ants via chemical suppression can result in greater numbers of other predator groups (Parr et al. 2016), which could potentially result in even greater predation pressure on monarchs. Finally, ecosystem management seeking to reduce monarch predation should be performed in such a way that does not compromise important biocontrol ecosystem services provided by predators within agricultural landscapes.

Current range-wide efforts to restore monarch butterfly breeding habitat presents a useful opportunity to engage in adaptive management to both enhance conservation measures and increase knowledge of monarch breeding ecology. Specifically, evidence is building for the use of summer grassland disturbance as a management strategy to both increase milkweed host plant attractiveness for monarch oviposition and to create an enemy-free window for eggs and larvae (Haan and Landis 2019b). Testing various types of disturbances including mowing, grazing, disking, and burning, the degree of disturbance intensity, and the spatial extent of disturbance for their effects on monarch egg laying and survival, aphid populations, predator populations, and host plant induced defenses traits would provide a wealth of knowledge about both grassland ecology and monarch conservation practices. Finally, although an effort is underway to determine how monarch oviposition is affected by landscape-level host plant distribution (Grant et al. 2018, Pitman et al. 2018), little is known about how habitat context influences monarch predation rates at the landscape scale. Experiments testing the relative predation pressure on monarch eggs and larvae as a function of landscape context could help optimize conservation efforts.

Although monarch butterflies are among the most widely recognized and beloved insects in North America, they are far from the most effective pollinators (Fishbein and Lawrence Venable 1996) or even the most imperiled butterfly species (Haddad 2019). Instead, they are tremendously valuable as a flagship species highlighting the continued erosion of biodiversity associated with agricultural intensification (Gustafsson et al. 2015). The attention received by the plight of monarchs represents an opportunity to draw attention to and implement conservation measures which benefit other imperiled species and ecological communities within agricultural landscapes. Research regarding how monarch conservation practices can best help achieve other parallel conservation goals is highly important and expedient for conservation practitioners. However, if we hope to stave off species loss and the deterioration of ecosystems in a more general and prolonged way, society must very soon grapple with the shortcomings of current economic system that depends on endless growth of production and consumption, discounts the future, and externalizes the costs of ecological degradation (Speth 2008). The loss of monarchs may be the tipping point to such a realization and the path toward a long-term sustainable future.

APPENDICES

APPENDIX A. RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were referenced in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2019-06

Author and Title of thesis:
Andrew Myers: The interacting influences of habitat context and predators on monarch butterfly (*Danaus plexippus* L.) oviposition and survival in agricultural landscapes

Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)
Specimens:

Order	**Family (if applicable)**	**Genus-species (if applicable)**	**Life stage**	**Quantity**	**Preservation**
Coleoptera	Cantharidae	n/a	larva	1	ethanol
Coleoptera	Coccinellidae	*Harmonia axyridis*	adult	2	pinned
Coleoptera	Carabidae	*Calleida* sp.	adult	1	pinned
Coleoptera	Curculionidae	*Rhyssomatus lineaticollis*	adult	2	pinned
Diptera	Tachinidae	n/a	adult	1	pinned
Hymenoptera	Formicidae	*Crematogaster cerasi*	adult	2	point mounted
Hymenoptera	Formicidae	*Tapinoma sessile*	adult	2	point mounted
Hymenoptera	Formicidae	*Formica subsericea*	adult	2	point mounted
Hymenoptera	Formicidae	*Lasius neoniger*	adult	2	point mounted
Hymenoptera	Formicidae	*Prenolepis imparis*	adult	2	point mounted
Hymenoptera	Formicidae	*Myrmica* sp.	adult	2	point mounted
Hymenoptera	Vespidae	*Vespula* sp.	adult	1	pinned
Lepidoptera	Erebidae	*Euchaetes egle*	larva	2	ethanol
Lepidoptera	Nymphalidae	*Danaus plexippus*	adult	2	pinned
Lepidoptera	Nymphalidae	*Danaus plexippus*	larva	1	ethanol
Hemiptera	Anthocoridae	n/a	adult	1	ethanol
Hemiptera	Aphididae	*Myzocallis asclepiadis*	adult	10+	ethanol
Hemiptera	Aphididae	*Aphis asclepiadis*	adult	10+	ethanol
Hemiptera	Aphididae	*Aphis nerii*	adult	10+	ethanol
Hemiptera	Aphididae	*Myzus persicae*	adult	5	ethanol
Hemiptera	Lygaeidae	*Lygaeus kalmii*	adult	2	pinned
Hemiptera	Miridae	n/a	adult	1	pinned
Hemiptera	Nabidae	n/a	adult	1	pinned

APPENDIX A. (CONT'D)

Hemiptera	Pentatomidae	*Podisus maculiventris*	adult	1	pinned
Orthoptera	Acrididae	n/a	nymph	2	ethanol
Orthoptera	Gryllidae	*Oecanthus* sp.	nymph	2	ethanol
Orthoptera	Gryllidae	subf. Nemobiinae	adult	2	pinned
Orthoptera	Tettigoniidae	*Neoconocephalus* sp.	adult	1	pinned
Orthoptera	Tettigoniidae	tribe Conocephalini	adult	2	ethanol
Neuroptera	Chrysopidae	n/a	adult	1	pinned
Neuroptera	Hemerobiidae	n/a	adult	1	pinned
Neuroptera	Hemerobiidae	n/a	larva	1	ethanol
Dermaptera	Forficulidae	*Forficula auricularia*	adult	2	pinned
Trombidiformes	Trombiidae	n/a	adult	1	ethanol
Araneae	Salticidae	n/a	adult	1	pinned
Opiliones	n/a	n/a	adult	1	pinned

APPENDIX B. SUPPLEMENTARY MATERIAL FOR CHAPTER 1

Table B1. Summary of research investigating monarch survival associated with natural enemy mortality.

Publication	Methods	Host Plant	Study Site	Survival Estimates for Life Stages						
				Egg–Instar 1	Instar 1–2	Instar 2–3	Instar 3–4	Instar 4–5	Instar 5–Pupa	Pupation
Cohen & Brower 1982	monitored 10 plants every 1–3 days for 20 days	*A. humistrata* (Walter)	grassy roadside, Cross Creek, Florida	42%	61%	54%	64%	44%	-	-
				overall survival 4%					-	-
Borkin 1982	1 summer of weekly observations of a breeding population	*A. syriaca*	old field, Saukville, Wisconsin	12%					-	11.5% lab-reared pupae parasitized by Tachinid flies
Zalucki & Kitching 1982	greenhouse grown plants at 2 sites in various patch sizes, checked 1–2 times/week	*A. fruticosa* L.	Griffith University campus, Queensland, Australia	18–38%	30–75%	57–78%	43–100%	67–100%	-	-
				overall survival 1–8%					-	-
Malcolm et al. 1987	plants searched weekly from March to mid-May, then monthly until October	*A. humistrata*	Cross Creek & Ordway, Florida	8.1–9.4%					-	-

Table B1. (Cont'd).

Zalucki et al. 1990	observed naturally laid eggs for 2–3 days until they disappeared	*A. humistrata*	sandhill habitat type, Cross Creek, Florida	64%	52%	67%	-	-	-	-
Zalucki & Brower 1992	added 10 eggs/ plant and monitored 1-3 days. also monitored wild oviposition	*A. humistrata*	sandhill habitat type, Cross Creek, Florida	65% (naturally laid only)	3.4 – 11.5%	-	-	-	-	-
Oberhauser et al. 2001	counted monarchs weekly May–August on 200–1400 ramets	*A. syriaca*	multiple sites in midwestern US	~1–15% (paper did not provide exact estimates)					-	-
Zalucki et al. 2001	monarch eggs placed on plants just prior to hatching and checked 5 days later	*A. syriaca*	Arcadia, Michigan	-	open: 24% plant exclosure: 72% leaf exclosure: 94%	-	-	-	-	-
Calvert 2004	3 experimental periods, compared outside vs. inside fire ant exclosures	*A. oenotheroides* (Cham. & Schltdl.) & *A. asperula* (Decaisne)	2 sites near Austin, TX; 1 pasture and 1 post oak savannah	open: 0; 0.5; 1.4% exclosure: 27%; 1.6%; 16%					-	-

Table B1. (Cont'd).

Prysby 2004	1. excluded: all predators; terrestrial only; & open. monitored daily for 7 days. 2. deployed eggs on milkweed with: ants & aphids; aphids only; control monitored daily for 6 days 3. lab-reared wild pupae	*A. syriaca*	old field, west-central Wisconsin	all preds. excluded: ~30% terrestrial pred. excluded: ~20% control: ~10% aphids & ants: ~ 10% aphids: ~ 30% no aphids or ants: ~ 40%	-	-	-	-	-	15–23% lab-reared pupae parasitized by tachinid flies
De Anda & Oberhauser 2015	2 breeding seasons of monitoring	*A. syriaca*	restored prairie at a city park, Falcon Heights, Minnesota	16%	36%	31%	-	-	-	-
Nail et al. 2015	MLMP egg–pupa field data & lab-reared pupa	various	north-central US	spring: 5.7% summer: 7.7%					summer: 76%	

Table B1. (Cont'd).

Oberhauser et al. 2015	1. prepupae and pupae placed in open cages exposed to natural enemies 2. comparison of *Petromalus* wasp parasitism on monarchs fed *A. syriaca* vs *A. currasavica* 3. pupae placed in various settings & on common milkweed with and without monarch frass present	*A. syriaca* & *A. currasavica* (used for feeding)	various garden and roadside sites near Minneapolis, MN	-	-	-	-	-	0–76% *Polistes* wasp predation across experiments 1 & 30–87% *Petromalus* parasitism across all experiments

APPENDIX C. SUPPLEMENTARY MATERIAL FOR CHAPTER 2

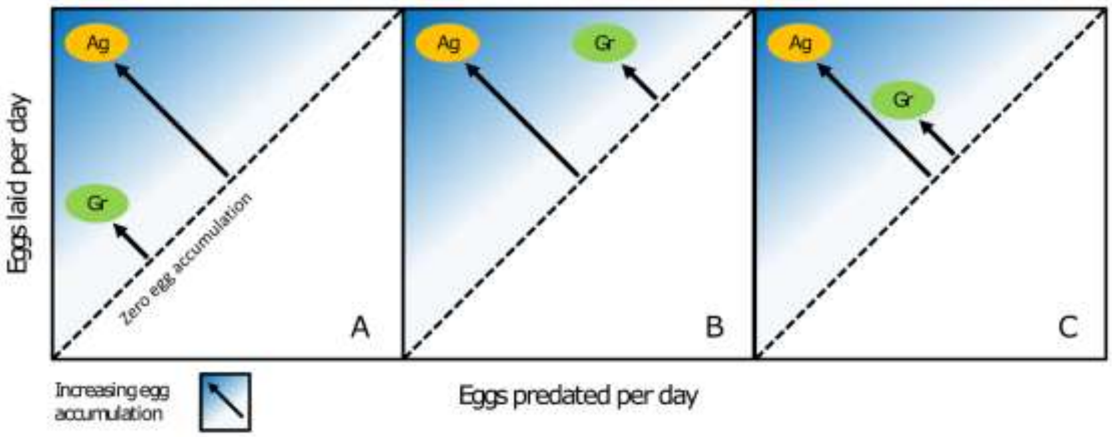

Figure C1. Conceptual diagram of alternative hypotheses explaining numbers of monarch eggs observed on common milkweed growing in agricultural and grassland habitats. The dashed diagonal line represents zero net egg accumulation (eggs laid equals eggs predated). Ovals represent agricultural (Ag) and grassland (Gr) habitats, with increasing length of the arrow representing greater egg accumulation. Identical patterns of egg accumulation can arise from different underlying processes. In A) predation is equal across the two habitats with more eggs accumulating in Ag driven by increased oviposition. In B) oviposition is equal across habitats with more eggs accumulating in Ag due to lower predation. In C) egg accumulation is driven by both differential predation and oviposition.

2016	June	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
	July	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
	August	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
2017	June	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
	July	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
	August	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31

plant deployment date
one oviposition check
two oviposition checks

Figure C2. Timeline of monarch butterfly (*Danaus plexippus* L.) oviposition experiments in 2016 and 2017 at the Kellogg Biological Station, Hickory Corners, MI. Dates highlighted in yellow indicate days on which experiments were deployed. Plants were checked for monarch eggs either once or twice per day (light and dark green, respectively).

Figure C3. Monarch survival experimental plant setup in Soybean treatment in 2016. Left plant is the sham exclosure, middle is open, and right is the full exclosure.

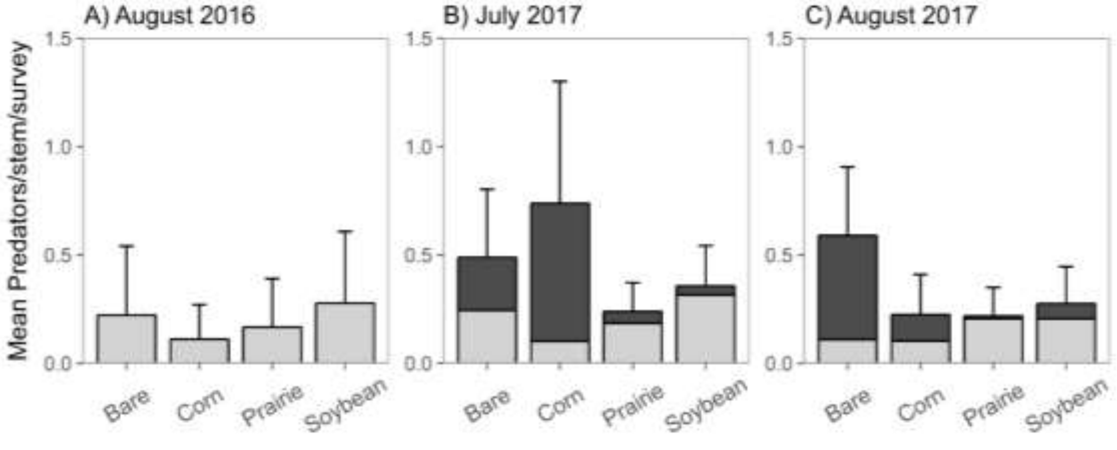

Figure C4. Results of predator surveys conducted during monarch egg survival checks. Means are the grand means of predators observed per milkweed stem averaged across all surveys conducted during the 72 h survival experiment (every 2–3 h for 24 h and at 48 h and 72 h) averaged across all 4 experimental blocks. Error bars are SEM of total predators. Ant data are not reported for August 2016, because ant numbers were not recorded.

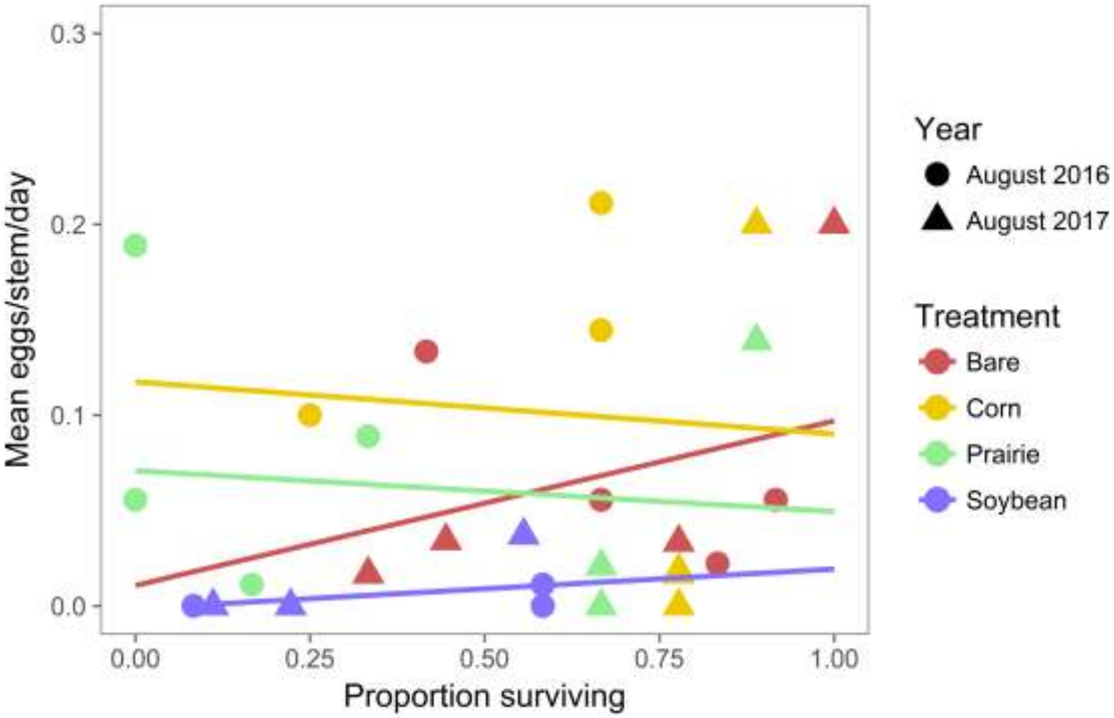

Figure C5. Monarch oviposition rates plotted against monarch survival to 72 h from oviposition and survival experiments performed in August 2016 and August 2017. Each point represents mean eggs/stem/day and average egg survival to 72 h for a given experimental plot and study year. The trend lines are best fit lines from linear models group by habitat treatment. One outlying point (corn, August 2016) was removed, changing the corn trend line from positive to negative.

APPENDIX D. SUPPLEMENTARY MATERIAL FOR CHAPTER 3

Table D1. Site locations and descriptions for monarch butterfly egg video surveillance studies in 2017 and 2018.

Site name	Coordinates	General description	Dominant vegetation[a]
KBS Site Corn treatment	42.407, -85.374	Four 9.1 x 27.4 m plots of conventionally managed corn in the Kellogg Biological Station Cellulosic Biofuels Diversity Experiment.	Corn (*Zea mays*)
KBS Site Prairie treatment	42.407, -85.374	Four 9.1 x 27.4 m plots of "low diversity prairie" in the Kellogg Biological Station Cellulosic Biofuels Diversity Experiment.	*Andropogon gerardii*, *Panicum virgatum*, *Sorghastrum nutans*, *Elymus canadensis*, *Schizacyrium scoparium*, *Koeleria cristata*, *Desmodium canadense*, *Rudbeckia hirta*, *Monarda fistulosa*, *Solidago rigida*
Garden	42.743, -84.486	Residential back yard garden adjacent to mowed lawn.	Poa sp., *Aegopodium podagraria*, *Cichorium intybus*, *Asclepias syriaca*, *Asclepias incarnata*, Taraxacum *officinale*
Corn edge	42.691, -84.490	Annually mowed strip of vegetation between a conventionally managed cornfield and a gravel road.	*Poa* sp., *Phalaris arundinacea*, *Asclepias syriaca*, *Amaranthus* sp., *Chenopodium berlandieri*, *Taraxacum officinale*
Old field	42.692, -84.491	Fallow field with cool season grasses and forbs.	*Poa* sp. and, *Solidago canadensis*, *Asclepias syriaca*, *Plantago lanceolata*, *Elytrigia repens*, *Taraxacum officinale*, *Cirsium arvense*
Marsh Edge	42.689, -84.475	Un-mowed cool season grasses (*Phalaris arundinacea*) adjacent to a cattail (*Typha* sp.) marsh.	*Phalaris arundinacea*, *Solidago canadensis*, *Symphyotrichum pilosum*, *Asclepias syriaca*, *Sonchus asper*, *Euthamia graminifolia*
Fencerow	42.679, -84.477	Un-mowed vegetation growing along a fence running between a seldom mowed lawn and a gravel road.	*Poa* sp., *Asclepias syriaca*, *Setaria* sp., *Daucus carota*, *Elaeagnus umbellata*, *Syringa vulgaris*

[a] In order of decreasing cover

Table D2. Detailed table of observation periods for monarch butterfly (*Danaus plexippus* L.) egg video surveillance with study sites, number of cameras deployed, and egg numbers per leaf used.

year	study site	deployed	removed	eggs monitored
2017	Garden	7/8	7/10	10
	KBS Corn Block 1	9/5	9/6	2
	KBS Corn Block 2	9/5	9/6	2
	KBS Corn Block 3	9/5	9/6	2
	KBS Corn Block 4	9/5	9/6	2
	KBS Prairie Block 1	9/5	9/6	2
	KBS Prairie Block 2	9/5	9/6	2
	KBS Prairie Block 3	7/12	7/13	3
	KBS Prairie Block 3	9/5	9/6	2
	KBS Prairie Block 4	9/5	9/6	2
2018	Corn edge	7/30	8/3	2
		7/30	8/3	2
		8/7	8/10	2
		8/7	8/10	2
		8/13	8/17	2
		8/13	8/17	2
		8/20	8/24	2
		8/20	8/24	2
		8/29	8/31	2
		8/29	8/31	2
		9/4	9/7	2
		9/4	9/7	2
	Fencerow	6/25	6/27	4
		7/3	7/5	2
		7/10	7/13	2
		7/10	7/13	2
		7/17	7/20	2
		7/17	7/20	2
	Garden	6/9	6/10	1
		6/11	6/12	1
		6/12	6/13	1

Table D2. (Cont'd).

Marsh edge	7/10	7/12	2
	7/17	7/20	2
	7/17	7/20	2
	7/30	8/3	2
	7/30	8/3	2
	8/7	8/10	2
	8/7	8/10	2
	8/13	8/17	2
	8/13	8/17	2
	8/20	8/24	2
	8/20	8/24	2
	8/29	8/31	2
	8/29	8/31	2
	9/4	9/5	2
	9/4	9/7	2
	9/5	9/7	2
Old field	7/3	7/5	2
	7/17	7/18	2
	7/17	7/20	2
	7/18	7/20	2
	7/30	8/3	2
	7/30	8/3	2
	7/30	8/3	2
	7/30	8/3	2
	8/7	8/9	2
	8/7	8/10	2
	8/7	8/10	2
	8/7	8/10	2
	8/13	8/17	2
	8/13	8/17	2
	8/13	8/17	2
	8/20	8/24	2
	8/20	8/24	2
	8/20	8/24	2
	8/29	8/31	2
	8/29	8/31	2
	8/29	8/31	2
	9/4	9/5	2
	9/4	9/7	2
	9/4	9/7	2
	9/5	9/7	2

APPENDIX E. SUPPLEMENTARY MATERIAL FOR CHAPTER 4

Table E1. Summary of models used to test aphid and ant predictors of monarch butterfly oviposition and neonate survival and the efficacy of aphid and ant treatments.

Model set description and purpose	**Model name**	**Response**	**Predictors**	**Model Fit Comparison**
Examining predictors of 96 h monarch neonate survival using generalized linear mixed effects models with binomial distribution and random effect of experimental plot	null	neonate survival	~ 1 + (1|plot)	
	aphid treatment only		~ aphid treatment+ (1|plot)	*
	ant treatment only		~ ant treatment + (1|plot)	
	additive		~ aphid treatment + ant treatment + (1|plot)	*
	interactive		~ aphid treatment*ant treatment + (1|plot)	**
Testing Tanglefoot treatment efficacy using generalized linear models with Poisson distribution	null	total ants observed/plant	~ 1	
	ant treatment		~ ant treatment	*
Testing *Myzocallis* treatment efficacy using generalized linear models with Poisson distribution	null	total *Myzocallis* observed/plant	~ 1	
	aphid treatment		~ aphid treatment	*
Testing *Aphis* treatment efficacy using generalized linear models with Poisson distribution	null	total *Aphis* observed/plant	~ 1	
	aphid treatment		~ aphid treatment	*
Testing the effect of ant presence on monarch butterfly oviposition using generalized linear models with Poisson distribution	null	total monarch eggs observed/plant	~ 1	
	ant treatment		~ ant treatment	*

Table E1. (Cont'd).

Testing the effects of aphid species and ant presence on non-ant predator numbers using generalized linear models with Poisson distribution and random effect of experimental plot	null	total non-ant predators/plant	~ 1	
	aphid treatment only		~ aphid treatment	
	ant treatment only		~ ant treatment	*
	additive		~ aphid treatment + ant treatment	*
	interactive		~ aphid treatment*ant treatment	**

* likelihood ratio test reveals significance compared with null model at $\alpha = 0.05$
** likelihood ratio test reveals best fit in multiple nested model comparison

Table E2. Mean numbers of non-ant predators observed per plant per day during monarch butterfly (*Danaus plexippus* L.) neonate survival study for three aphid treatments crossed with two ant treatments.

	Aphis		*Myzocallis*		No Aphids	
Taxon	Ants Excluded	Ants Present	Ants Excluded	Ants Present	Ants Excluded	Ants Present
Coccinellidae (lady beetles)	0	0	0	0.01	0	0
Carabidae: *Calleida* sp. (ground beetle)	0	0	0	0.01	0	0
Acrididae (grasshoppers)	0.08	0	0.04	0.01	0	0.04
Gryllidae: *Oecanthus* sp. (tree crickets)	0	0	0	0	0	0.01
Gryllidae: subfamily Nemobiinae (ground crickets)	0	0.01	0	0.01	0	0
Hemerobiidae & Chrysopidae (lacewings)	0	0.01	0	0.03	0.04	0.02
Parasitoid wasps (all families)	0	0.02	0	0.07	0	0.02
Pentatomidae: *Podisus maculiventris* (spined soldier bug)	0.04	0.02	0	0.05	0	0.04
Nabidae & Reduviidae (damsel & assassin bugs)	0	0	0	0.04	0	0.02
Miridae (plant bug, unknown sp.)	0.04	0	0	0	0	0.01
Araneae Thomisidae (crab spiders)	0	0	0	0	0	0.02
Araneae web-building spiders	0	0.01	0.04	0.02	0	0.03
Trombidiformes (mite, unknown sp.)	0	0.03	0	0	0	0.03
Gastropoda (slug, unknown sp.)	0	0	0	0.02	0	0
total predators	0.16	0.1	0.08	0.27	0.04	0.24

LITERATURE CITED

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Agrawal, A. A. 2019. Advances in understanding the long-term population decline of monarch butterflies. Proceedings of the National Academy of Sciences. 116: 8093–8095.

Agrawal, A. A., E. E. Kearney, A. P. Hastings, and T. E. Ramsey. **2012**. Attenuation of the jasmonate burst, plant defensive traits, and resistance to specialist monarch caterpillars on shaded common milkweed (Asclepias syriaca). Journal of Chemical Ecology. 38: 893–901.

Alexander, P., M. D. A. Rounsevell, C. Dislich, J. R. Dodson, K. Engström, and D. Moran. **2015**. Drivers for global agricultural land use change: The nexus of diet, population, yield and bioenergy. Global Environmental Change. 35: 138–147.

Ali, J. G., and A. A. Agrawal. **2014**. Asymmetry of plant-mediated interactions between specialist aphids and caterpillars on two milkweeds. Functional Ecology. 28: 1404–1412.

Altizer, S., and J. C. de Roode. **2015**. Monarchs and their debilitating parasites: immunity, migration and medicinal plant use, pp. 83–93. *In* Altizer, S.M., Nail, K.R. (eds.), Monarchs in a Changing World: Biology and Conservation of an Iconic Insect. Cornell University Press, Ithaca, NY.

De Anda, A., and K. S. Oberhauser. **2015**. Invertebrate natural enemies and stage-specific mortality rates of monarch eggs and larvae, pp. 60–70. *In* Altizer, S.M., Nail, K.R. (eds.), Monarchs in a Changing World: Biology and Conservation of an Iconic Butterfly. Cornell University Press, Ithaca, NY.

Anderson, J. B., and L. P. Brower. **1996**. Freeze-protection of overwintering monarch butterflies in Mexico: critical role of the forest as a blanket and an umbrella. Ecological Entomology. 21: 107–116.

Badgett, G., and A. K. Davis. **2015**. Population trends of monarchs at a northern monitoring site: Analyses of 19 years of fall migration counts at peninsula point, MI. Annals of the Entomological Society of America. 108: 700–706.

Bahlai, C. A., M. Colunga-Garcia, S. H. Gage, and D. A. Landis. **2013**. Long-term functional dynamics of an aphidophagous coccinellid community remain unchanged despite repeated invasions. PLoS ONE. 8: e83407.

Bartel, R. A., K. S. Oberhauser, J. C. De Roode, and S. M. Altizer. **2011**. Monarch butterfly migration and parasite transmission in eastern North America. Ecology. 92: 342–351.

Barton, B. T., and O. J. Schmitz.Opposite effects of daytime and nighttime warming on top-down control of plant diversity, Ecology. Wiley-Blackwell.

Batalden, R. V., and K. S. Oberhauser. **2015**. Potential changes in Eastern North American monarch migration in response to an introduced milkweed, Asclepias curassavica, pp. 215–

224. *In* Altizer, S.M., Nail, K.R. (eds.), Monarchs in a Changing World: Biology and Conservation of an Iconic Butterfly. Cornell University Press, Ithaca, NY.

Bates, D., M. Mächler, B. Bolker, and S. Walker. **2015**. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software. 67: 1–48.

Baum, K. A., and W. V. Sharber. **2012**. Fire creates host plant patches for monarch butterflies. Biology Letters. 8: 968–71.

Belsky, J., and N. K. Joshi. **2018**. Assessing role of major drivers in recent decline of monarch butterfly population in North America. Frontiers in Environmental Science. 6: 86.

Benton, T. G., J. A. Vickery, and J. D. Wilson. **2003**. Farmland biodiversity: is habitat heterogeneity the key? Trends in Ecology & Evolution. 18: 182–188.

Bhowmik, P., and J. D. Bandeen. **1976**. The biology of Canadian weeds 19. *Asclepias syriaca* L. Canadian Journal of Plant Science. 56.

Bianchi, F. J. J. A., C. J. H. Booij, and T. Tscharntke. **2006**. Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. Proceedings. Biological sciences / The Royal Society. 273: 1715–27.

Bishop, D. B., and C. M. Bristow. **2006**. Effects of the Presence of the Allegheny Mound Ant (Hymenoptera: Formicidae) in Providing Enemy-Free Space to Myrmecophilous Aphid and Soft Scale Populations. Annals of the Entomological Society of America. 96: 202–210.

Blader, T. 2018. Milkweed patch size effects on monarch butterfly oviposition within Iowa prairies and roadsides. Graduate Theses and Dissertations. 1–101.

Blitzer, E. J., C. F. Dormann, A. Holzschuh, A.-M. Klein, T. A. Rand, and T. Tscharntke. **2012**. Spillover of functionally important organisms between managed and natural habitats. Agriculture, Ecosystems & Environment. 146: 34–43.

Borkin, S. S. 1982. Notes on shifting distribution patterns and survival of immature *Danaus plexippus* (Lepidoptera: Danaidae) on the food plant *Asclepias syriaca*. Great Lakes Entomology. 15: 199–206.

Bowlin, M. S., I.-A. Bisson, J. Shamoun-Baranes, J. D. Reichard, N. Sapir, P. P. Marra, T. H. Kunz, D. S. Wilcove, A. Hedenström, C. G. Guglielmo, S. Åkesson, M. Ramenofsky, and M. Wikelski. **2010**. Grand challenges in migration biology. Integrative and comparative biology. 50: 261–79.

Boyle, J. H., H. J. Dalgleish, and J. R. Puzey. **2019**. Monarch butterfly and milkweed declines substantially predate the use of genetically modified crops. Proceedings of the National Academy of Sciences of the United States of America. 116: 3006–3011.

Brennan, L. A., and W. P. Kuvlesky. **2005**. Invited paper: North American grassland birds: an unfolding conservation crisis? Journal of Wildlife Management. 69: 1–13.

Brower, L. P. 1995. Understanding and misunderstanding the migration of the monarch butterfly (Nymphalidae) in North America. J. Lepidopt. Soc. 49: 304–385.

Brower, L. P., L. S. Fink, and P. Walford. **2006**. Fueling the fall migration of the monarch butterfly. Integrative and Comparative Biology. 46: 1123–1142.

Brower, L. P., D. R. Kust, E. R. Salinas, E. García-serrano, K. R. Kust, J. Miller, C. Ferdnandez del Rey, and K. Pape. **2004**. Catastrophic winter storm mortality of monarch butterflies in Mexico during January 2002, pp. 151–166. *In* Monarch Butterfly Biology and Conservation. Cornell University Press, Ithaca, NY.

Brower, L. P., O. R. Taylor, E. H. Williams, D. A. Slayback, R. R. Zubieta, and M. I. Ramírez. **2012**. Decline of monarch butterflies overwintering in Mexico: Is the migratory phenomenon at risk? Insect Conservation and Diversity. 5: 95–100.

Brower, L. P., E. H. Williams, P. Jaramillo-López, D. R. Kust, D. A. Slayback, and M. I. Ramírez. **2017**. Butterfly mortality and salvage logging: From the March 2016 storm in the monarch butterfly biosphere reserve in Mexico. American Entomologist. 63: 151–164.

Brussaard, L., P. Caron, B. Campbell, L. Lipper, S. Mainka, R. Rabbinge, D. Babin, and M. Pulleman. **2010**. Reconciling biodiversity conservation and food security: scientific challenges for a new agriculture. Current Opinion in Environmental Sustainability. 2: 34–42.

Calvert, W. H. 2004. The effect of fire ants on monarchs breeding in Texas, pp. 47–53. *In* Oberhauser, K.S., Solensky, M.J. (eds.), Monarch Butterfly Biology and Conservation. Cornell University Press, Ithaca, NY.

Chapin, F. S., E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavorel, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Díaz. **2000**. Consequences of changing biodiversity. Nature. 405: 234–42.

Chaplin-Kramer, R., M. E. O'Rourke, E. J. Blitzer, and C. Kremen. **2011**. A meta-analysis of crop pest and natural enemy response to landscape complexity. Ecology letters. 14: 922–32.

Chappell, M. J., and L. A. LaValle. **2009**. Food security and biodiversity: can we have both? An agroecological analysis. Agriculture and Human Values. 28: 3–26.

Cleary, E. C., and S. R. Craven. **1994**. Prevention and Control of Wildlife Damage, p. B-165–169. *In* Hygnstrom, S.E., Timm, R.M., Larson, G.E. (eds.), Prevention and Control of Wildlife Damage. University of Nebraska-Lincoln.

Cohen, J. A., and L. P. Brower. **1982**. Oviposition and larval success of wild monarch butterflies (Lepidoptera: Danaidae) in relation to host plant size and cardenolide concentration. Journal of the Kansas Entomological Society. 55: 343–348.

Davis, A. K. 2012. Are migratory monarchs really declining in eastern North America?

Examining evidence from two fall census programs. Insect Conservation and Diversity. 5: 101–105.

Derpsch, R., T. Friedrich, A. Kassam, and H. Li. **2010**. Current status of adoption of no-till farming in the world and some of its main benefits - Google Search. International Journal of Agricultural and Biological Engineering.

Dickson, T. L., and K. L. Gross. **2015**. Can the results of biodiversity-ecosystem productivity studies be translated to bioenergy production? PLOS ONE. 10: e0135253.

Diffendorfer, J. E., J. B. Loomis, L. Ries, K. Oberhauser, L. Lopez-Hoffman, D. Semmens, B. Semmens, B. Butterfield, K. Bagstad, J. Goldstein, R. Wiederholt, B. Mattsson, and W. E. Thogmartin. **2014**. National valuation of monarch butterflies indicates an untapped potential for incentive-based conservation. Conservation Letters. 7: 253–262.

Dixon, C. A., J. M. Erickson, D. N. Kellett, and M. Rothschild. **1978**. Some adaptations between *Danaus plexippus* and its food plant, with notes on *Danaus chrysippus* and *Euploea core* (Insecta: Lepidoptera). Journal of Zoology. 185: 437–467.

Van Dyck, H., A. J. Van Strien, D. Maes, and C. A. M. Van Swaay. **2009**. Declines in common, widespread butterflies in a landscape under intense human use. Conservation Biology. 23: 957–965.

Dyer, L. A., and M. L. Forister. **2016**. Wherefore and whither the modeler: Understanding the population dynamics of monarchs will require integrative and quantitative techniques. Annals of the Entomological Society of America. 109: 172–175.

Ellis, E. C., and N. Ramankutty. **2008**. Putting people in the map: anthropogenic biomes of the world. Frontiers in Ecology and the Environment. 6: 439–447.

Emani, C. 2014. The effects of transgenic crops on non-target organisms. *In* Ahuja, M.R., Ramawat, K.G. (eds.), Biotechnology and Biodiversity, Sustainable Development and Biodiversity. Springer International Publishing, Cham.

Feeny, P. 1976. Plant apparency and chemical defense. *In* Wallace, J.W., Mansell, R.L. (eds.), Biochemical Interaction Between Plants and Insects. Springer US, Boston, MA.

Fellers, J. H. 1989. Daily and seasonal activity in woodland ants. Oecologia. 78: 69–76.

Fernandez-Cornejo, J., and W. D. McBride. **2002**. Adoption of bioengineered crops. Economic Research Service/USDA.

Fiedler, A. K., and D. A. Landis. **2007**. Attractiveness of Michigan native plants to arthropod natural enemies and herbivores. Environmental Entomology. 36: 751–765.

Fischer, S. J., E. H. Williams, L. P. Brower, and P. A. Palmiotto. **2015**. Enhancing monarch butterfly reproduction by mowing fields of common milkweed. The American Midland Naturalist. 173: 229–240.

Fishbein, M., and D. Lawrence Venable. **1996**. Diversity and temporal change in the effective pollinators of *Asclepias tuberosa*. Ecology.

Flockhart, D. T. T., T. G. Martin, and D. R. Norris. **2012**. Experimental examination of intraspecific density-dependent competition during the breeding period in monarch butterflies (*Danaus plexippus*). PLoS ONE. 7: e45080.

Flockhart, D. T. T., J. B. Pichancourt, D. R. Norris, and T. G. Martin. **2015**. Unravelling the annual cycle in a migratory animal: Breeding-season habitat loss drives population declines of monarch butterflies. Journal of Animal Ecology. 84: 155–165.

Flockhart, D. T. T. T., L. P. Brower, M. I. Ramirez, K. A. Hobson, L. I. Wassenaar, S. Altizer, and D. R. Norris. **2017**. Regional climate on the breeding grounds predicts variation in the natal origin of monarch butterflies overwintering in Mexico over 38 years. Global change biology. 23: 2565–2576.

Flynn, D. F. B., M. Gogol-Prokurat, T. Nogeire, N. Molinari, B. T. Richers, B. B. Lin, N. Simpson, M. M. Mayfield, and F. DeClerck. **2009**. Loss of functional diversity under land use intensification across multiple taxa. Ecology letters. 12: 22–33.

Foley, J. A., N. Ramankutty, K. A. Brauman, E. S. Cassidy, J. S. Gerber, M. Johnston, N. D. Mueller, C. O'Connell, D. K. Ray, P. C. West, C. Balzer, E. M. Bennett, S. R. Carpenter, J. Hill, C. Monfreda, S. Polasky, J. Rockström, J. Sheehan, S. Siebert, D. Tilman, and D. P. M. Zaks. **2011**. Solutions for a cultivated planet. Nature. 478: 337–42.

Freese, B., and M. Crouch. **2015**. Monarchs in peril: herbicide-resistant crops and the decline of monarch butterflies in North America. Washington, D.C.

Glamann, J., J. Hanspach, D. J. Abson, N. Collier, and J. Fischer. **2017**. The intersection of food security and biodiversity conservation: a review. Regional Environmental Change. 17: 1303–1313.

Grant, T. J., H. R. Parry, M. P. Zalucki, and S. P. Bradbury. **2018**. Predicting monarch butterfly (*Danaus plexippus*) movement and egg-laying with a spatially-explicit agent-based model: The role of monarch perceptual range and spatial memory. Ecological Modelling. 374: 37–50.

Green, R. E., S. J. Cornell, J. P. W. Scharlemann, and A. Balmford. **2005**. Farming and the fate of wild nature. Science (New York, N.Y.). 307: 550–5.

Grieshop, M. J., B. Werling, K. Buehrer, J. Perrone, R. Isaacs, and D. Landis. **2012**. Big brother is watching: studying insect predation in the age of digital surveillance. American Entomologist. 58: 172–182.

Grixti, J. C., L. T. Wong, S. A. Cameron, and C. Favret. **2009**. Decline of bumble bees (*Bombus*) in the North American Midwest. Biological Conservation. 142: 75–84.

Guiney, M., and K. Oberhauser. **2009**. Insects as flagship conservation species. Terrestrial

Arthropod Reviews. 1: 111–123.

Gustafsson, K. M., A. A. Agrawal, B. V. Lewenstein, and S. A. Wolf. **2015**. The monarch butterfly through time and space: The social construction of an icon. BioScience. 65: 612–622.

Haan, N. L., and D. A. Landis. **2019a**. Grassland disturbance increases monarch butterfly oviposition and decreases arthropod predator abundance. Biological Conservation.

Haan, N. L., and D. A. Landis. **2019b**. The importance of shifting disturbance regimes in monarch butterfly decline and recovery. Frontiers in Ecology and Evolution.

Haddad, N. 2019. The last butterflies: A scientist's quest to save a rare and vanishing creature. Princeton University Press, Princeton, New Jersey.

Hallmann, C. A., M. Sorg, E. Jongejans, H. Siepel, N. Hofland, H. Schwan, W. Stenmans, A. Müller, H. Sumser, T. Hörren, D. Goulson, and H. de Kroon. **2017**. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLOS ONE. 12: e0185809.

Haribal, M., and J. A. A. Renwick. **1998**. Identification and distribution of oviposition stimulants for monarch butterflies in hosts and nonhosts. Journal of chemical ecology. 24: 891–904.

Hartzler, R. G. 2010. Reduction in common milkweed (*Asclepias syriaca*) occurrence in Iowa cropland from 1999 to 2009. Crop Protection. 29: 1542–1544.

Harvey, E., I. Gounand, C. L. Ward, and F. Altermatt. **2017**. Bridging ecology and conservation: from ecological networks to ecosystem function. Journal of Applied Ecology. 54: 371–379.

Hermann, S. L., C. Blackledge, N. L. Haan, A. T. Myers, and D. A. Landis. **n.d.** Predators of monarch butterfly eggs and neonate larvae are more diverse than previously recognized. Scientific Reports.

Inamine, H., S. P. Ellner, J. P. Springer, and A. A. Agrawal. **2016**. Linking the continental migratory cycle of the monarch butterfly to understand its population decline. Oikos. 125: 1081–1091.

Jacobsen, S.-E., M. Sørensen, S. M. Pedersen, and J. Weiner. **2013**. Feeding the world: genetically modified crops versus agricultural biodiversity. Agronomy for Sustainable Development. 33: 651–662.

Jones, I. M., S. Koptur, and J. E. Peña. **2017**. Exploring whether and how ants (Hymenoptera: Formicidae) affect reproductive fitness in Senna mexicana var. chapmanii (Fabaceae). Florida Entomologist. 100: 539–545.

Klein, A.-M., B. E. Vaissière, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C.

Kremen, and T. Tscharntke. **2007**. Importance of pollinators in changing landscapes for world crops. Proceedings. Biological sciences / The Royal Society. 274: 303–13.

Knight, S. M., D. R. Norris, R. Derbyshire, and D. T. T. Flockhart. **2019**. Strategic mowing of roadside milkweeds increases monarch butterfly oviposition. Global Ecology and Conservation.

Knop, E., L. Zoller, R. Ryser, C. Gerpe, M. Hörler, and C. Fontaine. **2017**. Artificial light at night as a new threat to pollination. Nature. 548: 206–209.

Koch, R. L., W. D. Hutchison, R. C. Venette, and G. E. Heimpel. **2003**. Susceptibility of immature monarch butterfly, *Danaus plexippus* (Lepidoptera: Nymphalidae: Danainae), to predation by Harmonia axyridis (Coleoptera: Coccinellidae). Biological Control. 28: 265–270.

Koch, R. L., R. C. Venette, and W. D. Hutchison. **2005**. Influence of alternate prey on predation of monarch Butterfly (Lepidoptera: Nymphalidae) larvae by the multicolored Asian lady beetle (Coleoptera: Coccinellidae). Environmental Entomology. 34: 410–416.

Kremen, C., N. M. Williams, and R. W. Thorp. **2002**. Crop pollination from native bees at risk from agricultural intensification. Proceedings of the National Academy of Sciences of the United States of America. 99: 16812–6.

Krischik, V., M. Rogers, G. Gupta, and A. Varshney. **2015**. Soil-applied imidacloprid translocates to ornamental flowers and reduces survival of adult *Coleomegilla maculata*, *Harmonia axyridis*, and *Hippodamia convergens* lady beetles, and larval *Danaus plexippus* and *Vanessa cardui* . PLoS ONE. 10.

Landis, D. A. 2017. Productive engagement with agriculture essential to monarch butterfly conservation. Environmental Research Letters. 12: 101003.

Landis, D. A., S. D. Wratten, and G. M. Gurr. **2000**. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annual review of entomology. 45: 175–201.

Lavoie, B., and K. S. Oberhauser. **2004**. Compensatory feeding in *Danaus plexippus* (Lepidoptera: Nymphalidae) in response to variation in host plant quality. Environmental Entomology. 33: 1062–1069.

Lenth, R., J. Love, and M. Herve. **2018**. Estimated marginal means, aka least-squares means. R Package Version 1.1.

Liebman, M., M. J. Helmers, L. A. Schulte, and C. A. Chase. **2013**. Using biodiversity to link agricultural productivity with environmental quality: Results from three field experiments in Iowa. Renewable Agriculture and Food Systems. 28: 115–128.

Lin, H., and D. Zelterman. **2002**. Modeling survival data: Extending the Cox model, Technometrics. Springer, New York.

Lynch, S. P., and R. A. Martin. **1993**. Milkweed host plant utilization and cardenolide sequestration by monarch butterflies in Louisiana and Texas. 107–123.

Macgregor, C. J., M. J. O. Pocock, R. Fox, and D. M. Evans. **2015**. Pollination by nocturnal Lepidoptera, and the effects of light pollution: A review. Ecological Entomology. 40: 187–198.

Malcolm, S. B. 1993. Conservation of monarch butterfly migration in North America: An endangered phenomenon, p. 357. *In* Zalucki, M.P. (ed.), Biology and Conservation of the Monarch Butterfly. Natural History Museum of Los Angeles.

Malcolm, S. B., and L. P. Brower. **1986**. Selective oviposition by monarch butterflies in a mixed stand of *Asclepias curassavica* L. and *A. incarnata* L. in south Florida. Journal of the Lepidopterists' Society (USA). 4: 255–263.

Malcolm, S. B., B. J. Cockrell, and L. P. Brower. **1987**. Monarch butterfly voltinism: effects of temperature constraints at different latitudes. Oikos. 49: 77.

Malcolm, S. B., B. J. Cockrell, and L. P. Brower. **1993**. Spring recolonization of eastern North America by the monarch butterfly: successive brood or single sweep migration? Biology and conservation of the monarch butterfly. 253–267.

Malcolm, S. B., M. P. Zalucki, B. J. Cockrell, and B. J. Mwakamela. **1999**. The lethal plant defense paradox and the influence of milkweed latex on larval feeding behavior of the monarch butterfly., pp. 139–149. *In* 1997 North American Conference on the Monarch Butterfly.

Marvier, M., C. McCreedy, J. Regetz, and P. Kareiva. **2007**. A meta-analysis of effects of Bt cotton and maize on nontarget invertebrates. Science.

Mckenna, D. D., K. M. Mckenna, S. B. Malcolm, and M. R. Berenbaum. **2001**. Mortality of lepidoptera along roadways in central Illinois. Journal of Lepidopterists' Society. 55: 63–68.

Meehan, T. D., B. P. Werling, D. A. Landis, and C. Gratton. **2011**. Agricultural landscape simplification and insecticide use in the Midwestern United States. Proceedings of the National Academy of Sciences of the United States of America. 108: 11500–5.

Mestre, L., R. Bucher, and M. H. Entling. **2014**. Trait-mediated effects between predators: Ant chemical cues induce spider dispersal. Journal of Zoology.

Mooney, K. A., and A. A. Agrawal. **2008**. Plant genotype shapes ant-aphid interactions: Implications for community structure and indirect plant defense. American Naturalist. 171: E195–E205.

Myers, A., C. A. Bahlai, and D. A. Landis. **2019**. Habitat type influences *Danaus plexippus* (Lepidoptera: Nymphalidae) oviposition and egg survival on *Asclepias syriaca* (Gentianales: Apocynaceae). Environmental entomology. 48: 675–684.

Myers, A. T., N. L. Haan, and D. A. Landis. **n.d.** Video surveillance reveals a diverse and largely nocturnal community of *Danaus plexippus* (L.) egg predators.

Nail, K. R., C. Stenoien, and K. S. Oberhauser. **2015**. Immature monarch survival: Effects of site characteristics, density, and time. Annals of the Entomological Society of America. 108: 680–690.

Naranjo, S. E. 2006. Long-term assessment of the effects of transgenic *Bt* cotton on the function of the natural enemy community. Environmental Entomology. 34: 1211–1223.

Nasr, M., J. Jalali Sendi, S. Moharramipour, and A. Zibaee. **2017**. Evaluation of *Origanum vulgare* L. essential oil as a source of toxicant and an inhibitor of physiological parameters in diamondback moth, *Plutella xylustella* L. (Lepidoptera: Pyralidae). Journal of the Saudi Society of Agricultural Sciences. 16: 184–190.

O., O. S., and M. P. Zalucki. **1990**. Cardiac glycosides and oviposition by *Danaus plexippus* on *Asclepias fruticosa* in south-east Queensland (Australia), with notes on the effect of plant nitrogen content. Ecological Entomology. 15: 177–185.

Oberhauser, K., and A. T. Peterson. **2003**. Modeling current and future potential wintering distributions of eastern North American monarch butterflies. Proceedings of the National Academy of Sciences. 100: 14063–14068.

Oberhauser, K. S. 2004. Overview of monarch breeding biology, pp. 3–7. *In* Oberhauser, K.S., Solensky, M.J. (eds.), The Monarch Butterfly: Biology and Conservation. Cornell University Press, Ithaca, NY.

Oberhauser, K. S., M. Anderson, S. Anderson, W. Caldwell, A. P. Deanda, M. D. Hunter, M. C. Kaiser, M. J. Solensky, A. De Anda, M. D. Hunter, M. C. Kaiser, and M. J. Solensky. **2015**. Lacewings, wasps and flies—oh my: insect enemies take a bite out of monarchs, pp. 71–82. *In* Altizer, S.M., Nail, K.R. (eds.), Monarchs in a Changing World: Biology and Conservation of an Iconic Insect. Cornell University Press, Ithaca, NY.

Oberhauser, K. S., M. D. Prysby, H. R. Mattila, D. E. Stanley-Horn, M. K. Sears, G. Dively, E. Olson, J. M. Pleasants, W. K. Lam, and R. L. Hellmich. **2001**. Temporal and spatial overlap between monarch larvae and corn pollen. Proceedings of the National Academy of Sciences of the United States of America. 98: 11913–11918.

Oberhauser, K., R. Wiederholt, J. E. Diffendorfer, D. Semmens, L. Ries, W. E. Thogmartin, L. Lopez-Hoffman, and B. Semmens. **2017**. A trans-national monarch butterfly population model and implications for regional conservation priorities. Ecological Entomology. 42: 51–60.

Oliver, T. H., I. Jones, J. M. Cook, and S. R. Leather. **2008**. Avoidance responses of an aphidophagous ladybird, *Adalia bipunctata*, to aphid-tending ants. Ecological Entomology. 33: 523–528.

Parr, C. L., P. Eggleton, A. B. Davies, T. A. Evans, and S. Holdsworth. **2016**. Suppression of savanna ants alters invertebrate composition and influences key ecosystem processes. Ecology. 97: 1611–1617.

Pfannenstiel, R. S. 2005. Nocturnal predators and their impact lepidopteran eggs in annual crops: What we don't see does help us!, pp. 463–471. *In* Second International Symposium on Biological Control of Arthropods; September 12-16, 2005, Davos, Switzerland.

Pierce, N. E., M. F. Braby, A. Heath, D. J. Lohman, J. Mathew, D. B. Rand, and M. A. Travassos. **2002**. The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). Annual Review of Entomology. 47: 733–771.

Pitman, G. M., D. T. T. Flockhart, and D. R. Norris. **2018**. Patterns and causes of oviposition in monarch butterflies: Implications for milkweed restoration. Biological Conservation. 217: 54–65.

Pleasants, J. 2017. Milkweed restoration in the Midwest for monarch butterfly recovery: estimates of milkweeds lost, milkweeds remaining and milkweeds that must be added to increase the monarch population. Insect Conservation and Diversity. 10: 42–53.

Pleasants, J. M. 2015. Monarch Butterflies and Agriculture. *In* Altizer, S.M., Nail, K.R. (eds.), Monarchs in a Changing World: Biology and Conservation of an Iconic Butterfly. Cornell University Press, Ithaca, NY.

Pleasants, J. M., and K. S. Oberhauser. **2013**. Milkweed loss in agricultural fields because of herbicide use: Effect on the monarch butterfly population. Insect Conservation and Diversity. 6: 135–144.

Pleasants, J. M., E. H. Williams, L. P. Brower, K. S. Oberhauser, and O. R. Taylor. **2016**. Conclusion of no decline in summer monarch population not supported. Annals of the Entomological Society of America. 109: 169–171.

Pleasants, J. M., M. P. Zalucki, K. S. Oberhauser, L. P. Brower, O. R. Taylor, and W. E. Thogmartin. **2017**. Interpreting surveys to estimate the size of the monarch butterfly population: Pitfalls and prospects. PLoS ONE. 12: e0181245.

Pocius, V. M., D. M. Debinski, K. G. Bidne, R. L. Hellmich, and F. K. Hunter. **2017**. Performance of early instar monarch butterflies (*Danaus plexippus* L.) on nine milkweed species native to Iowa. Journal of the Lepidopterists' Society. 71: 153–161.

Pocius, V. M., D. M. Debinski, J. M. Pleasants, K. G. Bidne, R. L. Hellmich, and L. P. Brower. **2017**. Milkweed matters: Monarch butterfly (Lepidoptera: Nymphalidae) survival and development on nine midwestern milkweed species. Environmental Entomology. 46: 1098–1105.

Pocius, V. M., J. M. Pleasants, D. M. Debinski, K. G. Bidne, R. L. Hellmich, S. P. Bradbury, and S. L. Blodgett. **2018**. Monarch butterflies show differential utilization of nine midwestern milkweed species. Frontiers in Ecology and Evolution. 6: 169.

Prysby, M. D. 2004. Natural Enemies and Survival of Monarch Eggs and Larvae, pp. 27–37. *In* Oberhauser, K.S., Solensky, M.J. (eds.), The Monarch Butterfly: Biology and Conservation. Cornell University Press, Ithaca, NY.

Purtauf, T., J. Dauber, and V. Wolters. **2005**. The response of carabids to landscape simplification differs between trophic groups. Oecologia. 142: 458–64.

Quinn, J. E. 2012. Sharing a vision for biodiversity conservation and agriculture. Renewable Agriculture and Food Systems. 28: 93–96.

Quinn, J. E., T. Awada, F. Trindade, L. Fulginiti, and R. Perrin. **2017**. Combining habitat loss and agricultural intensification improves our understanding of drivers of change in avian abundance in a North American cropland anthrome. Ecology and Evolution.

R Core Team. **2017**. R: A language and environment for statistical computing. http://www.R-project.org/. R Foundation for Statistical Computing, Vienna, Austria.

Rafter, J. L., A. A. Agrawal, and E. L. Preisser. **2013**. Chinese mantids gut toxic monarch caterpillars: Avoidance of prey defence? Ecological Entomology. 38: 76–82.

Ramírez, M. I., C. Sáenz, G. Rehfeldt, and L. Salas. **2015**. Threats to the availability of overwintering habitat in the Monarch Butterfly Biosphere Reserve, p. 312. *In* Altizer, S.M., Nail, K.R. (eds.), Monarchs in a Changing Word. Biology and Conservation of an Iconic Butterfly. Cornell University Press, Ithaca, NY.

Raudsepp-Hearne, C., G. D. Peterson, and E. M. Bennett. **2010**. Ecosystem service bundles for analyzing tradeoffs in diverse landscapes. Proceedings of the National Academy of Sciences of the United States of America. 107: 5242–7.

Rayor, L. 2004. Effects of monarch larval host plant chemistry and body size on *Polistes* wasp predatoin, pp. 39–46. *In* Oberhauser, K.S., Solensky, M.J. (eds.), The Monarch Butterfly: Biology and Conservation. Cornell University Press, Ithaca, NY.

Ries, L., D. J. Taron, and E. Rendón-Salinas. **2015**. The disconnect between summer and winter monarch trends for the eastern migratory population: Possible links to differing drivers. Annals of the Entomological Society of America. 108: 691–699.

Ries, L., E. F. Zipkin, and R. P. Guralnick. **2019**. Tracking trends in monarch abundances over the 20th century is currently impossible using museum records: a response to Boyle et al. (2019). bioRxiv. 581801.

Robinson, R. A., and W. J. Sutherland. **2002**. Post-war changes in arable farming and biodiversity in Great Britain. Journal of Applied Ecology. 39: 157–176.

Rusch, A., R. Chaplin-Kramer, M. M. Gardiner, V. Hawro, J. Holland, D. Landis, C. Thies, T. Tscharntke, W. W. Weisser, C. Winqvist, M. Woltz, and R. Bommarco. **2016**. Agricultural landscape simplification reduces natural pest control: A quantitative synthesis. Agriculture, Ecosystems & Environment. 221: 198–204.

Sánchez-Bayo, F., and K. A. G. Wyckhuys. **2019**. Worldwide decline of the entomofauna: A review of its drivers. Biological Conservation. 232: 8–27.

Satterfield, D. A., J. C. Maerz, and S. Altizer. **2015**. Loss of migratory behaviour increases infection risk for a butterfly host. Proceedings of the Royal Society B: Biological Sciences. 282: 20141734–20141734.

Saunders, M. E., J. K. Janes, and J. C. O'Hanlon. **2019**. Understanding the evidence informing the insect apocalypse myth. EcoEvoRxiv Preprints.

Saunders, S. P., L. Ries, N. Neupane, M. I. Ramírez, E. García-Serrano, E. Rendón-Salinas, and E. F. Zipkin. **2019**. Multiscale seasonal factors drive the size of winter monarch colonies. Proceedings of the National Academy of Sciences. 116: 8609–8614.

Saunders, S. P., L. Ries, K. S. Oberhauser, W. E. Thogmartin, and E. F. Zipkin. **2018**. Local and cross-seasonal associations of climate and land use with abundance of monarch butterflies *Danaus plexippus*. Ecography. 41: 278–290.

Schulte, L. A., J. Niemi, M. J. Helmers, M. Liebman, J. G. Arbuckle, D. E. James, R. K. Kolka, M. E. O'Neal, M. D. Tomer, J. C. Tyndall, H. Asbjornsen, P. Drobney, J. Neal, G. Van Ryswyk, and C. Witte. **2017**. Prairie strips improve biodiversity and the delivery of multiple ecosystem services from corn–soybean croplands. Proceedings of the National Academy of Sciences.

Schultz, C. B., L. M. Brown, E. Pelton, and E. E. Crone. **2017**. Citizen science monitoring demonstrates dramatic declines of monarch butterflies in western North America. Biological Conservation. 214: 343–346.

Semmens, B. X., D. J. Semmens, W. E. Thogmartin, R. Wiederholt, L. López-Hoffman, J. E. Diffendorfer, J. M. Pleasants, K. S. Oberhauser, and O. R. Taylor. **2016**. Quasi-extinction risk and population targets for the Eastern, migratory population of monarch butterflies (*Danaus plexippus*). Scientific Reports. 6: 23265.

Sendoya, S. F., A. V. L. Freitas, and P. S. Oliveira. **2009**. Egg-laying butterflies distinguish predaceous ants by sight. American Naturalist. 174: 134–140.

Smiley, J. 1986. Ant constancy at *Passiflora* extrafloral nectaries: effects on caterpillar survival. Ecology. 67: 516–521.

Smith, R. A., K. A. Mooney, and A. A. Agrawal. **2008**. Coexistence of three specialist aphids on common milkweed, *Asclepias syriaca*. Ecology. 89: 2187–2196.

Smithers, C. N. 1973. A note on natural enemies of *Danaus plexippus* (L.)(Lepidoptera: Nymphalide) in Australia. Australian Entomologist, The. 1: 37.

Sokal, R. R., and F. J. Rohlf. **1995**. The principles and practice of statistics in biological research, Biological Research.

Solensky, M. J. 2004. Overview of monarch migration, pp. 79–83. *In* Oberhauser, K.S., Solensky, M.J. (eds.), The Monarch Butterfly: Biology and Conservation. Cornell University Press, Ithaca, NY.

Speth, J. G. 2008. The bridge at the edge of the world. *In* New Haven: Yale University Press.

Stanton, R. L., C. A. Morrissey, and R. G. Clark. **2018**. Analysis of trends and agricultural drivers of farmland bird declines in North America: A review. Agriculture, Ecosystems and Environment. 254: 244–254.

Stenoien, C., K. R. Nail, and K. S. Oberhauser. **2015**. Habitat productivity and temporal patterns of monarch butterfly egg densities in the Eastern United States. Annals of the Entomological Society of America. 108: 670–679.

Stenoien, C., K. R. Nail, J. M. Zalucki, H. Parry, K. S. Oberhauser, and M. P. Zalucki. **2016**. Monarchs in decline: A collateral landscape-level effect of modern agriculture. Insect Science.

Stoate, C., A. Báldi, P. Beja, N. D. Boatman, I. Herzon, A. van Doorn, G. R. de Snoo, L. Rakosy, and C. Ramwell. **2009**. Ecological impacts of early 21st century agricultural change in Europe--a review. Journal of environmental management. 91: 22–46.

Stoate, C., N. D. Boatman, R. J. Borralho, C. R. Carvalho, G. R. de Snoo, and P. Eden. **2001**. Ecological impacts of arable intensification in Europe. Journal of Environmental Management. 63: 337–365.

Talbot, M. 1943. Response of the ant Prenolepis imparis Say to temperature and humidity changes. Ecology. 24: 345–352.

Taylor, C. 2009. Monarch population status. Monarch Watch Blog. (http://monarchwatch.org/%5Cnblog/2009/10/monarch-population-status-5/).

Taylor, C. 2010. Monarch Population Status. Monarch Watch Blog. (http://monarchwatch.org/blog/%5Cn2010/07/monarch-population-status-9/).

The Center for Biological Diversity, The Center for Food Safety, The Xerces Society, and L. Brower. **2014**. Petition to protect the monarch butterfly (*Danaus plexippus plexippus*) under the endangered species act. 1–159.

The Xerces Society. **2019**. Monarch butterflies in western North America in jeopardy. (http://xerces.org/2019/01/17/monarch-butterflies-in-western-north-america-in-jeopardy/).

Therneau, T. 2015. A Package for Survival Analysis in S.

Thogmartin, W. E., L. López-Hoffman, J. Rohweder, J. Diffendorfer, R. Drum, D. Semmens, S. Black, I. Caldwell, D. Cotter, P. Drobney, L. L. Jackson, M. Gale, D. Helmers, S. Hilburger, E. Howard, K. Oberhauser, J. Pleasants, B. Semmens, O. Taylor, P. Ward, J. F. Weltzin, and R. Wiederholt. **2017**. Restoring monarch butterfly habitat in the Midwestern US: “All hands on deck.” Environmental Research Letters. 12.

Thogmartin, W. E., R. Wiederholt, K. Oberhauser, R. G. Drum, J. E. Diffendorfer, S. Altizer, O. R. Taylor, J. Pleasants, D. Semmens, B. Semmens, R. Erickson, K. Libby, and L. Lopez-Hoffman. **2017**. Monarch butterfly population decline in North America: identifying the threatening processes. Royal Society Open Science. 4: 170760.

Tilman, D., K. G. Cassman, P. A. Matson, R. Naylor, and S. Polasky. **2002**. Agricultural sustainability and intensive production practices. Nature. 418: 671–7.

Tilman, D., J. Fargione, B. Wolff, C. D’Antonio, A. Dobson, R. Howarth, D. Schindler, W. H. Schlesinger, D. Simberloff, and D. Swackhamer. **2001**. Forecasting agriculturally driven global environmental change. Science (New York, N.Y.). 292: 281–4.

Tscharntke, T., Y. Clough, T. C. Wanger, L. Jackson, I. Motzke, I. Perfecto, J. Vandermeer, and A. Whitbread. **2012**. Global food security, biodiversity conservation and the future of agricultural intensification. Biological Conservation. 151: 53–59.

Tscharntke, T., A. M. Klein, A. Kruess, I. Steffan-Dewenter, and C. Thies. **2005**. Landscape perspectives on agricultural intensification and biodiversity - ecosystem service management. Ecology Letters. 8: 857–874.

Urquhart, F. A., and N. R. Urquhart. **2008**. Autumnal migration routes of the eastern population of the monarch butterfly (*Danaus p . plexippus* L.; Danaidae; Lepidoptera) in North America to the overwintering site in the Neovolcanic Plateau of Mexico. Canadian Journal of Zoology. 56: 1759–1764.

US Pollinator Health Task Force. **2015**. National strategy to promote the health of honey bees and other pollinators.

USDA. **2015**. Conservation and management of monarch butterflies: A strategic framework, Biological Conservation.

Venables, W. N., and B. D. Ripley. **2002**. Modern applied statistics with S., Issues of Accuracy and Scale. Springer, NY.

Wassenaar, L. I., and K. A. Hobson. **1998**. Natal origins of migratory monarch butterflies at wintering colonies in Mexico: New isotopic evidence. Proceedings of the National Academy of Sciences. 95: 15436–15439.

Werling, B. P., T. L. Dickson, R. Isaacs, H. Gaines, C. Gratton, K. L. Gross, H. Liere, C. M. Malmstrom, T. D. Meehan, L. Ruan, B. a Robertson, G. P. Robertson, T. M. Schmidt, A. C. Schrotenboer, T. K. Teal, J. K. Wilson, and D. a Landis. **2014**. Perennial grasslands enhance biodiversity and multiple ecosystem services in bioenergy landscapes. Proceedings of the National Academy of Sciences of the United States of America. 111: 1652–7.

Werling, B. P., T. D. Meehan, B. A. Robertson, C. Gratton, and D. A. Landis. **2011**.

Biocontrol potential varies with changes in biofuel-crop plant communities and landscape perenniality. GCB Bioenergy. 3: 347–359.

Wiklund, C. 1984. Egg-laying patterns in butterflies in relation to their phenology and the visual apparency and abundance of their host plants. Oecologia. 63: 23–29.

Wilcove, D. S., and M. Wikelski. **2008**. Going, going, gone: Is animal migration disappearing? PLoS Biology. 6: 1361–1364.

Wilcox, A. A. E., D. T. Tyler Flockhart, A. E. M. Newman, and D. R. Norris. **2019**. An evaluation of studies on the potential threats contributing to the decline of eastern migratory north American monarch butterflies (*Danaus plexippus*). Frontiers in Ecology and Evolution. 7: 99.

Wills, B. D., and D. A. Landis. **2018**. The role of ants in north temperate grasslands: a review. Oecologia.

Wolfenbarger, L. L., and P. R. Phifer. **2000**. The ecological risks and benefits of genetically engineered plants. Science. 290: 2088–2093.

Wood, S. A., D. S. Karp, F. DeClerck, C. Kremen, S. Naeem, and C. A. Palm. **2015**. Functional traits in agriculture: agrobiodiversity and ecosystem services. Trends in Ecology & Evolution. 30: 531–539.

Zalucki, M. P., and L. P. Brower. **1992**. Survival of first instar larvae of *Danaus plexippus* (Lepidoptera: Danainae) in relation to cardiac glycoside and latex content of *Asclepias humistrata* (Asclepiadaceae). Chemoecology. 3: 81–93.

Zalucki, M. P., L. P. Brower, and S. B. Malcolm. **1990**. Oviposition by *Danaus plexippus* in relation to cardenolide content of three Asclepias species in the southeastern U.S.A. Ecological Entomology. 15: 231–240.

Zalucki, M. P., and R. L. Kitching. **1982a**. Dynamics of oviposition in *Danaus plexippus* (Insecta: Lepidoptera) on milkweed, *Asclepias* spp. Journal of Zoology. 198: 103–116.

Zalucki, M. P., and R. L. Kitching. **1982b**. Temporal and spatial variation of mortality in field populations of *Danaus plexippus* L. and *D. chrysippus* L. Larvae (Lepidoptera: Nymphalidae). Oecologia. 53: 201–207.

Zalucki, M. P., S. B. Malcolm, T. D. Pauste, C. C. Hanlon, L. P. Brower, A. R. Clarke, T. D. Paine, C. C. Hanlon, L. P. Brower, and A. R. Clarke. **2001**. It's the first bites that count: Survival of first-instar monarchs on milkweeds. Austral Ecology. 26: 547–555.

Zaya, D. N., I. S. Pearse, and G. Spyreas. **2017**. Long-term trends in midwestern milkweed abundances and their relevance to monarch butterfly declines. BioScience. 67: 343–356.

Zhang, W., T. H. Ricketts, C. Kremen, K. Carney, and S. M. Swinton. **2007**. Ecosystem services and dis-services to agriculture. Ecological Economics. 64: 253–260.

Zipkin, E. F., L. Ries, R. Reeves, J. Regetz, and K. S. Oberhauser. **2012**. Tracking climate impacts on the migratory monarch butterfly. Global Change Biology. 18: 3039–3049.