The Effects and Importance of Natural Enemies on Immature Monarch (*Danaus plexippus*) Populations

A THESIS
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

Alma Patricia De Anda

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

Karen S. Oberhauser

August 2010

Acknowledgements

I would like to thank my family, especially my parents for always supporting my endeavors. My advisor, Karen Oberhauser, provided support and guidance throughout my graduate program and the writing of my thesis. My committee members, George Heimpel and Don Alstad, provided valuable advice and comments throughout my graduate program and when reviewing my thesis. I would like to thank the Oberhauser monarch lab group from 2005-2010, especially Leah Alstad, Reba Batalden, Grant Bowers, Dina Kountoupes, Wendy Macziewski and Lis Young-Isebrand for their help and support throughout this project. Claudia Neuhauser and Sandford Weisberg provided statistical advice; the University of Minnesota, Saint Paul Climatological Observatory for their climate data; and Michael W. Gates, Systematic Entomology Laboratory, Agricultural Research Services, US Department of Agriculture identified the parasitoid wasps from my experiments. Finally, a thank you to my friends in the graduate department for their support when I needed it the most.

Dedication

This thesis is dedicated to my parents, who never had the chance to receive an education themselves, but did everything they could to have all three of their daughters receive any education we desired. This is also dedicated to my sisters and nieces and nephew; I hope you enjoy the natural world and are inspired by it as much as I am.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

List of Tables

List of Figures

List of Figures (continued)

Chapter 1: Introduction

Predator-prey interactions and their impact on population dynamics have long been a focus of ecological studies. Monarch butterflies (*Danaus plexippus*) are excellent candidates for predation studies; their eggs and early instar larvae have low survival rates and are easily tracked. Additionally, plant-herbivore interactions due to plant secondary metabolites, are a large source of mortality for immature monarchs in southern species of milkweed, *Ascelpias humistrata* (Zalucki et.al., 1990; Zalucki and Brower, 1992). While milkweed is most common to the northern areas of monarch summer breeding generations, *A. syriaca* does not have high levels of these secondary metabolites, it does have high levels of latex (Malcolm and Zalucki, 1996; Agrawal, et.al, 2008). This can lead to immature monarch death due to starvation from gluing their mouthparts shut.

To determine the relative importance of predation, plant-herbivore interactions, and abiotic factors to Minnesota monarchs, I observed monarch survival in the field over three monarch summer breeding seasons. The first summer breeding season was for an undergraduate summer program that allowed me to observe that many immature monarchs were missing after a 24hr interval and that there were many potential invertebrate predators in the prairie habitat in which monarchs were found. My initial observations of monarch survival in the wild led to my questions about the effects of natural enemies on immature monarch survival in the field.

The following chapters describe my observational studies and the experiments that were later carried out based on my initial findings and interest in the dangers faced by all immature stages of the monarch butterfly. In Chapter 2, I report my findings after measuring stage-specific mortality rates and identifying key monarch predators over two summer breeding seasons. Chapter 3 details a two year experimental study that was aimed to identify if late pre-pupae and pupae were at risk by natural enemies, since many assume that later instars and pupae are protected by the toxins they sequester from their host plant. This chapter reports on the risks still posed for pre-pupae and pupae by natural enemies, including a never before reported parasitoid of monarchs in North America and the cues this parasitoid uses to find this non-pest host.

Chapter 2: Immature Monarch Field Survivorship in Minnesota

Introduction

Natural enemies have wide-ranging effects on insects. Understanding the extent to which predation affects the population dynamics of a species has been an important question in ecological studies, with early work to explain predator-prey dynamics by Lotka (1924), Voltera (1926) and Gause (Gilbert et.al., 1952). For example, predators drive population cycles in Larch budmoths (Turchin et al. 2003) and the southern pine beetle, *Dendroctonus frontalis* (Turchin, 2003), and alter larval foraging behavior in many species (Montllor and Bernays, 1994 and Stamp and Bowers, 2000). Monarch butterflies (*Danaus plexippus*) are organisms well suited for long-term population studies because of their multiple overlapping generations, their annual migration, and their continent-wide distribution (Prysby and Oberhauser, 2004). A 1997 North American Conference on the Monarch Butterfly identified several research priorities, to include the need to identify sources of variation in monarch abundance and distribution, especially sources of mortality (United States Fish and Wildlife Service, 1999).

Mortality is especially high in the immature stages, with approximately 5% to 10% survival rates from egg to the 5th instar (Oberhauser et al, 2001). For example, fire ants (*Solenopsis invicta*) kill up to 100% of monarch larvae in some locations in Texas (Calvert, 2004). Tachinid fly parasitoids cause high mortality rates that vary both with

location and year (Oberhauser et al., 2007). It is commonly believed that immature monarchs are protected from predators by toxicity derived from their milkweed host plants, and their aposematic coloration (Reichstein et al, 1968). Aposematism in monarch larvae indirectly supports the view that predators are or were a strong agent of natural selection for monarchs (Morin, 1999), however the extent to which aposematism prevents predation by invertebrates is not well understood (Prysby, 2004). I hypothesize that the effects of natural enemies on monarch eggs and larvae have a large impact on monarch numbers. Several researchers have studied how temperature (York and Oberhauser, 2002; Zalucki and Rochester, 2004) and host plant defenses (Malcolm and Zalucki, 1996) affect egg and larval survival. Natural enemies are likely to be equally or more important to monarch survival, particularly to eggs and first instars, which cannot or do not readily move off their host plant (pers. obs).

I conducted a two year observational study of immature monarchs in the wild to study mortality rates and causes of mortality (distinguishing between predation, abiotic factors, and host plant interactions) in monarchs' natural environment. With detailed tracking of individuals I was able to partition out the manner in which a specific individual egg or larva died. Studies done to identify rates and causes of mortality have either focused on short time scaled exclusion experiments (Prysby 2004, Calvert 2004) or on long term observational work where the data only allowed for inferential statements on the causes of mortality (Zalucki and Kitching, 1982). Multiple observations separated by short time

intervals over the entire monarch breeding season will help identify important monarch predators.

Methods

The Falcon Heights Community Park (Falcon Heights, MN) is a 5.9 hectare park that maintains a restored prairie habitat with naturally-occurring milkweed. This park has been a site for monarch monitoring for 8 years. Within this park, three sub-sites were chosen that each had more than 30 milkweed ramets. My research team and I monitored specific milkweed plants and tracked immature monarchs throughout the 2006 and 2007 summer breeding seasons. In 2006, we visited this site five days a week from 31-May through 28-July. In the three different sub-sites within the park, we began monitoring at approximately the same time each day, and used aluminum tags to identify milkweed ramets on which we observed naturally-laid monarchs. We monitored a total of 237 milkweed ramets for the presence of monarch individuals, with a total of 2,526 observations of individual eggs and larvae. Because we only observed on weekdays, I separated data by the time interval since the last observational period: either one day (Tuesday – Friday) or three days (Monday). For most analyses, I only used 24h interval observations.

Additionally, at each monitoring period, we noted/estimated 8 independent variables (Table 1) that I thought would influence immature monarch survival in the wild. The

remaining independent variables were either estimated after the observational study (Time in current stage and Sub-site differences) or measured by a climate station near the community park (Average air temperature, dew point, and precipitation) (Table 1).

In 2007, we visited the park from 29- May through 7- August, monitoring 10 days in a row with four days in between the observation periods. This was done to increase the number of consecutive 24h interval observation periods. We monitored 30 ramets per site, for a total sample size of 90. This was done in order to obtain a more accurate account of monarch individuals and their fates after a 24h interval. Based on the 2006 season, it became apparent that increasing the amount of ramets we monitored over the breeding season could potentially lead to human error in accurately recording monarch fates. Therefore, the only differences in the methodology of this study between the two breeding seasons are the observation interval lengths and the number of ramets observed during the breeding seasons. The rest of the monitoring protocol was consistent between years.

Each monarch observed during each observational period was assigned a fate that included: Survival: still present on the milkweed ramet. Death: missing from the milkweed ramet, "sucked out" (only chorion or exoskeleton remaining), chewed, larva dead by miring in latex or with mouthparts glued shut by plant latex, observed predation event or dead for unknown reasons. The short time intervals between observations made it reasonable to assume that "missing" eggs or first instars had suffered predation,

because eggs and first instar larvae do not move off milkweed plants on their own. To have further confidence that the “missing” individuals were truly missing due to predation, I went through all 5, 043 individual observations to be certain that the “missing” individuals were actually missing and not overlooked by the observer. If a monarch individual was observed to develop too quickly from one stage to the next, especially from an egg to a larva, then that individual was not “missing” but overlooked and this error was not included in the analysis.

I used a logistic regression analysis to determine the effects of 15 biotic and abiotic predictors on the probability of egg and larval survival over 24 hours. A backwards elimination method was used to choose the model that best fits the data. Table 1 gives a detailed explanation of each predictor; these included **Predator presence** on a milkweed plant, specifically invertebrate predators, **Aphid density** on a milkweed plant, **Percent of leaves consumed by herbivores** , **Monarch density on the ramet**, **Position of an individual** on a milkweed ramet (top, bottom, apex of a leaf) **Ramet reproductive status**, **Milkweed density**, **Start stage** of an individual immature monarch, **Age of individual** (the amount of time an individual immature monarch has been in a particular stage or stadium), **Date of year**, **Year, Average air temperature (°C)**, **Average dew point (°C), Average precipitation (mm) and Sub-site differences (block effect)**. A logistic regression was performed with both 2006 and 2007 data combined and also on each year separately.

I conducted a small pilot experiment in the summer of 2008 to test whether precipitation could account for 'missing' eggs (eggs being washed off a leaf) after a 24hr period. Monarch eggs were naturally laid on potted tropical milkweed (*A. curassavica*) plants and after 3 days, the experiment was carried out on August 19, 2008 at a residential garden backyard in St. Paul, MN. The eggs were exposed to 'rain' through a circular-spraying sprinkler system for 30 minutes. A rain gauge was used to measure how much water fell over the eggs over a 30 minute interval, with a total of approximately 254mm of rain.

Results

Observational study 2006 & 2007

Twenty four hour monarch eggs, first instars and second instar was similar both years. (Figure 1) Although second instar larvae fared worse in 2006, I am less confident in these results since second instar larvae are much more mobile. Extrapolating a 63% survival rate and assuming a constant survival for each day of that stage over the four day period that an egg remains in that stage, there would be approximately 16.3% of the original population surviving the egg stage. Similarly, applying a 60% 24h first instar survival rate to the remaining 16.3% of first instars, over a two day period, which is the approximate length of time of the first stadium, there would only be approximately 5.6% of larvae remaining in the population to continue on as second instars (Figure 2). Constant survival rates were for each stage were assumed because the predictor "time in

current stage" was found not to be significant in determining the chances of an individual surviving a 24hr interval.

Logistic regression analyses

In the combined 2006 and 2007 model, seven of the fifteen predictors have significant explanatory power to explain monarch survival in the wild. The signs of the coefficients in the logistic regression results in Table 2 suggest several interesting interpretations. The negative coefficient for the predictor 'date' has significant explanatory power, with more mortality occurring later in the season. Percent of leaves consumed by herbivores also has a negative effect; the chances a monarch egg or larva survives a 24h interval significantly decreases with the amount of herbivory experienced by the milkweed. Figures 3a and 3b show the progression of milkweed ramets with increased herbivory. Figure 3a is somewhat misleading because this year we continually added new milkweed ramets into our study and some of these plants were not as damaged by herbivores or they were younger milkweed ramets. Figure 3b shows the percent herbivory for 2007 and in this year, we did not continually add new milkweed ramets to our study. This figure highlights the fact that plants become more damaged as the weeks go by (5%-25% damaged), presumably by the increase in herbivore species or densities over the summer breeding season.

The level of aphids density (10-100) found on milkweed ramets significantly decreased the chances of immature monarchs surviving in the wild (Figure 4a, 4b, 4c and 4d). The

position of an egg or larva on a particular leaf affected its chances of surviving over 24 hours. An egg or larva on the bottom side of a milkweed leaf or on the apex of the plant was more likely to survive. Additionally, the bottom side of a milkweed leaf was the most common place to find monarch eggs, first instars and second instars (Figures 5a, 5b and 5c), however, the number of individuals observed on the plant in the 'apex' position varied from year to year in the first and second instar stadia. Finally, when compared to the survival of monarch eggs, first and second instar larvae fared worse over a 24h interval. The following predictors were not found to be significant in the combined model: predator presence, monarch density, plant reproductive status, milkweed density, time in current stage, sub-site differences (block effect), year, average air temperature, average dew point and average precipitation.

The results of the logistic analyses for each individual year demonstrated that spiders, in 2006 were significant predators for monarch eggs and larvae. The presence of spiders significantly decreased the likelihood of immature monarch survival (Table 3, Figure 6). Also important in 2006 were two abiotic predictors, average air temperature (°C) and average dew point (°C) (Table 3 and Figure 7). The warmer the ambient temperature was in the summer breeding months, the more likely an individual was to survive, although increased dew point reduced the chances of an individual surviving a 24h interval. Finally, the predictor 'reproductive plant status', was significant when the milkweed plant had finished flowering; monarch survival was lower (Table 3). In 2007, increasing density of other milkweed ramets surrounding the focal ramet, was found to significantly

improve the chances of an individual surviving a 24h period (Table 3). Unlike in 2006, ramets that had stopped flowering and had set seed positively affected an individual's chances of survival (Table 3).

Figures 8a and 8b show weekly monarch density over the two year study. Figures 9a and 9b highlight the high diversity of potential insect predators that were present over the two summers, specifically the prevalence of Hymenoptera (ants) and *Chrysopa spp* (Neuroptera). Finally, of n = 802 eggs naturally laid on 6 potted milkweed plants to simulate precipitation, all but one egg remained on the plant after a 30 minute interval of water exposure from a water sprinkler.

Sources of mortality

Eggs: The largest known cause of mortality in the wild in both years was the 'Gone' category. This was followed by the following categories in 2006: 'dead' category (12.1%), 'sucked out' (10.2%), 'chewed out' (1.6%), predation event (0.80%) and 'accidental' death (0.27%) (Figure 10). In 2007, the largest known causes of mortality for eggs after the 'Gone' category were: sucked out' category (18.7%), 'chewed out' (12.8%) and 'dead' (2.9%). There were no incidences of predation events or accidental deaths observed for this year (Figure 10). The single predation event we observed was by a stink bug nymph in 2006.

First instar larvae: The largest known cause of mortality in the wild in both years was the 'Gone' category. This was followed by the following categories in 2006: 'dead' category (2.9%), predation event by an unknown species of spider (2.2%), 'miring' death (1.4%), and 'accidental' death (0.72%). There were no incidents of larvae observed 'sucked out' or 'chewed out' this year (Figure 10). In 2007, the largest known causes of mortality for first instar larvae after the 'Gone' category was the 'predation event' category. This larva was bitten by an unknown predator (3.9%). No other causes of mortality were observed in the wild for first instar larvae (Figure 10).

Second instar larvae, 2006: The largest known cause of mortality in the wild in both years for second instars was the 'Gone' category. This was followed by the following categories in 2006: 'dead' (4.3%) and finally 'predation event' by a stink bug nymph(1.1%). No other causes of mortality were observed for second instar larvae that year (Figure 10). In 2007, the largest known causes of mortality for second instar larvae after the 'Gone' category was an equal chance of a 'predation event' (by an unknown predator; larva had been bitten by a chewing predator), 'sucked out' and 'chewed' mortality category (1.1%). No other causes of mortality were observed for second instar larvae this year (Figure 10).

Discussion

Mortality rates over 24 hours

Overall, monarch eggs, first instar larvae and second instar larvae each had similar survival rates each season (Figure 1), suggesting that the factors responsible for their survival were also similar each breeding season. At the restored prairie site I chose for this observational study, I found similar mortality rates in 2006 and 2007 for immature monarch eggs and larvae, specifically for monarch eggs, and first and second instar larvae. Daily monitoring rates for eggs suggest that approximately 25% become larvae, and less than 10% reach the second stadium. I hypothesized that predators, specifically invertebrate predators, would be a major factor for immature monarch survival. Monarch eggs and larvae are relatively similar in size and are 'soft bodied' (Oberhauser, 2004), therefore, one can assume that they may have the same predators. The fact that most eggs and larvae were reported as "gone" (Figure 10a and 10b) suggests that their predators had taken the entire individual, such as ants and spiders. Finally, for the combined years' data, when compared to the egg stage, monarch first and second instar survival rates were significantly lower (Table 2).

Types of Predators Observed

Spiders, with over 30,000 species are a group of organisms that are largely predaceous and feed almost exclusively on insects and rarely show specificity towards prey (Riechert and Lockley, 1984). For monarch eggs, predation due to spiders would have to come from spiders that search for prey, as opposed to spiders that either sit-and-wait or web-building spiders, such as jumping spiders (Salticidae). First and second instars could also

be preyed upon by similar spiders, but would also be likely to be preyed upon by sit-and-wait spiders, such as the crab spider (Thomisidae). Because spiders are generally not specialist predators, a milkweed community with its large assemblage of arthropods, can serve as both a potential risk to immature monarchs, such as was experienced in 2006, or it could also serve as a refuge for immature monarchs. The complexity of the milkweed community may allow spiders to actively search and find suitable prey, but it may not always be immature monarchs that they encounter.

Although ants were present throughout the summer breeding season over both years (Figures 9a and 9b) (pers. obs), their presence did not predict 24hr survival for monarch eggs and larvae. However, there was one instance in 2006 where 5 ants were observed attacking a third instar larva. Additionally, aphid presence was found to be significant in the combined model when the density of aphids was at 1-100 aphids. Aphid presence may affect monarch survival in several ways. Apparent competition could be occurring due to *A. nerii's* mutualistic relationship with ants (pers. obs.); in return for the honeydew that aphids provide to ants, the ants provide protection from predators. Thus, aphid presence may indirectly affect monarchs by increasing the presence of ant predators, thereby increasing a monarch's chance of being preyed on by ants. Alternatively, aphids may also have a negative impact on monarchs by decreasing the nutritional value of the milkweed plant since these aphids are phloem feeders (Malcolm, 1990). One reason why only this level of aphid density negatively affected monarchs and higher aphid densities did not could be that once there are high levels of aphids on milkweed plants, ants may

not find or be concerned with immature monarchs. The fact that aphid presence was associated with higher mortality suggests that the apparent competition scenario is a real possibility for immature monarch eggs and larvae to a certain extent.

Other than spiders in 2006 and aphids in the combined model, there were no associations between the presence of potential invertebrate predators on ramets and monarch survival. However, many known predators were present in fairly high densities on milkweed patches (Figures 9a and 9b). The predation events that were observed in this study were of a possible stink bug nymph (pers. obs), spiders and ants. Lacewing larvae are known to eat soft bodied insects, including monarchs, (Kaiser, in prep; pers. obs) and were often observed in the wild. Although I never observed syrphid fly larvae preying on immature monarchs, they do feed on soft bodied insects (Rank and Smiley, 1993) and are potential predators. Flies were not observed in 2006 because we did not think they would be possible invertebrate predators, however, after the first summer, we realized this was not the case. The imported Asian Lady Beetle, *Harmonia axyridis* does prey on immature monarchs (Koch et.al., 2003) and was observed often in milkweed patches, however, their presence did not predict immature monarch mortality. Finally, different insects in the order Hemiptera, primarily assassin bugs, were observed on milkweed plants, however, these did not comprise a significant source of mortality for immature monarchs.

Types of Mortality Observed: Broad Scale Mortality

Broad scale mortality can be thought of as all sources of death of both known and assumed predation versus abiotic sources of death. The logistic regression result for the combined years' data indirectly supports the hypothesis that invertebrate predators are important predictors of immature monarch survival. Percent herbivory, an estimate of percentage damage by herbivores to the focal milkweed ramet was negatively correlated with monarch survival. Herbivory is thought to attract predators and/or parasitoids from the volatile organic compounds (VOC's) emitted by the plant when attacked by herbivores (Tumlinson, W, et.al., 1993). Milkweed plant communities have a large invertebrate community of herbivores, including monarch larvae (Agrawal et.al., 2008). Generalist invertebrate predators and parasitoids might hone in on VOC's emitted by damaged milkweed ramets, or on visual cues associated with herbivory. Monarch eggs and larvae that were found on plants with any type of herbivory had a lower chance of surviving a 24h interval (Table 2 and 3). The increase in herbivory over the course of the summer in 2007, when we monitored the same milkweed ramets, may help to explain the increase in mortality with date.

The majority of monarchs observed throughout the years were found on the bottom side of a milkweed leaf, both eggs and larvae (Figures 5a, 5b and 5c Residing on the bottom of milkweed leaves can protect an individual from flying and possibly crawling, predators. However, residing on the bottom side of a leaf can protect monarchs from direct sunlight, which can lead to desiccation and reduce the chances of being washed off by rain. Precipitation, however, did not affect the chance of being washed off a plant. The

fact that almost all eggs remained on the milkweed plant during the experimental rain treatment, suggests missing eggs were probably taken by a predator. Monarch eggs and larvae had a better chance of survival when residing on the apex and bottom side of a milkweed leaf (Table 2). I hypothesize that the apex of the milkweed plant, before it flowers, contains milkweed leaves that are tightly bound and serve as a 'hiding' place for monarch eggs and larvae. Finally, an increase in milkweed density increases an individual's chances of survival over a 24hr interval (Table 3), suggesting that a positive effect is conferred to individuals when there are more milkweed plants that potential predators could search for.

Warmer temperatures did increase the chances of monarch survival in 2006 (Table 3), while more humid days negatively affectted survival (Table 3). Date also negatively affected monarch survival, with more mortality occurring later in the season during both years (Table 2).

The largest category of death experienced by both eggs and larvae was the 'missing' category. I assumed that these larvae had been consumed by invertebrate predators, but Zalucki et.al. (1991) and Zalucki and Brower, (1992) argue that many first instars die from miring and catelepsis; poisoning from cardenolide toxins in host plants, causing larvae to stop feeding and preventing them from laying silk to hold onto its leaf. Miring was observed a total of six times in two years, which is a very low rate occurrence, but catalepsis have occurred. *A. syriaca*, does not have high levels of cardenolides, but does

have high levels of latex (Agawral et.al, 2008). First instars then, should be more likely to die due to miring, not toxic shock, which would be more likely to be observed in the wild since first instar larvae would be glued to the milkweed plants by their mouthparts.

Types of Mortality Observed: Finer Scale Mortality

Deaths can also be partitioned into finer scale mortality by the kind of predation an individual experienced (i.e., chewed, sucked out). Eggs or larvae that had been sucked out were assumed to have been preyed on by organisms with sucking mouthparts, insects in the orders Hemiptera or Homoptera, or arachnids in the order Araneae. Chewed eggs or larvae were assumed to have been preyed on by insects in the orders Neuroptera, Coleoptera and possibly Hymenoptera. The spiders and insects mentioned above were all observed on milkweed plants throughout both breeding seasons (Figures 9a and 9b) and we know that these individuals died from natural enemies.

Conclusion

While predation occurs at the individual level, it provides a link to population level dynamics. This detailed observational study highlights the fact that invertebrate predators are very efficient at reducing monarch numbers in the summer breeding generations over such short time intervals. Characterizing how effective predators are at finding and consuming prey items (Hassell, 1978) can elucidate important population-level processes, such as density dependent (DD) mortality, or it may elucidate the importance of other

non-predatory factors affecting survival, such as abiotic factors. Looking at smaller scale interactions between insects and their environment can provide important information that could potentially be carefully extrapolated to improve the ability to predict the outcomes of alternative management strategies for organisms and their habitats (Steele and Forrester, 2005). It would be beneficial to quantify the rate of movement of later instars to other nearby plants/patches of milkweed to allow for the correct identification of the largest fate for later instars in my data set: the disappearance of larvae for unknown reasons. Additionally, functional response experiments could help to determine the potential effects of individual predator species on monarch mortality.

Table 1. Predictors used as independent variables in the logistic regression of factors affecting daily survival rates for immature monarchs in 2006 and 2007.

Predictor	**Explanation of predictors**
Predator presence	Categorical variable looking for presence/absence of the following predators: Hymenoptera- ants and wasps, Neuroptera- lacewing larvae, Coleoptera- *H. axyridis* adults and larvae, Diptera- Tachinidae, Syrphid fly larvae, Araneae- Salticidae, Thomisidae, Dermaptera- earwigs
Aphid density	Ordered variable with 5 levels estimating the number of aphids on a milkweed plant. Level 1: 0 aphids, Level 2: 1-10 aphids, Level 3: 11-100 aphids, Level 4: 101-1000 aphids, Level 5: >1000 aphids
Percent herbivory	Categorical variable with 4 levels estimating the percent plant damage by herbivory of a milkweed plant. Level 1: 0%, Level 2: <5%, Level 3: 5%-25%, Level 4: >25%
Monarch density	Log-transformed predictor that measures the number of other monarch individuals on a milkweed plant.
Position of an individual	Categorical variable with 4 levels, assigns a value to each individual's position on a milkweed plant. Level 1: top of leaf, Level 2: bottom of leaf, Level 3: apex of plant, Level 4: stem of plant, Level 6: flowers of plant.
Ramet reproductive status	Categorical variable with 4 levels, assigns a value to identify the reproductive state of a particular milkweed plant. Level 1: plant not flowering, Level 2: plant flowering, Level 3: plant finished flowering for the season, no seed pods visible, Level 4: plant finished flowering for the season, seed pods visible.
Milkweed density	Measurement of how many other milkweed plants surround the focal plant (m^2)
Start stage	Categorical variable with 6 levels, identifies what stage an individual monarch was in at the time of observation. Level 0: egg, Level 1: first instar, Level 2: second instar, Level 3: third instar, Level 4: fourth instar, Level 5: fifth instar.
Age of individual	Variable that determines the amount of time an individual spends in one particular stage or stadium.
Subsite differences	Categorical variable to analyze any subsite differences in survival
Date	The day of an observation
Year	
Average air temperature	In degrees Celsius, the average of 1h temperature readings over the 24h interval leading up to the date and time of the observation
Average dew point	In degrees Celsius, the average of 1h dew point readings over the 24h interval leading up to the date and time of the observation
Average precipitation	In millimeters, the average of 1h precipitation readings over the 24h interval leading up to the date and time of the observation

Table 2. Combined 2006 and 2007 logistic regression results determining significant predictors of immature monarch survival in the wild.

Parameter	Coefficient	Std error	P-value
2006 and 2007			
Date	-0.017	0.003	<0.0001
Herbivory	-0.296	0.071	<0.0001
Aphid presence	-0.305	0.102	0.0028
Position on plant (bottom)	0.459	0.116	0.0001
Position on plant (apex)	0.311	0.142	0.0279
1st instar	-0.254	0.0929	0.0062
2nd instar	-0.493	0.121	0.0001

Table 3. Logistic regression analyses, determining significant predictors in immature monarch survival for 2006 and 2007 separately.

Parameter	Coefficient	Std error	P-value
2006			
Constant	0.0506	0.6907	0.9416
Ave. air temp (°C)	0.1261	0.0382	0.001
Ave. dew point (°C)	-0.1265	0.0279	<0.0001
Spiders	-0.6088	0.2402	0.0113
Plant reproductive status (done flowering)	-0.506	0.2258	0.0251
Herbivory	-0.4717	0.1912	0.0136
Position on plant (apex)	0.5338	0.2166	0.0137
2nd instar	-0.6861	0.1784	0.0001
2007			
Constant	4.0274	0.7004	<0.0001
Date	-0.0241	0.00435	<0.0001
Milkweed density (sq.m)	0.425	0.1463	0.0037
Plant reproductive status (done flowering)	0.5941	0.2454	0.0155
Plant reproductive status (setting seed)	0.8038	0.2624	0.0022
Herbivory	-0.6501	0.1309	<0.0001
Position on plant (bottom)	0.3366	0.1563	0.0313
1st instar	-0.2179	0.9449	**0.0602**
2nd instar	-0.2843	0.1493	**0.0569**

Figure Legends

Figure 1. Percent survival of immature monarchs over a 24h interval during the 2006 and 2007 summer breeding seasons in St. Paul, MN. The error bars represent 95% Confidence Intervals. Eggs, first instar and second instar percent survival rates were not compared to each other; different letters above bars are only meant to show differences within each category of monarch development.

Figure 2. Extrapolating the more conservative estimates of daily immature monarch survival starting from the egg stage through the second stadium. Survival estimates based on observations of wild individuals suggest that approximately 1.7% of the initial population would survive through the second instar.

Figure 3a. Percent herbivory over the 2006 breeding season. The largest category of herbivory was less than 5%.

Figure 3b. Percent herbivory over the 2007 breeding season. The largest category of herbivory was less than 5%.

Figure 4a. Aphid presence over the 2006 breeding season. Increaed aphid presence is thought to increase the presence of ants throughout the season, indirectly decreasing the chances of monarch survival.

Figure 4b. Aphid presence over the 2007 breeding season. Increased aphid presence is thought to increase the presence of ants throughout the season, indirectly decreasing the chances of monarch survival.This year, aphid presence was markedly higher than the previous year, both in aphid numbers and length of aphid presence over the breeding season.

Figure 4c. The likelihood of surviving a 24h period for all eggs and larvae in 2006. N = 563 for Aphid 1 (Egg = 333, L1 = 175, L2 = 55), n = 405 for Aphid 2 (Egg = 254, L1 = 92, L2 = 59), n= 404 for Aphid 3 (Egg = 284, L1 = 62, L2 = 58), n = 80 for Aphid 4 (Egg = 55, L1 = 17, L2 = 8) and n = 7 for Aphid 5 (Egg = 6, L1 = 1).

Figure 4d. The likelihood of surviving a 24h period for all eggs and larvae in 2007. N = 344 for Aphid 1 (Egg = 233, L1 = 77, L2 = 34), n = 650 for Aphid 2 (Egg = 569, L1 = 132, L2 = 68), n= 662 for Aphid 3 (Egg = 488, L1 = 113, L2 = 61), n = 147 for Aphid 4 (Egg = 109, L1 = 18, L2 = 20) and n = 203 for Aphid 5 (Egg = 144, L1 = 37, L2 = 22).

Figure 5a. Absolute number of egg observations on each position on the plant during 2006 and 2007 with the majority of monarch eggs being laid on the bottom side of a milkweed leaf.

Figure 5b. Absolute number of first instar larvae observed in each position on the plant during 2006 and 2007, with most first instar larvae residing on the bottom side of a milkweed leaf.

Figure 5c. Absolute number of second instar larvae observed in each position on the plant during 2006 and 2007, with more variation of where second instars reside due to their increased mobility.

Figure 6. 24 hour survival of monarch eggs and all larval stadia over the 2006 summer breeding season. The association between the presence of spiders on milkweed ramets and the proportion of monarch survival is significant, $X^2 = 10.98$, $p = 0.000921$.

Figure 7. Comparison of monthly temperature, dew point (°C) and precipitation (mm) averages during the observational study.

Figure 8a. Weekly monarch density 2006. Number of milkweed plants/year varied this summer but over the entire season, approximately 237 milkweed ramets/week were monitored over 3 sites.

Figure 8b. Weekly monarch density per milkweed plant in 2007 Approximately 90 milkweed ramets/week were monitored over all 3 sites.

Figure 9a. Weekly presence of potential predators on milkweed plants over the summer breeding season in 2006. The category Hymenoptera included bees and wasps, however, the majority of observations in the wild were of ants (*Formica spp.*).

Figure 9b. Weekly presence of potential predators on milkweed plants over the summer breeding season in 2007. Included in this year that was not in 2006 were observations of fly species, including Dolichopodidae flies and syrphid flies.

Figure 10. 2006 and 2007 Egg and L1 and L2 mortality for each category of 'death'. First set of categories is of 2006 mortality and the next set of categories is for 2007mortality.

Figure 1.

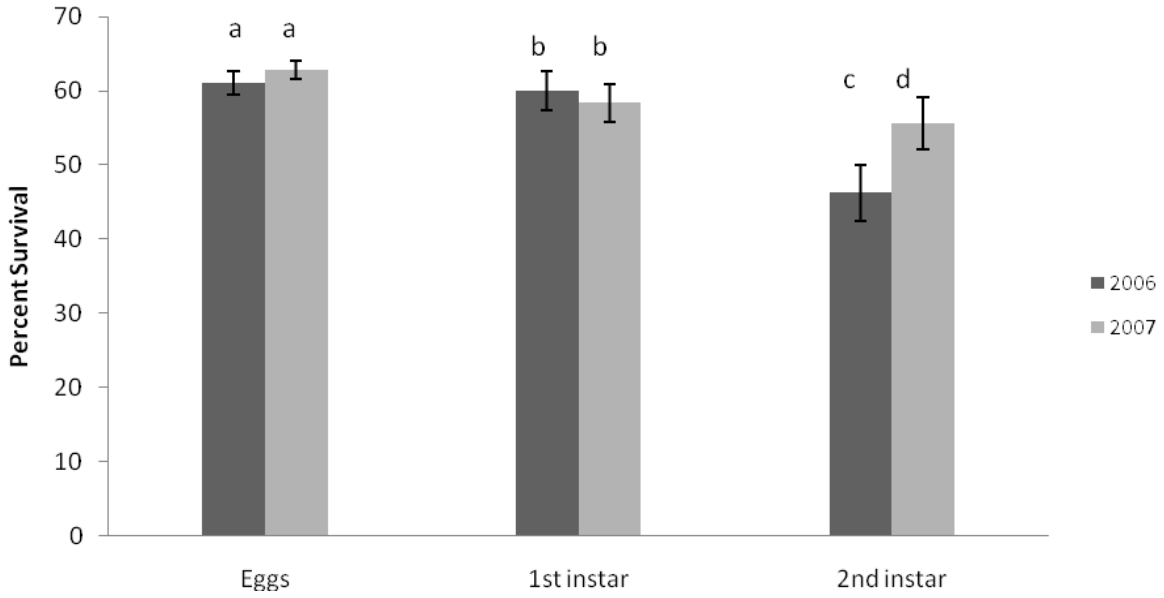


Figure 2.

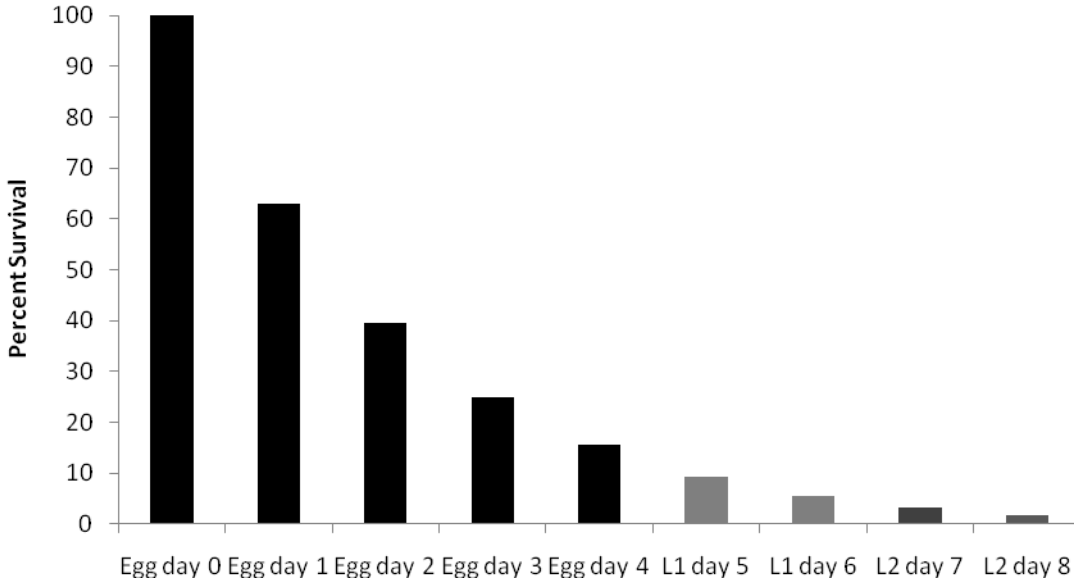


Figure 3a.

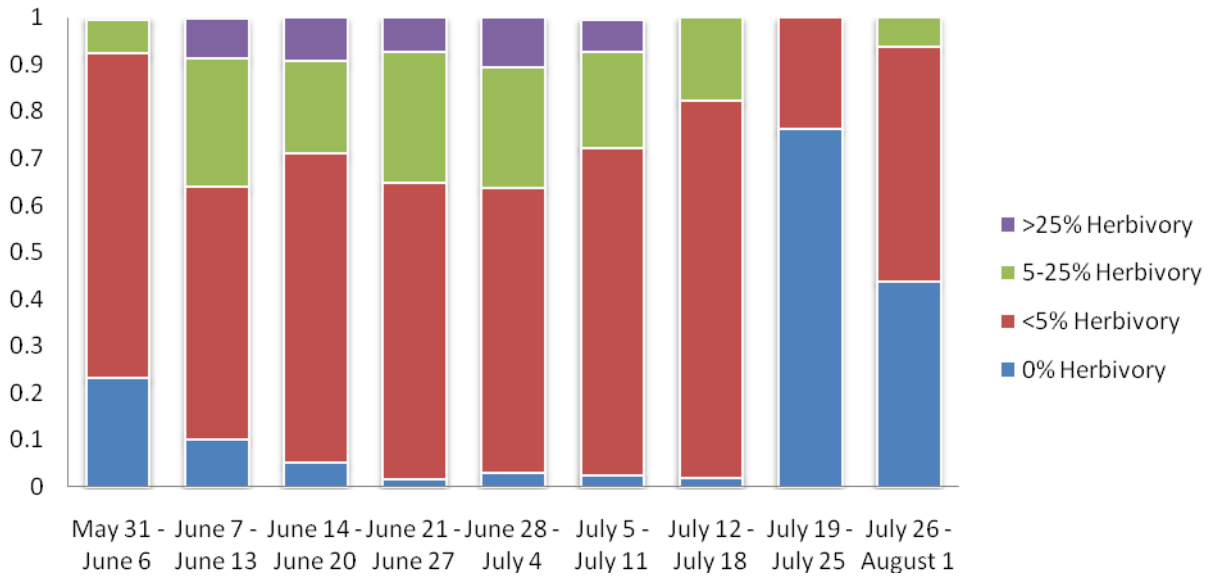


Figure 3b.

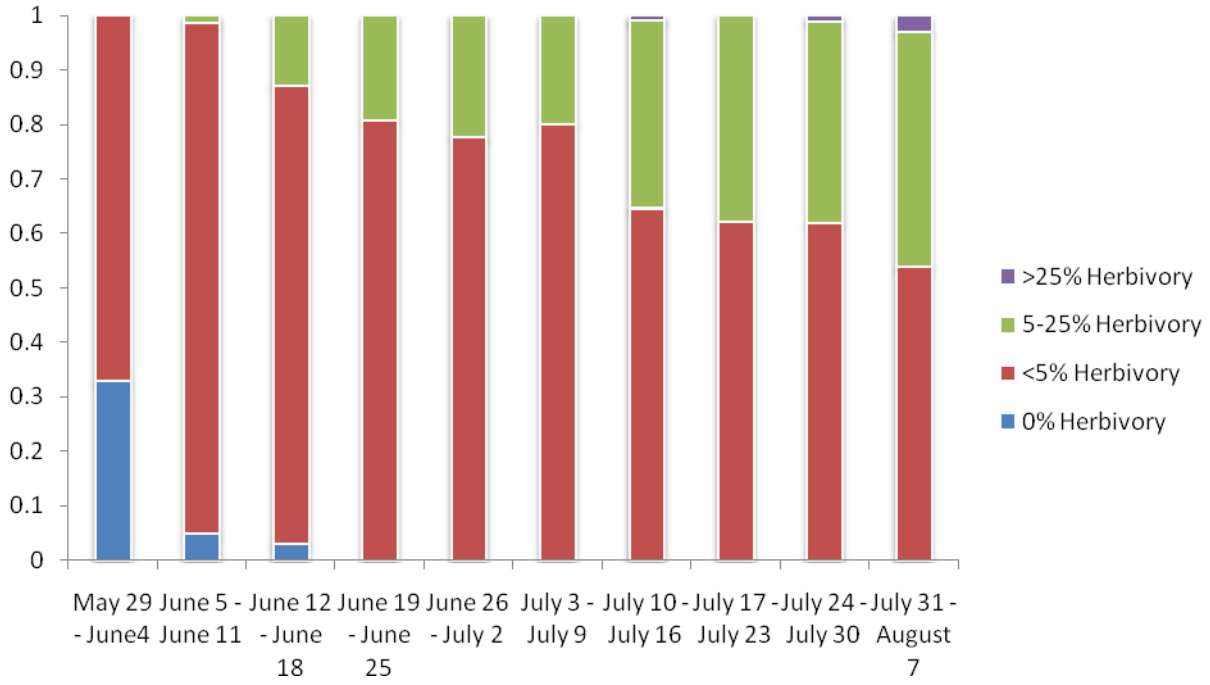


Figure 4a.

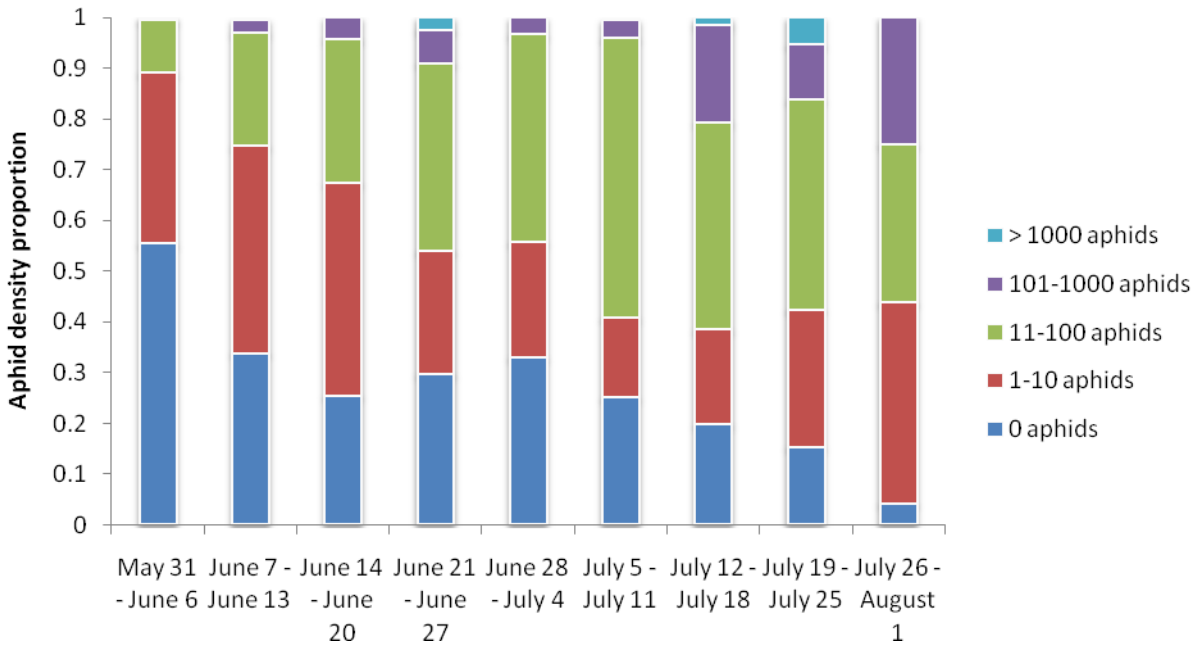


Figure 4b.

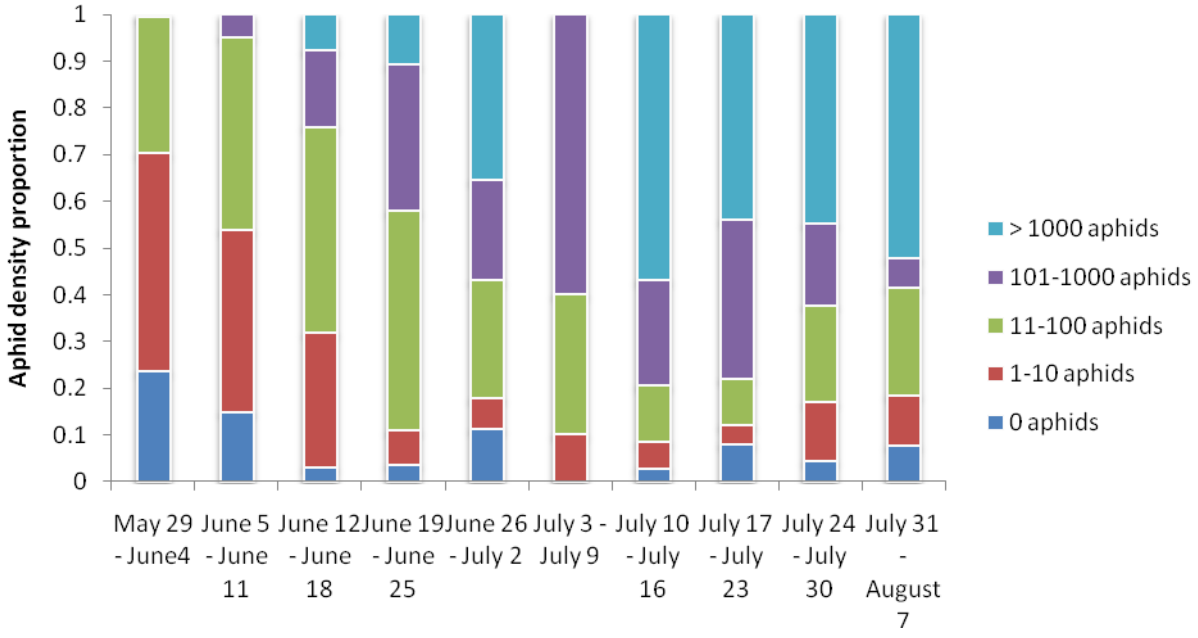


Figure 4c.

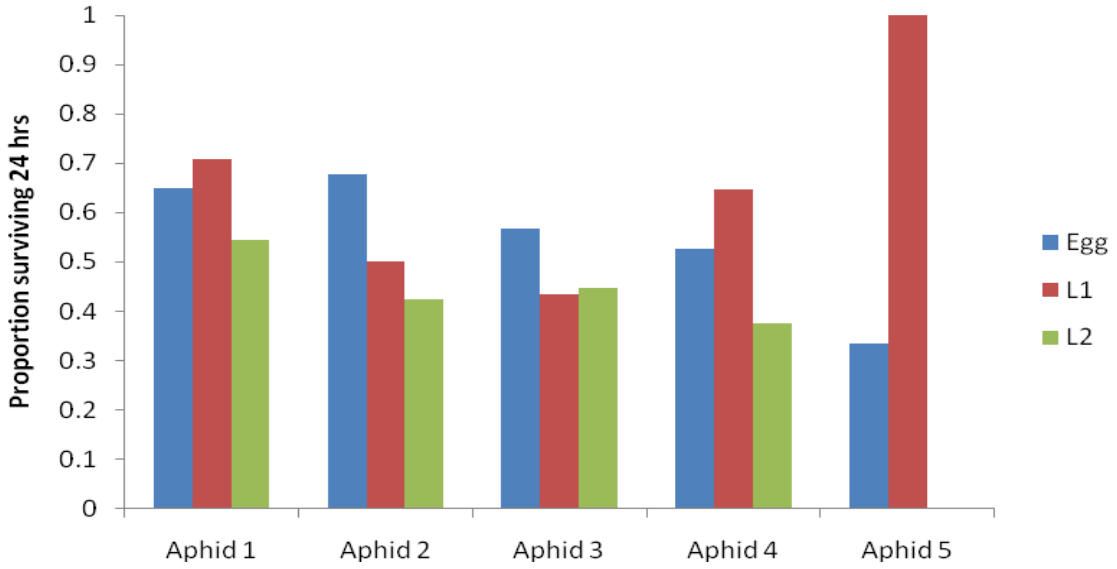


Figure 4d.

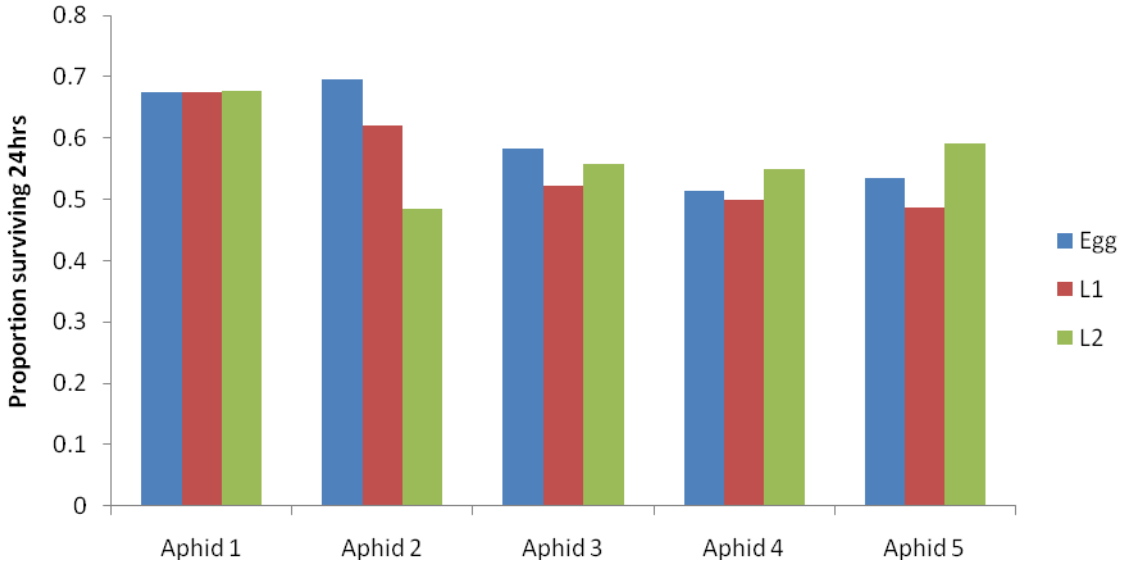


Figure 5a.

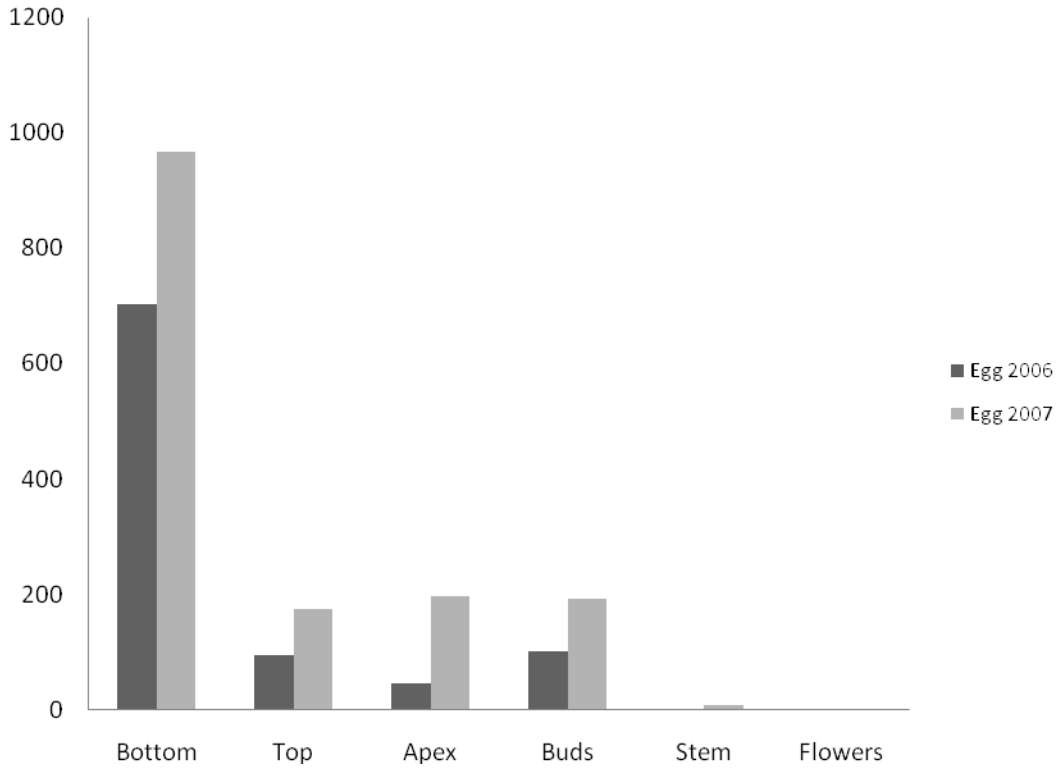


Figure 5b.

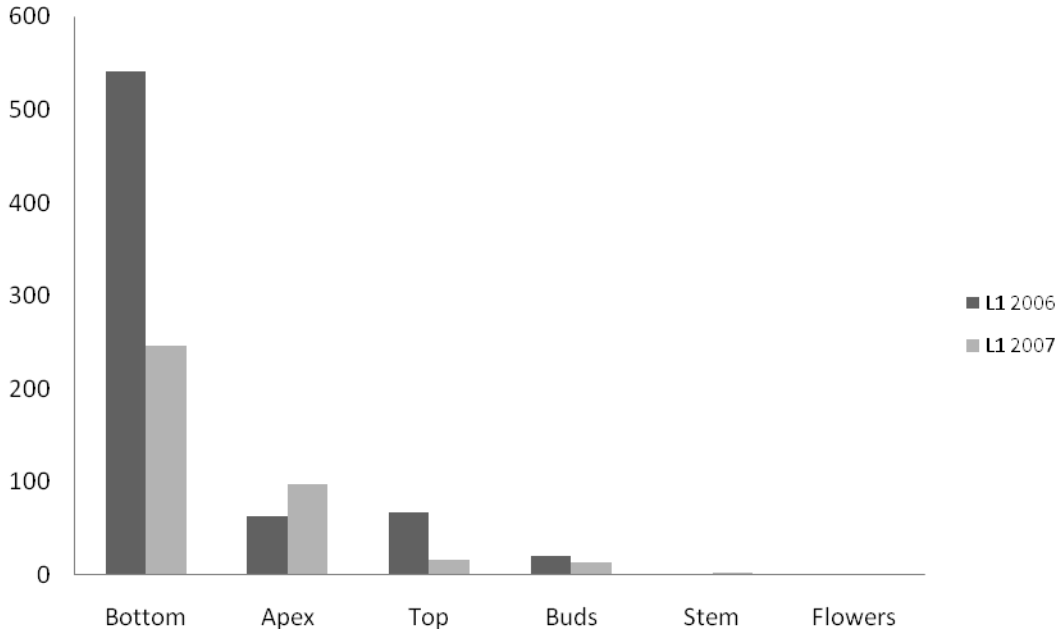


Figure 5c.

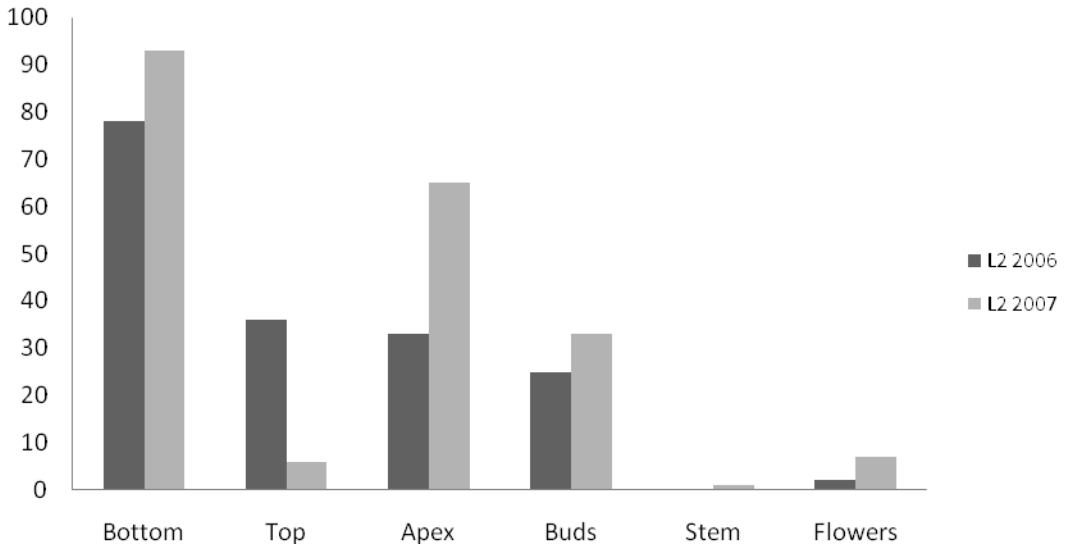


Figure 6.

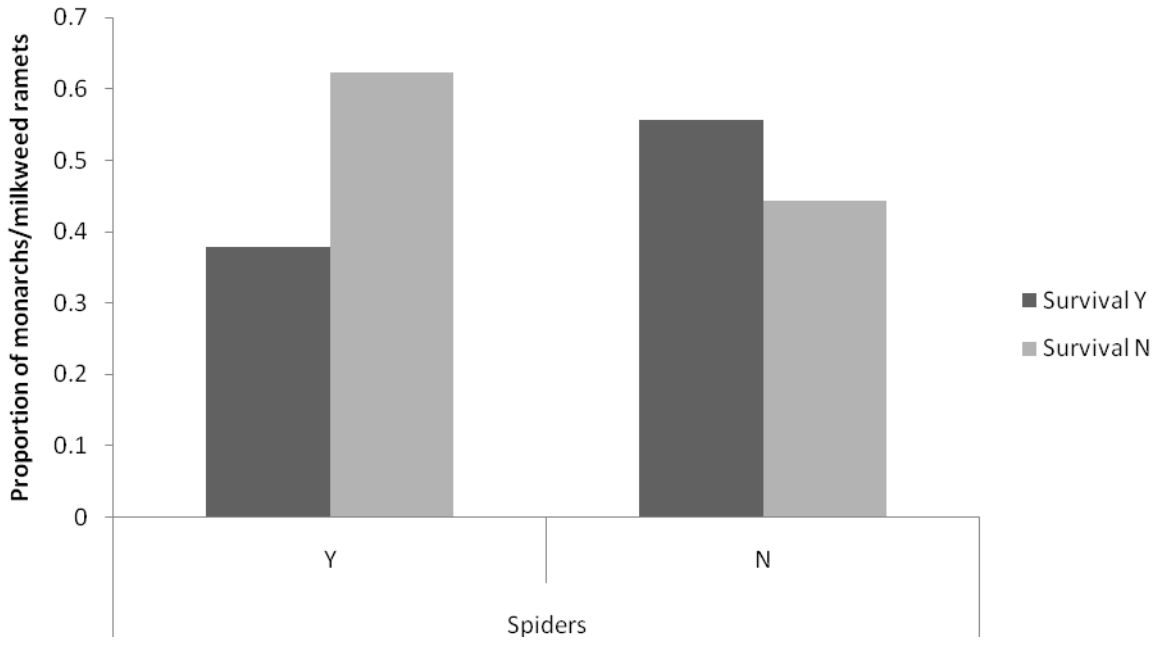


Figure 7.

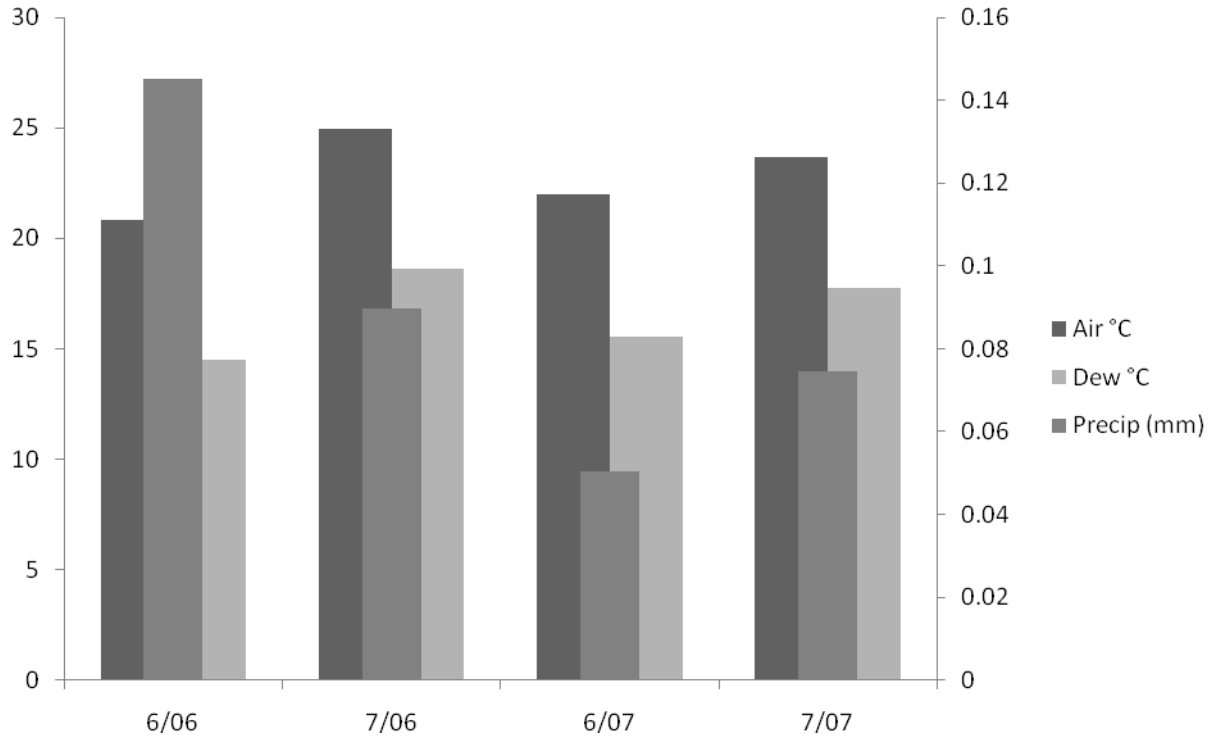


Figure 8a.

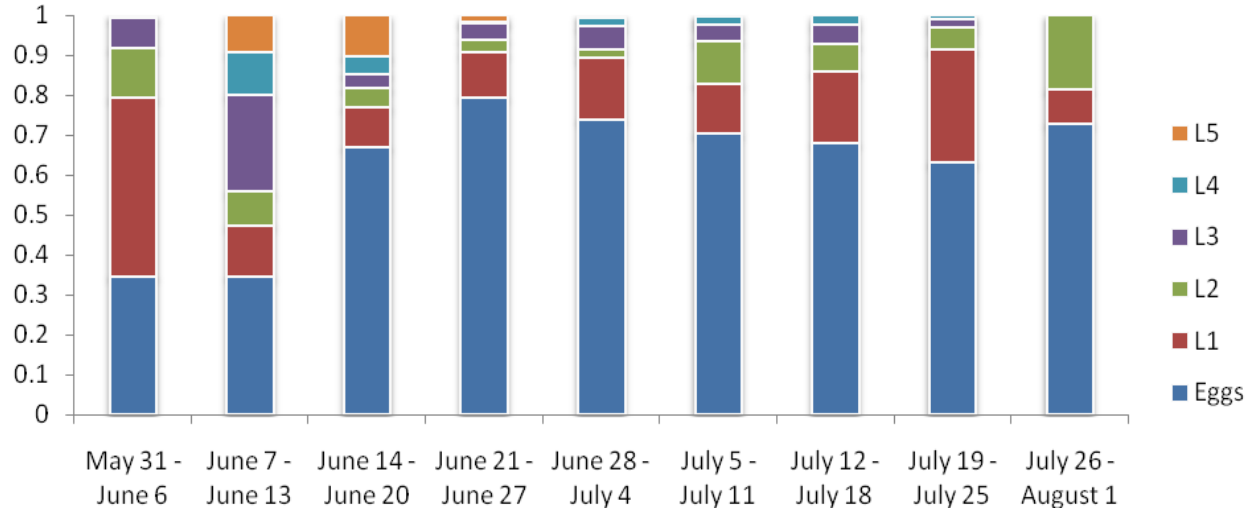


Figure 8b.

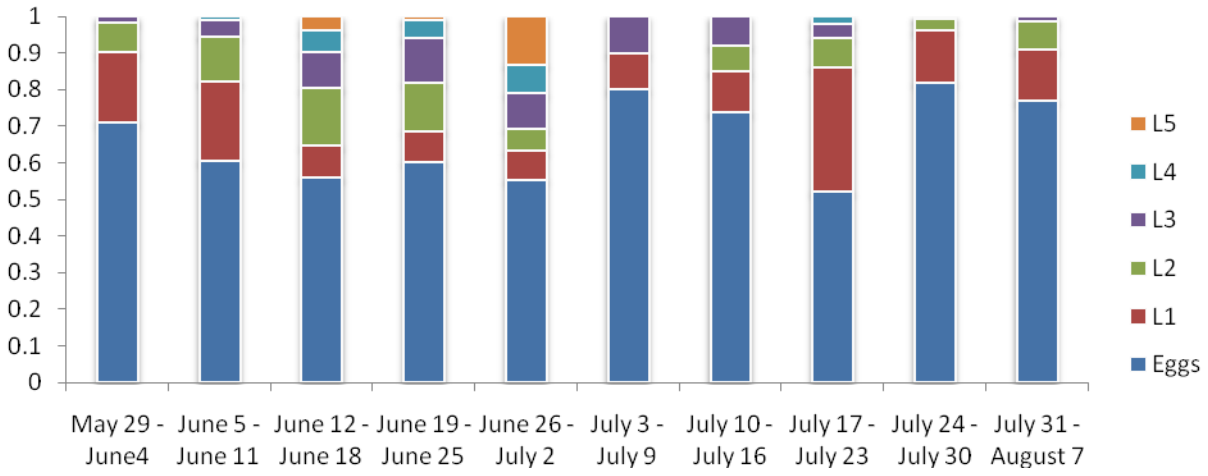


Figure 9a.

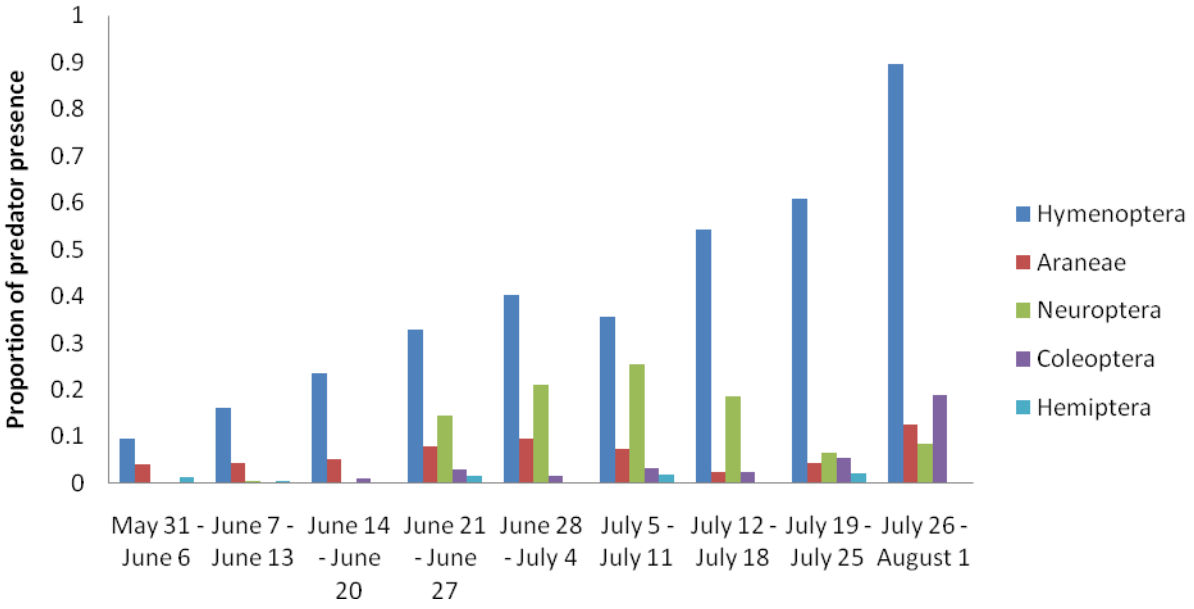


Figure 9b.

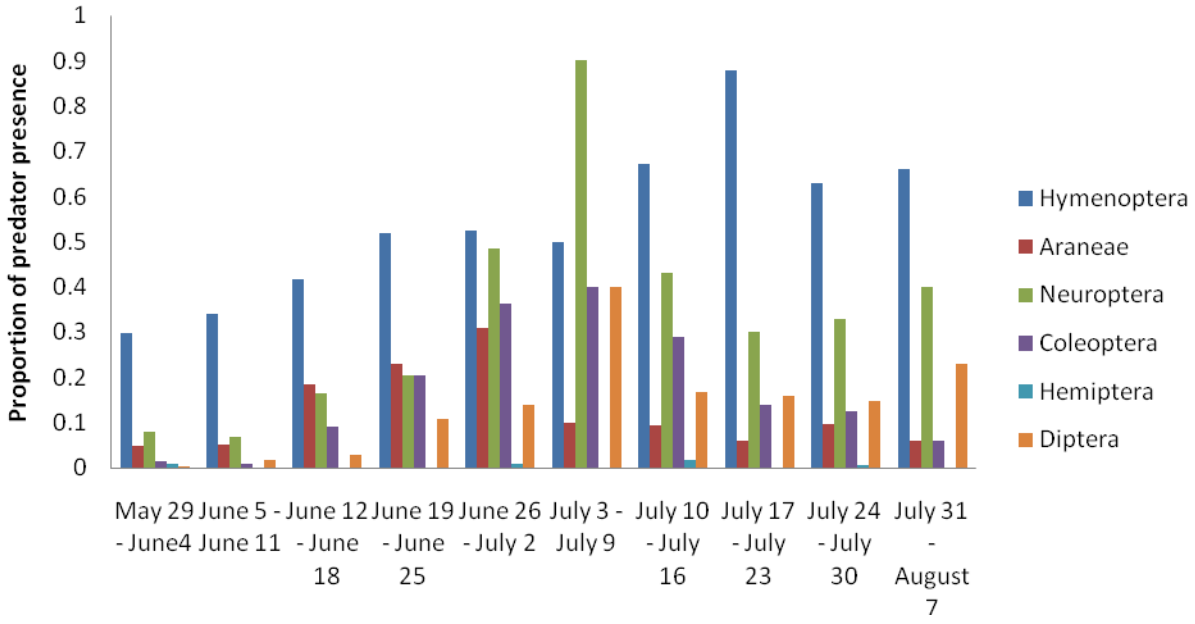
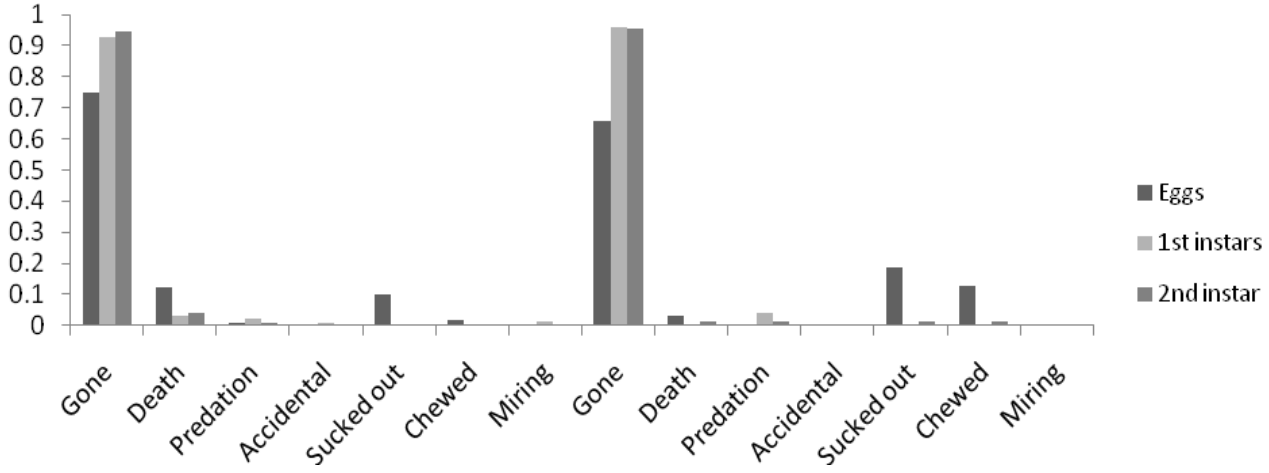


Figure 10.



Chapter 3: Parasitism of Immature Monarchs by a Parasitoid Wasp in Minnesota.

Introduction

Interest in hymenopteran parasitoids is primarily focused on their importance in biological control of many crop pest species (Eggleton and Belshaw, 1992). Parasitoids occur in several insect orders, including Diptera, Coleoptera and Neuroptera, but hymenopteran parasitoids are especially common, with recent estimates suggesting that 10% to 20% of all insects may be parasitoid wasps (Pennacchio and Strand, 2006). The chalcid wasps of the superfamily Chalcidoidea are among North America's most important insect groups for both natural and biological control. About 20 families and 2,000-plus species have been found on the continent — among the smallest of insects (Online, S.A.R.E, accessed May, 2010). *Pteromalus puparum* L. (Hymenoptera: Pteromalidae) is a gregarious pupal endoparasitoid that is used to control the small cabbage white butterfly, *Pieris rapae* L. (Lepidoptera: Pieridae) (Harvey et.al., 2007). It is considered to be an important biological control agent of *P. rapae*, with a parasitism rate that ranges from 18.9% - 84.2 % on *P. rapae* and 16.6% - 69.4% on two other lepidopteran cabbage pests (Lee and Heimpel, 2005). *P. puparum* is known also to attack pupae in other families of butterflies, including the Nymphalidae (Lei, GC et.al., 1997). Because of the strong link between oviposition and fitness, females of parasitoid species should maximize the number of offspring produced, therefore, the mechanisms that

determine how a female finds a suitable host patch and determines the time she spends searching for a host are subject to strong selective pressures (van Alphen et.al., 2003).

The monarch butterfly (*Danaus plexippus*) is a temperate insect herbivore that has a complex life history and produces multiple generations within a summer breeding season (Prysby and Oberhauser, 2004). Each generation can be subject to different environmental stressors due to weather differences, food-quality and abundance differences, and overlap with peak periods of natural enemy abundance (Lill, 2001). Parasitoids of monarchs previously identified are known to include 12 species of tachinid flies and at least 1 species of brachonid wasp (Arnaud 1978). Monarchs are frequently parasitized by the tachinid fly, *Lespesia archippivora* (Diptera: Tachinidae) (Oberhauser et al., 2007; pers. obs.) with published rates such as 1% in southern Ontario (Urquhart, 1960) and 11.15% in Wisconsin (Borkin, 1982).

There have been no other studies of the importance of wasp parasitoids in monarchs or if, parasitoids exploit volatile organic compounds (VOCs) emitted by plants in response to insect herbivory to find their hosts (Udayagiri, S. and R.L. Jones, 1992; De Moraes et.al., 1998), Here, I report on a two year experimental study that documents a previously thought unsuccessful monarch parasitoid (Ramsay, 1964).Wasp parasitoids in monarchs tend to attack their hosts during the pre-pupa, and thus studies that rely on wild hosts collected as larvae are unlikely to document wasp parasitism; Oberhauser et al. (2007)

reported on 1,471 of monarchs collected as eggs and larvae, with only 38 parasitized by wasps.

The focus of this study was to determine the importance and causes of pre-pupal and pupal monarch mortality due to parasitoids.

Methods

Experimental Round 1

Three sites with naturally occurring milkweed plants were chosen in the summer of 2008. The first site was a garden in Roseville, MN; the second site was a garden on the University of Minnesota (U of M) campus, St. Paul, MN; and the last site was an old field habitat near New Richmond, WI. The WI site and both sites in MN were approximately 80.5 km apart and the two MN sites were approximately 1mi apart.

Approximately 500 monarch eggs were reared in the laboratory to the late 5th stadium. On 21-24 July, 147 monarchs were exposed as pre-pupae or new pupae in the Roseville site; 124 at the WI site; and 117 at the St. Paul site. All larvae used this round and Round 2 came from wild-caught butterflies. Eggs and first and second instars were reared in petri dishes (100 x 15 mm) after which, they were moved to plastic containers (approximately 35 x 15 x 15 cm) until they were ready to pupate. All larvae were fed common milkweed (*Asclepias syriaca*).

Larvae were exposed on the lids of the plastic containers in which they had been reared. These lids were attached to ropes tied to four metal garden stakes approximately 1 m off the ground (Figure 1). The monarchs were left outside for 10 days, and then brought back into the lab where each pupa was put into an individual liter deli container. As a control, I reared 40 larvae in four rearing containers (10 larvae per container) in the laboratory.

I collected all parasitoids that emerged from each monarch, determining the number and sex ratio of the wasps. If monarch pupae died in the lab, I dissected them to determine the cause of death. If there were parasitoid larvae, pupae or adults present in the monarch pupae, I counted them and noted if they were dead or alive.

Experimental Round 2

Based on the results and methods of the first experiment, I conducted a second experiment in 2008, varying the experimental set up. The second round began on 31-August – 2 September. I used the Roseville (120 monarchs) and U of M campus (92 monarchs) sites from Round 1, and replaced the WI site with a garden in St. Paul, MN (192 monarchs). The St. Paul garden was approximately 4 km and 5 km from the U of M and Roseville site, respectively. Exposure time was the same as in Round 1.

Based on problems with the escape of pre-pupal larvae in Round 1, and a desire to determine if parasitoids used larval host plants or frass as cues, I varied the manner in which monarchs were exposed. I reared larvae in screen cages with wood frames (approximately 60 x 30 x 30 cm), and put the cages with larvae in the sites two to three days before they were ready to pupate. I kept the cages closed until all of the larvae were either pre-pupae or pupae. This procedure exposed any parasitoids to potential cues, such as the presence of frass and any plant volatile organic compounds (VOC's), which help parasitoids hone in on their hosts (Tumlinson et.al., 1993; De Moraes, et.al., 1998) (Figure 2a and 2b). After the cages had been open for 10 days, I brought them back to the lab and put them into individual containers as in round one. I raised 20 larvae, in two rearing containers, in the laboratory as controls.

Experimental Rounds 3-5

In the summer of 2009, I carried out a series of three experiments to better understand the role of VOCs released from damaged plants and host frass in helping parasitoids find their hosts. Because of a difference in parasitism rates in the two experimental rounds in 2008, I was also trying to determine how parasitism rates varied across the summer. I used the same methods as in round 2, except that all cages used to expose monarchs were autoclaved just before placing them outside.

As in 2008, I reared larvae under laboratory conditions, in plastic containers until early in the 4th stadium, after which they were put into wooden cages until they were ready to pupate. They were exposed to the outdoors for approximately 9 days. Each site had four cages of pre-pupae and pupae, with each cage representing one of the following treatments: 1) milkweed present, frass present; 2) milkweed, no frass; 3) no milkweed, frass; 4) no milkweed, no frass. Two of the sites used during the 2008 parasitoid experiments were used again in 2009: the garden in Roseville and the garden at the University of Minnesota. Sample sizes for each experimental round are shown in Table 1.

Round 3 began on 12 July – 21 July. Larvae used in this experiment were offspring of larvae obtained from Monarch Watch (University of Kansas). Differences in growth rates of individuals in this cohort led to different sample sizes in each treatment, since larvae had to be placed into individual experimental cages together. We did not have enough larvae for the milkweed/frass treatment at the U of M site. All monarchs were exposed as pre-pupae or new pupae, and when larvae were ready to be put into a cage, their treatment was randomly selected.

Larvae for Round 4 (27 July – 15 August) came from wild-caught adult butterflies raised in our laboratory, and we continued to experience variable growth rates and high mortality. We therefore only had enough viable larvae for a single site (Roseville garden).

Round 5 (19 August – 29 August) larvae were from the same parents as Round 4, but we reared larvae in the greenhouse. Beginning and end dates of exposure, treatment, location and end result of parasitism rates are seen in Table1. Tree, shrub and plant species at each site used repeatedly in this two year study are found in Tables2a and 2b.

All monarch pupae for all rounds in 2009 were dissected approximately three weeks after they were brought into the laboratory.

Results

Paper Wasp Predation

Paper wasp (Vespidae: *Polistes* spp.) predation was an unexpected source of mortality in this parasitoid study. In 2008, the garden in St. Paul, MN was not used in the statistical analysis due to the fact that many larvae escaped from the cage before they pupated or were preyed upon. We assumed that predators removed monarch pupae when a cremaster was left behind in the cage (Figure 3a). In 2009, my team and I observed paper wasp predation, particularly in the Roseville site. In Round 2, 19 of the 25 monarch pupae recovered from the St. Paul site (site not used in the analysis that round) were 'taken' as seen in Figure 3b. In Round 3 in the Roseville, MN site, 3 monarchs were taken in the no milkweed, no frass treatment, 2 from the milkweed, no frass treatment and 2 from the milkweed, frass treatment. Only 1 monarch was taken from the U of M site from the no milkweed, frass treatment. Round 4 (Roseville only) monarch individuals were taken in

the following treatments: 9 from the no milkweed, frass treatment, 26 from the milkweed, no frass treatment and 7 from the milkweed, frass treatment. Finally, in Round 5 (Roseville), 11 monarchs were taken in the no milkweed, frass treatment, 18 from the milkweed, no frass treatment and 28 from the milkweed, frass treatment. No monarchs were taken in the U of M site.

Round 1, 21 Jul – 2 Aug, 2008

Due to the predation that occurred by paper wasps on monarch prepupae and puape, parasitism rates are based on the number of monarch pupae recovered. I calculated a 90% binomial confidence interval for each treatment in each site. If these intervals did not overlap, I assumed that the proportions were different and, therefore, the outcomes of parasitism for each treatment per site were different. The observed percentage of parasitism in the Roseville, MN site was 0% (n = 143 of 147 recovered pupae), 90% confidence interval (0% - 2.06%). Two occurrences of parasitism occurred in the WI site: one with 113 female and 5 male wasps identified by M. W. Gates (Pteromalidae), Department of Entomology, Smithsonian Institution as *Pteromalis puparum* (Linnaeus). One monarch pupa had five unidentified fly maggots emerge (n = 103 of 124 recovered pupae), parasitism rate = 1.94%, 90% confidence interval (0.35% - 5.99%). One occurrence of parasitism occurred in U of M campus, site, with nine unidentified fly maggots emerging from one monarch pupa, n = 93/117. The observed percentage of parasitism was 1.08%, 90% binomial CI (0.06%, 5.00%). Parasitism rates did not vary among sites.

Round 2, 31 Aug – 10 Sep, 2008

The observed percentage of parasitism in the Roseville site was 53.98% (n = 113 of120 recovered pupae, 90% CI = 45.82% - 61.99%). The U of M site had an observed percentage of parasitism of 33.33% (n = 51 of 92 recovered pupae, 90% binomial confidence interval = 22.48% - 45.71%). There was a significant difference in occurrence of parasitism in the two sites ($X^2 = 16.4$, 3 d.f., $p < 0.001$). No monarch pupae were recovered from the residential St. Paul site; we believe they were all removed by paper wasps (Vespidae: *Polistes* spp.).

Parasitic wasp sex ratios

A female-biased sex ratio was observed from parasitized monarch pupae (Figure 4). Many wasp pupae did not develop successfully, and remained inside the monarch pupae; 88 of 164 recovered monarch pupae contained wasp pupae (53.66%). All pupae found inside each monarch pupa was dead (Figure 4). I dissected the remaining dead monarch pupae at least 3 weeks after the last wasps emerged

Round 3, 12 July – 21 July, 2009

No parasitism occurred in either the Roseville or U of M campus sites this round (Table 1).

Round 4, 27 July – 15 August, 2009 (Roseville, MN only)

There were no occurrences of parasitism for all treatments except in the milkweed/frass treatment; 4 monarch pupae were parasitized by *P. puparum* wasps, with an observed percent parasitism of 20% (Table 1). A Fisher's Exact contingency table analysis was performed on the combined treatment data of frass vs no frass on the chance of being parasitized due to low sample sizes from the no milkweed/frass treatment due to paper wasp predation. The probability of being parasitized depending on frass being present was significant, $p = 0.027$.

Parasitic wasp sex ratios

Two of the four monarch pupae recovered had *P. puparum* wasps successfully emerge. A female biased sex-ratio was observed (Figure 5). Overall, the range of emerging wasps of either sex was 3 – 117 parasitoid wasps per monarch pupa. All pupae inside monarch pupae were alive.

Round 5, 19 August – 29 August, 2009

In Roseville, parasitism only occurred in the milkweed/frass treatment (Table 1). Only 2 monarch pupae were recovered from this treatment, but both were parasitized. The chance of being parasitized based on the treatment effects was significant, $p = 4.8E\text{-}04$. To determine which treatments were significantly different from each other, the proportion of parasitized pupae were ranked and arcsin transformed in order to perform a Tukey-type multiple comparison tests for proportions (Zar, 1999). The treatments milkweed/frass vs. no milkweed/no frass proportions were not equal; all other treatments

failed to reject the null hypothesis of equal proportions when compared to each other (Table 3).

In the St. Paul campus site, parasitism occurred in the treatments no milkweed/ frass present and milkweed/frass (Table 1). To compare the treatments in which monarchs were parasitized to the treatments in which they were not parasitized, a 4 x 2 exact contingency table analysis was performed and it was found that the chance of being parasitized based on the treatment was significant, $p = 3.7E-05$. To determine which treatments were significantly different from each other, the proportion of parasitized pupae were ranked and arcsin transformed in order to perform a Tukey-type multiple comparison tests for proportions. The treatments milkweed/frass vs. no milkweed/no frass proportions were not equal, as well as the treatments no milkweed/frass vs. no milkweed/no frass. All other treatments fail to reject the null hypothesis of equal proportions when compared to each other (Table 3).

Parasitic wasp sex ratios

A female biased sex-ratio was observed at both sites (Figure 6). Overall, the range of emerging wasps of either sex was 1 – 132 in the U of M site, no live wasps emerged from the Roseville site.

Discussion

Natural Enemies Late in Immature Monarch Development

This is the first study to document natural enemies in late larval and pupal monarchs. The two instances of fly parasitism observed is most likely due to the tachinid fly, *L. archippivora. L. archippivora* has been documented to be the most common monarch parasitoid (Oberhauser, et.al., 2007) but found only in the first round of this two year study. Additionally, many pre-pupae were preyed upon by paper wasps in both 2008 and 2009. In Chapter 2, I demonstrated the high mortality experienced by early immature monarchs due to direct and indirect invertebrate predation in the wild. The tachinid fly parasitism and paper wasp predation observed in this study highlights the fact that despite their sequestration of cardenolide toxins, monarch larvae are still susceptible to predation by invertebrates late in their development.

Monarch Parasitoids

This is the first known account of parasitism by *Pteromalus puparum* (Linnaeus) on monarchs in North America; it was previously reported as an unsuccessful parasitoid in introduced monarch populations in New Zealand in 1868 (Ramsay, 1964). *P. puparum* is a gregarious generalist pupal parasitoid used as a biological control agent to control the cabbage white butterfly, *Pieris rapae*, on cabbage plants, *Brassica oleracea* L. (Capparales: Brassicacea) (Lee and Heimpel, 2005). This parasitoid was accidentally introduced into the United States from Europe in the late 1800s and is now found throughout the country, although its abundance varies in different geographical areas (Online, Mahr, accessed April, 2010). Pteromalidae (Chalcidoidea) species were introduced into Canada in 1949 to control the cabbage seed weevil, *Ceutrorhynchus*

obstrictus, (Marsham, 1802). Although introduced to Canada as *Habrocytus* sp, these were later identified as *P. puparum* species (Gibson et.al., 2006). *P. puparum* is no longer used as a biological control agent or commercially available (Hutchison, pers. comm), however, it was the predominant parasitoid of cabbage pests in Rosemount, MN (Dakota County) in the 1990's, approximately 32 km from my experimental sites (Wold-Burkness et.al., 2004). Often, introduced insect pests, such as *P. rapae* have large densities due to host plant monocultures and their interactions with parasitoids may not reflect the interactions between non-pest hosts and parasitoids (Oberhauser et.al., 2007). In this study, all wasp parasitism, and almost all pupal parasitism, was due to *P. puparum;* with the exception of the tachinid fly parasitism already mentioned. This is the first study to detail the extent of successful parasitism by an introduced parasitoid on non-pest monarch populations in North America.

Factors Affecting Parasitism Occurrence

Time of season

Parasitism was not commonly observed early in the experimental rounds (Round 1 and Round 3), however, parasitism occurrences generally increased in frequency later in the season (Rounds 2, 4 and 5). The time effect was naturally built into the experiment because we could only rear so many larvae at a time and synchronize their development to the right stadium. *P. puparum* may not be abundant early in the season, possibly due to the lower abundance of *P. rapae. P. puparum* is the dominant parasitoid of *P. rapae*, with up to 31% parasitism in Minnesota (Wold-Burkness et.al. 2005). *P. rapae* has 2-5

overlapping generations, during which time their numbers increase (Online, Hutchinson et.al., accessed March, 2010) This may be a possible explanation for the trend observed of low or no parasitism early on in the summer seasons.

Site differences

Parasitism occurred in both years and in all sites that had a combination of milkweed, frass or milkweed and frass present. All treatments that lacked frass had no observed parasitism (Table 1). Round 2 in 2008 was the only instance where an observable difference in the 90% CI rate of parasitism was observed at different sites (Roseville and U of M) with the same treatment, milkweed/frass. This difference suggests that with treatment being equal, these two sites are not equivalent in the chance of being parasitized. In that particular year, the Roseville site had a higher rate of parasitism than the U of M campus site. The Roseville site is a residential garden with various species of milkweed plants (*Asclepias spp*) and flowers common to both sites, however, the Roseville site is more diverse in its tree and shrub species (Table 2a, 2b). Although they are relatively close in proximity to each other and similar in vegetation type, parasitoids found monarch pupae more often in the Roseville site. Parasitoids are assumed to be patchily distributed (Hassel et.al., 1998) and influenced by patch exploitation strategies used to effectively increase their fitness (Spataro and Bernstein, 2007). This may explain why two sites, relatively similar in vegetation type and distance had different rates of parasitism.

Cues

The results of this study lend support to other findings identifying plant VOCs and host cues (frass) as signals parasitoids use to hone in on their hosts (Tumlinson et.al.,1993; De Moraes et.al., 1998). Plant VOCs disperse well into the atmosphere and can be detected by parasitoids over long distances, while kairomones (host cues) are only detected at a closer range (Tumlinson, et. al., 1993). In 2009, Round 4, an individual pupa was more likely to be parasitized if it was in a treatment that contained frass. In Round 5, there was an observable difference in both sites; in the Roseville site, a combination of cues (milkweed/frass only) led to an increased risk of parasitism while at the U of M site, being in the milkweed/frass and no milkweed/frass treatments led to an increased risk of parasitism (Table 3), suggesting that frass is an important cue that *P. puparum* uses to find its hosts. *P. puparum* has been known to ride on the back of their pre-pupae hosts in the field (Takagi, 1981), suggesting that they can hone into cues emitted by their hosts, possibly because female parasitoid wasps cannot oviposit their eggs into hardened pupae (Askew, 1971).

This is the first study to detail a never before reported monarch parasitoid in North America and factors that affect monarch pupae mortality, specifically frass, of this non-pest host.

Parasitoid Success and Sex Ratios

Additionally, we observed female biased sex ratios in successful emergence of *P. puparum* from monarch pupae. Indeed, this parasitoid exhibits female biased sex ratios of progeny (Takagi, 1986). Females were larger and a metallic-black color, while males were copper-colored. Females in many insects are larger, presumably due to the fitness advantage realized from a positive correlation between body size and fecundity, egg size and longevity (Ellers, J and M. Jervis, 2003). Also, the female biased sex ratio is common in parasitoid wasps that can control offspring sex and who experience local mate competition between siblings (J. Y. Wakano, 2005; Darrouzet et.al., 2008).

Although there were successful adult wasps that emerged from monarch pupae, undeveloped parasitoid pupae remained in the host pupae. *P. puparum* has the ability to regulate the number of eggs it will oviposit according to host size (Takagi, 1986), however, unsuccessful parasitoid development may have occurred for several reasons. In gregarious parasitoid species, as more larvae continue their development inside the host, intense competition is expected as resources diminish (Pexton et.al., 2008). Another reason for unsuccessful wasp emergence from their host could be because females wasps of this species take longer to develop than male wasps (Harvey et.al., 2007), with development in *P. puparum* lasting approximately three weeks. It is possible that we did not allow enough time between the exposure time outdoors and dissection time in Rounds 3-5. Another possibility is that wasps late in the season might have been in diapause. *P. puparum* wasps overwinter in their hosts as mature larvae, so this may be the case in the undeveloped wasps that remained inside monarch pupae towards the end of the season

(Online, Mahr, accessed April 2010) Finally, Desneux et.al., (2009) found support for the 'toxic plant hypothesis', a hypothesis explaining low host suitability due to high levels of toxins in host tissues. Support was found in the milkweed aphid (*Aphis asclepiadis*) and *Aphis nerii*, and its parasitoid, *Binodoxys communis*. Although this hypothesis was not tested for in this study, it is a possible explanation for the high number of unsuccessful parasitoid larvae.

A caveat about my findings is that the experimental set up for these experiments is not an accurate portrayal of monarch pupae in the wild. The pupal stage of this charismatic butterfly in the wild is poorly understood in terms of where they pupate and how far fifth instar larvae move off a host plant to pupate, or if they move off milkweed plants at all (Oberhauser, 2004). I observed one monarch pupae in the wild over a 2 year observational study hidden between stalks of butterfly weed (*A. tuberosa*) at a community park that has a restored prairie habitat (pers. obs.). To my knowledge, they do not aggregate in these numbers so it might be that their greatest form of protection in this stage is to be inconspicuous; however, what this study does exemplify is that if non-specific parasitoids are or were used as biological control agents in the wild, such as *P. puparum*, there is the possibility of parasitism of non-pest species.

Suggestions for future research

Most estimates of population densities for pupae and/or adults of lepidopteran species are derived from the egg to late larva stage because it is difficult to estimate densities in these

stages. The prevalence of natural enemies in the late developmental stages of the monarchs suggests that monarch population densities may be overestimated due to the mortality and parasitism rates experienced by monarchs in the wild. Future research should address the biology and relationships of both these species to further identify mechanisms that drive parasitism variation throughout the summer breeding season of the monarch butterfly. Exposing monarchs as pre-pupae evenly across all treatments would be beneficial in asserting the importance of host cues in the ability of parasitoids to find their hosts. Finally, it would be beneficial to study how parasitism is affected by the concentration of toxins found in host plants of herbivore hosts. This would help to clarify whether unsuccessful wasp emergence is due to toxins in host tissues. These studies will help to identify possible causes for the variation observed in wasp parasitism occurrences and the cues parasitoids use to find their hosts.

Table 1. Dates, locations, treatments, the number of pupae recovered per treatment, the percentage of pupae that were recovered that were parasitized and the respective 90% binomial confidence interval for each parasitism rate per treatment.

Round	Exposure Start Date	Exposure End Date	Location	Treatment	N	N recovered	% Parasitized	90% CI
3	12-Jul	21-Jul	Roseville	MW, F	19	15	0	0 - 18.10
3	12-Jul	21-Jul	Roseville	MW, No F	26	19	0	0 - 14.57
3	12-Jul	21-Jul	Roseville	No MW, F	28	19	0	0 - 14.57
3	12-Jul	21-Jul	Roseville	No, No	19	11	0	0 - 23.84
3	13-Jul	22-Jul	U of M	MW, No F	9	9	0	0 - 28.31
3	13-Jul	21-Jul	U of M	No MW, F	19	11	0	0 - 23.84
3	13-Jul	21-Jul	U of M	No, No	17	9	0	0 - 28.31
4	July 31 - August 6	August 7 - August 15	Roseville	MW, F	33	20	20	7.14 - 40.10
4	July 27 - August 3	August 3 - August 13	Roseville	MW, No F	51	10	0	0 - 25.89
4	29-Jul	N/A	Roseville	No MW, F	9	0	N/A	0 - 100
4	2-Aug	9-Aug	Roseville	No, No	17	17	0	0 - 16.16
5	20-Aug	27-Aug	Roseville	MW, F	30	2	100	22.36 - 100
5	21-Aug	26-Aug	Roseville	MW, No F	30	12	0	0 - 22.09
5	21-Aug	26-Aug	Roseville	No MW, F	30	19	0	0 - 14.59
5	21-Aug	26-Aug	Roseville	No, No	32	32	0	0 - 8.94
5	19-Aug	27-Aug	U of M	MW, F	30	29	23	11.5 - 39.39
5	21-Aug	28-Aug	U of M	MW, No F	30	30	0	0 - 9.5
5	20-Aug	27-Aug	U of M	No MW, F	30	30	13.3	4.69 - 27.96
5	22-Aug	29-Aug	U of M	No, No	31	31	0	0 - 9.21

Table 2a. Plants, trees and shrub species that were common for the majority of all sites used in this two year experimental study, but specifically to the two sites used repeatedly; Roseville, MN and U of M campus.

Perennials	**Shrubs**
Aster spp	Blueberry (Northblue)
Black-eyed Susan spp	Dogwood spp
Butterfly Weed	
Coneflower spp	
Joe Pye weed	
Meadow Blazing Star	
Rough Blazing Star spp	
Swamp Milkweed	

Table 2b. Tree, shrub and plant species that varied between both primary sites used over this two year experiment; Roseville, MN and the U of M campus.

Roseville Plant Spp	**U of M Campus Plant Spp**
Trees	**Trees**
Alleghany Serviceberry	Lilac
Honeycrisp Apple	
North Star Cherry	
Plum (Alderman)	
Plum (American)	
Plum (Mount Royal)	
Shrubs	**Shrubs**
Hazelnut (American)	Arctic Blue Willow
Snowberry (Red, White)	Butterfly bush
Sumac (Gro-Low)	
Winterberry spp	
Perennials	**Perennials**
Blue False Indigo	Yarrow
Blue Vervain	Whorled Milkweed
Canada Anemone	Bachelor’s button
Columbine	Turtle Head
Fern spp	Thread leaf
Grass spp	Globe Thistle
Iris spp	Blanket flower
Pasque Flower	Karl Foerster grass
Prairie spp	False Sunflower
Rattlesnake Master	Goldenrod
Sedge spp	Monarda spp
Shooting Star	Beardtongue
Solomon's Seal	Hollyhock
Wild Strawberry	
Trillium	
Virginia Blue Bells	
Walker's Low Catmint	
Wild Columbine	
Wild Geranium	
Wild Ginger	
Wild Golden Glow	
Wintergreen	

Table 3. Tukey-type multiple comparison of treatments. Only treatments with different outcomes of parasitism were compared to determine if the difference was significant.

Round	Site	Comparison	SE	q	q 0.05, ∞, 2	Conclusion
5	Roseville	M/F vs. -/-	13.295	5.069	2.772	Reject Ho: M/F ≠ -/-
5	Roseville	-/F vs. -/-	5.803	0.254	2.772	Fail to reject Ho: -/F = -/-
5	U of M	M/F vs. -/F	5.231	1.459	2.772	Fail to reject Ho: M/F = -/F
5	U of M	M/F vs. -/-	5.19	4.801	2.772	Reject Ho: M/F ≠ -/-
5	U of M	-/F vs. -/-	5.416	3.359	2.772	Reject Ho: -/F ≠ -/-

Figure Legends

Figure 1. Experimental set up of the first round of parasitoid experiment in 2008. The same set up was used at all 3 sites.

Figure 2a. Experimental set up of the second round of parasitoid experiment in 2008. The same set up was used in all 3 site . Cages were used instead of lids to expose monarchs to parasitoids, as was done in 2008.

Figure 2b.A closer view of the experimental set up of the second round of parasitoid experiment in 2008.

Figure 3a.An experimental cage from Round 2, 2008 where monarchs pupated but were not recovered because they were assumed to be taken by predatory paper wasps. Note the moanrch pupae attached to the top of the cage ceiling by their cremasters. This indicates that a monarch successfully pupate but was ‘taken’.

Figure 3b. Paper wasp in 2009 preying on a half eaten monarch pre-puapa in an experimental cage.

Figure 4. Adult and immature parasitoid wasps in monarch puape, Round 2, 2008 at each experimental site.

Figure 5. Adult and immature parasitoid wasps in monarch pupaee in Round 4, 2009.

Figure 6. Adult and immature parasitoid wasps in monarch puape in Round 5, 2009. Dead wasps are wasps that never emerged from a monarch pupa but were adults.

Figure 1.

Figure 2a.

Figure 2b.

Figure 3a.

Figure 3b.

Figure 4.

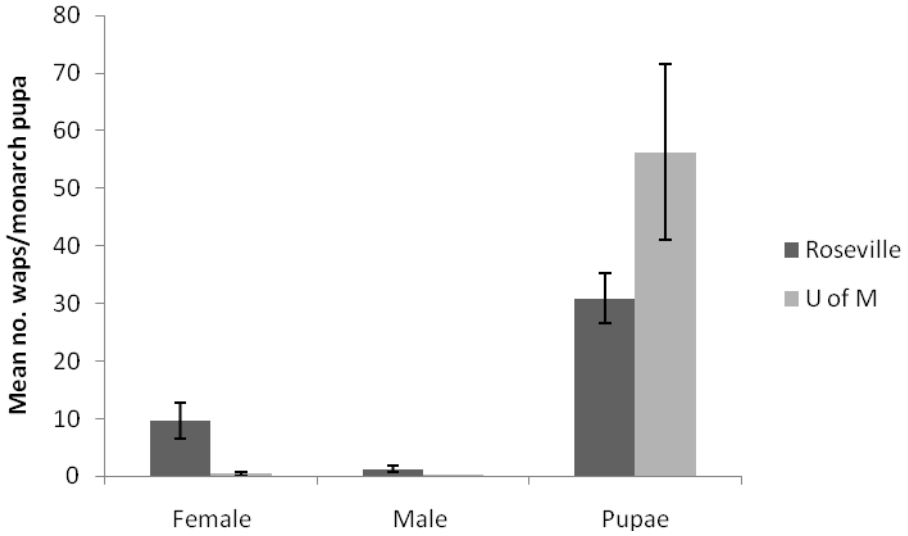


Figure 5.

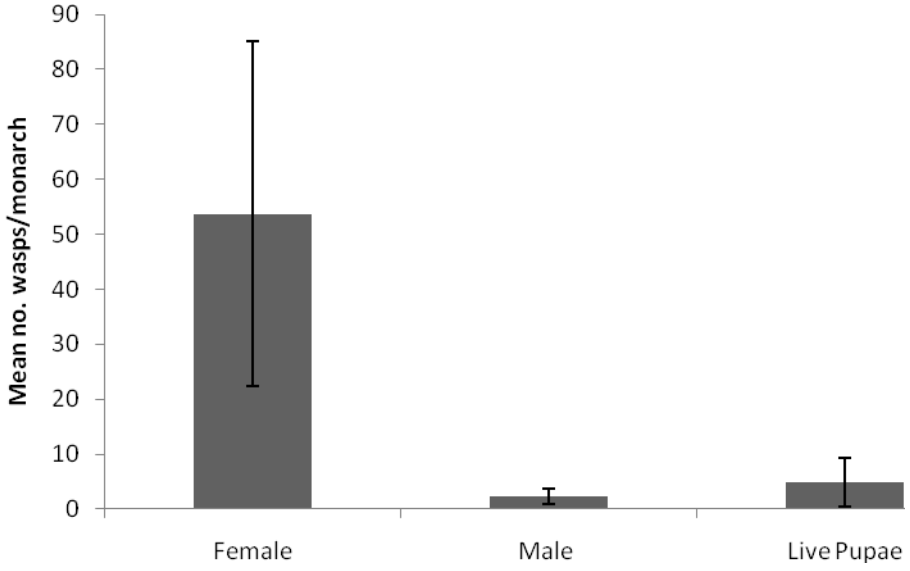
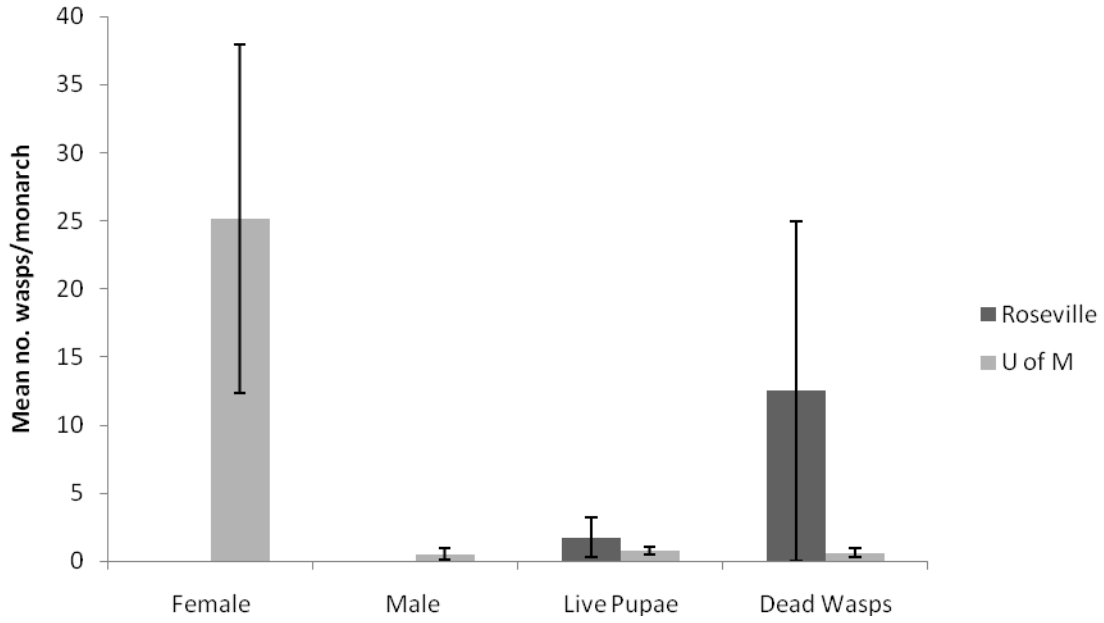


Figure 6.


Mean no. wasps/monarch
40
35
30
25
20
15
10
5
0
Female
Male
Live Pupae
Dead Wasps
Roseville
U of M

Bibliography

Chapter 1:

Agrawal. A. A., M. J. Lajeunesse and M. Fishbein, 2008. Evolution of latex and its constituent defensive chemistry in milkweeds (*Asclepias*): a phylogenetic test of plant defense escalation. *Enotomologia Experimentalis et Applicata.* **128(1)**: 126-138.

Malcom, S.B. and M.P. Zalucki.1996. Milkweed latex and cardenolide induction may resolve the lethal plant defence paradox. *Ent. Exper. Appl.* **80**: 193-196.

Zalucki, M.P. and L.P. Brower, 1991. Survival of first instar larvae of *Danaus plexippus* (Lepidoptera: Danainae) in relation to cardiac glycoside and latex content of *Asclepias humistrata* (Asclepiadaceae). *Chemoecology.* **3(2)**: 81-93.

Zalucki, M.P., L. P. Brower and A. Alonso-Mejia, 2001. Detrimental effects of latex and cardiac glycosides on survival and growth of first-instar monarch butterfly larvae *Danaus plexippus* feeding on the sandhill milkweed *Asclepias humistrata*. *Ecological Entomology.* **26(2)**: 212-224.

Chapter 2:

Agrawal. A.A., M. J. Lajeunesse and M. Fishbein, 2008. Evolution of latex and its constituent defensive chemistry in milkweeds (*Asclepias*): a phylogenetic test of plant defense escalation. *Entomologia Experimentalis et Applicata.* **128(1)**: 126-138.

Calvert, W.H. 2004. The effects of fire ants on monarch breeding in Texas, pp. 47-53. *In*: Oberhauser, K. S. and M. J. Solensky (eds.).The monarch butterfly biology and conservation. Cornell University Press, Ithaca NY.

Gilbert, O., T. B. Reynoldson, J. Hobart. 1952. Gause's Hypothesis: An Examination. *Journal of Animal Ecology*. **21(2):** 310-312.

Hassell, M. P. 1978. The Dynamics of Arthropod Predator-Prey Systems. New Jersey: Princeton University Press.

Kaiser, M. In preparation.

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.

Lotka, A. J. 1942. The progeny of an entire population. *The Annals of Mathematical Statistics*. **13(2):** 115-126.

Malcolm, S. B. 1990. Chemical defence in chewing and sucking insect herbivores: plant-derived cardenolides in the monarch butterfly and oleander aphid. *Chemoecology*. **1**: 12-21.

Malcom, S. B. and M. P. Zalucki. 1996. Milkweed latex and cardenolide induction may resolve the lethal plant defence paradox. *Entomologia Experimentalis et Applicata.* **80**: 193-196.

Montllor, C. B. and E. A. Bernays. 1994. Invertebrate predators and caterpillar foraging, p. 170-202. *In*: Stamp, N.E. and T.M. Casey (eds.). Caterpillars: Ecological and evolutionary constraints on foraging. Chapman and Hall, New York NY.

Morin, P. J. 1999. Models of predation in simple communities, p. 136-153. *In* Morin, P. J. Community Ecology. Blackwell Publishing Company, Malden, MA.

Oberhauser, K. S., M. D. Prysby, H. R. Mattila, D. E. Stanley-Horn, M. K. Sears, G. Dively, E. Olson, J. M. Pleasants, W. F. Lami, and R. L. Hellmich. 2001. Temporal and spatial overlap between monarch larvae and corn pollen. *Proceedings of the National Academy of Sciences.* **98** (21): 11913- 11918.

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

Oberhauser K. S., I.L. Gebhard, C. Cameron, and S. Oberhauser. 2007. Parasitism of monarch butterflies (*Danaus plexippus*) by *Lespesia archippivora* (Diptera: Tachinidae). *American Midland Naturalist*. **157**(2): 312-328.

Prysby, M. D. 2004. Natural enemies and survival of monarch eggs and larvae, p. 27-37. *In*: Oberhauser, K. S. and M. J. Solensky (eds.). The Monarch Butterfly: Biology and Conservation. Cornell University Press, Ithaca NY.

Reichert, S. E. and T. Lockey. 1984. Spiders as biological control agents. *Annual Review of Entomology.* **29**: 299-320.

Reichstein, T., J. von Euw, J. A. Parsons, and M. Rothschild. 1968. Heart poisons in the monarch butterfly. *Science*. **161**(3844): 861-866.

Stamp, N. E. and M. D. Bowers. 2000. Foraging behaviour of caterpillars given a choice of plant genotypes in the presence of insect predators. *Ecological Entomology*. **25**: 486-492.

Steele, M. A. and G. E. Forrester. 2005. Small-scaled field experiments accurately scale up to predict density dependence in reef fish populations at large scales. *Proceedings of the National Academy of Sciences.* **102**(38): 13513-13515.

Taylor, R. J. 1984. Predation. Chapman and Hall, New York NY.

Tumlinson, W., J. Lewis and L. E. M. Vet, 1993. How parasitic wasps find their hosts. *Scientific America*. **268(3)**: 100-106.

Turchin, P., S.N. Wood, S.P. Ellner, B.E. Kendall, W.W. Murdoch, A. Fischlin, J. Casas, E. McCauley, and C.J. Briggs. 2003. Dynamical effects of plant quality and parasitism on population cycles of larch budmoth. *Ecology*. **84**(5): 1207-1214.

Turchin, P. 2003. Complex population dynamics: A theoretical/empirical synthesis. New Jersey: Princeton University Press.

United States Fish and Wildlife Service, 1999. 1997 North American Conference on the Monarch Butterfly: round-table discussions and priority actions. Washington, D.C.: United States Fish and Wildlife Service, Office of International Affairs.

York, H. A. and K. S. Oberhauser, 2002. Effects of duration and timing of heat stress on monarch butterfly (*Danaus plexippus*) (Lepidoptera: Nymphalidae) development. *Journal of the Kansas Entomological Society*. **75(4)**: 290-298.

Zalucki, M. P. and W. A. Rochester. 2004. Spatial and temporal population dynamics of monarchs down-under: Lessons for North America *In*: Oberhauser, K. S. and M. J. Solensky (eds.). The Monarch Butterfly: Biology and Conservation. Cornell University Press, Ithaca NY.

Zalucki, M. P. and R.L. Kitching. 1982. Temporal and spatial variation of mortality in field populations of *Danaus plexippus* L. and *D. chrysippus* L. larvae (Lepidoptera: Nymphalidae). *Oecologia*. **53**(2): 201-207.

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(2)**: 81-93.

Zalucki, M. P, L. P. Brower and S. B. Malcolm. 1991. Oviposition by *Danaus plexippus* in relation to cardenolide content of three *Asclepias* species in the southeastern U.S.A. *Ecological Entomology*. **15(2):** 231-240.

Chapter 3:

Arnaud, P. H., Jr. 1978. A host-parasite catalog of North American Tachinidae (Diptera). U.S. Department of Agriculture Miscellaneous Publication No. 1319. Washington, D.C.: U.S. Department of Agriculture.

Askew, R. R., 1971. Parasitic insects. Heinemann Educational Books. London, GB. p.316.

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.

Darrouzet, E., G. Boivin and C. Chevrier. 2008. Sex allocation decision under superparasitism by the parasitoid wasp *Eupelmus vuilleti*. *Journal of Insect Behavior*. **21(3)**: 181 -191.

De Moraes, C. M., W. J. Lewis, P. W. Pare, H.T. Alborn and J. H. Tumlinson. 1998. Herbivore-infested plants selectively attract parasitoids. *Nature* **393:** 570-573.

Desneux, N., R. J. Barta, K. A. Hoelmer, K. R. Hopper and G. E. Heimpel, 2009. Multifaceted determinants of host specificity in an aphid parasitoid. *Oecologia*. **160**: 387-398.

Eggleton, P and R. Belshaw. 1992. Insect parasitoids: An evolutionary overview. *Philosophical Transactions: Biological Sciences*. **337(1279):** 1-20.

Ellers, J and M. Jervis, 2003. Body size and the timing of egg production in parasitoid wasps. *Oikos*. **102(1)**: 164-172.

Gibson, A. P., D. R. Gillespie and L. Dosdall. 2006. The species of Chalcidoidea (Hymenoptera) introduced to North America for biological control of the cabbage seedpod weevil, and the first recovery of *Stenomalina gracilis* (Chalcidoidea: Pteromalidae). *Canadian Entomology*. **138:** 285-291.

Grimaldi, D. and M. S. Engel. 2005. Evolution of the Insects. Cambridge University Press; Cambridge, UK.

Harvey, J. A., R. Gols, R. Wagenaar and T. M. Bezemer. 2007. Development of an insect herbivore and its pupal parasitoid reflect differences in direct plant defense. *J. Chem. Ecol.* **33**: 1556-1569.

Hassell, M. P. 1978. The Dynamics of Arthropod Predator-Prey Systems. New Jersey: Princeton University Press.

Hutchison, W. D., P. C. Bolin, and R. L. Hines. Imported Cabbageworm, http://www.vegedge.umn.edu/vegpest/colecrop/cabbworm.htm. April, 2010.

Lee, J. C. and G. E. Heimpel. 2005. Impact of flowering buckwheat on Lepidopteran cabbage pests and their parasitoids at two spatial scales. *Biological Control*. **34(3):** 290-301.

Lei, G. C., V. Vikberg, M. Nieminen and M. Kuussaari. 1997. The parasitoid complex attacking Finnish populations of the Glanville fritillary *Melitaea cinxia* (Lepidoptera: Nymphalidae), an endangered butterfly. *Journal of Natural History*. **31(4)**: 635-648.

Lill, J. T. and J. Marquis. 2001. Selection on herbivore life-history traits by the first and third trophic levels: The devil and the deep blue sea revisited. *Evolution*. **55(11)**: 2236-2247.

Mahr, S. *Pteromalus puparum*, Parasites of Imported Cabbageworm *http*://www.entomology.wisc.edu/mbcn/kyf312.html. April, 2010.

Marshall, S. A. 2006. Insects: Their Natural History and Diversity, With a Photographic Guide to Insects of Eastern North America. Buffalo, NY; Richmond Hill, Ontario.

Oberhauser K. S., I. L. Gebhard, C. Cameron, and S. Oberhauser. 2007. Parasitism of monarch butterflies (*Danaus plexippus*) by *Lespesia archippivora* (Diptera: Tachinidae). *American Midland Naturalist*. **157**(2): 312-328.

Pennacchio, F. and M. R. Strand. 2006. Evolution of developmental strategies in parasitic Hymenoptera. *Annual Review of Entomology*. **51**: 233-258.

Pexton, J. J., J. G. de Boer, G. E. Heimpel, L.E.M. Vet, J. B. Whitfield and P. J. Ode. 2008. Competition and brood reduction: testing alternative models of clutch-size evolution in parasitoids. *Behavioral Ecology*. 403-409.

Prysby, M. D. and K. S. Oberhauser. 2004. Temporal and geographic variation in monarch densities: Citizen scientists document monarch population pattersns. p. 9-20. *In*: Oberhauser, K. S. and M. J. Solensky (eds.). The Monarch Butterfly: Biology and Conservation. Cornell University Press, Ithaca NY.

Ramsay, G. W., 1964. Overwintering swarms of the monarch butterfly (*Danaus plexippus*) (L.) in New Zealand. *New Zealand Entomologist*. p. 10-16.

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

Rott, A. S. and H. C. J. Godfray. 2000. The structure of a leafminer-parasitoid community. *Journal of Animal Ecology.* **69**: 274-289.

Spataro, T and C**.** Bernstein, 2007. Influence of environmental conditions on patch exploitation strategies of parasitoids. *Behavioral Ecology.* **18(4)**:742-749.

Sustainable Argrigulture Research and Education. Manage Insects on Your Farm: A Guide to Ecological Strategies. http://www.sare.org/publications/insect/chapter5_principal_parasitoids.htm Accessed May, 2010.

Takagi, M., 1986. The reproductive strategy of the gregarious parasitoid, *Pteromalus puparum* (Hymenoptera: Pteromalidae). *Oecologia*. **70:** 321-325.

Tumlinson, J. H., W. J. Lewis and L.E.M. Vet. 1993. How parasitic wasps find their hosts. *Scientific America*. 100-106.

Udayagiri, S. and R. L. Jones. 1992. Role of plant odor in parasitism of European corn borer by braconid specialist *Macrocentrus grandii* Goidanich: Isolation and characterization of plant syndromes eliciting parasitoid flight response. *Journal of Chemical Ecology.* **18**: 1841-1855.

Urquhart, F. 1960. The Monarch Butterfly. Toronto: University of Toronto Press.

van Alphen J. J. M., C. Bernstein and G. Driessen. 2003. Information acquisition and time allocation in insect parasitoids. *Trends in Ecology & Evolution*. **18(2)**: 81-87.

Wakano, J. Y. 2005. Evolution of extraordinary female-biased sex ratios: The optimal schedule of sex ratio in local mate competition. *Journal of Theoretical Biology.* **237(2)**: 193 -202.

Wharton, R.A. 1993. Bionomics of the Braconidae. *Annual Review of Entomology.* **38**: 121-143.

Wold-Burkness, S.J., W.D. Hutchinson, J.C. Lee, R.L. Hines, P.C. Bolin and G.E. Heimpel. 2004. A long-term survey of parasitoid species composition and parasitism of *Trichoplusia ni* (Lepidoptera: Noctuidae), *Plutella xylostella* (Lepidoptera: Plutellidae), and *Pieris rapae* (Lepidoptera: Pieridae) in Minnesota cabbage. *Journal of Entomological Science*. **40(2)**: 211-221.

Zar, J.H., 1999. Biostatistical Analysis, 4th edition. New Jersey: Prentice-Hall. p. 563-565.