THE EFFECT OF COMMON MILKWEED (*ASCLEPIAS SYRIACA*) QUALITY ON MONARCH BUTTERFLY (*DANAUS PLEXIPPUS*) OVIPOSITION PREFERENCE AND LARVAL PERFORMANCE

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Thesis submitted to the University of Ottawa
in partial fulfillment of the requirements for the
Master of Sciences (M.Sc.)

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Abstract

Species are experiencing shifts in their phenology (i.e., seasonal timing of recurring biological events) due to climate change, leading to disruptions in the relative timing of interacting species. These shifts can be detrimental to the fitness of the consumer (e.g., herbivore) in the interaction. In its larval form, the monarch butterfly (*Danaus plexippus*) is a specialist herbivore that feeds on milkweed plants (*Asclepias* spp.). Given that plants generally experience seasonal declines in quality, it is hypothesized that if climate change disrupts the timing of the larval stage relative to the availability of younger milkweed plants, monarch performance will be negatively affected. In this thesis, I explore the potential for negative consequences for the eastern monarch population due to potential shifts in the timing of their interaction with milkweed—due to phenological shifts in either species. I used field surveys around Ottawa, ON to determine monarch oviposition preference on common milkweed (*Asclepias syriaca*) plants and the seasonal availability of their preferred plants. To determine the potential consequences for monarch fitness where females oviposit on non-preferred plants, I conducted a field experiment to assess the effect of milkweed size on monarch larval performance. Based on field surveys, females preferentially oviposited on smaller milkweed plants in earlier developmental stages with low levels of discolouration. Plants in early developmental stages were consistently available in large proportion over the summer season. These results suggest that even if the relative timing of the monarch-milkweed interaction in the eastern population is shifted due to climate change, there will likely be suitable milkweed plants available for oviposition throughout the breeding season, which could act as a buffer to disruptions in the relative timing of the interaction. I found that bigger plants exuded more latex and had thicker leaves than smaller plants. However, larval performance was unaffected by these plant quality differences. While it is unclear how the

relative timing of the monarch-milkweed interaction will change in the future, my results suggest that shifts in the relative timing of their interaction within the breeding season are unlikely to have negative consequences for larval performance in eastern Ontario. Future studies should determine how the relative timing of the interaction will change in the region and explore how climate change will affect the quality of milkweed plants.

Résumé

En raison des récents changements climatiques, plusieurs espèces subissent des changements dans leur phénologie (dans la période temporelle de leurs événements biologiques récurrents), ce qui entraîne des perturbations dans la coordination des interactions entre celles-ci. Ces changements peuvent être particulièrement néfastes pour des consommateurs tels que les herbivores lors de ces interactions. Par exemple, sous sa forme larvaire, le papillon monarque (*Danaus plexippus*) est un herbivore spécialisé qui se nourrit d'asclépiades (*Asclepias* spp.). Étant donné que les plantes subissent généralement des fluctuations saisonnières de qualité, on suppose que si les changements climatiques perturbent la synchronicité entre la transition au stade larvaire du monarque par rapport à la disponibilité de jeunes plants d'asclépiades, la performance du monarque peuvent être affectée négativement. Dans cette thèse, j'explore les conséquences négatives potentielles d'un asynchronisme dans l'interaction de la population de monarques de l'est avec l'asclépiade. Pour étudier cette interaction, j'ai conduit des recherches sur le terrain dans la région d'Ottawa, en Ontario. Les visites terrains avaient pour objectif de déterminer les préférences de site d'oviposition du monarque sur sa plante hôte l'asclépiade commune (*Asclepias syriaca*) et de fournir un inventaire qualitatif sur la disponibilité saisonnière de cette plante. Pour déterminer les conséquences sur les caractères reproductifs du monarque où les femelles pondraient sur des plantes non optimales, j'ai mené une expérience sur le terrain pour évaluer l'effet de la taille de l'asclépiade sur la performance des larves de monarque. Basé sur des recensements sur le terrain, les papillons femelles ont démontré une préférence pour les plantes de petites tailles, avec peu de décolorations et dans leurs premiers stades de développement. Les plantes aux premiers stades de développement étaient constamment disponibles au cours de la saison estivale. Ces résultats suggèrent que même si le moment relatif

de l'interaction monarque-asclépiade dans la population de l'est est déplacé en raison des changements climatiques, il y aura fort probablement des plants d'asclépiades disponibles pour la ponte tout au long de la saison de reproduction, ce qui pourrait servir de tampon aux perturbations dans la synchronicité entre les deux espèces. J’ai trouvé que les plantes de plus grande taille sécrètent davantage de latex et ont des feuilles plus épaisses que celles de petite taille. Cependant, la performance des larves ne fut pas affectée par ces différences de qualité entre les plantes. Bien que l'on ne sache pas comment la synchronicité entre le monarque et l'asclépiade changera à l'avenir, mes résultats suggèrent que les changements dans le moment relatif de leur interaction au cours de la saison de reproduction sont peu susceptibles d'avoir des conséquences négatives sur la performance des larves dans l'est de l'Ontario. Les études futures devraient prendre en compte la potentialité d’alimentation compensatoire et explorer comment les changements climatiques affecteront la disponibilité saisonnière et la qualité des plants d'asclépiades.

Acknowledgements

I would like to thank my supervisor, Dr. Heather Kharouba, for her guidance and endless support throughout this project. I am truly grateful to have had such a caring, dedicated, and patient supervisor. Thank you to all past and present Kharouba lab members for their help, encouragement, and for enriching my time at the University of Ottawa, particularly Felipe Dargent, Stephanie Rivest, Sharla Foster, Sarah Dolson, Philippe Tremblay, and Samantha Lalonde. Thank you to Stephanie Rivest and Jérémie Bouffard for their enormous help with the statistical analyses. I also want to give a special thank you to my lab mate, former roommate, and one of my best pals, Sharla Foster, who kept me laughing through it all, from late nights of course work to long days in the field.

Thank you to our 2019 lab coordinator, Dr. Mary Ann Perron, for her help and constant words of encouragement during the second field season. Thank you to my field assistant, Manon Veselovsky, for her extremely hard work and friendship. I also want to extend my gratitude to all volunteers: the Veselovsky family, Samantha Lalonde, Emma Brown, Sam Cotton, Cory Hogan, Avery Friedlander, Annelise Gray, Christine De Baets, Zoe Perkins, and Oliver Rind.

Thank you to the Forrest and Sargent lab, along with my committee members, Dr. Jessica Forrest and Dr. Andrew Simons, for their time, insightful questions, and helpful feedback. Thank you to all staff members at the MacSkimming Outdoor Education Centre, including Kevin Wallace for graciously allowing me to conduct both experiments in their beautiful space. I want to thank NSERC and the Ottawa Field Naturalists' Club for generously helping fund this research. Finally, I want to thank my friends and family for their encouragement and support. To Mom, Dad, Andrew, and Jacob, thank you for your love and support in everything I do, including this.

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1. Introduction

Climate change is altering species' phenology (i.e., seasonal timing of recurring biological events) at different rates (Parmesan and Yohe 2003; Root et al. 2003; Thackeray et al. 2016; Cohen et al. 2018), leading to shifts in the relative timing of key life cycle events of interacting species (e.g., Visser and Holleman 2001; McKinney et al. 2012; Mayor et al. 2017). Over the past 35 years, the relative timing of key life cycle events of interacting species has shifted in magnitude by 6.1 days/decade on average, which is consistent with recent climate change (Kharouba et al. 2018). In some contexts, these shifts have led to negative impacts on the fitness of consumers (Visser and Holleman 2001; Kudo et al. 2004; Reed et al. 2013b) and ecosystem function (Miller-Rushing et al. 2010; Yang and Rudolf 2010) but it remains difficult to predict when these negative impacts are likely to occur.

The likelihood of a consumer's fitness being negatively impacted by shifts in the relative timing of key life cycle events with their resource depends on multiple factors (Miller-Rushing et al. 2010; Kharouba and Wolkovich 2020; Samplonius et al. 2021). One key factor is the importance of the interaction of the consumer with its resource: the degree to which the fitness of the consumer is limited by the resource (Cury et al. 2003; Miller-Rushing et al. 2010). The greater the dependency of a consumer on a single resource, the less buffer there is to mitigate the impacts of a disruption in the relative timing of interacting species (e.g., lack of ability to use a different species; Miller-Rushing et al. 2010).

Another key factor is the length of time the resource is available within a given year (Dunn et al. 2011; Kharouba and Wolkovich 2020; Samplonius et al. 2021 and references therein). For example, if the timing of the interaction is disrupted, there will be more of a buffer for the consumer if its resource has a wider seasonal distribution than it does (Durant et al. 2007;

Dunn et al. 2011). Despite being a critical factor, the seasonal availability of a resource is rarely quantified (Samplonius et al. 2021). When it has been, there is variation across studies. Some studies have found that the resource is seasonally limited relative to the consumer (e.g., Lepidoptera larvae as prey for great tits (*Parus major*), Reed et al. 2013a, 2013b; plants as a resource for greater snow geese (*Chen caerulescens atlantica*), Doiron et al. 2015) while others have not found this to be the case (e.g., aerial insects as prey for tree swallows (*Tachycineta bicolor*), Dunn et al. 2011; Lepidoptera larvae as prey for black-throated blue warblers (*Setophaga caerulescens)*, Lany et al. 2016). This variation across studies underscores the need for direct tests of the seasonal availability of resources.

For herbivorous insects, both the quantity and quality of food available is important, as the quality of many plant species varies seasonally (Mattson 1980; Schroeder 1986). Typically, plant nutrient levels (e.g., nitrogen) decrease while physical and chemical defense levels (e.g., cardiac glycosides, tannins) increase over the season through maturation and senescence (Schroeder 1986). If an insect herbivore emerges too late within a season, its fitness may decrease due to the availability of lower quality plants (Feeny 1970; van Asch and Visser 2007). Given seasonal plant quality differences, choosing high quality plants for laying eggs (i.e., oviposition) is important for herbivorous offspring fitness, as many young larvae are incapable of travelling to new hosts until they have fed on the host plant chosen by their mother (Futuyma et al. 1984).

Migratory species may be particularly sensitive to mismatches between the timing of their life cycle and resource availability. Migratory species must adjust the timing of their arrival

from often distant, wintering grounds – increasing the potential for consumer-resource mismatch. (Hurlbert and Liang 2012; Chmura et al. 2019). In addition, phenological shifts due to climate change have often been greater for long-distance migratory bird species than for short-distance or non-migratory bird species (Butler 2003; Rubolini et al. 2007b; Doxa et al. 2012; Gill et al. 2013); however, this is not always the case (Jonzén et al. 2006; Rubolini et al. 2007a; Sullivan et al. 2016). It is less clear if and how migratory insects are shifting their phenology in response to climate change.

The North American monarch butterfly (*Danaus plexippus* L.) is well known for its migration between breeding grounds in the United States and Canada and overwintering sites in Mexico (Urquhart and Urquhart 1976; Cockrell et al. 1993; Malcolm et al. 1993). As a migratory species, the timing of the monarch butterfly's life cycle (e.g., timing of arrival to breeding grounds, oviposition) is likely to shift due to climate change. Citizen science data suggests that there have been changes in the timing of monarch arrivals to, and departures from, their breeding grounds over the past two decades (Howard and Davis 2015; Zipf et al. 2017). Unfortunately, these studies were not able to disentangle phenological changes from population declines in the species (Brower et al. 2012; Vidal and Rendón-Salinas 2014; Pelton et al. 2016) and thus, more research is needed. However, non-migratory North American butterflies have shifted their phenology over recent decades (Forister and Shapiro 2003; Zografou et al. 2021).

Monarchs may also be vulnerable to mismatches between the timing of their life cycle and their host plant given their high degree of dependency on milkweed (family: Apocynaceae) and the seasonal constraints on milkweed quality. As milkweed specialists, monarchs use milkweed plants exclusively to complete key stages of their life cycle (i.e., oviposition, larval stage) throughout their range (Ackery and Vane-Wright 1984). Research on the western monarch

population shows that larval performance can be constrained by seasonal changes in host plant quality (Yang and Cenzer 2020; Yang et al. 2020). Specifically, it has been found that monarch larvae grew faster on younger plants (Yang et al. 2020), and that milkweed defensive traits increased as the plants aged (Yang and Cenzer 2020; Yang et al. 2020). While findings (Yang and Cenzer 2020; Yang et al. 2020) suggest that there is a limited window where plants of high quality are available, work conducted has been experimental. The degree to which seasonal changes in quality constrain larval performance can be influenced by the availability of high quality milkweed throughout the season, which has not yet been assessed for the monarch.

In this thesis, I explore the potential for negative fitness consequences for the eastern monarch population around Ottawa, ON, Canada due to potential shifts in the relative timing of their interaction with common milkweed (*Asclepias syriaca* L.) caused by phenological shifts of either species. Common milkweed is the most important host plant species for eastern monarchs in their summer breeding range (Malcolm et al. 1993; Pocius et al. 2018). The timing of flowering of common milkweed has been shown to be sensitive to temperature (3.93 days/°C; Howard 2018), suggesting that the timing of flowering could change with climate warming. Here I assess: i) monarch oviposition preference; ii) the seasonal availability of their preferred plants; and iii) monarch larval performance on plants of different sizes. It has been previously demonstrated that female monarchs prefer to oviposit on plants that allow their offspring to achieve the highest performance (i.e., 'mother knows best' hypothesis; Thompson 1988; Thompson and Pellmyr 1991; Gripenberg et al. 2010; Jones and Agrawal 2019). If the timing of oviposition changes due to climate change but high quality (i.e., preferred) plants are available throughout the season, then offspring fitness, as well as ovipositing adults, is less likely to be

affected. However, if preferred plants are not available when females are deciding where to oviposit, then females may have to oviposit on lower quality plants, which could have negative fitness consequences for them and their offspring.

I conducted field surveys around Ottawa, ON, Canada to determine which common milkweed characteristics best predict monarch oviposition preference and to describe the seasonal availability of these plants. I considered the size, developmental stage, and condition of plants as most research on monarch oviposition preference for the eastern population has focused on factors at the patch- (e.g., patch size, stem density) or landscape- (e.g., land use type) level, rather than plant-level (Oberhauser et al. 2001; Pleasants and Oberhauser 2013; Blader 2018; Pitman et al. 2018). Recent studies on the eastern population report that monarchs preferentially oviposit on younger milkweed plants (Fischer et al. 2015; He and Agrawal 2020).

To test the hypothesis that plant size affects leaf quality, I also measured how milkweed defensive traits differed across plant size. If leaf quality declines as plants develop as suggested by research on the western population (Yang and Cenzer 2020; Yang et al. 2020), then larvae should perform better on smaller plants. I assume here that host plant quality is more important than its quantity. Relatively little work has been done on the eastern population. One study on the eastern population found that some milkweed defensive traits (leaf thickness and trichome density) negatively affected larval performance but not all (Agrawal and Fishbein 2006). Determining how sensitive larval performance is to plant size will give us a better sense of potential outcomes for the eastern population of monarch due to climate change.

2. Methods

2.1. Study System

2.1.1 Monarch Butterfly

The monarch butterfly (*D. plexippus*) is a migratory species in the family Nymphalidae. The Canadian population is migratory with two distinct pathways (COSEWIC 2016), with the eastern migratory group (hereafter referred to as 'eastern population') being the largest and the focus of this study. The eastern population has declined by more than 80% over the past two decades (Brower et al. 2012; Semmens et al. 2016). This has been attributed to threats such as habitat loss at overwintering (Brower et al. 2002) and breeding grounds (Flockhart et al. 2015), climate change and extreme weather events (Batalden et al. 2007; Brower et al. 2012), and decreased breeding habitats due to increased urbanization along with the use of glyphosate herbicides on milkweed plants (Brower et al. 2012; Flockhart et al. 2015). As a result, monarchs in Canada are listed as *Endangered* by COSEWIC (2016) and *Special Concern* under the Species at Risk Act (currently under consideration for status change; pers. comm from Ken Tuininga, Canadian Wildlife Service, Environment and Climate Change Canada). In the U.S., monarchs are a candidate for listing under the Endangered Species Act in the United States (U.S. Fish and Wildlife Service 2020).

The eastern monarch population has a long-distance autumn migration that is completed in a single generation from breeding grounds in southern Canada to overwintering grounds in central Mexico. Once there, they congregate in the Oyamel fir (*Abies religiosa*) forests in the mountains (Urquhart and Urquhart 1976) until the following spring, when they return to their breeding grounds in the northern United States and southern Canada in 3-4 generations (Prysby and Oberhauser 2004; Solensky 2004; Flockhart et al. 2019). Within southern Canada, they produce 2-3 generations (Cockrell et al. 1993; Malcolm et al. 1993).

Female monarchs use a combination of visual and olfactory cues to detect a suitable host plant for oviposition (Garlick 2007). Once detected, they will typically lay one single egg on the underside of a leaf (Urquhart 1960; Zalucki and Kitching 1982) but will lay an average of 300 to 400 eggs throughout their lives (Oberhauser and Kuda 1997). Eggs hatch 3-4 days after they are laid and larvae undergo five instars (Urquhart 1960). The larval stage lasts 10 to 21 days and then pupation takes 9 to 15 days (Urquhart 1960; Oberhauser 2004). Resident adults in the summer will live from 2-5 weeks, while the migratory final generation (i.e., in September) may live up to 9 months (Oberhauser 2004).

Monarch butterflies use milkweed plants exclusively to oviposit on as adults and feed on as larvae throughout their range (Ackery and Vane-Wright 1984). There are several milkweed species found across North America but common milkweed *(A. syriaca)* is the most important species for the eastern monarch population (Malcolm et al. 1993; Pocius et al. 2018). In my study region, other milkweed species are extremely rare: based on previous surveys conducted by the lab over the summer of 2017 at 6 sites of similar habitat around Ottawa (see details in part 1 methods), neither swamp (*A. incarnata*) nor butterfly (*A. tuberosa*) milkweed were found (Kharouba et al. unpubl.).

2.1.2 Common Milkweed

Common milkweed (*A. syriaca)* is a perennial herbaceous plant that is native to eastern North America (Fernald 1950). They reproduce by seed and through rootstocks with adventitious buds located either on the stem base or along lateral roots (Bhowmik and Bandeen 1976). They can be found along roadsides, and within open grasslands and croplands (Bhowmik and Bandeen 1976). In eastern Canada, shoots emerge in spring from April to May and continue to grow until

mid-August to mid-September when they begin to senesce (Bhowmik and Bandeen 1976). Their flowering stage begins in late June or early August, depending on factors such as initial growth, weather, and location (Bhowmik and Bandeen 1976).

Milkweed plants contain a number of physical (e.g., trichomes, leaf toughness) and chemical (e.g., cyanides and alkaloids) defensive traits (Agrawal and Fishbein 2006). Within their stems and leaves, they contain toxic latex, which is made up of high concentrations of cardiac glycosides (also referred to as cardenolides) and cysteine proteases (Agrawal and Fishbein 2006). Milkweed species vary in their profile of defensive compounds. In comparison to other species, common milkweed has a moderate level of trichomes, latex, and cardenolides (Agrawal et al. 2015).

2.1.3 Monarch Larval Response to Milkweed Defense Strategy

As milkweed specialists, monarch larvae have evolved various mechanisms that allow them to overcome the milkweed's defensive compounds. Behaviourally, young instars will bite trenches through the leaves, which halts the flow of latex, whereas later instars will first sever the petioles or midribs of the leaves before ingesting them (Zalucki and Brower 1992). Physiologically, they have sodium-potassium channels that are resistant to cardenolides (Dobler et al. 2012). Larvae will also sequester cardenolides within their body tissues, which provides them with protection from their natural enemies (Brower et al. 1967).

The survival rate of first instars range between 3 to 40%, depending on the host plant species (Zalucki and Kitching 1982; Zalucki and Malcolm 1999; Zalucki et al. 2001b). This low survival rate is likely due to plant defensive traits such as trichomes, which can prevent young instars from accessing the leaf surface (Rathcke and Poole 1975), leaf toughness, which can

make it difficult for small mandibles to pierce the plant's cuticle (Clissold et al. 2009), and plant secretions, which can drown larvae (Zalucki et al. 2001b). Ultimately, the ingestion of milkweed poses a trade-off between toxicity and protection against natural enemies (Despland 2017).

2.2 Observational Study

2.2.1 Field Surveys

I surveyed common milkweed plants for monarch eggs at 24 sites around Ottawa, Ontario, Canada from June 12th to September 8th, 2019 (Figure 1). This period represented the majority of the monarch breeding season according to the range of adult sightings from Journey North citizen science data for the same year (May 27-October 10; Journey North, https://journeynorth.org/sightings/). I chose sites that were well distributed across the area, covering a 23 km north-south and a 70 km east-west axis (Figure 1). Sites also spanned a land use gradient, differing in their landscape cover of urban, agriculture, and forest (Figure 1). Each site was visited six times (i.e., every 12-14 days) within the season.

To survey milkweed plants, I placed a 1 x 1 m^2 quadrat at five random locations within a predetermined 100 m transect. Within each quadrat, I made observations on the presence and absence of monarch eggs and larvae on each milkweed plant. I also measured the size, vegetative and flowering developmental stages, and herbivory damage on each plant. Following similar criteria and categorical levels used by Fischer et al. (2015), I measured height (from the root crown to the apical leaf), number of leaves, developmental stage (prebud, bud, anthesis, post-anthesis), herbivory (estimated as a percentage of leaf area missing based on four levels: 0%, <5%, 5-25%, >25%), and leaf discolouration (estimated as the percentage of yellowing leaf area based on four levels: <5%, 5-40%, 41-80%, 81-100%). These variables were chosen because they have either been found to affect monarch oviposition in one of the populations (e.g., Cohen

and Brower 1982; Zalucki and Kitching 1982; Bergström et al. 1994; Dixon 2003; Fischer et al. 2015; Blader 2018; Knight et al. 2019; He and Agrawal 2020) or because they relate to offspring performance (Yang et al. 2020). Given the complex, rhizomatous, root system of milkweed plants, I defined an individual plant as any stem that was separated from another stem by soil (Kasten et al. 2016).

To supplement these patch-level monarch egg surveys, I performed broader visual surveys for eggs at each site. For these surveys, two observers began at a random location within the 100 m predetermined transect and walked for five minutes at a constant speed along the transect, checking every milkweed plant within one meter to their side for monarch eggs and larvae. If a monarch was found, we paused the timer and recorded its location on the milkweed and the milkweed's characteristics. We then resumed the survey until we reached a total of five minutes on the timer.

2.2.2 Statistical Analyses

To determine the milkweed characteristics that best predict monarch oviposition preference, I used a binomial generalized linear mixed-effects model (package 'lme4') (Bates et al. 2015). I modelled monarch egg occurrence (presence/absence) on milkweed plants from both quadrat and visual surveys as a function of the following fixed effects: plant height (cm), developmental stage (ordered factor; four levels), level of leaf discolouration (ordered factor; five levels), level of herbivory (ordered factor; four levels), and number of leaves. To account for the seasonal patterns in monarch egg occurrence, the linear and quadratic terms of day of year were also included (monarch occurrence was better predicted by a quadratic relationship; Table

1; Figure 3). Number of leaves, height, and day of year were scaled to improve model convergence. Non-linearity of number of leaves and height was assessed.

Non-independence between predictor variables was assessed using the following models: linear mixed models for continuous-continuous, and continuous-categorical combinations, and ordinal logistic mixed regression models for categorical-categorical combinations (Table S1). In models with leaf number as a response, it was square root transformed to improve normality. Site was included as a random effect in all models to account for the multiple observations per visit. Given the seasonal pattern in all milkweed-specific variables (Table S1), day of year (linear and quadratic terms) was included in all models as a fixed effect. An optimizer ('bobyqa') was used to improve model convergence.

Given that the majority of predictor variables showed non-independence (Table S1), I tested the influence of each predictor on monarch occurrence separately. Since the milkweed-specific variables showed seasonal patterns (Table S1), I tested whether milkweed-specific variables explained additional variation in monarch occurrence after accounting for day of year. To do so, I compared the fit of seasonal models (day of year, linear and quadratic terms) to a model that also included a milkweed-specific variable. To compare models, I used the difference in Second-Order Akaike information criterion (AICc; <2 dAICc) and a chisq test (χ^2). I also assessed the overall fit of each model using conditional R^2 ("r.squaredGLMM" function). Post-hoc comparisons between levels of categorical variables were conducted using the emmeans function with a Tukey adjustment in the package emmeans (Lenth et al. 2018). All statistical analyses were performed using R 3.3-1 (R Core Team 2018).

2.3 Field Experiments

2.3.1 Experimental Overview

To assess the response of monarch larval performance to plants of different sizes, I conducted a field experiment in an old-field habitat at the MacSkimming Outdoor Education Centre, Cumberland, ON, Canada (Figure 1; 45°31′19.3368″ N, 75°20′50.4636″ W). At this site, milkweed is naturally occurring and found in high densities (e.g., 299 stems within a 6x6m^2 area). The area was last mowed in 2011 for hay but has been left undisturbed since then.

To obtain plants of different sizes, in early summer 2018, I mowed one patch (5x5 m^2) on June 18th and one on June 25th. In early summer 2019, I mowed three patches (patch=6x6 m^2) in total: one patch on each of June 25th, July 2nd, July 9th. I used a gas weed trimmer machine (STIHL-FS131R). This resulted in two milkweed size treatment levels in 2018 (small and medium) and three milkweed size treatment levels in 2019 (small, medium, and big). Given the life history and growth form of common milkweed, I do not assume that age and size are correlated in this experiment. Mowing has been shown to cause other aspects of plant quality to change (e.g., cardenolides) in the few days immediately after mowing (Malcolm and Zalucki 1996) in addition to plant size. However, the use of mowing to manipulate plant size did not affect my ability to compare the response of larval performance to plants of different sizes since all treatments were equally affected by mowing. In addition, previous studies have found that adult monarchs have oviposition preference for regenerating plants that have recently been mowed (Fischer et al. 2015; Alcock et al. 2016; Haan and Landis 2019; Knight et al. 2019) and that there were no detrimental impacts on larval survival (Haan and Landis 2019).

A one-week interval was chosen because I wanted to ensure there were differences in plant size across treatments but minimize differences in environmental conditions experienced by the larvae. Recent milkweed mowing studies have varied their mowing treatments between

one week and a month (Fischer et al. 2015; Haan and Landis 2019; Knight et al. 2019). Initial plant height (2018: $F_{1,11}$=102.33, p<0.00001; 2019: $F_{2,42}$=430.39, p<0.00001) was significantly different across treatments. Initial plant height differences across treatment levels at the start of the experiments reflected the height distribution of milkweed found across my observational sites surveyed the following day in 2019 (*n*=7; see part I of thesis for description of surveys; Figure 2).

Although only a single patch per size treatment level per year was mowed, I conducted the same experiment twice: in 2018 and 2019. Initial milkweed height of the different treatment levels was similar between years (see experimental details; Figure 2). Moreover, the two experiments (i.e., 2018 and 2019) were started only 11 days apart (July 30th, 2018 and August 10th, 2019) so environmental conditions experienced by the monarchs were likely similar (Ottawa mean temperature in August 2018 was 21.6° C vs. 19.6° C in August 2019; Environment and Natural Resources Canada, https://climate.weather.gc.ca/historical_data/search_historic_data_e.html).

Monarch eggs came from wild adults that were locally captured for both experiments. Within an experiment, all eggs were introduced to all plants on the same date.

2.3.2 Experimental Details

2.3.2.1 Monarch rearing

In July 2018, I collected monarch eggs and larvae from one site (Vanessa Honey Equestrian Centre, 45°25′36.6564″ N, 75°58′25.5396″ W) in the Ottawa region and kept them in a growth chamber (Biochambers model LTCB-19) on a 21°C/25 °C 15:9 L:D cycle with a peak in light intensity at 12:00pm. These conditions were chosen to represent the average

environmental conditions in July for this region. I fed the larvae fresh milkweed leaves every 1-2 days until they reached pupation.

On August 3rd, 2019, I collected four wild adult monarch butterflies (female *n* = 2, male *n* = 2) from two sites in the Ottawa region (45°22′01.1784″ N, 75°39′06.9588″ W, 45°28′55.3″ N, 75°47′20.5″ W).

Upon pupation (2018) and capture (2019), I placed each adult pair (2018: 1 pair, 2019: 2 pairs) in an enclosure (90 x 60 x 60 Pop-up Cage, Watkins & Doncaster) in a courtyard at the University of Ottawa, Ottawa, Canada (45°25′16.3884″ N, 75°40′52.644″ W). In each enclosure, I placed two potted hybrid coneflowers (*Echinacea purpurea* 'PowWow White" and *Echinacea purpurea* 'PowWow Wild Berry') and two potted milkweed plants (*A. syriaca* and *A. tuberosa*). Mated females oviposited on both milkweed species on August 9th, 2019, and within 24 hours of oviposition, eggs were transferred to the experimental site.

2.3.2.2 Experiment in 2018

On July 30th, I chose 15 random plants from each experimental patch, identified them, and measured their individual heights and number of leaves. On each marked milkweed plant (*n*=30), I placed two eggs on the underside of two leaves, respectively. If both eggs on a given plant hatched (this only occurred three times), then within 1-3 days of hatching, I randomly chose one larva to remain on the plant, and the other was placed on a plant without a larva within the same treatment. I placed bamboo stakes into the ground directly next to the plant and positioned polyethylene insect rearing bags (71 x 48 cm and 100 x 66 cm, Bug Dorm) over the stake and plant to enclose the egg. I fastened the bags tight at the bottom and held them secure with 2-3 metal tent pegs per bag.

2.3.2.3 Experiment in 2019

On August 10th, I chose 20 random plants from each experimental patch, and measured their individual heights and number of leaves. On each identified plant (n=60), one egg was placed on the underside of a leaf from the second upper whorl using the latex from the milkweed as an adhesive. I evenly divided the eggs from the two adult monarch pairs amongst treatment levels. Each plant was then enclosed with a sandbag-style exclusion bag (120 x 70 cm) (Thomson et al. 2011). These bags were sewn (Singer Model 3232) from polyester fabric (~300 μm aperture) and the bottom part of the bag was filled with sand (Quikrete Play Sand) directly in the field using a funnel.

2.3.2.4 Performance measurements

Every 1-3 days, I took measurements of larval stage and length. I used three estimates of larval performance: larval development time (days from larval emergence to pupation), final larval length (mm, defined by the last larval length measurement taken before pupation), and survival from larval emergence until pupation. In the western monarch population, larval length is highly correlated with larval weight (R^2=0.97; mass=0.0223*length$^{2.9816}$, R^2=0.97; Yang and Cenzer 2020) and larval weight is generally strongly correlated with fecundity in insects (Leather 1988; Honēk 1993).

2.3.3 Leaf Quality

To determine the influence of plant size on leaf quality, I measured leaf thickness and latex exudation following methods from Agrawal and Fishbein (2006). Both leaf thickness (Agrawal and Fishbein 2006) and latex exudation (Malcolm and Zalucki 1996; Zalucki et al.

2001b, 2001a; Van Zandt and Agrawal 2004) have been found to affect monarch larval performance. I randomly selected eight *A. syriaca* plants (those without monarchs) from each treatment level on September 13th, 2019. For each plant, I measured their height and recorded leaf thickness (mm) using a digital micrometer (Accusize, Model # MD71-0001). It was measured as the median of two readings from the first and second lateral veins of a single leaf on the third whorl of every plant. I measured latex exudation by cutting 5 mm off the tip of a leaf and collecting the quantity of exuding latex on 42.5 mm pre-weighed filter paper. Once all latex was collected on a pre-weighed disc of filter paper, I placed it on top of another dry pre-weighed filter paper disc and brought to the University of Ottawa. These discs were subsequently dried at 60°C for 72 hours and then weighed again using a microbalance (Sartorius Research R 160 P Electronic Semi-Microbalance), which provided the latex weight (g).

2.3.4 Statistical Analyses

To determine if milkweed size influences larval performance, I ran 4 separate models for each year (8 models total) with the following response variables: larval development time (days), final larval length (mm), absolute growth rate, and survival from larval emergence until pupation (excluding egg hatch failure). I used the mean initial plant height on the first day of the experiment as a measure of plant size. As preliminary analyses suggested an effect of year (environmental conditions and the rearing methods were not identical between years), I treated these years as two experiments rather than two treatments of an experimental factor. I calculated absolute growth rate as the slope of length vs. experimental day (mm/day). I estimated it based on experimental day 6 for 2018 and day 18 and 19 for 2019. I chose these growth periods to maximize sample size and capture the rapid growth phase of larvae. In 2019, I was not able to

check larvae in all treatment levels on the same day, so I had to use different final experimental days for the big and medium (day 18) treatment levels vs. for the small (day 19) treatment level.

Where data did not meet model assumptions, generalized linear models were used, otherwise linear models were used. To model differences in larval development time and the absolute growth rate as a function of milkweed size, I used a gamma error distribution. For survival, I used a binomial error distribution.

To determine potential differences in leaf quality amongst plants of different sizes, I modelled latex exudation and leaf thickness as a function of average milkweed height for each treatment level using linear models. To compare differences between levels of categorical variables, I used a TukeyHSD pairwise comparison test.

3. Results

3.1 Observational Study

Over the summer of 2019, my field assistants and I surveyed 2,851 milkweed plants and observed 62 monarch eggs and 51 larvae at 21 out of 24 sites (88%; 4.71 $\pm$ 0.8 SE individuals observed per location). We observed monarchs throughout the entire sampling period. There was a significant peak in monarch egg observations (Table 1; Figure 3), which corresponded to the third sampling period (n=41, 66% of total observations; July 15th, 2019 to July 26th, 2019). The fewest immature monarchs were observed during the sixth sampling period (n=1, 2% of total observations; August 27th, 2019 to September 8th, 2019) (Figure 3). During monarch surveys, we also observed adults (n=72) from June 18th to August 27th, 2019.

The variables that predicted egg occurrence were the following, in order of decreasing influence: (1) developmental stage; (2) height; and (3) level of leaf discolouration (Table 1;

Figure 4). Egg presence was highest on plants in earlier developmental stages (prebud, bud), shorter plants, and plants with lower levels of leaf discolouration (0, <5%) (Table S2, Figure 4). The probability of monarch occurrence only differed between prebud and anthesis, bud and anthesis, and bud and post-anthesis; between <5 and 40-80% discolouration (Table S2; Figure 4). Neither percentage of herbivory nor leaf number predicted monarch occurrence (Table 1).

The seasonal pattern of progression through stages differed across milkweed characteristics. The proportion of plants in later developmental stages (i.e., anthesis, post-anthesis) increased through the season (Figure 4a). However, the majority of plants surveyed through the entire season were in early developmental stages (mean: 52%) (prebud, bud). Even in the last sampling period, 52% of plants were prebud. Plants peaked in height mid-season (Table S1; Figure 4b); however, shorter plants, as defined by the average size of prebud plants (48cm $\pm$ 23SD), were available throughout the season (Figure 4b). Leaf discolouration increased through the season (Figure 4c). Consequently, the availability of plants with low levels of discolouration (0, <5%) decreased through the season (Figure 4c). The proportion of plants with low discolouration (0, <5%) ranged from 1% to 88% (mean: 22%) through the season.

Peak number of egg observations occurred in the 3rd sampling period (July 15th to July 26th, 2019; Figure 3) when 48% of plants had low discolouration (0, <5%), 57% of plants were in early developmental stages (prebud, bud; Figure 4), and the mean height of plants was 57 cm $\pm$ 1.7 SE.

3.2 Field Experiments (2018 and 2019)

Egg hatch failure within treatment levels was 27% in 2018 and varied between 10-35% across treatment levels in 2019. Survivorship from larval emergence to pupation varied from 45-

73% across treatment levels in 2018 and 77-94% in 2019. As expected, these values are high compared to the survival rates of naturally occurring monarchs (e.g., 12% from egg to pupation; Borkin 1982) but comparable to experiments that confined larvae with mesh bags as I did here (72% survival rate; Zalucki et al. 2001b).

Milkweed height did not have an effect on any of the larval performance estimates in either year (Table 2, Figure 6, 7).

Latex exudation ($F_{2,21}$=16.91, p<0.0001) and leaf thickness ($F_{2,21}$=34.6, p<0.0001) differed across milkweed size treatment. There was 76% more latex exuded from leaves on big plants than small (3.78 mg, $t_{21,2}$=5.78, p<0.0001) and 45% more than medium plants (1.37 mg, $t_{21,2}$=3.44, p=0.007) (Figure 8a). Big plants also had 33% thicker leaves than small (0.235 mm, $t_{21,2}$=7.85, p<0.0001) and 26% thicker leaves than medium plants (0.105 mm, $t_{21,2}$=6.31, p<0.0001) (Figure 8b).

4. Discussion

Using field observations and experiments, this study explored the effect of milkweed size, developmental stage and condition on monarch oviposition preference and larval performance. I found that plant developmental stage and condition influenced the number of eggs found on milkweed plants and that these plant types are available through the breeding season in Ottawa, Ontario. In my field experiment, I found that despite larger plants exuding more latex and having thicker leaves than smaller plants, larval performance was not reduced on larger plants.

4.1 Oviposition Preference

I found more eggs on shorter milkweed plants in earlier developmental stages (i.e., prebud, bud), and with less discolouration (i.e., fewer yellow leaves). This suggests that females prefer ovipositing on these plant types. However, to know with greater certainty that female monarchs prefer these plant types for oviposition, future researchers could conduct an experiment where females are given plants in different stages and conditions and allowed to choose a plant to oviposit.

I found more eggs on plants in earlier stages of development (i.e., prebud, bud) than those in later stages (i.e., anthesis, post-anthesis). My results are consistent with other studies that found monarchs prefer to oviposit on milkweed plants in earlier stages of development (*A. curassavica* and *A. fasicularis*: Dixon et al. 1978; *A. curassavica, A. fruticosa, A. physocarpa*: Zalucki and Kitching 1982; *A. syriaca*: Bergström et al. 1994). Bergström et al. (1994) linked this preference to the attractiveness of the volatile compounds emitted from young milkweed plant leaves compared to old milkweed plant leaves. The oviposition preference of monarchs for milkweed plants in earlier stages of development may be due to the decline in plant nutritional quality over the season, as observed on other milkweed species in California (Yang and Cenzer 2020; Yang et al. 2020), and for other plant species (Mattson 1980). As plants develop over a season, their defensive traits will increase while their nutritive content will decrease, thus causing a decline in their overall quality (Mattson 1980; Schroeder 1986).

Similarly, I found more eggs on milkweed plants with less discolouration. Other studies have also found leaf discolouration to be an important factor determining oviposition preference for monarch butterflies (Fischer et al. 2015; Blader 2018). The degree of leaf discolouration typically indicates how close a plant is to senescence (i.e., the final stage of plant development).

Senescence is characterized by changes in the metabolism and gene expression which leads to the degradation of chlorophyll in chloroplasts of older plants (Hill 1980), leading to the yellowing of leaves (Ougham et al. 2005). The process of senescence causes physiological changes such as the breakdown of proteins, redistribution of nutrients (e.g., nitrogen), and the inhibition of protein synthesis (Hill 1980; Guiboileau et al. 2010). Leaf discolouration can also indicate disease (e.g., pathogen infection) (Häffner et al. 2015), herbivory (e.g., milkweed stem weevil (*Rhyssomatus lineaticollis*) larvae) (Agrawal and Van Zandt 2003), nutrition deficiency (Noodén et al. 1997), or competition (which also causes nutrient deficiency) (Noodén et al. 1997). These factors can all decrease plant quality for herbivores (Mattson 1980). Given that levels of herbivory and leaf discolouration were independent (plants with greater discolouration were not plants with greater herbivory; Table S1) in my study, it is less likely that herbivory led to the observed milkweed leaf discolouration.

I also found more eggs on shorter plants. This is consistent with most previous studies that found monarchs prefer to oviposit on shorter plants (Zalucki and Kitching 1982; Baker and Potter 2018; Knight et al. 2019), though not all of these studies directly tested the effect of height (Zalucki and Kitching 1982: compared milkweed species of different heights , Knight et al. 2019: confounded height and disturbance). However, others have shown preference for taller plants (Cohen and Brower 1982; Malcolm and Brower 1986). When plants are grown from seed, size and age are generally correlated. As leaf quality has been shown to decline as plants age in other milkweed species (Yang and Cenzer 2020; Yang et al. 2020), it is also thought to decline with size.

I did not find an effect of leaf number on the occurrence of eggs on milkweed plants. To my knowledge, no other study has considered the effect of milkweed leaf number on oviposition

preference. However, milkweed plant biomass has been shown to affect developmental success of monarch larvae on younger plants (Yang et al. 2020). More leaves provide larva with more food, which is important for young instars that are often incapable of travelling to a new host until they are older (Futuyma et al. 1984; Fisher et al. 2020). Other measures of plant size, such as total leaf area, may be a better predictor of monarch oviposition preference on common milkweed. For example, Cohen and Brower (1982) found that the number of monarch eggs was positively correlated with milkweed total leaf area. Plants with larger leaves may be favoured by ovipositing monarchs given that larger leaves could provide their offspring with greater protection from direct sunlight, thus reducing the potential risk of desiccation (Cohen and Brower 1982). In any case, my results suggest that a simple measure of plant size like number of leaves is not the best predictor of oviposition preference on common milkweed in this area.

I also did not find a relationship between oviposition preference and herbivore damage. This result differs from previous studies which have found fewer monarch eggs (He and Agrawal 2020), and less oviposition in other Lepidoptera (*Trichoplusia ni*; Coapio et al. 2016), on herbivore-damaged plants. However, He and Agrawal (2020) did not quantify how much milkweed plants were damaged by herbivory in their damaged treatment. Therefore, it could be that the levels of herbivory in my study were not high enough on the plants at the time of oviposition to deter females. As plants experience increasing damage from herbivory, their quality as a food source for herbivorous larvae decreases (Karban and Baldwin 1997). Following herbivory, a plant often undergoes changes in their toxicity, leaf toughness, and/or trichome density (Karban and Baldwin 1997). This process increases the plant's defense against further damage and consequently can negatively affect subsequent herbivores (Van Zandt and Agrawal 2004). For example, common milkweed has been shown to increase latex production in response

to herbivory damage (Van Zandt and Agrawal 2004). Monarch larvae that fed on these herbivore-damaged plants developed to be 20% smaller than those reared on undamaged plants (Van Zandt and Agrawal 2004).

4.2 Seasonal Availability of Preferred Milkweed Plants

The seasonal availability of preferred plants differed depending on the characteristic. The availability of plants with low levels of discolouration (<5%) decreased through the season. However, the majority of plants surveyed throughout the season were in early developmental stages (i.e., prebud, bud) and were short (<50 cm; as defined by plants in a prebud stage). Therefore, regardless of the two-week window, plants with preferred characteristics were available, and in large proportion, throughout the season.

These results suggest that even if the relative timing of the monarch-milkweed interaction in the eastern population is shifted due to climate change, there will likely be suitable milkweed plants available for oviposition throughout the breeding season in this region. This seasonal availability of preferred plants could act as a buffer and mitigate the impacts of a disruption in the timing of the interaction with milkweed on the monarch. To my knowledge, this is the first assessment of seasonal availability of preferred plants for the monarch. Anecdotal observations of milkweed communities in Northern California suggest that milkweed plants there are more seasonally synchronized than in this region (i.e., young plants are only available early in the season; L. Yang, pers. comm.). As such, further study of seasonal patterns in milkweed development across other regions and populations of the monarchs is needed. In particular, future studies should broaden the types of sites surveyed and consider all habitats that have been previously found to be important for monarch oviposition (Oberhauser et al. 2001; Pleasants and

Oberhauser 2013; Blader 2018; Pitman et al. 2018). This will provide a greater understanding of the seasonal availability of milkweed across a larger landscape.

4.3 Larval Performance

I found that larger plants exuded more latex and had thicker leaves than smaller plants. This is consistent with other studies that have found that milkweed species, including common milkweed, increase the amount of latex exuded and other defensive traits over their ontogeny (Zalucki and Brower 1992; Agrawal 2005; Yang and Cenzer 2020). However, the higher expression of these defensive traits in larger plants did not translate into reduced larval performance on larger plants. This is inconsistent with previous work in the system. Other experimental studies have found higher larval performance on younger milkweed plants (Dixon et al. 1978; Yang and Cenzer 2020; Yang et al. 2020). My results are also inconsistent with studies that found higher latex and/or cardenolide concentrations (Zalucki et al. 1990, 2001a; Zalucki and Brower 1992; Malcolm and Zalucki 1996) and leaf thickness (Agrawal and Fishbein 2006) in milkweed plants directly reduce monarch larval performance.

My results may be inconsistent with previous experiments because of differences in the type and degree of manipulation used across experiments. First, previous studies (Yang and Cenzer 2020; Yang et al. 2020) directly tested the effect of plant age on performance since they propagated their plants from seed. However, I was not able to control for plant age with mowing. It could be that there are multi-generational effects on plant quality in common milkweed such that plant age and size are not well correlated (e.g., small, old plants). Additionally, mowing has been shown to affect this relationship. Dee and Baum (2019) found that when milkweed plants were aged in areas that had different mowing regimes, younger plants in less frequently mowed

areas had more stems and more flowering stems than younger plants in more frequently mowed areas. Second, previous studies used larger differences between plant treatment levels than I did (e.g., plants were a month apart in age: Yang et al. 2020; plants were 20-22 days apart in age: Yang and Cenzer 2020). Therefore, it is possible that the plant quality differences between my treatment levels were not great enough to affect larval performance.

Although there were differences in leaf quality, one reason why larval performance was not affected by plant size in this study may be that larvae compensated for lower leaf quality in the large size treatment level by consuming more leaves (i.e., compensatory feeding; Slansky and Wheeler 1991) Herbivores can exhibit compensatory feeding in several ways, including increasing their consumption of lower quality plants (i.e., plants with lower nitrogen and protein levels) to accumulate enough nutrients for growth and development (Slansky and Wheeler 1991). This strategy has been previously demonstrated in monarch larvae reared on common milkweed plants with lower nitrogen levels (Lavoie and Oberhauser 2004). Larvae reared on these lower quality plants had the same weight and development time as those reared on plants with higher nitrogen levels (Lavoie and Oberhauser 2004). In my study, the consumption of more leaves could have increased the performance of larvae in the large plant size treatment to the same level as the small plant size treatment, thus resulting in no difference in performance between treatment levels. Future studies should consider the potential for compensatory feeding and measure the amount of plant biomass consumed by each individual.

Finally, there are several factors that may have affected my ability to detect an effect. Firstly, I measured larval growth and development every 1-3 days, which could have decreased the precision of my estimates of final larval length and development time. Second, there were a high number of egg hatch failures, particularly in 2018 (average egg hatch failure: 26.6% in

2018, 22.5% in 2019), which decreased total sample size (Table 2), also decreasing the precision of my estimates of performance. Deploying more eggs to account for high hatch rate failure would have alleviated this issue. However, given the low abundance of monarchs in the region, there were constraints on the number of monarch eggs available in a short period of time. Finally, my experiment did not have true replication (i.e., multiple replicates per treatment level). True replication would have helped to obtain a better estimate of experimental error.

While it is unclear how the relative timing of the milkweed-monarch interaction will change in the future, my results suggest that shifts in the relative timing of their interaction within the breeding season may not have negative consequences for larval performance in Eastern Ontario. My results suggest that even if ovipositing monarchs are unable to find their preferred plants because of disruptions to the relative timing of their interaction with milkweed, larval performance may not be affected. However, further investigation on the potential effects of shifts in the relative timing of the milkweed-monarch interaction on monarch performance is needed. In particular, future work should consider the effects on adult performance, use seed-grown milkweed plants so that exact plant age is known, and avoid mechanical-induced changes in plant quality via chemistry.

4.4. Relationship Between Preference and Performance

Using a variety of visual and olfactory cues (Garlick 2007), ovipositing monarchs are thought to locate and then lay their eggs on high quality host plants that will allow their offspring to achieve the highest performance (Jones and Agrawal 2019). My results from the observational study suggest that females prefer to oviposit on shorter milkweed plants. Therefore, I should

have found higher performance on shorter plants. The plants in the small (32 cm ± 2.8 SE) and medium (52.7 cm ± 3.5 SE) treatment levels in the experiment were smaller than the size of the plants defined as prebud (47.64 cm ± 0.6 SE), and in-between the prebud and bud (72.2 cm ± 0.8 SE) plants in the observational study, respectively. Therefore, it could be that the experimental plants in the small and medium size treatments were too early in their development to have a differential impact on larval performance, as predicted by the oviposition preference study. Alternatively, it could be that ovipositing monarchs select common milkweed plants that reduce predation risk for their offspring rather than selecting them for their food quality (e.g., Haan and Landis 2019). Monarch predators (e.g., ants, arachnids, beetles, and true bugs) have been found to be more abundant on older milkweed plants than younger plants (Haan and Landis 2019). As my experiment controlled for predation, future studies should explore the relative importance of predation on larval performance, and measure oviposition preference and larval performance on the same plants.

5. Conclusions

Using a combination of field surveys and experiments, I explored monarch oviposition preference on common milkweed and the seasonal availability of these preferred plants. I also conducted field experiments to assess the effect of milkweed size on monarch larval performance. Determining how the availability of these preferred plants vary throughout the season and how monarch larval performance may be affected by feeding on non-preferred plants is essential for understanding how the eastern monarch population could be affected by climate change induced shifts in the timing of their interaction with milkweed in northern breeding grounds like eastern Ontario. My results suggest that even if climate change alters the timing of

the monarch's larval stage relative to milkweed development in this region, monarch larval performance is unlikely to be negatively affected. Future studies should expand this work to see whether adult performance could be impacted. It is also unknown how climate change will affect the seasonal availability and quality of milkweed plants.

In North America, milkweed populations have declined in recent decades due in part to land-use changes such as increased urbanization (Pleasants 2017; Lalonde et al. In review) and the use of glyphosate herbicides (Hartzler 2010; Pleasants and Oberhauser 2013). In an effort to increase milkweed populations, several milkweed restoration programs have been created and implemented throughout North America (e.g., Monarch Watch, University of Kansas). My findings suggest that shorter milkweed plants in earlier developmental stages are important for monarch oviposition in Eastern Ontario. Therefore, conservation programs in these areas should ensure there are plants in earlier developmental states when planning and implementing restoration projects.

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Tables

Table 1. The relative importance of milkweed characteristics in predicting monarch occurrence (presence and absence) on milkweed plants (*n*=2,851 surveyed plants, *n*=24 sites). Model fit was compared between a seasonal model (i.e., day of year and day of year^2) and a model that also included a milkweed-specific variable (leaf discolouration (%), herbivory (%), developmental stage, height (cm) and leaf number). Models are listed in order of decreasing influence as measured by the difference in AICc (dAICc). Also shown are conditional R^2 values.

Model	Estimate (SE)	dAICc	Chisq	P value (df)	R^2
Day of year + Day of year^2	Linear: -0.27 (0.4)	0	0.44*	0.51 (1)*	0.13
	Quadratic: -21.73 (3.14)	0	70.1**	<0.0001 (1)**	0.37
+ Developmental stage	N/A	23.39	29.39	<0.0001 (3)	0.59
+ Height (cm)	-1.61 (0.54)	6.82	8.82	0.003 (1)	0.52
+ Leaf discolouration (%)	N/A	5.71	13.71	0.01 (4)	0.51
+ Herbivory (%)	N/A	-0.68	5.32	0.15 (3)	0.51
+ Leaf number	2.28 (1.68)	-0.37	1.62	0.2 (1)	0.5

*Model fit is relative to an intercept-only model
**Model fit is relative to linear-only model

Table 2. Results from generalized linear and linear models predicting monarch performance based on milkweed size. Monarch performance was estimated as: development time (days), final larval length (mm), absolute growth rate (mm/days), and survival for both 2018 and 2019 experiments. Milkweed size is represented by size treatment levels. Generalized linear models were used for larval development time (gamma error distribution), absolute growth rate (gamma error distribution) and survival (binomial error distribution) and linear models were used for final larval length.

Year	**Response variable**	**Sample size (n)**				**Test statistic**	**P value (df)**
		Small treatment level	Medium treatment level	Big treatment level	Total sample size		
2018	Development time (days)	8	5	N/A	13	$\chi^2 = 0.01$	0.93 (1)
	Final larval length (mm)	8	5	N/A	13	$F_{1,11} = 0.06$	0.82
	Absolute growth rate (mm/day)	8	8	N/A	16	$\chi^2 = 2.09$	0.15 (1)
	Survival	8	5	N/A	13	$\chi^2 = 1.72$	0.19 (1)
2019	Development time (days)	18	17	10	45	$\chi^2 = 0.45$	0.5 (1)
	Final larval length (mm)	18	17	10	45	$F_{2,43} = 0.97$	0.33
	Absolute growth rate (mm/day)	20	17	11	48	$\chi^2 = 1.46$	0.23 (1)
	Survival	18	17	10	45	$\chi^2 = 0.98$	0.32 (1)

Figures

Figure 1. Location of observational survey sites (*n*=24; red dots) and experimental site (*n*=1; yellow diamond), and major land use types around Ottawa, ON, Canada. Axes represent the latitude and longitude coordinate plane (UTM coordinate system, meters).

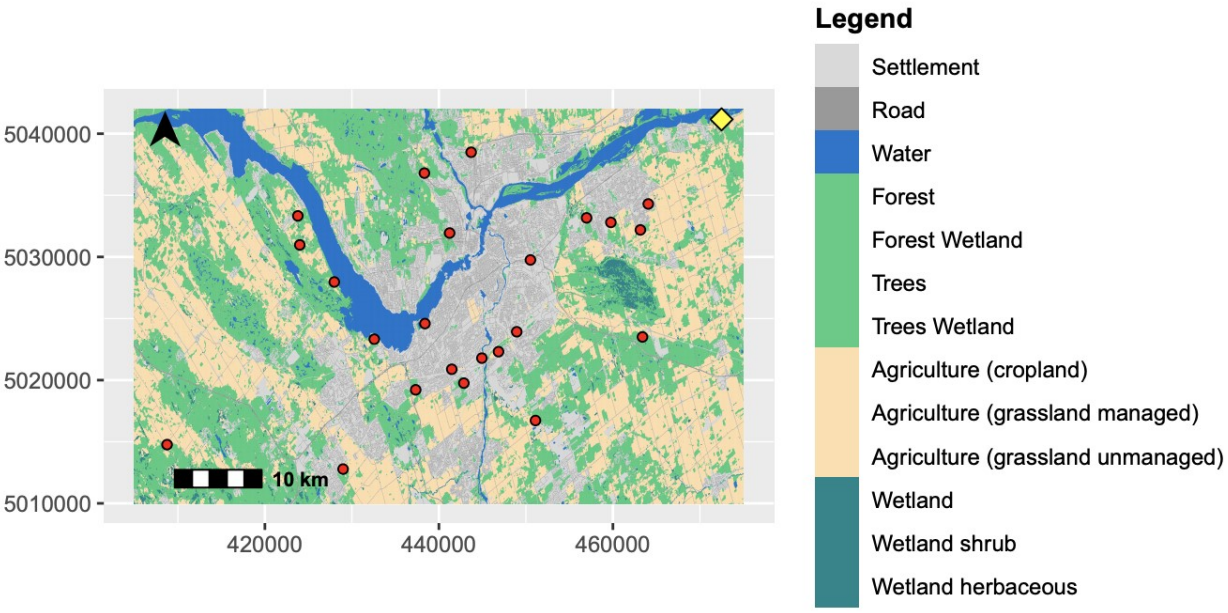


Figure 2. The height distribution of common milkweed from observational surveys in 2019 and initial heights from the field experiment in early August 2018 and 2019. Shown in grey is the height distribution (cm) from milkweed (*n*=114 plants) measured at seven sites around Ottawa on August 11th, 2019. Red, blue and black vertical lines represent the mean initial heights at the start of experiments from the small, medium and big plant treatment levels, respectively. Solid lines are mean initial heights at the start of the experiment on July 30th, 2018 (small plants (red) 39 cm ± 1.33 SE, medium plants (blue): 59 cm ± 0.75 SE). Dashed lines are mean initial heights at the start of the experiment on August 10th, 2019 (small treatment plants (red): 32.0 cm ± 2.8 SE, medium treatment plants (blue): 52.7 cm ± 3.5 SE, big treatment plants (black): 72.9 cm ± 4.4 SE).

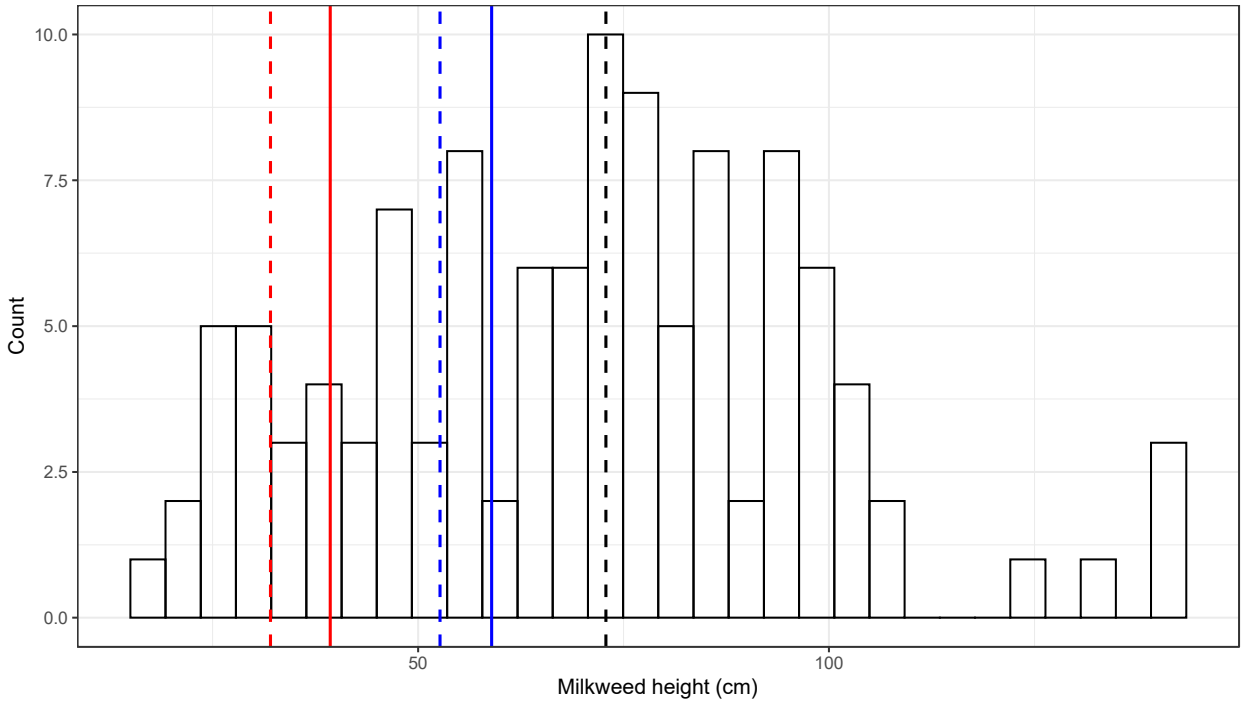


Figure 3. The number of monarch eggs observed (*n*=62) across the season. Shown are monarch egg counts for every 12-to-14-day sample period.

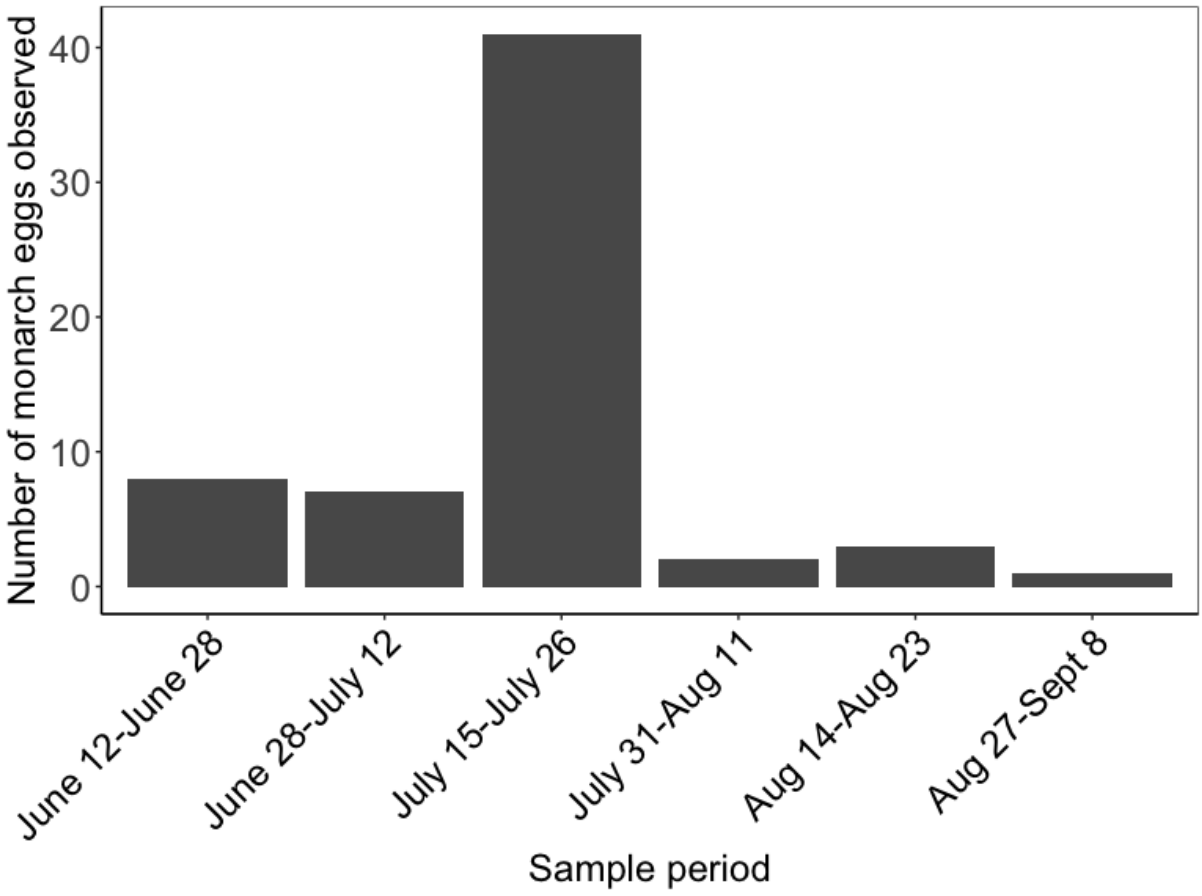


Figure 4. Common milkweed characteristics surveyed that best predict the occurrence of monarch eggs at 24 sites in Ottawa, Ontario. Predicted probability of egg occurrence on milkweed plants based on (a) developmental stage, (b) height (cm), and (c) level of leaf discolouration (%). In panels (a,c), shown are the predicted mean values (red dots) with 95% confidence intervals based on conditional effects of the model. Panel b shows the line of best fit with 95% confidence intervals based on the conditional effects of the model. Models were fit with day of year and its quadratic term (Table 1). Panels are ordered based on their relative importance in predicting monarch egg occurrence on milkweed plants (Table 1). Letters and stars represent significant pairwise comparisons ($p<0.05$; Table S2). Raw data is not shown to improve visual interpretation of figures.

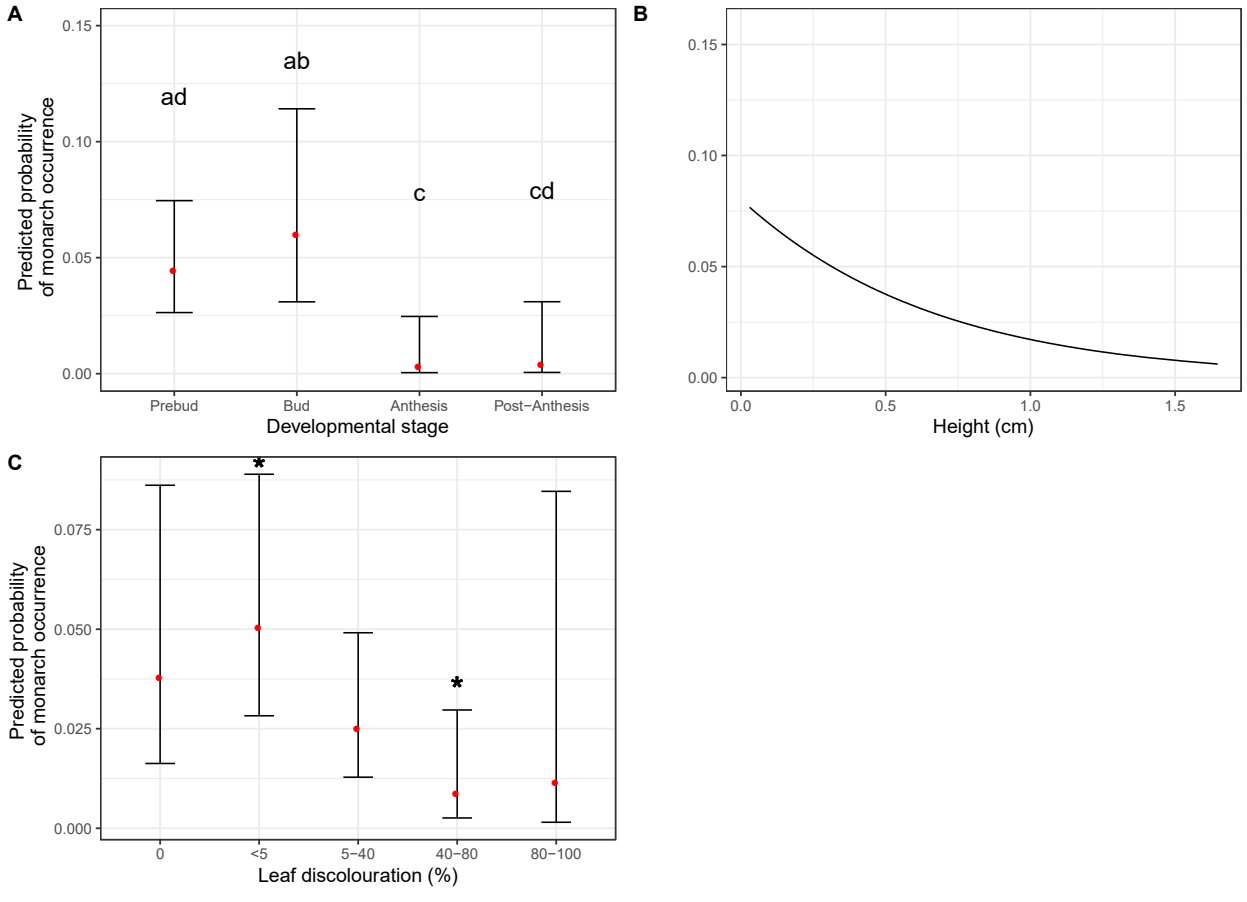


Figure 5. Seasonal patterns of common milkweed characteristics based on two-week sample periods (*n*=2,851 plants). Shown is the proportion of plants in each level of (a) development stage and (c) leaf discolouration (%). Panel (b) shows the mean value of plants in each level of height (cm). Panels are ordered based on their relative importance in predicting monarch egg occurrence on milkweed plants (Table 1).

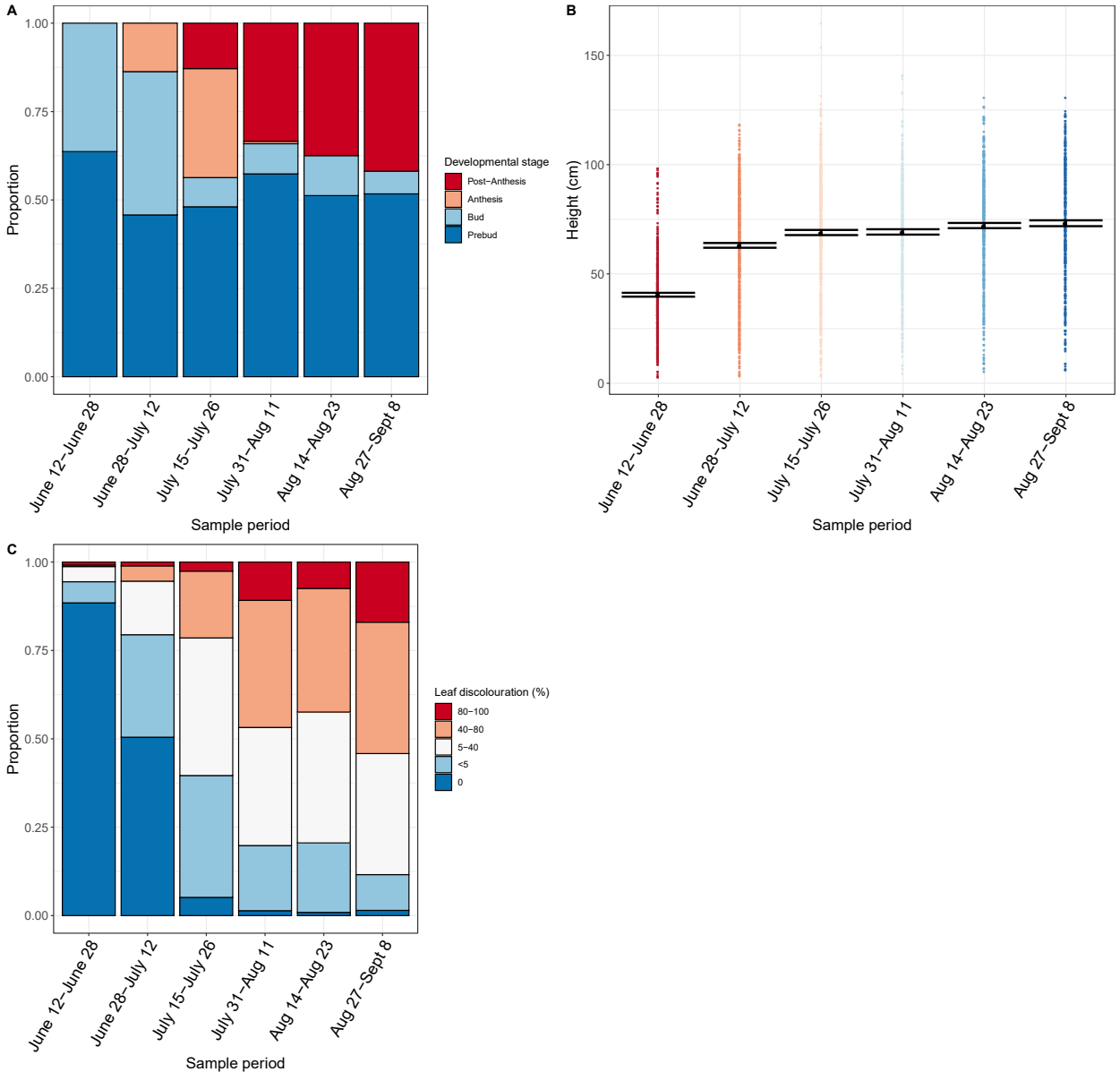


Figure 6. The effect of milkweed height (cm) on monarch larval performance in 2018 (Table 2). Larval performance is measured as development time (days) (a), final larval length (mm) (b), survival (c), and absolute growth rate (mm/day) (d). Milkweed height is represented as mean initial height per treatment level. Shown are predicted mean values (red dots) with 95% confidence intervals and individual data points (black dots, jittered).

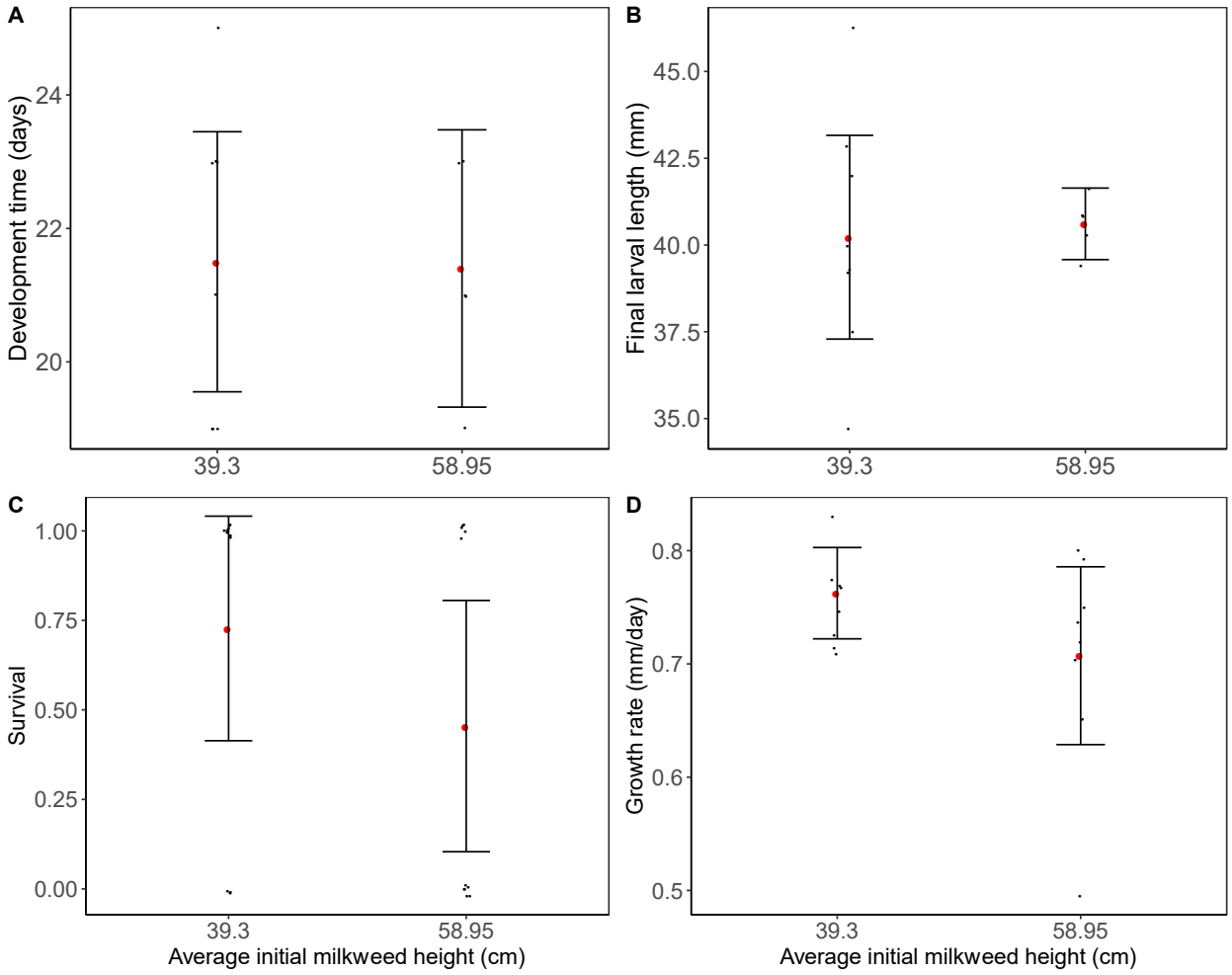


Figure 7. The effect of milkweed height (cm) on monarch larval performance in 2019 (Table 2). Larval performance is measured as (a) development time (days), (b) final larval length (mm), (c) survival, and (d) absolute growth rate (mm/day). Milkweed height is represented as mean initial height per size treatment level. Shown are predicted mean values (red dots) with 95% confidence intervals and individual data points (black dots, jittered).

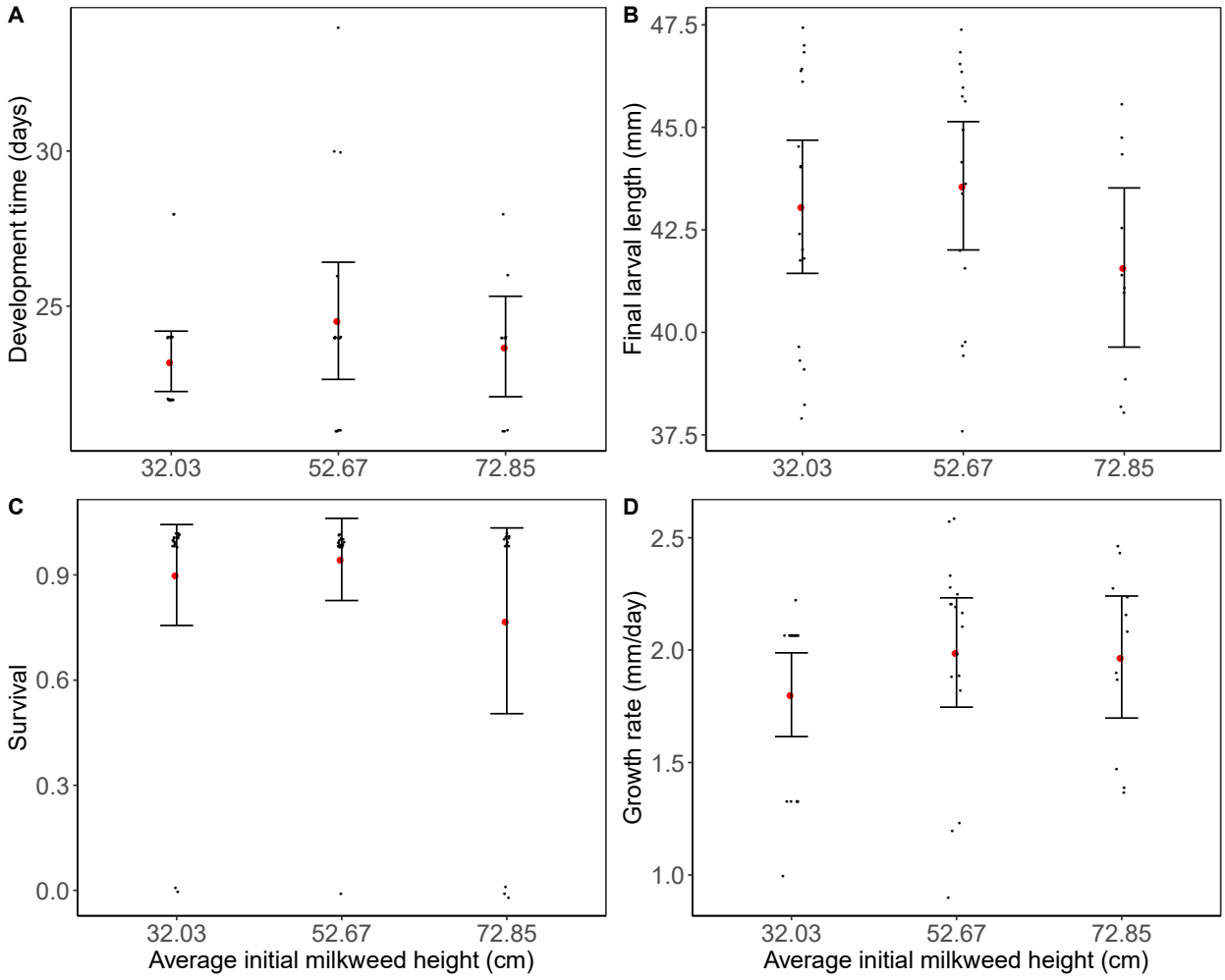
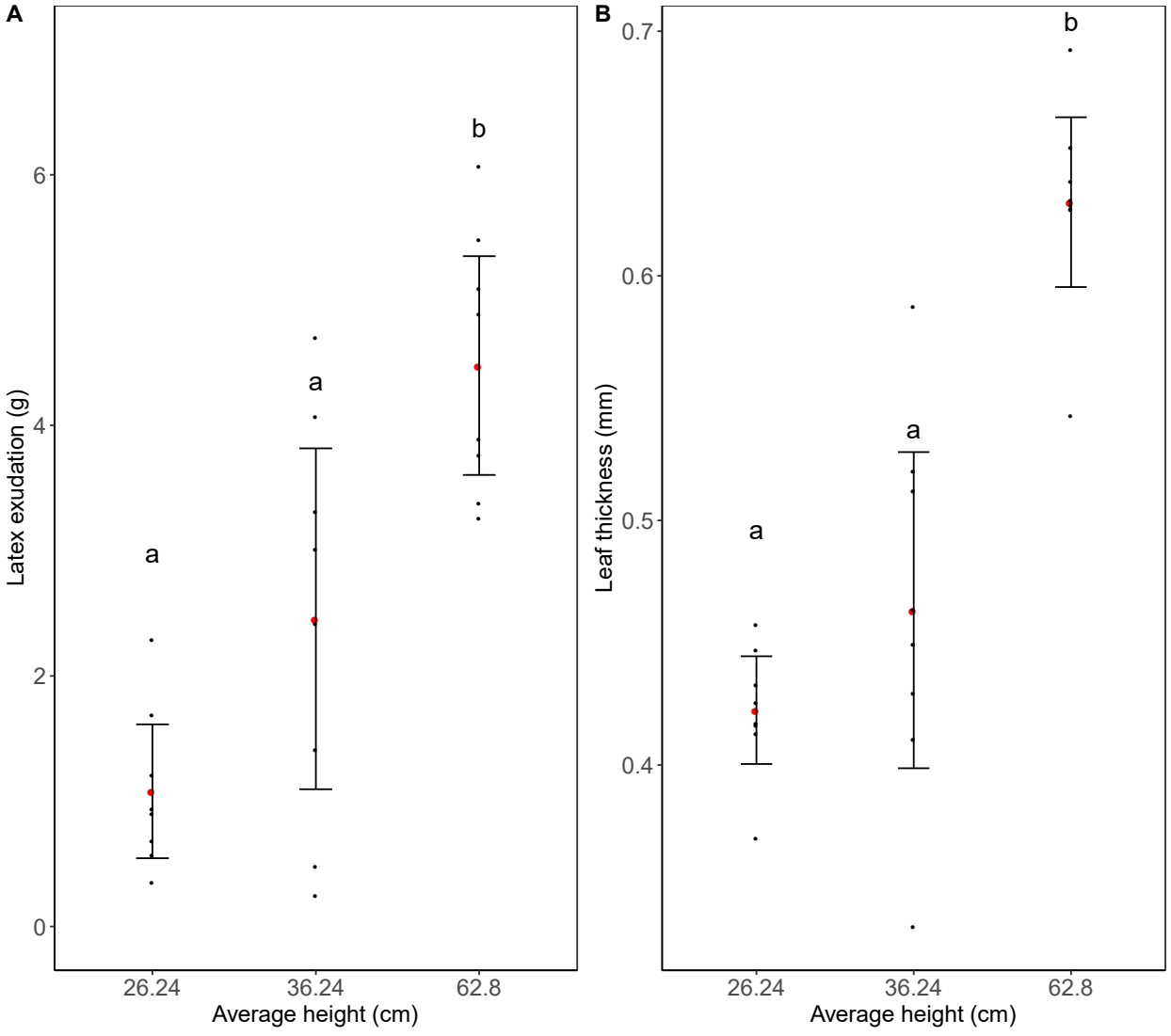


Figure 8. The effect of common milkweed plant size on leaf quality (*n*=8 plants per treatment level). Leaf quality is measured as (a) latex exudation (g) and (b) leaf thickness (mm) (b). Milkweed size is represented as mean height (cm) per treatment level. Heights were measured on September 13th, 2019. Shown are mean values (red dots) with 95% confidence intervals and individual data points (black dots). Letters represent significant pairwise comparisons ($p<0.05$).



Appendix

Table S1. Test of non-independence between milkweed characteristic predictor variables. Relationship between milkweed-specific variables and day of year (linear and its quadratic term), also shown. Each row represents an individual model. All models included day of year (linear and quadratic term) as a fixed effect and site as a random effect. Test statistics and model types are also shown.

Variable 1 (response)	Variable 2 (predictor)	Test statistic	P value	Variable types	Model
Height (cm)	Day of year + Day of year^2	LRT=198.8	<0.0001	Continuous vs. continuous	Linear mixed model
	Leaf number	$F_{1,2824}$=1169.25	<0.0001	Continuous vs. continuous	Linear mixed model
	Developmental stage	$F_{3,2822}$=738.39	<0.0001	Continuous vs. categorical	Linear mixed model
	Herbivory	$F_{3,2822}$=15.2	<0.0001	Continuous vs. categorical	Linear mixed model
	Leaf discolouration	$F_{4,2821}$=14.63	<0.0001	Continuous vs. categorical	Linear mixed model
Leaf number	Day of year + Day of year^2	LRT=525.41	<0.0001	Continuous vs. continuous	Linear mixed model
	Developmental stage	$F_{3,2822}$=85.95	<0.0001	Continuous vs. categorical	Linear mixed model
	Herbivory	$F_{3,2822}$=15.52	<0.0001	Continuous vs. categorical	Linear mixed model
	Leaf discolouration	$F_{4,2821}$=16.89	<0.0001	Continuous vs. categorical	Linear mixed model
Developmental stage	Day of year + Day of year^2	LRT=55.94	<0.0001	Categorical vs. continuous	Ordinal logistic mixed regression
	Herbivory	χ^2 =17.54	<0.001	Categorical vs. categorical	Ordinal logistic mixed regression
	Leaf discolouration	χ^2=40.7	<0.0001	Categorical vs. categorical	Ordinal logistic mixed regression
Herbivory	Day of year + Day of year^2	LRT=6	0.01	Categorical vs. continuous	Ordinal logistic mixed regression
	Leaf discolouration	χ^2=5.87	0.209	Categorical vs. categorical	Ordinal logistic mixed regression
Leaf discolouration	Day of year + Day of year^2	LRT=3.15	0.07	Categorical vs. continuous	Ordinal logistic mixed regression

Table S2. Post-hoc Tukey pairwise comparison results associated with Figure 4, the relationship between egg occurrence and milkweed plant characteristics. Estimates are on the logit scale and not response scale. Significant comparisons ($p<0.05$) are in bold.

Predictor variable	Comparisons between levels	Estimate ± SE	Z ratio	P value
Leaf discolouration (%)	0 and <5	-0.3 ± 0.38	-0.78	0.94
	0 and 5-40	0.42 ± 0.45	0.93	0.88
	0 and 40-80	1.49 ± 0.7	2.12	0.21
	0 and 80-100	1.21 ± 1.1	1.1	0.81
	<5 and 5-40	0.72 ± 0.35	2.08	0.23
	<5 and 40-80	**1.79 ± 0.63**	**2.84**	**0.04**
	<5 and 80-100	1.51 ± 1.05	1.44	0.6
	5-40 and 80-100	1.07 ± 0.65	1.65	0.47
	5-40 and 80-100	0.78 ± 1.06	0.74	0.95
	40-80 and 80-100	-0.28 ± 1.17	-0.24	1.0
Developmental stage	Prebud and bud	-0.32 ± 0.3	-1.06	0.71
	Prebud and anthesis	**2.62 ± 1.0**	**2.57**	**0.05**
	Prebud and post-anthesis	2.4 ± 1.0	2.34	0.0.9
	Bud and anthesis	**2.94 ± 1.03**	**2.84**	**0.02**
	Bud and post-anthesis	**2.72 ± 1.05**	**2.59**	**0.05**
	Anthesis and post-anthesis	-0.22 ± 1.43	-0.15	1.0