# HABITAT RESTORATION IN A CHANGING WORLD: DETERMINING THE INDIRECT EFFECTS OF WARMING ON MONARCH BUTTERFLIES (*DANAUS PLEXIPPUS)* AS MEDIATED BY CHANGES IN NECTAR QUALITY

**KATHERINE GRACE PEEL**

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

Department of Biology

Faculty of Science

University of Ottawa

**Abstract**

Loss of natural habitats is one of the most critical threats to biodiversity. Habitat restoration is a key strategy to re-establish degraded ecosystems and support the persistence and colonization of pollinators and insects. Pollinator-focused restoration includes introducing floral resources to landscapes to increase native insect pollinator abundance. However, climate change is a major, intensifying threat globally, and insects are among the most affected groups experiencing population declines across the biosphere. Habitat restoration projects and decisions must consider climate change to ensure the effective functioning and resilience of restored habitats, but anticipating the effects of climate change on insects is difficult. Both direct and indirect effects of warming temperatures are expected to impact insect populations, but the indirect effects remain poorly studied. It is unclear how nectar plants will respond to warming temperatures and how these responses may impact insects. Since floral seed mixes are a key component of habitat restoration, exploring how different floral species will respond to warming temperatures, and how those responses may impact pollinators, is essential. The endangered Eastern migratory population of monarch butterflies (*Danaus plexippus*) is at risk due to climate change. Monarchs rely on nectar from late-season flowering plants in their breeding range in Canada to fuel their migration south to Mexico. Thus, warming-induced declines in the nectar quality of these plants may have critical conservation repercussions. Here, I used a field warming experiment at the monarch's northern range limit in Ontario, Canada, to examine i) the vegetative, floral, and nectar responses of 3 highly visited flowering species to warming, ii) the variation of those responses across the 3 species, and iii) the subsequent impacts of these responses on the body composition of adult monarchs. I found that the warming treatment lowered nectar quality and availability of late-season flowering plants. These warming-induced

plant and nectar responses led to a decrease in the fat mass of monarchs who fed on the nectar of warmed plants. These body composition measurements are important metrics for monarch migration and overwintering survival. All three late-season flowering plant species experienced declines in nectar quality; it is unclear if a pattern of adaptive capacity exists in these plants. Therefore, habitat restoration projects should make planting decisions that are context-specific for the needs of the pollinators and plant communities in a specific area.

**Résumé**

La perte des habitats naturels constituent l'une des menaces les plus graves pour la biodiversité. La restauration des habitats est une stratégie clé pour rétablir les écosystèmes dégradés et soutenir la persistance et la colonisation des pollinisateurs et des insectes. La restauration axée sur les pollinisateurs comprend l'introduction de ressources florales dans les paysages pour augmenter l'abondance des insectes pollinisateurs indigènes. Cependant, le changement climatique est une menace majeure et croissante à l'échelle mondiale, et les insectes font partie des groupes les plus touchés par le déclin de leur population dans la biosphère. Les projets et les décisions de restauration des habitats doivent tenir compte du changement climatique pour assurer le fonctionnement efficace et la résilience des habitats restaurés, mais il est difficile d'anticiper les effets du changement climatique sur les insectes. Les effets directs et indirects du réchauffement des températures devraient avoir un impact sur les populations d'insectes, mais les effets indirects restent peu étudiés. On ne sait pas exactement comment les plantes nectarifères réagiront au réchauffement des températures et comment ces réponses peuvent avoir un impact sur les insectes. Étant donné que les mélanges de graines florales sont un élément clé de la restauration des habitats, il est essentiel d'explorer comment différentes espèces florales réagiront au réchauffement des températures et comment ces réponses peuvent avoir un impact sur les pollinisateurs. La population migratrice de l'Est du papillon monarque, une espèce en voie de disparition, est en danger en raison du changement climatique. Les monarques dépendent du nectar des plantes à fleurs de fin de saison dans leur aire de reproduction au Canada pour alimenter leur migration vers le sud, vers le Mexique. Ainsi, la baisse de la qualité du nectar de ces plantes induite par le réchauffement pourrait avoir des répercussions critiques sur la conservation. Ici, j'ai utilisé une expérience de réchauffement sur le

terrain à la limite nord de l'aire de répartition du monarque en Ontario, au Canada, pour examiner i) les réponses végétatives, florales et nectarifères de 3 espèces à fleurs très visitées au réchauffement, ii) la variation de ces réponses entre les 3 espèces et iii) les impacts ultérieurs de ces réponses sur la composition corporelle des monarques adultes. J'ai constaté que le traitement de réchauffement diminuait la qualité du nectar et la disponibilité des plantes à fleurs de fin de saison. Ces réponses des plantes et du nectar induites par le réchauffement ont conduit à une diminution de la masse lipide des monarques qui se sont nourris du nectar des plantes réchauffées. Ces mesures de la composition corporelle sont des paramètres importants pour la migration du monarque et la survie à l'hiver. Les trois espèces de plantes à fleurs de fin de saison ont connu une baisse de la qualité du nectar; il n'est pas certain que ces plantes présentent un modèle de capacité d'adaptation. Par conséquent, les projets de restauration des habitats doivent prendre des décisions de plantation adaptées au contexte et aux besoins des pollinisateurs et des communautés végétales d'une zone donnée.

## Acknowledgments

Thank you to my supervisor, Dr. Heather Kharouba, for your guidance and endless support through my undergraduate and master's theses. Thank you for sharing your knowledge and for creating and fostering a lab environment that is a safe, welcoming, supportive place for everyone. Finally, thank you for believing in me as a student and a researcher. To my lab mates, past and present, especially Sarah Dolson, Spencer Karau, Manon Veselovsky, Jenna Boomhower, Nicole Kester, Stephanie Rivest, Zoe Panchen, Hannah Keefe, and Maisy Roach-Krajewski, I am eternally thankful for your kindness, friendship, and emotional and mental encouragement. To Jenna, you made a challenging, hot field season bearable, and I am so grateful for your friendship.

To everyone at Fletcher Wildlife Garden, thank you for creating a beautiful wildlife refuge and allowing me to conduct my research in your gardens. I am especially grateful to Sandy Garland, without whom this project would have been impossible. Thanks to ECCC for funding the OTCs and the uOttawa Machine Shop for building them. To everyone who helped in the field, taking measurements and building the OTCs, I could not have done this without you. To Jacob Miall from Agriculture Canada, thank you for your generosity, for lending space and plants, and for sharing all your plant and insect knowledge.

Thanks to uOttawa staff, especially Sean Kirkwood, Alex Daniels, and Dave Needham from facilities, and Niki Nagy and Michelle Brazeau for equipment. Thanks to Dr. Christopher and Nora Guglielmo from the University of Western Ontario for analyzing the monarch body compositions. From my thesis advisory committee, thank you to Dr. Christina Davy, Dr. Julien Martin, and Dr. Greg Mitchell for taking the time to give me your constructive feedback, assistance, and kindness.

To my parents and my entire family, especially the Peel and Rees families, an extra special thank you for everything you've done for me. I felt your love, support, and encouragement all the way from Ottawa. To Stephen, thank you for being the composure to my worry, for being confident when I felt nervous, and for your never-ending patience and encouragement.

## Table of Contents

**List of Tables**

**Table 3.** Monarch body condition responses to indirect warming of the warming treatment from the Open-Top Chambers. All monarch responses are square root transformed and include wet mass (g) (n=59), fat mass (g) (n=59), lean mass (g) (n=59), and water mass (g) (n=59). All models are fit with linear models. The table shows the estimated effects (Estimate), standard error (SE), degrees of freedom (DF), F-values (F), R-squared values, and p-values (*p*). Fixed effects are warming treatment, sex, species, day of year (DOY), body length, and biologically significant two-way interactions ($p<0.08$). Bold font indicates statistical significance ($p<0.05$).

**List of Figures**

*canadensis* in the Canadensis Garden of Fletcher Wildlife Garden on August 20, 2023. Both chambers are wrapped in mesh tulle. Large monarch enclosures made of steel garden pegs with mosquito netting are built around the chambers with 30+ cm of clearance around all sides to allow space for the monarchs to move around in the enclosures. The open-top chambers and PVC control chambers are 1.5 meters tall and 1.2 meters wide at the base.

**Figure 4.** Average daytime (08:00 to 20:00) temperature (°C) variation across the study period from July 18 to September 22 across two treatments: control (blue) and warmed (red) chambers. The x-axis represents the day of the year, ranging from day 199 (July 18) to 265 (September 22). The thin lines represent average daytime temperatures across six replicates of each treatment. The bold lines indicate the overall temperature trend for each treatment during the observed period. Grey shading on bold lines represents 95% confidence interval.

**Figure 5.** Soil moisture measurements across two treatments: control (blue) and warmed (red) chambers from 2023 (a) and 2024 (b). The bold lines indicate the overall soil moisture trend for each treatment during the observed periods. Grey shading on bold lines represents 95% confidence interval.

**Figure 6.** Plant responses to temperature differences (Control and Warmed). Shown is: **a**) relative stem growth (cm), **b**) DOY of flower onset, **c**) number of flowers, and **d**) nectar sucrose concentration (°BRIX). The black symbols indicate the mean estimate based on the raw data with 95% confidence intervals for each species: *Monarda fistulosa* (circles)*, Solidago canadensis* (triangles)*,* and *Symphyotrichum novae-angliae* (squares). The red line represents the overall

trend (when present) of model predicted values, illustrating the significant general effect of the warming treatment across all species (Table 2). Means with 95% confidence intervals are shown. "*" denotes a statistically significant effect based on model output ($p<0.05$). Letters represent statistically significant pairwise comparisons between species ($p<0.05$).

**Figure 7.** Monarch body condition and composition responses to temperature differences (Control and Warmed). Shown is: **a**) monarch wet mass (g), **b**) monarch fat mass (g) **c**) monarch lean mass (g), and **d**) monarch water mass (g). The black symbols indicate the mean estimate based on raw data with 95% confidence intervals for each species: *Monarda fistulosa* (circles), *Solidago canadensis* (triangles), and *Symphyotrichum novae-angliae* (squares) under each treatment. The red line represents the overall trend (when present) of model predicted values, illustrating the significant general effect of the treatment across all species (Table 3). "*" denotes a statistically significant effect based on model output ($p<0.05$).

**List of Appendices**

**Table S1.** Incidence of each of the three plant species in each of the garden locations at Fletcher Wildlife Garden (Figure 1b).

**Table S2.** Summary of models used in statistical analysis including model type, response variables, predictor variables, random effects, and additional notes. The 'Type' column indicates function used for the main model. 'Response Variable' specifies the dependent variable being modeled, 'Predictor Variables' include the fixed effects in each model, and 'Random Effects' detail the random factors accounted for in each model.

**Table S3.** Sample size across all models. For model details, see Table S2. Sample sizes are given as number of total measurements, as well as size of each grouping, where appropriate. Sample sizes are given for each plant species (*Monarda fistulosa, Solidago canadensis, Symphyotrichum novae-angliae, Cirsium arvense, Asclepias syriaca, Sonchus oleraceus)* and sex (Female:Male), where necessary.

**Table S4**. Results of interaction effects of the warming treatment. Temperature model output is from a linear mixed effects model, relative stem growth and all monarch model output are from linear models, and flower onset, # of flowers, and nectar concentration outputs are from generalized linear mixed effects models. Species responses were estimated as: relative stem growth (%), day of year of flower onset, number of flowers, and nectar sucrose concentration (°BRIX). Monarch responses were estimated as square root transformations of: wet mass (g), fat

mass (g), lean mass (g), and water mass (g). The table shows the estimated effects (Estimate), standard error (SE), degrees of freedom (DF), F-values or z-values (F/z), and p-values ($p$) for each interaction. Bold font indicates potential biological significance of the interaction ($p<0.08$) and inclusion in the model.

**Table S5**. Post-hoc Tukey pairwise comparison results associated with Figure 6a, the relationship between species and relative stem growth; Figure 6b, the relationship between species and phenology; and Figure 6d, the relationship between species and nectar sucrose concentration. Estimates for flower onset and nectar sucrose concentration are on the logit scale and not response scale. Statistically significant comparisons ($p<0.05$) are in bold.

**Figure S1.** Average daytime (08:00 to 20:00) temperature (°C) variation across the study period from June 8 to July 18 across two treatments: control (blue) and warmed (red) chambers. The x-axis represents the day of the year, ranging from day 159 (June 8) to 199 (July 18). The thin lines represent average daytime temperatures across six replicates of each treatment. The bold lines indicate the overall temperature trend for each treatment during the observed period. Grey shading on bold lines represents 95% confidence interval. Over the time period, the average daytime temperature was 0.5°C lower in warmed chambers than control (0.1SE, $F_{1,2917}=15.1$, $p<0.001$).

## 1. Introduction

Climate change is increasingly becoming a major factor in biodiversity loss, and over the next 50-100 years, it is likely to rise to the forefront of threats to biodiversity (Arneth et al. 2020, McElwee 2021). Climate change has caused a wide range of impacts on biodiversity, ecosystem functions, and human welfare in recent decades (Habibullah et al. 2022, Shivanna 2022). Among the most affected groups of animals are insects (Harvey et al. 2022). Many, but not all, insect populations are declining across the biosphere due in part to climate change (Marshall et al. 2020, Harvey et al. 2022). However, it is difficult to anticipate the far-reaching community-level effects of these changes (e.g., declines in flowering plants; Scaven and Rafferty 2013, Harvey et al. 2022) because we do not have a good understanding of why insects' response to climate change has been so variable (Harvey et al. 2022).

The destruction of natural habitats is also an important threat to biodiversity (Fahrig 1997, IPBES 2019, Prakash and Verma 2022, Christian 2023). Loss of habitat results in reduced population sizes, leading to extinction (Fahrig 1997). To mitigate habitat loss, habitat restoration has been a key strategy over recent decades (Gilbert and Anderson 1998, Miller and Hobbs 2007). The goal of restoration has traditionally been to re-establish degraded ecosystems to a state that resembles their pre-disturbance condition. It can be an effective solution for recovering communities at risk of extinction (Gawecka and Bascompte 2023). For example, hedgerow restoration creates conditions of high floral resources that have been shown to increase the richness of insect populations by supporting the persistence and colonization of pollinators (M'Gonigle et al. 2015). Recent international agreements reaffirm the role of restoration in the context of global change; The United Nations (UN) General Assembly (New York) declared 2021–2030 the Decade on Ecosystem Restoration, and the 2022 UN Convention of Biological

Diversity set a target to restore at least 30% of degraded ecosystems globally by 2030. Estimates of the land area requiring restoration exceed 2 billion hectares globally, indicating an urgent need to prioritize restoration projects (Minnemeyer et al. 2011, Jacobs et al. 2015).

Given the growing threat of climate change, there are increasing calls to ensure that habitat restoration adequately considers it (Breed et al. 2013, Prober et al. 2019). Many restoration projects are now required to evaluate the ability of a restored system to withstand impacts from climate change and maintain its functioning and structure (climate-resilient restoration; Simonson et al. 2021; Kharouba 2024); yet it remains difficult to measure this aspect of restoration projects. An opportunity for maintaining plant-insect interactions lies in enhancing the climate resiliency of restoration (Prober et al. 2015).

A key aspect of pollinator-focused restoration is the introduction of floral resources to enhance landscapes and increase the diversity and abundance of native insect pollinators (Garibaldi et al. 2014). This approach is based on the evidence that insect species diversity is tightly linked with the diversity of floral resources (Benayas et al. 2009, Gordon and Kerr 2022). Since a critical component of pollinator-focused restoration is the choice of seed mix in the initial stages of restoration, effective pollinator restoration in a changing climate should consider how those plant species respond to warming temperatures and how those plant responses then influence the insects that interact with them.

The *direct* effects of warming, those that alter species' physical environments, have been relatively well documented in plants and insects. The physiology, phenology, and morphology of both taxa are sensitive to warming on average (Scaven and Rafferty 2013, Fu et al. 2015, Grainger and Gilbert 2017, Chmura et al. 2019). For example, consistent patterns of flowering advancements in response to recent climate change have been found across the Northern

Hemisphere (Menzel et al. 2006, Lorer et al. 2024). In insects, temperature is related to changes in biochemical and physiological rates (Gillooly et al. 2001, Kingsolver et al. 2013), as well as in metabolism and body size (Lemoine and Burkepile 2012, Lemoine et al. 2013, 2014). For example, insect body size has been found to decrease with warming temperatures (Tseng et al. 2018) due to a shortening of development time (Sheridan and Bickford 2011). Despite general trends in broad taxonomic groups, substantial variation in the type and magnitude of response to warming has occurred across species (Ge et al. 2015, Thackeray et al. 2016). For example, warming has been found to delay late-season flowering plants while advancing early-season ones (Sherry et al. 2007, Chmura et al. 2019), and larger-bodied insect species shrink disproportionately more than smaller-bodied ones under warmed conditions (Tseng et al. 2018, Wonglersak et al. 2021).

Part of this variation may relate to the multitude of ways that the indirect effects of warming can manifest. For insects, the *indirect* effects of warming, those that reflect changes mediated by at least one other interacting species (often plants), are complex and poorly understood (Jamieson et al. 2012, Kharouba and Yang 2021). One of the key indirect effects of warming on insects occurs via warming-driven changes in the availability of food resources provided by plants. For example, stronger climate change-driven phenological advancements in plants than insects increase the potential for phenological mismatch between plants and their insect pollinators and may reduce nectar availability for nectar feeders (Berggren et al. 2009, Ovaskainen et al. 2013). Warming-induced physiological stress in plants may also negatively impact pollinators and nectivores due to decreased floral rewards (Saavedra et al. 2003, Moss and Evans 2022, de Manincor et al. 2023).

It is uncertain whether warming-driven changes in nectar translate into changes in insect pollinator fitness and performance. Takkis et al. (2015) predicts that floral resources may increase when warming conditions still fall within a plant's optimal thermal range, thus benefitting pollinators. However, when warming leads to temperatures above the optimal thermal range, nectar volume and sugar content should decline, leading to negative consequences for pollinators (Takkis et al. 2015). Similarly, Moss and Evans (2022) found that a 1.5°C increase in temperature reduced total floral abundance by nearly 40% and reduced nectar volumes by over 60% in two wildflower species, *Lamium purpureum* and *Veronica persica.* There are several ways warming temperatures may negatively affect insect pollinators, such as lower floral attractiveness and fewer floral resources (Saavedra et al. 2003, Descamps et al. 2021a, de Manincor et al. 2023).

The monarch butterfly (*Danaus plexippus)*, an endangered migratory species in Canada and the United States, is primarily threatened by the loss of suitable habitat, declines in its host plant, milkweed (*Asclepias syriaca),* and climate change (Brower et al. 2012, Zylstra et al. 2021). Research on the effects of climate change on the monarch has focused primarily on the overall or direct effects (but see Kharouba and Yang 2021), which include larval relative growth rate, consumption, and mortality rates (Zalucki 1982, Lemoine et al. 2014, 2015, Kharouba and Yang 2021).

Despite growing research and public interest in monarch conservation, it remains unclear how their nectar plants respond to warming temperatures (Takkis et al. 2015, Descamps et al. 2021b) and how these changes impact monarchs. Since they convert sugar from nectar into fat in their bodies to use as a primary energy source (Alonso-Mejía et al. 1997, Brower et al. 2006, Sinclair and Marshall 2018), monarchs rely on high-quality nectar to fuel their fall migration to

Mexico and for energy reserves (Brower et al. 2006). Therefore, monarchs could encounter an increased risk of extinction if high-quality nectar sources are threatened by climate change.

Here, I use a field warming experiment in Ottawa, Canada, to determine the effects of warming on monarchs mediated through changes in nectar resources of three frequently used late-season nectar plants. I use a novel experimental design that allowed for the isolation of indirect effects from the direct effects of warming by allowing adult monarchs to feed on warmed nectar while spending non-feeding times outside of the warming treatment. I test a series of predictions concerning the effects of warming on three plant species and the monarch. First, if the magnitude of experimental warming goes beyond the plants' optimal thermal range, then the plant's vegetative and floral development and nectar production will be negatively impacted. Otherwise, warming will have no direct impact. Second, given the similarity in life history strategies of the three plant species and their co-occurrence in the same community, they likely have similar optimal thermal ranges and thus will respond similarly to the experimental warming. I use relative stem growth, timing of flowering onset, number of flowers, nectar sucrose concentration, and nectar volume as measures of vegetative and floral development and nectar production.

Finally, given that nectar quality has been related to insect flight performance, metabolism, and life history traits (Lebeau et al. 2016, Damien et al. 2020, Nicolson 2022), the direction of the effects of warming on the floral resources of the nectar plants will be the same for the monarchs. For example, if warming leads to declines in nectar quality and availability, then the body condition of adult monarchs will decline. I expect that the fat mass of the monarchs will track more closely with changes in nectar sucrose concentration than lean mass and water mass because sugar from nectar is primarily converted to fat mass (Brower et al.

2006). To test the prediction, I will use a feeding experiment and measure body composition metrics (wet and fat mass, protein, and water content) of the adult monarchs. These metrics are important for successful migration and reproductive success in monarchs (Sinclair and Marshall 2018, Enriquez and Visser 2023).

## 2. Methods

### 2.1 Study Area

I conducted a field warming experiment from June 8 to September 25, 2023, at the Fletcher Wildlife Garden (FWG)-Canadensis Botanical Garden (CBG) complex in Ottawa, Ontario, Canada (Figure 1a). FWG is a roughly 6-hectare wildlife refuge and garden managed by a volunteer committee of the Ottawa Field-Naturalists' Club since 1990. Before then, it was agricultural land managed by Agriculture and Agri-Food Canada. CBG is 13.8 ha of semi-managed meadow of which we used an approximate 4.5 ha portion of. The complex is located next to the Rideau Canal and 4 kilometres from the urban core of Ottawa.

### 2.2 Study System

#### *2.2.1 Monarch Butterfly*

The Eastern population of the monarch butterfly undergoes an annual long-distance migration from their breeding grounds in south-eastern Canada and the eastern United States to their overwintering grounds in oyamel fir forests in Mexico (Brower 1996). Population sizes of the monarch have fluctuated greatly over the last 30 years, but the general pattern is a consistent, dramatic decline (Brower et al. 2012, Semmens et al. 2016). As a result, it is currently listed as

'Endangered' under Canada's Species at Risk Act (Environment and Climate Change Canada, 2023).

Monarch population declines have been attributed to many factors, including the degradation of forests in overwintering areas in Mexico due to illegal logging (pre-1990s), loss of breeding habitat in the United States and Canada as a result of glyphosate use (mid-1990s to mid-2000s), and severe weather impacting resources and survival (Brower et al. 2012, Shirey and Ries 2023). Most recently (post-2005), climate change has been determined to be a primary driver of monarch declines (Shirey and Ries 2023). Zylstra et al. (2021) found that breeding-season weather conditions were the most important factors in explaining monarch summer population size (with extreme temperatures resulting in lower expected monarch counts), which is directly related to the size of the overwintering population. A recent study also suggested that the majority of forest degradation in monarch overwintering grounds in Mexico since 2007 is due to climate change challenges rather than illegal logging, as previously thought (Zylstra et al. 2020).

Monarchs are a multivoltine species with two to three distinct breeding generations in Southern Ontario and Quebec between June and September, followed by a third or fourth migratory generation that does not breed until the following spring (Brower et al. 2006). While adults of breeding generations have a life span of two to five weeks, the migratory generation of monarchs can live up to nine months (Brower et al. 2006). The females of this late-season, migratory generation are in reproductive diapause, a state that conserves energy by suppressing non-essential physiological functions (Koštál 2006). The monarchs in this study were reared during the late season to align with the migratory generation in reproductive diapause.

### *2.2.2 Nectar Plant Species*

I used three late-season nectar sources that are frequently used by the monarch butterfly in the Ottawa region (Figure 2; Pekos et al. sub.). They are also on native plant seed lists used in Ottawa for habitat restoration projects, such as by the Canadian Wildlife Federation. The species reproduce sexually via seeds and vegetatively via short rhizomes.

*Symphyotrichum novae-angliae (New England Aster)* is a forb in the family Asteraceae that flowers from August to late October. It is native to central and eastern North America and grows in typically moist habitats, including meadows, prairies, marshes, fens, forest edges, and disturbed anthropogenic sites (National Plant Data Team, 2024).

*Solidago canadensis (Canada Goldenrod)* is a tall forb in the family Asteraceae that flowers from July through October. It is native to northeastern and north-central North America and can grow under many conditions. It is often one of the first plants to colonize an area after a disturbance (National Plant Data Team, 2024).

*Monarda fistulosa (Wild Bergamot)* is a forb in the family Lamiaceae that flowers from July to September. It is native to much of North America, typically growing in dry fields, thickets, and clearings (National Plant Data Team, 2024).

Phylogenetically, *S. canadensis* and *S. novae-angliae* are more closely related, as both belong to the Asteraceae family.

## 2.3 Warming Experiment

### *2.3.1 Experimental Design*

On June 8, 2023, I placed 15 open-top chambers (OTC) and 15 control chambers on existing stems of *S. novae-angliae*, *S. canadensis,* and *M. fistulosa* (single species per chamber,

five chambers per species) at FWG. Each OTC was paired with a nearby (<2m) control chamber of the same plant species (known together as a 'pair') to ensure the same conditions for each. The 15 control chambers were built with the same dimensions as the OTCs using PVC piping. The distance between pairs was greater than 6 meters to minimize the risk of genetic similarity between replicates due to vegetative reproduction.

Location selection for enclosures was based on the availability of each nectar plant species to ensure relatively equal cover percentages of each plant species within the chambers and the avoidance of daytime shading from trees, which would affect the effectiveness of the OTCs. The experimental enclosures were located in four areas of FWG: the 'Butterfly Meadow', 'Milkweed Field', 'Old Field', and 'Canadensis Garden' (Figure 1b). The Butterfly Meadow is a well-maintained area (e.g., removal of invasives, targeted planting) with many wildflowers and is highly visited by the public. It is sheltered from the wind by rows of coniferous trees to the west and northwest. The Milkweed Field is a less-maintained area surrounded by coniferous trees on three sides and slightly away from a wide path often used by visitors. The Old Field is maintained with mowing to remain in the early stages of succession and is dominated by grasses and wildflowers. The Canadensis Garden is the least maintained of all the sites and is a large stand of mostly goldenrod. It is mowed once per year at the end of the season but otherwise unmaintained. Enclosures for *S. novae-angliae* were placed in the Old Field, Butterfly Garden, and Milkweed Field (Table S1, Figure 1b). Enclosures for *S. canadensis* were in the Canadensis Garden and Butterfly Garden (Table S1, Figure 1b). Enclosures for *M. fistulosa* were in the Milkweed Field and Butterfly Garden (Table S1, Figure 1b).

In early August, in preparation for the addition of monarchs to the experiment, a fine white mesh tulle was wrapped around the PVC frame of control chambers so that monarchs

(when added) would encounter the same physical barrier to get to the nectar in the flowers (i.e., monarchs would have to fly up over chamber sides to get into chamber) as they would with the OTCs. To ensure visual similarity between treatments, OTCs were also wrapped in the same fine white mesh tulle.

From August 15-30, 2023, I built 30 square 2-meter x 2-meter insect enclosures over the OTC and control chambers using steel garden poles, bamboo poles, and fine white mesh (Figure 3b). The design of the enclosures allowed the monarchs to spend their non-feeding time outside of the chambers. This allowed me to isolate the direct (i.e., warmed monarchs) and indirect effects of warming on monarchs (i.e., warmed nectar) and ensure that the monarchs were not directly warmed in non-feeding times. Any flowers in the space between the chamber and the mesh enclosure (~30 cm) were removed (and cut back throughout the season, as needed) so that nectar was only available inside the chamber.

### *2.3.2 Warming Treatment*

As described above, I used OTCs as a passive warming treatment on three plant species. OTCs have been shown to significantly alter temperature and minimize unwanted effects such as modified precipitation and humidity (Marion et al. 1997, De Frenne et al. 2010, Welshofer et al. 2018). While OTCs have been frequently used in plant ecological studies (Marion et al. 1997, Kudernatsch et al. 2008, De Frenne et al. 2010, Welshofer et al. 2018), they have not been widely used in insect studies (but see Dollery et al. 2006, Grainger and Gilbert 2017). It is even more rare to study flying insects the way I have here because it has been difficult to keep flying insects enclosed with OTCs without also including the direct impacts of warming on insects (e.g., Yang et al. 2021).

The OTCs used in this study had an aluminum frame and polycarbonate sides, measuring 1.2 $m^2$ at the bottom, tapering to a 0.9 m square opening at the top (Figure 3a). Photosynthetically active radiation (350 to 750 nm) spans the visible light portion (380 to 700 nm) of the electromagnetic spectrum (McCree 1971). The polycarbonate sides are estimated to allow 86% visible and ultraviolet light transmission and <5% infrared light transmission (De Frenne et al. 2010).

## 2.4 Measurements

### *2.4.1 Temperature and Soil Moisture*

Hourly temperature measurements were taken using 12 HOBO temperature data loggers (Onset HOBO Pendant Temperature/Light 64K Data Logger, ±0.53°C) in six treatment pairs across the 30 experimental units (Figure 1b). From June 6 to July 17, 2023, the loggers were pegged to the ground in each chamber. Temperature measurements from this time were quite variable, and I found inconsistent differences between warming and control chambers. After further analysis, there was a non-significant difference in temperature between the warmed and control chambers during this time period (Figure S1). This was likely because of ground temperature impacting logging, differences in the amount of ground cover, and the loggers' proximity to open air through the spaces at the bottom of the chambers. Therefore, after July 18, loggers were hung from 15 cm tall garden posts where there was greater distance from chamber openings and where ground temperature effects would be minimized. Only measurements taken after this position modification of the loggers are included in the analysis.

To compare soil moisture between warming treatments, I measured soil moisture in each of the 30 chambers using a bi-metal probe (RapiTest Moisture Meter) that measures conductivity

and indicates moisture content on a scale from 1-10. I placed the probe into the soil and allowed the meter to settle (~ 5 sec.) before I recorded the reading. I measured the soil moisture in each of the four corners and the centre of the chamber 1-2 times per week during the experiment. I then took the daily average of those values.

I also analyzed soil moisture measurements from June 19 to August 15, 2024. The data came from a field warming experiment in the same location and with the same OTC and control design as mine. Soil moisture was measured in each of the 30 chambers using a more accurate and precise moisture meter (Fieldscount TDR 100) which measures % volumetric water content. Otherwise, the sampling methods were the same as in 2023.

*2.4.2 Plants*

In early June 2023, I flagged and labelled 10 random stems from each chamber using a 1 $m^2$ quadrat square with 4 lengths of thin rope, strung horizontally and vertically, perpendicular to each other, creating a 5x5 grid. I flagged and labelled the 10 stems closest to the string intersections. I measured the height of each labelled stem 1-3 times per week throughout the season. Height measurements were taken from the root crown to the apical leaf of the main stem. I made 6466 stem height observations from June 9 to October 11, 2023.

Floral measurements of each flagged stem were taken from July 5 to October 11, 2023. I recorded the phenophase of all the flowers 1-3 times per week using flower phenophase classification protocols from the USA-National Phenology Network. Here, I considered 'flowering onset', or the date of first open flower, as my phenophase. I was not able to record the end of flowering, and thus the total flowering time, because the floral period for *S. canadensis* and *S. novae-angliae* ended after the experimental period (Figure 2). I also counted the number

of open flowers or floral units on each of the 10 labelled stems to get a measure of reproductive output. A flower was considered open when the petals revealed the reproductive structures. I only considered number of flowers for *S. novae-angliae* and *M. fistulosa* because of the difficulty in accurately counting the number of open *S. canadensis* flowers due to the small size of the flowering heads.

Most plant species that are known food sources for Lepidoptera are sucrose-dominant, with more than 60% sugar coming from sucrose (rather than glucose or fructose) (Wolff 2006). Therefore, I used nectar sucrose concentration as an estimate of nectar quality. I placed 15 cm x 20 cm mesh fruit protection Organza bags over open flowers 24 hours before nectar extraction in order to exclude nectar feeders from visiting the flower before nectar measurement and allow the flower to replenish nectar. I first extracted nectar from flowers using microcapillary tubes with tube volumes ranging from 1-8μL depending on the size of the flower (Drummond Microcaps). I avoided measuring nectar if it had rained within 24 hours, and all measurements were taken between 08:00 and 11:00 to minimize time of day effects. I measured nectar sucrose concentration by displacing the nectar from the microcapillary tubes onto a handheld low-volume refractometer (Eclipse 0-50 °BRIX Handheld Refractometer). Nectar sucrose concentration was measured in degrees BRIX, which is a measure of the dissolved solids in a liquid. One degree of BRIX is one gram of sucrose in 100 grams of solution. The sucrose concentrations reported in the nectar of the three species used here (see Figure 5d) are similar to those reported for butterfly-pollinated flowers, which generally have concentrations between 20-25°BRIX (Nicolson and Thornburg 2007, Willmer 2011).

I measured nectar volume by measuring the length of the nectar column in the microcapillary tube before displacement onto the refractometer, then calculating the volume of

the nectar from the known length and volume of the microcapillary tube and the length of the nectar column ($V_{nectar} = V_{tube}*L_{nectar}/L_{tube}$).

For *S. canadensis* and *S. novae-angliae,* the flowers were too small to extract nectar using standard-size microcapillary tubes. To overcome this, I used a Bunsen burner in the lab to melt the tubes and slowly pulled the glass using forceps to make the tube narrower in the middle before breaking it apart and using the narrow ends to extract nectar (methods from Corbet 2003). As a result, I could not calculate the nectar volume of *S. canadensis* or *S. novae-angliae* because the internal volume of the microcapillary tubes were no longer standardized. Further, despite modifications to the microcapillary tubes, the small size of the *S. canadensis* flowers made nectar collection difficult, resulting in a small sample size for measurements of nectar sucrose concentration (n=15, compared to *M. fistulosa* n=155, *S. novae-angliae* n=97).

### *2.4.3 Monarchs*

From July 18 to August 2, 2023, I caught five female and five male adult monarchs from the Canadensis Botanical Garden at FWG. I mated pairs outdoors in one of three enclosures (140 x 60 x 60 cm Pop-up Cage, Watkins & Doncaster) in a courtyard at the University of Ottawa. In each enclosure, I placed one male with one or two female adult monarchs at a time with 4-5 young, potted common milkweed (*Asclepias syriaca*) plants for oviposition. These plants were grown from seed in controlled growth chambers (CONVIRON GEN1000 and Biochambers model LTCB-19) at the University of Ottawa on a 20°C/25°C 16:8 L:D cycle. For food, I included a 10 cm plastic bowl filled with a 20% white sugar water solution and a sponge so that the adults could land and feed while in the enclosures. I replaced the sugar water daily to avoid fermentation.

I checked for eggs daily and transferred eggs immediately to 16 oz clear containers with mesh lids for ventilation in the growth chambers. Each larva was raised in its own container to avoid egg cannibalism. I reared the larvae on a 21°C/27°C 16:8 L:D cycle to mimic the photoperiod of late summer/early fall. I fed them daily with leaves from the same young *A. syriaca* plants grown in the growth chambers, as well as with leaves from established *A. syriaca* plants along the Ottawa River. Early instars (instars 1-3) were fed young leaves from the chamber-grown milkweed, and older instars were fed larger leaves from the river-grown milkweed. Larvae were allowed to feed *ab libitum*. Pupae remained in the growth chambers with the same environmental conditions until eclosion.

I introduced the first set of adults into the experiment on August 19, 2023. Once the monarchs' wings were fully expanded after eclosion (~5-12 hrs), I added them to the experimental enclosures at FWG. Until August 28, only female monarchs were used in the experiment to avoid sex effects. However, due to seasonal time constraints and rearing more males than females, males were also included in the experiment from August 28 to September 14. The adults were allowed to feed *ab libitum* on the nectar in the chambers for five days.

I weighed the mass of the adults on days 0, 1, 3, and 5 to ensure that I had weight measurements in case they did not survive to the end of the trial. To weigh them, I gently removed the butterflies from the enclosures and placed them immediately in a small container on a high-sensitivity scale (Sartorious Research R300S Analytical Balance Digital Laboratory Scale). The scale was tared with the small container before each monarch measurement. The scale was on-site at FWG to minimize travel, stress, and the length of time monarchs were removed from the experiment. On average, the monarchs were removed from the experiment for

less than one hour per day. In total, there were 59 completed 5-day feeding trials from August 20 to September 21, 2023.

At the end of the feeding trial, the adults were humanely euthanized and sent to Western University for further analysis to determine if nectar quality impacted the monarchs' body composition metrics. Monarch samples were analyzed for wet, fat, lean, and water masses following protocol from (Marden 1989). Wet mass is the body mass of the adults before analysis: the monarchs were placed in individual envelopes and weighed, with the envelope weight subtracted to obtain the total mass of the butterfly. Given the strong correlation between the weight of live adults on day 5 of the feeding trial and the weight of preserved samples ($R^2$=0.90), I used wet mass as an estimate of total mass instead of the weight measurements taken in the field. Wet mass was likely a more accurate body mass measurement because it was taken after the monarchs had been euthanized rather than in the field, where it was difficult to get them to stay still for the measurements.

After their wet mass was measured, the monarchs were transferred, in envelopes, to a drying oven set to 70°C, where they remained for three days until a constant mass was reached. Monarchs were re-weighed to obtain their dry mass. The difference between wet and dry mass represents the water mass, which was used here as an indicator for level of hydration. Given that monarchs are unable to land on bodies of water to drink without drowning, they rely on nectar as a major source of moisture and to avoid dehydration (Contreras et al. 2022). Their large ratio of surface area to volume means they face a high potential for desiccation, leading to mortality if water intake is low enough (O'Donnell 2022). Finally, monarchs need water to successfully migrate and survive overwintering because they feed and drink less through this period than during the breeding season (Brower et al. 2006, Sánchez-Tlacuahuac et al. 2023).

To measure dry fat mass, the monarchs then underwent petroleum ether extraction in a Soxhlet extractor. This extraction process removes all lipids except for the membrane phospholipids. The monarchs were re-dried for another 8 hours and re-weighed, with the difference in dry weight before and after extraction representing the butterfly's total lipid mass (or dry fat mass). Dry fat mass is one of the most important measurements of body composition for monarchs migrating south (Dockx 2012) because monarchs rarely feed over the winter, especially during the first half (Sánchez-Tlacuahuac et al. 2023). They manage their energy stores for migration and overwintering by entering reproductive diapause and depressing their metabolic rates, slowly consuming energy reserves (Sinclair and Marshall 2018, Enriquez and Visser 2023). They use lipids as their primary overwintering fuel (Sinclair and Marshall 2018), and then rely on those same lipid stores to fuel their initial spring flight from overwintering sites to their first breeding sites (Brower et al. 2006). Lipid conservation over the winter has been linked to spring fitness, with the persistence of many insect populations being linked to overwintering success (Sinclair and Marshall 2018). Fat content has also been frequently related to overall condition in insects, with higher fat mass generally resulting in better condition (Zwaan et al. 1991).

Finally, lean mass was measured as the remaining weight of the butterfly after water and lipid extraction. It is used as a proxy for the total amount of protein in the butterflies and may also be related to additional energy reserves for migration (Giulivi and Ramsey 2015). Lean mass is the muscle tissue equivalent of all the body parts containing water (O'Regan et al. 2012) and also includes chitin (exoskeleton) and non-lipid organ tissues.

## 2.5 Statistical Analysis

### *Overview*

All statistical analyses were performed using R 4.1.2 (R Core Team 2021). The analysis was split into three sections: environmental models, where I investigated the difference in environmental conditions between the OTCs and control, plant models, where I examined the effect of the warming treatment and other covariates on plant growth and development, and monarch models, where I examined the effect of the warming treatment and other covariates on monarch body composition. Linear mixed effects models (LME) using package lme4 (Bates et al. 2015, 2022) were used for the environmental models. For the 'number of flowers (integer)', 'flower onset (integer)', 'nectar sucrose concentration (percentage)', and 'soil moisture (percentage)' models, I used generalized linear mixed models (GLMM) using the glmmTMB package (Brooks et al. 2017, 2023). In all LMM and GLMM models, 'pair (n=15; an OTC and adjacent control)' was included as a random effect with a specified intercept to control for the within-visit correlation of observations. Given model overfitting, I used simple linear models (LM) for 'plant relative stem growth' and 'monarch body composition' models (R Core Team 2021).

For all models, I visually inspected residuals plots to check linearity, normality, and homoscedasticity assumptions. I applied data transformations (square root) as necessary to meet normality assumptions and used a GLMM where assumptions still could not be met. I checked the goodness of fit of the models by generating conditional and marginal R-squared values of LME models and conditional and marginal pseudo-R-squared values of GLMM using the performance package (Ludecke et al. 2021). I used the package 'DHARMa' (Hartig, 2022) to

test for under/overdispersion in the GLMMs. Figures 4-7 were created using ggplot2 (Wickham 2016, Wickham et al. 2023).

All two-way interaction terms were initially included in the models but were only retained in the final models and reported in the main body of the thesis if they were statistically significant ($P<0.05$) or potentially biologically significant ($P<0.08$). Otherwise, interactions were dropped from the final models and only reported in the supplemental material (Table S4). When DOY was included as an interaction term, it was centered on the mean to improve the interpretability and stability of the model estimates. To compare differences between levels of categorical variables, I used a TukeyHSD pairwise comparison test (Table S5). The sample size for all models is available in Table S3.

### *2.5.1 Environmental Models*

#### *2.5.1.1 Temperature*

To determine the mean daytime temperature difference between the OTC and control chambers over the season, I calculated the mean daily temperature (°C) between 8:00 and 20:00 in each chamber. I used an LME model with treatment and DOY as fixed effects and pair as a random effect (Table S2).

I also tested for differences in daily temperature fluctuation between warming treatments (maximum daily temperature—minimum daily temperature) and differences in nighttime temperatures (i.e., 20:00-8:00) using LME models with treatment as a fixed effect and pair as a random effect (Table S2).

*2.5.1.2 Soil Moisture*

To determine the soil moisture difference between the OTC and control chambers during each of the 2023 and 2024 seasons, I used the average soil moisture per chamber for both years. In 2023, soil moisture was measured on a scale from 1 to 10, and in 2024, soil moisture was measured by % volumetric water content. I used a GLMM with beta distribution and logit transformation to remove the constraints of bounded and percentage data for both years. I ran separate models for each year and included warming treatment and DOY as fixed effects (Table S2).

*2.5.2 Plant Models*

*2.5.2.1 Relative Stem Growth*

To estimate relative stem growth, I focused on the period of most rapid growth that occurred before flowering began (DOY 193 for *M. fistulosa,* 221 for *S. canadensis,* 243 for *S. novae-angliae;* Figure 2). I calculated the relative change in stem height from day 1 until the date of flower onset of each labelled plant stem ((final height-initial height)/initial height).

To determine if the warming treatment influenced relative stem growth, I used a linear model. I modelled relative stem growth as a function warming treatment and species (Table S2).

*2.5.2.2 Phenology*

To determine if the warming treatment influenced the timing of flowering onset, I used a GLMM with gamma distribution because of overdispersion (mean:variance = 221.9:412.5) and non-normality of the continuous variable. I used the day of year of flower onset for each of the

10 flagged stems in each chamber as the response variable and modelled it as a function of the interaction between warming treatment and species with pair as a random effect (Table S2).

*2.5.2.3 Number of Flowers*

To determine if the warming treatment influenced the number of flowers, I modelled the number of open flowers per stem as a function of warming treatment and species, with pair and DOY as crossed random effects to account for repeated sampling on different days throughout the season (Table S2). I used a GLMM with negative binomial distribution and logarithmic link because the number of open flowers was not normally distributed and was overdispersed (mean:variance = 5.2:30.8). Negative binomial probability distribution has been shown to be the best approach for overdispersed data (Welsh et al. 2000).

*2.5.2.4 Nectar Sucrose Concentration and Volume*

To determine if the warming treatment influenced nectar sucrose concentration, I modelled it as a function of warming treatment and species, with pair and DOY as crossed random effects to account for repeated sampling on different days throughout the season (Table S2). I used a GLMM with logit transformation to remove the constraints of percentage data. To determine if the warming treatment influenced nectar volume in *M. fistulosa*, I included it as a fixed effect, with pair and DOY as crossed random effects to account for repeated sampling on different days throughout the season (Table S2).

*2.5.3 Monarch Models*

To determine if the warming treatment indirectly influenced monarch body composition through nectar, I modelled each metric of body composition (n=4; wet mass, fat mass, lean mass, and water mass) as a function of warming treatment, plant species, sex (to account for physiological variation between sexes; 2-level factor), DOY of eclosion (to account for seasonal trends), and body length (to account for the monarch's inherent size upon eclosion) (Table S2), using a linear model. I checked for multicollinearity between independent factors using Variance Inflation Factor (VIF) and they did not warrant further investigation. All body composition metrics were square root transformed to meet normality assumptions. The interaction between warming treatment and sex was included in water mass model (Table S2, Table S4).

## 3. Results

3.1 Environmental Effects of Open-Top Chambers

The average daily range of temperatures for the OTCs was 17.6-32.8°C (range:15.2°C), and the average daily range of temperatures for the control chambers was 17.6-32.3°C (range: 14.7°C). The highest recorded temperature in the OTCs was 45.7°C, while the highest in the control chambers was 43.5°C.

The mean daytime temperatures of the OTCs were 0.57°C warmer than the control chambers (0.028SE, $F=4.18_{1,522}$, $p=0.04$, Table 1, Figure 4a). Daytime temperatures decreased throughout the season (-0.090°C/day (0.0081SE), $F=122.36_{1,537}$, $p<0.001$; Table S4, Figure 4a)

The OTCs and control chambers had the same level of daily temperature fluctuation (0.59°C (0.0044SE), $F=1.76_{1, 533}$, $p=0.19$; Table 1).

The nighttime temperatures of the OTCs were weakly warmer than the nighttime temperatures of the control chambers (0.45°C (0.24SE), $F=3.40_{1,481}$, $p=0.066$; Table 1, Figure 4b).

In 2023, there was no difference in soil moisture between OTCs and control plots (-1.1% (7.8SE), $z=-1.35_{1,179}$, $p=0.18$; Figure 5a). However, in 2024, OTCs were drier than control chambers (-1.3% (4.2SE), $z=-3.1_{1,171}$, $p<0.005$; Figure 5b).

In 2023, soil moisture increased over the season (2.4%/day (0.19SE), $z=12.29_{1,179}$, $p<0.001$; Figure 5a), whereas it decreased over the 2024 season (-0.51%/day (0.1SE), $z=-3.29_{1,169}$, $p<0.001$; Figure 5b).

## 3.2 Plant Responses

### *3.2.1 Relative Stem Growth*

Overall, the OTCs did not affect the relative stem growth of the plants (18% (3.1SE), $F=0.5_{1,270}$, $p=0.48$; Table 2, Figure 6a). There was no interaction between warming treatment and species ($F=0.22_{2,268}$, $p=0.81$). Relative stem growth differed across species ($F=29.38_{2,270}$, $p<0.001$; Table 2, Figure 6a): *S. novae-angliae* grew more than both *M. fistulosa* (1.8% (0.036SE), $t=-5.04_{1,270}$, $p<0.0001$) and *S. canadensis* (2.9% (0.039SE), $t=-7.50_{1,270}$, $p<0.0001$; Table S5, Figure 6a). *M. fistulosa* grew more than *S. canadensis* (1.1% (0.038SE), $t=2.78$, $p<0.0001$; Table S5, Figure 6a).

*3.2.2 Phenology*

The timing of flowering onset was not different between the warming treatments (0.0021 days (0.003SE), $z=0.7_{1,177}$, $p=0.48$; Table 2, Figure 6b). There was no interaction between the warming treatment and species ($z=0.40_{1,175}$, $p=0.68$; Table S4). The timing of flowering onset was different between species ($z=8.0_{2,177}$, $p<0.005$; Table 2, Figure 6b): *M. fistulosa* flowered before *S. canadensis* (-0.094 days (0.012SE) $z=-5.07_{inf}$, $p<0.0001$), and *S. novae-angliae* (-0.19 days (0.017SE), $z=-10.99_{inf}$, $p<0.0001$; Table S5, Figure 6b). *S. canadensis* flowered before *S. novae-angliae* (-0.098 days (0.019SE), $z=-5.24_{inf}$, $p<0.0001$; Table S5, Figure 6b).

*3.2.3 Number of Flowers*

The number of flowers on stems in the OTCs was lower than in the control chambers (-0.25 flowers (0.055SE), $z=-4.62_{1,531}$, $p<0.001$; Table 2, Figure 6c). There was no interaction between warming treatment and species ($z=-0.18_{1,530}$, $p=0.86$; Table S4). *Symphyotrichum novae-angliae* had more flowers than *M. fistulosa* (1.35 flowers (0.30SE) $z=3.76_{1, 531}$, $p<0.001$; Table 2, Figure 6c).

*3.2.4 Nectar Sucrose Concentration and Nectar Volume*

Nectar sucrose concentration was lower in warmed plants than in control plants (-0.27°BRIX (0.066SE), $z=-4.03_{1,229}$, $p<0.001$; Table 2, Figure 6d). There was no interaction between the warming treatment and species ($p=0.84$; Table S4). Species differed in their nectar sucrose concentration ($z=-3.0_{2,229}$, $p<0.005$; Table 2): *M. fistulosa* had higher sucrose

concentration than *S. canadensis* (0.99°BRIX (0.33SE), z=2.99, $p<0.01$) and *S. novae-angliae* (0.70°BRIX (0.21SE), z=2.58, $p<0.05$; Table S5, Figure 6d).

The nectar volume of *M. fistulosa* was the same between warming treatments (0.072µL (0.057SE), $F=1.61_{1,143}$, $p=0.21$; Table 2).

## 3.3 Monarch Responses

### *3.3.1 Wet Mass*

The warming treatment did not influence adult monarch wet mass (-0.028g (0.018SE), $F=1.18_{1,51}$, $p=0.18$; Table 3; Figure 7a). There was no interaction between the warming treatment and plant species ($F=0.48_{2,50}$, $p=0.62$), nor with DOY ($F=0.16_{2,50}$, $p=0.69$), nor with sex ($F=1.78_{2,50}$, $p=0.19$; Table S4).

Wet mass was weakly predicted by body length (0.015g (0.0065SE), $F=3.64_{1,51}$, $p=0.063$; Table 3), with larger body lengths leading to higher wet mass. Wet mass did not differ across plant species ($F=0.59_{2,51}$, $p=0.56$; Table 3, Figure 7a), nor across sexes (0.012g (0.026SE), $F=2.57_{1,51}$, $p=0.12$; Table 3), nor with seasonality (0.0030g (0.0027SE), $F=0.19_{1,51}$, $p=0.89$; Table 3).

### *3.3.2 Fat Composition*

Adult fat mass was lower in the OTCs than in the control treatment (-0.038g (0.016SE), $F=3.17_{1,51}$, $p<0.05$; Table 3, Figure 7b). There was no interaction between the warming treatment and plant species ($F=0.55_{2,50}$, $p=0.59$), nor with DOY ($F=0.14_{2,50}$, $p=0.71$), nor with sex ($F=0.12_{2,50}$, $p=0.73$; Table S4).

Monarchs who eclosed later in the season had higher fat mass (0.0052g (0.0024SE), $F=4.68_{1,51}$, $p<0.05$; Table 3). Adult fat mass did not differ across plant species ($F=0.22_{2,51}$, $p=0.80$; Table 3, Figure 7b), nor across sexes ($F=0.03_{1,51}$, $p=0.87$; Table 3), nor across body lengths ($F=0.30_{1,51}$, $p=0.59$; Table 3).

### *3.3.3 Lean Composition*

Adult lean mass was not influenced by the warming treatment (-0.015g (0.0011SE), $F=0.41_{1,51}$, $p=0.41$; Table 3, Figure 7c). There was no interaction between the warming treatment and plant species ($F=1.83_{2,50}$, $p=0.17$), nor with DOY ($F=0.78_{2,50}$, $p=0.38$), nor with sex ($F=0.69_{2,50}$, $p=0.42$).

Adult monarchs with larger body lengths had higher lean mass (0.013g (0.0039SE), $F=11.74_{1,51}$, $p<0.005$; Table 3). Adult lean mass did not differ across plant species ($F=0.92_{2,51}$, $p<0.53$; Table 3, Figure 7c), nor across sexes ($F=0.63_{1,51}$, $p=0.56$; Table 3), nor with seasonality ($F=0.99_{1,51}$, $p=0.37$; Table 3).

### *3.3.4 Water Composition*

Adult water mass was not influenced by the warming treatment (-0.0046g (0.013SE), $F=1.56_{1,51}$, $p=0.22$; Table 3, Figure 7d). There was no interaction between the warming treatment and plant species ($F=0.15_{2,50}$, p=0.86; Table S4), nor with DOY ($F=0.074_{2,50}$, $p=0.79$; Table S4). There was a weak interaction between warming treatment and sex, where the warming effect was lower on males than on females (-0.064g (0.036SE) $F=3.08_{1,51}$, $p<0.08$; Table 3).

The water mass of monarchs depended on sex (0.037g (0.020SE) $F=6.37_{1,51}$, $p<0.05$; Table 3), where males had higher water mass than females. Adult water mass weakly depended

on body length, with larger body length leading to higher water mass (0.010g (0.0047SE) $F=3.23_{1,51}$, $p=0.07$; Table 3. Adult water mass did not differ across plant species ($F=0.35_{2,51}$, $p=0.84$; Table 3, Figure 7d) nor with seasonality ($F=0.20_{1,51}$, $p=0.62$; Table 3).

## 4. Discussion

Using a field experiment, this study explored the direct effects of a warming treatment on the stem growth, phenology, and nectar characteristics of three late-season flowering plants and the subsequent impact of these changes on monarch wet mass and body composition. The warming treatment led to a decrease in nectar sucrose concentration and fewer flowers on each stem. I showed that adult monarchs who fed on the nectar of plants in the warmed treatment had lower overall fat mass compared to monarchs who fed on control nectar. This suggests that the warming treatment led to lower nectar quality and availability for the monarch. Lastly, I found that the effects of the warming treatment were consistent across all three plant species.

### 4.1 Plant Responses to Warming Treatment

#### *4.1.1 Decrease in nectar sucrose concentration*

The warming treatment led to a decrease in nectar sucrose concentration. This finding is in line with my prediction and consistent with some studies that found high temperatures decrease the nectar sucrose content (Scaven and Rafferty 2013, Takkis et al. 2015); however, it is inconsistent with a study that found the opposite relationship (de Manincor et al. 2023). One explanation for this inconsistency may be the environmental context of the studies. The summers in Ottawa are humid (Environment and Natural Resources, n.d.), whereas summers in Riverside, California, USA, where de Manincor et al. (2023) took place, are dry. Drier conditions could

increase evaporation, resulting in higher nectar sucrose concentrations and reduced nectar volume. Indeed, in the one species where I was able to measure nectar volume (*M. fistulosa*), nectar volume was not influenced by the warming treatment. Therefore, it may be important to consider ambient humidity in combination with warming when predicting how nectar sucrose concentrations will change in the face of climate change.

Heat stress, especially during thermally sensitive developmental periods such as floral development and flowering, may result in physiological responses such as fewer flowers, modified floral features, smaller corolla, and changes in biomass per flower, which can lead to metabolic responses such as decreased nectar sucrose concentration (Takkis et al. 2018, Borghi et al. 2019, Jagadish 2020). If these changes disrupt pollination, it could lead to negative consequences for plant fitness and cascading effects on pollinators who feed on the nectar.

### *4.1.2 Decrease in number of flowers*

I found that the average number of flowers per stem was lower in the OTCs relative to the control chambers. This was consistent with my prediction that warmer temperatures would negatively impact floral development. High temperatures can be a source of heat stress for plants, which can cause them to produce fewer flowers (Saavedra et al. 2003). Increased temperatures resulting in fewer flowers are consistently found across warming experiments (Liu et al. 2012, Moss and Evans 2022, de Manincor et al. 2023).

As flower number strongly influences the reproductive success of animal-pollinated plants (Sun et al. 2018), my results suggest that climatic warming may impact the fecundity of flowering plants. This may have cascading effects on the pollinators that depend on them (Saavedra et al. 2003). As a caveat, while I found that the number of flowers per stem was lower

in OTCs relative to the control, I did not measure the total number of stems in the chambers, so it is possible that stem abundance increased (see Moss and Evans 2022) leading to a higher number of total flowers in the community. Though, anecdotally, I did not notice any dramatic changes in the density of plants, and the length of the warming treatment was likely not long enough to see that effect. Future studies should investigate the total number of stems in each chamber and the number of flowers per stem to determine if the relationship between warming treatment and flower number per stem is related to the number of stems.

### *4.1.3 No effect on relative stem growth*

The warming treatment did not change the relative stem growth of the three plant species used here. This was not consistent with my prediction that warmer temperatures would negatively impact vegetative development. However, warmer temperatures that are not above a species' optimal thermal range generally accelerate plant growth by lengthening the growing season and increasing metabolic rates, leading to higher rates of primary production (Kudernatsch et al. 2008, Jamieson et al. 2012, Tait and Schiel 2013). It could be that the magnitude of the warming effect from the OTCs was not large enough and/or above the species' optimal thermal ranges to impact the relative stem growth of the plants. It could also be that the respiration rates of the plants also increased with the warming treatment. Respiration rates exponentially increase with experimentally warmed conditions, and if respiration increases more strongly than photosynthesis, increased respiration may diminish the effects of increased photosynthesis (Atkin et al. 2005, King et al. 2006).

*4.1.4 No effect on flower onset*

The warming treatment did not change the date of flower onset. This was contrary to my prediction, as temperature is a primary driver in regulating plant phenology (Parmesan and Yohe 2003, Jamieson et al. 2012, Thackeray et al. 2016). There is considerable evidence from observational and experimental studies that there has been an advancement in the timing of flowering with climate warming (e.g., Hollister and Webber 2000, McEwan et al. 2011, Rafferty et al. 2020). However, substantial interspecific variation in the degree and direction of change in timing has also been observed (Stuble et al. 2021). It is possible that I did not find responses because the i) magnitude of the warming effect of the OTCs was not great enough, ii) the start of the warming treatment was not early enough, or iii) the study species are not considered early-season species. The cues plants use for the timing of flowering often occur earlier in the season and sometimes more than a year earlier (Evers et al. 2021). For example, the timing of flowering onset is strongly influenced by the timing of budburst, so it is possible that the warming treatment was not started early enough, before budburst occurred, to influence flower phenology (Ettinger et al. 2018). Many studies show that earlier-season species (e.g., those that flower before the peak of summer heat) have experienced larger phenological shifts due to climate change than later-season species in the same community (Sherry et al. (2007); Chmura et al. 2019). Since the plant species in this study are late-season species that flower in the second half of summer, this may explain why I did not find an effect of the warming treatment on the timing of flowering.

#### *4.1.5 Consistent responses across plant species*

The effect of the warming treatment on the responses I measured was consistent across plant species. I did not find any responses where the effect of the warming treatment depended on species. This aligns with my prediction that since these three plant species share similar life history strategies, such as seasonality, reproductive methods, and seeding strategies, it is reasonable to expect similar responses to warming. Future studies should consider species with more divergent life history strategies, phenological patterns, and physiological tolerances to heat stress. They should explore whether the consistent responses observed in this study extend to a broader range of species and if certain traits offer resilience against climatic changes.

It is likely that I am underestimating the response of *S. canadensis* to the warming treatment since I was unable to count the number of flowers, and extraction of nectar from the flowers was very difficult. Thus, I did not find effects of the warming treatment as hypothesized. However, in comparison, a study that was successful in extracting nectar from the small flowers of *S. canadensis* found much higher levels of nectar sucrose concentration than I did (Jachuła et al. 2020).

### 4.2 Monarch Responses to the Warming Treatment

#### *4.2.1 Mixed effects on body composition components*

I found that monarchs who fed on warmed nectar had lower fat mass. This was expected given that I found a decrease in nectar sucrose concentration and the number of flowers in the warmed treatments. Monarchs convert sugar from nectar into fat in their bodies to use as a primary energy source (Alonso-Mejía et al. 1997, Brower et al. 2006, Sinclair and Marshall 2018).

Notably, the decreases in fat content measured in individuals who fed on warmed nectar could be biologically significant. The fat content of non-reproductive monarchs, like the ones used here, has previously been measured to be between 0.019 and 0.037 g and was found to decrease by 24-51% during the overwintering period (James 1984). A similar decline in fat mass during migration was also seen by Gibo and McCurdy (1993). The average fat content of monarchs who fed on control nectar in this experiment was 0.026 g, which falls in this expected range. Conversely, monarchs who fed on warmed nectar had an average fat content of 0.017 g, which is below the minimum measured by James (1984). Given that stored lipids are important fuel for the fall migration, for survival during their overwintering period (Brower et al. 2006), and to fuel the start of the spring migration (Enriquez and Visser 2023), a reduction of fat mass in monarchs who feed on lower-quality nectar from the warming treatment has the potential to put additional stress on the population.

In contrast, the lean mass of monarchs who fed on warmed nectar was not different compared to those who fed on control nectar. This was expected, given that nectar is not typically a good source of protein. Most of the protein in adult monarchs is thought to be accumulated during the larval stage while feeding on milkweed leaves, which are a good source of protein (Campbell and Carr 1987). Though not a high protein source, nectar does contain 16 of the 20 amino acids, which some butterflies are capable of consuming and using to build protein (Nepi et al. 2012, Borghi et al. 2019). Monarchs may use this protein as additional energy reserves for migration (Giulivi and Ramsey 2015). My results suggest that climatic warming will not have an impact on the availability of key amino acids in nectar and thus no impact of monarch lean mass.

Third, the water mass of monarchs who fed on warmed nectar was not different than those who fed on control nectar. This is inconsistent with my prediction that the water mass of monarchs would decrease with warming. I expected that the warming treatment might increase nectar water evaporation, since humidity is inversely proportional to the rate of water evaporation, and nectar tends to equilibrate with ambient humidity (Villarreal and Freeman 1990, Nicolson 2022). This would result in a decrease in the water content available to monarchs. However, this effect may have been minimized here due to Ottawa's high ambient humidity levels. My results suggest that monarch populations preparing for migration in Southern Ontario and Ottawa may not be at high risk of dehydration due to warming effects on plants.

### *4.2.2 Dependency of water mass on sex*

I found that the effect of the warming treatment on water mass was slightly lower for males than females and that males had higher water mass than females. This finding may be the result of differences in hydration or tissue water retention needs or energy metabolism differences between the sexes because of reproductive investments. For example, spermatophores are composed of 89.5% water and 10.5% organic compounds, and there is evidence that males place water directly in the spermatophores to minimize the energy expenditure that females would incur to do so (Pham 1997). This indicates that water may be an important resource for males. While water may also be a limiting component for egg production (Marshall 1982), the selection on females to maintain water reserves may not be as strong as in males, especially given that monarchs used were in reproductive diapause.

## 4.3 Monarch Responses to Other Variables

### *4.3.1 Consistent responses across plant species*

The body composition components did not respond differently to different plant species. This was surprising given that species differed in their nectar sucrose concentration and number of flowers regardless of treatment. A potential explanation for this discrepancy is the possibility of compensatory feeding of nectar by the monarchs; consuming more food in response to decreases in dietary nutrients (Simpson et al. 1989). Monarchs may do this either by eating larger meals or by feeding more often when nectar quality is lower (e.g., on *S. canadensis* but see section 4.1.5; Simpson et al. 1989). Given that the nectar sucrose concentration differed across species, adult monarchs may have compensated for lower quality nectar by consuming more and thus increasing their fat or water mass, eliminating any species-level differences. In the larval stage, monarchs are known to compensate for host plant quality by consuming more milkweed when quality is low (Lavoie and Oberhauser 2004). Nectarivorous birds will increase the volume of nectar consumed when the nectar has lower sucrose concentrations (Nicolson and Fleming 2003). Despite potential compensatory feeding, monarchs who fed on warmed nectar were unable to compensate to overcome differences in nectar sucrose concentration, as evidenced by a decrease in fat mass due to the warming treatment.

### *4.3.2 Seasonal changes in fat mass*

The fat mass of monarchs who eclosed later in the season was higher than those who eclosed earlier in the season. This effect may be in part due to greater pressure later in the season to accumulate energy stores in preparation for migration. Higher fat mass in later-season

monarchs may also be the result of seasonal changes in the milkweed leaves they were fed as larvae. Most monarch larvae in the study were fed milkweed leaves grown from seed in growth chambers during their first three instars and milkweed leaves collected by the Ottawa River in their later instars. However, the earliest monarchs that emerged from their eggs were exclusively fed milkweed from growth chambers while it was still abundant. In contrast, the latest monarchs were fed diets of Ottawa River milkweed leaves after the potted plants had been fully depleted. It is important to note that this does not necessarily indicate a seasonal effect of milkweed on monarchs. Throughout the season, monarch larvae were fed young milkweed as they have been shown to grow fastest on younger plants (Yang et al. 2020), but the source of the milkweed was different at the beginning and the end of the experiment. While I have yet to find evidence that the fat content of milkweed leaves may differ between potted and wild plants, this is a possible explanation for this relationship.

### *4.3.3 Body length related to lean and wet masses*

Adult monarchs with larger body lengths had higher wet and lean masses. The strong relationship between monarch body length and lean mass suggests that it may be a reliable predictor, potentially allowing for noninvasive estimates of lean mass without the need to sacrifice individuals for analysis. However, this body length relationship is weaker for wet mass and does not exist for fat mass or water mass, thus limiting the utility of body length as a predictor for body composition overall.

Body mass was included in the models to control for the inherent size of the monarchs upon eclosion. While there are many factors that influence insect body length (temperature, latitude, metabolic rate; Atkinson 1996, Sheridan and Bickford 2011), one important factor is

larval diet (Nicholls et al. 2021), particularly for monarchs. Since milkweed is the main source of protein for monarchs (Campbell and Carr 1987), it follows that larvae with larger body lengths also have higher lean mass as adults, the result of the milkweed they fed on as larvae.

## 4.4 Effectiveness of OTCs

The magnitude of the daytime warming effect of the chambers used in this study was, on average, 0.57°C, which, with current global warming predictions, is likely to occur within the next few decades, likely by the middle of the century (Calvin et al. 2023). Other studies using passive-warmed OTCs report a wide range in the magnitude of the temperature increase (0.7-1.8°C) (Marion et al. 1997, Dollery et al. 2006, Kudernatsch et al. 2008, Welshofer et al. 2018). The OTC warming effect found here is less than what is predicted by climate projections in Ontario, which estimate that average temperatures will rise by as much as 3°C to 8°C over the next century (Gough et al. 2016). Climate projections from the Climate Atlas of Canada (2019) indicate that under a High Carbon Scenario (RCP 8.5), the typical hottest day in Ottawa will rise from 33.7°C (1976-2005) to anywhere between 35.6°C and 42.4°C (2051-2080). Therefore, my results represent a conservative picture of the effects of future climatic warming.

As for virtually all field-warming experiments, my warming treatment likely altered environmental variables I addition to temperature (e.g., $CO_2$ concentrations, UV-B radiation; Wolkovich et al. 2012). Most notably, it is likely that the OTCs reduced soil moisture to some extent, as demonstrated by the results from 2024. This is consistent with a previous study that used a similar-sized OTC, albeit the effect in the study differed across sites (Welshofer et al. 2018). In any case, the effects of soil moisture possibly confounds the effects of warming on plant and monarch responses to the warming treatment (Borghi et al. 2019). However, several

lines of reasoning suggest minimal soil moisture-related effects of the warming treatment including finding no change in nectar volume in *M. fistulosa,* nor in monarch water mass with the warming treatment. Anecdotally, I did not find any indications of drought stress in plants in the warming treatment based on the most commonly documented symptoms, such as signs of scorching, yellowing or limp leaves, leaf rolling or brittleness, flower sagging, wilting, or premature fall (Seleiman et al. 2021). Thus, it could be that the decreases in soil moisture in the warming treatment may not be biologically meaningful. Nonetheless, to be precautionary, I have tried not to attribute OTC effects to be simply due to their warming effect but to the effect of the treatment overall.

### 4.5 Limitations

As the focus of this experiment was monarchs, I did not disentangle the direct effects of warming from the indirect effects on the focal plants included in the study. Plants were exposed to the existing insect community until the mesh enclosures were added in mid-August. Therefore, for part of the season, plants would have experienced direct effects of warming (and other environmental effects) from the OTCs and indirect effects of warming experienced by pollinators and herbivores that entered the chambers or were already present at the start of the experiment. However, the plant responses I observed here are comparable to many other OTC experiments, which also only measured the response of plants to the overall effects of warming (Marion et al. 1997, De Frenne et al. 2010, Welshofer et al. 2018).

I am not able to differentiate the effects of seasonality from plant species in the study. Despite all three plant species being late-season species, they had different flowering times (Table S5, Figure 2). As a result, seasonality is likely confounded with plant species. However,

as this was a field experiment with the aim of closely mimicking natural conditions, my results can provide important lessons for management (see next section). Future experiments that select species with more overlapping flowering periods are needed.

### 4.6 Implications for Habitat Restoration

Most habitat restoration has focused on the restoration of vegetation (i.e., bottom-up), with few targets centered on fauna and even fewer on animal-plant interactions (Genes and Dirzo 2022). My results have implications for pollinator-focused restoration. First, nectar availability and quality could decrease with climate change. As such, habitat restoration projects should aim to select plants that thrive in warming conditions and continue to provide optimal nectar resources for pollinators. These may include introduced plants with high adaptive capacity or those from more southern regions that have already adapted to warmer temperatures (Liu et al. 2017, Schlaepfer and Lawler 2023, Kharouba 2024).

Second, all three of the plant species used here responded to the warming treatment negatively and consistently. Therefore, until further studies on nectar quality and warming can be completed, the priority should be planting a high diversity of species to increase the likelihood that monarchs and other pollinators will have high-quality nectar available. Continuous monitoring of restored habitats and the quality of the nectar of plants found there may be beneficial to understanding the response of the community to climate change and would allow land managers to adjust planting strategies as needed.

Land managers should also consider butterfly and pollinator requirements to make effective restoration decisions. For example, while high sucrose nectar is important for creating fat stores to fuel migration, butterflies may choose to consume more dilute nectars with lower

sucrose concentrations to increase hydration (Willmer 1988, Nicolson 1998), especially as climate change increases the risk of hydration (Chown et al. 2011, Arnold and Michaels 2017). Therefore, choosing a variety of flowering plants with different sucrose concentrations will be increasingly important under climate change scenarios.

## 5. Conclusion

I found that the experimental warming treatment significantly reduced the nectar quality of late-season flowering plants. I show that monarchs that fed on this lower-quality warmed nectar had negative implications for fat mass. Fat mass is an important energy source for monarchs, fueling their migration to Mexico and sustaining them during overwintering. These results suggest that monarch populations may experience reduced survival and lower migration and overwintering success rates due to indirect effects of warming temperatures, mediated by changes in nectar plant quality and availability.

These results have important implications for informing habitat restoration practices. Future studies should take into account suggestions made here and also consider the responses of additional plant-insect interactions to warming temperatures. The consideration of strategies for habitat restoration, including planting a high diversity of flowering nectar plants as well as the potential use of 'non-native' plants from more Southern regions that may be better adapted to warming, may help mitigate the effects of climate change on pollinators (Kharouba 2024). Careful ecological assessment is important to ensure long-term climate resiliency of restored habitats.

**References**

Alonso-Mejía, A., E. Rendon-Salinas, E. Montesinos-Patiño, and L. P. Brower. 1997. Use of lipid reserves by monarch butterflies overwinteri ng in Mexico: Implications for conservation. Ecological Applications 7:934–947.

Arneth, A., Y.-J. Shin, P. Leadley, C. Rondinini, E. Bukvareva, M. Kolb, G. F. Midgley, T. Oberdorff, I. Palomo, and O. Saito. 2020. Post-2020 biodiversity targets need to embrace climate change. Proceedings of the National Academy of Sciences 117:30882–30891.

Arnold, P. M., and H. J. Michaels. 2017. Nectar Sampling for Prairie and Oak Savanna Butterfly Restoration. Applications in Plant Sciences 5:1600148.

Atkin, O. K., D. Bruhn, V. M. Hurry, and M. G. Tjoelker. 2005. The hot and the cold: unravelling the variable response of plant respiration to temperature. Functional Plant Biology 32:87.

Atkinson, D. 1996. Ectotherm life-history responses to developmental temperature. Pages 183–204 *in* I. A. Johnston and A. F. Bennett, editors. Animals and Temperature. First edition. Cambridge University Press.

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

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2022. lme4: Linear Mixed-Effects Models using Eigen and S4.

Benayas, J. M. R., A. C. Newton, A. Diaz, and J. M. Bullock. 2009. Enhancement of Biodiversity and Ecosystem Services by Ecological Restoration: A Meta-Analysis. Science 325:1121–1124.

Berggren, Å., C. Björkman, H. Bylund, and M. P. Ayres. 2009. The distribution and abundance of animal populations in a climate of uncertainty. Oikos 118:1121–1126.

Borghi, M., L. Perez de Souza, T. Yoshida, and A. R. Fernie. 2019. Flowers and climate change: a metabolic perspective. New Phytologist 224:1425–1441.

Breed, M. F., M. G. Stead, K. M. Ottewell, M. G. Gardner, and A. J. Lowe. 2013. Which provenance and where? Seed sourcing strategies for revegetation in a changing environment. Conservation Genetics 14:1–10.

Brooks, M., B. Bolker, K. Kristensen, M. Maechler, A. Magnusson, H. Skaug, A. Nielsen, C. Berg, and K. van Bentham. 2023. glmmTMB: Generalized Linear Mixed Models using Template Model Builder.

Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. Maechler, and B. M. Bolker. 2017. glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. The R Journal 9:378–400.

Brower, L. P. 1996. Monarch Butterfly Orientation: Missing Pieces of a Magnificent Puzzle. Journal of Experimental Biology 199:93–103.

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

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

Calvin, K., D. Dasgupta, G. Krinner, A. Mukherji, P. W. Thorne, C. Trisos, J. Romero, P. Aldunce, K. Barrett, G. Blanco, W. W. L. Cheung, S. Connors, F. Denton, A. Diongue-Niang, D. Dodman, M. Garschagen, O. Geden, B. Hayward, C. Jones, F. Jotzo, T. Krug, R. Lasco, Y.-Y. Lee, V. Masson-Delmotte, M. Meinshausen, K. Mintenbeck, A. Mokssit, F. E. L. Otto, M. Pathak, A. Pirani, E. Poloczanska, H.-O. Pörtner, A. Revi, D. C. Roberts, J. Roy, A. C. Ruane, J. Skea, P. R. Shukla, R. Slade, A. Slangen, Y. Sokona, A. A. Sörensson, M. Tignor, D. Van Vuuren, Y.-M. Wei, H. Winkler, P. Zhai, Z. Zommers, J.-C. Hourcade, F. X. Johnson, S. Pachauri, N. P. Simpson, C. Singh, A. Thomas, E. Totin, P. Arias, M. Bustamante, I. Elgizouli, G. Flato, M. Howden, C. Méndez-Vallejo, J. J. Pereira, R. Pichs-Madruga, S. K. Rose, Y. Saheb, R. Sánchez Rodríguez, D. Ürge-Vorsatz, C. Xiao, N. Yassaa, A. Alegría, K. Armour, B. Bednar-Friedl, K. Blok, G. Cissé, F. Dentener, S. Eriksen, E. Fischer, G. Garner, C. Guivarch, M. Haasnoot, G. Hansen, M. Hauser, E. Hawkins, T. Hermans, R. Kopp, N. Leprince-Ringuet, J. Lewis, D. Ley, C. Ludden, L. Niamir, Z. Nicholls, S. Some, S. Szopa, B. Trewin, K.-I. Van Der Wijst, G. Winter, M. Witting, A. Birt, M. Ha, J. Romero, J. Kim, E. F. Haites, Y. Jung, R. Stavins, A. Birt, M. Ha, D. J. A. Orendain, L. Ignon, S. Park, Y. Park, A. Reisinger, D. Cammaramo, A. Fischlin, J. S. Fuglestvedt, G. Hansen, C. Ludden, V. Masson-Delmotte, J. B. R. Matthews, K. Mintenbeck, A. Pirani, E. Poloczanska, N. Leprince-Ringuet, and C. Péan. 2023. IPCC, 2023: Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. IPCC, Geneva, Switzerland. First. Intergovernmental Panel on Climate Change (IPCC).

Campbell, T. A., and M. E. Carr. 1987. Variation in vigor, crude protein, and extract yields among individual common milkweed (asclepias syriaca L.) plants. Biomass 12:293–299.

Chmura, H. E., H. M. Kharouba, J. Ashander, S. M. Ehlman, E. B. Rivest, and L. H. Yang. 2019. The mechanisms of phenology: the patterns and processes of phenological shifts. Ecological Monographs 89:e01337.

Chown, S. L., J. G. Sørensen, and J. S. Terblanche. 2011. Water loss in insects: An environmental change perspective. Journal of Insect Physiology 57:1070–1084.

Christian, H.-M. 2023. The Main Drivers of Biodiversity Loss: A Brief Overview. Journal of Ecology & Natural Resources 7.

Contreras, H. L., J. Goyret, C. T. Pierce, R. A. Raguso, and G. Davidowitz. 2022. Eat, Drink, Live: Foraging behavior of a nectarivore when relative humidity varies but nectar resources do not. Journal of Insect Physiology 143:104450.

Corbet, S. A. 2003. Nectar sugar content: estimating standing crop and secretion rate in the field. Apidologie 34:1–10.

Damien, M., S. Llopis, N. Desneux, J. Van Baaren, and C. Le Lann. 2020. How does floral nectar quality affect life history strategies in parasitic wasps? Entomologia Generalis 40:147–156.

De Frenne, P., A. De Schrijver, B. J. Graae, R. Gruwez, W. Tack, F. Vandelook, M. Hermy, and K. Verheyen. 2010. The use of open-top chambers in forests for evaluating warming effects on herbaceous understorey plants. Ecological Research 25:163–171.

Descamps, C., A. Jambrek, M. Quinet, and A.-L. Jacquemart. 2021a. Warm Temperatures Reduce Flower Attractiveness and Bumblebee Foraging. Insects 12:493.

Descamps, C., M. Quinet, and A.-L. Jacquemart. 2021b. Climate Change–Induced Stress Reduce Quantity and Alter Composition of Nectar and Pollen From a Bee-Pollinated Species (Borago officinalis, Boraginaceae). Frontiers in Plant Science 12:755843.

Dollery, R., I. D. Hodkinson, and I. S. Jónsdóttir. 2006. Impact of warming and timing of snow melt on soil microarthropod assemblages associated with *Dryas*- dominated plant communities on Svalbard. Ecography 29:111–119.

Enriquez, T., and B. Visser. 2023. The importance of fat accumulation and reserves for insect overwintering. Current Opinion in Insect Science 60:101118.

Ettinger, A. K., S. Gee, and E. M. Wolkovich. 2018. Phenological sequences: how early-season events define those that follow. American Journal of Botany 105:1771–1780.

Evers, S. M., T. M. Knight, D. W. Inouye, T. E. X. Miller, R. Salguero-Gómez, A. M. Iler, and A. Compagnoni. 2021. Lagged and dormant season climate better predict plant vital rates than climate during the growing season. Global Change Biology 27:1927–1941.

Fahrig, L. 1997. Relative Effects of Habitat Loss and Fragmentation on Population Extinction. The Journal of Wildlife Management 61:603.

Fu, G., Z.-X. Shen, W. Sun, Z.-M. Zhong, X.-Z. Zhang, and Y.-T. Zhou. 2015. A Meta-analysis of the Effects of Experimental Warming on Plant Physiology and Growth on the Tibetan Plateau. Journal of Plant Growth Regulation 34:57–65.

Garibaldi, L. A., L. G. Carvalheiro, S. D. Leonhardt, M. A. Aizen, B. R. Blaauw, R. Isaacs, M. Kuhlmann, D. Kleijn, A. M. Klein, C. Kremen, L. Morandin, J. Scheper, and R. Winfree. 2014. From research to action: enhancing crop yield through wild pollinators. Frontiers in Ecology and the Environment 12:439–447.

Gawecka, K. A., and J. Bascompte. 2023. Habitat restoration and the recovery of metacommunities. Journal of Applied Ecology 60:1622–1636.

Ge, Q., H. Wang, T. Rutishauser, and J. Dai. 2015. Phenological response to climate change in China: a meta-analysis. Global Change Biology 21:265–274.

Genes, L., and R. Dirzo. 2022. Restoration of plant-animal interactions in terrestrial ecosystems. Biological Conservation 265:109393.

Gibo, D. L., and J. A. McCurdy. 1993. Lipid accumulation by migrating monarch butterflies ( *Danaus plexippus* L.). Canadian Journal of Zoology 71:76–82.

Gilbert, O. L., and P. Anderson. 1998. Habitat creation and repair. Oxford University Press, Oxford ; New York.

Gillooly, J. F., J. H. Brown, G. B. West, V. M. Savage, and E. L. Charnov. 2001. Effects of Size and Temperature on Metabolic Rate. Science 293:2248–2251.

Giulivi, C., and J. Ramsey. 2015. On fuel choice and water balance during migratory bird flights. International Biology Review 2015:58.

Gordon, S. C. C., and J. T. Kerr. 2022. Floral diversity increases butterfly diversity in a multitrophic metacommunity. Ecology 103:e3735.

Gough, W., V. Anderson, and K. Herod. 2016. Ontario Climate Change and Health Modelling Study. Ministry of Health and Long-Term Care, Public Health Policy and Programs Branch, Toronto, Ontario.

Grainger, T. N., and B. Gilbert. 2017. Multi-scale responses to warming in an experimental insect metacommunity. Global Change Biology 23:5151–5163.

Habibullah, M. S., B. H. Din, S.-H. Tan, and H. Zahid. 2022. Impact of climate change on biodiversity loss: global evidence. Environmental Science and Pollution Research 29:1073–1086.

Harvey, J. A., K. Tougeron, R. Gols, R. Heinen, M. Abarca, P. K. Abram, Y. Basset, M. Berg, C. Boggs, J. Brodeur, P. Cardoso, J. G. de Boer, G. R. De Snoo, C. Deacon, J. E. Dell, N. Desneux, M. E. Dillon, G. A. Duffy, L. A. Dyer, J. Ellers, A. Espíndola, J. Fordyce, M. L. Forister, C. Fukushima, M. J. G. Gage, C. García-Robledo, C. Gely, M. Gobbi, C. Hallmann, T. Hance, J. Harte, A. Hochkirch, C. Hof, A. A. Hoffmann, J. G. Kingsolver, G. P. A. Lamarre, W. F. Laurance, B. Lavandero, S. R. Leather, P. Lehmann, C. Le Lann, M. M. López-Uribe, C. Ma, G. Ma, J. Moiroux, L. Monticelli, C. Nice, P. J. Ode, S. Pincebourde, W. J. Ripple, M. Rowe, M. J. Samways, A. Sentis, A. A. Shah, N. Stork, J. S. Terblanche, M. P. Thakur, M. B. Thomas, J. M. Tylianakis, J. Van Baaren, M. Van de Pol, W. H. Van der Putten, H. Van Dyck, W. C. E. P. Verberk, D. L. Wagner, W. W. Weisser, W. C. Wetzel, H. A. Woods, K. A. G. Wyckhuys, and S. L. Chown. 2022. Scientists' warning on climate change and insects. Ecological Monographs.

Hollister, R. D., and P. J. Webber. 2000. Biotic validation of small open-top chambers in a tundra ecosystem. Global Change Biology 6:835–842.

IPBES. 2019. Summary for policymakers of the global assessment report on biodiversity and ecosystem services. Zenodo.

Jachuła, J., B. Denisow, and M. Strzałkowska-Abramek. 2020. Does an invader have a bright side? Floral reward in two *Solidago* species. Journal of Apicultural Research 59:599–608.

Jacobs, D. F., J. A. Oliet, J. Aronson, A. Bolte, J. M. Bullock, P. J. Donoso, S. M. Landhäusser, P. Madsen, S. Peng, J. M. Rey-Benayas, and J. C. Weber. 2015. Restoring forests: What constitutes success in the twenty-first century? New Forests 46:601–614.

Jagadish, S. V. K. 2020. Heat stress during flowering in cereals – effects and adaptation strategies. New Phytologist 226:1567–1572.

James, D. G. 1984. Phenology of weight, moisture and energy reserves of Australian monarch butterflies, *Danaus plexippus*. Ecological Entomology 9:421–428.

Jamieson, M. A., A. M. Trowbridge, K. F. Raffa, and R. L. Lindroth. 2012. Consequences of Climate Warming and Altered Precipitation Patterns for Plant-Insect and Multitrophic Interactions. Plant Physiology 160:1719–1727.

Kharouba, H. M. 2024. Shifting the paradigm: The role of introduced plants in the resiliency of terrestrial ecosystems to climate change. Global Change Biology 30:e17319.

Kharouba, H. M., and L. H. Yang. 2021. Disentangling the direct, indirect, and combined effects of experimental warming on a plant–insect herbivore interaction. Ecosphere 12.

King, A. W., C. A. Gunderson, W. M. Post, D. J. Weston, and S. D. Wullschleger. 2006. Plant Respiration in a Warmer World. Science 312:536–537.

Kingsolver, J. G., S. E. Diamond, and L. B. Buckley. 2013. Heat stress and the fitness consequences of climate change for terrestrial ectotherms. Functional Ecology 27:1415–1423.

Koštál, V. 2006. Eco-physiological phases of insect diapause. Journal of Insect Physiology 52:113–127.

Kudernatsch, T., A. Fischer, M. Bernhardt-Römermann, and C. Abs. 2008. Short-term effects of temperature enhancement on growth and reproduction of alpine grassland species. Basic and Applied Ecology 9:263–274.

Lavoie, B., and K. S. Oberhauser. 2004. Compensatory Feeding in *Danaus plexippus* (Lepidoptera: Nymphalidae) in Response to Variation in Host Plant Quality. Environmental Entomology 33:1062–1069.

Lebeau, J., R. A. Wesselingh, and H. Van Dyck. 2016. Nectar resource limitation affects butterfly flight performance and metabolism differently in intensive and extensive agricultural landscapes. Proceedings of the Royal Society B: Biological Sciences 283:20160455.

Lemoine, N. P., and D. E. Burkepile. 2012. Temperature-induced mismatches between consumption and metabolism reduce consumer fitness. Ecology 93:2483–2489.

Lemoine, N. P., D. E. Burkepile, and J. D. Parker. 2014. Variable effects of temperature on insect herbivory. PeerJ 2:e376.

Lemoine, N. P., J. N. Capdevielle, and J. D. Parker. 2015. Effects of *in situ* climate warming on monarch caterpillar ( *Danaus plexippus* ) development. PeerJ 3:e1293.

Lemoine, N. P., W. A. Drews, D. E. Burkepile, and J. D. Parker. 2013. Increased temperature alters feeding behavior of a generalist herbivore. Oikos 122:1669–1678.

Liu, Y., J. Mu, K. J. Niklas, G. Li, and S. Sun. 2012. Global warming reduces plant reproductive output for temperate multi-inflorescence species on the Tibetan plateau. New Phytologist 195:427–436.

Liu, Y., A. M. O. Oduor, Z. Zhang, A. Manea, I. M. Tooth, M. R. Leishman, X. Xu, and M. Van Kleunen. 2017. Do invasive alien plants benefit more from global environmental change than native plants? Global Change Biology 23:3363–3370.

Lorer, E., K. Verheyen, H. Blondeel, K. De Pauw, P. Sanczuk, P. De Frenne, and D. Landuyt. 2024. Forest understorey flowering phenology responses to experimental warming and illumination. New Phytologist 241:1476–1491.

de Manincor, N., A. Fisogni, and N. E. Rafferty. 2023. Warming of experimental plant–pollinator communities advances phenologies, alters traits, reduces interactions and depresses reproduction. Ecology Letters 26:323–334.

Marden, J. H. 1989. Bodybuilding Dragonflies: Costs and Benefits of Maximizing Flight Muscle. Physiological Zoology 62:505–521.

Marion, G. M., G. H. R. Henry, D. W. Freckman, J. Johnstone, G. Jones, M. H. Jones, E. Lévesque, U. Molau, P. Mølgaard, A. N. Parsons, J. Svoboda, and R. A. Virginia. 1997. Open-top designs for manipulating field temperature in high-latitude ecosystems. Global Change Biology 3:20–32.

Marshall, K. E., K. Gotthard, and C. M. Williams. 2020. Evolutionary impacts of winter climate change on insects. Current Opinion in Insect Science 41:54–62.

McCree, K. J. 1971. The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. Agricultural Meteorology 9:191–216.

McElwee, P. 2021. Climate Change and Biodiversity Loss. Current History 120:295–300.

McEwan, R. W., R. J. Brecha, D. R. Geiger, and G. P. John. 2011. Flowering phenology change and climate warming in southwestern Ohio. Plant Ecology 212:55–61.

Menzel, A., T. H. Sparks, N. Estrella, E. Koch, A. Aasa, R. Ahas, K. Alm-Kübler, P. Bissolli, O. Braslavská, A. Briede, F. M. Chmielewski, Z. Crepinsek, Y. Curnel, Å. Dahl, C. Defila, A. Donnelly, Y. Filella, K. Jatczak, F. Måge, A. Mestre, Ø. Nordli, J. Peñuelas, P. Pirinen, V. Remišová, H. Scheifinger, M. Striz, A. Susnik, A. J. H. Van Vliet, F. Wielgolaski, S. Zach, and A. Zust. 2006. European phenological response to climate change matches the warming pattern. Global Change Biology 12:1969–1976.

M'Gonigle, L. K., L. C. Ponisio, K. Cutler, and C. Kremen. 2015. Habitat restoration promotes pollinator persistence and colonization in intensively managed agriculture. Ecological Applications 25:1557–1565.

Miller, J. R., and R. J. Hobbs. 2007. Habitat Restoration—Do We Know What We're Doing? Restoration Ecology 15:382–390.

Minnemeyer, S., L. Laestadius, N. Sizer, C. Saint-Laurent, and P. Potapov. 2011. A World of Opportunity. World Resource Institute, Washington, DC.

Moss, E. D., and D. M. Evans. 2022. Experimental Climate Warming Reduces Floral Resources and Alters Insect Visitation and Wildflower Seed Set in a Cereal Agro-Ecosystem. Frontiers in Plant Science 13:826205.

National Plant Data Team, Greensboro, NC USA. 2024. The PLANTS Database.

Nepi, M., C. Soligo, D. Nocentini, M. Abate, M. Guarnieri, G. Cai, L. Bini, M. Puglia, L. Bianchi, and E. Pacini. 2012. Amino acids and protein profile in floral nectar: Much more than a simple reward. Flora - Morphology, Distribution, Functional Ecology of Plants 207:475–481.

Nicholls, E., M. Rossi, and J. E. Niven. 2021. Larval nutrition impacts survival to adulthood, body size and the allometric scaling of metabolic rate in adult honeybees. Journal of Experimental Biology 224:jeb242393.

Nicolson, S. W. 1998. The Importance of Osmosis in Nectar Secretion and its Consumption by Insects.

Nicolson, S. W. 2022. Sweet solutions: nectar chemistry and quality. Philosophical Transactions of the Royal Society B: Biological Sciences 377:20210163.

Nicolson, S. W., and P. A. Fleming. 2003. Energy balance in the Whitebellied Sunbird *Nectarinia talatala* : constraints on compensatory feeding, and consumption of supplementary water. Functional Ecology 17:3–9.

Nicolson, S. W., and R. W. Thornburg. 2007. Nectar Chemistry in Nectaries and Nectar. Springer, Dordrecht, The Netherlands.

O'Donnell, M. J. 2022. A perspective on insect water balance. Journal of Experimental Biology 225:jeb242358.

O'Regan, S. M., C. G. Guglielmo, and G. M. Taylor. 2012. Measurement of arthropod body composition using quantitative magnetic resonance. Invertebrate Biology 131:216–223.

Ovaskainen, O., S. Skorokhodova, M. Yakovleva, A. Sukhov, A. Kutenkov, N. Kutenkova, A. Shcherbakov, E. Meyke, and M. D. M. Delgado. 2013. Community-level phenological response to climate change. Proceedings of the National Academy of Sciences 110:13434–13439.

Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421:37–42.

Pham, E. Y. 1997, June. Chemical composition of the spermatophores of overwintering monarch butterflies (Danaus plexippus [L].). California Polytechnic State University, San Luis Obispo, California, USA.

Prakash, S., and A. K. Verma. 2022. Anthropogenic activities and biodiversity threats. International Journal of Biological Innovations 04:94–103.

Prober, S. M., M. Byrne, E. H. McLean, D. A. Steane, B. M. Potts, R. E. Vaillancourt, and W. D. Stock. 2015. Climate-adjusted provenancing: a strategy for climate-resilient ecological restoration. Frontiers in Ecology and Evolution 3.

Prober, S. M., V. A. J. Doerr, L. M. Broadhurst, K. J. Williams, and F. Dickson. 2019. Shifting the conservation paradigm: a synthesis of options for renovating nature under climate change. Ecological Monographs 89:e01333.

R Core Team. 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rafferty, N. E., J. M. Diez, and C. D. Bertelsen. 2020. Changing Climate Drives Divergent and Nonlinear Shifts in Flowering Phenology across Elevations. Current Biology 30:432-441.e3.

Saavedra, F., D. W. Inouye, M. V. Price, and J. Harte. 2003. Changes in flowering and abundance of *Delphinium nuttallianum* (Ranunculaceae) in response to a subalpine climate warming experiment. Global Change Biology 9:885–894.

Sánchez-Tlacuahuac, N., J. L. Pimentel-Equihua, V. Espinosa-Hernández, and H. Vibrans. 2023. What do monarchs feed on in winter? Nectar sources at hibernation sites. Journal of Insect Conservation 27:181–191.

Scaven, V. L., and N. E. Rafferty. 2013. Physiological effects of climate warming on flowering plants and insect pollinators and potential consequences for their interactions. Current Zoology 59:418–426.

Schlaepfer, M. A., and J. J. Lawler. 2023. Conserving biodiversity in the face of rapid climate change requires a shift in priorities. WIREs Climate Change 14:e798.

Seleiman, M. F., N. Al-Suhaibani, N. Ali, M. Akmal, M. Alotaibi, Y. Refay, T. Dindaroglu, H. H. Abdul-Wajid, and M. L. Battaglia. 2021. Drought Stress Impacts on Plants and Different Approaches to Alleviate Its Adverse Effects. Plants 10:259.

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

Sheridan, J. A., and D. Bickford. 2011. Shrinking body size as an ecological response to climate change. Nature Climate Change 1:401–406.

Sherry, R. A., X. Zhou, S. Gu, J. A. Arnone, D. S. Schimel, P. S. Verburg, L. L. Wallace, and Y. Luo. 2007. Divergence of reproductive phenology under climate warming. Proceedings of the National Academy of Sciences 104:198–202.

Shirey, V., and L. Ries. 2023. Population dynamics and drivers of the eastern monarch (Danaus plexippus) across its full annual cycle: a cross-scale synthesis of a model migratory species. Current Opinion in Insect Science 60:101132.

Shivanna, K. R. 2022. Climate change and its impact on biodiversity and human welfare. Proceedings of the Indian National Science Academy 88:160–171.

Simonson, W. D., E. Miller, A. Jones, S. García-Rangel, H. Thornton, and C. McOwen. 2021. Enhancing climate change resilience of ecological restoration — A framework for action. Perspectives in Ecology and Conservation 19:300–310.

Simpson, S. J., L. B. Browne, and A. C. M. V. Gerwen. 1989. The patterning of compensatory sugar feeding in the Australian sheep blowfly. Physiological Entomology 14:91–105.

Sinclair, B. J., and K. E. Marshall. 2018. The many roles of fats in overwintering insects. Journal of Experimental Biology 221:jeb161836.

Stuble, K. L., L. D. Bennion, and S. E. Kuebbing. 2021. Plant phenological responses to experimental warming—A synthesis. Global Change Biology 27:4110–4124.

Sun, H., B. Huang, X. Yu, C. Tian, Q. Peng, and D. An. 2018. Pollen limitation, reproductive success and flowering frequency in single-flowered plants. Journal of Ecology 106:19–30.

Tait, L. W., and D. R. Schiel. 2013. Impacts of Temperature on Primary Productivity and Respiration in Naturally Structured Macroalgal Assemblages. PLoS ONE 8:e74413.

Takkis, K., T. Tscheulin, and T. Petanidou. 2018. Differential Effects of Climate Warming on the Nectar Secretion of Early- and Late-Flowering Mediterranean Plants. Frontiers in Plant Science 9:874.

Takkis, K., T. Tscheulin, P. Tsalkatis, and T. Petanidou. 2015. Climate change reduces nectar secretion in two common Mediterranean plants. AoB Plants 7:plv111.

Thackeray, S. J., P. A. Henrys, D. Hemming, J. R. Bell, M. S. Botham, S. Burthe, P. Helaouet, D. G. Johns, I. D. Jones, D. I. Leech, E. B. Mackay, D. Massimino, S. Atkinson, P. J. Bacon, T. M. Brereton, L. Carvalho, T. H. Clutton-Brock, C. Duck, M. Edwards, J. M. Elliott, S. J. G. Hall, R. Harrington, J. W. Pearce-Higgins, T. T. Høye, L. E. B. Kruuk, J.

M. Pemberton, T. H. Sparks, P. M. Thompson, I. White, I. J. Winfield, and S. Wanless. 2016. Phenological sensitivity to climate across taxa and trophic levels. Nature 535:241–245.

Tseng, M., K. M. Kaur, S. Soleimani Pari, K. Sarai, D. Chan, C. H. Yao, P. Porto, A. Toor, H. S. Toor, and K. Fograscher. 2018. Decreases in beetle body size linked to climate change and warming temperatures. Journal of Animal Ecology 87:647–659.

Villarreal, A. G., and C. E. Freeman. 1990. Effects of Temperature and Water Stress on Some Floral Nectar Characteristics in Ipomopsis longiflora (Polemoniaceae) under Controlled Conditions. Botanical Gazette 151:5–9.

Welsh, A. H., R. B. Cunningham, and R. L. Chambers. 2000. Methodology for Estimating the Abundance of Rare Animals: Seabird Nesting on North East Herald Cay. Biometrics 56:22–30.

Welshofer, K. B., P. L. Zarnetske, N. K. Lany, and L. A. E. Thompson. 2018. Open-top chambers for temperature manipulation in taller-stature plant communities. Methods in Ecology and Evolution 9:254–259.

Wickham, H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Wickham, H., W. Chang, L. Henry, T. L. Pedersen, K. Takahashi, C. Wilke, K. Woo, H. Yutani, and D. Dunnington. 2023. ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics.

Willmer, P. G. 1988. The role of insect water balance in pollination ecology: Xylocopa and Calotropis. Oecologia 76:430–438.

Willmer, P. G. 2011. Rewards 2: The Biology of Nectar in Pollination and floral ecology. Princeton University Press, Princeton, New Jersey, USA.

Wolff, D. 2006. Nectar Sugar Composition and Volumes of 47 Species of Gentianales from a Southern Ecuadorian Montane Forest. Annals of Botany 97:767–777.

Wonglersak, R., P. B. Fenberg, P. G. Langdon, S. J. Brooks, and B. W. Price. 2021. Insect body size changes under future warming projections: a case study of Chironomidae (Insecta: Diptera). Hydrobiologia 848:2785–2796.

Yang, L. H., M. L. Cenzer, L. J. Morgan, and G. W. Hall. 2020. Species-specific, age-varying plant traits affect herbivore growth and survival. Ecology 101:e03029.

Yang, P. F., C. Markmann, K. Voigt, and R. Braverman. (n.d.). Passive Warming Chambers Decrease Abundance of Flying Insects.

Zalucki, M. P. 1982. Temperature and rate of development in Danaus plexippus L. and D. chrysippus L. (Lepidoptera:Nymphalidae). Australian Journal of Entomology 21:241–246.

Zwaan, B. J., R. Bijlsma, and R. F. Hoekstra. 1991. On the developmental theory of ageing. I. Starvation resistance and longevity in Drosophila melanogaster in relation to pre-adult breeding conditions. Heredity 66:29–39.

Zylstra, E. R., L. Ries, N. Neupane, S. P. Saunders, M. I. Ramírez, E. Rendón-Salinas, K. S. Oberhauser, M. T. Farr, and E. F. Zipkin. 2021. Changes in climate drive recent monarch butterfly dynamics. Nature Ecology & Evolution 5:1441–1452.

Zylstra, E. R., W. E. Thogmartin, M. I. Ramirez, and E. F. Zipkin. 2020. Summary of available data from the monarch overwintering colonies in central Mexico, 1976-1991. Open-File Report, U.S. Geological Survey.

## Tables

**Table 1.** Responses of mean daytime temperature (°C), nighttime temperature (°C), and temperature fluctuation (°C) based on the warming effect of the open-top chambers (n=560 measurements from 12 loggers over 66 days) fit as linear mixed effects models. The table shows the estimated effects (Estimate), standard error (SE), degrees of freedom (DF), F-values (F), conditional (first) and marginal (second) $R^2$ values, and p-values (*p*) for the predictors warming treatment, and DOY for mean daytime temperature (°C), with pair as a random effect. Bold font indicates statistical significance ($p<0.05$).

| Response Variable | Predictor | Estimate | SE | DF | F | $R^2$ | *p* |
|---|---|---|---|---|---|---|---|
| Mean Daytime Temperature (°C) | **Treatment** | **0.57** | **0.28** | **1, 552** | **4.18** | **0.22, 0.19** | **<0.05** |
| | **DOY** | **-0.090** | **0.0081** | **1, 537** | **122.36** | | **<0.001** |
| Mean Temperature Fluctuation (°C) | Treatment | 0.59 | 0.044 | 1, 553 | 1.76 | 0.004, 0.03 | 0.19 |
| Mean Nighttime Temperature (°C) | Treatment | 0.45 | 0.24 | 1, 481 | 3.40 | 0.02, 0.01 | 0.066 |

**Table 2.** Vegetative and floral development and nectar production responses of three plant species to the warming treatment of the Open-Top Chambers. Plant responses are relative stem growth (%) (n=301), day of year of flower onset (n=183), number of flowers (n=147), nectar sucrose concentration (°BRIX) (n=250), and nectar volume (μL) (n=155). Relative stem growth is fit with a linear model, and nectar volume with a linear mixed effects model. Flower onset, number of flowers, and nectar concentration are fit with generalized linear mixed effects models. The table shows the estimated effects (Estimate), standard error (SE), degrees of freedom (DF), F-values (F) for linear mixed effects models, the z-values for generalized linear mixed effects models, and p-values (*p*). Conditional $R^2$ (first) and marginal $R^2$ (second) are reported for each model. Fixed effects are warming treatment and species. Only warming treatment is included as a fixed effect for nectar volume. Pair is a random effect for all models except for relative stem growth. Day of year (DOY) is included as a random effect in the number of flowers, nectar concentration, and nectar volume models. Bold font indicates statistical significance ($p<0.05$).

| Response Variable | Predictor | Estimate | SE | DF | F/z | $R^2$ | *p* |
|---|---|---|---|---|---|---|---|
| Relative Stem Growth (%) | Treatment | 1.8 | 3.1 | 1, 270 | 0.5 | 0.18 | 0.48 |
| | **Species** | **NA** | **NA** | **2, 270** | **29.38** | | **<0.001** |
| Flower onset (DOY) | Treatment | 0.0021 | 0.0030 | 1, 177 | 0.7 | 0.96, 0.86 | 0.48 |
| | **Species** | **NA** | **NA** | **2, 177** | **NA** | | **<0.05** |
| # of Flowers | **Treatment** | **-0.25** | **0.055** | **1, 531** | **-4.62** | **0.54, 0.31** | **<0.001** |
| | **Species** | **NA** | **NA** | **2, 531** | **3.76** | | **<0.001** |
| Nectar Concentration (°BRIX) | **Treatment** | **-0.27** | **0.066** | **1, 229** | **-4.03** | **0.78, 0.26** | **<0.001** |
| | **Species** | **NA** | **NA** | **2, 229** | **NA** | | **<0.01** |
| Nectar Volume (μL) | Treatment | -0.072 | 0.057 | 1, 143 | 1.61 | 0.35, 0.01 | 0.21 |

**Table 3.** Monarch body condition responses to indirect warming of the warming treatment from the Open-Top Chambers. All monarch responses are square root transformed and include wet mass (g) (n=59), fat mass (g) (n=59), lean mass (g) (n=59), and water mass (g) (n=59). All models are fit with linear models. The table shows the estimated effects (Estimate), standard error (SE), degrees of freedom (DF), F-values (F), R-squared values, and p-values (*p*). Fixed effects are warming treatment, sex, species, day of year (DOY), body length, and biologically significant two-way interactions (p<0.08). Bold font indicates statistical significance (*p*<0.05).

| Response Variable | Predictor | Estimate | SE | DF | F | $R^2$ | *p* |
|---|---|---|---|---|---|---|---|
| Wet Mass (g) | Treatment | -0.028 | 0.018 | 1, 51 | 1.18 | 0.17 | 0.18 |
| | Sex | 0.012 | 0.026 | 1, 51 | 2.57 | | 0.12 |
| | Species | NA | NA | 2, 51 | 0.59 | | 0.56 |
| | DOY | 0.0030 | 0.0027 | 1, 51 | 0.19 | | 0.89 |
| | Body Length | 0.015 | 0.0065 | 1, 51 | 3.64 | | 0.063 |
| Fat Mass (g) | **Treatment** | **-0.038** | **0.016** | 1, 51 | **3.17** | **0.15** | **<0.05** |
| | Sex | -0.021 | 0.023 | 1, 51 | 0.027 | | 0.87 |
| | Species | NA | NA | 2, 51 | 0.22 | | 0.80 |
| | **DOY** | **0.0052** | **0.0024** | 1, 51 | **4.68** | | **<0.05** |
| | Body Length | 0.0037 | 0.0057 | 1, 51 | 0.30 | | 0.59 |
| Lean Mass (g) | Treatment | -0.015 | 0.011 | 1, 51 | 0.41 | 0.24 | 0.41 |
| | Sex | -0.0010 | 0.016 | 1, 51 | 0.63 | | 0.56 |
| | Species | NA | NA | 2, 51 | 0.92 | | 0.53 |
| | DOY | 0.0016 | 0.0016 | 1, 51 | 0.99 | | 0.37 |
| | **Body Length** | **0.013** | **0.0039** | 1, 51 | **11.74** | | **<0.005** |
| Water Mass (g) | Treatment | -0.0046 | 0.013 | 1, 51 | 1.56 | 0.23 | 0.22 |
| | **Sex** | **0.037** | **0.020** | **1, 51** | **6.37** | | **<0.05** |
| | Species | NA | NA | 2, 51 | 0.35 | | 0.84 |
| | DOY | 0.0012 | 0.0020 | 1, 51 | 0.20 | | 0.62 |
| | Body Length | 0.010 | 0.0047 | 1, 51 | 3.23 | | 0.07 |
| | *Warming * Sex* | -0.064 | 0.036 | 1, 51 | 3.08 | | 0.08 |

## Figures

**Figure 1.** Locations of experimental sites in Ottawa, ON, Canada. **a)** Map of Ottawa. Fletcher Wildlife Garden is highlighted in red. **b)** Map of Fletcher Wildlife Garden. A (brown) represents the Discovery Centre, B (pink) represents the Butterfly Meadow (*Monarda fistulosa* n=4, *Solidago canadensis* n=1, *Symphyotrichum novae-angliae* n=1), C (yellow) represents Canadensis Garden (*M. fistulosa* n=0, *S. canadensis* n=4, *S. novae-angliae* n=0), D (purple) represents the Old Field (*M. fistulosa* n=0, *S. canadensis* n=0, *S. novae-angliae* n=2), E (orange) represents the Milkweed Garden (*M. fistulosa* n=1, *S. canadensis* n=0, *S. novae-angliae* n=1) (Table S1). Black thermometer symbols represent the approximate location of a pair of HOBO temperature loggers.

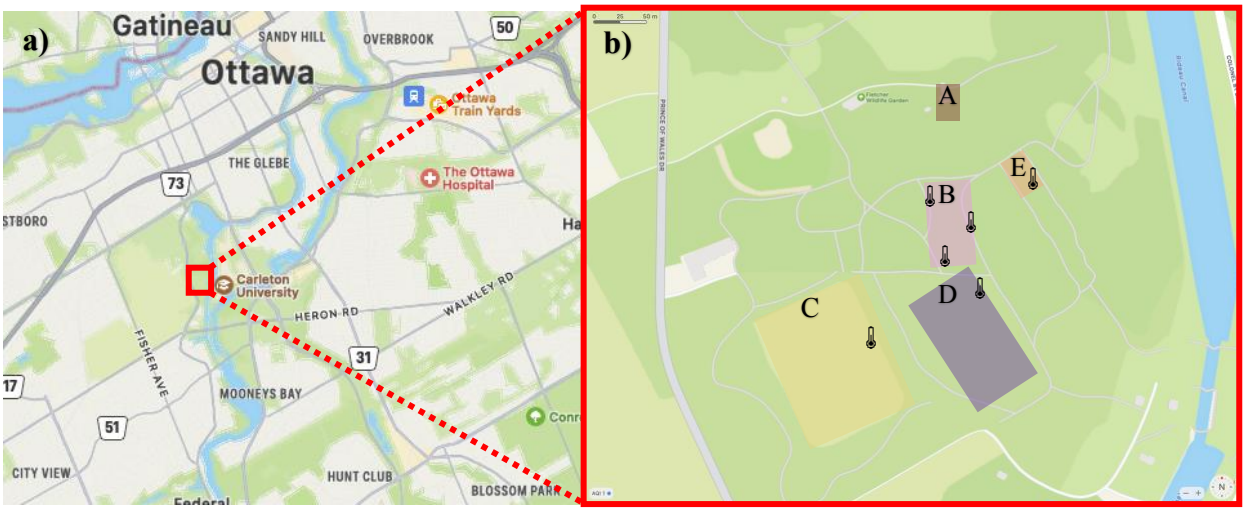


**Figure 2.** Timeline of the field experiment from July 15 to October 15, 2023. The pink line indicates the flowering period of *Monarda fistulosa*, the yellow line indicates the flowering period of *Solidago canadensis,* and the purple line indicates the flowering period of *Symphyotrichum novae-angliae.* The end of flowering for *S. canadensis* and *S. novae-angliae* was not captured in the experiment, indicated by the arrows. Flowering end dates of all species are estimates because the floral periods ended after the experimental period. Orange line and shading indicate period of adult monarch inclusion in the experiment and time of monarch feeding on each plant species.

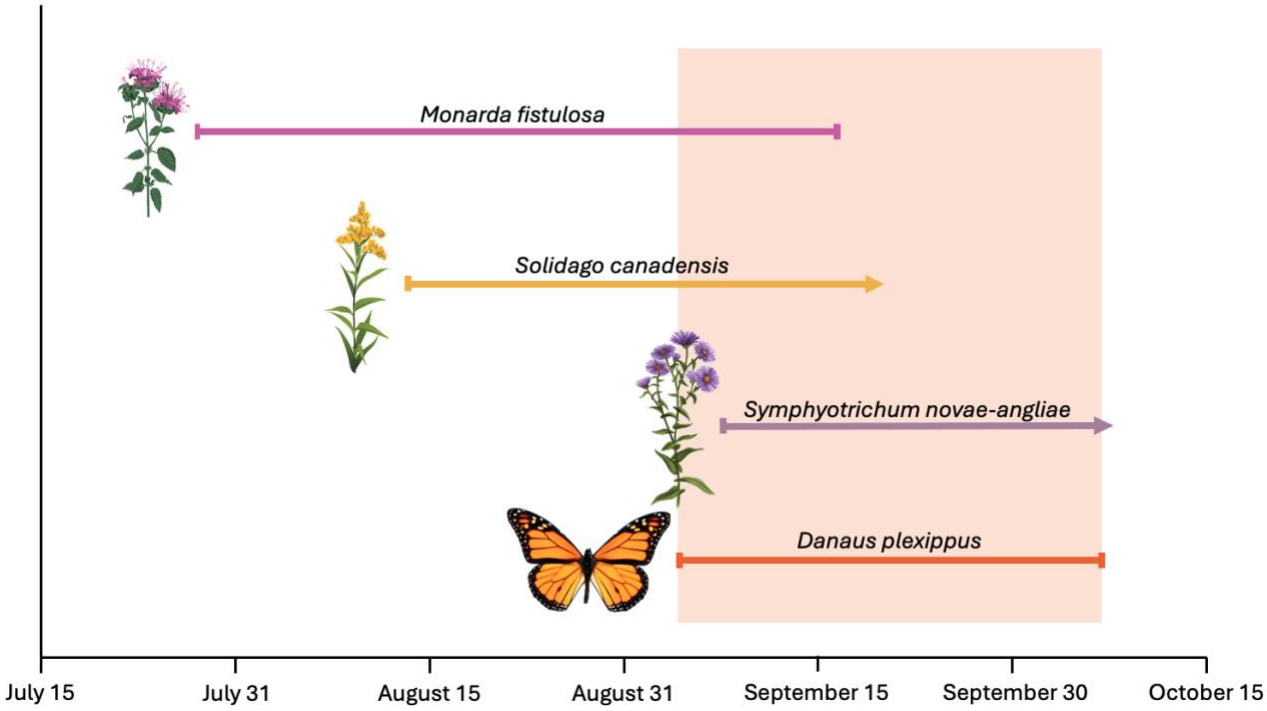


**Figure 3.** Experimental design of warming chambers and enclosures. **a)** open-top chambers on stems of *Solidago canadensis* in the Canadensis Garden of Fletcher Wildlife Garden on June 9, 2023. **b)** an example of a pair of an open-top chamber (left) and control chamber made of PVC piping (right) on stems of *S. canadensis* in the FWG-CBG complex on August 20, 2023. Both chambers are wrapped in mesh tulle. Large enclosures made of steel garden pegs with mosquito netting are built around the chambers with 30+ cm of clearance around all sides to allow space for the monarchs to move around in the enclosures. The open-top chambers and PVC control chambers are 1.5 meters tall and 1.2 meters wide at the base.

**Figure 4.** Temperature (°C) variation across the season and between temperature treatments in summer 2023. Shown is the **(a)** average daytime (08:00 to 20:00) temperature across the season and two treatments (control (blue) and warmed (red) chambers); and **(b)** average nighttime temperature (20:00 to 8:00) differences between temperature treatments. There was no seasonal change in nighttime temperature (Table 1). In panel **(a)**, the thin coloured lines represent daily averages across six replicates of each treatment. The trendlines indicate the overall temperature trend for each treatment during the observed period. Shading on bold lines represents 90% confidence interval. Shown in panel **(b)** is the mean with 95% confidence intervals.

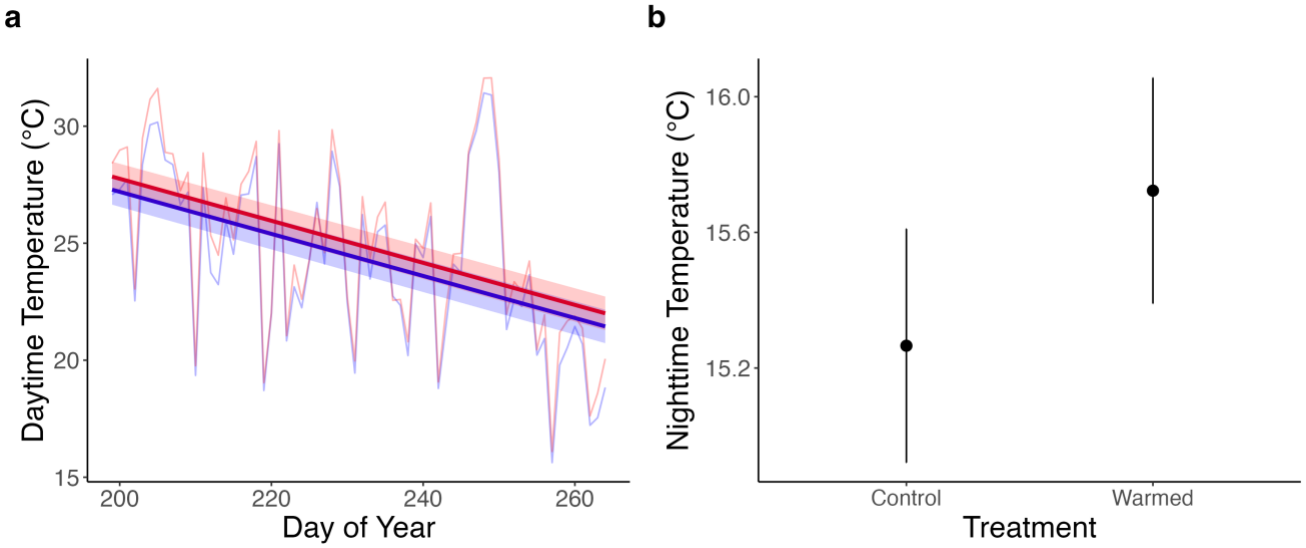


**Figure 5.** Soil moisture responses to the temperature treatment (control (blue) and warmed (red) chambers) and over the season in 2023 **(a)** and 2024 **(b)**. In 2023, soil moisture was measured as conductivity from 0 to 10. In 2024, soil moisture was measured as a ratio (volumetric water content (VWC); the volume of water:volume of soil). In both panels, the bold line indicates the seasonal soil moisture trend. For 2023, the predicted fit is based on a GLMM with soil moisture as a function of DOY. In 2024, the predicted fit is based on a model with temperature treatment and DOY. Shading around lines represent the 90% confidence interval.

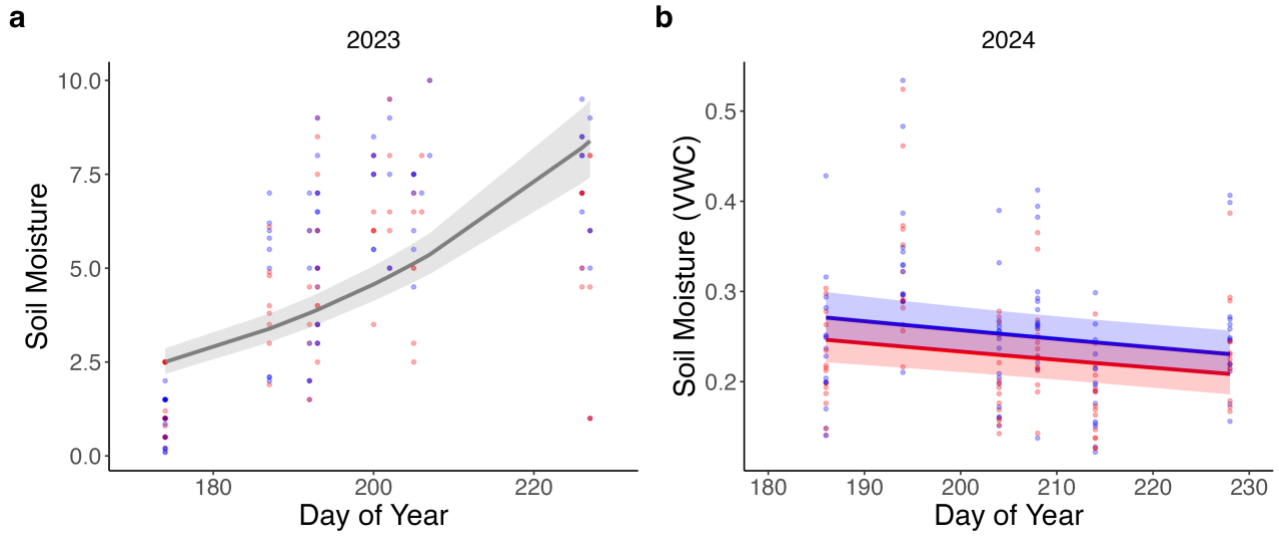


**Figure 6.** Plant responses to temperature differences (Control and Warmed). Shown is: **a**) relative stem growth (cm), **b**) DOY of flower onset, **c**) number of flowers, and **d**) nectar sucrose concentration (°BRIX). The black symbols indicate the mean estimate based on the raw data with 95% confidence intervals for each species: *Monarda fistulosa* (circles), *Solidago canadensis* (triangles), and *Symphyotrichum novae-angliae* (squares). The red line represents the overall trend (when present) of model predicted values, illustrating the significant general effect of the warming treatment across all species (Table 2). Means with 95% confidence intervals are shown. "*" denotes a statistically significant effect based on model output ($p$<0.05). Letters represent statistically significant pairwise comparisons between species ($p$<0.05).

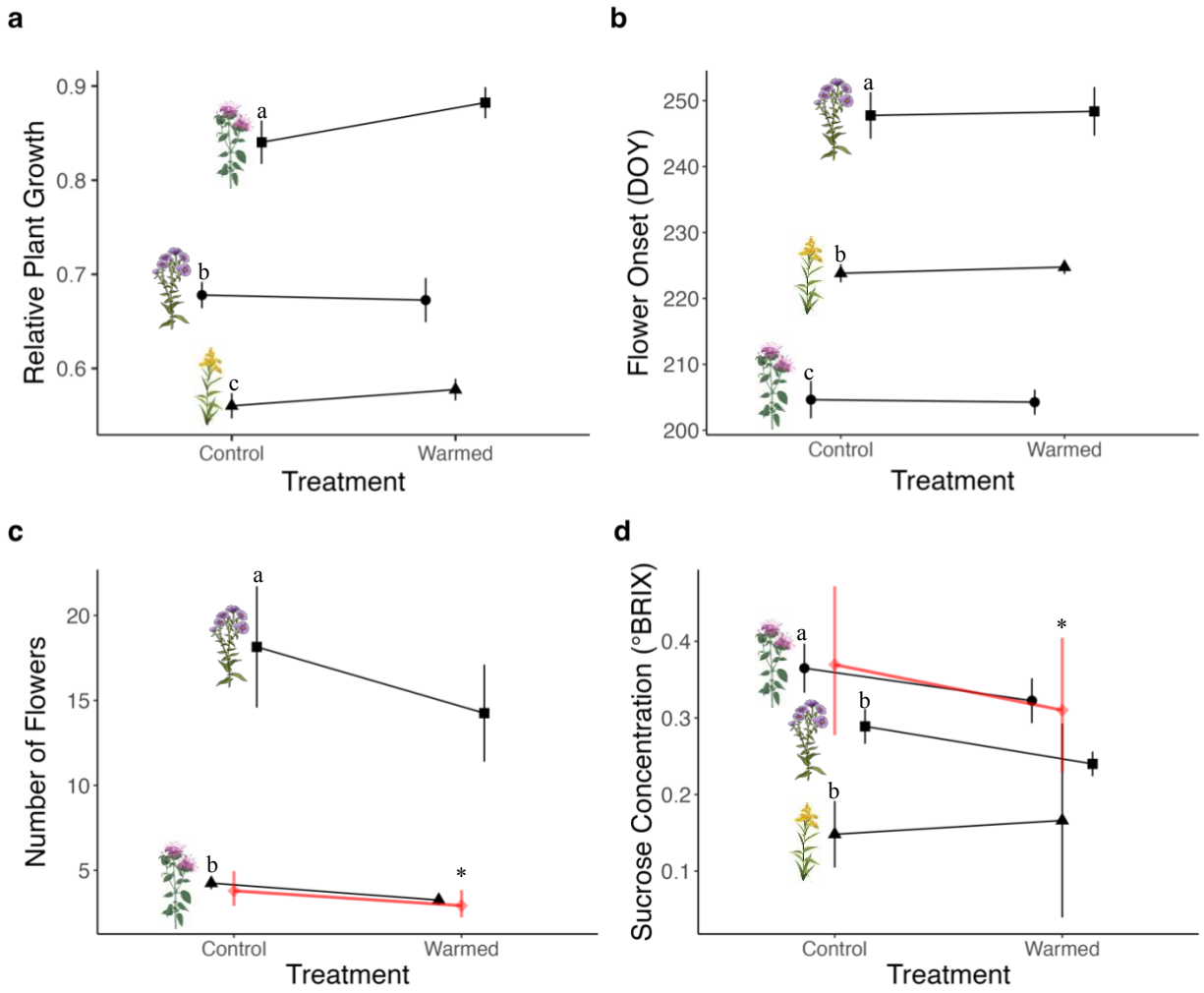
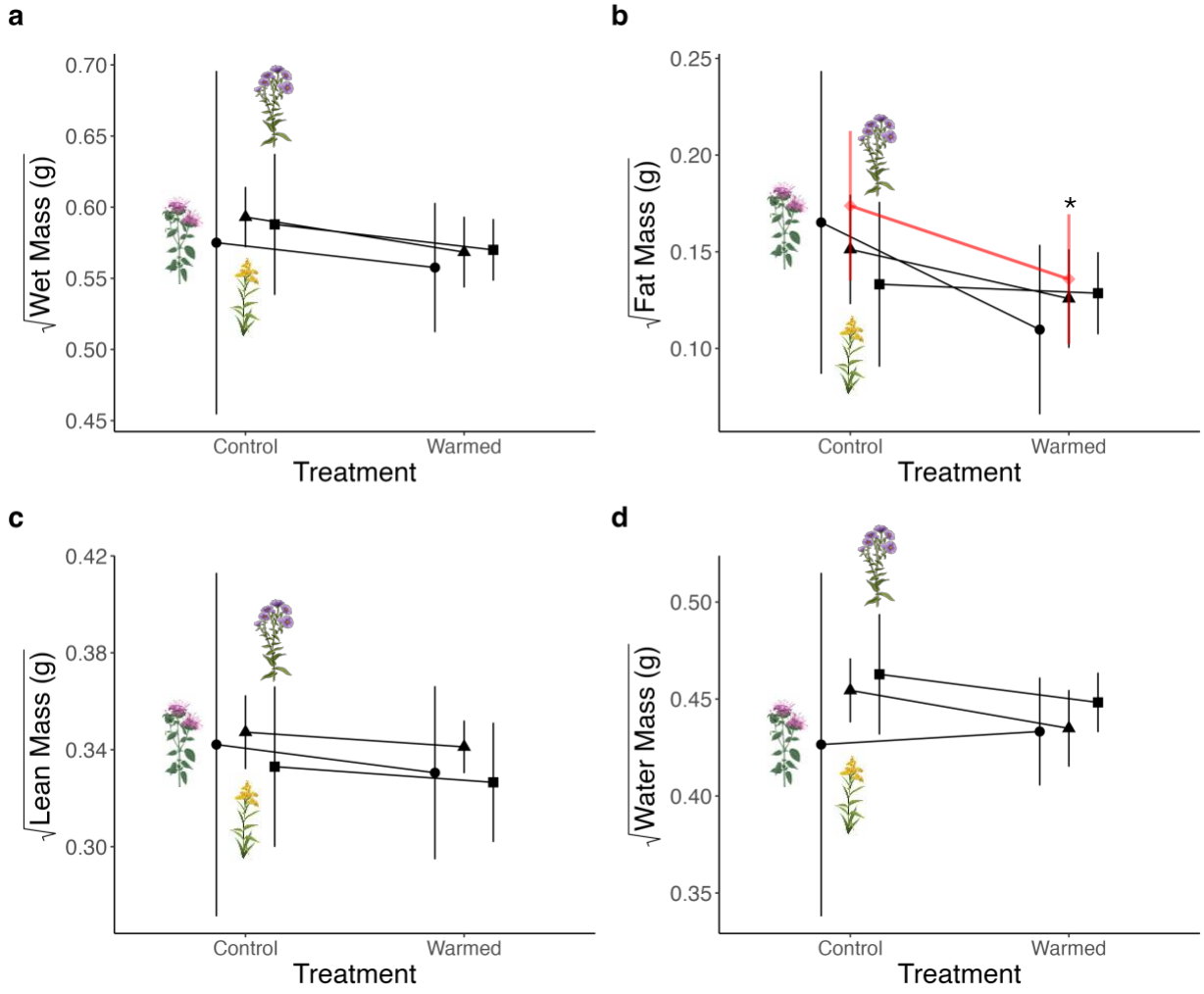


**Figure 7.** Monarch body condition and composition responses to temperature differences (Control and Warmed). Shown is: **a**) monarch wet mass (g), **b**) monarch fat mass (g) **c**) monarch lean mass (g), and **d**) monarch water mass (g). The black symbols indicate the mean estimate based on raw data with 95% confidence intervals for each species: *Monarda fistulosa* (circles), *Solidago canadensis* (triangles), and *Symphyotrichum novae-angliae* (squares) under each treatment. The red line represents the overall trend (when present) of model predicted values, illustrating the significant general effect of the treatment across all species (Table 3). "*" denotes a statistically significant effect based on model output ($p$<0.05).



## Appendix

**Table S1.** Incidence of each of the three plant species in each of the garden locations at Fletcher Wildlife Garden (Figure 1b).

| Fletcher Wildlife Garden Locations | *Monarda fistulosa (n=)* | *Solidago canadensis (n=)* | *Symphyotrichum novae-angliae (n=)* |
|---|---|---|---|
| Butterfly Meadow | 4 | 1 | 1 |
| Canadensis Garden | 0 | 4 | 0 |
| Old Field | 0 | 0 | 2 |
| Milkweed Garden | 1 | 0 | 1 |

**Table S2.** Summary of models used in statistical analysis including model type, response variables, predictor variables, random effects, and additional notes. The 'Type' column indicates function used for the main model. 'Response Variable' specifies the dependent variable being modeled, 'Predictor Variables' include the fixed effects in each model, and 'Random Effects' detail the random factors accounted for in each model. For sample size of each model, see Table S3.

| Model | Type | Response Variable | Predictor Variables | Random Effects |
|---|---|---|---|---|
| OTC Treatment | LMM | Mean daytime temperature (°C) | Treatment + DOY | Pair |
| | | Mean temperature fluctuation (°C) | Treatment | Pair |
| | | Mean nighttime temperature (°C) | Treatment | Pair |
| Soil Moisture | GLMM (beta distribution, logit transformation) | Soil moisture | Treatment + DOY | Pair |
| Relative Stem Growth | LM | Relative stem growth | Treatment + species | |
| Flower Onset (DOY) | GLMM (gamma distribution) | DOY of flower onset per stem | Treatment + species | Pair |
| # of Flowers | GLMM (negative binomial) | # of open flowers per stem | Treatment + species | Pair + DOY |
| Nectar Concentration (°BRIX) | GLMM (beta distribution, logit transformation) | Nectar concentration | Treatment + species | Pair + DOY |
| Nectar Volume (μL) | LMM | Nectar volume | Treatment | Pair + DOY |
| Wet Mass (g) | LM | Wet mass (g) | Treatment + species + sex + DOY + body length | |
| Fat Mass (g) | LM | Fat mass (g) | Treatment + species + sex + DOY + body length | |
| Lean Mass (g) | LM | Lean mass (g) | Treatment + species + sex + DOY + body length | |
| Water Mass (g) | LM | Water mass (g) | Treatment * sex + species + DOY + body length | |

**Table S3.** Sample size across all models. For model details, see Table S2. Sample sizes are given as number of total measurements, as well as size of each grouping, where appropriate. Sample sizes are given for each plant species (*Monarda fistulosa, Solidago canadensis, Symphyotrichum novae-angliae, Cirsium arvense, Asclepias syriaca, Sonchus oleraceus)* and sex (Female:Male), where necessary.

| Model Type | Response Variable | Total Sample Size (n=) | Species (n=) | Control (n=) | Warmed (n=) |
|---|---|---|---|---|---|
| Temperature | Mean Temperatures (°C) | 560 measurements across 12 loggers | *M. fistulosa* = 110 measurements, 4 loggers | 55 measurements, 2 loggers | 55 measurements, 2 loggers |
| | | | *S. canadensis* = 118 measurements, 2 loggers | 59 measurements, 1 logger | 59 measurements, 1 logger |
| | | | *S. novae-angliae* = 332 measurements, 6 loggers | 165 measurements, 3 loggers | 167 measurements, 3 loggers |
| Soil Moisture | 2023 Soil Moisture (%) | 186 measurements across 26 chambers | *M. fistulosa* = 48 measurements, 10 chambers | 26 measurements, 5 chambers | 20 measurements, 5 chambers |
| | | | *S. canadensis* = 88 measurements, 7 chambers | 40 measurements, 4 chambers | 48 measurements, 3 chambers |
| | | | *S. novae-angliae* = 50 measurements, 9 chambers | 24 measurements, 5 chambers | 26 measurements, 4 chambers |
| | 2024 Volumetric Water Content (%) | 176 measurements across 26 chambers | *C. arvense* = 56 measurements, 10 chambers | 28 measurements, 5 chambers | 28 measurements, 5 chambers |
| | | | *A. syriaca* = 60 measurements, 10 chambers | 30 measurements, 5 chambers | 30 measurements, 5 chambers |
| | | | *S. oleraceus* = 60 measurements, 10 chambers | 30 measurements, 5 chambers | 30 measurements, 5 chambers |
| Plant | Relative Stem Growth (%) | 4563 observations across 300 stems | *M. fistulosa* = 1117 observations, 100 stems | 560 observations, 50 stems | 557 observations, 50 stems |
| | | | *S. canadensis* =1901 observations, 100 stems | 951 observations, 50 stems | 950 observations, 50 stems |
| | | | *S. novae-angliae* = 1987 observations, 100 stems | 1000 observations, 50 stems | 987 observations, 50 stems |
| | Flower Onset (DOY) | 183 observations across 26 chambers | *M. fistulosa* = 90 observations, 10 chambers | 47 observations, 5 chambers | 43 observations, 5 chambers |
| | | | *S. canadensis* = 36 observations, 7 chambers | 16 observations, 4 chambers | 20 observations, 3 chambers |
| | | | *S. novae-angliae* = 57 observations, 9 chambers | 33 observations, 5 chambers | 24 observations, 4 chambers |
| | # of Flowers | 537 observations across 19 chambers | *M. fistulosa* = 479 observations, 10 chambers | 257 observations, 5 chambers | 222 observations, 5 chambers |
| | | | *S. novae-angliae* = 58 observations, 9 chambers | 34 observations, 5 chambers | 24 observations, 4 chambers |
| | Nectar Sucrose Concentration (°BRIX) | 250 measurements | *M. fistulosa =139* measurements | 67 measurements | 72 measurements |
| | | | *S. canadensis* =15 measurements | 10 measurements | 5 measurements |
| | | | *S. novae-angliae =97* measurements | 40 measurements | 57 measurements |
| | Nectar Volume (μL) | 155 measurements | *M. fistulosa* =155 measurements | 74 measurements | 81 measurements |
| Monarch | Body Composition Analyses (g) | 59 monarchs | *M. fistulosa* =14 monarchs | 7 monarchs (F:M=7:0) | 7 monarchs (F:M=7:0) |
| | | | *S. canadensis* = 27 monarchs | 11 monarchs (F:M=8:3) | 16 monarchs (F:M=15:1) |
| | | | *S. novae-angliae* = 18 monarchs | 11 monarchs (F:M=5:6) | 7 monarchs (F:M=6:1) |

**Table S4**. Results of interaction effects of the warming treatment. Temperature model output is from a linear mixed effects model, relative stem growth and all monarch model output are from linear models, and flower onset, # of flowers, and nectar concentration outputs are from generalized linear mixed effects models. Species responses were estimated as: relative stem growth (%), day of year of flower onset, number of flowers, and nectar sucrose concentration (°BRIX). Monarch responses were estimated as square root transformations of: wet mass (g), fat mass (g), lean mass (g), and water mass (g). The table shows the estimated effects (Estimate), standard error (SE), degrees of freedom (DF), F-values or z-values (F/z), and p-values (*p*) for each interaction. Bold font indicates potential biological significance of the interaction ($p<0.08$) and inclusion in the model.

| Model type | Response Variable | Predictor | Estimate | SE | DF | F/z | *p* |
|---|---|---|---|---|---|---|---|
| Temperature | Mean Daytime (°C) | *Treatment * DOY* | -0.0097 | 0.016 | 1, 551 | 0.38 | 0.54 |
| Plant | Relative Stem Growth (%) | *Treatment * species* | NA | NA | 2, 268 | 0.22 | 0.81 |
| | Flower Onset (DOY) | *Treatment * species* | NA | NA | 1, 175 | 0.4 | 0.68 |
| | # of Flowers | *Treatment * species* | NA | NA | 1, 530 | -0.18 | 0.86 |
| | Nectar Concentration (°BRIX) | *Treatment * species* | NA | NA | 2, 227 | NA | 0.84 |
| Monarch | Wet Mass (g) | *Treatment * species* | NA | NA | 2, 50 | 0.55 | 0.59 |
| | | *Treatment * DOY* | -0.00086 | 0.0021 | 1, 50 | 0.16 | 0.69 |
| | | *Treatment * sex* | -0.068 | 0.051 | 1, 50 | 1.78 | 0.19 |
| | Fat Mass (g) | *Treatment * species* | NA | NA | 2, 50 | 0.55 | 0.59 |
| | | *Treatment * DOY* | 0.00070 | 0.0019 | 1, 50 | 0.14 | 0.71 |
| | | *Treatment * sex* | -0.016 | 0.045 | 1, 50 | 0.12 | 0.73 |
| | Lean Mass (g) | *Treatment * species* | NA | NA | 2, 50 | 1.83 | 0.17 |
| | | *Treatment * DOY* | -0.0011 | 0.0013 | 1, 50 | 0.78 | 0.38 |
| | | *Treatment * sex* | -0.025 | 0.031 | 1, 50 | 0.69 | 0.42 |
| | Water Mass (g) | *Treatment * species* | NA | NA | 2, 50 | 0.15 | 0.86 |
| | | *Treatment * DOY* | -0.00042 | 0.0015 | 1, 50 | 0.074 | 0.79 |
| | | ***Treatment * sex*** | **-0.064** | **0.036** | **1, 50** | **3.08** | **<0.08** |

**Table S5**. Post-hoc Tukey pairwise comparison results associated with Figure 6a, the relationship between species and relative stem growth; Figure 6b, the relationship between species and phenology; and Figure 6d, the relationship between species and nectar sucrose concentration. Estimates for flower onset and nectar sucrose concentration are on the logit scale and not response scale. Statistically significant comparisons ($p$<0.05) are in bold.

| Predictor Variable | Comparison between levels | Estimate | SE | t/z | *p* |
|---|---|---|---|---|---|
| Relative Stem Growth (%) | ***M. fistulosa – S. canadensis*** | **0.11** | **0.038** | **2.78** | **<0.05** |
| | ***M. fistulosa – S. novae-angliae*** | **-0.18** | **0.036** | **-5.04** | **<0.0001** |
| | ***S. canadensis – S. novae-angliae*** | **-0.29** | **0.039** | **-7.50** | **<0.0001** |
| Flower Onset (DOY) | ***M. fistulosa – S. canadensis*** | **-0.094** | **0.012** | **-5.07** | **<0.0001** |
| | ***M. fistulosa – S. novae-angliae*** | **-0.19** | **0.017** | **-10.99** | **<0.0001** |
| | ***S. canadensis – S. novae-angliae*** | **-0.098** | **0.019** | **-5.24** | **<0.0001** |
| Nectar Sucrose Concentration (°BRIX) | ***M. fistulosa – S. canadensis*** | **0.99** | **0.33** | **2.99** | **<0.01** |
| | ***M. fistulosa – S. novae-angliae*** | **0.70** | **0.21** | **2.58** | **<0.05** |
| | *S. canadensis – S. novae-angliae* | -0.29 | 0.41 | -0.72 | 0.75 |

**Figure S1.** Average daytime (08:00 to 20:00) temperature (°C) variation across the study period from June 8 to July 18 across two treatments: control (blue) and warmed (red) chambers. The x-axis represents the day of the year, ranging from day 159 (June 8) to 199 (July 18). The thin lines represent average daytime temperatures across six replicates of each treatment. The bold lines indicate the overall temperature trend for each treatment during the observed period. Grey shading on bold lines represents 95% confidence interval. Over the time period, the average daytime temperature was 0.5°C lower in warmed chambers than control (0.1SE, $F_{1,2917}$=15.1, $p$<0.001).