EXPERIMENTAL IDENTIFICATION OF HIGH-REWARD NECTAR PLANTS FOR THE MONARCH BUTTERFLY (*DANAUS PLEXIPPUS*)

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Abstract

Monarch butterfly (*Danaus plexippus*) populations have significantly declined in the last 30 years. As a result, monarchs have been listed as 'Endangered' under Canada's Species at Risk Act, meaning conservation strategies must be developed and implemented to slow and reverse this decline. Monarchs migrate up to 5000 km from their breeding grounds in Canada to their wintering grounds in Central Mexico, with habitat loss and degradation occurring throughout their range. Consequently, restoration work is needed to increase monarch success across the continent of North America. Current work in Canada is focused on restoring grassland habitats with *Asclepias spp.* (milkweed species), the monarch's larval host plant, and nectar plants. While nutrient concentrations in nectar vary between plant species, little is known about the effects of nectar quality from different plant species on the performance of adult monarchs. For the most effective use of limited conservation resources, work must be done to identify the most beneficial nectar plant species for monarchs. For the migratory generation which does not breed, the effects of nectar on body composition have never been studied. This generation undergoes several physiological changes to build and maintain large fat stores for this journey, indicating that fat is a key factor for migration success. Consequently, I conducted an experimental study evaluating monarch butterflies' body condition (wet mass, fat, lean mass, and water mass) when foraging on one of seven nectar plant species for seven days. I found that nectar plant species significantly affected monarch fat mass, lean mass, and overall wet mass. These results indicate a need to carefully choose plants for restoration, as these choices could have implications for migration success. This study represents the first experimental test of the effects of nectar plants on monarch butterfly body composition, providing an approach for future studies to evaluate nectar plants across the monarch's range.

Résumé

Les populations de papillons monarques (*Danaus plexippus*) ont considérablement diminué au cours des 30 dernières années. En conséquence, les monarques ont été inscrits sur la liste des espèces en voie de disparition en vertu de la loi canadienne sur les espèces en péril, ce qui signifie que des stratégies de conservation doivent être élaborées et mises en œuvre pour ralentir et inverser ce déclin. Les monarques migrent sur une distance pouvant aller jusqu'à 5 000 km entre leurs aires de reproduction au Canada et leurs aires d'hivernage dans le centre du Mexique. La perte et la dégradation de leur habitat se produisent dans toute leur aire de migration. Par conséquent, des travaux de restauration sont nécessaires pour accroître le succès des monarques sur l'ensemble du continent nord-américain. Au Canada, les travaux actuels se concentrent sur la restauration des habitats de prairies avec *Asclepias* spp. (asclépiade), la plante hôte des larves de monarques, et des plantes à nectar. Bien que les concentrations de nutriments dans le nectar varient d'une espèce végétale à l'autre, on sait peu de choses sur les effets de la qualité du nectar provenant de différentes espèces végétales sur les performances des monarques adultes. Afin d'optimiser les ressources de conservation limitées, il convient d'identifier les espèces de plantes à nectar les plus bénéfiques pour les monarques. Pour la génération migratoire qui ne se reproduit pas, les effets du nectar sur la composition corporelle n'ont jamais été étudiés. Cette génération subit plusieurs changements physiologiques pour construire et maintenir de grandes réserves de graisse pour ce voyage, ce qui indique que la graisse est un facteur clé pour le succès de la migration. Par conséquent, j'ai mené une étude expérimentale évaluant l'état corporel des papillons monarques (masse humide, masse grasse, masse maigre et masse d'eau) lorsqu'ils se nourrissent de l'une des sept espèces de plantes à nectar pendant sept jours. J'ai constaté que les espèces de plantes à nectar affectaient de manière significative la masse grasse, la masse maigre et la masse humide globale des monarques. Ces résultats montrent qu'il est

nécessaire de choisir avec soin les plantes à restaurer, car ces choix pourraient avoir des conséquences sur le succès de la migration. Cette étude représente le premier test expérimental des effets des plantes à nectar sur la composition corporelle des papillons monarques, fournissant une approche pour de futures études visant à évaluer les plantes à nectar dans l'ensemble de l'aire de répartition du monarque.

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models with a random effect, marginal R^2 (R^2_{marg}) quantifies the model fit without the random effect, and conditional R^2 (R^2_{cond}) quantifies the model fit with the random effect included. Significant results ($p < 0.05$) are marked in bold.

Table 3: Tukey HSD-adjusted pairwise comparisons of nectar plant species' effect on wet mass, fat mass, and lean mass of foraging butterflies in the greenhouse experiment. The difference estimate, standard error (SE), degrees of freedom (df), t-ratio, and p-value (*p*) are presented for each plant species pair contrast. The wet mass contrast compares the slope of butterfly weight through the sampling period (log_e(g)/day), while the fat (log_e(g)) and lean mass (g) contrasts compare the single measurement of those variables at the end of each butterfly's trial. Positive estimate values indicate a higher mean estimate for the first plant mentioned in the pairing. The total number of observations [N] is shown for each model response, with the number of trials for each plant species [N_{sp}] next to their first reference. Full model effects can be found in Table 2. Significant effects ($p < 0.05$) are marked in bold.

Table 4: Results of linear models evaluating differences in adult monarch weight and body composition after foraging on *Solidago altissima* in the field or greenhouse experiments. Included here are the wet mass (n=27 butterflies) and final body composition metrics (n=23 butterflies): fat mass (g), lean mass (g), water mass (g), and the total number of measurements taken [N]. All analyses included location (categorical, 2 levels) and butterfly sex (categorical, 2 levels) as predictor variables. The weight model included forewing length (continuous), an interaction term between plant species and sampling day, and a random effect for butterfly ID. Body composition models included the butterfly's starting weight (continuous). For enclosure

location and sex, the reference levels are “field” and “female”, respectively. The mean estimate, standard error (SE), test statistic (t), degrees of freedom (df), and p-value (*p*) are presented. Also included are R^2 and R^2_{adj} for linear models; the latter indicates the model fit adjusted for the number of parameters included. For models with a random effect, marginal R^2 (R^2_{marg}) quantifies the model fit without the random effect, and conditional R^2 (R^2_{cond}) quantifies the model fit with the random effect included. Significant effects ($p < 0.05$) are marked in bold.

Table 5: Results of linear models evaluating differences in adult monarch weight and body composition after foraging in the field on *Eutrochium maculatum* or *Solidago altissima*. Included here are wet mass (n=17 butterflies) and final body composition metrics (n=14 butterflies): fat mass (g), lean mass (g), water mass (g), and the total number of measurements taken [N]. All analyses included plant species (categorical, 2 levels) and total floral surface area (continuous) as predictor variables. Wet mass model included an interaction term between plant species and sampling day and a random effect of butterfly ID. Body composition models included the butterfly's starting weight (continuous). The reference level for plant species is “*E. maculatum*”. The mean estimate, standard error (SE), t-value (t), degrees of freedom (df), and p-value (*p*) are presented. Also included are R^2 and R^2_{adj} for linear models; the latter indicates the model fit adjusted for the number of parameters included. For models with a random effect, marginal R^2 (R^2_{marg}) quantifies the model fit without the random effect, and conditional R^2 (R^2_{cond}) quantifies the model fit with the random effect included. Significant effects ($p < 0.05$) are marked in bold.

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shading representing the 95% confidence interval. N = 196 weight measurements of 55 butterflies.

Figure 4: Predicted effects of six nectar plant species on (a) fat mass, (c) lean mass, and (d) water mass of monarch butterflies, and effect of (b) floral surface area on fat mass. Plant species are ordered from most visited by monarchs (left) to least visited (right) based on community science data (Moczula et al., In Prep). Data were analyzed using linear models (Table 2). Raw data (grey points), 95% confidence interval (a, c, d: error bars; b: grey shading), and predicted mean values (black points) are presented for each species. Letters above the upper confidence limit are included where significant differences between groups were found. Letters common to different plant species indicate no significant differences between those species, while unique letters between species indicate significant differences (Table 3). The dependent variable of (a & b) fat mass was log-transformed for statistical analysis, and the predicted values here have been transformed back to the observed scale. N = 46 butterflies analyzed for body composition.

Figure 5: Comparison of butterfly wet mass, fat mass, lean mass, and water mass after foraging on *S. altissima* in either the field or the greenhouse. Butterflies foraged on their treatment plant for seven to ten days and were weighed throughout their trial (N = 107 observations on 27 butterflies). Butterflies that foraged for seven days were analyzed for body composition (fat, lean, and water mass; N = 23). These data were analyzed using (a) a linear mixed effects model with butterfly ID as a random effect, or (b-d) linear models. Full model parameters can be found in Table 4. Mean (black dot) and 95% confidence intervals are shown (a: shaded colour; b-d: error bars), with raw data overlaid (grey points). The dependent variable of (a) wet mass was log-

transformed for statistical analysis, and the predicted values here have been transformed back to the observed scale. Significant relationships are indicated with stars (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

Figure 6: Temperatures in enclosures containing Solidago altissima (tall goldenrod) in the field (blue) and the greenhouse (red) between August 31st and September 10th, 2022, in Ottawa, Ontario, Canada. Panel (a) shows the raw data of daily average temperatures over the measuring period. Panel (b) shows the model-predicted results of hourly pairwise comparisons in the two locations with the 95% confidence interval (N = 286 temperature readings).

1. Introduction

Populations of both common and at-risk insect species are experiencing significant declines globally (Hallmann et al., 2017; Sánchez-Bayo & Wyckhuys, 2019; Jactel et al., 2020; Montgomery et al., 2020). Numerous threats are hypothesized to drive insect population declines, including habitat loss and degradation, climate change, disease, and pesticide use (Capinera, 2018; Sánchez-Bayo & Wyckhuys, 2019; Janousek et al., 2023). To mitigate the threats of habitat loss and degradation, remaining natural habitats must be preserved and degraded habitats must be restored (Shuey, 2013).

The eastern migratory monarch butterfly (*Danaus plexippus*) is a migratory insect species that has declined by approximately 80% since monitoring began in 1994 (Stenoien et al., 2018; Saunders et al., 2019; Zylstra et al., 2021). As a result of this decline, monarchs are currently listed as 'Endangered' under Canada's Species at Risk Act (Canada, 2024). Consequently, a Recovery Strategy and Action Plan are being developed to reverse its decline. Some key threats monarchs face include loss of suitable habitat, declines in their host plants, milkweed (*Asclepias* spp.), loss of nectar flowers, and climate change (Stenoien et al., 2018; Zylstra et al., 2021). Monarchs are open habitat generalists, using grasslands, agricultural fields and margins, riparian areas, rights-of-way, parklands, and even urban gardens during both breeding and migration (Davis et al., 2012; Stenoien et al., 2015; Thogmartin et al., 2017). Therefore, monarch butterfly conservation will require habitat restoration across many open land cover types (Takkis et al., 2015, 2018; McCombs et al., 2022).

Currently, monarch recovery work is focused on restoring grassland habitats with plant species that increase monarch breeding success, such as *Asclepias spp.* (milkweed species), the

monarch's larval host plants, and breeding-season nectar plants. However, the "floral nectar limitation hypothesis" postulates that nectar availability during the migratory period may also limit monarch population growth (Agrawal & Inamine, 2018; Saunders et al., 2019; Fisher et al., 2023). In support of this hypothesis, recent evidence found that fall normalized difference vegetation index (NDVI), a measure of landscape greenness and a proxy for nectar flower availability during migration (Zylstra et al., 2021), positively correlates with eastern migratory monarch migratory roost size and overwintering monarch population size in Mexico (Saunders et al., 2019; Davis et al., 2024). Consequently, monarch conservation strategies should also consider the restoration of nectar flowers used by monarchs to fuel their expensive fall migration and winter survival (Brower et al., 2006; Saunders et al., 2019; Fyson et al., 2023; Davis et al., 2024).

The monarch's physiological strategies during the migratory and wintering periods further emphasize the importance of energy sources (Brower et al., 2006). Individuals from the monarch's migratory generation emerge in a state of reproductive diapause (the suspension of reproductive organ development and reproductive behaviour) in the fall (Goehring & Oberhauser, 2002). This re-routes energy from reproductive processes towards migration (Alonso-Mejía et al., 1997; Lehmann et al., 2016; Enriquez & Visser, 2023). Monarchs rely on fat reserves to fuel their flight to Mexico and survive the winter staging period, as they do not forage once at their wintering grounds (Alonso-Mejía et al., 1997). Overall, accumulating energy stores and being efficient with those stores appear key to migration success and overwinter survival (Brown & Chippendale, 1974; Alonso-Mejía et al., 1997; Brower et al., 2006).

Monarchs, like most butterflies, are considered nectar-foraging generalists, meaning they can forage on a wide variety of flower species (Dumroese et al., 2016, Moczula et al., In Prep).

Despite this life-history strategy, recent surveys have found that monarchs prefer to forage on certain species of flowers, even when the availability of these species is relatively low (Fisher et al., 2023; Pekos et al., 2025). Experimental feeding trials have also found monarchs can assess nectar quality from artificial flowers and respond by changing their foraging habits to increase use of high-reward nectars (Blackiston et al., 2011; Cepero et al., 2015). Together, these studies suggest that despite being open habitat generalists, monarchs are selective when foraging, and I hypothesize their foraging choices may be linked to nectar quality.

While studies have shown that variation in nectar quality can affect monarch fecundity during the breeding season (Mevi-Schutz & Erhardt, 2005), we have little idea which species provide the greatest energy benefit for monarchs (Arnold & Michaels, 2017; Moczula et al., In Prep). The nectar reward gained from a given species will depend on several factors, including nutrient concentrations (i.e., sugars, amino acids), nectar viscosity, and flower tube length (Gardener & Gillman, 2001; Arnold & Michaels, 2017; Klumpers et al., 2019; Lois-Milevicich et al., 2021). Variations in nutrient concentrations can affect a nectar's quality as a food source (Mevi-Schutz & Erhardt, 2005; Cahenzli & Erhardt, 2012), while nectar viscosity and flower tube length can affect the ability of pollinators to obtain nectar (Klumpers et al., 2019). Overall, these traits could lead to differences in the relative benefit of plant species for monarch energy acquisition, potentially affecting muscle mass, fat mass, and/or the hydration of monarchs. While differences in amino acid and sugar composition of nectar have been explored (e.g. Arnold & Michaels, 2017; Venjakob et al., 2022), monarch feeding trials have focused on the effect of amino acids on fecundity during the breeding season (Mevi-Schutz & Erhardt, 2005; e.g. Arnold, 2016), but not on how different flower species affect the fat stores of monarchs during the migratory period. To my knowledge, no study has tested the effect of nectar from plants of

different species on adult monarch body composition (wet mass, fat mass, lean mass, water mass). Ultimately, for effective monarch migratory habitat restoration, it is important to determine which flowers are the most beneficial for energy gain in the form of fat mass.

1.1 Objectives

The main objective of this study is to assess the value of key nectar plant species for monarch energy gain during their fall migratory period. To meet this objective, I conducted feeding trials with adult monarchs foraging on seven different nectar plant species in Ottawa, Ontario, Canada. Six species were tested in a greenhouse setting, and two in a field setting (one of these species being common with the greenhouse). I evaluated the effects of nectar quality from these species on the body composition of adult monarchs, including wet, fat, lean, and water mass. I used data from Moczula et al. (In Prep) to determine the frequency of flower use by monarchs and guide species selection. Given that monarchs choose to feed on some flowers more than others (Fisher et al., 2023; Pekos et al., In Review) and can learn which flowers contain high-reward nectar in an experimental setting (Blackiston et al., 2011), the frequency of monarchs foraging on plants in the wild should correspond to high-reward species. I predicted that monarchs that forage on previously determined highly visited flowering plants will have higher body condition than monarchs that forage on seldom-visited plants.

2. Methods:

2.1 Study system

The monarch butterfly has four distinct populations in North America: two resident populations; one around the Gulf of Mexico and the other in Southern California, and two migratory populations separated by the Rocky Mountains (Oberhauser, K. & Solensky, 2004).

North America's eastern population of monarchs is the largest and undertakes the longest migration, traveling up to 5000 km from eastern Canada to their overwintering roosts in the oyamel forests of Central Mexico (Williams et al., 2015). Eastern monarchs have 2-3 distinct breeding generations through the spring and summer, and a fourth migratory generation that does not breed (Oberhauser et al., 2017). Eastern migratory monarchs typically arrive and breed in Ottawa, Ontario, Canada between July 1st and September 1st (Fyson et al., In Review). Individuals of the migratory generation emerge from mid-August to mid-September (Taylor et al., 2019). This generation lives up to 9 months, traveling to Mexico for the winter, overwintering, and journeying north in the spring (Oberhauser et al., 2017).

During breeding and migration, monarchs utilize open habitats for fuel in both their larval and adult life stages (Grant et al., 2018; Fyson et al., 2023; Diffendorfer et al., 2024). Adult monarchs forage on nectar from flowering plants for energy, while larval monarchs gain their energy from consuming the leaves of their host plant, *Asclepias sp.* (milkweed) (Brower et al., 2012; Pleasants & Oberhauser, 2013).

2.2 Overall experimental design

I conducted two feeding experiments from July to September 2022 in Ottawa, Ontario, Canada, to compare the quality of seven nectar plant species. The first experiment was conducted in a greenhouse at Carleton University (Figure 1; Figure 2a) with 6 potted plant species. This greenhouse was built in the 1960s, and while it contained fans for air circulation, there were no other environmental controls (temperature, humidity, etc.). The second experiment took place in an old field habitat with naturally available plants. In the field (Figure 1; Figure 2b & 2c), I compared the quality of two species: one that could not be grown in the greenhouse, and one available in both the greenhouse and the field. This second species, common to both

experimental locations, allowed me to determine if the results of the field experiments could be directly compared with those in the greenhouse.

2.3 Experimental details

2.3.1 Plants details

To test plant quality for monarch energy acquisition, I selected species and genera that varied in the degree of visitation by monarchs. This information was gathered from Moczula et al. (In Prep), who evaluated community science images of monarchs foraging on different flower species across land cover types in eastern Canada between 2012 and 2018. Specifically, Moczula et al. (In Prep) tallied the number of monarchs pictured nectaring (extending their proboscis into the flower) on flowers throughout summer and fall. From these counts, I selected species along a gradient of visitation in the August-September period, from highly visited to never visited.

Because most plants I used in my study first flower in their second year following germination, I attempted to source my species of interest from Ontario nurseries instead of growing them from seed. However, all non-native species of interest, save one, were not available for purchase from nurseries. Consequently, I attempted transplantation from the field to the greenhouse for several species, to varying degrees of success (see Table S1 for full list). I ultimately tested six species in the greenhouse, listed here from most to least visited in Moczula et al. (In Prep): *Symphyotrichum ericoides* (white heath aster), *Solidago altissima* (tall goldenrod), *Buddleja davidii* (butterfly bush), *Echinacea purpurea* (purple coneflower), *Rudbeckia hirta* (black-eyed Susan), and *Heliopsis helianthoides* (ox-eye sunflower). All species were native to North America, save *Buddleja davidii* (butterfly bush), which represents a well-visited species native to central China that is commonly found in gardens in Canada and the United States (Moczula et al., In Prep; Table 1). Finally, I also tested two well-visited species of

plants in the field: *S. altissima*, common to both experimental locations, and *Eutrochium maculatum* (spotted joe-pye weed).

2.3.1.2 Greenhouse plants

Mature plants were obtained from NVK Nurseries Inc. (*S. ericoides*, *B. davidii*, *E. purpurea*, *R. hirta*), Agriculture and Agri-Food Canada (*H. helianthoides*), or transplanted from field sites (*S. altissima*; Table 1). All species that were obtained before they began flowering were repotted into 7.6 L (2-gallon) pots with Promix BX Black general potting soil (Table 1). Those species already flowering when obtained were not repotted, as the weight of flowering heads increased the risk of damage to the plants during this process, and the stress of repotting could affect nectar production (Pacini & Nepi, 2007). *E. purpurea* and *H. helianthoides* plants were already flowering when obtained and were not repotted (Table 1). All species were introduced to the greenhouse by July 5^{th,} apart from *E. purpurea*, which was introduced on August 25th (See Table 1 for full list of dates). *E. purpurea* was not included at the start of the experiment, because it was only available for purchase in late July. Greenhouse feeding trials ran from August 19th to September 10th, 2022. Plant species were placed in black mesh and white mesh enclosures (i.e., one species per enclosure; both colours: 58 cm x 58 cm x 142 cm; purchased from RaisingButterflies.org) (Figure 2). Four to seven black enclosures and one to two white enclosures were used per species, apart from *B. davidii*, which had no white enclosures. All plants were watered daily.

Enclosures were randomly distributed on greenhouse benches, and each enclosure could house a maximum of four 7.6 L pots. For all species except *B. davidii*, 3-4 pots were placed in each enclosure to maximize the number of flowers available to each butterfly. *B. davidii* plants were too large to fit three potted plants in an enclosure, so only two per enclosure were used. To

control for insect pest species, I applied *Safer's* insecticidal soap to the leaves and stems of all plants in the greenhouse weekly from July 6th until August 5th.

2.3.1.2 Field plants

Concurrent to the greenhouse experiment, I conducted a field experiment from August 25th to September 10th at Fletcher Wildlife Gardens (Ottawa, ON; Figure 1, Figure 2b & 2c). Fletcher's is approximately 6 hectares of land formerly used for agriculture. Since 1990, it has been actively managed by volunteers from the Ottawa Field Naturalists' Club seeking to provide refuge for wildlife in urban Ottawa, planting native flowers and removing invasive species. The main purpose of the field study was to test an additional nectar flower species that I could not source for my greenhouse experiment, i.e., *E. maculatum*. Additionally, by testing *S. altissima* in the field and the greenhouse, I could assess if the *E. maculatum* results could be directly compared to greenhouse results.

In the field, I placed 1.2 m x 1.2 m x 1.8 m open-bottomed mesh tents (Pop-Up Net Shelter, Lee Valley, Canada) over each species on August 22nd to house the plants and the butterflies (Figure 2b & 2c). Non-treatment flowers in the enclosures were cut and removed before butterflies were added. Once butterflies were introduced, each enclosure was checked daily for budding non-treatment flowers, and any found were removed.

2.4 Monarch details

Three adult males and three adult females were captured from three field sites around Ottawa (Figure 1) from the 18th to the 22nd of July 2022. Single mating pairs were kept in a sheltered courtyard at the University of Ottawa in the same white mesh enclosures used in the greenhouse. Each pair was provided with flowering *E. purpurea* for nectar and *Asclepias incarnata* (swamp milkweed) for both nectar and oviposition. Oviposited eggs were collected

daily from each enclosure and placed in mesh-topped insect-rearing containers. Given naturally high levels of larval mortality during the early rearing period (Nail et al., 2015), I collected eggs and caterpillars from the field between July 27th and August 5th to increase the number of butterflies tested. Ultimately, 56 of the butterflies came from the mating pairs (n=41 greenhouse, n=16 field), with the remaining 21 coming from larvae collected from the field (n=14 greenhouse, n=7 field). Offspring from wild-caught mating pairs versus field-collected larvae were randomly distributed among plant species treatments.

Eggs and larvae were raised in a single growth chamber (*Biochambers* model TPC-19) at the University of Ottawa. The growth chamber was set to a 27 °C daytime and 21°C nighttime temperature cycle. Day length was changed daily and set to match the regional time of sunset and sunrise in Ottawa, ON, Canada. Day length started at 14.94 h when mating pairs were obtained on July 26th and decreased to 12.80 h on September 10th, the last day of experimental trials. These temperature and light conditions mimicked the natural late-season solar conditions necessary to induce diapause (Goehring & Oberhauser, 2002).

Larvae were fed daily with *ad libitum* fresh *A. syriaca* (common milkweed) leaves, either from a site close to the university (Figure 1) or from one of several field sites across Ottawa used in Pekos et al. (2025) that came from urban and natural meadows. Milkweed leaves were sourced from a single location each day.

Once eclosed, each adult butterfly was released into an enclosure with a single species of nectar plant in bloom. While I attempted to minimize differences in the amount of nectar across enclosures and species (e.g., same size and number of pots), inevitably there was variation in floral surface area (see next section for details). However, by allowing the monarchs to feed *ad*

libitum and controlling for differences in nectar availability in our models, any potential effects of nectar availability on the body condition of monarchs should be minimized.

Given a limited number of field enclosures, some enclosures had a second female butterfly added to increase sample sizes. Specifically, 4 enclosures contained two butterflies at a time for *E. maculatum* trials (totaling 8 females) and 3 enclosures contained two butterflies for *S. altissima* trials (totaling 6 females). Males involved in the field trials (n = 5) were tested individually to avoid within-sex competition and mating behaviour between sexes. I marked all field butterflies with an ID on the bottom of their hindwing using a fine-tip permanent marker (Microperm 0.45 mm, Sakura, Japan) to distinguish individuals and track individual wet mass change over the trial period. Overall, 55 foraging trials (29 males, 26 females) took place in the greenhouse, and 23 (18 females, 5 males) took place in the field (See Table S2 for all sample sizes). Of those in the greenhouse, 13 were on *S. ericoides*, 14 on *S.* altissima, 5 on *B. davidii*, 10 on *E. purpurea*, 5 on *R. hirta*, and 8 on *H. helianthoides*. Of those in the field, 10 were on *E. maculatum*, and 13 were on *S. altissima.*

2.5 Measurements

2.5.1 Plant Measurements

To control for variation in nectar amount in enclosures in my statistical models, I counted number of flowering units available to each butterfly in its enclosure on the day it began its trial. Flowers were classified as simple flowers, with one flower per flowering unit, or as an inflorescence with more than one flower per flowering unit. The surface area of simple flowers was calculated as a circle, using the radius of the flower, and surface area of inflorescences was calculated as a cylinder (Table 1; Figure S1). Three to five representative flowers per species were measured to obtain an average surface area of a flowering unit. Surface area, in conjunction

with the number of flowering units available to each butterfly at the start of its trial, allowed me to calculate the total floral surface area in each enclosure, a proxy for the total nectar available to the butterfly (Galetto & Bernardello, 2004; Hicks et al., 2016; Tavares et al., 2016).

2.5.2 Monarch Measurements

Initially, feeding trials in both experiments lasted for 10 days. However, because of the limited number of enclosures, the narrow window of eclosion across individuals, and the uncertainty of how the butterflies would fare in the greenhouse, trials were shortened to 7 days. This allowed some enclosures to be used a second time, after the first butterfly trial was finished, increasing the number of feeding trials conducted (refer to Table S2 for sample sizes in statistical test).

To assess nectar quality for each monarch, I measured butterfly weight (i.e., wet mass) over the feeding trial and body composition (fat, lean, and water mass) upon trial completion. I recognize that when weighing monarchs, I am technically taking weight measurements; however, to be consistent with the body composition literature, I refer to these weight measurements as mass. None of the butterflies measured on day 10 were included in the body composition analysis because of potential differences in body composition resulting from an additional three days of foraging. Consequently, body composition results for 46 of the 55 greenhouse butterflies were analyzed (i.e., n=22 males, n=24 females). The release of captive-reared butterflies is discouraged because of possible negative impacts on the monarch gene pool (Tenger-Trolander, 2023). Consequently, no butterflies studied here were released.

2.5.2.1 Wet mass

Initially, all butterflies were weighed on days 0, 5, 7, and 10. Day 10 wet mass measurements were not included in any analysis. When the first set of butterflies (n=23) reached

day 5 of the trial in the greenhouse, I noticed a decline in wet mass between day 0 and 5 measurements. Butterflies normally lose wet mass following eclosion in the form of excess body water (Molleman et al., 2011). Consequently, I began measuring and recording wet mass on day 1 for the remaining (n=32) butterflies measured in the greenhouse for greater resolution on wet mass measurements across time. Day 1 wet mass measurements were taken for all butterflies from the field experiment. All wet mass measurements for greenhouse and field butterflies were taken using a scale (PA114 scale, Ohaus, USA) in the greenhouse accurate to 0.0001 g. All butterflies were weighed in a tared insect-rearing container between 9:00 am and 12:00 pm. Similarly, field butterflies were transported to the greenhouse (~1 km drive) in insect-rearing containers and weighed in a tared container. Wet mass was recorded when the butterfly had settled on the top or bottom of the container and the 0.001 g reading on the scale remained stable. Additionally, I recorded each butterfly's sex, forewing length, and date of the wet mass measurement.

2.5.2.2 Body composition

Immediately after their trial, each butterfly was frozen in individual 11 cm x 6.5 cm glassine envelopes. Body composition analysis (i.e. total fat, lean, water mass) was conducted at Western University (London, Ontario, Canada). All weight measurements were taken to the nearest 0.0001 g with the butterfly in the envelope, and the envelope weight was subtracted. Butterflies were weighed before the procedure, which represents the butterfly's wet mass at the end of the feeding trial. After wet mass measurements, monarchs were then transferred to a drying oven set to 70°C, where they remained for 3 days until reaching a constant mass. Butterflies were weighed again to obtain their dry mass, and water mass was calculated as the difference between wet and dry masses. Butterflies then underwent petroleum ether extraction in

a Soxhlet extractor to remove all lipids apart from membrane phospholipids (Christie & Han, 2012; Saini et al., 2021). The butterflies were re-dried for 8 hours and re-weighed, with the difference in dry masses before and after extraction representing the fat mass of the butterfly. Finally, lean mass was measured as the remaining weight of the butterfly after drying and lipid extraction, and represents the weight of dry muscle, organ, wing, and exoskeleton mass.

2.5.3 Environmental measurements

To compare field and greenhouse conditions, hourly temperature was recorded by two *HOBO* dataloggers (Onset HOBO Pendant Temperature/Light 64K Data Logger, ±0.53°C), each one placed inside one mesh enclosure containing *S. altissima* in both the greenhouse and the field from August 31st to September 10th. Loggers were suspended in inverted plastic baskets to raise them off the ground. Baskets were placed at ground level in the field, and at bench level (level with the bottom of the pots) in the greenhouse.

2.6 Statistical analyses

2.6.1 Overview

All analyses were conducted in R Version 4.2.1 (R Core Team, 2022-06-23). Statistical significance was assessed at an $\alpha = 0.05$. To evaluate the effect of plant species on butterfly wet mass tested in the greenhouse and field, I fit linear mixed-effects models using the lme4 package (Bates et al., 2015). Significance values for fixed effects were obtained using the lmerTest package (Kuznetsova et al., 2017). Butterfly ID was included as a random intercept for all wet mass models to control for multiple measurements taken on each butterfly through time. I used linear models to evaluate how plant species affected the body composition of butterflies from the greenhouse trials. For the wet mass of butterflies in the field, butterfly ID was included as a

nested factor within the random intercept of enclosure ID, to account for multiple butterflies being tested in each enclosure. All other field models included enclosure ID as a random intercept. One individual was found dead on day 7 of their trial. For this individual, weight measurements were retained for all days before day 7, and the individual was excluded from body composition analysis.

Model assumptions were evaluated using the performance (Lüdecke et al., 2021) and car (Fox & Weisberg, 2019) packages. Model fit was visually assessed by evaluating homogeneity of variance and normality of residuals. All model residuals met assumptions except for fat measurements from the greenhouse, which did not meet the homogeneity of variance assumption. To meet this assumption, I log-transformed fat and used this as the response variable for the greenhouse trials. Variance inflation factors (VIFs) and generalized variance inflation factors (GVIFs) were evaluated to ensure low collinearity across predictors in all models. Collinearity is considered low when VIFs are below 5 for continuous variables and GVIF indices ($GVIF^{(1/(2*df))}$) are below 2 for categorical variables (Fox & Monette, 1992). All model variables were below these thresholds and were consequently retained. For each statistical analysis where the effect of plant species was significant, a multiple comparisons test with a Tukey HSD (honestly significant difference) post hoc adjustment was conducted to determine which species pairing(s) were significantly different.

2.6.2 Greenhouse models

To determine the effect of plant species on butterfly wet mass over the trial period, I evaluated wet mass as a function of the two-way interaction between sampling day (continuous) and plant species (categorical, 6 levels). I assessed this interaction because I expected differences between plant species to increase over the trial period, resulting in different slopes for the change

in wet mass for each plant species. I also included the following four covariates: (1) forewing length (continuous) to account for the structural size of the butterfly; (2) sex (factor; two levels with reference level = female), as males are known to have greater mass than females (Brower et al., 2006; Davis & Holden, 2015); (3) total floral surface area (continuous), to account for variation in the amount of nectar across enclosures and plant species; and (4) enclosure colour (factor; two levels with reference level = black) as I suspected mesh colour could affect temperature and light conditions in the enclosure. The response of wet mass was log-transformed as weight change was non-linear throughout the trial. In total, 55 butterflies were tested for their wet mass until day 7 of the greenhouse trial, totaling 196 wet mass measurements.

For body composition analyses, the effects of plant species on fat, lean, and water mass were tested separately. In addition to plant species, each model included four covariates: (1) forewing length (continuous), (2) sex (factor; two levels with reference level = female), (3) total floral surface (continuous), and (4) starting wet mass (continuous) of the butterfly, to account for inherent body condition upon eclosion. Because body composition analyses were based on a single measurement from day 7, sampling day was not included in these models. Overall, body composition was evaluated for 46 butterflies (n=22 males, n=24 females).

2.6.3 Field analyses

I evaluated the effect of plant species on wet mass as a function of a two-way interaction between sampling day (continuous) and plant species (factor, 2 levels with reference level = *E. maculatum*) because I expected weight differences between species to increase as butterflies progressed through their trial. I included total floral surface area as a covariate in this model. Males were excluded from this analysis as only one male was tested on *E. maculatum*. The response of wet mass was log-transformed as weight change was non-linear throughout the trial.

Overall, 68 weight measurements were taken on 17 female butterflies (n=9 on *E. maculatum*, n=8 on *S. altissima*).

For body composition analyses, in addition to plant species, I included 2 covariates: (1) initial wet mass (continuous), and (2) total floral surface area (continuous). Again, because body composition analyses were based on a single measurement from day 7, the sampling day was not included in these models. Forewing length was not included as a covariate to reduce the number of model parameters, given the reduced sample size for this analysis, and because initial wet mass should similarly account for the inherent size of the butterfly. Body composition was evaluated for 14 female butterflies (n=7 on *E. maculatum*, n=7 on *S. altissima*).

2.6.4 Greenhouse versus field analyses

To compare the effect of experimental location (greenhouse vs. field) on butterfly wet mass, I used butterflies raised on *S. altissima* in both experimental locations. I evaluated the effect of experimental location on wet mass via a two-way interaction between sampling day (continuous) and experimental location (categorical, 2 levels). I included two covariates in this model: (1) sex (factor, 2 levels with reference level = female) and (2) forewing length (continuous). I did not include total floral surface area or enclosure color, as these inherently differed between experimental locations, as field enclosures were larger and were only one colour (black). Again, the response of wet mass was log-transformed as weight change was non-linear throughout the trial. Overall, I evaluated 102 weight observations on 27 butterflies in this model (Greenhouse: 8 males and 6 females; Field: 4 males and 9 females).

To compare body composition between experimental locations, I included two covariates: (1) initial wet mass (continuous) and (2) sex (factor, 2 levels with reference level = female). Again, given the limited sample size, forewing length was not included as a covariate to

reduce the number of model parameters. Overall, 23 butterflies were evaluated for their body composition (Greenhouse: 6 females and 7 males; Field: 8 females and 2 males).

Temperature data were analyzed using a linear mixed-effects model. The variables included were (1) location and (2) the random intercept of the date and time of the temperature reading. This is analogous to a paired test by grouping simultaneous readings at both locations. This comparison was narrowed to daytime temperatures, between 8:00 am to 8:00 pm, totaling 286 hourly temperature measurements.

3. Results:

3.1. Greenhouse trials

3.1.1 Wet mass

Changes in butterfly wet mass depended on plant species (sampling day*species: $\chi^2_{5,196}$ = 37.6, $p < 0.001$; Table 2, Figure 3). Tukey-adjusted pairwise comparisons revealed several differences between species (Table 3; Figure 3); butterflies foraging on *B. davidii* lost more wet mass than those foraging on *E. purpurea* (-0.044 log(g)/day, SE = 0.010, t_{136} = -4.6, $p < 0.001$), *S. altissima* (-0.027 $\log_e$(g)/day, SE = 0.009, t_{136}= 2.9, p = 0.049), and *S. ericoides* (-0.028 $\log_e$(g)/day, SE = 0.009, t_{136} = 3.0, p = 0.043). Additionally, butterflies foraging on *H. helianthoides* and *R. hirta* each, respectively, lost significantly more wet mass than those foraging on *E. purpurea* (-0.040 $\log_e$(g)/day, SE = 0.008, t_{136} = 4.9, $p < 0.001$; -0.036 $\log_e$(g)/day, t_{136} = 3.6, p = 0.006). Butterflies foraging on *H. helianthoides* also lost significantly more weight than those raised on *S. ericoides* (-0.023 $\log_e$(g)/day, SE = 0.009, t = 3.0, p = 0.043). Overall, *E. purpurea*, *S. altissima*, and *S. ericoides* supported the highest wet mass through the sampling period, followed in decreasing order by *R. hirta*, *H. helianthoides*, and *B. davidii*.

I also found wet mass was significantly associated with several covariates in my model (Table 2). Firstly, butterfly wet mass was positively associated with forewing length (0.044 $\log_e$(g)/mm, SE = 0.005, t_{136} = 9.0, p < 0.001). Secondly, males were heavier than females (0.108 $\log_e$(g), SE = 0.027, t_{136} = 4.0, p < 0.001). Finally, butterflies foraging in white enclosures were heavier than those foraging in black enclosures (0.066 $\log_e$(g), SE = 0.032, t_{136} = 2.1, p-value = 0.038). I did not find any evidence of an effect of total floral surface area on monarch butterfly wet mass.

3.1.2 Fat mass

Fat mass differed across plant species ($F_{5,46}$ = 5.55, p < 0.001; Table 2, Figure 4a). Tukey-adjusted pairwise comparisons (Table 3; Figure 4a) showed that butterflies foraging on *B. davidii* had lower fat than those foraging on *E. purpurea* (-2.60 $\log_e$(g), SE = 0.632, t_{36} = -4.11, p = 0.003) and *S. altissima* (-2.66 $\log_e$(g), SE = 0.67, t_{36} = -3.97, p = 0.004). Fat mass was negatively associated with total floral surface area (-5.7*e-5 $\log_e$(g/cm^2), SE = 2.5*e-5, t_{36} = -2.24, p = 0.031; Table 3, Figure 4b), but none of the other covariates (Table 2). Overall, *S. altissima* and *E. purpurea* supported the highest fat mass, followed in decreasing order by *S. ericoides*, *R. hirta*, *H. helianthoides*, and *B. davidii*.

3.1.3 Lean mass

Butterfly lean mass also differed across species (Table 2; Figure 4c). Similar to fat mass, Tukey-adjusted pairwise comparisons of lean mass (Table 3) showed that butterflies foraging on *B. davidii* had lower lean mass than *E. purpurea* (-0.027 g, SE = 0.006, t_{36} = -4.4, p = 0.003) and *S. altissima* (-0.022 g, SE = 0.007, t_{36} = -3.3, p = 0.025). Butterflies foraging on *R. hirta* also had lower lean mass than those foraging on *E. purpurea* (-0.027 g, SE = 0.007, t_{36} = -4.0; p = 0.004). Butterfly lean mass was positively associated with forewing length (0.004 g/mm, SE = 0.001, t_{36}

= 3.6, p = 0.001; Table 2) and wet mass at the start of the trial (0.126 g_{lean}/g_{wet}, SE = 0.035, t_{36} = 3.6, p = 0.001; Table 2). Overall, *E. purpurea* and *S. altissima* supported the highest lean mass, followed in decreasing order by *H. helianthoides*, *S. ericoides*, *R. hirta*, and *B. davidii.*

3.1.4 Water mass

Butterfly water mass was not associated with plant species. Water mass was only significantly positively associated with forewing length (0.012 g/mm, SE = 0.003, t_{36} = 3.9, p < 0.001; Table 2; Figure 4d).

3.2 Field trials

There was no difference in any metric of butterfly body condition between *E. maculatum* and *S. altissima* (Table 5). Field butterfly wet mass was significantly negatively associated with sampling day (-0.036 $\log_e$(g)/day, t_{60} = -9.66, p < 0.001), while lean mass and water mass were significantly positively correlated with initial wet mass (0.276 g_{lean}/g_{wet}, t_8 = 7.04, p < 0.001 and 0.424 g_{water}/g_{wet}, t_8 = 3.15, p = 0.014, respectively). Total floral surface area was not associated with any body condition metric, and initial wet mass was not significantly associated with fat mass (Table 5).

3.3 Greenhouse versus field

Between August 31st and September 10th, average daily temperatures in the greenhouse varied from 21.5°C to 27.8°C, while those in the field varied from 14.9°C to 23.2°C. Overall, average temperatures were significantly higher in the greenhouse (4.64°C, t_{284} = 6.6, p < 0.001; Figure 6) than in the field.

Butterflies foraging on *S. altissima* in the field versus in the greenhouse differed in their wet mass and body composition (Table 4; Figure 5). Over the trial, butterflies in the greenhouse

lost significantly more wet mass than those in the field (-0.020 $\log_e$(g)/day, t_{94} = -4.3, p = 0.001). Similarly, butterflies in the greenhouse had lower fat (-0.027 g, t_{19} = -4.2, p < 0.001) and lean mass (-0.009 g, t_{19} = -3.5, p = 0.002). Water mass did not differ between locations.

Butterfly wet mass was positively associated with forewing length (0.027 $\log_e$(g)/mm, t_{94} = 3.2, p = 0.002; Table 4). Males were again significantly heavier than females (0.052 g, t_{94} = 3.1, p = 0.003). Additionally, the butterfly's starting wet mass was positively associated with butterfly fat mass (0.126 g_{fat}/g_{wet}, t_{19} = 2.11, p = 0.049), lean mass (0.253 g_{lean}/g_{wet}, t_{19} = 10.5, p < 0.001) and water mass (0.283 g_{water}/g_{wet}, t_{19} = 2.23, p = 0.038).

4. Discussion

Data on the nectar rewards provided by different flower species for monarchs is lacking, limiting our ability to make evidence-based restoration decisions concerning nectar species for the monarch. Using an experimental approach, my study revealed that nectar plant species on which adult monarchs forage affect fat mass, a proxy for overall migratory fitness (Brown & Chippendale, 1974; Alonso-Mejía et al., 1997; Brower et al., 2006), and lean mass, indicating potential implications for building and maintaining flight muscles (Marden, 2000). The significant differences in these composition metrics were generally reflected in overall weight differences across plant species. The influence of nectar plant species on fat and lean mass highlights the need to carefully consider the plants used in monarch habitat restoration activities, as these could have implications for monarch fitness during the migratory period. I did not find strong evidence that the frequency of nectar plant use from community science data indicates nectar plant quality.

4.1 Monarch body composition in response to plant treatment

Fat mass of monarchs may have varied across plant species in my study because of different nectar sucrose content and/or concentrations. Sucrose is the primary nectar component that butterflies need to produce fat as adults (Brower et al., 2006). Flowers can vary in the total amount of sucrose available per flower, termed sucrose "content", and in sucrose concentration (Arnold & Michaels, 2017). Arnold & Michaels (2017) surveyed 19 species of plants for nectar sucrose concentrations, including *Symphyotrichum ericoides* which I tested in my study, and *Solidago rigida* (rough goldenrod), a species closely related to *Solidago altissima* also tested in my study. Arnold & Michaels (2017) found *S. ericoides* to have the 2nd highest sucrose concentration at 63%, significantly greater than 7 other species, and *S. rigida* the 6th highest at ~57%, significantly greater than 4 other species. Additionally, Chen et al. (2014) found that the nectar sucrose concentration of *B. davidii*, which I also tested in my study, varied from 17%-33.5%, considerably lower than found for *S. rigida* and *S. ericoides* from the Arnold and Michaels (2017) study. The relatively high sucrose concentrations of the *Solidago sp.* and *S. ericoides* match the relatively high fat content I measured in butterflies in my study, while the relatively low sucrose concentration measured in *B. davidii* matches the relatively low fat content for butterflies that foraged on this species in my study. This suggests that the interspecific species differences I found in fat content could be related to nectar sucrose concentration. Also, in support of this hypothesis, I did not find evidence for a positive relationship between fat mass and floral surface area, a proxy for nectar volume/availability (Galetto & Bernardello, 2004; Hicks et al., 2016; Tavares et al., 2016). Importantly, while the mechanism cannot fully be determined from my experiment, my results provide strong evidence that not all nectar plants are equal for monarch butterfly fat accumulation.

Like my hypothesized mechanism linking variation in sucrose concentration among flower species to fat mass, interspecific variation in amino-acid profiles and concentration (Baker & Baker, 1986; Gardener & Gillman, 2001) could explain the differences in lean mass I found. Lean mass comprises an individual's exoskeleton, organs, and muscles. Exoskeletons are fixed in size post-eclosion (Flockhart et al., 2017; Freedman & Dingle, 2018) and would have partly been controlled for in my models via forewing length. Therefore, variation in lean mass likely reflects organ and/or muscle size differences. While generally not considered as important as fat for migration, higher lean mass in insects is associated with larger flight muscles (Marden, 2000). This could result in longer and more efficient flights for monarchs and ultimately higher migration success. Future research should examine the specific components of lean mass affected by nectar and determine their consequences for monarch flight performance.

In contrast to fat and lean mass findings, plant treatment did not affect the water mass of monarchs. For monarch butterflies, water mass may play a role in optimal balance for flight (Gibo & McCurdy, 1993), and their body morphology leaves them prone to desiccation (O'Donnell, 2022). Consequently, to maintain hydration, monarchs rely on water obtained from nectar and seek additional water sources, such as roadway puddles and dew or rain droplets (Frey et al., 2002; Hobson et al., 2023). In the greenhouse, I observed monarchs drinking spilled water droplets after watering the plants, and rainwater and/or dew were likely periodically available to butterflies in the field. This supplementary water could negate differences in the water content of nectar across species, leading to no effect of plant species on monarch water mass.

Beyond this experiment, the water content of different nectar species may be an important consideration in more arid environments. In the southern United States and Mexico, monarchs traverse large areas of land that are prone to drought and have little to no

supplementary water sources, resulting in decreased water mass (Hobson, 2005; Descamps et al., 2021; Kuppler & Kotowska, 2021). In contrast, in temperate southern Canada, many sources of supplementary freshwater exist, and monarchs in this region have been found to have higher mass relative to monarchs from further south (Hobson, 2005). Thus, a monarch's ability to obtain water in this region may be a minor concern compared to obtaining sugar and amino acids.

4.2 Relationship between monarch body condition, plant visitation rates based on community science data, and nectar quality

Visitation rates of flowers observed by community scientists (refer to Moczula et al., In Prep) appear to roughly match the nectar rewards received by monarchs from the six species of plants evaluated in this study. Qualitatively evaluating my results, the two species of lowest visitation, *R. hirta* and *H. helianthoides*, are among the lowest quality for overall wet and fat mass, and in *R. hirta*'s case, lean mass. However, the lowest quality species, *B. davidii*, for each of these metrics was the 3rd most visited species in Moczula et al. (In Prep). The highest quality species based on my study in order were *E. purpurea* (4th most visited in Moczula et al., In Prep), *S. altissima* (2nd most visited), and *S. ericoides* (1st most visited). In summary, *B. davidii* seems to be the major break in visitation predicting quality, despite the ability of monarchs to identify and learn high-value nectar rewards (Blackiston et al., 2011; Cepero et al., 2015). It is important to note that *B. davidii* is an outlier from the other species tested in two factors: (1) it was the only non-native species, and (2) it is the only species not from the family Asteraceae. These outlying factors should not change the prediction that monarchs can learn nectar rewards and adjust foraging habits accordingly unless they interact with the monarch's ability to assess nectar rewards. To truly determine if plant origin or family affects the monarch's ability to evaluate

nectar rewards, tests with many more species of flowering plants (native and non-native, Asteraceae and non-Asteraceae) are needed.

Apart from these potential outlying factors, the observation that *B. davidii* is of worse quality than predicted by its overall visitation could be because butterfly observations on flowers from Moczula et al. (In Prep) were not standardized to the relative availability of these plants in the area. In other words, frequent use of a plant species in community science data could result from its high relative availability on the landscape rather than a preference for that species. To truly determine whether monarchs seek to use nectar plants of higher benefit, surveys of use relative to availability, such as those done by Pekos et al. (2025) and Fisher et al. (2023) are needed in combination with experimental foraging trials like those described here. Future studies should determine if the preferred plants from the aforementioned studies represent high-quality nectar plants. If this is demonstrated, then surveys of use relative to availability could be used in place of experimental tests to select plant species for monarch habitat restoration across the monarch's range and generations.

4.3 Experimental location and floral surface area effects

When comparing butterflies foraging on *S. altissima* in the greenhouse versus the field, I found that field butterflies had significantly higher fat and lean mass by the end of the trial than greenhouse butterflies. I posit this is in part a result of temperature differences. Temperature was on average 4.64°C warmer in the greenhouse over the course of the experiment. This likely increased butterfly metabolism and fat usage (Shah et al., 2021; Harvey et al., 2023). Warmer temperatures in the greenhouse could have also had direct effects on plants, leading to decreased total nectar production (Takkis et al., 2015, 2018; McCombs et al., 2022), lower sucrose concentration (Peel et al, In Prep), and fewer flowers produced (Liu et al., 2012; de Manincor et

al., 2023). With sucrose being the main building block for fat (Brower et al., 2006), lower nectar sucrose concentration and/or overall availability of nectar could have led to decreased fat mass. Additionally, higher temperatures can alter nectar amino acid concentrations, increasing non-essential amino acid concentrations while decreasing essential amino acids for bees (Descamps et al., 2021). While amino acids are not all similarly affected, changes in amino acid concentrations due to warming could have led to the differences in lean mass between locations.

Beyond temperature-induced flower changes, differences in body composition between locations could have resulted from field enclosures containing more flowers than greenhouse enclosures, as they were overall larger and housed more plant stems. However, there is no indication that nectar availability was limiting in the greenhouse. If butterfly fat or lean mass were limited by the total nectar volume available in the greenhouse, I would have expected the total floral surface area to be a significant positive predictor of these variables in the greenhouse, whereas, I found the opposite in the greenhouse for a single fitness metric – an overall negative relationship between surface area and butterfly fat in the greenhouse – and no relationship between surface area and any fitness metric in the field. I suggest the negative relationship found between total floral surface area and fat mass in the greenhouse should be interpreted with caution given it appears to be driven by three observations (Fig. 4b). A larger sample size of flowers with larger surface areas would be needed to have more confidence in this pattern.

4.4 Experimental framework and limitations

Greenhouse trials presented several challenges when sourcing plants for this experiment, ultimately making this experiment highly labour intense. Many species I attempted to test do not flower in their first year, and therefore, could not be grown from seed within a single season. Consequently, I tried to source these plants from Ontario nurseries or transplant them from field

sites. Only three of the six species initially survived transplantation (Table S1), and of those three, two became heavily infested with insect herbivores that I could not control. Despite these challenges, the greenhouse did allow me to thoroughly test six different plant species under the same environmental conditions and identify that plant species can affect the fat mass of monarchs.

When considering the effort involved in my two experimental locations, I suggest field studies as an attractive alternative to greenhouse studies to evaluate the quality of nectar plants. Enclosures can be placed over existing healthy plants close to the trial period, eliminating the risk of plant deterioration, and the costs of obtaining plants and renting greenhouse space. The drawbacks of this method are the limited opportunities for environmental control, as many variations are likely to exist in the field, such as sunlight availability, soil moisture, and other microclimatic factors. Reducing this variation by using sites with various plant species in close proximity could present a suitable solution to evaluate the relative benefit of nectar plants for monarch fitness.

4.5 Implications for habitat restoration

In 2024, the Government of Canada announced that it would invest $623,000 over a two-year period into monarch habitat restoration projects in Ontario, all of which involve use of native seed mixes (NCC; Government of Canada, 2024). Ultimately, for these and future restoration projects to be most effective for all parts of the monarch life cycle, it is important to identify which nectar sources best contribute to fat stores and migration success of monarchs.

The experimental approach I used in this study represents a novel, holistic test of different nectar plant species' value to monarchs, identifying key species that benefit the monarch in Canada during its migratory period. This approach can be extended to test flowering

plants in other periods of the monarch's lifecycle, such as the breeding period, and at different latitudes to determine the best species to use in monarch habitat restoration across the monarch's range. Combined with relative use surveys, general patterns of nectar plant quality with monarch preference could help identify important flowers that should be considered in monarch habitat restoration plans. With limited restoration resources, identifying and restoring habitats with high-quality plants will help maximize these resources' benefits for monarch populations. This experimental approach can also easily be extended to other species of at-risk Lepidoptera.

5. Conclusions:

I evaluated the benefit of seven nectar plant species for adult monarch body condition. My experiment specifically examined how flower species influence the body condition of the migratory, non-breeding generation of monarchs that travel to Mexico – a generation that remains understudied. I found that different nectar plant species can significantly impact adult monarch body condition in the form of fat mass and lean mass, and these impacts could have significant implications for monarch migration success. Ultimately, restoration activities should include species of plants that are highly beneficial for monarch body condition, such as those identified here, and species that have yet to be evaluated. Furthermore, my experiment represents an approach that can be used to quickly and efficiently assess the fitness of monarchs foraging on live plants. It can easily be extended to other flower species and periods of the monarch's annual cycle.

Tables

Table 1: Information about the plant species tested in field and greenhouse experiments from August 19th to September 10th, 2022. Presented are the species name, common name, origin (native [N] or non-native [NN]), floral surface area formula, number of monarch visits to that species (Observations) from Moczula et al. (In Prep), the size of their plant pot (L), and the date the plants were introduced to the greenhouse (Greenhouse date). Note, as described above, monarchs were not introduced into the experiment until August 19th.

Scientific name	Common Name	Origin	Surface area formula	Observations	Pot size (L)	Greenhouse date
Buddleja davidii	Butterfly bush	NN	$2\pi rh+2\pi r^2$	114	7.6	June 21st
Echinacea purpurea	Purple coneflower	N	$2\pi rh+2\pi r^2$	64	3.8	August 25th
Eutrochium maculatum	Spotted joe-pye weed	N	$2\pi rh+2\pi r^2$	168	NA	Field
Heliopsis helianthoides	Ox-eye sunflower	N	πr^2	0	1 L	July 5th
Rudbeckia hirta	Black-eyed Susan	N	$2\pi rh+2\pi r^2$	28	7.6	June 21st
Solidago altissima	Tall goldenrod	N	$2\pi rh+2\pi r^2$	161	7.6	June 27th
Symphyotrichum ericoides	White heath aster	N	πr^2	185	7.6	June 21st

Table 2: Results of linear models evaluating differences in adult monarch weight and body composition after foraging on one of six different nectar plant species in the greenhouse experiment. Included here are $\log_e$(wet mass) (n=55 butterflies) and final body composition metrics (n=52 butterflies): $\log_e$(fat mass (g)), lean mass (g), water mass (g), and the total number of measurements taken [N]. All analyses included plant species (categorical, 6 levels), forewing length (continuous), butterfly sex (categorical, 2 levels), and total floral surface area (continuous) as predictor variables. The weight model included the butterfly's enclosure colour (categorical, 2 levels), an interaction term between plant species and sampling day, and a random effect for butterfly ID. Body composition models included the butterfly's starting weight (continuous). For enclosure colour and sex, the reference levels are "black" and "female", respectively. The mean estimate, standard error (SE), test statistic (t), degrees of freedom (df), and p-value (*p*) are presented. For the 'plant' predictor, the p-value of the overall effect is given, and pairwise comparisons between species can be found in Table 3. Also included are R^2 and R^2_{adj} for linear models; the latter indicates the model fit adjusted for the number of parameters included. For models with a random effect, marginal R^2 (R^2_{marg}) quantifies the model fit without the random effect, and conditional R^2 (R^2_{cond}) quantifies the model fit with the random effect included. Significant results ($p < 0.05$) are marked in bold.

Response [N]	Predictor	Estimate	SE	df	t	R^2_{marg} / R^2_{cond}	*p*
$\log_e$(Wet Mass (g)) [196]	Plant species	NA	NA	178,5	6.38	0.753 / 0.842	0.271
	Forewing length	**0.044**	**0.005**	**178,1**	**9.04**		**<0.001**
	Sex [Male]	**0.108**	**0.027**	**178,1**	**4.00**		**<0.001**
	Sampling day	**-0.053**	**0.005**	**178,1**	**-11.0**		**<0.001**
	Enclosure colour [White]	**0.066**	**0.032**	**178,1**	**2.09**		**0.038**
	Total floral surface area	-1.26e-4	2.1e-3	178,1	-0.60		0.549
	Plant x Sampling day	**NA**	**NA**	**178,5**	**37.6**		**<0.001**
						R^2 / R^2_{adj}	
$\log_e$(Fat Mass (g)) [46]	**Plant species**	**NA**	**NA**	**36,5**	**5.32**	0.461 / 0.327	**<0.001**
	Sex [Male]	0.126	0.386	36,1	0.32		0.747
	Forewing length	0.009	0.112	36,1	0.08		0.936
	Starting weight	0.265	3.581	36,1	0.07		0.941
	Total floral surface area	**-5.7*e-5**	**2.6*e-5**	**36,1**	**-2.24**		**0.031**
Lean Mass (g) [46]	**Plant species**	**NA**	**NA**	**36,5**	**5.99**	0.835 / 0.794	**<0.001**
	Sex [Male]	0.007	0.004	36,1	1.86		0.071
	Forewing length	**0.004**	**0.001**	**36,1**	**3.55**		**0.001**
	Starting weight	**0.126**	**0.035**	**36,1**	**3.56**		**0.001**
	Total floral surface area	-2.1e-7	2.5e-7	36,1	-0.85		0.403
Water Mass (g) [46]	Plant species	NA	NA	36,5	2.26	0.529 / 0.412	0.069
	Sex [Male]	0.015	0.011	36,1	1.47		0.150
	Forewing length	**0.012**	**0.003**	**36,1**	**3.92**		**<0.001**
	Starting weight	-0.117	0.097	36,1	-1.20		0.239
	Total floral surface area	-6.7e-7	7.0e-7	36,1	-0.96		0.342

Table 3: Tukey HSD-adjusted pairwise comparisons of nectar plant species' effect on wet mass, fat mass, and lean mass of foraging butterflies in the greenhouse experiment. The difference estimate, standard error (SE), degrees of freedom (df), t-ratio, and p-value (*p*) are presented for each plant species pair contrast. The wet mass contrast compares the slope of butterfly weight through the sampling period (log_e(g)/day), while the fat (log_e(g)) and lean mass (g) contrasts compare the single measurement of those variables at the end of each butterfly's trial. Positive estimate values indicate a higher mean estimate for the first plant mentioned in the pairing. The total number of observations [N] is shown for each model response, with the number of trials for each plant species [N_{sp}] next to their first reference. Full model effects can be found in Table 2. Significant effects ($p < 0.05$) are marked in bold.

Model Response [N]	Plant species contrast [N_{sp}]	Estimate	SE	df	t-ratio	*p*
Log_e (Wet mass (g)) [196]	*S. ericoides* [13] - *S. altissima* [14]	0.001	0.007	136	0.10	1.000
	***S. ericoides - B. davidii* [6]**	**0.028**	**0.009**	**136**	**2.95**	**0.043**
	S. ericoides - E. purpurea [10]	-0.016	0.007	136	-2.29	0.205
	S. ericoides - R. hirta [6]	0.020	0.010	136	2.01	0.342
	***S. ericoides - H. helianthoides* [9]**	**0.023**	**0.008**	**136**	**2.95**	**0.043**
	S. altissima - B. davidii	**0.027**	**0.009**	**136**	**2.90**	**0.049**
	S. altissima - E. purpurea	-0.017	0.007	135	-2.42	0.156
	S. altissima - R. hirta	0.019	0.010	135	1.95	0.375
	S. altissima - H. helianthoides	0.022	0.008	136	2.90	0.049
	B. davidii - E. purpurea	**-0.044**	**0.010**	**136**	**-4.56**	**<0.001**
	B. davidii - R. hirta	-0.008	0.012	136	-0.68	0.984
	B. davidii - H. helianthoides	-0.005	0.010	136	-0.46	0.997
	E. purpurea - R. hirta	**0.036**	**0.010**	**135**	**3.58**	**0.006**
	E. purpurea - H. helianthoides	**0.040**	**0.008**	**136**	**4.85**	**<0.001**
	R. hirta – H. helianthoides	0.003	0.011	136	0.32	1.000
Log_e(Fat (g)) [46]	*S. ericoides - S. altissima*	-0.97	0.604	36	-1.61	0.599
	S. ericoides - B. davidii	1.69	0.641	36	2.63	0.115
	S. ericoides - E. purpurea	-0.91	0.548	36	-1.67	0.561
	S. ericoides - R. hirta	0.89	0.682	36	1.31	0.781
	S. ericoides - H. helianthoides	0.85	0.598	36	1.43	0.712
	S. altissima - B. davidii	**2.66**	**0.67**	**36**	**3.97**	**0.004**
	S. altissima - E. purpurea	0.06	0.578	36	0.10	1.000
	S. altissima - R. hirta	1.86	0.736	36	2.53	0.143
	S. altissima - H. helianthoides	1.82	0.65	36	2.81	0.080
	B. davidii - E. purpurea	**-2.60**	**0.632**	**36**	**-4.11**	**0.003**
	B. davidii - R. hirta	-0.80	0.766	36	-1.04	0.900
	B. davidii - H. helianthoides	-0.84	0.702	36	-1.19	0.838
	E. purpurea - R. hirta	1.80	0.681	36	2.65	0.112
	E. purpurea - H. helianthoides	1.77	0.626	36	2.82	0.077
	R. hirta - H. helianthoides	-0.04	0.712	36	-0.05	1.000
	S. ericoides - S. altissima	-0.010	0.006	36	-1.76	0.501

Lean mass (g) [46]	*S. ericoides - B. davidii*	0.011	0.006	36	1.79	0.487
	S. ericoides - E. purpurea	-0.016	0.005	36	-2.96	0.056
	S. ericoides - R. hirta	0.011	0.007	36	1.57	0.621
	S. ericoides - H. helianthoides	-0.006	0.006	36	-0.98	0.920
	S. altissima - B. davidii	**0.022**	**0.007**	**36**	**3.30**	**0.025**
	S. altissima - E. purpurea	-0.005	0.006	36	-0.96	0.927
	S. altissima - R. hirta	0.021	0.007	36	2.91	0.064
	S. altissima - H. helianthoides	0.005	0.006	36	0.73	0.976
	B. davidii - E. purpurea	**-0.027**	**0.006**	**36**	**-4.37**	**0.001**
	B. davidii - R. hirta	-0.001	0.008	36	-0.10	1.000
	B. davidii - H. helianthoides	-0.017	0.007	36	-2.47	0.160
	E. purpurea - R. hirta	**0.027**	**0.007**	**36**	**3.95**	**0.004**
	E. purpurea - H. helianthoides	0.010	0.006	36	1.65	0.572
	R. hirta - H. helianthoides	-0.016	0.007	36	-2.33	0.208

Table 4: Results of linear models evaluating differences in adult monarch weight and body composition after foraging on *Solidago altissima* in the field or greenhouse experiments. Included here are the wet mass (n=27 butterflies) and final body composition metrics (n=23 butterflies): fat mass (g), lean mass (g), water mass (g), and the total number of measurements taken [N]. All analyses included location (categorical, 2 levels) and butterfly sex (categorical, 2 levels) as predictor variables. The weight model included forewing length (continuous), an interaction term between plant species and sampling day, and a random effect for butterfly ID. Body composition models included the butterfly's starting weight (continuous). For enclosure location and sex, the reference levels are "field" and "female", respectively. The mean estimate, standard error (SE), test statistic (t), degrees of freedom (df), and p-value (*p*) are presented. Also included are R^2 and R^2_{adj} for linear models; the latter indicates the model fit adjusted for the number of parameters included. For models with a random effect, marginal R^2 (R^2_{marg}) quantifies the model fit without the random effect, and conditional R^2 (R^2_{cond}) quantifies the model fit with the random effect included. Significant effects ($p < 0.05$) are marked in bold.

Model response [N]	Predictor	Estimate	SE	df	t	R^2_{marg} / R^2_{cond}	*P*
Log_e(Wet Mass (g)) [102]	Location [Greenhouse]	-0.005	0.039	94	-0.12	0.702 / 0.871	0.906
	Sampling day	**-0.033**	**0.003**	**94**	**-10.1**		**<0.001**
	Forewing length	**0.027**	**0.009**	**94**	**3.15**		**0.002**
	Sex [Male]	**0.109**	**0.036**	**94**	**3.03**		**0.003**
	Location x Sampling day	**-0.020**	**0.005**	**94**	**-4.28**		**<0.001**
						R^2 / R^2_{adj}	
Fat Mass (g) [23]	**Location [Greenhouse]**	**-0.027**	**0.006**	**19**	**-4.19**	0.563 / 0.494	**<0.001**
	Starting weight	**0.126**	**0.060**	**19**	**2.11**		**0.049**
	Sex [Male]	-0.002	0.008	19	-0.25		0.807
Lean Mass (g) [23]	**Location [Greenhouse]**	**-0.009**	**0.003**	**19**	**-3.54**	0.890 / 0.872	**0.002**
	Starting weight	**0.253**	**0.024**	**19**	**10.5**		**<0.001**
	Sex [Male]	-0.003	0.003	19	-1.06		0.301
Water Mass (g) [23]	Location [Greenhouse]	-0.005	0.014	19	-0.36	0.241 / 0.121	0.726
	Starting weight	**0.283**	**0.127**	**19**	**2.23**		**0.038**
	Sex [Male]	-0.008	0.02	19	-0.45		0.659

Table 5: Results of linear models evaluating differences in adult monarch weight and body composition after foraging in the field on *Eutrochium maculatum* or *Solidago altissima*. Included here are wet mass (n=17 butterflies) and final body composition metrics (n=14 butterflies): fat mass (g), lean mass (g), water mass (g), and the total number of measurements taken [N]. All analyses included plant species (categorical, 2 levels) and total floral surface area (continuous) as predictor variables. Wet mass model included an interaction term between plant species and sampling day and a random effect of butterfly ID. Body composition models included the butterfly's starting weight (continuous). The reference level for plant species is "*E. maculatum*". The mean estimate, standard error (SE), t-value (t), degrees of freedom (df), and p-value (*p*) are presented. Also included are R^2 and R^2_{adj} for linear models; the latter indicates the model fit adjusted for the number of parameters included. For models with a random effect, marginal R^2 (R^2_{marg}) quantifies the model fit without the random effect, and conditional R^2 (R^2_{cond}) quantifies the model fit with the random effect included. Significant effects ($p < 0.05$) are marked in bold.

Model response [N]	Predictor	Estimate	SE	df	t	R^2_{marg} / R^2_{cond}	*p*
Log_e(Wet Mass (g)) [68]	Plant species [*S. altissima*]	0.055	0.028	60	0.87	0.466 / 0.866	0.387
	Sampling day	**-0.036**	**0.002**	**60**	**-9.66**		**<0.001**
	Total floral surface area	2.5e-6	1.3e-6	60	1.95		0.056
	Plant species x Sampling day	0.004	0.005	60	0.671		0.505
Fat Mass (g) [14]	Plant species [*S. altissima*]	0.001	0.015	8	0.063	0.203 / 0.344	0.951
	Total floral surface area	4.4e-7	3.7e-7	8	1.17		0.274
	Starting weight	0.043	0.150	8	0.287		0.782
Lean Mass (g) [14]	Plant species [*S. altissima*]	0.005	0.003	8	1.39	0.891 / NA	0.203
	Total floral surface area	-2.0e-8	8.9e-8	8	-0.22		0.831
	Starting weight	**0.276**	**0.039**	**8**	7.04		**<0.001**
Water Mass (g) [14]	Plant species [*S. altissima*]	0.015	0.012	8	1.32	0.664 / NA	0.224
	Total floral surface area	-2.1e-8	3.1e-7	8	-0.069		0.946
	Starting weight	**0.424**	**0.135**	**8**	**3.15**		**0.014**

Figures

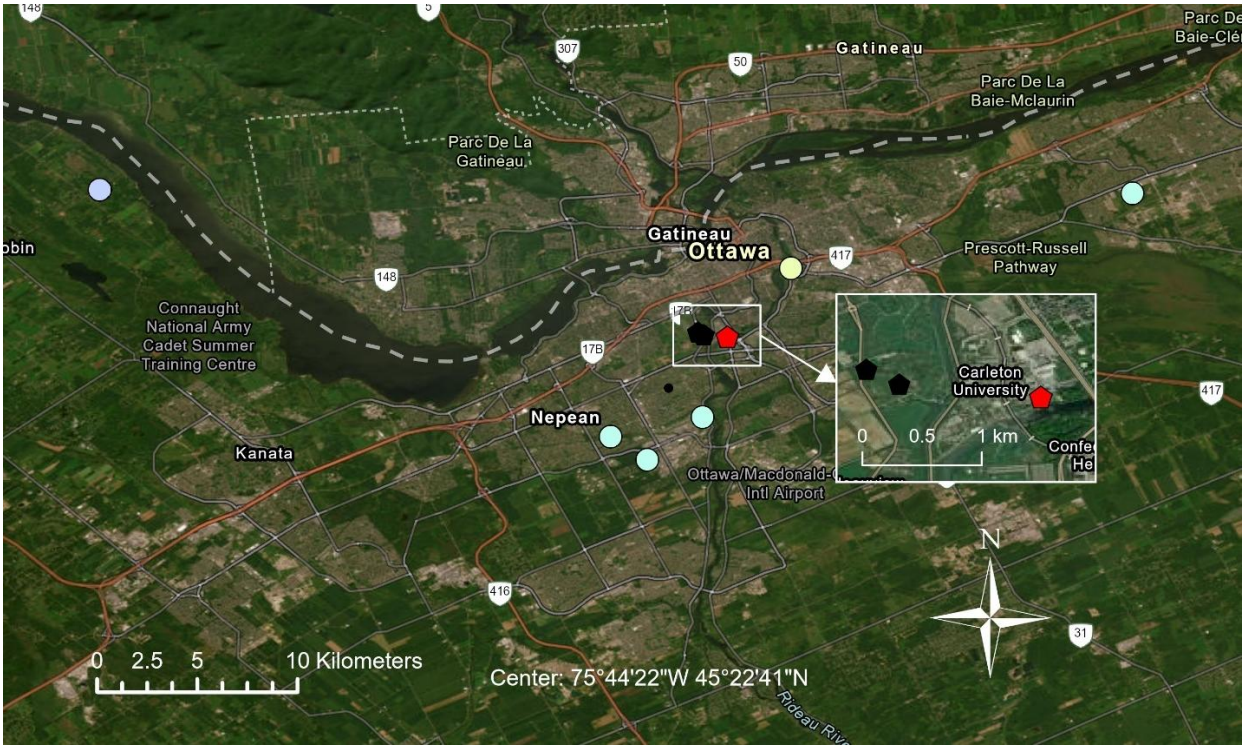

Figure 1: Sites where monarchs were collected (blue, purple) and experimental procedures took place (black, red) in Ottawa, Ontario, Canada. Blue circles: sites where adult monarch butterflies from mating pairs were captured. Purple circle: site where larval monarchs were collected. Yellow circle: A site where milkweed was collected to feed larval monarchs (Further sites from Pekos et al., In Review). Pentagons: sites where experiments took place, black representing field sites, red representing the greenhouse. A scale bar of 10 kilometres and the longitude and latitude of the centre of the image area are included. The white rectangle enlarges the experimental locations and shows an adjusted scale bar.

Figure 2: Experimental enclosures for butterfly feeding trials in (a) the Carleton University, Ottawa, Ontario, Canada greenhouse and (b-c) Fletcher Wildlife Garden (FWG) fields, Ottawa, Ontario, Canada. Shown are a) mesh enclosures filled with plants on greenhouse benches; b) a field enclosure containing *Eutrochium maculatum* (spotted joe-pye weed) at FWG; c) field enclosures containing *Solidago altissima* (tall goldenrod) at FWG.

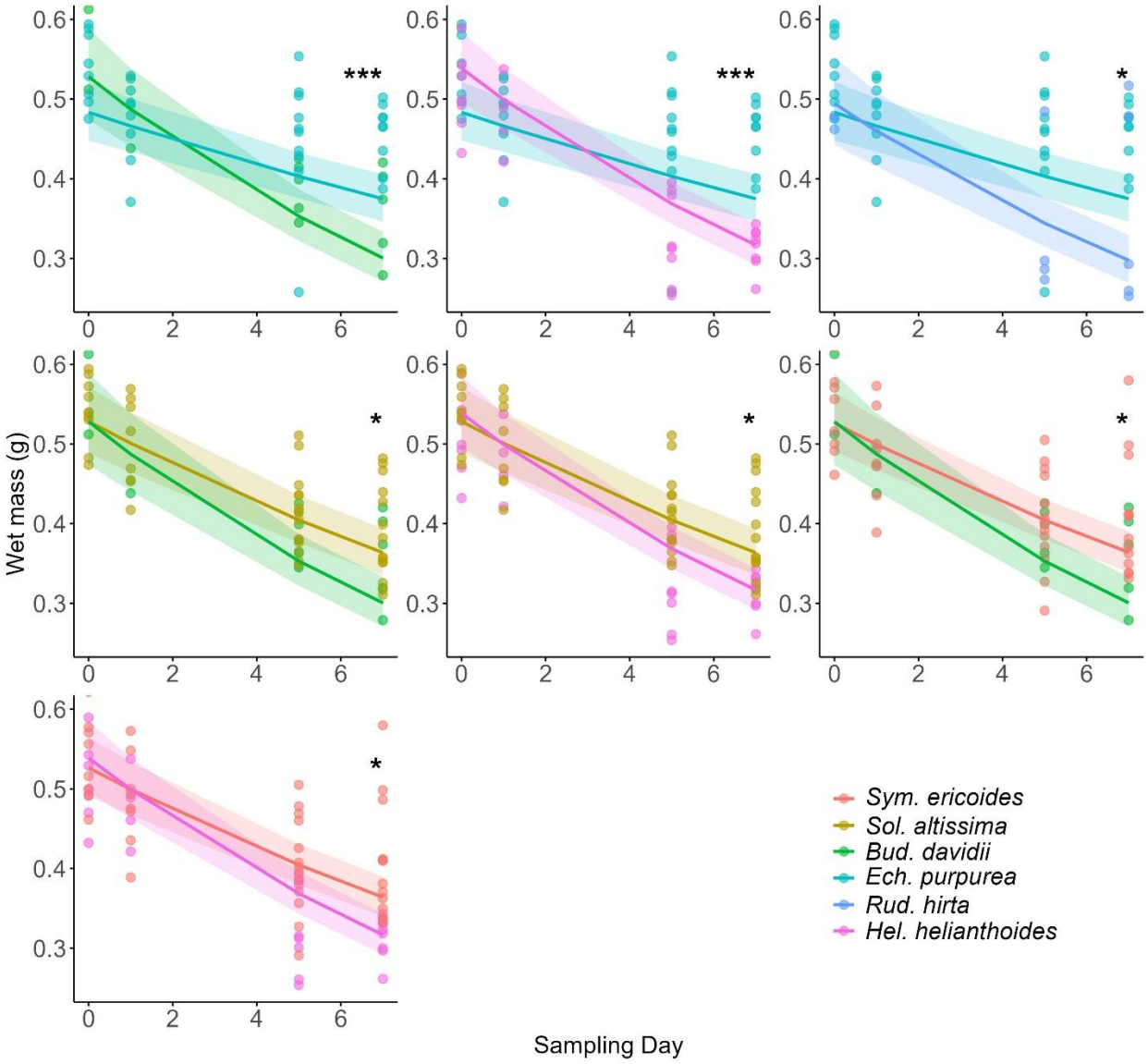

Figure 3: Predicted effects from linear models of plant species on butterfly wet mass through experimental trials. Each panel shows two species that significantly differed in their effect on butterfly weight through the sampling period, as determined by a linear mixed effects model (Table 3). The dependent variable of wet mass was log-transformed for statistical analysis, and the predicted values here have been transformed back to the observed scale. Significance levels for the interaction (i.e. differences in slope) are indicated by stars (* < 0.05, ** < 0.005, *** < 0.001). Mean predicted weights through sampling day are shown (central lines) surrounded by shading representing the 95% confidence interval. N = 196 weight measurements of 55 butterflies.

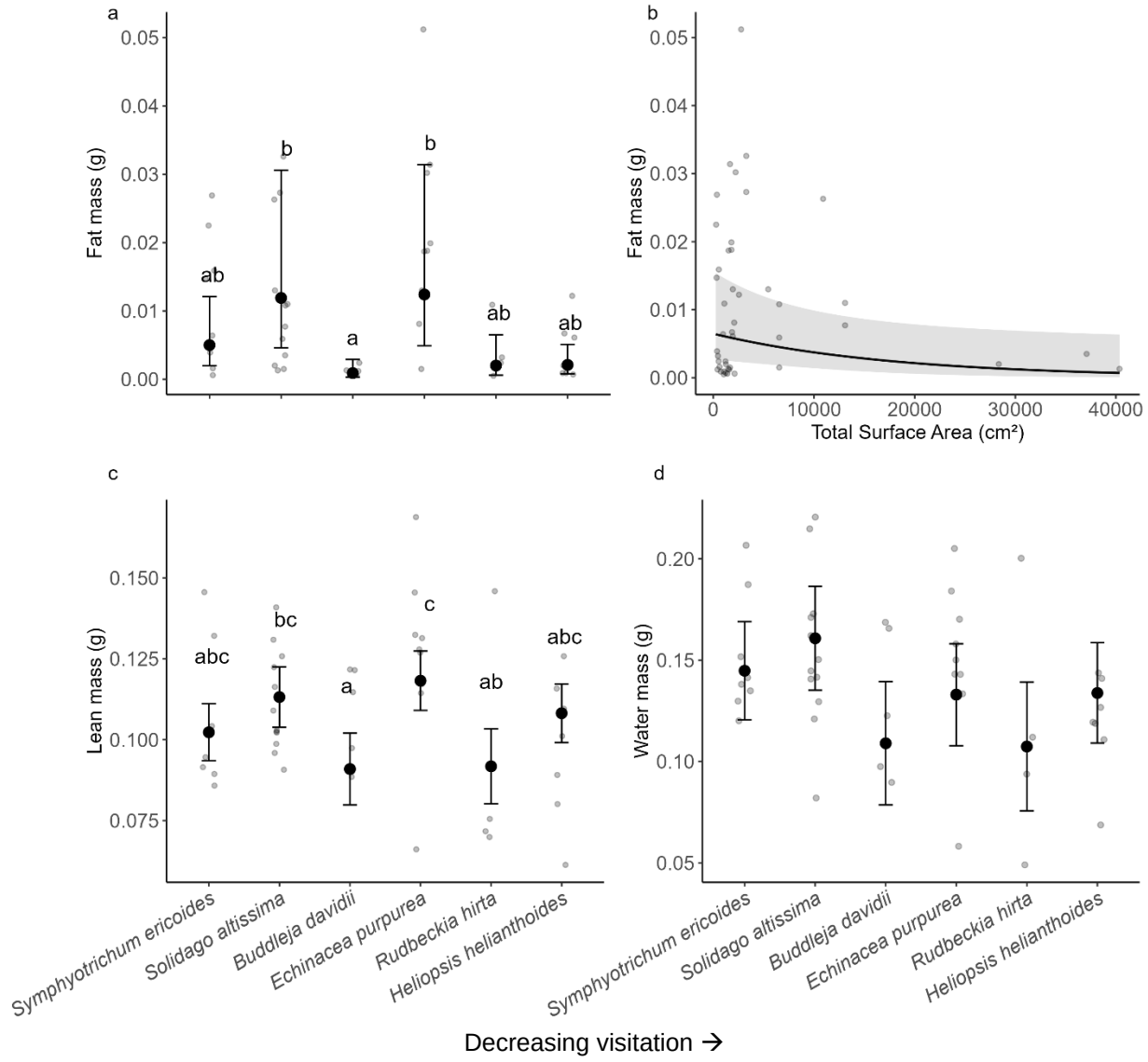

Figure 4: Predicted effects of six nectar plant species on (a) fat mass, (c) lean mass, and (d) water mass of monarch butterflies, and effect of (b) floral surface area on fat mass. Plant species are ordered from most visited by monarchs (left) to least visited (right) based on community science data (Moczula et al., In Prep). Data were analyzed using linear models (Table 2). Raw data (grey points), 95% confidence interval (a, c, d: error bars; b: grey shading), and predicted mean values (black points) are presented for each species. Letters above the upper confidence limit are included where significant differences between groups were found. Letters common to different plant species indicate no significant differences between those species, while unique letters between species indicate significant differences (Table 3). The dependent variable of (a & b) fat mass was log-transformed for statistical analysis, and the predicted values here have been transformed back to the observed scale. N = 46 butterflies analyzed for body composition.

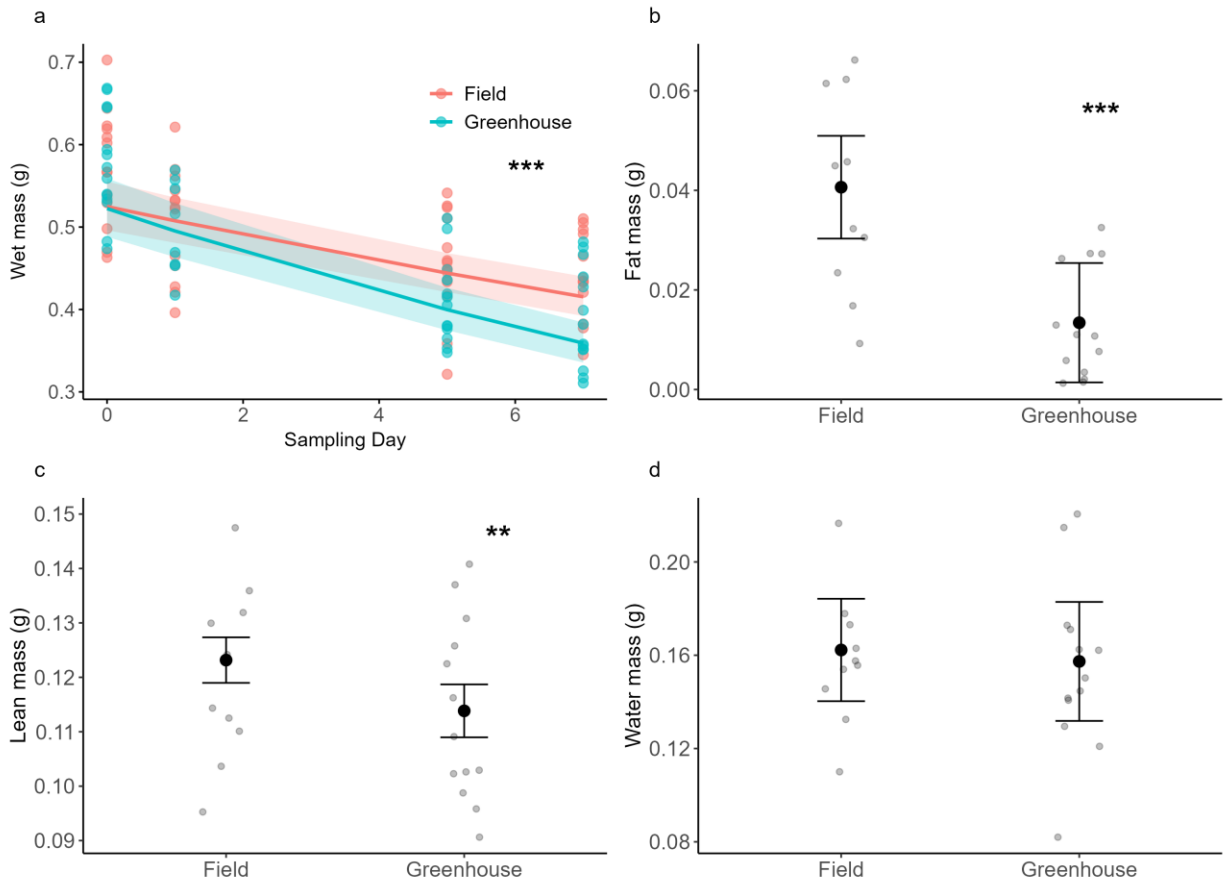

Figure 5: Comparison of butterfly wet mass, fat mass, lean mass, and water mass after foraging on *S. altissima* in either the field or the greenhouse. Butterflies foraged on their treatment plant for seven to ten days and were weighed throughout their trial (N = 107 observations on 27 butterflies). Butterflies that foraged for seven days were analyzed for body composition (fat, lean, and water mass; N = 23). These data were analyzed using (a) a linear mixed effects model with butterfly ID as a random effect, or (b-d) linear models. Full model parameters can be found in Table 4. Mean (black dot) and 95% confidence intervals are shown (a: shaded colour; b-d: error bars), with raw data overlaid (grey points). The dependent variable of (a) wet mass was log-transformed for statistical analysis, and the predicted values here have been transformed back to the observed scale. Significant relationships are indicated with stars (*p < 0.05, **p < 0.01, ***p < 0.001).

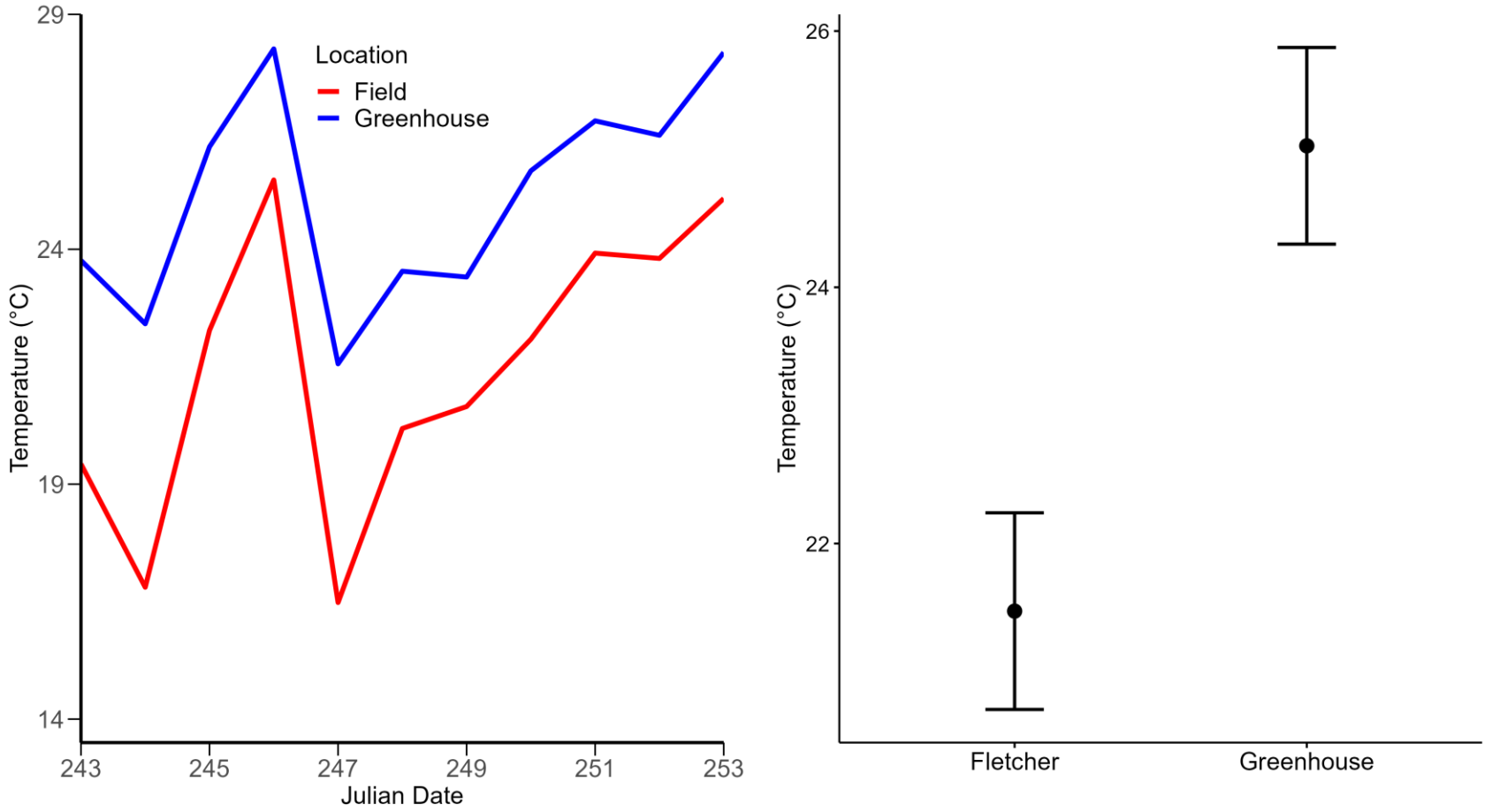

Figure 6: Temperatures in enclosures containing *Solidago altissima* (tall goldenrod) in the field (blue) and the greenhouse (red) between August 31st and September 10th, 2022, in Ottawa, Ontario, Canada. Panel (a) shows the raw data of daily average temperatures over the measuring period. Panel (b) shows the model-predicted results of hourly pairwise comparisons in the two locations with the 95% confidence interval (N = 286 temperature readings).

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Appendix

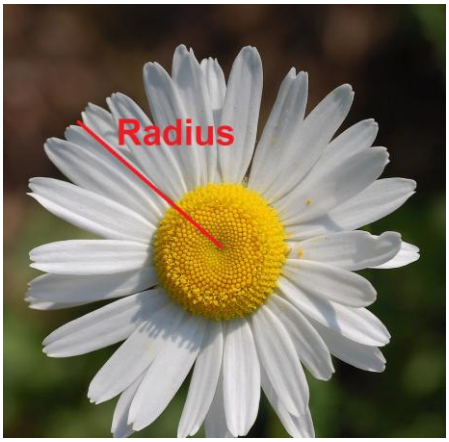
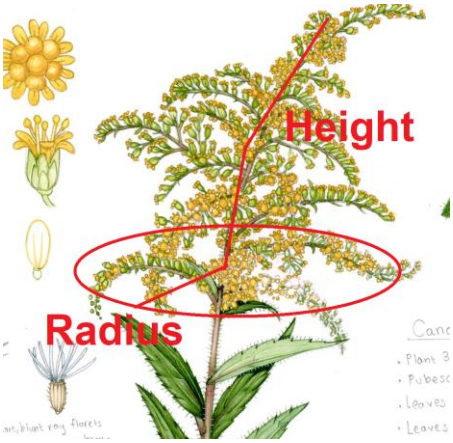
Simple flower (Circular)	Complex flower (Circular with depth)
English daisy 	Goldenrod 

Figure S1: Illustration of the methods used to obtain the floral surface area for different species, developed by Dr. Stephanie Rivest. For flowers with a complex shape, the radius at the widest point and the height of the flowering head (compound unit) were taken. For flowers with a simple circular shape, the radius of the flowering head was taken. These were used to calculate the total surface area of flowering units.

Table S1: Species of flowers I attempted to grow in the greenhouse for monarch foraging experiments in 2022. Some species failed to survive due to transplantation, insect infestation, or both. Species that were successful and used in my greenhouse feeding trials are bolded. Presented are the common and scientific names for each species, the habitat type where these species occur, and the number of times monarchs visited that plants' genus in Moczula et al. (In Prep). Around the beginning of July, I found powdery mildew on *R. hirta*, aphids on *Symphyotrichum novae-angliae* (New England asters) and *Eutrochium maculatum*, and *Galerucella spp*. larvae on all *Lythrum salicaria* (purple loose-strife) plants.

Scientific name	**Common Name**	**Origin**	**# of visits**	**Source**	**Greenhouse date**	Condition
Buddleja davidii	**Butterfly bush**	**Non-native**	**114**	**NVK Nurseries; Agriculture & Agri-Food Canada**	**June 21st**	**Good**
Cirsium vulgare	Bull thistle	Non-native	88	Field transplant	June 22nd; July 12th	Died after transplantation
Echinacea purpurea	**Purple coneflower**	**Native**	**64**	**NVK Nurseries**	**August 25th**	**Good**
Eutrochium maculatum	Spotted joe-pye weed	Native	168	NVK Nurseries	June 21st	Aphid infestation
Helianthus annuus	Common sunflower	Native	35	Seed	June 6th	Too tall for enclosures
Helianthus tuberosus	Jerusalem artichoke	Native	35	Field transplant	July 12th	Too few plants of good condition
Heliopsis helianthoides	**Ox-eye sunflower**	**Native**	**0**	**Agriculture & Agri-Food Canada**	**July 5th**	**Good**
Lythrum salicaria	Purple loose-strife	Non-native	45	Field transplant	June 27th	*Gallerucella* infestation
Rudbeckia hirta	**Black-eyed Susan**	**Native**	**28**	**NVK Nurseries**	**June 21st**	**Powdery mildew**
Solidago altissima	**Tall goldenrod**	**Native**	**161**	**Field transplant**	**June 27th**	**Good**

Symphyotrichum ericoides	**White heath aster**	**Native**	**185**	**NVK Nurseries**	**June 21st**	**Good**
Symphyotrichum novae-angliae	New England aster	Native	185	Field transplant	June 10th	Aphid infestation
Trifolium pratense	Red clover	Non-native	178	Field transplant	June 22nd	Flowers senesced before experiment

Table S2: Number of individual butterflies in each statistical test for greenhouse and field analyses. Comparison denotes the key variable of interest evaluated by the model, with the number of butterflies analyzed per group presented for models of (a) wet mass and (b) body composition (fat, lean, and water mass). The effect of plant species was compared (1) within the greenhouse and (3) within the field. Finally, a single plant species (*S. altissima*) was used in both locations to compare butterfly fitness in (2) the greenhouse versus the field.

		Wet Mass		**Body composition**
Comparison	**Group**	**# Individuals**	**# Measurements**	**# Individuals**
(1) Greenhouse plant species	*Buddleja davidii*	5	16	5
	Echinacea purpurea	10	40	9
	Heliopsis helianthoides	9	28	7
	Rudbeckia hirta	4	15	4
	Solidago altissima	14	50	13
	Symphyotrichum ericoides	13	47	8
	Total:	**55**	**196**	**46**
(2) Experimental location	Greenhouse	14	50	13
	Field	13	52	10
	Total:	**27**	**102**	**23**
(3) Field plant species	*Solidago altissima*	8	32	7
	Eutrochium maculatum	9	36	7
	Total:	**17**	**68**	**14**