NECTAR RESOURCE QUALITY OF OAK SAVANNA POLLINATOR HABITATS

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ABSTRACT

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Efforts to restore critically imperiled midwestern oak savanna habitat are commonly guided by the living requirements of the Federally endangered Karner blue butterfly (*Plebejus melissa samuelis*). Studies often correlate butterfly abundance with nectar species abundance; however, the resource and habitat characteristics that determine population persistence are poorly understood. We quantified the floral abundance, nectar volume and sugar concentration for twenty-two species and calculated their average nectar availability per stem. Species average nectar volume and sugar concentrations per flower were measured along with environmental variables to examine sources of nectar variation. Species-specific average nectar quality estimates were subsequently combined with previously determined stem density estimates (Walsh, 2017) in oak savanna habitats associated with Karner blue butterfly conservation. Vegetation surveys were conducted once in the spring and summer to assess patterns of potential nectar resource availability over time.

This study examined how seasonal nectar availability influenced habitat quality for nectar consuming pollinators within oak savanna habitats. We found that species identity reliably predicted nectar volume and sugar concentrations with marginal variation from relative humidity and canopy cover. Species average nectar characteristics ranged between 0.02 - 2.20 μL and 3.06 - 61.26% Brix per flower. Combining nectar sugar concentration per flower with floral availability allowed us to estimate a species' nectar sugar contribution to a landscape. *Rubus flagellaris* and *Ceanothus americanus* contributed the most nectar sugar per stem in the spring and summer, respectively. The application of nectar quality data to vegetation surveys of 15 sites

identified differences between site nectar sugar availability not previously detected by flowering stem density. Further investigation demonstrated sites associated with natural Karner blue butterfly occupancy had more nectar sugar available in the spring than previous release locations no longer occupied. We show it is essential to assess the nectar resources available during both spring and summer to fully quantify the resource dynamics between seasons. These results can be used to improve understanding of the seasonal distribution and abundance of oak savanna nectar resources to aid future habitat restoration planning and conservation efforts for pollinators of this critically imperiled habitat.

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INTRODUCTION

Oak savanna habitats historically covered a substantial portion of the Midwestern United States. Characterized by sparsely dispersed mature oak trees with an understory of grasses and forbs, these ecosystems are predominately maintained through fire and grazing disturbances (Olson, 1996; Sankaran *et al*., 2004; Anderson *et al.*, 2007). Since European settlement, oak savannas have become highly fragmented due to fire suppression, agriculture, and urbanization (Nuzzo, 1986; Grossmann & Mlandenoff, 2007) causing severe decline in the diversity and abundance of native wildlife populations (Swengel & Swengel, 1999; Kocher & Williams, 2000; Meehan *et al.*, 2013; Archer *et al*., 2014). With approximately 0.02% of the historic range intact (Nuzzo, 1986) Midwest oak savannas are classified as a critically imperiled habitat in the United States (Noss *et al.*, 1995). Oak savannas also preserve high levels of biodiversity relative to neighboring habitats (Leach & Givnish, 1999), making them an important focus for conservation efforts.

Common habitat restoration practices for oak savanna remnants include prescribed burning, planting native savanna species, and selective removal of woody species. Historical records are a valuable tool to direct restoration management for any habitat type (Landres *et al.*, 1999; Swetnam *et al.*, 1999). Unfortunately, it is uncommon for Midwest oak savannas to have significant records of ecosystem features (Brudvig & Asborjornsen, 2007), limiting our understanding of expected community composition. Inherent spatial variation in habitat composition and structure is caused by historical dependence on fire disturbances, which increases the difficulty of defining management goals (Asbjornsen *et al.*, 2005; Anderson *et al.*, 2007). To gain insight on appropriate habitat conditions, management can be guided by the living requirements of an indicator species that requires high-quality habitat to survive.

Frequently extirpated following habitat degradation, the reestablishment of a previously persisting indicator species is a good sign of a successful ecological restoration (Chan & Packer, 2006).

A commonly used indicator species for oak savanna habitats is the Karner blue butterfly (*Plebejus melissa samuelis*) (Shuey, 1997; Chan & Packer, 2006). The Karner blue is federally endangered (U.S. Fish and Wildlife Service, 1992) and can be found scattered throughout the Midwest oak savanna range through six states from Minnesota to New Hampshire (Haack, 1993; USFW, 2003, 2011). Karner blue butterflies thrive in disturbed and semi-open oak savannas (USFW, 2003) that contain its exclusive larval host plant Wild lupine (*Lupinus perennis)* (Opler & Malilul, 1998). Habitat with a combination of sun and shade benefits the Karner blue by supporting an optimal quality of Wild lupine (Herms, 1996; Grundel *et al.*, 1998a; Walsh, 2017) and providing various foraging environments for adult males and females (Grundel *et al.*, 1998b). As a bivoltine insect the Karner blue annually produces two generations. The first generation will emerge in April from eggs that were laid in the previous summer. Karner blue larvae then feed exclusively on Wild lupine leaves until pupating into adult butterflies in early May and June. These adults will feed on nectar, mate, and oviposit eggs that will emerge as second-generation adults in July and August (Grundel *et al*., 2000). To assess the flowering resources in oak savanna habitats we compared the quality of nectar available between sites categorized by Karner blue butterfly occupancy.

Karner blue butterflies have a wingspan of 2.7 cm and can move several hundred meters within a week (King, 2003), allowing for mobility between populations only if relatively close together. Due to regional extirpation, the Karner blue butterfly cannot naturally recolonize most locations (Brown & Bedford, 1997; Lundholm & Simser, 1999; Nienhuis *et al.*, 2002) therefore

human assisted reintroduction is common. The success of Karner blue butterfly reintroduction depends on the availability of suitable habitat, the characteristics of which are not fully understood (Pickens & Root, 2008). Studies often correlate butterfly abundance with host-plant abundance (Fred & Brommer, 2003), nectar species abundance (Schultz & Dlugosch, 1999; Holl, 1995), and the area of the habitat (Moilaen & Hanski, 1998; Bergman & Kindvall, 2004). However, it has been acknowledged that more detailed factors affect the habitat quality for butterfly species (Moilanen & Hanski, 1998; Schultz & Dlugosch, 1999; Ellis, 2003; Fred & Brommer, 2003). Walsh (2017) assessed the biotic and abiotic factors impacting the persistence of Karner blue butterfly populations following a drought event in 2012. This study reported that sites continuously occupied by Karner blue butterflies are associated with increased density of lupine plants, increased symbiotic ant mound entrances, and intermediate heat load and canopy cover levels when compared to sites no longer occupied. No difference was found in nectar plant density between sites occupied and no longer occupied. In most cases, fluctuating abiotic factors like weather can drive population trends while resource availability often limits population size (Begon *et al.* 1996; Roulston & Goodell, 2011; Hicks *et al*., 2016).

Vegetation surveys evaluating the flowering plants available to pollinators commonly measure the density of flowering stems within a management unit (Williams, 1988; Chan & Packer, 2006; Walsh, 2017). Estimating resource availability by plant density, or even individual flower numbers, can lead to errors due to varying species nectar content (Schultz & Dlugosch, 1999). Schultz and Dlugosch (1999) worked to more accurately estimate the adult resources available to populations of Fender's blue butterfly (*Icarioides fenderi*). They concluded the abundance of individual flowers did not predict butterfly abundance but the abundance of nectar sugar from all native species present was associated with population size. Fender's blue

butterflies are known to preferentially nectar on native flowers over non-native flowers (Wilson *et al.*, 1997), further supporting Schultz and Dlugosch's findings. Categorized as an opportunistic forager, the Karner blue butterfly feeds on the nectar of almost any flowering plant available (Savignano & Zaremba, 1993) but also observed frequently selecting species with yellow or white flowers (Grundel *et al.*, 2000). Many habitat assessments do not consider the nutritional quality of flower nectar when categorizing the resources utilized. Flowering species community composition and a more detailed understanding of resources available could reveal stronger associations with the abundance of nectar feeding pollinators.

Nectar is mostly composed of water but also contains nutritional compounds, such as sugars, lipids, and amino acids (Cahenzli & Erhardt, 2012a; Nicolson & Thornburg, 2007; Willmer, 2011). Different plant species can vary in the concentration and composition of nectar sugars, but sucrose, fructose, and glucose are the most common (Baker & Baker, 1975; Dafni, 1992; Kearns & Inouye, 1993; Nicolson & Thornburg, 2007). Plants producing nectar high in sucrose concentration, in comparison to glucose or fructose, are broadly correlated with butterflies, moths, long-tongued bees, and hummingbirds (Baker & Baker, 1982, 1983). Nectar is derived from photosynthesis and therefore nectar composition varies depending on a plant's exposure to light, water, and temperature (Freeman & Head, 1990; Pacini *et al.*, 2003). In both field and laboratory conditions nectar sugar concentrations decline as temperature increases but are unaffected by water stress (Freeman & Head, 1990; Villarreal & Freeman, 1990). However, water stress typically results in fewer and/or smaller flowers produced (Plowright, 1981; Cresswell & Galen, 1991). Humidity conditions can also account for influences on nectar secretory changes due to volume equilibration with air moisture (Willmer, 2011). Nectar chemistry and production characteristics are not yet fully understood as genetic traits,

nevertheless, they are thought to have strong genetic components (Kearns & Inouye, 1993). As plants age the changes observed in sugar proportions tend to be minor compared distinct species differences observed (Nicolson & van Wyk, 1998). This suggests that even with variations in nectar production due to environmental differences or plant age, relative uniformity of nectar composition within a species is expected (Nicolson & Thornburg, 2007).

Nectar is primarily made of water (35-85%; Seeley, 2009) and is often utilized by individual bees and colonies to meet their water intakes needs (Ostwald *et al.*, 2016). Bumblebees and honeybees assess the profitability of standing crop by nectar volume and greater volumes can support foraging activity (Heinrich, 1976; Silva *et al.*, 2004). While bees typically prefer nectar with high sugar concentrations (35-65% Brix) individuals have been observed storing dilute nectar in a swollen crop (Park, 1923). To reduce body temperatures bees will evaporate regurgitated dilute crop contents onto the proboscis (Heinrich, 1980; Nicolson, 2009). Honeybee colonies employ similar mechanisms by evaporating large volumes of water from stored nectar to buffer against hive overheating (Lindauer, 1955; Nicolson, 2009). Nectar volume plays a meaningful role in foraging behavior and the health of a local bee colony. The abundance of nectar feeders within an ecosystem can be inferred from the measure of nectar volume and sugar concentration flowering resources provide (Roubik, 1989).

The quality of resources available to adult butterflies is important to replenishing larval reserves as they decline with time (Boggs, 2009). When fed nectar with low sugar concentrations (5%) adult butterflies preferentially consumed sugar-rich nectar (30%) when possible to compensate for nutrient deficiencies (Cahenzli & Erhardt, 2012b). Butterflies not only display behavioral preferences for sugar but also exhibit quantitative improvements in reproduction. Female butterflies fed nectar containing 20% sugar increased body weight maintenance and total

egg production in later oviposition (Murphy *et al.*, 1983). Male butterflies consuming sugar rich diets also benefit from fitness increases by producing more nourishing spermatophores resulting in greater larval hatching mass (Murphy *et al.*, 1983). Overall, butterflies consuming adequate quantities of sugar often experience improved fecundity, longevity, and increased fitness over a lifetime (Hill & Pierce, 1989; Mevi-Schutz & Erhardt, 2005; Bauerfeind & Fischer, 2009; Cahenzli & Erhardt, 2012a). The reproductive benefits butterflies receive from sugar-rich floral nectar further supports the co-evolutionary relationship between butterflies and flowers dependent on butterfly pollination (Cahenzli & Erhardt, 2012a). Nectar quality is an important resource to assess for butterfly conservation due to clear benefits to population dynamics through increased fitness.

Successful reintroduction of the Karner blue butterfly into restored oak savanna habitats would indicate critical community features have been reestablished. For most North American pollinator species, long-term population data are lacking, and knowledge of their basic ecology is incomplete (National Research Council, 2007). Continued efforts for Karner blue butterfly conservation can improve habitat management and provide simultaneous protection for a variety of oak savanna pollinators (Shuey, 1997; Rodger, 1998; Roberge & Angelstam, 2004). Conservation planning focused on nectar resources can benefit other oak savanna Lepidopteran species of concern such as the Dusted skipper (*Atryonopsis hianna*), Frosted elfin (I*ncisalia irus*), and Persius dusky wing (*Erynnis persius*) (Ohio Department of Natural Resources, 2019). The sugar content of nectar resources has shown to be beneficial to butterfly populations and also has a direct effect on the fitness of social bees (Brodschneider & Crailsheim, 2010; Vaudo *et al*., 2015; Vaudo *et al.*, 2016), and likely solitary bees to some extent (Pamminger *et al*., 2019).

Even though relatively few plants rely on a single pollinator species, the loss of a species lowers the redundancy, or security, of pollination services in an ecosystem (NRC, 2007).

The aim of this study is to provide a better understanding of how nectar resource distribution in space and time may influence habitat quality for Lepidoptera and other nectar consuming pollinators within oak savanna habitats. Our approach was to couple nectar quality characteristics and floral abundance data for twenty-two oak savanna forbs with existing nectar plant density data in oak savanna habitats. Our goal was to examine whether sites categorized by Karner blue butterfly occupancy differ in nectar resource quality due to species-specific variation in relative abundance and floral nectar traits. Specifically, we focused on the following questions: 1) How do environmental factors affect nectar composition, and can nectar quality be reliably associated with species identity? 2) Which species contributes the most hydration and sugar potential when considering variation in floral abundance, nectar volume and sugar availability per stem? and 3) How does the quality of habitat nectar resources (sugar density and total per site) vary among sites that differ in Karner blue occupancy or change between spring and summer seasons? To answer these questions, we quantified species nectar and floral characteristics, which were subsequently combined with previously determined flowering stem density estimates (Walsh, 2017) in oak savanna habitats associated with Karner blue butterfly conservation. Vegetation surveys were conducted once in the spring and summer to assess patterns of potential nectar resource availability over time. Species average nectar volume and sugar concentrations were measured along with environmental variables to examine sources of variation in nectar quality within and among a subset of sites. Nine species were raised in greenhouse conditions to improve nectar collection sample sizes because frequent rainfall events limited field measurements. Understanding these characteristics and temporal variations in nectar

resources will aid habitat restoration planning and benefit conservation efforts for nectar feeding pollinators of this critically imperiled habitat.

MATERIALS AND METHODS

Nectar Species Selection

To select oak savanna species for nectar characterization, we utilized recent vegetation surveys of fifteen oak savanna habitats conducted by Walsh (2017) within the Oak Openings Region of northwest Ohio (n = 8) and the Allegan State Game Area in western Michigan (n = 7). Because this vegetation survey was designed to better understand habitat characteristics conducive to Karner blue butterfly survival, sites had been chosen based on Karner blue presence (1) Currently Occupied; before and after a drought in 2012, (2) Formerly Occupied; no longer occupied after the drought, (3) Previous Release; previous release sites no longer occupied, and (4) Priority Restoration; high priority restoration sites for future releases (Table 1). All sites formerly occupied by the Karner blue were naturally occurring populations and not reintroduced by managers. Priority restoration sites were not associated with Karner blue occupancy but were assessed for oak savanna health and potential to become a future release location. One 25 m transect was randomly placed for every 850 m^2 of habitat. Surveys recorded spring (late May/early June) and summer (early July) density of flowering plants defined as the number of flowering stems in the ground per m^2.

Twenty-two flowering species frequently appearing across sites and at a greater density were selected from the survey data for characterization of floral availability and nectar quality (Table 2). Species from the vegetation survey with previously determined nectar composition (*Asclepias tuberosa, Coreopsis lanceolata, Helianthus divaricatus, Lespedeza capitata, Liatris aspera, Monarda fistulosa, Monarda punctata*) (Arnold & Michaels, 2017) were further characterized for floral availability (described below). Twenty-five species from the survey were not characterized for floral and nectar resources due to low densities across few sites. Plants

recorded within the Walsh (2017) survey as 'Unknown' or not identified to species were excluded. Records of *Hieracium* spp. were an exception to this rule because of its presence in nine of the fifteen sites at a relatively high frequency (1-370 stems). *Hieracium scabrum* (Rough Hawkweed) was chosen as a representative for all survey recordings of *Hieracium* spp. As an alternative native species, *Hieracium scabrum* is found is prairie savannas, open woodlands, and clearings with a recorded presence throughout Allegan State Game Area, Michigan and the Oak Openings Region of Ohio (Voss, 1996; U.S. Department of Agriculture, 2019). To improve nectar sample sizes *Hieracium scabrum* plants were also grown to flowering from seeds in the BGSU greenhouse, thanks to donation by the Toledo Metroparks, Blue Creek Seed Nursery.

To evaluate whether species chosen for further study were representative of the standing nectar crop, we assessed the proportion of plants characterized for floral and nectar attributes within each site for each season (Table 3). Using the twenty-two species selected, six of the fifteen sites had greater than 90% of stems characterized for both spring and summer surveys. An additional three sites had 70-90% of the stems characterized within each season. For the remaining six sites, at least one season had less than 60% of stems present characterized. This was likely due to a combination of unknown species identifications, sites having high species richness, and/or species occurring only within a single site at a high density. For example, the spring R3 survey (55.56% characterized, Table 3) was primarily made up of *Chamaecrista fasciculata* (42%) which was not recorded in any other site. The spring R1 survey (40.00% characterized, Table 3) had the greatest species richness (n = 20) with six species characterized, five only occurring in the R1 survey, and four unknowns.

Nectar Sampling

Floral abundance and nectar data were collected between May and August in 2018 and 2019 from sites in the Oak Openings Region of Toledo, Ohio. Environmental factors were recorded at the time of flower collection to measure their potential effects on nectar production and impacts of site characteristics. Time and monetary constraints prevented data collection from Allegan State Game Area, Michigan sites. Pamminger *et al*. (2019) reanalyzed data from Simidtschiev (1988) depicting nectar quality of fifty-two sunflower varieties (*Helianthus annuus*) grown for five years in sites 300 kilometers/186.41 miles apart and concluded a more limited effects on nectar quality than previously thought. Oak Openings Region survey sites in Toledo, Ohio are comparably separated from Allegan State Game Area, Michigan (approximately 298 kilometers/185 miles southeast) and in similar USDA Hardiness zones (U.S. Department of Agriculture, 2020). Regional differences in nectar quality are therefore expected to be minimal.

Beginning at a random GPS coordinate within the property boundary, we placed a 25 m transect through the nearest patch of selected flowering species. Plants were chosen for nectar sampling along the 25 m transect and within 3 m laterally. We sampled from each transect only once with GPS coordinates recorded at the beginning and end. Sampled plants of the same species were a minimum of 0.5 m apart. Species too rare to appear in these transects were sampled when encountered and recorded with a GPS coordinate. To prevent possible contamination or removal of floral resources by visitors (Wyatt *et al.*, 1992), unopened or newly opened flowers were bagged with bridal-mesh netting. The netting was removed for sampling within 24-48 hours or when the flower had opened. If a rainfall event occurred, sampling was delayed 24-hours to ensure that nectar was not washed out of the flower.

Environmental factors recorded at the time of collection included temperature, relative humidity, soil moisture, and canopy cover. Local temperature and relative humidity were measured with a psychrometer (Mengshen Digital Thermometer & Hygrometer, M350). We used a time-domain reflectometer (Spectrum Technologies, Inc., Field Scout TDR 300) to document the soil moisture at the base of each plant. The percent of canopy cover was a proxy measurement for direct sunlight and measured by taking a picture of the canopy above each plant within the mobile application HabitApp (Mobile application, HabitApp v. 1.1, Scrufster). To ensure nectar sampling attempts were not compromised by rainfall events, we grew several species in the greenhouse at Bowling Green State University (*Achillea millefolium, Ceanothus americanus, Coreopsis lanceolata*, *Dianthus armeria, Fragaria virginiana, Hieracium scabrum, Hypericum perforatum, Rosa carolina,* and *Rubus flagellaris)* (Table 4). To induce flowering, we gave all plants a sixty-day vernalization treatment in a growth chamber (8 hr photoperiod, 10º C) and then returned them to the greenhouse to grow until flowers could be sampled. Plants were not provided supplemental lighting following vernalization and watered as needed.

We collected nectar with microcapillary tubes when flowers were large enough. For small flowers a centrifuge technique was used to remove nectar without damaging the floral tissue (Arnold & Michaels, 2017). A single inflorescence was collected and stored in a cooler on ice (Bertsch, 1983) until the entire transect was collected and the samples could be processed on-site. A microcentrifuge (HF120 NanoFuge with 6 × 1.5-mL rotor; Tomy Seiko Co., Tokyo, Japan) was field-powered using a car inverter (EverStart plus 400W, 120W cigarette plug). Each inflorescence was placed facedown into a 1.5 mL Eppendorf tube partially filled with glass wool (0.15 g ± 0.05), leaving 5 mm of space in the bottom. To increase the probability of detecting

trace amounts of nectar the maximum number of flowers that could fit in a single layer were placed face-down within the Eppendorf tube.

Centrifuge spinning duration was tested to verify method procedures. Five microliters of a 20% Brix sucrose solution were centrifuged for one minute (n = 20) and two minutes (n = 20). Samples were spun at 6,000 rpm for 2 minutes, which pulled nectar from the flowers to the bottom of the Eppendorf tube and collected pollen in the glass wool to prevent contamination of nectar composition (Nicolson & Thornburg, 2007). The number of open flowers was counted before centrifuging to allow calculation of the volume of nectar produced per flower. The glass wool was removed, and the Eppendorf tube was placed into another cooler with ice for transport to the Bowling Green State University lab. Total nectar volume was measured with a 2 µL (±0.05-µL accuracy) or 10 µL micropipette (±0.5-µL accuracy) depending on estimated sample volume. Sugar concentrations were estimated using a handheld refractometer (Bellingham + Stanley, model 45-81, 0–50% Brix, low volume) calibrated to grams of sucrose per 100 grams of solution (Brix) (Bolten *et al.*, 1979). Nectar samples exceeding 50% Brix were diluted by 50% using distilled water (Kearns and Inouye, 1993).

Species Floral Availability

Floral availability is defined here as the number of flowers per stem accessible to a pollinator at any given time of encounter. When a species was encountered in the field the total number of open flowers per stem in the ground was recorded. To remain efficient data collecting procedures varied depending on the complexity of a species' flowering arrangement. For species that presented relatively few open flowers at a time, 1-30 flowers, the total number of flowers per stem was counted (*Comandra umbellata, Dianthus armeria, Fragaria virginiana, Hypericum perforatum, Lithospermum canescens, Potentilla simplex, Rosa carolina, Rubus flagellaris*). For

species with greater floral availability, complicated floral arrangements, and/or many florets in a capitulum, as in the Asteraceae, (*Achillea millefolium, Asclepias tuberosa, Baptisia tinctoria, Coreopsis lanceolata, Ceanothus americanus, Euphorbia corollata, Hieracium scabrum, Helianthus divaricatus, Krigia virginica, Lespedeza capitata, Liatris aspera, Monarda fistulosa, Monarda punctata, Rudbeckia hirta*), flowers were counted for the total number of branches, the number of inflorescences available on three branches, and/or the number of open flowers available on three inflorescences present on the stem. Data collected throughout the season was averaged to estimate the number of flowers likely available to visiting pollinators.

Nectar Quality Analysis

To evaluate potential impacts on resources for nectar feeding pollinators within the spring and summer vegetation surveys, we determined nectar volume (µL) and sugar concentration (Brix) per flower for each species. Nectar sugar content was converted from Brix to mg of sugar using the equation $s = 10dvC$ where d = density of sucrose in a sucrose solution at concentration C (g sucrose per 100 g solution) and v = volume of nectar (ml). The density of sucrose was calculated as $d = 0.0037921C + 0.0000178C^2 + 0.9988603$ (Bolten *et al*., 1979; Hicks *et al*., 2016). For each species, the average nectar volume (µL) and sugar (mg) per flower were multiplied by the average number of flowers available per stem. Species-specific nectar volume and sugar availability per stem were compared to determine potential sugar provisions of each species for effective pollinator conservation.

Species-specific sugar per stem estimates were applied directly to the number of stems recorded within the Walsh (2017) spring and summer vegetation surveys to estimate quadrat level nectar resources. Plants not characterized for nectar quality, not identified to species, or recorded as ‘Unknown’ were not included in determining average quadrat nectar content. All

non-flowering quadrats were recorded as providing zero resources. The total number of quadrats surveyed within a site was used as a proxy measurement for site size because one transects was randomly placed for every 850 m^2 of habitat. Examining resources per quadrat allowed us to evaluate resources at the scale nectar-feeders would encounter them in the field and provide insight on foraging behavior.

Average stem level resources for each species was applied to the number of stems present in the entire survey area of each site. Site total nectar quality was estimated by summing the nectar volume and sugar contribution of all species. The following equations summarize how site total nectar volume and sugar availability were estimated during each season:

$$TV_a = \Sigma_{i=1}(V_i \times N_i) \qquad TS_a = \Sigma_{i=1}(S_i \times N_i)$$

where TV_a/TS_a = total volume (μL)/sugar (mg) within site *a*, V_i/S_i = average volume/sugar available per stem for species *i*, N_i = number of stems within site *a* corresponding to species *i*. The total number of quadrats surveyed was incorporated into modeling to compare total resources available amoung sites. Similar upscaling of flower level nectar quality measures to the landscape level has been conducted by Schultz and Dlugosch (1999), Hicks *et al*. (2016), and Pamminger *et al*. (2019).

Statistical Analysis

Unless otherwise noted, all analyses were performed using JMP (JMP®, Student Edition 14, SAS Institute Inc., Cary, NC, 2019). Residual plots and normal quantile plots of Log10 transformed data were used to assess and improve fit to assumptions of normally distributed errors and equal variance. Analyses of environmental effects on nectar composition of Ohio field samples was completed for thirteen of twenty-two species (excluding seven species previously

characterized by Arnold and Michaels (2017) that lacked corresponding environmental data). We used non-parametric Spearman's correlation to explore underlying relationships during preliminary analyses of environmental variables and nectar quality (Table 5). Across all nectar samples, volume and sugar concentration per flower were not significantly correlated (Spearman's $r_s = 0.009$, $p = 0.87$, Table 5), therefore analyses were conducted separately. A generalized linear model (GLM) was used to evaluate the relationship of environmental factors (canopy cover, relative humidity, temperature, soil moisture) and dependent variables (nectar volume and sugar). To determine which environmental factors were most related to nectar volume and sugar concentration, we created a set of potential models with various combinations of predictors and compared them using AICc. For each response, we identified the model with lowest AICc value with a difference greater than two units from others. To evaluate how nectar volume (μL) and sugar availability (mg) per plant may be affected by environmental factors, we also used combinations of environmental predictors to compare models predicting species-specific nectar quality per stem based on the nectar volume (μL) and sugar concentration (Brix) per flower and their estimated floral abundance. We additionally compared the quality of nectar samples between our study vs. Arnold and Michaels (2017) as well as sampling locations (field vs. greenhouse) to confirm consistent sampling methods.

Flowering community composition was compared between site categories (currently occupied, formerly occupied, previous release, or priority restoration) using non-metric multidimensional scaling (NMDS) using the 'vegan' package from R version 3.4.3. (Oksanen *et al.*, 2018). NMDS is an indirect gradient analysis that creates ordination plots based on a dissimilarity matrix. Sites with more similar flowering community composition would be expected to be oriented closer together. Ordination plots for spring and summer vegetation

surveys were determined by the average density of all flowering species recorded. We used permutational multivariate analysis of variance (PERMANOVA) to test for significant differences in community composition between site categories within each season.

All twenty-two characterized species were used to assess quadrat and site level resources. When species-specific nectar volume (μL) and sugar availability (mg) per stem were applied directly to number of stems recorded within vegetation surveys, a strong correlation was observed between total nectar volume and sugar availability across sites (Pearson's $r = 0.98$, $p < 0.0001$, Figure 1). Therefore, sites were assessed for nectar volume and sugar resources but due to this strong correlation only analyses for sugar are reported. Corresponding nectar volume analyses can be found within the modeling tables.

To assess the effects of site category and season on the availability sugar (mg) per 0.5 m^2 quadrat and on the total sugar availability within site level, we used linear mixed models with site as a random effect. The best models were determined by p-value with significance of the model and each fixed effect established through Kenward-Roger approximation. We excluded any quadrats for which < 90% of flowering stems had been characterized to minimize potential measurement error. A simple alternative method for zero modified lognormal distribution was not available. Category and seasonal availability of flowering stems and sugar per quadrat were compared between site categories and seasons using Tukey HSD all pairs comparisons.

RESULTS

Nectar Variation

A low rate of nectar removal occurred across species grown in both field and greenhouse conditions (Table 6). No significant difference was found in centrifugate volume or sugar concentration between the spin durations (1 minute: $X^2 = 0.07$, DF = 1, p = 0.79; 2 minutes: $X^2 = 0.75$, DF = 1, p = 0.39; Table 7). Attempted samples that provided zero nectar did not contribute to species-specific nectar quality. With no immediate explanation for low rates of nectar removal, all flower samples that yielded measurable volumes were used to provide the species average nectar quality per flower.

Nectar volume and sugar concentration per flower were best predicted by species identity, relative humidity, and canopy cover ($F_{14,77} = 23.75$, $p < 0.0001$, Adj $R^2 = 0.78$, AICc = 128.47, Table 8a; $F_{14,75} = 8.03$, $p < 0.0001$, Adj $R^2 = 0.52$, AICc = 93.75, Table 8b). The inclusion of site as a predictor variable did not improve either model's AICc value, suggesting site-specific environmental effects not recorded were a negligible factor influencing nectar quality per flower. Species identity was the most significant predictor of both nectar volume and sugar concentration per flower ($p < 0.0001$, LogW = 21.59, Table 8a; $p < 0.0001$, LogW = 7.57, Table 8b). Consistent with expectations, an increase in relative humidity was associated with increased nectar volume, likely due to reduced nectar evaporation (LogW = 4.74, $p < 0.0001$, Table 8a). Greater relative humidity was also associated with slightly decreased sugar concentration, which would be expected due to dilution by an increase of volume (p = 0.009, Table 8b). Sugar concentration decreased weakly as canopy cover increased (p = 0.0008, Table 8b).

To assess potential maternal environmental effects on nectar composition, samples collected from field sites and the Bowling Green State University greenhouse were compared using Wilcoxon Kruskal-Wallis testing (Table 9). *Rubus flagellaris* samples collected from the field and greenhouse had similar nectar volumes (X^2 = 1.59, DF = 1, p = 0.21). However, sugar concentration was significantly different between *R. flagellaris* collection sources (X^2 = 8.80, DF = 1, p = 0.003). An increase in nectar sugar concentration is expected due to a lack of canopy cover and increased temperature or humidity within the greenhouse setting. Field locations with 0% canopy ranged between 6 – 50% Brix while greenhouse samples ranged between 23 – 96% Brix. Samples collected for *R. flagellaris* (n = 52) had no outliers present within the data; therefore, greenhouse samples were included in the species nectar analyses.

Coreopsis lanceolata samples collected from the field (n = 5) and greenhouse (n = 9) did not show significant differences in volume or sugar concentration (X^2 = 1.00, DF = 1, p = 0.32; X^2 = 3.39, DF = 1, p = 0.06; Table 9). *C. lanceolata* was one of seven species in this study previously characterized for nectar quality by Arnold and Michaels (2017). All samples collected for this study of *C. lanceolata* (n = 14) were compared to those collected in the field by Arnold (n = 5) to ensure consistent collection methods and compare potential maternal environmental effects (Table 10). No significant difference was found between study samples in volume nor sugar concentration (X^2 = 0.27, DF = 1, p = 0.60; X^2 = 0.002, DF = 1, p = 0.96) supporting the comparison of new nectar data with those by Arnold.

The nectar quality per flower was incorporated with floral display size (species average number of open flowers per stem) to analyze predictors of nectar volume (µL) and sugar availability (mg) on a per stem basis. As expected, we found similar results when extrapolating nectar quality to the stem level (Volume: $F_{14,77}$ = 8.98, p < 0.0001, Adj R^2 = 0.55, AICc =

128.47, Table 11a; Sugar: $F_{14,75} = 18.09$, $p < 0.0001$, Adj $R^2 = 0.72$, AICc = 146.58, Table 11b). Linear regression models confirmed that species identity remained the most important predictor of nectar volume and sugar availability relative to other environmental factors ($p < 0.0001$, LogW = 8.17, Table 11a; $p < 0.0001$, LogW = 18.19, Table 11b). Nectar volume per stem remained significantly influenced by relative humidity ($p < 0.0001$, LogW = 4.74) but not canopy cover ($p = 0.15$, Table 11a). Sugar per stem was significantly affected by canopy cover ($p = 0.05$, Table 11b). Some variables with non-significant parameter effects were retained in the final model as they did improve the fit by lowering the AICc value. Overall, species identity had the most influence in predicting variation in nectar volume and sugar availability per stem with moderate influences of relative humidity and canopy cover.

Species Characterization

On average each flowering species had a sample size of fifteen, ranging from one to fifty-two nectar samples. Average nectar volume ranged from 0.02 - 2.20 µL per flower (Table 12, Figure 2). *Baptisia tinctoria* and *Rubus flagellaris* had the greatest nectar volume per flower (2.20 and 1.83 µL, respectively). The largest volumes were approximately one-hundred times greater than the smallest nectar volumes of *Comandra umbellata* and *Dianthus armeria* (0.02 and 0.38 µL, respectively). Species average sugar concentration ranged from 3.06 - 61.26% Brix (Table 12, Figure 3). *Monarda punctata a*nd *Liatris aspera* had the highest sugar concentrations (61.26 and 59.75% Brix, respectively). These highest sugar concentrations were approximately fifteen times greater than the lower concentrations of *Hypericum perforatum* and *Achillea millefolium* (3.06 and 5.33% Brix, respectively). Because *Comandra umbellata* and *Rosa carolina* each had only one nectar sample successfully collected, their actual variation in nectar composition could not be determined; but these were included in the site assessment in order to

characterize a greater proportion of the community. Nectar removal was consistently unsuccessful for *Krigia virginica* via microcapillary tubes, centrifugation, or paper wicks. Therefore, *Krigia virginica* was recorded as producing no nectar for this study and included in the site assessment accordingly.

Ceanothus americanus is a shrub that on average provided the most flowers per stem (Table 13). Each inflorescence held approximately 120 flowers, adding up to an estimated 3,001 flowers per stem in the ground. When encountered in the field *C. americanus* were typically matured and averaging between 2-3 feet in height. The species providing the second greatest number of flowers was *Rudbeckia hirta* with an estimated 255 flowers per stem. The lowest number of flowers per stem was *Potentilla simplex* which provided one flower on average.

Estimates of nectar volume and sugar concentration per stem in the ground for each species provide insights into nectar resources that are particularly relevant for habitat restoration (Table 14). Due to extraordinarily high floral availability, *C. americanus* provided the most hydration potential and sugar per stem (442.79 μL, Figure 4; 134.20 mg, Figure 5). The species with the second greatest nectar volume per stem was *Baptisia tinctoria* (55.75 μL). *Monarda punctata* provided the next highest sugar concentration per stem (40.84 mg). At the opposite end of the range, eight of the twenty-two species provided < 3 μL of nectar and < 1 mg of sugar per stem in the ground (*Comandra umbellata, Dianthus armeria, Euphorbia corollata, Fragaria virginiana, Helianthus divaricatus, Krigia virginica, Potentilla simplex,* and *Rosa carolina*). When considering species that occurred in the spring vegetation survey, *Rubus flagellaris* provided the highest sugar concentration and most nectar sugar per stem (Table 12, Figure 6).

Site Assessment

Flowering community composition was compared between site categories using non-metric multidimensional scaling (NMDS). NMDS is an indirect gradient analysis that creates ordination plots based on a dissimilarity matrix. All sites had been categorized by Walsh (2017) as either currently occupied by Karner blue butterflies (n = 5), occupied before a drought in 2012 but not after (formerly occupied, n = 3), previous release site no longer occupied (n = 4), or high priority restoration site (n = 3). Ordination plots for spring and summer vegetation surveys were determined by the average density of all flowering species recorded (n = 36 and n = 45, respectively). In the spring, NMDS analysis showed that spring flowering community composition was significantly different between category types (DF = 14, F = 1.88, p = 0.03, Table 15a). Sites currently occupied and formerly occupied by the Karner blue appeared to have more similar spring flowering communities than previous release locations and restoration sites (Figure 7). Interestingly, the occupied site closest in the ordination to another release and restoration site is the only active Karner blue butterfly habitat in the Oak Openings region of Ohio. In the summer, we found a non-significant trend for occupied and formerly occupied sites to be oriented away from release and restoration sites (DF = 14, F = 1.37, p = 0.09, Table 15b, Figure 8).

Walsh (2017) found no significant difference in nectar plant density between sites occupied and formerly occupied by the Karner blue but found that both location categories had greater nectar plant densities than previous release sites. When we analyzed each season independently, there was no difference in the number of flowering stems per 0.5 m^2 quadrat among categories in the spring (Wilcoxon/Kruskal-Wallis test, p = 0.30) but found a significant difference in the summer (p < 0.0001, Table 16). Further analysis of summer floral stem density

using Tukey-Kramer HSD all pairs comparison revealed priority restoration sites had significantly more flowering stems than all other site categories (Table 17). On average, priority restoration sites had fewer empty, non-flowering quadrats in summer than other categories (22%, Table 18). When we evaluated whether there was a significant change in flowering stem abundance between seasons within a category type (Table 19), currently occupied and previous release sites had significantly greater flowering stem density in the spring than summer (Wilcoxon each-pairs comparison, $p < 0.0001$ and $p = 0.003$, respectively). No difference was found between seasonal flowering resources of formerly occupied sites ($p = 0.33$) or priority restoration sites ($p = 0.51$).

Quadrat-level analyses with site as a random effect revealed that sugar availability (mg) per 0.5 m^2 was best predicted by the interaction between category and season ($F = 0.08$, $p < 0.0001$, Adj $R^2 = 0.16$, AICc = 1682.57, Table 20a). The most significant predictive factor for sugar per quadrat was the interaction between category and season ($p < 0.0001$; Figure 9). Parameter estimates showed that spring had less sugar per quadrat available than in the summer (Spring mean = 20.55 mg (± 1.9), Summer mean = 92.42 mg (± 18.23); $p = 0.03$, Figure 10). Site size did not have a significant influence on sugar availability per quadrat ($p = 0.21$).

Similar models of the total sugar present within a site to provide insight into landscape-level patterns of total resources between categories (Table 21). Due to the small numbers of replicates for sites within each category (n = 3-5) and the incomplete assessment of nectar species, these site level models cannot provide robust conclusions. Total nectar sugar (g) within a site was best predicted by the interaction between category and season ($F_{7,22} = 3.08$, $p = 0.005$, Adj $R^2 = 0.33$, AICc = 83.48, Table 22a). Model parameter estimates showed that occupied sites contained the greatest spring total sugar available across categories.

Comparisons of the number of flowering stems and nectar sugar (mg) availability per quadrat within site categories (Table 23) found that sites currently occupied by the Karner blue butterfly had the strongest correlation between flowering stems and sugar per quadrat (Spearman's correlation $r_s = 0.75$, $p < 0.0001$). Formerly occupied and priority restoration sites expressed moderate correlations between flowering stem density and resource availability ($r_s = 0.68$, $p < 0.0001$ and $r_s = 0.64$, $p = < 0.0001$, respectively). Previous release sites no longer occupied showed a moderate correlation between stem and sugar resources but was the weakest of all the categories ($r_s = 0.48$, $p < 0.0001$). This varying degree of association between flowering stems density and estimated sugar availability indicates extraneous factors such as habitat management, species-specific floral abundance or nectar quality could impact the accuracy of nectar resource assessment via flowering stem density.

The most important predictive factor for sugar per quadrat was the interaction between category and season ($p < 0.0001$, Table 20a). Therefore, we used post-hoc testing to further analyze how resources vary among occupancy categories within each season using Tukey-Kramer HSD all pairs testing (Table 24). In the spring sample population, previous release sites provided fewer resources than occupied and formerly occupied sites for flowering stem availability (Release mean = 4.69 stems ± 0.52, Occupied mean = 11.26 stems ± 1.73, Former mean = 11.67 stems ± 1.86; $p = 0.0003$ and $p < 0.0001$, respectively) and sugar availability per 0.5 m^2 quadrat (Release mean = 14.03 mg ± 3.07, Occupied mean = 23.69 mg ± 2.66, Former mean = 13.82 mg ± 2.19; $p < 0.0001$ and $p = 0.001$). Due to the skewed distribution of sugar availability within each category the data median was used to compare central tendency (Figure 11). In the spring, sites with a history of Karner blue butterfly occupation (occupied and formerly

occupied) on average provided a median sugar density eleven times greater than release locations or restoration sites (Figure 12).

In the summer sampled communities, it was more apparent that the magnitude of difference between category resources depended on the resource being measured. Among four pairwise category comparisons (occupied/release, occupied/restoration, former/release, and former/restoration) there was no difference in summer stem density, but we found a significant difference among site categories when nectar sugar available was analyzed (Table 24). Occupied and formerly occupied sites had lower sugar availability in the summer compared to release sites ($p < 0.0001$ and $p < 0.0001$, respectively) and restoration sites ($p < 0.0001$ and $p < 0.0001$, respectively). These results indicate that species-specific floral abundance and nectar sugar quality provided an improved assessment of the resources available and revealed association not previously measured.

Category pairwise comparisons suggested that sites related to Karner blue occupation were associated with greater spring sugar availability (Table 24). Therefore, we used further post-hoc testing to assess how the distribution of quadrat scale sugar resources differed among categories within each season. On average, each stem within the spring vegetation survey provided 2.46 mg of sugar. Therefore, we expected each stem to provide at least 2 mg sugar to estimate weather flowering patches (quadrats) provide high or low quantity of nectar sugar. The distribution of spring stem and sugar resources within sampled quadrats in occupied sites were highly similar with relatively even distribution of low, medium, and high quality patches (Figure 13, 42% ≤ 5 stems, 37% between 6-20 stems, 21% > 20 stems; Figure 14, 39% < 10 mg, 35% between 10 - 40 mg, 26% > 40 mg). The success of Karner blue butterfly conservation within occupied sites may be linked to this even spatial distribution of stem and nectar sugar resources

($p < 0.0001$, Table 24). In comparison, population release sites had a greater proportion of low flowering stem and sugar patches (Figure 13, 66% < 5 stems, 32% between 6-20 stems, 2% > 20 stems; Figure 14, 78% < 10 mg, 10% between 10 – 40 mg, and 12% > 40 mg; Table 24). The summer occupied, formerly occupied, and release sites sampled had similar flowering stem availability (Figure 13, Table 24) with a high proportion of low stem densities (≤ 5 stems; occupied = 77%; former = 76%; release = 63%). Applying the minimum expectation of 2 mg of sugar per stem, release sites had a greater proportion of high-quality sugar patches in the summer than occupied or formerly occupied sites (Figure 14; Table 24). While the distribution of stem density estimates would predict occupied, formerly occupied, and release sites to provide similar flowering resources, the application of species-specific nectar sugar revealed finer differences in resource availability.

Spring nectar sugar resources were directly compared between naturally occupied sites and previous release locations to further investigate differences revealed in pairwise and post-hoc testing. All eight sites relevant to Karner blue butterfly occupancy had 70-100% of stems present characterized within the spring survey (Table 3). The median sugar available per quadrat ranged between 0.82 - 24.20 mg of sugar and between 73.32 - 786.82 mg of sugar available sitewide (Table 21). Two of the four sites categorized as previous release locations had > 90% of stems successfully characterized (P2 and P4; Table 3). The median sugar available per quadrat in these locations, respectively, was 0.32 mg and 0.29 mg of sugar with 39.91 mg and 258.05 mg total of sugar in each site (Table 21). P2 and P4 appeared to provide total sugar quantities comparable to sites relevant to Karner blue occupancy but fell short in sugar available within individual quadrats. The previous release location, P1, had 44% of the stems present characterized yet provided a median of 27.62 mg of sugar per quadrat with a site total of 904.66 mg of sugar in the

spring (Table 21). While sugar density and total sugar availability within P1 was comparable to occupied sites, other habitat features could be responsible for the Karner blue disappearance. For example, the average canopy cover over sampling transects in P1 was 5.61% while the only location currently occupied nearby in the Oak Openings had an average canopy cover of 47.34%.

DISCUSSION

Nectar Variation

Our findings are consistent with literature suggesting that species identity can reliably predict nectar volume and sugar concentrations with marginal variation from environmental factors (Kearns & Inouye, 1993; Mitchell, 2004). Over the range of environmental conditions sampled, species was more important than environment. Developing a pollinator friendly habitat frequently incorporates increases in the abundance of nectar species (Holl, 1995; Schultz & Dlugosch, 1999). Demonstrating species-specific nectar traits further supports the application of nectar quality data to vegetation surveys within our study and future habitat assessments. This study found that relative humidity was the environmental factor with the greatest impact on nectar volume and sugar concentration (Farkas *et al*., 2012). Canopy cover influenced sugar concentration and nectar volume to a lesser extent. Understanding environmental impacts on nectar quality can help anticipate foraging challenges pollinators may experience in the face of climate change and aid conservation planning. More immediately, the identity of flowering species within a habitat can be used to inform land managers about the quality of nectar resources available to pollinators within a habitat.

Nectar is a valuable resource that can affect ecological community structure of both plants and animals (Feinsinger, 1978; Brown & Kodric-Brown, 1979; Blüthgen *et al*., 2000; Apple & Feener 2001; Neuhauser *et al*., 2003; Whitham *et al*., 2003). Plants can separately regulate nectar volume and sugar concentration after secretion to achieve a certain degree of homeostasis to ensure pollinator visits (Nicolson, 1995; Nepi & Stpiczynska, 2008). Little investigation has been completed for the heritability of other nectar traits such as concentration of sugars, amino acids, age effects, temporal patterns, taste, or scent (Mitchell, 2004). It is

believed that environmental variation has less effect on these traits than production rates and are therefore more heritable (Pleasants, 1983; Mitchell & Shaw, 1993). But nectar production is a complex process and our study just breaks the surface. Species-specific nectar production can also be influenced by floral age (Farkas *et al*., 2012; Kato & Sakai, 2008), can vary daily, and have different twenty-four hour production patterns (Mohr & Jay, 1990; Burquez & Corbet, 1991; Gilbert *et al*., 1991; Pham-Deleque *et al*., 1990; Stern *et al*., 1996). The longevity of individual flowers could not be assessed in this study due to time constraints and limited field assistance.

Nectar is derived from photosynthesis and therefore nectar composition can vary depending on a plant's exposure to light, water, and temperature (Freeman & Head, 1990; Pacini *et al*., 2003). The amount of light an individual plant received was measured through the percent of overhead tree canopy. Increased canopy slightly decreased floral sugar concentration, likely due to a decrease in photosynthesis and therefore sugar for nectar production (Pacini *et al*., 2003). The volume of nectar present within a flower decreased as local relative humidity decreased because nectar evaporated to equilibrate with the surrounding air moisture (Bertsch, 1983; Wyatt *et al*., 1992; Willmer, 2011). Our data was collected on a relatively small scale but can be used to inform how nectar-feeding pollinators may be impacted by large scale problems, such as climate change. As the global and local climates begin to shift towards higher temperature with more frequent rainfalls, relative humidity is expected to decrease over land (Byrne & O'Gorman, 2018). Theoretically, a decrease in relative humidity would decrease nectar volume and increase nectar viscosity for most flowering species. An increase in viscosity would require pollinators to adapt foraging behaviors to meet new energy balance requirements.

Data analysis limitations were experienced due to relatively small sample sizes. Species-specific responses to environmental factors could not be fully assessed nor nectar quality differences between collection locations. Further work is needed to examine species-specific floral longevity and nectar production responses to other factors in the environment (e.g. nutrients, management practices). Changes in spatial and temporal patterns of nectar volume and sugar concentration have been shown to affect the visitation rate of bees and butterflies (Frankie, 1983; May,1985; Hainsworth & Hamill, 1993; Klinkhamer *et al*., 2001). More detailed characterization of flowering species' nectar resources available within a habitat could meaningfully improve the assessment of resource availability over time (Schultz & Dlugosch, 1999; Hicks *et al.*, 2016).

Species Characterization

Our study characterized twenty-two flowering species for floral abundance, nectar volume and sugar concentration per flower. Species-specific nectar quality can be used to suggest the type of floral visitors that would most benefit. Within the spring vegetation survey *Fragaria virginiana* and *Rubus flagellaris* provide sugar concentrations suitable for butterfly and bee feeding, respectively; in the summer *Ceanothus americanus* and *Monarda punctata* provided concentrations best suited for butterfly and bee feeding, respectively. The inclusion of nectar sugar concentration per flower along with floral availability allowed us to estimate a species' nectar sugar contribution to a landscape. *Rubus flagellaris* and *Ceanothus americanus* contributed the most nectar sugar per stem in the spring and summer, respectively. When restoring or evaluating pollinator habitats land managers can use the results of our study to construct a more quantitative guide to floral resources provided by flowering species in different seasons.

Review of experimental studies indicates that the recommendations regarding the 'optimal concentration' for nectar feeding doesn't incorporate body size, quantity of intake, or species but exclusively depends on the feeding mechanism (Kim *et al*., 2011). Lepidopterans use active suction to pull nectar through a proboscis (Kingsolver & Daniel, 1979; Pivnick & McNeil, 1985) while most bees, and some ants, dip their tongue into more vicious nectar (Kingsolver & Daniel, 1995; Paul & Roces, 2003). Theoretically, butterfly-pollinated flowers generally provide nectar between 20–25% Brix (Nicolson and Thornburg, 2007) and optimal feeding sugar concentrations for social bees is 35-65% Brix (Pamminger, 2019). This optimal feeding range is the suggested nectar concentration for effortless feeding, but pollinators can physically consume nectar outside of this range when necessary and may actively seek variety to balance feeding from non-optimal sources when foraging in a diverse nectar community. Studies have shown that locations with greater nectar resource diversity were more likely to support larger butterfly populations and more diverse bee communities (Williams, 1988; Britten & Riley, 1994; Shultz & Dlogosch, 1999; Potts *et al*., 2004).

When considering species that occurred in the spring vegetation survey, *Rubus flagellaris* and *Lithospermum canescens* provided the highest sugar concentrations (39.80% and 30.33% Brix, respectively) suitable for bee visitors and within the upper range considered optimal for butterflies. The only species that supplied an optimal butterfly-feeding sugar concentration was *Fragaria virginiana* (23.67%). In the summer vegetation survey, *Monarda punctata a*nd *Liatris aspera* had the highest sugar concentrations sutiable for bee visitors (61.26% and 59.75% Brix, respectively). *Ceanothus americanus* (22.44%) was the only summer species that produced nectar within the optimal range for butterfly feeding.

Volume directly impacts nectar concentration and therefore the type pollinator visitors. Bumblebees and honeybees assess the profitability of standing crop by nectar volume and greater volumes can support foraging activity (Heinrich, 1976; Silva *et al*., 2004). When considering species that occurred in the spring vegetation survey, *Rubus flagellaris* and *Potentilla simplex* provided the highest nectar volumes per flower (2.46 µL and 1.8 µL, respectively). In the summer vegetation survey, *Baptisia tinctoria a*nd *Hypericum perforatum* had the greatest nectar volumes (2.20 µL and 1.69 µL, respectively). The abundance of nectar feeders within an ecosystem is often linked to the energy value of resources, calculated through nectar volume and concentration (Roubik, 1989). Other floral resources important to pollinator community structure include flower morphology (Neal *et a*l., 1998), pollen availability (Stone *et al*., 1999), and the presence of micro-constituents in nectar (Vogel, 1983).

Floral availability was very important when evaluating a species' total nectar availability. For example, of the twenty-two species examined *Ceanothus americanus* provided a median sugar concentration per flower but an astounding amount of sugar per stem due to having approximately 3,000 small flowers. For the spring survey, *Rubus flagellaris* and *Lithospermum canescens* remained the highest sugar contributors per stem. From the summer vegetation survey *C. americanus* yielded the most nectar sugar per stem followed by *Monarda punctata*. The inclusion of species-specific nectar sugar concentration per flower, floral abundance, and stem counts from Walsh (2017) enabled us to estimate the resource contribution of each species. The nectar resources per stem reported form our study is a valuable contribution to oak savanna habitat assessment and similar procedures can be applied to future habitat assessments to complete more detailed evaluation.

Site Assessment

Our study established a quantitative approach to evaluate oak savanna habitat nectar resources which revealed temporal differences not initially observed. Nectar resources are typically evaluated through flowering stems density, but this approach can be misleading due to variations in species floral abundance and nectar quality (Schultz & Dlugosch, 1999). We identified significant differences between site nectar sugar availability not previously measured by flowering stem density. Further investigation demonstrated sites associated with natural Karner blue butterfly occupancy had significantly more nectar sugar available in the spring than previous release locations no longer occupied. As an indicator and umbrella taxon (Shuey, 1997; Fleishman *et al*., 2000; Chan & Packer, 2006) the habitat requirements of the Karner blue butterfly can guide effective oak savanna habitat restoration and pollinator conservation. Our study found it is essential to assess the nectar resources available during both spring and summer to fully understand the resource dynamics between seasons.

The oak savanna vegetation surveys utilized for this study were categorized by Karner blue butterfly occupancy related to a severe reginal drought (Walsh, 2017). Sites were categorized as occupied by Karner blue butterflies before and after the drought, no longer occupied after the drought (formerly occupied), previous release sites no longer occupied, and high priority restoration sites. Walsh (2017) found that occupied sites that successfully supported Karner blue populations through the drought had a greater flowering lupine density, ant entrance density, and heat loads. Walsh's findings coincide with the existing concept that fluctuating abiotic factors like weather can drive overall insect population trends (Begon *et al.* 1996). While Walsh (2017) did not find nectar plant density had a consistent effect on Karner blue presence

further examination of resource nectar availability may distinguish general limitations to population size (Roulston & Goodell, 2011; Hicks *et al*., 2016).

Our analyses revealed an interactive relationship between category and nectar sugar availability that changed between seasons. The impact of category associated with season is likely because each site had a unique of seasonal flowering community composition associated with habitat features such as canopy cover and conservation management history. As a result, the establishment of different flowering communities in each category contributed towards to differences in species-driven nectar sugar availability. When the species flowering stem densities in oak savanna vegetation surveys were compared between site category types, in both spring and summer vegetation surveys sites currently occupied and formerly occupied by the Karner blue butterfly appeared to have more similar flowering communities than previous release locations and restoration sites.

Studies often correlate butterfly abundance with nectar species abundance (Schultz & Dlugosch, 1999; Holl, 1995); however, it has been acknowledged that more detailed factors such as metapopulation dynamics (Moilanen & Hanski, 1998; Fred & Brommer, 2003), vegetation height (Ellis, 2003), and nectar quality (Schultz & Dlugosch, 1999) affect the habitat quality for butterfly species. Estimating resource availability by plant density alone, or even individual flower numbers, fails to capture resource effects from differences in species nectar quality. Non-parametric testing showed no difference in summer flowering stem density between four category comparisons (occupied v. release; occupied v. restoration; former v. release; former v. restoration), although there were significant differences in nectar sugar availability. These results demonstrate that species-specific floral abundance and nectar sugar quality together can provide an improved assessment of the resources available and revealed associations not previously

measured (Schultz & Dlugosch, 1999; Hicks *et al*., 2016). Estimating nectar sugar per plant allowed us to evaluate sugar availability at the quadrat and site level to provide new insights on the magnitude and distribution of nectar resources available to oak savanna pollinators.

Sites currently occupied and formerly occupied, before the drought, had significantly more nectar sugar available in the spring than previous release sites that are no longer occupied. The naturally occupied locations provided approximately eleven times more sugar per quadrat in the spring than previous release sites. Our results highlight the importance of oak savanna sugar availability in the spring (late May/early June). Consuming enough nectar sugar is an important factor in butterfly nutrition with direct effects on individual fitness (Murphy *et al*., 1983). Increasing nectar sugar resources available in the spring for first generation Karner blue butterflies would likely increase the population size of the second generation. In the late summer, second generation butterflies are faced with a short window of time for larval development and nectar plant turnover as Wild lupine and flowering plants begin to senesce (Grundel, 1998). A larger clutch size of Karner blue butterflies laid in the spring, to hatch in the summer, may buffer the effects of smaller summer clutch sizes if second generation juvenile and adult resources are insufficient. It is suggested that viable populations of Karner blue butterflies should have 1,000 first-generation individuals leading to approximately 3,000 second-generation individuals, occurring throughout 5 subpopulations (Haack, 1993). Ensuring enough sugar is available in the spring to the first-generation may be an important factor to support population viability.

Further discrepancies in spring nectar sugar availability between categories suggest spring resource spatial distribution may be an additional important feature of Karner blue butterfly habitats. The distribution of sugar resources in sites currently occupied exhibited a more even distribution of low, medium, and high-quality patches of flowering stems and sugar

availability. The success of Karner blue butterfly conservation within occupied sites may also be linked to this similar distribution of stem and nectar sugar resources in the spring. Studies have found that wild bumble bees exhibit optimal foraging when landscape resources permit traveling shorter distances for higher quality patches (Redhead *et al*., 2016). In the summer, occupied, formerly occupied, and previous release sites had highly similar distribution of flowering stems, which could explain the assumed resource similarity between these sites. However, the distribution of sugar availability was no longer similar between these categories. As landscape-level resources decline bumble bees become more willing to travel further distances and spend more time in search of higher quality floral patches (Westphal *et al*., 2006; Woodard & Jha, 2017). Such foraging behavior creates the risk of expending energy that may not be readily replenished. Social bees may not mirror the foraging behavior of all pollinators, but modeling foraging and pollination services require criteria for habitat foraging distances and an estimate of resource value (Cresswell *et al*., 2000; Lonsdorf *et al*.; 2009, Raine *et al*.; 2009). Describing the quality and distribution of floral resources within oak savanna landscapes is the first step to understanding how to better manage habitats to meet pollinator foraging requirements.

Our study was able to show that measuring flowering stem density alone is an incomplete evaluation of resources and can create misleading information. Evaluating floral abundance and nectar resource quality are necessary to improve habitat restoration and pollinator conservation. The twenty-two species characterized for this study are the first step to guide oak savanna species selection and habitat assessment. Measuring flowering stem density is a preferred method for resource assessment because collecting nectar quality characteristics can be very time intensive (Schultz & Dlugosch, 1999). To reduce future time spent in the field, work should be continued to develop a catalog of oak savanna flowering resource characteristics that include

floral availability, pollen count, phenology, nectar volume and sugar concentration. Establishing a quantitative approach to nectar resource assessment will increase habitat assessment accuracy, create measurable restoration goals, and further improve habitats for local pollinators.

By using sites currently occupied by the Karner blue butterfly to represent desired oak savanna resource requirements, we were able to show that a greater abundance of spring nectar resources is important. Typically, habitat assessments are completed during the summer before Karner blue butterflies are released into a new site as second-generation adults. Our study shows it is essential to assess the nectar resources available during both adult generations to fully understand the resource dynamics between seasons. Further analyses suggest the spatial distribution of nectar quality within habitats may be a valuable feature to quantify in the future. Other features such as habitat size (Moilaen & Hanski, 1998; Bergman & Kindvall, 2004), host plant abundance (Fred & Brommer, 2003; Walsh, 2017), and canopy cover (Grundel *et al*., 1998a, 1998b) should also be incorporated into oak savanna restoration and Karner blue butterfly reintroduction habitat standards (Chan & Packer, 2006; Walsh, 2017). The results of this study can be used to improve our understanding of the characteristics and temporal variations of oak savanna nectar resources in order to aid future habitat restoration planning and conservation efforts for pollinators of this critically imperiled habitat.

REFERENCES

Archer, C. R., Pirk, C. W. W., Carvalheiro, L. G., & Nicolson, S. W. (2014). Economic and ecological implications of geographic bias in pollinator ecology in the light of pollinator declines. *Oikos*, *123*(4), 401-407.

Arnold, P. M., & Michaels, H. J. (2017). Nectar sampling for prairie and oak savanna butterfly restoration. *Applications in Plant Sciences*, 5(6), 1600148.

Anderson, R. C., Fralish, J. S., & Baskin, J. M. (eds). (2007). Savannas, Barrens, and Rock Outcrop Plant Communities of North America. *Cambridge University Press*.

Apple, J., & Feener, D. (2001). Ant visitation of extrafloral nectaries of Passiflora: the effects of nectary attributes and ant behavior on patterns in facultative ant-plant mutualisms. *Oecologia*, *127*(3), 409-416.

Asbjornsen, H., Brudvig, L. A., Mabry, C. M., Evans, C. W., & Karnitz, H. M. (2005). Defining reference information for restoring ecologically rare tallgrass oak savannas in the Midwestern United States. *Journal of Forestry*, *103*(7), 345-350.

Baker, H. G., & Baker, I. (1975). Studies of nectar-constitution and pollinator plant coevolution. In: Gilbert LE, Raven PH (eds) Coevolution of animals and plants. *University of Texas Press*, pp 100–140.

Baker, I., & Baker, H. G. (1982). Some chemical constituents of floral nectars of Erythrina in relation to pollinators and systematics. *Allertonia,* 3, 25–37.

Baker, H. G., & Baker, I. (1983). Floral nectar sugar constituents in relation to pollinator type. In: C.E. Jones, & R.J. Little (Eds.), Handbook of experimental pollination biology (pp. 117–141). New York: Van Nostrand Reinhold.

Bateson, M. (2002). Recent advances in our understanding of risk-sensitive foraging preferences. *Proceedings of the Nutrition Society*, *61*(4), 509-516.

Baude, M., Leloup, J., Suchail, S., Allard, B., Benest, D., Meriguet, J., ... & Raynaud, X. (2011). Litter inputs and plant interactions affect nectar sugar content. *Journal of Ecology*, *99*(3), 828-837.

Bauerfeind, S. S., & Fischer, K. (2009). Effects of larval starvation and adult diet-derived amino acids on reproduction in a fruit-feeding butterfly. *The Netherlands Entomological Society,* 130, 229-237.

Begon, M., Harper., J. L., & Townsend, C. R. (1986). *Ecology: individuals, populations and communities.* Blackwell Scientific Publications.

Bergman, K. O., & Kindvall, O. (2004). Population viability analysis of the butterfly *Lopinga achine* in a changing landscape in Sweden. *Ecography,* 27, 49-58.

Bertsch, A. (1983). Nectar production of *Epilobium angustifolium* L. at different air humidities; nectar sugar in individual flowers and the optimal foraging theory. *Oecologia*, *59*(1), 40-48.

Blüthgen, N., Verhaagh, M., Goitía, W., Jaffé, K., Morawetz, W., & Barthlott, W. (2000). How plants shape the ant community in the Amazonian rainforest canopy: the key role of extrafloral nectaries and homopteran honeydew. *Oecologia*, *125*(2), 229-240.

Boggs, C. L. (2009). Understanding insect life histories and senescence through a resource allocation lens. *Functional Ecology*, 23, 27–37.

Bolten, A. B., Feinsinger, P., Baker, H. G., & Baker, I. (1979). On the calculation of sugar concentration in flower nectar. *Oecologia,* 41, 301–304.

Britten, H. G., & Riley, L. (1994). Nectar source diversity as an indicator of habitat suitability for the endangered Uncompahgre fritillary, *Bolaria acrocnema* (Nymphalidae). *Journal of the Lepidopterists' Society*, 48(3), 173-179.

Brodschneider, R., & Crailsheim, K. (2010). Nutrition and health in honey bees. *Apidologie*, *41*(3), 278-294.

Brown, J. H., & Kodric-Brown, A. (1979). Convergence, competition, and mimicry in a temperate community of hummingbird-pollinated flowers. *Ecology*, *60*(5), 1022-1035.

Brown, S. C., & Bedford, B. L. (1997). Restoration of wetland vegetation with transplanted wetland soil: an experimental study. *Wetlands*, *17*(3), 424-437.

Brudvig, L. A., & Asbjornsen, H. (2007). Stand structure, composition, and regeneration dynamics following removal of encroaching woody vegetation from Midwestern oak savannas. *Forest Ecology and Management*, *244*(1-3), 112-121.

Burkle, L. A., & Irwin, R. E. (2009). The effects of nutrient addition on floral characters and pollination in two subalpine plants, *Ipomopsis aggregata* and *Linum lewisii*. *Plant Ecology*, *203*(1), 83-98.

Burquez, A., & Corbet, S. A. (1991). Do flowers reabsorb nectar? *Functional ecology*, 369-379.

Byrne, M. P., & O'Gorman, P. A. (2018). Trends in continental temperature and humidity directly linked to ocean warming. *Proceedings of the National Academy of Sciences*, *115*(19), 4863-4868.

Cahenzli, F., & Erhardt, A. (2012a). Enhancing offspring quality or quantity? Different ways for using nectar amino acids in female butterflies. *Oecologia,* 169, 1005-1014.

Cahenzli, F., & Erhardt, A. (2012b). Nectar sugars enhance fitness in male *Coenonympha pamphilus* butterflies by increasing longevity or realized reproduction. *Oikos*, 121(9), 1417-1423.

Chan, P. K., & Packer, L. (2006). Assessment of potential Karner Blue butterfly (*Lycaeides melissa samuelis*)(family: Lycanidae) reintroduction sites in Ontario, Canada. *Restoration Ecology*, *14*(4), 645-652.

Cresswell, J. E., & Galen, C. (1991). Frequency-dependent selection and adaptive surfaces for floral character combinations: the pollination of *Polemonium viscosum*. *American Naturalist*, 138, 1342–1353.

Cresswell, J. E., Osborne, J. L., & Goulson, D. (2000). An economic model of the limits to foraging range in central place foragers with numerical solutions for bumble bees. *Ecological Entomology,* 25, 249–255.

Dafni, A. (1992). Rewards in flowers. In Rickwood, D., & Hames, B. D. [eds.], Pollination ecology: A practical approach, 127–161. *Oxford University Press*, New York, New York, USA.

Dirig, R. (1994). Historical notes on wild lupine and the Karner blue butterfly at the Albany Pine Bush, New York. *Karner Blue Butterfly–a symbol of a vanishing landscape*, 23-36.

Ellis, S. (2003). Habitat quality and management for the northern brown argus butterfly Aricia Artaxerxes (Lepidoptera: Lycaenidae) in North East England. *Biological Conservation,* 113, 285-294.

Falconer, D. S. (1989). Introduction to Quantitative Genetics 3rd ed. *Harlow: Longman Scientific & Technical*.

Farkas, Á., Molnár, R., Morschhauser, T., & Hahn, I. (2012). Variation in nectar volume and sugar concentration of *Allium ursinum* L. ssp. *ucrainicum* in three habitats. *The Scientific World Journal*.

Feinsinger, P. (1978). Ecological interactions between plants and hummingbirds in a successional tropical community. *Ecological monographs*, *48*(3), 269-287.

Frankie, G. W. (1983). Why bees move among mass-flowering neotropical trees. *Handbook of experimental pollination biology*, 360-372.

Fred, M. S., & Brommer, J. E. (2003). Influence of habitat quality and patch size on occupancy and persistence in two populations of the Apollo butterfly (*Parnassius apollo*). *Journal of Insect Conservation,* 7, 85-98.

Freeman, C. E., & Head, K. C. (1990). Temperature and sucrose composition of floral nectars in *Ipomopsis longiflora* under field conditions. *Southwestern Naturalist*, 35, 423–426.

Gilbert, F. S., Haines, N., & Dickson, K. (1991). Empty flowers. *Functional Ecology*, 29-39.

Grossmann, E. B., & Mladenoff, D. J. (2007). Open woodland and savanna decline in a mixed-disturbance landscape (1938 to 1998) in the Northwest Wisconsin (USA) Sand Plain. *Landscape Ecology,* 22, 43-55.

Grundel, R., Pavlovic, N. B., & Sulzman, C. L. (1998a). The effect of canopy cover and seasonal change on host plant quality for the endangered Karner blue butterfly (*Lycaeides melissa samuelis*). *Oecologia*, *114*(2), 243-250.

Grundel, R., Pavlovic, N. B., & Sulzman, C. L. (1998b). Habitat use by the endangered Karner blue butterfly in oak woodlands: the influence of canopy cover. *Biological conservation*, *85*(1-2), 47-53.

Grundel, R., Pavlovic, N. B., & Sulzman, C. L. (2000). Nectar plant selection by the Karner blue butterfly (*Lycaeides melissa samuelis*) at the Indiana Dunes National Lakeshore. *The American Midland Naturalist*, 144(1), 1-10.

Haack, R. A. (1993). The endangered Karner blue butterfly (Lepidoptera: Lycaenidae): biology, management considerations, and data gaps. United States Department of Agriculture, Forest Service General Technical Report NC, 83-100.

Hainsworth, F. R., & Hamill, T. (1993). Foraging rules for nectar: food choices by painted ladies. *The American Naturalist*, *142*(5), 857-867.

Heinrich, B., & Raven, P. H. (1972). Energetics and pollination ecology. *Science*, *176*(4035), 597-602.

Heinrich, B. (1976). The foraging specializations of individual bumblebees. *Ecological monographs*, *46*(2), 105-128.

Heinrich, B. (1980). Mechanisms of body-temperature regulation in honeybees, Apis mellifera: I. Regulation of head temperature. *Journal of Experimental Biology*, *85*(1), 61-72.

Herms, C. P. (1996). The endangered Karner blue butterfly (Lepidoptera: Lycaenidae) in Michigan: Habitat suitability, potential impacts of gypsy moth (Lepidoptera: Lymantriidae) suppression, and laboratory rearing (*Lycaeides melissa samuelis, Lymantria dispar, Lupinus perennis*).

Hicks, D. M., Ouvrard, P., Baldock, K. C., Baude, M., Goddard, M. A., Kunin, W. E., ... & Osgathorpe, L. M. (2016). Food for pollinators: quantifying the nectar and pollen resources of urban flower meadows. *PloS one*, *11*(6).

Hill, C. J., & Pierce N. E. (1989). The effect of adult diet on the biology of butterflies. *Oecologia,* 81, 249-257.

Holl, K. D. (1995). Nectar resources and their influence on butterfly communities on reclaimed coal surface mines. *Restoration Ecology*, 3(2), 76-85.

IUCN, International Union for Conservation of Nature. (1998). “IUCN guidelines for re-introductions". IUCN, Cambridge, United Kingdom.

Kato, S., & Sakai, S. (2008). Nectar secretion strategy in three Japanese species: changes in nectar volume and sugar concentration dependent on flower age and flowering order. *Botany*, *86*(4), 337-345.

Kearns, C. A., & Inouye, D. W. (1993). Techniques for pollination biologists. *University Press of Colorado*, Niwot, Colorado, USA.

King, R. S. (2003). Habitat Management: Habitat Management for the Karner Blue Butterfly (*Lycaeides melissa samuelis*) Evaluating the Short-Term Consequences. *Ecological Restoration*, *21*(2), 101-106.

Kingsolver, J. G., & Daniel, T. L. (1979). On the mechanics and energetics of nectar feeding in butterflies. *Journal of Theoretical Biology*, *76*(2), 167-179.

Kingsolver, J. G., & Daniel, T. L. (1995). Regulatory mechanisms in insect feeding. *New York: Champman & Hall*. pp 32–53.

Klinkhamer, P. G., De Jong, T. J., & Linnebank, L. A. (2001). Small-scale spatial patterns determine ecological relationships: an experimental example using nectar production rates. *Ecology Letters*, *4*(6), 559-567.

Kocher, S. D., & Williams, E. H. (2000). The diversity and abundance of North American butterflies vary with habitat disturbance and geography. *Journal of Biogeography*, *27*(4), 785-794.

Lande, R., & Arnold, S. J. (1983). The measurement of selection on correlated characters. *Evolution*, *37*(6), 1210-1226.

Landres, P. B., Morgan, P., & Swanson, F. J. (1999). Overview of the use of natural variability concepts in managing ecological systems. *Ecological applications*, *9*(4), 1179-1188.

Lawson, D. A., & Rands, S. A. (2019). The effects of rainfall on plant–pollinator interactions. *Arthropod-Plant Interactions*, 1-9.

Leach, M. K., & Givnish, T. J. (1999). Gradients in the composition, structure, and diversity of remnant oak savannas in southern Wisconsin. *Ecological Monographs,* 69, 353-374.

Lindauer, M. (1955). The water economy and temperature regulation of the honeybee colony. *Bee World*, *36*(5), 81-92.

Lonsdorf, E., C. Kremen, T. Ricketts, R. Winfree, N. Williams, and S. Greenleaf. (2009). Modelling pollination services across agricultural landscapes. *Annals of Botany,* 103, 1589–1600.

Lundholm, J. T., & Simser, W. L. (1999). Regeneration of submerged macrophyte populations in a disturbed Lake Ontario coastal marsh. *Journal of Great Lakes Research*, *25*(2), 395-400.

May, P. G. (1985). Nectar uptake rates and optimal nectar concentrations of two butterfly species. *Oecologia*, *66*(3), 381-386.

Meehan, T. D., Glassberg, J., & Gratton, C. (2013). Butterfly community structure and landscape composition in agricultural landscapes of the central United States. *Journal of insect conservation*, *17*(2), 411-419.

Mevi-Schutz, J., & Erhardt, A. (2005). Amino acids in nectar enhance butterfly fecundity: a long awaited link. *The American Naturalist,* 164(4), 411-419.

Mitchell, R. J., & Shaw, R. G. (1993). Heritability of floral traits for the perennial wildflower *Penstemon centranthifolius* (Scrophulariaceae): clones and crosses. *Heredity*, *71*(2), 185-192.

Mitchell, R. J. (2004). Heritability of nectar traits: why do we know so little? *Ecology*, *85*(6), 1527-1533.

Mohr, N. A., & Jay, S. C. (1990). Nectar production of selected cultivars of *Brassica campestris* L. and *Brassica napus* L. *Journal of Apicultural Research*, *29*(2), 95-100.

Moilanen, A., & Hanski, I. (1998). Metapopulation dynamics: effects of habitat quality and landscape structure. *Ecology,* 79, 2503-2515.

Murphy, D. D., Launer, A. E., & Ehrlich, P.R. (1983). The role of adult feeding in egg production and population dynamics of the checkerspot butterfly *Euphydryas editha. Oecologia,* 56, 257–263.

National Research Council. (2007). *Status of Pollinators in North America*. The National Academies Press.

Neal, P. R., Dafni, A., & Giurfa, M. (1998). Floral symmetry and its role in plant-pollinator systems: terminology, distribution, and hypotheses. *Annual Review of Ecology and Systematics*, *29*(1), 345-373.

Nepi, M., & Stpiczynska, M. (2008). Do plants dynamically regulate nectar features through sugar sensing? *Plant signaling & behavior*, *3*(10), 874-876.

Neuhauser, C., Andow, D. A., Heimpel, G. E., May, G., Shaw, R. G., & Wagenius, S. (2003). Community genetics: expanding the synthesis of ecology and genetics. *Ecology*, *84*(3), 545-558.

Nicolson, S. W. (1995). Direct demonstration of nectar reabsorption in the flowers of *Grevillea robusta* (Proteaceae). *Functional Ecology*, 584-588.

Nicolson, S. W., & van Wyk, B. (1998). Nectar sugars in Proteaceae: patterns and processes. *Australian Journal of Botany*, 46, 489–504.

Nicolson, S. W., & Thornburg, R. W. (2007). Nectar Chemistry. In *Nectaries and Nectar (*pp 215-263). Springer Netherlands.

Nicolson, S. W. (2009). Water homeostasis in bees, with the emphasis on sociality. *Journal of Experimental Biology*, *212*(3), 429-434.

Nienhuis, P. H., Bakker, J. P., Grootjans, A. P., Gulati, R. D., & De Jonge, V. N. (2002). The state of the art of aquatic and semi-aquatic ecological restoration projects in the Netherlands. In *Ecological Restoration of Aquatic and Semi-Aquatic Ecosystems in the Netherlands (NW Europe)* (pp. 219-233). Springer, Dordrecht.

Noss, R. F., LaRoe III, E. T., & Scott, J. M. (1995). Endangered ecosystems of the United States: a preliminary assessment of loss and degradation. Biological Report 28, National Biological Service, Washington, D.C.

Nuzzo, V. (1986). Extent and status of Midwest oak savanna: presettlement and 1985. *Natural Areas Journal,* 6, 6-36.

Ohio Department of Natural Recourses, Division of Wildlife. (2019). Ohio's listed species. Retrieved from http://wildlife.ohiodnr.gov/species-and-habitats/state-listed-species

OKBBRT, Ohio Karner blue butterfly Recovery Team. (1998). Ohio Karner blue butterfly conservation plan. Report to the Ohio Department of Natural Resources, Division of Wildlife, Columbus, Ohio.

Olson, S. D. (1996). The historical occurrence of fire in the Central Hardwoods, with emphasis on southcentral Indiana. *Natural Areas Journal*, 16, 248–256.

Opler, P., & Malilul, V. (1998). A field guide to eastern butterflies. Houghton Mifflin Co., Boston.

Ostwald, M. M., Smith, M. L., & Seeley, T. D. (2016). The behavioral regulation of thirst, water collection and water storage in honey bee colonies. *Journal of Experimental Biology*, *219*(14), 2156-2165.

Pacini, E., Nepi, M., & Vesprini, J. L. (2003). Nectar biodiversity: a short review. *Plant Systematics and Evolution*, *238*(1-4), 7-21.

Pamminger, T., Becker, R., Himmelreich, S., Schneider, C. W., & Bergtold, M. (2019). The nectar report: quantitative review of nectar sugar concentrations offered by bee visited flowers in agricultural and non-agricultural landscapes. *PeerJ*, *7.*

Park, O. W. (1923). Water stored by bees. *Am. Bee J*, *63*, 348-349.

Paul, J., & Roces, F. (2003). Fluid intake rates in ants correlate with their feeding habits. *Journal of Insect Physiology*, *49*(4), 347-357.

Pham-Delègue, M. H., Mesquida, J., Marilleau, R., Le Métayer, M., & Renard, M. (1990). Quantitative and qualitative analysis of rapeseed flower nectar (*Brassica napu*s L. var. *oleifera Metzger*). In *VI International Symposium on Pollination 288* (pp. 430-434).

Pickens, B. A., & Root, K. V. (2008). Factors affecting host-plant quality and nectar use for the Karner blue butterfly: implications for oak savanna restoration. *Natural Areas Journal*, *28*(3), 210-217.

Pivnick, K. A., & McNeil, J. N. (1985). Effects of nectar concentration on butterfly feeding: measured feeding rates for *Thymelicus lineola* (Lepidoptera: Hesperiidae) and a general feeding model for adult Lepidoptera. *Oecologia*, *66*(2), 226-237.

Pleasants, J. M., & Chaplin, S. J. (1983). Nectar production rates of *Asclepias quadrifolia*: causes and consequences of individual variation. *Oecologia*, *59*(2-3), 232-238.

Plowright, R. C. (1981). Nectar production in the boreal forest lily *Clintonia borealis*. *Canadian Journal of Botany*, 59, 156–160.

Potts, S. G., Vulliamy, B., Roberts, S., O'Toole, C., Dafni, A., Ne'eman, G., & Willmer, P. G. (2004). Nectar resource diversity organises flower-visitor community structure. *Entomologia experimentalis et applicata*, *113*(2), 103-107.

Pyke, G. H., & Waser, N. M. (1981). The production of dilute nectars by hummingbird and honeyeater flowers. *Biotropica*, 260-270.

Raine, N. E., Rossmo, D. K., & Le Comber, S. C. (2009). Geographic profiling applied to testing models of bumblebee foraging. *Journal of The Royal Society Interface,* 6, 307–319.

Redhead, J. W., Dreier, S., Bourke, A. F., Heard, M. S., Jordan, W. C., Sumner, S., ... & Carvell, C. (2016). Effects of habitat composition and landscape structure on worker foraging distances of five bumble bee species. *Ecological Applications*, *26*(3), 726-739.

Roberge, J. M., & Angelstam, P. E. R. (2004). Usefulness of the umbrella species concept as a conservation tool. *Conservation biology*, *18*(1), 76-85.

Rodger, L. (1998). *Tallgrass communities of Southern Ontario: a recovery plan*. World Wildlife Fund.

Roubik, D. W. (1989). *Ecology and natural history of tropical bees*. Cambridge University Press.

Roulston, T. A. H., & Goodell, K. (2011). The role of resources and risks in regulating wild bee populations. *Annual review of entomology*, *56*, 293-312.

Sankaran, M., Ratnam, J., & Hanan, J. P. (2004). Tree-grass coexistence in savannas revisited—insights from an examination of assumptions and mechanisms invoked in existing models. Ecology Letters, 7, 480–490.

Savignano, D. A., & Zaremba, R. (1993). Element stewardship abstract: the Karner blue butterfly. *The Nature Conservancy, Albany, NY*.

Schultz, C. B., & Dlugosch, K. M. (1999). Nectar and hostplant scarcity limit populations of an endangered Oregon butterfly. *Oecologia*, 119, 231–238.

Seeley, T. D. (2009). THE WISDOM OF THE HIVE: THE SOCIAL PHYSIOLOGY OF HONEY BEE COLONIES. Harvard University Press.

Shafir, S., Bechar, A., & Weber, E. U. (2003). Cognition-mediated coevolution–context-dependent evaluations and sensitivity of pollinators to variability in nectar rewards. *Plant Systematics and Evolution*, *238*(1-4), 195-209.

Shuey, J. A. (1997). Dancing with fire: ecosystem dynamics, management, and the Karner blue (*Lycaeides melissa samuelis* Nabokov)(Lycaenidae). *Lepidopterists Society*, 51, 263-268.

Silva, E. M., Dean, B. B., & Hiller, L. (2004). Patterns of floral nectar production of onion (*Allium cepa* L.) and the effects of environmental conditions. *Journal of the American Society for Horticultural Science*, *129*(3), 299-302.

Simidtschiev, T (1988). Nektarproduktivitat von Sonnenblumensorten, Hybriden und Linien verschiedener Herkunft. *Die Biene,* 12, 637-641.

Stern, R. A., & Gazit, S. (1996). Lychee pollination by the honeybee. *Journal of the American Society for Horticultural Science*, *121*(1), 152-157.

Stone, G. N., Gilbert, F., Willmer, P., Potts, S., Semida, F., & Zalat, S. (1999). Windows of opportunity and the temporal structuring of foraging activity in a desert solitary bee. *Ecological Entomology*, *24*(2), 208-221.

Swengel, S. R., & Swengel, A. B. (1999). Correlations in abundance of grassland song bird sand prairie butterflies. *Biological Conservation*, *90*(1), 1-11.

Swetnam, T. W., Allen, C. D., & Betancourt, J. L. (1999). Applied historical ecology: using the past to manage for the future. *Ecological applications*, *9*(4), 1189-1206.

U. S. Department of Agriculture. (2020). *USDA Plant hardiness Zone Map.* Retrieved from https://planthardiness.ars.usda.gov/PHZMWeb/.

U. S. Department of Agriculture, Natural Resource Conservation Service. (2019, July 15). *Plant Database*. Retrieved from https://plants.usda.gov/java/.

U.S. Fish and Wildlife Service. (1992). Endangered and threatened wildlife and plants; determination of endangered status for the Karner blue butterfly. Final rule. *Federal Register,* 57(240), 59236– 59244.

Vaudo, A. D., Patch, H. M., Mortensen, D. A., Tooker, J. F., & Grozinger, C. M. (2016). Macronutrient ratios in pollen shape bumble bee (*Bombus impatiens*) foraging strategies and floral preferences. *Proceedings of the National Academy of Sciences*, 113(28).

Vaudo, A. D., Tooker, J. F., Grozinger, C. M., & Patch, H. M. (2015). Bee nutrition and floral resource restoration. *Current Opinion in Insect Science*, *10*, 133-141.

Villarreal, A. G., & Freeman, C. E. (1990). Effects of temperature and water stress on some floral nectar characteristics in *Ipomopsis longiflora* (Polemoniaceae) under controlled conditions. *Botanical Gazette*, 151, 5–9.

Vogel, S. (1983). Ecophysiology of zoophilic pollination. Physiological Plant Ecology III – Responses to the Chemical and Biological Environment (ed. by OL Lange, PS Nobel, CB Osmond & H Ziegler), pp. 560–624. Springer-Verlag, Berlin

Voss, E. G. (1996). MICHIGAN FLORA PART III: DICOTS CONCLUDED. *Cranbrook Institute of Science Bulletin*, 61, p 363.

Walsh, R. P. (2017). Microclimate and biotic interactions affect Karner blue butterfly occupancy and persistence in managed oak savanna habitats. *Journal of Insect Conservation*, *21*(2), 219-230.

Westphal, C., Steffan-Dewenter, I., & Tscharntke, T. (2006). Foraging trip duration of bumblebees in relation to landscape-wide resource availability. *Ecological Entomology*, *31*(4), 389-394.

Whitham, T. G., Young, W. P., Martinsen, G. D., Gehring, C. A., Schweitzer, J. A., Shuster, S. M., ... & Woolbright, S. (2003). Community and ecosystem genetics: a consequence of the extended phenotype. *Ecology*, *84*(3), 559-573.

Williams, E. H. (1988). Habitat and range of *Euphydryas gillettii* (Nymphalidae). *Journal of the Lepidopterists' Society*, *42*(1), 37-45.

Willmer, P. G., & Stone, G. N. (2004). Behavioral, ecological, and physiological determinants of the activity patterns of bees. *Advances in the Study of Behavior*, *34*(34), 347-466.

Willmer, P. G. (2011). Reward 2: *The Biology of Nectar*. In *Pollination and Floral Ecology.* Princeton University Press, 190- 220.

Wilson, M. V., Hammond, P. C., & Schultz, C. B. (1997). The interdependence of native plants and Fender's blue butterfly. *Conservation and management of native flora and fungi. Native Plant Society of Oregon, Corvallis*, 83-87.

Wolff, D. (2006). Nectar sugar composition and volumes of 47 species of Gentianales from a southern Ecuadorian montane forest. *Annals of Botany*, *97*(5), 767-777.

Woodard, S. H., & Jha, S. (2017). Wild bee nutritional ecology: predicting pollinator population dynamics, movement, and services from floral resources. *Current Opinion in Insect Science*, *21*, 83-90.

Wyatt, R., Broyles, S. B., & Derda, G. S. (1992). Environmental influences on nectar production in milkweeds (*Asclepias syriaca* and *A. exaltata*). *American Journal of Botany*, *79*(6), 636-642.

APPENDEIX A. FIGURES

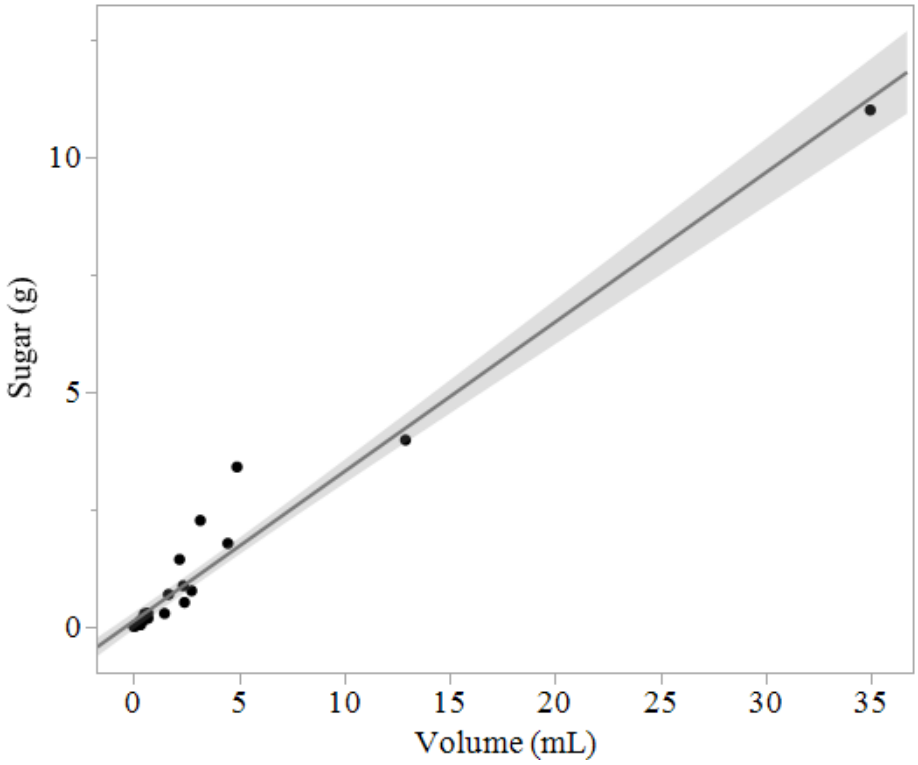


Figure 1. Site total nectar volume (mL) and sugar (g) measured within fifteen sites displayed positive correlation (Pearson's $r = 0.98$, $p < 0.0001$).

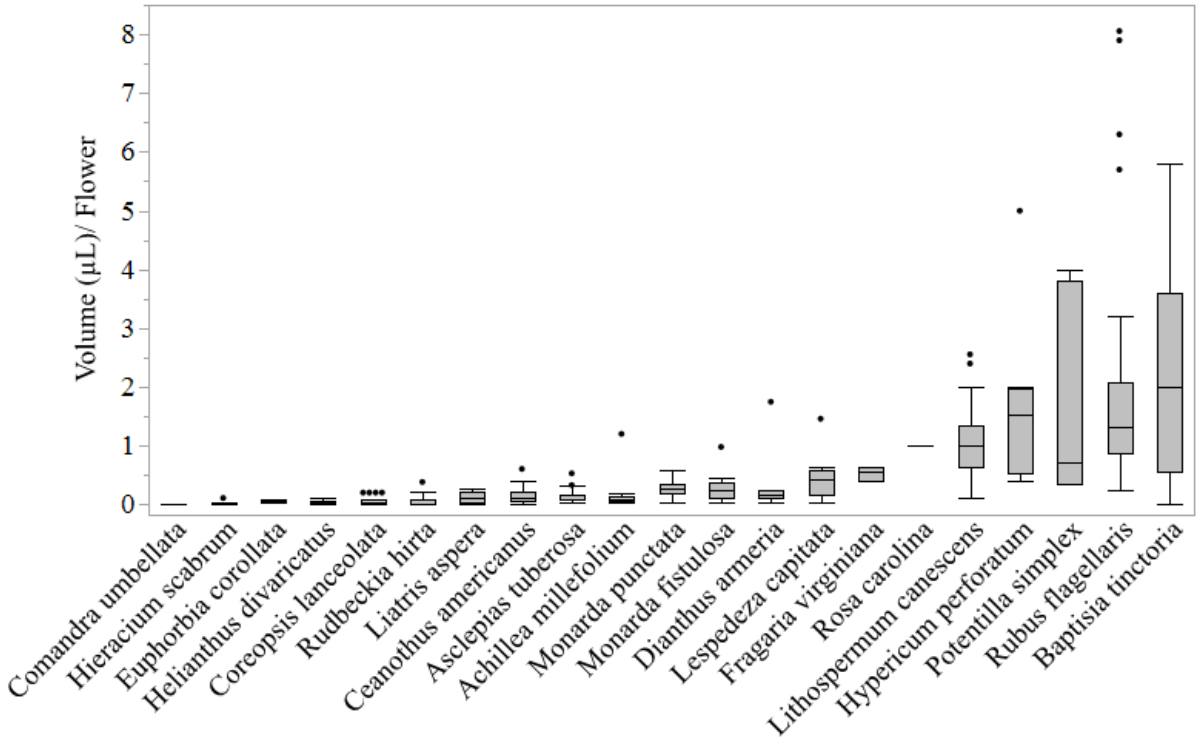


Figure 2. Variation in total volume (µL) per flower among species, ordered from lowest average volume to greatest. Error bars represent the range of nectar sample volume within that species.

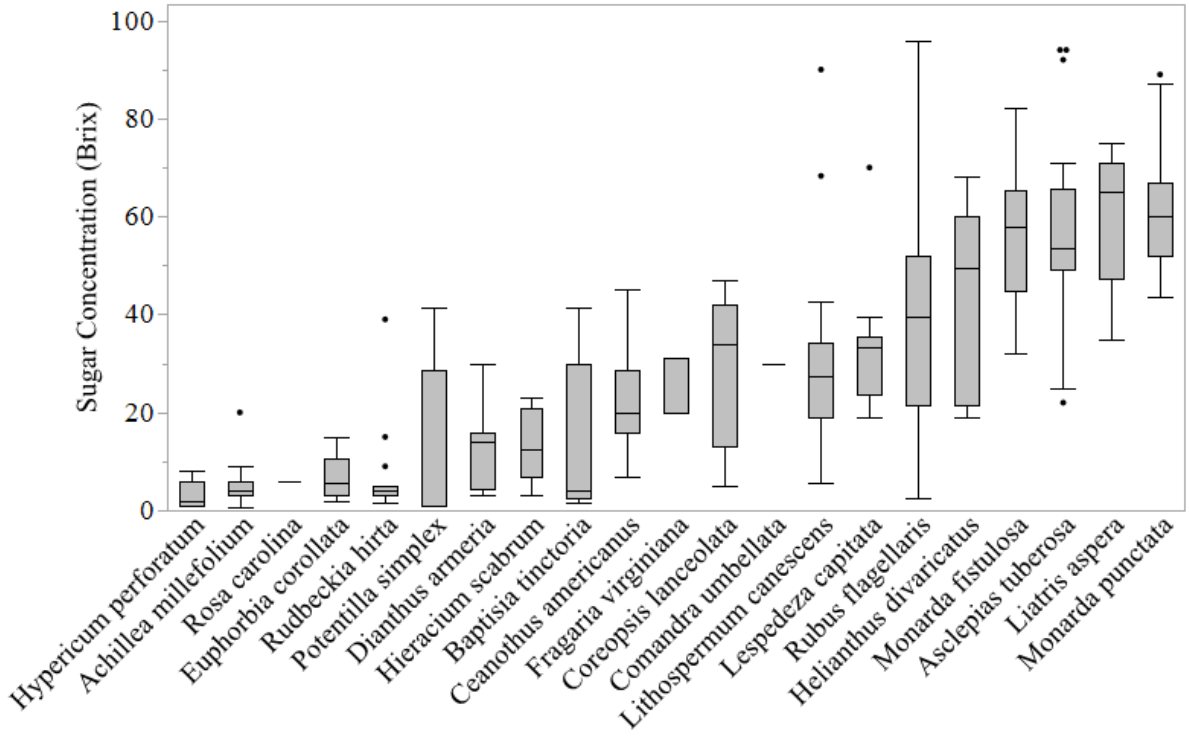
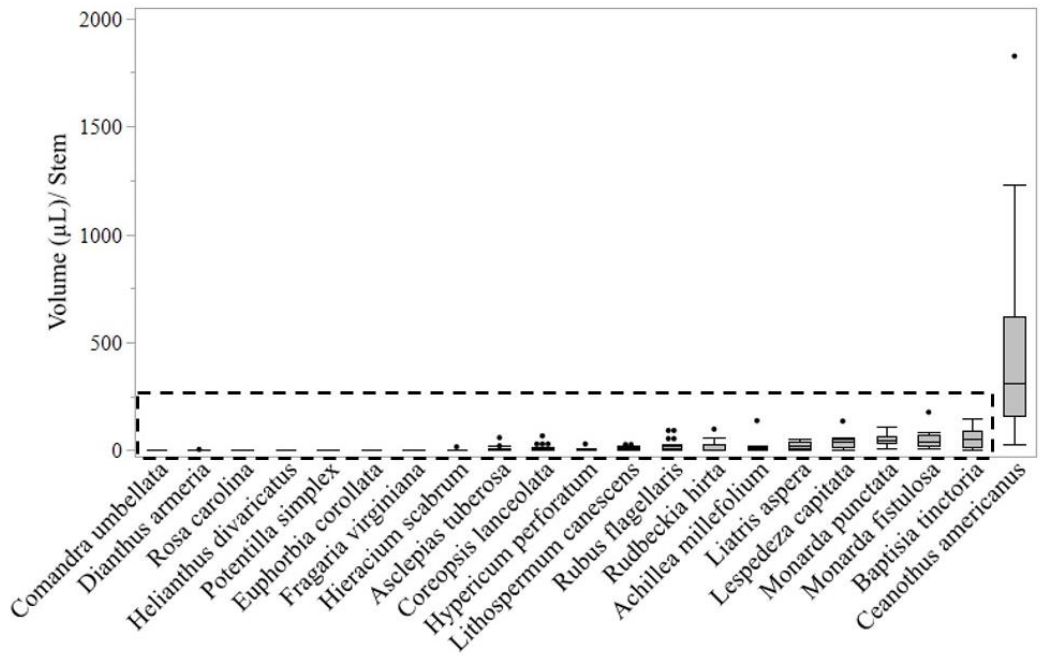


Figure 3. Variation in sugar concentration (Brix = g sucrose/ 100 g solution) per flower among species, ordered from lowest average concentration to greatest. Error bars represent the range of nectar sample sugar concentration within that species.

(a)



(b)

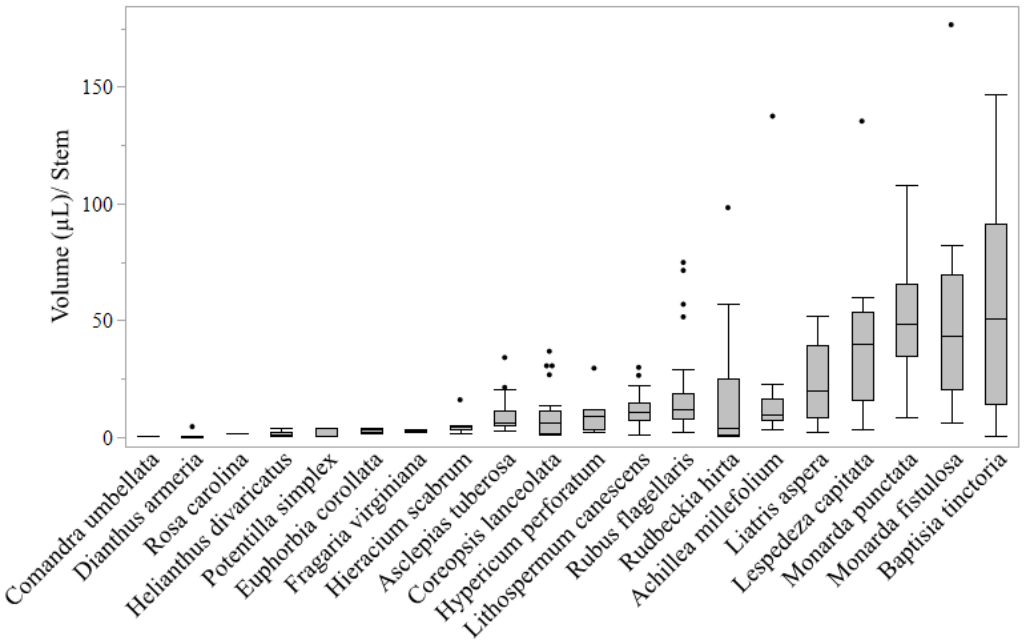
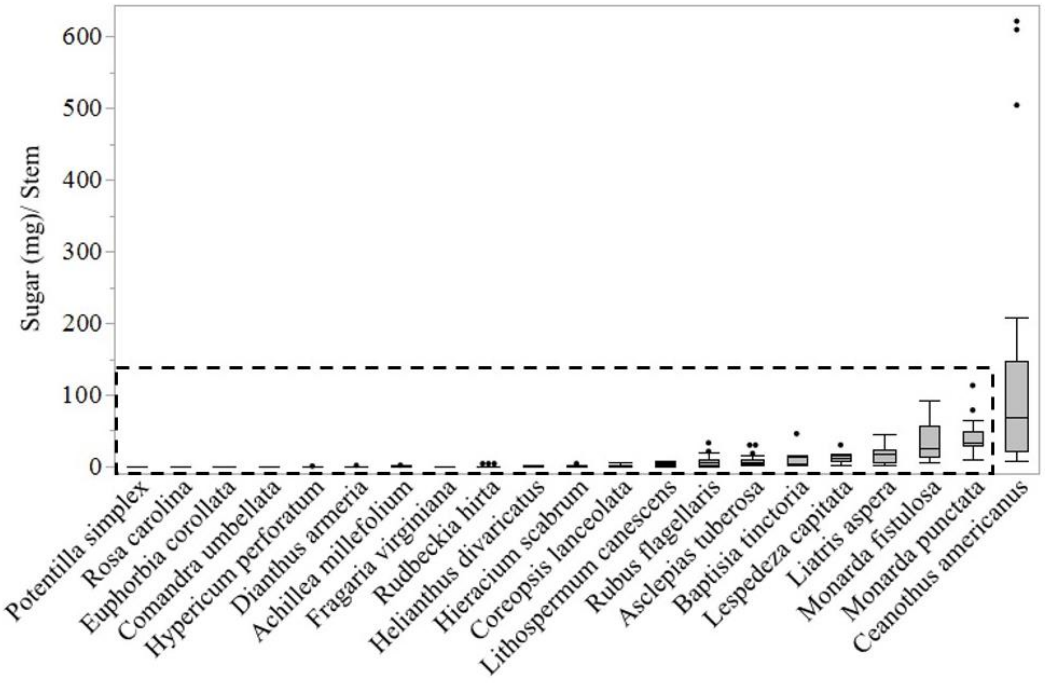


Figure 4. Variation in total volume (µL) per stem among species, ordered from lowest average volume to greatest. The volume of each nectar sample was applied to the average number of flowers per stem for each species. Error bars represent the estimated range of nectar volume per stem within that species. Area identified in graph 'a' is shown in graph 'b' with modified scale to improve species comparison.

(a)



(b)

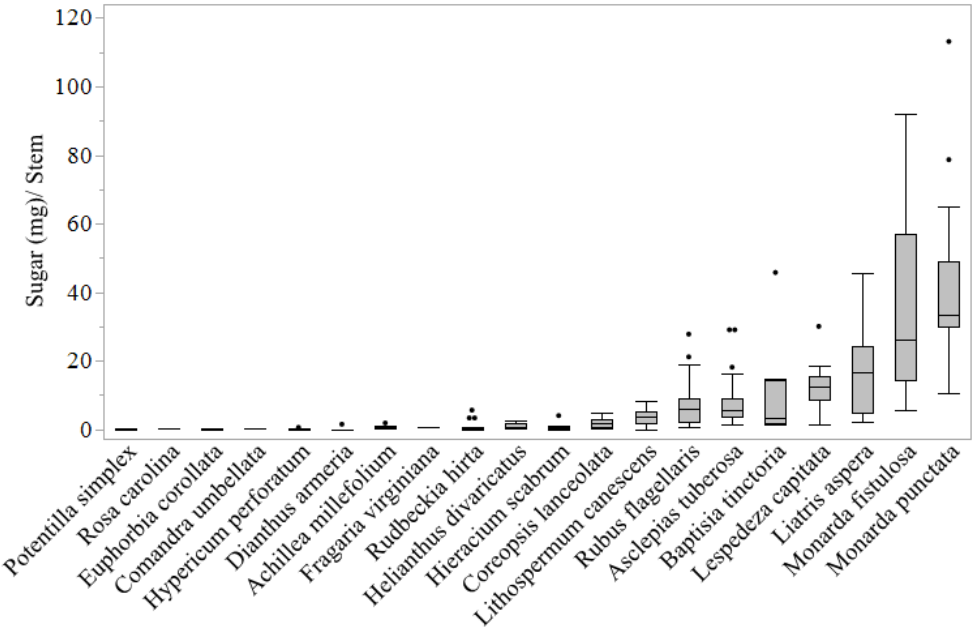


Figure 5. Variation in total sugar (mg) per stem among species, ordered from lowest average sugar availability to greatest. The sugar concentration (Brix**)** of each nectar sample was converted to milligrams and applied to the average number of flowers per stem for each species. Error bars represent the estimated range of total sugar per stem within that species. Area identified in graph (a) is shown in graph (b) with modified scale to improve species comparison.

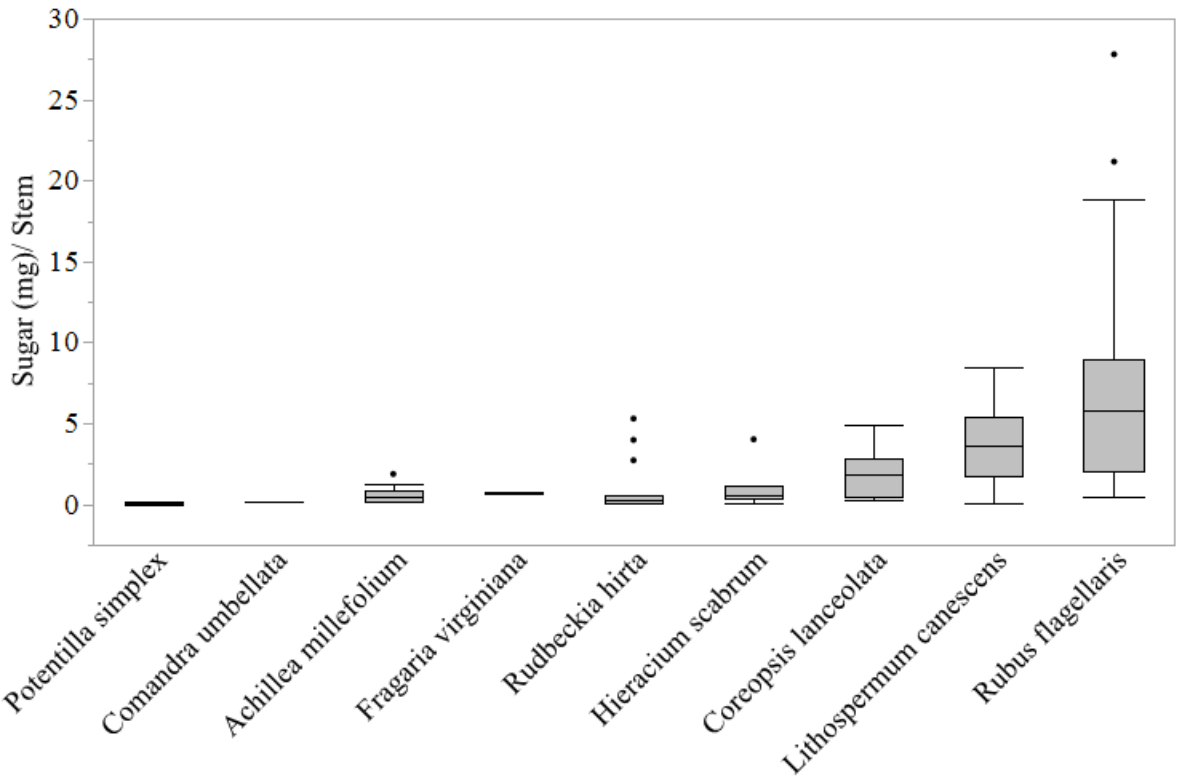


Figure 6. Variation in sugar (mg) per stem of species within spring vegetation survey. The sugar concentration (Brix**)** of each nectar sample was converted to milligrams and applied to the average number of flowers per stem for each species. Error bars represent the estimated range of total sugar per stem within that species.

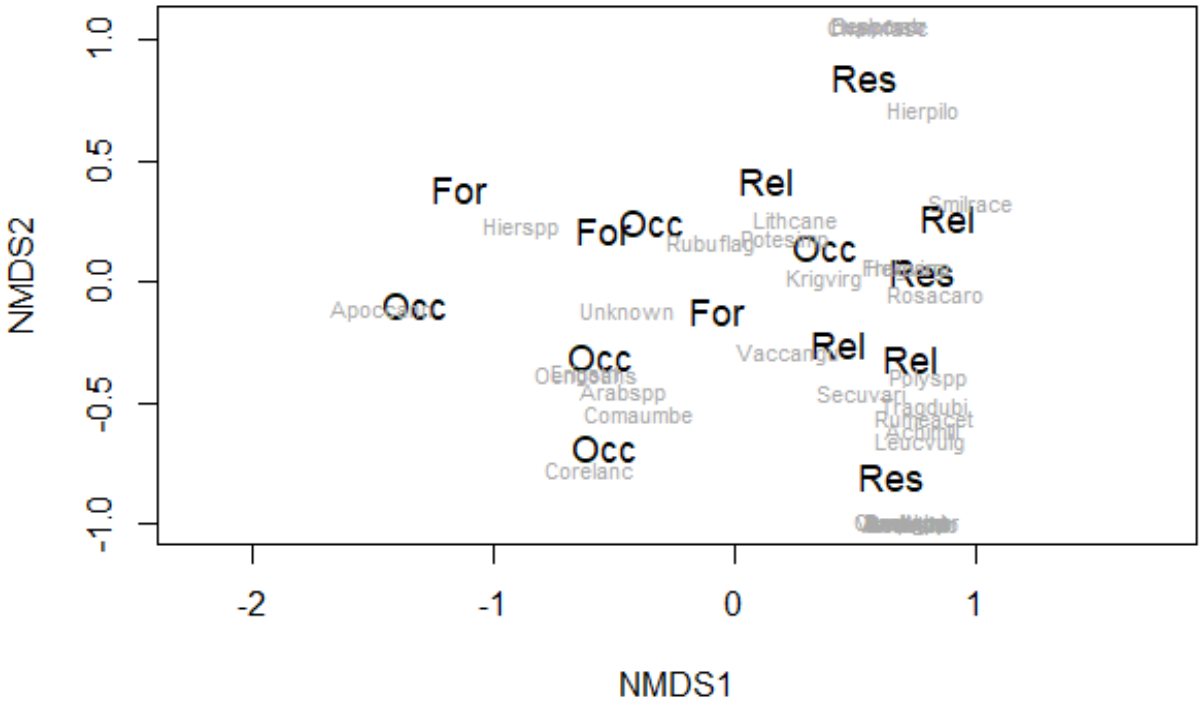


Figure 7. Non-metric multidimensional scaling analysis to compare spring flowering community composition (n = 36 species) between site categories; currently occupied (Occ), previously occupied (Prev), release (Rel), restoration (Res). Site categories were found to have significantly different community composition predicted by species stem abundance (p = 0.03). Occupied and formerly occupied sites oriented away from release and restoration sites.

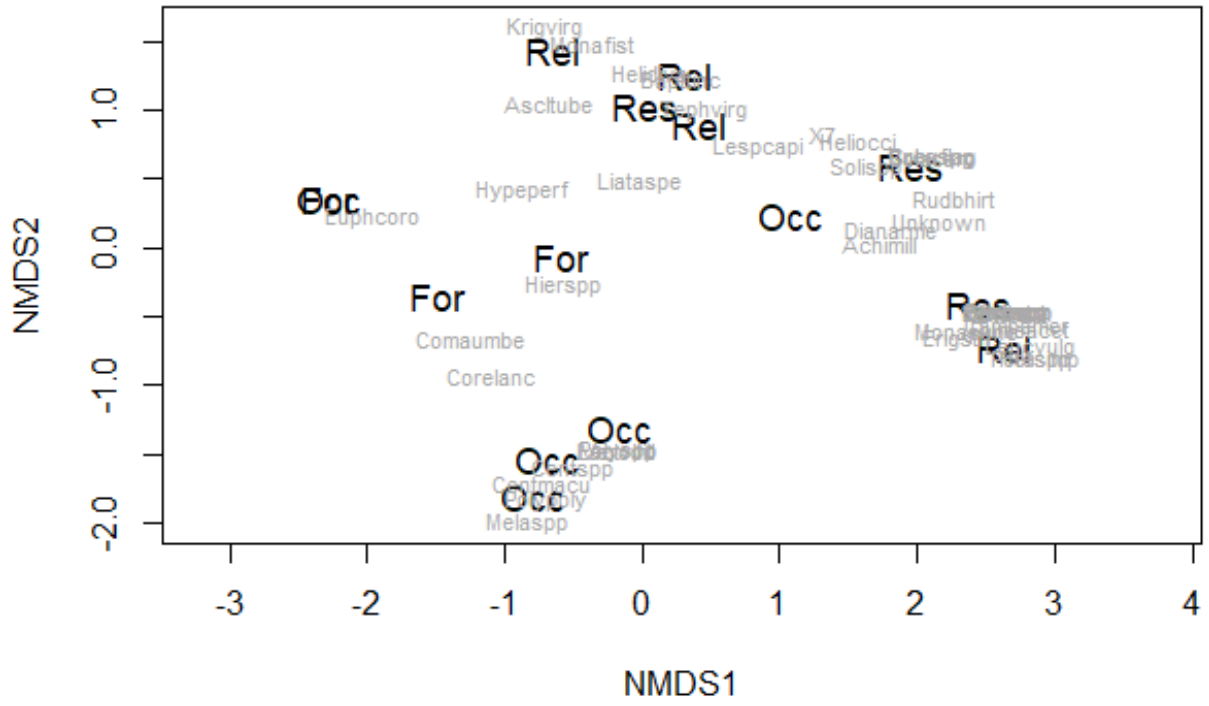


Figure 8. Non-metric multidimensional scaling analysis to compare summer flowering community composition (n = 45 species) between site categories; currently occupied (Occ), previously occupied (Prev), release (Rel), restoration (Res). Site categories were found to have a non-significant trend between with occupied and formerly occupied sites oriented away from release and restoration sites (p = 0.09).

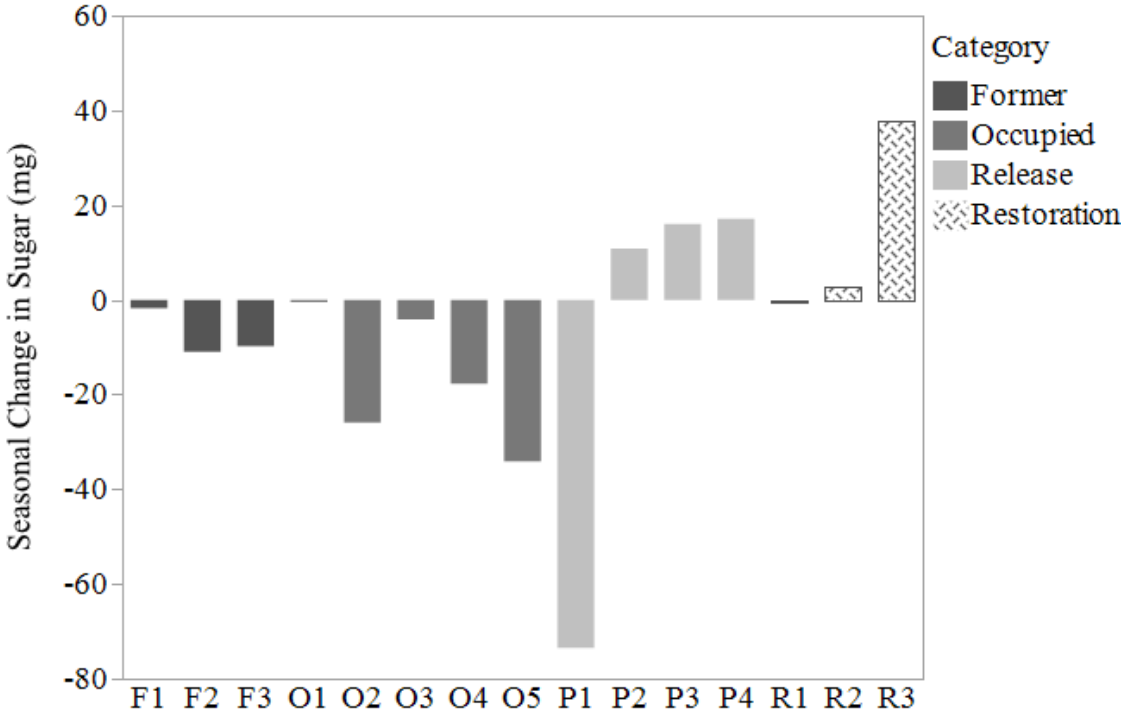


Figure 9. Sugar dynamics between seasons represented by the difference between median sugar (mg) per quadrat from spring into the summer. The data median best represents central statistic due to skewed distribution of sugar availability.

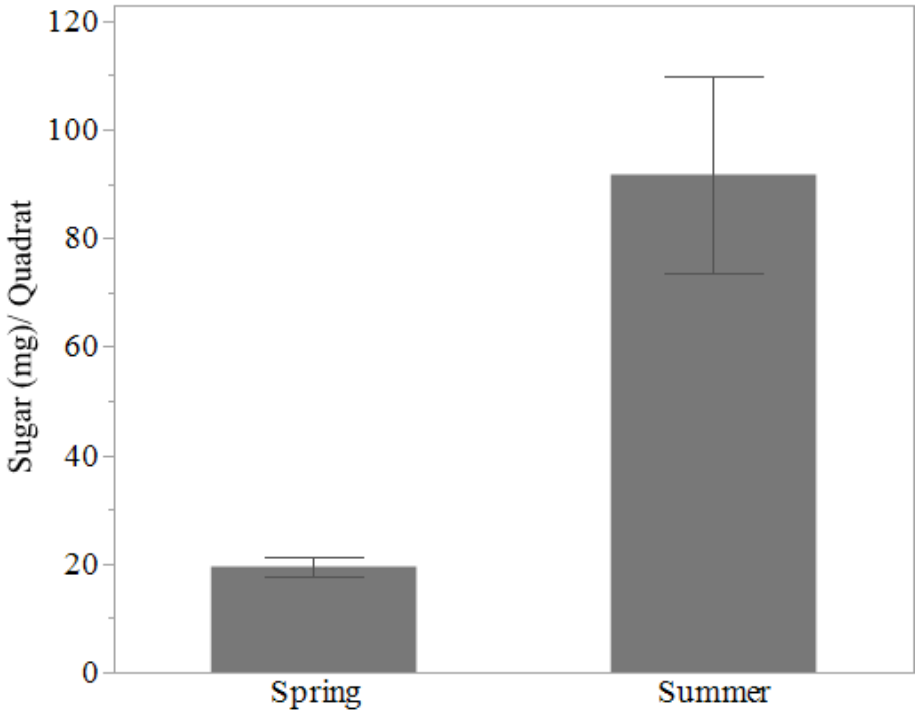
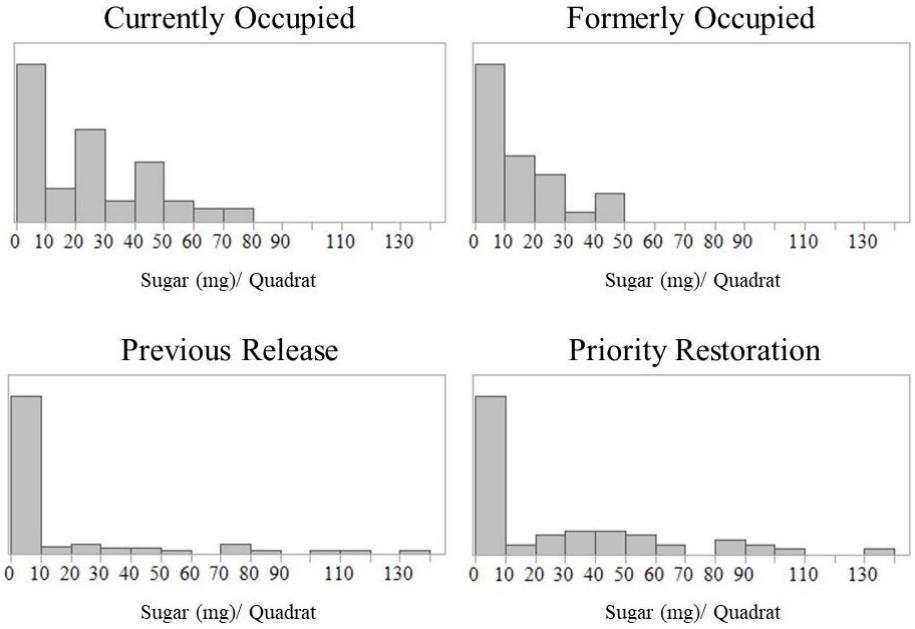


Figure 10. Average **s**ugar availability (mg) per quadrat between the spring and summer across all sites (Linar mixed model, p = 0.03, Table 20)

(a) Spring



(b) Summer

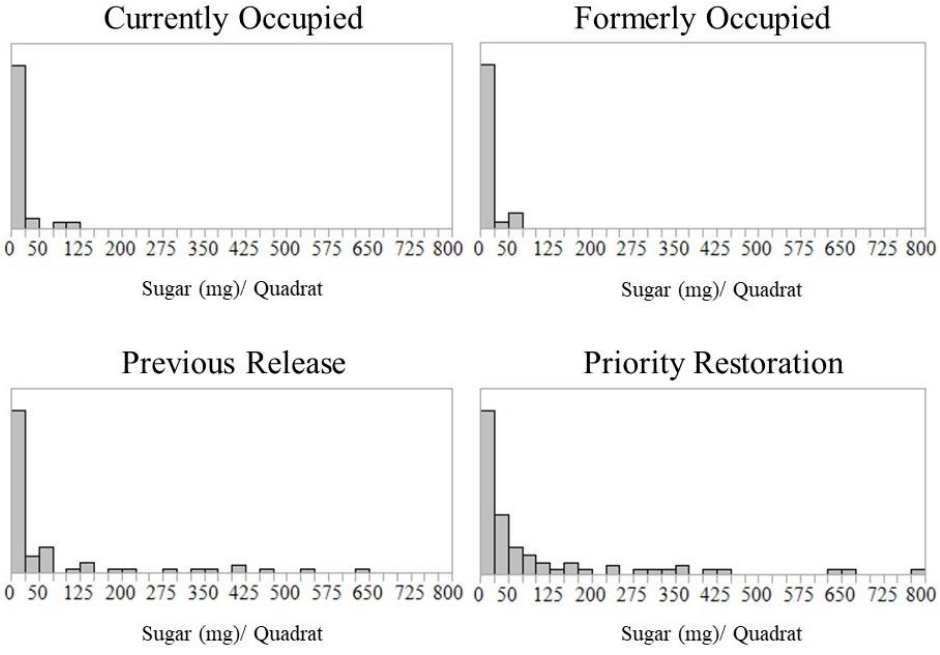


Figure 11. Seasonal distribution of sugar (mg) per 0.5 m^2 quadrat in the (A) spring and (B) summer within category type; currently occupied by Karner blue butterflies, occupied before a drought but not after (former), previous 'release' sites no longer occupied, and high priority restoration sites. Summer sugar availability outliers in release sites (n = 3; maximum = 1,210.21 mg) and restoration sites (n = 3; maximum = 2,689.36 mg) were not represented in graph to improve visual comparison of resources between site categories.

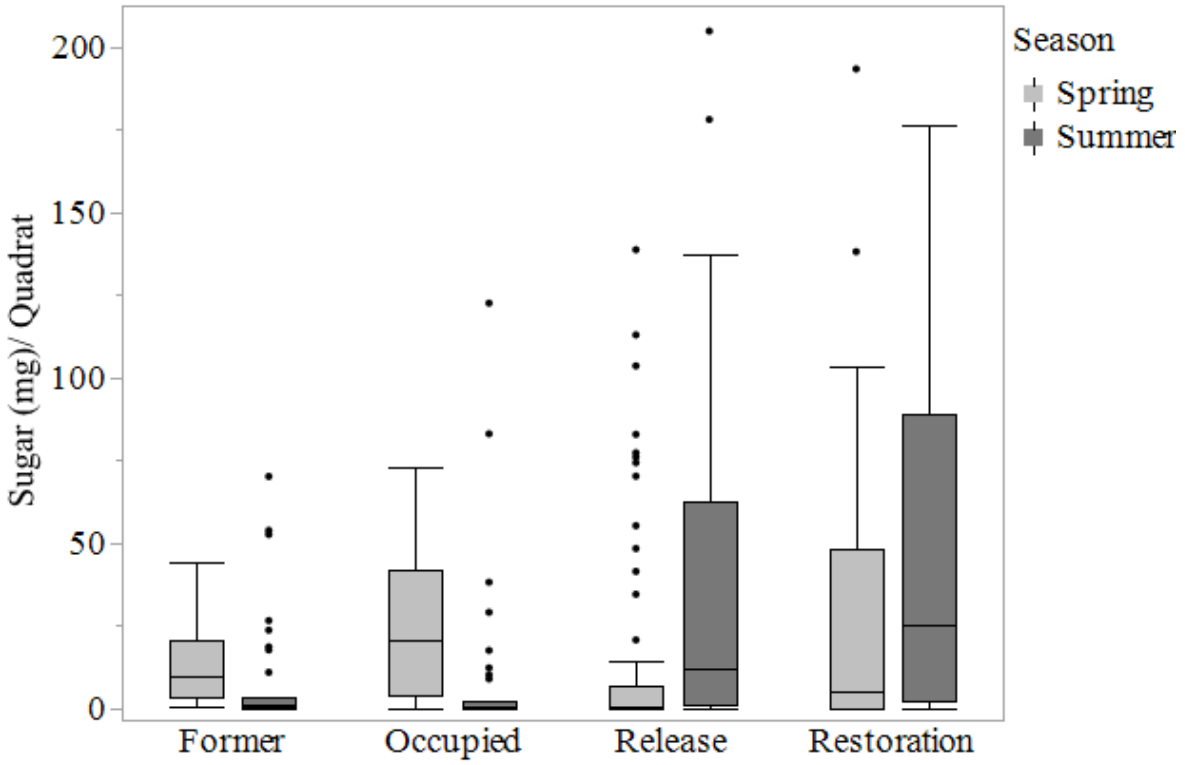


Figure 12. Seasonal median sugar (mg) per 0.5 m^2 quadrat within sites categorized as currently occupied by Karner blue butterflies, occupied before a drought but not after (former), previous 'release' sites no longer occupied, and high priority restoration sites. In both seasons, occupied and former sites are statistically similar (spring p = 0.30, summer p = 0.93, Table 20) and release and restoration are statistically similar (spring p = 0.44, summer p = 0.35, Table 20). Summer sugar availability outliers in release sites (n =11) and restoration sites (n = 15) were not represented in graph to improve visual comparison of resources between site categories.

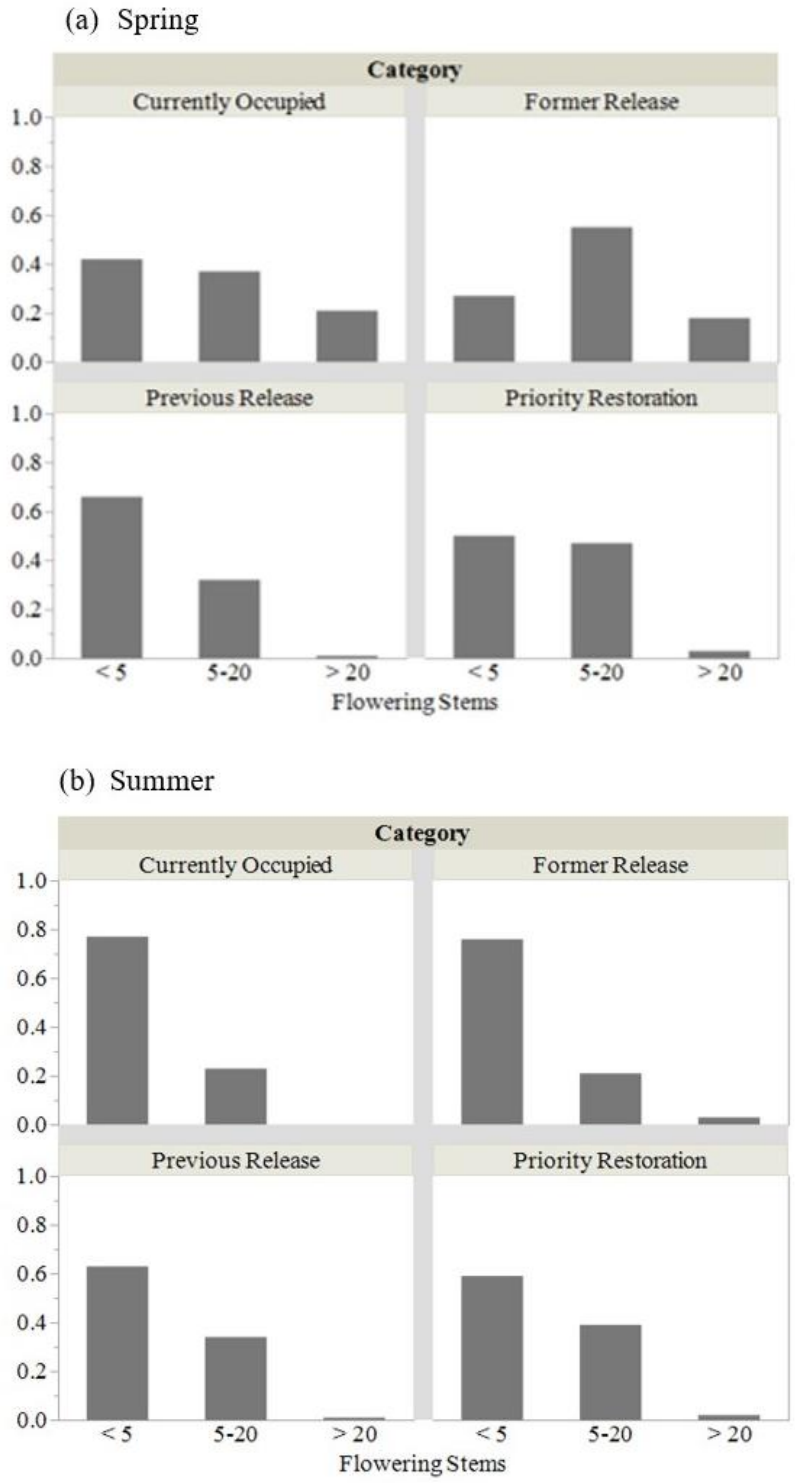


Figure 13. Proportion of quadrats providing low (< 5 stems), medium (5-20 stems), or high (> 20 stems) quantity of flowering stems in (a) spring and (b) summer within category type; currently occupied by Karner blue butterflies, occupied before a drought but not after (former), previous 'release' sites no longer occupied, and high priority restoration sites.

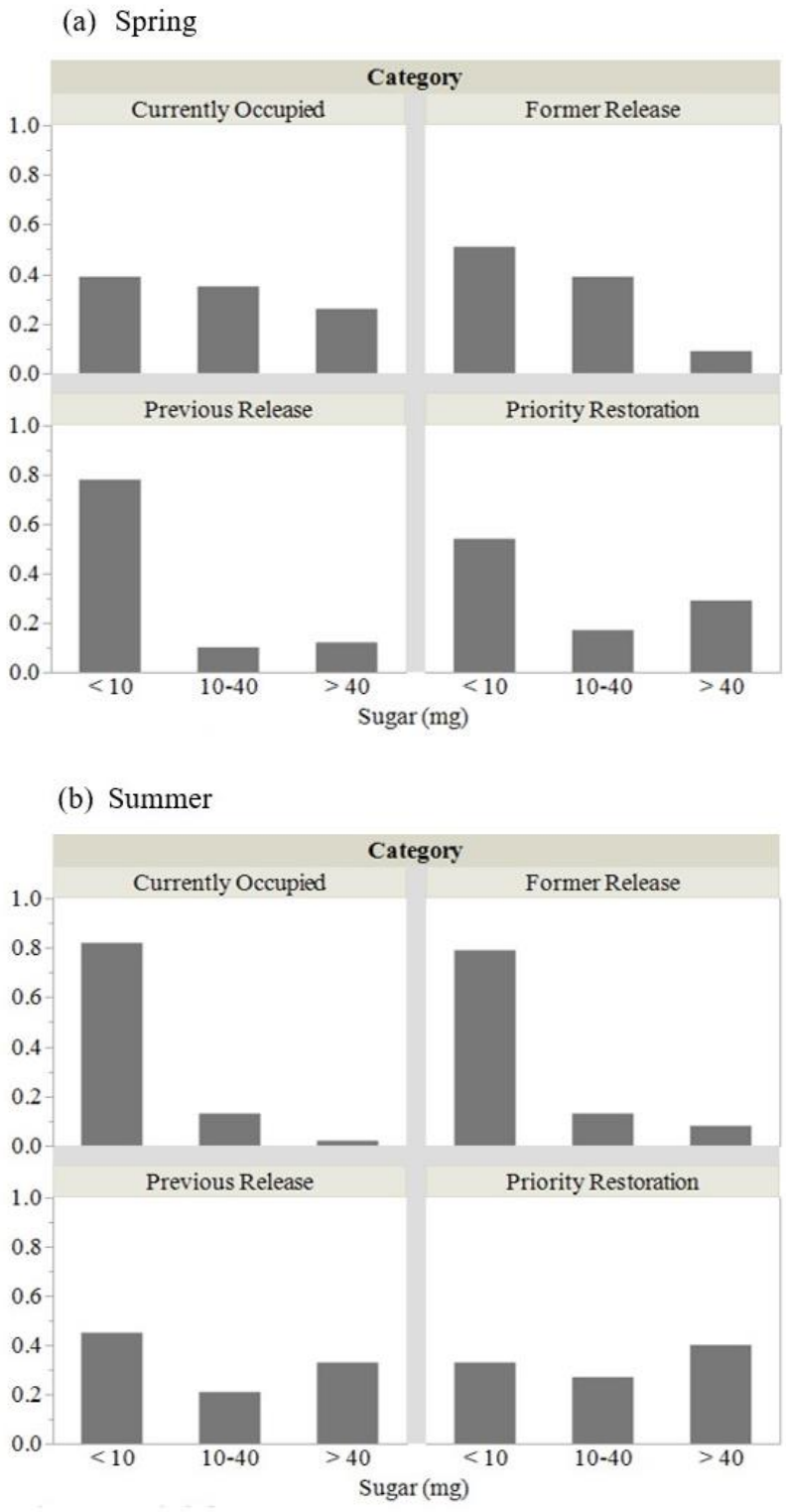


Figure 14. Proportion of quadrats providing low (< 10 mg), medium (10-40 mg), and high (> 40 mg) quantity of sugar in (a) spring and (b) summer within category type; currently occupied by Karner blue butterflies, occupied before a drought but not after (former), previous 'release' sites no longer occupied, and high priority restoration sites.

APPENDIX B. TABLES

Table 1. Sites surveyed by Walsh (2017) categorized as (1) currently occupied by Karner blue butterflies, (2) occupied before a drought but not after (former), (3) previous release site no longer occupied, and (4) high priority restoration site. In parenthesis is the state where each site is located followed by the number of quadrats sampled during each season as an indicator of habitat size.

(1) Currently Occupied	(2) Formerly Occupied	(3) Previous Release	(4) Priority Restoration
O1 (MI), 24	F1 (MI), 24	P1 (OH), 24	R1 (OH), 72
O2 (MI), 30	F2 (MI), 24	P2 (OH), 42	R2 (OH), 30
O3 (MI), 18	F3 (MI), 30	P3 (OH), 72	R3 (OH), 60
O4 (MI), 24		P4 (OH), 24	
O5 (OH), 30			

Table 2. Species selected for nectar and floral characterization, presence during spring and summer surveys or both; number of sites where a species occurred in during specified season; and their abundance (average number of stems in the ground) within a site during each season. When appearing in both spring and summer surveys, sites and average number of stems are listed as spring followed by summer. Survey data was collected by Walsh (2017).

Species Name	**Common Name**	**Brood**	**# Sites**	**Avg. # Stems**
Achillea millefolium L.	Yarrow	Both	2;4	13; 8
Asclepias tuberosa L.	Butterfly milkweed	Summer	2	2.5
Baptisia tinctoria L. R. Br.	Yellow indigo	Summer	4	7.5
Ceanothus americanus L.	New jersey tea	Summer	2	45
Comandra umbellata L. Nutt.	Bastard toadflax	Both	3;2	6;3
Coreopsis lanceolata L.	Lance-leaved coreopsis	Both	2; 5	5; 11
Dianthus armeria L.	Deptford pink	Summer	4	16
Euphoria corollata L.	Flowering spurge	Both	1; 11	8; 17
Fragaria virginiana Duchesne	Virginia strawberry	Spring	5	9
Helianthus divaricatus L.	Woodland sunflower	Summer	4	50
Hieracium pilosella L.	Mouse-ear hawkweed	Spring	2	1
Hieracium spp.	Hawkweed spp.	Both	7; 2	125; 8
Hypericum perforatum L.	Perforate St. John's wort	Summer	2	3
Krigia virginica L.	Dwarf dandelion	Both	6; 1	14; 1
Lespedeza capitata Michx.	Round-headed bush clover	Summer	6	33
Liatris aspera Michx.	Rough blazing star	Summer	5	4
Lithospermum canescens Michx.	Hoary puccoon	Spring	3	5
Monarda fistulosa L.	Wild bergamot	Summer	2	22
Monarda punctata L.	Dotted horsemint	Summer	4	39
Potentilla simplex Michx.	Cinquefoil	Both	11; 1	18; 1
Rosa carolina L.	Carolina rose	Spring	3	22
Rubus flagellaris Willd.	Dewberry	Spring	10	57
Rudbeckia hirta L.	Black-eyed susan	Both	1; 2	2; 7

Table 3. The proportion of non-flowering/empty quadrats and proportion of plants characterized for floral and nectar attributes within each site and season. P3, R2, R3 (spring), and P1 (spring and summer) had high densities of a species not frequently found in other sites. O4 and R3 (summer) had a high density of species unidentified in Walsh (2017). R1 (spring and summer) had the greatest species richness of all sites surveyed for both seasons, making assessment of nectar resources there likely to be an underestimate.

	Spring		Summer	
Site	% Empty Quadrats	% Stems Characterized	% Empty Quadrats	% Stems Characterized
O1	37.50	71.83	50.00	75.00
O2	30.00	92.82	23.33	72.41
O3	66.67	96.97	88.89	100.00
O4	66.67	100.00	83.33	16.00
O5	20.00	91.20	80.00	91.67
F1	58.33	93.22	75.00	71.15
F2	70.83	98.65	54.17	100.00
F3	40.00	100.00	30.00	100.00
P1	41.67	43.66	20.83	58.65
P2	45.24	95.74	35.71	91.27
P3	27.78	33.56	56.94	100.00
P4	54.17	100.00	58.33	100.00
R1	25.00	40.00	31.67	40.55
R2	20.00	41.27	36.67	100.00
R3	86.67	55.56	10.00	54.68

Table 4. List of species cultivated in the Bowling Green State University greenhouse, where they originated, life stage when purchased, and how they were acquired.

Species	**Source**	**Age**	**Acquired**
Coreopsis lanceolata	Wood County Park District	Mature	Purchased
Ceanothus americanus	Prairie Moon Nursery	Mature	Purchased
Rosa Carolina	Prairie Moon Nursery	Mature	Purchased
Fragaria virginiana	St. John's Nature Preserve	Mature	Transplanted
Rubus flagellaris	St. John's Nature Preserve	Mature	Transplanted
Achillea millefolium	Blue Creek Seed Nursery	Seed	Donated
Hieracium scabrum	Blue Creek Seed Nursery	Seed	Donated
Hypericum perforatum	Blue Creek Seed Nursery	Seed	Donated
Dianthus armeria	St. John's Nature Preserve	Seed	Collected

Table 5. List of Spearman's rank correlation between nectar composition variables and environmental factors. Asterisks indicate statistical significance at the $\alpha = 0.05$ level.

Variables			**Direction of Correlation**	**Spearman's Coefficient**	**p - value**
Volume	x	Canopy Cover	+	0.10	0.34
Volume	x	Relative Humidity	+	0.31	0.0005*
Volume	x	Soil Moisture	+	0.34	0.0008*
Volume	x	Temperature	-	0.34	< 0.0001*
Volume	x	Sugar	+	0.009	0.89
Sugar	x	Canopy Cover	-	0.19	0.07
Sugar	x	Relative Humidity	-	0.10	0.26
Sugar	x	Soil Moisture	+	0.07	0.89
Sugar	x	Temperature	-	0.08	0.38

Table 6. Total number of attempted nectar samples for each species from field sites and the BGSU greenhouse. n > 0 corresponds to the number of samples with nectar successfully removed for each species and n% > 0 corresponds to the overall percentage. The final row is totaled across all species.

Scientific Name	Common Name	Total	n > 0	n% > 0
Achillea millefolium L.	Yarrow	85	15	17.65
Asclepias tuberosa L.	Butterfly weed	11	10	90.91
Baptisia tinctoria L. R. Br.	Yellow indigo	26	13	50.00
Ceanothus americanus L.	New jersey tea	48	25	52.08
Comandra umbellata L. Nutt.	Bastard toadflax	21	1	4.76
Coreopsis lanceolata L.	Lance-leaf coreopsis	28	15	53.57
Dianthus armeria L.	Deptford pink	89	7	7.87
Euphorbia corollata L.	Flowering spurge	84	5	5.95
Fragaria virginiana Duchesne	Strawberry	26	3	11.54
Helianthus divaricatus L.	Woodland sunflower	19	7	36.84
Hieracium scabrum L.	Rough hawkweed	92	9	9.78
Hypericum perforatum Michx	St. Johnswort	110	8	7.27
Krigia virginica L.	Dwarf dandelion	15	0	0.0
Lespedeza capitata Michx.	Round-headed bush clover	2	2	100.0
Liatris aspera Michx.	Rough blazing star	4	1	25.0
Lithospermum canescens Michx.	Hoary puccoon	61	23	37.7
Monarda fistulosa L.	Wild bergamot	7	1	14.29
Monarda punctata L.	Dotted horsemint	6	1	16.67
Potentilla simplex Michx.	Cinquefoil	43	5	11.63
Rosa carolina L.	Carolina rose	46	3	6.52
Rubus flagellaris Willd.	Dewberry	115	54	46.96
Rudbeckia hirta L.	Black-eyed susan	67	21	31.34
TOTAL		1,005	229	29.02

Table 7. Wilcoxon/Kruskal-Wallis test to compare the nectar volume (µL) removed and sugar concentration (Brix) between 1 minute and 2 minutes centrifugation periods. Parenthesis indicates standard deviation for 20 replicates. No significant differences were found between nectar composition and spin duration.

Variable	Duration	Mean	DF	X^2	Prob > X^2
Volume (µL)	1 minute	4.46 (0.86)	1	0.07	0.79
	2 minutes	4.36 (1.23)			
Brix	1 minute	22.10 (1.33)	1	0.75	0.39
	2 minutes	21.8 (4.00)			

Table 8. General linear model showing the relationship between (a) nectar volume (µL) and (b) sugar concentration (Brix) per flower with species identity, canopy cover (%), relative humidity (%), temperature (°F), and soil moisture (%). The best model is listed at the bottom and identified in bold. ΔAICc compares all models to the best model. All models were significant ($p < 0.0001$). Parameter estimate of species identity was averaged across all species. Statistical significance set at the $\alpha = 0.05$ level.

a) Nectar Volume per Flower

Dependent Variable	**Fixed Effect**	**Estimate**	**p-value**	**Adj R^2**	**AICc**	**ΔAICc**
Log10 (µL per flower)	Species Identity	0.10	< 0.0001	0.78	132.64	4.17
	Relative Humidity	0.01	0.001			
	Canopy Cover	0.004	0.12			
	Soil Moisture	0.004	0.25			
	Temperature	-0.004	0.67			
Dependent Variable	**Fixed Effect**	**Estimate**	**p-value**	**Adj R^2**	**AICc**	**ΔAICc**
Log 10 (µL per flower)	Species Identity	0.08	< 0.0001	0.77	157.83	29.36
	Relative Humidity	0.02	< 0.0001			
Dependent Variable	**Fixed Effect**	**Estimate**	**p-value**	**Adj R^2**	**AICc**	**ΔAICc**
Log 10 (µL per flower)	Species Identity	0.10	< 0.0001	0.78	128.47	--
	Relative Humidity	0.02	< 0.0001			
	Canopy Cover	0.003	0.15			

b) Sugar Concentration per Flower

Dependent Variable	**Fixed Effect**	**Estimate**	**p-value**	**Adj R^2**	**AICc**	**ΔAICc**
Log10 (Brix per flower)	Species Identity	0.04	< 0.0001	0.52	99.16	5.41
	Canopy Cover	-0.01	0.002			
	Relative Humidity	-0.01	0.01			
	Soil Moisture	0.002	0.49			
	Temperature	-0.003	0.74			
Dependent Variable	**Fixed Effect**	**Estimate**	**p-value**	**Adj R^2**	**AICc**	**ΔAICc**
Log10 (Brix per flower)	Species Identity	0.02	< 0.0001	0.49	98.92	5.17
	Canopy Cover	-0.01	0.005			
Dependent Variable	**Fixed Effect**	**Estimate**	**p-value**	**Adj R^2**	**AICc**	**ΔAICc**
Log10 (Brix per flower)	Species Identity	0.02	< 0.0001	0.52	93.75	--
	Canopy Cover	-0.006	0.001			
	Relative Humidity	-0.008	0.01			

Table 9. Wilcoxon/Kruskal-Wallis test to compare the difference between mean nectar volume (µL) and sugar concentration (Brix) per flower collected from field sites versus a greenhouse. Parenthesis indicates standard deviation. *Coreopsis lanceolata* had similar nectar volume or sugar concentration for both sample sources. *Rubus flagellaris* had similar nectar volume between sources but significantly different sugar concentrations.

Variable	Source	n	Mean	DF	X^2	Prob > X^2
Coreopsis lanceolata						
Volume (µL)	Field	5	0.11 (0.11)	1	1.00	0.32
	Greenhouse	9	0.04 (0.03)			
Brix	Field	5	20.2 (14.23)	1	3.49	0.06
	Greenhouse	9	32.94 (13.03)			
Rubus flagellaris						
Volume (µL)	Field	21	2.46 (2.38)	1	1.59	0.21
	Greenhouse	31	1.40 (0.79)			
Brix	Field	21	28.31 (19.35)	1	8.80	0.003
	Greenhouse	31	47.58 (20.71)			

Table 10. Wilcoxon/Kruskal-Wallis test to compare the difference between mean nectar volume and sugar concentration per flower collected within this study (Day) versus Arnold and Michaels (2017). Parenthesis indicates standard deviation. *Coreopsis lanceolata* had similar nectar volume and sugar concentration for between studies.

Variable	Source	Mean	DF	X^2	Prob > X^2
Volume (µL)	Day	0.07 (0.07)	1	0.27	0.60
	Arnold	0.06 (0.09)			
Brix	Day	28.39 (14.39)	1	0.003	0.96
	Arnold	30.63 (15.37)			

Table 11. General linear model analyses showing the relationship between (a) nectar volume (µL) and (b) sugar availability (mg) per stem with species identity, canopy cover (%), relative humidity (%), temperature (°F), and soil moisture (%). The best model is listed at the bottom and identified in bold. ΔAICc compares all models to the best model. All models were significant ($p < 0.0001$). Parameter estimate of species identity was averaged across all species. Statistical significance set at the $\alpha = 0.05$ level.

a) Nectar Volume per Stem

Dependent Variable	**Fixed Effect**	**Estimate**	**p-value**	**Adj R^2**	**AICc**	**ΔAICc**
Log10 (µL per stem)	Species Identity	0.01	< 0.0001	0.55	132.64	4.17
	Relative Humidity	0.02	0.001			
	Canopy Cover	0.004	0.11			
	Soil Moisture	0.004	0.25			
	Temperature	-0.004	0.67			
Dependent Variable	**Fixed Effect**	**Estimate**	**p-value**	**Adj R^2**	**AICc**	**ΔAICc**
Log10 (µL per stem)	Species Identity	0.001	< 0.0001	0.58	157.83	29.36
	Relative Humidity	0.02	< 0.0001			
Dependent Variable	**Fixed Effect**	**Estimate**	**p-value**	**Adj R^2**	**AICc**	**ΔAICc**
Log10 (µL per stem)	Species Identity	0.10	< 0.0001	0.55	128.47	--
	Relative Humidity	0.02	< 0.0001			
	Canopy Cover	0.01	0.15			

b) Sugar Availability per Stem

Dependent Variable	**Fixed Effect**	**Estimate**	**p-value**	**Adj R^2**	**AICc**	**ΔAICc**
Log 10 (Sugar (mg) per stem)	Species Identity	-0.08	< 0.0001	0.73	151.39	5.08
	Canopy Cover	-0.004	0.15			
	Relative Humidity	0.005	0.33			
	Soil Moisture	0.004	0.31			
	Temperature	-0.003	0.80			
Dependent Variable	**Fixed Effect**	**Estimate**	**p-value**	**Adj R^2**	**AICc**	**ΔAICc**
Log10 (Sugar (mg) per stem)	Species Identity	-0.07	< 0.0001	0.73	146.58	0.27
	Canopy Cover	-0.004	0.10			
	Relative Humidity	0.006	0.14			
Dependent Variable	**Fixed Effect**	**Estimate**	**p-value**	**Adj R^2**	**AICc**	**ΔAICc**
Log10 (Sugar (mg) per stem)	Species Identity	-0.07	< 0.0001	0.72	146.31	--
	Canopy Cover	-0.004	0.05			

Table 12. Means and standard errors (in parentheses) of sugar (Brix) and nectar volume (µL) per flower for each taxon. Sampling Sites (and abbreviations) from Lucas Co.: Bond (B), Cactus Hill (CH), Julia's Savanna (JS), Oak Dune (OD), South Piel (SP), and Wahl (W) from Kitty Todd Nature Conservancy; Blue Creek Seed Nursery (BC), Corridor 15 (C15), Flying Tigers (TMG), Jeffers (TMJ), Lark Sparrow Meadow (TML), Parkway (TPK), and Wabash (TMW) from the Toledo Metroparks; Central (LCC) and Entrance (LCE) from Lou Campbell State Nature Preserve; Southview Savanna (SS) from the Olander Park System; Visitor's Service (VS) from the Toledo Zoo; Helen's Yard (HY); and Crissey Road (CR); in Wood Co.: Rudolph Savanna (RS) from the Wood County Park District; Greenhouse (GH), Ecological Research Station (ERS), and Poe Prairie (PP) at Bowling Green State University; Wintergarden (WG) from the Bowling Green Parks and Recreation. N_{Total} = total sample size across all locations. Asterisk indicates species nectar composition characterized by Arnold (2017).

Scientific name	Common name	Locations sampled	Mean sugar conc. (Brix)	Mean volume (µL)
Achillea millefolium L.	Yarrow	GH (12), W (1), WG (2), N_{Total} = 15	5.33 (1.21)	0.17 (0.075)
Asclepias tuberosa L.*	Butterfly milkweed	LCC (5), RS (6), TMJ (6), BC (10) PR (2), SP (1), N_{Total} = 30	57.23 (17.57)	0.29 (0.018)
Baptisia tinctoria L. R. Br.	Yellow indigo	B (3), JS (4), LCC (1), OD (2), W (3), N_{Total} = 9	14.56 (5.46)	2.20 (0.50)
Ceanothus americanus L.	New jersey tea	CH (3), GH (21), N_{Total} = 24	22.44 (2.17)	0.15 (0.030)
Comandra umbellata L. Nutt.	Bastard toadflax	LCC (1), N_{Total} = 1	30 (--)	0.02 (--)
Coreopsis lanceolata L.	Lance-leaved coreopsis	CR (2), GH (9), HY (1), VS (2), N_{Total} = 14	28.39 (3.85)	0.064 (0.019)
Coreopsis lanceolata L.*	Lance-leaved coreopsis	RS (5), N_{Total} = 5	33.60 (14.88)	0.07 (0.007)
Dianthus armeria L.	Deptford pink	GH (6), SP (1), N_{Total} = 7	12.64 (3.50)	0.38 (0.23)
Euphorbia corollata L.	Flowering spurge	C15 (1), SP (2), WG (2), N_{Total} = 5	6.50 (2.24)	0.048 (0.009)
Fragaria virginiana Duchesne	Virginia strawberry	GH (3), N_{Total} = 3	23.67 (3.67)	0.53 (0.065)
Helianthus divaricatus L.*	Woodland sunflower	TMW (6), LCC (6), LCE (6), SP (1), N_{Total} = 19	43.61 (17.81)	0.06 (0.022)
Hieracium scabrum Michx.	Rough hawkweed	GH (7), N_{Total} = 7	13.79 (2.77)	0.041 (0.013)
Hypericum perforatum L.	St. Johnswort	GH (6), SS (1), W (1), N_{Total} = 8	3.06 (0.99)	1.69 (0.52)
Lespedeza capitata Michx.*	Round-headed bush clover	SP (12), N_{Total} = 12	32.67 (13.58)	1.14 (0.22)
Liatris aspera Michx. *	Rough blazing star	SP (9), LCC (7), N_{Total} = 16	59.75 (13.15)	0.12 (0.007)
Lithospermum canescens Michx.	Hoary puccoon	CH (3), LCC (11), SP (8), N_{Total} = 22	30.33 (4.02)	1.05 (0.14)
Monarda fistulosa L.*	Wild bergamot	TML (8), ERS (4), SP (1), N_{Total} = 13	56.12 (13.69)	0.03 (0.009)
Monarda punctata L.*	Dotted horsemint	TMJ (7), RS (9), SP (4), N_{Total} = 20	61.28 (13.11)	0.13 (0.014)
Potentilla simplex Michx.	Cinquefoil	C15 (1), LCE (3), WG (1), N_{Total} = 5	12.08 (7.88)	1.80 (0.82)
Rosa carolina L.	Pasture rose	OD (1), N_{Total} = 1	6 (--)	1.00 (--)
Rubus flagellaris Willd.	Dewberry	CH (5), C15 (4), GH (31), LCE (2), SP (1), WG (9), N_{Total} = 52	39.80 (3.07)	1.83 (0.23)
Rudbeckia hirta L.	Black-eyed susan	BC (1), C15 (1), LCE (3), OD (2), W (5), WG (4), N_{Total} =16	6.78 (2.30)	0.068 (0.028)

Table 13. The number of open flowers per plant as recorded when encountered in the field. Species with relatively few open flowers at a time (1-30) the total number of flowers per stem was counted. For species with greater floral availability the average number of branches, inflorescences per branch, and/or open flowers per inflorescence present on the stem was multiplied to estimate total floral availability. Sample size (n) is in reference to the number of stems in the ground counted for floral availability.

	Flowers per Plant	
Scientific Name	**Total**	**n**
Achillea millefolium L.	114.13	108
Asclepias tuberosa L.	63.84	107
Baptisia tinctoria L. R. Br.	25.34	50
Ceanothus americanus L.	3,000.78	55
Comandra umbellata L. Nutt	24.23	39
Coreopsis lanceolata L.	151.15	31
Dianthus armeria L.	2.57	48
Euphorbia corollata L.	48.56	112
Fragaria virginiana Duchesne	5.06	111
Helianthus divaricatus L.	31.37	195
Hieracium scabrum Michx.	140.87	95
Hypericum perforatum L.	5.90	134
Krigia virginiana L.	20.88	131
Lespedeza capitata Michx.	92.55	86
Liatris aspera Michx.	184.49	107
Lithospermum canescens Michx.	11.01	365
Monarda fistulosa L.	179.87	121
Monarda punctata L.	180.87	108
Potentilla simplex Michx.	1.03	265
Rosa carolina L.	1.64	140
Rubus flagellaris Willd.	9.03	92
Rudbeckia hirta L.	254.93	107

Table 14. Estimated floral resources available per stem for each species. Mean number of open flowers, nectar volume (μL) and sugar (mg) available per stem for each species. Standard error indicates by parenthesis.

Scientific Name	Common Name	# Flowers	Volume	Sugar
Achillea millefolium L.	Yarrow	114.13	18.99 (8.59)	0.58 (0.12)
Asclepias tuberosa L.	Butterfly milkweed	63.84	9.51 (1.17)	7.68 (1.33)
Baptisia tinctoria L. R. Br.	Yellow indigo	25.34	55.75 (12.62)	10.60 (4.74)
Ceanothus americanus L.	New jersey tea	3,000.78	442.78 (88.96)	134.20 (36.97)
Comandra umbellata L. Nutt.	Bastard Toadflax	24.23	0.48 (--)	0.16 (--)
Coreopsis lanceolata L.	Lance-leaved coreopsis	151.15	9.87 (2.44)	2.04 (0.34)
Dianthus armeria L.	Depford pink	2.57	0.98 (0.59)	0.26 (0.21)
Euphorbia corollata L.	Flowering spurge	48.56	2.33 (0.42)	0.15 (0.05)
Fragaria virginiana Duchesne	Virginia strawberry	5.06	2.70 (0.33)	0.68 (0.03)
Helianthus divaricatus L.	Woodland sunflower	31.37	1.77 (0.21)	0.98 (0.17)
Hieracium scabrum Michx.	Rough hawkweed	140.87	5.72 (1.77)	1.08 (0.51)
Hypericum perforatum L.	St. Johnswort	5.90	9.96 (3.10)	0.22 (0.06)
Krigia virginiana L.	Dwarf dandelion	20.88	0	0
Lespedeza capitata Michx.	Round-headed bush clover	92.55	41.81 (10.03)	12.72 (2.06)
Liatris aspera Michx.	Rough blazing star	184.49	23.34 (4.03)	17.53 (3.21)
Lithospermum canescens Michx.	Hoary puccoon	11.01	11.61 (1.52)	3.69 (0.50)
Monarda fistulosa L.	Wild bergamot	179.87	53.39 (12.26)	35.61 (7.11)
Monarda punctata L.	Dotted horsemint	180.87	51.89 (5.28)	40.84 (5.20)
Potentilla simplex Michx.	Cinquefoil	1.03	1.86 (0.85)	0.06 (0.03)
Rosa carolina L.	Pasture rose	1.64	1.64 (--)	0.10 (--)
Rubus flagellaris Willd.	Dewberry	9.03	16.53 (2.12)	6.90 (0.83)
Rudbeckia hirta L.	Black-eyed susan	254.93	17.37 (7.10)	0.92 (0.40)

Table 15. Permutational multivariate analysis of variance (PERMANOVA) was completed to test for significant differences in community composition between site categories within the (a) spring and (b) summer vegetation survey.

a) Spring

	Sum Sq	**Mean Sq**	**Df**	**F**	**Pr(>F)**
Category	1.58	0.53	3	1.88	0.03
Residuals	3.09	0.28	11		
Total	4.67		14		

b) Summer

	Sum Sq	**Mean Sq**	**Df**	**F**	**Pr(>F)**
Category	1.45	0.48	3	1.37	0.09
Residuals	3.88	0.35	11		
Total	5.32		14		

Table 16. Wilcoxon/Kruskal-Wallis test to compare the number of flowering stems per 0.5 m^2 quadrat between categories within the spring and summer vegetation surveys. Analysis includes quadrats void of flowering stems. Parenthesis indicates standard deviation. Asterisks indicate statistical significance at the $\alpha = 0.05$ level.

Season	**Category**	**Mean**	**DF**	X^2	**Prob >** X^2
Spring	Occupied	4.86 (9.72)	3	3.67	0.30
	Former	4.79 (8.78)			
	Release	3.82 (6.30)			
	Restoration	3.63 (4.95)			
Summer	Occupied	1.28 (2.59)	3	72.23	< 0.0001*
	Former	1.63 (3.01)			
	Release	2.14 (3.28)			
	Restoration	3.46 (5.73)			

Table 17. Tukey-Kramer HSD all pairs test to compare the number of flowering stems per 0.5 m^2 quadrat between categories within the spring and summer vegetation surveys. Analysis includes quadrats void of flowering stems. Data received a Log10 transformation. The difference in mean shows the actual absolute difference in the means minus the honest significant difference (HSD).

Season	Category	Δ Mean	p-value
Spring	Occupied - Former	0.07	0.77
	Occupied - Release	0.004	0.99
	Occupied - Restoration	0.02	0.99
	Former - Release	0.08	0.71
	Former - Restoration	0.10	0.57
	Release - Restoration	0.02	0.99
Summer	Occupied - Former	0.07	0.60
	Occupied - Release	0.15	0.007*
	Occupied - Restoration	0.36	< 0.0001*
	Former - Release	0.08	0.44
	Former – Restoration	0.29	< 0.0001*
	Release - Restoration	0.21	< 0.0001*

Table 18. Category percent of non-flowering quadrats appearing with spring and summer vegetation survey.

Category	Spring	Summer
Currently Occupied	41.27%	62.70%
Formerly Occupied	55.13%	51.28%
Previous Release	38.27%	46.91%
Priority Restoration	52.47%	22.22%

Table 19. Wilcoxon/Kruskal-Wallis test to compare the number of flowering stems per 0.5 m^2 quadrat between seasons within each category. Analysis includes quadrats void of flowering stems. Parenthesis indicates standard deviation. Asterisks indicate statistical significance at the $\alpha = 0.05$ level.

Category	**Category**	**Mean**	**DF**	X^2	**Prob > X^2**
Occupied	Spring	4.86 (9.72)	1	22.89	< 0.0001*
	Summer	1.28 (2.59)			
Former	Spring	4.79 (8.78)	1	0.84	0.36
	Summer	1.63 (3.01)			
Release	Spring	3.82 (6.30)	1	8.57	0.003*
	Summer	2.14 (3.28)			
Restoration	Spring	3.63 (4.95)	1	0.44	0.51
	Summer	3.46 (5.73)			

Table 20. Linear mixed effects models showing the relationship between (a) sugar availability (mg) and (b) nectar volume (μL) per quadrat with category, season (spring and summer), the interaction between category and season, and site as a random effect. Parameter estimates were averaged across all interaction effects. Quadrat containing < 90% characterized stems were removed from all models. Statistical significance set at the $\alpha = 0.05$ level.

a) Sugar availability per quadrat

Dependent Variable	**Fixed Effect**	**Estimate**	**p-value**	**Adj R^2**	**Model p-value**
Log10 (sugar/ quadrat +1)	Category* Season	0.10	< 0.0001	0.16	< 0.0001
	Season	-0.05	0.03		
	Category	-0.03	0.75		
	Total Quadrats	0.01	0.21		
	Random Effect	**Variance Ratio**	**DF**		
	Site	0.08	14		

b) Nectar volume per quadrat

Dependent Variable	**Fixed Effect**	**Estimate**	**p-value**	**Adj R^2**	**Model p-value**
Log10 (volume/ quadrat +1)	Category* Season	0.12	< 0.0001	0.13	< 0.0001
	Season	-0.05	0.09		
	Category	-0.35	0.92		
	Total Quadrats	0.01	0.22		
	Random Effect	**Variance Ratio**	**DF**		
	Site	0.07	14		

Table 21. Site estimated median sugar availability (mg) per quadrat and site total sugar (mg) within the spring and summer. Sites surveyed by Walsh (2017) were categorized as (1) currently occupied by Karner blue butterflies, (2) occupied before a drought but not after (former), (3) previous release site no longer occupied, and (4) high priority restoration site.

Category	Site	Spring		Summer	
		Quadrat	Total	Quadrat	Total
Currently Occupied	O1	0.82	73.32	0.38	37.03
	O2	11.20	590.55	1.08	342.90
	O3	4.30	123.84	0.15	2.72
	O4	6.90	321.76	1.10	26.33
	O5	24.16	786.82	0.36	201.71
Formerly Occupied	F1	2.69	132.93	1.10	114.89
	F2	11.29	453.80	0.30	10.87
	F3	11.83	368.36	1.08	206.52
Previous Release	P1	27.62	904.66	0.58	2,708.35
	P2	0.32	39.91	9.77	5,744.54
	P3	0.32	234.85	10.60	1,114.87
	P4	0.29	258.05	3.91	2,362.01
Priority Restoration	R1	20.71	2,156.63	19.08	6,785.90
	R2	0.10	7.45	0.98	112.18
	R3	0.26	21.76	25.44	9,989.92

Table 22. Generalized linear modeling showing the relationship between site (a) total sugar (g) and (b) total nectar volume (mL) with site category (occupied, formerly occupied, previous release, and restoration), season (spring and summer), and the interaction between site category and season. Parameter estimates were averaged across all effects. Statistical significance set at the $\alpha = 0.05$ level.

(a) Total sugar availability

Dependent Variable	Fixed Effect	Estimate	p-value	Adj R^2	Model p-value
Log10 (Total Sugar)	Category*Season	0.59	0.002	0.33	0.005
	Category	-0.06	0.04		
	Season	-0.14	0.24		

(b) Total nectar volume

Dependent Variable	Fixed Effect	Estimate	p-value	Adj R^2	Model p-value
Log10 (Total Volume)	Category*Season	0.40	0.002	0.33	0.005
	Category	-0.06	0.05		
	Season	-0.09	0.30		

Table 23. List of Spearman's rank correlation between the number of flowering stems per $0.5m^2$ quadrat and the corresponding sugar (mg) per $0.5m^2$ quadrat within each category across both spring and summer vegetation surveys. Asterisks indicate statistical significance at the $\alpha = 0.05$ level.

Category	Spearman's Coefficient	p - value
Currently Occupied	0.75	< 0.0001*
Formerly Occupied	0.68	< 0.0001*
Previous Release	0.48	< 0.0001*
Priority Restoration	0.64	< 0.0001*

Table 24. Tukey-Kramer HSD all pairs test to compare the number of flowering stems and nectar sugar (mg) per 0.5 m^2 quadrat between categories within the spring and summer vegetation survey. Analysis of sample populations with flowering stems characterized for floral and sugar abundance. Empty quadrats and species not characterized for resource availability were not included. Data received a Log10 transformation. The difference in mean shows the actual absolute difference in the means minus the honest significant difference (HSD).

Season	Category	# Stems Δ Mean	# Stems p-value	Sugar Δ Mean	Sugar p-value
Spring	Occupied - Former	0.10	0.67	0.11	0.94
	Occupied - Release	0.28	0.0003*	0.81	< 0.0001*
	Occupied - Restoration	0.19	0.06	0.39	0.09
	Former - Release	0.38	< 0.0001*	0.70	0.001*
	Former - Restoration	0.28	0.008*	0.28	0.49
	Release - Restoration	0.10	0.53	0.42	0.04
Summer	Occupied - Former	0.08	0.77	0.09	0.98
	Occupied - Release	0.14	0.21	1.00	< 0.0001*
	Occupied - Restoration	0.16	0.09	1.21	< 0.0001*
	Former - Release	0.06	0.85	0.90	< 0.0001*
	Former - Restoration	0.08	0.65	1.12	< 0.0001*
	Release - Restoration	0.02	0.97	0.21	0.45