

Elevated temperature and periodic water stress alter growth and quality of common milkweed (*Asclepias syriaca*) and monarch (*Danaus plexippus*) larval performance

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Abstract In this study, we examined the independent and interactive effects of temperature and water availability on the growth and foliar traits of common milkweed (*Asclepias syriaca*) and performance of a specialist herbivore, larvae of the monarch butterfly (*Danaus plexippus*). Milkweed from multiple population sources collected across a latitudinal gradient in Wisconsin, USA, were grown under all combinations of ambient or elevated temperature and the presence or absence of periodic water stress. Elevated temperature marginally increased, while water stress decreased plant growth. Milkweed from more northerly latitudes experienced larger growth responses to elevated temperature and were more resistant to water stress, especially under higher temperatures. Elevated temperature and water stress also altered milkweed composite foliar trait profiles. Elevated temperature generally increased leaf nitrogen and structural compounds, and decreased leaf mass per area. Water stress also elevated foliar nitrogen, but reduced defensive traits. Monarch larvae performed well on milkweed under elevated temperature and water stress, but gained the most mass on plants exposed to both treatments in combination. Our

findings suggest that milkweed populations from more northerly latitudes in the upper Midwest may benefit more from rising temperatures than those in southerly locations, but that these beneficial effects depend on water availability. Monarch larvae grew larger on plants from all experimental treatments relative to ambient condition controls, indicating that future changes in milkweed presence on the landscape will likely influence monarch populations more than the effects of future changes in plant quality on larval performance.

Keywords *Asclepias* · Common milkweed · *Danaus plexippus* · Elevated temperature · Monarch butterfly · Water stress · Plant–insect interactions

Introduction

Climate change is among the most pressing environmental factors currently affecting ecosystem stability on Earth. Anthropogenic activities, such as land-use change and fossil fuel consumption, have affected the Earth's climate system, with global average temperatures markedly increasing over the past 150 years and the greatest rate of increase in the last quarter century (IPCC 2013). By the end of this century, global average temperatures are predicted to increase by as much as 5 °C relative to pre-industrial levels (IPCC 2013). The magnitude and timing of precipitation events are also expected to change under future climate predictions, but with patterns of change more variable and regionally dependent. In the Midwestern USA, the frequency of heavy precipitation events is expected to increase, along with the length of time in between these events, suggesting an increase in the periodic occurrence of drought conditions (U S Global Change Research Program

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2009). Climate change is already affecting numerous ecosystems globally (Parmesan 2006; Pelini et al. 2009), and future shifts in climate will likely have continued consequences for ecosystem functioning and stability.

Temperature and precipitation play influential roles in plant–insect interactions (Zvereva and Kozlov 2006; Pelini et al. 2009; Cornelissen 2011; Jamieson et al. 2012). Elevated temperature can enhance plant and insect growth rates directly by accelerating metabolic activity, assuming a lack of growth-limiting factors, and that the increase does not exceed physiological constraints. Conversely, plants generally decrease growth under persistent moisture stress (as well as associated changes in atmospheric vapor pressure deficit) and employ a number of biochemical and physiological mechanisms to tolerate water deficits (Chaves et al. 2003). Indirectly, increased temperature and water stress influence plant–insect interactions through altered foliar quality, with consequences for herbivore performance (Zvereva and Kozlov 2006; Gutbrodt et al. 2011; Jamieson et al. 2013).

The monarch (*Danaus plexippus*) is one of the most recognized insects on Earth. In central and eastern North America in the fall, monarch butterflies migrate from southern Canada and northern midwest and eastern USA to overwintering habitat in Mexico. In the spring, monarchs return northward to breeding grounds in the USA and continue to move northward, across multiple generations, into the northern USA and southern Canada. Given the monarch's charisma and global recognition, it acts as a flagship insect species, providing awareness and education about anthropogenic impacts on the environment, including habitat degradation and loss and nontarget effects of pesticides (Guiney and Oberhauser 2009). While not currently endangered, loss of overwintering sites in Mexico and host plant habitat in the USA, in combination with altered climatic events, have been implicated in reductions in monarch population sizes (Oberhauser and Peterson 2003; Zalucki and Lammers 2010; Brower et al. 2012; Pleasants and Oberhauser 2013).

Two environmental factors that strongly influence monarch populations are temperature and precipitation (Malcolm et al. 1987). A number of studies have modeled the effects of temperature and precipitation patterns on population-level responses of monarchs (Zalucki and Rochester 2004; Batalden et al. 2007; Zipkin et al. 2012). Fewer studies, though, have examined the effects of environmental variation on the physiological responses by juvenile monarchs themselves (York and Oberhauser 2002; Serratore et al. 2013). To date, no studies have examined how predicted future changes in temperature and precipitation will affect the growth and quality of a main host plant for the monarch, *Asclepias syriaca*, the common milkweed.

Monarchs are specialists on host plants in the subfamily Asclepiadoideae, with *A. syriaca* most widely utilized by breeding populations in the Midwestern USA (Malcolm et al.

1989). There is a long, rich ecological and evolutionary history of plant–insect interactions within the milkweed system (Malcolm 1992, 1995; Mooney et al. 2010; Agrawal et al. 2012) with a central focus being on phytochemical variation. A suite of plant traits influences milkweed–insect interactions (Agrawal and Fishbein 2006). Foliar nitrogen and the ratio of carbon to nitrogen (C/N) are often limiting factors for insect growth and development (Mattson 1980). Foliar structural and morphological traits [e.g., fiber, lignin, and leaf mass per area (LMA)] also influence insect performance by increasing leaf recalcitrance or by altering the balance of nutrients with other constituents (Coley 1983; Agrawal and Fishbein 2006; Clissold et al. 2009).

Milkweeds display additional characteristics targeted to reduce herbivory (i.e., chemical and physical defenses), which include cardenolide production, latex mobilization, and physical structures such as trichomes. Cardenolides are nonvolatile, phloem-mobile steroidal toxins that disrupt cellular ion transport (Malcolm 1992; Agrawal et al. 2012) and are rapidly induced in response to herbivory (Malcolm and Zalucki 1996; Agrawal et al. 2012). Trichomes and latex present physical barriers to herbivory and generally have negative relationships with larval monarch performance (Malcolm and Zalucki 1996; Zalucki et al. 2001; Agrawal 2005; Zalucki et al. 2012). Importantly, plant traits influencing herbivory in common milkweed are environmentally plastic and vary considerably among genotypes of *A. syriaca* (Vannette and Hunter 2011; Tao and Hunter 2012; Agrawal et al. 2012).

Our primary objectives in this study are to (1) assess how predicted changes in temperature and water stress affect the growth and quality of common milkweed, (2) determine whether responses to the treatments are consistent among common milkweed from different populations and geographic locations, and (3) measure how changes in plant quality affect monarch performance. Specifically, we predicted that (1) elevated temperature would increase and water stress would decrease plant growth, (2) elevated temperature and water stress would alter the composite trait profiles of *A. syriaca*, (3) elevated temperature and water stress would both independently and interactively alter individual plant traits, (4) plant responses to temperature and water stress would vary between northern and southern populations, and (5) changes in plant traits would influence monarch larval growth.

Methods

Plant and insect culture

We collected seeds of *A. syriaca* from five separate open-field locations in Wisconsin (WI) and Michigan (MI),

USA, in the fall of 2011. To represent “northern” populations, two sets of pods were collected in northern WI near Cedar (CED; N 46.5, W –90.5) and Woodruff (KMP; N 45.8, –89.6), respectively, and one set of pods was collected in northern MI near Bonds Falls (BFS; N 46.4, W –89.1). To represent “southern” populations, two sets of seed pods were collected in south-central WI (TRU; N 43.0, –89.4 and MWH; N 43.1, –89.5). To ensure we had enough plants for appropriate replication, we collected four seed pods from two closely (<0.5 m) positioned ramets of *A. syriaca* to ensure that the pods were taken from a single genet at each location. Pods were stored in a paper bag and cold-stratified for 3 months. Seeds were germinated in a greenhouse in MetroMix potting medium #300 in 61 × 30 cm plastic flats for 4 weeks, after which plants were removed, weighed, and transferred to individual 1L pots containing the same medium.

The study was conducted in February–April 2012 in WI, USA. As such, monarch larvae were not available from naturally occurring populations. We purchased newly hatched first-instar monarch larvae from Shady Oak Butterfly Farm (Brooker, FL). Larvae were fed leaves of *A. curassavica* during shipping and, upon arrival, were immediately removed from the shipping container, starved for 24 h to void gut contents, and weighed prior to the feeding trial.

Experimental design

This study was performed in environmentally controlled rooms at the University of Wisconsin Biotron. Average summer (June, July, and August) temperature in WI from 2000 to 2010 was approximately 24 °C (www.aos.wisc.edu/~sco/clim-watch/archives.html). While we acknowledge that considerable variation exists along the latitudinal gradient from which we collected seed pods, it was not logistically possible to grow each plant population in the average summer temperature from the location they were collected. Thus, to standardize temperatures across the experiment, we used a statewide average summer temperature and maintained two rooms at “ambient” temperatures (25:18 °C, day/night). Summer temperatures in WI are projected to increase as much as 5 °C (WICCI 2011); therefore, we simulated a warming environment using two rooms at “elevated” temperatures (30:23 °C, day/night). The four rooms were separated into two blocks, with an ambient and elevated room in each block. To simulate changes in precipitation patterns, half of the plants in each room for each temperature regime were exposed to periodic water stress. The water stress treatment involved watering plants to saturation at the onset of the experiment and allowing them to desiccate until symptoms of water stress were observed (e.g., minor wilting and leaf curling). After symptoms of water stress were apparent, the plant

was thoroughly watered until saturation. The timing of watering events in the periodic water stress treatments varied between the temperature regimes: watering was needed less frequently at ambient temperatures (average 7.5 days) than under elevated temperatures (average 5 days), given the larger evaporative demand in the warmer rooms.

Asclepias syriaca growth and quality

Plants (total $n = 453$) from each population were randomly assigned to one of four possible treatment combinations: ambient temperature, no water stress; ambient temperature, periodic water stress; elevated temperature, no water stress; and elevated temperature, periodic water stress. Because of different germination success rates among plant population sources, the number of plants from each population source used in each treatment combination in each room varied ($n = 5–15$). Plants were grown under their respective treatment condition for 60 days, after which plants were harvested and the roots, stems, and leaves were dried at 50 °C. Initial plant dry mass was calculated based on a wet/dry mass ratio ($R^2 = 0.95$) determined from a subset of plants ($n = 20$) distributed evenly across the five plant population sources during transplanting. Final total plant growth was calculated as final dry mass minus initial dry mass.

Fresh leaves of *A. syriaca* were assayed for a suite of chemical and morphological properties that influence plant and insect growth. Foliar carbon, nitrogen, fiber, lignin, cardenolide concentrations, LMA, and water content were estimated using reflectance spectroscopy. Spectroscopy is emerging as an effective tool to rapidly and accurately characterize a wide variety of plant traits (Foley et al. 1998; Asner and Martin 2011; Serbin et al. 2014). Reflectance spectroscopy exploits specific structural and leaf biochemical absorption features within and on the surface of leaves across the visible, near infrared, and shortwave infrared wavelengths to nondestructively estimate the value of foliar traits. Importantly, reflectance spectroscopy provides the ability to repeatedly measure real-time, in vivo responses by plants to biological stress and environmental variation (e.g., Asner and Martin 2008; Serbin et al. 2012; Couture et al. 2013). The ability of reflectance spectroscopy to rapidly and nondestructively determine plant traits makes the technique an invaluable tool with a wide variety of ecologically based applications. An underappreciated benefit of reflectance spectroscopy is the ability to obtain information on multiple foliar traits, simultaneously and nondestructively, from a single spectral measurement.

Foliar carbon (C), nitrogen (N), fiber, lignin concentrations (% dry mass), and LMA (g m^{-2}) were generated from

calibrations from Serbin (2012). Foliar cardenolide concentrations ($\mu\text{g mg}^{-1}$) were generated from calibrations from Couture et al. (2013), amended to expand the dynamic range of the calibration model. The modeling approach used in the current study was similar to that from Couture et al. (2013), and model diagnostic statistics for cardenolide predictions using spectroscopy can be found in supporting information (Fig. S1). Foliar water content was determined using the normalized differential water index (NDWI) and calculated as the relative difference between reflectance at wavelengths 857 and 1241 nm (Gao 1996).

Leaf reflectance was measured using a high-spectral-resolution ASD FieldSpec 3 Full-Range (350–2500 nm) spectroradiometer (Analytical Spectral Devices, Boulder, CO, USA). All measurements were taken from the leaf adaxial surface using a leaf-clip assembly that was attached to a plant probe having an internal, calibrated light source. For consistency among foliar measurements over time, reflectance was measured on the four uppermost fully expanded leaves of individual plants, with five spectra averaged per leaf, and the spectra from the four leaves were averaged to determine the mean leaf reflectance of the plant. Spectral measurements were taken at weeks four and eight of the experiment.

Plant quality traits measured in this study not currently able to estimate using spectroscopy were measured using standard approaches. Foliar trichomes (mm^2) were quantified by counting all trichomes on hole punches collected from leaves. Two 6-mm-diameter hole punches were collected from the two uppermost fully expanded leaves, and trichomes were counted using a microscope at $4\times$ magnification. Trichome counts were averaged to produce a single estimate of foliar trichome densities. Latex (mg) was determined gravimetrically by collecting latex exuding from the hole punches on preweighed filter paper disks (6 mm diameter), then dried to a constant mass at 50°C , and reweighed.

Monarch larval growth

Early-stage, first-instar larvae ($n = 137$) of similar size were individually weighed and placed on single plants from each possible combination of the five population sources and four treatments. Monarch larvae were reared under the same environmental conditions as plants. Due to differing numbers of plants, the number of plants per population source in each treatment combination for the larval bioassay varied ($n = 2\text{--}5$) for each block. Monarchs were placed on an upper fully expanded leaf, the plant was then covered in a fine mesh bag, and larvae were allowed to feed for 7 days. After 7 days, larvae were removed from the plant and kept in a rearing cup for 48 h to void gut contents, then frozen, lyophilized, and weighed. Initial

larval dry mass was calculated using a wet/dry mass ratio ($R^2 = 0.94$) determined from a subset of larvae ($n = 10$) at the onset of larval bioassay. Larval growth was calculated as final dry mass minus initial dry mass.

Statistical analyses

We determined the influence of temperature, water stress, and plant population source on the composite trait profiles of *A. syriaca* using permutational multivariate analysis of variance (PERMANOVA; Anderson 2001), employing Bray–Curtis measurements of dissimilarity and 999 permutations. Composite foliar trait responses were visualized with nonmetric multidimensional scaling (NMS) with Bray–Curtis dissimilarities. Traits included in these analyses were foliar nitrogen, fiber, lignin, cardenolide concentrations, C/N ratios, LMA, trichome densities, latex amount, and water content. Trait data used in these analyses were averaged by plant population, and traits that were repeatedly measured (nitrogen, C/N, fiber, lignin, cardenolide concentrations, LMA, and foliar water content) were also averaged across time periods. If PERMANOVA indicated a statistically significant shift in the composite trait profile under one of the factors, we further examined the percent contribution of each plant trait to the overall profile shift using similarity percentage analysis (SIMPER; Clarke 1993).

To determine the effects of temperature, water stress, plant population source, and time on foliar nitrogen, fiber, lignin, cardenolide concentrations, C/N ratios, and water content (NDWI), we used a repeated measures analysis of variance (ANOVA) with a multi-split-plot design following the model $Y_{ijklm} = b_i + T_j + e_{ij} + W_k + TW_{jk} + \varepsilon_{ijk} + P_l + TP_{jl} + WP_{kl} + TWP_{jkl} + \varepsilon_{ijkl} + C_m + TC_{jm} + WC_{km} + TWC_{jkm} + PC_{lm} + TPC_{jlm} + WPC_{klm} + TWPC_{jklm} + E_{ijklm}$. In this model, b represents block i , T represents temperature j , e represents whole-plot error, W represents water treatment k , ε represents split-plot error, P represents seed population source l , ε represents the split-split-plot error, C represents time m , and E represents the split-split-split-plot error. Y_{ijkl} represents the average response of block i , temperature j , water treatment k , and seed population source l . Total plant growth, foliar trichomes, and latex were analyzed using a similar model, except with time removed as an effect. In the case of monarch larval growth, we used an analysis of covariance (ANCOVA) with a similar model as above, again removing time as an effect but including initial dry weight as a covariate. F tests were conducted with degrees of freedom assigned using the Satterthwaite approximation. The low replication of main effects in this statistical design, while most appropriate, increases the potential for type II errors. To balance between the potential for committing type I versus type II

errors, we report P values $0.05 < 0.10$ as “marginally” significant and P values < 0.05 as “significant.”

Relationships between temperature and foliar traits with monarch growth were examined using partial least squares regression (PLSR) following Couture and Lindroth (2012). Larger mean regression coefficients indicate greater contributions by predictor variables to the response, and the sign indicates the direction of the influence of the variable on the larval performance variable. No growth or chemical data were collected from plants that monarchs fed on directly; as such, relationships between plant traits and monarch growth were based on plant traits collected from the larger subset of plants (described above). Foliar trait information used in this analysis was from the second collection time only. Plant trait and monarch growth data were averaged within each room by water treatment and population ($n = 40$).

Examination of residuals confirmed that data met assumptions of normality. Statistical analyses were performed using R (www.r-project.org) or JMP version 10.0 statistical software (SAS Incorporated, 2012, Cary, NC, USA).

Results

Plant growth

Milkweed growth was influenced by temperature (T), water stress, plant population source (pop.), and their interactions (Table 1; Fig. 1). Overall, elevated temperature marginally increased total plant growth by $\sim 32\%$, while water stress decreased total plant growth by $\sim 76\%$ (Fig. 1). Northerly plant populations had larger growth responses to elevated

temperature (average $\sim 80\%$) than southerly plant populations (average $\sim 10\%$; Fig. 1), but substantial variation existed in this response across the three northerly plant populations (range of increase: 31–111 %, marginally significant $T \times \text{pop.}$ interaction, Table 1). The effect of water stress also varied among plant populations, but depended on temperature, such that the negative effect of water stress on plant growth was greatest in plants from southerly, compared to northerly, populations under elevated temperature (marginally significant $T \times \text{water stress} \times \text{pop.}$ interaction, Table 1).

Plant traits

PERMANOVA revealed that elevated temperature (pseudo- $P < 0.001$) and periodic water stress (pseudo- $P < 0.008$) both altered the composite phytochemical profile of *A. syriaca* (Fig. 2). Relationships of individual foliar traits with NMS axes revealed that the presence of either stressor generally increased nutritional quality. Elevated temperature decreased LMA and trichome densities and increased latex production, while water stress reduced foliar C/N ratios, water content, latex, and trichomes and increased cardenolide levels. Subsequent SIMPER analysis revealed that changes in foliar morphological, structural, and nutritional characteristics (i.e., LMA, trichomes, fiber, lignin, and C/N) accounted for over 90 % of the variation in composite foliar trait profiles under elevated temperatures and water stress (Table 2).

Plant quality variables repeatedly measured over time were influenced by temperature, water stress, plant population source, time, and their interactions (Fig. 3; SI Tables 1 and 2). On average, foliar nitrogen levels were $\sim 10\%$ higher under elevated temperature (Fig. 3; SI Table 1). Elevated temperatures offset a decline in nitrogen levels under ambient temperature conditions later in the study (i.e., leaves lost nitrogen with time, except under elevated temperatures, significant $T \times \text{time}$ interaction, SI Table 2), but this response varied among plant populations (significant $T \times \text{pop.} \times \text{time}$ interaction, SI Table 2). While statistically significant, the magnitude of difference in foliar nitrogen levels among populations was small ($\leq 5\%$). Overall, periodic water stress increased foliar nitrogen levels by 20 % (Fig. 3; SI Table 1). The magnitude of increase, however, varied among temperature levels, plant populations, and across time (significant $T \times \text{water stress} \times \text{pop.} \times \text{time}$ interaction, SI Table 2). Opposite of foliar nitrogen, C/N levels were lower in elevated temperature (8 %) and under periodic water stress (14 %; Fig. 3; SI Table 1). Elevated temperature offset an increase in C/N ratios with time (significant $T \times \text{time}$ interaction, SI Table 2), and the magnitude of this response varied among plant populations (significant $T \times \text{pop.} \times \text{time}$

Table 1 Summary of F and P values for the effects of temperature, water stress, plant population source, and their interactions on total plant growth

| Treatments and interactions | df | Total plant growth | |
|---|-------|--------------------|------------------|
| | | F | P |
| Temperature | 1,2 | 9.1 | <i>0.071</i> |
| Water stress | 1,2 | 110.2 | 0.005 |
| Temperature \times water stress | 1,2 | 2.2 | 0.257 |
| Population | 4,248 | 11.6 | <0.001 |
| Temperature \times population | 4,248 | 2.4 | <i>0.050</i> |
| Water stress \times population | 4,248 | 9.3 | <0.001 |
| Temperature \times water stress \times population | 4,248 | 2.1 | <i>0.078</i> |

P values < 0.05 are bolded, and P values $0.05 < P < 0.10$ are italicized

Numerator and denominator degrees of freedom (df numerator, denominator) were calculated using the Satterthwaite approximation

Fig. 1 Response magnitudes of total plant growth relative to control plant growth. Data presented were both separated for each plant population and averaged across populations for all possible treatment combinations of ambient temperature, water stress (H_2O stress); elevated temperature, no water stress (eTemp.); or the combination of elevated temperature and water stress (eTemp. + H_2O stress). Error bars represent ± 1 SE. Northern populations: BFS (Bonds Falls, MI); CED (Cedar, WI); KMP (Woodruff, WI). Southern populations: MWH (Madison, WI); TRU (Madison, WI). Latitudinal designation is under population code

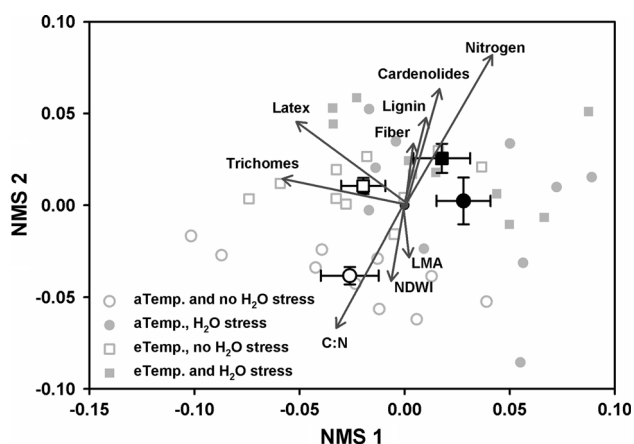
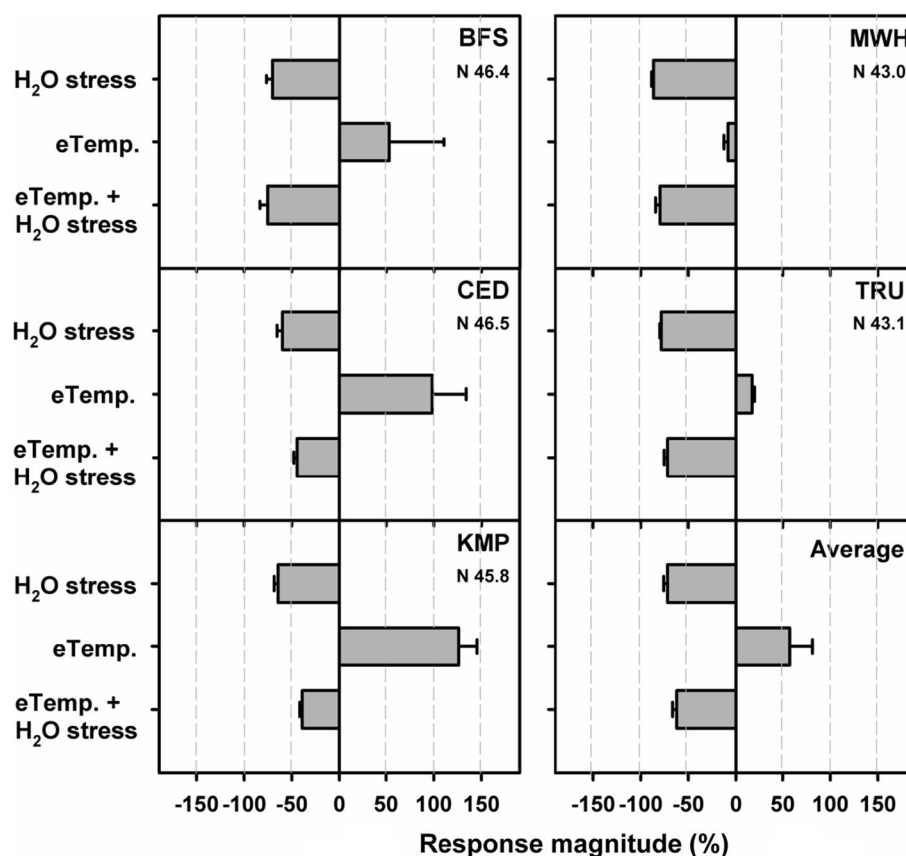


Fig. 2 Nonmetric multidimensional scaling (NMS) ordination plots demonstrating differences between composite plant trait profiles of common milkweed exposed to all possible combinations of ambient (aTemp.) and elevated (eTemp.) temperature and the absence (no H_2O stress) or presence (H_2O stress) of water stress. Gray symbols represent average for each population by each water treatment, across time, within each room (i.e., temperature treatment; $n = 40$), and black points represent mean treatment effects, \pm SE, averaged across time and populations. Solid arrows show relationships of foliar traits with NMS axes. Units: nitrogen, fiber, lignin (% dry mass); cardenolides ($\mu\text{g mg}^{-1}$); LMA (g m^{-2}); trichomes (mm^2); latex (mg)

interaction, SI Table 2). Variation in C/N levels among plant populations was statistically significant; yet, the magnitude of response was minimal ($\leq 5\%$). Overall,

water stress decreased C/N levels by $\sim 14\%$ (Fig. 3; SI Table 1); yet, the magnitude of decrease varied among temperature levels, plant populations, and time points (significant $T \times$ water stress \times pop. \times time interaction, SI Table 2). The effects of elevated temperature on water content varied among populations and across time, such that water content decreased under elevated temperatures in more northerly populations, but increased in more southerly populations (significant $T \times$ pop. interactions, SI Table 2), and the responses were more pronounced earlier, compared with later, in the study, and greater under water stress conditions (significant T water stress \times pop. \times time interaction, SI Table 2). Overall, water stress decreased foliar water content by $\sim 16\%$ (Fig. 3; SI Table 1). The negative effect of water stress on foliar water content was larger in more northerly populations more pronounced earlier in the study (significant water stress \times pop. \times time interaction, SI Table 2).

Fiber and lignin levels increased $\sim 10\%$ under elevated temperature (Fig. 3; SI Table 1). The effects of higher temperatures were generally larger earlier in the study, and under conditions of water stress (significant $T \times$ water stress \times time interaction, SI Table 2), the magnitude and direction of the responses varied across times and among plant populations (significant $T. \times$ water stress \times pop. \times time interaction, SI Table 2). On average, LMA decreased $\sim 23\%$

Table 2 Mean values of individual plant traits exposed to either ambient or elevated temperature (top panel) of no water stress or water stress (bottom panel)

| Plant trait | Ambient temperature mean trait value | Elevated temperature mean trait value | Contribution (%) |
|-------------------------------------|--------------------------------------|---------------------------------------|------------------|
| LMA (g m ⁻²) | 63.1 | 48.3 | 52.5 |
| Trichomes (mm ²) | 11.9 | 14.1 | 15.6 |
| Fiber (% dm) | 30.4 | 33.1 | 11.3 |
| Lignin (% dm) | 14.9 | 16.4 | 9.1 |
| C/N | 14.3 | 13.2 | 6.2 |
| Latex (mg) | 1.2 | 1.4 | 2.1 |
| Nitrogen (% dm) | 3.3 | 3.5 | 1.6 |
| Cardenolides (μg mg ⁻¹) | 1.7 | 2.0 | 1.2 |
| NDWI | 0.03 | 0.03 | 0.03 |

| Plant trait | No water stress mean trait value | Water stress mean trait value | Contribution (%) |
|-------------------------------------|----------------------------------|-------------------------------|------------------|
| LMA (g m ⁻²) | 56.3 | 55.1 | 46.6 |
| Trichomes (mm ²) | 14.7 | 11.4 | 17.5 |
| Fiber (% dm) | 31.7 | 31.8 | 11.0 |
| Lignin (% dm) | 15.2 | 16.1 | 10.0 |
| C/N | 14.9 | 12.6 | 8.6 |
| Latex (mg) | 1.4 | 1.1 | 2.4 |
| Nitrogen (% dm) | 3.1 | 3.7 | 2.2 |
| Cardenolides (μg mg ⁻¹) | 1.8 | 2.0 | 1.3 |
| NDWI | 0.04 | 0.03 | 0.03 |

Repeatedly measured data are averaged across time, and traits are ranked in order of largest contribution (%) to the overall dissimilarity between leaves from ambient compared with elevated temperature and no water stress compared with water stress, as determined by SIMPER analyses

LMA leaf mass per area, C/N ratio of carbon to nitrogen, NDWI normalized differential water index, % dm percent dry mass

under elevated temperature (Fig. 3; SI Table 1), and elevated temperature offset an increase in LMA with time, but the response magnitude varied among plant populations (significant T × pop. × time interaction, SI Table 2), and was observed only under conditions of high water availability (significant T × water stress × time interaction, SI Table 2). Increases in LMA later, compared with earlier, in the study, were largest under periodic water stress (significant water stress × time interaction, SI Table 2); the magnitude of this response, however, varied among plant populations (significant water stress × pop. × time interaction, SI Table 2).

Water stress generally increased cardenolide levels (Fig. 3; SI Table 1), but the response was greatest under elevated temperatures (significant T × water stress interaction, SI Table 2). This response was most pronounced earlier than later in the study (significant T × water

stress × time interaction, SI Table 2), but the response magnitude varied among plant populations (significant T × water stress × pop. × time interaction, SI Table 2). Elevated temperatures had little effect on foliar trichome densities, while water stress decreased trichome levels by ~30 % (Fig. 3; SI Table 3); the response magnitude, however, varied among plant populations (significant water stress × pop. interaction, SI Table 4). Overall, the effect of temperature on latex was dependent on water stress, such that latex production was greatest in plants in elevated temperature with high water availability (significant T × water stress interaction, SI Table 4). While plants with high water availability generally had higher latex levels than plants experiencing periodic water stress, substantial variation in the response magnitude existed among plant populations (significant water stress × pop. interaction, SI Table 4).

Monarch growth

Monarch larval growth was influenced by temperature, water stress, plant population source, and the temperature × population interaction. (Figure 4; Table 3). Elevated temperature increased monarch larval growth by 35 %; the magnitude of this response, however, varied among plant populations. The increase in larval growth under elevated temperature was greater on plants from southerly (38 % average increase) compared with northerly populations (28 % increase). Monarch larvae grew ~13 % larger on water-stressed plants, a response that was consistent among plant population sources. Monarch larval growth varied substantially among plant population sources, and the magnitude of growth increase under elevated temperature, but not water stress, varied among plant population source (Fig. 4; Table 3). PLSR analysis relating monarch growth with plant traits produced a significant relationship ($R^2 = 0.67$, $P \leq 0.001$). Temperature and nitrogen concentrations had the most positive relationships, while C/N ratios and leaf water content had the most negative relationships with larval growth (Table 4).

Discussion

Our study examined the influence of elevated temperature and water stress on the growth and foliar quality of common milkweed and larval performance of the monarch butterfly. Both elevated temperature and water stress altered milkweed growth and foliar quality, although the response varied across plants from different geographic populations. Monarch larvae grew larger under elevated temperatures and on plants that experienced water stress. Elevated temperature had the strongest positive effect on

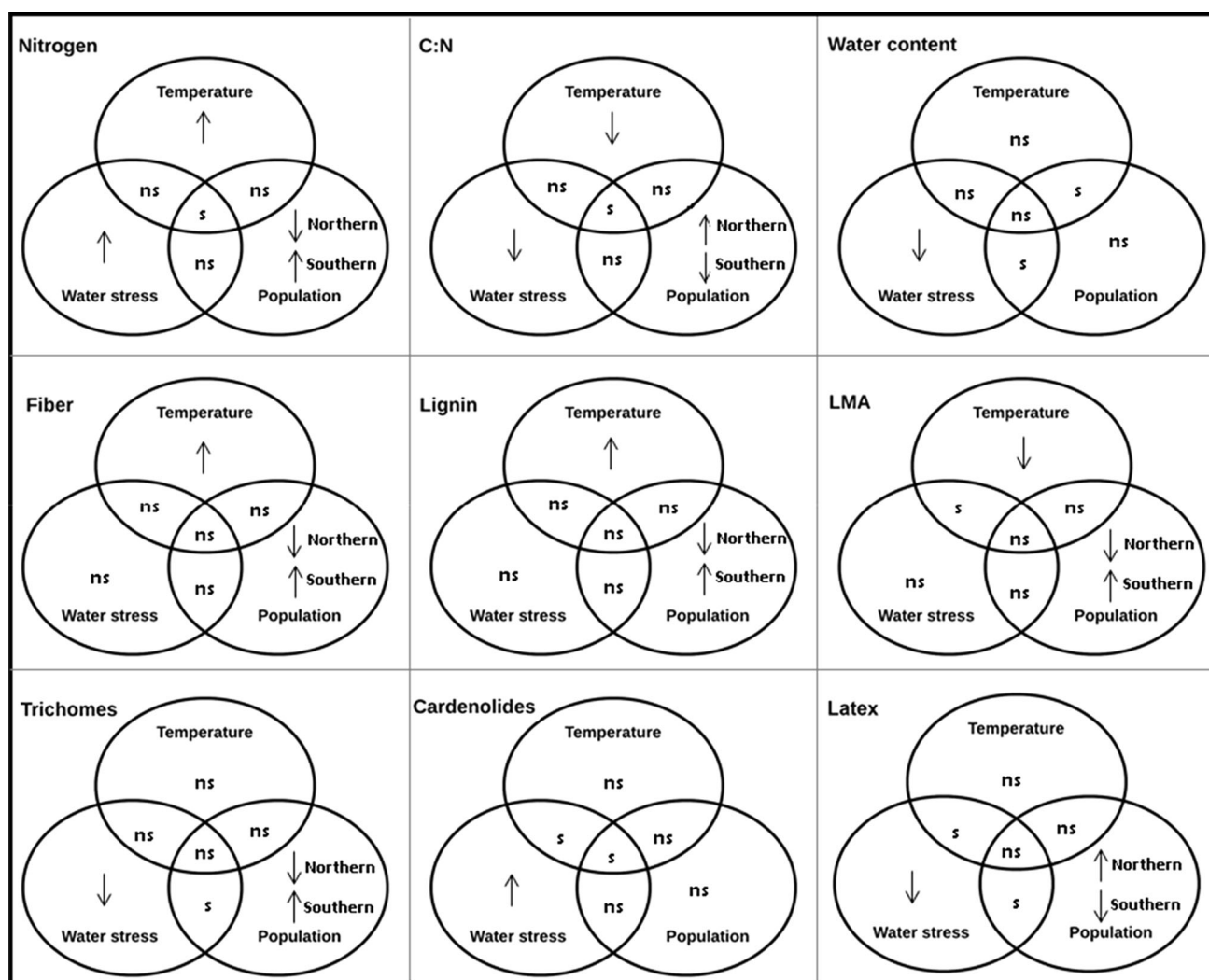


Fig. 3 Independent and interactive effects of elevated temperature, water stress, and population on foliar traits of common milkweed. C/N, ratio of carbon to nitrogen; LMA, leaf mass per area; water

content determined by NDWI. Arrows or “s” indicate a significant response at $P < 0.10$, and the *direction of the arrow* indicates the trait response; ns, nonsignificant. Units are the same as Fig. 2

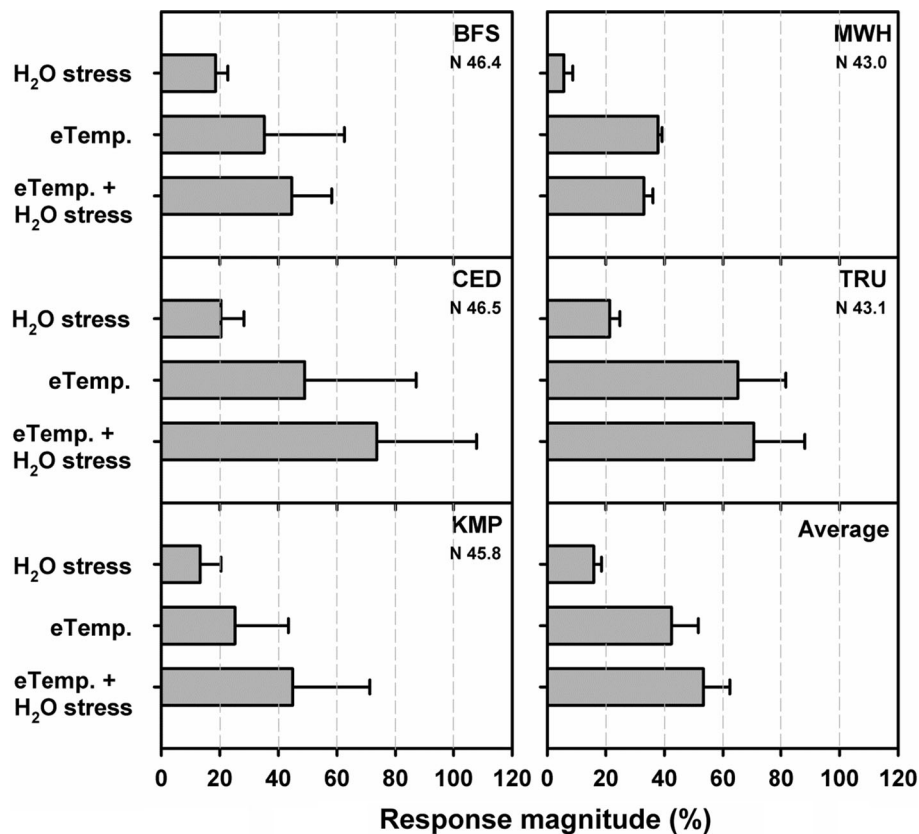
monarch growth, followed by increased foliar nitrogen levels, while decreased water content and increased C/N had the largest negative influences on monarch growth. While extrapolating population growth responses from the performance of individuals is risky (Awmack and Leather 2002), a generally positive relationship exists between insect mass and fecundity (Honěk 1993). The enhanced growth of monarch larvae under all treatments, relative to the control, suggests that monarch populations may be less affected by the influence of changes in plant quality on larvae performance in future environments and more affected by the loss of milkweed habitat across the landscape.

Milkweed growth and foliar traits

As we expected, relative to control treatments, milkweed grew larger under elevated temperatures and grew less

when water stressed. Studies examining the effects of temperature and drought on plant growth generally report that elevated temperature increases (e.g., Veteli et al. 2002; Nybakken et al. 2012; Lavola et al. 2013) and water stress limits plant growth (e.g., Gutbrodt et al. 2011; Jamieson et al. 2013). In agreement with our second hypothesis, elevated temperature and water stress altered the composite trait profiles of *A. syriaca*. Confirming our third hypothesis, elevated temperature and water stress influenced individual plant traits. Elevated temperatures caused foliar nitrogen concentrations to increase and thus C/N levels to decrease. While foliar nutrient levels are thought to be largely unaffected by elevated temperature (Zvereva and Kozlov 2006), studies have reported increased nitrogen levels with elevated temperatures for a range of species (Tingey et al. 2003; Serbin et al. 2012). Tingey et al. (2003) reported increased foliar nitrogen levels in response to elevated

Fig. 4 Response magnitudes of monarch larval growth relative to growth of larvae fed plants from control treatments. Data presented were both separated for each plant population and averaged across populations for all possible treatment combinations of ambient temperature, water stress (H₂O stress); elevated temperature, no water stress (eTemp.); or the combination of elevated temperature and water stress (eTemp. + H₂O stress). Error bars represent ± 1 SE. Northern populations: BFS (Bonds Falls, MI); CED (Cedar, WI); KMP (Woodruff, WI). Southern populations: MWH (Madison, WI); TRU (Madison, WI). Latitudinal designation of plant location is under population code



temperatures as a consequence of an increase in protein-containing structural fractions (i.e., cellulose and lignin). Similarly, we also found that foliar structural components (i.e., fiber and lignin) increased at higher temperatures, providing a parallel mechanism by which elevated temperature may alter leaf nutrient status.

In agreement with a meta-analysis by Huberty and Denno (2004), water stress also increased nitrogen concentrations, lowering C/N ratios, and reduced foliar water content. On average, LMA declined under elevated temperatures, but a large increase in LMA was observed in the combination of water stress and elevated temperatures later in the current study, potentially a mechanism to enhance water use efficiency while maintaining accelerated growth under higher temperatures. In addition, stressed plants generally had lower physical defenses. With the exception of lower foliar water content, these findings suggest that predicted changes in temperature and water availability may result in plants of higher quality, with higher nutrient value and lower defense levels, for herbivores utilizing these plants as a resource.

Different populations of *A. syriaca* exhibited contrasting responses in plant traits to the experimental treatments. A number of other studies have reported geographic- and population-level variation in numerous traits of *A. syriaca* (Malcolm 1995; Agrawal 2004, 2005; Vannette and Hunter

Table 3 Summary of *F* and *P* values for the effects of temperature, water stress, plant population source, and their interactions on monarch larval growth

| Treatments and interactions | <i>df</i> | Larval growth | |
|---|-----------|---------------|------------------|
| | | <i>F</i> | <i>P</i> |
| Temperature | 1,2 | 31.9 | 0.028 |
| Water stress | 1,2 | 36.9 | 0.013 |
| Temperature × water stress | 1,2 | 2.0 | 0.267 |
| Population | 4,111 | 66.3 | <0.001 |
| Temperature × population | 4,111 | 5.0 | <0.001 |
| Water stress × population | 4,111 | 1.0 | 0.429 |
| Temperature × water stress × population | 4,111 | 0.7 | 0.564 |
| Initial dry mass | 1,106 | 0.0 | 0.958 |

P values < 0.05 are bolded, and *P* values 0.05 < *P* < 0.10 are italicized

Numerator and denominator degrees of freedom (*df*: numerator, denominator) were calculated using the Satterthwaite approximation

2011; Woods et al. 2012; Wason et al. 2013; Wason and Hunter 2014). In agreement with Woods et al. (2012), plants from more northerly populations produced lower absolute amounts of aboveground biomass than plants from more southerly populations (SI Fig. 2). In contrast to Woods et al. (2012), however, plants from more northerly

Table 4 Standardized coefficients calculated using partial least squares regression relating milkweed foliar traits and monarch larval growth

| Foliar trait or environmental variable | Monarch larval growth |
|--|-----------------------|
| Temperature | 2.48 |
| Nitrogen (% dm) | 1.53 |
| C/N | -0.22 |
| Fiber (% dm) | 0.15 |
| Lignin (% dm) | -0.14 |
| LMA (g m ⁻²) | 0.34 |
| Cardenolides (µg mg ⁻¹) | -0.07 |
| Latex (mg) | 0.83 |
| Trichomes (mm ²) | 0.04 |
| NDWI | -0.71 |

Negative or positive weighted coefficients indicate a negative or positive influence, respectively, of foliar traits or environmental variables on herbivore performance. Two largest positive and negative weighted coefficients are in bold

C/N ratio of carbon to nitrogen, LMA leaf mass per area, NDWI normalized differential water index, % dm percent dry mass

populations exhibited greater growth responses to increased temperature than plants from more southerly populations. Plants from more northerly populations, which historically experience more drought conditions (WICCI 2011), were also less affected by water stress, especially at elevated temperatures, than plants from more southerly populations. Although changes in phenology will play a role in the growth of different *A. syriaca* populations in different geographic environments (Woods et al. 2012), and our sample size was limited, our findings suggest that milkweed from more northerly populations may perform better under future predicted climatic conditions than milkweed from more southerly populations.

As with other studies, we found substantial population-level variation in plant nutritional, structural, morphological, and physical defense characteristics (i.e., latex and trichomes). In contrast, we did not detect population-level variation in foliar cardenolides, potentially a consequence of the limited number of populations included in our study. Nevertheless, our findings do support the idea that including intraspecific variation in plant traits is necessary to advance spatially based ecological theory (Hunter et al. 1996) especially in the context of climate change.

To date, few studies have examined whether foliar traits of *A. syriaca* respond to predicted environmental change at the population-specific level. We found that several plant traits, including growth, plant nutritional status, morphology, and defense, were governed by complex interaction of multiple environmental change factors, and that these responses varied among plant populations. Our

findings are in line with those of Vannette and Hunter (2011) who found increased CO₂ levels altered numerous defense responses differently depending on plant genotype. The fact that *A. syriaca* populations respond differently to environmental drivers is unsurprising, given that many plant species exhibit genotype-specific responses to environmental change (Julkunen-Tiitto et al. 1993; Lindroth et al. 2001; Bidart-Bouzat et al. 2005; Peltonen et al. 2010; Lavola et al. 2013). Population-level consequences of responses of individuals to future predicted climate change will likely play an important role in the distribution and abundance of *A. syriaca* in North America.

Monarch larval growth

Monarch larvae grew larger on plants exposed to elevated temperature and water stress. Increased growth was related to both the positive direct effect of temperature on monarch physiology and the positive indirect effect of improvements in plant nutritional status under temperature and water stress. And while reduced water content and higher foliar C/N had negative influences on larval growth, it was not enough to offset the positive influences of elevated temperature and enhanced foliar nitrogen levels. We did not evaluate herbivore-induced changes in foliar quality, however, so we are uncertain whether any foliar induction responses affected monarch growth.

Temperature directly affects numerous aspects of insect life cycles, and elevated temperatures are predicted to accelerate physiology and development and alter behavior and survival (Bale et al. 2002; Pelinei et al. 2009; Cornelissen 2011) generally having positive effects on insect performance (Zvereva and Kozlov 2006). However, accelerated development rates under elevated temperature can only be sustained if growth-limiting factors respond positively in concert, and we found that as larval growth increased under elevated temperatures that plant nutritional status improved as well. Optimal temperature for monarch larval development is approximately 27 °C (Zalucki 1982), just below the maximum temperature to which larvae were exposed, but at the high end of projected mean temperature increases for the end of the century.

Monarch growth varied substantially among plant populations, and moreover, increases in growth under elevated temperature by monarchs varied among plant populations. Monarch larvae grew larger on more southerly, compared with more northerly, milkweed populations (SI Fig. 3). This finding has potential implications for localized monarch populations, depending on the ability of specific plant populations to persist under future climate scenarios. However, considering that monarch performance improved over control treatments on all plant populations, our results suggest that monarch larvae will

likely benefit from both the effects of temperature and the resulting changes in host plant quality under future predicted changes in temperature and water availability.

Our study revealed positive effects of warming on monarch larval growth, but we did not assess temperature treatments at which growth would likely be negatively impacted [e.g., consistent temperatures above 33 °C (Zalucki 1982)]. In addition, the spatial and temporal patterns of temperature and precipitation changes across our study region are unlikely to be uniform (Kucharik et al. 2010), such that both milkweed and monarchs at different locations across the latitudinal gradient will experience different degrees of climatic change. Testing extensive levels of detailed interacting climatic variability, however, was not logistically possible. Regardless of these limitations, we present the first study, to our knowledge, demonstrating population-level variability, across a latitudinal gradient, of milkweed growth and foliar trait changes and monarch larval growth in response to predicted future increases in temperature and changes water availability.

In summary, our findings demonstrate that future predicted levels of temperature and changes in water availability will likely alter the growth and quality of the primary host plant for larvae of the monarch butterfly. Importantly, we also show substantial population-level variation in *A. syriaca* in response to changes in temperature and water stress, specifically that population from northerly latitudes experienced greater increases in growth than southerly populations. Monarch larvae grew larger under all the experimental treatments, compared to the control, and this response was consistent on all plant populations.

The ecological niche space of monarchs is predicted to move northward with climate change (Zalucki and Rochester 2004; Batalden et al. 2007). Importantly, if *A. syriaca* populations are also able to move northward in concert, monarch populations may be somewhat buffered from aspects of predicted climate change via positive direct (i.e., temperature-mediated) and indirect (i.e., host-mediated nutritional) effects. Our findings suggest that the observed loss of habitat for northward-bound populations (Brower et al. 2012; Pleasants and Oberhauser 2013) is critical to monitor, since future changes in the presence or absence, including distances among populations, of milkweed across landscapes may have a larger influence on monarch populations than changes in plant quality.

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