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Climate change and an invasive, tropical milkweed: an ecological trap for monarch butterflies

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Abstract. While it is well established that climate change affects species distributions and abundances, the impacts of climate change on species interactions has not been extensively studied. This is particularly important for specialists whose interactions are tightly linked, such as between the monarch butterfly (*Danaus plexippus*) and the plant genus *Asclepias*, on which it depends. We used open-top chambers (OTCs) to increase temperatures in experimental plots and placed either nonnative *Asclepias curassavica* or native *A. incarnata* in each plot along with monarch larvae. We found, under current climatic conditions, adult monarchs had higher survival and mass when feeding on *A. curassavica*. However, under future conditions, monarchs fared much worse on *A. curassavica*. The decrease in adult survival and mass was associated with increasing cardenolide concentrations under warmer temperatures. Increased temperatures alone reduced monarch forewing length. Cardenolide concentrations in *A. curassavica* may have transitioned from beneficial to detrimental as temperature increased. Thus, the increasing cardenolide concentrations may have pushed the larvae over a tipping point into an ecological trap; whereby past environmental cues associated with increased fitness give misleading information. Given the ubiquity of specialist plant–herbivore interactions, the potential for such ecological traps to emerge as temperatures increase may have far-reaching consequences.

Key words: *Asclepias*; *cardenolide*; *Danaus plexippus*; *global warming*; *Lepidoptera*; *plant defense*.

INTRODUCTION

As global temperatures continue to rise, species may respond to climate change in a variety of ways. For instance, species may shift their distributions by migrating to unaffected or climatically similar areas (Parmesan and Yohe 2003, Moritz et al. 2008). Alternatively, species may undergo phenotypic change that ameliorates negative climate-induced impacts or takes advantage of potential positive effects (i.e., increase in population growth at higher latitudes; Schlaepfer et al. 2002, Deutsch et al. 2008, Angilletta 2009). Regardless of the mechanism, climate change research has often focused on the responses of single species to changes in global climate. While this research provides valuable insight into the effects of global warming on generalist consumers, the impacts of climate change on dietary specialists are not as readily apparent (Gough et al. 2015). Thus, it has become increasingly recognized that species interactions, especially interactions between tightly packed species, need to be considered when trying to understand the full impacts of climate change on ecological dynamics (O'Connor et al. 2012, Urban et al. 2013, Elder and Reilly 2014).

Whenever rapid environmental change reduces the quality of an organism's habitat, including the quality of its diet, there is potential for the species to be caught in an ecological trap (Schlaepfer et al. 2002, Battin 2004). Ecological traps occur when organisms make maladaptive habitat choices and/or experience negative phenotypic responses based on environmental cues that once correlated positively with habitat quality and/or evolutionarily stable phenotypic traits (Schlaepfer et al. 2002, Robertson and Hutto 2006). In an altered environment, formerly reliable signals may no longer correspond to positive adaptive outcomes and the organism becomes “trapped” by their responses. This may result in a decline in fitness (Schlaepfer et al. 2002, Van Dyck et al. 2015). Ecological traps due to anthropogenic actions have become increasingly prevalent. For example, off the coast of Western Africa, climate-change-induced environmental variability and overfishing have created cool, chlorophyll dense waters, usually indicative of healthy fish populations, that are devoid of fish (Sherley et al. 2017). This has created an ecological trap for endangered African penguins, which use chlorophyll density as an indicator of good fishing grounds (Sherley et al. 2017). However, effects of climate change on species interactions that generate ecological traps represent a recognized but surprisingly little-studied problem (Urban et al. 2013). For herbivores, and particularly specialists, rapid changes in the quality of their plant hosts under

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environmental change may generate ecological traps if the plants upon which they rely become unsuitable.

Many specialists feed either on a single plant species or multiple species within a single genus, and an herbivore's fitness may vary depending upon the type of species and quality of the species being consumed (Ali and Agrawal 2012). For instance, the monarch butterfly (*Danaus plexippus*) feeds almost exclusively on milkweed species within the genus *Asclepias*. *Asclepias* species vary widely in their production of cardenolides, secondary chemical defenses that the monarch sequesters as an anti-predator (Brower et al. 1967) or an anti-parasite defense (de Roode et al. 2008). Furthermore, *Asclepias* species differ in latex production (Agrawal and Konno 2009), physical defenses, leaf morphology (Agrawal et al. 2009a), and phenologies (Woodson 1954). Individual monarch fitness varies non-linearly with cardenolide production, where more toxic milkweeds confer a greater defense against predators, but can be too toxic to monarchs at high concentrations, such that intermediate levels result in higher fitness (Malcolm 1994, Sternberg et al. 2012). Consequently, any changes, either positive or negative, to milkweed chemistry due to global warming could have corresponding indirect effects on monarch performance.

Even if plant quality is unaffected by increased temperatures, monarch physiology, development, and cardenolide metabolism may change with different temperatures. Monarch larvae exposed to constant, elevated temperatures experience increased mortality, longer developmental times, and weigh less as adults (York and Oberhauser 2002). Additionally, survival and development rates of monarch larvae are maximized at temperatures around 29°C (Zalucki 1982), and increasing temperatures decrease monarch time to pupation (Lemoine et al. 2015). While these studies help us to understand the impacts of different temperature regimes on monarch development, little research has been conducted to examine temperature-mediated effects on resource quality. To quantify the potential indirect effects of climate change on herbivore fitness and to gauge whether a warmer planet will result in the creation of an ecological trap, we focused on the interaction between monarch butterflies (*D. plexippus*) and two of their milkweed host plants, *Asclepias curassavica* and *Asclepias incarnata*.

Asclepias curassavica is an exotic, commercially planted milkweed species found predominantly in the southeastern United States that can negatively affect monarchs by providing a year-round source of food, reducing the propensity to migrate, and thereby increasing disease prevalence in non-migratory populations (Satterfield et al. 2015). A majority of monarchs that do not overwinter in Mexico do so in the southern United States (Howard et al. 2010), and southern females prefer to reproduce on *A. curassavica* in the fall (Batalden and Oberhauser 2015). In recent years, monarchs have established year-round populations on introduced, invasive *A. curassavica* in the southern United States, potentially to their detriment (Satterfield et al. 2015). In contrast, *A. incarnata* is a common, native milkweed species found throughout the eastern and southeastern portion of the monarch migratory range that senesces during the winter months (Ladner and Altizer 2005, Agrawal et al. 2009a).

If *A. curassavica* quality were to improve due to environmental change, populations of sedentary, non-migratory

monarchs could increase. But, if the quality of *A. curassavica* foliage were to decline under environmental change, sedentary monarch populations could fall into an ecological trap. Here, we investigated whether increased temperatures will negatively or positively affect the foliar quality of *A. curassavica* and *A. incarnata* and, subsequently, impact monarch fitness. Because relative differences in host quality can generate ecological traps, comparing our results with those from *A. incarnata* allows us to show that the invasive *A. curassavica* represents a potential ecological trap under warmer climatic conditions.

MATERIAL AND METHODS

Study system

Monarch butterflies have a wide distributional range across North America, spanning from central Canada south through central Mexico, with isolated island populations in the Caribbean and Hawaii (Altizer and Davis 2010). Most eastern U.S. monarch butterflies make an annual, multi-generational migration spanning 3,500 km between breeding grounds and overwintering sites (Brower and Malcolm 1991), although sedentary populations have established on *A. curassavica* in Florida, Texas, and Louisiana (Satterfield et al. 2015). For our experiment, the monarchs used were from the non-inbred F2 generation of lab-reared butterflies. Parent monarchs were collected in Baton Rouge, Louisiana and Katy, Texas, USA from migratory monarch populations. Their offspring (the F1 generation) were reared on *A. tuberosa* to ensure F2 offspring naivety to the two focal experimental species, *A. curassavica* and *A. incarnata*. Offspring from the F2 generation were from a single parental pair. Unless infected with parasites, ovipositing monarch females and monarch larvae show no preference between these two milkweed species (Lefevre et al. 2010). Furthermore, monarchs in this study were uninfected with the parasite protozoan parasite, *Ophryocystis elektroscirrha* (OE), based on methods described in Altizer and Oberhauser (1999) and Altizer (2001).

To protect against herbivory, milkweeds have a variety of defensive mechanisms, including latex exudation and production of toxic cardiac glycosides (cardenolides). Latex is a sticky, viscous substance that is exuded upon tissue damage and can trap early instar monarchs and gum-up larval mouth parts (Agrawal et al. 2009b). *Asclepias incarnata* exudes slightly more latex than *A. curassavica* on average (Agrawal and Konno 2009). Cardenolides are toxic steroidal compounds that disrupt the Na⁺/K⁺ ATPase system in cell membranes (Malcolm 1991, Bingham and Agrawal 2010). *Asclepias curassavica* is known to have total cardenolide concentrations 11-times higher than those in *A. incarnata*, and *A. curassavica* also contains a much larger number of chemically distinct cardenolides than *A. incarnata* (de Roode et al. 2008). Although monarchs sequester cardenolides for their own defense, particularly high cardenolide concentrations can impose significant fitness costs (Zalucki et al. 2001, Sternberg et al. 2012, Tao et al. 2016).

For the experiments described below, all milkweed plants were grown from seeds retrieved from the USDA-NPGS (National Plant Germplasm System). Milkweed seedlings were grown in CMP6010 environmental growth chambers (Convion, Winnipeg, MB, Canada) set at 16-h photoperiods

at 28°C. The seeds were sown in a mixture of Sun Gro professional growing soil (Sun Gro, Seba Beach, AB, Canada), vermiculite, and Scotts 14-14-14 osmocote fertilizer (The Scotts Company, Marysville, OH, USA). At the time of the experiment, the individual milkweed plants were 4 months old.

Experimental setup

Experimental design.—We conducted a fully factorial experiment to examine how increased temperature and milkweed species identity affect monarch growth and development. We crossed ambient vs. elevated temperature with the two milkweed species (*A. incarnata* and *A. curassavica*), and we established 10 replicates of each of the four treatments. To warm the experimental sites, we constructed open-top chambers (OTCs; Godfree et al. 2011, Elderd and Reilly 2014). OTCs were constructed with plexiglass plates (Solar Components Corporation, Manchester, New Hampshire, USA) that slant inward to focus solar energy within the plot (Godfree et al. 2011). A single, hexagonal OTC consisted of six trapezoidal sections attached with fencing brackets and PVC piping. Each trapezoidal section was supported by a thin, wooden skeleton spanning the outer edges, and was covered by the solar plexiglass. In the center of each plot, we planted a single potted milkweed, which was covered with a butterfly bag (Appendix S1: Fig. S1). The amount of plant biomass for each species in each plot was approximately the same, as milkweeds used were the same age and size. Plots were spaced approximately 3.5 m apart. In a subset of the plots, we placed iButtons (Maxim Integrated, San Jose, California, USA), which recorded temperature and humidity every 10 min. The iButtons were enclosed in a small mesh bag made of the same material covering the individual plants. The bag containing the iButton was then encased in reflective material and placed approximately 15 cm north of the plant and approximately 15 cm aboveground (Brooks et al. 2012). The placement of the iButtons, along with being enclosed in a mesh bag covered in reflective material, minimized the chance that the iButtons were exposed to direct sunlight, which can cause large temperature fluctuations. The iButton data allowed us to determine the extent to which the OTCs raised temperature and humidity in experimental warming plots as compared to control plots. Control plots were left in ambient conditions, uncovered by an OTC. The experiment was conducted at Louisiana State University, Innovation Park (Baton Rouge, Louisiana, USA).

There has been some criticism of the use of OTCs as described above since they only raise temperature during the daylight hours when the sun is shining (Godfree et al. 2011). To alleviate this concern, Godfree et al. (2011) advocated the use of thermal masses (i.e., water-filled PVC pipes) lining the perimeter within the OTCs to maintain treatment differences during the nighttime. Trial runs using thermal masses indicated they did not help to significantly regulate either temperature or humidity compared to non-thermal mass lined OTCs (M. J. Faldyn, *unpublished data*). This is likely due to the fact that in southern Louisiana, average summer humidity stays consistently high (usually above 80%) compared to Central NSW Australia where the thermal masses were first tested. Thus, the thermal masses had less of a regulatory effect on humidity and subsequently temperature.

To acclimatize the plants, plants were placed in their appropriate temperature treatments for 72 h prior to the beginning of the experiment. After 72 h, 80 first-instar monarchs (the F2 generation from the lab reared colony), were placed on the plants, sealed within the insect-mesh bag, and allowed to feed normally. Plants were watered every morning and checked daily. After two weeks in the field, all surviving monarchs had pupated. The developing monarchs had adequate plant tissue to support their development to pupation, given that plants had remaining leaf tissue at the end of the experiment. Pupae were brought into the lab once they were observed in the field. Collected pupae were then weighed, sexed, allowed to eclose, and the fate of each larva recorded (i.e., whether or not it survived from first instar to adulthood). Adult monarchs were weighed one day after eclosion (wet mass), sexed, and their forewings measured following Van Hook et al. (2012).

Plant trait measurements.—To measure plant traits that may be affected by warming, we collected data before and after placing monarch larvae within each of the plots. After the 72-h acclimatization period, initial samples for carbon, nitrogen, latex, and cardenolide measurements were taken by either measuring the trait in the field (latex) or collecting leaf tissue for subsequent analysis. Once the experiment was concluded and all pupae returned to the lab, we completed a second set of measurements to quantify chemical changes in the host plants.

Milkweed foliar carbon and nitrogen concentrations were analyzed on a LECO TruSpec CN analyzer (LECO Corporation, Saint Joseph, MI, USA) and reported in ppm (equivalent to mg/kg of plant samples). Milkweed latex measurements were collected following methods similar to (Agrawal 2005), wherein a fully expanded, intact leaf was clipped (0.5 cm) and the exuding latex was collected on a dried, preweighed 1-cm disk of filter paper, then placed and sealed inside a dried, preweighed Eppendorf vial. The vial was promptly weighed in the lab, and the resulting difference in mass was the “wet” latex mass. The vial was opened and dried overnight at 60°C, and weighed again to collect a “dry” latex mass. Milkweed foliar cardenolide concentrations were quantified using methods modified from Malcolm and Zalucki (1996) and described by Zehnder and Hunter (2007). Leaf tissue was frozen in liquid N₂, and stored in an ultra cold (−80°C) freezer. Leaf tissue was dried, ground using a mini-ball mill, weighed, and then extracted in 100% methanol. The supernatant from the samples in methanol was vacufuged at 45°C until dry. Samples were then resuspended in either 150 μL of methanol or 75 μL of methanol depending on the dry mass of plant tissue available (dry mass <20 mg was resuspended in 75 μL of methanol). Samples were spiked with 0.15 mg/mL digitoxin as an internal standard and analyzed using reverse phase high-performance liquid chromatography (UPLC; Waters, Milford, Massachusetts, USA). Running time for each sample was approximately 8 min. Peaks were detected by absorption at 218 nm using a diode array detector and absorbance spectra were recorded from 200 to 300 nm. Peaks with symmetrical absorption maxima between 217 and 222 nm were recorded as cardenolides. Total cardenolide concentration was calculated as the sum of all separated cardenolide peaks, corrected by the concentration of

the internal standard (digitoxin) and the estimated sample mass.

Statistical analysis

Open-top chambers and monarchs.—The effects of OTCs on plot temperatures were analyzed using a repeated-measures ANOVA across days. Temperature measurements were recorded every 10 min, with daytime temperatures averaged between 08:00 and 20:00 and nighttime temperatures averaged between 20:00 and 08:00. A base-10 log-transformation was applied to ensure normality. Both daytime and nighttime average temperatures were analyzed to assess OTC performance. Monarch pupal masses, adult masses, and adult forewing lengths were analyzed using a three-way ANOVA between *A. curassavica* and *A. incarnata* host plants, ambient or warmed plots, and monarch sex. Monarch survivorship was analyzed using a chi-squared analysis between *A. curassavica* and *A. incarnata* host plants and ambient or warmed plots. The repeated-measures ANOVAs, three-way ANOVAs, and chi-squared analysis for the OTCs, monarch, and milkweed data were conducted in SAS 9.4 using the Proc Mixed and Proc Freq procedures (SAS Institute, 2013). All data were tested to ensure normality.

Milkweed.—Milkweed latex exudation, plant carbon : nitrogen ratios, and total cardenolide concentration were analyzed using a repeated-measures ANOVA comparing initial (pre-treatment) and final (post-treatment) milkweed tissue. To ensure normality, carbon : nitrogen ratios were base-10 log-transformed and total cardenolide concentrations were square-root transformed. Milkweed cardenolide composition (relative abundance of different molecular types) was analyzed using a permutational MANOVA performed in R using using adonis in the vegan package (Oksanen et al. 2015). This acts as an analysis of variance by partitioning among sources of variation and fitting linear models to calculated distance matrices based on these partitions (Oksanen et al. 2015). To assess differences in the cardenolide composition of the milkweed, we used metaMDS in vegan for Nonmetric Multidimensional Scaling (NMDS) (McCune and Grace 2002) with 999 permutations per model run and a maximum of 20 runs per dimension. Model stress declined rapidly from a one-dimensional to a two-dimensional model, declining only slightly thereafter in a three-dimensional model. Model stress is a goodness of fit statistic for the observations, defined so that the sum of squared values is equal to squared stress where large stress values indicate a poor model fit (e.g., stress value between 0.5 and 0.15 is a fair fit) (Oksanen et al. 2015). We therefore used a two-dimensional model (model stress = 0.1063083), indicating a good ordination fit. We used the NMDS coordinates from this analysis to plot the position of the milkweed cardenolides in multidimensional space.

RESULTS

Open top chambers (OTCs)

Overall, the OTCs significantly raised temperatures in the experimental plots ($F_{1,56} = 636.02$, $P < 0.0001$). During the daytime, temperatures in the OTC enclosed plots were raised

by 3°C, maintaining an average temperature around 35°C, compared to ambient plots with an average temperature of 32°C ($F_{1,28} = 576.12$, $P < 0.0001$, Appendix S1: Fig. S2). In daytime hours, monarchs in the OTC plots experienced brief peaks in temperature up to a maximum of 46°C, and in open, ambient plots monarchs experienced temperature peaks of up to 38°C. Nighttime ambient temperatures were lower than nighttime OTC plot temperatures ($F_{1,28} = 60.98$, $P < 0.0001$), with an average temperature of 23°C. On average, nighttime temperatures were raised by roughly 0.2°C in OTC covered plots. Additionally, there were significant differences between daytime and nighttime temperatures ($F_{1,56} = 39,170.4$, $P < 0.0001$), differences across experimental days ($F_{13,56} = 39,175.22$, $P < 0.0001$), and an interaction between experimental day and OTC applications ($F_{1,56} = 441.10$, $P < 0.0001$). In general, the increase in temperature in our experimental plots reflects the projected increase in temperature expected at our experimental site by 2080 (Karl et al. 2009).

Monarch

Warmer temperatures had strikingly different effects on monarch survival to adulthood depending on host plant. Specifically, survivorship was five times lower on *A. curassavica* at warmer temperatures than on *A. curassavica* at ambient temperatures, whereas no differences were seen in monarch survivorship on *A. incarnata* between temperatures (species \times temperature interaction, $\chi^2 = 4.38$, $P = 0.0363$, Fig. 1A). As expected, pupal weights varied significantly with gender, with male pupae weighing 16% more than female pupae ($F_{1,30} = 6.77$, $P = 0.0143$). Marginally significant differences in adult monarch weight were driven by the interaction between the host milkweed plant species and the temperature treatment ($F_{1,23} = 3.07$, $P = 0.0929$, Fig. 1B), with no observed differences in adult mass between sexes. Adult monarchs forewing lengths decreased by 2.5 mm, on average, when exposed to warmer temperatures ($F_{1,20} = 11.4$, $P = 0.003$, Fig. 1C), with male monarchs having marginally longer forewings overall ($F_{1,20} = 3.99$, $P = 0.0594$).

Milkweed

Across all temperature treatments, the introduced *A. curassavica* exuded more than three times the amount of latex produced by the native *A. incarnata* ($F_{1,37.9} = 43.05$, $P < 0.0001$, Fig. 2A). After two weeks in the field, both plant species produced more latex by an average of 70% ($F_{1,38.2} = 10.53$, $P = 0.0024$). There was no significant main or interaction effect of warming on latex exudation in this experiment. *A. incarnata* had a foliar C:N ratio that was 14% higher than *A. curassavica* ($F_{1,59} = 8.22$, $P = 0.0057$, Fig. 2B), while foliar C:N ratios declined by 13% in both species over the two week period ($F_{1,59} = 8.7$, $P = 0.0045$, Fig. 2B).

On average, *A. curassavica* produced 13-fold higher foliar cardenolide concentrations than *A. incarnata* ($F_{1,39} = 299.41$, $P < 0.0001$, Fig. 2C). Foliar cardenolide concentrations more than doubled in both species over time ($F_{1,39.1} = 25.94$, $P < 0.0001$, Fig. 2C). Importantly, the temporal increases in foliar cardenolide concentrations in *A. curassavica* were higher in the warming treatment, reaching 4 mg/g dry mass ($F_{1,39.1} = 13.02$, $P = 0.0009$, Fig. 2C).

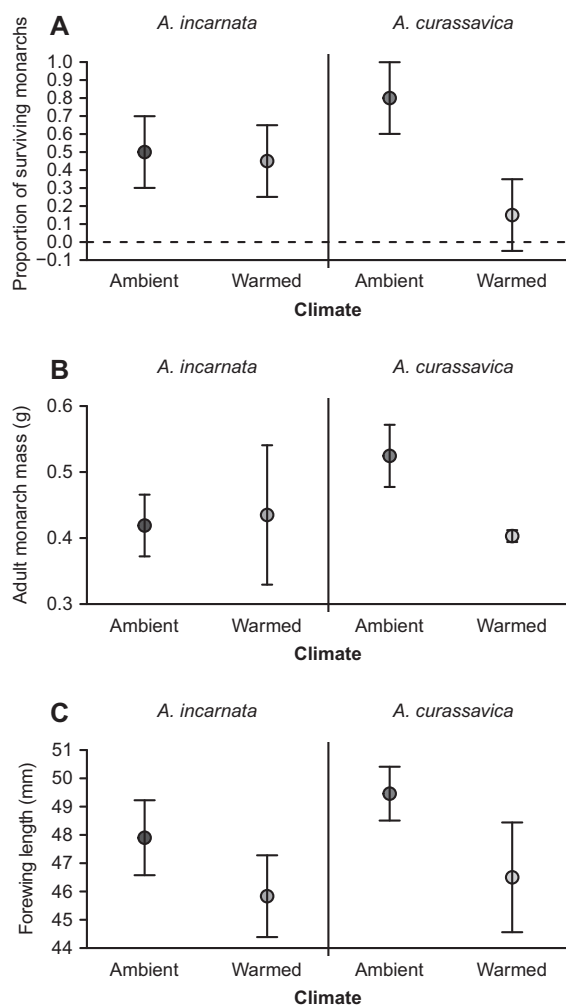


FIG. 1. The survival (A), adult mass (B), and forewing length (C) of monarch butterflies reared on two milkweed species under ambient and elevated temperatures. (A) The proportion of surviving adult monarchs, with 95% confidence intervals. Note the significant interaction between the warming treatment and milkweed species. (B) Average adult monarch mass, with 95% confidence intervals, follows the same significant patterns as the survivorship results. (C) Average forewing length with 95% confidence intervals. Note the significant effect of the warming treatment. Darker colors indicate the ambient treatment, while lighter colors indicate the warmed treatment.

A. curassavica produced a five times greater variety of cardenolides than did *A. incarnata* (PerMANOVA, $F_{1,55} = 28.7645$, $P = 0.001$), with cardenolide composition changing significantly over time (PerMANOVA, $F_{1,55} = 21.7170$, $P = 0.001$). The temporal changes in cardenolide composition were more variable among individual *A. incarnata* plants than among individual *A. curassavica* (PerMANOVA interaction, $F_{1,55} = 12.9588$, $P = 0.001$, Appendix S1: Fig. S3). Temperature treatment had no effect on milkweed cardenolide composition (PerMANOVA, $F_{1,55} = 1.0704$, $P = 0.349$).

DISCUSSION

The exotic, invasive *A. curassavica* represents a potential ecological trap for monarchs, given their markedly reduced performance under warmer conditions as compared to

current conditions (Fig. 1). The dramatic drop in performance may have been driven by increases in total cardenolide production, especially in combination with increased temperatures (Fig. 2). Interestingly, this pattern was not driven by changes in the chemical composition of the cardenolides, as the two milkweed species have distinctive profiles (Appendix S1: Fig. S3). Temperature alone did not influence cardenolide composition in either milkweed species (Appendix S1: Fig. S3). We suspect that monarchs performed better on *A. curassavica* than on *A. incarnata* under ambient conditions because the latter has lower foliar N concentrations (Fig. 2B). However, the substantial increase in foliar cardenolide concentrations in *A. curassavica* under warming temperatures (Fig. 2C) may cause the dramatic decline in monarch performance illustrated in Fig. 1. Beyond temperature effects on monarchs mediated by diet quality, increased temperatures also decreased monarch forewing lengths (Fig. 1C), which may negatively impact monarch flight potential. Alterations in forewing lengths can change wing loading, affecting butterfly flap-glide efficiency, flight speed, and maneuverability (Betts and Wootton 1988). Previous work has noted substantial declines in monarch fitness as cardenolide concentrations approach 3 mg/g dry mass (Sternberg et al. 2012, Tao et al. 2016). Here, by the end of our experiment, foliar cardenolide concentrations exceeded 4 mg/g dry mass in *A. curassavica*. Given that neither monarch larvae nor parasite-free, ovipositing female adults appear to choose among milkweed species based on cardenolide concentration (Lefevre et al. 2010, 2012), warming temperatures may cause *A. curassavica* to function as an ecological trap.

Interestingly, while there were temperature-induced changes in the overall production of defensive compounds by *A. curassavica*, elevated temperatures did not influence the types of compounds produced given that each milkweed species produces a distinctive cardenolide signal (Appendix S1: Fig. S3). Because all experimental bags had larvae within them, we cannot determine whether temporal changes in foliar quality (Fig. 2) resulted from ontogenetic change in milkweeds or from induction via herbivory. Previous trials exposing *A. curassavica* and *A. incarnata* to ambient and warmed environments without herbivory have shown that latex exudation decreases with increased temperatures (M. J. Faldyn, *unpublished data*), in contrast to the results reported here in which temperature had no effect on latex exudation. Furthermore, previous studies in other systems have shown that plants with inducible defenses often experience a decrease in inducibility when exposed to increased temperatures (Zhu et al. 2010, DeLucia et al. 2012). For *Asclepias*, warmer environmental conditions may lead to increased transpiration, which affects cellular turgor pressure, subsequently impacting latex production as latex exudation is dependent on turgor pressure (Agrawal and Konno 2009). Whether the result of induction or ontogeny, it is clear that milkweed total cardenolide production reaches deleterious concentrations in *A. curassavica* foliage when plants and larvae are reared under warmer temperatures.

Our work explores how temperature influences the interaction between monarchs and milkweeds and compliments previous work that considered independent effects of temperature on monarch development and on milkweed distributions. For example, projected climate change may force

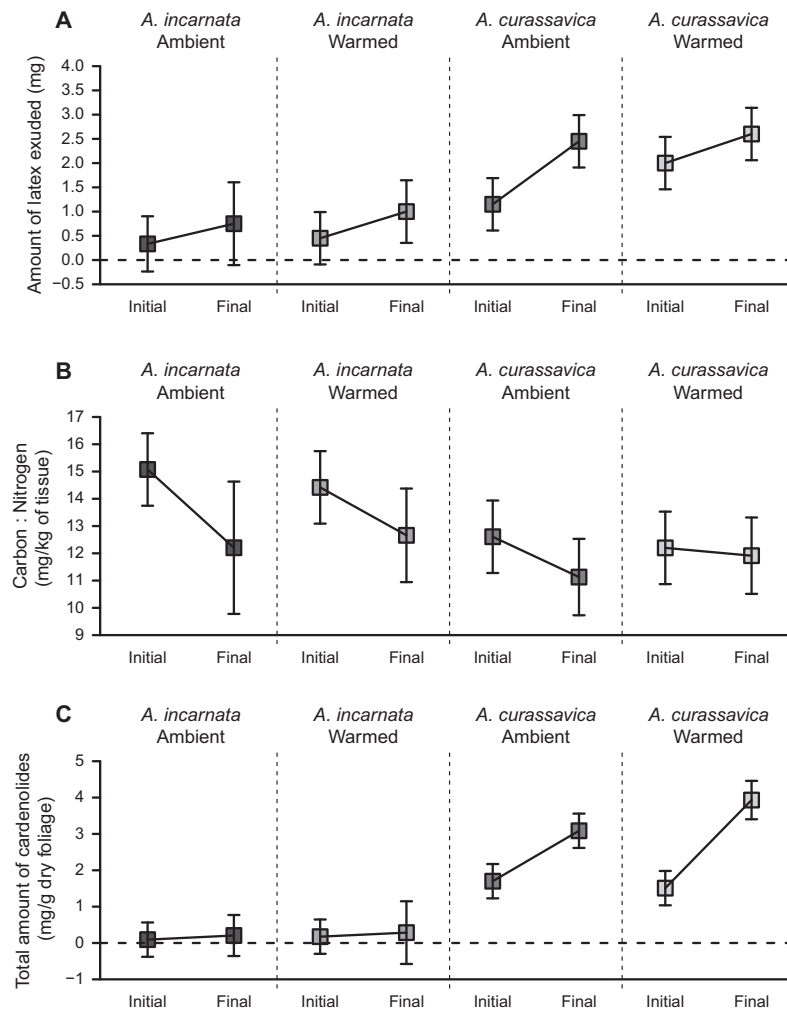


FIG. 2. Indices of foliar quality of milkweeds grown under ambient and elevated temperatures measured before (initial) and after (final) hosting monarch caterpillars. (A) The average amount of latex exuded prior to and at the conclusion of the experiment for each experimental treatment with 95% confidence intervals. (B) The average carbon : nitrogen ratios with 95% confidence intervals. (C) The average total cardenolide concentration with 95% confidence intervals. Darker colors indicate the ambient treatment, while lighter colors indicate the warmed treatment.

breeding niches for monarch butterflies northward (Batalden et al. 2007), and current winter range may become inadequate for monarchs due to increased cool weather precipitation (Oberhauser and Peterson 2003). Furthermore, predicted northward shifts of *Asclepias* sp. into Canada may lead to northward shifts in monarch summer distributions (Lemoine 2015). Understanding changes in host plant distributions for tightly coupled, insect–plant interactions (e.g., the monarch–milkweed system) is crucial, but understanding changes in host resource quality is equally important to consider. Other environmental drivers may also influence these interactions, including water availability (Andrews and Hunter 2015), nutrient deposition, (Zehnder and Hunter 2008, Tao et al. 2014), and elevated atmospheric concentrations of carbon dioxide (Vannette and Hunter 2014). Biotic interactions with other species may also need to be considered. For example, *A. curassavica* may delay or eliminate migration due to the year round availability of leaf tissue, and loss of migration increases the monarch's exposure to the protozoan parasite, *Ophryocystis elektroscirrha* (OE; Satterfield et al. 2015). Additional pathogens can interact in complex

ways with OE infections, potentially affecting monarch performance more than temperature increases alone (Nifosi and Hunter 2015). While temperature induced changes in milkweed chemistry may benefit monarchs by decreasing parasite loads, it seems unlikely that they could compensate for the dramatic declines in monarch performance illustrated in Fig. 1A. Adding the cascading effects of global climate change and other environmental change to the mix may further complicate these interactions.

While our experimental design addresses monarch performance on two distinct host plants at different temperatures, it does not address host plant selection by ovipositing females. Female monarchs may preferentially oviposit on more toxic milkweed plants to reduce parasitic OE virulence in their offspring (Lefevre et al. 2010). Furthermore, in mixed groups of *A. curassavica* and *A. incarnata*, female monarchs selectively oviposit on *A. curassavica* so their offspring can sequester more potent cardenolides (Malcolm and Brower 1986). As some milkweed species increase in total cardenolide concentrations with increasing temperatures, monarchs may oviposit on more potent milkweed that will help medicate against OE infections

and improve sequestered defenses. Our experiments may have imposed a substantial stress on milkweeds, potentially inducing changes in foliar quality different from those that may accompany more gradual climate change. However, in addition to increases in average annual temperature, climate models predict concomitant increases in climatic variability, including a higher frequency of heat waves (Karl et al. 2009). Higher annual temperatures and more frequent heat waves may combine to intensify the ecological trap that results from elevated cardenolide concentrations in *A. curassavica*. Ultimately, the combination of direct and indirect effects of multiple drivers will determine the overall effects of environmental change on monarchs and their milkweed hosts. Nonetheless, warming alone appears sufficient to generate an ecological trap for the populations of monarchs feeding on *A. curassavica*.

In general, research continues to show the importance of indirect effects in determining how species respond to climate change (O'Connor et al. 2012, Elderd and Reilly 2014, Cerrato et al. 2016). The direction and the strength of such interactions may have important fitness consequences regardless of whether or not individual species are consigned to an ecological trap. However, there is generally a temperature optimum at which individual fitness is maximized (Angilletta 2009). If that optimum is surpassed as the Earth warms (Deutsch et al. 2008), the species may eventually fall into a trap. Given current trends in planting of *A. curassavica* to alleviate habitat loss, best gardening practices should be reevaluated to reinforce the notion that native milkweed species should be preferentially planted. Additionally, nurseries should work to increase the number of locally native milkweed species sold and work to deemphasize the selling of *A. curassavica*. Overall, we have shown the importance of examining how species interactions may respond to abiotic changes due to climatic drivers. This is particularly true for specialists and their response to global warming. Without gaining proper insight into how these interactions shift as the planet warms, we may be unwittingly setting ecological traps.

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