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6 **Climate change and an invasive, tropical milkweed: An**
7 **ecological trap for monarch butterflies**

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Summary

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 non-native *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 weights when feeding on *A. curassavica*. However, under future conditions, monarchs fared much worse on *A. curassavica*. The decrease in adult survival and weight 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.

Keywords: *Danaus plexippus*, lepidoptera, *Asclepias*, global warming, cardenolide, 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 & 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-linked species, need to be considered when trying to understand the full impacts of climate change on ecological dynamics (O'Connor *et al.*, 2012; Elderd & Reilly, 2014; Urban *et al.*, 2013).

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 (Battin, 2004; Schlaepfer *et al.*, 2002). 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 (Robertson & Hutto, 2006; Schlaepfer *et al.*, 2002). 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 pen-

guins 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 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 & 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 & 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 of 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 & 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*.

A. 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 & 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 south-eastern portion of the monarch migratory range that senesces during the winter months (Ladner & 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 & Davis, 2010). Most eastern United States monarch butterflies make an annual, multi-generational migration spanning 3500 km between breeding grounds and overwintering sites (Brower & 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, LA and Katy, TX, 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 & 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). *A. incarnata* exudes slightly more latex than *A. curassavica* on average (Agrawal & Konno, 2009). Cardenolides are toxic steroidal compounds that disrupt the Na⁺/K⁺ ATPase system in cell membranes (Bingham & Agrawal, 2010; Malcolm, 1991). *A. 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 cardenolides 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 environmental growth chambers (Conviron CMP6010) set at 16-hr photoperiods at 28°C. The seeds were sown in a mixture of SunGro professional growing soil (www.sungro.com), vermiculite, and Scotts 14-14-14 osmocote fertilizer (www.scotts.com). 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 versus elevated temperature with the two milkweed species (*A. incarnata* and *A. curassavica*), and we established ten replicates of each of the four treatments. To warm the experimental sites, we constructed open-top chambers (OTCs) (Godfree *et al.*, 2011; Elder & Reilly, 2014). OTCs were constructed with plexiglass plates (Solar Components Corporation, Manchester, NH, 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 meters apart. In a subset of the plots, we placed iButtons (Maxim Integrated, San Jose, CA, USA), which recorded temperature and humidity every ten minutes. 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, LA, 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 (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 hours prior to the beginning of the experiment. After 72 hours, 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 1st instar to adulthood). Adult monarchs were weighed one day after eclosion (wet weight), 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-hour 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 (<http://www.leco.com>) 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 weight was the “wet” latex weight. The vial was opened and dried overnight at 60°C, and weighed again to collect a “dry” latex weight. Milkweed foliar cardenolide concentrations were quantified using methods modified from Malcolm & Zalucki (1996) and described by Zehnder & Hunter (2007). Leaf tissue was frozen in liquid N₂, and stored in an UltraCold (-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 weight of plant tissue available (dry weight less than 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 Inc., Milford, MA, 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 & Monarchs:

The effects of OTCs on plot temperatures were analyzed using a repeated measures ANOVA across days. Temperature measurements were recorded every 10-minutes, with daytime temperatures averaged between 8am and 8pm, and nighttime temperatures averaged between 8pm and 8am. A base-10 log-transformation was applied to ensure normality. Both daytime and nighttime average temperatures were analyzed to assess OTC performance. Monarch pupal weights, adult weights, 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 Inc., 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 ‘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 & 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 0.5-0.15 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 x 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 weight between sexes. Adult monarchs forewing lengths decreased by 2.5mm, 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* ($F_{1,59}=8.22$, $p=.0057$, 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). *A. curassavica* produced a 5-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 & Wootton, 1988). Previous work has noted substantial declines in monarch fitness as cardenolide concentrations approach 3mg/g dry mass (Sternberg *et al.*, 2012; Tao *et al.*, 2016). Here, by the end of our experiment, foliar cardenolide concentrations exceeded 4mg/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 (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 & 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 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 & 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 & Hunter, 2015), nutrient deposition, (Zehnder & Hunter, 2008; Tao *et al.*, 2014), and elevated atmospheric concentrations of carbon dioxide (Vannette & 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 & 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 & 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 (Elder & Reilly, 2014; O'Connor *et al.*, 2012; Cerreto *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

400 sold and work to deemphasize the selling of *A. curassavica*. Overall, we have shown the
401 importance of examining how species interactions may respond to abiotic changes due to
402 climatic drivers. This is particularly true for specialists and their response to global warm-
403 ing. Without gaining proper insight into how these interactions shift as the planet warms,
404 we may be unwittingly setting ecological traps.

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Figure Captions

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 weight, 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.
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.

Fig. 1

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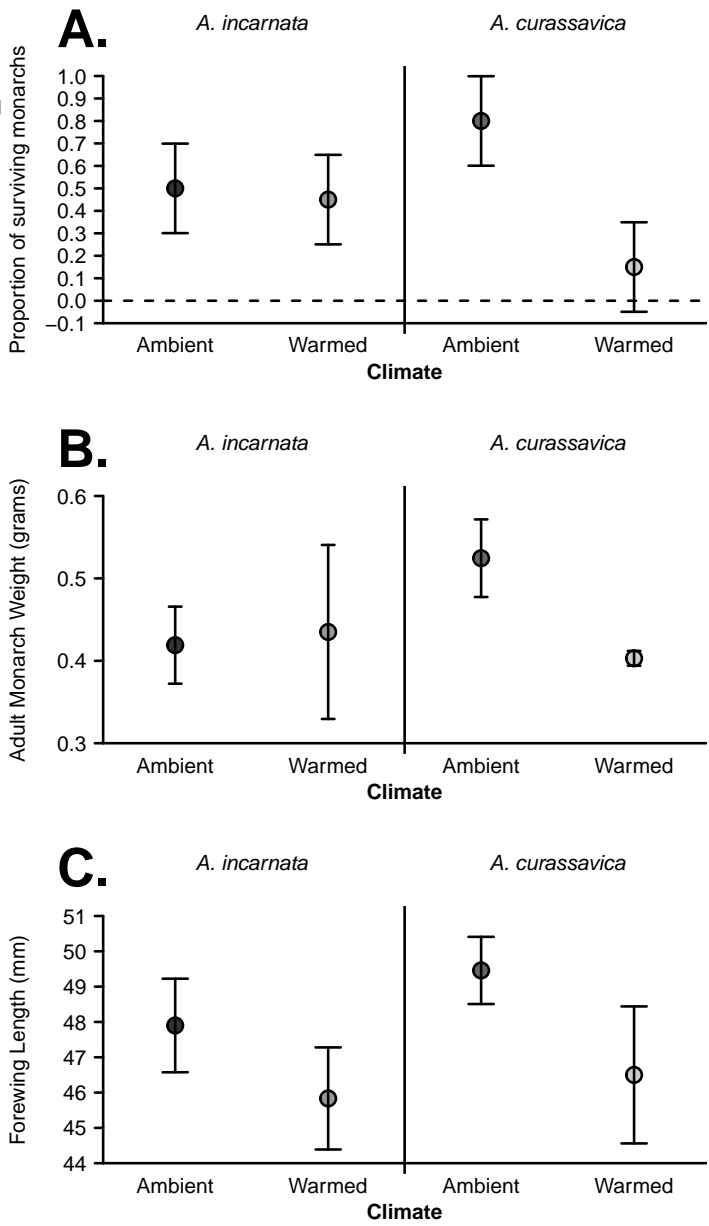


Fig. 2

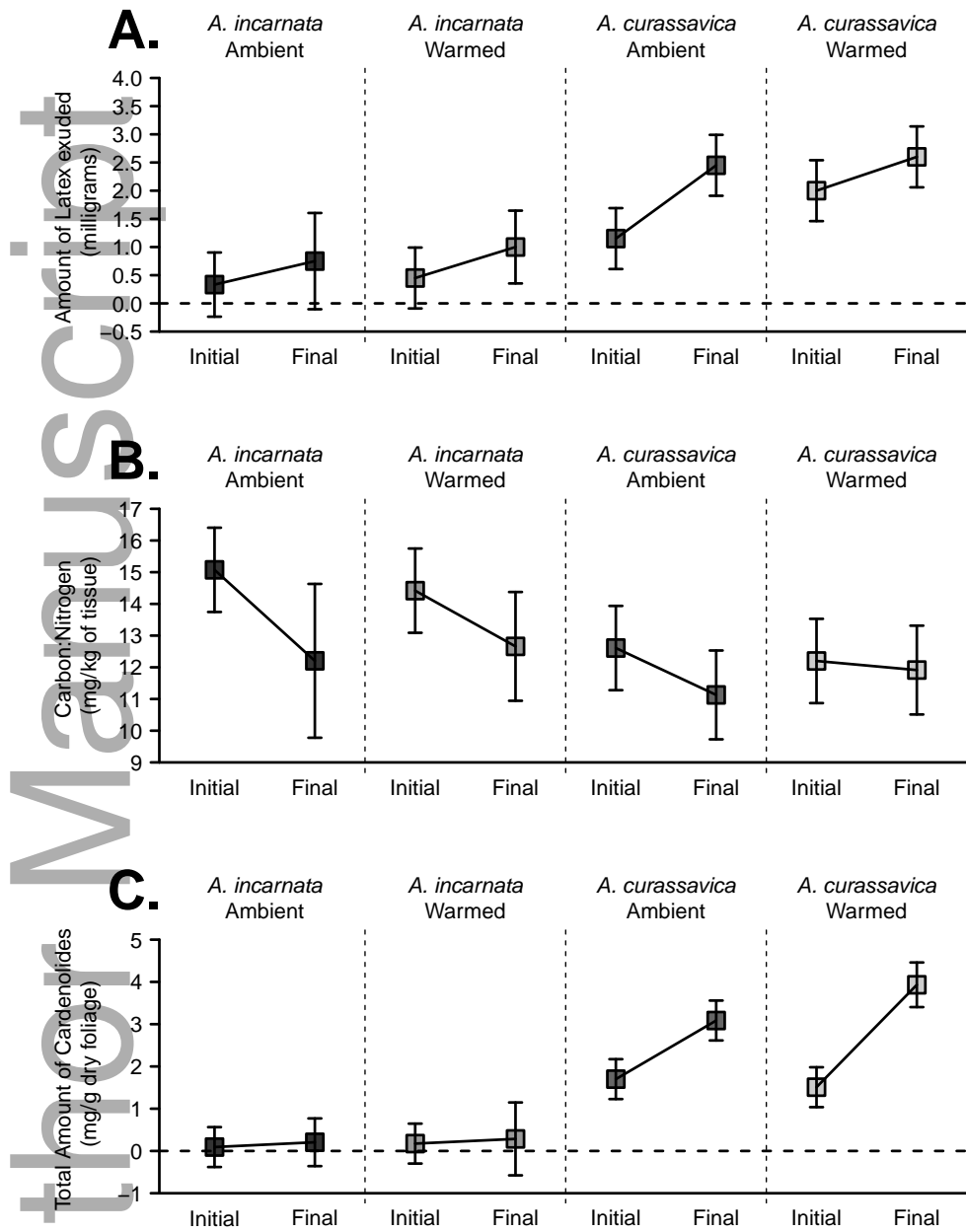




Fig. S1 Field site and experimental layout at LSU Innovation Park, Baton Rouge, Louisiana, USA.

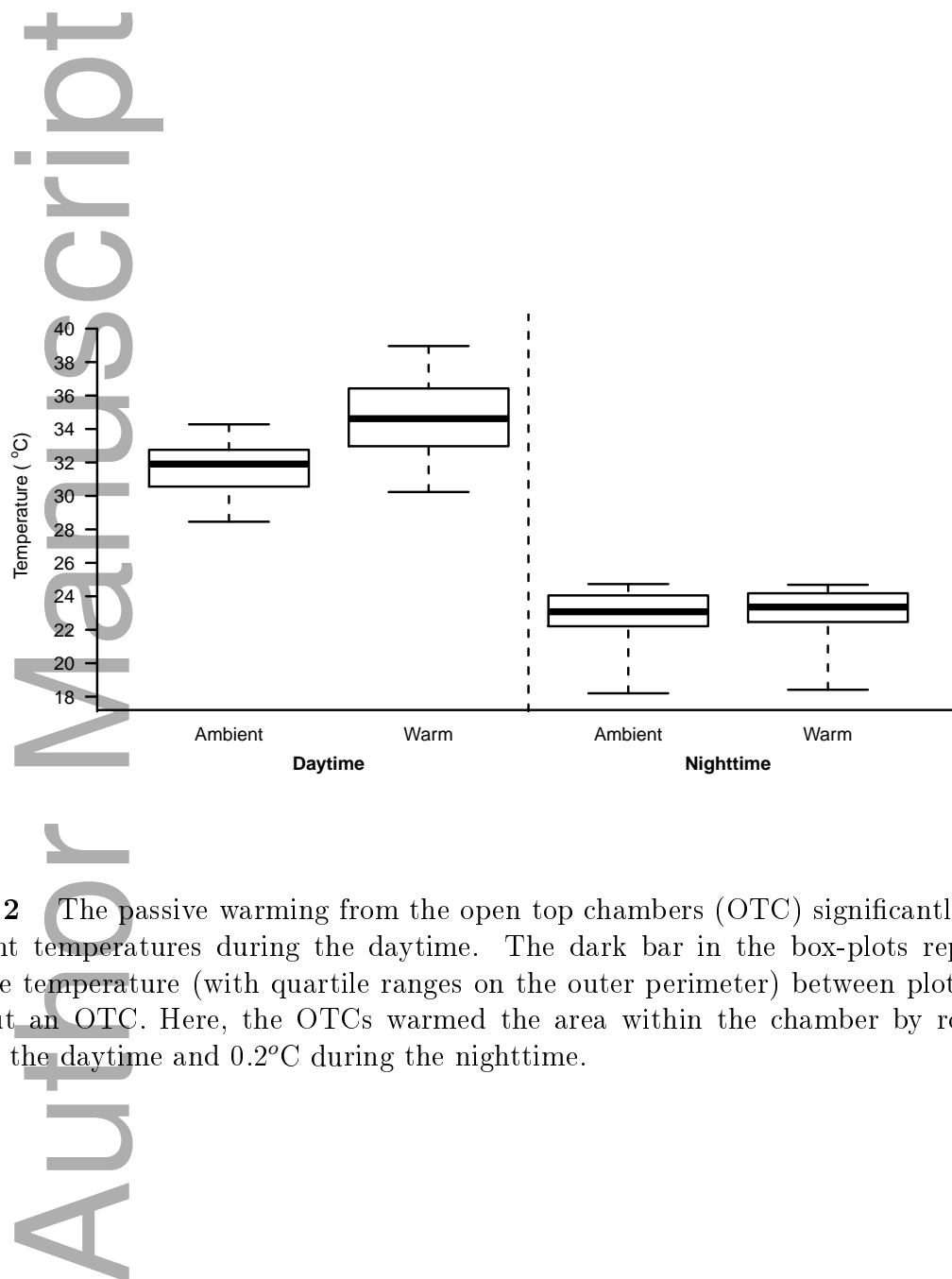


Fig. S2 The passive warming from the open top chambers (OTC) significantly increased ambient temperatures during the daytime. The dark bar in the box-plots represent the average temperature (with quartile ranges on the outer perimeter) between plots with and without an OTC. Here, the OTCs warmed the area within the chamber by roughly 3°C during the daytime and 0.2°C during the nighttime.

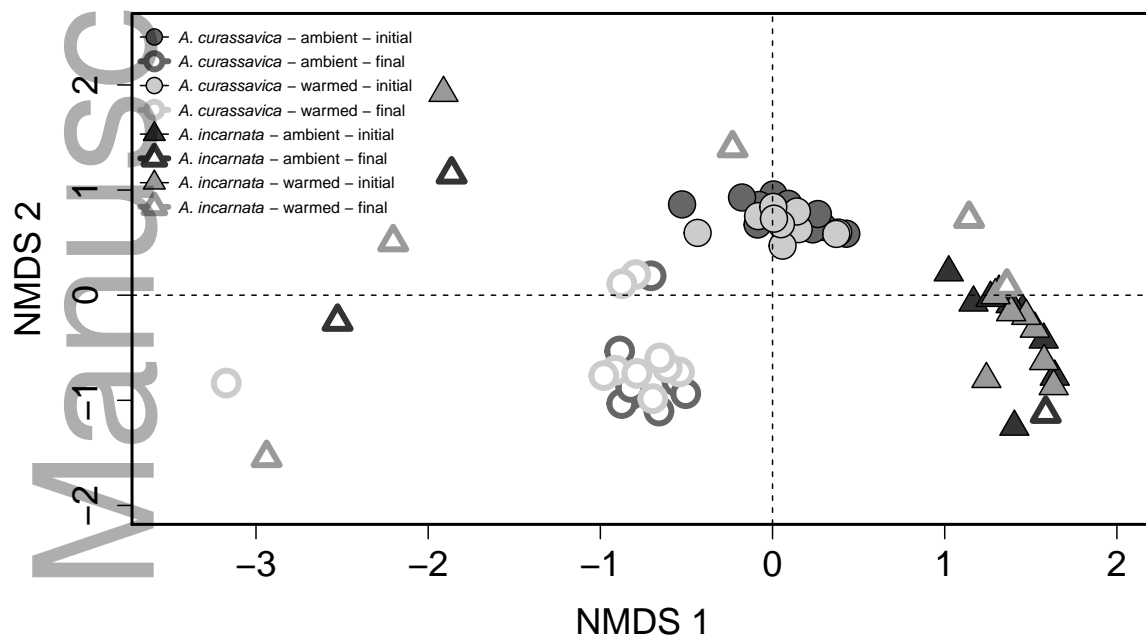
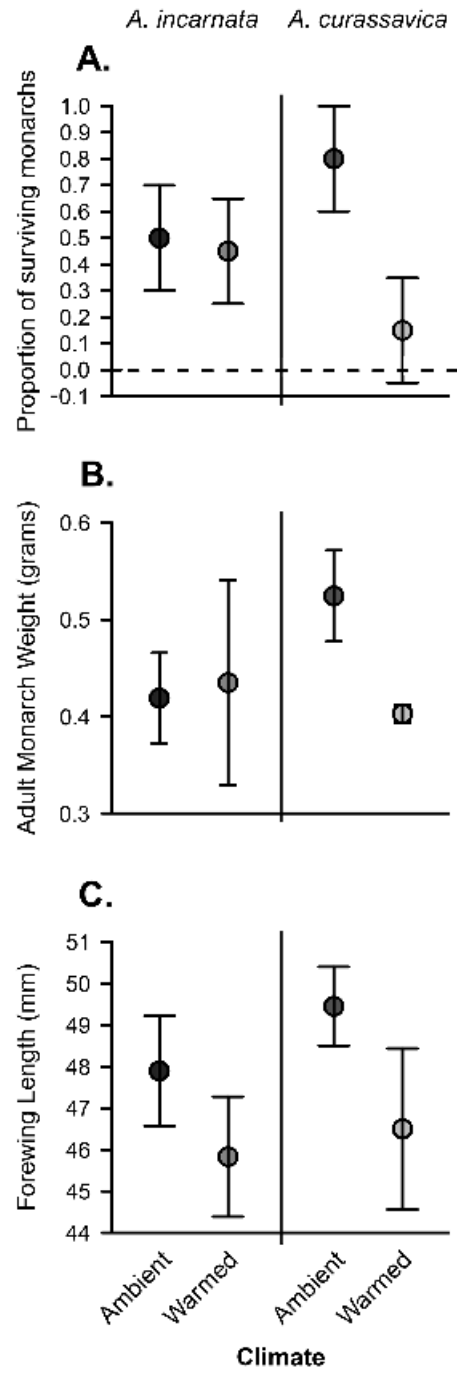
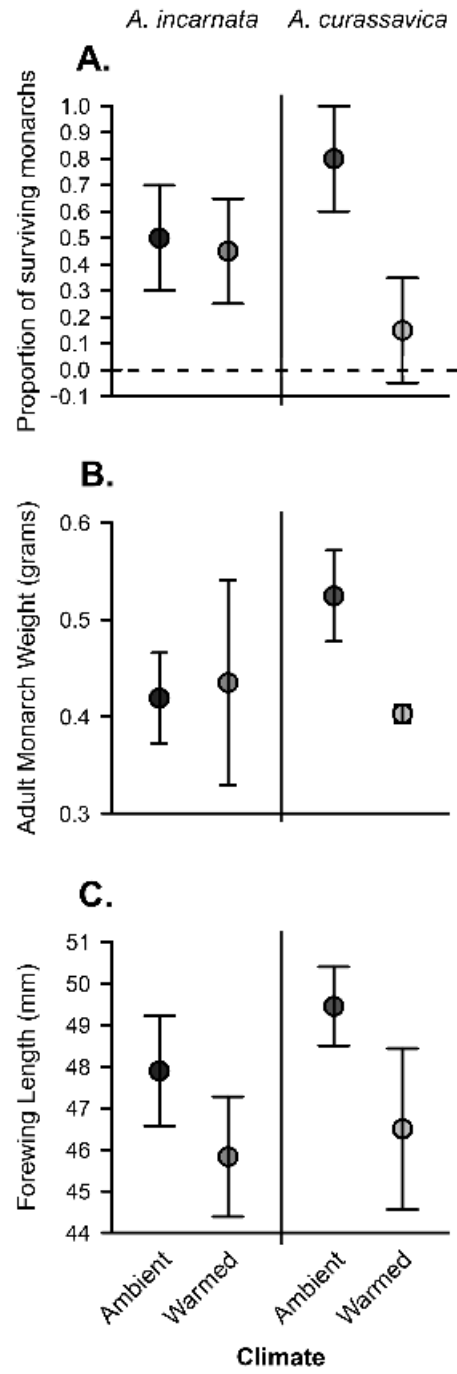


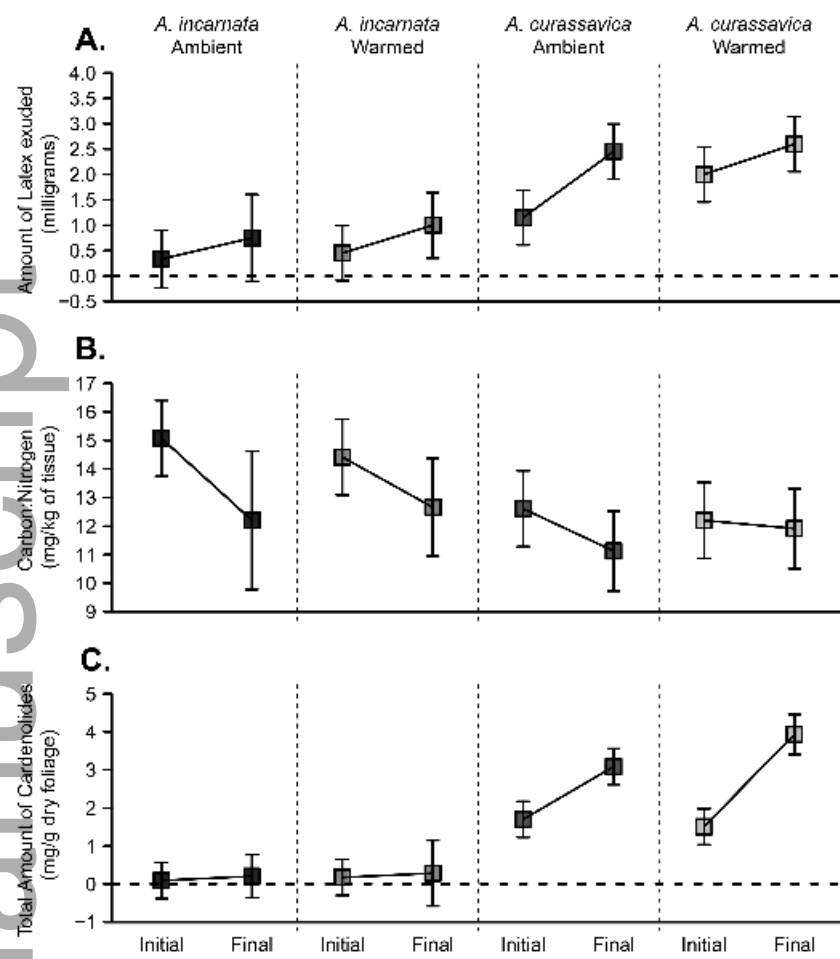
Fig. S3 Shapes represent cardenolide composition of individual milkweed plants placed in ordination space. NMDS axis 1 and NMDS axis 2 aid in visualizing the differences that occur in the composition of the cardenolides produced by both *A. curassavica* and *A. incarnata* between the treatments. From the clustering, cardenolide composition is different between *A. curassavica* and *A. incarnata* and changes during the two weeks between the initial and final plant trait measurements. Together, cardenolide composition reflects the interaction between milkweed plant species and sampling date.



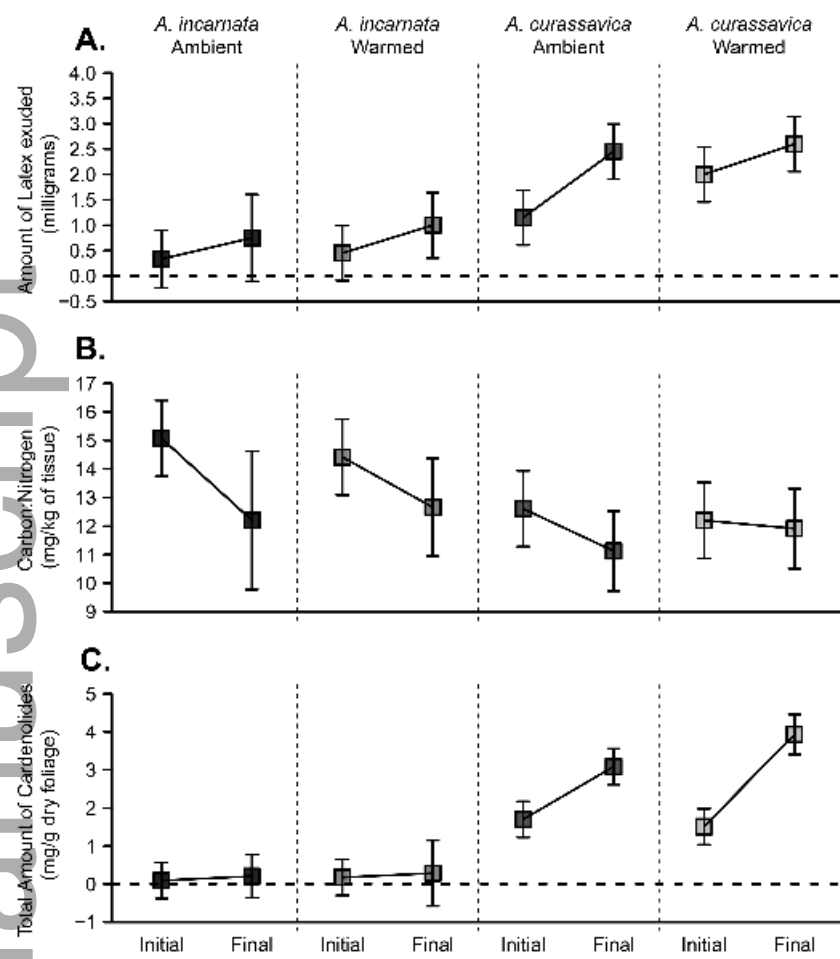
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