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The timing of heat waves has multiyear effects on milkweed and its insect community

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Abstract

Extreme heat events are becoming more frequent and intense as climate variability increases, and these events inherently vary in their timing. We predicted that the timing of a heat wave would determine its consequences for insect communities owing to temporal variation in the susceptibility of host plants to heat stress. We subjected common milkweed (*Asclepias syriaca*) plants to in-field experimental heat waves to investigate how the timing of heat waves, both seasonally and relative to a biotic stressor (experimental herbivory), affected their ecological consequences. We found that heat waves had multiyear, timing-specific effects on plant–insect communities. Early-season heat waves led to greater and more persistent effects on plants and herbivore communities than late-season heat waves. Heat waves following experimental herbivory had reduced consequences. Our results show that extreme climate events can have complex, lasting ecological effects beyond the year of the event—and that timing is key to understanding those effects.

KEYWORDS
aphids, *Asclepias speciosa*, climate change, extreme climate event, herbivory, temporal ecology

INTRODUCTION

In tandem with overall warming, anthropogenic climate change has led to an increase in the frequency and intensity of heat waves and other extreme climatic events (Fischer & Knutti, 2015; Perkins-Kirkpatrick & Gibson, 2017). More frequent and intense heat waves have the potential to disrupt ecological processes and threaten ecosystem resilience (Maxwell et al., 2019; Smith, 2011). In plants, limited evidence from field experiments shows

that the impacts of a heat wave on growth and other traits can linger for weeks to months after the extreme event (Siebers et al., 2015, 2017; Wang et al., 2008).

A critical feature of heat waves, separating them from warming more generally, is that they have an inherent element of timing (Jentsch et al., 2007). Heat waves occur at varying times within and among years, meaning that they coincide with different stages of organismal development, seasonal community assembly, and weather patterns. It follows that a similar heat wave event could have different effects depending solely on its seasonal timing. For example, we know that the susceptibility of both plants and insects to heat stress depends on their developmental stage (Chiu et al., 2015; Siebers et al., 2015, 2017; Zhang et al., 2015). Previous studies have manipulated the frequency and amplitude of heat fluctuations (Gillespie et al., 2012; Sentis et al., 2013), which are important aspects related to timing—but we have a poorer understanding of the role of seasonal timing of heat waves. Given the particular timing of heat waves, ecological responses may be transient or persistent (Wolkovich et al., 2014). No studies to date have tested whether the timing of heat waves influences their community-level consequences on plant–herbivore systems over multiple years, leaving us with little understanding of temporal variation in heat wave effects.

A second gap in our understanding of heat wave effects is the community-level response of herbivorous insects and other plant-associated arthropods. For plant-associated communities, the effects of heat waves are likely to extend beyond direct effects of heat on individual arthropods; they will also depend heavily on host plant responses, because plant characteristics are key to structuring these communities (Koricheva & Hayes, 2018). This has been true in gradual-warming research, where effects of higher temperature on host plant chemistry and growth generally benefit herbivores (Jamieson et al., 2012). Plant responses to heat waves have indeed been shown to impact interacting species and communities in short-term tests. For example, in growth chamber studies, discrete heat waves led to signaling conflict between induced defenses and heat stress responses (Havko et al., 2020), reducing defense expression, and daily heat fluctuations at higher amplitudes increased the top-down control of aphids by lady beetles relative to constant temperatures (Sentis et al., 2013). Because most past studies were conducted in growth chambers, which limited their duration and the number of species involved, our understanding of both the longer-term ecological consequences and the multispecies community-level consequences of extreme heat events is limited (Hamann et al., 2021).

Owing to the connection between plant phenotypes and associated communities, the greatest community-level effects from extreme events are likely to occur when plants themselves show the greatest responses to such events.

Younger, rapidly growing plants are thought to be more susceptible to the negative impacts of extreme events (Jentsch et al., 2007), so we predict greater and more persistent community-level effects from heat waves with earlier timing relative to plant development. We also expect that plants experiencing additional stressors such as herbivory will be more susceptible to negative impacts from extreme events (Bansal et al., 2013), leading to greater effects on communities associated with those plants. Accordingly, here we present the results of a field experiment testing the short- and long-term impacts of heat wave timing, both within a season and relative to experimental herbivory, on plant performance and insect herbivore communities.

We employed common milkweed (*Asclepias syriaca*) and open-top heating chambers with infrared heaters to elevate temperatures around the clock for 96 h, mimicking a natural extreme heat event. We experimentally varied the timing of heat waves as well as whether they were immediately preceded by experimental herbivory by a specialist leaf-chewing beetle (*Tetraopes tetrophthalmus*). Because milkweed is perennial, we were able to follow the impacts of the single heat wave on plant growth and aphid communities for multiple years.

MATERIALS AND METHODS

We conducted a field experiment in a recently tilled old field at Kellogg Biological Station (Hickory Corners, MI, USA). Milkweed plants were propagated from rhizome cuttings collected on 16–18 May 2018, from 10 *A. syriaca* genets growing naturally at Kellogg Biological Station. We ensured each genet was distinct by collecting only from the centers of patches isolated by >100 m. Rhizome pieces were cut to 5–10 cm and planted into an even randomized grid (2-m spacing) at a depth of approximately 10 cm between 18 and 21 May 2018. We checked plants daily and, as they emerged, enclosed each individually in a mesh cage (19 L, 200-μm white nylon mesh). We randomly assigned the 173 plants that successfully emerged to six treatment groups representing a factorial manipulation of temperature (control, early- season heat wave, or late-season heat wave) and the presence and absence of an early season bout of experimental herbivory.

Experimental beetle herbivory treatments

Plants in our herbivory treatment received two female and two male adult red milkweed beetles (*T. tetrophthalmus*) in their enclosure on 13–16 July 2018. Beetles were locally collected and maintained on fresh milkweed foliage until their use. We maintained four

beetles per plant by checking cages daily and replacing dead or missing beetles. Beetles were removed from a cage when approximately 10% of that plant's leaf area had been consumed.

Heat wave treatments

We simulated heat wave conditions for individual plants using open-top chambers combined with electric infrared heaters, allowing us to achieve heating during cloudy conditions and at night (Appendix S1). This contrasts with most in-field warming experiments, which use only open-top chambers. Nighttime warming is characteristic of heat waves (De Boeck et al., 2010) and is known to contribute to biological impacts such as yield losses (García et al., 2015). Our chambers were tetrahedrons of anticondensate greenhouse plastic (6 mil, 91% transmittance, Ply-Ag Corp., CA), with a base of 1.5 m^2, a height of 1.2 m, and an upper opening of 0.6 m^2 (Appendix S1). We wired 300-W electric heating elements (Tempco, Inc., IL) into the open top of each heating chamber, turning these on at night as well as during the daytime when the sun had been obscured for at least 60 min.

We applied heat waves to 18 plants at a time (nine herbivory and nine herbivory-control), with each heat wave lasting 96 h. Our early heat wave treatment included three 18-plant cohorts that received heat wave treatments on 16, 23, and 27 July. Our late heat wave treatment included another three 18-plant cohorts on 13, 20, and 24 August.

During each heat wave treatment, we placed procedural control chambers of the same design but constructed from metal screens over six randomly selected control plants (three per herbivory treatment) to account for any chamber effects on plants or insects. Procedural controls did not differ from open-air controls in plant size ($\chi_1^2 = 0.34$, $p = 0.56$; Appendix S1), aphid abundance ($\chi_1^2 = 2.95$, $p = 0.09$; Appendix S1), chewing herbivory ($\chi_1^2 = 1.04$, $p = 0.31$; Appendix S1), or cardenolides ($\chi_1^2 = 1.54$, $p = 0.21$; Appendix S1), indicating that our results were not due to chamber effects. Outside of the 96-h treatments, all plants were exposed to ambient conditions in the field.

We recorded air temperatures every 15 min with HOBO Pendant data loggers (Onset Computer Corporation, MA) in solar radiation shields. Our heat wave treatments elevated daytime highs 9.0°C above an ambient high of 36.8°C, nighttime lows 3.1°C above an ambient low of 10.7°C during the early heat wave treatment, daytime highs 9.0°C above an ambient high of 35.3°C, and nighttime lows 3.4°C above an average ambient of 12.5°C during the late heat wave treatment (Appendix S1).

Plant trait and insect community sampling

We measured milkweed stem heights, visually estimated percentage damage by chewing herbivores, and censused the herbivore community weekly for each plant during the year of our heat wave treatments (2018) and for the two following growing seasons (2019–2020). At the end of the first growing season, we examined the within-year effects of our treatments on plant resistance by collecting eight leaves per plant to be analyzed for the concentration of cardenolides, toxic compounds thought to be important for milkweed defense (Agrawal et al., 2012). Four of the eight leaves were top-most mature leaves, which would have formed after the heat wave treatment, while the other four were bottom-most leaves, which were present during the heat treatment. At the end of each growing season, we measured plant fecundity by collecting, drying, and massing fruits. Only plants that produced fruits in a given year (0% in Year 1, 21% in Year 2, and 8% in Year 3) were included in fecundity analysis. The milkweed herbivore community was dominated by three species of specialist aphid that commonly co-occur on *A. syriaca*: *Aphis nerii*, *Aphis asclepiadis*, and *Myzocallis asclepiadis*. Other herbivores, including monarch caterpillars (*Danaus plexippus*), were observed rarely in our weekly surveys. We therefore did not include their counts in our analyses, but we analyze their effects on plants via our visual estimates of total leaf herbivory by chewers.

Cardenolide analysis

We quantified foliar cardenolides from leaf extracts on an Agilent 1100 HPLC with diode array detector and a Gemini C18 reversed-phase, 3-μm, 150-mm × 4.6-mm column. Frozen (−20°C) leaf tissue was oven dried at 60°C and then ground to a fine powder (50 mg) and extracted with 1.5 mL 100% methanol and spiked with 20 μg of digitoxin as an internal standard. Cardenolides were then extracted by adding 20 FastPrep beads and agitating twice on a FastPrep-24 homogenizer for 45 s at 6.5 m/s and then centrifuged at 20,800 × g for 12 min. Supernatants were dried in a vacuum concentrator at 35°C, resuspended in 200 μL methanol, and filtered using 0.45 μm hydrophilic membranes. We injected 15 μL into the HPLC running a constant flow of 0.7 mL/min with a gradient of acetonitrile and water as follows: 0–2 min at 16% acetonitrile, 2–25 min from 16% to 70%, 25–30 min from 70% to 95%, and 30–35 min at 95%, followed by 10 min reconditioning at 16% acetonitrile. Because the most polar (and dominant) cardenolide of *A. syriaca* was not detected in these samples, we dried down the

extracts, resuspended them in 16% acetonitrile, 16% methanol, and 68% water, and refiltered and injected them in the HPLC. Cardenolides from both runs were recorded at 218 nm, and absorbance spectra were recorded between 200 and 300 nm. Peaks showing a single absorption maximum between 214 and 222 nm correspond to the unsaturated lactone indicative of cardenolides (Malcolm & Zalucki, 1996). Concentrations were standardized by peak area to the digitoxin internal standard with known concentration. We report the total cardenolide concentrations (sum of the three peaks) on a dry mass basis.

Statistical analysis

We tested the independent and interactive effects of heatwave timing and experimental herbivory on plant growth and fecundity, aphid abundance, and chewing herbivore damage using linear mixed models and generalized linear mixed models in the lme4 R package (Bates et al., 2015; R Core Team, 2021). For plant responses, we analyzed the log fruit mass, log maximum stem length achieved by each plant in a given year, and log cardenolide concentrations with normal error distributions. Cardenolide concentrations were analyzed separately for old (heat wave-concurrent) and new (post-heat wave) leaves. For chewing damage, we used square root percentage damage and a normal distribution. For aphids, we totaled counts across weeks within species each year for each plant and used a negative binomial distribution. Each model included early heat wave, late

heat wave, and herbivory treatment fixed effects along with early HW:herbivory and late HW:herbivory interaction terms. We analyzed each year separately and included genet as a random intercept. Type III ANOVA from the car package was used for significance testing (Fox & Weisberg, 2019). Sample size was 22–27 individuals per treatment combination (see Appendix S1 for details).

RESULTS

Plants

Our heat wave and herbivory treatments had no effect on plant growth, measured as the maximum annual stem height achieved (Appendix S1), but they had substantial effects on plant fecundity and cardenolide concentration. In particular, early-season heat waves increased fecundity (fruit mass per plant) for plants that produced fruits by 254% in Year 2 (the year following the event; Figure 1; $\chi_1^2 = 3.96$, $p = 0.047$), while late-season heat waves had no effect ($\chi_1^2 = 0.56$, $p = 0.46$). Treatment groups did not differ in the probability of a given plant producing fruits (Appendix S1).

Early heat waves decreased cardenolide content in both old leaves that had been present during the heat wave (Figure 2; 7% decrease; $\chi_1^2 = 9.39$, $p = 0.002$) and in new leaves that were formed after the heat wave (17% decrease; $\chi_1^2 = 16.93$, $p < 0.001$). For old leaves, this decrease in cardenolides was absent in plants that also experienced the experimental herbivory treatment
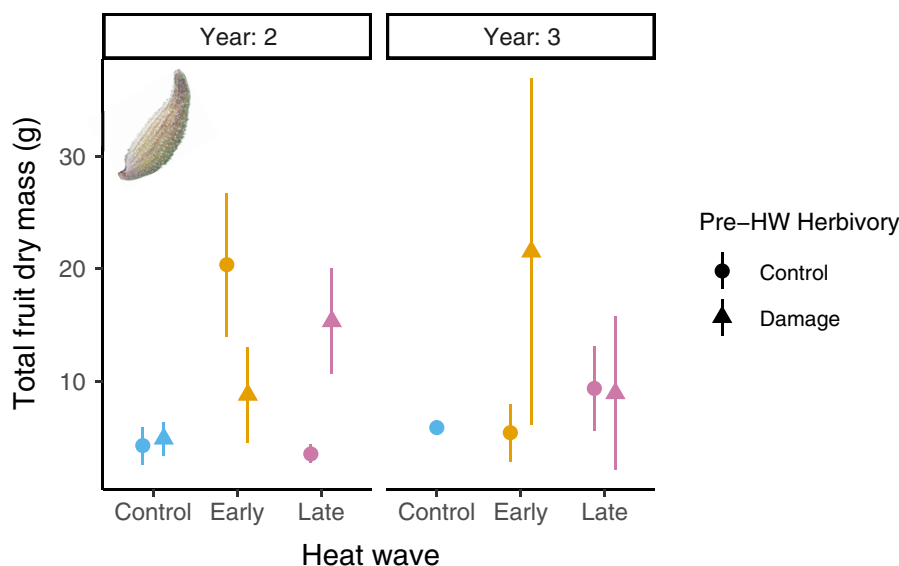

FIGURE 1 Fecundity of milkweed plants, measured as total dry mass of fruit per plant. Points represent mean of each treatment group, whiskers are SEs of mean. Figure excludes individuals that did not produce fruit. Year 1 of experiment is not shown because plants did not reproduce until their second year. Sample size is one to eight plants per treatment combination.
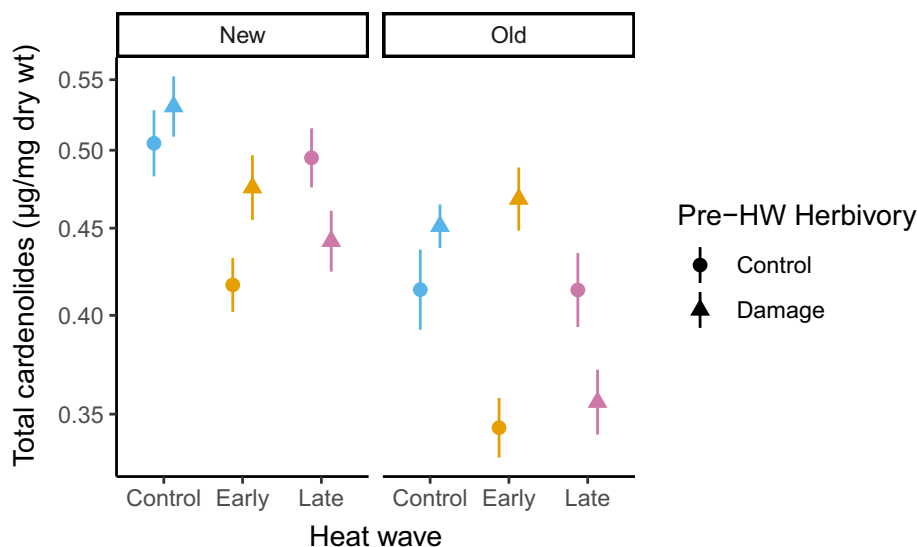
FIGURE 2 Total leaf cardenolide content after heat wave treatments in Year 1. New leaves are those formed after heat waves, old leaves are those that were present during heat waves. Points represent mean of each treatment group, whiskers are SEs of mean. Please note log scale on *y*-axis. Sample size is 22–26 plants per treatment combination.

(Figure 2; early HW:herbivory interaction: $\chi_1^2 = 7.09$, $p = 0.008$), suggesting that heat wave responses and defense induction canceled each other out. In contrast, late heat waves did not affect cardenolide content in new (Figure 2; $\chi_1^2 = 2.27$, $p = 0.13$) or old ($\chi_1^2 = 0.0058$, $p = 0.94$) leaves, suggesting that cardenolide production is less plastic later in the growing season.

Herbivores

Heat waves' effects on milkweed aphid communities depended on both the timing of the original heat wave and how many years had passed since the heat wave. Heat waves did not affect total aphid abundance in Year 1 (Figure 3; early HW: $\chi_1^2 = 0.024$, $p = 0.88$, late HW: $\chi_1^2 = 0.72$, $p = 0.78$). By Year 2, early heat waves in Year 1 had no effect (Figure 3; $\chi_1^2 = 1.48$, $p = 0.22$), while late heat waves caused a 362% increase in total aphid abundance ($\chi_1^2 = 17.65$, $p < 0.001$). In contrast, both early and late heat waves led to increases in aphid abundance in Year 3, by 207% and 144%, respectively (Figure 3; early HW: $\chi_1^2 = 14.15$, $p < 0.001$, late HW: $\chi_1^2 = 9.61$, $p = 0.002$). The early-season effect on Year 3 was diminished in plants that had also undergone Year 1 experimental herbivory treatments (Figure 3; early HW:herbivory interaction: $\chi_1^2 = 9.61$, $p = 0.002$), suggesting that pre-heat wave beetle herbivory protected plants from heat wave-related increases in aphid abundance 2 years later.

During the year of the heat wave, there were no direct effects of treatment on species-level abundance (Figure 3) of the three aphid species present: *A. asclepiadis* (early

HW: $\chi_1^2 = 0.31$, $p = 0.58$, late HW: $\chi_1^2 = 0.45$, $p = 0.50$), *M. asclepiadis* (early HW: $\chi_1^2 = 0.0046$, $p = 0.95$, late HW: $\chi_1^2 = 0.57$, $p = 0.45$), or *A. nerii* (early HW: $\chi_1^2 = 2.59$, $p = 0.11$, late HW: $\chi_1^2 = 0.16$, $p = 0.69$). Survey data from before and after the late heat wave indicate that aphids did not experience substantial direct mortality from the heat wave treatment; treated plants averaged 8.06 aphids/plant 3 days before treatments began and 35.01 aphids/plant on the day treatments ended. Pre-heat wave herbivory treatments decreased *A. nerii* abundance by 59% (Figure 3; $\chi_1^2 = 9.17$, $p = 0.002$), but this effect disappeared when plants had also experienced an early-season heat wave (early HW:herbivory interaction: $\chi_1^2 = 5.87$, $p = 0.015$). In contrast, experimental herbivory treatments did not impact same-year abundance of *A. asclepiadis* (Figure 3; $\chi_1^2 = 0.86$, $p = 0.35$) or *M. asclepiadis* ($\chi_1^2 = 0.018$, $p = 0.89$).

The multiyear effects of the heat waves on aphid species abundance differed strikingly from the same-year effects. Multiyear effects also depended on the species of aphid: early-season heat waves had more positive effects on abundance *A. asclepiadis* over the 3-year study period, whereas late-season heat waves had more positive effects on *M. asclepiadis*. For Year 2, early-season heat waves the previous year led to 121% higher *M. asclepiadis* abundance (Figure 3; $\chi_1^2 = 6.91$, $p = 0.009$) but no change in the abundance of *A. asclepiadis* ($\chi_1^2 = 0.45$, $p = 0.50$) or *A. nerii* ($\chi_1^2 = 0.022$, $p = 0.88$). Late-season heat waves led to 477% higher Year 2 abundance for *A. asclepiadis* (Figure 3; $\chi_1^2 = 6.15$, $p = 0.013$) and 236% higher abundance of *M. asclepiadis* ($\chi_1^2 = 3.76$, $p = 0.052$) but no change in abundance of *A. nerii* ($\chi_1^2 = 0.018$, $p = 0.89$). For Year 3, early-season heat waves led to a 1279% higher
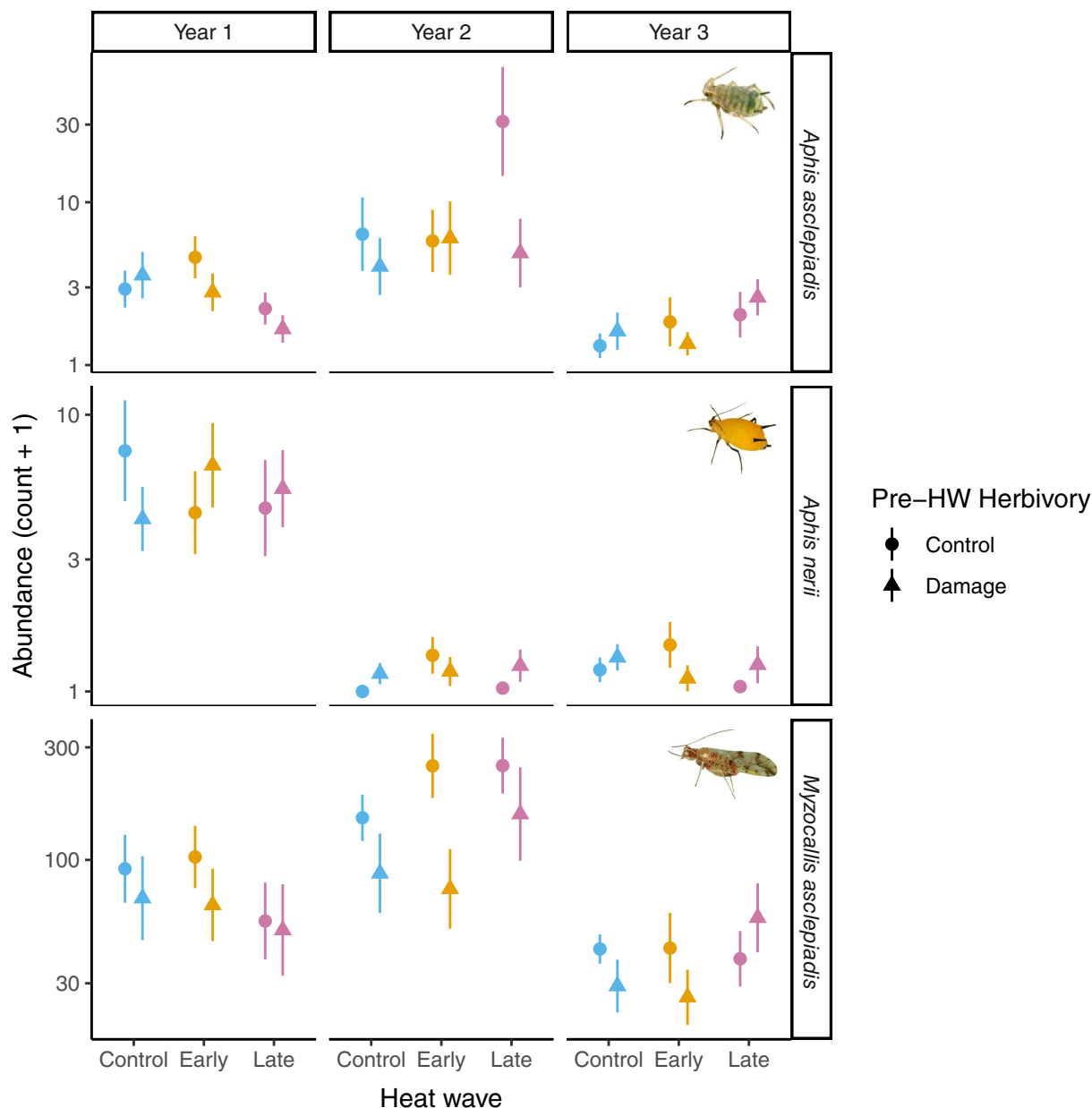
FIGURE 3 Annual abundance counts for each species of aphid, based on weekly surveys. Points represent mean of each treatment group, whiskers are SEs of mean. Note log(count +1) scale on *y*-axis. Sample size is 22–26 plants per treatment combination.

abundance of *A. asclepiadis* (Figure 3; $\chi_1^2 = 11.49$, $p < 0.001$) and 46% higher abundance of *M. myzocallis* ($\chi_1^2 = 4.15$, $p = 0.04$) but no change in the abundance of *A. nerii* ($\chi_1^2 = 0.25$, $p = 0.62$). Late-season heat waves led to 645% higher Year 3 abundance of *A. asclepiadis* (Figure 3; $\chi_1^2 = 8.22$, $p = 0.004$) and 70% higher abundance of *M. asclepiadis* ($\chi_1^2 = 3.77$, $p = 0.052$), but again no change in the abundance of *A. nerii* ($\chi_1^2 = 2.34$, $p = 0.13$).

Pre-heat wave herbivory treatment did not directly impact abundance in later years of *A. asclepiadis* (Figure 3; Year 2: $\chi_1^2 = 0.0526$, $p = 0.82$; Year 3: $\chi_1^2 = 1.76$, $p = 0.18$), *M. asclepiadis* (Year 2: $\chi_1^2 = 0.085$, $p = 0.77$; Year 3: $\chi_1^2 = 0.0004$, $p = 0.95$), or *A. nerii* (Figure 3; Year 2:

$\chi_1^2 = 0.021$, $p = 0.89$; Year 3: $\chi_1^2 = 1.57$, $p = 0.21$); however, it did interact with heat wave effects. Specifically, heat wave-related increases in *A. asclepiadis* abundance were diminished in plants that had also undergone Year 1 experimental herbivory treatments (Figure 3; early HW:herbivory interaction, Year 3: $\chi_1^2 = 11.64$, $p = 0.001$; late HW:herbivory interaction, Year 2: $\chi_1^2 = 3.18$, $p = 0.074$, Year 3: $\chi_1^2 = 6.49$, $p = 0.011$), again suggesting that plants subjected to the herbivory treatment were protected from heat wave-related increases in aphid abundance.

Damage on plants from chewing herbivores did not respond as strongly to heat wave events as did aphid
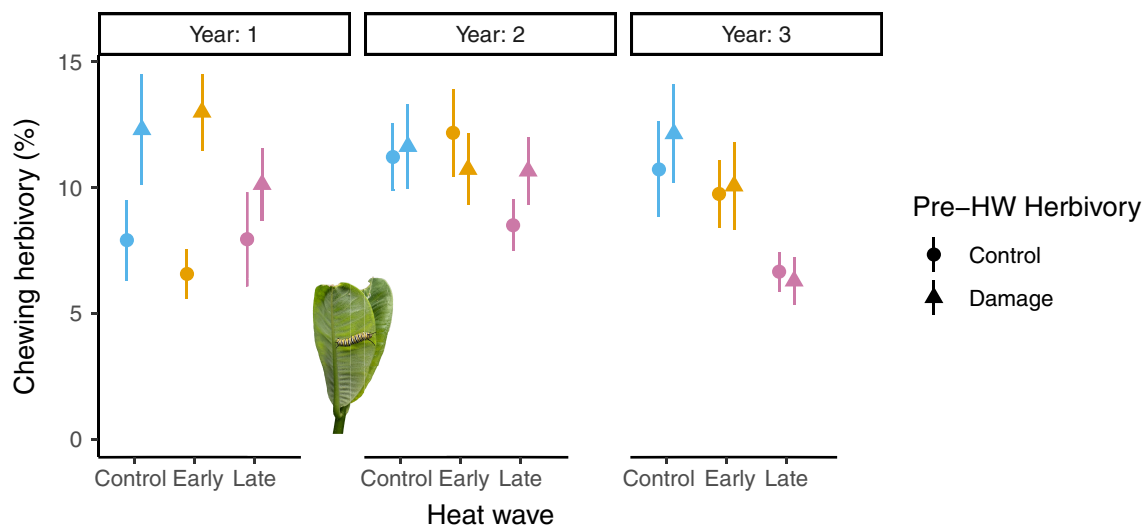
FIGURE 4 Average percentage leaf area lost to chewing herbivores. Points represent mean of each treatment group, whiskers are SEs of mean. Sample size is 22–26 plants per treatment combination.

abundance. We did, however, find a trend for damage by ambient chewing herbivores in Year 3 to be 39% lower for plants that had experienced a late-season heat wave in Year 1 (Figure 4; $\chi^2_1 = 3.04$, $p = 0.081$).

DISCUSSION

Climate change, in its effects on organismal phenology and metabolic rates, has been described as the "manipulation of time by humans" (Wolkovich et al., 2014). Indeed, a wealth of research has shown that gradual warming can alter the timing of species interactions, with wide-ranging effects (Renner & Zohner, 2018). Our study shows that changes in metabolic rates or interaction timing are not the only ways time and climate change are linked. The timing (e.g., seasonality) of extreme events associated with climate change can also alter ecological interactions, and the manifestation of those impacts has its own element of timing. Although many have called for investigation of timing as it relates to extreme weather events (Jentsch et al., 2007; Thompson et al., 2013), our study advances knowledge by directly manipulating extreme event timing for an ecological community in the field.

Our results show that heat waves—by definition events that last only days—can impact plant and insect herbivore communities in ways that last multiple years, an example of discrete events with persistent impacts (Wolkovich et al., 2014). Unlike in other multiyear warming studies, our experimental warming events were short term to the extent that the tissues and herbivores responding to the heat wave were, for the most part, not

present during the actual event. The community-level effects of extreme events instead reverberated long afterward due to their impact on plant individuals as hosts for species interactions. Moreover, the multiyear impacts differed depending on two aspects of timing: within-season timing of events (relative to the calendar and to other biotic events) and among-season timing of impacts (years since the heat wave occurred). In what follows, we explain how heat wave effects on plants and their herbivore communities may be modulated by within-season timing and persist across years.

While other studies emphasize the direct negative effects of heat waves on plants (Bisbis et al., 2018; Ciais et al., 2005), our results suggest that heat waves do not have to kill or severely damage plants in order to be significant ecologically. In fact, we saw substantial community-level impacts from heat wave events that were neutral to positive from a plant fitness perspective. Plant growth, in terms of maximum annual height achieved, was not impacted by either early or late heat waves, and fecundity increased in the year following an early-season heat wave. The observed changes in insect communities must therefore be the result of more subtle changes in host plant traits rather than changes in overall host plant availability or productivity. This result suggests that climate change monitoring studies that focus on detecting signs of direct stress may miss ecological consequences of extreme events. Ambient warming also has a positive effect on milkweed growth (Couture et al., 2015; Kharouba & Yang, 2021), suggesting that a singular focus on stressed plants may obscure the ecological consequences of higher mean temperatures as well.

As expected, plants were more responsive to heat waves occurring earlier in the season (Jagadish

et al., 2021; Jentsch et al., 2007), with early-season heat waves leading to increases in fecundity and decreases in foliar cardenolides, while late-season heat waves had no such effects. Studies of gradual warming have found both increases and decreases in foliar defenses depending on the compound and species (Jamieson et al., 2012), with milkweed cardenolides increasing slightly with elevated ambient temperature (Couture et al., 2015; Faldyn et al., 2018). Our results therefore indicate that foliar chemical responses to extreme events can vary with timing and differ from responses to gradual warming. In addition, we found that heat wave effects at the herbivore community level were later developing and more persistent for early-season heat waves, again indicating greater susceptibility to extreme events for earlier plant stages.

Overall, we found that any short-term negative effects of heat waves on herbivore populations were compensated for by long-term positive effects via changes in host plants. Heat waves increased total aphid abundance on milkweed plants, particularly in the year following heat wave treatments. Nonetheless, the overall positive trend in aphid abundance concealed that heat waves had differential impacts on three aphid species within the community. Rather than across-the-board increases or decreases in herbivore abundance following heat waves, we observed a rebalancing of community composition based on individual aphid species' responses to heat wave effects on their host plants. We also found that the timing of heat wave impacts on aphids (whether effects were observed in Year 1, 2, or 3) varied by species, altering temporal dynamics of community composition and potentially of intraguild interactions.

Our results suggest that plant responses to extreme heat events are an additional factor at play in aphid competitive interactions, which were previously found to be influenced by plant genotype and the presence of ants that tend *A. asclepiadis* but antagonize *A. nerii* and *M. asclepiadis* (Mooney et al., 2008; Smith et al., 2008). Differences in colonization timing among aphid species may also contribute to the observed species-specific effects. *A. nerii* is the only species of the three that cannot overwinter in Michigan, so it colonizes milkweed plants later in the growing season than the locally overwintering *A. asclepiadis* and *M. asclepiadis* (Mooney et al., 2008). Given that *A. asclepiadis* and *M. asclepiadis* abundances were more responsive to heat wave treatments, it may be the case that heat wave effects on early-season plant phenotypes, which are only experienced by locally overwintering aphid species, may be more pronounced or more important for aphid abundance than the later-season phenotypes encountered by *A. nerii*. Because these heat wave effects on aphid abundance were positive, increased frequency of heat waves in the future may lead to community patterns favoring the locally overwintering *A. asclepiadis* and *M. asclepiadis*, to the detriment of the annually recolonizing *A. nerii*. In herbivore communities more generally, the life history of particular species may be an important factor in shaping community composition in the face of intensifying heat waves.

Heat wave effects on herbivore communities were also modulated, in this case attenuated, by prior biotic interactions of the host plant. Specifically, heat wave-induced abundance increases of *A. nerii* and *A. asclepiadis* were rendered slim or nonexistent when the heat wave was preceded by herbivory by a leaf-chewing beetle. This regulation of aphid populations after beetle herbivory could be due to prolonged induction of plant defenses. Previous work, primarily on *A. nerii*, showed reduced aphid numbers on high-cardenolide milkweed species and genotypes (Agrawal, 2004; Züst & Agrawal, 2016) and on common milkweed plants previously damaged by chewing herbivores (Ali & Agrawal, 2014; Van Zandt & Agrawal, 2004). We found a similar result, which is that prior herbivory offset heat wave-induced decreases in leaf cardenolide content. In other words, heightened plant defenses in response to chewing herbivory counteracted increases in plant susceptibility resulting from heat waves, both in terms of defense production in the same year and herbivore abundance in future years. This result is an example of species interactions attenuating the impacts of climate change on plants. In contrast to our results for heat waves, elevated CO_2 tends to diminish induced defense responses, magnifying rather than attenuating climate change-induced increases in plant susceptibility (DeLucia et al., 2012; Karowe & Grubb, 2011).

Our results make it clear that slight variation in the timing of extreme climate events can have significant impacts on ecological communities. Annual-scale warming due to climate change is already thought to decrease the stability and predictability of communities (Gilman et al., 2010; Kreyling et al., 2011). The ecological variability arising from short-term events, coupled with the increasing frequency of extreme events themselves, indicates that community predictability may decrease even more quickly than previously thought. Rather than seeing the timing of extreme events as just one more layer of complexity in the already complex topology of global change; however, we see timing as a key that could unlock explanations for previously unexplained variation in community responses to changing climates. Taken together, our results highlight the particular importance of early-season extreme events for plant-associated communities, the persistent effects of heat waves on community composition, and the overarching need to consider timing when designing and interpreting heat wave studies.

AUTHOR CONTRIBUTIONS

William C. Wetzel designed the study. Luke N. Zehr implemented the experiment and collected field data. Anurag A. Agrawal performed chemical analysis. Olivia L. Cope performed data analysis and wrote the first draft of the manuscript. All authors contributed to revising the manuscript and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data (Cope et al., 2023) are available in figshare at https://doi.org/10.6084/m9.figshare.21867828.v1.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.