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Seasonal assembly of arthropod communities on milkweeds experiencing simulated herbivory

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ABSTRACT

1. The seasonal assembly of arthropod communities is shaped by biotic and abiotic aspects of the habitat that limit the appearance or activity phenology of potential community members. In addition, previous interactions within the community, such as herbivore-induced plant defensive responses, aggregation, and predator avoidance also likely affect the assembly of arthropod communities on individual plants.

2. We observed the phenology of arthropod communities and defensive plant traits on 100 milkweed (*Asclepias eriocarpa*)

individuals at monthly intervals over a growing season. We experimentally wounded a subset of plants each month (April-August) to observe the effect of simulated herbivore damage on the seasonal assembly of these arthropod communities.

3. All plant traits and measures of arthropod communities changed over the season. The observed response to experimental leaf damage suggested a trend of induced susceptibility in early months, but not late months.

4. Plants receiving early-season simulated herbivory experienced more subsequent leaf damage than unmanipulated plants.

5. We observed several lagged correlations in our study indicating that blue milkweed beetle (*Chrysochus cobaltinus)* abundance was lower in months following high natural leaf damage, and that the abundance of a secondary omnivore (*Lygaeus kalmii)* and total predator abundance tended to follow months with high *C. cobaltinus* abundance.

6. This study suggests that ahistoric habitat factors determined much of the observed seasonality of arthropod communities, but induced responses to simulated herbivory also contributed historical effects that influenced arthropod community assembly.

KEYWORDS milkweed, phenology, community assembly, historical contingency, environmental filter, induced responses to herbivory

INTRODUCTION

The processes that drive community assembly have been of central interest since the foundation of ecology as a discipline (Belyea & Lancaster, 1999). Numerous studies have documented community assembly in the years following a disturbance (Bazzaz, 1975; Thibault & Brown, 2008) or invasion (Fridley *et al.*, 2007). A key question arising from such studies is the degree to which historical processes, such as previous species interactions and the order of species colonization, affect the trajectory of community assembly compared to habitat ‘filters’, ahistorical factors in the habitat which prevent the establishment of some species in the community without regard to priority effects (Kraft *et al.*, 2015). This framework was originally developed to study the assembly of communities in space (Diamond, 1975; Keddy, 1992; Chase, 2003; Maire *et al.*, 2012), but some aspects of this framework may also be usefully applied to the study of seasonal community assembly, the phenological process by which species appear in a defined community throughout the season (Benbow *et al.*, 2013). In the context of seasonal phenology, we adapt the habitat filter concept to examine aspects of the environment that limit the appearance or activity of a species at a particular point in time throughout the season, independent of previous interactions in the community. Here, we are applying a broad definition of the habitat filter, which includes both biotic and abiotic factors in the environment.

Recognizing the difficulty of separating biotic and abiotic effects (Chase, 2003; Kraft *et al.*, 2015), our goal is to emphasize the key distinction between historical and ahistorical factors, regardless of whether they are biotic or abiotic.

Examples of ahistorical habitat filters could include seasonal changes in temperature (abiotic) or seasonal changes in the constitutive defenses of host plants (biotic e.g. Boege & Marquis, 2005) that are not driven by past interactions in the community. For example, degree-day models accurately described both the phenology of plants and their pollinators in the Rocky Mountains, suggesting that seasonal temperature patterns are highly correlated with the assembly of plant and pollinator communities (Forrest & Thomson, 2011). Degree-day models have been similarly successful at describing plant phenophases (Dunne *et al.*, 2003) and the phenology of herbivorous insects (Strathdee *et al.*, 1993; Nufio *et al.*, 2010; Buckley *et al.*, 2015), suggesting that seasonal temperature patterns are also correlated with the assembly of plant-herbivore interactions. Seasonally varying plant traits that are independent of previous interactions can also limit the herbivores present on plants. For example, leaf defensive traits such as toughness and tannin content often increase endogenously over the season, so herbivores that are deterred by these traits may be confined to the early season (Feeny, 1970; Forkner *et al.*, 2004).

On the other hand, studies of species interactions have also found evidence that interactions among the species already present in a community can sometimes affect the trajectory of community assembly. In these cases, the composition of the arthropod community is historically contingent, as the direct interactions or habitat modifications of one species could affect the phenology of others. For example, herbivore damage on a particular plant may induce defenses or attractants that affect herbivore populations at a later time point (Van Zandt & Agrawal, 2004; Ali & Agrawal, 2014). Accordingly, herbivores on the same plant can compete by altering plant habitat or attracting predators (Kaplan & Denno, 2007) even if most of these interactions are lagged in time (i.e., temporally asymmetric, Lawton & Hassell, 1981; Yang, 2012). If arthropods aggregate or have positive interactions, the presence of an arthropod in a community may increase the likelihood of establishment of other arthropods. For example, leaf-tying caterpillars increase the subsequent colonization of a variety of arboreal insects by creating habitat structures (Lill & Marquis, 2003). Trophic cascades and predator avoidance can also affect community assembly over annual timescales (Piovia-Scott *et al.*, 2017). For example, the persistence of overwintering predators in dried leaf galls decreased the abundance of herbivorous insects on oak trees in the following spring (Wetzel *et al.*, 2016). Similarly, the adhesion of dead insects to tarweed plants increased early-season predator abundance on those plants, which led to a subsequent decrease in herbivory (Krimmel & Pearse, 2013).

Historical interactions and habitat filters may interact to determine the phenology and seasonal assembly of arthropod communities. For example, induced plant defenses act as a historical effect by linking past herbivore damage with future herbivore communities. However, the efficacy of inducible defenses can also change over the season independently of past damage (Hougen-Eitzman & Karban, 1995; Shiojiri & Karban, 2008; McMunn, 2017). Likewise, in aquatic ecosystems, size refuges from predation often occur as prey grow larger, so the effect of predators (a historical effect) may change over the season independently of historical effects as potential prey grow (Rudolf & Armstrong, 2008).

The study of phenological shifts has received renewed interest in the past decade due to concerns about the ongoing effects of climate change (Parmesan & Yohe, 2003; Polgar & Primack, 2011). One particular concern is that differences in the relative magnitude of phenological shifts could affect species interactions. In evaluating the phenological shifts of different organisms, it may be particularly important to consider the relative importance of habitat filtering (i.e., ahistorical factors) versus historical species interactions. When the phenologies of organisms are largely driven by habitat filters, an understanding of their effects can help predict phenological shifts that might arise from a changed environment. When the phenologies of organisms are largely driven by contingent historical effects within communities, a change in the environment may have more complex consequences for phenological shifts and species interactions.

We conducted an observational study and manipulative experiment on milkweed (*Asclepias eriocarpa*) arthropod communities in order to determine how the timing of herbivore damage influences the seasonal community assembly of arthropods on milkweed. We surveyed arthropod communities as well as a suite of milkweed defensive traits over the growing season. On a subset of plants each month, we mechanically damaged leaves to simulate herbivore damage. Using this combined observational and experimental study, we asked the following questions: (1) To what degree do arthropod communities change over the season irrespective of damage treatments? (2) Are arthropod communities affected by experimental damage to the plant in the previous month? (3) Does the effect of experimental damage on arthropod communities depend on the month in which it happens? (4) Is there correlative evidence for lagged effects of arthropod communities on arthropod communities of the same plant one month later? With this design, we sought to compare the amount of variation in arthropod communities described by historical interactions between arthropods and the modifications that they make to the plant, and ahistorical habitat filters, including plant or seasonality-based factors unaffected by the arthropods.

METHODS

Study Site

We conducted our study in a grassland habitat on Arnold Hill at Hastings Reservation, near Carmel Valley, California (36.361 N, 121.563 W). The site had a history of grazing and hay production until 1937, when the property was acquired by the University of California (Griffin 1990). In addition to milkweed, the grasslands at the site included a variety of non-native grasses including *Avena* sp., *Bromus hordeaceus*, *B. diandrus*, *Hordeum* sp., *Stipa pulchra,* and *Aira caryophyllea,* as well as forbs such as *Madia gracilis*, *Plagiobothrys* sp., *Amsinckia menziesii,* and *Lupinus* sp , and *Lepidium nitidum.* Grasslands were bordered by oak savannas and woodlands comprised of *Quercus lobata*, *Q. douglasii*, *Q. chrysolepis*, *Q. agrifolia*, and *Q. kelloggii* as well as *Toxicodendron diversilobum*. Slopes and aspects of sites varied, but were generally on hilly terrain.

Milkweed and arthropod natural history

We conducted surveys of arthropod interactions with woolypod milkweed (*Asclepias eriocarpa*). *Asclepias eriocarpa* is found throughout California where it occurs commonly in coast range and

Sierra foothill grasslands, chaparral, and savannas. *Asclepias eriocarpa* grows in distinct patches, which likely consist of genetically identical ramets with a limited degree of below-ground connection. Patches of *A. eriocarpa* ramets were common throughout grasslands at Hastings Reservation.

Many milkweeds, including *A. eriocarpa*, are known for their leaf traits that cause resistance to herbivores, such as latex production, toxicity due to cardiac glycosides, and physical leaf characteristics, such as tomentose trichomes (Agrawal, 2004, 2017; Agrawal *et al.*, 2009; Rasmann *et al.*, 2009). The herbivore community associated with the milkweeds at Hastings Reservation is specialized on the genus and includes many species with bright coloration such as monarch butterflies (*Danaus plexippus*), milkweed leaf beetles (*Chrysochus cobaltinus*), small milkweed bugs (*Lygaeus kalmii*), and milkweed longhorned beetles (*Tetraopes basalis*). All of these herbivores sequester cardiac glycosides (Farrell, 2001).

Experimental and observational design

In April 2013, we selected ten patches of milkweed for our study based on the presence of >10 milkweed individuals in early April 2013. All patches were within 1 km of one another, but separated from each other by at least 20 m. Within each patch we selected ten milkweed individuals spaced >1 meter apart from one another and >10 cm in height, resulting in a total of 100 milkweed plants surveyed each month until September 2013. Ramets within 5 cm of one another were considered to be the same individual, though we do not know the actual extent of vascular connection between ramets of this species. In each patch, we randomly assigned one plant to each of five treatments that received mechanical damage in either April, May, June, July, or August and five plants to a control treatment, which received no experimental damage. Experimental damage consisted of six longitudinal strips of puncture wounds from a fabric pattern wheel on ½ of the plant’s leaves. One plant (J97) was qualitatively different than all others in the population, because it was an order of magnitude larger, produced early flowers, and had an arthropod abundance that was an order of magnitude higher than all other plants. We removed this plant from all experimental comparisons, which rely on comparisons between plants. We retained information from this plant for comparisons made on repeated observations of plants over time (such as lagged correlations).

Plant Traits

For all plants in months April-September, we visually assessed the amount of leaf tissue missing due to herbivory, recording the percentage of original leaf tissue remaining (0-100). Plants with green tissue aboveground were considered to be alive in that month. Each month we also recorded a series of invasively measured plant traits on the randomly chosen plant that received damage in that month (one plant per patch –see above). On this plant, we measured leaf toughness averaged between two leaves (puncture force in grams to push a 13.9 mm^2 hexagonal pin through the leaf lamella) using a penetrometer (Pesola spring scale). We measured latex exudation averaged between two leaves by cutting the distal one cm of each leaf and absorbing the exuded latex onto a preweighed piece of filter paper (Whatman #1). The mass of exuded latex was calculated as the difference in pre- and post-measurement mass of the filter paper. Because of differences in moisture or measurement error, a few negative values were obtained, so we subtracted the minimum negative value from all measurements to standardize values to zero. At the same time, we measured trichome density averaged between two leaves. Leaf disks were cut using a 3.3 mm leaf borer. We photographed both sides of each leaf disk under 30X magnification. Using ImageJ software (NIH, Bethesda, MD), we used image thresholding to estimate the total trichome density as trichome area / area of leaf disk. Latex exudation was also recorded in the month following damage in order to assess inducibility.

Arthropod Surveys

Each month, we recorded all arthropods observed in contact with each milkweed plant. We initially recorded each arthropod as a morphospecies and collected a voucher specimen from each. The majority of common arthropod morphospecies from the survey were identified to species. We assigned each arthropod to a trophic guild (herbivore, omnivore, predator, transient) based on natural history records for those species. We calculated a trophic index of arthropod communities based on the following abundances: (Predators + 0.5*Omnivores) / (Herbivores + 0.5*Omnivores).

Statistical Analysis

We analyzed the seasonal change in leaf traits using a set of linear mixed models, predicting leaf toughness and trichome density from a fixed effect of month of the survey and a random effect of patch (block) to account for variation due to genotype or microhabitat. For latex, we included a second fixed effect, induction in the previous month, and its interaction with calendar month in order to assess the inducibility of latex and its seasonality.

We analyzed univariate estimates of arthropod communities (abundance, species richness, abundance of particular species or guilds) as well as percent leaf damage using a linear mixed effects models. These models described the arthropod community or damage estimates with the fixed effects of month*induction. We included patch as a random effect to account for spatial differences in arthropod communities and potential host genotype effects. Supporting this, the inclusion of the patch factor in models consistently lowered the AIC of models. The abundance of individual arthropod species other than *C. cobaltinus* was low enough that we could not assess the effects of induction on them. All univariate measures of arthropod communities and percent leaf damage estimates were square root transformed prior to analysis in order to fit linear model assumptions.

In a final set of analyses, we looked for lagged relationships between aspects of arthropod communities. In these models, we related an aspect of arthropod communities or a plant trait at month *t* (for example predator abundance) with aspects of the arthropod community on the same plant in the previous month *t-1* using generalized linear mixed effects models with Poisson error distributions. The one exception was an analysis of plant mortality, where we used a binomial error distribution. We included plant individual and month as random effects in these models to account for seasonal and between-individual variation in arthropod communities. The specific hypotheses we tested involving lagged relationships are shown in Table 1 and were chosen based on *a priori* expectations about the lagged consequences of leaf damage, abundance of a key herbivore (*C. cobaltinus*), and predator abundance.

All statistics and graphics were generated in R. v. 3.0.3 (R Core Development Team 2014) using packages, *vegan* for multivariate community analysis (Oksanen *et al.*, 2010) and *lme4* for mixed models (Bates *et al.*, 2015). Model fit (R^2) statistics of fixed effects were calculated based on a comparison of log-likelihood ratios between models with and without specified fixed effects (Nakagawa & Schielzeth, 2013). In most cases, significance of terms in linear mixed effects models were assessed based on a likelihood ratio test between the full model and a null model lacking the specified term. For *lme4* models of lagged correlations, significance tests were drawn from model output.

RESULTS

Phenology of milkweed defensive traits and simulated herbivory

Trichome density on *A. eriocarpa* generally declined over the season (LR = 19.08, P = 0.002), with mean trichome densities highest in April and lowest at the end of the summer (Fig. 1a). Latex production varied over the season (LR= 20.06, P = 0.018), where latex production was highest in July (Fig. 1b). Latex production after simulated damage was highly variable relative to control plants, and we found no consistent evidence for significant latex induction by mechanical damage in *A. eriocarpa* over the season (LR = 6.63, P = 0.249, Fig. 1b). However, latex production was lower in months following high natural herbivore damage (lagged linear model, LR=22.54, P < 0.001). Leaf toughness (puncture force) changed over the season (LR=52.83, P < 0.001), where mean toughness was lowest in April and peaked from June until August (Fig. 1c).

Phenology of arthropod community

The most commonly encountered arthropod on *A. eriocarpa* was *Chrysochus cobaltinus*, which was encountered an order of magnitude more often than any other arthropod. The six most common arthropods beyond *C. cobaltinus* were the spider *Mallos pallidus*, milkweed bug *Lygaeus kalmii* (Hemiptera), *Scolops* sp. (Hemiptera), milkweed longhorn beetle *Tetraopes basilis* (Coleoptera), *Mecaphesa* sp. (Aranea), and big-eyed bug *Geocoris pallens* (Hemiptera) (Fig. 4).

The community of arthropods changed over the season in terms of arthropod abundance (LR = 152.74, P < 0.001), and species richness (LR = 247.46, P < 0.001), (Figs. 2,3). In April, arthropod communities were relatively depauperate (Fig. 2b) and sparse (Fig. 2a). The species richness and abundance of herbivores peaked from May through July. During this time, the community of arthropods expanded to include late-season arthropods, and shifted toward a community more similar to the latest season (August) community. Both the species richness and abundance of arthropods decreased in August and September

(Fig.2) when the arthropod community collapsed to a depauperate community comprised mostly of milkweed specialists, such as *C. cobaltinus* and *L. kalmii* (Fig. 4).

We divided the arthropod community into trophic guilds (herbivores, predators, and omnivores; Fig. 3). The abundance of arthropods in each guild changed over the season (herbivores: LR = 117.1, $P < 0.001$; predators: LR = 132.2, $P<0.001$; omnivores: LR=41.5, $P<0.001$). Herbivore abundance peaked later in the season (July) than predators (May), suggesting that the mean trophic level of the community may decrease over the season. In order to test this, we calculated a trophic index, whose values are greater with increasing trophic position of individuals. Accordingly, the trophic index (TI) of the community varied over the season (LR=164.5, $P<0.001$) was highest in May (0.51), and decreased to 0.12 in June, where it remained until predators fell out of the community at the end of the season. The phenology of *C. cobaltinus*, the key herbivore in the community, largely drove the overall abundance of herbivores (Figs 3,4). The abundance of *C. cobaltinus* adults peaked in May, and abundance of their resulting egg masses peaked in July. The phenologies of the next most abundant herbivores, *L. kalmii* and *T. basilis*, were slightly later than *C. cobaltinus* adults (Fig. 4). The most abundant predators were all spiders, though a variety of uncommon predators were seen on plants. The hemipteran, *G. pallens*, was the most common omnivore, and its phenology on milkweed was relatively early (Fig. 4).

Percent leaf damage varied over the season (LR = 147.4, P <0.001), where leaf damage peaked from June-July (Fig. 5a). While evidence of leaf damage was usually persistent, percent leaf damage could decrease over time due to growth of the plant or abscission of damaged leaves. By September, there was 60% mortality of plants (Fig. 5b). The highest rate of mortality occurred between the July and August censuses, shortly after the peak of herbivore damage. Because milkweeds have a perennial root system, mortality of a ramet indicates the inability of that ramet to reproduce or acquire resources, but regrowth of another ramet from the same root system may occur in subsequent years.

Effects of experimental leaf damage) on arthropods and further damage throughout the growing season.

The effect of experimental leaf damage on subsequent leaf damage depended on the month of simulated herbivory (LR = 12.13, P = 0.016, Fig. 5). Damage in April led to a marginal increase in leaf damage in May (LR = 2.48, P = 0.114), and damage in June led to a significant increase in leaf damage in July (LR = 23.01, P<0.001). The experimental leaf damage treatment did not result in greater plant mortality in the subsequent month (LR=1.35, P=0.245).

Experimental leaf damage had no effect on total arthropod abundance (LR = 0.10, P = 0.751) and species richness (LR = 0.18, P=0.668, Fig. 2). Experimental leaf damage decreased the abundance of predatory arthropods (LR=4.03, P=0.045), but it had no significant effect on herbivores (LR=0.01, P=0.96) or omnivores (LR = 0.06, P=0.812). This led to a 20% lower trophic index (i.e. numbers of predators per herbivore) of the arthropod community on experimentally damaged plants compared to control plants (LR = 4.59, P=0.032).

Lagged correlations of herbivores and predators on arthropod communities

Natural leaf damage in a given month preceded lower herbivore abundance and *C. cobaltinus* abundance in the following month (Table 1). Leaf damage had no lagged correlation with predator abundance, *L. kalmii* abundance, or the mortality of plants (Table 1). A higher abundance of *C. cobaltinus* individuals in a given month resulted in a higher abundance of *L. kalmii* and predatory arthropods in the following month (Table 1). The abundance *of C. cobaltinus* had no lagged correlation with total herbivore abundance, and we observed no temporal autocorrelation in *C. cobaltinus* populations on a given plant (Table 1). Predator abundance in a given month had no correlation with total herbivore abundance, *C. cobaltinus* abundance, *L. kalmii* abundance, or predator abundance in the following month (Table 1).

Predator abundance was greater on plants in months following high herbivore abundance (Table 1).

DISCUSSION

A key goal of our study was to assess the relative importance of historical interactions and habitat filters on the seasonal assembly of arthropod communities on milkweed. While we found evidence for the importance of both, the majority of variation in all measures of herbivory or arthropod community could be explained by ‘month’ alone without respect to prior experimental or natural herbivore damage. For example, while we found that both experimental leaf damage and naturally occurring leaf damage could influence herbivore damage in the following month, ‘month’ without respect to any aspect of the biotic community explained 73% of variation in herbivore damage, whereas experimental leaf damage only explained 7%. This suggests that historical interactions within this arthropod community had a relatively small effect on the phenology of herbivory.

Interestingly, the effects of experimental damage and the lagged correlations between aspects of the arthropod community were mostly positive, suggesting herbivore facilitation, induced susceptibility, and aggregation are likely particularly important in arthropod communities on milkweed (c.f. Ali & Agrawal, 2014). This contrasts with the idea of phenological priority effects that have been found in other systems

(Wolkovich & Cleland, 2011). For example, the early germination of invasive grasses reliably excludes late-germinators in Californian grasslands (Wainwright *et al.*, 2012), suggesting that in other systems, species interactions may outweigh the role of ahistorical habitat filters on community assembly. On the other hand, effects attributed to habitat filters in this study (and most studies of phenology) can be confounded with predicable changes in community interactions or undetected community interactions.

A notable pattern in our study was that experimental leaf damage affected subsequent leaf damage in some months, but not in others (Fig. 5). Specifically, we observed induced susceptibility to a greater extent in early months (April – June) than later months (Fig. 5). While there are surprisingly few studies on the phenology of defensive induction, our observed pattern of greater inducibility in early months is consistent with that literature. For example, induction of grapevines by Willamette mites reduced subsequent damage by a heterospecific mite, but only when the Willamette mites were introduced to the plant early in the season (Hougen-Eitzman & Karban, 1995). Likewise, an interplant cue reduced herbivore damage to sagebrush, but only when that cue was provided early in the growing season (Shiojiri & Karban, 2008). It has been suggested that early-developmental plant tissues are inherently more plastic than late-developmental tissues (Boege &

Marquis, 2005), so greater early-season inducibility may be a general pattern.

One finding of our study is that experimental leaf damage increased the leaf damage in some following months, suggestive of induced susceptibility (Fig. 3). However, when we looked for correlations between a previous month’s natural leaf damage and herbivore abundance, we found a negative relationship, a trend more consistent with induced resistance (Table 1). One possible reason for this is that our mechanical leaf damage treatment may have been an insufficiently realistic proxy for actual herbivore damage in this system. In a study of the effects of inducible responses to milkweed herbivores, induction by different herbivores had differing effects on subsequent herbivore feeding (Van Zandt & Agrawal, 2004; Ali & Agrawal, 2014), suggesting that plant species may recognize cues specific to particular herbivores, and elicit different defensive responses based on those cues (Halitschke *et al.*, 2001). Similarly, the way in which some herbivores feed reduces plant defenses. In other milkweed species, milkweed beetles and monarch caterpillars trench leaf vasculature, reducing latex pressure in the rest of the leaf (Dussourd, 1999; Helmus & Dussourd, 2005). Consistent with this pattern, we found that experimental damage did not reduce latex production (Fig. 1), but naturally occurring damage in a previous month did. Another possibility is repulsion from natural herbivore damage could be mediated by herbivore, not plant, cues. For example, many insects mark territory with olfactory cues, which deter colonization by future conspecifics (Roitberg & Prokopy, 1987).

We observed a pattern in which the abundance of many arthropods showed lagged positive correlations with each other (Table 1). For example, the presence of the key herbivore, *C. cobalitinus*, increased the abundance of a secondary herbivore, *L. kalmii*, as well as the abundance of predatory arthropods in the subsequent month. Likewise, experimental damage to plants resulted in greater herbivore abundance and subsequent leaf damage (Figs. 3,5). Each of these patterns suggests a tendency toward aggregation in milkweed arthropods and a preference for feeding on already damaged plants. This is consistent with some past work in milkweed systems. For example, monarch feeding increased subsequent leaf damage and the abundance of at least two specialist herbivores on *A. syriaca* (Van Zandt & Agrawal, 2004), probably because monarchs diffuse latex defenses in milkweed. Consistent with this, we found that latex production was lower in months following high natural herbivore damage (Table 1). Similarly, Ali and Agrawal (2024) found that monarch caterpillars (*Danaus plexippus*) benefited from prior herbivory by oleander aphids (*Aphis nerii*), but prior damage by monarchs negatively affected aphid growth. Interestingly, we found that a higher abundance of predatory insects tended to follow herbivorous insects, suggesting that predators show a bottom-up response to consumer abundance (Table 1). However, we did not see any effect of predatory insect abundance on the abundance of herbivores in the subsequent month, suggesting a lack of top-down control of herbivores in this system (Table 1). This is consistent with the observation that each of the key milkweed herbivores on *A. eriocarpa* displays conspicuous warning coloration and sequesters toxic milkweed alkaloids (Farrell, 2001).

In conclusion, we found that the majority of variation in the communities of arthropods on milkweed was due to predictable changes in arthropods over the season, consistent with a large role for ahistorical habitat filters. However, we also found evidence that species interactions within a community affect the composition of herbivores present on a given milkweed plant. These interactions were largely positive, where secondary herbivores and predators were in higher abundance on plants following a high population of the key herbivore, *C. cobaltinus*. It is likely that ahistorical habitat factors set the template for seasonal community assembly in this system, with a smaller changes in the trajectory of community assembly determined prior species interactions within the community.

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TABLES

Table 1: Lagged (1-month) correlations in the assembly of arthropod communities on *A. eriocarpa*.

Fixed effects model (response ~ predictor)	Random effects	Error distribution	z-value	*P*
Lagged correlation with leaf damage				

herbivore abundance ~ lag1(leaf damage)	month + plant	Poisson	-4.83	<0.001
predator abundance ~ lag1(leaf damage)	month + plant	Poisson	-0.35	0.724
***C. cobaltinus* abundance ~ lag1(leaf damage)**	month + plant	Poisson	-2.18	0.029
L. kalmii abundance ~ lag1(leaf damage)	month + plant	Poisson	-0.91	0.363
mortality ~ lag1(leaf damage)	month + plant	Binomial	-0.25	0.805
Lagged correlation with* C. cobaltinus *abundance (adults + eggs)				
herbivore abundance ~ lag1(*C. cobaltinus* abundance)	month + plant	Poisson	-1.5	0.13
C. cobaltinus abundance ~ lag1(*C. cobaltinus* abundance)	month + plant	Poisson	0.06	0.95
***L. kalmii* abundance ~ lag1(*C. cobaltinus* abundance)**	month + plant	Poisson	3.37	0.002
predator abundance ~ lag1(*C. cobaltinus* abundance)	month + plant	Poisson	4.39	<0.001
Lagged correlation with* predator *abundance				

herbivore abundance ~ lag1(predator abundance)	month + plant	Poisson	-0.72	0.47
C. cobaltinus abundance ~ lag1(predator abundance)	month + plant	Poisson	1.26	0.207
L. kalmii abundance ~ lag1(predator abundance)	month + plant	Poisson	1.37	0.171
predator abundance ~ lag1(predator abundance)	month + plant	Poisson	0.13	0.895
Lagged correlation with **herbivore** ***abundance***				
predator abundance ~ lag1(herbivore abundance)	month + plant	Poisson	4.4	<0.001

FIGURE HEADINGS

Fig. 1. Milkweed defenses (trichomes, latex exudation, and leaf toughness) over the growing season. We measured latex exudation both on both undamaged plants (black squares) and experimentally damaged induced plants (red triangles). We found no evidence for latex induction. Bars represent means +/- SE.

Fig. 2: Arthropod abundance (a) and species richness (b) on milkweed individuals that were induced or not induced in the prior month over the growing season. Bars represent means+/- SE.

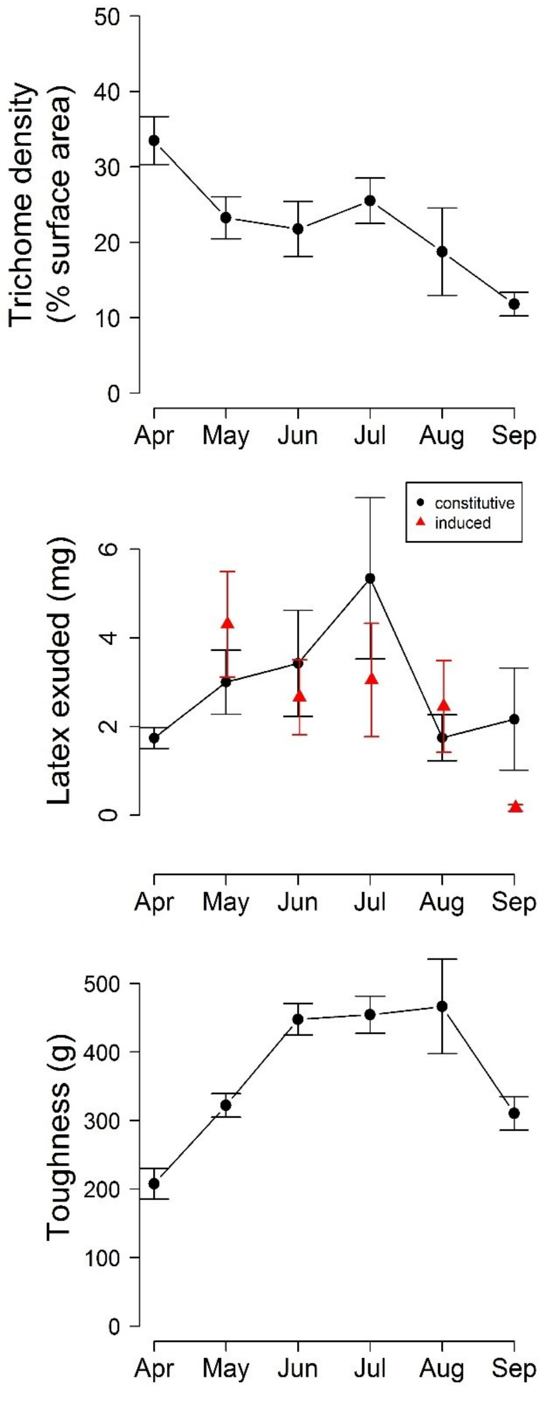
Fig. 3: The abundance of arthropods by guild: herbivores (a), predators (b), omnivores (c) on milkweed individuals that were induced or not induced in the prior month over the growing season. Bars represent means +/- SE.

Fig. 4: The abundance of the most common arthropods: The coleopteran herbivore, *Chrysochus cobaltinus* (adults and eggs) (a), spiders *Mallos pallidus* and *Mecaphesa* sp. (b), hemipterans, *Lygeaus kalmii*, *Scolops* sp., and *Geocrois pallens* (c), and the coleopteran herbivore *Tetraopes basilis* (d) on milkweed individuals over the growing season. Bars represent means +/- SE.

Fig. 5: Percent leaf damage on milkweed individuals over the growing season (a). Cumulative mortality of milkweed ramets over the growing season (b). Bars represent means +/- SE.

FIGURES

Fig. 1



Trichome density (% surface area)
50
40
30
20
10
0
Apr
May
Jun
Jul
Aug
Sep
constitutive
induced
Latex exuded (mg)
6
4
2
0
Apr
May
Jun
Jul
Aug
Sep
Toughness (g)
500
400
300
200
100
0
Apr
May
Jun
Jul
Aug
Sep

686

687**Fig. 2.**

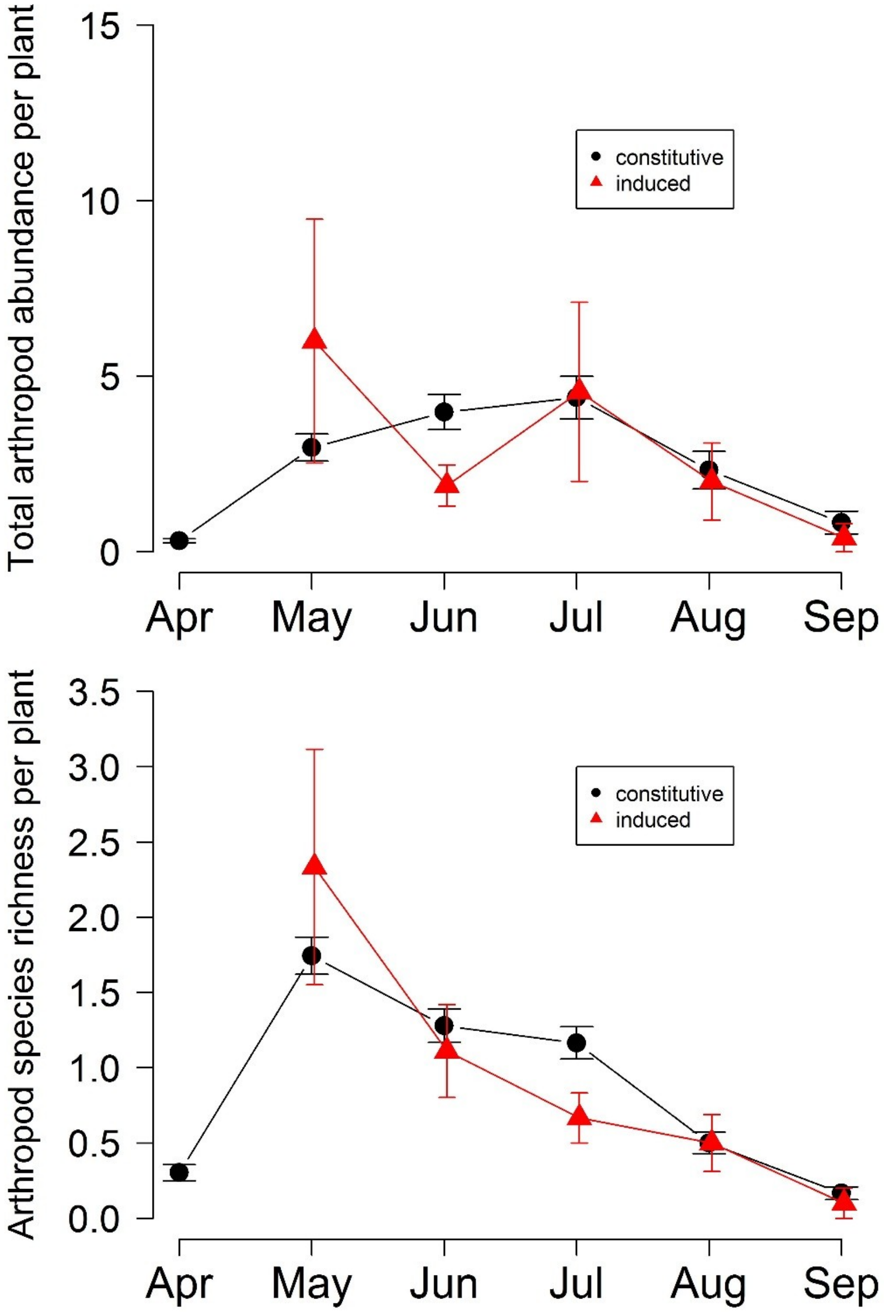
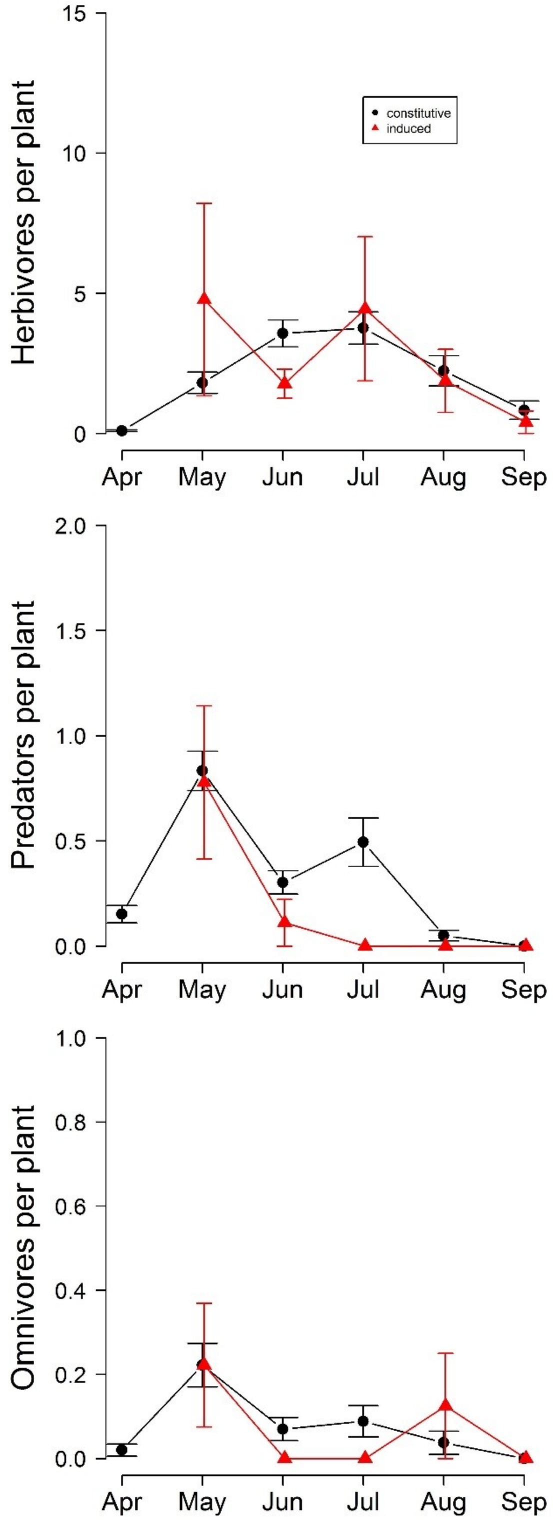

Total arthropod abundance per plant
15
10
5
0
constitutive
induced
Apr
May
Jun
Jul
Aug
Sep
Arthropod species richness per plant
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
constitutive
induced
Apr
May
Jun
Jul
Aug
Sep

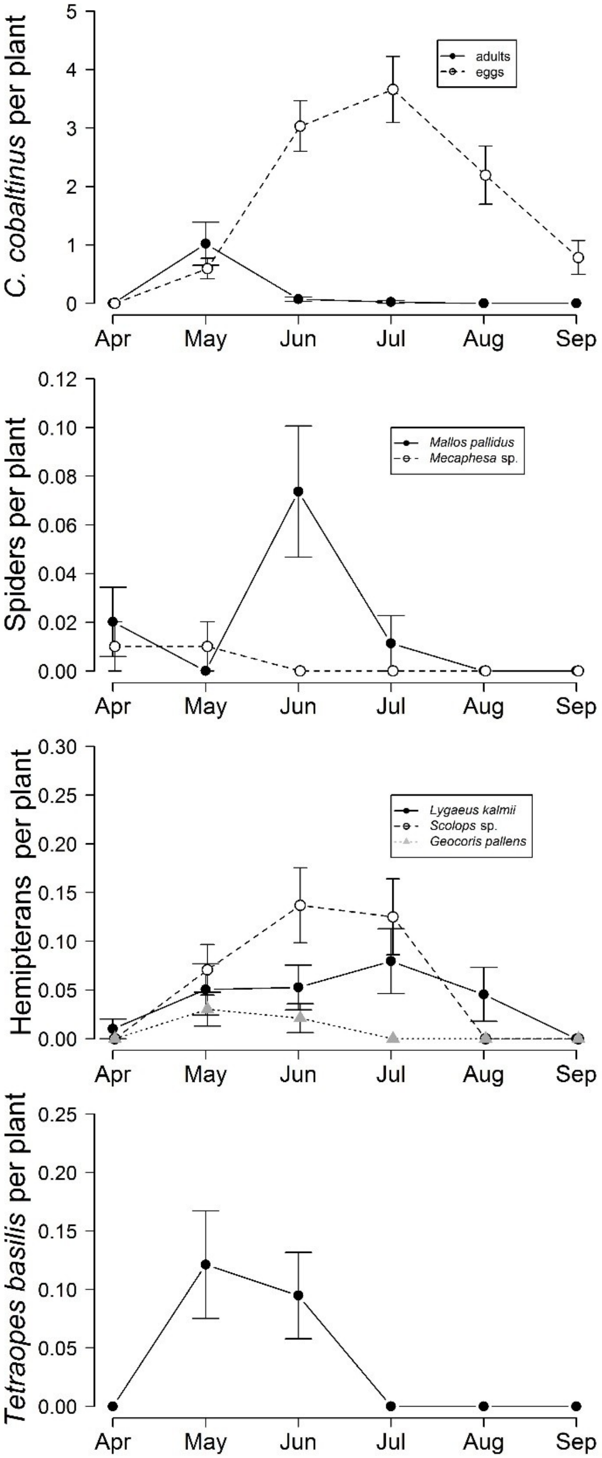
Fig. 3.



Herbivores per plant
15
10
5
0
constitutive
induced
Apr
May
Jun
Jul
Aug
Sep
Predators per plant
2.0
1.5
1.0
0.5
0.0
Apr
May
Jun
Jul
Aug
Sep
Omnivores per plant
1.0
0.8
0.6
0.4
0.2
0.0
Apr
May
Jun
Jul
Aug
Sep

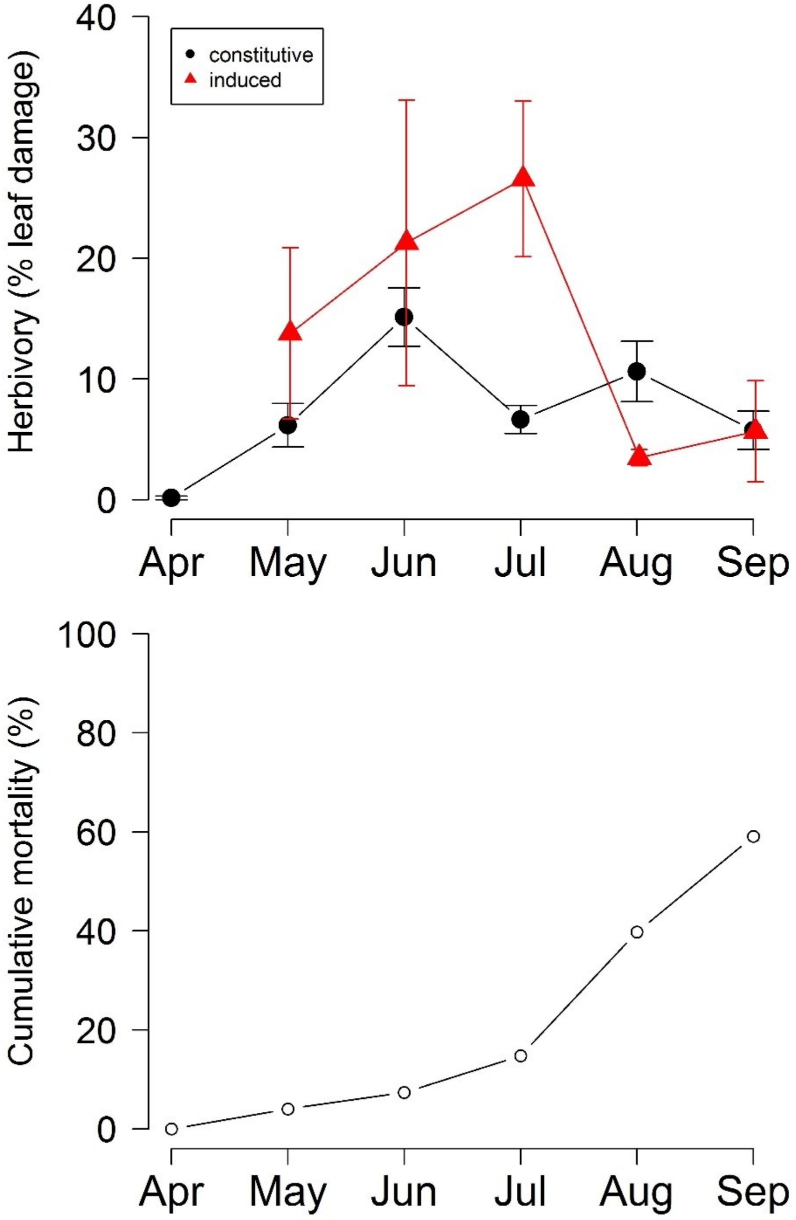
691

692**Fig. 4.**


C. cobaltinus per plant
adults
eggs
Spiders per plant
Mallos pallidus
Mecaphesa sp.
Hemipterans per plant
Lygaeus kalmii
Scolops sp.
Geocoris pallens
Tetraopes basilis per plant
Apr
May
Jun
Jul
Aug
Sep
0
1
2
3
4
5
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.05
0.15
0.20
0.25
0.30

693

694**Fig. 5.**


Herbivory (% leaf damage)
40
30
20
10
0
● constitutive
▲ induced
Apr
May
Jun
Jul
Aug
Sep
Cumulative mortality (%)
100
80
60
40
20
0
Apr
May
Jun
Jul
Aug
Sep