

**1Seasonal assembly of arthropod communities on milkweeds  
2experiencing simulated herbivory**

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12

**13ABSTRACT**

141. The seasonal assembly of arthropod communities is shaped by biotic  
15and abiotic aspects of the habitat that limit the appearance or activity  
16phenology of potential community members. In addition, previous  
17interactions within the community, such as herbivore-induced plant  
18defensive responses, aggregation, and predator avoidance also likely  
19affect the assembly of arthropod communities on individual plants.

202. We observed the phenology of arthropod communities and  
21defensive plant traits on 100 milkweed (*Asclepias eriocarpa*)

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

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

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

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

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

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

### 43INTRODUCTION

44 The processes that drive community assembly have been of  
45central interest since the foundation of ecology as a discipline (Belyea  
46& Lancaster, 1999). Numerous studies have documented community  
47assembly in the years following a disturbance (Bazzaz, 1975; Thibault  
48& Brown, 2008) or invasion (Fridley *et al.*, 2007). A key question arising  
49from such studies is the degree to which historical processes, such as  
50previous species interactions and the order of species colonization,  
51affect the trajectory of community assembly compared to habitat  
52'filters', ahistorical factors in the habitat which prevent the  
53establishment of some species in the community without regard to  
54priority effects (Kraft *et al.*, 2015). This framework was originally  
55developed to study the assembly of communities in space (Diamond,  
561975; Keddy, 1992; Chase, 2003; Maire *et al.*, 2012), but some aspects  
57of this framework may also be usefully applied to the study of seasonal  
58community assembly, the phenological process by which species  
59appear in a defined community throughout the season (Benbow *et al.*,  
602013). In the context of seasonal phenology, we adapt the habitat filter  
61concept to examine aspects of the environment that limit the  
62appearance or activity of a species at a particular point in time  
63throughout the season, independent of previous interactions in the  
64community. Here, we are applying a broad definition of the habitat  
65filter, which includes both biotic and abiotic factors in the environment.

66Recognizing the difficulty of separating biotic and abiotic effects  
67(Chase, 2003; Kraft *et al.*, 2015), our goal is to emphasize the key  
68distinction between historical and ahistorical factors, regardless of  
69whether they are biotic or abiotic.

70Examples of ahistorical habitat filters could include seasonal changes  
71in temperature (abiotic) or seasonal changes in the constitutive  
72defenses of host plants (biotic e.g. Boege & Marquis, 2005) that are not  
73driven by past interactions in the community. For example, degree-day  
74models accurately described both the phenology of plants and their  
75pollinators in the Rocky Mountains, suggesting that seasonal  
76temperature patterns are highly correlated with the assembly of plant  
77and pollinator communities (Forrest & Thomson, 2011). Degree-day  
78models have been similarly successful at describing plant phenophases  
79(Dunne *et al.*, 2003) and the phenology of herbivorous insects  
80(Strathdee *et al.*, 1993; Nufio *et al.*, 2010; Buckley *et al.*, 2015),  
81suggesting that seasonal temperature patterns are also correlated with  
82the assembly of plant-herbivore interactions. Seasonally varying plant  
83traits that are independent of previous interactions can also limit the  
84herbivores present on plants. For example, leaf defensive traits such as  
85toughness and tannin content often increase endogenously over the  
86season, so herbivores that are deterred by these traits may be  
87confined to the early season (Feeny, 1970; Forkner *et al.*, 2004).

88On the other hand, studies of species interactions have also found  
89evidence that interactions among the species already present in a  
90community can sometimes affect the trajectory of community  
91assembly. In these cases, the composition of the arthropod community  
92is historically contingent, as the direct interactions or habitat  
93modifications of one species could affect the phenology of others. For  
94example, herbivore damage on a particular plant may induce defenses  
95or attractants that affect herbivore populations at a later time point  
96(Van Zandt & Agrawal, 2004; Ali & Agrawal, 2014). Accordingly,  
97herbivores on the same plant can compete by altering plant habitat or  
98attracting predators (Kaplan & Denno, 2007) even if most of these  
99interactions are lagged in time (i.e., temporally asymmetric, Lawton &  
100Hassell, 1981; Yang, 2012). If arthropods aggregate or have positive  
101interactions, the presence of an arthropod in a community may  
102increase the likelihood of establishment of other arthropods. For  
103example, leaf-tying caterpillars increase the subsequent colonization of  
104a variety of arboreal insects by creating habitat structures (Lill &  
105Marquis, 2003). Trophic cascades and predator avoidance can also  
106affect community assembly over annual timescales (Piovia-Scott *et al.*,  
1072017). For example, the persistence of overwintering predators in  
108dried leaf galls decreased the abundance of herbivorous insects on oak  
109trees in the following spring (Wetzel *et al.*, 2016). Similarly, the  
110adhesion of dead insects to tarweed plants increased early-season

111predator abundance on those plants, which led to a subsequent  
112decrease in herbivory (Krimmel & Pearse, 2013).

113Historical interactions and habitat filters may interact to determine the  
114phenology and seasonal assembly of arthropod communities. For  
115example, induced plant defenses act as a historical effect by linking  
116past herbivore damage with future herbivore communities. However,  
117the efficacy of inducible defenses can also change over the season  
118independently of past damage (Hougen-Eitzman & Karban, 1995;  
119Shiojiri & Karban, 2008; McMunn, 2017). Likewise, in aquatic  
120ecosystems, size refuges from predation often occur as prey grow  
121larger, so the effect of predators (a historical effect) may change over  
122the season independently of historical effects as potential prey grow  
123(Rudolf & Armstrong, 2008).

124The study of phenological shifts has received renewed interest in the  
125past decade due to concerns about the ongoing effects of climate  
126change (Parmesan & Yohe, 2003; Polgar & Primack, 2011). One  
127particular concern is that differences in the relative magnitude of  
128phenological shifts could affect species interactions. In evaluating the  
129phenological shifts of different organisms, it may be particularly  
130important to consider the relative importance of habitat filtering (i.e.,  
131ahistorical factors) versus historical species interactions. When the  
132phenologies of organisms are largely driven by habitat filters, an

133 understanding of their effects can help predict phenological shifts that  
134 might arise from a changed environment. When the phenologies of  
135 organisms are largely driven by contingent historical effects within  
136 communities, a change in the environment may have more complex  
137 consequences for phenological shifts and species interactions.

138 We conducted an observational study and manipulative experiment on  
139 milkweed (*Asclepias eriocarpa*) arthropod communities in order to  
140 determine how the timing of herbivore damage influences the seasonal  
141 community assembly of arthropods on milkweed. We surveyed  
142 arthropod communities as well as a suite of milkweed defensive traits  
143 over the growing season. On a subset of plants each month, we  
144 mechanically damaged leaves to simulate herbivore damage. Using  
145 this combined observational and experimental study, we asked the  
146 following questions: (1) To what degree do arthropod communities  
147 change over the season irrespective of damage treatments? (2) Are  
148 arthropod communities affected by experimental damage to the plant  
149 in the previous month? (3) Does the effect of experimental damage on  
150 arthropod communities depend on the month in which it happens? (4)  
151 Is there correlative evidence for lagged effects of arthropod  
152 communities on arthropod communities of the same plant one month  
153 later? With this design, we sought to compare the amount of variation  
154 in arthropod communities described by historical interactions between  
155 arthropods and the modifications that they make to the plant, and

156ahistorical habitat filters, including plant or seasonality-based factors  
157unaffected by the arthropods.

## 158**METHODS**

### 159***Study Site***

160We conducted our study in a grassland habitat on Arnold Hill at  
161Hastings Reservation, near Carmel Valley, California (36.361 N,  
162121.563 W). The site had a history of grazing and hay production until  
1631937, when the property was acquired by the University of California  
164(Griffin 1990). In addition to milkweed, the grasslands at the site  
165included a variety of non-native grasses including *Avena* sp., *Bromus*  
166*hordeaceus*, *B. diandrus*, *Hordeum* sp., *Stipa pulchra*, and *Aira*  
167*caryophyllea*, as well as forbs such as *Madia gracilis*, *Plagiobothrys* sp.,  
168*Amsinckia menziesii*, and *Lupinus* sp , and *Lepidium nitidum*.  
169Grasslands were bordered by oak savannas and woodlands comprised  
170of *Quercus lobata*, *Q. douglasii*, *Q. chrysolepis*, *Q. agrifolia*, and *Q.*  
171*kelloggii* as well as *Toxicodendron diversilobum*. Slopes and aspects of  
172sites varied, but were generally on hilly terrain.

### 173***Milkweed and arthropod natural history***

174We conducted surveys of arthropod interactions with woolypod  
175milkweed (*Asclepias eriocarpa*). *Asclepias eriocarpa* is found  
176throughout California where it occurs commonly in coast range and

177Sierra foothill grasslands, chaparral, and savannas. *Asclepias eriocarpa*  
178grows in distinct patches, which likely consist of genetically identical  
179ramets with a limited degree of below-ground connection. Patches of  
180*A. eriocarpa* ramets were common throughout grasslands at Hastings  
181Reservation.

182Many milkweeds, including *A. eriocarpa*, are known for their leaf traits  
183that cause resistance to herbivores, such as latex production, toxicity  
184due to cardiac glycosides, and physical leaf characteristics, such as  
185tomentose trichomes (Agrawal, 2004, 2017; Agrawal *et al.*, 2009;  
186Rasman *et al.*, 2009). The herbivore community associated with the  
187milkweeds at Hastings Reservation is specialized on the genus and  
188includes many species with bright coloration such as monarch  
189butterflies (*Danaus plexippus*), milkweed leaf beetles (*Chrysochus*  
190*cobaltinus*), small milkweed bugs (*Lygaeus kalmii*), and milkweed  
191longhorned beetles (*Tetraopes basalus*). All of these herbivores  
192sequester cardiac glycosides (Farrell, 2001).

### 193**Experimental and observational design**

194In April 2013, we selected ten patches of milkweed for our study based  
195on the presence of >10 milkweed individuals in early April 2013. All  
196patches were within 1 km of one another, but separated from each  
197other by at least 20 m. Within each patch we selected ten milkweed  
198individuals spaced >1 meter apart from one another and >10 cm in

199height, resulting in a total of 100 milkweed plants surveyed each  
200month until September 2013. Ramets within 5 cm of one another were  
201considered to be the same individual, though we do not know the  
202actual extent of vascular connection between ramets of this species. In  
203each patch, we randomly assigned one plant to each of five treatments  
204that received mechanical damage in either April, May, June, July, or  
205August and five plants to a control treatment, which received no  
206experimental damage. Experimental damage consisted of six  
207longitudinal strips of puncture wounds from a fabric pattern wheel on  
208½ of the plant's leaves. One plant (J97) was qualitatively different than  
209all others in the population, because it was an order of magnitude  
210larger, produced early flowers, and had an arthropod abundance that  
211was an order of magnitude higher than all other plants. We removed  
212this plant from all experimental comparisons, which rely on  
213comparisons between plants. We retained information from this plant  
214for comparisons made on repeated observations of plants over time  
215(such as lagged correlations).

### 216***Plant Traits***

217For all plants in months April-September, we visually assessed the  
218amount of leaf tissue missing due to herbivory, recording the  
219percentage of original leaf tissue remaining (0-100). Plants with green  
220tissue aboveground were considered to be alive in that month. Each

221month we also recorded a series of invasively measured plant traits on  
222the randomly chosen plant that received damage in that month (one  
223plant per patch -see above). On this plant, we measured leaf  
224toughness averaged between two leaves (puncture force in grams to  
225push a 13.9 mm<sup>2</sup> hexagonal pin through the leaf lamella) using a  
226penetrometer (Pesola spring scale). We measured latex exudation  
227averaged between two leaves by cutting the distal one cm of each leaf  
228and absorbing the exuded latex onto a preweighed piece of filter paper  
229(Whatman #1). The mass of exuded latex was calculated as the  
230difference in pre- and post-measurement mass of the filter paper.  
231Because of differences in moisture or measurement error, a few  
232negative values were obtained, so we subtracted the minimum  
233negative value from all measurements to standardize values to zero.  
234At the same time, we measured trichome density averaged between  
235two leaves. Leaf disks were cut using a 3.3 mm leaf borer. We  
236photographed both sides of each leaf disk under 30X magnification.  
237Using ImageJ software (NIH, Bethesda, MD), we used image  
238thresholding to estimate the total trichome density as trichome area /  
239area of leaf disk. Latex exudation was also recorded in the month  
240following damage in order to assess inducibility.

#### 241***Arthropod Surveys***

242 Each month, we recorded all arthropods observed in contact with each  
243 milkweed plant. We initially recorded each arthropod as a  
244 morphospecies and collected a voucher specimen from each. The  
245 majority of common arthropod morphospecies from the survey were  
246 identified to species. We assigned each arthropod to a trophic guild  
247 (herbivore, omnivore, predator, transient) based on natural history  
248 records for those species. We calculated a trophic index of arthropod  
249 communities based on the following abundances:  $(\text{Predators} +$   
250  $0.5 * \text{Omnivores}) / (\text{Herbivores} + 0.5 * \text{Omnivores})$ .

### 251 ***Statistical Analysis***

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

259 We analyzed univariate estimates of arthropod communities  
260 (abundance, species richness, abundance of particular species or  
261 guilds) as well as percent leaf damage using a linear mixed effects  
262 models. These models described the arthropod community or damage  
263 estimates with the fixed effects of month\*induction. We included patch

264as a random effect to account for spatial differences in arthropod  
265communities and potential host genotype effects. Supporting this, the  
266inclusion of the patch factor in models consistently lowered the AIC of  
267models. The abundance of individual arthropod species other than *C.*  
268*cobaltinus* was low enough that we could not assess the effects of  
269induction on them. All univariate measures of arthropod communities  
270and percent leaf damage estimates were square root transformed prior  
271to analysis in order to fit linear model assumptions.

272In a final set of analyses, we looked for lagged relationships between  
273aspects of arthropod communities. In these models, we related an  
274aspect of arthropod communities or a plant trait at month  $t$  (for  
275example predator abundance) with aspects of the arthropod  
276community on the same plant in the previous month  $t-1$  using  
277generalized linear mixed effects models with Poisson error  
278distributions. The one exception was an analysis of plant mortality,  
279where we used a binomial error distribution. We included plant  
280individual and month as random effects in these models to account for  
281seasonal and between-individual variation in arthropod communities.  
282The specific hypotheses we tested involving lagged relationships are  
283shown in Table 1 and were chosen based on *a priori* expectations  
284about the lagged consequences of leaf damage, abundance of a key  
285herbivore (*C. cobaltinus*), and predator abundance.

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

## 297 **RESULTS**

### 298 ***Phenology of milkweed defensive traits and simulated***

#### 299 ***herbivory***

300 Trichome density on *A. eriocarpa* generally declined over the season  
301 (LR = 19.08, P = 0.002), with mean trichome densities highest in April  
302 and lowest at the end of the summer (Fig. 1a). Latex production varied  
303 over the season (LR = 20.06, P = 0.018), where latex production was  
304 highest in July (Fig. 1b). Latex production after simulated damage was  
305 highly variable relative to control plants, and we found no consistent  
306 evidence for significant latex induction by mechanical damage in *A.*  
307 *eriocarpa* over the season (LR = 6.63, P = 0.249, Fig. 1b). However,

308 latex production was lower in months following high natural herbivore  
309 damage (lagged linear model, LR=22.54,  $P < 0.001$ ). Leaf toughness  
310 (puncture force) changed over the season (LR=52.83,  $P < 0.001$ ),  
311 where mean toughness was lowest in April and peaked from June until  
312 August (Fig. 1c).

### 313 **Phenology of arthropod community**

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

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

330(Fig.2) when the arthropod community collapsed to a depauperate  
331community comprised mostly of milkweed specialists, such as *C.*  
332*cobaltinus* and *L. kalmii* (Fig. 4).

333We divided the arthropod community into trophic guilds (herbivores,  
334predators, and omnivores; Fig. 3). The abundance of arthropods in  
335each guild changed over the season (herbivores: LR = 117.1,  $P <$   
3360.001; predators: LR = 132.2,  $P < 0.001$ ; omnivores: LR=41.5,  
337 $P < 0.001$  ). Herbivore abundance peaked later in the season (July) than  
338predators (May), suggesting that the mean trophic level of the  
339community may decrease over the season. In order to test this, we  
340calculated a trophic index, whose values are greater with increasing  
341trophic position of individuals. Accordingly, the trophic index (TI) of the  
342community varied over the season (LR=164.5,  $P < 0.001$ ) was highest in  
343May (0.51), and decreased to 0.12 in June, where it remained until  
344predators fell out of the community at the end of the season. The  
345phenology of *C. cobaltinus*, the key herbivore in the community,  
346largely drove the overall abundance of herbivores (Figs 3,4). The  
347abundance of *C. cobaltinus* adults peaked in May, and abundance of  
348their resulting egg masses peaked in July. The phenologies of the next  
349most abundant herbivores, *L. kalmii* and *T. basilis*, were slightly later  
350than *C. cobaltinus* adults (Fig. 4). The most abundant predators were  
351all spiders, though a variety of uncommon predators were seen on

352plants. The hemipteran, *G. pallens*, was the most common omnivore,  
353and its phenology on milkweed was relatively early (Fig. 4).

354Percent leaf damage varied over the season (LR = 147.4,  $P < 0.001$ ),  
355where leaf damage peaked from June-July (Fig. 5a). While evidence of  
356leaf damage was usually persistent, percent leaf damage could  
357decrease over time due to growth of the plant or abscission of  
358damaged leaves. By September, there was 60% mortality of plants  
359(Fig. 5b). The highest rate of mortality occurred between the July and  
360August censuses, shortly after the peak of herbivore damage. Because  
361milkweeds have a perennial root system, mortality of a ramet indicates  
362the inability of that ramet to reproduce or acquire resources, but  
363regrowth of another ramet from the same root system may occur in  
364subsequent years.

**365Effects of experimental leaf damage) on arthropods and  
366further damage throughout the growing season.**

367The effect of experimental leaf damage on subsequent leaf damage  
368depended on the month of simulated herbivory (LR = 12.13,  $P = 0.016$ ,  
369Fig. 5). Damage in April led to a marginal increase in leaf damage in  
370May (LR = 2.48,  $P = 0.114$ ), and damage in June led to a significant  
371increase in leaf damage in July (LR = 23.01,  $P < 0.001$ ). The  
372experimental leaf damage treatment did not result in greater plant  
373mortality in the subsequent month (LR=1.35,  $P=0.245$ ).

374Experimental leaf damage had no effect on total arthropod abundance  
375(LR = 0.10, P = 0.751) and species richness (LR = 0.18, P=0.668, Fig.  
3762). Experimental leaf damage decreased the abundance of predatory  
377arthropods (LR=4.03, P=0.045), but it had no significant effect on  
378herbivores (LR=0.01, P=0.96) or omnivores (LR = 0.06, P=0.812). This  
379led to a 20% lower trophic index (i.e. numbers of predators per  
380herbivore) of the arthropod community on experimentally damaged  
381plants compared to control plants (LR = 4.59, P=0.032).

### 382**Lagged correlations of herbivores and predators on arthropod** 383**communities**

384Natural leaf damage in a given month preceded lower herbivore  
385abundance and *C. cobaltinus* abundance in the following month (Table  
3861). Leaf damage had no lagged correlation with predator abundance, *L.*  
387*kalmii* abundance, or the mortality of plants (Table 1). A higher  
388abundance of *C. cobaltinus* individuals in a given month resulted in a  
389higher abundance of *L. kalmii* and predatory arthropods in the  
390following month (Table 1). The abundance of *C. cobaltinus* had no  
391lagged correlation with total herbivore abundance, and we observed no  
392temporal autocorrelation in *C. cobaltinus* populations on a given plant  
393(Table 1). Predator abundance in a given month had no correlation  
394with total herbivore abundance, *C. cobaltinus* abundance, *L. kalmii*  
395abundance, or predator abundance in the following month (Table 1).

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

## 398 **DISCUSSION**

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

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

418(Wolkovich & Cleland, 2011). For example, the early germination of  
419invasive grasses reliably excludes late-germinators in Californian  
420grasslands (Wainwright *et al.*, 2012), suggesting that in other systems,  
421species interactions may outweigh the role of a historical habitat filters  
422on community assembly. On the other hand, effects attributed to  
423habitat filters in this study (and most studies of phenology) can be  
424confounded with predictable changes in community interactions or  
425undetected community interactions.

426A notable pattern in our study was that experimental leaf damage  
427affected subsequent leaf damage in some months, but not in others  
428(Fig. 5). Specifically, we observed induced susceptibility to a greater  
429extent in early months (April - June) than later months (Fig. 5). While  
430there are surprisingly few studies on the phenology of defensive  
431induction, our observed pattern of greater inducibility in early months  
432is consistent with that literature. For example, induction of grapevines  
433by Willamette mites reduced subsequent damage by a heterospecific  
434mite, but only when the Willamette mites were introduced to the plant  
435early in the season (Hougen-Eitzman & Karban, 1995). Likewise, an  
436interplant cue reduced herbivore damage to sagebrush, but only when  
437that cue was provided early in the growing season (Shiojiri & Karban,  
4382008). It has been suggested that early-developmental plant tissues  
439are inherently more plastic than late-developmental tissues (Boege &

440Marquis, 2005), so greater early-season inducibility may be a general  
441pattern.

442One finding of our study is that experimental leaf damage increased  
443the leaf damage in some following months, suggestive of induced  
444susceptibility (Fig. 3). However, when we looked for correlations  
445between a previous month's natural leaf damage and herbivore  
446abundance, we found a negative relationship, a trend more consistent  
447with induced resistance (Table 1). One possible reason for this is that  
448our mechanical leaf damage treatment may have been an insufficiently  
449realistic proxy for actual herbivore damage in this system. In a study of  
450the effects of inducible responses to milkweed herbivores, induction by  
451different herbivores had differing effects on subsequent herbivore  
452feeding (Van Zandt & Agrawal, 2004; Ali & Agrawal, 2014), suggesting  
453that plant species may recognize cues specific to particular herbivores,  
454and elicit different defensive responses based on those cues  
455(Halitschke *et al.*, 2001). Similarly, the way in which some herbivores  
456feed reduces plant defenses. In other milkweed species, milkweed  
457beetles and monarch caterpillars trench leaf vasculature, reducing  
458latex pressure in the rest of the leaf (Dussourd, 1999; Helmus &  
459Dussourd, 2005). Consistent with this pattern, we found that  
460experimental damage did not reduce latex production (Fig. 1), but  
461naturally occurring damage in a previous month did. Another  
462possibility is repulsion from natural herbivore damage could be

463mediated by herbivore, not plant, cues. For example, many insects  
464mark territory with olfactory cues, which deter colonization by future  
465conspecifics (Roitberg & Prokopy, 1987).

466We observed a pattern in which the abundance of many arthropods  
467showed lagged positive correlations with each other (Table 1). For  
468example, the presence of the key herbivore, *C. cobalitinus*, increased  
469the abundance of a secondary herbivore, *L. kalmii*, as well as the  
470abundance of predatory arthropods in the subsequent month. Likewise,  
471experimental damage to plants resulted in greater herbivore  
472abundance and subsequent leaf damage (Figs. 3,5). Each of these  
473patterns suggests a tendency toward aggregation in milkweed  
474arthropods and a preference for feeding on already damaged plants.  
475This is consistent with some past work in milkweed systems. For  
476example, monarch feeding increased subsequent leaf damage and the  
477abundance of at least two specialist herbivores on *A. syriaca* (Van  
478Zandt & Agrawal, 2004), probably because monarchs diffuse latex  
479defenses in milkweed. Consistent with this, we found that latex  
480production was lower in months following high natural herbivore  
481damage (Table 1). Similarly, Ali and Agrawal (2024) found that  
482monarch caterpillars (*Danaus plexippus*) benefited from prior herbivory  
483by oleander aphids (*Aphis nerii*), but prior damage by monarchs  
484negatively affected aphid growth. Interestingly, we found that a higher  
485abundance of predatory insects tended to follow herbivorous insects,

486suggesting that predators show a bottom-up response to consumer  
487abundance (Table 1). However, we did not see any effect of predatory  
488insect abundance on the abundance of herbivores in the subsequent  
489month, suggesting a lack of top-down control of herbivores in this  
490system (Table 1). This is consistent with the observation that each of  
491the key milkweed herbivores on *A. eriocarpa* displays conspicuous  
492warning coloration and sequesters toxic milkweed alkaloids (Farrell,  
4932001).

494In conclusion, we found that the majority of variation in the  
495communities of arthropods on milkweed was due to predictable  
496changes in arthropods over the season, consistent with a large role for  
497ahistorical habitat filters. However, we also found evidence that  
498species interactions within a community affect the composition of  
499herbivores present on a given milkweed plant. These interactions were  
500largely positive, where secondary herbivores and predators were in  
501higher abundance on plants following a high population of the key  
502herbivore, *C. cobaltinus*. It is likely that ahistorical habitat factors set  
503the template for seasonal community assembly in this system, with a  
504smaller changes in the trajectory of community assembly determined  
505prior species interactions within the community.

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659

## 660TABLES

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

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

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	month +			<0.0
<b>herbivore abundance ~ lag1(leaf damage)</b>	plant	Poisson	-4.83	01
	month +			0.72
predator abundance ~ lag1(leaf damage)	plant	Poisson	-0.35	4
<b>C. cobaltinus abundance ~ lag1(leaf damage)</b>	month +			0.02
	plant	Poisson	-2.18	9
	month +			0.36
<i>L. kalmii</i> abundance ~ lag1(leaf damage)	plant	Poisson	-0.91	3
	month +			0.80
mortality ~ lag1(leaf damage)	plant	Binomial	-0.25	5

---

**Lagged correlation with C. cobaltinus abundance (adults + eggs)**

---

herbivore abundance ~ lag1( <i>C. cobaltinus</i> abundance)	month +			
	plant	Poisson	-1.5	0.13
<i>C. cobaltinus</i> abundance ~ lag1( <i>C. cobaltinus</i> abundance)	month +			
	plant	Poisson	0.06	0.95
<b><i>L. kalmii</i> abundance ~ lag1(<i>C. cobaltinus</i> abundance)</b>	month +			0.00
	plant	Poisson	3.37	2
<b>predator abundance ~ lag1(<i>C. cobaltinus</i> abundance)</b>	month +			<0.0
	plant	Poisson	4.39	01

---

**Lagged correlation with predator abundance**

herbivore abundance ~ lag1(predator abundance)	month + plant	Poisson	-0.72	0.47
<i>C. cobaltinus</i> abundance ~ lag1(predator abundance)	month + plant	Poisson	1.26	0.20 7
<i>L. kalmii</i> abundance ~ lag1(predator abundance)	month + plant	Poisson	1.37	0.17 1
predator abundance ~ lag1(predator abundance)	month + plant	Poisson	0.13	0.89 5

---

***Lagged correlation with herbivore abundance***

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<b>predator abundance ~ lag1(herbivore abundance)</b>	month + plant	Poisson	4.4	<0.0 01
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## 661 **FIGURE HEADINGS**

662 **Fig. 1. Milkweed defenses (trichomes, latex exudation, and leaf**  
663 **toughness) over the growing season.** We measured latex  
664 exudation both on both undamaged plants (black squares) and  
665 experimentally damaged induced plants (red triangles). We found no  
666 evidence for latex induction. Bars represent means  $\pm$  SE.

667 **Fig. 2:** Arthropod abundance (a) and species richness (b) on milkweed  
668 individuals that were induced or not induced in the prior month over  
669 the growing season. Bars represent means  $\pm$  SE.

670 **Fig. 3:** The abundance of arthropods by guild: herbivores (a),  
671 predators (b), omnivores (c) on milkweed individuals that were induced  
672 or not induced in the prior month over the growing season. Bars  
673 represent means  $\pm$  SE.

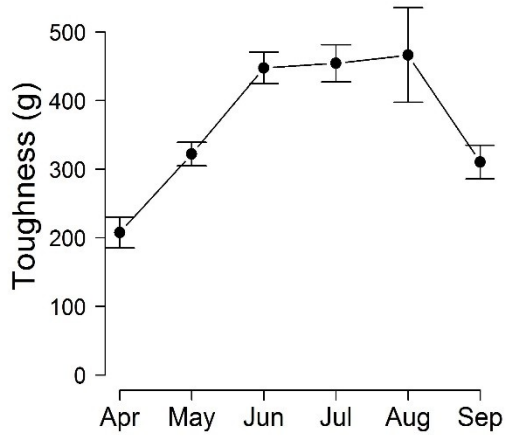
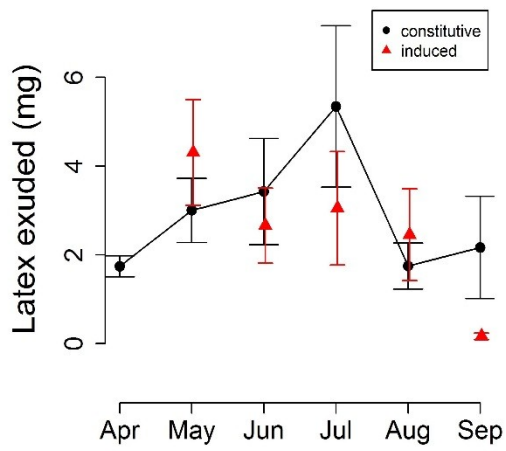
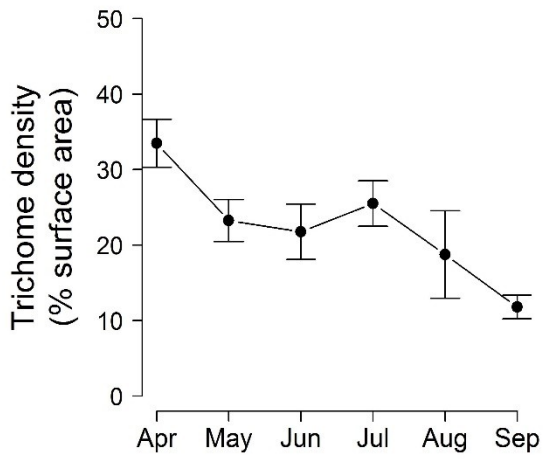
674 **Fig. 4:** The abundance of the most common arthropods: The  
675 coleopteran herbivore, *Chrysochus cobaltinus* (adults and eggs) (a),  
676 spiders *Mallos pallidus* and *Mecaphesa* sp. (b), hemipterans, *Lygeus*  
677 *kalmii*, *Scolops* sp., and *Geocrois pallens* (c), and the coleopteran  
678 herbivore *Tetraopes basilis* (d) on milkweed individuals over the  
679 growing season. Bars represent means  $\pm$  SE.

680**Fig. 5:** Percent leaf damage on milkweed individuals over the growing  
681season (a). Cumulative mortality of milkweed ramets over the growing  
682season (b). Bars represent means +/- SE.

683

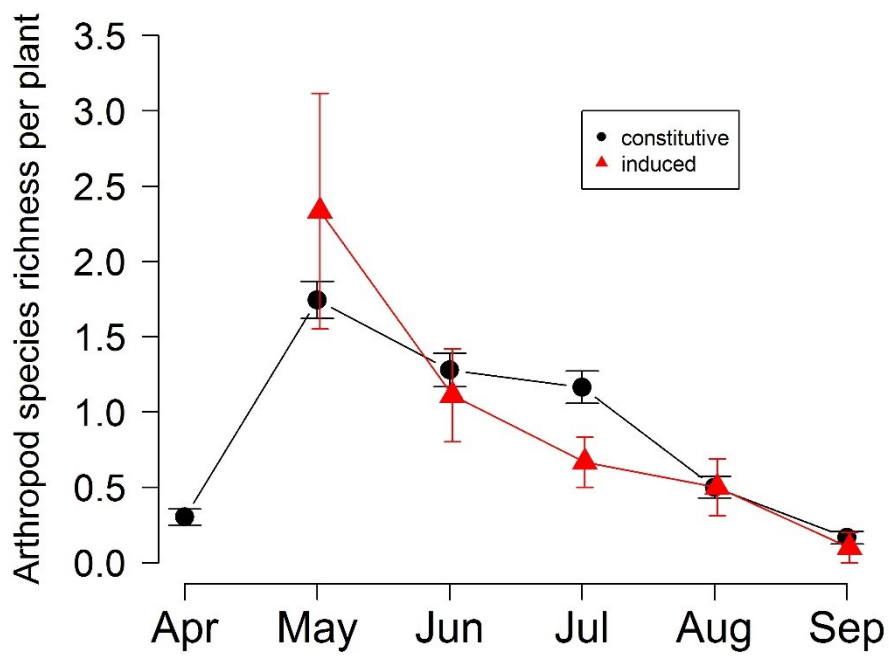
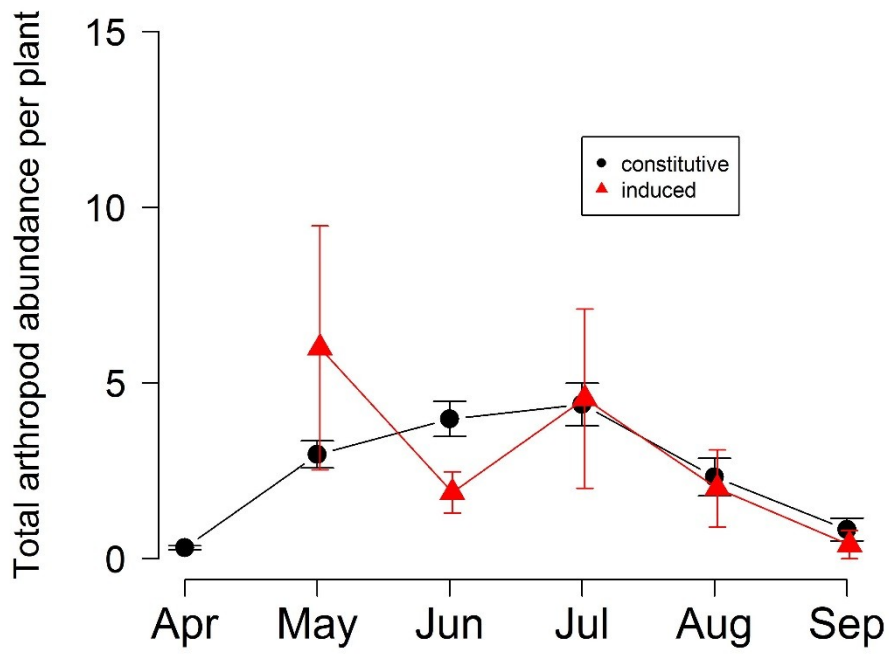
684**FIGURES**

685**Fig. 1**



686

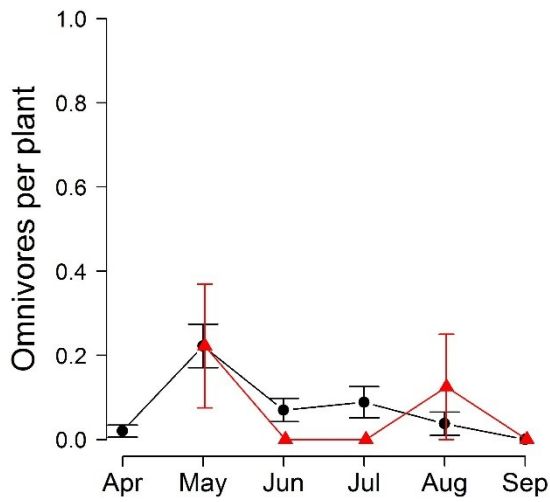
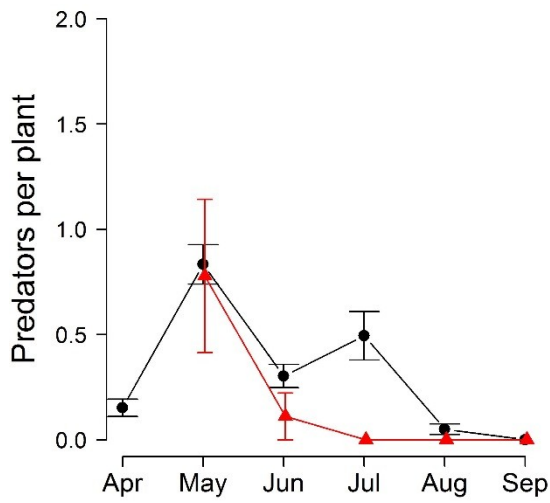
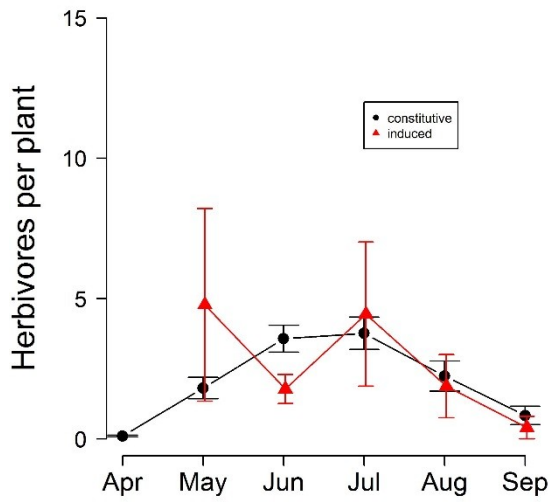
687 **Fig. 2.**



688

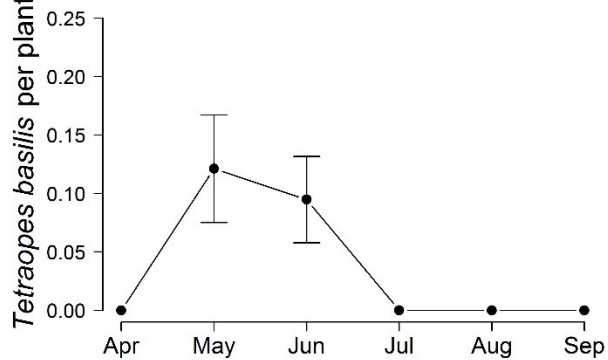
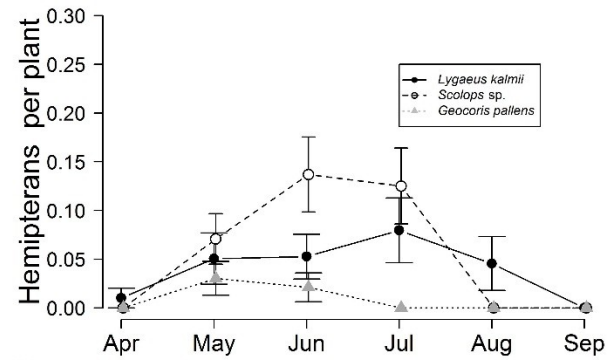
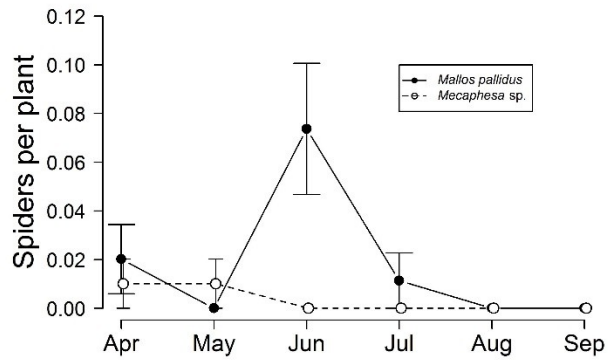
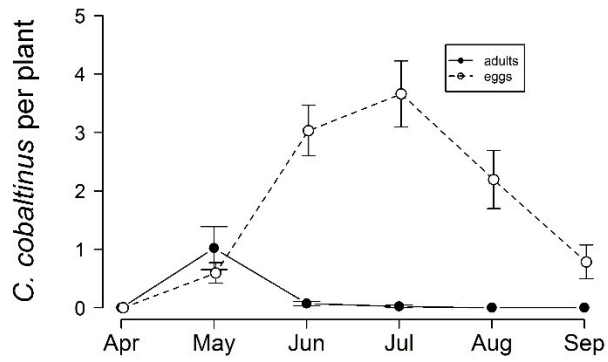
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690 **Fig. 3.**



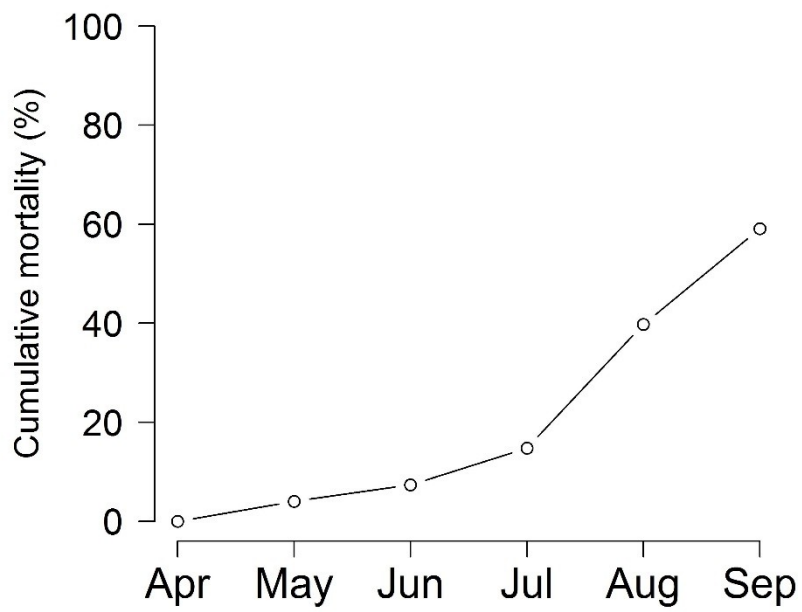
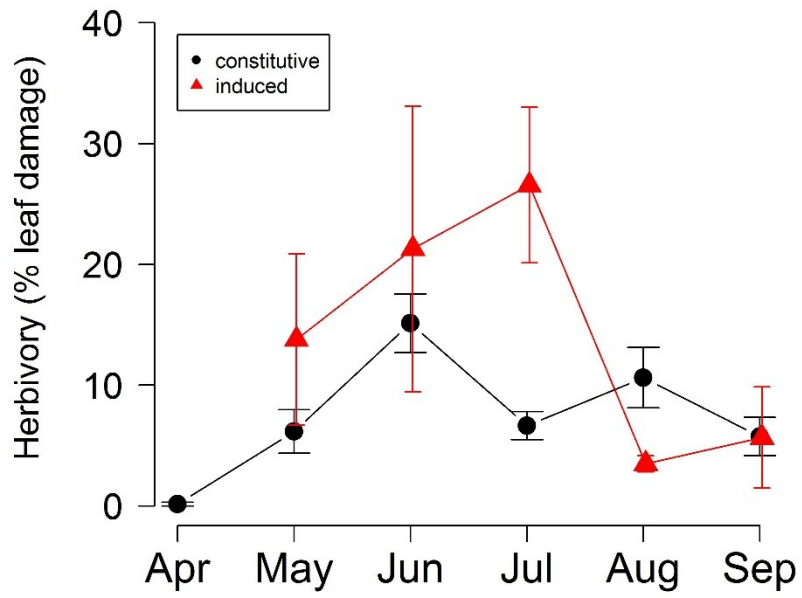
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692 **Fig. 4.**



693

694 **Fig. 5.**



695