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

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13**ABSTRACT**

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

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

138We conducted an observational study and manipulative experiment on
139milkweed (*Asclepias eriocarpa*) arthropod communities in order to
140determine how the timing of herbivore damage influences the seasonal
141community assembly of arthropods on milkweed. We surveyed
142arthropod communities as well as a suite of milkweed defensive traits
143over the growing season. On a subset of plants each month, we
144mechanically damaged leaves to simulate herbivore damage. Using
145this combined observational and experimental study, we asked the
146following questions: (1) To what degree do arthropod communities
147change over the season irrespective of damage treatments? (2) Are
148arthropod communities affected by experimental damage to the plant
149in the previous month? (3) Does the effect of experimental damage on
150arthropod communities depend on the month in which it happens? (4)
151Is there correlative evidence for lagged effects of arthropod
152communities on arthropod communities of the same plant one month
153later? With this design, we sought to compare the amount of variation
154in arthropod communities described by historical interactions between
155arthropods 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 (*Chrysomelid*
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 mm² 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.

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

297**RESULTS**

298***Phenology of milkweed defensive traits and simulated*** 299***herbivory***

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

365***Effects of experimental leaf damage) on arthropods and***
366***further 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, $P=0.245$).

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. 3762). 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 3861). 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).

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 ahistorical 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 predicable 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. cobalitus*, 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

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

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

	month +			<0.0
herbivore abundance ~ lag1(leaf damage)	plant	Poisson	-4.83	01
	month +			0.72
predator abundance ~ lag1(leaf damage)	plant	Poisson	-0.35	4
C. cobaltinus abundance ~ lag1(leaf damage)	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
<i>L. kalmii</i> abundance ~ lag1(<i>C. cobaltinus</i> abundance)	month +			0.00
	plant	Poisson	3.37	2
predator abundance ~ lag1(<i>C. cobaltinus</i> abundance)	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
<hr/> Lagged correlation with herbivore abundance				
predator abundance ~ lag1(herbivore abundance)	month + plant	Poisson	4.4	<0.0 01

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 $\pm$ SE.

683

684**FIGURES**

685**Fig. 1**

686

687**Fig. 2.**

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689

690**Fig. 3.**

691

692**Fig. 4.**

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694**Fig. 5.**