Title

Timing of a plant—herbivore interaction alters plant growth and reproduction

Journal

Ecology, 104(1)

ISSN

0012-9658

Authors

Rasmussen, Nick L

Yang, Louie H

Publication Date

2023

DOI

10.1002/ecy.3854

Running head: Subsequent effects of interaction timing

Timing of a plant-herbivore interaction alters plant growth and reproduction

Nick L. Rasmussen[1,2] and Louie H. Yang[1,3]

[1] Department of Entomology and Nematology, University of California, Davis, CA 95616 USA

[2] Division of Integrated Science and Engineering, California Department of Water Resources, West Sacramento, CA 95691 USA

[3] corresponding author: *lhyang@ucdavis.edu*

Open Research statement: Data from this study have been permanently archived on Dryad: Rasmussen, Nick; Yang, Louie (2021), The timing of herbivory: early-season herbivory affects plant size and late-season herbivory affects seed production, Dryad, Dataset, https://doi.org/10.5061/dryad.69p8cz93c

ABSTRACT

Phenological shifts have the potential to change species interactions, but relatively few studies have used experimental manipulations to examine the effects of variation in timing of an interspecific interaction across a series of life-stages of a species. While previous experimental studies have examined the consequences of phenological timing in plant-herbivore interactions for both plants and their herbivores, less is known about their effects on subsequent plant reproduction. Here, we conducted an experiment to determine how shifts in the phenological timing of monarch (*Danaus plexippus*) larval herbivory affected milkweed (*Asclepias fascicularis*) host plant performance, including effects on growth and subsequent effects on flower and seed pod phenology and production. We found that variation in the timing of herbivory affected both plant growth and reproduction, with measurable effects several weeks to several months after herbivory ended. The timing of herbivory had qualitatively different effects on vegetative and reproductive biomass: early-season herbivory had the strongest effects on plant size, while late-season herbivory had the strongest effects on the production of viable seeds. These results show that phenological shifts in herbivory can have persistent and qualitatively different effects on different life stages across the season.

Keywords: phenological mismatch, phenological shifts, *Danaus plexippus*, *Asclepias fascicularis*, flowering phenology, herbivory, ontogeny, seasonal windows of opportunity, climate change, pollination

INTRODUCTION

The timing of phenological events, such as bird migration, plant flowering, and amphibian breeding, varies among years (Parmesan 2007, Thackeray et al. 2010a). Determining the ecological consequences of this variation is important for understanding the temporal dynamics of natural communities as well as how these dynamics are altered by climate change (Yang and Rudolf 2010, Miller-Rushing et al. 2010, Sutherland et al. 2013). Phenological shifts mediated by climate change have been documented in nearly all major taxonomic groups examined and in habitats across the globe (Parmesan and Yohe 2003, Root et al. 2003, Thackeray et al. 2016). However, there is a great deal of variation in the direction and magnitude of phenological responses across species and trophic levels (Both et al. 2009, Ovaskainen et al. 2013, Thackeray et al. 2016). This interspecific variation in phenological shifts could affect how those species interact, but research is only beginning to explore these effects (Kharouba et al. 2018).

Phenological shifts could change interspecific interactions by altering which ontogenetic stages of species co-occur in time (Yang and Rudolf 2010, Rasmussen et al. 2014, Rasmussen and Rudolf 2016). Many traits change over ontogeny, including size, behavior, nutrient requirements, resource allocation, and defense strategies (Werner and Gilliam 1984, Boege and Marquis 2005, Barton and Koricheva 2010, Yang and Cenzer 2020, Yang et al. 2020). The suite of traits that organisms exhibit at the time they encounter one another could significantly affect the strength of the interaction and even whether an interaction occurs. For example, a prey organism could reach a body size refuge before it encounters a predator (Rasmussen et al. 2014), or a pollinator could encounter a plant before the initiation of flower production (Hegland et al. 2009). If climate

change reduces and/or eliminates temporal overlap of life stages of species that evolved to interact, it could cause significant changes to demographic rates and even community structure.

Interactions between plants and their herbivores represent one of the most studied types of interspecific interaction, but relatively few studies have investigated the consequences of variation in timing of herbivory for plant growth and reproduction (Maschinski and Whitham 1989, Bale et al. 2002, Singer and Parmesan 2010, Barton 2013, Thackeray et al. 2016). Many studies have documented phenological shifts in plants and insects (Fitter and Fitter 2002, Visser and Both 2005, Singer and Parmesan 2010, Cook et al. 2012, CaraDonna and Inouye 2014), and some studies have demonstrated that herbivores are not always shifting in sync with their host plants (Visser and Both 2005, Both et al. 2009, Singer and Parmesan 2010, Thackeray et al. 2010b, 2016). While some studies have looked at the effects of phenological timing on herbivores (Che-Castaldo et al. 2019, Yang et al. 2020), relatively few use experimental manipulations to isolate mechanisms (Chmura et al. 2019). If plant and herbivore phenologies diverge, the resulting erosion of the herbivory interaction could create a net benefit to the plant, while increased phenological overlap could create stronger negative effects on the plant (e.g., Singer and Parmesan 2010). The outcomes of phenological shifts in nature can be complex. For example, Liu et al (2011) found that experimental warming of meadow plots caused increased temporal overlap between the herbivorous larval stage of a moth and the flowering stage of a plant. The plant suffered significantly higher florivory and lower fruit production relative to un-warmed plots. In general, the ecological repercussions of phenological shifts may depend on the relative change in phenologies, the type of interaction, and the biotic and abiotic context of the interaction.

77 The consequences of phenological shifts may depend in part on the temporal and ontogenetic
78 trajectories of plant tolerance and resistance traits (Boege and Marquis 2005, Barton and
79 Koricheva 2010, Che-Castaldo et al. 2019). The effects of herbivory on plant performance could
80 be strongest early in ontogeny if early season herbivory removes a greater proportion of the
81 plant's total resources, or if these plants lack the resources for effective tolerance or resistance
82 (Strauss and Agrawal 1999, Boege and Marquis 2005). In these cases, early herbivory during a
83 vulnerable and critical life stage could have longer-lasting effects on plants. Alternatively, early
84 herbivore attack could have relatively limited effects on plant fitness if early herbivory allows
85 the plant sufficient time to recover prior to reproduction. In some systems, the effects of
86 herbivory could be strongest later in the season as resource allocation shifts away from growth
87 toward reproduction (Trumble et al. 1993). For example, if herbivory occurs immediately prior
88 to flowering, herbivores could remove the resources or structures a plant needs to reproduce,
89 leaving insufficient time for the plant to recover before the seasonal window for reproduction has
90 passed. Thus, expectations for how the timing of herbivory affects plant size and subsequent
91 reproductive traits such as flowering and fruiting remain unclear.

92 In this study, we conducted an experiment to determine how shifts in the phenological timing of
93 herbivory affected host plant performance. Specifically, we manipulated the timing of herbivory
94 by larvae of the monarch butterfly (*Danaus plexippus*) to determine the consequences for growth
95 and reproduction of one of its host plant species, narrow-leaf milkweed (*Asclepias fascicularis*).
96 We answer three main questions: (1) How does timing of herbivory affect the growth trajectories
97 of host plants?, (2) How does timing of herbivory affect the reproductive traits of host plants?,
98 and (3) Are the effects of herbivory timing on plant reproductive traits qualitatively similar to the
99 effects on plant growth?

METHODS

Study system

Narrow-leaf milkweed *(Asclepias fascicularis)* is a native long-lived herbaceous perennial distributed throughout the western United States. It generally occurs in open areas below 2,134 m, including riparian corridors, roadsides, and margins of agricultural fields (Munz and Keck 1959), and is patchily distributed throughout the California Central Valley. Timing of phenological events for this milkweed species can vary considerably among years and locations. In Davis, CA, plants generally begin producing new shoots in March and April, flower from June to September, and produce fruits from June to November.

Monarch larvae are specialist herbivores of plants in the subfamily Asclepiadoideae, particularly those in the genus *Asclepias*. Monarch larva can inflict intense herbivory damage, sometimes completely defoliating their host plants. This occurs despite a suite of constitutive and induced anti-herbivore traits exhibited by *A. fascicularis* and other *Asclepias* species, including trichomes, pressurized latex, and toxic cardenolides (Rasmann et al. 2009b, 2009a). The intensity of monarch damage appears to be strongly modulated by both host plant species and host plant age, with particularly strong effects on small or young host plants with relatively weak defensive traits (Yang and Cenzer 2020, Yang et al. 2020). Damage by other specialist herbivores can have a variety of effects on milkweed growth and reproduction, including negative effects on growth, but not reproduction (Matter 2001), increased stem mortality (Agrawal and Van Zandt 2003), and reduced reproduction (Erwin et al. 2014).

120 The western population of North American monarch butterflies overwinters in aggregations
121 along the Pacific coast of California. This population migrates inland in the spring, eventually
122 expanding their range across a broad region of North American west of the Rocky Mountains
123 (Dingle et al. 2005, Yang et al. 2016). Adult butterflies often arrive in the Central Valley of
124 California between February and April (Dingle et al. 2005). Variation in the migration and
125 breeding phenology of monarchs appears to be driven by complex patterns of precipitation and
126 temperature across space (overwintering, stopover, and breeding habitats) and time (across
127 seasons and years) (Forister and Shapiro 2003, Zipkin et al. 2012, Espeset et al. 2016, Forister et
128 al. 2018). Western monarch butterflies populations have declined significantly during the past
129 few decades (Pelton et al. 2019), and phenological mismatches between monarchs and their
130 milkweed host plants mediated by climate change have been hypothesized as a possible cause
131 (Thogmartin et al. 2017, Yang and Cenzer 2020). The phenological variation associated with this
132 strong plant-herbivore interaction makes this system a useful and potentially important model for
133 understanding the consequences of phenological shifts for the outcome of species interactions.

134 **Experimental design**

135 We used four experimental treatments to determine the effects of shifts in the phenological
136 timing of monarch larvae herbivory on milkweed plant performance. This included an herbivory-
137 free control and three treatments manipulating the timing of monarch herbivory (i.e., early, mid,
138 and late). For the early monarch herbivory treatment, each plant received one second-instar larva
139 on May 11, 2015 when plants were 60 days old. A larva was allowed to feed until it pupated or
140 defoliated its plant (a larva abandons its host at this point in nature). The mid and late herbivory
141 timing treatments received second-instar larvae two and four weeks later (May 25 and June 8,

142 2015), respectively. These introduction dates spanned an observed seasonal window of relatively
143 high oviposition rates in a wild, nonexperimentally manipulated population monarchs in Davis,
144 CA in 2015 (Yang et al., *in press*).

145 A core group of 112 plants (4 treatments × 28 replicates) was used to quantify the effects of
146 experimental treatments throughout the entire growing season, including plant size, flowering,
147 and fruiting. Two additional groups of plants were grown under the same conditions as the core
148 group but were sacrificed at specific times to quantify metrics of plant size, including dry mass.
149 The first set contained 21 plants, seven of which were harvested at each of the three monarch
150 introduction dates. These plants experienced no herbivory damage before sacrificed for biomass
151 measurements. The second set contained 28 plants (4 treatments × 7 replicates) and was
152 harvested on June 21, 2015 to determine plant size after all experimentally-induced herbivory
153 was completed but before initiation of flowering. Plants were maintained in a randomized
154 complete spatially blocked design in an outdoor lathhouse at the Orchard Park Research
155 Greenhouse Complex at the University of California, Davis, CA USA.

156 **Experimental setup**

157 Seeds of local genotypes of narrow-leaved milkweed (seed collection location: north of Winters,
158 Yolo Co., CA, USA) were acquired from Hedgerow Farms (Winters, CA, USA). We stratified
159 the seeds for 17 days, starting February 24, 2015, by wrapping them in moist paper towels and
160 keeping them in a plastic bag inside a dark refrigerator at 3.5°C. Seeds were planted into seedling
161 trays filled with potting mix (1 part compost : 1 part coarse sand : 1 part milled peat, by volume).
162 Trays were kept in a greenhouse and incubated on a heating pad (~26°C) and misted for 2
163 minutes every 50 minutes. On April 11, we transplanted seedlings with their first set of true

leaves to plastic square tree pots (30.5 cm tall ×12.7 cm wide, CP512CH, Stuewe and Sons, Inc., Tangent, OR) using the same potting mix. Plants were then maintained at temperatures that closely approximated outdoor temperatures. They were watered three times per week, once with reverse osmosis water and twice with a dilute fertilizer solution (N:P:K 2:1:2, 200 ppm N). On May 11, all plants in the experiment were covered with bags composed of fine (~97 holes per cm^2) polyester netting (Skeeta, Bradenton, FL) secured around the pots with elastic cord. These bags retained larvae and prevented colonization by predators and other herbivores. Then, all plants were moved to a 50% shade lathhouse, where they experienced natural abiotic conditions. On this same day, May 11, we initiated the early herbivory treatment.

Monarch eggs were obtained from a large, local insectary population (Utterback Farms, Woodland, CA), which was re-established from local monarch genotypes each year and regularly supplemented with new migrant adults to maintain genetic diversity. Adult butterflies in this population were previously screened for the presence of a protozoan parasite, *Ophryocystis elektroscirrha (*H. Kaya*, pers. comm.).* In addition, we surface sterilized eggs in 10% bleach solution (v/v) to minimize vertical transmission. For each of the three monarch herbivory treatments, eggs were incubated at 28°C for 48 h prior to hatch. Neonate larvae were allowed to develop through the first instar on a diet of freshly-cut milkweed foliage *(A. curassavica)*. The following day, each plant within a given treatment received a single second-instar larva (see 'Experimental design'). Plants strongly differed in size among these three dates (Fig. 1A; $\chi^2 = 63.26$, $p < 0.0001$), regardless of the size metric used. All plants were maintained in the lathhouse throughout the period of time they received monarch herbivory. If a larva died, it was replaced with one of similar size and age (2-4 occurrences per treatment). Larvae generally took about two weeks to complete development, though development time declined somewhat with

later arrival, likely due to increasing ambient temperature ($\chi^2 = 97.61$, $p < 0.0001$; all pairwise comparisons: $p < 0.05$; early: 14.8 d ± 1.06 SD, mid: 13.85 d ± 1.82 SD, late: 11.8 d ± 0.43 SD). However, there were no differences among treatments in pupa dry mass ($\chi^2 = 3.40$, $p = 0.1830$; early: 247.45 mg ± 28.51 SD, mid: 258.96 mg ± 29.31 SD, late: 258.44 mg ± 21.66 SD). Therefore, we concluded that plants in the three herbivory timing treatments experienced herbivory exposure that was fairly standardized but realistic.

On June 22, the day after the last monarch larva completed development, all plants were moved (4 treatments × 28 replicates = 112 plants) to a field plot at the Russell Ranch Sustainable Agriculture Facility, a research site owned by the University of California-Davis and located 12 km west of Davis, CA. At this point, no plants had yet developed open flowers, though some had flower buds. We buried pots so that the soil in the pots was level with the surrounding ground. We maintained the plants in pots rather than transplanting them directly into the soil to reduce transplant shock and retain a barrier against gopher damage. These pots had large drainage holes instead of corners on the bottom, providing plant roots access to the surrounding soil. Plants were watered twice within the first few weeks of planting via irrigation lines to promote root establishment (June 24 and July 9). Roots extending into soil outside the pot were evident within a few weeks, as determined by three test plants designated for this purpose (i.e., not replicates from the experiment).

The natural conditions at this field site allowed us to determine how the initial monarch herbivory timing treatments would affect subsequent plant life stages and their interactions with the environment and ecological communities. Arthropods commonly associated with *A. fascicularis* began to colonize these plants immediately, many of which likely originated from a

nearby long-term experimental array populated with the same milkweed species. This included specialist herbivores (e.g., large milkweed bug, *Oncopeltus fasciatus;* small milkweed bug, *Lygaeus kalmii*) and predators (e.g., spiders, assassin bugs). We did not observe monarch caterpillars or blue milkweed beetles *(Chrysochus cobaltinus)*, the two primary folivores, perhaps because the seasonal window of opportunity for these two species had largely passed by the time milkweeds were planted in the field (late June) (Yang and Cenzer 2020). We managed the non-native invasive oleander aphid *(Aphis nerii)* at low levels by routinely (every 2-3 d) dislodging them from plants using cans of compressed air to prevent this species from overwhelming our experimental treatments. We did not collect data on pollinator visits but did make casual observations of the pollinator community during our regular visits to measure plant traits. Honeybees were the most commonly observed pollinators throughout the flowering season, but a variety of other pollinator were also frequently observed including butterflies, flies, and native bees.

Measured responses

We determined the consequences of variation in the phenological timing of monarch herbivory on milkweeds by measuring a suite of traits related to plant growth and reproduction. We measured plant size because it is the trait most directly affected by herbivory and is an important determinant of resources available for flower and fruit production. Based on the two groups of plants sacrificed to quantify size metrics (see 'Experimental design'), we determined that all size metrics were highly correlated ($r \geq 0.90$ in all cases), including total dry mass, shoot dry mass, mean stem length, total stem length, and maximum stem length. Therefore, we decided that stem

length measurements of live plants represented an appropriate, non-destructive metric to evaluate size. On September 9, we evaluated final plant size by determining total, mean, and maximum stem length. We chose this date because this is when plants first began to show signs of senescence, indicating that maximum size for the year had been attained. As with the two groups of sacrificed plants, we found that the three size metrics based on stem lengths were highly correlated ($r \geq 0.90$ in all cases). Many plants were still maturing seed pods at the time of these final plant size measurements, so plants could not be harvested for biomass measurement.

We evaluated potential subsequent effects of herbivory timing on flowering traits. We measured traits relating to flower quantity (number of umbels per plant, number of flowers per umbel), and also examined a suite of phenological traits for each plant, all measured relative to the seed germination date. This included first flowering age (plant age at which first flower on first umbel opened), mean flowering age (mean age at which first flower opened on all umbels), flowering duration (time from first flower opening on first umbel to last flower closing on last umbel), and within-plant flowering synchrony (coefficient of variation [CV] for plant age at which the first flower on all umbels opened). To collect these data, we used green labeling tape to number all umbels and recorded for each umbel the number of flowers, the date the first flower opened, and the date the last flower closed. Previous work has demonstrated that flower quantity and flowering phenology are important in determining the composition and abundance of visiting pollinators, which can in turn, affect seed production (Rafferty and Ives 2012).

We also determined if herbivory timing affected fruit production by counting the number of pods matured per plant and documenting a suite of fruiting phenology traits for each plant, again measured relative to the date that plants first started growing. This included timing of first pod

253 matured, mean timing of pod maturation, fruiting duration (time between initiation of first pod
254 and maturation of last pod), and within-plant fruiting synchrony (CV for plant age at which all
255 pods matured). We used permanent markers to number pods. The number and timing of seed pod
256 production likely determined the strength of interactions with seed predators. The experiment
257 was ended when the last pod in the experiment reached maturity (November 11, 2015).

258 After all pods were collected, we examined the viability of all seeds in the laboratory and used
259 this trait as a proxy for annual reproductive success. The seeds from a given pod were placed
260 between layers of moist paper towels in a petri dish. Seeds were cold stratified for 14 days in the
261 dark at 3.5°C and then incubated for ten days in the dark at 28°C, following methods similar to
262 those of other milkweed studies (Woods et al. 2011, Erwin et al. 2014, Züst et al. 2015). Paper
263 towels were changed on day five to prevent mold accumulation. We counted the seeds and
264 determined the proportion that germinated. Failure to germinate for a given seed could be due to
265 inviability, damage by seed predators, or delayed germination. We assumed that experimental
266 treatments could cause differences in seed viability and/or predation but were unlikely to cause
267 variation in the time required for germination of viable seeds.

268 **Statistical analysis**

269 All analyses were performed using the R statistical computing environment (R Development
270 Core Team 2019). We ran generalized linear mixed models to analyze the fixed effect of the
271 herbivory treatment (one factor with four levels) on responses relating to plant performance.
272 Spatial block was included as a random effect in all models. All models were run using the
273 'lme4' package, except for those with a negative binomial error structure, which we ran using the
274 'glmmADMB' package. For all analyses, we evaluated significance of fixed effects using

likelihood ratio tests, which compared the model with the herbivory treatment to the intercept only model. For models that indicated a significant treatment effect, we used the 'lsmeans' package to generate pairwise comparisons of treatment levels and adjusted for the false discovery rate using the Benjamini-Hochberg procedure (Benjamini and Hochberg 1995). We analyzed treatment effects on the proportion of foliage removed by monarch larvae (binomial error structure). We analyzed final plant size based on maximum stem length (Gaussian error structure; excluded four plants because of intense, late-season rabbit herbivory). We analyzed treatment effects on whether a plant produced at least one umbel (binomial error structure) as well as the number of umbels produced (negative binomial error structure). For the remaining flowering responses, including the number of flowers per umbel, first flowering, mean flowering, flowering duration, and within-plant flowering synchrony, we used a Gaussian error structure and excluded plants with no flowers. The number of plants without any mature pods was high and exhibited a treatment effect (see 'Results'), so we employed a hurdle model approach. We modelled the effect of treatment on whether a plant produced at least one mature seed pod using a binomial error structure. Then, we used a zero-truncated negative binomial error structure to analyze the effect of treatment on responses of plants that did produce mature pods, including number of mature pods, total seeds per plant, and germinated seeds per plant. We analyzed number of seeds per umbel using a Gaussian error structure. For fruiting phenology, we used a Gaussian error structure and excluded plants without pods to analyze day of first pod matured, day of mean pod matured, fruiting duration, and within-plant fruiting synchrony.

RESULTS

Herbivore damage and plant size

297 Across all three herbivory treatments, the mean percent foliage removed by monarch larvae was
298 59.8%. The percentage of foliage removed from plants by monarchs declined with later larval
299 arrival (Fig. 1B; $\chi^2 = 87.13$, $p < 0.0001$). In the early herbivory treatment, 81% of foliage was
300 removed on average (Fig. 1B), and 100% of foliage was removed in 26% of these plants. The
301 mid and late treatments experienced 72% and 27% mean foliage removal, respectively.

302 At the end of the growing season, plants differed significantly in size based on maximum stem
303 length (Fig. 1C; $\chi^2 = 34.07$, $p < 0.0001$). Plants in the control and late treatments were
304 indistinguishable from each other in size but were significantly larger than those in both the early
305 (1.31× larger), and mid (1.11× larger) treatments. In addition, the early and mid-season
306 treatments differed significantly from each other. The pattern of treatment effects on plant size in
307 September were the same qualitatively as those observed in June, three months earlier (June size
308 vs. September size: $r = 0.43$, $p < 0.0001$, d.f. = 106).

309 **Flower quantity**

310 Of the 112 plants, 62% produced at least one umbel. Treatment did not significantly affect
311 whether a plant flowered ($\chi^2 = 5.67$, $p = 0.1285$; flowering plants: control = 79%, early = 54%,
312 mid = 61%, late = 54%). However, herbivory strongly reduced the number of umbels (Fig. 2A;
313 $\chi^2 = 14.54$, $p = 0.0023$). Control plants produced 2.37×, 3.48×, and 2.78× more umbels than
314 early, mid, and late herbivory plants, respectively. Umbel size (i.e., mean number of flowers per
315 umbel) showed no treatment effects ($\chi^2 = 4.00$, $p = 0.2612$; control: 14.59 flowers ± 4.73 SD,
316 early: 13.81 flowers ± 2.68 SD, mid: 15.48 flowers ± 4.78 SD, late: 12.28 flowers ± 5.11 SD).

317 **Flowering phenology**

There was a significant treatment effect on plant age at first flowering (Fig. 2B; $\chi^2 = 10.42$, $p = 0.0153$). Plants in the control treatment initiated flowering at the youngest age (mean age of first flowering = 110 d old), significantly earlier than plants in the early and mid-season herbivory treatments, which took 1.20× and 1.16× longer to initiate flowering, respectively. Timing for plants in the late herbivory treatment was intermediate between the control and other herbivory treatments (i.e., mean age of first flowering = 121 d old), and therefore did not differ from any other treatments. Plant age at mean flowering was significantly correlated with age at first flowering ($r = 0.73$, $p < 0.0001$, d.f. = 67), but there were no significant treatment differences ($\chi^2 = 2.22$, $p = 0.5285$; control: 134.12 d ± 23.01 SD, early: 145.98 d ± 23.97 SD, mid: 135.71 ± 23.75 SD, late: 133.29 d ± 23.79 SD). Flowering duration showed a significant treatment effect (Fig. 2C; $\chi^2 = 14.52$, $p = 0.0023$), driven by the difference between the control and mid-season herbivory plants. Control plants flowered over the longest duration (43 d), which was 1.45×, 2.31×, and 1.46× longer than the early, mid, and late-season herbivory treatments, respectively. Within-plant flowering synchrony, based on CV, was significantly correlated with flowering duration ($r = 0.83$, $p < 0.0001$, d.f. = 64) but showed a marginally non-significant effect ($\chi^2 = 7.66$, $p = 0.0535$; control: 0.08 ± 0.05 SD, early: 0.07 ± 0.07 SD, mid: 0.03 ± 0.05 SD, late: 0.08 ± 0.06 SD).

Fruit quantity

Of the 112 plants, 46% produced at least one mature pod. There was a treatment effect on whether a plant produced mature pods ($\chi^2 = 15.87$, $p = 0.0012$; fruiting plants: control: 71%, early: 39%, mid: 25%; late: 39%), driven by the difference between the control and the three herbivory treatments. Based on the plants that produced mature pods, there was not a significant

effect of treatment on the number of pods produced ($\chi^2 = 5.57$, $p = 0.1344$; control: 18.43 pods ± 25.01 SD, early: 8.33 pods ± 10.02 SD, mid: 12.57 pods ± 10.39 SD, late: 13.17 pods ± 22.98 SD).

Fruiting phenology

There were no treatment differences in plant age at first pod maturation ($\chi^2 = 5.40$, $p = 0.1444$; control: 162.10 d ± 17.61 SD, early: 176.75 d ± 30.15 SD, mid: 178.86 d ± 27.17 SD, late: 177.58 d ± 24.94 SD) or in plant age at mean pod maturation ($\chi^2 = 1.09$, $p = 0.7806$; control: 182.83 d ± 26.07 SD, early: 188.30 d ± 25.94 SD, mid: 187.27 d ± 25.04 SD, late: 189.12 d ± 25.41 SD). There was a treatment effect on fruiting duration (Fig. 3A; $\chi^2 = 8.67$, $p = 0.0340$), driven by the difference between the control and mid treatment. Fruiting duration for the control was 1.34×, 1.65×, and 1.37× longer compared to the early, mid, and late treatments, respectively. There was no treatment effect on the synchrony of pod maturation, based on CV ($\chi^2 = 3.07$, $p = 0.3810$; control: 0.06 ± 0.05 SD, early: 0.04 ± 0.06 SD, mid: 0.03 ± 0.03 SD, late: 0.05 ± 0.05 SD).

Seed production and germination

Based on the 52 plants with mature pods, there were no treatment differences in total seeds produced ($\chi^2 = 5.99$, $p = 0.1220$; control: 792.48 seeds ± 1200.22 SD, early: 375.17 seeds ± 482.33 SD, mid: 463.43 seeds ± 376.49 SD, late: 484.00 seeds ± 820.97 SD). There also were no treatment differences in total seeds per umbel ($\chi^2 = 5.14$, $p = 0.1617$). However, there was a significant effect of treatment on the number of germinated seeds (Fig. 4A; $\chi^2 = 8.13$, $p = 0.0433$), driven primarily by the difference between the control and late herbivory treatment. The

number of germinated seeds in the control treatment was 2.22×, 1.72×, and 2.99× higher compared to the early, mid, and late treatments, respectively.

DISCUSSION

We found that timing of herbivory by monarch larvae had a variety of effects on growth and reproduction of narrow-leaf milkweed. Herbivory timing had direct effects on plant size that persisted for months after the herbivory had occurred and indirect effects on a number of traits related to flowering, fruiting, and seed production. Importantly, the effects of herbivory timing on reproductive traits were qualitatively different from those on plant size, demonstrating that the former cannot necessarily be inferred from the latter. Taken together, these findings suggest that shifts in the phenological timing of interactions between plants and their herbivores can have lasting and complex consequences for the host plant.

A large body of research has demonstrated that herbivory can have strong effects on host plants (e.g., Maron and Crone 2006), but relatively few studies have looked at how shifts in the timing of plant-herbivore interactions affect plant performance. We found that timing of herbivory determined not only whether there was an effect of herbivory on plant size but also the magnitude of this effect. Plants subjected to early herbivory remained small compared to the no herbivory control plants throughout the growing season (Fig. 1C) likely because they were small when attacked (Fig. 1A), which resulted in monarch larvae consuming a large proportion of their foliage (Fig. 1B). All plants from the early herbivory treatment survived this intense herbivore damage but could not regrow to fully compensate for this biomass loss. This finding is consistent with previous work showing that herbivory on small plants results in stronger negative impacts than for larger plants (e.g., Hanley et al. 1995, Haukioja and Koricheva 2000) but is at odds with

the idea that established plants are generally better able to compensate for early season versus late season herbivory (Maschinski and Whitham 1989, Strauss and Agrawal 1999, see also, Barton and Koricheva 2010). The observed pattern suggests that the compensatory responses of these plants were more likely to have been resource-constrained than time-constrained, or (non-exclusively) that our early season herbivory treatment damaged these plants during an important and transient window of opportunity for growth.

Conversely, plants in the late herbivory treatment, which were attacked a month later than those in the early herbivory treatment, were indistinguishable in size from plants in the no herbivory control treatment by the end of the growing season (and 1.3× larger than those in the early herbivory treatment; Fig. 1C). This is likely because the late herbivory plants were significantly larger at the time of attack compared to those in the early herbivory treatment (3.4× larger; Fig. 1A), and therefore these late herbivory plants generally lost a much smaller proportion of their foliage (Fig. 1B). In parallel with the effects of increasing plant size, increasing defensive traits with plant age could also have contributed to observed differences in the direct effects of herbivory. While previous studies have documented relatively small plant age-associated increases in defensive traits in narrow-leaved milkweed compared with other milkweed species, even these small increases can significantly reduce monarch growth and survival (Yang et al. 2020) and may have contributed to reduced rates of damage for plants that interacted with monarch herbivores later in the season.

Plants with the mid-season herbivory were intermediate in size between early and late herbivory plants at the end of the growing season (Fig. 1C). The proportion of foliage these plants lost to monarch herbivory was nearly as high as that of the early herbivory plants (Fig. 1B), but these

405 plants were larger at the time of herbivory initiation compared to the early herbivory plants (Fig.
406 1A). Therefore, the total amount of photosynthetic tissue remaining after herbivory was higher
407 for mid-season herbivory plants, which may explain why they were better able to compensate for
408 herbivory with regrowth (though not as well as late herbivory plants).

409 The effects of herbivory timing on plant size lasted throughout the growing season, leaving a
410 signature of effects 81 to 110 days after the experimental herbivory ended, depending upon the
411 treatment (Fig. 1C). These persistent effects on plant size could potentially affect subsequent size
412 and stage-structured species interactions (Yang and Rudolf 2010), including the risk or intensity
413 of future herbivory. For example, these effects on plant size could affect the likelihood of further
414 herbivore damage if larger plants are more conspicuous or attractive to ovipositing monarch
415 butterflies or other herbivores (Cohen and Brower 1982, Zalucki and Kitching 1982, Matter
416 1996, Zalucki et al. 2016), or if plant defensive traits vary with plant size (Yang et al. 2020).

417 Effects of herbivory timing on reproductive life-stages of the milkweed plants were more
418 variable than those on plant size. For two responses, number of umbels per plant (Fig. 2A) and
419 whether a plant produced any fruits, plants showed an effect of herbivory but not herbivory
420 timing. For several other responses, the timing of herbivory determined whether there was any
421 effect of herbivory, including timing of first flowering, flowering duration, fruiting duration, and
422 number of germinating seeds. These more variable effects of herbivory timing on reproductive
423 traits is not surprising given that these life-stages took place in the field, weeks to months after
424 the herbivore treatments were completed.

425 Importantly, these effects of herbivory timing on reproductive traits were qualitatively different
426 than effects on plant size. Late herbivory did not significantly affect final plant size, but it

strongly reduced the number of umbels (Fig. 1C vs. Fig. 2A), the likelihood of fruiting, and the number of germinated seeds. This suggests that these plants may have prioritized defense and compensatory growth of photosynthetic biomass over production and defense of reproductive structures. *A. fascicularis* is a long lived perennial, so they may have reduced investment in reproduction during this first year to store energy for potentially increased growth and reproduction in the following years.

Early herbivory significantly reduced final plant size, but early herbivory plants were virtually indistinguishable from late herbivory plants for nearly all flowering and fruiting traits (Fig. 2, Fig. 3), suggesting that early herbivory plants prioritized reproduction over growth. Plants with intermediate herbivory timing were intermediate in final size between early and late herbivory plants, but their reproductive traits were, in some cases, the most impacted relative to the control plants. Specifically, these mid season herbivory plants exhibited the fewest umbels, shortest flowering duration, lowest proportion of plants producing pods, and shortest fruiting duration (Fig. 2, Fig. 3). This outcome perhaps demonstrating the difficulties of balancing regrowth and reproduction with the timing of herbivory that these plants experienced.

Conclusions

The key finding of this study is that variation in the timing of herbivory affected both plant growth and reproduction, with measurable effects manifesting several weeks to months after herbivory treatments ended. The timing of herbivory had qualitatively different effects on vegetative and reproductive biomass. Early herbivory had the strongest effects on plant size, while late herbivory had the strongest effects on the production of viable seeds. These complex responses by plants that differed in herbivory timing indicate that measuring only the direct

449 effects on plant growth does not necessarily provide an accurate understanding of subsequent
450 indirect effects on plant reproduction.

451 Studies examining how phenological shifts affect species interactions have generally focused on
452 the life stage when the two species interact directly but not on the potential for more persistent
453 effects on subsequent life stages. For example, studies of competition often focus on
454 establishment and growth but not reproduction, while studies of pollination often focus on
455 visitation more than seed set. In part, this may be because studies that examine responses across
456 multiple life stages can be difficult and time-consuming, and increasingly delayed or indirect
457 effects could potentially be more difficult to identify, quantify, or interpret. While it seems clear
458 that variation in the seasonal timing of a species interaction can affect subsequent life-stages, a
459 wide range of outcomes are plausible. In some cases, compensatory responses could attenuate
460 the effects of early season herbivory, while in other cases, the effects of early season damage
461 appear to persist or amplify over time.

462 Future studies could potentially expand upon several aspects of this study. For example, data on
463 the number and type of pollinators visiting plants exposed to different herbivory timing could
464 provide a more detailed understanding of the relative contributions of plant resource limitation
465 versus pollinator limitation in fruit and seed production. Also, this study was conducted with
466 first-year plants. However, these milkweeds are long-lived perennials, and it is likely that
467 milkweed populations are largely composed of older plants. It would be useful to document
468 herbivory and timing-of-herbivory effects across multiple years to determine if they attenuate,
469 persist, or amplify over time. While this study focused on the sexual component of host plant
470 reproduction, it would also be useful to document similar effects on asexual reproduction. In

addition, while previous studies have documented wide-ranging effects of prior herbivory on subsequent herbivores across multiple species (Van Zandt and Agrawal 2004a, 2004b, Rasmann et al. 2009a, Erwin et al. 2014, Ali and Agrawal 2014), studies into the heterospecific effects of variation in the timing of herbivory remain relatively few.

The study of phenological shifts in the timing of herbivory could improve our understanding of monarch-milkweed interactions, with potential implications for conservation. In particular, the western monarch population has declined dramatically in recent years (Pelton et al. 2019), likely driven by multiple correlated drivers (Crone et al. 2019). While the ultimate roles of climatic factors and phenological shifts remain unclear, our current study illustrates some of the potentially complex effects on milkweed growth and reproduction that could result from shifts in the timing of herbivory. As counterparts to this current study, previous studies have examined how age-varying plant traits affect monarch growth and survival (Yang and Cenzer 2020, Yang et al. 2020). Together, these studies suggest that even relatively small changes in the timing of milkweed-monarch interactions have the potential to affect both interacting species in complex and significant ways.

More generally, the findings of our study suggest the value of measuring responses to perturbation across the multiple life stages to more fully capture their subsequent effects. These longer timescale responses may be especially important in the context of climate change and phenological shifts, where studies are rapidly moving away from a historical focus on single life history stages or events towards the examination of phenological changes and consequences over longer seasonal trajectories (Yang 2020).

Acknowledgements

Thanks to Utterback Farms, Inc. for providing monarch eggs for this experiment, staff of the Orchard Park Greenhouse Facility, NSF CAREER grant to LHY (DEB-1253101), Israel Herrera and his staff for access to and support for the site where the experiment was conducted, and assistance from the following undergraduate students in collecting data: Vivian Le, Darren Wong, Nicholas Wintaro, Joseph Darosa, Allyson Earl, Helena Vogel, and Juan Andres de Domini.

Literature Cited

Agrawal, A. A., and P. A. Van Zandt. 2003. Ecological play in the coevolutionary theatre: genetic and environmental determinants of attack by a specialist weevil on milkweed. Journal of Ecology 91:1049–1059.

Ali, J. G., and A. A. Agrawal. 2014. Asymmetry of plant-mediated interactions between specialist aphids and caterpillars on two milkweeds. Functional Ecology 28:1404–1412.

Bale, J. S., G. J. Masters, I. D. Hodkinson, C. Awmack, T. M. Bezemer, V. K. Brown, J. Butterfield, A. Buse, J. C. Coulson, J. Farrar, J. E. G. Good, R. Harrington, S. Hartley, T. H. Jones, R. L. Lindroth, M. C. Press, I. Symrnioudis, A. D. Watt, and J. B. Whittaker. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. Global Change Biology 8:1–16.

Barton, K. E. 2013. Ontogenetic patterns in the mechanisms of tolerance to herbivory in Plantago. Annals of Botany:mct083.

Barton, K. E., and J. Koricheva. 2010. The Ontogeny of Plant Defense and Herbivory: Characterizing General Patterns Using Meta-Analysis. The American Naturalist 175:481–493.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B-Methodological 57:289–300.

Boege, K., and R. J. Marquis. 2005. Facing herbivory as you grow up: the ontogeny of resistance in plants. Trends in Ecology & Evolution 20:441–448.

Both, C., M. van Asch, R. G. Bijlsma, A. B. van den Burg, and M. E. Visser. 2009. Climate change and unequal phenological changes across four trophic levels: constraints or adaptations? Journal of Animal Ecology 78:73–83.

CaraDonna, P. J., and D. W. Inouye. 2014. Phenological responses to climate change do not exhibit phylogenetic signal in a subalpine plant community. Ecology 96:355–361.

Che-Castaldo, C., C. M. Crisafulli, J. G. Bishop, E. F. Zipkin, and W. F. Fagan. 2019. Disentangling herbivore impacts in primary succession by refocusing the plant stress and vigor hypotheses on phenology. Ecological Monographs 0:e01389.

Chmura, H. E., H. M. Kharouba, J. Ashander, S. M. Ehlman, E. B. Rivest, and L. H. Yang. 2019. The mechanisms of phenology: the patterns and processes of phenological shifts. Ecological Monographs 89:e01337.

Cohen, J. A., and L. P. Brower. 1982. Oviposition and Larval Success of Wild Monarch Butterflies (Lepidoptera: Danaidae) in Relation to Host Plant Size and Cardenolide Concentration. Journal of the Kansas Entomological Society 55:343–348.

Cook, B. I., E. M. Wolkovich, and C. Parmesan. 2012. Divergent responses to spring and winter warming drive community level flowering trends. Proceedings of the National Academy of Sciences of the United States of America 109:9000–9005.

537 Crone, E. E., E. M. Pelton, L. M. Brown, C. C. Thomas, and C. B. Schultz. 2019. Why are
538 monarch butterflies declining in the West? Understanding the importance of multiple
539 correlated drivers. Ecological Applications 29:e01975.
540 Dingle, H., M. P. Zalucki, W. A. Rochester, and T. Armijo-Prewitt. 2005. Distribution of the
541 monarch butterfly, Danaus plexippus (L.) (Lepidoptera: Nymphalidae), in western North
542 America. Biological Journal of the Linnean Society 85:491–500.
543 Erwin, A. C., T. Züst, J. G. Ali, and A. A. Agrawal. 2014. Above-ground herbivory by red
544 milkweed beetles facilitates above- and below-ground conspecific insects and reduces
545 fruit production in common milkweed. Journal of Ecology 102:1038–1047.
546 Espeset, A. E., J. G. Harrison, A. M. Shapiro, C. C. Nice, J. H. Thorne, D. P. Waetjen, J. A.
547 Fordyce, and M. L. Forister. 2016. Understanding a migratory species in a changing
548 world: climatic effects and demographic declines in the western monarch revealed by
549 four decades of intensive monitoring. Oecologia 181:819–830.
550 Fitter, A. H., and R. S. R. Fitter. 2002. Rapid changes in flowering time in British plants. Science
551 296:1689–1691.
552 Forister, M. L., J. A. Fordyce, C. C. Nice, J. H. Thorne, D. P. Waetjen, and A. M. Shapiro. 2018.
553 Impacts of a millennium drought on butterfly faunal dynamics. Climate Change
554 Responses 5:3.
555 Forister, M. L., and A. M. Shapiro. 2003. Climatic trends and advancing spring flight of
556 butterflies in lowland California. Global Change Biology 9:1130–1135.
557 Hanley, M., M. Fenner, and P. Edwards. 1995. The Effect of Seedling Age on the Likelihood of
558 Herbivory by the Slug Deroceras-Reticulatum. Functional Ecology 9:754–759.

559 Haukioja, E., and J. Koricheva. 2000. Tolerance to herbivory in woody vs. herbaceous plants.
560 Evolutionary Ecology 14:551.
561 Hegland, S. J., A. Nielsen, A. Lázaro, A.-L. Bjerknes, and Ø. Totland. 2009. How does climate
562 warming affect plant-pollinator interactions? Ecology Letters 12:184–195.
563 Kharouba, H. M., J. Ehrlén, A. Gelman, K. Bolmgren, J. M. Allen, S. E. Travers, and E. M.
564 Wolkovich. 2018. Global shifts in the phenological synchrony of species interactions
565 over recent decades. Proceedings of the National Academy of Sciences:201714511.
566 Liu, Y., P. B. Reich, G. Li, and S. Sun. 2011. Shifting phenology and abundance under
567 experimental warming alters trophic relationships and plant reproductive capacity.
568 Ecology 92:1201–1207.
569 Maron, J. L., and E. Crone. 2006. Herbivory: effects on plant abundance, distribution and
570 population growth. Proceedings of the Royal Society B: Biological Sciences 273:2575–
571 2584.
572 Maschinski, J., and T. G. Whitham. 1989. The Continuum of Plant-Responses to Herbivory - the
573 Influence of Plant-Association, Nutrient Availability, and Timing. American Naturalist
574 134:1–19.
575 Matter, S. F. 1996. Interpatch Movement of the Red Milkweed Beetle, Tetraopes
576 tetraophthalmus: Individual Responses to Patch Size and Isolation. Oecologia 105:447–
577 453.
578 Matter, S. F. 2001. Effects of Above and Below Ground Herbivory by Tetraopes tetraophthalmus
579 (Coleoptera: Cerambycidae) on the Growth and Reproduction of Asclepias syriaca
580 (Asclepidacae). Environmental Entomology 30:333–338.

581 Miller-Rushing, A. J., T. T. Hoye, D. W. Inouye, and E. Post. 2010. The effects of phenological
582 mismatches on demography. Philosophical Transactions of the Royal Society B:
583 Biological Sciences 365:3177–3186.
584 Munz, P. A., and D. D. Keck. 1959. A California Flora. University of California Press, Berkeley,
585 CA.
586 Ovaskainen, O., S. Skorokhodova, M. Yakovleva, A. Sukhov, A. Kutenkov, N. Kutenkova, A.
587 Shcherbakov, E. Meyke, and M. del M. Delgado. 2013. Community-level phenological
588 response to climate change. Proceedings of the National Academy of Sciences
589 110:13434–13439.
590 Parmesan, C. 2007. Influences of species, latitudes and methodologies on estimates of
591 phenological response to global warming. Global Change Biology 13:1860–1872.
592 Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts
593 across natural systems. Nature 421:37–42.
594 Pelton, E. M., C. B. Schultz, S. J. Jepsen, S. H. Black, and E. E. Crone. 2019. Western monarch
595 population plummets: status, probable causes, and recommended conservation actions.
596 Frontiers in Ecology and Evolution 7.
597 R Development Core Team. 2019. R: A language and environment for statistical computing. R
598 Foundation for Statistical Computing, Vienna, Austria.
599 Rafferty, N. E., and A. R. Ives. 2012. Pollinator effectiveness varies with experimental shifts in
600 flowering time. Ecology 93:803–814.
601 Rasmann, S., A. A. Agrawal, S. C. Cook, and A. C. Erwin. 2009a. Cardenolides, induced
602 responses, and interactions between above- and belowground herbivores of milkweed
603 (Asclepias spp.). Ecology 90:2393–2404.

604 Rasmann, S., M. D. Johnson, and A. A. Agrawal. 2009b. Induced Responses to Herbivory and
605 Jasmonate in Three Milkweed Species. Journal of Chemical Ecology 35:1326–1334.
606 Rasmussen, N.L and L.H. Yang. 2021. The timing of herbivory: early-season herbivory affects
607 plant size and late-season herbivory affects seed production, Dryad,
608 Dataset, https://doi.org/10.5061/dryad.69p8cz93c
609 Rasmussen, N. L., and V. H. W. Rudolf. 2016. Individual and combined effects of two types of
610 phenological shifts on predator–prey interactions. Ecology 97:3414–3421.
611 Rasmussen, N. L., B. G. Van Allen, and V. H. W. Rudolf. 2014. Linking phenological shifts to
612 species interactions through size-mediated priority effects. Journal of Animal Ecology
613 83:1206–1215.
614 Root, T. L., J. T. Price, K. R. Hall, S. H. Schneider, C. Rosenzweig, and J. A. Pounds. 2003.
615 Fingerprints of global warming on wild animals and plants. Nature 421:57–60.
616 Singer, M. C., and C. Parmesan. 2010. Phenological asynchrony between herbivorous insects
617 and their hosts: signal of climate change or pre-existing adaptive strategy? Philosophical
618 Transactions of the Royal Society B: Biological Sciences 365:3161–3176.
619 Strauss, S. Y., and A. A. Agrawal. 1999. The ecology and evolution of plant tolerance to
620 herbivory. Trends in Ecology & Evolution 14:179–185.
621 Sutherland, W. J., R. P. Freckleton, H. C. J. Godfray, S. R. Beissinger, T. Benton, D. D.
622 Cameron, Y. Carmel, D. A. Coomes, T. Coulson, M. C. Emmerson, R. S. Hails, G. C.
623 Hays, D. J. Hodgson, M. J. Hutchings, D. Johnson, J. P. G. Jones, M. J. Keeling, H.
624 Kokko, W. E. Kunin, X. Lambin, O. T. Lewis, Y. Malhi, N. Mieszkowska, E. J. Milner-
625 Gulland, K. Norris, A. B. Phillimore, D. W. Purves, J. M. Reid, D. C. Reuman, K.

626 Thompson, J. M. J. Travis, L. A. Turnbull, D. A. Wardle, and T. Wiegand. 2013.
627 Identification of 100 fundamental ecological questions. Journal of Ecology 101:58–67.
628 Thackeray, S. J., P. A. Henrys, D. Hemming, J. R. Bell, M. S. Botham, S. Burthe, P. Helaouet,
629 D. G. Johns, I. D. Jones, D. I. Leech, E. B. Mackay, D. Massimino, S. Atkinson, P. J.
630 Bacon, T. M. Brereton, L. Carvalho, T. H. Clutton-Brock, C. Duck, M. Edwards, J. M.
631 Elliott, S. J. G. Hall, R. Harrington, J. W. Pearce-Higgins, T. T. Høye, L. E. B. Kruuk, J.
632 M. Pemberton, T. H. Sparks, P. M. Thompson, I. White, I. J. Winfield, and S. Wanless.
633 2016. Phenological sensitivity to climate across taxa and trophic levels. Nature 535:241–
634 245.
635 Thackeray, S. J., T. H. Sparks, M. Frederiksen, S. Burthe, P. J. Bacon, J. R. Bell, M. S. Botham,
636 T. M. Brereton, P. W. Bright, L. Carvalho, T. Clutton-Brock, A. Dawson, M. Edwards, J.
637 M. Elliott, R. Harrington, D. Johns, I. D. Jones, J. T. Jones, D. I. Leech, D. B. Roy, W. A.
638 Scott, M. Smith, R. J. Smithers, I. J. Winfield, and S. Wanless. 2010a. Trophic level
639 asynchrony in rates of phenological change for marine, freshwater and terrestrial
640 environments. Global Change Biology 16:3304–3313.
641 Thackeray, S. J., T. H. Sparks, M. Frederiksen, S. Burthe, P. J. Bacon, J. R. Bell, M. S. Botham,
642 T. M. Brereton, P. W. Bright, L. Carvalho, T. Clutton-Brock, A. Dawson, M. Edwards, J.
643 M. Elliott, R. Harrington, D. Johns, I. D. Jones, J. T. Jones, D. I. Leech, D. B. Roy, W. A.
644 Scott, M. Smith, R. J. Smithers, I. J. Winfield, and S. Wanless. 2010b. Trophic level
645 asynchrony in rates of phenological change for marine, freshwater and terrestrial
646 environments. Global Change Biology 16:3304–3313.
647 Thogmartin, W. E., R. Wiederholt, K. Oberhauser, R. G. Drum, J. E. Diffendorfer, S. Altizer, O.
648 R. Taylor, J. Pleasants, D. Semmens, B. Semmens, R. Erickson, K. Libby, and L. Lopez-

Hoffman. 2017. Monarch butterfly population decline in North America: identifying the threatening processes. Royal Society Open Science 4:170760.

Trumble, J. T., D. M. Kolodny-Hirsch, and I. P. Ting. 1993. Plant Compensation for Arthropod Herbivory. Annual Review of Entomology 38:93–119.

Van Zandt, P. A., and A. A. Agrawal. 2004a. Community-wide impacts of herbivore-Induced plant responses in milkweed (Asclepias syriaca). Ecology 85:2616–2629.

Van Zandt, P. A., and A. A. Agrawal. 2004b. Specificity of Induced Plant Responses to Specialist Herbivores of the Common Milkweed Asclepias Syriaca. Oikos 104:401–409.

Visser, M. E., and C. Both. 2005. Shifts in phenology due to global climate change: the need for a yardstick. Proceedings of the Royal Society B-Biological Sciences 272:2561–2569.

Werner, E. E., and J. F. Gilliam. 1984. The ontogenetic niche and species interactions in size-structured populations. Annual Review of Ecology and Systematics 15:393–425.

Woods, E. C., A. P. Hastings, N. E. Turley, S. B. Heard, and A. A. Agrawal. 2011. Adaptive geographical clines in the growth and defense of a native plant. Ecological Monographs 82:149–168.

Yang, L. H. 2020. Towards a more temporally explicit framework for community ecology. Ecological Research 35:445–462.

Yang, L. H., and M. L. Cenzer. 2020. Seasonal windows of opportunity in milkweed–monarch interactions. Ecology 101:e02880.

Yang, L. H., M. L. Cenzer, L. J. Morgan, and G. W. Hall. 2020. Species-specific, age-varying plant traits affect herbivore growth and survival. Ecology 107:e03029.

Yang, L. H., D. Ostrovsky, M. C. Rogers, and J. M. Welker. 2016. Intra-population variation in the natal origins and wing morphology of overwintering western monarch butterflies Danaus plexippus. Ecography 39:998–1007.

L. H. Yang, K. Swan, E. Bastin, J. Aguilar, M. Cenzer, A. Codd, N. Gonzalez, T. Hayes, A. Higgins, X. Lor, C. Macharaga, M. McMunn, K. Oto, N. Winarto, D. Wong, T. Yang, N. Afridi, S. Aguilar, A. Allison, A. Ambrose-Winters, E. Amescua, M. Apse, N. Avoce, K. Bastin, E. Bolander, J. Burroughs, C. Cabrera, M. Candy, A. Cavett, M. Cavett, L. Chang, M. Claret, D. Coleman, J. Concha, P. Danzer, J. DaRosa, A. Dufresne, C. Duisenberg, A. Earl, E. Eckey, M. English, A. Espejo, E. Faith, A. Fang, A. Gamez, J. Garcini, J. Garcini, G. Gilbert-Igelsrud, K. Goedde-Matthews, S. Grahn, P. Guerra, V. Guerra, M. Hagedorn, K. Hall, G. Hall, J. Hammond, C. Hargadon, V. Henley, S. Hinesley, C. Jacobs, C. Johnson, T. Johnson, Z. Johnson, E. Juchau, C. Kaplan, A. Katznelson, R. Keeley, T. Kubik, T. Lam, C. Lansing, A. Lara, V. Le, B. Lee, K. Lee, M. Lemmo, S. Lucio, A. Luo, S. Malakzay, L. Mangney, J. Martin, W. Matern, B. McConnell, M. McHale, G. McIsaac, C. McLennan, S. Milbrodt, M. Mohammed, M. Mooney-McCarthy, L. Morgan, C. Mullin, S. Needles, K. Nunes, F. O'Keeffe, O. O'Keeffe, G. Osgood, J. Padilla, S. Padilla, I. Palacio, V. Panelli, K. Paulson, J. Pearson, T. Perez, B. Phrakonekham, I. Pitsillides, A. Preisler, N. Preisler, H. Ramirez, S. Ransom, C. Renaud, T. Rocha, H. Saris, R. Schemrich, L. Schoenig, S. Sears, A. Sharma, J. Siu, M. Spangler, S. Standefer, K. Strickland, M. Stritzel, E. Talbert, S. Taylor, E. Thomsen, K. Toups, K. Tran, H. Tran, M. Tuqiri, S. Valdes, G. VanVorhis, S. Vue, S. Wallace, J. Whipple, P. Yang, M. Ye, D. Yo, Y. Zeng. *in press.* Different factors limit

692 early- and late-season windows of opportunity for monarch development. Ecology and
693 Evolution. doi: 10.1002/ece3.9039

694 Yang, L. H., and V. H. W. Rudolf. 2010. Phenology, ontogeny and the effects of climate change
695 on the timing of species interactions. Ecology Letters 13:1–10.

696 Zalucki, M. P., and R. L. Kitching. 1982. Dynamics of oviposition in Danaus plexippus (Insecta:
697 Lepidoptera) on milkweed, Asclepias spp. Journal of Zoology 198:103–116.

698 Zalucki, M. P., H. R. Parry, and J. M. Zalucki. 2016. Movement and egg laying in Monarchs: To
699 move or not to move, that is the equation. Austral Ecology 41:154–167.

700 Zipkin, E. F., L. Ries, R. Reeves, J. Regetz, and K. S. Oberhauser. 2012. Tracking climate
701 impacts on the migratory monarch butterfly. Global Change Biology 18:3039–3049.

702 Züst, T., S. Rasmann, and A. A. Agrawal. 2015. Growth–defense tradeoffs for two major anti-
703 herbivore traits of the common milkweed Asclepias syriaca. Oikos 124:1404–1415.

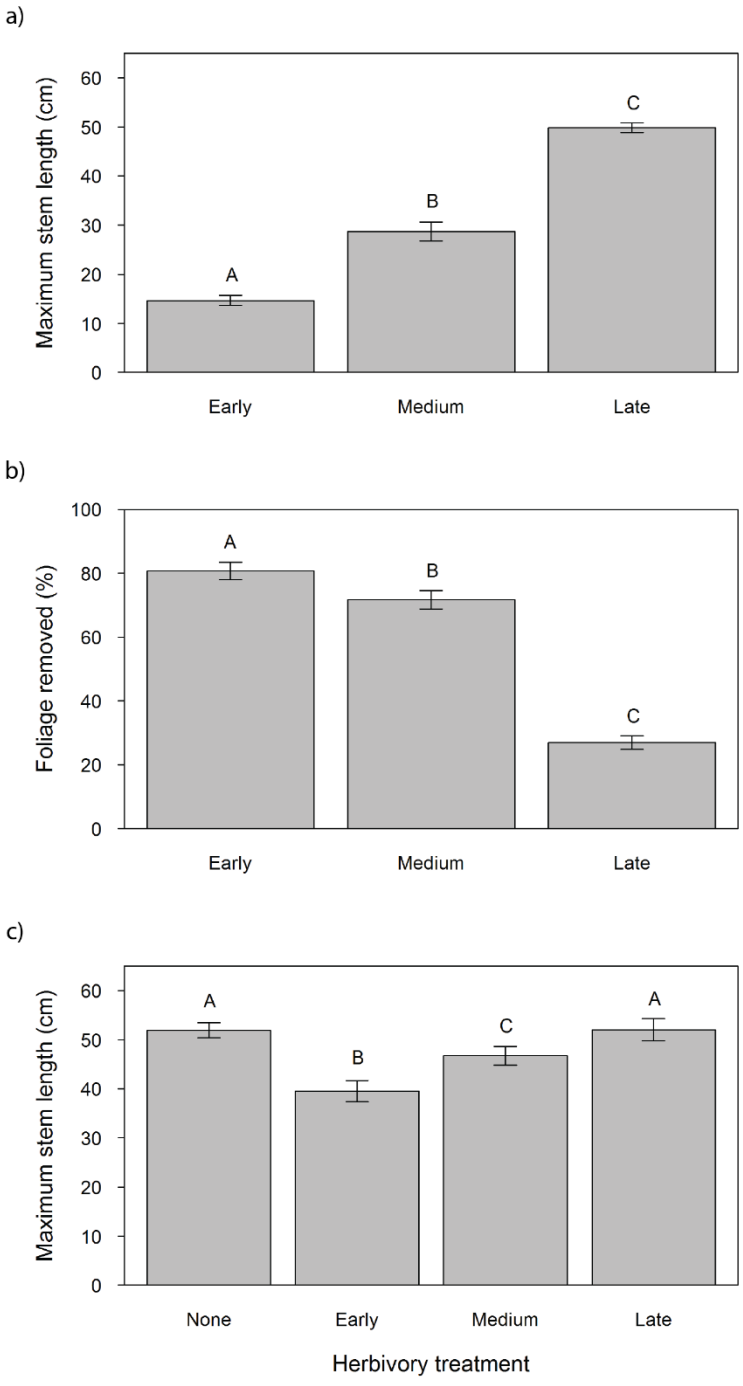
704

Figure 1. (a) Plant size at the time of herbivory treatment initiation, (b) percentage of foliage removed from plants by herbivory in each group, and (c) plant size at the end of the growing season. None = no monarchs added, early = monarchs added to 60-d old plants (May 11, 2015), mid = monarchs added to 74-d old plants, Late = monarchs added to 88-d old plants. Bars represent raw means ± SE. Treatment levels labeled with the same letter do not differ ($\alpha = 0.05$).

Figure 2. Effects of herbivory timing on milkweed flowering traits. (a) Number of umbels produced, (B) age of plants when flowers on the first umbel opened, and (C) number of days between flowers on the first umbel opening and flowers of the last umbel closing (i.e., flowering duration). None = no monarchs added, early = monarchs added to 60-d old plants (May 11, 2015), mid = monarchs added to 74-d old plants, Late = monarchs added to 88-d old plants. Bars represent raw means ± SE. Treatment levels labeled with the same letter do not differ ($\alpha = 0.05$).

Figure 3. Effects of herbivory timing on fruiting traits. (A) Number of days between initiation of first fruit and maturation of last fruit (i.e., fruiting duration) and (B) number of seeds germinated of all seeds produced, used as a proxy for seed viability. None = no monarchs added, early = monarchs added to 60-d old plants (May 11, 2015), mid = monarchs added to 74-d old plants, Late = monarchs added to 88-d old plants. Bars represent raw means ± SE. Treatment levels labeled with the same letter do not differ ($\alpha = 0.05$).

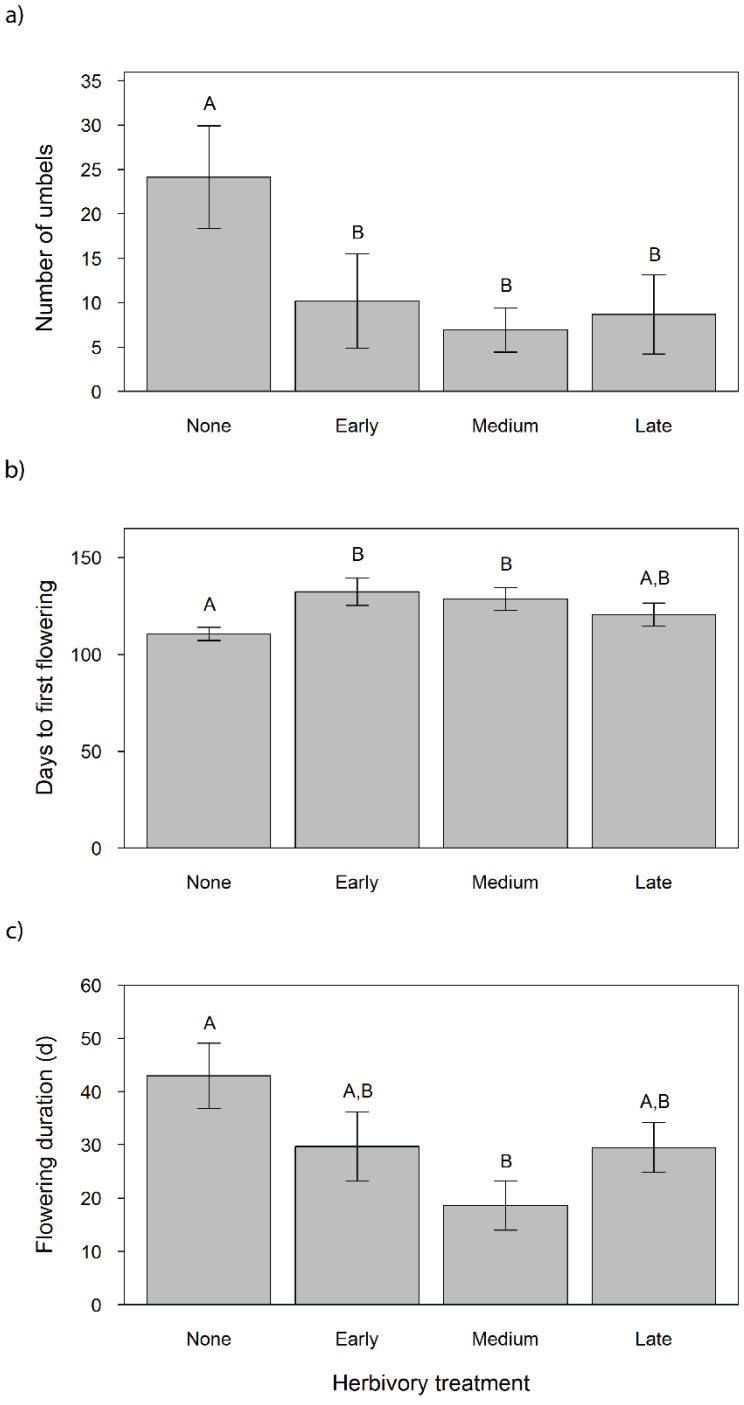
722 **Figure 1**



723

724

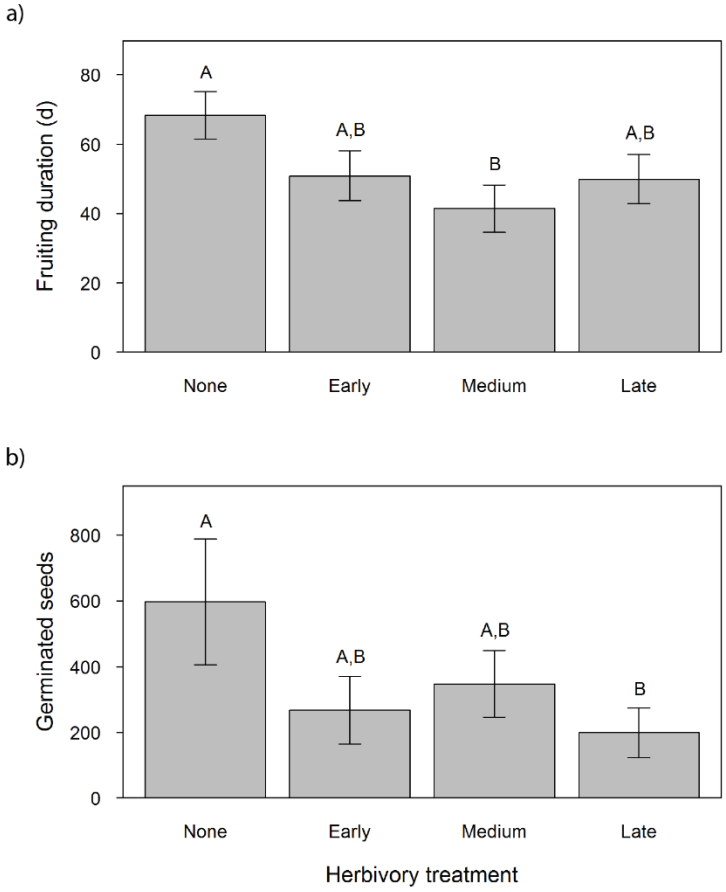
725 **Figure 2**



726

727

728 **Figure 3**



729