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Seasonal windows of opportunity in milkweed-monarch interactions

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Abstract

Many organisms experience seasonal windows of opportunity for growth and reproduction. These windows represent intervals in time when organisms experience improved prospects for advancing their life history objectives, constrained by the combined effects of seasonally variable biotic and abiotic conditions acting independently or in combination. While seasonal windows of opportunity are likely to be widespread in nature, relatively few studies have conducted the repeated observations necessary to identify them or suggest the factors that structure them in time. Here, we present the results of three experimental studies conducted at different field sites in three different years in which we manipulated the phenology of monarch caterpillars (*Danaus plexippus*) throughout the growing season. The primary aims of these experiments were to a) identify seasonal windows of opportunity for successful larval development on milkweed (*Asclepias* spp.), and b) to suggest which factors are most likely to constrain these windows of opportunity in time. We found strong seasonal windows of opportunity in the developmental success of monarchs, with distinct periods of higher developmental prospects during each study year. We evaluated the role of seasonal variation in abiotic thermal stress, host plant density, host plant defensive traits, and natural enemy risk as potential factors that may limit seasonal windows of opportunity. By comparing the seasonal patterns of larval success and potential explanatory factors across all three years, we find patterns that are consistent with seasonally variable abiotic conditions, host plant availability, host plant traits, and natural enemy risk factors. These results suggest the potential for seasonal variation in the factors that limit monarch larval development and population growth. More generally, this study also highlights the value of temporally explicit experimental studies that can identify and examine seasonal patterns in species interactions.

Keywords: windows of opportunity, phenological shift, temporally explicit ecology, seasonal fitness landscape, phenology-ontogeny landscape, match-mismatch, monarch caterpillars, *Danaus plexippus*, *Asclepias*

Introduction

Many organisms have life histories that utilize seasonal windows of opportunity. We define seasonal windows of opportunity as intervals of time in which an organism has improved prospects for achieving key life history objectives such as growth or reproduction. The term "windows of opportunity" has been used more broadly in other contexts to describe diurnal periods of improved foraging opportunity (e.g., Stone et al. 1999), rare opportunities for the recruitment of plants (e.g., Eriksson and Fröborg 1996) and animals (e.g., Yang et al. 2008), and episodic periods that allow for rapid ecosystem recovery (e.g., Balke et al. 2014) or invasion (e.g., DeGasperis and Motzkin 2007). Here, we focus on windows of opportunity that occur within a single year and provide improved conditions for growth or reproduction on a seasonal timescale. Identifying these windows of opportunity provides a key step towards developing a temporally explicit understanding of seasonal life histories and associated species interactions (Yang and Rudolf 2010, Rafferty and Ives 2011, Farzan and Yang 2018).

Seasonal windows of opportunity represent the combined effects of seasonally varying biotic and abiotic conditions on organismal development in dynamic environments. Independently or in combination, these conditions create constraining factors that limit windows of opportunity for successful development, with potential fitness consequences. When the combined effects of these constraining factors change gradually, seasonal windows of opportunity can reflect incremental improvements in developmental prospects. If the combined effects of these constraining factors change more quickly, seasonal windows of opportunity can represent discrete periods to capitalize on transient favorable conditions. While the study of phenology has long investigated the seasonal timing of single life history events such as peak flowering or clutch initiation, phenological studies are increasingly focused on integrated life histories and longer developmental trajectories in a multispecies context (Pau et al. 2011, Nakazawa and Doi 2012, Chuine and Régnière 2017, Carter et al. 2018). In this context, seasonal windows of opportunity can also describe the intervals of time required to complete longer life history trajectories, such as an organism's growth from egg to adulthood.

Efforts to describe the occurrence of seasonal windows of opportunity in time can also help to disentangle the specific constraining factors that limit successful development and population growth. Identifying seasonal windows of opportunity is possible with structured observations or experimental manipulations that quantify how organismal success rates vary throughout the season; these repeated measurements provide a way to visualize the fitness landscape across a season (Yang and Rudolf 2010). Hypotheses about potential constraining factors can be evaluated via temporally explicit correlations and comparisons with specific biotic and abiotic conditions. In particular, phenological manipulations (i.e., experimental phenological shifts) effectively change the relative timing of species interactions and the climatic conditions experienced by organisms, providing a valuable step towards disentangling the causal factors that constrain seasonal windows of opportunity. However, relatively few studies have experimentally manipulated phenology across an entire season to identify seasonal windows of opportunity of organisms in the field (but see Rafferty and Ives 2011, Farzan and Yang 2018).

In this study, we investigate the seasonal windows of opportunity available for the successful development of monarch butterfly (*Danaus plexippus*) larvae on their milkweed (*Asclepias* spp.) host plants. The strong interactions between milkweed host plants and their monarch herbivores provides a good context to assess seasonal windows of opportunity. Milkweeds support relatively simple and well-described arthropod communities (e.g., Agrawal 2005) with strong, pairwise plant-herbivore interactions that are known to have important seasonal dynamics (e.g., Nelson et al. 1981, Root 1986, Van Zandt and Agrawal 2004). Native temperate milkweeds are long-lived perennials that re-emerge from rhizomes each spring, and milkweed-associated communities reassemble each year, providing repeated and replicated opportunities to examine phenological shifts. Although the specific drivers of migratory phenology in monarchs are likely to be complex (Zipkin et al. 2012), monarchs may be particularly likely to experience phenological mis-matches with their destination community due to their migratory life history, as has been observed in other migratory species (e.g., Inouye et al. 2000, Thorup et al. 2007, Chmura et al. 2019). Interactions between milkweeds and larval monarchs occur throughout a long growing season each year in natural communities near our study sites (*unpubl. obs.*), and manipulations of their interaction phenology are realistic across a wide range of the season. Finally, monarch butterflies have experienced long-term population declines over several decades that have dramatically accelerated for the western population in recent years (Frey and Schaffner 2004, Thogmartin et al. 2017, Pelton et al. 2019), and efforts to identify seasonal windows of opportunity and the constraining factors that limit them could potentially inform our understanding of monarch population dynamics and conservation priorities.

We used phenological manipulations conducted in three years to quantify the seasonal windows of opportunity available for monarch larval development. Our primary question asked: Q1) How do the developmental prospects of monarch larvae change throughout the season? In particular, we sought to identify when seasonal windows of opportunity for successful larval development occur. In addition, we also aimed to evaluate hypothesized constraining factors that could potentially structure these windows of opportunity. Specifically, we asked: Q2) To what extent are seasonal patterns of developmental success consistent with constraining factors based on seasonally varying abiotic conditions, seasonally variable changes in host plant availability or quality, or seasonal variation in the risk of natural enemy attack? We hypothesized that the developmental prospects of monarch caterpillars are fundamentally constrained by limited host plant availability in the early season, and host plant senescence in the late season. Thus, we hypothesized that greater milkweed patch densities would allow caterpillars to overcome limitations of host plant quantity in the early season, potentially creating earlier seasonal windows of opportunity. We further expected that milkweed species-specific differences in the seasonal expression of plant defensive traits would strongly shape the seasonal fitness landscape, with host plant species that show faster increases in defensive traits over development also having more constrained seasonal windows of opportunity. We anticipated that abiotic stress could limit larval success, potentially with low temperatures limiting growth rates in the early season and high temperatures limiting survival in the mid-summer. We did not have strong initial expectations for the seasonal pattern of natural enemy risk at the outset of this experiment, though our observations in the first year of this study informed our hypotheses in subsequent years.

Methods

Study system

We studied the development of western monarch butterflies (*Danaus plexippus*) on two species of milkweeds (narrow-leaved milkweed, *Ascelpias fascicularis* and showy milkweed, *Asclepias speciosa*) native to the Central Valley of California, USA at multiple field sites near Davis, CA. Both species of milkweeds are summer-growing perennials that emerge from belowground rhizomes in the spring, before senescing their aboveground parts in the late summer or early fall. Western monarch butterflies spend the winter in climatically moderated overwintering aggregations along the California coast before migrating inland to find milkweed host plants and reproduce over multiple generations. The duration of the larval generation is typically 10 to 21 days, dependent on multiple factors (Urquhart 1960, Zalucki 1982). Declining photoperiod in the fall generally triggers reproductive diapause and return migration from natal habitats across the range to overwintering sites in California and Mexico (Goehring and Oberhauser 2002, Morris et al. 2015, Yang et al. 2016).

Seasonal fitness landscape experiments

During three seasons between 2010 and 2014, we conducted a series of experiments in which we manipulated the seasonal phenology (i.e. simulated oviposition) of monarch eggs on milkweeds. In each study, cohorts of eggs were introduced to random, unique subsets of a milkweed population at multiple intervals throughout each field season (Fig. 1). Each cohort represents an experimental manipulation of appearance (i.e., oviposition) phenology, with repeated measurements of larval development throughout the growing season. The length of each monarch was measured with dial calipers to the nearest 0.1 mm every 2-3 days to assess growth and development throughout the season. Caterpillar mass was determined based on a power law regression of caterpillar length and mass data collected from 73 unmanipulated caterpillars measured in 2014 ($mass=0.0223*length^{2.9816}$, $R^2=0.97$). The maximum size attained by each caterpillar provided a metric of individual-level developmental success, integrating aspects of both survival and growth rate. We compared the maximum size attained by monarch caterpillars in each cohort in order to assess how monarch developmental success changed throughout the year; this metric integrates larval growth and survival and serves as an integrated proxy of larval developmental success. These experiments aimed to characterize the seasonal fitness landscape in way that is consistent with the phenology-ontogeny seasonal fitness landscape approach (Yang and Rudolf 2010), but focused on a single dimension of fitness, monarch developmental success.

2010

Experiments in 2010 were conducted with seven cohorts of 32 even-aged monarch eggs, introduced to milkweed plants at 20-22 day intervals between May 20, 2010 and September 23, 2010. Each of two locally distributed milkweed species (narrow-leaved milkweed, *A. fascicularis* and showy milkweed, *A. speciosa*) was represented by 16 replicates in each cohort. This experimental design included an additional factor to test for the effects of host plant density on developmental success, with eight replicates in each species*cohort group consisting of individual plants (density=1), and eight replicates consisting of three plants bundled together with overlapping foliage (density=3). This treatment was intended to evaluate the hypothesis that higher density patches might lift host plant quantity limitations early in the season. Milkweeds were propagated from locally sourced even-aged seedlings (Hedgerow Farms, Winters, CA, USA) transplanted into 2.5 L containers on April 1, 2010 and maintained with regular drip irrigation on open outdoor benches at a UC Davis plant research facility (38.542415° N, 121.763263° W).

Each cohort of monarch eggs was obtained from a large, local insectary population (Utterback Farms, Woodland, CA, USA). This population was re-established from local monarch genotypes each year, maintained in large greenhouses, regularly supplemented with new adults to maintain genetic diversity, and had been previously assessed for parasites and pathogens (H.K. Kaya, *pers. comm.*). For each cohort, we used a 6.4 mm hole-punch to generate a set of 32 leaf discs with single monarch eggs attached, and attached them to the underside of apical leaves on their experimental host plants with a drop of milkweed latex. This procedure minimized direct handling of the egg and allowed caterpillars to hatch directly on their experimental host plant *in situ*. In this study, the developmental stage (i.e., egg, larval instar or pupa) and length of each monarch caterpillar was measured with dial calipers to the nearest mm three times per week until death or pupation. We recorded the presence of other members of the arthropod community that were observed on each plant throughout the study. In order to maximize unconstrained seasonal community assembly, plants were kept uncaged until caterpillars approached the 5th instar, at which point they were bagged with coarse mesh to prevent caterpillar movement off the host plant during the pre-pupal wandering phase.

In addition to the milkweed plants used in the main experiment, we measured seasonally variable plant traits on an additional 6-7 non-experimental plants in each species*cohort combination (42 *A. fascicularis*, and 45 *A. speciosa*) at each interval. Plant height, latex exudation, trichome density, and leaf toughness were measured once for each of these plants throughout the season. Plant height was measured as the maximum distance from the apical leaf petiole to the soil surface above the root crown. Latex exudation was measured as the mean dry mass of latex collected on pre-weighed filter paper discs after cutting 5 mm from the distal tip of two fully expanded upper leaves. Trichome density was counted visually under magnification from 3 mm diameter leaf discs punched from fully expanded upper leaves. Leaf toughness was measured as the mean of four penetrometer readings from four fully expanded upper leaves using spring scales (Pesola Präzisionswaagen AG, Switzerland) with a 13.86 mm^2 hexagonal penetrometer attachment. Measurements of plant defense traits were adapted from Agrawal and Fishbein

(2006), and references therein. Seasonal and species-associated variation in plant traits were analyzed with standard linear models including cohort, host plant species and their interaction as factors.

This analysis used permutational linear models (the *lmp* function in the *lmPerm* package, Wheeler and Torchiano 2016) to evaluate the effects of cohort, host plant species, density and their two-way interactions on the maximum mass attained by each monarch. The permutational framework was chosen because it does not assume any particular underlying data distribution (Wheeler and Torchiano 2016). We used 999,999 permutational iterations of the data to test each factor in the model using unique (Type II) sums of squares, using a null model that excludes related higher order interaction terms when present to assess significance (using the *Anova* function in the *car* package, Fox and Weisberg 2011). Cohort was included in these models as a categorical factor, allowing for responses in monarch size over the season consistent with hypothesized windows of opportunity for monarch development. Because many of these analyses investigate categorical explanatory factors, we assess effect sizes as ΔR^2, the change in proportion of variance explained relative to a null model without the explanatory factor. We also present comparisons of means for some analyses in order to convey these effect sizes on a biologically relevant scale, though many of these data are not normally distributed. To test the *a priori* hypothesis that higher host plant densities would allow greater larval success in the early season, we analyzed planned directional contrasts of the density factor for each host species at the beginning of the first window of opportunity with a one-tailed test (Cho and Abe 2013). We present analyses based on both the original length data and on more generalizable biomass measures in order to make them more comparable across studies, and present both scales in figures (*ggplot2*, version 3.1.1.9, Wickham 2016); these two analyses generally yielded very similar results.

We conducted a survival analysis to generate age and stage-specific Kaplan-Meier survival curves for monarchs on each host plant species in each cohort. These curves present detailed larval survival rates on a daily scale following egg introduction and on the scale of larval instars. We estimated the timing of mortality for all caterpillars as the midpoint between the date it was last observed alive and the date of subsequent observation when it was first observed dead or missing; we rechecked for missing caterpillars on at least three successive observation days. Caterpillars that survived to pupation were right-censored in the survival analysis. The data for these curves were compiled using the *survival* (Therneau and Grambsch 2000) and *survminer* (Kassambara and Kosinski 2019) packages in R. Daily survival curves were approximately linear on semi-log plots, suggesting relatively constant daily survival rates, which we estimated using linear regression. The overall daily survival rates of caterpillars on narrow-leaved and showy milkweed host plants was estimated across all cohorts from the back-transformed slope coefficient of the linear regression of log-transformed survival rates against caterpillar developmental age in days. We applied a similar approach to estimate host species and cohort-specific daily survival rates. Some host species by cohort combinations showed steep survivorship curves whose slope coefficients could not be estimated by log-linear regression because there was only one non-zero survivorship observation; in these cases, we estimated the daily survivorship rate directly as $e^{\log(s)/t}$, where s is the survivorship at time t. We compared the overall differences between the survival curves of caterpillars on each host plant species and among cohorts using a log-rank test.

2011

In 2011, we conducted a similar experiment using a population of 96 narrow-leaved milkweeds established in two 6 x 500 m multispecies wildflower strips along an agricultural margin at UC Davis Russell Ranch Sustainable Agriculture Facility (38.541714° N, 121.878196° W). We established this milkweed population by transplanting dormant plants from 2.5 L containers into the field on February 5, 2011, and allowing the plants to emerge the following spring. All milkweeds were propagated from local seeds (Hedgerow Farms, Winters, CA, USA) in the previous year. Plants were transplanted in transects that bisected each wildflower strip with at least 5 m between adjacent milkweeds. The surrounding plant community included mostly CA native flowering species such as *Phacelia tanacetifolia*, *Lupinus succulentus*, *Lupinus densiflorus*, *Phacelia californica*, *Eschscholzia californica*, *Grindelia camporum*, *Helianthus bolanderi*, *Lupinus formosus*, and *Trichostema lanceolatum* (K. Ward, *pers. comm.*).

In this experiment, monarch eggs were introduced to random subsets of the milkweed population in five cohorts at 35 day intervals throughout the season (May 4, 2011: N=24; June 8, 2011: N=22; July 13, 2011: N=17; August 17, 2011: N=18; and September 21, 2011: N=15). For each cohort throughout the season, plant height, trichome density, latex exudation and leaf toughness was measured on each experimental plant immediately prior to monarch introduction. Monarch introduction and plant trait measurements were conducted using the same methods as in 2010. Sample sizes varied throughout the season due to milkweed mortality (i.e., we did not deploy monarch eggs onto dead or senescent plants). We measured the developmental stage and caterpillar length of each introduced monarch three times per week, as in 2010. We generated age- and stage-specific survival curves for each cohort, and estimated overall daily survival rates using the same methods as with the 2010 data, but with narrow-leaved milkweed only. In addition to these measurements, we also conducted a standardized 2-minute visual survey of the arthropod community on each non-senescent plant in the cohort three times per week, whether or not that plant still hosted a surviving monarch caterpillar. The goal of this survey was to assess seasonal patterns in the density of natural enemies for monarchs. We recorded the identity and count of each arthropod observed during each standard census. We identified arthropods at multiple taxonomic levels in the field, then aggregated these data into three broad functional groups (predator/parasitoid, competitor or other/unknown) based on their primary interactions with monarch caterpillars.

This experiment was initially designed with a fully factorial open-bottomed cage treatment to exclude predators from a random subset of plants during each interval, using identical cages with an additional opened side panel as a cage control. In this design, all milkweed plants were uncaged for the majority of the season, and a set of cages was erected around the plants in each focal cohort every 35 days, after all visible members of the arthropod community were removed. However, this exclusion manipulation appeared to be largely ineffective, likely due to incomplete removal of the arthropod community and gaps in the cage barrier that allowed for recolonization. We evaluated the effect of the exclosure treatment by comparing the density of predators, competitors and other arthropods in both groups. We also compared monarch developmental success in exclosure versus open treatments. As in 2010, the analysis of these data used permutational linear models to evaluate the role of cohort and predator exclosure on maximum monarch mass attained.

2014

We conducted another field experiment in 2014 to evaluate seasonal windows of opportunity for monarch developmental success in patches of different densities. This experiment was conducted in a 20 x 300 m agricultural margin at the UC Davis Russell Ranch Sustainable Agriculture Facility, where *A. fascicularis* milkweeds were established in a 3 x 50 patch grid (38.540038° N, 121.876000° W). Half of these patches included a single milkweed (density=1), while the other half had three milkweeds planted with approximately 20 cm spacing (density=3). Patch densities alternated across the grid to ensure spatial interspersion (Hurlbert 1984).

All plants were initially germinated in the greenhouse from local seeds (Hedgerow Farms, Winters, CA, USA), then transplanted into buried wire mesh gopher baskets (Diggers, Santa Cruz, CA, USA) as dormant seedlings in the winter of 2013-2014. All gopher baskets were constructed of 19 mm hexagonal wire mesh to prevent gopher herbivory but allow free root access and water drainage. The density=1 patches were protected with 30 cm diameter 18.9 L gopher baskets, and the density=3 patches were protected with 43 cm diameter 56.8 L gopher baskets. All plants received drip irrigation as needed, at the same rate across the experiment (3.8 L per hour per emitter, one emitter per plant). We measured total milkweed stem length for each patch at each interval, and the effect of patch density on total stem length was evaluated with a linear model.

Six cohorts of monarch eggs were introduced to randomized subsets of both milkweed densities at 28 day intervals (May 8, 2014: *N*=26; June 5, 2014: *N*=26; July 3, 2018: *N*=26; July 31, 2018: *N*=24; August 28, 2014: *N*=24; September 25, 2014: *N*=24). As in previous years, we monitored the developmental stage and size of experimental monarch caterpillars three times per week until death or pupation, and used a permutational linear model with cohort, patch density and their interaction to analyze these data. We generated age- and stage-specific survival curves for each cohort, and estimated overall daily survival rates using the same methods as with the 2010 and 2011 data. In order to maintain exposure to natural biotic and abiotic conditions throughout larval development, these larvae were not enclosed at any point in development. In order to inform the right-censoring of the survival analysis, we estimated the pupation rate by combining direct observations of pupation on the focal patch with counts of 5th instar larvae that reached a threshold size and were not observed again. The approximate threshold size for pupation (895 mg or 35 mm) was determined by assessing the maximum larval size attained by all (N=6) pupating caterpillars in 2010 and 2011, and among 248 caterpillars reared in the laboratory in 2014 and 2015. We hypothesized *a priori* that the positive effect of host plant density on developmental success would be greatest in the early season, and conducted a planned directional contrast of the first cohort comparing the maximum size of monarchs in the two density treatments.

The experiments conducted in 2010, 2011 and 2014 all manipulated the timing of monarch cohorts on milkweed host plants, but were conducted at different field sites, and with additional differences in the specific milkweed species examined, the specific timing of monarch cohorts, and other experimental aspects. We summarize these differences in Table S1.

Results

2010

In 2010, monarch developmental success varied strongly throughout the season (cohort, p=0.014 for mass [p=0.002 for length], ΔR^2=0.14 for mass [ΔR^2=0.15 for length]), showing two seasonal windows of opportunity for successful larval development on narrow-leaved milkweed and one early window of opportunity on showy milkweed (Figs. 2a-b, *cohort*species*, p=0.054 for mass [p=0.15 for length], ΔR^2=0.053 for mass [ΔR^2=0.040 for length]). For example, the mean attained size of caterpillars introduced to *A. fascicularis* during the apparent windows of opportunity (June 9, July 1, September 2 and September 23) was 144 mg [19 mm], whereas the expected size of caterpillars introduced to *A. fascicularis* in other cohorts (May 20, July 22 and August 12) was 0.07 mg [1.5 mm]. Monarchs developed more successfully on narrow-leaved milkweed compared to showy milkweed (Figs. 2a-b, *species*, p=0.031 for mass [p=0.029 for length], ΔR^2=0.073 for mass [ΔR^2=0.060 for length]), especially in the late season. For example, the mean attained size of caterpillars introduced in the early summer (June 9 and July 1) was 116 mg [18 mm] on *A. fascicularis*, and 67 mg [15 mm] on *A. speciosa*, but caterpillars on *A. fascicularis* experienced a second window of opportunity in the early fall (September 2 and September 23) with mean attained sizes of 172 mg [20 mm], whereas caterpillars on *A. speciosa* experienced much lower prospects for successful development during that same interval, with mean attained sizes of 0.3 mg [2.3 mm]. Milkweed density and density-associated interactions did not affect developmental success overall (*density*, p=0.81 for mass [p=0.85 for length], ΔR^2=0.019 for mass [ΔR^2=0.018 for length]; *cohort*density*, p=0.61 for mass [p=0.65 for length], ΔR^2=0.019 for mass [ΔR^2=0.017 for length]; *species*density*, p=0.75 for mass [p=0.74 for length], ΔR^2=0.0004 for mass [ΔR^2=0.0004 for length]), but monarchs at the beginning of the first window of opportunity performed marginally better on high density patches of narrow-leaved milkweed compared with caterpillars on low-density patches (Fig. S1, June cohort, one-tailed test, *density*, p=0.13 for mass [p=0.029 for length], ΔR^2=0.089 for mass [ΔR^2=0.23 for length], 203 mg [21 mm] vs. 9 mg [7.5 mm]). This pattern was not observed on showy milkweed

(June cohort, one-tailed test, *density*, p=0.82 for mass [p=0.37 for length], ΔR^2=0.061 for mass [ΔR^2=0.057 for length]).

This seasonal pattern of overall larval success (maximum size attained) was consistent with the seasonal pattern of stage- and age-specific survivorship (Figs. 3 and S2), with both showing windows of opportunity in the early summer, and a second window of opportunity for caterpillars on narrow-leaved milkweed in the early fall. Survivorship curves were approximately log-linear suggesting relatively consistent mortality rates over development (i.e. Type II survivorship curves consistent with exponential declines in cohort numbers over time). This pattern of survival is consistent with past studies of age- and-stage specific survivorship in this system (De Anda et al. 2015). Combining all cohorts, larval survival was significantly higher on narrow-leaved milkweed (92% daily survivorship) than on showy milkweed (79% daily survivorship, log-rank test, p=0.042). However, the substantial variation in survivorship curves attributable to cohort (log-rank test, p<0.0001, Figs. 3 and S2) illustrates the importance of seasonal windows of opportunity. This is mirrored in the host plant and cohort-specific daily survivorship rates on narrow-leaved milkweed: (May 20, 63%; June 9, 83%; July 1, 92%; July 22, 0%; August 12, 44%; September 2, 89%; September 23, 98%) and on showy milkweed (May 20, 68%; June 9, 85%; July 1, 85%; July 22, 44%; August 12, 58%; September 2, 55%; September 23, 59%). Only 2.2% (5 of 224) of eggs introduced in this experiment survived to pupation: one in the July 1 cohort, one in the September 2 cohort, and 3 in the September 23 cohort; all developed on narrow-leaved milkweed.

The pattern of differential success on the two host plants was also consistent with the seasonal pattern of defensive traits, where showy milkweed showed greater increases through the season than narrow-leaved milkweed for latex exudation (Fig. 2c; *cohort*, p<0.00001, ΔR^2=0.35; *species*, p<0.00001, ΔR^2=0.44; *cohort*species*, p=0.00024, ΔR^2=0.16) and trichome density (Fig. 2c*; cohort*, p=0.00029, ΔR^2=0.21; *species*, p<0.00001, ΔR^2=0.55; *cohort*species*, p=0.0059, ΔR^2=0.086). Leaf toughness also increased throughout the season (*cohort*, p<0.00001, ΔR^2=0.36) and was generally higher in showy milkweed (*species*, p<0.00001, ΔR^2=0.24), but seasonal changes in leaf toughness were not significantly different between the two host plant species (Fig. S3, *cohort*species*, p=0.50, ΔR^2=0.03). Hatch failure rates were highest in July and August, during a period of low larval success overall (Fig. 2d, *cohort*, p<0.00001, ΔR^2=0.25), and the effect of cohort differed between the two milkweed species (*cohort*species*, *p*=0.027, ΔR^2=0.053). Natural enemies were most abundant in July and August before declining 85% in the two September cohorts (Fig. 2e).

2011

Developmental success varied significantly in 2011 (*cohort*, *p*=0.050 for mass [*p*=0.004 for length], ΔR^2=0.12 for mass [ΔR^2=0.18 for length]), with a single window of opportunity for successful larval development in the first half of the season (Fig. 4a); the mean attained size of caterpillars in the first three cohorts (started on May 4, June 8, and July 13) was 181 mg [20 mm], while the mean attained size of caterpillars in the final two cohorts (started on August 17 and September 21) was 0.02 mg [1 mm].

The stage- and age-specific survivorship of caterpillars were consistent with this seasonal pattern of overall larval success, with reduced survivorship in the final two cohorts (Fig. 5 and S4). Survivorship curves differed significantly by cohort (log-rank test, *p*<0.0001), with daily survival rates of 88% overall and 91% in the May 4 cohort, 88% in the June 8 cohort, 92% in the

July 13 cohort, 12% in the August 17 cohort, and 0% in the September 21 cohort. Of the 96 eggs included in this experiment, only one (1%) survived to pupation; this pupa was observed in the May 4 cohort.

The defensive traits of the narrow-leaved milkweed host plants also increased significantly throughout the season (Fig. 4b, latex exudation: *cohort*, $p<0.0001$, $\Delta R^2=0.29$; trichome density: *cohort*, $p<0.0001$, $\Delta R^2=0.27$; leaf toughness: *cohort*, $p<0.0001$, $\Delta R^2=0.33$), though the scale of this seasonal variation was smaller than that observed in showy milkweed in 2010 (e.g., Fig. 2c, Fig. 4b). The window of opportunity for larval development was consistent with the observed decline in hatch rate at the end of the season (Fig. 4c). The exclosure treatment did not significantly affect the density of other arthropods in the community (predators, $p=0.11$, $\Delta R^2=0.02$; competitors, $p=0.44$, $\Delta R^2=0.005$; other arthropods, $p=0.48$, $\Delta R^2=0.004$; all arthropods, $p=0.85$, $\Delta R^2=0.0003$, Supporting Information), or monarch developmental success (*exclosure*, $p=0.66$, $\Delta R^2=0.017$; *exclosure*cohort*, $p=0.83$, $\Delta R^2=0.016$). We retained the exclosure treatment factor in this analysis to conservatively reflect the complete *a priori* design, although we did not attempt to experimentally assess the effect of the biotic community from this manipulation. Instead, the primary contribution of this experiment was to identify the seasonal window of opportunity at this site in 2011; the significant effect of cohort on monarch developmental success was qualitatively unchanged with or without the inclusion of the exclosure factors in the model. The density of predaceous arthropods on milkweeds declined approximately four-fold throughout the season (Fig 4d), possibly reflecting the rapid colonization and subsequent loss of arthropods from the surrounding spring-flowering plant community.

2014

In 2014, we observed two seasonal windows of opportunity for successful monarch development on narrow-leaved milkweed (Fig. 6a). Caterpillars in the May 8 cohort developed well (mean attained size 88 mg [16mm]), and developmental success declined into the mid-season (June 5, July 3 and July 31 cohorts, mean attained size 0.17 mg [2 mm]) before increasing again at the end of the season (September 25 cohort, mean attained size 226 mg [22 mm])(Fig. 6a, *cohort*, p=0.0027 for mass [p<0.0001 for length], ΔR^2=0.14 for mass [ΔR^2=0.20 for length]). This pattern is consistent with the higher hatch failure rates observed in the mid-summer cohorts (Fig. 5b), which contributed to reduced survivorship overall (Fig. 7 and S5). The overall daily survival rate was 84%. As in previous years, survivorship curves differed significantly by cohort (log-rank test, p<0.0001), with daily survival rates of 87% overall and 83% in the May 8 cohort, 5% in the June 5 cohort, 69% in the July 3 cohort, 33% in the July 31 cohort, 84% in the August 28 cohort and 91% in the September 25 cohort. We directly observed one caterpillar reach pupation in this experiment, and additionally estimate that two more reached pupation based on reaching a threshold size; all of these developed in the September 25 cohort. The availability of milkweed was significantly higher in the high-density patches throughout the season, with 283% greater total stem lengths compared with the low-density patches (Fig. S6, *density*, p<0.0001, ΔR^2=0.09). The increased milkweed density did not affect the developmental success of monarch caterpillars (*density*, p=0.55 for mass [p=0.13 for length], ΔR^2=0.017 for mass [ΔR^2=0.026 for length]) or alter their seasonal windows of opportunity (*cohort*density*, p=0.81 for mass [p=0.79 for length], ΔR^2=0.014 for mass [ΔR^2=0.014 for length]) in the overall analysis. However, monarchs in the high-density patches showed marginally greater developmental success than monarchs in the low-density patches at the beginning of the first window of opportunity (Fig. S1, May cohort, one-tailed test, *density*, p=0.06 for mass [p=0.13 for length], ΔR^2=0.096 for mass

[ΔR^2=0.054 for length]), with mean attained sizes of 159 mg [20 mm] in the high density milkweed patches versus 17 mg [9 mm] in the low density patches.

Discussion

These experiments identified seasonal windows of opportunity for successful larval development of monarch caterpillars on milkweed host plants. In 2010, we observed two distinct windows of opportunity for successful larval development on narrow-leaved milkweed, and a single early-season window of opportunity on showy milkweed. In 2011, we observed a single broad window of opportunity on narrow-leaved milkweed across three months in the first half of the growing season, followed by reduced developmental success in remainder of the season. In 2014, we again saw two distinct windows of opportunity, with distinct peaks in larval success observed in early May and late September. In particular, our finding of a substantial window of opportunity for fall breeding represents a notable departure from the historic pattern of spring and summer breeding in the Central Valley (Art Shapiro, *pers. comm.*), and is consistent with recent observations of wild monarch breeding in this system (L.H. Yang, *unpubl. data*).

The results of this study suggest that the seasonal windows of opportunity for successful larval development are context-dependent; we observed different patterns in different years, at different field sites and on different host plants. However, the variation observed in this study suggests factors which could structure these windows of opportunity. These constraining factors likely fall into three broad classes acting independently or in combination: 1) seasonal changes in abiotic conditions, 2) seasonal changes in host plant quality or availability, and 3) seasonal changes in the risk posed by natural enemies, such as predators, parasites and pathogens. For example, in 2010 and 2014, we observed distinct periods of reduced mid-summer larval developmental success. These mid-summer slumps occurred in July and August, during a period of sustained hot and dry conditions at our study sites (Fig. S7). This seasonal pattern could suggest a potential abiotic constraint on larval development, consistent with previous studies of thermal tolerance in monarchs (Zalucki 1982, Malcolm et al. 1987, Nail et al. 2015a). However, these periods of reduced larval success overall were also associated with periods of reduced hatch rates (Figs. 2d and 6b); while the lower thermal limits of monarch eggs have been well-studied (Nail et al. 2015a), the degree to which hot and dry conditions are associated with reduced hatch rates is unknown. In 2010, the period of reduced hatch rates was also coincident with a period of increased predator density on our experimental plants, including many egg predators. Terrestrial predators have been previously shown to have substantial negative effects on the survival of monarch eggs and larvae in other milkweed-monarch systems (Prysby 2004, De Anda et al. 2015). However, our experiment in 2011 showed a different seasonal pattern than in 2010 and 2014, with a single early seasonal window of opportunity, and both hatch rates and predator densities declining throughout the field season. Proximately, the pattern of declining predator densities likely reflects the phenology of the surrounding native vegetation which was unique to this study year; many of the wildflowers in this surrounding community grew most actively in the early season, and likely harbored abundant and diverse communities of natural enemies that colonized the milkweeds upon emergence. Consistent with our observations at the time, we speculate that the phenology of the surrounding plant community could have extended the early season window of opportunity by creating a cooler and moister microclimate initially, but exposing the monarchs to hotter and drier conditions as the surrounding vegetation senesced. Thus, while this study suggests several constraining factors, future experimental work will be necessary to assess causation, and to assess the relative importance of different constraining factors across multiple contexts.

The experiment in 2010 does suggest a potentially strong role of host plant defensive traits in structuring seasonal windows of opportunity. The seasonal patterns of larval development on narrow-leaved and showy milkweed suggest that monarchs were able to successfully use mature narrow-leaved milkweed plants in the late season, but were less successful feeding on late-season showy milkweed (Figs. 2 and 3). This comparison is consistent with the seasonal changes in defensive traits we observed on these two host species; for example, while narrow-leaved milkweed showed consistently moderate defensive traits throughout the season, the defensive traits of showy milkweed increased rapidly with plant size into August, before declining with senescence. Previous studies have shown that milkweed defensive traits such as latex exudation can reduce caterpillar success (Zalucki et al. 2001, Agrawal et al. 2015). In this case, the seasonal patterns of larval development and defensive traits on narrow-leaved and showy milkweed are consistent with host plant quality as a factor constraining seasonal windows of opportunity for monarchs.

Density manipulations in 2010 and 2014 suggest the possibility of host plant limitation in the early season. In both years, monarchs had greater success in high-density milkweed patches than low-density patches. Although these effects were only marginally significant, this pattern is consistent with our *a priori* expectation that the small size of milkweed plants in the early season could constrain early season windows of opportunity, as negative density dependence has been observed in previous analyses at the site-level across years (Nail et al. 2015b). We hypothesize that these patch size effects are strongest in the early season because this is when individual plants are smallest; as plants became large enough to support complete monarch development, patch density may become less important. We speculate that the importance of this density effect in nature could depend on the relative phenology and growth of milkweeds and monarchs in the early season, as well as seasonal patterns of monarch oviposition density. We expect that the potential for seasonal host plant limitation would be greatest in the early season when individual plants are small, and under conditions that combine small patch sizes and high oviposition densities. While this current experiment introduced single monarch eggs, future studies will be necessary to further assess the possibility of seasonal milkweed limitation in this system.

As in any population with high reproductive potential, we expect the mortality of individual monarchs to be high. Monarchs face a multitude of hurdles in their development from eggs to adults, including abiotic conditions (Zalucki 1982, Malcolm et al. 1987, Nail et al. 2015a), host plant defenses (Zalucki et al. 2001, Agrawal et al. 2015), host plant limitation (Nail et al. 2015b), and natural enemies (Altizer and Oberhauser 1999, Prysby 2004, De Anda et al. 2015). Even if western monarch populations were stable, we would expect developmental success to be strongly constrained, with mortality occurring throughout their life history. The overall survival curves and daily survival rates observed in our current study reinforce this observation, and share many fundamental similarities with age- and stage-specific patterns observed in previous studies (De Anda et al. 2015). In our study, larvae show an approximately Type II survivorship curve with relatively similar proportional mortality hazards throughout their development prior to pupation. However, the substantial variation in survivorship curves associated with host plant species and seasonal cohort suggests the importance of a host-specific and temporally explicit perspective for understanding monarch development and population dynamics. For example, these findings suggest that phenological mismatches between the migrating population of monarchs and the windows of opportunity constrained by the developmental trajectory of milkweeds could have population-level consequences.

Recent population surveys indicate that western monarch populations have declined precipitously in recent years, with an 86% decline in overwintering densities documented in the Western Monarch Thanksgiving Count between 2017 and 2018 (Pelton et al. 2019). These observations suggest that annual recruitment in the western monarch population is now occurring well below the replacement level. There has been considerable discussion of the myriad possible factors contributing to long-term declines in both the eastern and western monarch populations (e.g., Stevens and Frey 2010, Zipkin et al. 2012, Pleasants and Oberhauser 2013, Badgett and Davis 2015, Ries et al. 2015, Pleasants et al. 2016, Espeset et al. 2016, Inamine et al. 2016, Thogmartin et al. 2017, Boyle et al. 2019), with some studies pointing particularly to anthropogenic declines in breeding-season milkweed availability (Pleasants and Oberhauser 2013, Flockhart et al. 2015, Pleasants et al. 2016), while others point to seasonal population declines that begin with the fall migration (Badgett and Davis 2015, Ries et al. 2015, Inamine et al. 2016, Agrawal and Inamine 2018). Although monarch populations have declined particularly rapidly in the western population over several decades (Frey and Schaffner 2004, Schultz et al. 2017, Pelton et al. 2019), relatively few studies have investigated the specific mechanisms and drivers of the western population decline specifically (but see Stevens and Frey 2010, Jepsen and Black 2015, Espeset et al. 2016, Pelton et al. 2019).

The goal of this current study was to examine seasonal windows of opportunity for monarch larval development at a local scale, with experimental manipulations of oviposition phenology across multiple seasons. As such, we are cautious about overstating the broader implications of these findings for the population dynamics and conservation of monarch butterflies on a continental scale. Even after decades of detailed study, a great deal about the ecology of this species remains unknown, especially in the western population. However, this current study may contribute to a more detailed understanding of the factors affecting monarch populations. If there are strong limitations on larval development and success, how do we expect those limitations to be structured seasonally? What is the relative importance of abiotic, bottom-up and top-down factors in constraining the developmental success of monarchs? Do we expect migrating monarchs to break reproductive diapause in order to capitalize on a fall window of opportunity (Perez and Taylor 2004), and how would substantial fall breeding affect the natal origins of the overwintering population (Yang et al. 2016)? Identifying seasonal windows of opportunity is a useful step towards understanding organismal development from a temporally explicit perspective, and particularly underscores the importance of temporally explicit metrics of growth and survivorship for modeling population dynamics. These seasonal windows of opportunity also suggest that the phenological diversity of milkweed host plants and other seasonally variable resources could have important effects on monarch populations at key points during their annual cycle. With a clearer understanding of the seasonal fitness landscape, we can begin to understand how seasonal patterns at the individual level could scale up to affect population dynamics and species interactions throughout the year.

More broadly, this study illustrates an experimental approach to the study of seasonal windows of opportunity that could be applied in many other systems. While further studies will be necessary to disentangle the specific factors that structure windows of opportunity, studies like this one readily suggest seasonally detailed hypotheses for subsequent examination, and demonstrate the variable nature of species interactions within, as well as across, seasons. The key challenge of these studies is also their greatest strength – repeated observations on a seasonal scale. However, these approaches allow us to develop a temporally explicit perspective on the factors that affect development, population dynamics and species interactions.

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Figure 1. Generalized schematic models of the phenological manipulation experiments used in this study. a) In each year, we randomly assigned a population of milkweeds (green arrows) to receive experimental cohorts of monarchs at several intervals (orange bars) throughout the season. We introduced monarchs as eggs and monitored the success of each monarch throughout its development. We also measured seasonal variation in plant traits and abiotic conditions. b) In 2010 and 2014, we also manipulated the density of milkweed patches, giving monarchs access to patches of 1 or 3 plants per patch. A single monarch egg was introduced to each patch.

Figure 2. Monarch larval developmental success measured across seven seasonal cohorts in 2010 on a) *A. fascicularis* (in red) and b) *A. speciosa* host plants (in blue). Monarchs reared on both milkweeds showed low success in the earliest cohort, followed by a window of opportunity for successful larval development in June and early July, followed by a period of reduced success in mid-summer. Point shape reflects patch density (circles = one milkweed per patch, triangles = three plants per patch); in the June cohort, monarchs performed marginally better on high-density patches than on low-density patches. Point size is proportional to larval size. Caterpillars on *A. fascicularis* showed a second window of opportunity for larval development in September and October which was not observed for caterpillars on *A. speciosa*. c) Defensive traits mean latex exudation (solid lines, on the left axis) and trichome density (dashed lines, on the right axis) changed throughout the season, and differed between the two host species. d) The hatch rate of caterpillars was lowest in July and August, and differed between host plant species, reflecting potential differences in the abiotic microclimate or the biotic community of egg predators. e) Predator abundance was highest in July and August. All fit lines represent smoothed conditional means from LOESS regression.

Figure 3. Age-specific survivorship curves for each of seven cohorts developing on *A. fascicularis* and *A. speciosa* in 2010. Age is reported in development days, the number of days between the date of introduction and the date of larval death or pupation. Tick marks on the survivorship curve indicate pupation. A stage-specific version of this figure is available in Fig. S2, and tabular survivorship data is available in Data S1.

Figure 4. Monarch developmental success measured across five seasonal cohorts in 2011 on *A. fascicularis* in a native wildflower strip. a) Monarch caterpillars experienced the greatest larval success in May, June and July, with reduced developmental success in later season. b) Mean latex exudation (solid lines, on the left axis) and trichome density (dashed lines, on the right axis) increased throughout the season, but remained relatively low compared with other milkweed host species. c) Hatch rate declined in the last two cohorts of this experiment, as did d) observed predator densities. All fit lines represent smoothed conditional means from LOESS regression.

Figure 5. Age-specific survivorship curves for each of five cohorts developing on *A. fascicularis* in 2011. Age is reported in development days, the number of days between the date of introduction and the date of larval death or pupation. Tick marks on the survivorship curve indicate pupation. A stage-specific version of this figure is available in Fig. S4, and tabular survivorship data is available in Data S1.

Figure 6. Monarch developmental success measured across six seasonal cohorts in 2014 on *A. fascicularis* in agricultural margin habitat. Monarch caterpillars showed two windows of opportunity, with one window for larval success in early May, and a second window for larval success in late August and September. Milkweeds patch density (circles = one milkweed per patch, triangles = three plants per patch) did not significantly affect the developmental success of

caterpillars across the entire season, but monarchs in the high-density patches showed marginally greater developmental success than monarchs in the low-density patches at the beginning of the first window of opportunity. The fit line represents smoothed conditional means from LOESS regression.

Figure 7. Age-specific survivorship curves for each of six cohorts developing on *A. fascicularis* in 2014. Age is reported in development days, the number of days between the date of introduction and the date of larval death or last sighting. Tick marks on the survivorship curve indicate pupation. A stage-specific version of this figure is available in Fig. S5, and tabular survivorship data is available in Data S1.

Figure 1

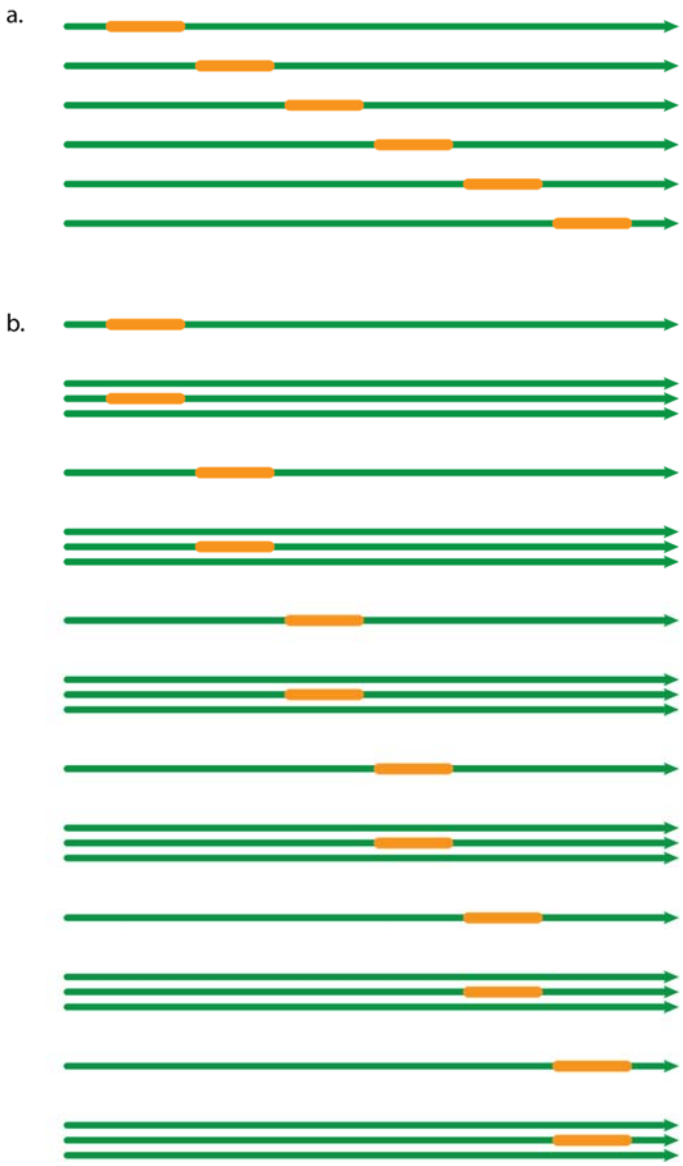

Figure 2

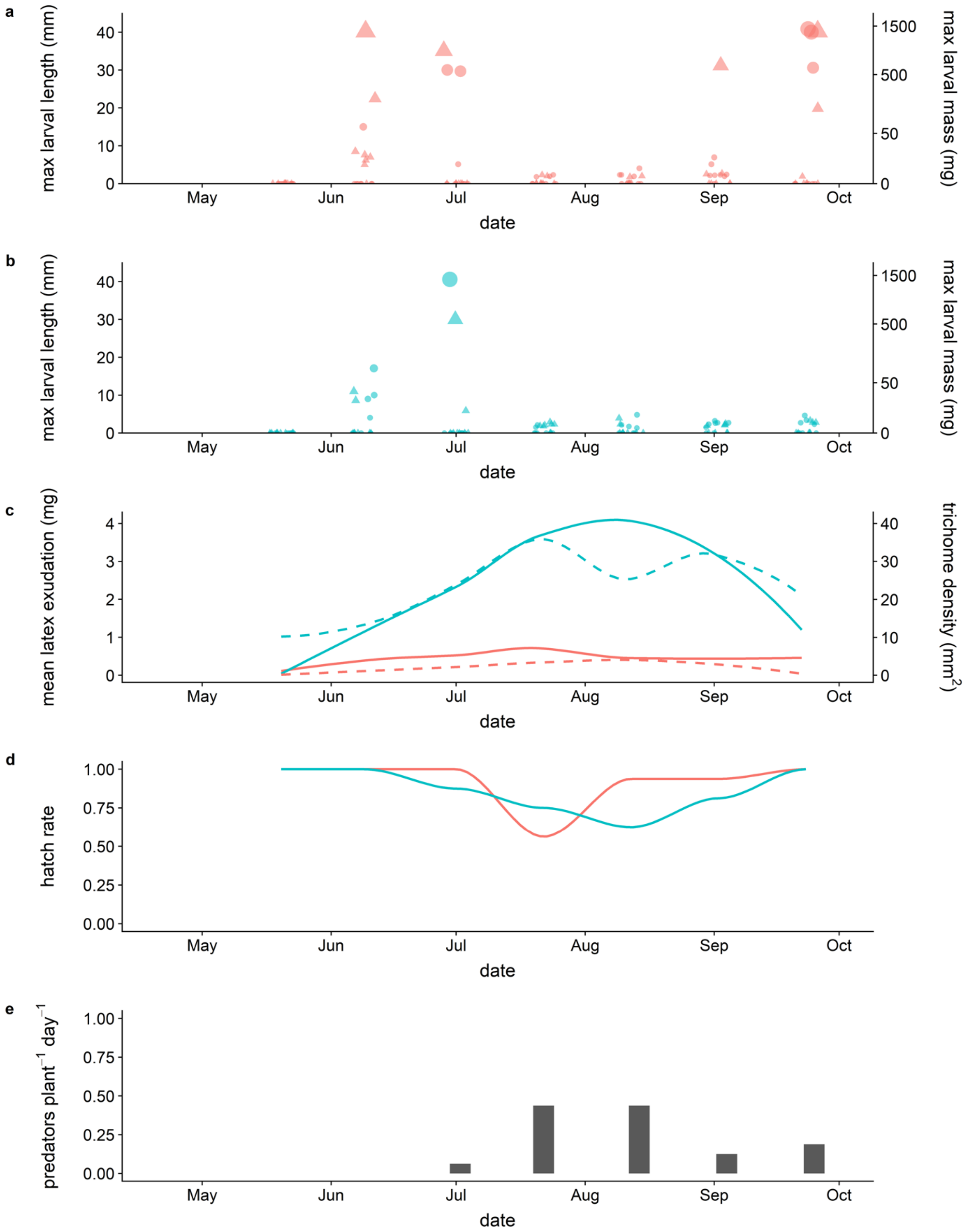
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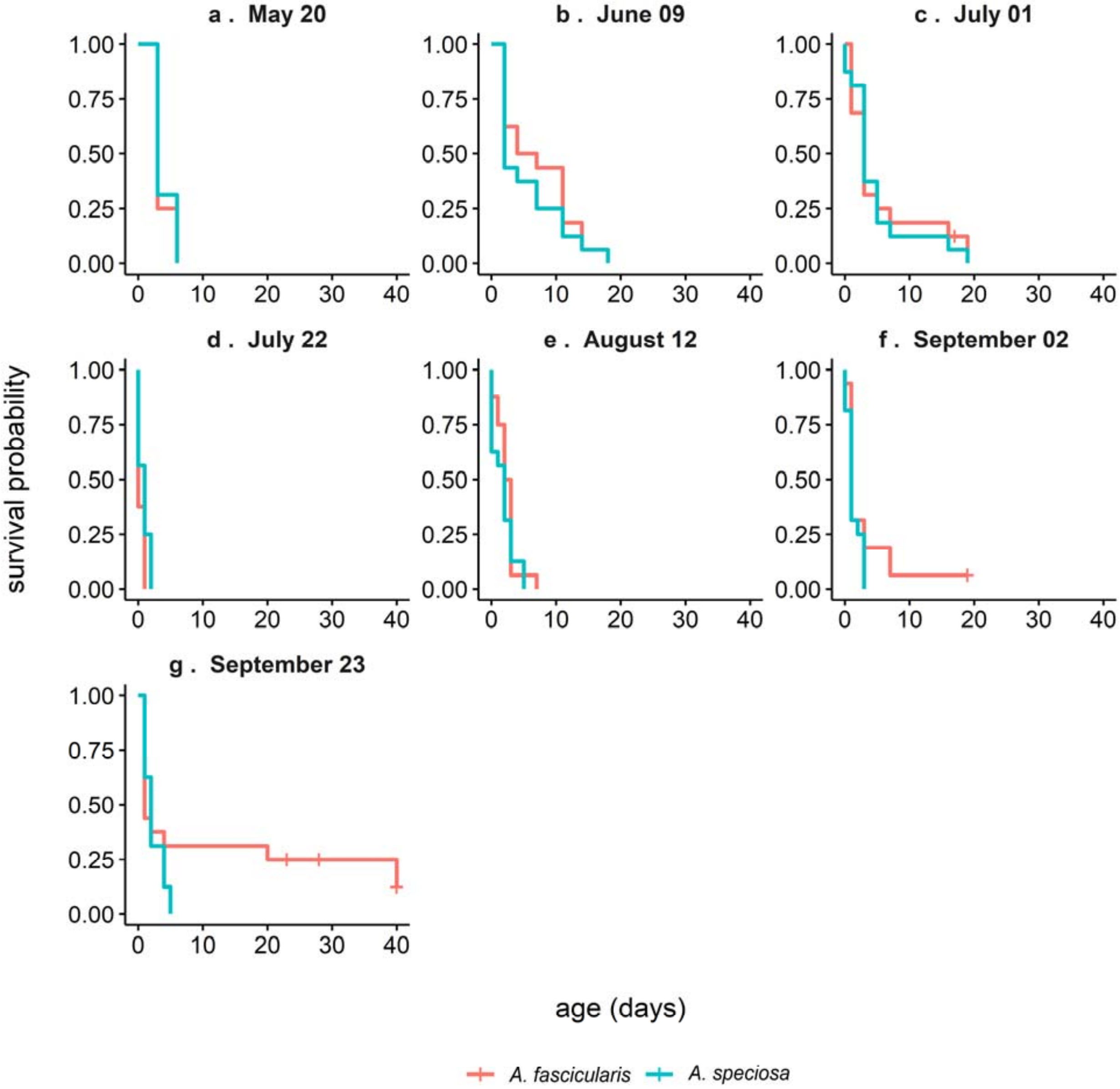
Figure 3


a . May 20
b . June 09
c . July 01
d . July 22
e . August 12
f . September 02
g . September 23
survival probability
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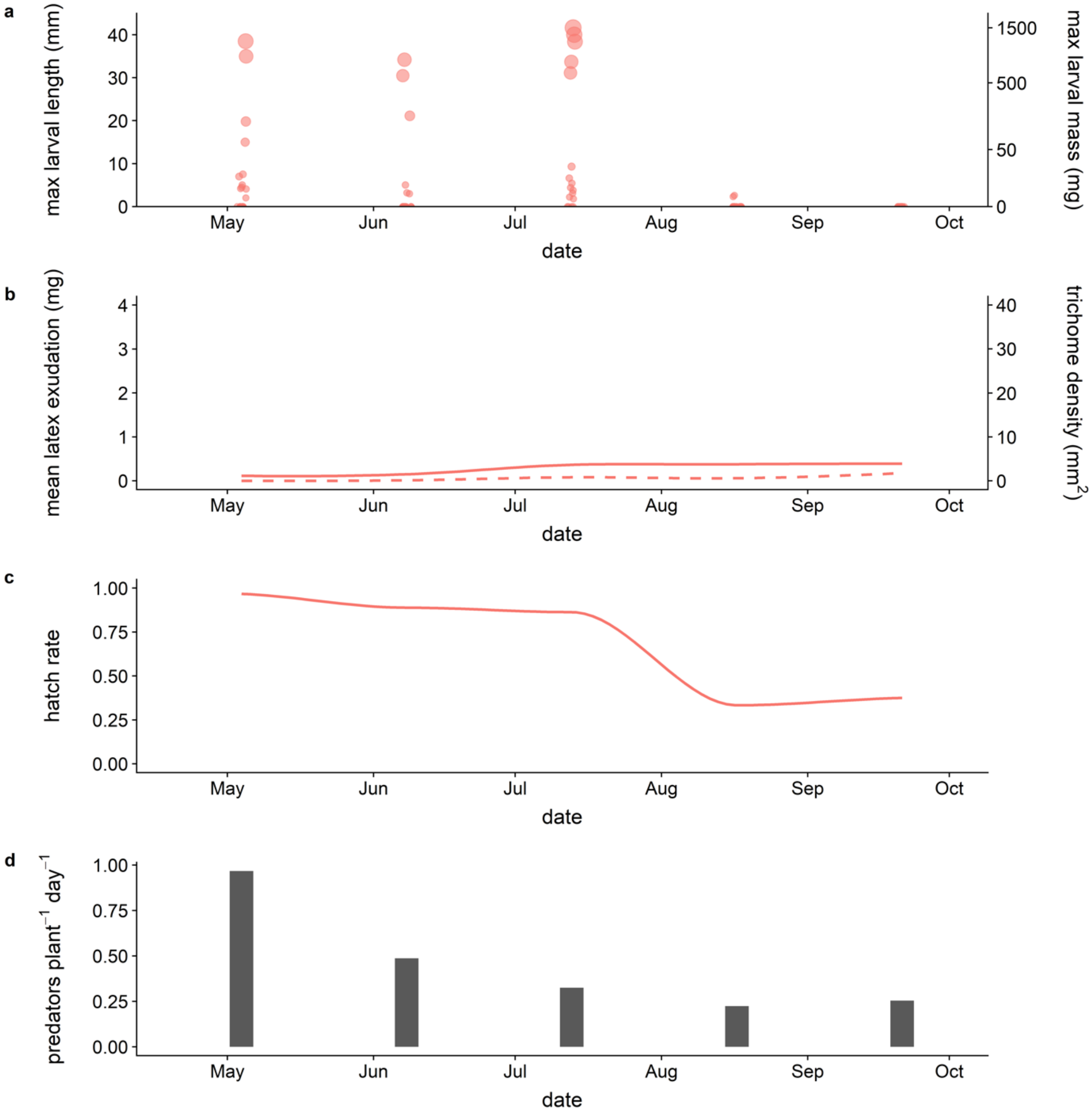
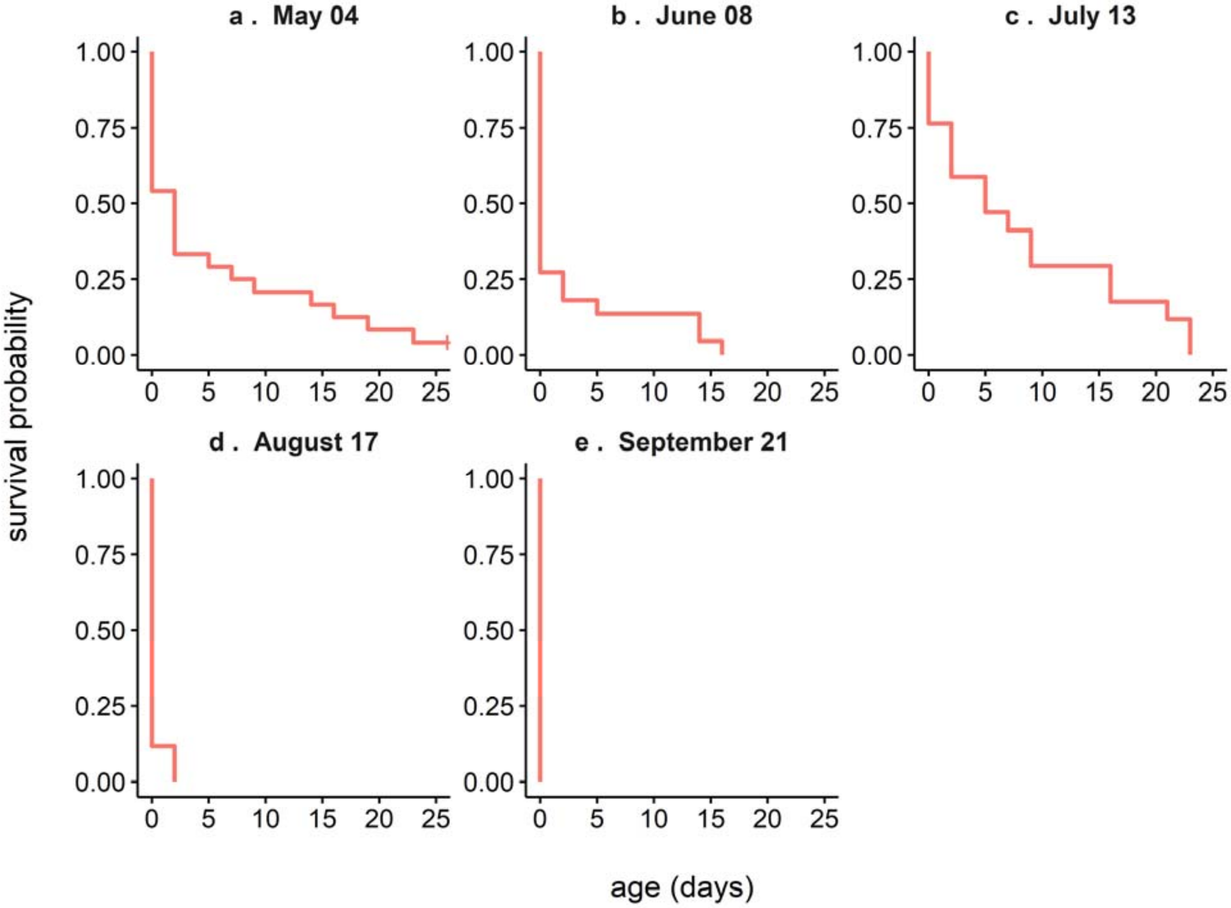
0.75
0.50
0.25
0.00
0
10
20
30
40
age (days)
A. fascicularis
A. speciosa

Figure 4


a
max larval length (mm)
0
10
20
30
40
max larval mass (mg)
0
50
500
1500
May
Jun
Jul
Aug
Sep
Oct
date
b
mean latex exudation (mg)
0
1
2
3
4
trichome density (mm^2)
0
10
20
30
40
May
Jun
Jul
Aug
Sep
Oct
date
c
hatch rate
0.00
0.25
0.50
0.75
1.00
May
Jun
Jul
Aug
Sep
Oct
date
d
predators plant^{-1} day^{-1}
0.00
0.25
0.50
0.75
1.00
May
Jun
Jul
Aug
Sep
Oct
date

777

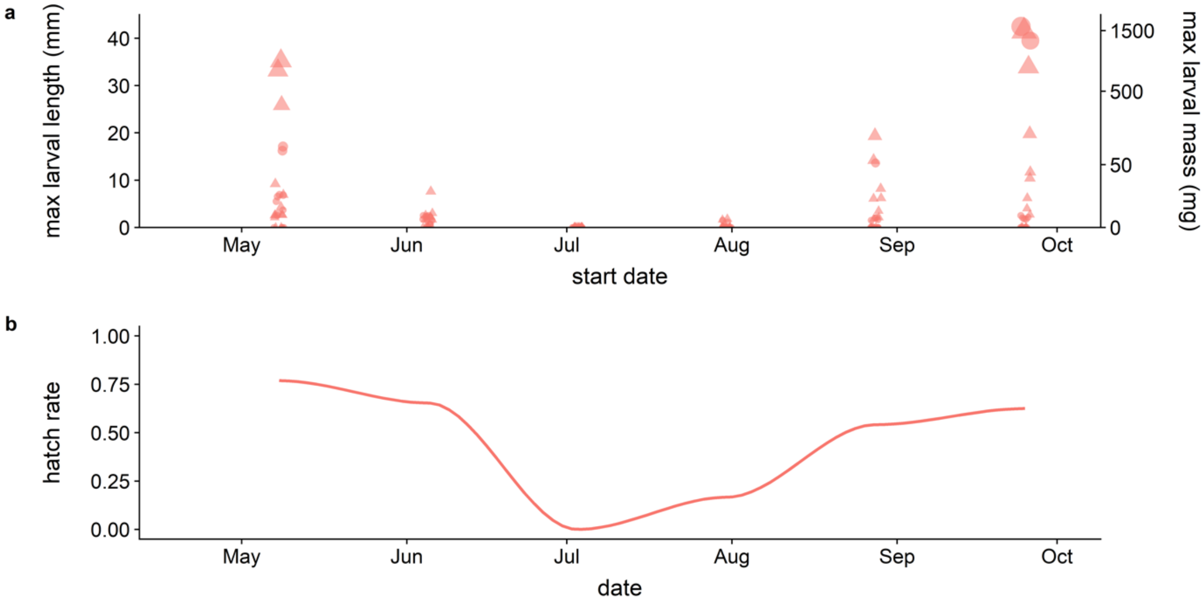
Figure 5



778

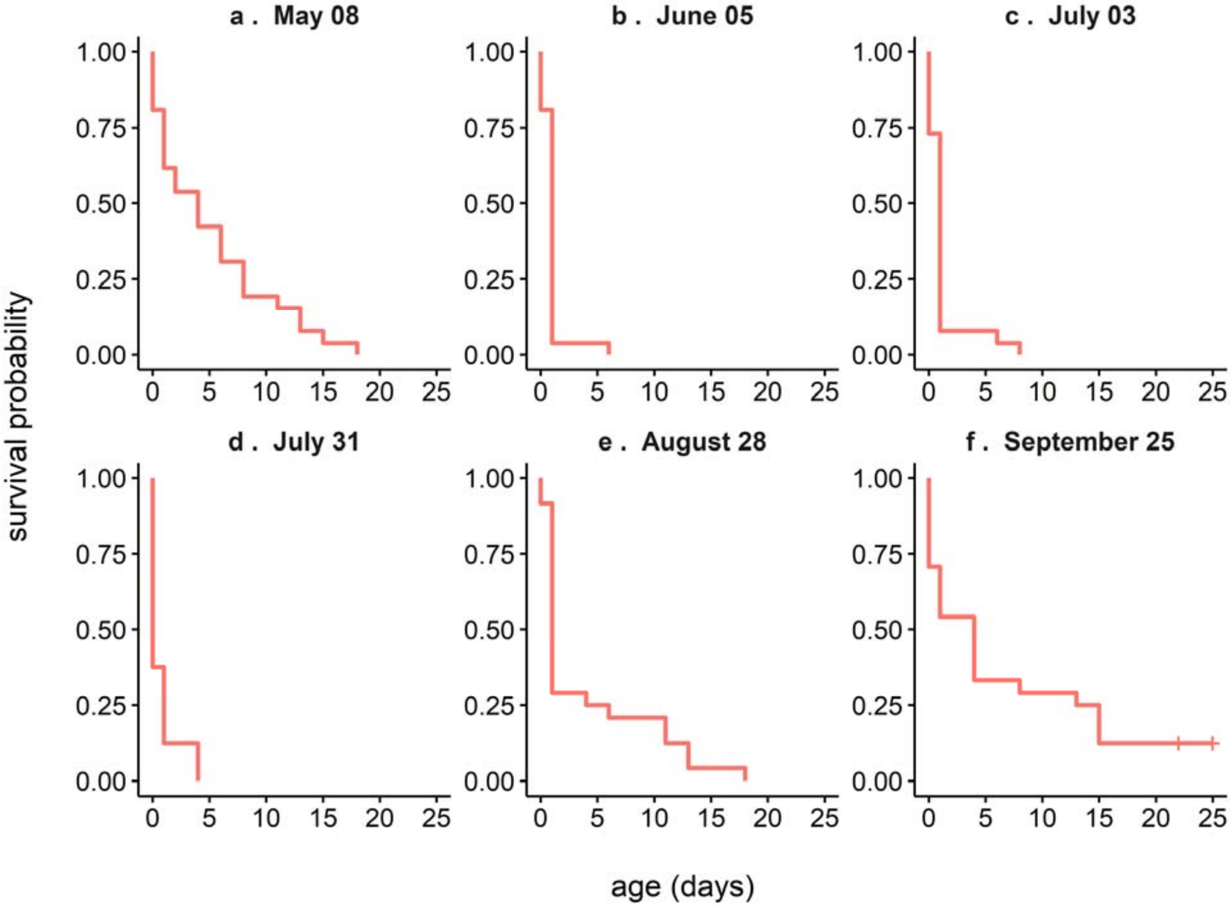
779

Figure 6


a
max larval length (mm)
40
30
20
10
0
1500
500
50
0
max larval mass (mg)
May
Jun
Jul
Aug
Sep
Oct
start date
b
hatch rate
1.00
0.75
0.50
0.25
0.00
May
Jun
Jul
Aug
Sep
Oct
date

785

Figure 7



786

787

Seasonal windows of opportunity in milkweed-monarch interactions

Louie H. Yang and Meredith L. Cenzer

2019, *Ecology*

Appendix S1.

Figure S1. Effect of milkweed patch density (1 vs. 3 plants per patch) on maximum size attained for early season cohorts on *A. fascicularis* in a) 2010 and b) 2014.

Figure S2. Stage-specific survivorship curves for each of seven cohorts developing on *A. fascicularis* and *A. speciosa* in 2010. Stages are abbreviated as follows: "E" = egg, "L1" to "L5" = 1st to 5th larval instars, "P" = pupa. An age-specific version of this figure is available in Fig. 3, and tabular survivorship data is available in Data S1.

Figure S3. Mean leaf toughness as measured with a penetrometer in the 2010 experiment. Measurements for *A. speciosa* are shown in blue and measurements for *A. fascicularis* are shown in red. The large points represent the mean for each species*cohort combination; error bars represent 95% CI. Fit lines represent smoothed conditional means from LOESS regression. The late season increase in leaf toughness coincided with leaf senescence.

Figure S4. Stage-specific survivorship curves for each of five cohorts developing on *A. fascicularis* in 2011. Stages are abbreviated as follows: "E" = egg, "L1" to "L5" = 1st to 5th larval instars. An age-specific version of this figure is available in Fig. 5, and tabular survivorship data is available in Data S1.

Figure S5. Stage-specific survivorship curves for each of six cohorts developing on *A. fascicularis* in 2014. Stages are abbreviated as follows: “E” = egg, “L1” to “L5” = 1st to 5th larval instars. An age-specific version of this figure is available in Fig. 7, and tabular survivorship data is available in Data S1.

Figure S6. a) Total stem length for milkweed patches in the 2014 experiment. As expected, the total availability of milkweed was 283% higher in the high density patches with three plants compared with the low density patches with a single plant. b) In the second panel, these same data are presented on a semi-log scale, showing a consistent proportional difference in patch size throughout the season.

Figure S7. Climatic data for Davis, CA in a) 2010, b) 2011 and c) 2014. For year, the top panel shows the daily maximum temperature (in degrees C) and the daily precipitation total (in mm).

Figure S1

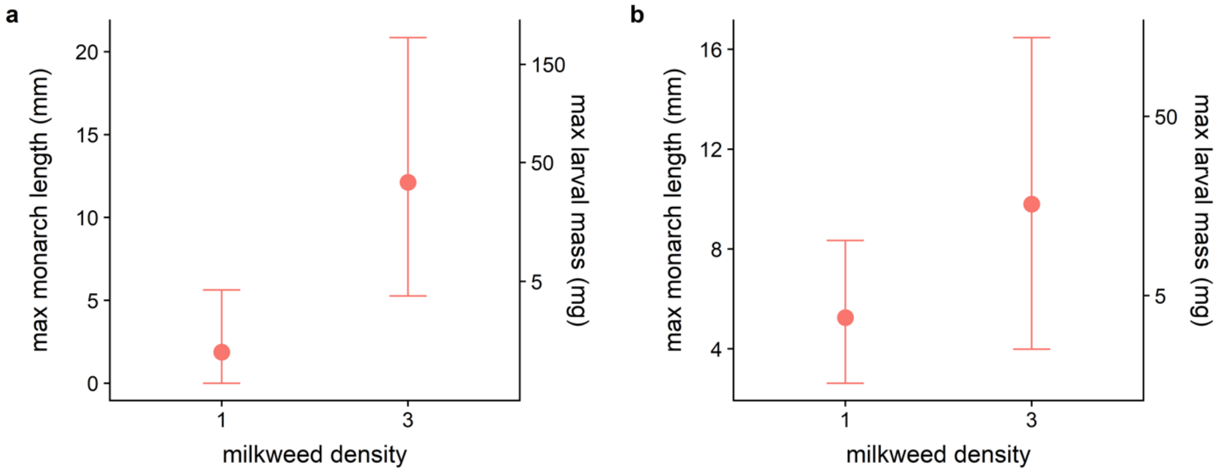

a
max monarch length (mm)
20
15
10
5
0
max larval mass (mg)
150
50
5
1
3
milkweed density
b
max monarch length (mm)
16
12
8
4
max larval mass (mg)
50
5
1
3
milkweed density

Figure S2

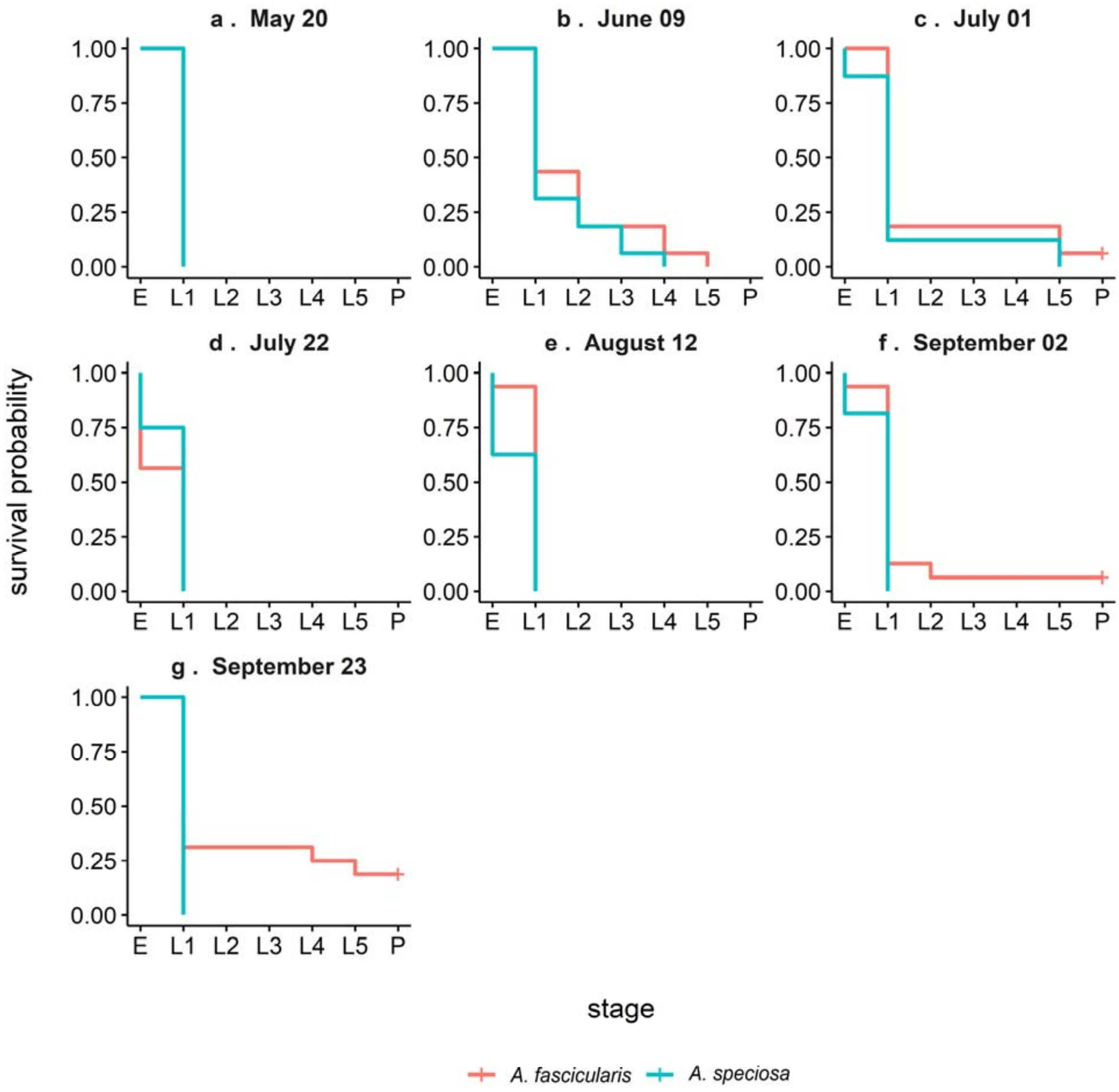

a . May 20
b . June 09
c . July 01
d . July 22
e . August 12
f . September 02
g . September 23
survival probability
1.00
0.75
0.50
0.25
0.00
E
L1
L2
L3
L4
L5
P
stage
A. fascicularis
A. speciosa

Figure S3

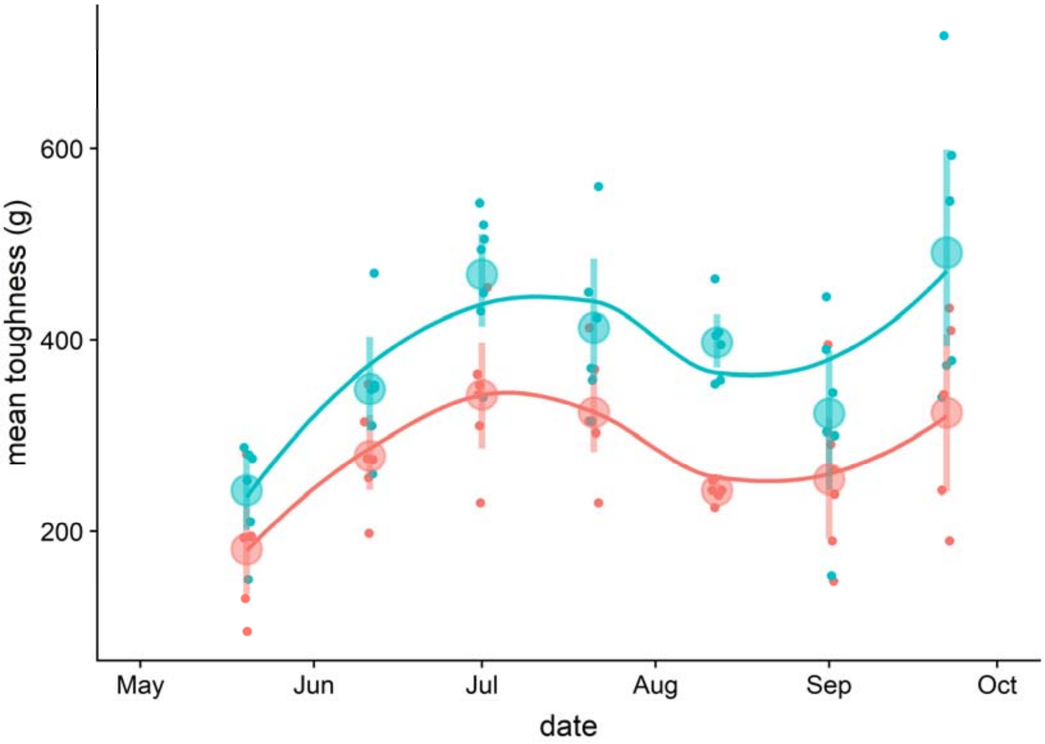


Figure S4

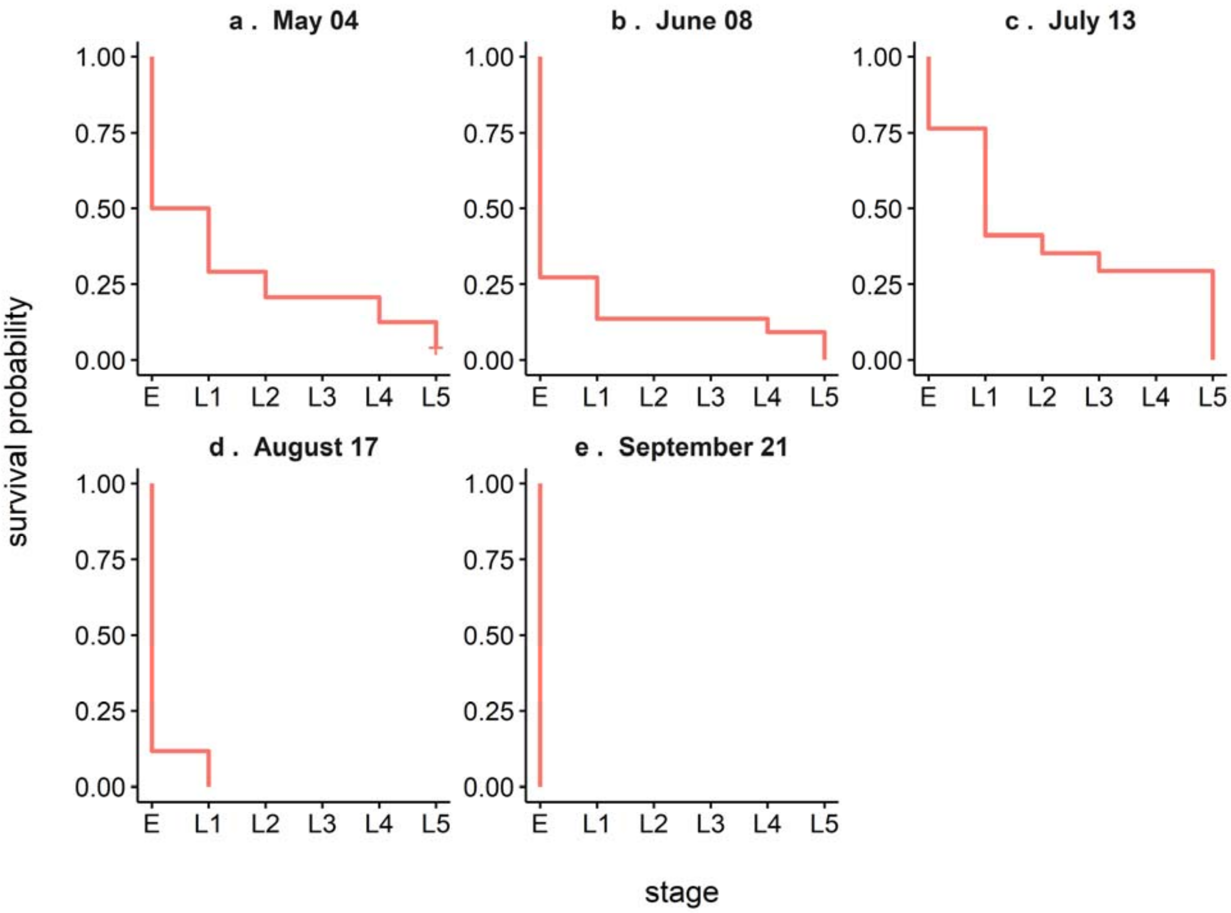

a . May 04
b . June 08
c . July 13
d . August 17
e . September 21
survival probability
1.00
0.75
0.50
0.25
0.00
E
L1
L2
L3
L4
L5
stage

Figure S5

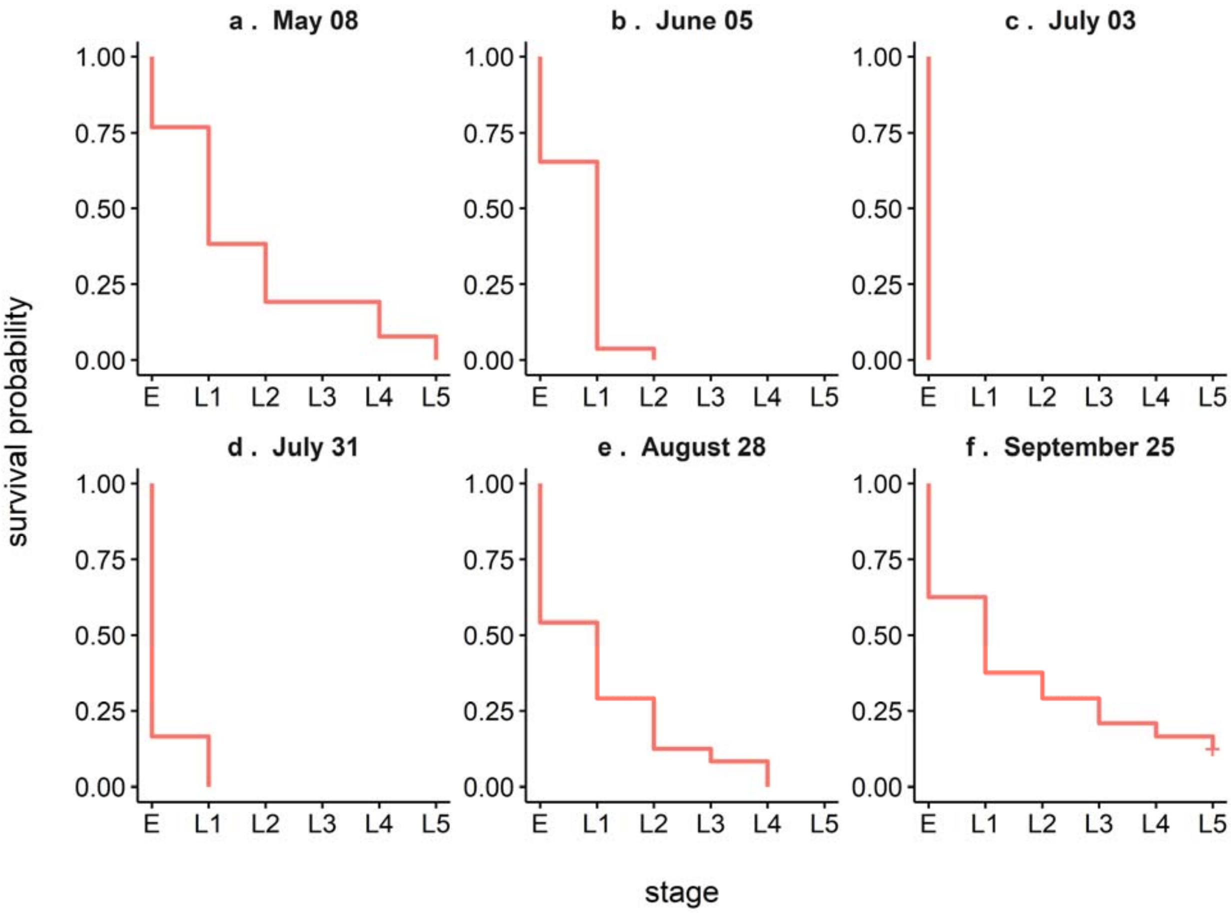

a . May 08
b . June 05
c . July 03
d . July 31
e . August 28
f . September 25
survival probability
1.00
0.75
0.50
0.25
0.00
E
L1
L2
L3
L4
L5
stage

Figure S6

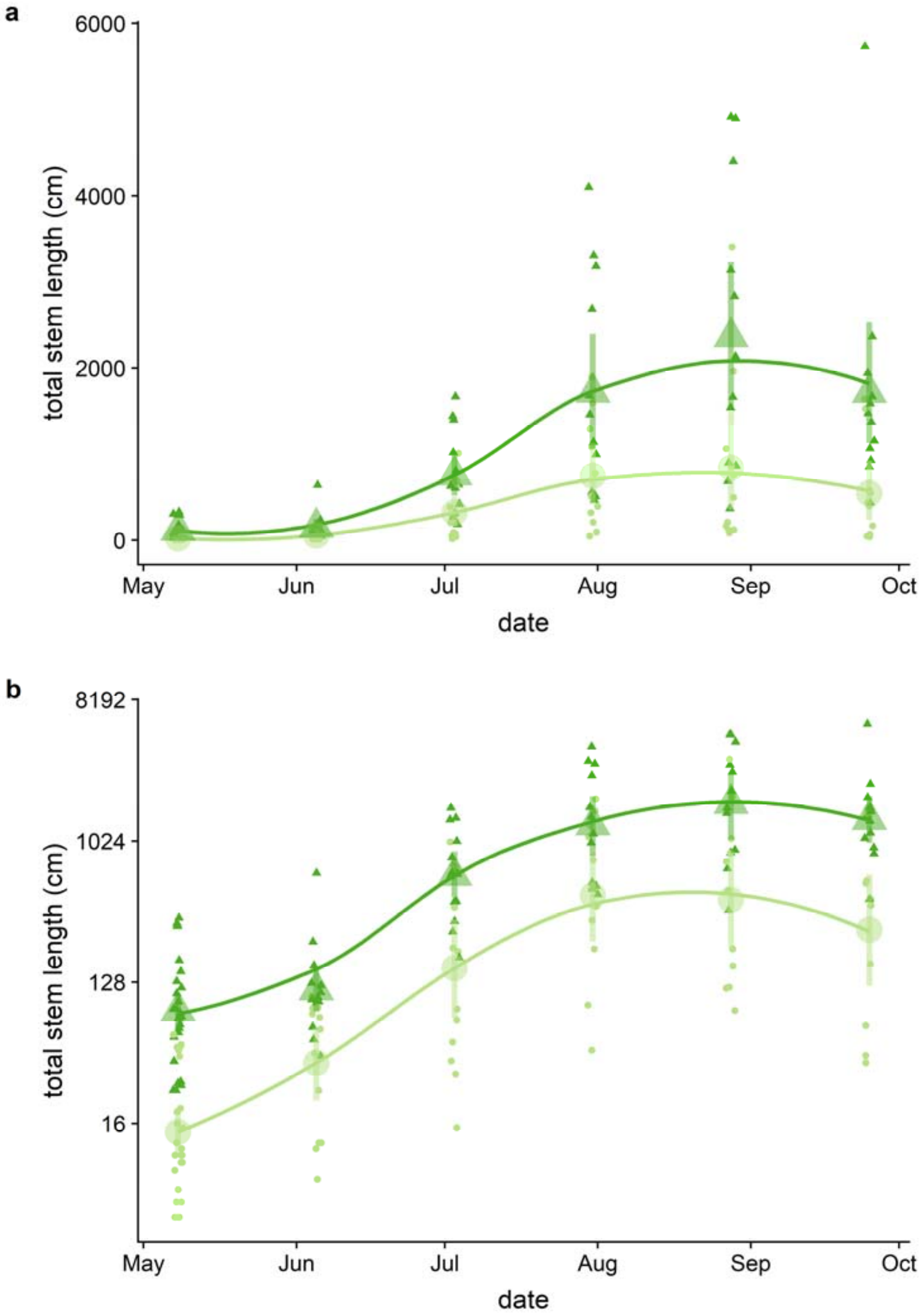
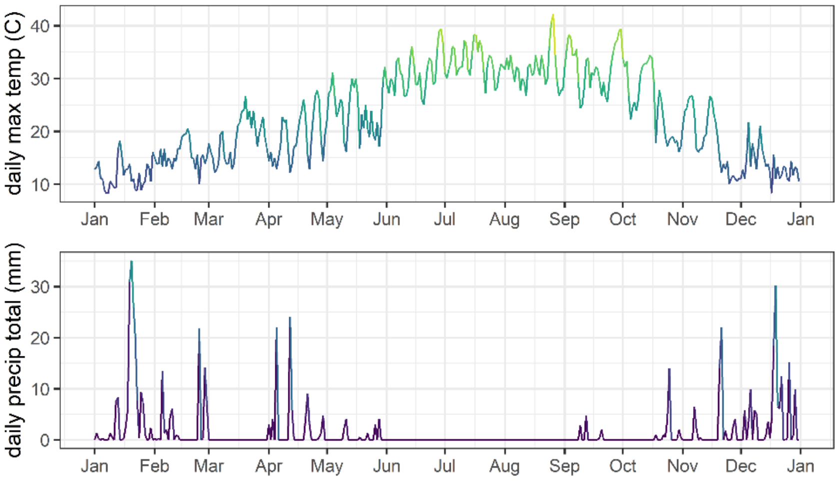
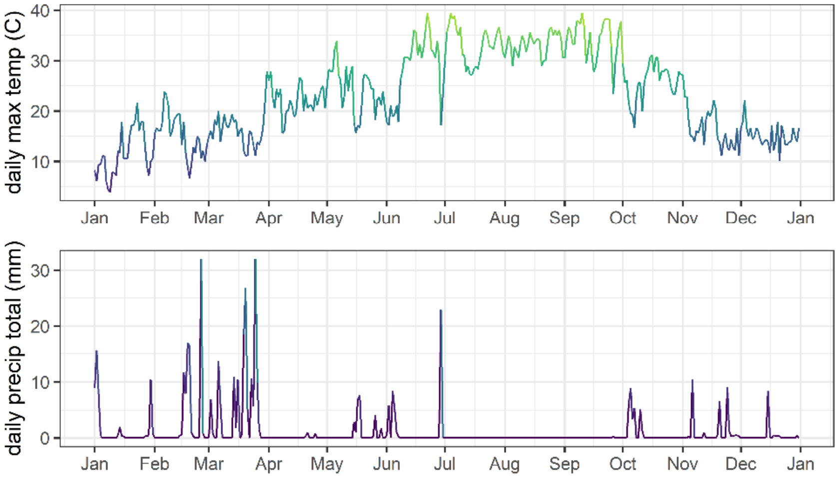

a
6000
4000
2000
0
total stem length (cm)
May
Jun
Jul
Aug
Sep
Oct
date
b
8192
1024
128
16
total stem length (cm)
May
Jun
Jul
Aug
Sep
Oct
date

Figure S7

a) 2010



b) 2011



c) 2014

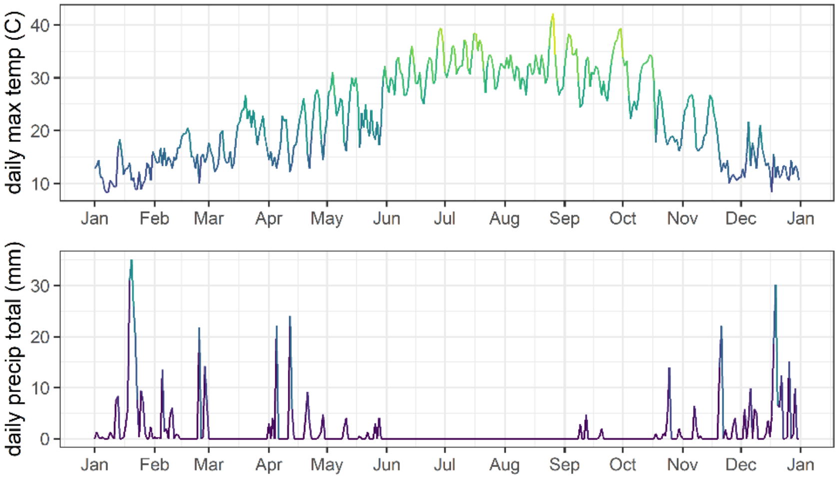


Table S1

year	**site description**	**milkweed species**	**cohorts (*N*)**	**additional treatments**	**pupal enclosure**
2010	container plants on outdoor benches	*A. fascicularis*, *A. speciosa*	May 20 (*32*), June 09 (*32*), July 1 (*32*), July 22 (*32*), August 12 (*32*), September 2 (*32*); September 23 (*32*)	milkweed density	enclosed after late 4th instar
2011	outdoor plantings in native wildflower strips along an agricultural margin	*A. fascicularis*	May 4 (*24*), June 8 (*22*), July 13 (*17*), August 17 (*18*), September 21 (*15*)	exclosure	enclosed after late 4th instar
2014	outdoor plantings in a tilled agricultural margin	*A. fascicularis*	May 8 (*26*), June 5 (*26*), July 3 (*26*), July 31 (*24*), August 28 (*24*), September 25 (*24*)	milkweed density	none

Metadata S1

Seasonal windows of opportunity in milkweed-monarch interactions

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[2]Department of Ecology and Evolution, University of Chicago, Chicago, IL 60637 USA

Dataset identity

DataS1.zip contains the following files:

1. “2010 milkweed data.csv” : milkweed data from 2010
2. “2010 monarch growth data 2017-07-03.csv”: monarch growth data from 2010
3. “2010 predator observations 2018-05-22.csv”: predator observations from 2010
4. “2011 milkweed-monarch experiment community data 2018-05-24.csv”: arthropod community data from 2011
5. “2011 milkweed-monarch experiment data 2018-05-24.csv”: milkweed and monarch data from 2011
6. “2014Aug28.csv”: milkweed and monarch data from 2014-08-28
7. “2014Jul03.csv”: milkweed and monarch data from 2014-07-03
8. “2014Jul31.csv”: milkweed and monarch data from 2014-07-31
9. “2014Jun04.csv”: milkweed and monarch data from 2014-06-04
10. “2014May08.csv”: milkweed and monarch data from 2014-05-08
11. “2014Sept25.csv”: milkweed and monarch data from 2014-09-25
12. “monarch length analysis 2019-08-02 for Ecology.Rmd”: Rmarkdown analysis script of monarch length
13. “monarch mass analysis 2019-08-02 for Ecology.Rmd”: Rmarkdown analysis script of monarch mass
14. “monarch_length_analysis_2019-08-02_for_Ecology.html”: Rmarkdown analysis report of monarch length analyses
15. “monarch_mass_analysis_2019-08-02_for_Ecology.html”: Rmarkdown analysis report of monarch mass analyses

Data structural descriptors

Dataset file: header information

“2010 milkweed data.csv” contains the following columns:

1. plant ID: unique plant ID (destructively sampled)

2. species: milkweed species
3. date: date
4. cohort: numerical cohort
5. status: plant live or dead
6. plant height: plant max height
7. A: penetrometer reading on apical leaf A (g)
8. B: penetrometer reading on apical leaf B (g)
9. C: penetrometer reading on apical leaf C (g)
10. D: penetrometer reading on apical leaf D (g)
11. mean penetrometer: mean of four penetrometer readings (g)
12. trichome count: number of trichomes on 3mm diameter leaf punches
13. trichome density: trichomes per mm^2
14. B tare: mass of dry filter disc for leaf B
15. D tare: mass of dry filter disc for leaf D
16. B final: mass of dry filter disc and latex exudate for leaf B
17. D final: mass of dry filter disc and latex exudate for leaf D
18. B latex: mass of dry latex exudate for leaf B
19. D latex: mass of dry latex exudate for leaf D
20. mean latex: mean latex exudate mass
21. notes: notes

Dataset file: header information

“2010 monarch growth data 2017-07-03.csv” contains the following columns:

1. rep: unique patch ID
2. density: 1 or 3 plants per patch
3. species: milkweed species
4. start date: cohort initiation date
5. hatch date: egg hatch date
6. cohort: numerical cohort designation
7. term stage: final observed stage
8. num stage: numerical final observed stage
9. term size: final observed size
10. term date: final observed date
11. dev time: difference between egg hatch date and final observed date (d)
12. max size: largest observed monarch length (mm)
13. max date: date of largest observed monarch length (mm)
14. hatch: did the egg hatch (1 or 0)
15. pupae: did the egg develop into a pupae (1 or 0)
16. pupal mg: pupal mass (mg)

Dataset file: header information

“2010 predator observations 2018-05-22.csv” contains the following columns:

1. Aranae : counts of observed Aranae
2. parasitoid wasps: counts of observed parasitoid wasps
3. Reduviidae: counts of observed Reduviidae
4. Orius: counts of observed *Orius* spp.
5. Chrysopidae: counts of observed Chrysopidae
6. unknown: counts of unidentified predators

Dataset file: header information

“2011 milkweed-monarch experiment community data 2018-05-24.csv” contains the following columns:

1. plant: unique plant ID
2. date: date
3. number: raw count data
4. number2: corrected count data
5. taxon: taxon names (see data cleaning in analysis script)
6. interval: numeric cohort ID

Dataset file: header information

“2011 milkweed-monarch experiment data 2018-05-24.csv” contains the following columns:

1. transect: east (E) or west (W) parallel transect
2. num: numeric ID
3. tagID: concatenated transect and number
4. species: milkweed species
5. rand: random number (legacy from randomization)
6. treatment: open or exclosure cage
7. date: date
8. interval: numeric cohort
9. height: milkweed height (cm)
10. notes: notes
11. aphids: aphid count
12. total latex: total dry latex mass (mg)
13. toughness: mean penetrometer reading (g)
14. trichome: mean trichome count
15. hatch: did the egg hatch (1 or 0)
16. maxsize: largest observed monarch length (mm)
17. pupal weight: pupal mass (mg)
17. devdays: difference between egg hatch date and final observed date (d)
18. PlantID/Date: unique plant ID (redundant)
19. Obsdays: count of observation days

Dataset file: header information

“2014Aug28.csv”, “2014Jul03.csv”, “2014Jul31.csv”, “2014Jun04.csv”, “2014May08.csv” and “2014Sept25.csv” contain the following columns:

1. patch.ID: unique patch ID
2. species: milkweed species (Af = *Asclepias fascicularis*)
3. density: 1 or 3 plants per patch
4. obs.date: observation date
5. cohort: cohort initiation date
6. monarch.stage: stage code (depreciated, see data clean up in analysis script)
7. monarch.length: length in mm
8. num.stems: number of milkweed stems
9. ht.by.stem: height of stems, up to 10 stems sampled
10. lvs.by.stem: leaves per stem, up to 10 stems sampled
11. stem.dia: stem diameter, up to 10 stems sampled
12. dam: % leaf area removed by herbivores
13. num.flowers: count of fresh flowers
14. old.flowers: count of senescent flowers
15. num.pods: count of mature pods
16. notes: notes