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1 Running head: Plant traits limit opportunity windows

2 **Species-specific, age-varying plant traits affect herbivore growth**
3 **and survival**

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10 **Abstract**

11 Seasonal windows of opportunity represent intervals of time within a year
12 during which organisms have improved prospects of achieving life history
13 aims such as growth or reproduction, and may be commonly structured by
14 temporal variation in abiotic factors, bottom-up factors, and top-down
15 factors. Although seasonal windows of opportunity are likely to be common,
16 few studies have examined the factors that structure seasonal windows of
17 opportunity in time. Here, we experimentally manipulated host plant age in
18 two milkweed species (*Asclepias fascicularis* and *Asclepias speciosa*) in order
19 to investigate the role of plant species-specific and plant age-varying traits
20 on the survival and growth of monarch caterpillars (*Danaus plexippus*). We
21 show that the two plant species showed diverging trajectories of defense
22 traits with increasing age. These species-specific and age-varying host plant
23 traits significantly affected the growth and survival of monarch caterpillars
24 through both resource quality- and resource quantity-based constraints. The
25 effects of plant age on monarch developmental success were comparable to
26 and sometimes larger than those of plant species identity. We conclude that
27 species-specific and age-varying plant traits are likely to be important factors
28 with the potential to structure seasonal windows of opportunity for monarch
29 development, and examine the implications of these findings for both
30 broader patterns in the ontogeny of plant defense traits and the specific
31 ecology of milkweed-monarch interactions in a changing world.

32 **Keywords:** seasonal windows of opportunity, phenology, ontogeny,
33 *Asclepias*, *Danaus plexippus*, climate change, host plant age, host plant
34 species, defense trajectories, herbivore growth and survival, plant vigor
35 hypothesis, plant stress hypothesis

36

37 **Introduction**

38 Seasonal windows of opportunity are intervals of time within a year during
39 which organisms have improved prospects of achieving life history aims such
40 as growth or reproduction (Yang and Censer 2020). Seasonal windows of
41 opportunity are likely to occur in a wide range of systems (e.g., Yang and
42 Rudolf 2010, Anderson et al. 2012, Wright et al. 2013, Carter et al. 2018,
43 Farzan and Yang 2018, Yang and Censer 2020), resulting from commonplace
44 temporal variation in biotic and abiotic factors. However, while phenology
45 examines the *realized* seasonal timing of an organism's life history, seasonal
46 windows of opportunity represent transient periods of time with the *potential*
47 for improved developmental or fitness outcomes. Because underlying
48 windows of opportunity may not always be reflected in observed phenology,
49 experimental manipulations provide a particularly useful approach for
50 identifying seasonal windows of opportunity (Yang and Rudolf 2010). Despite
51 this, relatively few studies have experimentally identified seasonal window of
52 opportunity in nature (but see Van Asch et al. 2007, Liu et al. 2011, Rafferty
53 and Ives 2011, Warren et al. 2011, Kharouba et al. 2015, Farzan and Yang
54 2018, Yang and Censer 2020), and even fewer have experimentally
55 examined the specific factors that define these windows of opportunity in
56 time.

57 Seasonal windows of opportunity are defined by the co-occurrence of factors
58 that, in combination, have a positive effect on growth or reproduction.

59 Broadly, many seasonal windows of opportunity are likely to be structured by
60 temporal variation in abiotic factors, bottom-up factors, and top-down factors
61 (Yang and Cezner 2020). When the combined effects of these factors present
62 adverse conditions, they constrain the seasonal timing of development.
63 When the combined effects of these factors are favorable, they create
64 seasonal windows of opportunity. However, separating and evaluating the
65 role of specific factors in structuring seasonal windows of opportunity is
66 challenging due to the multiple correlated factors that often change
67 simultaneously across a seasonal timescale.

68 The interactions between herbivores, their host plants, and their surrounding
69 community provide unique opportunities to examine seasonal windows of
70 opportunities. For herbivores, these windows of opportunity are likely to be
71 structured by a variety of seasonally varying factors, including climatic
72 conditions, natural enemy communities and plant traits. Questions about the
73 ontogeny of plant defense traits have received particular attention as
74 ecologists have sought to understand the specific mechanisms (Barton 2013,
75 2016, Quintero et al. 2013) and general patterns (Boege and Marquis 2005,
76 Barton and Koricheva 2010, Barton and Boege 2017) that explain how plant-
77 herbivore interactions change across development. Broadly, these studies
78 document a diversity of ontogenetic trajectories (including both increasing
79 and declining trends) in a wide range of plant defense traits (including both
80 tolerance and chemical, physical, and indirect resistance traits). While
81 specific patterns of change differ with both plant and herbivore identity

82 (Barton and Koricheva 2010), the observation of significant ontogenetic
83 changes in plant defense traits is both general and robust (Barton and
84 Koricheva 2010, Barton and Boege 2017). In addition, plant phenology has
85 recently been suggested as a key factor that could unify the hypothesis that
86 herbivores generally prefer and perform better on vigorously growing plants
87 (i.e., the *plant vigor hypothesis*, Price 1991) and the hypothesis that
88 herbivore outbreaks are more likely on stressed plants (i.e., the *plant stress*
89 *hypothesis*, White 1974); phenological changes in plant traits can change the
90 quality of plant resources in ways that are consistent with both hypotheses
91 (White 2009, Che-Castaldo et al. 2019). However, while seasonal changes in
92 plant defense traits are likely to be a common consequence of plant
93 ontogenetic trajectories in many systems, few studies have examined the
94 ecological consequences of these temporally variable plant defense traits for
95 the developmental prospects of herbivores.

96 Here, we present an experiment designed to isolate and examine the role of
97 plant traits in constraining seasonal windows of opportunity for larval
98 monarchs (*Danaus plexippus*) feeding on two milkweed host plants
99 (*Asclepias fascicularis* and *Asclepias speciosa*). While previous studies have
100 identified seasonal windows of opportunity in the interactions between
101 milkweed host plants and their monarch caterpillar herbivores (Yang and
102 Censer 2020), more specific experiments are necessary to identify the
103 factors that structure these windows of opportunity in time. In this
104 experiment, we isolated the species-specific effects of age-varying plant

105 traits on the developmental prospects of monarch caterpillars by presenting
106 plants of two milkweed species and three age classes synchronously to a
107 single cohort of monarch caterpillars. This design aimed to control for the
108 effects of seasonally variable abiotic conditions and natural enemy
109 communities while isolating the effects of species-specific and age-varying
110 plant traits. The key questions we address in this study are: a) How do plant
111 traits, including measures of both size (i.e., resource *quantity*) and defensive
112 traits (i.e., resource *quality*), change with plant age in two species of
113 milkweed host plants? b) How do these species-specific and age-varying
114 changes in plant traits affect the growth and survival of larval monarchs?

115 **Methods**

116 *Plant establishment*

117 We started three cohorts of narrow-leaved milkweed (*A. fascicularis*) and
118 showy milkweed (*A. speciosa*) from seed on April 8, May 7 and June 8, 2014.
119 These two milkweed species are native to the California Central Valley, and
120 the seeds used in this study were propagated from local source populations
121 (Hedgerow Farms, Winters, CA, USA). Each cohort of seeds was started
122 directly into 2.5 L containers filled with potting soil (1:1:1 ratio of sand,
123 compost and peat moss by volume with 1.78 kg/m³ dolomite), which were
124 irrigated and fertilized (electrical conductivity, EC = 1.5-1.6 mS cm⁻¹) via drip
125 emitters as necessary to prevent water and nutrient limitation. Plants from
126 each cohort were randomly interspersed in a single greenhouse

127 (approximately 20-35° C) at the University of California, Davis Orchard Park
128 Research Greenhouse Facility (38.543129° N, 121.763425° W) with
129 individual plants spaced on open grate wire benches to prevent contact
130 between the leaves of neighboring plants. These three cohorts were started
131 approximately 4 weeks apart to yield three distinct age classes of milkweed
132 (25-day, 57-day and 86-day-old plants, hereafter, the *4*, *8* and *12-week*
133 cohorts) for each species ($N=18$ plants of each species in each age class,
134 $N=108$ plants total) at the start of the experiment.

135 *Measuring plant traits*

136 We measured the size (total stem length, total leaf count, total stem cross-
137 sectional area and total leaf area) and defensive traits (mean latex exudation
138 and trichome density) of each plant at the start of the experiment (July 3,
139 2014). All plants were actively growing at the start of the experiment, and
140 two of the 12-week-old plants had begun developing flowers (reflecting
141 *seedling*, *vegetative juvenile* and *juvenile-mature transition stages*, *sensu*
142 Barton and Koricheva 2010). In the context of this experiment, plant age
143 provides a proxy for both plant phenology and ontogeny; i.e., older plants
144 represent plants that are more phenologically advanced and
145 developmentally mature. Total stem length was measured as the product of
146 the total stem count (all stems > 5 cm), and the mean stem length
147 (averaged from a subsample of up to 10 stems > 5 cm in length). Total leaf
148 counts included all fully expanded leaves on each plant. Total stem cross-

149 sectional area is the cumulative cross-sectional area of stems, calculated
150 from the total stem count (all stems >5 cm) and the mean stem diameter
151 measured from a subsample of up to 10 stems >5 cm in length. Total leaf
152 area was estimated as the product of the total leaf count and the mean area
153 per leaf for each plant species × plant age combination. The mean area per
154 leaf was estimated as the area of an ellipse using measurements of the
155 length and width of $N=5$ fully expanded leaves randomly selected from each
156 group. Latex exudation was measured as the mean dry mass of latex
157 collected on pre-weighed filter paper discs after cutting 5 mm from the distal
158 tip of two fully expanded upper leaves, following Agrawal (2005). Trichome
159 density was assessed from the upper surface of 3 mm diameter leaf discs
160 punched from fully expanded apical leaves using digital analysis of
161 magnified images to determine the proportion of the leaf area obscured by
162 trichomes based on manual color thresholding (Abramoff et al. 2004).

163 *Monarch introduction and monitoring*

164 A single monarch egg was introduced to each plant on July 3, 2014
165 (experimental day 0). In order to minimize direct handling of the eggs, we
166 punched 6.4 mm leaf discs from oviposition host plants with single monarch
167 eggs attached, and attached them to the apical leaves on their experimental
168 host plants with a drop of milkweed latex. Monarch eggs were obtained from
169 a large, local insectary population (Utterback Farms, Woodland, CA, USA)
170 which was re-established from local monarch genotypes each year,

171 maintained in large greenhouses, regularly supplemented with new adults to
172 maintain genetic diversity, and had been previously assessed for parasites
173 and pathogens (H.K. Kaya, *pers. comm.*). All monarch eggs in this
174 experiment were selected haphazardly from a single oviposition time-
175 restricted cohort to minimize variation in hatch timing. Each monarch egg
176 was checked 24 h after its initial introduction (experimental day 1) to assess
177 hatch rate and larval length. Afterwards, we re-measured caterpillars every
178 2-3 days until they died or left the plant ($N=1034$ observations). All larvae
179 were measured to the nearest 0.1 mm using dial calipers; eggs were
180 assumed to have a length of zero. Larval mass was estimated from a power
181 law regression of caterpillar length and mass, parameterized from a dataset
182 describing 73 unmanipulated caterpillars measured in 2014 ($\text{mass}=0.0223 *$
183 $\text{length} + 2.9816, R^2=0.97$). During each observation, we also visually
184 estimated the proportion of leaf area that was removed due to herbivory
185 (hereafter, *percent damaged*). Caterpillars were intentionally not bagged or
186 constrained at any point in this experiment so that we could assess when
187 caterpillars left their host plants (in terms of caterpillar age, caterpillar size,
188 and host plant herbivory). Caterpillars that left their host plant below a
189 minimum threshold size for pupation (35 mm length, or 895 mg) were
190 assumed to have been unable to complete their larval development on a
191 single host plant; in the context of a single plant patch, we considered these
192 to be “dead” in our survival analyses. Caterpillars that left their host plant
193 after attaining this threshold size were considered to be seeking pupation

194 sites, and were considered to be right-censored in survival analyses. The
195 threshold size for pupation (895 mg or 35 mm) was determined by assessing
196 the larval size attained by all pupating caterpillars in previous field
197 experiments, and among 248 caterpillars reared in the laboratory in 2014
198 and 2015 (Yang and Censer 2019). In 2.8% ($N=29$) of observations, we
199 observed a second non-focal caterpillar that had moved onto an
200 experimental plant; in the majority of these cases, we were able to
201 unambiguously identify the focal caterpillar and remove the non-focal
202 caterpillar. In three instances (0.3% of observations), the identity of the focal
203 caterpillar could not be determined; although the qualitative conclusions of
204 this study were unaffected by the inclusion or exclusion of these plants, we
205 removed all observations from these three plants for the analyses presented
206 here.

207 *Analyses of plant traits*

208 We analyzed plant traits (total stem length, total stem cross-sectional area,
209 total leaf area, mean latex exudation and trichome density) using linear
210 models with likelihood ratio tests to assess the significance of plant species,
211 plant age and their interaction as explanatory categorical factors (R Core
212 Team 2018). These analyses allowed us to examine how plant traits changed
213 with age in each milkweed species.

214 *Survival analyses*

215 We analyzed the survival of monarchs for each plant species and age cohort
216 to generate species- and age-specific Kaplan-Meier survivorship curves
217 (Therneau and Grambsch 2000, Therneau 2015, Kassambara and Kosinski
218 2019). We compared curves using a log-rank test procedure for right-
219 censored data (Harrington and Fleming 1982) implemented in the *survdiff*
220 function in the *survival* package in R (Therneau 2015). We quantified the
221 overall daily survivorship rates for each group of interest using the slope
222 coefficient of a log-linear regression of survival rates over time, with visual
223 inspection to confirm model fit assumptions. In addition, we used a Cox
224 proportional hazards model in order to combine plant species and plant age
225 effects into a single survival model (using the *coxph* function in the *survival*
226 package, Therneau 2015) and estimate the proportional hazard ratios
227 associated with the specific levels of each factor (using the *ggforest* function
228 in the *survminer* package, Kassambara and Kosinski 2019).

229 *Estimation of larval growth rates*

230 We estimated overall larval growth rates as the slope of the log-linear fit of
231 experimental day vs. $\log(\text{mass})$ for each individual caterpillar; i.e., as a
232 relative growth rate. In order to estimate the slope of a log-linear regression
233 in a dataset that included zero values, we added a small constant equal to
234 the minimum observed mass across the dataset to all mass data in the log-
235 linear analysis. We used a log-linear fit of mass (as opposed to length) data
236 because visual inspection indicated that caterpillar masses show a more log-

237 linear (i.e. exponential) pattern of increase over time, although these two
238 metrics of monarch size yield qualitatively identical results. To avoid
239 inaccurate overall slope estimates resulting from insufficient data, we
240 excluded caterpillars that died before reaching 10 mm length.

241 In addition, we also estimated overall larval growth rates as the mass of
242 caterpillars on experimental day 8; i.e., as the absolute growth rate. When
243 assessing caterpillar size attained over this interval, all caterpillars that did
244 not survive to the end of that interval were necessarily excluded. We chose
245 day 8 for these growth rate estimates in order to achieve a balance between
246 maximizing the length of time considered, and minimizing the number of
247 caterpillars excluded.

248 For simplicity, we primarily present relative growth rates based on the slope
249 of the log-linear regression here because this estimate is informed by more
250 observations for each summary growth rate, and because this approach can
251 be more easily generalized to examine a range of interval-specific growth
252 rates. Because both of these overall growth rate estimates are measured
253 relative to size on day 0, they are mathematically similar and yield
254 qualitatively similar results; in addition, although they use different criteria
255 for data exclusion, they both summarize the growth rates of a similar
256 number of caterpillars ($N=74$ for the log-linear approach, and $N=71$ for the
257 size on day 8 approach). For completeness, the analysis of absolute growth
258 rates is presented in Appendix S1.

259 We also estimated the interval-specific relative growth rates of caterpillars
260 using log-linear regression on two timescales: a) for all possible intervals;
261 i.e., between all available adjacent experimental days (0, 1, 4, 6, 8, 11, 13,
262 15, and 18) and b) comparing early (between days 0 and 1) and late
263 (between days 1 and 11) growth rates.

264 *Analyses of plant species and plant age effect sizes on larval growth rates*

265 We calculated the size of the plant species effect for each cohort as the fixed
266 effect coefficient of the plant species factor in a linear model with the overall
267 relative growth rate as the response variable. This effect size metric
268 describes the expected proportional change in the relative growth rate for
269 caterpillars reared on showy milkweed relative to narrow-leaved milkweed.
270 An effect sizes of would zero indicate that caterpillars showed similar relative
271 growth rates on narrow-leaved and showy milkweed; negative effect sizes
272 indicate that growth rates were slower on showy milkweed than on narrow-
273 leaved milkweed. For example, an effect size of -0.05 for a given cohort
274 would indicate that the caterpillars in that cohort showed relative growth
275 rates that are 5% lower on showy milkweed than on narrow-leaved
276 milkweed.

277 We also calculated the size of the plant age effect for each available
278 experimental day (0, 1, 4, 6, 8, 11, 13, and 15) and plant species
279 combination using the fixed effect coefficient of the plant age explanatory
280 factor in a linear model with log-transformed mass as the response variable.

281 This effect size metric describes the effect of plant age on the overall relative
282 growth rate of caterpillars on each plant species for each day of the
283 experiment in units of proportional change in mass per week. In this analysis,
284 an effect size of zero would indicate that caterpillar mass was uncorrelated
285 with plant age on a given experimental day; negative effect sizes indicate
286 that plant age was negatively correlated with caterpillar mass. For example,
287 an effect size of -0.05 in this analysis would indicate that the expected mass
288 of surviving caterpillars on a given experimental day, developing on a given
289 host plant species was reduced by 5% for each week of increasing host plant
290 age.

291 *Analyses of maximum larval size attained*

292 We analyzed the maximum larval size attained using linear models and
293 likelihood ratio tests to evaluate the significance of plant species, plant age
294 and their interaction effects as explanatory categorical factors (R Core Team
295 2018). Maximum larval size provides an integrated measurement of larval
296 developmental success including aspects of both growth and survival.

297 *Analyses of plant damage*

298 We analyzed the maximum percent damaged using linear models and
299 significance tests with plant species, plant age and their interaction as
300 explanatory categorical factors (R Core Team 2018), as in the analysis of

301 maximum larval size. Maximum percent damaged indicates the maximum
302 level of herbivory before the caterpillar died or left the plant.

303 **Results**

304 *Plant traits varied with plant species and age*

305 The size and defensive traits of both milkweed species changed over time in
306 species-specific ways. Across all cohorts, narrow-leaved milkweed showed
307 total stem lengths that were 3.1 times greater than those of showy milkweed
308 (*plant species*: $F_{1,106}=76.7$, $p<0.0001$, Fig. 1a). While both species increased
309 their total stem length across the three cohorts (*plant age*: $F_{1,106}=128.5$,
310 $p<0.0001$), total stem length increased more quickly in narrow-leaved
311 milkweed than in showy milkweed (*plant species* \times *plant age*: $F_{1,105}=117.3$,
312 $p<0.0001$), reflecting differences in the architecture of these two species. In
313 4-week-old plants, the mean total stem length of narrow-leaved milkweeds
314 was only 1.2 times that of showy milkweed (12.5 vs. 10.4 cm) , but this
315 difference increased to 3.3 times (44.9 vs. 13.7 cm) in 9-week-old plants, and
316 to 3.6 times in 12-week-old plants (116.3 vs. 31.9 cm). Total leaf count
317 showed a similar pattern (Fig. 1b). The total cross-sectional stem area was
318 also greater in narrow-leaved milkweed overall (*plant species*: $F_{1,106}=14.6$,
319 $p=0.0002$, Fig. 1c), increased with plant age (*plant age*: $F_{1,106}=180.4$,
320 $p<0.0001$); and increased more in narrow-leaved milkweed relative to showy
321 milkweed (*plant species* \times *plant age*: $F_{1,105}=4.2$, $p=0.041$), though this
322 weaker interaction effect suggests that this metric of plant size did not

323 continue to diverge over plant ontogeny (Fig 1c). By comparison, total leaf
324 area increased with plant age (*plant age*: $F_{1,106}=285.3$, $p<0.0001$, Fig. 1d),
325 but did not differ between species overall (*plant species*: $F_{1,106}=0.028$,
326 $p=0.867$, Fig. 1d); while narrow-leaved milkweed showed an accelerating
327 trajectory of increasing leaf area with age, showy milkweed showed a
328 decelerating trajectory of increasing leaf area with age (*plant species* × *plant*
329 *age*: $F_{1,105}=8.6$, $p=0.0041$, Fig. 1d).

330 In contrast, both defense traits showed a significant diverging pattern with
331 plant age (Fig 1e and 1f). Overall, mean latex exudation was 11 times
332 greater in showy milkweed compared to narrow-leaved milkweed (*plant*
333 *species*: $F_{1,106}=57.3$, $p<0.0001$, Fig. 1e), and the mass of exuded latex
334 increased with plant age for both species (*plant age*: $F_{1,106}=55.8$, $p<0.0001$,
335 Fig. 1e). However, the pattern of increased latex exudation with plant age
336 differed strongly by plant species (*plant species* × *plant age*: $F_{1,105}=77.6$,
337 $p<0.0001$, Fig. 1e); while the mean mass of exuded latex increased more
338 than four-fold between 4 and 12 week-old narrow-leaved milkweeds (0.19
339 mg to 0.80 mg), it increased by almost 19 times between 4 and 12 week-old
340 showy milkweeds (0.64 mg to 12.00 mg). Among 4-week-old plants, showy
341 milkweed exuded 3.4 times more latex than narrow-leaved milkweed (0.64
342 vs. 0.19 mg); among 12-week-old plants, showy milkweed exuded 14.9 times
343 more latex than narrow-leaved milkweed (12.00 vs. 0.80 mg). Trichome
344 densities showed a similar pattern; overall, trichomes were 4.2 times denser
345 on showy milkweed compared with narrow-leaved milkweed (*plant species*:

346 $F_{1,106}=19.2$, $p<0.0001$, Fig. 1f), and plants showed generally increasing mean
347 trichome densities with plant age across both species (2.2% among 4-week-
348 old plants to 10.2% among 12-week-old plants, *plant age*: $F_{1,106}=19.5$,
349 $p<0.0001$, Fig. 1f). Trichome densities increased faster on showy milkweed
350 than on narrow-leaved milkweed (*plant species* × *plant age*: $F_{1,105}=22.3$,
351 $p<0.0001$, Fig. 1f).

352 Plant age explained more of the observed variation in total stem length, total
353 stem cross-sectional area and total leaf area than plant species ($\Delta R^2=0.41$ vs
354 $\Delta R^2=0.25$ for total stem length, $\Delta R^2=0.60$ vs $\Delta R^2=0.05$ for total stem cross-
355 sectional area, $\Delta R^2=0.73$ vs $\Delta R^2=0.0001$ for total stem length). The variance
356 explained by plant age and plant species was comparable for total leaf count
357 ($\Delta R^2=0.31$ for plant age vs. $\Delta R^2=0.35$ for plant species), latex exudation
358 ($\Delta R^2=0.26$ for plant age vs $\Delta R^2=0.26$ plant species) and trichome density
359 ($\Delta R^2=0.14$ for plant age vs $\Delta R^2=0.13$ plant species).

360 *Plant species and plant age effects on larval survival*

361 Across all cohorts, the survival curves of monarch larvae differed on narrow-
362 leaved and showy milkweed ($\chi^2_1=4.8$, $p=0.028$), with caterpillars on narrow-
363 leaved milkweed showing 10.4% higher daily survival rates (91.6% vs 82.9%,
364 Fig. 2). This result is consistent with the increased hazard ratio (1.59, 95% CI
365 1.04-2.5, $p=0.034$) observed on showy milkweed relative to narrow-leaved
366 milkweed (Fig. S1). This effect of plant species on survival became stronger
367 with plant age; while the survival curves of caterpillars on both host plant

368 species are largely overlapping for 4-week-old plants ($\chi^2_1=0$, $p=0.99$, Fig.
369 2a), they are more different on 8- and 12-week-old plants (8-week-old plants:
370 $\chi^2_1=2.9$, $p=0.089$, Fig. 2b; 12-week-old plants: $\chi^2_1=2.9$, $p=0.086$, Fig. 2c). For
371 example, caterpillars showed 2.4% greater daily survival rate on showy
372 milkweed among 4-week-old plants (Fig. 2a), but showed 10.1% and 8.4%
373 greater daily survival on narrow-leaved milkweed in weeks 8 and 12,
374 respectively (Fig. 2b and 2c). We did not observe a statistically significant
375 overall effect of plant age on the survival curves of larvae developing on
376 either host plant species using log-rank tests (narrow-leaved milkweed,
377 $\chi^2_2=2.8$, $p=0.247$; showy milkweed, $\chi^2_2=0.8$, $p=0.684$), although a
378 comparison between the youngest and oldest plant age groups suggested a
379 stronger pattern of lower survival on younger plants of narrow-leaved
380 milkweed ($\chi^2_1=2.9$, $p=0.0885$) compared to showy milkweed ($\chi^2_1=0.4$,
381 $p=0.523$). However, we did observe a trend towards reduced survival on
382 younger plants across both species, which was consistent with the estimated
383 hazard ratios for 8-week-old plants (0.93, 95% CI 0.57-1.5, $p=0.792$) and 12-
384 week-old plants (0.70, 95% CI 0.41-1.2, $p=0.195$) relative to 4-week-old
385 plants (Fig. S1). Overall, caterpillars on both host plants species showed the
386 lowest daily survival rates on the youngest host plants (Fig. 2 and S2, 79.5%
387 on narrow-leaved milkweed, 81.5% on showy milkweed), with increasing
388 daily survival rates on older plants (8-week-old plants: 92.8% on narrow-
389 leaved milkweed, 84.3% on showy milkweed; 12-week-old plants: 96.6% on
390 narrow-leaved milkweed, 89.1% on showy milkweed).

391 *Plant species and plant age effects on larval growth rates*

392 Across all host plant cohorts, larval growth was 5.7% higher on narrow-
393 leaved milkweed than on showy milkweed (0.79 mg/mg/day vs. 0.74 mg/mg/
394 day; *plant species*, $F_{1,71}=4.0$, $p=0.049$, Fig. 3-4, Fig. S2), with no significant
395 differences in the effects of plant age on larval growth across species (*plant*
396 *species* \times *plant age*: $F_{2,70}=1.53$, $p=0.22$). However, developing on showy
397 milkweed (instead of narrow-leaved milkweed) had negative effects on
398 relative growth rate that were 4.2 times greater in 12-week-old plants
399 compared with 4-week-old plants (4-week-old plants, -0.027 mg/mg/day; 8-
400 week-old plants, -0.016 mg/mg/day; 12-week-old plants, -0.114 mg/mg/day,
401 Fig. 4a). This result suggests that species-specific differences in plant traits
402 on monarch growth are stronger in older plants than in younger plants.
403 Overall, plant age explained 5 times more variation in overall larval growth
404 rate than plant species ($\Delta R^2=0.207$ for plant age, $\Delta R^2=0.043$ for plant
405 species).

406 Caterpillars grew fastest on the youngest host plants in both species (Fig. 3-
407 4, Fig. S2, *plant age*: $F_{2,72}=9.6$, $p=0.0002$). The overall relative growth rates
408 of caterpillars were fastest on 4-week-old plants (0.82 mg/mg/day), and
409 declined consistently on older host plants (8-week-old plants, 0.75
410 mg/mg/day; 12-week-old plants, 0.70 mg/mg/day, Fig. 3, see also Fig. S2 to
411 S6). These differences in larval growth rates were established early, with
412 diverging trajectories for caterpillars on plants of different ages appearing

413 after the first experimental day (Fig. 3 and S2). The effect of plant age on
414 monarch growth rates was stronger in the first 24h of the experiment than in
415 the subsequent 10 days (Fig. 3, *plant age* × *interval*: $\chi^2_9=6.7$; $p=0.0099$, see
416 also Fig. S2), though this short, transient period of increased growth created
417 persistent differences in caterpillar size throughout development (Fig. 3 and
418 S2). Relative growth rates on 4-week-old plants were 1.9 times greater than
419 those on 12-week-old plants across both plant species when looking at the
420 interval from day 0 to day 1 (*plant age*: $F_{1,96}=17.2$, $p<0.0001$, Fig. 3), and
421 plant species identity did not have a significant effect on these growth rates
422 (*plant species*: $F_{1,96}=0.4$, $p=0.53$, Fig. 3). In contrast, in the interval from day
423 1 to day 11, caterpillars growth rates did not differ significantly among host
424 plants of different ages (*plant age*: $F_{1,38}=0.58$, $p=0.45$, Fig. 3), but did grow
425 9.1% faster on narrow-leaved milkweed compared with showy milkweed
426 (*plant species*: $F_{1,38}=4.1$, $p=0.051$, Fig. 3).

427 The effects of plant age on the realized growth rates of surviving larvae
428 changed over the course of the experiment, as caterpillars died or left their
429 host plant due to insufficient resources. The effects of plant age on
430 caterpillar growth rates were variable but consistently negative throughout
431 the experiment for showy milkweed, but these effects showed larger
432 changes for caterpillars feeding on narrow-leaved milkweed (Fig. 4b). On
433 narrow-leaved milkweed, the magnitude of the negative plant age effect
434 declined throughout the experiment, and the few ($N=4$) caterpillars that
435 survived to experimental day 15 showed a positive effect of plant age on

436 larval growth rate (Fig 4b). This result suggests that while monarch
437 caterpillars initially grew faster on younger plants, continued growth
438 throughout the experiment was increasingly limited by host plant size.

439 *Analyses of maximum larval size*

440 The expected maximum larval size attained, integrating both larval survival
441 and growth, was greatest for caterpillars developing on larger, older plants
442 across both host plant species (263 mg on 4-week-old plants, 317 mg on 8-
443 week-old plants, 578 mg on 12-week old plants, *plant age*: $F_{1,103}=3.0$,
444 $p=0.053$, Fig. 5).

445 Caterpillars also attained larger sizes growing on narrow-leaved milkweed
446 than on showy milkweed. Across all cohorts, monarch larvae attained masses
447 2.7 times larger on narrow-leaved milkweed compared with showy milkweed
448 (570 mg vs. 210 mg; *plant species*: $F_{1,102}=10.2$, $p=0.0018$, Fig. 5). The
449 difference between the maximum larval sizes attained on the two host plant
450 species increased with plant age, from a 1.2-fold mean difference for 4-week-
451 old plants to a 3-fold mean difference in 12-week-old plants, though these
452 responses were variable and not statistically significant (*plant species* ×
453 *plant age*: $F_{1,101}=77.6$, $p=0.13$). Comparable proportions of observed
454 variation in maximum larval size were explained by plant species
455 ($\Delta R^2=0.087$) and plant age ($\Delta R^2=0.052$).

456 *Analyses of plant damage*

457 Caterpillars feeding on the youngest plants consumed a large proportion of
458 available leaf area before leaving their host plant (Fig. 6a and 6b, *plant age*:
459 $F_{1,103}=3.4$, $p=0.038$), and caterpillars that stayed on the youngest host plants
460 longer consumed nearly all available leaf material (Fig. 6c and 6d). The effect
461 of plant age was particularly evident on showy milkweed; caterpillars left 4-
462 week-old showy milkweed after consuming 26.1% of available leaf area,
463 while caterpillars left 12-week-old showy milkweed after consuming only
464 5.6% of leaf area (Fig. 6b). Across all plant ages, percent damage was 1.4
465 times greater in narrow-leaved milkweed compared with showy milkweed
466 (*plant species*: $F_{1,102}=1.4$, $p=0.24$), and older showy milkweed deterred
467 herbivory more strongly than younger plants. Among 4-week-old plants, the
468 percent damage was 1.2 times higher in showy milkweed compared with
469 narrow-leaved milkweed, but this pattern reversed in 8- and 12-week-old
470 plants (2 times more herbivory in narrow-leaved milkweed among 8-week-
471 old plants, and 2.5 times more herbivory in narrow-leaved milkweed among
472 12-week-old plants, *plant species* \times *plant age*: $F_{2,101}=1.2$, $p=0.30$).

473 **Discussion**

474 Taken together, these results show that species-specific and age-varying
475 host plant traits significantly affect the growth and survival of monarch
476 caterpillars. The plant traits that herbivores experience changed significantly
477 over seasonal time following species-specific trajectories, and those changes
478 in plant traits had strong effects on the developmental success of monarch

479 larvae. Potentially in combination with seasonal changes in abiotic conditions
480 and the biotic natural enemy community, these species-specific and age-
481 varying changes in plant traits are likely to be important factors structuring
482 seasonal windows of opportunity for monarch development.

483 Plant traits showed consistent differences between species and were
484 strongly structured by plant age (Fig. 1). The species-specific differences
485 between host plants increased with plant age for total stem length (Fig. 1a)
486 and total number of leaves (Fig. 1b), reflecting species-specific differences in
487 plant architecture. By comparison, total stem cross-sectional area (Fig. 1c)
488 and total leaf area (Fig. 1d) showed relatively non-diverging ontogenetic
489 trajectories suggesting that, despite large differences in their architecture,
490 the plant biomass available to herbivores did not diverge between species as
491 markedly over ontogeny as other species-specific traits, including defensive
492 traits (Fig. 1e and 1f). Broadly, these seasonal changes in plant defense
493 traits could result from the intrinsic ontogenetic trajectory of constitutive
494 traits (Barton and Koricheva 2010), the accumulation of plastic traits over
495 time (e.g., induced resistance or susceptibility, Rasmann et al. 2009), or
496 some combination of these processes. While induced responses to herbivory
497 could contribute to the realized seasonal patterns of plant traits observed in
498 other contexts, the observed patterns in our current study seem unlikely to
499 represent induced responses to prior herbivory because we did not detect
500 any non-monarch herbivores in the system throughout the study. While host
501 plant species identity was also informative in our study, plant age often

502 explained a comparable proportion of the observed variation in plant traits.
503 Older plants showed more strongly differentiated species-specific plant traits
504 in this study, while younger plants of both species were unexpectedly
505 similar. These two milkweed species express distinct plant defense
506 syndromes as mature plants (Agrawal and Fishbein 2006). In our study,
507 species-level differences emerged over ontogeny as the defensive traits of
508 these species diverged with increasing plant age (Fig. 1e and 1f). These
509 findings extend the meta-analytic dataset described by Barton and Koricheva
510 (2010) which documented generally increasing constitutive chemical
511 defenses from the seedling stage to maturity in herbaceous plants, but
512 lacked a sufficient sample size of studies to identify general ontogenetic
513 patterns in physical defense traits with herbaceous plants (but see Traw and
514 Feeny 2008). The results of this current study show significant changes in
515 both types of plant defense traits over ontogeny, with trajectories that
516 differed strongly between the two milkweed species. Though future studies
517 will be necessary to capture seasonal patterns in a broader set of plant traits
518 that could potentially affect windows of opportunity for monarchs (e.g., water
519 content, specific leaf area, secondary compounds and C:N content, Agrawal
520 and Fishbein 2006), the patterns observed in this study are consistent with
521 seasonal patterns previously observed for trichome density and leaf
522 toughness in these two species (Yang and Cenzler 2020), and suggest that
523 several traits relevant to monarch development may change with plant age.

524 In this study, plant age explained substantially more variation in overall
525 larval growth rate than plant species (Fig. 3). Across larval development,
526 monarch caterpillars grew fastest on the youngest plants of both species,
527 and this overall pattern was strongly (and unexpectedly) driven by large
528 differences in growth rate during the first 24h of larval development (Fig. 3).
529 Plant age-associated differences in larval growth rate during the first day
530 after egg introduction created substantial differences in larval size that
531 persisted throughout the rest of larval development (Fig. 3 and S2). This
532 result is consistent with a previous study showing that monarch caterpillars
533 grew faster on milkweed leaves with partially severed petioles (and thus
534 reduced latex pressure) during the first 2-4 days of larval development on
535 four out of nine species of milkweed examined (Zalucki et al. 2001); in both
536 studies, early instar caterpillars grew faster on host leaves with reduced
537 latex exposure. These findings are also consistent with studies indicating
538 that adult monarchs preferentially oviposit on younger host plants (Zalucki
539 and Kitching 1982), as well as the recent vegetative regrowth of host plants
540 that have been strategically mowed for habitat management (Fischer 2015,
541 Haan and Landis 2019, Knight et al. 2019). Similar preferential herbivory on
542 rapid regrowth has been observed in other systems in response natural
543 disturbance regimes (e.g., Spiller and Agrawal 2003). Our results suggest
544 that plant age is a key determinant of variation in this defensive trait, and
545 show that the strongest effects of these age-associated differences in plant
546 traits on growth rate occur in the first 24h of larval development.

547 Overall, monarch caterpillars experienced greater developmental success
548 (i.e., faster growth, Fig. 4a; higher survivorship, Fig. 2c; and larger maximum
549 larval sizes, Fig. 5) on narrow-leaved milkweed than on showy milkweed, and
550 the difference between host plant species was particularly strong for older
551 host plants (Figs. 2c, 4a and 5). These findings are consistent with our
552 observation that of increasing species-associated trait differentiation with
553 increasing plant age. These patterns are also consistent with the different
554 seasonal windows of opportunity that have been previously observed for
555 monarchs feeding on these two host plants (Yang and Censer 2020): while
556 monarchs showed two seasonal windows of opportunity on narrow-leaved
557 milkweed, those feeding on showy milkweed only showed the early season
558 window. We suggest that increasing plant defense traits over ontogeny could
559 limit late season windows of opportunity in showy milkweed. The findings of
560 our current study are also consistent with the hypothesis that the two
561 seasonal window of opportunity observed on narrow-leaved milkweed could
562 correspond to a “double-dipping” herbivore strategy (*sensu* White 2015,
563 Che-Castaldo et al. 2019) in which monarch larvae successfully use both
564 vigorously growing and senescing plant tissues. Future studies will be
565 necessary to more specifically examine how increasingly senescent plant
566 traits affect larval success in the second window of opportunity observed in
567 this system.

568 Our findings indicate that the early season window of opportunity may be
569 influenced by temporal variation in both resource *quantity* (i.e., plant size)

570 and *quality* (as affected by age-varying defensive traits). Younger plants of
571 both species provided higher quality resources that allowed for faster larval
572 growth rates initially (Fig. 3), but for narrow-leaved milkweed, older plants
573 provided greater resource quantity over a longer developmental timescale
574 (Fig. 4b). These changes in the developmental limitations imposed by
575 seasonally varying resource quality and quantity are further supported by
576 observed patterns of herbivore damage and larval survival. On the youngest
577 plants, the developmental success of larval monarchs appeared to be
578 ultimately limited by the availability of host plant biomass (*i.e.*, resource
579 quantity). Caterpillars on the youngest plants fed on less-defended (*i.e.*,
580 higher-quality) resources and grew fast (Figs. 1 and 3); they often consumed
581 a substantial proportion of their host plants before starving or attempting to
582 disperse to a second host plant (Fig. 6). As a result, these caterpillars showed
583 steep and short survivorship curves on both host plant species; in general,
584 these caterpillars grew fast and died young (Figs. 2 and 3). In comparison,
585 caterpillars developing on the oldest host plants seemed to be limited by the
586 *quality* of host plant biomass as constrained by plant defense traits. These
587 caterpillars showed the slowest growth rates (Fig. 3), but rarely consumed
588 their entire host plant (Fig. 6), and showed the longest survivorship curves
589 (Fig. 2).

590 The relative importance of milkweed *quality* and *quantity* as factors that
591 structure seasonal windows of opportunity for monarch development could
592 also depend on the density of milkweeds in available habitat patches, as well

593 as the density of monarch oviposition. This experiment was conducted with
594 singular host plants as replicates, where attempted dispersal by larvae below
595 the pupation threshold size was assumed to be fatal. This assumption is
596 likely to be a reasonable one in habitats where individual plants are widely
597 spaced, where biotic or abiotic conditions limit the ability of monarch
598 caterpillars to move between neighboring plants (e.g., due to increased
599 thermal stresses or predation risk), or if monarchs show limited abilities to
600 locate second host plants. Alternatively, high-density patches of young
601 milkweed plants could potentially provide high-quality host plant resources
602 with reduced plant-quantity constraints; this suggests that higher density
603 patches could potentially allow for earlier seasonal windows of opportunity,
604 consistent with the results of previous field experiments (Yang and Censer
605 2020). Further studies specifically examining the risk of plant-to-plant
606 movement would be valuable to better understand the role of plant density
607 on seasonal window of opportunity for monarch development. Moreover,
608 while this study investigated the effects of plants traits in two milkweed
609 species during their first growing season, additional studies assessing other
610 host plant species, additional plant traits (including physical, chemical and
611 indirect defense traits), and a wider range of plant ages (especially
612 considering plants in their second growing season and beyond) will be
613 necessary to assess the generality of the patterns observed here. In addition,
614 the role of seasonal variation in climatic conditions and natural enemy

615 interactions remains uncertain, and both of these factors have the potential
616 to interact with the effects of plant trait variation in nature

617 More broadly, these findings contribute to the general observation that
618 temporal variation in plant traits can strongly affect plant-herbivore
619 interactions (e.g., Van der Wal et al. 2000, Van Asch et al. 2007, Barton and
620 Koricheva 2010, Che-Castaldo et al. 2019). The results of this study indicate
621 that the effects of plant age on monarch developmental success are
622 comparable to and sometimes larger than those of plant species identity.
623 Acknowledging substantial temporal variation in plant traits does not
624 diminish the importance of species-level trait assessments; expectations
625 about how plant traits affect herbivores are often usefully structured around
626 species-level characterizations, and such studies can identify clusters of
627 species that share key traits (Agrawal and Fishbein 2006). In combination
628 with such species-level trait assessments, the temporal dimensions of plant
629 age and seasonal variation provide additional orthogonal axes to examine
630 variation in plant defense traits and their effects on herbivores.

631 These results may also suggest some specific implications for our
632 understanding of milkweed-monarch interactions in a changing world, and
633 the potential for milkweed limitation in the population dynamics of monarchs
634 (Nail et al. 2015, Pleasants et al. 2016, Inamine et al. 2016, Thogmartin et al.
635 2017), and especially in western North America (Espeset et al. 2016, Pelton
636 et al. 2019). If age-varying plant traits have strong effects on the

637 developmental prospects of monarchs generally, monarchs may experience
638 changing constraints on larval development as their host plant traits develop
639 through the season. In particular, the development of monarch larvae in the
640 early season could potentially be limited by small host plant size, even in
641 habitats with abundant host plant resources later in the season. The
642 potential for milkweed limitation in the early season would potentially be
643 exacerbated if seasonal intervals where growth is limited by host plant
644 quantity coincided with periods of high oviposition density. Conversely, if
645 later-season milkweeds generally present stronger defensive traits than
646 early-season plants, monarchs could potentially experience reduced growth
647 rates during periods of lower resource quality even when the apparent
648 availability of host plant resources is high. Because these potential seasonal
649 limitations are mediated by changes in resource quality as much as resource
650 quantity, estimates of milkweed abundance and spatial distribution by
651 themselves may not capture a key temporal dimension of the dynamic
652 resource landscape. If a wider range of milkweed species show the kinds of
653 species-specific and age-varying traits observed in this current study, it
654 would suggest that migrating monarchs face a complex and dynamic
655 landscape of potential host plants with traits that are affected by phenology
656 and ontogeny as much as species distributions. The complexity of this
657 dynamic resource landscape likely presents a challenge for migrating
658 monarchs as well as the ecologists that aim to study them. Developing a
659 more temporally explicit approach may be necessary to assess the combined

660 effects of plant age and species identity on the spatial distribution and
661 temporal availability of milkweed resources on a continental scale. Further, it
662 is unclear how monarch migrations and the dynamics of this seasonally
663 variable landscape will change with global warming. The age of host plants
664 that migrating monarchs encounter each year is likely to be affected by both
665 the environmental cues that influence milkweed phenology, as well as the
666 continental-scale drivers of monarch migration. The potential for significant
667 mis-matches in the relative phenologies of milkweeds and monarchs remains
668 uncertain, though the magnitude of observed plant-age effects in this study
669 suggests that the consequences of such phenological mis-matches, if
670 realized, could be substantial. Further studies will be necessary to identify
671 the environmental cues that drive phenological responses in a range of
672 milkweed species, and how phenological variation across different species
673 distributions affects the overall spatiotemporal availability of milkweed
674 resources throughout each season.

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811

813 **Figure legends**

814 **Figure 1.** Plant traits a) mean total stem length, b) mean total leaf count, c)
815 total stem cross-sectional area, d) total leaf area, e) mean latex exudation,
816 and d) mean trichome density changed over plant ontogeny and differed
817 between plant species. Color represents plant species, and point shape
818 represents plant age. Error bars represent 95% confidence intervals.

819

820 **Figure 2.** Survival of larval monarchs on a) 4-week-old, b) 8-week-old and c)
821 12-week-old plants. Tick marks on the survivorship curve indicate pupation.
822 Color represents plant species.

823

824 **Figure 3.** The effects of plant age on mean relative growth rates by plant
825 species and experimental day. The effects of plant age on the overall
826 (lifetime) relative growth rates of caterpillars are shown for a) narrow-leaved
827 milkweed and b) showy milkweed. The interval-specific relative growth rates
828 for caterpillars during experimental days 0 to 1 are shown for c) narrow-
829 leaved milkweed and d) showy milkweed. The interval-specific relative
830 growth rates for caterpillars during experimental days 1 to 11 are shown for
831 e) narrow-leaved milkweed and f) showy milkweed. These figures show that
832 the persistent negative effects of plant age on caterpillar size shown in Figs 3
833 and 4 emerges from growth differences that occur in the first 24h of larval

834 development. Point color and point shape represent plant age. Error bars
835 represent 95% confidence intervals.

836

837 **Figure 4.** a) The mean plant species effect size for each plant age. These
838 effect sizes represent the linear model coefficients for the effect of showy
839 milkweed relative to narrow-leaved milkweed on surviving larval mass. Bar
840 color represents plant age. Showy milkweed had a negative effect on larval
841 mass in each plant age cohort, but this effect was larger in the oldest cohort.
842 b) The mean plant age effect size for the surviving population on each
843 experimental day, separated by host plant species. These effect sizes
844 represent the linear model coefficient for plant age effects on surviving larval
845 mass. Bar color represents plant species. The effects of plant age are
846 consistently negative on showy milkweed. On narrow-leaved milkweed, the
847 effect of plant age is generally negative, but the magnitude of these effects
848 declines over time.

849

850 **Figure 5.** The maximum size (mass and length) attained by caterpillars
851 developing on two host plant species of different ages. Color represents
852 plant species, and point shape represents plant age. Error bars represent
853 95% confidence intervals.

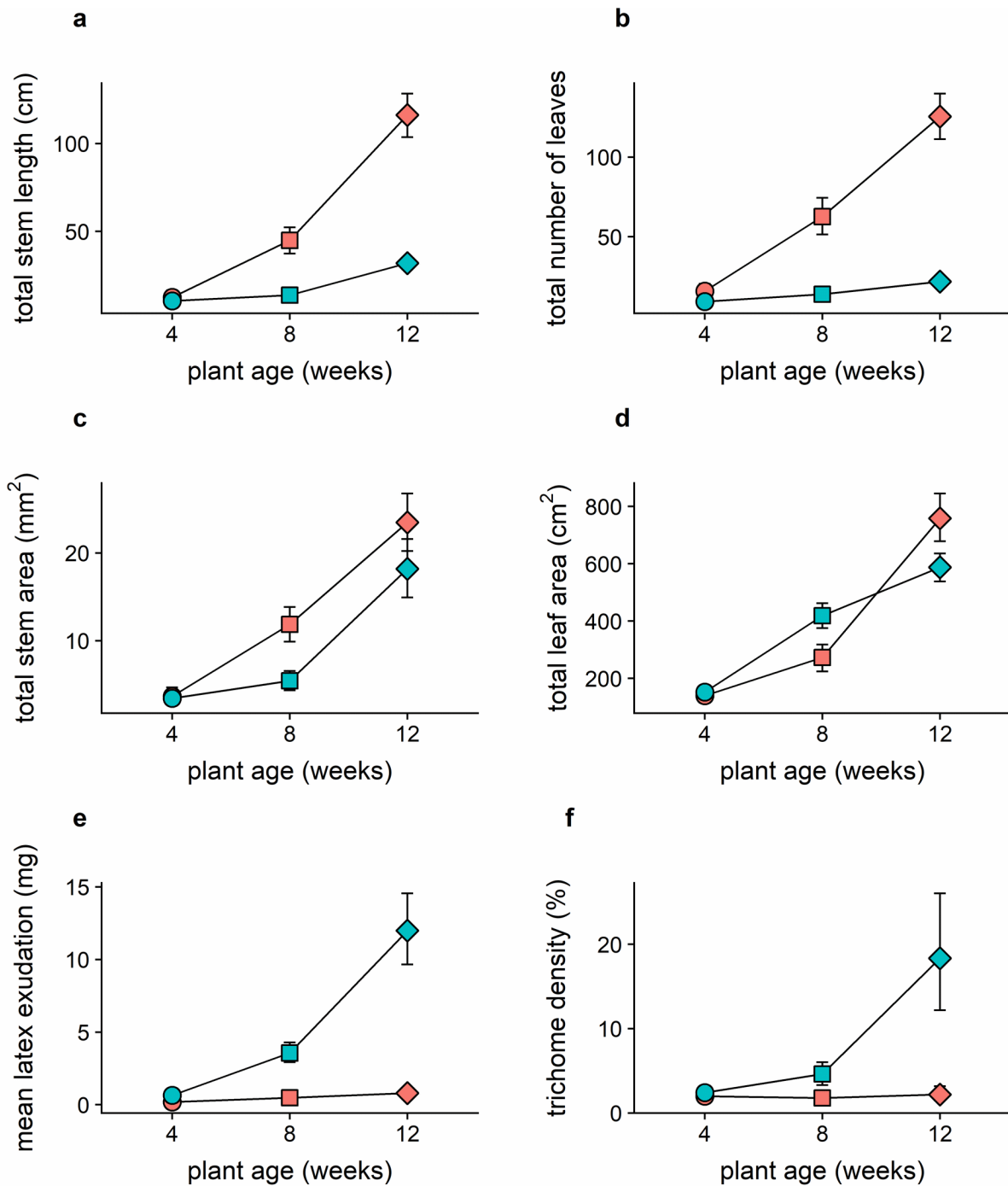
854

855 **Figure 6.** Plant damage by host plant species and age. Mean maximum
856 herbivore damage for plants of each age cohort for a) narrow-leaved

857 milkweed and b) showy milkweed. Mean maximum damage represents the
858 percent of leaf area consumed by monarchs before leaving their host plant.
859 Point color and point shape represent plant age. Error bars represent 95%
860 confidence intervals. b) Percent damage on plants over time, showing the
861 subset of plants with surviving caterpillars present at each time point. Point
862 color and point shape represent plant age. Point size reflects the size of the
863 surviving caterpillar population. Error bars represent 95% confidence
864 intervals.

865

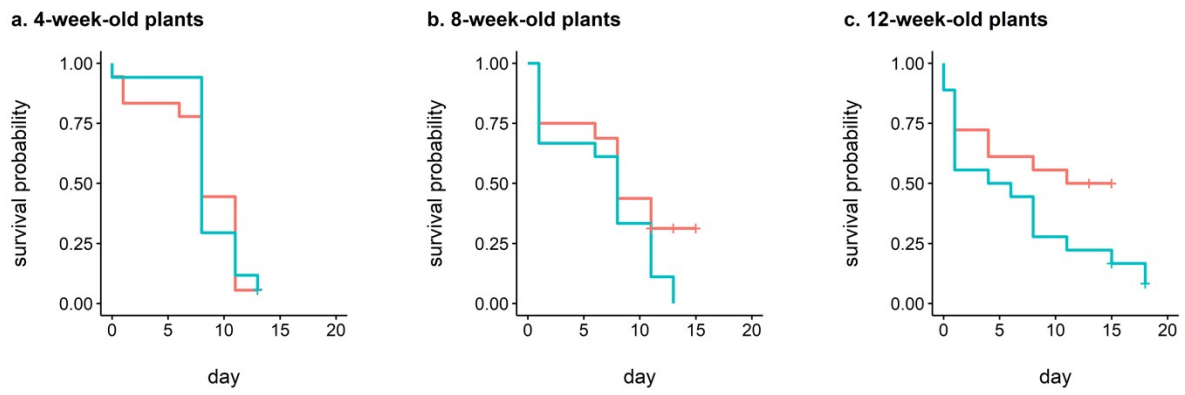
866 **Figure 1**



plant species ● *A. fascicularis* ● *A. speciosa*

867

869 **Figure 2**



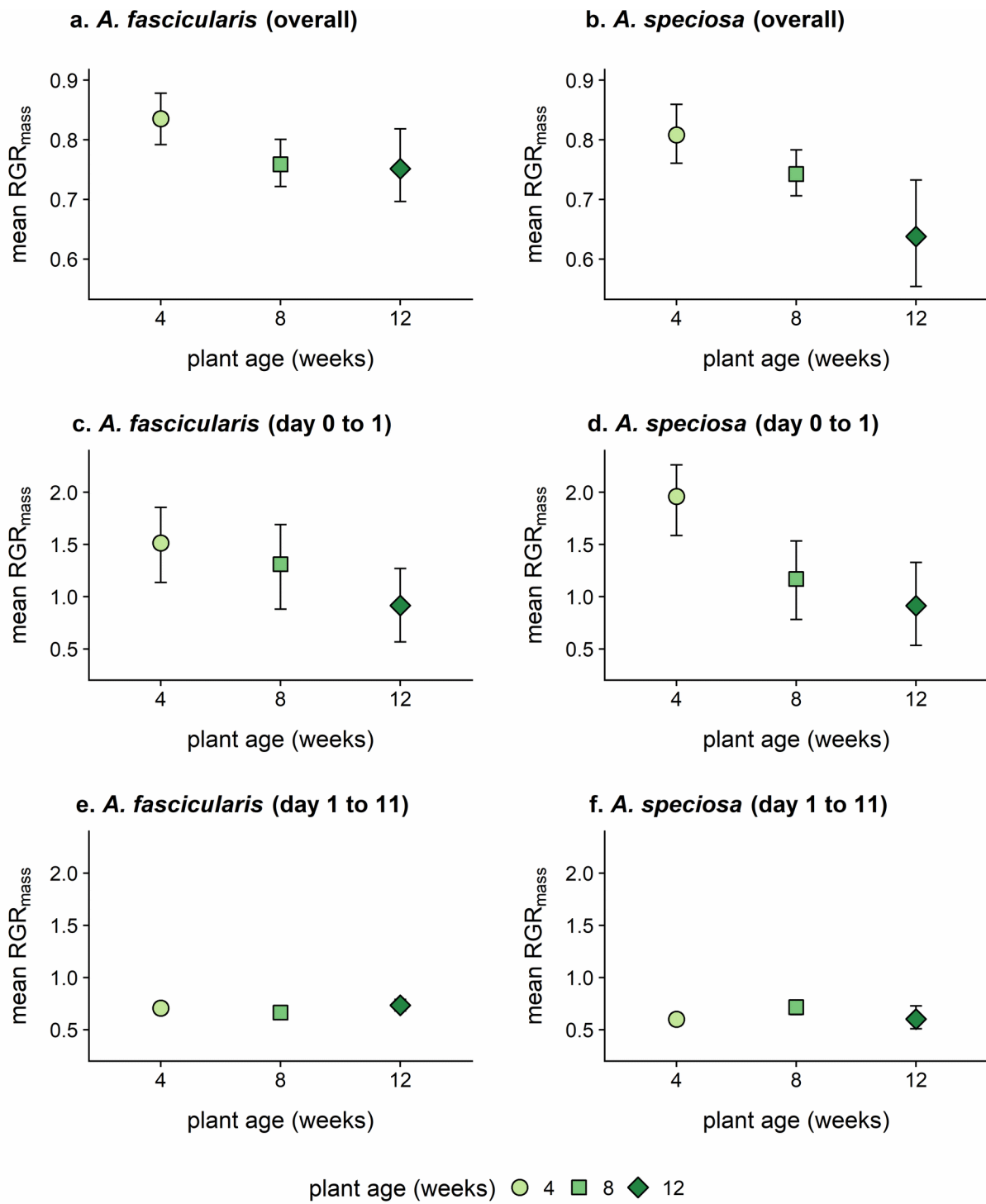
870

plant species — *A. fascicularis* — *A. speciosa*

871

872

873 **Figure 3**

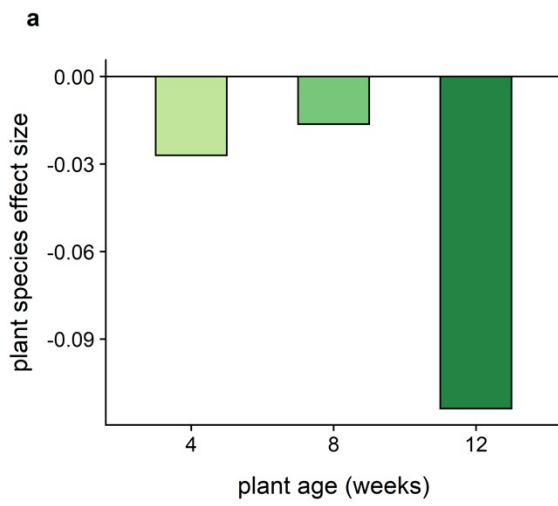


874

875

876 **Figure 4**

877

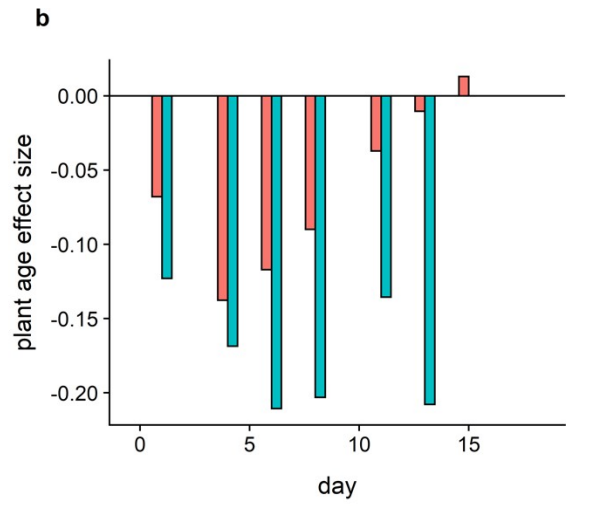


plant age (weeks) ■ 4 ■ 8 ■ 12

878

879

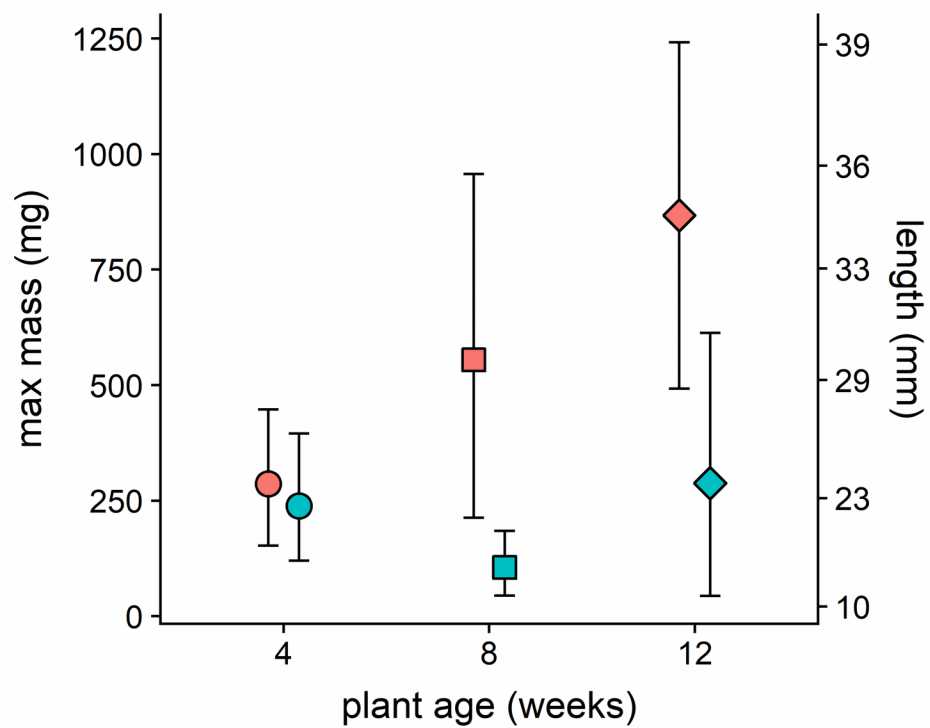
880



plant species ■ *A. fascicularis* ■ *A. speciosa*

881 **Figure 5**

882



plant species ● *A. fascicularis* ● *A. speciosa*

883

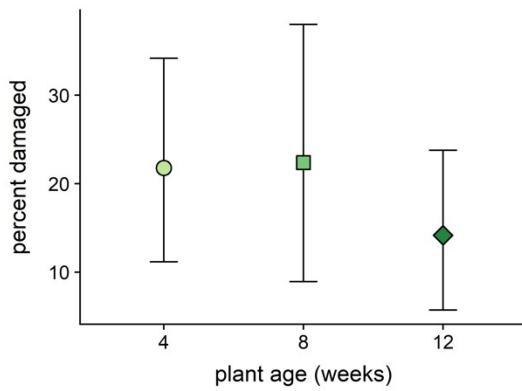
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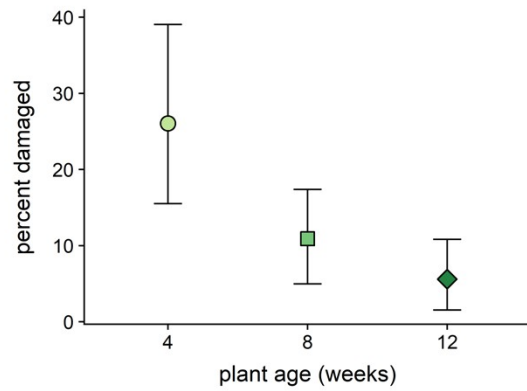
886 **Figure 6**

887

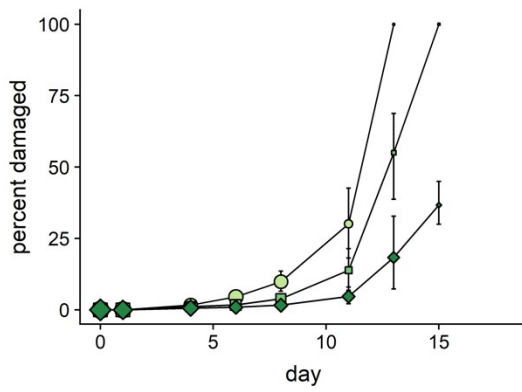
a. *A. fascicularis* mean max damage



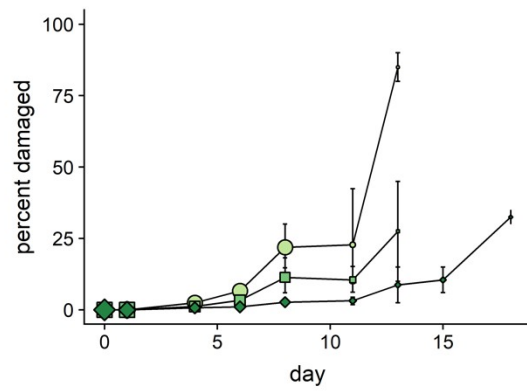
b. *A. speciosa* mean max damage



c. *A. fascicularis* damage by day



d. *A. speciosa* damage by day



plant age (weeks) ○ 4 ■ 8 ◆ 12

888

889

890

891

892