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1 Running head: Plant traits limit opportunity windows

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

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8 **Abstract**

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

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

29

30 **Introduction**

31 Seasonal windows of opportunity are intervals of time within a year during which organisms
32 have improved prospects of achieving life history aims such as growth or reproduction (Yang
33 and Cenzer 2020). Seasonal windows of opportunity are likely to occur in a wide range of
34 systems (e.g., Yang and Rudolf 2010, Anderson et al. 2012, Wright et al. 2013, Carter et al.
35 2018, Farzan and Yang 2018, Yang and Cenzer 2020), resulting from commonplace temporal
36 variation in biotic and abiotic factors. However, while phenology examines the *realized* seasonal
37 timing of an organism's life history, seasonal windows of opportunity represent transient periods
38 of time with the *potential* for improved developmental or fitness outcomes. Because underlying
39 windows of opportunity may not always be reflected in observed phenology, experimental
40 manipulations provide a particularly useful approach for identifying seasonal windows of
41 opportunity (Yang and Rudolf 2010). Despite this, relatively few studies have experimentally
42 identified seasonal window of opportunity in nature (but see Van Asch et al. 2007, Liu et al.
43 2011, Rafferty and Ives 2011, Warren et al. 2011, Kharouba et al. 2015, Farzan and Yang 2018,
44 Yang and Cenzer 2020), and even fewer have experimentally examined the specific factors that
45 define these windows of opportunity in time.

46 Seasonal windows of opportunity are defined by the co-occurrence of factors that, in
47 combination, have a positive effect on growth or reproduction. Broadly, many seasonal windows
48 of opportunity are likely to be structured by temporal variation in abiotic factors, bottom-up
49 factors, and top-down factors (Yang and Cenzer 2020). When the combined effects of these
50 factors present adverse conditions, they constrain the seasonal timing of development. When the
51 combined effects of these factors are favorable, they create seasonal windows of opportunity.

52 However, separating and evaluating the role of specific factors in structuring seasonal windows
53 of opportunity is challenging due to the multiple correlated factors that often change
54 simultaneously across a seasonal timescale.

55 The interactions between herbivores, their host plants, and their surrounding community provide
56 unique opportunities to examine seasonal windows of opportunities. For herbivores, these
57 windows of opportunity are likely to be structured by a variety of seasonally varying factors,
58 including climatic conditions, natural enemy communities and plant traits. Questions about the
59 ontogeny of plant defense traits have received particular attention as ecologists have sought to
60 understand the specific mechanisms (Barton 2013, 2016, Quintero et al. 2013) and general
61 patterns (Boege and Marquis 2005, Barton and Koricheva 2010, Barton and Boege 2017) that
62 explain how plant-herbivore interactions change across development. Broadly, these studies
63 document a diversity of ontogenetic trajectories (including both increasing and declining trends)
64 in a wide range of plant defense traits (including both tolerance and chemical, physical, and
65 indirect resistance traits). While specific patterns of change differ with both plant and herbivore
66 identity (Barton and Koricheva 2010), the observation of significant ontogenetic changes in plant
67 defense traits is both general and robust (Barton and Koricheva 2010, Barton and Boege 2017).
68 In addition, plant phenology has recently been suggested as a key factor that could unify the
69 hypothesis that herbivores generally prefer and perform better on vigorously growing plants (i.e.,
70 the *plant vigor hypothesis*, Price 1991) and the hypothesis that herbivore outbreaks are more
71 likely on stressed plants (i.e., the *plant stress hypothesis*, White 1974); phenological changes in
72 plant traits can change the quality of plant resources in ways that are consistent with both
73 hypotheses (White 2009, Che-Castaldo et al. 2019). However, while seasonal changes in plant
74 defense traits are likely to be a common consequence of plant ontogenetic trajectories in many

75 systems, few studies have examined the ecological consequences of these temporally variable
76 plant defense traits for the developmental prospects of herbivores.

77 Here, we present an experiment designed to isolate and examine the role of plant traits in
78 constraining seasonal windows of opportunity for larval monarchs (*Danaus plexippus*) feeding
79 on two milkweed host plants (*Asclepias fascicularis* and *Asclepias speciosa*). While previous
80 studies have identified seasonal windows of opportunity in the interactions between milkweed
81 host plants and their monarch caterpillar herbivores (Yang and Censer 2020), more specific
82 experiments are necessary to identify the factors that structure these windows of opportunity in
83 time. In this experiment, we isolated the species-specific effects of age-varying plant traits on the
84 developmental prospects of monarch caterpillars by presenting plants of two milkweed species
85 and three age classes synchronously to a single cohort of monarch caterpillars. This design aimed
86 to control for the effects of seasonally variable abiotic conditions and natural enemy
87 communities while isolating the effects of species-specific and age-varying plant traits. The key
88 questions we address in this study are: a) How do plant traits, including measures of both size
89 (i.e., resource *quantity*) and defensive traits (i.e., resource *quality*), change with plant age in two
90 species of milkweed host plants? b) How do these species-specific and age-varying changes in
91 plant traits affect the growth and survival of larval monarchs?

92 **Methods**

93 *Plant establishment*

94 We started three cohorts of narrow-leaved milkweed (*A. fascicularis*) and showy milkweed (*A.*
95 *speciosa*) from seed on April 8, May 7 and June 8, 2014. These two milkweed species are native

106 to the California Central Valley, and the seeds used in this study were propagated from local
107 source populations (Hedgerow Farms, Winters, CA, USA). Each cohort of seeds was started
108 directly into 2.5 L containers filled with potting soil (1:1:1 ratio of sand, compost and peat moss
109 by volume with 1.78 kg/m³ dolomite), which were irrigated and fertilized (electrical
110 conductivity, EC = 1.5-1.6 mS cm⁻¹) via drip emitters as necessary to prevent water and nutrient
111 limitation. Plants from each cohort were randomly interspersed in a single greenhouse
112 (approximately 20-35° C) at the University of California, Davis Orchard Park Research
113 Greenhouse Facility (38.543129° N, 121.763425° W) with individual plants spaced on open
114 grate wire benches to prevent contact between the leaves of neighboring plants. These three
115 cohorts were started approximately 4 weeks apart to yield three distinct age classes of milkweed
116 (25-day, 57-day and 86-day-old plants, hereafter, the 4, 8 and 12-week cohorts) for each species
117 ($N=18$ plants of each species in each age class, $N=108$ plants total) at the start of the experiment.

118 *Measuring plant traits*

109 We measured the size (total stem length, total leaf count, total stem cross-sectional area and total
110 leaf area) and defensive traits (mean latex exudation and trichome density) of each plant at the
111 start of the experiment (July 3, 2014). All plants were actively growing at the start of the
112 experiment, and two of the 12-week-old plants had begun developing flowers (reflecting
113 *seedling*, *vegetative juvenile* and *juvenile-mature transition stages*, *sensu* Barton and Koricheva
114 2010). In the context of this experiment, plant age provides a proxy for both plant phenology and
115 ontogeny; i.e., older plants represent plants that are more phenologically advanced and
116 developmentally mature. Total stem length was measured as the product of the total stem count
117 (all stems > 5 cm), and the mean stem length (averaged from a subsample of up to 10 stems > 5

118 cm in length). Total leaf counts included all fully expanded leaves on each plant. Total stem
119 cross-sectional area is the cumulative cross-sectional area of stems, calculated from the total
120 stem count (all stems >5 cm) and the mean stem diameter measured from a subsample of up to
121 10 stems >5 cm in length. Total leaf area was estimated as the product of the total leaf count and
122 the mean area per leaf for each plant species × plant age combination. The mean area per leaf
123 was estimated as the area of an ellipse using measurements of the length and width of $N=5$ fully
124 expanded leaves randomly selected from each group. Latex exudation was measured as the mean
125 dry mass of latex collected on pre-weighed filter paper discs after cutting 5 mm from the distal
126 tip of two fully expanded upper leaves, following Agrawal (2005). Trichome density was
127 assessed from the upper surface of 3 mm diameter leaf discs punched from fully expanded apical
128 leaves using digital analysis of magnified images to determine the proportion of the leaf area
129 obscured by trichomes based on manual color thresholding (Abramoff et al. 2004).

130 *Monarch introduction and monitoring*

131 A single monarch egg was introduced to each plant on July 3, 2014 (experimental day 0). In
132 order to minimize direct handling of the eggs, we punched 6.4 mm leaf discs from oviposition
133 host plants with single monarch eggs attached, and attached them to the apical leaves on their
134 experimental host plants with a drop of milkweed latex. Monarch eggs were obtained from a
135 large, local insectary population (Utterback Farms, Woodland, CA, USA) which was re-
136 established from local monarch genotypes each year, maintained in large greenhouses, regularly
137 supplemented with new adults to maintain genetic diversity, and had been previously assessed
138 for parasites and pathogens (H.K. Kaya, *pers. comm.*). All monarch eggs in this experiment
139 were selected haphazardly from a single oviposition time-restricted cohort to minimize variation

140 in hatch timing. Each monarch egg was checked 24 h after its initial introduction (experimental
141 day 1) to assess hatch rate and larval length. Afterwards, we re-measured caterpillars every 2-3
142 days until they died or left the plant ($N=1034$ observations). All larvae were measured to the
143 nearest 0.1 mm using dial calipers; eggs were assumed to have a length of zero. Larval mass was
144 estimated from a power law regression of caterpillar length and mass, parameterized from a
145 dataset describing 73 unmanipulated caterpillars measured in 2014 ($\text{mass}=0.0223 * \text{length} +$
146 $2.9816, R^2=0.97$). During each observation, we also visually estimated the proportion of leaf area
147 that was removed due to herbivory (hereafter, *percent damaged*). Caterpillars were intentionally
148 not bagged or constrained at any point in this experiment so that we could assess when
149 caterpillars left their host plants (in terms of caterpillar age, caterpillar size, and host plant
150 herbivory). Caterpillars that left their host plant below a minimum threshold size for pupation
151 (35 mm length, or 895 mg) were assumed to have been unable to complete their larval
152 development on a single host plant; in the context of a single plant patch, we considered these to
153 be “dead” in our survival analyses. Caterpillars that left their host plant after attaining this
154 threshold size were considered to be seeking pupation sites, and were considered to be right-
155 censored in survival analyses. The threshold size for pupation (895 mg or 35 mm) was
156 determined by assessing the larval size attained by all pupating caterpillars in previous field
157 experiments, and among 248 caterpillars reared in the laboratory in 2014 and 2015 (Yang and
158 Cenzler 2019). In 2.8% ($N=29$) of observations, we observed a second non-focal caterpillar that
159 had moved onto an experimental plant; in the majority of these cases, we were able to
160 unambiguously identify the focal caterpillar and remove the non-focal caterpillar. In three
161 instances (0.3% of observations), the identity of the focal caterpillar could not be determined;
162 although the qualitative conclusions of this study were unaffected by the inclusion or exclusion

163 of these plants, we removed all observations from these three plants for the analyses presented
164 here.

165 *Analyses of plant traits*

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

171 *Survival analyses*

172 We analyzed the survival of monarchs for each plant species and age cohort to generate species-
173 and age-specific Kaplan-Meier survivorship curves (Therneau and Grambsch 2000, Therneau
174 2015, Kassambara and Kosinski 2019). We compared curves using a log-rank test procedure for
175 right-censored data (Harrington and Fleming 1982) implemented in the *survdif* function in the
176 *survival* package in R (Therneau 2015). We quantified the overall daily survivorship rates for
177 each group of interest using the slope coefficient of a log-linear regression of survival rates over
178 time, with visual inspection to confirm model fit assumptions. In addition, we used a Cox
179 proportional hazards model in order to combine plant species and plant age effects into a single
180 survival model (using the *coxph* function in the *survival* package, Therneau 2015) and estimate
181 the proportional hazard ratios associated with the specific levels of each factor (using the
182 *ggforest* function in the *survminer* package, Kassambara and Kosinski 2019).

183 *Estimation of larval growth rates*

184 We estimated overall larval growth rates as the slope of the log-linear fit of experimental day vs.
185 $\log(\text{mass})$ for each individual caterpillar; i.e., as a relative growth rate. In order to estimate the
186 slope of a log-linear regression in a dataset that included zero values, we added a small constant
187 equal to the minimum observed mass across the dataset to all mass data in the log-linear analysis.
188 We used a log-linear fit of mass (as opposed to length) data because visual inspection indicated
189 that caterpillar masses show a more log-linear (i.e. exponential) pattern of increase over time,
190 although these two metrics of monarch size yield qualitatively identical results. To avoid
191 inaccurate overall slope estimates resulting from insufficient data, we excluded caterpillars that
192 died before reaching 10 mm length.

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

198 For simplicity, we primarily present relative growth rates based on the slope of the log-linear
199 regression here because this estimate is informed by more observations for each summary
200 growth rate, and because this approach can be more easily generalized to examine a range of
201 interval-specific growth rates. Because both of these overall growth rate estimates are measured
202 relative to size on day 0, they are mathematically similar and yield qualitatively similar results;
203 in addition, although they use different criteria for data exclusion, they both summarize the
204 growth rates of a similar number of caterpillars ($N=74$ for the log-linear approach, and $N=71$ for

205 the size on day 8 approach). For completeness, the analysis of absolute growth rates is presented
206 in Appendix S1.

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

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

212 We calculated the size of the plant species effect for each cohort as the fixed effect coefficient of
213 the plant species factor in a linear model with the overall relative growth rate as the response
214 variable. This effect size metric describes the expected proportional change in the relative growth
215 rate for caterpillars reared on showy milkweed relative to narrow-leaved milkweed. An effect
216 sizes of would zero indicate that caterpillars showed similar relative growth rates on narrow-
217 leaved and showy milkweed; negative effect sizes indicate that growth rates were slower on
218 showy milkweed than on narrow-leaved milkweed. For example, an effect size of -0.05 for a
219 given cohort would indicate that the caterpillars in that cohort showed relative growth rates that
220 are 5% lower on showy milkweed than on narrow-leaved milkweed.

221 We also calculated the size of the plant age effect for each available experimental day (0, 1, 4, 6,
222 8, 11, 13, and 15) and plant species combination using the fixed effect coefficient of the plant
223 age explanatory factor in a linear model with log-transformed mass as the response variable. This
224 effect size metric describes the effect of plant age on the overall relative growth rate of
225 caterpillars on each plant species for each day of the experiment in units of proportional change

226 in mass per week. In this analysis, an effect size of zero would indicate that caterpillar mass was
227 uncorrelated with plant age on a given experimental day; negative effect sizes indicate that plant
228 age was negatively correlated with caterpillar mass. For example, an effect size of -0.05 in this
229 analysis would indicate that the expected mass of surviving caterpillars on a given experimental
230 day, developing on a given host plant species was reduced by 5% for each week of increasing
231 host plant age.

232 *Analyses of maximum larval size attained*

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

237 *Analyses of plant damage*

238 We analyzed the maximum percent damaged using linear models and significance tests with
239 plant species, plant age and their interaction as explanatory categorical factors (R Core Team
240 2018), as in the analysis of maximum larval size. Maximum percent damaged indicates the
241 maximum level of herbivory before the caterpillar died or left the plant.

242 **Results**

243 *Plant traits varied with plant species and age*

244 The size and defensive traits of both milkweed species changed over time in species-specific
245 ways. Across all cohorts, narrow-leaved milkweed showed total stem lengths that were 3.1 times

246 greater than those of showy milkweed (*plant species*: $F_{1,106}=76.7$, $p<0.0001$, Fig. 1a). While both
247 species increased their total stem length across the three cohorts (*plant age*: $F_{1,106}=128.5$,
248 $p<0.0001$), total stem length increased more quickly in narrow-leaved milkweed than in showy
249 milkweed (*plant species* \times *plant age*: $F_{1,105}=117.3$, $p<0.0001$), reflecting differences in the
250 architecture of these two species. In 4-week-old plants, the mean total stem length of narrow-
251 leaved milkweeds was only 1.2 times that of showy milkweed (12.5 vs. 10.4 cm) , but this
252 difference increased to 3.3 times (44.9 vs. 13.7 cm) in 9-week-old plants, and to 3.6 times in 12-
253 week-old plants (116.3 vs. 31.9 cm). Total leaf count showed a similar pattern (Fig. 1b). The
254 total cross-sectional stem area was also greater in narrow-leaved milkweed overall (*plant*
255 *species*: $F_{1,106}=14.6$, $p=0.0002$, Fig. 1c), increased with plant age (*plant age*: $F_{1,106}=180.4$,
256 $p<0.0001$); and increased more in narrow-leaved milkweed relative to showy milkweed (*plant*
257 *species* \times *plant age*: $F_{1,105}=4.2$, $p=0.041$), though this weaker interaction effect suggests that this
258 metric of plant size did not continue to diverge over plant ontogeny (Fig 1c). By comparison,
259 total leaf area increased with plant age (*plant age*: $F_{1,106}=285.3$, $p<0.0001$, Fig. 1d), but did not
260 differ between species overall (*plant species*: $F_{1,106}=0.028$, $p=0.867$, Fig. 1d); while narrow-
261 leaved milkweed showed an accelerating trajectory of increasing leaf area with age, showy
262 milkweed showed a decelerating trajectory of increasing leaf area with age (*plant species* \times *plant*
263 *age*: $F_{1,105}=8.6$, $p=0.0041$, Fig. 1d).

264 In contrast, both defense traits showed a significant diverging pattern with plant age (Fig 1e and
265 1f). Overall, mean latex exudation was 11 times greater in showy milkweed compared to narrow-
266 leaved milkweed (*plant species*: $F_{1,106}=57.3$, $p<0.0001$, Fig. 1e), and the mass of exuded latex
267 increased with plant age for both species (*plant age*: $F_{1,106}=55.8$, $p<0.0001$, Fig. 1e). However,
268 the pattern of increased latex exudation with plant age differed strongly by plant species (*plant*

269 *species* × *plant age*: $F_{1,105}=77.6$, $p<0.0001$, Fig. 1e); while the mean mass of exuded latex
270 increased more than four-fold between 4 and 12 week-old narrow-leaved milkweeds (0.19 mg to
271 0.80 mg), it increased by almost 19 times between 4 and 12 week-old showy milkweeds (0.64
272 mg to 12.00 mg). Among 4-week-old plants, showy milkweed exuded 3.4 times more latex than
273 narrow-leaved milkweed (0.64 vs. 0.19 mg); among 12-week-old plants, showy milkweed
274 exuded 14.9 times more latex than narrow-leaved milkweed (12.00 vs. 0.80 mg). Trichome
275 densities showed a similar pattern; overall, trichomes were 4.2 times denser on showy milkweed
276 compared with narrow-leaved milkweed (*plant species*: $F_{1,106}=19.2$, $p<0.0001$, Fig. 1f), and
277 plants showed generally increasing mean trichome densities with plant age across both species
278 (2.2% among 4-week-old plants to 10.2% among 12-week-old plants, *plant age*: $F_{1,106}=19.5$,
279 $p<0.0001$, Fig. 1f). Trichome densities increased faster on showy milkweed than on narrow-
280 leaved milkweed (*plant species* × *plant age*: $F_{1,105}=22.3$, $p<0.0001$, Fig. 1f).

281 Plant age explained more of the observed variation in total stem length, total stem cross-sectional
282 area and total leaf area than plant species ($\Delta R^2=0.41$ vs $\Delta R^2=0.25$ for total stem length, $\Delta R^2=0.60$
283 vs $\Delta R^2=0.05$ for total stem cross-sectional area, $\Delta R^2=0.73$ vs $\Delta R^2=0.0001$ for total stem length).
284 The variance explained by plant age and plant species was comparable for total leaf count
285 ($\Delta R^2=0.31$ for plant age vs. $\Delta R^2=0.35$ for plant species), latex exudation ($\Delta R^2=0.26$ for plant age
286 vs $\Delta R^2=0.26$ plant species) and trichome density ($\Delta R^2=0.14$ for plant age vs $\Delta R^2=0.13$ plant
287 species).

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

289 Across all cohorts, the survival curves of monarch larvae differed on narrow-leaved and showy
290 milkweed ($\chi^2_1=4.8$, $p=0.028$), with caterpillars on narrow-leaved milkweed showing 10.4%

291 higher daily survival rates (91.6% vs 82.9%, Fig. 2). This result is consistent with the increased
292 hazard ratio (1.59, 95% CI 1.04-2.5, $p=0.034$) observed on showy milkweed relative to narrow-
293 leaved milkweed (Fig. S1). This effect of plant species on survival became stronger with plant
294 age; while the survival curves of caterpillars on both host plant species are largely overlapping
295 for 4-week-old plants ($\chi^2_1=0$, $p=0.99$, Fig. 2a), they are more different on 8- and 12-week-old
296 plants (8-week-old plants: $\chi^2_1=2.9$, $p=0.089$, Fig. 2b; 12-week-old plants: $\chi^2_1=2.9$, $p=0.086$, Fig.
297 2c). For example, caterpillars showed 2.4% greater daily survival rate on showy milkweed
298 among 4-week-old plants (Fig. 2a), but showed 10.1% and 8.4% greater daily survival on
299 narrow-leaved milkweed in weeks 8 and 12, respectively (Fig. 2b and 2c). We did not observe a
300 statistically significant overall effect of plant age on the survival curves of larvae developing on
301 either host plant species using log-rank tests (narrow-leaved milkweed, $\chi^2_2=2.8$, $p=0.247$; showy
302 milkweed, $\chi^2_2=0.8$, $p=0.684$), although a comparison between the youngest and oldest plant age
303 groups suggested a stronger pattern of lower survival on younger plants of narrow-leaved
304 milkweed ($\chi^2_1=2.9$, $p=0.0885$) compared to showy milkweed ($\chi^2_1=0.4$, $p=0.523$). However, we
305 did observe a trend towards reduced survival on younger plants across both species, which was
306 consistent with the estimated hazard ratios for 8-week-old plants (0.93, 95% CI 0.57-1.5,
307 $p=0.792$) and 12-week-old plants (0.70, 95% CI 0.41-1.2, $p=0.195$) relative to 4-week-old plants
308 (Fig. S1). Overall, caterpillars on both host plants species showed the lowest daily survival rates
309 on the youngest host plants (Fig. 2 and S2, 79.5% on narrow-leaved milkweed, 81.5% on showy
310 milkweed), with increasing daily survival rates on older plants (8-week-old plants: 92.8% on
311 narrow-leaved milkweed, 84.3% on showy milkweed; 12-week-old plants: 96.6% on narrow-
312 leaved milkweed, 89.1% on showy milkweed).

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

314 Across all host plant cohorts, larval growth was 5.7% higher on narrow-leaved milkweed than on
315 showy milkweed (0.79 mg/mg/day vs. 0.74 mg/mg/day; *plant species*, $F_{1,71}=4.0$, $p=0.049$, Fig. 3-
316 4, Fig. S2), with no significant differences in the effects of plant age on larval growth across
317 species (*plant species* \times *plant age*: $F_{2,70}=1.53$, $p=0.22$). However, developing on showy
318 milkweed (instead of narrow-leaved milkweed) had negative effects on relative growth rate that
319 were 4.2 times greater in 12-week-old plants compared with 4-week-old plants (4-week-old
320 plants, -0.027 mg/mg/day; 8-week-old plants, -0.016 mg/mg/day; 12-week-old plants, -0.114
321 mg/mg/day, Fig. 4a). This result suggests that species-specific differences in plant traits on
322 monarch growth are stronger in older plants than in younger plants. Overall, plant age explained
323 5 times more variation in overall larval growth rate than plant species ($\Delta R^2=0.207$ for plant age,
324 $\Delta R^2=0.043$ for plant species).

325 Caterpillars grew fastest on the youngest host plants in both species (Fig. 3-4, Fig. S2, *plant age*:
326 $F_{2,72}=9.6$, $p=0.0002$). The overall relative growth rates of caterpillars were fastest on 4-week-old
327 plants (0.82 mg/mg/day), and declined consistently on older host plants (8-week-old plants, 0.75
328 mg/mg/day; 12-week-old plants, 0.70 mg/mg/day, Fig. 3, see also Fig. S2 to S6). These
329 differences in larval growth rates were established early, with diverging trajectories for
330 caterpillars on plants of different ages appearing after the first experimental day (Fig. 3 and S2).
331 The effect of plant age on monarch growth rates was stronger in the first 24h of the experiment
332 than in the subsequent 10 days (Fig. 3, *plant age* \times *interval*: $\chi^2_9=6.7$; $p=0.0099$, see also Fig. S2),
333 though this short, transient period of increased growth created persistent differences in caterpillar
334 size throughout development (Fig. 3 and S2). Relative growth rates on 4-week-old plants were
335 1.9 times greater than those on 12-week-old plants across both plant species when looking at the
336 interval from day 0 to day 1 (*plant age*: $F_{1,96}=17.2$, $p<0.0001$, Fig. 3), and plant species identity

337 did not have a significant effect on these growth rates (*plant species*: $F_{1,96}=0.4$, $p=0.53$, Fig. 3).
338 In contrast, in the interval from day 1 to day 11, caterpillars growth rates did not differ
339 significantly among host plants of different ages (*plant age*: $F_{1,38}=0.58$, $p=0.45$, Fig. 3), but did
340 grow 9.1% faster on narrow-leaved milkweed compared with showy milkweed (*plant species*:
341 $F_{1,38}=4.1$, $p=0.051$, Fig. 3).

342 The effects of plant age on the realized growth rates of surviving larvae changed over the course
343 of the experiment, as caterpillars died or left their host plant due to insufficient resources. The
344 effects of plant age on caterpillar growth rates were variable but consistently negative throughout
345 the experiment for showy milkweed, but these effects showed larger changes for caterpillars
346 feeding on narrow-leaved milkweed (Fig. 4b). On narrow-leaved milkweed, the magnitude of the
347 negative plant age effect declined throughout the experiment, and the few ($N=4$) caterpillars that
348 survived to experimental day 15 showed a positive effect of plant age on larval growth rate (Fig
349 4b). This result suggests that while monarch caterpillars initially grew faster on younger plants,
350 continued growth throughout the experiment was increasingly limited by host plant size.

351 *Analyses of maximum larval size*

352 The expected maximum larval size attained, integrating both larval survival and growth, was
353 greatest for caterpillars developing on larger, older plants across both host plant species (263 mg
354 on 4-week-old plants, 317 mg on 8-week-old plants, 578 mg on 12-week old plants, *plant age*:
355 $F_{1,103}=3.0$, $p=0.053$, Fig. 5).

356 Caterpillars also attained larger sizes growing on narrow-leaved milkweed than on showy
357 milkweed. Across all cohorts, monarch larvae attained masses 2.7 times larger on narrow-leaved

358 milkweed compared with showy milkweed (570 mg vs. 210 mg; *plant species*: $F_{1,102}=10.2$,
359 $p=0.0018$, Fig. 5). The difference between the maximum larval sizes attained on the two host
360 plant species increased with plant age, from a 1.2-fold mean difference for 4-week-old plants to a
361 3-fold mean difference in 12-week-old plants, though these responses were variable and not
362 statistically significant (*plant species* \times *plant age*: $F_{1,101}=77.6$, $p=0.13$). Comparable proportions
363 of observed variation in maximum larval size were explained by plant species ($\Delta R^2=0.087$) and
364 plant age ($\Delta R^2=0.052$).

365 *Analyses of plant damage*

366 Caterpillars feeding on the youngest plants consumed a large proportion of available leaf area
367 before leaving their host plant (Fig. 6a and 6b, *plant age*: $F_{1,103}=3.4$, $p=0.038$), and caterpillars
368 that stayed on the youngest host plants longer consumed nearly all available leaf material (Fig.
369 6c and 6d). The effect of plant age was particularly evident on showy milkweed; caterpillars left
370 4-week-old showy milkweed after consuming 26.1% of available leaf area, while caterpillars left
371 12-week-old showy milkweed after consuming only 5.6% of leaf area (Fig. 6b). Across all plant
372 ages, percent damage was 1.4 times greater in narrow-leaved milkweed compared with showy
373 milkweed (*plant species*: $F_{1,102}=1.4$, $p=0.24$), and older showy milkweed deterred herbivory
374 more strongly than younger plants. Among 4-week-old plants, the percent damage was 1.2 times
375 higher in showy milkweed compared with narrow-leaved milkweed, but this pattern reversed in
376 8- and 12-week-old plants (2 times more herbivory in narrow-leaved milkweed among 8-week-
377 old plants, and 2.5 times more herbivory in narrow-leaved milkweed among 12-week-old plants,
378 *plant species* \times *plant age*: $F_{2,101}=1.2$, $p=0.30$).

379 **Discussion**

380 Taken together, these results show that species-specific and age-varying host plant traits
381 significantly affect the growth and survival of monarch caterpillars. The plant traits that
382 herbivores experience changed significantly over seasonal time following species-specific
383 trajectories, and those changes in plant traits had strong effects on the developmental success of
384 monarch larvae. Potentially in combination with seasonal changes in abiotic conditions and the
385 biotic natural enemy community, these species-specific and age-varying changes in plant traits
386 are likely to be important factors structuring seasonal windows of opportunity for monarch
387 development.

388 Plant traits showed consistent differences between species and were strongly structured by plant
389 age (Fig. 1). The species-specific differences between host plants increased with plant age for
390 total stem length (Fig. 1a) and total number of leaves (Fig. 1b), reflecting species-specific
391 differences in plant architecture. By comparison, total stem cross-sectional area (Fig. 1c) and
392 total leaf area (Fig. 1d) showed relatively non-diverging ontogenetic trajectories suggesting that,
393 despite large differences in their architecture, the plant biomass available to herbivores did not
394 diverge between species as markedly over ontogeny as other species-specific traits, including
395 defensive traits (Fig. 1e and 1f). Broadly, these seasonal changes in plant defense traits could
396 result from the intrinsic ontogenetic trajectory of constitutive traits (Barton and Koricheva 2010),
397 the accumulation of plastic traits over time (e.g., induced resistance or susceptibility, Rasmann et
398 al. 2009), or some combination of these processes. While induced responses to herbivory could
399 contribute to the realized seasonal patterns of plant traits observed in other contexts, the observed
400 patterns in our current study seem unlikely to represent induced responses to prior herbivory
401 because we did not detect any non-monarch herbivores in the system throughout the study.
402 While host plant species identity was also informative in our study, plant age often explained a

403 comparable proportion of the observed variation in plant traits. Older plants showed more
404 strongly differentiated species-specific plant traits in this study, while younger plants of both
405 species were unexpectedly similar. These two milkweed species express distinct plant defense
406 syndromes as mature plants (Agrawal and Fishbein 2006). In our study, species-level differences
407 emerged over ontogeny as the defensive traits of these species diverged with increasing plant age
408 (Fig. 1e and 1f). These findings extend the meta-analytic dataset described by Barton and
409 Koricheva (2010) which documented generally increasing constitutive chemical defenses from
410 the seedling stage to maturity in herbaceous plants, but lacked a sufficient sample size of studies
411 to identify general ontogenetic patterns in physical defense traits with herbaceous plants (but see
412 Traw and Feeny 2008). The results of this current study show significant changes in both types
413 of plant defense traits over ontogeny, with trajectories that differed strongly between the two
414 milkweed species. Though future studies will be necessary to capture seasonal patterns in a
415 broader set of plant traits that could potentially affect windows of opportunity for monarchs (e.g.,
416 water content, specific leaf area, secondary compounds and C:N content, Agrawal and Fishbein
417 2006), the patterns observed in this study are consistent with seasonal patterns previously
418 observed for trichome density and leaf toughness in these two species (Yang and Censer 2020),
419 and suggest that several traits relevant to monarch development may change with plant age.

420 In this study, plant age explained substantially more variation in overall larval growth rate than
421 plant species (Fig. 3). Across larval development, monarch caterpillars grew fastest on the
422 youngest plants of both species, and this overall pattern was strongly (and unexpectedly) driven
423 by large differences in growth rate during the first 24h of larval development (Fig. 3). Plant age-
424 associated differences in larval growth rate during the first day after egg introduction created
425 substantial differences in larval size that persisted throughout the rest of larval development (Fig.

426 3 and S2). This result is consistent with a previous study showing that monarch caterpillars grew
427 faster on milkweed leaves with partially severed petioles (and thus reduced latex pressure)
428 during the first 2-4 days of larval development on four out of nine species of milkweed examined
429 (Zalucki et al. 2001); in both studies, early instar caterpillars grew faster on host leaves with
430 reduced latex exposure. These findings are also consistent with studies indicating that adult
431 monarchs preferentially oviposit on younger host plants (Zalucki and Kitching 1982), as well as
432 the recent vegetative regrowth of host plants that have been strategically mowed for habitat
433 management (Fischer 2015, Haan and Landis 2019, Knight et al. 2019). Similar preferential
434 herbivory on rapid regrowth has been observed in other systems in response natural disturbance
435 regimes (e.g., Spiller and Agrawal 2003). Our results suggest that plant age is a key determinant
436 of variation in this defensive trait, and show that the strongest effects of these age-associated
437 differences in plant traits on growth rate occur in the first 24h of larval development.

438 Overall, monarch caterpillars experienced greater developmental success (i.e., faster growth, Fig.
439 4a; higher survivorship, Fig. 2c; and larger maximum larval sizes, Fig. 5) on narrow-leaved
440 milkweed than on showy milkweed, and the difference between host plant species was
441 particularly strong for older host plants (Figs. 2c, 4a and 5). These findings are consistent with
442 our observation that of increasing species-associated trait differentiation with increasing plant
443 age. These patterns are also consistent with the different seasonal windows of opportunity that
444 have been previously observed for monarchs feeding on these two host plants (Yang and Censer
445 2020): while monarchs showed two seasonal windows of opportunity on narrow-leaved
446 milkweed, those feeding on showy milkweed only showed the early season window. We suggest
447 that increasing plant defense traits over ontogeny could limit late season windows of opportunity
448 in showy milkweed. The findings of our current study are also consistent with the hypothesis that

449 the two seasonal window of opportunity observed on narrow-leaved milkweed could correspond
450 to a “double-dipping” herbivore strategy (*sensu* White 2015, Che-Castaldo et al. 2019) in which
451 monarch larvae successfully use both vigorously growing and senescing plant tissues. Future
452 studies will be necessary to more specifically examine how increasingly senescent plant traits
453 affect larval success in the second window of opportunity observed in this system.

454 Our findings indicate that the early season window of opportunity may be influenced by
455 temporal variation in both resource *quantity* (i.e., plant size) and *quality* (as affected by age-
456 varying defensive traits). Younger plants of both species provided higher quality resources that
457 allowed for faster larval growth rates initially (Fig. 3), but for narrow-leaved milkweed, older
458 plants provided greater resource quantity over a longer developmental timescale (Fig. 4b). These
459 changes in the developmental limitations imposed by seasonally varying resource quality and
460 quantity are further supported by observed patterns of herbivore damage and larval survival. On
461 the youngest plants, the developmental success of larval monarchs appeared to be ultimately
462 limited by the availability of host plant biomass (*i.e.*, resource quantity). Caterpillars on the
463 youngest plants fed on less-defended (*i.e.*, higher-quality) resources and grew fast (Figs. 1 and
464 3); they often consumed a substantial proportion of their host plants before starving or attempting
465 to disperse to a second host plant (Fig. 6). As a result, these caterpillars showed steep and short
466 survivorship curves on both host plant species; in general, these caterpillars grew fast and died
467 young (Figs. 2 and 3). In comparison, caterpillars developing on the oldest host plants seemed to
468 be limited by the *quality* of host plant biomass as constrained by plant defense traits. These
469 caterpillars showed the slowest growth rates (Fig. 3), but rarely consumed their entire host plant
470 (Fig. 6), and showed the longest survivorship curves (Fig. 2).

471 The relative importance of milkweed *quality* and *quantity* as factors that structure seasonal
472 windows of opportunity for monarch development could also depend on the density of
473 milkweeds in available habitat patches, as well as the density of monarch oviposition. This
474 experiment was conducted with singular host plants as replicates, where attempted dispersal by
475 larvae below the pupation threshold size was assumed to be fatal. This assumption is likely to be
476 a reasonable one in habitats where individual plants are widely spaced, where biotic or abiotic
477 conditions limit the ability of monarch caterpillars to move between neighboring plants (e.g., due
478 to increased thermal stresses or predation risk), or if monarchs show limited abilities to locate
479 second host plants. Alternatively, high-density patches of young milkweed plants could
480 potentially provide high-quality host plant resources with reduced plant-quantity constraints; this
481 suggests that higher density patches could potentially allow for earlier seasonal windows of
482 opportunity, consistent with the results of previous field experiments (Yang and Censer 2020).
483 Further studies specifically examining the risk of plant-to-plant movement would be valuable to
484 better understand the role of plant density on seasonal window of opportunity for monarch
485 development. Moreover, while this study investigated the effects of plants traits in two milkweed
486 species during their first growing season, additional studies assessing other host plant species,
487 additional plant traits (including physical, chemical and indirect defense traits), and a wider
488 range of plant ages (especially considering plants in their second growing season and beyond)
489 will be necessary to assess the generality of the patterns observed here. In addition, the role of
490 seasonal variation in climatic conditions and natural enemy interactions remains uncertain, and
491 both of these factors have the potential to interact with the effects of plant trait variation in nature
492 More broadly, these findings contribute to the general observation that temporal variation in
493 plant traits can strongly affect plant-herbivore interactions (e.g., Van der Wal et al. 2000, Van

494 Asch et al. 2007, Barton and Koricheva 2010, Che-Castaldo et al. 2019). The results of this study
495 indicate that the effects of plant age on monarch developmental success are comparable to and
496 sometimes larger than those of plant species identity. Acknowledging substantial temporal
497 variation in plant traits does not diminish the importance of species-level trait assessments;
498 expectations about how plant traits affect herbivores are often usefully structured around species-
499 level characterizations, and such studies can identify clusters of species that share key traits
500 (Agrawal and Fishbein 2006). In combination with such species-level trait assessments, the
501 temporal dimensions of plant age and seasonal variation provide additional orthogonal axes to
502 examine variation in plant defense traits and their effects on herbivores.

503 These results may also suggest some specific implications for our understanding of milkweed-
504 monarch interactions in a changing world, and the potential for milkweed limitation in the
505 population dynamics of monarchs (Nail et al. 2015, Pleasants et al. 2016, Inamine et al. 2016,
506 Thogmartin et al. 2017), and especially in western North America (Espeset et al. 2016, Pelton et
507 al. 2019). If age-varying plant traits have strong effects on the developmental prospects of
508 monarchs generally, monarchs may experience changing constraints on larval development as
509 their host plant traits develop through the season. In particular, the development of monarch
510 larvae in the early season could potentially be limited by small host plant size, even in habitats
511 with abundant host plant resources later in the season. The potential for milkweed limitation in
512 the early season would potentially be exacerbated if seasonal intervals where growth is limited
513 by host plant quantity coincided with periods of high oviposition density. Conversely, if later-
514 season milkweeds generally present stronger defensive traits than early-season plants, monarchs
515 could potentially experience reduced growth rates during periods of lower resource quality even
516 when the apparent availability of host plant resources is high. Because these potential seasonal

517 limitations are mediated by changes in resource quality as much as resource quantity, estimates
518 of milkweed abundance and spatial distribution by themselves may not capture a key temporal
519 dimension of the dynamic resource landscape. If a wider range of milkweed species show the
520 kinds of species-specific and age-varying traits observed in this current study, it would suggest
521 that migrating monarchs face a complex and dynamic landscape of potential host plants with
522 traits that are affected by phenology and ontogeny as much as species distributions. The
523 complexity of this dynamic resource landscape likely presents a challenge for migrating
524 monarchs as well as the ecologists that aim to study them. Developing a more temporally explicit
525 approach may be necessary to assess the combined effects of plant age and species identity on
526 the spatial distribution and temporal availability of milkweed resources on a continental scale.
527 Further, it is unclear how monarch migrations and the dynamics of this seasonally variable
528 landscape will change with global warming. The age of host plants that migrating monarchs
529 encounter each year is likely to be affected by both the environmental cues that influence
530 milkweed phenology, as well as the continental-scale drivers of monarch migration. The
531 potential for significant mis-matches in the relative phenologies of milkweeds and monarchs
532 remains uncertain, though the magnitude of observed plant-age effects in this study suggests that
533 the consequences of such phenological mis-matches, if realized, could be substantial. Further
534 studies will be necessary to identify the environmental cues that drive phenological responses in
535 a range of milkweed species, and how phenological variation across different species
536 distributions affects the overall spatiotemporal availability of milkweed resources throughout
537 each season.

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650

651

652 **Figure legends**

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

657

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

660

661 **Figure 3.** The effects of plant age on mean relative growth rates by plant species and
662 experimental day. The effects of plant age on the overall (lifetime) relative growth rates of
663 caterpillars are shown for a) narrow-leaved milkweed and b) showy milkweed. The interval-
664 specific relative growth rates for caterpillars during experimental days 0 to 1 are shown for c)
665 narrow-leaved milkweed and d) showy milkweed. The interval-specific relative growth rates for
666 caterpillars during experimental days 1 to 11 are shown for e) narrow-leaved milkweed and f)
667 showy milkweed. These figures show that the persistent negative effects of plant age on
668 caterpillar size shown in Figs 3 and 4 emerges from growth differences that occur in the first 24h
669 of larval development. Point color and point shape represent plant age. Error bars represent 95%
670 confidence intervals.

671

672 **Figure 4.** a) The mean plant species effect size for each plant age. These effect sizes represent
673 the linear model coefficients for the effect of showy milkweed relative to narrow-leaved
674 milkweed on surviving larval mass. Bar color represents plant age. Showy milkweed had a

675 negative effect on larval mass in each plant age cohort, but this effect was larger in the oldest
676 cohort. b) The mean plant age effect size for the surviving population on each experimental day,
677 separated by host plant species. These effect sizes represent the linear model coefficient for plant
678 age effects on surviving larval mass. Bar color represents plant species. The effects of plant age
679 are consistently negative on showy milkweed. On narrow-leaved milkweed, the effect of plant
680 age is generally negative, but the magnitude of these effects declines over time.

681

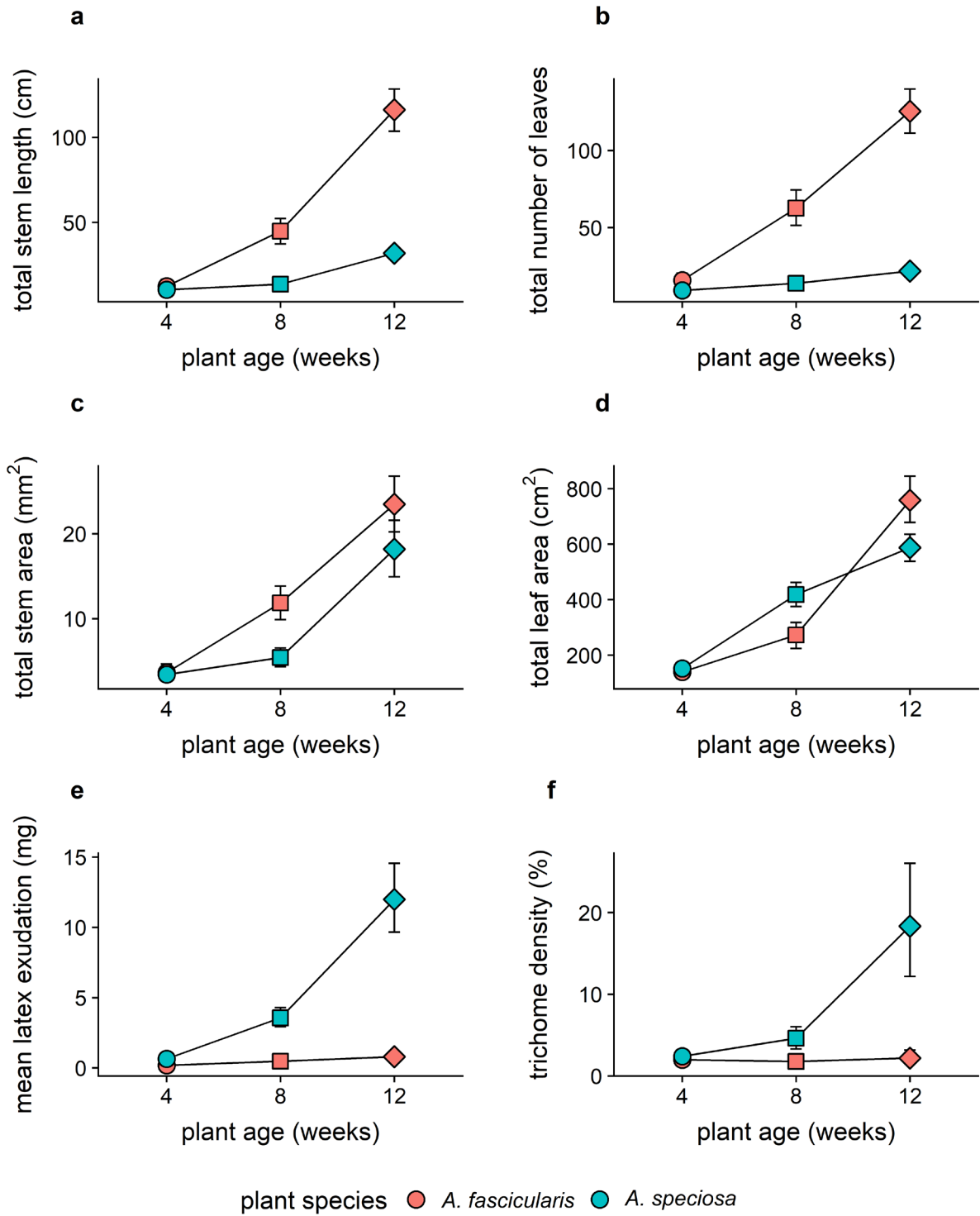
682 **Figure 5.** The maximum size (mass and length) attained by caterpillars developing on two host
683 plant species of different ages. Color represents plant species, and point shape represents plant
684 age. Error bars represent 95% confidence intervals.

685

686 **Figure 6.** Plant damage by host plant species and age. Mean maximum herbivore damage for
687 plants of each age cohort for a) narrow-leaved milkweed and b) showy milkweed. Mean
688 maximum damage represents the percent of leaf area consumed by monarchs before leaving their
689 host plant. Point color and point shape represent plant age. Error bars represent 95% confidence
690 intervals. b) Percent damage on plants over time, showing the subset of plants with surviving
691 caterpillars present at each time point. Point color and point shape represent plant age. Point size
692 reflects the size of the surviving caterpillar population. Error bars represent 95% confidence
693 intervals.

694

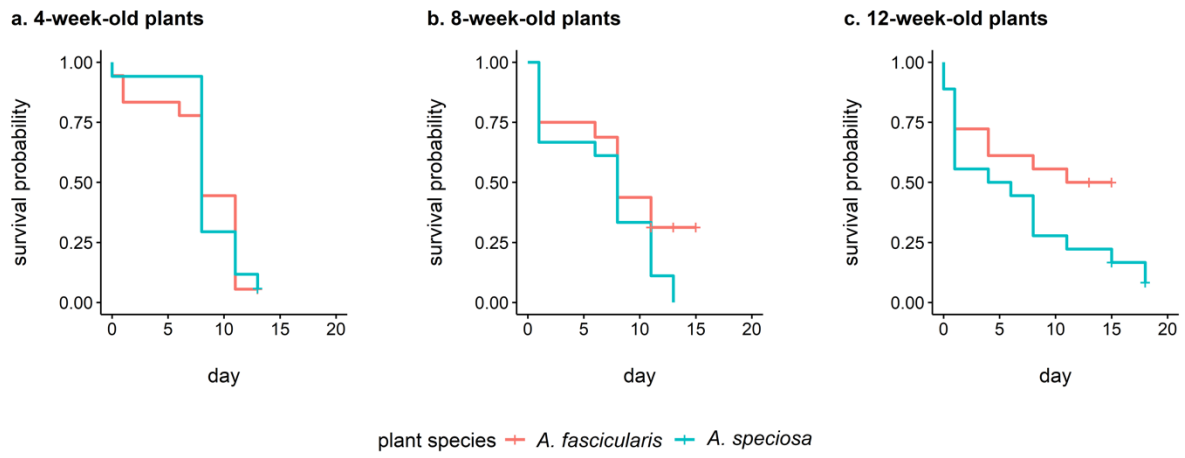
695 **Figure 1**



697

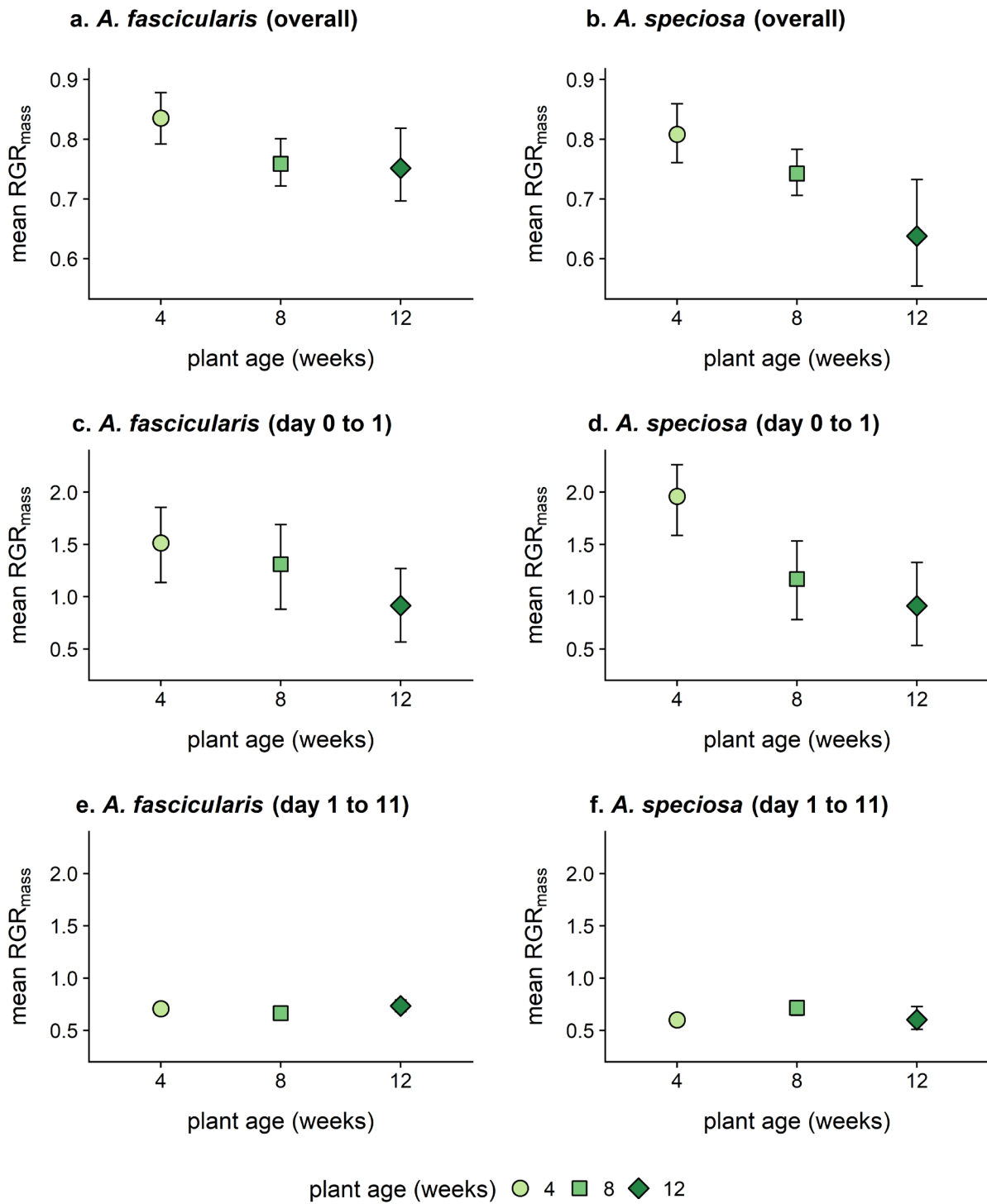
698

699 **Figure 2**



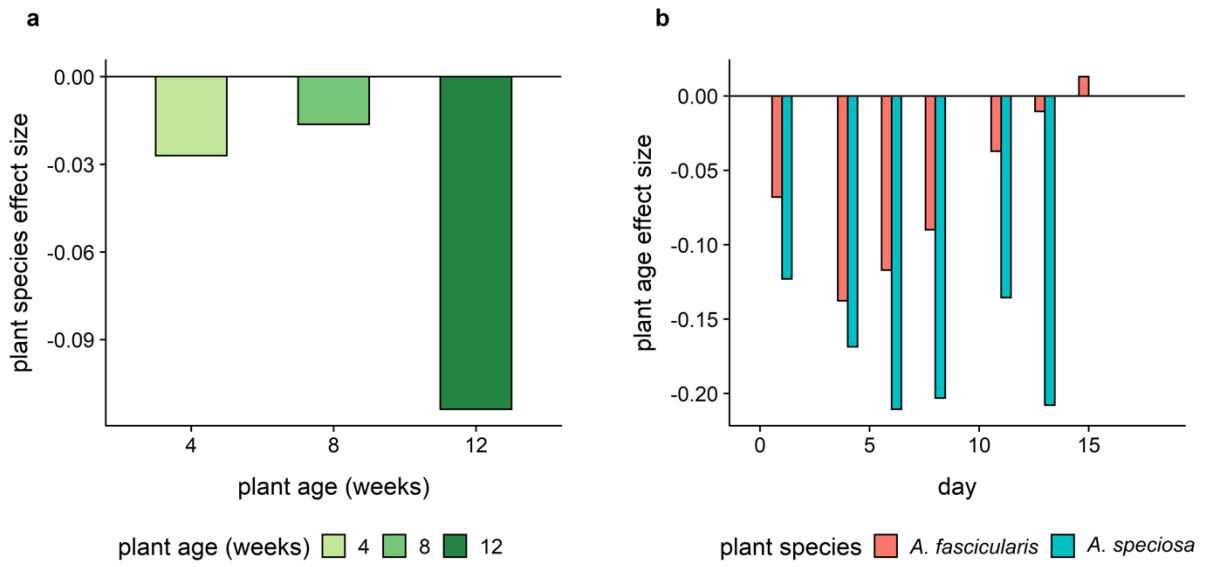
701

702



706 **Figure 4**

707

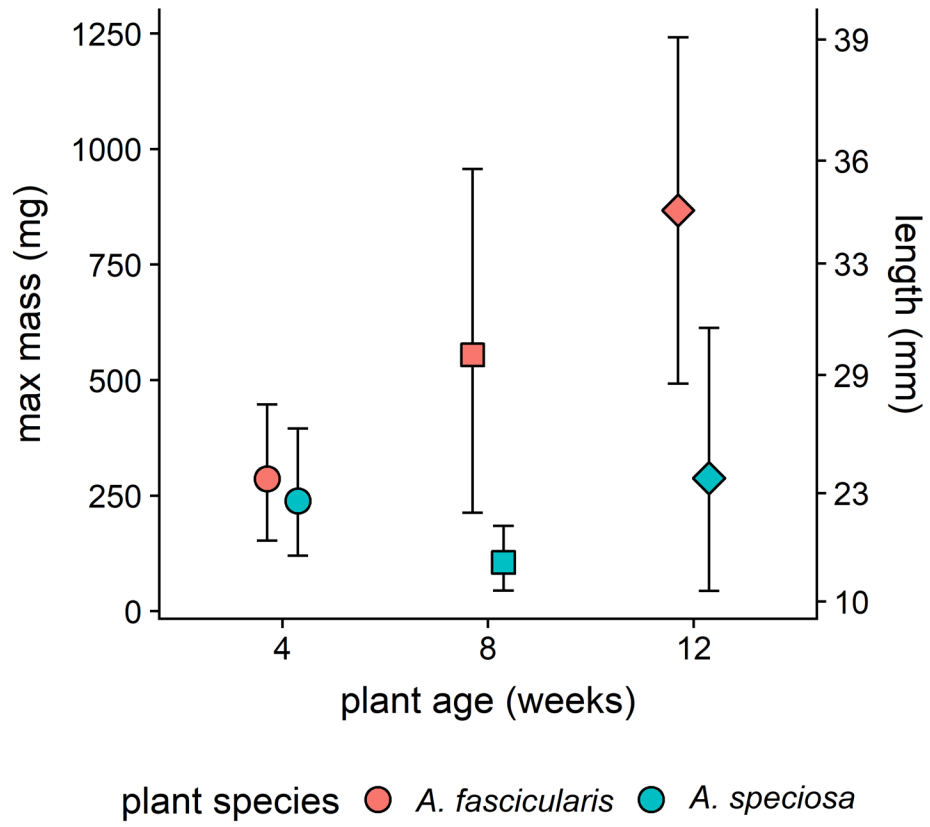


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711 **Figure 5**

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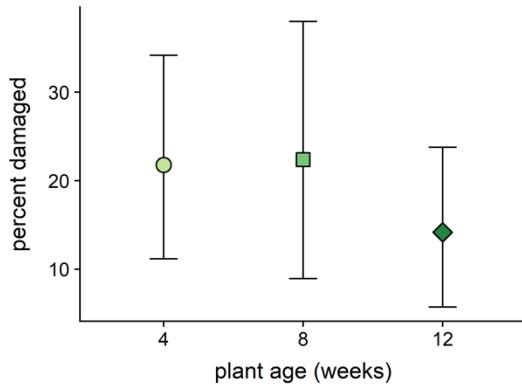
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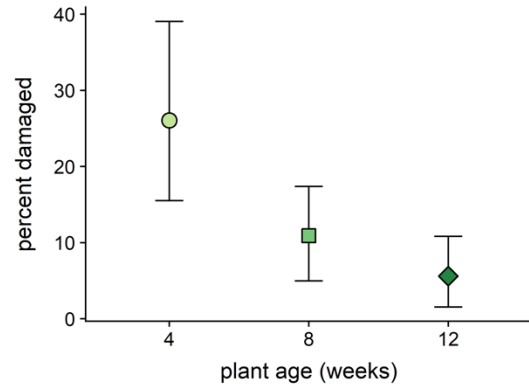
716 **Figure 6**

717

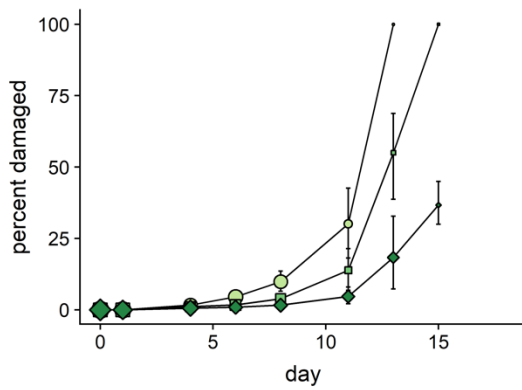
a. *A. fascicularis* mean max damage



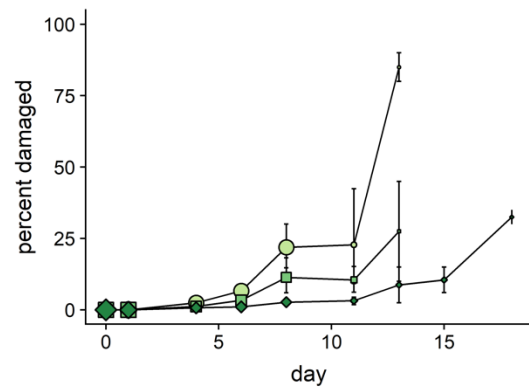
b. *A. speciosa* mean max damage



c. *A. fascicularis* damage by day



d. *A. speciosa* damage by day



plant age (weeks) ○ 4 ■ 8 ◆ 12

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