

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 Cezner 2019). 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 Cezner 2019), 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 Cezner 2019), 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 Cezner 2019). 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 2019), 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

96 to the California Central Valley, and the seeds used in this study were propagated from local  
97 source populations (Hedgerow Farms, Winters, CA, USA). Each cohort of seeds was started  
98 directly into 2.5 L containers filled with potting soil (1:1:1 ratio of sand, compost and peat moss  
99 by volume with 1.78 kg/m<sup>3</sup> dolomite), which were irrigated and fertilized (electrical  
100 conductivity, EC = 1.5-1.6 mS cm<sup>-1</sup>) via drip emitters as necessary to prevent water and nutrient  
101 limitation. Plants from each cohort were randomly interspersed in a single greenhouse  
102 (approximately 20-35° C) at the University of California, Davis Orchard Park Research  
103 Greenhouse Facility (38.543129° N, 121.763425° W) with individual plants spaced on open  
104 grate wire benches to prevent contact between the leaves of neighboring plants. These three  
105 cohorts were started approximately 4 weeks apart to yield three distinct age classes of milkweed  
106 (25-day, 57-day and 86-day-old plants, hereafter, the 4, 8 and 12-week cohorts) for each species  
107 (N=18 plants of each species in each age class, N=108 plants total) at the start of the experiment.

### 108 *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  $\times$  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 Censer 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 *survdiff* 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 5), with no significant differences in the effects of plant age on larval growth across species  
317 (*plant species*  $\times$  *plant age*:  $F_{2,70}=1.53$ ,  $p=0.22$ ). However, developing on showy milkweed  
318 (instead of narrow-leaved milkweed) had negative effects on relative growth rate that were 4.2  
319 times greater in 12-week-old plants compared with 4-week-old plants (Fig. 6a; 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. 6a). 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. 3-5, *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. 4, see also Fig. S2 to S5). 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 5).  
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. 5, *plant age*  $\times$  *interval*:  $\chi^2_9=6.7$ ;  $p=0.0099$ , see also Fig. S6),  
333 though this short, transient period of increased growth created persistent differences in caterpillar  
334 size throughout development (Fig. 3). Relative growth rates on 4-week-old plants were 1.9 times  
335 greater than those on 12-week-old plants across both plant species when looking at the interval  
336 from day 0 to day 1 (*plant age*:  $F_{1,96}=17.2$ ,  $p<0.0001$ , Fig. 5), and plant species identity did not



337 have a significant effect on these growth rates (*plant species*:  $F_{1,96}=0.4$ ,  $p=0.53$ , Fig. 5). In  
338 contrast, in the interval from day 1 to day 11, caterpillars growth rates did not differ significantly  
339 among host plants of different ages (*plant age*:  $F_{1,38}=0.58$ ,  $p=0.45$ , Fig. 5), but did grow 9.1%  
340 faster on narrow-leaved milkweed compared with showy milkweed (*plant species*:  $F_{1,38}=4.1$ ,  
341  $p=0.051$ , Fig. 5).

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. 6b). 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 6b). 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. 7).

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. 7). 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. 8a and 8b, *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 8c and 8d). 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. 8b). 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. Because the growth form of showy milkweed is largely single-  
392 stemmed with large leaves, whereas narrow-leaved milkweed is generally branching with smaller  
393 leaves, total stem cross-sectional area and total leaf area are probably more indicative of the plant  
394 biomass available to herbivores than total stem length and total number of leaves. By  
395 comparison, total stem cross-sectional area (Fig. 1c) and total leaf area (Fig. 1d) showed  
396 relatively non-diverging ontogenetic trajectories suggesting that, despite large differences in their  
397 architecture, the plant biomass available to herbivores did not diverge between species as  
398 markedly over ontogeny as other species-specific traits, including defensive traits (Fig. 1e and  
399 1f). Broadly, these results indicate that the traits experienced by herbivores are strongly  
400 influenced by plant age. While host plant species identity was also informative, plant age often  
401 explained a comparable proportion of the observed variation in plant traits in this study. These  
402 findings extend the meta-analytic dataset described by Barton and Koricheva (2010) which

403 documented generally increasing constitutive chemical defenses from the seedling stage to  
404 maturity in herbaceous plants, but lacked a sufficient sample size of studies to identify general  
405 ontogenetic patterns in physical defense traits with herbaceous plants (but see Traw and Feeny  
406 2008). The results of this current study show significant changes in both plant defense traits over  
407 ontogeny, but the trajectories of these traits also differed strongly between the two milkweed  
408 species.

409 By comparison, plant age explained substantially more variation in overall larval growth rate  
410 than plant species (Fig. 4). Across larval development, monarch caterpillars grew fastest on the  
411 youngest plants of both species, and this overall pattern was strongly (and unexpectedly) driven  
412 by large differences in growth rate during the first 24h of larval development (Fig. 5). Plant age-  
413 associated differences in larval growth rate during the first day after egg introduction created  
414 substantial differences in larval size that persisted throughout the rest of larval development (Fig.  
415 3). This result is consistent with a previous study showing that monarch caterpillars grew faster  
416 on milkweed leaves with partially severed petioles (and thus reduced latex pressure) during the  
417 first 2-4 days of larval development on four out of nine species of milkweed examined (Zalucki  
418 et al. 2001); in both studies, early instar caterpillars grew faster on host leaves with reduced latex  
419 exposure. These findings are also consistent with studies indicating that adult monarchs  
420 preferentially oviposit on younger host plants (Zalucki and Kitching 1982), as well as the recent  
421 vegetative regrowth of host plants that have been strategically mowed for habitat management  
422 (Fischer 2015, Haan and Landis 2019, Knight et al. 2019). Similar preferential herbivory on  
423 rapid regrowth has been observed in other systems in response natural disturbance regimes (e.g.,  
424 Spiller and Agrawal 2003). Our results suggest that plant age is a key determinant of variation in

425 this defensive trait, and show that the strongest effects of these age-associated differences in  
426 plant traits on growth rate occur in the first 24h of larval development.

427 Monarch caterpillars experienced greater developmental success (i.e., attained larger maximum  
428 larval sizes) on narrow-leaved milkweed than on showy milkweed (Fig. 7), and the difference  
429 between host plant species was particularly strong for older host plants (Fig. 7). These findings  
430 are consistent with our observation that older plants showed more strongly differentiated species-  
431 specific plant traits in this study, while younger plants of both species were unexpectedly similar  
432 in their traits. These two milkweed species express distinct plant defense syndromes as mature  
433 plants (Agrawal and Fishbein 2006). In our study, species-level differences emerged over  
434 ontogeny as the defensive traits of these species diverged with increasing plant age (Fig. 1e and  
435 1f). On the oldest host plants, both growth (Fig. 4 and 6a) and survivorship (Fig 2c) were  
436 strongly structured by plant species; on both counts, caterpillars developing on narrow-leaved  
437 milkweed fared better than those developing on showy milkweed. These patterns are consistent  
438 with the different seasonal windows of opportunity that have been previously observed for  
439 monarchs feeding on these two host plants (Yang and Censer 2019): while monarchs showed  
440 two seasonal windows of opportunity on narrow-leaved milkweed, those feeding on showy  
441 milkweed only showed the early season window. We suggest that increasing plant defense traits  
442 over ontogeny could limit late season windows of opportunity in showy milkweed. The findings  
443 of our current study are also consistent with the hypothesis that the two seasonal window of  
444 opportunity observed on narrow-leaved milkweed could correspond to a “double-dipping”  
445 herbivore strategy (*sensu* White 2015, Che-Castaldo et al. 2019) in which monarch larvae  
446 successfully use both vigorously growing and senescing plant tissues. Our findings indicate that  
447 the bounds of the early season window of opportunity may be influenced by temporally varying

448 resource quantity (*i.e.*, plant size) and quality (as affected by age-varying defensive traits).  
449 Future studies will be necessary to more specifically examine how increasingly senescent plant  
450 traits affect larval success in the second window of opportunity observed in this system.

451 In this study, differences between plant species in the effect of plant age became more apparent  
452 as the experiment progressed (Fig. 6b); while caterpillars generally developed better on younger  
453 plants than on older plants, the effect of plant age was more consistently negative throughout  
454 monarch development on showy milkweed (Fig. 6b). In comparison, when developing on  
455 narrow-leaved milkweed, caterpillars early in the experiment showed a smaller negative effect of  
456 plant age relative to those developing on showy milkweed, and these negative effects became  
457 weaker throughout the experiment (Fig 6b). This plant age effect trajectory on narrow-leaved  
458 milkweed illustrates the multiple and potentially conflicting effects of plant age: while younger  
459 plants provided higher *quality* resources that allowed for faster larval growth rates initially, older  
460 plants provided greater resource *quantity* over a longer developmental timescale. These changes  
461 in the developmental limitations imposed by seasonally varying resource quality and quantity are  
462 further supported by observed patterns of herbivore damage and larval survival. On the youngest  
463 plants, the developmental success of larval monarchs appeared to be ultimately limited by the  
464 availability of host plant biomass (*i.e.*, resource quantity). Caterpillars on the youngest plants fed  
465 on less-defended (*i.e.*, higher-quality) resources and grew fast (Figs. 1 and 4); they often  
466 consumed a substantial proportion of their host plants before starving or attempting to disperse to  
467 a second host plant (Fig. 8). As a result, these caterpillars showed steep and short survivorship  
468 curves on both host plant species; in general, these caterpillars grew fast and died young (Fig. 2).  
469 In comparison, caterpillars developing on the oldest host plants seemed to be limited by the  
470 *quality* of host plant biomass as constrained by plant defense traits. These caterpillars showed the

471 slowest growth rates (Fig. 4), but rarely consumed their entire host plant (Fig. 8), and showed the  
472 longest survivorship curves (Fig. 2).

473 The relative importance of milkweed *quality* and *quantity* as factors that structure seasonal  
474 windows of opportunity for monarch development could also depend on the density of  
475 milkweeds in available habitat patches. This experiment was conducted with singular host plants  
476 as replicates, where attempted dispersal by larvae below the pupation threshold size was assumed  
477 to be fatal. This assumption is likely to be a reasonable one in habitats where individual plants  
478 are widely spaced, where biotic or abiotic conditions limit the ability of monarch caterpillars to  
479 move between neighboring plants (e.g., due to increased thermal stresses or predation risk), or if  
480 monarchs show limited abilities to locate second host plants. Alternatively, high-density patches  
481 of young milkweed plants could potentially provide high-quality host plant resources with  
482 reduced plant-quantity constraints; this suggests that higher density patches could potentially  
483 allow for earlier seasonal windows of opportunity, consistent with the results of previous field  
484 experiments (Yang and Censer 2019). Further studies specifically examining the context-  
485 dependent risk of plant-to-plant movement in milkweed patches of varying density, in different  
486 habitats, at different larval sizes, and at different times of year would be valuable to better  
487 understand how plant density could affect seasonal window of opportunity for monarch  
488 development. Moreover, while this study investigated the effects of plants traits in two milkweed  
489 species during their first growing season, additional studies assessing other host plant species,  
490 additional plant traits (including physical, chemical and indirect defense traits), and a wider  
491 range of plant ages (especially considering plants in their second growing season and beyond)  
492 will be necessary to assess the generality of the patterns observed here.

493 The results of this study indicate that age-varying plant traits likely play a strong role in  
494 structuring seasonal windows of opportunity for monarch caterpillars. However, because this  
495 experiment was designed to isolate the effects of age-varying plant traits without the contributing  
496 effects of temporally variable abiotic and top-down factors, the role of seasonal variation in  
497 climatic conditions and natural enemy interactions remains uncertain. Both additional factors are  
498 likely to interact with the effects of plant trait variation in nature; for example, delayed growth  
499 rates on lower quality host plants could expose larvae to greater predation risk. While both  
500 additional factors are likely to affect seasonal windows of opportunity for larval development,  
501 either independently or interactively with seasonal changes in host plant quality or quantity,  
502 separate experimental studies will be necessary to quantify their effects.

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



514 These results may also suggest some specific implications for our understanding of milkweed-  
515 monarch interactions in a changing world, and the potential for milkweed limitation in the  
516 population dynamics of monarchs (Nail et al. 2015, Pleasants et al. 2016, Inamine et al. 2016,  
517 Thogmartin et al. 2017), and especially in western North America (Espeset et al. 2016, Pelton et  
518 al. 2019). If age-varying plant traits have strong effects on the developmental prospects of  
519 monarchs generally, monarchs may experience changing constraints on larval development as  
520 their host plant traits develop through the season. In particular, the development of monarch  
521 larvae in the early season could potentially be limited by small host plant size, even in habitats  
522 with abundant host plant resources later in the season. Conversely, if later-season milkweeds  
523 generally present stronger defensive traits than early-season plants, monarchs could potentially  
524 experience reduced growth rates during periods of lower resource quality even when the  
525 apparent availability of host plant resources is high. Because these potential seasonal limitations  
526 are mediated by changes in resource quality as much as resource quantity, estimates of milkweed  
527 abundance and spatial distribution by themselves may not capture a key temporal dimension of  
528 the dynamic resource landscape. If a wider range of milkweed species show the kinds of species-  
529 specific and age-varying traits observed in this current study, it would suggest that migrating  
530 monarchs face a complex and dynamic landscape of potential host plants with traits that are  
531 affected by phenology and ontogeny as much as species distributions. The complexity of this  
532 dynamic resource landscape likely presents a challenge for migrating monarchs as well as the  
533 ecologists that aim to study them. Developing a more temporally explicit approach may be  
534 necessary to assess the combined effects of plant age and species identity on the spatial  
535 distribution and temporal availability of milkweed resources on a continental scale. Further, it is  
536 unclear how monarch migrations and the dynamics of this seasonally variable landscape will

537 change with global warming. The age of host plants that migrating monarchs encounter each  
538 year is likely to be affected by both the environmental cues that influence milkweed phenology,  
539 as well as the continental-scale drivers of monarch migration. The potential for significant mis-  
540 matches in the relative phenologies of milkweeds and monarchs remains uncertain, though the  
541 magnitude of observed plant-age effects in this study suggests that the consequences of such  
542 phenological mis-matches, if realized, could be substantial. Further studies will be necessary to  
543 identify the environmental cues that drive phenological responses in a range of milkweed  
544 species, and how phenological variation across different species distributions affects the overall  
545 spatiotemporal availability of milkweed resources throughout each season.

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### 655 **Figure legends**

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

660

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

663

664 **Figure 3.** Mean surviving larval size over time for caterpillars developing on a) narrow-leaved  
665 milkweed and b) showy milkweed host plants, plotted on a log scale. The minimum observed  
666 mass value (0.75 mg) was added to each observation to allow the plotting of observed zero  
667 values. Host plant age affects larval size on both plant species throughout the experiment. Point  
668 area reflects the size of the surviving population, color and point shape represent plant age. Error  
669 bars represent 95% confidence intervals.

670

671 **Figure 4.** Overall mean relative growth rates for caterpillars developing on each plant age cohort  
672 of a) narrow-leaved milkweed and b) showy milkweed. This figures shows an effect of plant age  
673 on the overall (lifetime) growth rates of caterpillars. Point color and point shape represent plant  
674 age. Error bars represent 95% confidence intervals.

675

676 **Figure 5.** Interval-specific relative growth rates for caterpillars developing on narrow-leaved  
677 milkweed during a) experimental days 0 to 1 and b) experimental days 1 to 11, and for  
678 caterpillars developing on showy milkweed during c) experimental days 0 to 1 and d)  
679 experimental days 1 to 11. These figures show that the persistent negative effects of plant age on  
680 caterpillar size shown in Figs 3 and 4 emerges from growth differences in the first 24h of larval  
681 development. Point color and point shape represent plant age. Error bars represent 95%  
682 confidence intervals.

683

684 **Figure 6.** a) The mean plant species effect size for each plant age. These effect sizes represent  
685 the linear model coefficients for the effect of showy milkweed relative to narrow-leaved  
686 milkweed on surviving larval mass. Bar color represents plant age. Showy milkweed had a  
687 negative effect on larval mass in each plant age cohort, but this effect was larger in the oldest  
688 cohort. b) The mean plant age effect size for the surviving population on each experimental day,  
689 separated by host plant species. These effect sizes represent the linear model coefficient for plant  
690 age effects on surviving larval mass. Bar color represents plant species. The effects of plant age  
691 are consistently negative on showy milkweed. On narrow-leaved milkweed, the effect of plant  
692 age is generally negative, but the magnitude of these effects declines over time.

693

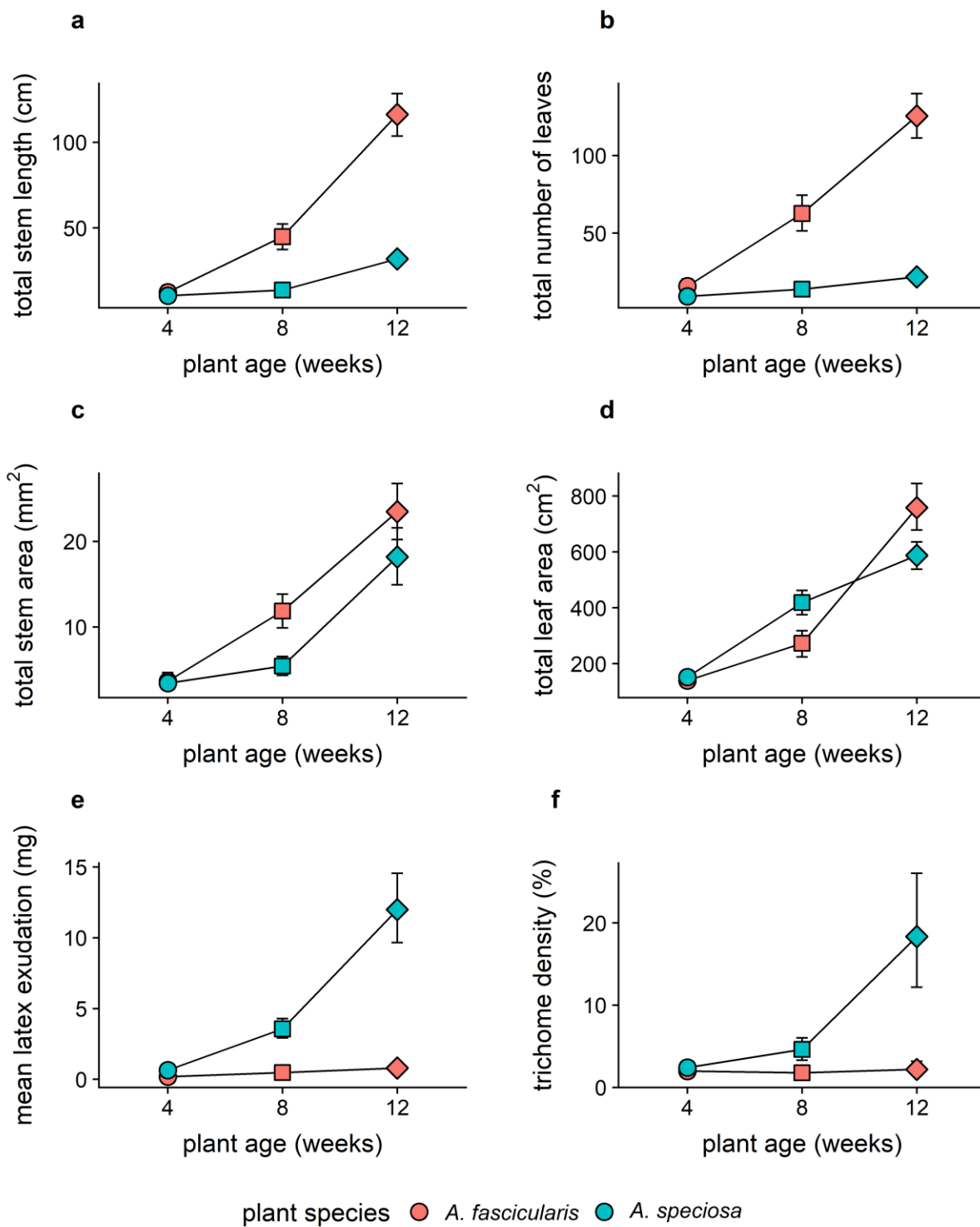


694 **Figure 7.** The maximum size (mass and length) attained by caterpillars developing on two host  
695 plant species of different ages. Color represents plant species, and point shape represents plant  
696 age. Error bars represent 95% confidence intervals.

697  
698 **Figure 8.** Plant damage by host plant species and age. Mean maximum herbivore damage for  
699 plants of each age cohort for a) narrow-leaved milkweed and b) showy milkweed. Mean  
700 maximum damage represents the percent of leaf area consumed by monarchs before leaving their  
701 host plant. Point color and point shape represent plant age. Error bars represent 95% confidence  
702 intervals. b) Percent damage on plants over time, showing the subset of plants with surviving  
703 caterpillars present at each time point. Point color and point shape represent plant age. Point size  
704 reflects the size of the surviving caterpillar population. Error bars represent 95% confidence  
705 intervals.

706

707 **Figure 1**

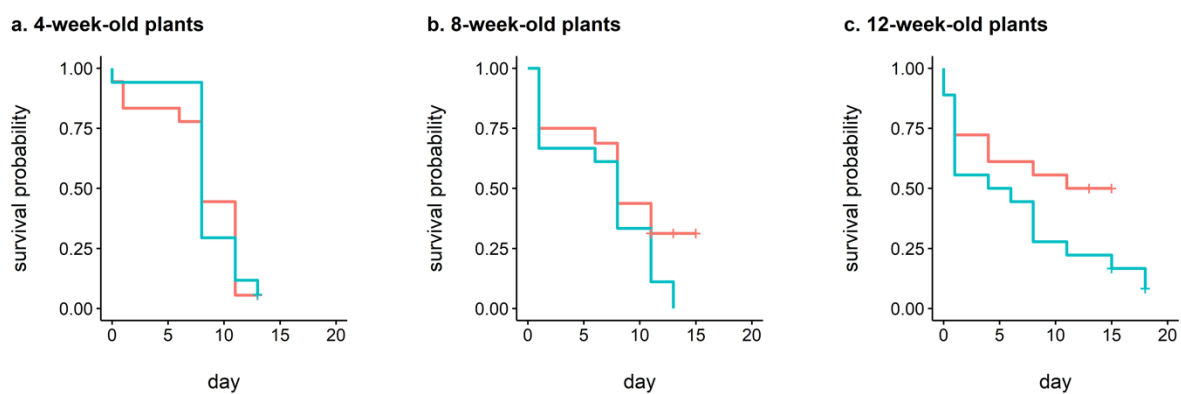


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710

711 **Figure 2**



712

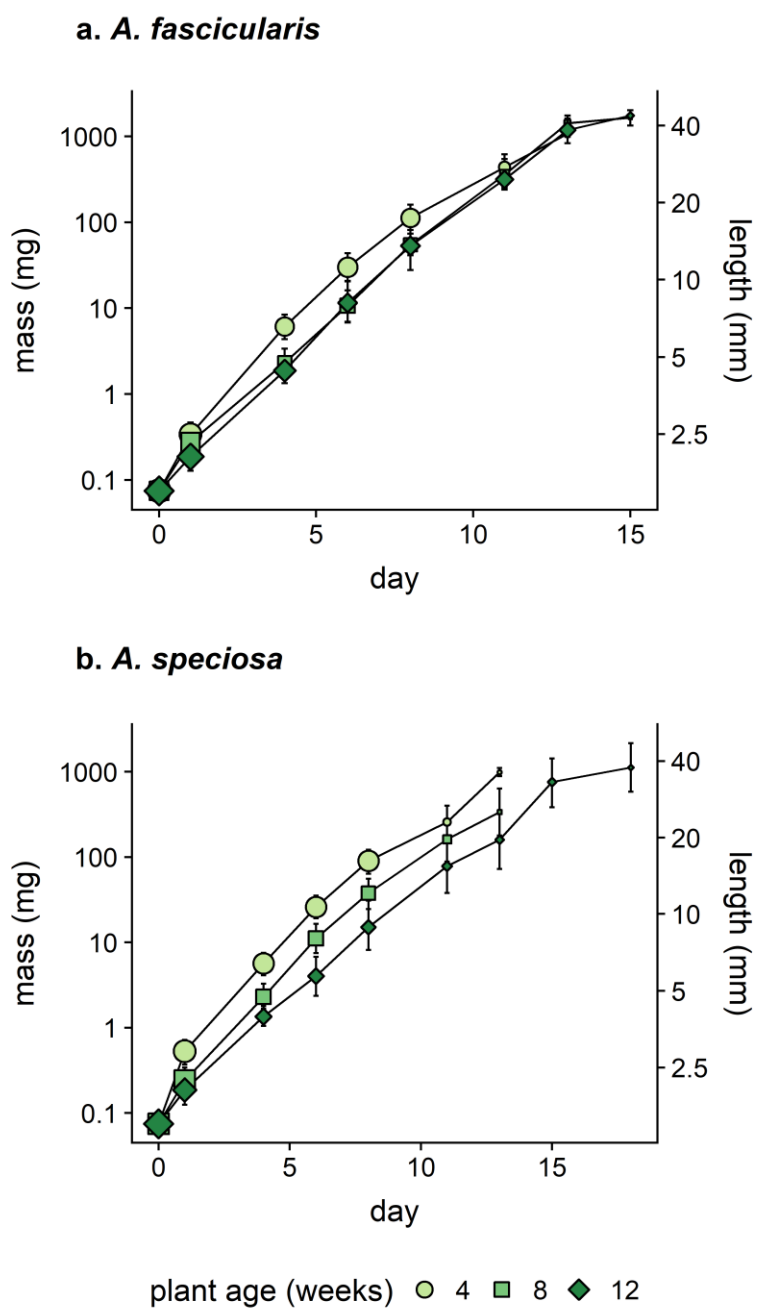
plant species — *A. fascicularis* — *A. speciosa*

713

714

715 **Figure 3**

716



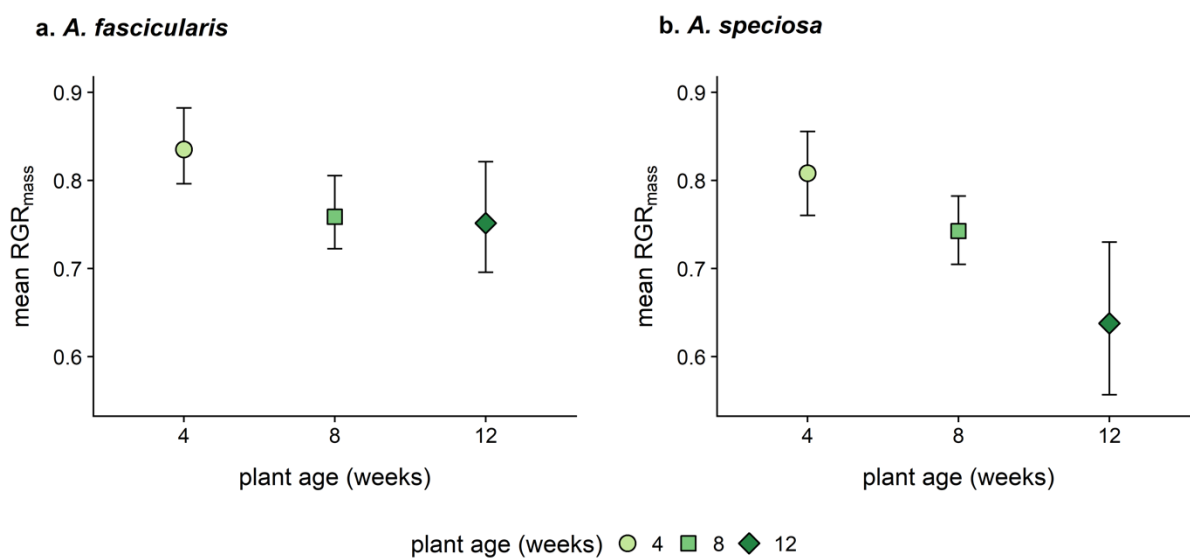
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719

720 **Figure 4**

721



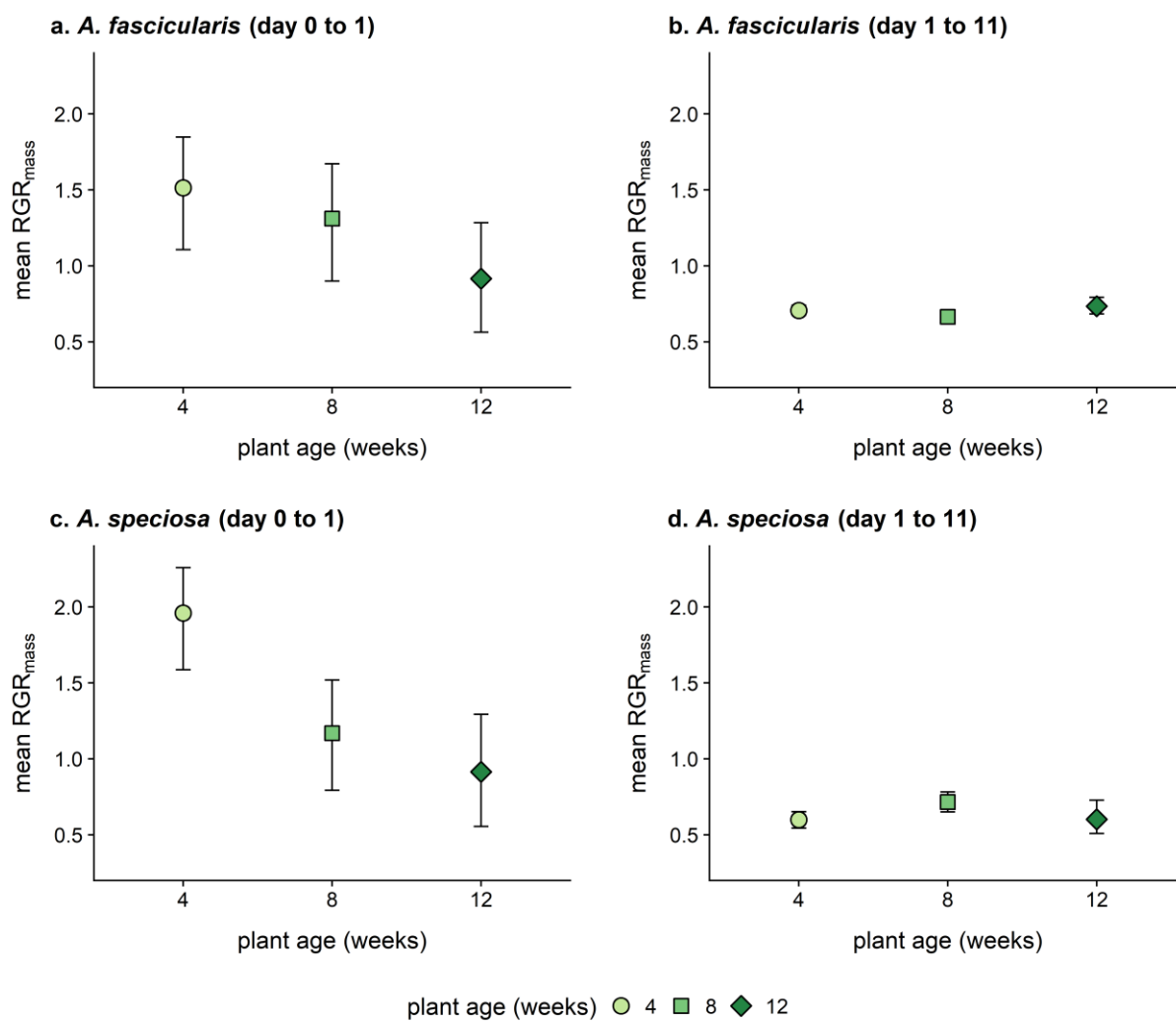
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724

725 **Figure 5**

726



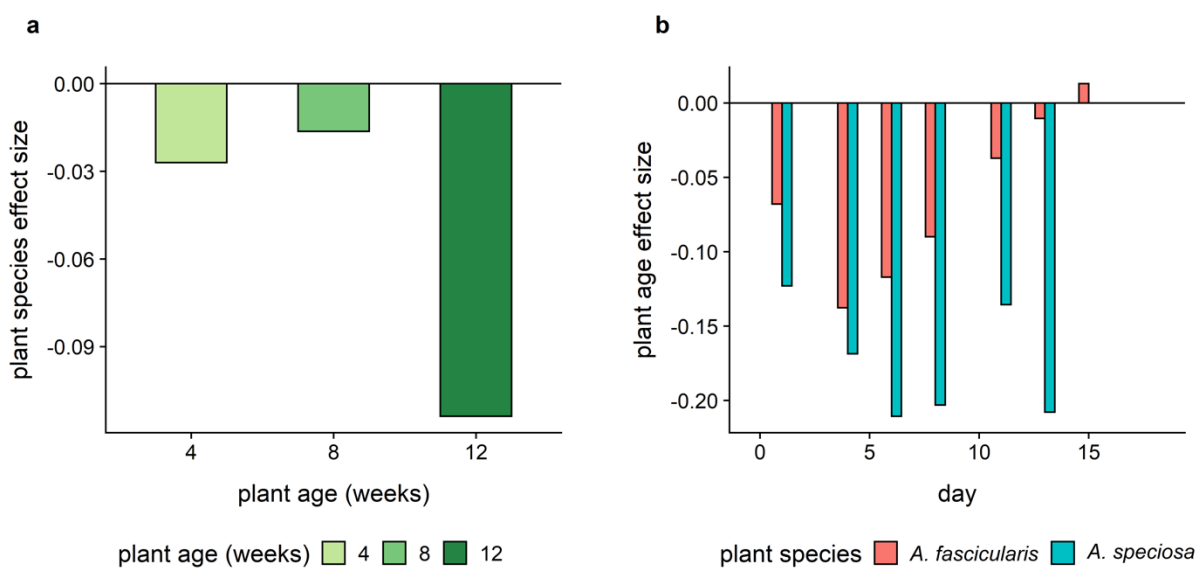
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730 **Figure 6**

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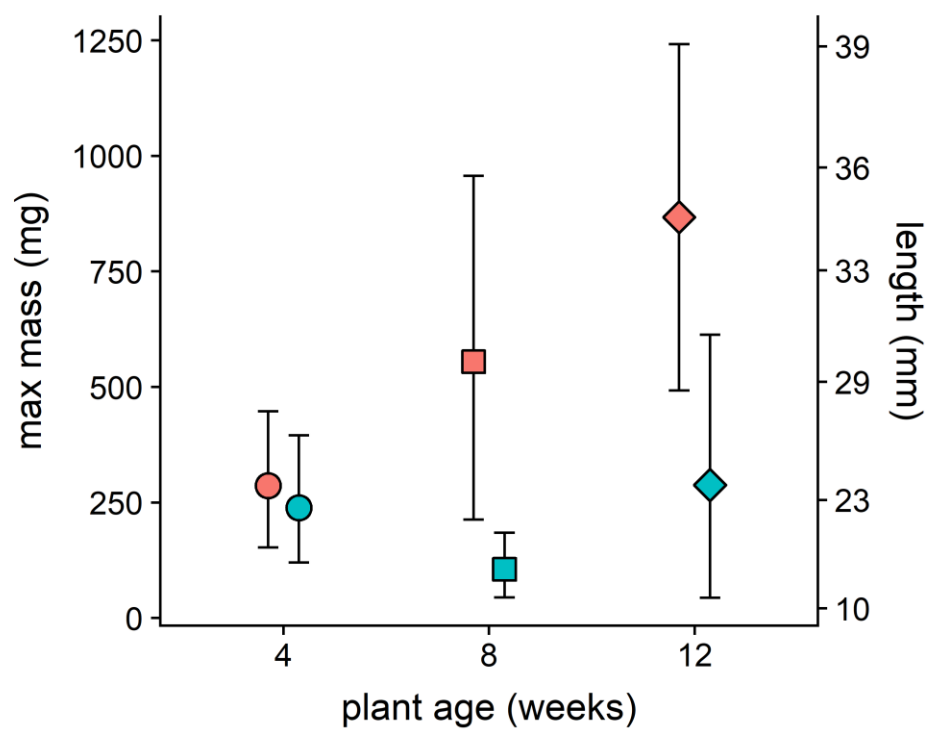
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733

734

735 **Figure 7**

736



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

737

738

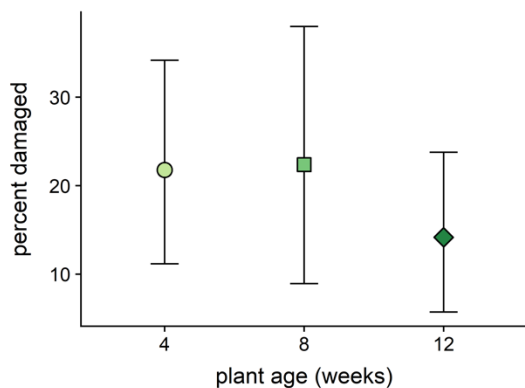
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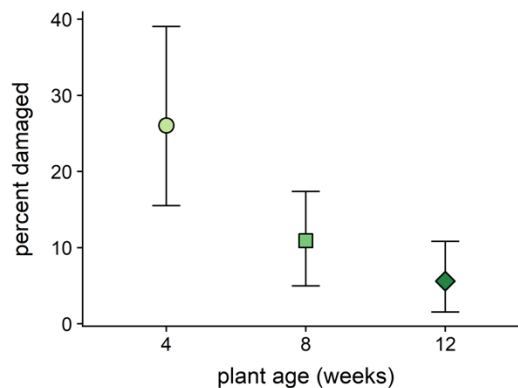
740 **Figure 8**

741

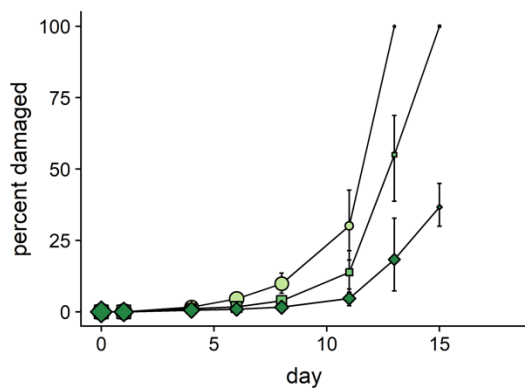
**a. *A. fascicularis* mean max damage**



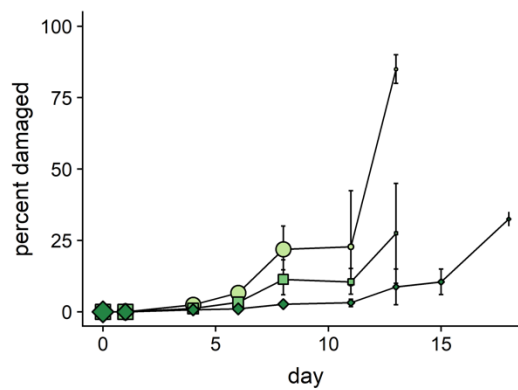
**b. *A. speciosa* mean max damage**



**c. *A. fascicularis* damage by day**



**d. *A. speciosa* damage by day**



plant age (weeks) ○ 4 ■ 8 ◆ 12

742

743

744

745

746

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

748 Louie H. Yang, Meredith L. Cenzer, Laura J. Morgan, and Griffin W. Hall

749 2019, *Ecology*

750 **Appendix S1.**

751 **Figure S1.** Hazard ratios from a Cox proportional hazard (survivorship) model with plant species  
752 and plant age as explanatory factors. The first column indicates the explanatory factor, the  
753 second column indicates the levels of each factor, the third column represents the estimated  
754 hazard ratio with 95% confidence intervals in parentheses, the fourth column shows the  
755 estimated hazard ratio and confidence intervals graphically, and the fifth column shows the  $p$ -  
756 value for each non-reference factor level.

757 **Figure S2.** Individual growth trajectories of caterpillar size in each plant species and age cohort;  
758 each line presents the size of an individual caterpillar. Panels in the left column (a, c and d)  
759 present data from caterpillars reared on narrow-leaved milkweed, while panels on the right  
760 column (b, d and f) present data from caterpillars reared on showy milkweed. Line color  
761 represents plant age. Compared with Fig. 3, these data are shown on an untransformed axis, and  
762 with length on the primary axis and mass on the secondary axis, in order to present a minimally  
763 processed overview of individual caterpillar growth.

764 **Figure S3.** Mean mass on day 8 for caterpillars that developed on each plant species at each  
765 plant age. This metric is similar to the overall relative growth rate calculated via log-linear  
766 regression and shown in Fig. 4 because both metrics assess growth from the beginning of the

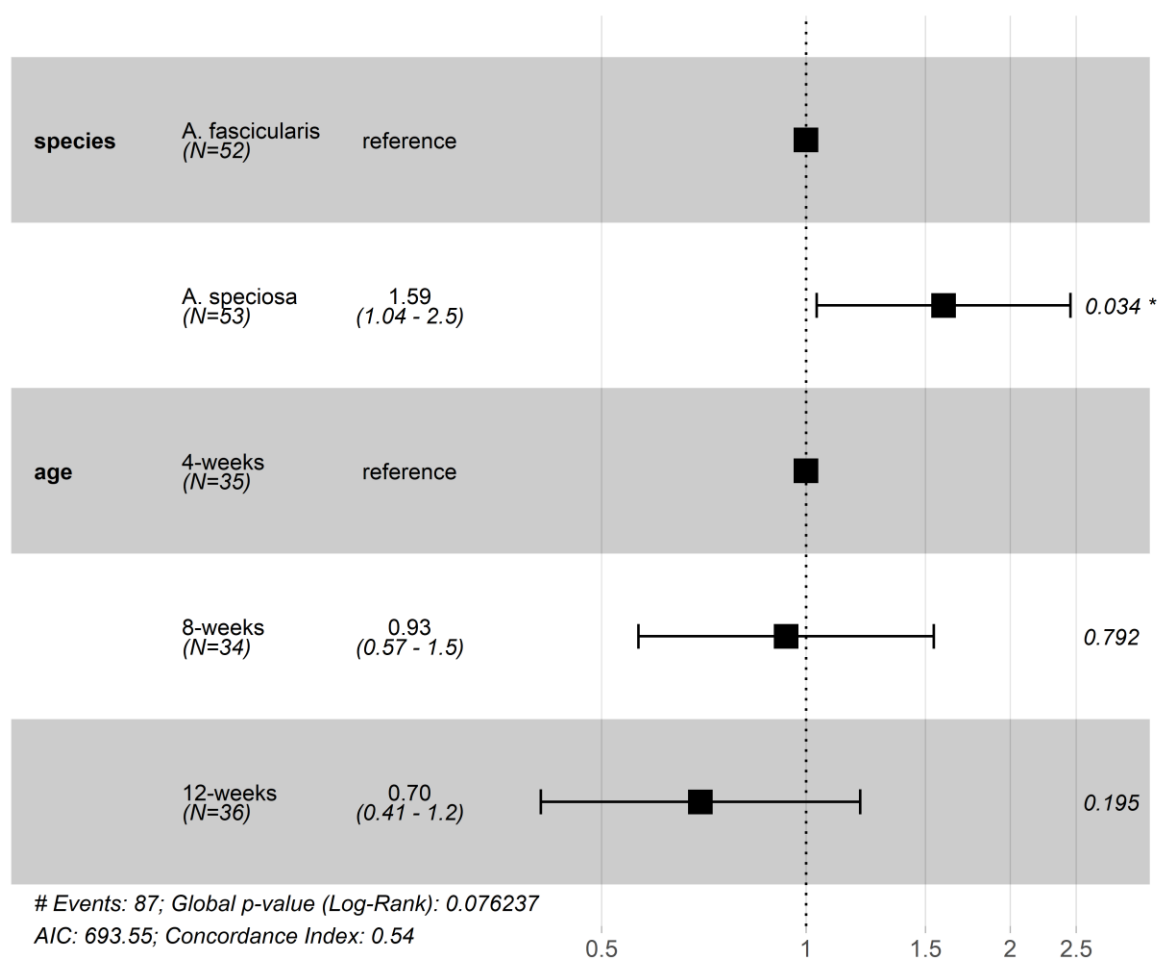
767 experiment, when initial sizes were zero, and both represent a similar overall sample size ( $N=71$   
768 in this figure vs  $N=74$  in Fig. 4). Point color and shape represent plant age. Error bars represent  
769 95% confidence intervals.

770 **Figure S4.** Individual log-linear fits for caterpillars reared on narrow-leaved milkweed. Point  
771 color and shape represents plant age, and the black line represents the best fit log-linear  
772 regression.

773 **Figure S5.** Individual log-linear fits for caterpillars reared on showy milkweed. Point color and  
774 shape represents plant age, and the black line represents the best fit log-linear regression.

775 **Figure S6.** Interval-specific relative growth rates calculated for all adjacent observations on a)  
776 narrow-leaved milkweed and b) showy milkweed. This figure is similar to Fig. 5, but presents  
777 growth data for all possible adjacent intervals. Point size reflects the size of the surviving  
778 population. Point color and shape represent plant age. Error bars represent 95% confidence  
779 intervals.

780 **Figure S1**

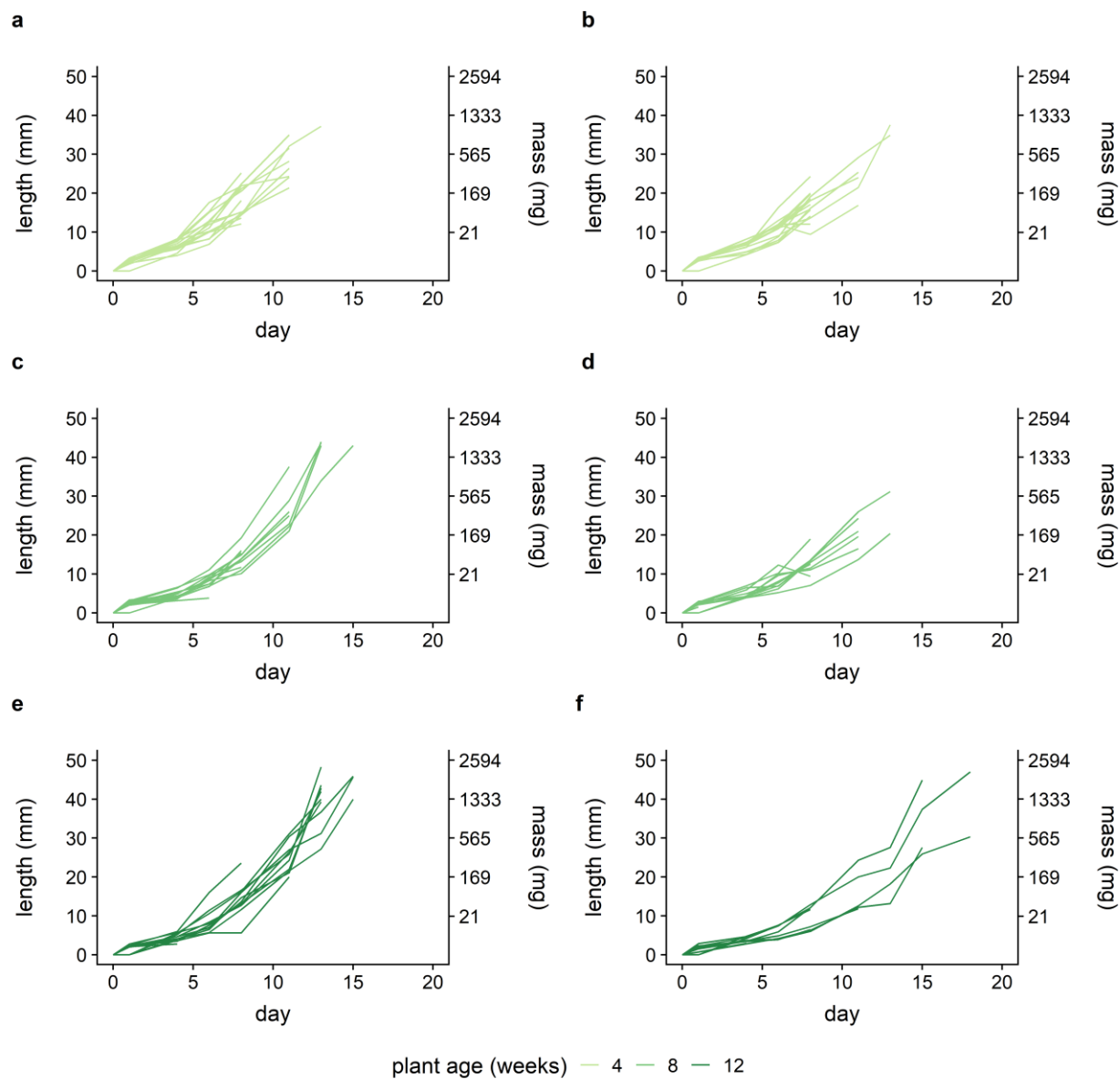


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782

783 **Figure S2**

784

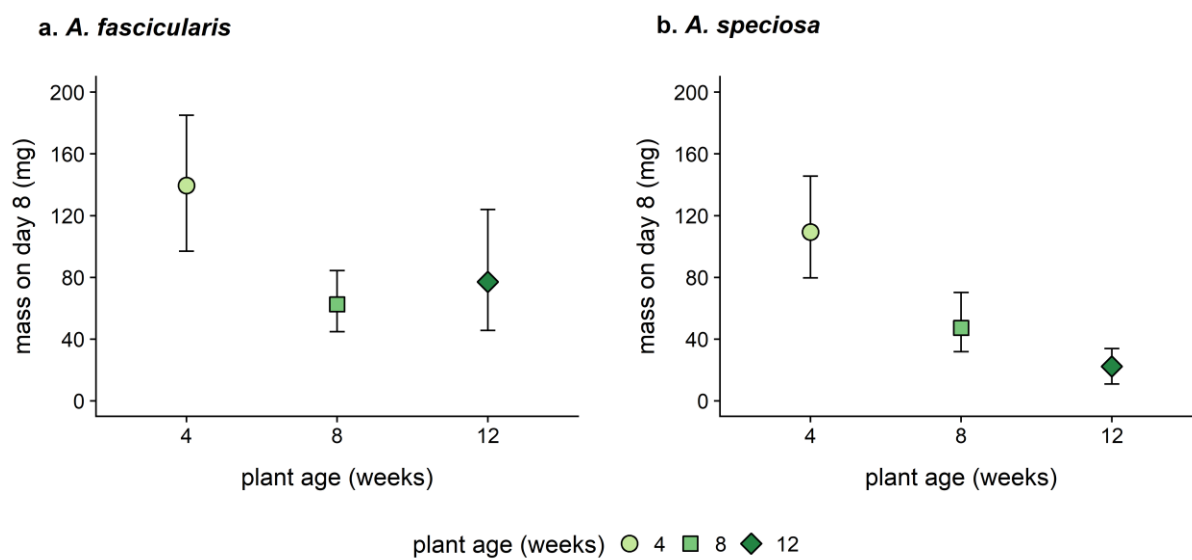


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787 **Figure S3**

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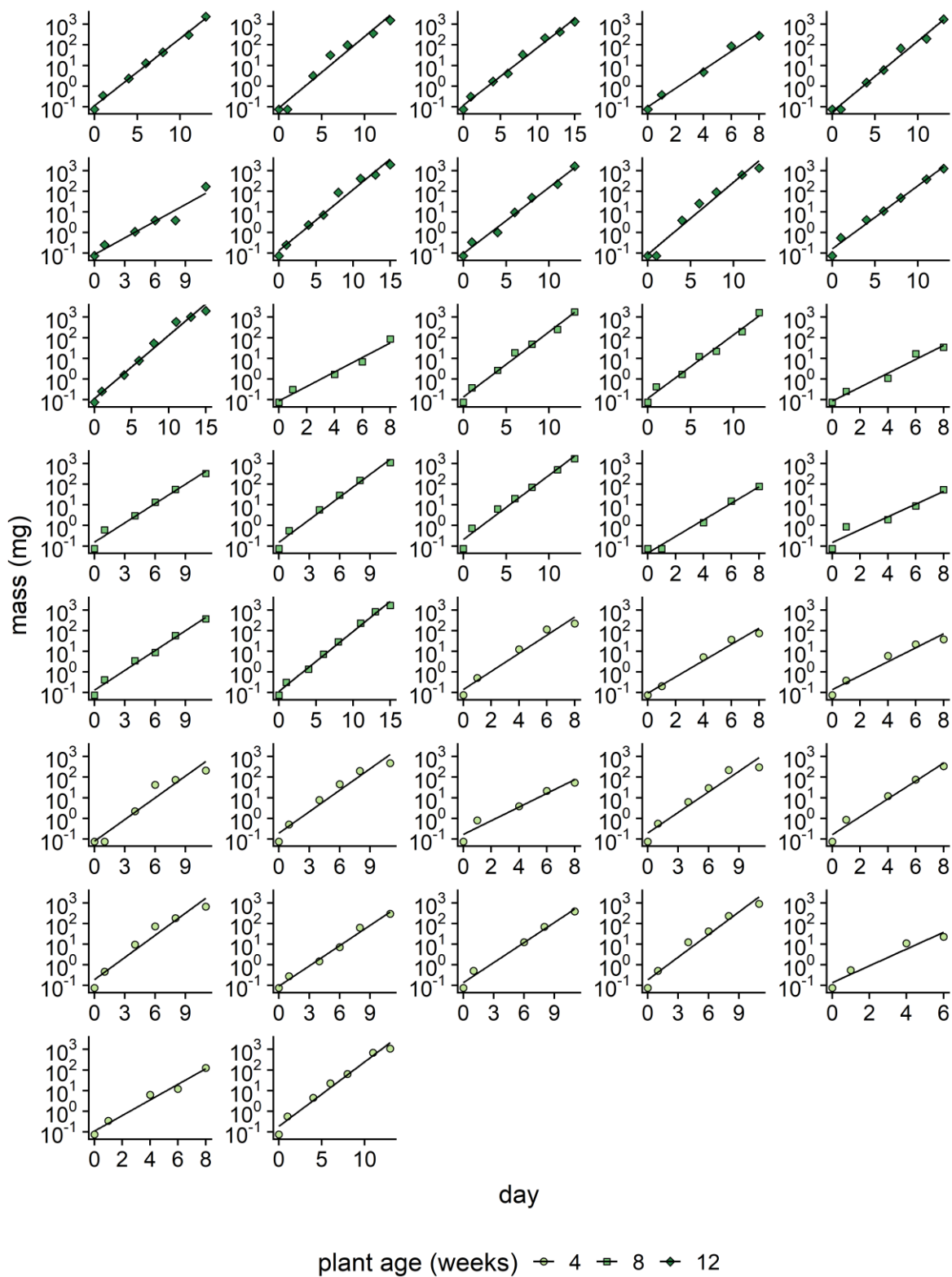


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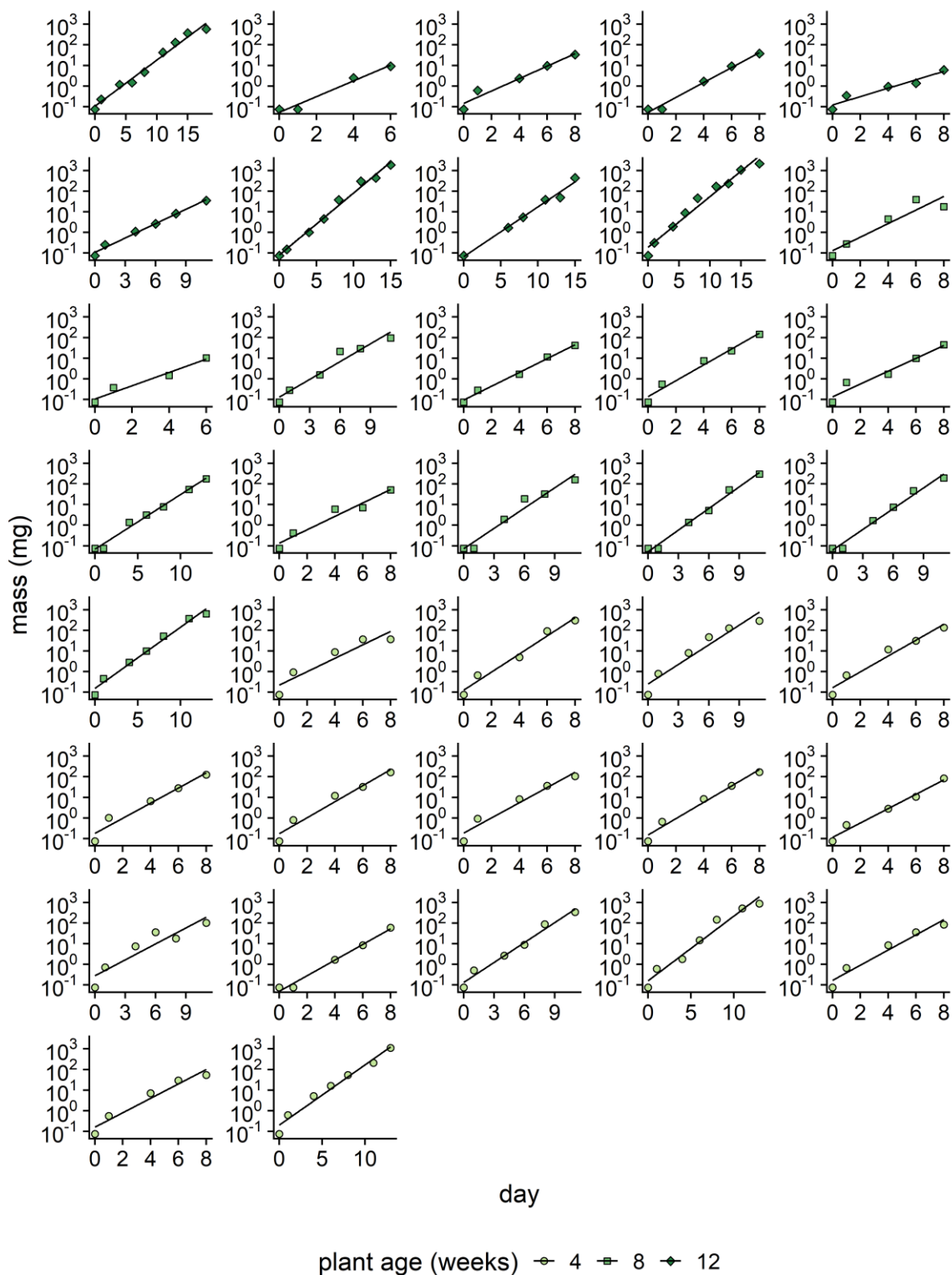
791

792 **Figure S4**



793

794 **Figure S5**

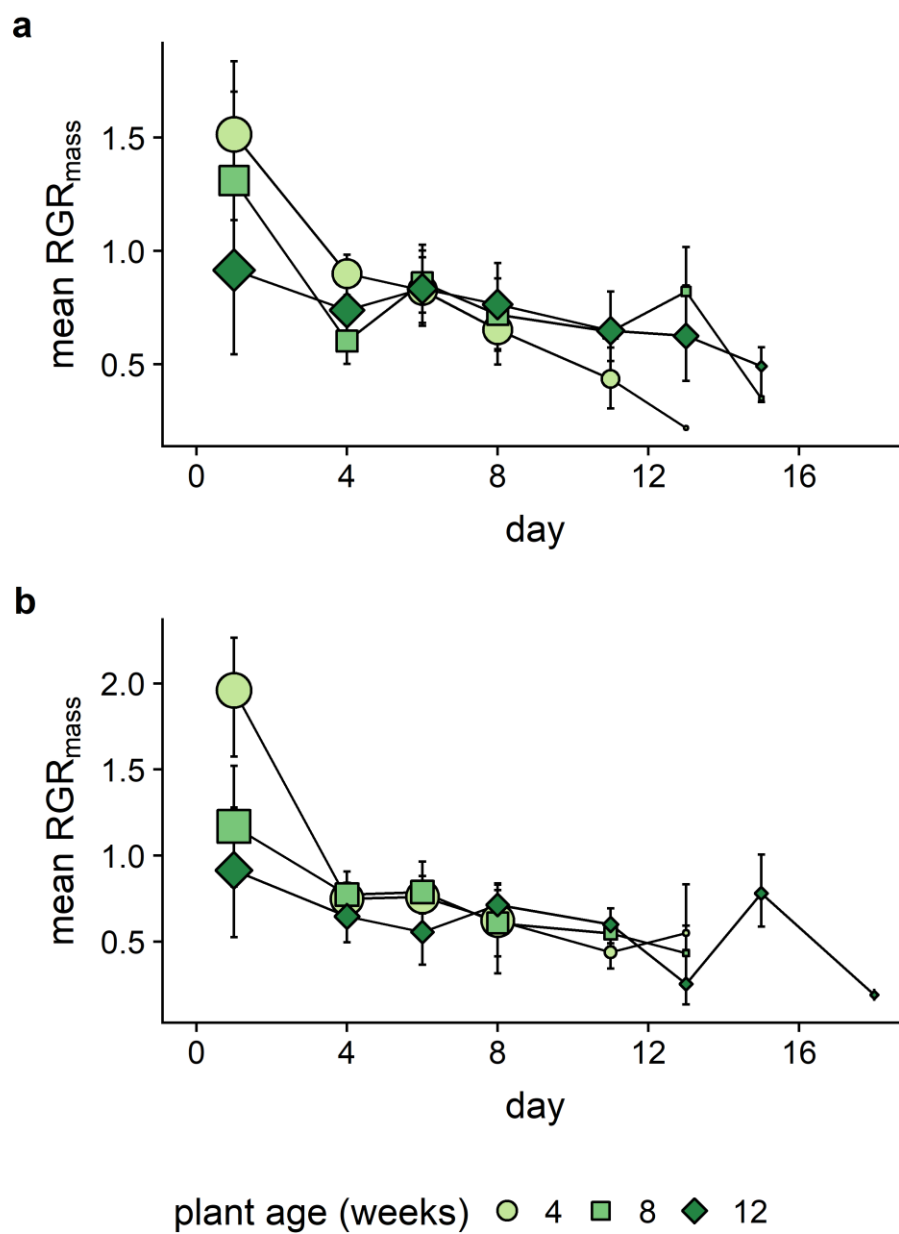


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796 **Figure S6**

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