1 **Fine-scale plant defense variability increases top-down control of an herbivore**

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31 **Abstract**

32 Herbivore populations are regulated by plant defenses and natural enemies. While plant defense

33 can suppress herbivore populations, these defenses adversely affect natural enemies thereby

34 releasing herbivores from top-down control. Over their lifespans, herbivores and their natural

35 enemies may experience substantial variation in plant defense. Defense variability can suppress

36 the growth of herbivores, but the impacts of defense variability on natural enemies and top-down

37 control of herbivores are unknown. We independently manipulated the mean and variation of a

38 plant toxin experienced by individual *Trichoplusia ni* caterpillars and its parasitoid *Copidosoma*

39 *floridanum.* Increases in the mean toxin concentration, but not its variance, experienced by

40 individual *T. ni* and *C. floridanum* decreased the fitness of *C. floridanum*, whereas both mean

41 and variance impacted *T. ni* fitness. Thus, increased defense variability for individual herbivores

42 suppressed herbivore fitness with no perceptible cost to top-down control. However, impacts of

43 variability depend heavily on scale of variability.

Introduction

Herbivores experience both bottom-up pressures from defense traits of their host plants as well as top-down pressure from their predators, parasitoids, and pathogens (Hunter & Price 1992). The combination of bottom-up and top-down impacts must be considered when examining the factors regulating herbivore populations (Price *et al.* 1980; Vidal & Murphy 2018). Yet, often only one set of bi-trophic interactions are examined. Plant defensive chemicals are important bottom-up factors in suppressing herbivore fitness (Stamp 2003), but can also impact natural enemies, such as parasitoids (Ode 2006, 2013; Gols 2014). These chemicals can reduce fitness of parasitoids developing in herbivores by decreasing host quality (Lampert & Bowers 2010; Harvey *et al.* 2011), increasing herbivore immunity against parasitoids (Kaplan et al. 2016), and directly exposing of developing parasitoids to plant defense chemicals (Barbosa *et al.* 1986; McGovern *et al.* 2006; Lampert *et al.* 2008). In other cases, defense chemicals may increase parasitoid performance by inhibiting the immune response of the herbivore (Bukovinszky *et al.* 2009; Smilanich *et al.* 2009; Lampert 2012; Quintero *et al.* 2014; Ode 2019). When examining the consequences of plant traits on herbivores, the effect of plant traits on top-down controls such as parasitoids should be considered as well (Poelman *et al.* 2008; Pearse *et al.* 2020).

Herbivores encounter variability in defenses both spatially and temporarily throughout development (Hakes & Cronin 2011; Quintero *et al.* 2014; Hunter 2016; Cope *et al.* 2020). Herbivores can experience variation moving across different parts of the same plant or between individual plants (Ruhnke *et al.* 2009; Wetzel *et al.* 2016), through ontogenetic variation in plant defense production (Quintero *et al.* 2014; Cope *et al.* 2020; Ochoa-López *et al.* 2020), or through induction (Karban *et al.* 1997). From the perspective of an herbivore, variation in plant defensive

67 traits can occur at radically different scales, and the scale at which variation occurs can have

68 profound consequences for herbivores. For example, by comparing no-choice rearing studies,

69 Wetzel *et al.* (2016) found that population-level herbivore performance was greater with

70 increasing plant trait variability. However, at a finer spatial scale in which individual herbivores

71 experience variation in plant defense, defense variation suppressed herbivore performance and

72 population growth rate (Pearse *et al.* 2018), probably because herbivores could not acclimate to

73 changing plant defenses (Wetzel & Thaler 2016).

74   To date, we know very little about how variable defenses impact the natural enemies of

75 herbivores and the top-down control of herbivores. Furthermore, some studies have suggested

76 that the ability of herbivores to metabolize plant defense toxins may change ontogenetically

77 (Quintero & Bowers 2018; Boege *et al.* 2019). Given the widespread occurrence of spatial and

78 temporal variation in plant defenses, and the documented effects on herbivores (Wetzel *et al.*

79 2016; Pearse *et al.* 2018), variation in plant defenses likely affects higher trophic levels and top-

80 down control of herbivore populations. For instance, herbivores that are less mobile than their

81 natural enemies may experience increased top-down attack of herbivores if their natural enemies

82 readily move between high quality patches with abundant herbivores and low-quality patches

83 with fewer herbivores (Riolo *et al.* 2015). Another possibility, which we test here, is that defense

84 variability at fine scales might suppress herbivore performance to a greater degree than that of

85 natural enemies, resulting in greater top-down control.

86   In this study, we explored the impacts of toxin variation in the diet of the cabbage looper

87 *Trichoplusia ni* (Lepidoptera: Noctuidae) on development of its parasitoid *Copidosoma*

88 *floridanum* (Hymenoptera: Encyrtidae). Cabbage loopers can be reared on artificial diets in

89 which precise concentrations of plant toxins can be incorporated, making them well-suited for

90   studies of the impacts of defenses on *T. ni* caterpillars (Pearse *et al.* 2018) and *C. floridanum*

91   (Lampert & Bowers 2010, 2013; Lampert *et al.* 2011). Using an artificial diet, we examined the

92   effect of xanthotoxin, a plant defense produced by many plants in the Apiaceae, on herbivore and

93   parasitoid performance across a range of mean xanthotoxin concentrations without otherwise

94   changing nutritional content of the diet. We then fed individual parasitized caterpillars varying

95   concentrations of xanthotoxin in their diet to explicitly test the effects of variation in toxin

96   concentration and compare them to the effects of mean concentrations. This design allowed us to

97   measure the emergent effects of toxin variability by removing other variables that might obscure

98   these effects.

99   We hypothesized that the negative effects of toxin variability in the diet of the herbivore

100 would decrease host quality resulting in negative impacts on the parasitoid. Alternatively, the

101 effects of toxin variability may diminish at higher trophic levels and variability in host diet may

102 not be strongly experienced by parasitoids and, consequently, may enable increased top-down

103 control of herbivores. Because parasitoids experience plant defenses via their herbivorous host,

104 they may not be exposed to the same degree of variation in a defensive trait as the herbivore.

105 Such differential effects may be very important in the regulation of herbivore populations, as

106 negative effects of a plant resistance trait on natural enemies can effectively negate the benefit of

107 that trait as a plant defense (Ode 2006; Peterson *et al.* 2016). Furthermore, we explore the

108 importance of the scale of variability for parasitoid performance by manipulating variability of

109 xanthotoxin in individual diets and between individuals by averaging performance across

110 treatments of herbivores fed constant xanthotoxin levels. We also compared the effects of

111 xanthotoxin variability on the potential for top-down control of herbivores by examining the

112 number of parasitoids (emerged from parasitized hosts) per host in a subsequent generation.

113   While diet variability can reduce herbivore fitness (Wetzel *et al.* 2016; Pearse *et al.* 2018), it

114   remains unknown whether this will also limit the potential for top down control through negative

115   effects on higher trophic levels.

116   **METHODS**

117   **Insect colonies**

118   *Trichoplusia ni* (Lepidoptera: Noctuidae) is polyphagous, feeding on plants from over 40

119   families, including several members of the Apiaceae (Sutherland & Green 1984) that contain

120   xanthotoxin. *Copidosoma floridanum* (Hymenoptera: Encyrtidae) is a polyembryonic egg-larval

121   parasitoid of plusiine noctuid moths (Noyes 1988). Polyembryonic parasitoids lay either one egg

122   (male or female) or two eggs (one male and one female) inside their host egg. Once the host egg

123   hatches, embryos produced by these parasitoid eggs clonally multiply forming as many as a few

124   thousand individuals per host before finally pupating within the mummified cuticular remains of

125   the host's final instar (Strand 1989; Ode *et al.* 2018).

126   Laboratory colonies of unparasitized *T. ni* and *C. floridanum*-parasitized *T. ni* were

127   maintained in separate Sanyo MIR-554 environmental chambers set at 25° C, a 16L:8D

128   photoperiod, and 30% RH and reared for several generations on artificial diet before use in

129   experiments. Approximately 25 adult *T. ni* were kept in 3.78 L plastic containers and provided

130   with cotton soaked in 10% honey water as a food source. The top of each container was covered

131   with a sheet of paper towel on which *T. ni* adults laid eggs. Egg sheets were changed daily to

132   provide *C. floridanum* females young host eggs, which are preferred for oviposition (Strand

133   1989). Some egg sheets were exposed to adult female *C. floridanum* and then placed in petri

134   dishes and incubated at 25° C until hatching. Unparasitized egg sheets were surface sterilized

135   with a 10% bleach solution and then air-dried before incubation. After hatching, first instar *T. ni*

136   larvae (both parasitized and unparasitized) were transferred to 37 mL plastic cups (SOLO®

137   soufflé cups [Dart Container Corp., Mason, MI, USA]; two larvae per cup) containing ~15 ml

138   artificial diet (modified from Shorey and Hale 1965; Table S1), and reared until pupation or

139   mummy formation. Moth pupae were collected and placed in the adult rearing containers where

140   they were allowed to emerge. Mummies formed from parasitized caterpillars were kept in 15 ml

141   glass test tubes plugged with a cotton stopper and allowed to emerge. Because *C. floridanum*

142   adults generally only live a few days, mated females were usually used for culture maintenance

143   or experiments within six hours of emergence.

144   **Experimental design**

145   Newly emerged (< 6 hours old) adult *C. floridanum* females were allowed to oviposit on

146   egg sheets containing less than 12-hour old *T. ni* eggs. *Copidosoma floridanum* lays mostly

147   mixed-sex and all-female broods when mated (Hardy *et al.* 1993; Ode & Strand 1995). To ensure

148   that some all-male broods were produced, some of the eggs were parasitized with unmated

149   females. Unfertilized eggs develop into haploid males due to the haplodiploid development of

150   wasps. Paper towels with parasitized eggs were then placed in petri dishes at 25° C until

151   hatching.

152   *Constant diet experiment:* To establish the overall impact of xanthotoxin concentration

153   on parasitoid fitness, individual parasitized first instar caterpillars were placed individually in

154   37mL SOLO® soufflé cups containing artificial diet supplemented with either 0, 0.5, 1.0, 1.5, or

155   2.0 mg xanthotoxin/g diet (n = 54, 55, 55, 52, 52 respectively). Artificial diets used in the

156   experiments only differed from the diet used for colony maintenance (see above) in the addition

157   of xanthotoxin. Caterpillars were weighed on the sixth day of development to measure growth

158   differences across the diet treatments. Larvae were reared on the same diet until mummies were

159   formed. The values from this experiment were used to construct curves of the mean effect of

160   xanthotoxin concentration on *C. floridanum* fitness measures to use as a baseline comparison for

161   the fitness effects of variable diets (below).

162   *Diet-switching experiment:* To test the effects of xanthotoxin variability on *C. floridanum*

163   performance, larvae were switched every three days between two diets that had a combined

164   average of 1.0 mg xanthotoxin/g diet. Diet pairs represented either high variability (0 and 2.0 mg

165   xanthotoxin/g diet), low variability (0.5 and 1.5 mg xanthotoxin/g diet, or no variability (1.0 and

166   1.0 mg xanthotoxin/g diet) (n = 80 per treatment). Therefore, treatments varied in the magnitude

167   of variation, but not the mean, of xanthotoxin concentration experienced by the caterpillars. Half

168   of the larvae started on the higher concentration of xanthotoxin first, while the other half were

169   placed on the lower diet first to account for any initial diet effects. On the sixth day (as with the

170   constant diet experiment, above), prior to switching diets, caterpillars were weighed to measure

171   growth rate differences between treatments. Larvae that died after day six were dissected to

172   confirm parasitism status. The small number of larvae that were not parasitized (3/240 in

173   variability, 17/268 in mean experiment) were removed from any further analyses.

174   *Parasitoid fitness measures:* For both the constant diet and diet-switching experiments,

175   after mummy formation, mummies were placed individually in a glass test tube fitted with a

176   cotton stopper and kept at 25° C until adult eclosion. Adult wasps were allowed to emerge for 24

177   hours, after which the emerged adults and mummified host remains were frozen at -20˚C.

178   Survival was based on whether any adults from the brood successfully emerged. In each brood of

179   *C. floridanum*, the first 200 wasps were sexed and the remaining adults were counted. Mummies

180   were dissected to determine the number of unemerged larvae, pupae, and pharate adults and

181   combined with the number of emerged adults to determine total brood size. Brood size, the total

182   combined number of emerged and unemerged *C. floridanum* individuals from a single host, was

183   used as a direct measure of parasitoid fitness. Emergence was defined as the proportion of

184   emerged adults out of the total individuals per brood.

185   **Statistical analyses**

186   Statistical analyses were performed using R version 3.6.2. Survival (coded as yes or no

187   for each brood) was analyzed using logistic regression with xanthotoxin concentration (or

188   treatment for the variability experiment) as a predictor. Analyses were performed with

189   xanthotoxin concentration as a single predictor variable for baseline fitness curves using the

190   linear model (lm) function in R. Variability treatment effects on day six mass, pupation time, and

191   brood size (response variables) were compared with one-way ANOVA, with treatment as the

192   predictor, using the lm function analyzed with type III sum of squares. Emergence was analyzed

193   using a logistic regression with proportion of emerged individuals out of the brood total as the

194   response variable. Brood type was included as a fixed effect in each model but was not

195   significant except for some brood size analyses and was therefore removed from the other

196   models. To explore the effects of variability at the population level (rather than individual diet

197   cups), performance values from the constant xanthotoxin experiment were combined using

198   nonlinear averaging to calculate performance values corresponding with the same treatments as

199   the variability experiment. This was used to simulate a population where individuals feed on

200   separate diets, thus comparing variability across rather than within individuals. Nonlinear

201   averages were calculated for low variability and high variability using the same two diet

202   treatments for each as in the diet-switching experiment (above). Calculated nonlinear averages

203   for low, high, and no variability were then compared using pairwise t-tests using propagated

204   errors following the methods of Pearse et al. (2018). Results are presented with mean and

205   standard error (mean ± SE).

206   We also wanted to project the effects of variable diets on the population dynamics of the

207   parasitoid and host using an estimate of subsequent generation parasitoid-host ratio (i.e. adult

208   wasps per host egg). Lifetime egg production data of *T. ni* (from Pearse et al. 2018) was

209   combined with the brood size and survivorship data presented here on *C. floridanum.* We

210   projected the effects of variable toxins on herbivore population dynamics using the combined

211   impacts of variable defense on herbivores and parasitoids. Parasitized hosts failing to yield any

212   adult parasitoids were considered as having an emerged brood size of zero. These data were

213   combined with the number of emerged adult parasitoids to calculate average emerged brood size.

214   Since only female wasps contribute to parasitism of hosts, we multiplied the brood size by the

215   overall proportion of females in mixed-sex broods. We did not adjust the estimate based on the

216   number of all-male broods since the number of unmated females producing these broods was

217   controlled in the experiment. The final average brood size in each mean and variability treatment

218   was then divided by the corresponding lifetime egg production of *T. ni* to calculate adult wasps

219   per host egg. This calculation uses both the subsequent generation of adult wasps (number

220   emerged from a parasitized caterpillar) and the eggs laid by an unparasitized individual of the

221   same generation (from Pearse et al. 2018). This accounts for loss of fitness in the next generation

222   through brood size or egg production due to xanthotoxin effects. Standard error was propagated

223   based on the individual errors from the respective data sets.

224   **RESULTS**

225   The likelihood that any *C. floridanum* successfully emerged significantly declined when

226   their hosts fed on diets with higher mean concentrations of xanthotoxin (Figure 1A), but was not

10

227 impacted by the magnitude of variability in xanthotoxin (Figure 1B). Only 8.7% of the

228 parasitized hosts produced any adult wasps when fed diets containing the highest concentrations

229 of xanthotoxin (2.0 mg/g). Most mortality was caused by death of the host prior to mummy

230 formation with the host being 13.5 times more likely to die on 2.0 mg/g diet than the no

231 xanthotoxin diet ($\chi^2 = 38.00$, df = 1, p < 0.0001) in the mean experiment. Only 13.1% of total

232 brood mortality in all mean treatments combined occurred after mummy formation. Similarly,

233 mortality in the variability experiment also occurred primarily before parasitoid pupation (84.1%

234 of total mortality). Survival was highest on constant diets containing no xanthotoxin, where

235 60.9% of hosts survived to form mummies. Survivorship in the variability experiment was 41.5%

236 overall which was consistent with the 1.0 mg/g constant diet treatment and did not vary between

237 variability treatments.

238   Increasing concentrations of xanthotoxin had sublethal effects on parasitoid fitness, but

239 the magnitude of variability had no significant effects on parasitoid fitness. Day six mass of host

240 caterpillars decreased logarithmically with increasing xanthotoxin levels (Figure 2A). Average

241 host mass ranged from 9.02 ± 0.79 mg on diets with no xanthotoxin to only 1.39 ± 0.25 mg when

242 fed with 2 mg/g xanthotoxin. There was no effect of variability on host growth (Figure 2B) with

243 a combined treatment average day six mass of 3.1 ± 0.15mg which was very similar to the

244 average on the 1.0 mg xanthotoxin treatment in the mean experiment. Starting diet did not

245 influence day six mass for any treatment in the variability experiment ($F_{4,82} = 1.68$, p = 0.1571).

246   Xanthotoxin concentration also increased the amount of time to pupation in the mean

247 experiment but pupation time was not impacted by variability (Figure 2C-D). Pupation time

248 ranged from 16.9 ± 0.22 days on average on 0 mg xanthotoxin diet to 22.8 ± 0.61 days on 2.0 mg

249 xanthotoxin diet. Average pupation time with all variability treatments combined was 20.81 ±

250 0.26 days. Larger larvae tended to pupate faster in both experiments (Figure S1) and the

251 difference in growth rate due to larva size was greater on higher mean xanthotoxin diets.

252   Dietary xanthotoxin concentration had no impact on brood size until concentrations were

253 greater than 1.0 mg/g, forming two distinct groups divided above 1.0 mg/g xanthotoxin (Figure

254 3A). Brood size on 1.0 mg/g or lower xanthotoxin diets averaged 1366.4 ± 59.5 individuals per

255 host while the average of the higher xanthotoxin diets was about 40% less with only 797.7 ± 82.7

256 individuals per host. Despite the effect of high constant xanthotoxin on brood size, this was not

257 affected by variability either (Figure 3B). Average brood size across all variability treatments

258 combined was nearly 1100 individuals per host.

259   Parasitoid brood emergence was affected by dietary xanthotoxin concentration similarly

260 to brood size, with broods having similar emergence at 1.0 mg/g or lower concentration diets and

261 far lower emergence on the two higher xanthotoxin diets (Figure 3C), but this was also

262 unaffected by the variability treatments (Figure 3D). This is likely because brood size was

263 strongly correlated with emergence (logistic regression: $\chi^2 = 24.197$, df = 1, p < 0.0001). Smaller

264 broods tend to have poor emergence, since these broods often leave too much host tissue

265 unconsumed preventing the adult wasps from successfully emerging (Ode & Strand 1995).

266   The results of individual parasitoid performance on the constant diet was also used to

267 determine the effects of variability in diet between individuals of a population through nonlinear

268 averaging (Figure 4). Variability between individuals affected all parasitoid fitness parameters

269 except development time, since the relationship between mean dietary xanthotoxin and

270 development time was linear (Figure 2C). Parasitoid broods had the highest survival without

271 variability across individual diets (Figure 4A). However, high variability positively affected host

272 growth (Figure 4B) compared to low variability, though the difference was not significant

273   compared to no variability. The population had an average day six mass of 5.25 ± 1.02 mg with

274   high variability between individual diets compared to just 2.60 ± 0.729 mg with low variability

275   and 3.23 ± 0.63 mg with no variability. However, brood size suffered heavily with variability

276   between individual diets (Figure 4C). Variability between individual diets led to approximately

277   one-third less total brood size (low: 1015.71 ± 251.85, high: 980.54 ± 200.70) than no variability

278   (1518.09 ± 105.61). Due to the correlation of brood size and emergence, variability at this scale

279   affected emergence in the same way, with more variability greatly decreasing emergence

280   success.

281   **Calculated effects on top-down control in the next generation**

282   By combining these results and those from Pearse et al. (2018) on the egg production of

283   *T. ni,* we calculated the parasitoid pressure on the next generation of herbivores in variable

284   systems as the number of adult wasps per host egg. The parasitoid pressure resulting from

285   caterpillars feeding on variable diets was dependent on the scale of variability. There were many

286   more parasitoids per host when individual caterpillars consumed more variable diets, but the

287   opposite was true when diets varied between but not within individuals. Low variability and high

288   variability diets had nearly twice the parasitoid pressure of non-variable diets with variability at

289   the individual scale (Figure 5). However, parasitoid pressure with no variability was three times

290   that of high variability when variability was experienced by populations, not individuals.

291   **DISCUSSION**

292   Our results highlight the importance of scale in considering the impacts of variability on

293   higher trophic levels. When individual *T. ni* caterpillars experienced variability of xanthotoxin in

294   their diet, that variability suppressed caterpillar performance (Pearse et al. 2018), but had no

295   detectable impact on parasitoid (*C. floridanum*) performance. This effect held true for numerous

296   measures of parasitoid performance, including survival, brood size, and emergence success. This

297   was despite the clear negative effects of high levels of xanthotoxin on parasitoid fitness including

298   slower development, decreased brood size, poor emergence, and low overall survivorship of

299   hosts and broods.

300   In contrast, variation in plant defenses might not be experienced by an individual,

301   whereas it may be experienced by a population of herbivores. When individual caterpillars did

302   not experience variability in xanthotoxin, but a population of caterpillars did, there was little

303   population-level effect of xanthotoxin on caterpillar performance (Pearse et al. 2018). However,

304   population-level variability in xanthotoxin had a strong negative effect on parasitoid

305   performance. This population-level effect of variability was consistent when considering several

306   parasitoid performance measures including survival, brood size, and emergence success.

307   When individual caterpillars feed on variable diets, it is possible that the individual

308   parasitoid broods do not actually experience the variability in the host. Parasitoids feed

309   primarily on the hemolymph and at least some portion of xanthotoxin in the caterpillar's diet

310   enters the hemolymph (Lampert *et al.* 2011). However, parasitoids may influence the host

311   feeding in a way that reduces the amount of variability experienced. Regardless of whether

312   parasitoids experience variability, the host was expected to still be negatively affected by

313   variability as previously shown (Pearse et al. 2018). Day six parasitized caterpillar mass among

314   constant xanthotoxin levels followed an extremely similar trend to that of unparasitized

315   caterpillars (Pearse et al. 2018). Despite this, no negative effect of variability was observed for

316   day six mass of parasitized caterpillars, while a negative impact of variability was observed in

317   unparasitized caterpillars (Pearse et al. 2018). This suggests parasitism changes the effect on the

318   host as well, possibly by regulating the feeding rates of the host to mitigate the effects of a

319 changing diet and *C. floridanum* parasitism is known to cause changes in host physiology

320 (Strand 1989; Strand *et al.* 1990, 1991). Alternatively, it is possible that the weight increase from

321 *C. floridanum* broods inside the host outweighed any effects of variability, but we find this

322 unlikely as the parasitoids are still only embryos at such an early stage in development (Strand

323 1989).

324   There is substantial evidence that plant defenses can reduce performance of parasitoids

325 (Ode 2006). Our study is in line with these findings, because we find clear negative impacts of

326 high xanthotoxin concentrations on fitness and survival of *C. floridanum*. Yet we present novel

327 evidence that the negative effects of *variation* in plant defenses decrease with increasing trophic

328 levels when that variation is experienced by individuals. This could have large consequences for

329 the role of variation in tri-trophic interactions and population dynamics. Herbivores may

330 experience increased bottom-up control from variable defenses while maintaining the top-down

331 effects of parasitoids which are relatively unaffected by variable defense. Thus, population

332 growth of herbivore populations would be severely limited in environments where individual

333 herbivores experience variation in plant defenses.

334   However, the scale of variability will greatly impact the potential of defense variation to

335 limit population growth of herbivores. Parasitoid populations may suffer heavily from defense

336 variability experienced by herbivore populations, but not individuals, despite suffering little

337 consequence from variability within individual host diets. Thus, herbivore suppression may be

338 greater with increased variability within individual plants, where a single herbivore is likely to

339 experience that variability. This may be the case for plants that induce defenses against

340 herbivores. Variation in defense between plants may actually favor herbivores when parasitoids

341 are present as the impacts of highly defended plants are severe for parasitoids but the benefits of

342   low defense plants are not as strong for parasitoids as they are for herbivores, mainly due to

343   mortality effects. Interestingly, a model that considered the dispersal of herbivores and natural

344   enemies also found that small scale variation in plant defense increased top-down control of

345   herbivores because aggregations of herbivores on high-quality hosts subsidized natural enemy

346   population (Riolo *et al.* 2015). It appears that fine-scale variation in plant defense may increase

347   top-down control of herbivores because of multiple advantages to natural enemies in a variable

348   defensive landscape.

349   The decreased diversity of most agriculture systems contributes to the heightened

350   pressure from herbivorous pests (Altieri and Nicholls 2004), and to a reduction in top-down

351   control of these herbivores (Letourneau *et al.* 2011). There are numerous hypotheses to explain

352   why a loss of plant diversity might increase herbivory and limit top-down control. Our study

353   suggests that defense variability may be an important explanation for why more diverse systems

354   experience less herbivory. Defense variability can cause a direct reduction in herbivore

355   performance, and, when defense variability is not experienced as acutely at higher trophic levels,

356   it can result in a proportionately greater increase in population growth of predators compared to

357   herbivores. However, our study also suggests that defense variability at very fine scales, those

358   that are experienced by an individual herbivore, are needed for enhanced top-down control.

359   There is mounting evidence that defense variation, particularly at small spatial scales, contributes

360   to the regulation of herbivores through a combination of bottom-up and top-down effects.

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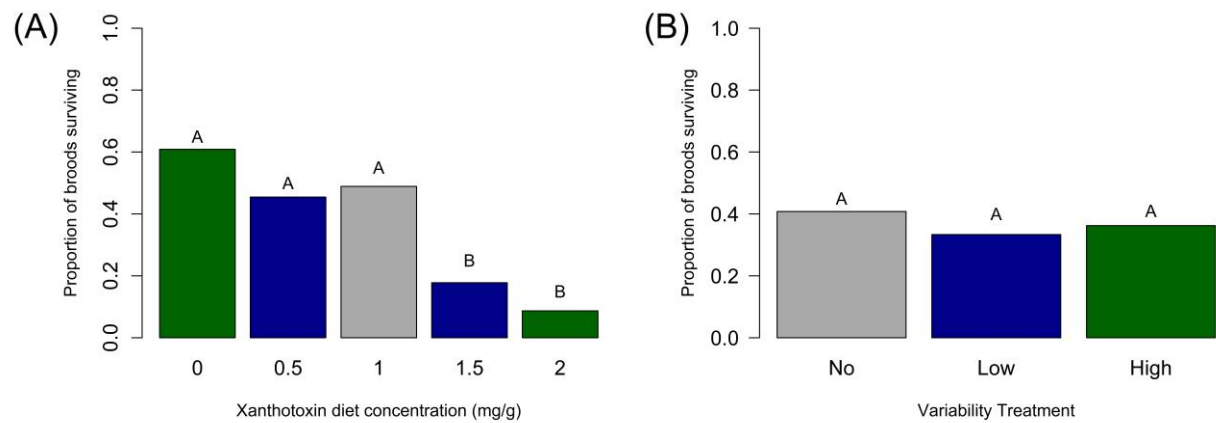
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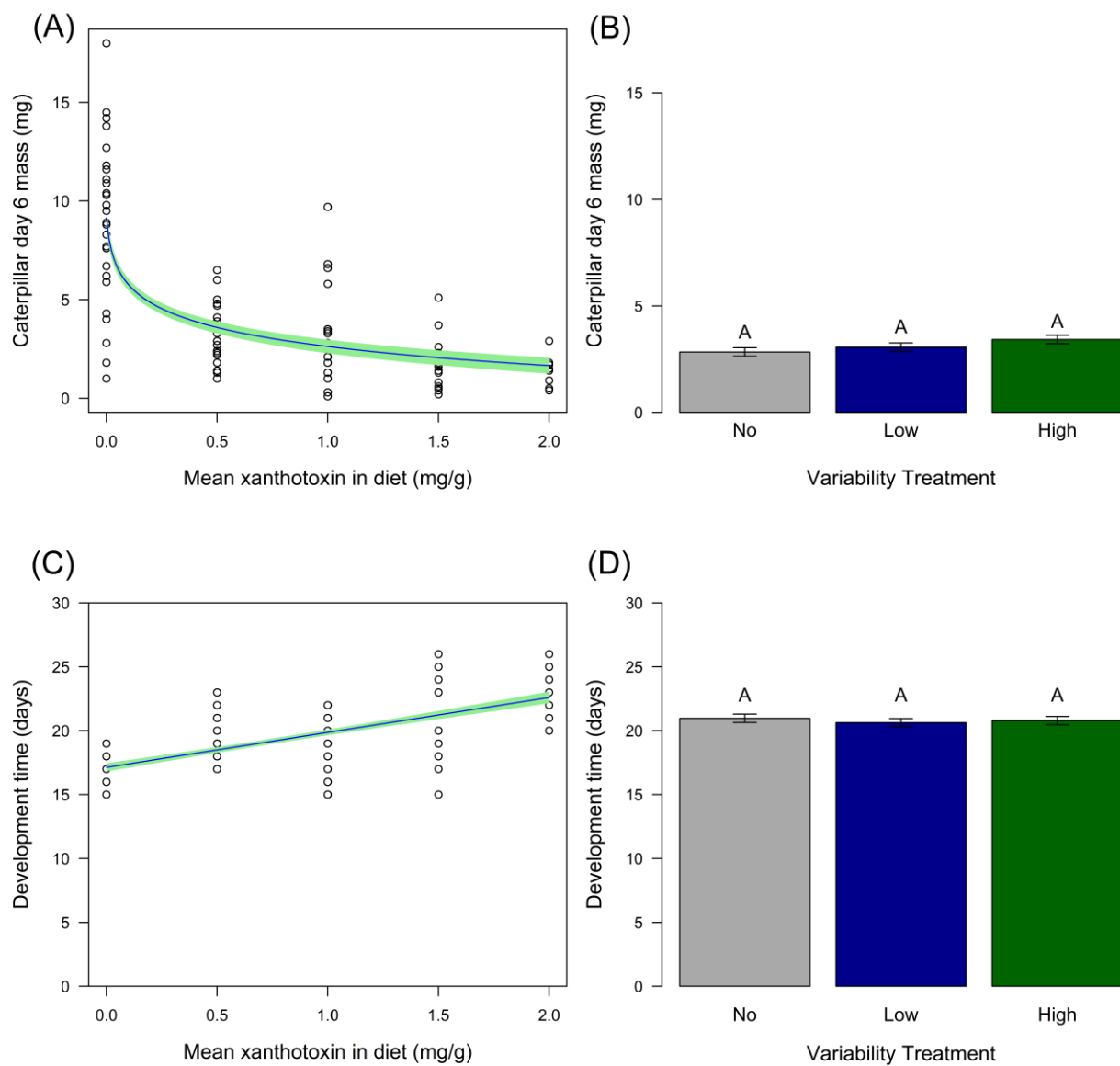
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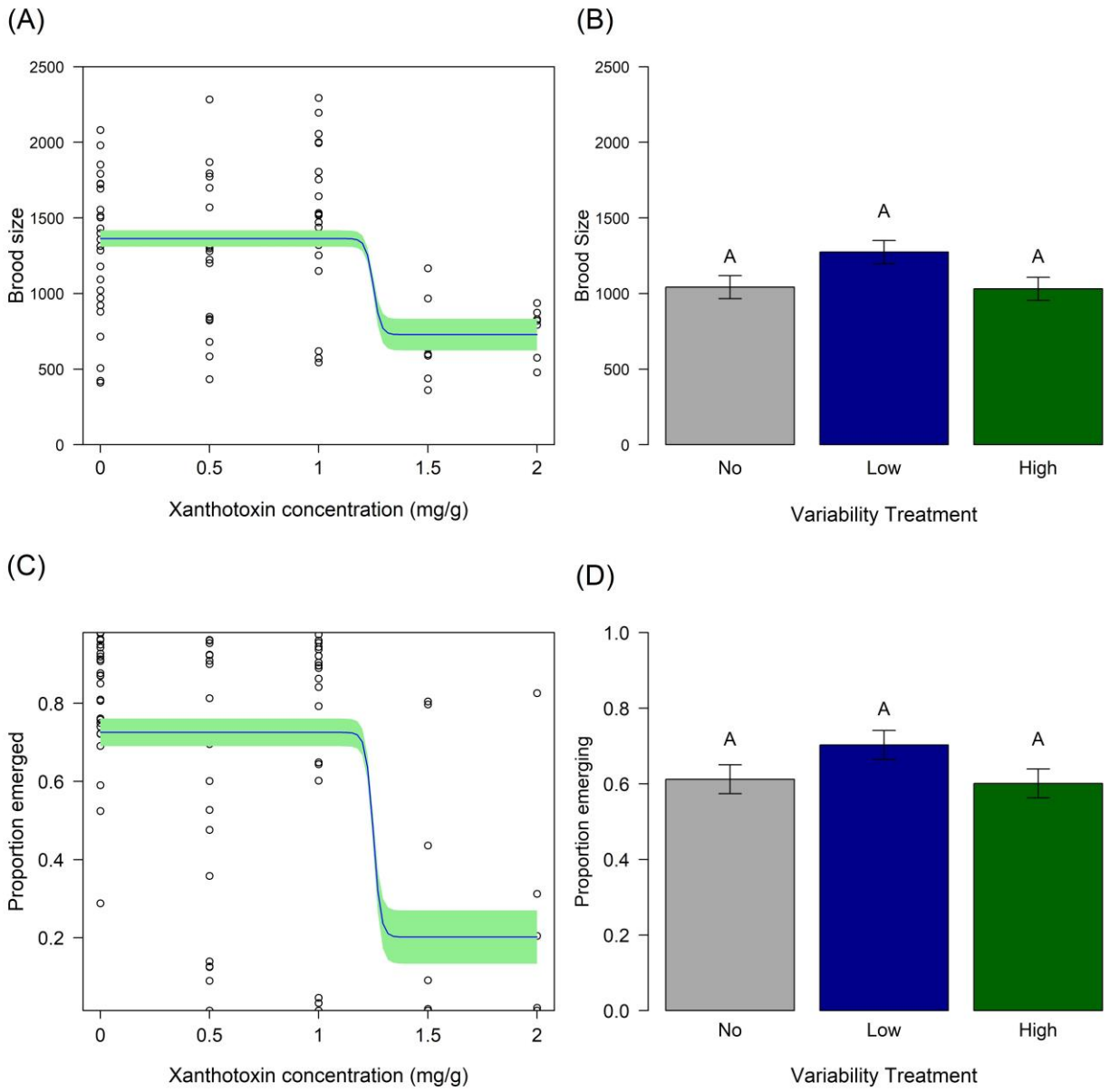
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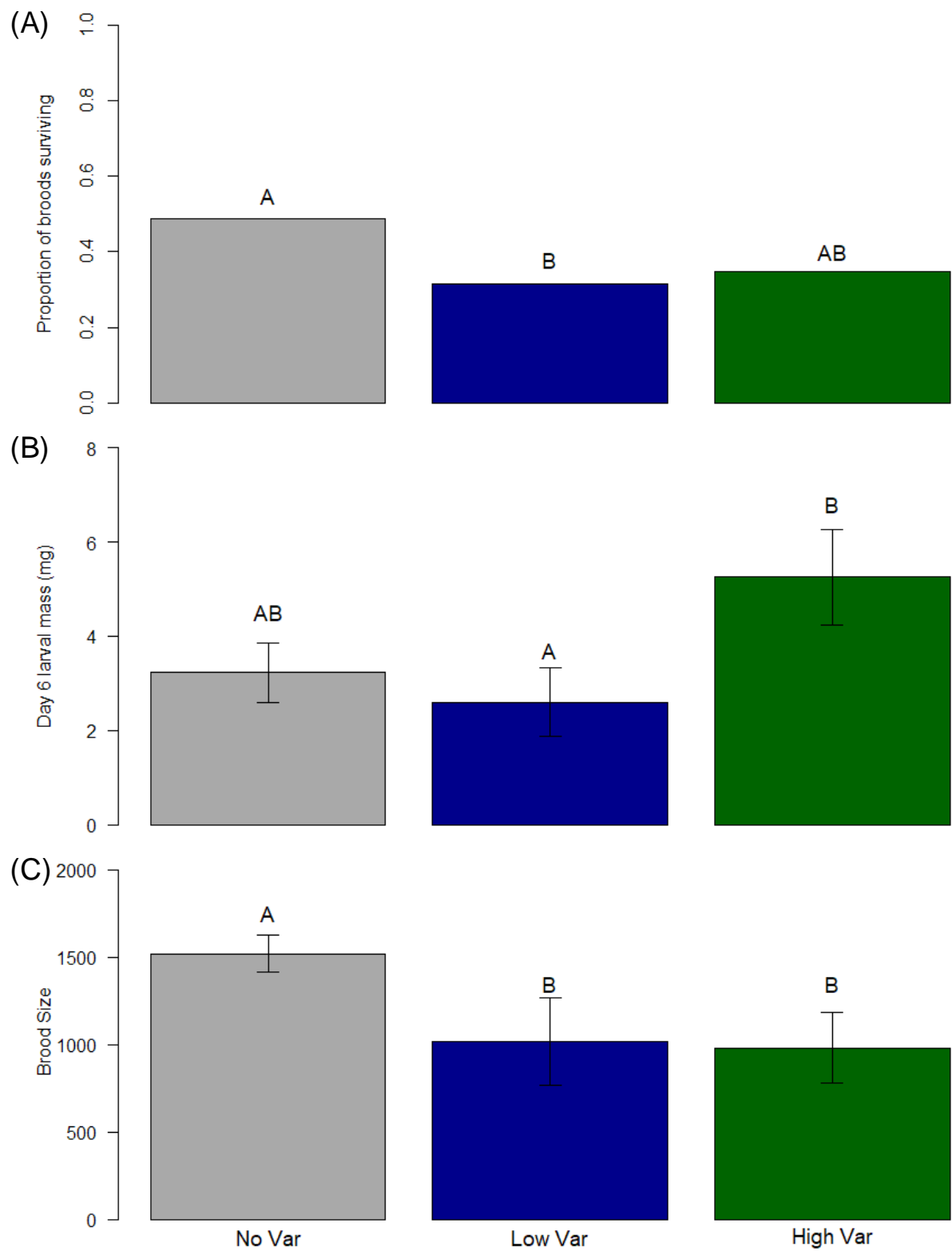495   Figure 3:

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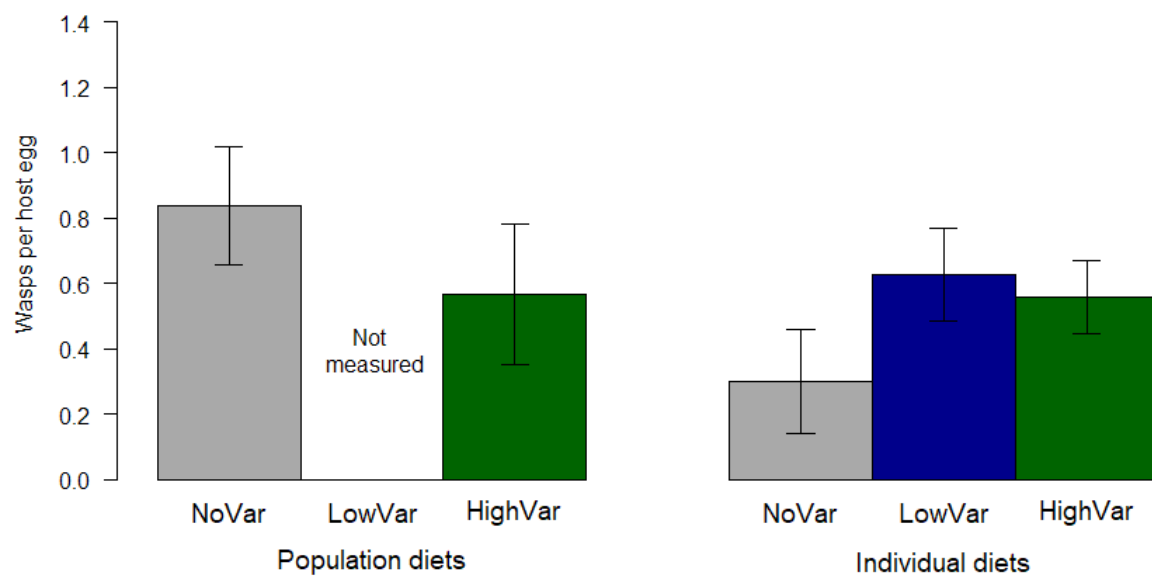
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499   Figure 4:

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504   Figure 1. *C. floridanum* brood survivorship on constant xanthotoxin diets (A) and variable diets

505   with a mean of 1.0 mg xanthotoxin/g of diet (B). Bars are calculated from exact proportions of

506   broods surviving out of the total. Pairwise comparisons were performed as post-hoc analysis of

507   odds ratios following logistic regression (A: LR χ^2 = 38.71, df = 1, p < 0.0001; B: LR χ^2 = 1.416,

508   df = 2, p = 0.4926). Bars with different letters above are significantly different groups.

509

510   Figure 2. (A) Day six mass of caterpillars on constant xanthotoxin diet. Regression line is a

511   logarithmic function of xanthotoxin concentration with a small added value (0.01) to account for

512   zero values and mass as the response variable ($F_{1,82}$ = 104.01, p < 0.0001, R2 = 0.559). (B) Day

513   six mass of caterpillars on variable xanthotoxin diets. There were no significant differences

514   between variable diet treatments (one-way ANOVA; $F_{2,189}$ = 1.24, p = 0.2918). (C)

515   Development time for parasitoid broods when hosts fed on constant xanthotoxin diet. Line fit is

516   based on a linear regression of xanthotoxin concentration versus development time ($F_{1,39}$ = 55.44,

517   p < 0.0001, R^2 = 0.587). (D) Development time for parasitoid broods when hosts fed on variable

518   diet. There were no significant differences between treatments in the of variable diets (one-way

519   ANOVA, $F_{2,96}$ = 0.14, p = 0.873). Error bars in each plot represent standard error.

520

521   Figure 3. (A) Parasitoid brood size when hosts fed on constant xanthotoxin diets. A sigmoid

522   curve was fit in the mean experiment for brood size, and brood type included as a fixed-effect

523   ($F_{3,71}$ = 3.10, p = 0.032, R^2 = 0.116), though brood type did not have a significant effect. (B)

524   Parasitoid brood size when hosts fed on variable diets. Variability treatments were analyzed with

525   brood type included as a fixed-effect (ANOVA, $F_{4,68}$ = 6.55, p = 0.0002). Since the brood type

526 did not affect the relationship between treatments (broodtype*treatment: $F_{4,68} = 0.76$, $p = 0.558$),

527 bars are displayed as averages of all brood types combined with standard error. (C) Emergence

528 success of individual broods on constant xanthotoxin diet. Proportions were calculated as the

529 number of individuals emerging divided by the number of unemerged individuals per host.

530 Proportions were analyzed using logistic regression of proportion with treatment as a predictor

531 ($\chi^2 = 13.07$, df = 1, $p = 0.0003$). (D) Emergence success of individual broods on variable diets.

532 Pairwise comparisons were performed as post-hoc analysis of odds ratios following logistic

533 regression ($\chi^2 = 1.71$, df = 2, $p = 0.4251$). No significant differences were found between

534 variable diet treatments. Error bars show standard error.

535

536 Figure 4. Effect of variability in xanthotoxin between, rather than within, individual caterpillar

537 diets. Values are calculated based on nonlinear averaging of the observed values in the constant

538 diet experiment for mortality (A), day six caterpillar mass (B), and parasitoid brood size (C).

539 Different letters above the bars indicate significant differences between groups. (A) Proportion

540 of broods surviving to emergence when diets vary between individuals. Comparisons: chi-square

541 test of proportions, $\chi^2 = 6.46$, df = 2, $p = 0.0396$. (B) Effect of variability between individuals on

542 caterpillar mass at day six. Comparisons based on pairwise t-test, $p < 0.05$. (C) Effects of

543 variability in host diet between individuals on parasitoid brood size. Comparisons based on

544 pairwise t-test, $p < 0.05$.

545

546 Figure 5. Parasitoids per host egg in second generation after feeding on variable diets. The two

547 sets of bars represent the scale of variability, either between individual (variability in population

548   diets, left) or within individual diets (right). Values were calculated by combining parasitoid

549   brood data from the current study and lifetime fecundity data from Pearse *et al.* 2018. Population

550   variability was calculated from nonlinear averaging of observed performance in the constant

551   diets from the respective studies. Values for individual diet variability were calculated from the

552   experimental results of the variability treatments in each study. Bars are displayed with standard

553   error.