

# Host-mediated, cross-generational intraspecific competition in a herbivore species

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Conspecific insect herbivores co-occurring on the same host plant interact both directly through interference competition and indirectly through exploitative competition, plant-mediated interactions and enemy-mediated interactions. However, the situation is less clear when the interactions between conspecific insect herbivores are separated in time within the same growing season, as it is the case for multivoltine species. We hypothesized that early season herbivory would result in reduced egg laying and reduced performance of the next generation of herbivores on previously attacked plants. **We tested this hypothesis in a choice experiment with box tree moth females (*Cydalima perspectalis* Walker, Lepidoptera: Crambidae). These females were exposed to box trees (*Buxus sempervirens* L., Buxaceae) that were either undamaged or attacked by conspecific larvae earlier in the season.** We then compared the performance of the next generation larvae on previously damaged *vs* undamaged plants. Previous herbivory had no effect on oviposition behaviour, but the weight of next generation larvae was significantly lower in previously damaged plants. There was a negative correlation between the number of egg clutches **laid on plants by the first generation and the performance of the next generation larvae**. Overall, our findings reveal that early season herbivory reduces the performance of conspecific individuals on the same host plant later in the growing season, and that this time-lagged intraspecific competition results from a mismatch between the oviposition preference of females and the performance of its offspring.

## 1 Main text

### 1.1 Introduction

**Biotic interactions are strong factors affecting the fitness of interacting individuals, even when interactions are delayed in time or do not imply direct contact between individuals. Such interactions can be found in both plants through plant-soil feedbacks (Putten et al., 2016) and in animals (Fisher et al., 2019; Pfennig & Pfennig, 2020).** For instance, insect herbivores exploiting the same plant can compete for food, even when interactions among individuals are separated in time (Kaplan & Denno, 2007). Insects may reduce the impact of interspecific competition by avoiding crowded plants, or plants that have been previously consumed by herbivores, which assumes that they can detect competitors or their effects on plants (Shiojiri & Takabayashi, 2003; De Moraes et al., 2001). For many species, the choice of the oviposition site by mated females is crucial in this respect. The preference-performance hypothesis — *aka* the ‘*mother knows best hypothesis*’ — states that female insects evolved host searching behaviour that leads

41 them to oviposit on hosts where their offspring do best (Gripenberg et al., 2010). A good match between  
42 the preference of a mated female for a given plant and the performance of its offspring developing on the  
43 same plant implies that females can recognize cues that correlate with larval performance, for instance those  
44 related to plant defenses and nutritional quality. Yet, these cues can be largely modified by the simultaneous  
45 or sequential presence of other competing herbivores (Bultman & Faeth, 1986; Nykänen & Koricheva, 2004;  
46 Abdala-Roberts et al., 2019; Visakorpi et al., 2019). Therefore, initial herbivory **by a given species** may  
47 have time-lagged consequences on the preference and performance of herbivores **of another species** that  
48 subsequently attack the same plant in the same growing season (Poelman et al., 2008; Stam et al., 2014).  
49 However, while such time-lagged *interspecific* interactions between herbivores have long been documented  
50 (Faeth, 1986), surprisingly much less is known about delayed *intraspecific* interactions in multivoltine species  
51 having several generations per year.

52 Previous herbivory generally reduces the performance of later arriving herbivores on the same plant (Moreira  
53 et al., 2018), **although the opposite effect can also be observed (Sarmiento et al., 2011; Godinho  
54 et al., 2016). Reduced performance of herbivores on previously damaged plants could occur  
55 through both exploitative competition — whereby the first attacking herbivore depletes the  
56 resource available to forthcoming herbivores (Kaplan & Denno, 2007) — or through changes in  
57 plant traits (Hilker & Fatouros, 2015; Abdala-Roberts et al., 2019; Marchand & McNeil, 2004;  
58 Blenn et al., 2012; Fatouros et al., 2012). By triggering an hormonal response and inducing the  
59 production of anti-herbivore defenses as well as resource reallocation in plant tissues, herbivory  
60 may induce changes in plant quality that generally reduce the performance of late coming herbivores (Agrawal,  
61 1999; Abdala-Roberts et al., 2019; Wratten et al., 1988). Such an affect has long been documented in  
62 interspecific interactions (Kaplan & Denno, 2007; Moreira et al., 2018), but also in intraspecific  
63 interactions.** For instance, prior damage by the western tent caterpillar *Malacosoma californicum* Packard  
64 (Lepidoptera: Lasiocampidae) induces the regrowth of tougher leaves acting as physical defenses and reducing  
65 the fitness of the next tent caterpillars generation (Barnes & Murphy, 2018).

66 Previous herbivory can influence the oviposition preference of later herbivores. Several studies have demon-  
67 strated that mated females can discriminate host plants that have been previously attacked by insect  
68 herbivores (Wise & Weinberg, 2002; Stam et al., 2014; Moura et al., 2017; Barnes & Murphy, 2018; Moreira  
69 et al., 2018; Weeraddana & Evenden, 2019), thereby reducing competition between herbivores separated  
70 in time. Mated females can directly detect the present, past and possibly future presence of competitors  
71 themselves. For instance, Averill & Prokopy (1987) showed that female *Rhagoletis pomonella* Walsh (Diptera:  
72 Tephritidae) marks its oviposition site with an epideictic pheromone that deters conspecific females from  
73 laying eggs, thus reducing intraspecific competition at the larval stage. The frass of several Lepidoptera  
74 species was also found to act as an oviposition deterrent (Jones & Finch, 1987; Hashem et al., 2013; Molnár  
75 et al., 2017). **Mated females may also detect herbivory-induced changes in the physical and  
76 chemical characteristics of attacked plants, and consequently avoid laying eggs on less suitable  
77 plants.** However, several authors reported a mismatch between prior herbivory effects on female oviposition  
78 preference *vs* larval growth, consumption or survival of their offspring (Wise & Weinberg, 2002; Bergamini  
79 & Almeida-Neto, 2015; Martinez et al., 2017). For instance, Weeraddana and Evenden (2019) found that  
80 herbivory by the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) on canola plants  
81 (*Brassica napus* L.) had no effect on subsequent oviposition by the bertha armyworm, *Mamestra configurata*  
82 Walker (Lepidoptera: Noctuidae) whereas its larvae had reduced growth on previously damaged plants.  
83 **Thus, in order to quantify the effect of prior herbivory on subsequent herbivore performance,  
84 we need to assess how it affects both female choice and progeny performance in attacked and  
85 non-attacked hosts.**

86 In the present study, we investigated the consequences of box tree (*Buxus* spp.) defoliation by the first  
87 generation of the box tree moth (BTM) *Cydalima perspectalis* Walker (Lepidoptera: Crambidae) larvae  
88 on (i) the oviposition behaviour of **the adults emerging from those larvae** and (ii) on the larval  
89 performance in the next generation. **Specifically, we hypothesized that plants that had previously  
90 been attacked by conspecific larvae would (i) receive fewer eggs (i.e. reduced preference) and  
91 (ii) host smaller larvae and chrysalis (i.e. reduced performance) of the next generation than  
92 previously undamaged plants. Our experimental design allowed us to separate the effects of**

93 **previous herbivory on both preference and performance of conspecific herbivores attacking**  
94 **the same plant in sequence.** By doing so, our study brings new insights into the understanding of  
95 cross-generational intraspecific competition in insect herbivores and further challenges the ‘*mother knows best*  
96 *hypothesis*’.

## 97 **1.2 Materials and methods**

### 98 **1.2.1 Natural history**

99 The BTM is a multivoltine moth species introduced to Europe in 2007 from Asia (Wan et al., 2014). In  
100 its native range, BTM larvae can feed on different host genera, whereas in Europe they feed exclusively on  
101 box trees (Wan et al., 2014). In the introduced area, **BTM larvae overwinter in cocoons tied between**  
102 **two adjacent leaves, mainly in the third instar. Therefore, defoliation restarts in early spring**  
103 at the beginning of the growing season. In Europe, damage is aggravated by the fact that the BTM has 3-4  
104 generations a year (Kenis et al., 2013; Matošević et al., 2017). When several pest generations successively  
105 defoliate the same box tree, there are no leaves left to eat and the caterpillars then feed on the bark, which  
106 can lead to the death of the host tree (Kenis et al., 2013; Wan et al., 2014; Alkan Akıncı & Kurdoğlu, 2019).

### 107 **1.2.2 Biological material**

108 In spring 2019, we obtained box trees from a commercial nursery and kept them in a greenhouse at INRAE  
109 Bordeaux forest research station. Box trees were on average 25 cm high and 20 cm wide. We transferred  
110 them into 5 L pots with horticultural loam. For two months, we watered them every four days from the  
111 above (*i.e.* watering leaves too) to remove any potential pesticide remain.

112 We initiated BTM larvae rearing with caterpillars collected in the wild in early spring 2019, **corresponding**  
113 **to those that had overwintered.** We reared them at room temperature in 4320 cm<sup>3</sup> plastic boxes, and  
114 fed them *ad libitum*, with branches collected on box trees around the laboratory. **We used the next**  
115 **generation larvae to induce herbivory on box tree plants (experimental treatment, see below)**  
116 **and the subsequent adults for the oviposition experiment.** At 25°C, the larval phase lasts for about  
117 30 days and the BTM achieves one generation in 45 days. Adults live 12-15 days. A single female lays on  
118 average 800 eggs.

### 119 **1.2.3 Experimental design**

120 On June 18<sup>th</sup> 2019, we haphazardly assigned box trees to *control* and *herbivory* experimental groups. The  
121 *herbivory* treatment consisted of  $n = 60$  box trees that received five L3 larvae each. Larvae were allowed to  
122 feed freely for one week, after which we removed them all from plants. In order to confirm that the addition  
123 of BTM larvae caused herbivory, we visually estimated BTM herbivory as the percentage of leaves consumed  
124 by BTM larvae per branch, looking at every branch on every plant. We then averaged herbivory at the plant  
125 level. **In 8 plants, herbivory data were missing and was imputed as the average of herbivory**  
126 **measured in other plants.** In the herbivory treatment, the percentage of leaves consumed by BTM larvae  
127 ranged from 2.2 to 17.2% and was on average 9.1%. The *control* group ( $n = 61$ ) did not receive any BTM  
128 larva. On July 8<sup>th</sup>, we randomly distributed plants of the *herbivory* and *control* treatments on a 11 × 11 grid  
129 in a greenhouse (*i.e.* total of 121 plants). We left 40 cm between adjacent pots, which was enough to avoid  
130 any physical contact between neighbouring plants (**Figure 1, Figure 2**).

131 The same day, we released *ca* 100 BTM moths that had emerged from chrysalis less than two days before (*i.e.*,  
132 an uncontrolled mix of males and females). **We released moths at the four corners of the experiment**  
133 **to reduce the risk of spatial aggregation.** Moths were allowed to fly freely within the greenhouse. They  
134 could feed on small pieces of cotton imbibed with a sugar-water solution, disposed on the ground in the  
135 greenhouse.

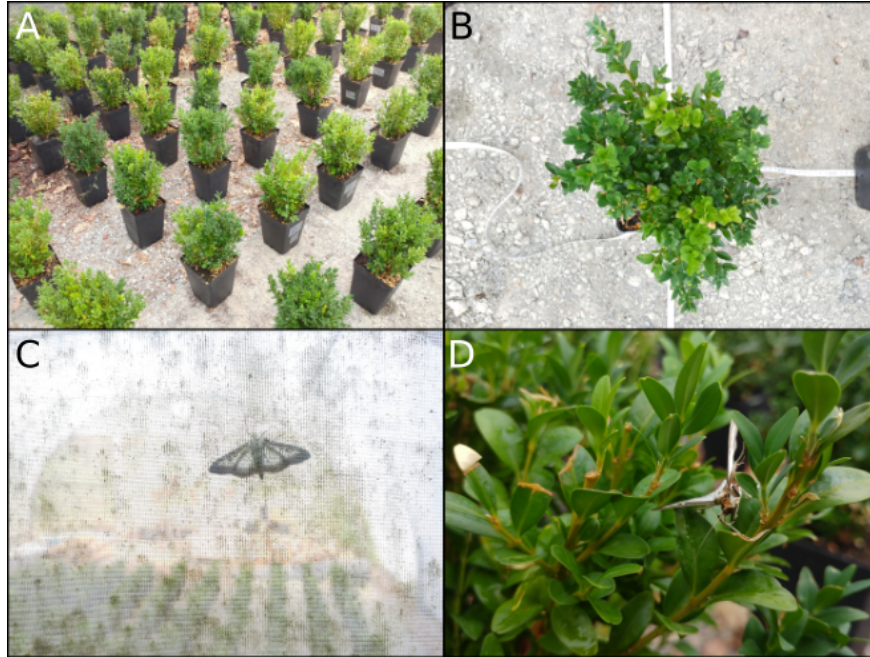


Figure 1: Some photos, because it is nice to see what an experiment looked like. The two top photos (A, B) illustrate the experimental design and in particular distance among potted plants. Photo C is a view of the greenhouse from the outside, with an adult box tree moth in the foreground, and potted plants in the background. Photo D shows an adult box tree moth on a box tree branch, shortly after it was released.

136 It is important to note that at the time we released moths, there were no larvae feeding on experimental box  
 137 trees anymore. In addition, at this time, plants in the herbivory treatment had been cleared of caterpillars for  
 138 three weeks (**corresponding to the duration of the chrysalis stage**) during which they were watered  
 139 every two to three days from above. Although larval frass may have been present in pots submitted to the  
 140 herbivory treatment, it should have been washed out from leaves. Finally, we carried out our experiment in  
 141 an enclosed greenhouse in which the potential effect of natural enemies on BTM behaviour can be neglected.  
 142 The consequences are that any effect of prior herbivory on subsequent oviposition behaviour and larval  
 143 performance should have been independent of cues emitted by BTM larvae themselves or by their frass (Sato  
 144 et al., 1999; Molnár et al., 2017) and therefore were only plant-mediated.

#### 145 1.2.4 BTM host choice

146 In order to test whether initial defoliation of focal plants influenced host choice for oviposition by BTM  
 147 females, we counted egg clutches on every branch of every box tree on July 17<sup>th</sup>. Once eggs were counted, we  
 148 moved box trees to another greenhouse. In order to prevent larvae from moving from one potted plant to  
 149 another, we installed box trees in plastic saucers filled with a few centimeters of water (renewed regularly).

#### 150 1.2.5 BTM growth rate

151 Fifteen days later (July 31<sup>st</sup>), we haphazardly collected up to five L3 BTM larvae per box tree (**only 6%**  
 152 **of plants hosted less than five larvae**). We kept them in Petri dishes without food for 24h **to make**  
 153 **larvae empty their gut** and weighed them to the closest 10 µg. In some Petri dishes, we observed cases  
 154 of cannibalism such that in some instances we could only weight two larvae (Schillé and Kadiri, *personal*  
 155 *observation*). For each plant, we therefore calculated the average weight of a L3 larva, dividing the total mass

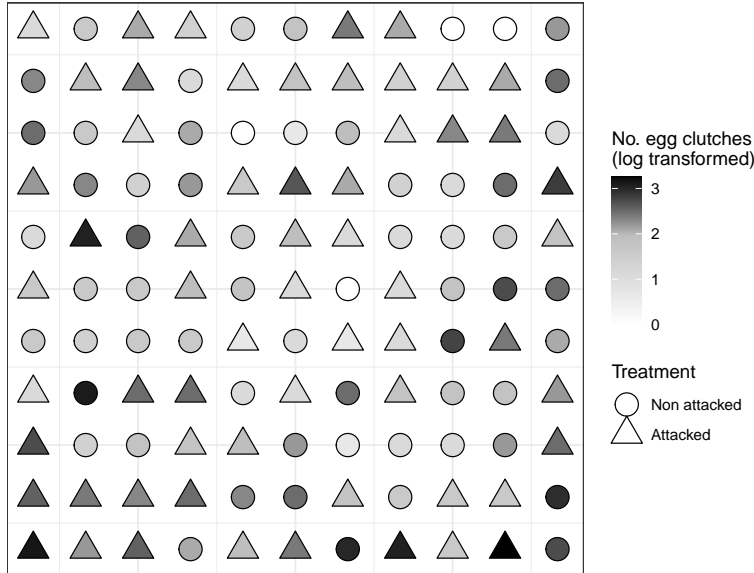


Figure 2: Experimental design. Pots were 40 cm apart. Circles and triangles represent non-attacked (control) and attacked trees. Scale colour represents the number of egg clutches per box tree (log-transformed).

156 by the number of larvae. Because we did not record the day every single egg hatched, we could not quantify  
 157 the number of days caterpillars could feed and therefore simply analysed the average weight of a L3 larva.

158 Larvae were allowed to complete their development on the potted box trees. After every larvae pupated, we  
 159 counted the number of chrysalis per box tree and weighted them to the closest 10  $\mu\text{g}$ .

### 160 1.2.6 Analyses

161 All analyses were ran in R using libraries *nlme* and *car* (Team, 2018; Pinheiro et al., 2020; Fox et al., 2016).

162 We first looked for spatial patterns in female BTM oviposition. **We ran a generalized least square model**  
 163 **(GLS) testing the effect of potted tree location in the experimental design (through their  $x$**   
 164 **and  $y$  coordinates, Figure 2))** on the number of clutches per plant (*log-transformed*) from which we  
 165 explored the associated variogram using the functions *gls* and *Variogram* in the *nlme* library. There was  
 166 evidence that oviposition was spatially structured, with strong spatial autocorrelation between 1 and 3m  
 167 (Figure S1).

168 We tested the effect of prior herbivory on female BTM oviposition (***log-transformed number of egg***  
 169 ***clutches***) while controlling for spatial non-independence using two independent sets of GLS models. **In**  
 170 **the first one, we considered prior herbivory as a two-levels factor (attacked vs non-attacked)**  
 171 **and used the full data set, whereas in the second one, we treated herbivory as a continuous**  
 172 **variable, excluding data from the control treatment. In both cases, we had no particular hypothesis**  
 173 regarding the shape of the spatial correlation structure. We therefore ran separate models with different  
 174 spatial correlation structures (namely, exponential, Gaussian, spherical, linear and rational quadratic), and  
 175 compared them based on their AIC (Zuur, 2009). For each model, we computed the  $\Delta AIC$  (*i.e.*,  $\Delta_i$ ) as  
 176 the difference between the AIC of each model  $i$  and that of the model with the lowest AIC (Burnham &  
 177 Anderson, 2002). We report and interpret the results of the model with the lowest AIC (see *Results*).

178 We then tested the effect of prior herbivory on BTM performance using a two-steps approach. We first used  
 179 two separate ordinary least square models, **with the mean weight of L3 larvae (*log-transformed*) or**  
 180 **the mean weight of chrysalis (untransformed) as a response variable, the herbivory treatment**  
 181 **(non-attacked vs attacked) as a two-levels factor and the number of egg clutches as a covariate.**  
 182 **Then, we restricted the analyses to plants from the herbivory treatment to test the effect of**

183 the percentage of prior herbivory, number of egg clutches and their interaction on the mean  
 184 weight of L3 larvae (*log*-transformed) and chrysalis, separately. We deleted non-significant  
 185 interactions prior to the estimation of model coefficient parameters. Finally, we tested the  
 186 correlation between mean BTM larval weight and mean BTM chrysalis weight at the plant  
 187 level using Pearson’s correlation.

### 188 1.3 Results

189 We counted a total of 818 egg clutches and 593 larvae on 117 out of 121 plants (*i.e.* 96.7%). The  
 190 presence of egg clutches was comparable between control (plants with eggs, *i.e.* 47.1%) and  
 191 herbivory treatments (49.6%). However, at individual plant level, the number of egg clutches varied  
 192 from 0 to 25 (mean  $\pm$  SD:  $6.76 \pm 5.11$ , **Figure 2**).

193 When modelling the effect of prior herbivory on the number of egg clutches using the full data set, the  
 194 best model (*i.e.*, model 5 with  $\Delta_i = 0$ , **Table 1**) was the model with a rational quadratic spatial correlation.  
 195 It was competing with three other models with  $\Delta_i < 2$  (**Table 1**). When the analysis excluded data  
 196 from control plants, the best model was that with a Gaussian spatial correlation (**Table 1**).  
 197 It was competing with three other models, including that with a rational quadratic spatial  
 198 correlation ( $\Delta AIC = 0.2$ ). For sake of consistency, we therefore used this spatial correlation  
 199 in further analyses, for it was common to the two analyses. The results were comparable with  
 200 other spatial correlation structures.

201 The herbivory treatment had no significant effect on the number of egg clutches per plant, regardless  
 202 of whether herbivory was treated as a categorical (model 5, full data set:  $F_{1,119} = 2.91$ ,  $P =$   
 203 **0.09**, **Figure 3A**) or continuous variable (model 5, herbivory treatment only:  $F_{1,53} = 0.88$ ,  $P =$   
 204 **0.353**).

205 The mean weight of BTM larvae varied from 6 to 54 mg (mean  $\pm$  SD:  $20 \pm 9$  mg). There was a significant,  
 206 negative relationship between the number of egg clutches on a box tree and subsequent larval weight (**Table**  
 207 **2**, **Figure 3B**), suggesting intraspecific competition for food. BTM larval weight was lower on box trees that  
 208 had been previously defoliated (**Table 2**, **Figure 3B**), regardless of the amount of herbivory (**Table**  
 209 **2**). There was no significant interaction between the herbivory treatment and the number of egg clutches,  
 210 indicating that intraspecific competition was independent of prior herbivory (**Table 2**). The results were  
 211 the same regardless of whether herbivory was treated as a categorical or continuous variable  
 212 (**Table 2**).

213 The mean weight of BTM chrysalis varied from 52 to 210 mg (mean  $\pm$  SD:  $145 \pm 35$  mg,  $n$   
 214 104). There was a significant positive correlation between the mean weight of BTM larvae  
 215 and the mean weight of chrysalis (Pearson’s  $r = 0.34$ ,  $t$ -value = 3.67,  $P$ -value =  $< 0.001$ ).  
 216 The effects of herbivory treatment and number of egg clutches on mean chrysalis weight were  
 217 very comparable to those observed for BTM larvae: BTM chrysalis weight was lower on box  
 218 trees that had been previously defoliated (**Table 2**, **Figure 3C**), regardless of the amount of

Table 1: Summary of AIC of GLS models testing the effect of prior herbivory on the number of egg clutches with different spatial correlation structures, for the full dataset and the data set excluding plants from the control treatment.

Model	Correlation structure	Full model		Herbivory treatment	
		AIC	\Delta	AIC	\Delta
Model 1	Exponential	249.8	0.4	116.6	0.3
Model 2	Gaussian	250.2	0.8	116.3	0.0
Model 3	Spherical	250.9	1.5	117.7	1.4
Model 4	Linear	255.1	5.7	121.2	4.9
Model 5	Rational quadratic	249.4	0.0	116.5	0.2

219 herbivory. There was a significant, negative relationship between the number of egg clutches  
 220 on a box tree and subsequent chrysalis weight (Table 2, Figure 3C). There was no significant  
 221 interaction between the herbivory treatment and the number of egg clutches (Table 2).

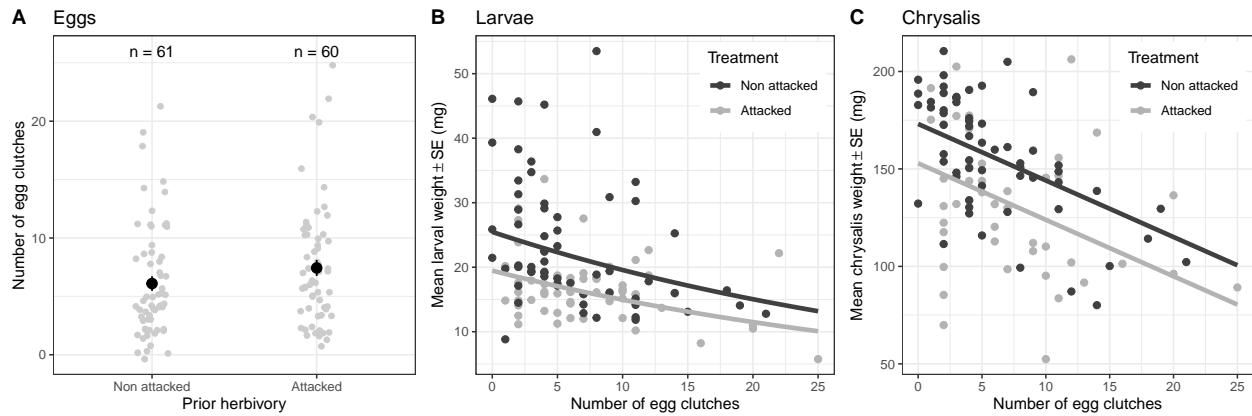


Figure 3: Effects of prior herbivory and conspecific density on (A) the number of egg clutches, (B) L3 larva weight and (C) chrysalis weight. In A, grey dots represent raw data. Black dots and vertical bars represent raw means (+/- SE). In B and C, dots represent raw data. Black and grey curves represent model predictions for control and herbivory treatments, respectively.

## 222 1.4 Discussion

223 Our findings reveal that early season herbivory reduces the performance of conspecific individuals that  
 224 subsequently attack the same host plant later in the plant growing season. This time-lagged intraspecific  
 225 competition results from a mismatch between female oviposition preference and the performance of its  
 226 offspring.

227 *Prior herbivory had no effect BTM oviposition choice.* One possibility for female BTM not choosing among  
 228 plants may be that that prior herbivory had no effect on box tree characteristics, or that female BTM were  
 229 indifferent to them at the time we conducted the experiment.

230 The first explanation seems unlikely as **we found clear evidence that prior herbivory reduced the**  
 231 **performance of BTM larvae latter in the season.** This is fully in line with the numerous studies that  
 232 have established that insect herbivory induces changes in plant physical and chemical traits, which have  
 233 profound consequences on herbivores or herbivory on the same host plant later in the season (Poelman et al.,  
 234 2008; Abdala-Roberts et al., 2019; Wise & Weinberg, 2002; Stam et al., 2014; but see Visakorpi et al., 2019).  
 235 We cannot dismiss the second explanation that BTM females were indifferent to box tree cues related to  
 236 earlier herbivory. This may be particularly true in species whose females individually lay several hundred eggs,  
 237 **for which spreading eggs among several host plants may be an optimal strategy** (Root & Kareiva,  
 238 1984; Hopper, 1999). Consistently, Leuthardt and Baur (2013) observed that BTM females evenly distributed  
 239 egg clutches among leaves and branches, and that oviposition preference was not dictated by the size of the  
 240 leaves. Assuming that this behavior is reproducible, the close distance between box-trees that we used in  
 241 the present experiment (40 cm) could explain the lack of effect of initial defoliation on BTM oviposition  
 242 behavior. In addition, Leuthardt *et al.* (2013) showed that BTM larvae are able to store or metabolise highly  
 243 toxic alkaloid present in box tree leaves. Last, BTM larvae proved to be unable to distinguish between box  
 244 tree leaves infected or not by the box rust *Puccinia buxi*, while their growth is reduced in the presence of  
 245 the pathogenic fungus (Baur et al., 2019). Altogether, these results suggest that BTM female moths are not  
 246 influenced by the amount of intact leaves and probably not either by their chemical quality when choosing the  
 247 host plant, perhaps because of their strong ability to develop on toxic plants. **It remains however possible**  
 248 **that BTM adults use other cues to select their hosts such as the presence of conspecific eggs,**  
 249 **larvae or chrysalis.**

Table 2: Summary of models testing the effect of prior herbivory (with the full data set or the data set restricted to the herbivory treatment) and initial egg clutch density on mean BTM larvae and chrysalis weight

Data set	Response	Predictor	df	F-value	P-value	R <sup>2</sup>	Estimate (SE)
Full	Larvae	Number of egg clutches	1, 117	26.31	< 0.001	0.27	-0.026 (0.006)
		Herbivory	1, 117	20.30	< 0.001		-0.269 (0.06)
		Eggs x Herbivory	1, 117	0.73	0.396		
	Chrysalis	Number of egg clutches	1, 100	33.74	< 0.001	0.31	-0.003 (0.001)
		Herbivory	1, 100	12.23	< 0.001		-0.02 (0.006)
		Eggs x Herbivory	1, 100	3.14	0.079		
Herbivory subset	Larvae	Number of egg clutches	1, 56	10.55	0.002	0.14	-0.022 (0.007)
		Herbivory	1, 56	0.16	0.691		-0.003 (0.009)
		Eggs x Herbivory	1, 56	1.74	0.193		
	Chrysalis	Number of egg clutches	1, 41	4.28	0.045	0.06	-0.002 (0.001)
		Herbivory	1, 41	1.08	0.306		-0.001 (0.001)
		Eggs x Herbivory	1, 41	0.39	0.535		

250 *Prior box tree defoliation by the spring generation of BTM larvae reduced the performance of the next*  
251 *generation.* Two alternative, non-mutually exclusive mechanisms can explain this phenomenon. First, the  
252 reduced performance of individuals of the second generation can have resulted from induced plant defenses.  
253 This explanation is in line with studies that have documented in several plant species reduced herbivore  
254 performance and changes in plant-associated herbivore communities linked to induced defenses after prior  
255 herbivory (Nykänen & Koricheva, 2004; Karban, 2011; Stam et al., 2014). In the case of multivoltine  
256 species, negative relationship between prior herbivory and subsequent larva growth rate could indicate  
257 intraspecific plant-mediated cross-generation competition between cohorts of herbivores separated in time  
258 (Barnes & Murphy, 2018), which could influence herbivore population dynamics and distribution across  
259 host individuals. However, **BTM is thought to have broad tolerance to variability in host traits,**  
260 **as suggested by previous observations that BTM larva growth rate did not differ significantly**  
261 **among box-tree varieties (Leuthardt et al., 2013).** It is unknown whether herbivory induced  
262 **changes in host traits are of the same order of magnitude as trait variability among varieties.**  
263 **However, assuming variability among varieties is greater, this result goes against the view that**  
264 **reduced performance of larvae of the summer generation resulted from box tree response to**  
265 **prior herbivory.** Secondly, reduced performance on previously defoliated plants may partly result from  
266 food shortage and increased exploitative competition among larvae of the same cohort. Although free living  
267 mandibulate herbivores were described to be less sensitive to competition (Denno et al., 1995), the effect of  
268 food shortage may have been exacerbated by the small size of box trees and interference competition (Kaygin  
269 & Taşdeleler, 2019).

270 *We detected a negative relationship between the number of egg clutches laid by BTM female moths and the*  
271 *subsequent growth of BTM larvae.* This suggests the existence of intraspecific competition for food within  
272 the same cohort. Such competition has already been reported, particularly in leaf-miners (Bultman &  
273 Faeth, 1986; Faeth, 1992), which are endophagous insect herbivores whose inability to move across leaves  
274 makes them particularly sensitive to the choice of oviposition sites by gravid female. In our study, we  
275 prevented larvae from moving from one plant to another and noticed that some box trees were completely  
276 defoliated by the end of the experiment. Although we did not record this information, it is very likely that  
277 larvae first ran out of food in plants on which several egg clutches were laid. **We are however unable to**  
278 **determine whether the observed intraspecific competition in this cohort was determined by**  
279 **food shortage, or by herbivore-induced changes in resource quality, or both.** In addition, we  
280 noticed that the number of chrysalis in 32 control plants (out of 61, *i.e.* 52%) was greater than  
281 the number of larvae, whereas this only happened in only one previously attacked plant (*i.e.*  
282 2%). This indicates that in spite of our precautions some larvae could move from attacked to  
283 control plants (Table 3). Together with the fact that patterns of chrysalis weight were very  
284 similar to patterns of larval weight, these findings can be seen as another argument in favor



285 of larvae escaping from intraspecific competition on previously attacked plants. However, this  
286 idea should be taken with caution as it is possible that such an experimental setup with small  
287 potted trees overestimated the effect of intraspecific competition.

288 Our findings may have profound implications on our understanding of BTM population dy-  
289 namics. In many Lepidoptera species, all eggs are present in the ovarioles as the adult molt and  
290 larva body mass is proportional to fecundity (i.e., ‘capital breeders’, (Honěk, 1993; Awmack  
291 & Leather, 2002)). As a consequence, host plant quality during larval growth and develop-  
292 ment is a key determinant of individuals fitness (Awmack & Leather, 2002). Although the  
293 relationship between plant quality and herbivore fitness may vary among species (Moreau et  
294 al., 2006; Awmack & Leather, 2002; Colasurdo et al., 2009), we speculate that herbivory by  
295 the first BTM larva generation reduces the fitness of the second BTM generation, and that  
296 this effect may be further strengthened where high population density increases intra-specific  
297 cross-generational competition (Tammaru & Haukioja, 1996). We may therefore predict a  
298 strong density dependent feedback on population growth.

## 299 1.5 Conclusion

300 Our greenhouse experiment provides evidence for negative interaction across and within BTM generations,  
301 which are independent of BTM female choice for oviposition site. Such interactions may have consequences  
302 on BTM population dynamics and damage on box-trees. On the one hand, the slow-growth-high-mortality  
303 hypothesis states that any plant trait reducing the growth rate of herbivores can be seen as a resistance  
304 trait, because slow-growing herbivores are longer exposed to their enemies such as parasitoids, spiders or  
305 insectivorous birds (Benrey & Denno, 1997; Coley et al., 2006; Uesugi, 2015). It is therefore possible that  
306 a stronger top-down control can be exerted by generalist enemies on BTM larvae feeding on previously  
307 defoliated hosts which could reduce damage on box-trees. On the other hand, if herbivores take a longer  
308 time to complete development, they may cause more damage to plants, in particular to those with low  
309 nutritional quality as a result of compensatory feeding (Simpson & Simpson, 1990; Milanovic et al., 2014).  
310 The consequences of time-lagged intraspecific competition on the spread of and damage by BTM remain  
311 however to be investigated in the field. Particular efforts should be dedicated to the identification of host  
312 traits controlling the performance of BTM larvae and the interaction between these traits and the higher  
313 trophic level.

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318 Costa-Pereira and Sara Magalhães for their helpful comments on earlier version of this manuscript.

## 319 1.7 Data accessibility

320 Raw data as well as codes of statistic analysis are available in supplementary material and on the INRA  
321 dataverse: Castagneyrol, Bastien; van Halder, Inge; Kadiri, Yasmine; Schillé, Laura; Jactel, Hervé, 2020,  
322 "Raw data for the paper ‘Host-mediated, cross-generational intraspecific competition in a herbivore species’,  
323 <https://doi.org/10.15454/KMUX39>, Portail Data INRAE, V3.0.

## 324 1.8 Conflict of interest

325 The authors of this preprint declare that they have no financial conflict of interest with the content of this  
326 article. Bastien Castagneyrol is one of the *PCI Ecology* recommenders.

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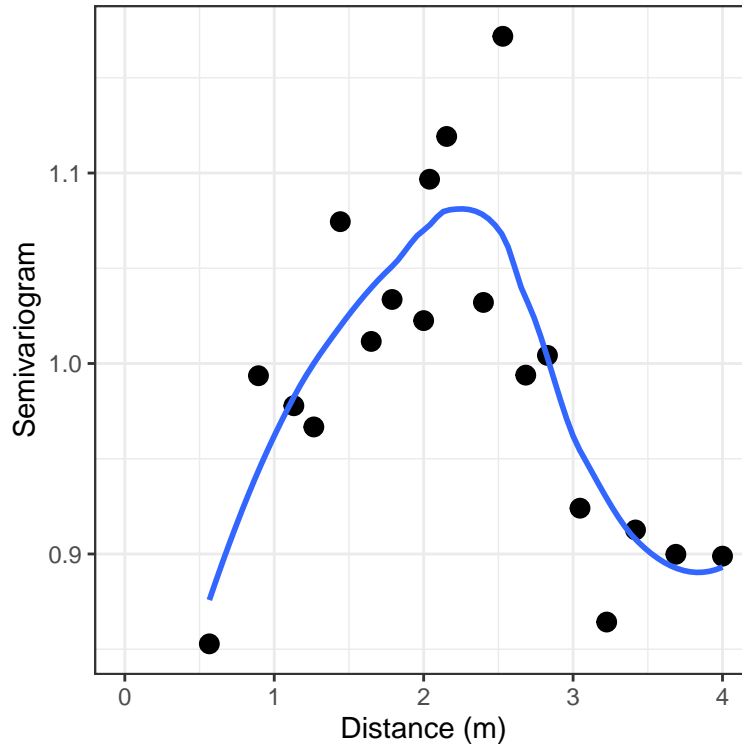
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## 471 2 Appendix

### 472 2.1 Supplementary material

473 **Figure S1** - Semivariogram of the number of egg clutches as a function of distance among box trees.



474

Table 3: Repartition of egg clutches, larvae and chrysalis across box trees with or without prior herbivory. Numbers correspond to mean ( $\pm$  sd) and total number of egg clutches, larvae or chrysalis (n).

Response variable	Control	Herbivory treatment
Egg clutches	6.1 (4.87), n = 372	7.43 (5.3), n = 446
Larvae	4.84 (0.61), n = 295	4.97 (0.18), n = 298
Chrysalis	6.8 (5.78), n = 415	1.85 (1.79), n = 111

## 475 2.2 Raw data

476 **Table S2** - Raw data used in the present manuscript: `x` and `y` are the position of each box tree in the  
477 green house; `Treatment` is the prior herbivory treatment; `Clutch.number` is the total number of egg clutches  
478 counted on a given box tree; `N.L3` is the number of retrieved L3 larvae, `L3.mean` is the mean weight of a  
479 L3 larvae (g); `N.chrysalids` is the number of retrieved chrysalis; `Chrysalid.mean` is the mean weight of a  
480 chrysalis. `Herbivory` is the % of leaves consumed by box tree moth larvae, which was either measured or  
481 estimated where raw data was missing (`Herbivory_source`).

x	y	Treatment	Clutch.number	N.L3	L3.mean	N.chrysalids	Chrysalid.mean	Herbivory	Herbivory_s
1	1	Attacked	22	5	0.0221740	0	NaN	7.3	Estimated
2	1	Attacked	8	5	0.0183980	0	NaN	8.8	Estimated
3	1	Attacked	12	5	0.0187360	1	0.1020100	8.8	Imputed
5	1	Attacked	6	5	0.0146140	0	NaN	16.4	Estimated
6	1	Attacked	10	5	0.0165620	2	0.1101750	10.5	Estimated
8	1	Attacked	20	5	0.0110140	1	0.0961900	8.7	Estimated
9	1	Attacked	4	5	0.0132300	1	0.1438500	7.4	Estimated
10	1	Attacked	25	5	0.0057520	1	0.0891900	11.7	Estimated
1	2	Attacked	12	5	0.0226500	2	0.2062350	6.2	Estimated
2	2	Attacked	10	5	0.0162200	1	0.0524100	0.0	Imputed
3	2	Attacked	9	5	0.0200760	0	NaN	4.2	Estimated
4	2	Attacked	11	5	0.0211200	5	0.1465000	4.8	Estimated
7	2	Attacked	5	5	0.0112560	0	NaN	9.0	Estimated
9	2	Attacked	4	5	0.0161760	1	0.1338800	15.1	Estimated
10	2	Attacked	4	5	0.0173680	1	0.1706800	9.4	Estimated
1	3	Attacked	14	5	0.0159000	4	0.1686525	8.6	Imputed
4	3	Attacked	5	5	0.0159420	2	0.1380100	0.0	Imputed
5	3	Attacked	6	5	0.0121100	1	0.1319100	8.6	Imputed
11	3	Attacked	11	5	0.0101960	1	0.0836300	7.2	Estimated
1	4	Attacked	2	5	0.0111600	2	0.1224050	11.4	Estimated
3	4	Attacked	11	5	0.0157420	0	NaN	8.6	Imputed
4	4	Attacked	11	5	0.0158140	4	0.1557575	6.8	Estimated
6	4	Attacked	2	5	0.0238660	2	0.1728600	11.7	Estimated
8	4	Attacked	5	5	0.0187260	2	0.1527050	10.0	Estimated
11	4	Attacked	8	5	0.0181900	0	NaN	10.3	Estimated
5	5	Attacked	1	5	0.0201820	1	0.1914500	2.7	Estimated
7	5	Attacked	1	5	0.0148320	3	0.1752800	8.9	Estimated
8	5	Attacked	2	5	0.0176160	1	0.0853400	9.1	Estimated
10	5	Attacked	10	4	0.0171925	1	0.1453700	9.0	Estimated
1	6	Attacked	4	5	0.0158160	2	0.1774000	2.2	Estimated
4	6	Attacked	6	5	0.0161500	0	NaN	6.8	Estimated
6	6	Attacked	2	4	0.0148600	0	NaN	10.9	Estimated
8	6	Attacked	2	5	0.0273120	2	0.0698850	17.0	Estimated
2	7	Attacked	20	5	0.0104900	4	0.1364600	3.6	Estimated
4	7	Attacked	7	5	0.0275520	5	0.1299800	12.5	Estimated
6	7	Attacked	6	5	0.0143660	2	0.1127850	9.7	Estimated
7	7	Attacked	2	5	0.0145880	0	NaN	17.2	Estimated
11	7	Attacked	5	5	0.0129260	3	0.1438500	9.5	Estimated
1	8	Attacked	8	5	0.0161140	0	NaN	9.5	Estimated
5	8	Attacked	4	5	0.0336620	4	0.1512050	0.0	Imputed
6	8	Attacked	13	5	0.0136940	1	0.0916800	5.6	Imputed
7	8	Attacked	7	5	0.0119960	0	NaN	7.6	Estimated
11	8	Attacked	16	5	0.0082180	5	0.1013240	5.6	Imputed
3	9	Attacked	2	5	0.0124840	2	0.1309350	0.0	Imputed
8	9	Attacked	2	5	0.0140740	1	0.0996800	5.6	Imputed
9	9	Attacked	9	5	0.0147260	3	0.1120367	15.1	Estimated
10	9	Attacked	10	5	0.0121140	3	0.1454233	7.9	Estimated
2	10	Attacked	6	5	0.0183400	3	0.1203367	0.0	Imputed
3	10	Attacked	9	5	0.0159820	3	0.1078233	6.8	Estimated
5	10	Attacked	2	5	0.0291080	2	0.1450000	10.5	Estimated
6	10	Attacked	5	5	0.0185740	0	NaN	8.6	Estimated
7	10	Attacked	6	5	0.0177680	0	NaN	12.4	Estimated
8	10	Attacked	3	5	0.0149260	1	0.2025200	9.4	Estimated
9	10	Attacked	3	5	0.0195980	2	0.1319950	8.2	Estimated
10	10	Attacked	7	5	0.0157780	2	0.0985400	16.2	Estimated
1	11	Attacked	2	5	0.0161540	5	0.1175720	8.6	Imputed
3	11	Attacked	7	5	0.0190760	5	0.1385900	8.5	Estimated
4	11	Attacked	2	5	0.0170000	2	0.1571000	5.2	Estimated

## 2.3 R codes used to generate this report

```

library(tidyverse)
library(knitr)
library(kableExtra)
library(nlme)
library(car)
library(here)

wd = here()

my.ggplot <- function(){
  theme_bw() + theme(legend.key = element_blank())
}

knitr::opts_chunk$set(echo= F, warning= F, message = F, results = "hide",
  fig.width=4, fig.height=4, dpi= 100, fig.pos = "H",
  fig.path = paste0(wd, '/Figures/'),
  output.dir = paste0(wd, '/Outputs/'))

d = read.csv(paste0(wd, '/Data/Castagneyrol_cydalima_data.csv'), header = T)

str(d)

d =
  d %>% mutate(L3.mean = Weight.L3 / N.L3) %>%
  mutate(Treatment = factor(Treatment, levels = c("Non attacked", "Attacked")))

knitr::include_graphics(paste0(wd, '/Figures/Figure 1.png'))

d %>%
  ggplot(aes(x, y, shape = Treatment, fill = log1p(Clutch.number))) +
  # geom_rect(aes(xmin = 1.5, ymin = 1.5, xmax = 10.5, ymax = 10.5), fill = 'grey90') +
  geom_point(size = 6) +
  scale_shape_manual(values = c(21, 24)) +
  my.ggplot() +
  labs(x = "", y = "") +
  scale_fill_gradientn(colours = c('white', 'grey', 'black'),
    values = c(0,0.6,1), name = 'No. egg clutches\n(log transformed)') +
  theme(axis.ticks.x = element_blank(),
    axis.text.x = element_blank(),
    axis.ticks.y = element_blank(),
    axis.text.y = element_blank())

d.center = droplevels(d[d$x > 1 & d$x < 11 & d$y > 1 & d$y < 11,])
m1a = gls(log1p(Clutch.number) ~ Treatment,
  correlation = corExp(form = ~x + y, nugget = TRUE), data = d, na.action = "na.omit")
m1b = gls(log1p(Clutch.number) ~ Treatment,
  correlation = corGaus(form = ~x + y, nugget = TRUE), data = d, na.action = "na.omit")
m1c = gls(log1p(Clutch.number) ~ Treatment,
  correlation = corSpher(form = ~x + y, nugget = TRUE), data = d, na.action = "na.omit")
m1d = gls(log1p(Clutch.number) ~ Treatment,
  correlation = corLin(form = ~x + y, nugget = TRUE), data = d, na.action = "na.omit")
m1e = gls(log1p(Clutch.number) ~ Treatment,

```



```

correlation = corRatio(form = ~x + y, nugget = TRUE), data = d, na.action = "na.omit")

l1 = list(m1a, m1b, m1c, m1d, m1e)

l2 = list(
m1a_2 = update(m1a, ~ Herbivory, data = d[d$Herbivory > 0,]),
m1b_2 = update(m1b, ~ Herbivory, data = d[d$Herbivory > 0,]),
m1c_2 = update(m1c, ~ Herbivory, data = d[d$Herbivory > 0,]),
m1d_2 = update(m1d, ~ Herbivory, data = d[d$Herbivory > 0,]),
m1e_2 = update(m1e, ~ Herbivory, data = d[d$Herbivory > 0,])
)

AIC_1 = round(unlist(lapply(l1, function(x){AIC(x)})),1)
delta_1 = AIC_1 - min(AIC_1)
AIC_2 = round(unlist(lapply(l2, function(x){AIC(x)})),1)
delta_2 = AIC_2 - min(AIC_2)

Table_AIC = data.frame(AIC_1, delta_1, AIC_2, delta_2)
plot.resid = function(m){
df = data.frame(f = fitted(m), r = residuals(m))
A = df %>%
  ggplot(aes(f, r)) + my.ggplot() + geom_point() +
  geom_smooth(method = "lm", se = F) + geom_hline(yintercept = 0)
B = df %>%
  ggplot(aes(r)) + my.ggplot() + geom_histogram()
cowplot::plot_grid(A,B)
}

m_larvae = lm(log(L3.mean) ~ Clutch.number * Treatment , d)
m_larvae2 = lm(log(L3.mean) ~ Clutch.number * Herbivory, d[d$Treatment == "Attacked",])
# plot.resid(m_larvae2)

d =
d %>%
mutate(Chrysalid.mean = Weight.chrysalids / N.weighted.chrysalids)

m_chrys = lm(Chrysalid.mean ~ Clutch.number * Treatment , d)
m_chrys2 = lm(Chrysalid.mean ~ Clutch.number * Herbivory, d[d$Treatment == "Attacked",])
#plot(m_chrys2)
# lapply(l1, function(x) anova(x))
# lapply(l2, function(x) anova(x))
Table_AIC %>%
mutate(Model = paste('Model', 1:5),
'Correlation structure' = c('Exponential', 'Gaussian', 'Spherical',
'Linear', 'Rational quadratic')) %>%
select(Model, `Correlation structure`, everything()) %>%
kable(col.names = c("Model", "Correlation structure", "AIC", "\\Delta", "AIC", "\\Delta"),
caption = "Summary of AIC of GLS models testing the effect of prior herbivory on the number of
kableExtra::kable_styling() %>%
add_header_above(c(" " = 2, "Full model" = 2, "Herbivory treatment" = 2))
anova(m1e)

```

```

Fig_3A =
  d %>%
  ggplot(aes(Treatment, Clutch.number)) +
  my.ggplot() +
  geom_point(position = position_jitter(0.1), colour = "grey80") +
  stat_summary(size = 0.6) +
  labs(x = "Prior herbivory", y = "Number of egg clutches") +
  annotate(geom = 'text', x = 1:2, y = 26, label = paste("n =", c(61, 60)))
res_larvae = anova(m_larvae, test = "m")
n = expand.grid(Treatment = levels(d$Treatment), Clutch.number = seq(0, 25))
p = predict(update(m_larvae, ~.- Clutch.number:Treatment), newdata = n)
n$Fit = 1000 * exp(p)

Fig_3B =
  d %>%
  ggplot(aes(Clutch.number, 1000 * L3.mean, colour = Treatment)) +
  my.ggplot() +
  geom_point(size = 2) +
  scale_colour_manual(values = c("grey25", "grey70")) +
  labs(x = "Number of egg clutches",
       y = expression("Mean larval weight" %+-% "SE (mg)")) +
  geom_line(data = n, aes(Clutch.number, Fit), size = 1.5) +
  theme(legend.position = c(0.8, 0.85))
r = with(d, cor.test(Chrysalid.mean, L3.mean))
r_corr = round(r$estimate,2)
r_tval = round(r$statistic,2)
r_pval = ifelse(round(r$p.value,3) < 0.001, "< 0.001", round(r$p.value,3))
res_chrys = anova(m_chrys)
n = expand.grid(Treatment = levels(d$Treatment), Clutch.number = seq(0, 25))
p = predict(update(m_chrys, ~.- Clutch.number:Treatment), newdata = n)
n$Fit = 1000 * p

Fig_3C =
  d %>%
  ggplot(aes(Clutch.number, 1000 * Chrysalid.mean, colour = Treatment)) +
  my.ggplot() +
  geom_point(size = 2) +
  scale_colour_manual(values = c("grey25", "grey70")) +
  labs(x = "Number of egg clutches",
       y = expression("Mean chrysalis weight" %+-% "SE (mg)")) +
  geom_line(data = n, aes(Clutch.number, Fit), size = 1.5) +
  theme(legend.position = c(0.8, 0.85))

cowplot::plot_grid(
  Fig_3A + labs(title = 'Eggs'),
  Fig_3B + labs(title = 'Larvae'),
  Fig_3C + labs(title = 'Chrysalis'),
  ncol = 3,
  labels = c('A', 'B', 'C')
)
f = function(model, response, data_set){
  ANOVA = anova(model, test = "m")
  Fval = function(ANOVA) {round(ANOVA[,4], 2)}
}

```

```

Pval = function(ANOVA) {ifelse(ANOVA[,5] < 0.001, '< 0.001', round(ANOVA[,5], 3))}

if(Pval(ANOVA)[3] < 0.05){
  b = round(summary(model)$coefficients[-1,1], 3)
  b_se = round(summary(model)$coefficients[-1,2], 3)
  Estimate = paste(b, ' (', b_se, ')', sep = '')

}

}

form = formula(paste("~",
                    paste(attr(model$terms, "variables")[[3]],
                          attr(model$terms, "variables")[[4]],
                          sep = "+")))
b = round(summary(update(model, formula. = form))$coefficients[-1,1], 3)
b_se = round(summary(update(model, formula. = form))$coefficients[-1,2], 3)
Estimate = c(paste(b, ' (', b_se, ')', sep = ''), '')
}

return(df =
  data.frame(
    Data = c(data_set, "", ""),
    Response = c(response, '', ''),
    Predictor = c("Number of egg clutches", "Herbivory", "Eggs x Herbivory"),
    df = paste(ANOVA$Df[1:3], rep(ANOVA$Df[4], 3), sep = ", "),
    `F-value` = Fval(ANOVA)[-4],
    `P-value` = Pval(ANOVA)[-4],
    R2 = c(round(summary(model)$adj.r.squared, 2), "", ""),
    Estimate = Estimate))
}

rbind(
  f(m_larvae, response = "Larvae", data_set = "Full"),
  f(m_chrys, response = "Chrysalis", data_set = ""),
  f(m_larvae2, response = "Larvae", data_set = "Herbivory subset"),
  f(m_chrys2, response = "Chrysalis", data_set = "")) %>%
kable(caption = "Summary of models testing the effect of prior herbivory (with the full data set or t
      col.names = c("Data set", "Response", "Predictor", "df", "F-value", "P-value", "R2", "Estimate
      escape = T, digit = 2) %>%
kable_styling() %>%
collapse_rows(columns = 1:2, valign = "top")

m0 = gls(log1p(Clutch.number) ~ x + y, data = d, na.action = "na.omit")
variog0 <- Variogram(m0, form = ~x + y, resType = "pearson", nugget = T)
variog0 %>%
  ggplot(aes(0.4*dist, variog)) +
  my.ggplot() +
  geom_point(size = 3) +
  geom_smooth(se = F) +
  labs(x = "Distance (m)", y = "Semivariogram") +
  xlim(0, 4)
d %>%

```

```

select(Treatment, Clutch.number, N.L3, N.chrysalids) %>%
gather(Resp, Val, 2:4) %>%
group_by(Treatment, Resp) %>%
summarise(N = sum(Val), M = round(mean(Val),2), SD = round(sd(Val),2)) %>%
mutate(Val = paste(M, ' (', SD, ')', ', n = ', N, sep = '')) %>%
select(- N, - M, - SD) %>%
mutate(Resp = factor(Resp, levels = c('Clutch.number', 'N.L3', 'N.chrysalids'),
                      labels = c('Egg clutches', 'Larvae', 'Chrysalis'))) %>%
spread(Treatment, Val) %>%
kable(col.names = c('Response variable', 'Control', 'Herbivory treatment'),
      caption = "Repartition of egg clutches, larvae and chrysalis across box trees with or without p
kable_styling()
d %>%
rename(N.chrysalids = N.chrysalids) %>%
select(x, y, Treatment, Clutch.number, N.L3, L3.mean, N.chrysalids, Chrysalid.mean, Herbivory, Herbivory) %>%
kable() %>% kable_styling()

```

### 3 Responses to reviewers' comments

Dear Dr Magalhães,

We would like to thank you for your constructive and helpful comments. We revised the original manuscript accordingly. Significant changes in the manuscript are highlighted in bold characters. You may find our responses to your comments and to the two reviewers below, highlighted with bold characters. Wherever only minor changes were requested, we simply indicated “[R] — Done”, otherwise, we justified what we changed, or not, in the manuscript.

We hope that the revised version of our manuscript has addressed every concerns and will be suitable for recommendation.

Best regards,

Bastien Castagneyrol, on behalf of co-authors.

---

Dear authors,

First of all, I deeply apologize for having taken so long to comment on this manuscript. I hope that the quality of the reviews compensates for this long wait... I found this article interesting and straightforward. I particularly appreciated the scale and nature of the experiment, being an intermediate between a lab and a field experiment. The thorough and insightful comments of the two reviewers also point in the same direction: they both enjoyed the manuscript very much. They do, however, suggest a number of changes that I think should be addressed in the revised version of the manuscript. In particular, they both suggest including more variables in your analysis, which they (and I) believe you can do based on the data you already have. If that is the case, I think it is a cost-effective means to make your article more complete.

**[Response] — We followed recommendations made by the two reviewers and yourself and provide below detailed answers wherever necessary**

Below I place my own comments, and I would be happy to look at a revised version of this paper soon. I only have two relatively major and a lot of minor comments.

1. I agree with one of the reviewers in that the introduction could be a bit further streamlined. If I understand correctly, the first paragraph is about preference-performance correlations, the second about preference, and the third a bit about performance and then another bit about preference-performance. You also go a bit back and forth concerning the effects of conspecifics and that of heterospecifics. I

512 don't want to impose my view on the introduction of a paper that is not mine, but I would like to  
513 feel that, whatever the structure chosen, it is apparent to the reader. In any case, I would exchange  
514 the order of second and third paragraph, first differences in performance and then oviposition choice,  
515 because the latter does not make sense without the former and the reverse is not true.

516 **[R] — We changed the order of the two paragraphs as suggested and modified several sen-**  
517 **tences to improve the flow. Please note that we did not highlight every single change in the**  
518 **manuscript, only the most important additions.**

519 2. I think the reader needs some information on how larval weight correlates (or may correlate) with fitness  
520 in this (or related) species. This would allow discriminating among the two main possible interpretations  
521 for this data set, namely (a) they don't discriminate because the consequences for fitness are not strong  
522 enough or (b) they don't discriminate because they don't have access to reliable cues.

523 **[R] — We now discuss this question:**

524 *Our findings may have profound consequences on BTM population dynamics. In most*  
525 *of Lepidoptera species, all the eggs are present in the ovarioles as the adult molt and*  
526 *larva body mass is proportional to fecundity (i.e., 'capital breeders', (Honěk, 1993;*  
527 *Awmack & Leather, 2002)). As a consequence, host plant quality during larval growth*  
528 *and development is the key determinant of individuals fitness (Awmack & Leather, 2002).*  
529 *Although the relationship between plant quality and herbivore fitness may vary among*  
530 *species (Moreau et al., 2006; Awmack & Leather, 2002; Colasurdo et al., 2009), we*  
531 *speculate that herbivory by the first BTM larva generation reduces the fitness of the*  
532 *second BTM generation, and that this effect may be further strengthened where high*  
533 *population density increase intra-specific cross-generational competition (Tammaru &*  
534 *Haukioja, 1996).*

535 3. Minor comments:

536 4. I would remove "multivoltine" from the title. The cross-generational already gives the idea. . . .

537 **[R] — Done**

538 5. Line 19: replace "proposed" by "offered".

539 **[R] — Done**

540 6. Line 23: unclear if this number of eggs is from the previous or the current brood.

541 **[R] — Changed**

542 7. Line 37: replace "their" by "its".

543 **[R] — Done**

544 8. Line 54: "deters" instead of "deter".

545 **[R] — Done**

546 9. Line 60: incidentally, previous herbivory can also lead to increased performance in subsequent infestations  
547 (e.g., Sarmiento et al 2011 Ecol Lett, Godinho et al 2016 Oecologia). This is just a side comment, you  
548 don't need to include this in the paragraph. . .

549 **[R] — Thank you for these references.**

550 10. Lines 94-100. This paragraph is a bit confusing. First, I suggest placing the sentences on the biological  
551 details of the system (lines 96-98) elsewhere, maybe in the very beginning of the Material and Methods  
552 section. Second, it is not very clear to me when were the moths placed on the experimental trees. Is  
553 "the overwintering generation" the same as the "caterpillars collected in the wild"? If so, please be clear  
554 about this. Also, I guess that by "their" adults you mean "the adults emerging from those larvae"?  
555 (also commented by one of the reviewers).

556 [R] — We restructured this paragraph, adding a new subsection (“*natural history*”) and clar-  
557 ifying the description of first and second BTM generations (section “biological material”).

558 11. Line 104: I would state “plants” instead of “plant individuals”.

559 [R] — Done

560 12. Line 111: you seem to use “chrysalis” and “chrysalids” interchangeably. If these terms refer to the same  
561 thing (I guess they do...) please choose one.

562 [R] — Done

563 13. Results: Did you count the number of egg clutches per plant with at least one clutch or per experimental  
564 plant in general? That is, could there be a difference in the number of plants with no egg clutches  
565 among treatments?

566 [R] — We screened every single plant and counted the number of egg clutches on all plants.  
567 We added the information on the presence/absence of eggs to the ‘*results*’ section.

568 14. I would not discard the data concerning chrysalids so easily. It is indeed a pity that you cannot  
569 discriminate whether more larvae died in one treatment vs the other or if there was active migration, as  
570 you state. But in any case, this means that more individuals of this second ‘generation’ are eventually  
571 found on the previously clean plants, and this is an interesting result per se. I would at least discuss  
572 this a bit further in the Discussion.

573 [R] — We do agree this is very unfortunate we have not been satisfyingly efficient in preventing  
574 larvae from moving among plants. Although we have been reluctant to present this data in  
575 first instance, we now reinjected them back into the manuscript, with words of caution in the  
576 discussion. Changes can be seen throughout the text in the “methods / analyses”, “*results*”  
577 and “*discussion*” sections.

578 15. Lines 180-183: Maybe rephrase as to use a more fluid text style. Ex: One possibility for female BTM  
579 not choosing among plants may be that...

580 [R] — Done. Indeed, it reads smoother.

581 16. Lines 182-190: I think the main argument against this hypothesis comes from your own data: larval  
582 weight differs among treatments. Assuming this is correlated with fitness, there are consequences for  
583 the moths of their mothers’ choice.

584 [R] — Yes! thank you for mentioning this. We have added this obvious argument.

585 17. Line 193: I don’t understand why laying 200 eggs corresponds necessarily to a bet-hedging strategy.  
586 Maybe rephrase?

587 [R] — Done.

588 18. Lines 203-204: This paragraph is about the possible absence of cues, not about the possible absence of  
589 fitness consequences, so this sentence is best placed in the previous paragraph.

590 [R] — This paragraph, starting with “*Prior box tree defoliation by the spring generation of*  
591 *BTM larvae reduced the performance of the next generation*” is about herbivore growth. We  
592 dealt with possible absence of cues in the previous paragraph.

593 19. Line 206: I would remove “trait-mediated” from this sentence because I am not convinced that the  
594 dichotomy between the two explanations rests on this. Instead, I think that the two possible explanations  
595 are past vs current competition. Also is there a possibility to obtain the density of larvae in the two  
596 treatments? That is, the number of larvae per intact lead?

597 [R] — We removed “*trait-mediated*”. Although we acknowledge that this would have been a  
598 powerful way to further address competition, we did not precisely count the number of larvae  
599 per plant or per shoot, mostly to avoid disturbance.

600 20. Lines 213-215: does this mean that larval weight has no effect on fitness / population growth? Please  
601 clarify.

602 [R] — Done:

603 *the BTM is thought to have broad tolerance to variability in host traits, as suggested*  
604 *by previous observations that BTM larva growth rate did not differ significantly among*  
605 *box-tree varieties (Leuthardt et al., 2013). It is unknown whether herbivory induced*  
606 *changes in host traits are of the same order of magnitude as trait variability among*  
607 *varieties. However, assuming variability among varieties is greater, this result goes*  
608 *against the view that reduced performance of larvae of the summer generation resulted*  
609 *from box tree response to prior herbivory*

610 21. Line 219: although I agree with one of the reviewers that the possibility that food shortage may lead to  
611 cannibalism is fascinating, I would remove this sentence unless you have hard data on which to base  
612 this statement. In particular, if this were to be true, you would need to explain (a) why you still find  
613 the same overall number of larvae alive between the two treatments and (b) whether it is expected that  
614 this cannibalism does not compensate for food shortage in terms of larval weight. Overall, I think that  
615 this observation opens too many doors, so either it is solid or it better be left out of the Discussion.

616 [R] — We deleted the mention to cannibalism, because this is true we do not have hard data  
617 ta back it up.

618 22. Lines 223-225: These sentences fit best in the next paragraph.

619 [R] — We completely modified the corresponding paragraph to account for the several com-  
620 ments on the results and discussion.

621 23. Line 245: replace “in particular to plants” by “in particular to those”.

622 [R] — Done.

623 24. Lines 359-361: please check formatting here.

624 [R] — Done.

625

626 Reviewed by Inês Fragata, 2020-09-08 23:55

627 In this manuscript the authors test whether female choice for oviposition impacts intraspecific competition  
628 across generations. In order to do this, the authors compare oviposition and larvae weight of box tree moths  
629 on box trees previously exposed to conspecific herbivores or un-attacked controls. They observe that previous  
630 herbivory does not affect where female choose to lay eggs, but it affects larvae weight. This suggests that there  
631 is a mismatch between female choice and larvae performance, which is against the preference-performance  
632 hypothesis. The question that the authors are trying to answer is very interesting and can help us to  
633 understand better how species avoid intra and interspecific competition, even across different generations.  
634 Unfortunately, the methodological problems with the chrysalids made it more difficult to fully explore the  
635 potential of the question and experimental design. I have some questions/suggestions that may allow to  
636 explore better the data set that you have here, and go a bit deeper into your questions.

637 [R] — Thank you for comments.

638 Questions/suggestions:

- 639 • If I understood correctly, you have the percentage of damage per plant that was done by the first  
640 infesting larvae. You could use this as a covariate in your choice experiment to see if damage was a  
641 better way of predicting female choice, in addition to your spatial correlation structure. I think it could  
642 also be interesting to use the initial percentage of herbivory on the analysis of the larvae weight.

643 [R] — We have now added this information and re-ran models accordingly. We therefore  
644 made appropriate changes in the *methods* | *Experimental design* and *Results* sections (but did

645 not list all changes here). The reason we did not consider herbivory data in first instance  
646 was that potted plants were initially used in a completely separate experiment, for another  
647 purpose. We could not match every pot tags between the two projects, leading to missing  
648 data. However, because we agree that testing BTM response to actual herbivory rather than  
649 to a qualitative attacked/non-attacked factor, we decided to bring original data back into the  
650 main text.

- 651 • Besides the effect on choice of the egg laying females, herbivory could also affect plant quality and  
652 manifest in other life stages. In addition to looking at the weight of the caterpillars, since you have a  
653 measure of egg to caterpillar mortality, it would be interesting to see if egg to larvae mortality was  
654 higher in attacked or control trees.

655 [R] — We agree that this would have been a great addition to the paper. However, we only  
656 counted and measured a subset of larvae and chrysalis (up to five), and therefore are not able  
657 to follow this advice

- 658 • Regarding the data on the chrysalids, why do you assume that it was the number of chrysalids that  
659 was wrong, and not the number of eggs or larvae (i.e. you could miss some larvae/eggs)? Does this  
660 excess occur more in non-damaged trees? Also, does the number of extra chrysalids match the number  
661 of missing caterpillars from nearby trees/attacked trees? Because it would be interesting to see if there  
662 was more dispersal for pre-attacked trees than for control trees. It is quite striking to have half your  
663 controls and 1 one attacked plant where this happens, so I wonder if there is something biologically  
664 interesting underlying this pattern. However, if you are planning to not analyse the data or speculate  
665 on it, I think it would be better to remove the chrysalid part, as the usefulness of the information is  
666 unclear.

667 [R] — Please see our response to Dr Magalhães, above\*

- 668 • L148 - Did you release the moths in this region of the plots (between the 1 and 3)? because that  
669 could be a reason for the spatial structure to occur? alternatively did it had a source of light/heat or  
670 something alike? because it is rather strange that they clustered around that region.

671 [R] — We released moths at the four corners of the experiment to reduce the risk of spatial  
672 aggregation (information now added to the manuscript). We have no data to support any  
673 explanation regarding the aggregation of eggs in one particular part of the experiment. This  
674 could actually be because of light (the part of the greenhouse received more sunlight in the  
675 afternoon) or because of fresher air arriving from the doors.

- 676 • L152 – Why did you use these different spatial correlation structures? and what does it mean to have  
677 these different spatial correlation structures? This is important to explain what are you accounting for  
678 in the analysis.

679 [R] — We simply followed textbook recommendations when there is no *a priori* hypothesis on  
680 the shape of potential patterns.

681 *we had no particular hypothesis regarding the shape of the spatial correlation structure.*  
682 *We therefore ran separate models with different spatial correlation structures (namely,*  
683 *exponential, Gaussian, spherical, linear and rational quadratic), and compared them*  
684 *based on their AIC (Zuur, 2009)*

- 685 • L163:166 – From table 2 you have 3 models (and not two) that have similar performance, and they are  
686 not significantly better or worse compared to the quadratic one, as you need at least a difference of 2 in  
687 the AIC, using the rule of thumb from Burnham & Anderson 2004

688 [R] — Thank you for noticing, we corrected the text.

- 689 • In the first section of the discussion, I think two hypotheses that you don't mention are that 1) the  
690 moths may need cues from other life stages, such as female conspecific oviposition or the chrysalids; 2)  
691 you let too much time pass and the cues related with the conspecific were not present anymore.



692 [R] — Thank you for these suggestions. We adapted the manuscript accordingly. As for (1):

693 *or that female BTM were indifferent to them at the time we conducted the experiment.*  
694 *[...] It remains however possible that BTM adults use other cues to select their hosts*  
695 *such as the presence of conspecific eggs, larvae or chrysalis.*

696 as for (2):

697 *it is also possible that induced defense reactions were delayed in box trees, or that*  
698 *they were already relaxed when we released BTM moths three weeks after the herbivory*  
699 *treatment (Karban, 2011), which remains to be evaluated.*

700 Text comments

701 • L17:20 – This sentence is a bit weird, suggestion: “We tested this hypothesis in a choice experiment  
702 with box tree moth females (*Cydalima perspectalis* Walker, Lepidoptera: Crambidae). These females  
703 were exposed to box trees (*Buxus sempervirens* L., Buxaceae) previously defoliated by conspecific larvae  
704 earlier in the season.”

705 [R] — Changed.

706 • L30 – remove the thus from “Insects may thus reduce”

707 [R] — Done.

708 • L32 – I think you mean assumes instead of supposes

709 [R] — Changed.

710 • L38 – I don’t understand why you say “in particular” here, are those the only traits that will be  
711 important for females to detect that correlate with larval performance? for example presence of  
712 predators and competitors could be other factors that females may detect before ovipositing.

713 [R] — We replaced *in particular* by *for instance*.

714 • L41 – “time-lagged consequences on the preference”

715 [R] — Done

716 • L49 – I would substitute a mix of, with both

717 [R] — Done

718 • L60 – I would replace the “later herbivores” by later arriving/appearing herbivores

719 [R] — Done

720 • L61 – I would replace the “late coming herbivores” by later arriving/appearing herbivores

721 [R] — We preferred keeping this sentence unchanged to avoid repetition with the previous  
722 one.

723 • L71:73 – This last sentence is not very clear. Maybe: “Thus, in order to quantify the effect of prior  
724 herbivory on subsequent herbivore performance, we need to assess how it affects both female choice and  
725 progeny performance in attacked and non-attacked hosts.”

726 [R] — Done

727 • L99:100 – “Their adults...” whose adults? you mean that the larvae were used on the preference test  
728 and the adult stage on the performance test? maybe something like: “The adult stage of these larvae  
729 were used in...”

730 [R] — we clarified this point:

731 \_We initiated BTM larvae rearing with caterpillars collected in the wild in early  
732 spring 2019, corresponding to those that had overwintered\*\*. We reared them at room

733 temperature in 4320  $cm^3$  plastic boxes, and fed them *ad libitum*, with branches  
734 collected on box trees around the laboratory. We used the next generation larvae to induce  
735 herbivory on box tree plants (experimental treatment, see below) and the subsequent  
736 adults for the oviposition experiment.

- 737 • L112 – feed on missing a space

738 [R] — Done

- 739 • L117 – Any specific reason for waiting the three weeks? is it the amount of time that they would take  
740 to lay eggs again?

741 [R] — We have added this information to the revised version:

742 *In addition, at this time, plants in the herbivory treatment had been cleared of caterpillars*  
743 *for three weeks (corresponding to the duration of the chrysalis stage) during*  
744 *which they were watered every two to three days from above.*

- 745 • L132 – Why did you wait 24h for weighting them? and not measured them right away? Also was there  
746 a high variance in larvae weight?

747 [R] — We have added this information to the revised version:

748 *We kept them in Petri dishes without food for 24h to make larvae empty their gut*  
749 *and weighted them to the closest 10  $\mu g$ .*

750 We present variability in larval weight in the ‘Results’ section:

751 *The mean weight of BTM larvae varied from 6 to 54 mg (mean  $\pm$  SD:  $20 \pm 9$  mg).*

- 752 • L140 – 61 instead of 60 (or otherwise you have the number wrong above)

753 [R] — Changed

- 754 • L140:141 – maybe “and only 1 previously attacked plant” instead of “(and only in 1 previously attacked  
755 plant)”

756 [R] — We rephrased this sentence.

- 757 • L146 – x and y coordinates of what?

758 [R] — Changed

759 *We ran a generalized least square model (GLS) testing the effect of potted tree location*  
760 *in the experimental design (through their x and y coordinates, Figure 2) on the number*  
761 *of clutches per plant (log-transformed) from which we explored the associated variogram*  
762 *using the functions *gls* and *Variogram* in the *nlme* library.*

- 763 • L166 – I would not call them competing models. Additionally, you should add whether they show  
764 similar results, since you cannot say which one is best.

765 [R] — *x* and *y* coordinates referred to the design of the experiment (Figure 2). We referred  
766 to ‘*competing models*’ after Burnham & Anderson textbook (2002). We now state that the  
767 results would have been the same regardless of the spatial correlation structure. However,  
768 because this information is not essential, we preferred not reporting the detailed model out-  
769 puts. Interested or skeptical readers will have access to raw data and codes and will be able  
770 to simple uncomment the corresponding lines of codes.

- 771 • Fig1 – I would put this figure as supplementary material.

772 [R] — We agree that this figure is not essential, but on the other hand we value this kind  
773 of illustration showing what the experiment looked like, because the reality is sometimes  
774 substantially different from what a ‘Methods’ section give to imagine.

- 775 • Fig 2- I would like to know what are the x and y axis? meters? random unities?

776 [R] — Pots were installed 40 cm apart. The numbers on axes were misleading. We removed  
777 them.

778

779 Reviewed by Raul Costa-Pereira, 2020-09-06 15:59

780 Castagneyrpol et al. present results from a well-designed experiment aiming to test the detrimental effects  
781 between conspecific individuals that have never meet. They studied a peculiar system where consumers  
782 (box tree moth larvae) have limited mobility, relying on their mother’s oviposition decision to occupy good  
783 food patches (i.e., host plants). Interestingly, food patches are dynamic, and consumers can reduce resource  
784 quality to next-generation conspecifics by triggering defensive responses in host plants. Therefore, even if  
785 conspecific individuals never coexisted on the same individual host plant, the legacy of past “tenants” can  
786 reverberate negatively on current and future ones. Motivated by this interesting conceptual basis, the authors  
787 set up a greenhouse experiment to test how past herbivory affects preference (i.e., selection of oviposition sites  
788 by mothers, quantified as the number of egg clutches) and performance (i.e., individual consumer growth,  
789 quantified as average larvae body mass). Surprisingly, mothers did not avoid laying eggs on plants previously  
790 consumed by larvae, but larvae feeding on these plants with the legacy of past conspecifics were smaller.  
791 These results are exciting because they shed new light on the mechanisms shaping temporal dynamics of  
792 antagonistic interactions both between (plant-herbivore) and within (herbivore-herbivore) species.

793 [R] — Thank you for this very nice summary and positive appreciation!

794 I enjoyed reading the manuscript and think it is well-written and the figures are well-presented. The  
795 experimental design is creative and statistical analyses are solid (I particularly appreciated how the authors  
796 accounted for the underlying spatial structure of their experiment in the models). Below I describe a few  
797 major points that came up while reading the manuscript, as well as some minor points that I believe can be  
798 helpful. Please let me know if you have any questions, I am happy to clarify.

799 [R] — Thank you for your valuable and clear comments.

800 First, I feel that the conceptual framing of the manuscript is fascinating and could be contextualized and  
801 motivated more broadly in the introduction. Indeed, multivoltine insect herbivores are a great example of how  
802 individuals can affect conspecifics they have never met. Still, similar types of time-lagged interactions between  
803 conspecifics occur across diverse taxa (e.g., squirrels [Fisher et al. 2019 Ecology Letters], frogs [Pfennig &  
804 Pfennig 2020 Copeia]) and via different mechanisms (e.g., extend phenotypes, ecosystem engineers). Therefore,  
805 although the current structure of the introduction works well, I think that opening the manuscript with a  
806 more general view of ecological interactions among individuals separated in time would call the attention of a  
807 wider and more diverse readership. This approach could also help to reduce some overlap in ideas across the  
808 1st and 2nd paragraphs of the introduction.

809 [R] — We really appreciated this suggestion. We added a couple of opening sentences to  
810 broaden the scope of the paper

811 *Biotic interactions are strong factors affecting the fitness of interacting individuals,*  
812 *even interactions are delayed in time and do not imply direct contact among individuals.*  
813 *Examples of such interactions can be found in both plants through plant-soil feedbacks*  
814 *(Putten et al., 2016) and in animals (???) (Fisher et al., 2019)*

815 Hypotheses and respective predictions could be more thoroughly presented to readers. The last paragraph of  
816 the introduction is concise and nicely describes the general hypotheses of the study (lines 84-85). However,  
817 readers will only find out how the authors investigated their hypotheses in the methods (e.g., lines 123-135),  
818 which creates a certain gap in the narrative flow. Thus, the authors could include their respective predictions  
819 as well at the end of the introduction (including ‘operational variables’ - e.g., We expect that plants that  
820 previously hosted larvae should [i] have fewer eggs and [ii] host smaller larvae). Moreover, as the experimental  
821 design allows inferring the contribution of purely spatial effects on oviposition patterns, the authors could  
822 at least mention this at the end of the introduction. By the way, I think that measuring and accounting  
823 for spatial structure in oviposition patterns is an exciting novelty of the manuscript. Thus, maybe the

824 biological causes and implications of this spatial non-independence in mothers' oviposition choices could be  
825 more explored in the discussion.

826 [R] — We have modified the end of the introduction in order to introduce “*operational vari-*  
827 *ables*” as suggested. As for the discussion on spatial analyses, we agree that our results could  
828 pave the way for further investigation. However, the experiment was not designed to explore  
829 such spatial effects. We only aimed at controlling possible bias in the design. Therefore, we  
830 preferred not putting too much emphasis on this issue as it would have been very speculative.

831 Finally, I have a quick suggestion about a potential additional dimension of performance that could be  
832 considered. To quantify the effects of past herbivory on offspring performance, the authors compared the  
833 average body mass of larvae across treatments. I fully agree this is a key aspect of *per capita* offspring  
834 performance, and results are exciting in this regard. In light of the natural history of the system, as the  
835 authors also measured the number of L3 larvae per plant (lines 130-131), I wonder whether the conversion  
836 rate egg → L3 larva (e.g., number of eggs/number of L3 larvae) could not be used as an additional metric of  
837 performance. Is there evidence in the literature that past herbivory can affect egg eclosion rates and/or early  
838 larval development (L1 → L2 → L3)? This alternative metric would capture a different facet of offspring  
839 performance not necessarily correlated with mean larvae weight (e.g., larvae mortality associated with lower  
840 foraging rates and/or increased toxins).

841 [R] — This would have been a great addition to the paper indeed. Unfortunately, we did not  
842 have such an information at hand for we counted *up to* five larvae per plant. The phrasing of  
843 the original version was ambiguous in this respect. We modified it accordingly.

844 Minor comments:

845 • Lines 1-2. The title is solid and general, but I am not sure if all readers will be familiar with the concept  
846 of ‘multivoltine’ (I guess it depends on the target journal).

847 [R] — We deleted the reference to *multivoltine* species.

848 • Lines 39-40. Given the idea of the last sentence in this paragraph, it would be good to emphasize here  
849 that “competing herbivores” refers to different species of herbivores (i.e., interspecific competition). I  
850 would say the same about Line 42.

851 • Lines 46-50. I feel that most of these ideas were already presented to readers in the previous paragraph.  
852 The argument presented in Line 50 sounds like an exciting way to begin this paragraph.

853 [R] — We deeply modified the introduction, please see our response to Dr Magalhães’ com-  
854 ments.

855 • Line 50. I follow the meaning of ‘passage of competitors’ but it’s possible that some readers may find  
856 it a bit confusing. One potential alternative (maybe not that accurate) would be something like ‘the  
857 legacy’ of past herbivores on host plants.

858 [R] — The corresponding sentence was deleted.

859 • Line 51. Maybe the authors could provide a brief view of what ‘direct’ detection means in this context,  
860 e.g. “...mated females can directly detect (e.g., via visual or olfactory cues) the present...”.

861 [R] — We added “themselves” to make it clear that the female can detect herbivores, or  
862 herbivory-induced changes in plant traits.

863 • Line 61. Adding a ‘triggering’ or ‘stimulating’ before “defenses that generally” could make this idea  
864 clearer to readers.

865 [R] — We rephrased this sentence.

866 • Line 60. I am not an expert in plant-herbivore systems, but a first intuitive, simple mechanism seems  
867 to be the reduction of food biomass by previous consumers. Does it make sense?

868 [R] — Yes it does! We now mention interference competition and resource depletion.

869 • Lines 65-66. This is very interesting, and the following example illustrates well this mismatch between  
870 selection by mothers vs. impacts on offspring. However, I feel a follow-up conceptual sentence would  
871 help readers to crystallize this idea by clarifying that not necessarily the effects on preference and  
872 performance are congruent.

873 [R] — **We restructured the introduction to improve the reading.**

874 • Lines 74-75. Considering the broad readership of ecologists and evolutionary biologists this manuscript  
875 has the potential to reach, I recommend the authors to define the concept of ‘multivoltine’. Not all  
876 readers may be familiar with it.

877 [R] — **Done.**

878 • Line 75. Tiny detail: remove the italic from spp.

879 [R] — **Done.**

880 • Lines 84-86. This is a matter of writing style, but one possibility here is to ‘change the pace’ of this key  
881 sentence to emphasize the potential effects on both preference and performance encapsulated by this  
882 hypothesis. A simple way to do this would be: "...early herbivory would (i) reduce oviposition..., and  
883 also (ii) reduce the performance ...

884 [R] — **Done.**

885 • Lines 85-86. As the last sentence of the introduction is often one of the most ‘visited’ by readers, I feel  
886 this one could deliver a ‘self-standing’, stronger message. For instance, instead of ‘By addressing the  
887 above’, one alternative could be ‘By addressing the effects of previous herbivory by conspecifics on both  
888 preference and performance of subsequent...’

889 [R] — **Done.**

890 • Line 94. I think this initial sentence could be moved down in this paragraph.

891 [R] — **The sentence was moved to the “natural history” new paragraph.**

892 • Line 107. It is clear from the previous sentence, but it would be helpful to clarify that this plant-level  
893 herbivory metric represents the mean frequency of attacked leaves/branch.

894 [R] — **Done:**

895 \_\_ In order to confirm that the addition of BTM larvae caused herbivory, we visually  
896 estimated BTM herbivory as the percentage of leaves consumed by BTM larvae, looking  
897 at every branch on every plant. We then averaged herbivory at the plant level. In  
898 8 plants, herbivory data was missing and was imputed as the average of herbivory  
899 measured in other plants. In the herbivory treatment, the percentage of leaf area  
900 consumed by BTM larvae ranged from 2.2 to 17.2% and was on average 9.1%.\_\_

901 • Figure 1. These photos are great for illustrating the experimental design and study system! I would  
902 just suggest adding more details in the legend.

903 [R] — **Done.**

904 • Line 113. feed on.

905 [R] — **Done.**

906 • Lines 115-116. I wonder if this difference of three weeks between caterpillars being removed (from the  
907 herbivory treatment) and moths oviposition reflect the phenology of this species in natural ecosystems.  
908 In other words, in light of the biology of BTM, a given box tree in nature could experience two separate  
909 groups/generations of caterpillars within three weeks?

910 [R] — **Yes, the duration of the pupal stage in the wild is long enough to have two separate**  
911 **generations.**

912 • Line 118. Just to make it more straightforward: "... washed out from leaves".

913 [R] — **We deleted this sentence.**

914 • Line 120-121. This is an important point that could be briefly mentioned in the last paragraph of the  
915 introduction, e.g., "our experimental design allows us to quantify... of plant-mediated".

916 [R] — **Done.**

917 • Lines 126-128. I think that first presenting 'why' (i.e., "to prevent larvae from moving from one potted  
918 plant to another") and then 'how' (i.e., "we installed box trees in plastic saucers and interspaced plants  
919 and filled saucers with a few centimeters of water").

920 [R] — **Done.**

921 • Line 131. All plants had at least five larvae?

922 [R] — **Done: "only 6% of plants hosted less than five larvae".**

923 • Line 133. It would be helpful to emphasize that this value represents the average weight of larvae from  
924 one plant individual.

925 [R] — **Done.**

926 • Line 142. Thanks for such a careful explanation; I appreciate your transparency!

927 [R] — **Thanks.**

928 • Line 150. Could you please describe in more detail the structure of these models? (i.e., define response  
929 variable and predictors).

930 [R] — **Done.**

931 • Line 165. With other three models ( $\Delta i < 2$  - Models 2, 3, and 4), right?

932 [R] — **Yes, corresponding information is now available in Table 1.**

933 • Line 177. How about finishing this sentence after 'season' and then start a new sentence with 'This  
934 time-lagged'?

935 [R] — **Done.**

936 • Line 182-183. Maybe the fact that larval frass was washed out could be a potential explanation?

937 [R] — **We added the following sentence:**

938 *However, we cannot exclude that some cues were mediated by larva frass, which was*  
939 *watched out from leaves when we watered plants.*

940 • Lines 201-203. This an interesting explanation! I wonder if host plants in the native range of BTM  
941 have even higher toxic alkaloids than box trees.

942 [R] — **This is an interesting question that would be worth digging further. We are not aware**  
943 **of dedicated studies.**

944 • Lines 206-207. I do not follow this idea, could you please clarify? I feel that 'reduced performance of  
945 individuals... have been trait-mediated' requires some further explanation.

946 [R] — **"Trait mediated" was misleading. We deleted these two words and believe the sentence**  
947 **reads better now.**

948 • Line 219-220. The fact that food limitation can trigger cannibalism in this system is fascinating!

949 [R] — **yes, we have been quite surprised to observe this, but did not try to investigate it**  
950 **further. However, in the absence of back-up data, we eventually deleted reference to this**  
951 **possible phenomenon.**

952 • Line 221. To avoid repetition, I suggest replacing the first ‘Herbivore’ by ‘Consumer’.

953 [R] — **This sentence was deleted.**

954 • Line 228. Would it be ‘with’ or ‘within’?

955 [R] — **Changed to “within”.**

956 • Line 237. Perhaps ‘negative interactions... generations’ could communicate more clearly the results.

957 [R] — **Done.**

958 • Line 241. Because this idea expands to the next sentence, it would be good to mention their main  
959 enemies (e.g., parasitoids, predators).

960 [R] — **Done.**

961 • Line 244. It seems that a verb is missing in this sentence, ‘causing more damage’ is one option.

962 [R] — **Done.**

963 • Line 247. be investigated

964 [R] — **Done.**

965 • Line 248. dedicated

966 [R] — **Done.**

967 I hope the authors find these comments helpful. Best wishes, Raul.

968 [R] — **We did, thanks!**