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Larval food influences temporal oviposition and egg quality traits in females of *Lobesia botrana*.

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27 **Abstract**

28 Many phytophagous insects are agricultural pests, and control methods require accurate
29 monitoring and decisions based on the determination of population age structure. The
30 reproductive output (fecundity, egg size and percent egg hatch) is a central life history trait
31 because it determines the offspring number, and temporal oviposition patterns are of primary
32 importance in conditioning larval hatching and the occurrence of later larval instars in time. In
33 turn, these phenomena determine the window for natural enemy attack and thus impact the
34 context of biological control programs. In addition, for most phytophagous insects, the quality
35 of the host plants that larvae consume determines the insects' reproductive output. The
36 purpose of the present study was to determine whether the number of eggs laid, egg size and
37 egg hatch percentage vary with female age and the cultivar on which females develop as
38 larvae, as well as the temporal effects of these parameters. This determination was performed
39 in laboratory experiments where larvae were reared on artificial diets based on dried fruits of
40 seven cultivars. Our results showed that the cultivars had a significant effect on female
41 temporal oviposition. Independent of the food tested, the numbers of oviposited eggs, their
42 size and percent egg hatch decreased with daily oviposition rank. Such temporal patterns must
43 be incorporated in age-structured mathematical models used in the design of control
44 strategies. Temporal oviposition and variation in egg quality traits will also be useful in
45 biological control programs, especially when based on egg or larval parasitoids, which is thus
46 discussed.

47

48 **Keywords:** *Lobesia botrana*, fecundity, egg size, percent egg hatch, cultivars, female age

49

50 **Key message**

- 51 • In insects, temporal oviposition and hatching success are critical to reproductive
52 success and in determining population age structure and the windows in which
53 juvenile and other instars are available to parasitoids.
- 54 • We hypothesized that variability in larval food would influence the temporal
55 characteristics of female oviposition and several egg quality traits of *Lobesia botrana*.
- 56 • We found a significant effect of grape cultivar on the temporal oviposition and egg
57 quality traits.
- 58 • These results could be useful for adjusting the release of the natural enemies of eggs in
59 biological control strategies and for age structure population temporal models.

60

61

62

63 **Introduction**

64

65 The abundance of phytophagous insects and the optimal regulation of their interactions with
66 their trophic resources are determined by numerous interacting biotic and abiotic factors.
67 Consequently, substantial literature on phytophagous insects has been devoted to
68 understanding the factors that govern female reproductive output because this output
69 determines the potential number of offspring produced. The female reproductive output
70 depends on at least three determinant life history traits: fecundity, egg size and percent egg
71 hatch. The number of eggs laid by a female is clearly important to determine their
72 reproductive potential, but egg size (often correlated to percent egg hatch) is also considered a
73 crucial reproductive parameter. Numerous studies have examined the relationship between
74 egg size and fitness components of the progeny. Such studies often demonstrate that within a
75 species, small eggs are less likely to hatch (Fox and Czesak 2000 and references therein), and
76 hatching individuals from larger eggs have higher fitness than those from smaller eggs
77 (Karlsson 1989; Fox and Czesak 2000; Roff 2002; Torres-Vila and Rodriguez-Molina 2002).

78 Many insect species are pests and are responsible for huge annual losses in global crop
79 production (Thacker 2002). Most of the control methods currently used to increase crop
80 production rely on accurate monitoring and decisions, which require precise information on
81 the biology and the ecology of the pest (Tammaru and Javois 2000). In capital breeder insects
82 (whose individual reproductive potential is limited by the nutrition ingested during the larval
83 stages), larval food quality is one of the most important factors that determine female
84 reproductive output (Awmack and Leather 2002). Several studies have shown the influence of
85 plant quality on larval development, larval survival, and female reproductive output (Awmack
86 and Leather 2002; Thiéry and Moreau 2005; Moreau et al. 2006a; 2006b; 2007). However,
87 most studies have considered the effect of the host plant on fecundity, egg size and percent

88 egg hatch without considering the temporal effect on these life history traits. This information
89 is lacking because for the same fecundity, the temporal oviposition pattern could be
90 dramatically different. For instance, all eggs could be laid in a single session, or an equal
91 number of eggs could be laid each day throughout the life of the female. These different
92 temporal oviposition patterns can lead to differences in population growth rate and the timing
93 of further larval instars. They can also influence biological control management by modifying
94 the optimal windows of attack for natural enemies (e.g., egg parasitoids or predators and
95 larval parasitoids). Information on temporal oviposition is needed for progress in the study of
96 pest population age structure determination and the construction of basic life tables (Carey
97 2001 for a review; Ainseba et al. 2011; Farahani et al. 2012). To our knowledge, only a few
98 studies have examined the effect of the host plant on temporal oviposition (see Hafiz 2006;
99 Samih and Izadi 2006), and no studies have examined the egg size and percent egg hatch
100 throughout the oviposition period, which are necessary for a complete understanding of the
101 variation in insect oviposition and integration into life history tables.

102 The European grapevine moth, *Lobesia botrana* (Denis and Schiffermueller,
103 Lepidoptera: Tortricidae), is certainly the most harmful grape pest in Europe, north Africa and
104 west Asia (Bovey 1966; Roehrich and Boller 1991; Iorati et al. 2011; Thiéry et al. 2014;), and
105 its recent introduction to Chilean and California vineyards highlights the problem of pest
106 management (Gutierrez et al. 2012; Varela et al. 2013). *Lobesia botrana* may cause serious
107 damage to the grape directly by consuming flower clusters and fruits, or by facilitating
108 infection by pathogenic fungi such as grey mould disease, *Botrytis cinerea* (Persoon,
109 Helotiales: Sclerotiniaceae), or black mould, *Aspergillus* spp. (Micheli, Eurotiales:
110 Trichocomaceae) (Cozzi et al. 2006; Thiéry 2008; Delbac and Thiéry 2015). It may also
111 facilitate attack by secondary pests, including fruit flies (Barata et al. 2012) such as
112 *Drosophila melanogaster* (Meigen, Diptera: Drosophilidae) (Gravot et al. 2001) and *D.*

113 *suzukii* (Matsumura, Diptera: Drosophilidae) (Rouzes et al. 2012). Therefore, *L. borana* is a
114 highly problematic pest in vineyards and requires permanent monitoring and control (Thiéry
115 2008; 2011; Ortega-Lopez et al. 2014). This pest is an ideal candidate for testing the effect of
116 the host plant on temporal oviposition because (i) previous studies have shown strong effects
117 of cultivars where larvae feed on both larval developmental and reproductive life history traits
118 (Moreau et al. 2006a; 2006b; 2006c; 2007; Thiéry et al. 2014) and (ii) information on
119 temporal oviposition is needed for advancements in grape pest mathematical models (Ainseba
120 et al. 2011).

121 The present work focuses on the temporal oviposition of the main pest of European
122 vineyards and on how the host plant can affect this temporal pattern. Therefore, we determine
123 whether the cultivar on which the females feed as larvae affect the number of eggs laid, egg
124 size, and egg hatch percentage, as well as the temporal patterns of these three life history
125 traits. To examine this dependence, we conducted laboratory experiments by rearing larvae on
126 artificial diets derived from seven different cultivars (Chardonnay, Chasselas,
127 Gewurztraminer, Grenache, Merlot, Pinot and Riesling). We then measured individual female
128 oviposition based on time, egg size and percent egg hatch.

129

130 **Materials and Methods**

131

132 **Study system, origin and maintenance of moths**

133

134 The strain of *L. botrana* (INRA Bordeaux) used for this study originated from individuals
135 collected in a French Sauternes vineyard (cultivar Semillon) in 1997, to which wild adults are
136 periodically added. This rearing line is maintained with a substantial number of caged adults
137 (several thousand a week) to avoid genetic drift. This laboratory strain has conserved genetic

138 variability because considerable variation is found in the larval and adult behaviours and in
139 larval immune parameters (Vogelweith et al. 2011). The stock colony is maintained without
140 diapause on a semi-artificial diet (as described in Thiéry and Moreau 2005), with the
141 following composition: 150 ml water, 3 g agar, 9 g maize flour, 11 g wheat germ, 9 g yeast,
142 0.9 g ascorbic acid, 0.3 g benzoic acid, 0.3 ml maize oil, 0.3 g nipagin, and 0.2 g iprodione, at
143 24 ± 1 °C, $60 \pm 10\%$ RH with a photoperiod 15: 8 h light/dark + 1 of dusk. The first 15-
144 photophase hours were at 1000-lux luminosity, and the last hour (dusk) was at 25 lux. All
145 tests were performed under these conditions.

146

147 **Larval diet treatments and general procedure**

148

149 The influence of different grape cultivars on *L. botrana* was tested using a standardized
150 procedure (Thiéry and Moreau 2005; Moreau et al. 2006a; 2006b). Compared to direct
151 feeding on bunches **in the laboratory or in the field**, this procedure has at least three main
152 advantages: a) feeding isolated larvae prevents competition and subsequent food deprivation;
153 b) it prevents differences in grape bunch compactness, which impact larval feeding behaviour
154 (our unpublished observations) and the climatic environment (**temperature and insolation**) of
155 the larvae (Pieri and Fermaud 2005); and c) it prevents infections by fungi on grapes, which
156 may affect larval fitness as shown by Savoupoulou-Soultani and Tzanakakis (1988) and
157 Mondy and Corio-Costet (2000).

158 To avoid immature competition, larvae were reared individually to pupation in
159 Eppendorf tubes filled with 1.5 ml of a medium containing the following (for 100
160 Eppendorfs): **150 ml water, 5 g agar, 6 g cellulose powder, 4 g vitamin-free casein, 3.5 g**
161 **glucose, 2 g mineral salt, 0.12 g cholesterol, 0.12 g maize oil, 0.25 g benzoic acid, 0.1 g**
162 **nipagin** and 12 g freeze-dried grape fruit powder. Freeze dried material was obtained from

163 freshly collected **grape flower clusters** within 12 h of collection using a Christ alpha 1-4 LD
164 plus device. This classic procedure preserves fresh foods (Ratti 2001) and is typically used for
165 secondary metabolites in grapes (see Michalczyk et al. 2009). The grape cultivar dried
166 powders were obtained from grape flower clusters of *V. vinifera* cv. Chardonnay, Chasselas,
167 Gewurztraminer, Grenache, Merlot, Pinot and Riesling, all of which were harvested from our
168 gene collection of grape plants “Domaine de la Grande Ferrade”, INRA-Bordeaux. The
169 insecticide-free grape flower clusters were collected at the beginning of the growing season
170 (beginning of May 2003) at phenological stages 23-27 (Eichhorn and Lorenz 1977), which
171 correspond to the grape phenology on which the first annual generation of *L. botrana* larvae
172 feeds.

173 The Eppendorf lids were pierced to allow air circulation. Using a fine brush, newly
174 hatched larvae (age < 8 h) were transferred individually to the diets in each Eppendorf, with
175 100 larvae per diet (cultivar). Neonate larvae from eggs produced by thousands of caged
176 females were randomly chosen and assigned to the different diets. Eppendorf tubes were
177 randomized in the Eppendorf racks, which were moved within the climatic chamber every
178 three days to minimize the effect of possible climatic gradients.

179 The larvae from each diet were monitored daily until pupation. **Two-day-old** pupae
180 were then carefully removed from the diet and weighed to the nearest 0.1 mg. Because it is
181 difficult to weigh adult moths with sufficient accuracy, we used the mass of living pupae as an
182 index of adult body size. Pupae were then placed individually in glass tubes (70 mm x 9 mm
183 diameter) covered with cotton plugs and stored in the test room until emergence **under the**
184 **same conditions previously described. Adults were sexed after emergence by checking their**
185 **ventral abdominal extremity (Thiéry, 2008).**

186 All newly emerged female adults resulting from the eight larval diets were used to
187 evaluate the temporal effect of (1) egg laying (2) egg size and (3) larval hatching. **Because it**

188 has been reported that the cultivars on which larvae develop can modify the female
189 oviposition preferences (Moreau et al. 2008), we decided not to run the oviposition
190 experiments on one specific cultivar, which may have interfered with the temporal
191 oviposition. Thus, we used an inert substrate (i.e., considered equal for all females) where
192 females could deposit their eggs. Newly emerged females (less than 1 day old) were
193 individually confined to 0.5 litre transparent cellophane bags as mating and oviposition
194 chambers, and they were provided with water *ad libitum* through a soaked cotton dental wick.
195 One- or two-day-old virgin males originating from the same diet were added to each caged
196 virgin female 1 h before dusk, which is just before their sexual activity (Bovey 1966). Only
197 one male was randomly assigned to each female. Pairs were caged in these bags until the
198 death of both sexes.

199 Females could behave and oviposit freely inside the cellophane bag until death. Each
200 morning, the cellophane bags were checked, and new eggs laid during the previous night were
201 marked with a specific colour outside the bags. For the analyses, only females that laid a
202 sufficient number of eggs (> 7 eggs) during their lifetime (because non-mated females can lay
203 only a few eggs) and that began to lay at the beginning of her life were considered to obtain a
204 representative picture of the temporal oviposition in this species. We thus obtained the
205 temporal oviposition for a variable number of females that depended on the sex ratio, larval
206 survival and mating success: Chardonnay n = 33, Chasselas n = 24, Gewurztraminer n = 24,
207 Grenache n = 20, Merlot n = 35, Pinot n = 34 and Riesling n = 29.

208 At the end of the experiment (i.e., when the females were found dead in the bags), we
209 randomly selected a sample of females that had laid a sufficient number of eggs each day in
210 each cultivar to assess egg size and percent egg hatch: Chardonnay n = 14, Chasselas n = 9,
211 Gewurztraminer n = 12, Grenache n = 9, Merlot n = 17, Pinot n = 15 and Riesling n = 10. For
212 each selected female, three eggs from the walls of the cellophane bags were randomly

213 selected per oviposition day, when possible. Indeed, some females sometimes laid less than
214 three eggs per day, particularly at the end of life. **Previous studies we have done (results not**
215 **shown in this paper) showed that measuring three eggs per oviposition day and per female**
216 **gives the same results than if we measured more eggs whatever the oviposition rank.** Each
217 egg was then measured with an ocular micrometre. The egg surface (estimated as an elliptic
218 surface, $S = \pi \times a \times b$ in mm^2 , where a and b were the ellipse semi axes) was used as an index
219 of egg size. The mean egg size per day was estimated for each female from this sample. To
220 estimate daily fertility (i.e., hatching success), the measured eggs were incubated at 22°C for
221 10 days until hatching.

222

223 **Statistical Analysis**

224

225 For all analyses, mixed effects models were performed, including the identity of the females
226 as a random effect. The pupal mass of the females was also included to control for its
227 potential effect. The number of eggs laid by day and cultivar was compared using negative
228 binomial generalized linear mixed effects model (GLMM) accounting for zero-inflated data.
229 The statistical significance of each parameter was tested with χ^2 -statistics for unbalanced
230 design (Fox and Weisberg 2011). The proportion of hatching eggs was arcsine square root
231 transformed to normalize. However, the transformed variable was not normally distributed
232 and did not meet the assumption of homoscedasticity, as was the case for the size of eggs laid.
233 Therefore, the proportion of hatched eggs and their size were compared among cultivars and
234 days using a linear mixed effects model based on rank transformation (as a more powerful
235 alternative to the classical nonparametric Friedman test, Baguley 2012). The statistical
236 significance of each parameter was tested with Wald χ^2 -statistics for unbalanced design. In
237 each case, pairwise Wilcoxon multiple comparison post hoc tests were performed (P -values

238 were adjusted for multiple comparisons using the Benjamini-Yekutieli step-up procedure,
239 Benjamini and Yekutieli 2001). The same procedure was used to analyse differences in egg
240 size.

241 All statistical analyses were performed using R software (v. 3.1.1, R Development
242 Core Team 2014) and implemented using the following packages: *lme4* (linear mixed effects
243 models), *glmmADMB* (negative binomial generalized linear mixed effects model with zero-
244 inflated data) and *car* (deviance analysis for unbalanced design).

245

246 **Results**

247

248 **1) Number of eggs laid throughout the oviposition period**

249

250 The total number of eggs laid did not differ among females fed on the different cultivar diets
251 (GLMM: $\chi^2 = 4.07$, $df = 6$, $P = 0.67$) but varied positively according to the mass of the pupae
252 ($\chi^2 = 18.77$, $df = 1$, $P < 0.0001$). The number of eggs laid decreased with oviposition day
253 (female age) with the maximum of eggs laid in the first day of oviposition and with only few
254 eggs laid at the end of the oviposition period ($\chi^2 = 231.57$, $df = 6$, $P < 0.0001$, Fig. 1). A
255 significant interaction between cultivar and day was found (interaction cultivar x day, $\chi^2 =$
256 62.51 , $df = 36$, $P < 0.01$, Fig. 2), indicating that larval diets with different grape cultivars had
257 an effect on female temporal oviposition. For some cultivars, such as Gewürztraminer and
258 Pinot noir, the number of eggs laid remained stable for the first four days of oviposition and
259 decreased for the remaining oviposition days, whereas for other cultivars, such as
260 Chardonnay, the numbers of eggs laid decreased linearly with oviposition days (Fig. 2).

261

262 **2) Egg size variation throughout the oviposition period**

263
264 The size of the eggs laid by females fed with different cultivars during their larval period did
265 not differ (linear mixed effect model: $\chi^2 = 11.45$, $df = 6$, $P = 0.08$) and did not vary with
266 female pupal mass ($\chi^2 = 0.62$, $df = 1$, $P = 0.43$). However, the size of eggs decreased with
267 increasing days ($\chi^2 = 340.92$, $df = 6$, $P < 0.0001$, Fig. 3). There was also a significant
268 interaction between day and cultivar ($\chi^2 = 132.26$, $df = 36$, $P < 0.0001$): for some cultivars,
269 such as Chardonnay or Riesling, the size of eggs laid remained stable for the first four days of
270 oviposition and then decreased, whereas for other cultivars, such as Pinot noir, the size of
271 eggs laid decreased progressively (Fig. 4).

272

273 **3) Percent egg hatch throughout the oviposition period**

274

275 The percent of hatching eggs did not vary according to the female mass (linear mixed effects
276 model: $\chi^2 = 0.40$, $df = 1$, $P = 0.53$) or among cultivars ($\chi^2 = 10.44$, $df = 6$, $P = 0.11$). However,
277 the percent of hatched eggs decreased with increasing oviposition days ($\chi^2 = 93.77$, $df = 6$, P
278 < 0.0001 , Fig. 5), with no interaction with cultivar (interaction cultivar x day, $\chi^2 = 38.80$, $df =$
279 35 , $P = 0.30$). At the beginning of the oviposition period, approximately 90% of eggs hatched,
280 whereas only approximately 60% of eggs hatched at the end of the oviposition period.

281

282 **Discussion**

283 One of the goals of the present study was to assess the temporal effect on three major
284 life history reproductive traits of *L. botrana* (fecundity, egg size and percent egg hatch). Our
285 results show that all three parameters decreased over time. The second goal of this study was
286 to determine whether larval feeding on a diet containing different cultivars had an effect on
287 the temporal oviposition patterns and on egg size and percent egg hatch throughout the

288 oviposition period. Our results clearly showed that the cultivar on which females developed as
289 larvae had a significant effect on the temporal oviposition and egg size. However, no effect on
290 percent egg hatch was detected. This result indicates that food characteristics associated with
291 the immature stages affect how adult females distribute eggs over time. **This phenomenon,**
292 **combined with the effect of consuming different cultivars on the larval growth rates, may**
293 **explain how the distribution of the next adult generation varies over time (Thiéry et al, 2014).**

294 In the context of our study, the distinction between income and capital breeding
295 insects is important. Income breeder females mature their eggs throughout their adult life,
296 whereas capital breeders emerge with a nearly fixed number of ovocytes (Papaj 2000).
297 *Lobesia botrana* is considered a capital breeder (**species with non-feeding adults**); its female
298 reproductive potential is thus limited by the resources accumulated during the larval stage
299 (Slansky and Rodriguez 1987; Awmack and Leather 2002). In our experimental design,
300 females lacked access to any additional food except clear water, so the resources mobilized
301 for the egg production were derived from reserves accumulated during the larval stage
302 (Awmack and Leather 2002). Previous studies showed that pupal mass is a good predictor of
303 fecundity in *L. botrana* (Moreau et al. 2007). Egg-laying activity is under physiological
304 pressure from the oogenesis process and oocyte formation (McDonald and Borden 1995;
305 Chapman et al. 2013). Females used in the present study had an average egg load of $144.6 \pm$
306 37.8 eggs and laid approximately 60% of its eggs in the two first days of the laying period.
307 Subsequently, the number of eggs laid decreased daily until the death of the female. The large
308 number of eggs laid during the first night could be adaptive because under natural conditions
309 *L. botrana* adults have a relatively short life expectancy (ca. one week according to the
310 climatic conditions) (Moreau et al. 2006a; Moreau et al. 2006b; Thiéry 2008). In addition, the
311 predation risk for a moth is high because nocturnal insectivores often prey on moths; these
312 predators include bats, species of owls and other species of birds (Arlettaz et al. 2000). Given

313 that there is a delay between encountering a mate and the first egg laid of approximately four
314 days for a female from our rearing strain (Moreau et al. 2006a) or in the field (Moreau et al.
315 2007), females have only a few days to lay eggs. In that case, laying a maximum number of
316 eggs as quickly as possible could be adaptive. This phenomenon was observed, for example,
317 in *Dryas iulia* (Fabricius, Lepidoptera: Nymphalidae), whose females laid the greatest number
318 of eggs at the beginning of the laying sequence (Dunlap-Pianka et al. 1977).

319 We showed that eggs laid on the first day of oviposition are larger than eggs laid on
320 the subsequent days. This size decrease is consistent with previous results performed in the
321 same species (Moreau et al. 2009). Lepidoptera ovaries structure and morphological aspects
322 of oogenesis are well known (see Swevers et al. 2005 for a review or Chapman et al. 2012 for
323 a book). Briefly, oocytes are produced in the germarium and begin to move down the
324 ovariole, enlarging as they pass through the vitellarium, where yolk containing both protein
325 and lipids is deposited on them (vitellogenesis). The fat body is the principal site of
326 production of the major yolk protein precursor (YPP), which is vitellogenin (Vg) in most
327 insects (Swevers et al. 2005). In many butterflies and moths, ovaries contain only
328 previtellogenic oocytes, and vitellogenesis starts at eclosion. Therefore, their ovarioles usually
329 contain a series of oocytes in successive stages of development. In *L. botrana*, we found a
330 delay of approximately 4 days between encountering a mate and the first egg laid (Moreau et
331 al. 2006a; Moreau et al. 2007). During this time, the first eggs have time to mature and
332 enlarge with yolk. The only reason for the differential size is thus the amount of yolk, which
333 is related to the decreased synthesis by the fat body or its depletion.

334 The proportion of larvae that hatched from eggs was strongly dependent on the
335 oviposition days; fewer eggs hatched at the end of the life of females independently of the
336 cultivars, which is consistent with the amount of yolk in the egg. This decline can be
337 interpreted as a result of the egg size decrease, which was clearly associated with the egg size

338 in that species (Moreau et al. 2006; 2007), most likely because larger eggs have the largest
339 nutritional provisions inside, as already shown in other species (Berrigan 1991; Fox and
340 Czesak 2000). This decrease is not due to a decrease in sperm numbers because we showed
341 that in *L. botrana*, one male ejaculate contains much more spermatozooids than necessary to
342 fertilize all female eggs (Muller et al. 2015).

343 Our results show that the effect of the cultivar on which females developed as larvae
344 had a significant effect on the temporal oviposition and egg size. For some cultivars, the
345 number and the size of eggs laid remained stable for the first four days of oviposition and
346 decreased for the remaining oviposition days, whereas for other cultivars, the numbers of eggs
347 laid or the size of eggs decreased through oviposition days. However, no effect of cultivar was
348 detected for percent egg hatch. This effect is very weak in comparison to the profound effect
349 of cultivar on total number of eggs laid, total mean egg size and mean percent egg hatch that
350 we have previously demonstrated (Moreau et al. 2006a; 2006b; 2007).

351 We found here that time within the oviposition period affects the number of eggs laid
352 and the probability that an egg hatches. These individual temporal effects also influence the
353 population scale. The data provided here on time-dependent offspring production in *L.*
354 *botrana* could also be useful for the development of age-structured mathematical models of
355 vineyard infestation such as that developed by Ainseba et al. (2011). Therefore, we also
356 believe that this feature needs to be incorporated in future *L. botrana* population age-
357 structured mathematical models. These effects of time would also influence biological control
358 management based on egg parasitoids, predators and larval parasitoids to determine the
359 optimal windows in which natural enemies should be released. As an example, several species
360 of *Trichogramma* can be used in inundative biological control programs in a variety of crops
361 against numerous pests (Morrison 1985; Reda Abd el-Monsef 2004; Agamy 2010; Andrade et
362 al. 2011) including *L. botrana* with irregular results (Barnay 1999; Hommay et al. 2002; El-

363 Wakeil et al. 2010). Parasitism efficiency in *Trichogramma* parasitoids is, of course,
364 influenced by environmental factors (Calvin et al. 1984; Pizzol et al. 2010) and by intrinsic
365 factors (Schmidt 1994; Pizzol et al. 2012) such as egg size, quality and age (Roriz et al. 2006;
366 Moreau et al. 2009; Pizzol et al. 2012). Indeed, large and numerous eggs are often more
367 conspicuous for the searching females and more suitable for the subsequent development of
368 their progeny (Van Huis and De Rooy 1998; Moreau et al. 2009; Pizzol et al. 2012). With our
369 results, the optimal window for parasitoids or predator release must occur when the first eggs
370 are laid (bigger eggs and more eggs). This optimal window could be calculated because we
371 showed that for each grape variety, the emergence of *L. botrana* females occurred as a single
372 wave after approximately ten days (Thiéry et al. 2014). In addition, considering the cultivar
373 effect on temporal oviposition could be useful when monitoring eggs in vineyards.
374 Insecticides against the larvae (growth regulators or *Bt*) are mostly applied at a typical egg
375 stage called ‘black head’, which corresponds to 2/3 of the egg incubation period. Shorter or
376 longer oviposition periods as a function of previous generation food (*i.e.*, the cultivar on
377 which the female fed as larva) would thus cause, at the population and the vineyard scales,
378 important variation in these typically monitored stages. We strongly believe that this
379 information concerning temporal oviposition is needed to advance our knowledge of pest
380 ecology with the aim of better biological control.

381

382 **Author contribution**

383 Conceived and designed the experiments: JM and DT. Performed the experiments: JM and
384 DT. Analysed the data: KM and JM. Contributed reagents/materials/analysis tools: JM, DT
385 and KM. Wrote the paper: JM, KM and DT.

386

387

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390 Regional Council of Bourgogne. The authors thank Marc-Etienne Toulouse for experimental
391 contributions in rearing *Lobesia botrana*.

392

393 **Conflict interest**

394 The authors declare that they have no conflict of interest.

395

396 **Ethical approval**

397 All applicable international, national, and/or institutional guidelines for the care and use of
398 animals were followed.

399

400 **Informed consent**

401 This article does not contain any studies with human participants performed by any of the
402 authors.

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640 **Figure legends**

641 **Fig. 1** Number of eggs laid by a *Lobesia botrana* female according to the day of oviposition
642 for all cultivars. Bold line: median; box: middle two quartiles; dashed lines: $1.5 \times$ interquartile
643 range; open circle: extreme value. The numbers inside the parentheses indicate the number of
644 females. Columns with the same letter are not significantly different ($P > 0.05$) based on
645 pairwise Wilcoxon multiple comparison post hoc tests.

646

647 **Fig. 2** Number of eggs laid by *Lobesia botrana* female according to the day of oviposition and
648 for each cultivar where the female came from. Bold line: median; box: middle two quartiles;
649 dashed lines: $1.5 \times$ interquartile range; open circle: extreme value. The numbers inside the
650 parentheses indicate the number of females. Columns with the same letter are not significantly
651 different ($P > 0.05$) based on pairwise Wilcoxon multiple comparison post hoc tests.

652

653 **Fig. 3** Egg size according to the day of oviposition for all cultivars. Bold line: median; box:
654 middle two quartiles; dashed lines: $1.5 \times$ interquartile range; open circle: extreme value. The
655 numbers inside the parentheses indicate the number of measured eggs. Columns with the
656 same letter are not significantly different ($P > 0.05$) based on pairwise Wilcoxon multiple
657 comparison post hoc tests.

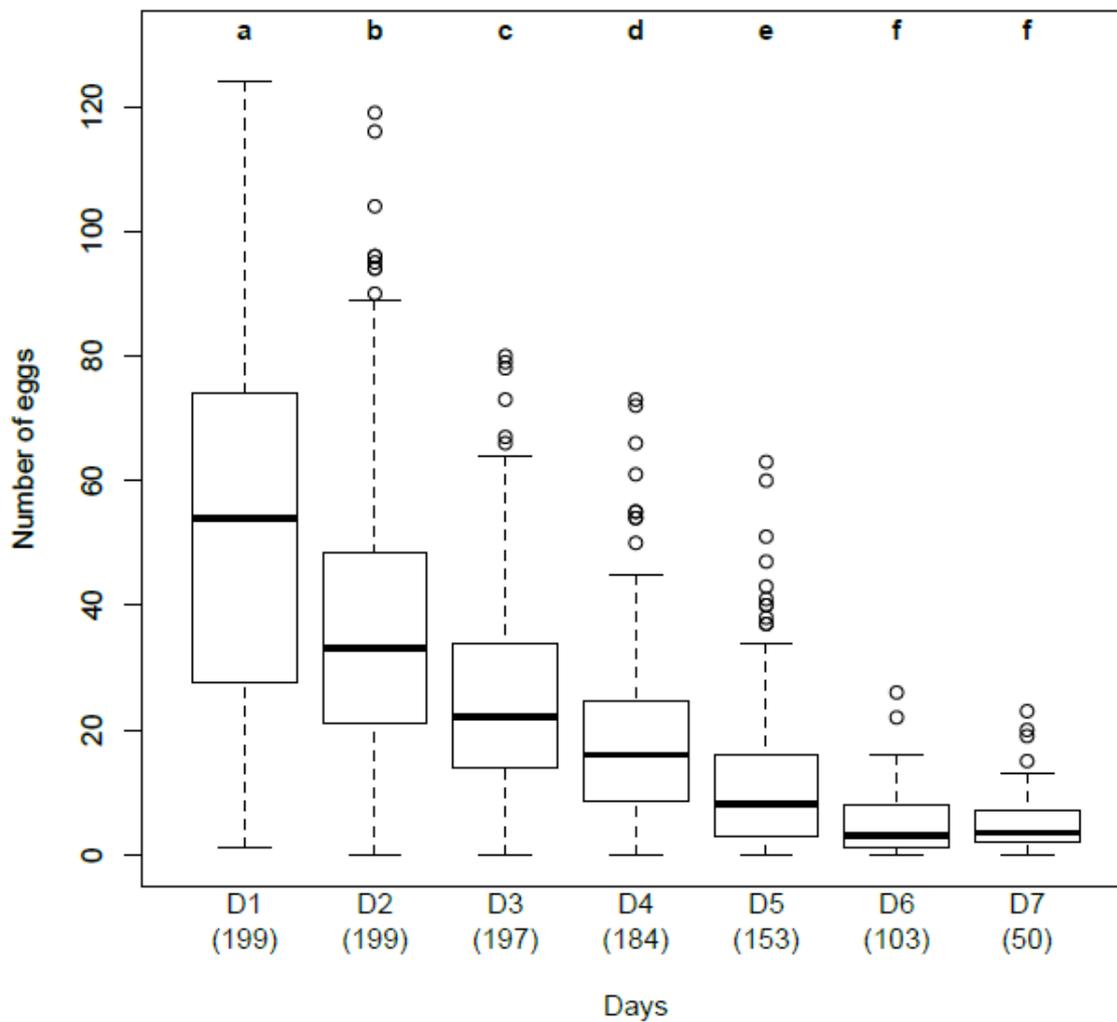
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659 **Fig. 4** Egg size according to the day of oviposition and for each cultivar where the female
660 come from. Bold line: median; box: middle two quartiles; dashed lines: $1.5 \times$ interquartile
661 range; open circle: extreme value. The numbers inside the parentheses indicate the number of
662 measured eggs. Columns with the same letter are not significantly different ($P > 0.05$) based
663 on pairwise Wilcoxon multiple comparison post hoc tests.

664

665 **Fig. 5** Proportion of hatching eggs according to the day of oviposition for all cultivars. Bold
666 line: median; box: middle two quartiles; dashed lines: $1.5 \times$ interquartile range; open circle:
667 extreme value. The numbers inside the parentheses indicate the number of eggs checked for
668 percent egg hatch. Columns with the same letter are not significantly different ($P > 0.05$)
669 based on pairwise Wilcoxon multiple comparison post hoc tests.
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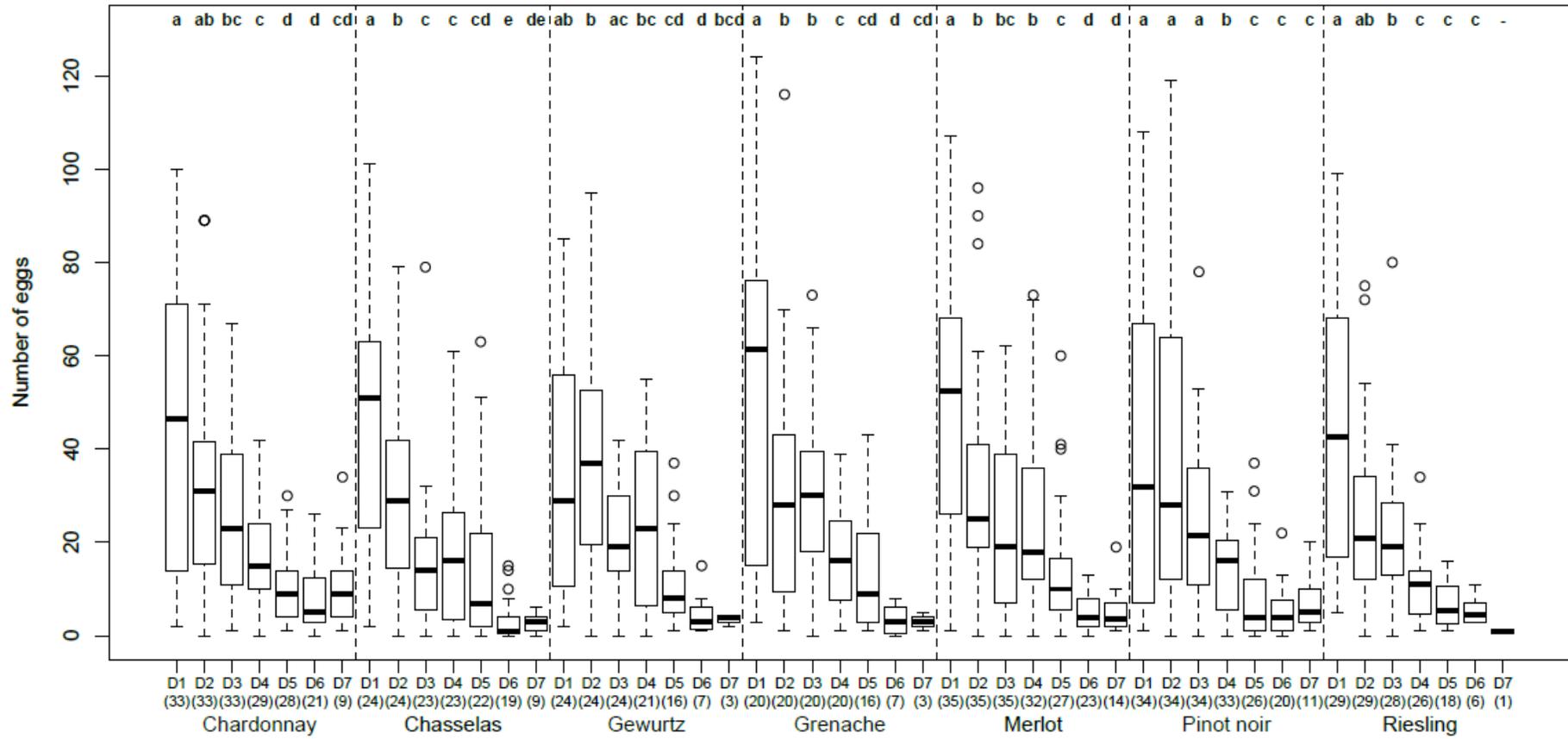
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Figure 1

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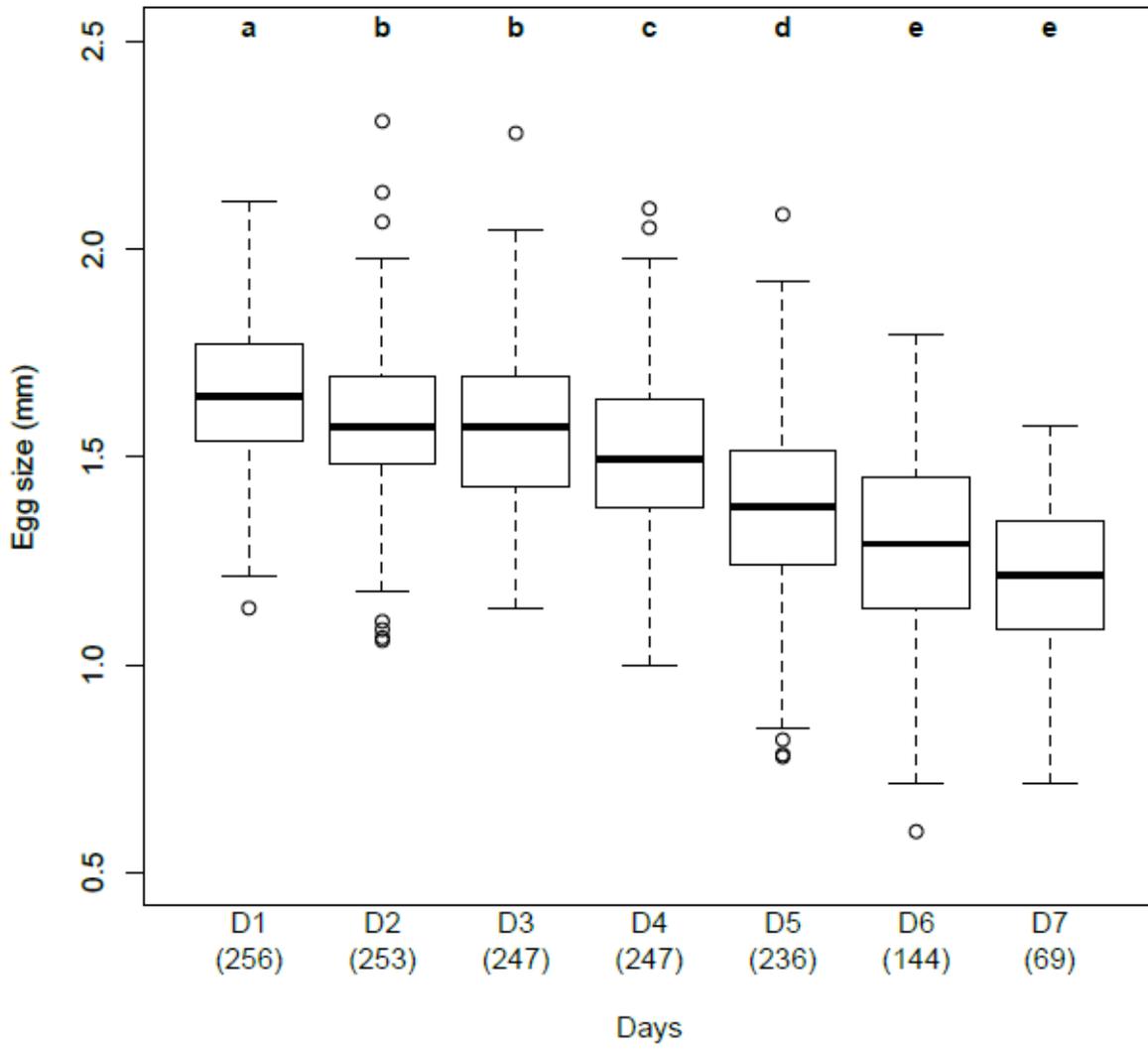
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Figure 2
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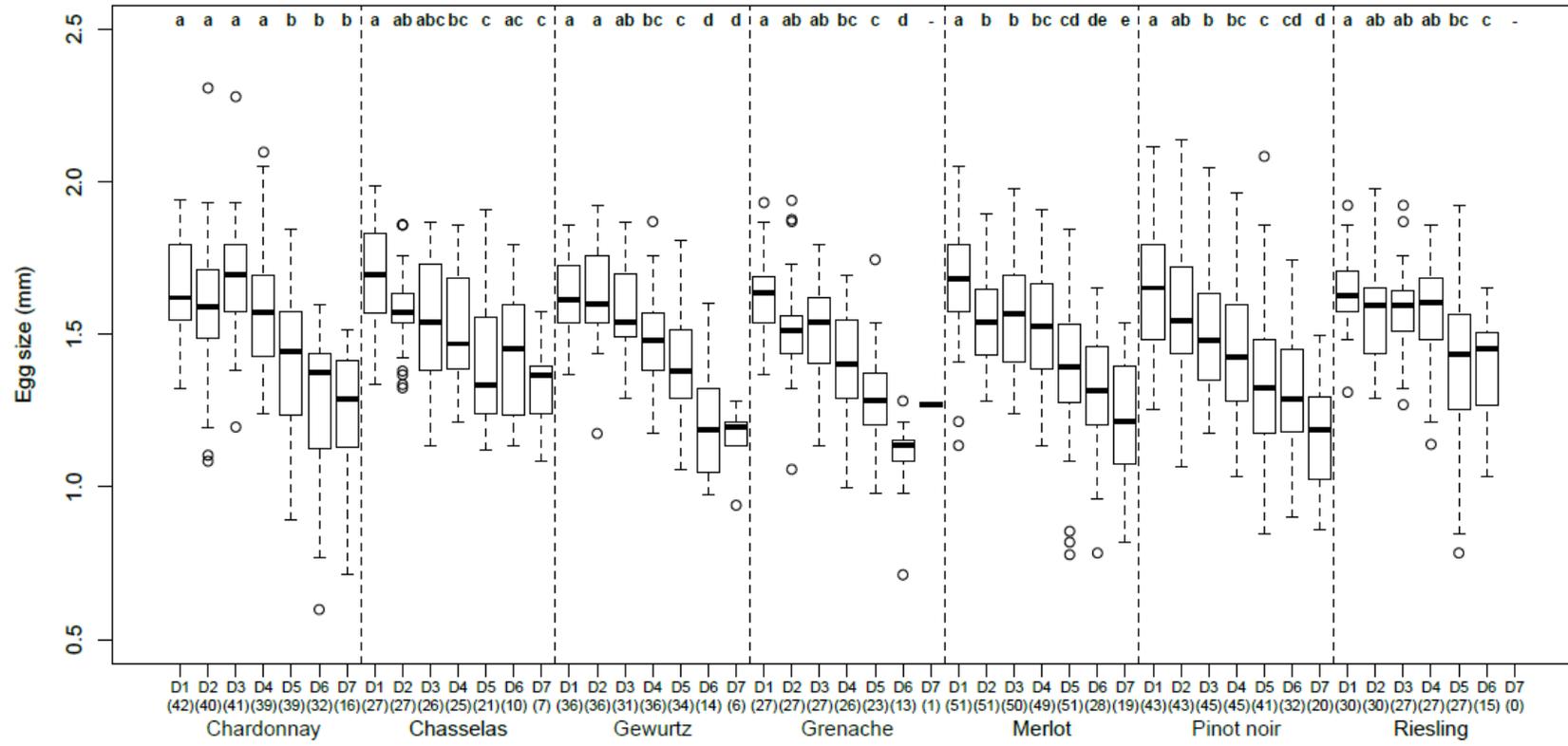
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Figure 3
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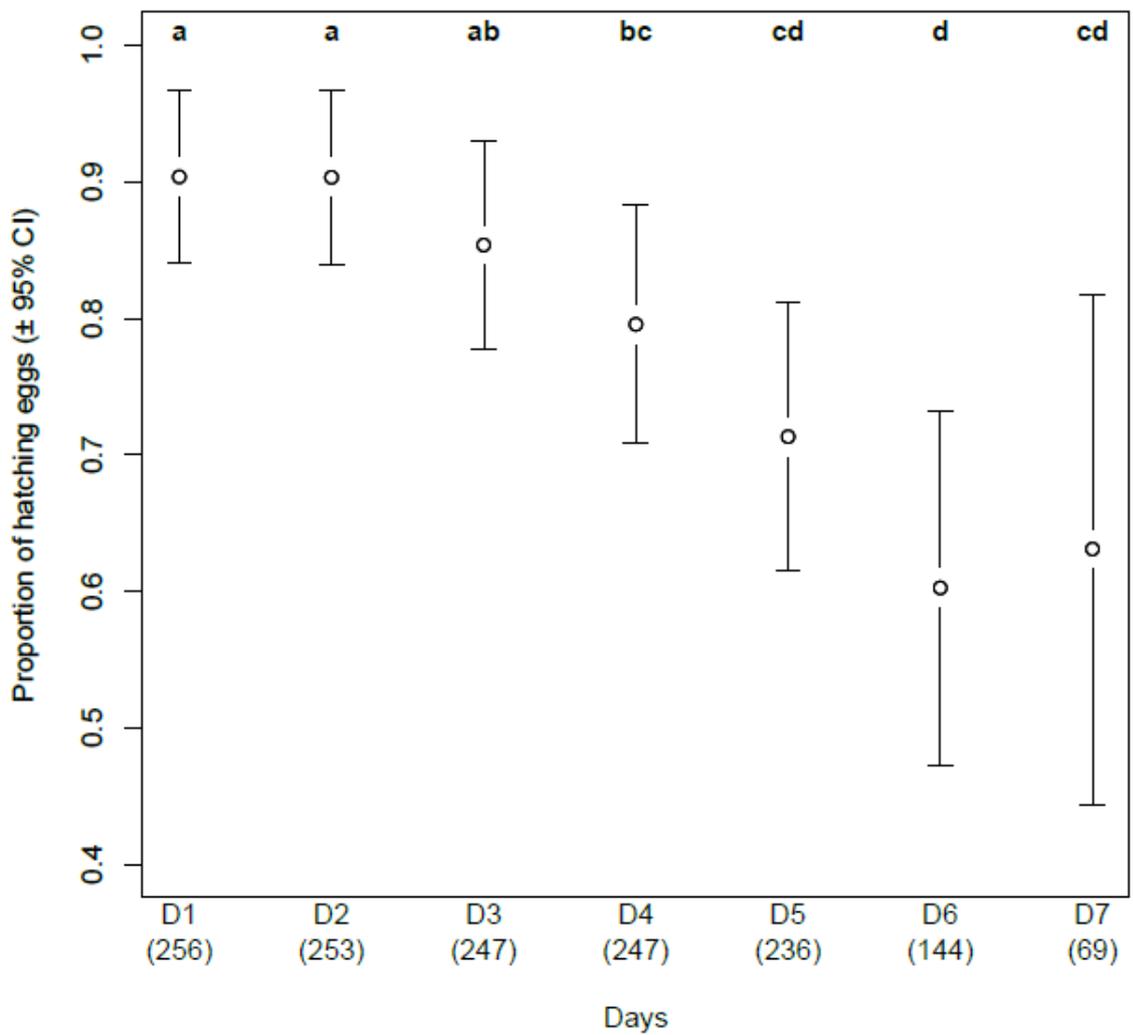
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Figure 4
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Figure 5
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