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1 **Aphid honeydew may be the predominant sugar source for *Aphidius***
2 **parasitoids even in nectar-providing intercrops**

3

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16

17 **ABSTRACT**

18

19 The nectar provision hypothesis predicts that the introduction of nectar-producing plants in
20 agroecosystems benefits parasitoid populations in the field and enhances biological control.
21 Intercropping is a common crop diversification scheme that may bring complementary nectar
22 sources for parasitoids and increase herbivore pest control. For instance, intercropping cereals with
23 faba beans introduces nectar sources in usually sugar-devoid systems (*i.e.* cereal single crops).
24 However, the nectar provision hypothesis has never been evaluated at the field scale in such
25 intercropping systems. To test this hypothesis, we evaluated if sugar is a limiting factor for *Aphidius*
26 parasitoids in single triticale crops and if their nectar feeding activity increases in faba bean-triticale
27 intercrops. *Aphidius* feeding patterns were evaluated from their sugar profiles, using high-
28 performance liquid chromatography (HPLC). In parallel, aphid density and parasitism rates were
29 estimated at the edge and in the centre of single crops and intercrops. Sugar analyses revealed that
30 honeydew was always the main sugar source for parasitoids, and although a significant proportion of
31 parasitoid populations were recorded to feed on nectar, this proportion did not increase in
32 intercrops. Besides, parasitism rates did not increase in intercrops, nor were aphid populations
33 reduced. Thus, our results do not support the nectar provision hypothesis, but rather suggest that
34 although nectar provision benefits parasitoid populations in some systems, its effects on biological
35 control are highly context-dependent. They also confirm that honeydew can be a major food source
36 for parasitoids, which may not necessarily be sugar limited at the field scale.

37

38 **Keywords:** Biological control, Crop diversification, Extrafloral nectar, Nectar provision hypothesis,
39 Nutritional ecology

1. INTRODUCTION

40
41

42 Biodiversity provides a wide range of ecosystemic services in agroecosystems, one of which
43 being pest regulation (Altieri et al. 1999, Bommarco et al. 2013). Particularly, the diversification of
44 cropping systems is thought to increase biological control, *i.e.* the top-down regulation of pest
45 populations by their natural enemies (the enemy hypothesis - Root 1973, Landis et al. 2000, Heimpel
46 & Mills 2017). Indeed, several meta-analyses suggest that increased plant diversity at several spatial
47 scales is generally associated to higher populations of pest's natural enemies (predators and
48 parasitoids), and/or higher parasitism or predation rates (Bommarco & Banks 2003, Chaplin-Kramer
49 et al. 2011, Letourneau et al. 2011). Despite this general relationship between biodiversity and pest
50 regulation, attempts to increase biological control in agroecosystems through habitat manipulation
51 have shown variable and inconsistent results (Heimpel & Jervis 2005, Heimpel 2019). Understanding
52 the underlying ecological processes is required to predict the responses of natural enemies to
53 diversification and to implement successful conservation biological control programmes.

54 The main mechanism mentioned to explain why cropping system diversification is expected
55 to benefit natural enemies is that it allows providing them with additional resources (Root 1973,
56 Landis et al. 2000). In particular, diversification of cultivated areas may provide plant-produced sugar
57 for natural enemies, such as floral or extrafloral nectar (Wilkinson & Landis 2005, Jones et al. 2017),
58 or guttation (Urbaneja-Bernat et al. 2020). Such resources are essential for biological control agents
59 that feed on pests as larvae, but rely on sugar sources during their adult stage for metabolic
60 maintenance, survival and dispersion (Wäckers & van Rijn, 2005). These 'life-history omnivores'
61 include some predators such as syrphids and lacewings, and most parasitoids (Wäckers & van Rijn
62 2005, Hogervorst et al. 2007a). Many conventional monocultures are nectar-depleted: in such
63 systems, food is likely to be limiting for these organisms (Heimpel & Jervis 2005). Providing
64 complementary food sources should thus allow increasing natural enemy performances, as well as
65 their attraction and retention (*e.g.* reduced emigration) (Kean et al. 2003, van Rijn & Sabelis 2005).
66 Consequently, nectar provision *via* crop diversification should support higher natural enemy
67 populations, resulting in enhanced biological pest control. Heimpel & Jervis (2005) proposed the
68 *nectar provision hypothesis* to formalize this idea and to list the conditions required for its
69 expression.

70 A few studies have provided elements in favour of the nectar provision hypothesis. Notably,
71 significant effort has been made to evaluate the potential of field margins surrounding crops to
72 provide sugar subsidies for natural enemies, mainly parasitoids. Several studies have established that
73 these field margins may serve as a food reservoir for parasitoids (Olson & Wäckers 2007, Lee &
74 Heimpel 2008, Winkler et al. 2009, Kishinievsy et al. 2018), and that the presence of resource
75 subsidies in such margins is correlated with increased parasitoid populations and parasitism rates
76 (Tylianakis et al. 2004, Lavandero et al. 2005). However, these effects are often restricted to the field
77 border: parasitoid populations and parasitism usually decrease exponentially when moving away
78 from the field edge towards the centre of the field (Tylianakis et al. 2004, Bianchi & Wäckers 2008,
79 Pollier et al. 2018). Thus, it has been suggested that within-field diversification should be a more
80 efficient way to improve biological control, through a more regularly distributed parasitism within
81 fields (Mills & Wajnberg 2008). Indeed, simulation experiments conducted by Vollhardt *et al.* (2010a)
82 suggest that the effects of nectar provision on parasitoid feeding patterns and parasitism could be

83 greater when flowers are randomly distributed in the field rather than when they are restricted to
84 the field borders.

85 Even though the provision of food sources directly in the field seems very promising for
86 biological control, its effects on parasitoid resource exploitation patterns have scarcely been
87 evaluated. Experiments in semi-natural conditions have shown that providing extrafloral nectar close
88 to host patches may favour attraction and retention of parasitoids into the field (Jamont et al. 2014).
89 Some studies have also evidenced that artificial sugar food resources in the field are effectively
90 consumed by parasitoids and lead to increased parasitoid fitness, population abundance and
91 parasitism rate (Winkler et al. 2006, Lee & Heimpel 2008, Tena et al. 2015). However, up to now, no
92 study has fully tested the nectar provision hypothesis in diversified crops. Indeed, its unambiguous
93 evaluation requires demonstrating several elements. First, it has to be shown that sugar food is
94 actually a limiting factor for biological control in undiversified crops. This may not always be the case
95 as even in simplified systems, parasitoids may find alternative resources such as honeydew (Heimpel
96 & Jervis 2005, Lee et al. 2006, Hogervorst et al. 2007a, Tena et al. 2016). A second step is to
97 determine if diversification leads to higher nectar consumption by parasitoids, which may not always
98 be the case even when sugar food is limiting, depending on nectar accessibility and parasitoid
99 behavioural responses (Heimpel & Jervis 2005) - on the other hand, nectar consumption could
100 increase even if sugar is not limiting, as nectar is often expected to be a high quality food resource
101 for parasitoids (Wäckers et al. 2008, Vollhardt et al. 2010a). Then, a higher nectar consumption
102 should increase parasitism rates, and ultimately reduce the density of pest populations (Heimpel &
103 Jervis 2005, Heimpel & Mills 2017). This does not follow automatically as enhanced biological control
104 is not the only possible outcome of increased nectar consumption: nectar feeding could also
105 stimulate parasitoid emigration from the field, as proposed by Heimpel (2019).

106 Intercropping is an ancestral agricultural practice that consists in simultaneously growing
107 several crops in the same field (Vandermeer 1989). These cropping systems offer multiple
108 ecosystemic services, including increased production, enhanced stability of yield and higher
109 competition over weeds (Bedoussac et al. 2015). As they exhibit higher diversity within the field,
110 intercrops could also benefit biological control. In particular, pests attacking a crop that does not
111 produce nectar could be better regulated if their natural enemies are complemented with sugar
112 produced by another crop. Previous work tends to show that intercrops are one of several
113 diversification schemes that affect natural enemies the most (Letourneau et al. 2011). However, the
114 processes by which they affect parasitoid resource use and their consequences on ecological
115 interactions are largely unknown. It has been suggested that the difficulty to elucidate these
116 processes comes from methodological challenges (Heimpel & Mills 2017): however, recent
117 methodological developments make it much easier to quantify resource use by parasitoids in the
118 field (Wäckers & Steppuhn 2003, Lavandero et al. 2005, Hogervorst et al. 2007a, Luquet et al. 2021).

119 An example of systems varying in the food sources available for parasitoids are cereal
120 monocrops and legume-cereal intercrops, which are a common intercropping scheme in many
121 regions of the world (Malézieux et al. 2009, Yu et al. 2015), mainly to take advantage of nitrogen
122 fixation by legumes and for weed control purposes (Bedoussac et al. 2015). Cereal crops are notably
123 attacked by several aphid species, such as *Sitobion avenae*, *Metopolophium dirhodum* and
124 *Rhopalosiphum padi*, which all are regulated by parasitoids belonging to the *Aphidius* genus. These
125 cereal crops do not produce nectar, and parasitoids could thus be food limited, especially in the
126 centre of the field. They may still feed on aphid honeydew (Hogervorst et al. 2007a), but this food
127 source is sometimes limiting as a sole food source (Tena et al. 2015), and often of an inferior quality

128 than nectar (Wäckers et al. 2008). Intercropping a cereal with a nectar producing plant may give
129 parasitoids access to high-quality food in the whole field. In particular, legumes such as faba bean
130 (*Vicia faba* L) produce a sugar-rich extrafloral nectar, highly accessible and suitable for parasitoids
131 (Jamont et al. 2013, 2014). Faba bean may be a particularly interesting crop for biological control, as
132 it produces extrafloral nectar early and during most of the growing season, and may thus provide
133 high quality food for parasitoids during extended periods (Lu et al. 2014, Jones et al. 2017).

134 In this study, we tested the nectar provision hypothesis in faba bean-triticale intercrops. We
135 evaluated if i/sugar use by parasitoids is limited in pure cereal crops, especially in the centre of fields,
136 ii/ intercropping the cereal with a legume leads to increased nectar consumption by parasitoids,
137 resulting in iii/ higher rates of nectar feeding and, iv/ reduced pest populations. To do so, we
138 compared the feeding patterns and parasitism rates by *Aphidius* spp. parasitoids, as well as aphid
139 densities, in single triticale crops and triticale-faba bean intercrops.

140

141 2. MATERIAL AND METHODS

142

143 To evaluate parasitoid sugar feeding patterns in the field, we used an inferential approach
144 based on sugar profiles developed in previous studies (Wäckers & Steppuhn 2003, Heimpel et al.
145 2004, Hogervorst et al. 2007a, Luquet et al. 2021). First, we set up a laboratory experiment to
146 evaluate the relationships between parasitoid sugar profiles and their feeding history (*i.e.* if they had
147 fed recently or not) and food source (nectar or honeydew). These data were used as a reference
148 baseline to evaluate the feeding status of insects captured in different field treatments (in the centre
149 and close to the edge of single and mixed crop fields). Feeding patterns observed in the field were
150 then analysed in relation to observed parasitism rates and aphid densities.

151

152 2.1 Lab experiment – Reference sugar profiles

153 2.1.1 Biological material

154 All insects and plants used to define the reference sugar profiles were collected from our
155 own laboratory rearing. Faba bean plants (*Vicia faba* L.) var. Divine were used for extrafloral nectar
156 collection. As spontaneous vetch (*Vicia sativa* L.) producing high amounts of extrafloral nectar was
157 commonly encountered in the fields we investigated, we also reared *V. sativa* var. Pépite plants for
158 nectar sampling. *Acyrtosiphon pisum* aphids came from the LSR1 lineage (The International Aphid
159 Genomics Consortium, 2010) and were reared on faba bean. *Sitobion avenae* were collected on
160 wheat crops around Angers (France) in May 2016 and were reared on barley (*Hordeum vulgare* L.
161 var. Prestige). *Aphis fabae* aphids were collected on faba bean crops around Angers in May 2018 and
162 were reared on faba bean. *Aphidius ervi* parasitoids were collected as mummies from *A. pisum* and *S.*
163 *avenae* in faba bean and wheat fields around Angers in 2016. In the laboratory, these two lineages
164 were reared separately, respectively on the *A. pisum*-faba bean complex and *S. avenae*-barley
165 complex, for over 50 generations before being used in the experiments. Plants, aphids and
166 parasitoids were reared under long-day conditions (16L:8D) at 22 °C (day) and 20 °C (night). Humidity
167 conditions were 80% relative humidity (RH) during the day and 90% RH (night) for aphids, and 70%
168 RH for parasitoids.

169 2.1.2 Sugar food collection

170 Honeydew samples were collected from *A. pisum*, *A. fabae* and *S. avenae* aphids, which were
171 the most abundant honeydew producers observed in the studied fields (Table D.1). Samples were
172 collected between December 2017 and January 2018 (*A. pisum*, *S. avenae*) and in August 2018 (*A.*
173 *fabae*). To collect honeydew, Parafilm® cages were placed during 24h around aphid colonies, on their
174 respective plants. Cages were then removed and honeydew droplets were collected directly from the
175 Parafilm®, using micropipettes with capillary 2 µL tips. Droplets from the same colony were pooled to
176 obtain 2 µL samples. Extrafloral nectar samples were collected from *V. faba* plants between
177 December 2017 and January 2018 and from *V. sativa* plants between February and March 2019.
178 Droplets were collected directly from the plant nectaries, using a micropipette. Droplets from the
179 same plant were pooled to obtain 2 µL samples.

180 Samples from each food source were stored at -20°C and used in the following 48h for
181 feeding experiments. Other samples were prepared for sugar analysis (15 samples each for *A. pisum*
182 and *S. avenae* honeydew, 8 samples for *A. fabae* honeydew, 12 samples for each extrafloral nectar).
183 These samples were diluted 50 times in 80% methanol (MeOH), and the solutions obtained were
184 diluted 4000 times in Milli-Q® ultra-pure water. 100 µL samples from each solution were extracted
185 for HPLC analysis.

186

187 2.1.3 Feeding experiment

188 Within 1 day after emergence, unfed *A. ervi* females reared on *A. pisum* or *S. avenae* were
189 individually placed in 2 mL Eppendorf® tubes. Females were then given a feeding treatment following
190 the experimental procedure described by Tian et al. (2016). Briefly, about two hours after being
191 placed in the tube, they were given water during 30 min and were then put in another tube
192 containing one of the following feeding treatments: extrafloral nectar (*V. faba* or *V. sativa*),
193 honeydew (*A. pisum*, *S. avenae* or *A. fabae*), or no food source (unfed insects). Females were
194 observed for at least three minutes, to ensure that they had fed continuously at least for 5 seconds.
195 After feeding, they were either frozen immediately, or kept starving for 1, 12, 24 or 48h before being
196 frozen, in order to follow their sugar metabolic dynamics. Starved females were placed in 200 mL
197 plastic tubes where they were able to fly. After being frozen, insects were kept at -80 °C for further
198 HPLC analysis. 278 individuals were obtained in total. Parasitoids fed on a given food source were
199 collected in the same week as the respective food material.

200

201 2.1.4 HPLC Analysis of sugar profiles

202 Sampled females were lyophilized, weighed, crushed and the powder obtained was diluted in
203 80% methanol. After a 15 min incubation period (76°C), methanol was evaporated in a speed-vac and
204 samples were diluted in 500 µl Milli-Q water. They were then centrifuged to isolate the supernatant
205 containing all soluble sugars, and water was evaporated. Finally, samples were saved in a final
206 volume of 100 µl of Milli-Q water for HPLC analysis. Eighty µl of each diluted sample were injected in
207 a DIONEX ICS 3000 system (Dionex Corp., Sunnyvale, CA, USA) equipped with a CarboPac PA1 column
208 for the HPLC analysis. The column was eluted with 100 mM NaOH and kept at 20 °C. Every ten
209 samples a standard was run to check for deviation from the calibrated values. The sugar content of
210 each individual was analyzed using the Chromeleon™ Chromatography Data System. The standards

211 used for these analyses were glucose (2.5 mg/l), fructose (2.5 mg/l), sucrose (5 mg/l), raffinose (10
212 mg/l), stachyose (10 mg/l), melezitose (10 mg/l), erlose (10 mg/l) and maltose (40 mg/l). Food
213 samples (nectar, honeydew) were analysed the same way, using the same standards.

214

215 2.2 Field experiment

216 2.2.1 Study sites

217 The study sites were located in the Maine-et-Loire department (West of France), in the
218 municipalities of Beaucouzé, Bouchemaine, Savennières and La Possonnière. Six pure triticale fields
219 and six triticale-faba bean intercrop fields from three farmers were investigated (see Appendix A for
220 coordinates). Triticale was sown in rows in all fields. In intercrops, faba beans were sown using
221 broadcast seeding, without a particular spatial arrangement, except for one field where they were
222 sown in rows (Table A.1). Both plants were sown so that they occupied the same surface, although
223 much variability was observed during the season and across fields (triticale: 33 ± 20 % surface, $65 \pm$
224 53 tillers/m² ; faba bean: 29 ± 19 % surface, 14 ± 9 plants/m²). All fields were organically managed
225 and received no pesticide or biopesticide treatment. They were all bordered by woody field margins
226 containing abundant spontaneous vegetation (grasses, flowers), including nectar-producing flowers
227 (plants from the Apiaceae family were predominant). Experiments were carried out between weeks
228 17 (24th April) and 24 (14th June) in 2018. Temperature during the experiments ranged from 21.8 °C
229 to 30.8 °C and humidity ranged from 47% to 81% RH.

230

231 2.2.2 Insect sampling and surveys

232 Two 20-m sampling lines were established in each field: one at 5 m from the field edge and
233 one at 50 m from the field edge. Along each line, insect samples and surveys were repeated at six
234 dates, covering most of the aphid and parasitoid seasonal activity (weeks 17, 18, 19, 21, 22, 24).

235 *Parasitoid samplings:* Along each sampling line, insects were captured using a STIHL® SH 86C
236 shredder vac. A net bag was installed inside the vac pipe to collect the insects without damaging
237 them. The capture was done by vacuuming each plant from the bottom to the top along the sampling
238 line. After sampling, collected insects were placed in a freezer bag and immediately transferred to a
239 portable freezer at -20°C. Bags from seven fields (three intercrops, four single crops) were then
240 opened in the laboratory and insects were sorted on a plate in an ice box to retrieve Aphidiinae
241 parasitoids. These insects were then quickly identified and sexed under a stereoscopic microscope
242 (80X), still using ice to avoid as much as possible unfreezing the samples. All identified *Aphidius*
243 females were stored at -80 °C before being prepared for sugar extraction and HPLC analysis as
244 described above.

245 *Insect surveys:* At each sampling date, 50 triticale tillers were randomly chosen along each
246 sampling line in each field, at more than five meters from the row where the aspirations were carried
247 out. All aphids and mummies were counted on each tiller. Aphids were identified at the species level
248 and mummy shape and colour were used to identify the parasitoid genus (*Aphidius*, *Ephedrus* or
249 *Praon* - Praslicka et al. 2003). Parasitism rates at each date, for each field and distance from the edge
250 were estimated by dividing the total number of mummies counted on a sampling line by the sum of
251 aphids and mummies on this line.

252

253

2.3 Data analysis

254

2.3.1 Feeding pattern predictions from HPLC data

255 To evaluate insect feeding patterns in the field, we referred to the method developed by
256 Luquet et al. (2020). The lab dataset, containing the sugar profiles of insects with known feeding
257 history and food source, was used as a *training dataset* to learn a *classifier*, *i.e.* a procedure to
258 predict an insect feeding status from its sugar profile. This classifier allowed inferring feeding history
259 and food sources from field-collected insects. In particular, we used it to perform *prevalence*
260 *estimation*, *i.e.* estimating the relative proportion of insects with different feeding histories and food
261 sources in each field treatment.

262 *Defining feeding classes:* In order for comparisons to be meaningful, we evaluated a
263 maximum time after a feeding event, which would define an insect as « fed ». This 'detection time'
264 was set to 12 h, which corresponds to the average rate of feeding by *Aphidius ervi* (Azzouz et al.
265 2004, L rault et al., unpublished data). This choice was reinforced by the fact that a significant shift
266 can be observed in the sugar profiles of insects that have last fed more than 12 hours ago, in our own
267 data and in previously published datasets (Hogervorst et al. 2007a). Thus, insects were assigned to
268 one of the three following classes: "nectar-fed" (insects fed from nectar within less than 12 hours),
269 "honeydew-fed" (insects fed from honeydew for less than 12 hours) and "unfed" (insects that never
270 fed or that were starved for more than 12 hours).

271 *Training dataset construction:* To ensure the quality of predictions, we subsampled our lab
272 data to obtain a balanced training dataset (Barranquero et al. 2015, Chicco et al. 2017). We randomly
273 selected data to obtain a 183-sample training dataset that contained about the same number of
274 samples from each class. In addition, we balanced the data as much as possible between the
275 different time treatments (*i.e.* the periods for which insects had been fed), parasitoid origin (*A.*
276 *pisum*, *S. avenae*), and the different food sources (*i.e.* different honeydews, different nectars).

277 *Choice of predictor variables:* We used all individual sugars detected in parasitoids as
278 variables to predict their feeding class. We also included the ratio Glucose/(Glucose + Fructose) or
279 'GF Ratio' as a potential predictor, which is known to be a key marker of insect feeding (Steppuhn &
280 W ckers 2004, Hogervorst et al. 2007a, Tena et al. 2013). Given the high number of null values for
281 melezitose, we did not use the Erlose/Melezitose ratio (Hogervorst et al. 2007a, Tena et al. 2013) as a
282 food source marker for HPLC datasets. Instead, we computed the ratio (Maltose + Erlose +
283 Melezitose)/Total Sugar (Luquet et al. 2021). We named this variable the 'H Ratio' (H for
284 'Honeydew').

285 *Method choice and prevalence estimation:* We used the heatmap suggested by Luquet et al.
286 (2020) to choose the most appropriate method for prediction. From the 'noise index' (0.58) and the
287 size (183) of our dataset, it appeared that the best approach for prevalence estimation was to use a
288 Random Forest classifier (Breiman, 2001) coupled with an Adjusted Counting correction method
289 (Forman, 2008). These methods were used to predict the relative frequency of insects from each
290 class in each field type (Single crop, Intercrop) and distance from the field edge (5m, 50m). The
291 algorithm was trained and used on R software version 3.5.2 (R Core Team, 2018) with the help of the
292 *randomForest* package (Liaw & Wiener 2002).

293

294

2.3.2 Comparison between field treatments

295 *Lab data:* The overall sugar levels of different food sources, as well as insects receiving
296 different feeding treatments, were compared using generalised linear models (GLM) with a Gamma
297 error structure. To estimate how much variation in insect sugar profiles is due to their feeding
298 treatment, we applied a redundancy analysis using the *vegan* package (Oksanen *et al.* 2018).

299 *Field data:* To evaluate the differences between the feeding patterns of insects in each field
300 type and distance from the field edge, we compared the relative frequency of insects from each
301 feeding class by using chi-squared tests. In addition, we used GLM with a Gamma distribution to
302 compare their mean overall sugar levels. To analyse aphid densities and parasitism rates according to
303 field type and distance, we fitted generalized linear mixed models to the data (GLMM), using *lme4*
304 package on R (Bates *et al.* 2015). Aphid density and parasitism rates were setup as response
305 variables, while field type, field distance and the interaction between them were included as fixed
306 explanatory variables. The field in which measures were done was included as a random variable, to
307 account for site effects. The week during which measures were done was added as a second random
308 variable, to account both for temporal sources of variation (*e.g.* climatic fluctuations) and temporal
309 autocorrelation. The model for aphid density was fitted using a negative-binomial error structure and
310 log link, while the model for parasitism rates was fitted using a binomial error structure and logit link.
311 Appropriate checks were done to assess sufficient model validity (Zuur *et al.* 2016, Hartig & Lohse
312 2020).

313

314

3. RESULTS

315

316

3.1 Lab experiment

317

3.1.1 Sugar spectrum of food sources

318 Fructose, glucose, sucrose and melezitose were the only sugars found in the analysed nectars
319 and honeydews. Melezitose was found only in *Aphis fabae* honeydew. The different food sources
320 varied both in their relative and absolute amounts of sugars (Fig. 1, Table 1A). *A. pisum* honeydew, *S.*
321 *avenae* honeydew and *V. faba* nectar were dominated by glucose, while *A. fabae* honeydew was
322 dominated by melezitose and *V. sativa* nectar was dominated by sucrose. Overall, sugar
323 concentration in extrafloral nectar was about ten times higher than in honeydew (Table 1A).

324

325

3.1.2 Parasitoid sugar profiles

326 Fructose, glucose, sucrose, melezitose, erlose, stachyose and maltose were found in all
327 feeding treatments. Sugar profiles were dominated by glucose (>50 % of total sugars for all
328 treatments, except for insects fed on *V. sativa* nectar) (Table 1B).

329 Both qualitative and quantitative differences were observed between insects that had just
330 emerged and insects that had just fed from different food sources. All 'just-fed' insects had a higher
331 total sugar level than unfed emerging insects (GLM, *Feeding treatment effect*: $LR \chi^2 = 204.8$, $df = 5$, P
332 $< 1e^{-16}$), except insects fed with *S. avenae* honeydew (Table 1B). Besides, insects feeding on more
333 sugar-rich sources also exhibited higher sugar levels (Table 1). Some correspondences were observed
334 between the relative sugar composition of insects and the sugar spectrum of the food sources they

335 fed on: for instance, insects fed on *V. sativa* nectar had the highest relative sucrose concentration
336 and insects fed on *A. fabae* honeydew had the highest relative melezitose concentration (Fig.1, Table
337 1).

338 Insect sugar profiles varied quickly after emergence and/or after feeding. Sugar metabolic
339 dynamics followed the same trend for all feeding treatments (Fig. 2, Appendix B). A quick decrease in
340 the total sugar level was observed in the first hour after a feeding event, followed by a stable period
341 (Fig. 2). After 12h, sugar amounts decreased continuously and 48h after feeding, honeydew-fed
342 insects had the same sugar levels as insects that never fed. However, after 48h insects fed with *V.*
343 *faba* nectar still had higher amounts of sugars than unfed insects (Fig. 2). Globally, the trend
344 observed for the total sugar amount was the same for the individual sugars (Appendix B). However,
345 sugar amounts did not change following the same rate: sugar profiles of fed insects became more
346 glucose-dominated with time after feeding (Appendix B). Besides, parasitoid origin (*A. pisum* or *S.*
347 *avenae*) did not appear to influence sugar profiles (permutation tests for RDA, 1000 permutations, F
348 = 1.7, $df = 1$, $P = 0.3$).

349

350 3.1.3 Model training

351 Some general patterns of variation could be observed when plotting the data according to
352 the chosen predictive variables, such as the fructose amount and the Glucose-Fructose Ratio (Fig. 3).
353 However, a sizeable degree of overlap was observed for all combinations of variables. This was partly
354 due to the fact that many honeydew-fed insects had similar profiles as unfed insects (even after
355 applying a 12h detection time), and that some nectar-fed insects still had high levels of sugars after
356 24 or 48h although they were considered as unfed (Appendix C). According to the redundancy
357 analysis, 42% of the lab dataset sugar profile variation was explained by the insect feeding treatment
358 ('noise index' = 58%).

359 The classification error was estimated as 35% on the lab dataset. Errors were mainly
360 confusions between unfed and honeydew-fed insects, due to the high similarity between the sugar
361 profiles of these classes (Table 2). However, the size of the dataset justified using the 'Adjusted
362 Counting' method, to temperate these prediction errors. According to the classifier, variable relative
363 importance was by decreasing order: Fructose, Glucose, GF Ratio, Sucrose, H ratio, Stachyose,
364 Maltose, Melezitose and Erlose.

365

366

367 3.2 Field experiment

368 3.2.1 Feeding patterns in single crop and intercrop fields

369 Our prediction algorithm ('Random Forest coupled with Adjusted Counting') revealed that all
370 *Aphidius* spp. females captured had recently fed from honeydew or nectar (Fig. 4). Honeydew was
371 the most common food source in all field treatments (type and distance from the edge). The
372 treatment in which the most nectar-fed females were captured was in intercrops at 50m (45%, *i.e.* 17
373 of 38 individuals), while the treatment with the fewest nectar-fed females was single crops at 50m
374 (26%, *i.e.* 17 of 68 individuals). However, observed differences were not statistically significant ($\chi^2 =$
375 4.5 $df = 3$, $P = 0.2$; see Fig.4). Besides, no difference was observed in the overall sugar levels of

376 females in the different field treatments (Fig. D.1) (GLM, *Field distance*: $LR \chi^2 = 0.2$, $df = 1$, $P = 0.6$,
 377 *Field type*: $LR \chi^2 = 2.6$, $df = 1$, $P = 0.1$, *Field distance * Field type*: $LR \chi^2 = 0.8$, $df = 1$, $P = 0.4$).

378
 379

380 3.2.3 Aphid density and parasitism rates in single crop and intercrop fields

381 Out of the 1758 aphids counted in total on triticale, 1616 were identified as *S. avenae* (92%),
 382 98 as *M. dirhodum* (5%) and 44 as *R. padi* (3% - see also Table D.1). The total number of aphids
 383 observed in the different field types and distances from the field edge ranged from 0 to 110, with a
 384 lot of variation according to the week and the field (Fig. 5A, see Fig. D.2 for the raw data). The same
 385 dynamics were observed in all field treatments: populations increased until weeks 21 and 22, and
 386 then decreased quickly until week 24 (Fig. 5A). Overall, no difference in aphid numbers was found
 387 according to field type ($\chi^2 = 0.08$, $df = 1$, $P = 0.8$), position ($\chi^2 = 0.8$, $df = 1$, $P = 0.4$), or the interaction
 388 between them ($\chi^2 = 0.02$, $df = 1$, $P = 0.9$).

389 298 mummies were observed in total. They were mainly mummies from *Aphidius* parasitoids
 390 (254, *i.e.* 92%), but we also observed 23 *Ephedrus* mummies (only at the end of the season, weeks 22
 391 and 24) and 1 *Praon* mummy. Weekly aphid parasitism rates by *Aphidius* parasitoids ranged from 0%
 392 to 100% with a lot of inter-field variation, particularly in the centre of the field (Fig. 5B, see Fig. D.3
 393 for the raw data). For all field treatments, the highest parasitism rates were observed at the end of
 394 the season (week 24, Fig. 5B). Overall, no difference in parasitism rates was found according to field
 395 type ($\chi^2 = 0.1$, $df = 1$, $P = 0.7$), position ($\chi^2 = 2.1$, $df = 1$, $P = 0.15$), or the interaction between them (χ^2
 396 = 0.06, $df = 1$, $P = 0.8$).

397

398 4. DISCUSSION

399

400 To evaluate rigorously the effects of sugar complementation on biological control in
 401 agroecosystems, Heimpel & Jervis (2005) have formalized the nectar provision hypothesis, which
 402 proposes a set of premises that must be addressed together. Here, we tested the nectar provision
 403 hypothesis in triticale-faba bean intercrops. However, no evidence was found for any of its premises;
 404 whether it be sugar limitation for *Aphidius* parasitoids, increased nectar feeding in intercrops,
 405 increased aphid parasitism rates on aphids, or reduced aphid density. These results raise new
 406 questions for evaluating the potential of diversified systems to enhance biological control.

407

408 4.1 Sugar profile variability: implications for feeding detection

409 A large variability was found in the sugar spectrum of food sources, in our laboratory
 410 experiment. Both the absolute concentration and relative composition of honeydew sugars were
 411 highly dependent on the honeydew producer, as already evidenced by Hogervorst et al. (2007b).
 412 Extrafloral nectar sugar composition also showed important variation, both between different plants
 413 of the same species and between two phylogenetically close nectar-producing Fabaceae. This
 414 supports previous results showing that nectar composition is highly variable (Koptur 1994, Wäckers
 415 2005). This variability in food sugar composition resulted in high variability in the sugar profiles of
 416 insects from different feeding treatments. These results have several implications for feeding

417 detection methods using metabolic data. In particular, variability between insects feeding on
418 different resources of the same type (*e.g.* different honeydews) indicate that an insect feeding on a
419 given resource may not be representative of all insects feeding on this type of resource. For instance,
420 the sugar profile of an insect feeding on honeydew A will sometimes poorly predict the food source
421 of an insect feeding on honeydew B. These results underline the fact that for predictions to be
422 trustworthy, important attention should be given to the composition of the reference lab dataset:
423 this includes considering insects feeding on different resources of the same type. They also justify the
424 use of powerful supervised learning algorithms that deal efficiently with different sources of
425 variability (Luquet et al. 2021). On the other hand, sugar composition variation between nectars or
426 between honeydews could be taken advantage of in future studies: given sufficient knowledge of
427 resources present in the field, relevant differences in sugar profiles could be used to discriminate
428 insects feeding on different nectars or different honeydews, as already suggested by Wäckers &
429 Steppuhn (2003).

430

431 4.2 Sugar limitation in single crops

432 All parasitoid females captured in the field were found to be fed in triticale single crops, both
433 near the field edge and in the field centre. These results suggest that food may not be limiting in such
434 crops. This contradicts the first premise of the nectar provision hypothesis, which states that sugar
435 should be a limiting resource for parasitoids in nectar-depleted systems. However, this is not the first
436 time that such an observation is made: Lee et al. (2006) had already shown that flower resources do
437 not increase sugar feeding by *Diadegma insulare* in cabbage fields, and suggested that sugar
438 resource is not limited in such fields either. In our case, HPLC results showed that females mainly fed
439 from honeydew (two thirds of captured insects), suggesting that this food source alone may be
440 sufficient to support parasitoid populations in triticale fields. These results are consistent with
441 previous studies that showed that honeydew is an important food source for parasitoids (Lee et al.
442 2006, Wäckers et al. 2008, Tena et al. 2016), including in cereal fields (Hogervorst et al. 2007a). In
443 other contexts, however, honeydew alone has been shown to be limiting for parasitoid population
444 growth (Tena et al. 2013, 2015). It is thus likely that the capacity of nectar-deprived systems to
445 support parasitoid populations is highly context-dependent, *i.e.* depending on the community of
446 honeydew producers present in the system (Tena et al. 2016), but also on seasonal variations (Segoli
447 & Rosenheim 2013, Tena et al. 2013). Besides, even if our field data indicated that the prevalence of
448 nectar-fed females was the lowest in the centre of single crop fields, we still found that one quarter
449 of the females caught in the centre had recently fed on nectar, despite the expected absence of
450 nectar sources at this location. This result can be explained in two ways. First, some parasitoids may
451 manage to exploit the nectar sources present in field margins and then forage for aphids at the
452 centre, as suggested by several studies showing that when sugar resources are scarce, parasitoids are
453 able to travel several tenths of meters to find food (Lavandero et al. 2005, Lee et al. 2006). Second,
454 the centre of the field may not have been completely depleted in nectar sources. Indeed, it is rare
455 that agroecosystems are completely devoid of weeds, notably in extensively managed and/or organic
456 crops (Petit et al. 2011). Such spontaneous vegetation can provide high-quality, accessible nectar for
457 parasitoids (Wäckers 2004, Araj et al. 2019). Although they were much less abundant than faba bean
458 plants in intercrops, we observed the presence of vetch in many of our investigated fields, which
459 grew during the season. Carbohydrate analysis revealed that this weed produces a particularly sugar-
460 rich extrafloral nectar: it is likely that such spontaneous nectar-producing plants are exploited by

461 parasitoids in the field. Several studies have shown that vetch extrafloral nectar increases the
462 lifespan and fecundity of different parasitoid species (Géneau et al. 2012, Irvin et al. 2014), and the
463 presence of this plant in field borders may lead to higher parasitism rates (Pollier et al. 2018). Further
464 work will be needed to quantify to what extent nectar-producing weeds may support parasitoid
465 populations.

466

467 4.3 Parasitoid feeding patterns in intercrops vs single crops

468 Feeding patterns in intercrops were overall the same as what we observed in single crops,
469 either at the field edge or in the centre: all parasitoid females were fed, and more than half of them
470 (58%) had fed from honeydew. Besides, their sugar levels were sensibly the same as in single crops.
471 Thus, contrary to the predictions of the nectar provision hypothesis, we did not find clear evidence
472 that nectar feeding increases in intercrops, compared to single crops. This result is surprising as
473 nectar is generally a higher quality food source than honeydew (Wäckers 2000, Wäckers et al. 2008).
474 Consequently, even if sugar food is not limiting due to honeydew availability, parasitoids are
475 expected to forage for nectar rather than honeydew when given the choice (Vollhardt et al. 2010b).
476 However, recent results tend to challenge this idea. Lenaerts et al. (2016) notably showed that
477 *Aphidius ervi* prefers to feed on sugars that are over-represented in honeydew rather than nectar-
478 related sugars. Thus, there may be some level of gustatory preference for honeydew in *Aphidius*
479 parasitoids, which should be evaluated in further research. Charles & Paine (2016) also showed that
480 extrafloral nectar may sometimes be an inferior food source compared to honeydew: in their
481 experiments, *Aphidius colemani* parasitoids survived longer on *Aphis gossypii* honeydew than on
482 zucchini extrafloral nectar. Several other cases, where honeydew had similar, or more pronounced
483 positive effects than nectar or honey on parasitoid fitness were also reported in recent studies
484 (Benelli et al. 2017, Monticelli et al. 2020, Rand & Waters 2020), indicating that foraging for
485 honeydew rather than nectar may sometimes be adaptive. However, in our case it is difficult to know
486 if honeydew was a resource with higher nutritional quality, without further investigation on fitness
487 effects from each food source.

488 Alternatively, the fact that we did not observe increased nectar feeding in intercrop fields
489 may be due to the relative quantity of available resources. Indeed, simulations run by Vollhardt et al.
490 (2010a) showed that above a certain aphid density, nectar-feeding may become marginal compared
491 to honeydew-feeding. In intercrop fields, aphid densities were particularly dense as we observed
492 huge colonies of *Aphis fabae* on faba bean plants, which can continuously produce high amounts of
493 honeydew (Fischer et al. 2005). Thus, given the potential massive honeydew production in intercrop
494 fields, parasitoids may have fed on honeydew as their main sugar source simply because it is the
495 most abundant. In future studies, resource density (in addition to resource diversity) may be an
496 important parameter to consider, in order to better understand parasitoid feeding patterns. Finally,
497 it has to be considered that we may have missed nectar feeding events because some insects have
498 fed on both sugar sources.

499

500 4.4 Implications for biological control

501 Our measures of parasitism rates were consistent with other studies in cereal fields in the
502 same region and season (Pollier et al. 2018, 2019, Jeavons et al., in prep.). Contrary to our

503 hypothesis, we did not find any effect of intercropping on the parasitism rates nor on the density of
504 triticale aphids, which suggests that the aphid biological control service provided by parasitoids
505 remained unchanged compared to single crops. These results do not support the hypothesis that
506 providing nectar enhances pest regulation in faba bean-cereal intercrops. This is consistent with our
507 observations of parasitoid feeding activity: as sugar food appeared to be non-limiting in single crops,
508 parasitoid feeding patterns and sugar levels did not change in intercrops, leading to the absence of
509 consequence on their parasitism activity. Again, the fact that our results contrast with previous
510 studies validating the nectar provision hypothesis in other systems (Winkler et al. 2006, Tena et al.
511 2015) emphasizes the context-dependency of the outcome of sugar complementation on biological
512 control patterns (Jones et al. 2017), depending on the initial potential of agroecosystems to support
513 parasitoid populations. In the future, biochemical analyses using tools such as HPLC will be useful to
514 quantify to what extent food may be limiting in various systems, and thus to estimate the potential
515 for biological control improvement through nectar complementation for parasitoid populations.

516 Even if our experimental methodology made us miss an increasing effect of intercropping on
517 parasitoid nectar consumption, different issues may explain why we still do not observe enhanced
518 biological control. First, it is possible that more parasitoids benefited from nectar in intercrops, but
519 that they quickly emigrated from the field. Heimpel (2019) has indeed suggested that patch-leaving
520 decisions after nectar feeding may often be adaptive, *e.g.* because of risks of self-superparasitism or
521 to avoid inbreeding depression. Second, we did not consider the fourth trophic level, which may also
522 benefit from field diversification (Araj et al. 2009). In the same system as ours (legume-cereal
523 intercrops), a very recent study has found that the ratio between hyperparasitoids and primary
524 parasitoids increases in intercrops, suggesting that hyperparasitoids may benefit more from
525 diversification than primary parasitoids (Jeavons et al., *in prep.*). Thus, even if parasitoids have
526 benefited from diversification, this effect may have been counterbalanced by increased top-down
527 regulation by hyperparasitoids. This result calls for a better integration of trophic networks to better
528 understand the effects of diversification schemes on ecosystemic services.

529 Our study is not the first to find no evidence that providing nectar increases biological
530 control. In 2005, Heimpel & Jervis reviewed published and unpublished studies that compared
531 parasitism levels and pest densities in control plots vs nectar-enhanced plots. They showed that only
532 7 out of 20 studies had found increased parasitism rates, and only one of these found decreased pest
533 density. These results, along with ours, tend to demonstrate that sugar food may often not be the
534 key limiting factor for biological control in agroecosystems. Parasitoids and other biological control
535 agents actually have many other requirements to maintain their populations and ensure high levels
536 of pest regulation: these include alternative hosts or preys, overwintering habitats or refuges
537 (Gillespie et al. 2016). Further research will be needed to precisely evaluate what actually limits the
538 population growth and pest suppression by natural enemies in particular contexts, in order to set up
539 adapted management for conservation biological control.

540

541 Declaration of interest

542 The authors declare no competing interest.

543

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555

556 Data availability statement

557 All data presented and scripts needed to reproduce analyses can be found on the following
558 repository: [https://github.com/MartinLuquetEcology/Parasitoid-sugar-feeding-and-parasitism-in-](https://github.com/MartinLuquetEcology/Parasitoid-sugar-feeding-and-parasitism-in-intercrops)
559 [intercrops](https://github.com/MartinLuquetEcology/Parasitoid-sugar-feeding-and-parasitism-in-intercrops).

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746 Table legends

747

748 **Table 1.** Average HPLC sugar spectrum of **A.** Food sources (honeydews and nectars) and **B.** Female
749 parasitoids that just emerged ('emerging') or that just fed from different food sources. ApH =
750 *Acyrtosiphon pisum* honeydew, AfH = *Aphis fabae* honeydew, SaH = *Sitobion avenae* honeydew, VfN
751 = *Vicia faba* extrafloral nectar, VsN = *Vicia sativa* extrafloral nectar. Average values for all sugars are
752 displayed, along with standard errors in brackets. Mean total sugar amounts and their standard
753 errors are also displayed for all food sources and insect feeding treatments. Same letters indicate no
754 significant difference (alpha = 0.05).

755

756 **Table 2.** Confusion matrix from the Random Forest classification (based on out-of-bag error as
757 described in Breiman, 2001). Values correspond to the actual versus predicted feeding class of
758 parasitoids from the lab data. Percentages indicate what fraction of individuals from each class was
759 predicted to belong to each class.

760

761 Figure legends

762

763 **Figure 1.** Relative sugar concentrations of sugar food sources (top) and *Aphidius ervi* females that
 764 have just fed on these resources (bottom), as well as emerging unfed insects ('no food'). *AfH* = *Aphis*
 765 *fabae* honeydew, *ApH* = *Acyrtosiphon pisum* honeydew, *SaH* = *Sitobion avenae* honeydew, *VfN* =
 766 *Vicia faba* extrafloral nectar, *VsN* = *Vicia sativa* extrafloral nectar.

767

768 **Figure 2.** Overall sugar metabolic dynamics of *Aphidius ervi* females after receiving a feeding
 769 treatment. Triangles represent nectar-fed insects, circles represent honeydew-fed insects and
 770 squares represent unfed insects. Colours correspond to the different feeding treatments. *AfH* = *Aphis*
 771 *fabae* honeydew, *ApH* = *Acyrtosiphon pisum* honeydew, *SaH* = *Sitobion avenae* honeydew, *VfN* =
 772 *Vicia faba* extrafloral nectar, *VsN* = *Vicia sativa* extrafloral nectar. Bars represent standard errors.

773

774 **Figure 3.** Two dimensional representation of the lab dataset, according to the Fructose content of
 775 *Aphidius ervi* females ($\mu\text{g}/\text{mg}$ insect) vs the Glucose/(Glucose + Fructose) Ratio (GF Ratio). Colours
 776 refer to the different feeding classes. These classes were assigned to insects after setting a 12h
 777 'detection time': insects that have last fed for more than 12 hours are labelled as 'unfed'.

778

779 **Figure 4.** Estimated relative frequencies of *Aphidius* spp. females from each feeding class in
 780 intercrops and in single crops, at 5m and 50m from the border. These frequencies were computed by
 781 classifying field-caught individuals using a Random Forest algorithm, followed by the Adjusted
 782 Counting prevalence estimation method.

783

784 **Figure 5. A.** Total aphid abundance and **B.** Parasitism rate by *Aphidius* spp. parasitoids estimated at
 785 different sampling dates in pure crops (blue) and intercrops (red) (mean \pm SE), at 5m (left) or 50m
 786 (right) from the field border. Aphid abundance was estimated as the sum of aphids from all species
 787 counted on 50 triticale tillers. Parasitism rate was computed as the number of mummies counted on
 788 50 triticale tillers, divided by the sum of mummies and aphids found on the same tillers.

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791

792 Table 2

	Food source	<i>n</i>	Glucose	Fructose	Sucrose	Melezitose	Erlucose	Maltose	Stachyose	Raffinose	Total
A. Food	Honeydew	AfH	8	19.6 (2.8)	37.2 (4.0)	0 (0)	63.7 (21.4)	0 (0)	0 (0)	0 (0)	120.4^b (20.8)
		ApH	1 5	52.4 (14.7)	22.8 (7.8)	23.2 (12.4)	0 (0)	0 (0)	0 (0)	0 (0)	98.4^a ^b (32.9)

B. Parasitoids ($\mu\text{g}/\text{mg insect}$)	Nectar	SaH	1 5	13.6 (2.0)	9.7 (1.9)	13.1 (6.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	36.4^a (8.4)
		VfN	1 2	408.4 (65.1)	276.2 (47.0)	156.2 (99.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	840. 8^{bc} (183.8)
		VsN	1 2	209.4 (30.7)	187.2 (22.1)	541.9 (93.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	938. 5^c (118.4)
	/	Emerging	3 1	20.3 (2.2)	2.73 (0.7)	0.83 (0.3)	0.07 (0.02)	0.3 (0.2)	0.99 (0.3)	0.13 (0.04)	0 (0)	25.4^a (3.0)
	Honeydew	AfH	1 0	32.5 (8.1)	12.1 (3.8)	1.6 (0.5)	11.8 (4.6)	0.6 (0.3)	1.8 (0.9)	3.4 (1.7)	0 (0)	63.9^c d (15.9)
		ApH	1 9	28.7 (3.1)	10.6 (2.2)	3.3 (0.7)	0.1 (0.02)	1.7 (0.6)	1.7 (0.4)	0.1 (0.03)	0 (0)	46.1^b c (5.9)
SaH		2 1	19.2 (2.1)	4.6 (1.6)	1.7 (0.8)	0.1 (0.02)	1.1 (0.5)	0.7 (0.2)	0.1 (0.04)	0 (0)	27.5^a b (4.3)	
Nectar	VfN	2 0	66.0 (7.1)	35.9 (5.2)	4.6 (1.3)	0.1 (0.03)	0.1 (0.05)	1.3 (0.2)	0.3 (0.07)	0 (0)	103. 3^d (13.0)	
	VsN	2 0	75.5 (3.8)	46.3 (2.8)	61.4 (8.9)	1.1 (0.1)	2.6 (0.4)	7.6 (0.7)	0.3 (0.1)	0 (0)	194. 9^e (13.5)	

793

794

795 *Table 3*

<i>Actual</i> \ <i>Predicted</i>	Honeydew	Nectar	Unfed
Honeydew	36 (60%)	3 (5%)	21 (35%)
Nectar	3 (5%)	50 (83%)	7 (12%)
Unfed	23 (37%)	7 (11%)	33 (52%)

796

797

798 **Highlights**

799

- 800 - Parasitoids were not sugar-limited in nectar-depleted triticale crops
801 - Parasitoids fed mainly on honeydew in single crops and nectar-providing intercrops
802 - Biological control was not enhanced in nectar-providing intercrops

803

804 **Martin Luquet:** Conceptualization, Methodology, Investigation, Formal analysis, Visualization,
805 Writing - Original Draft. **Ainara Peñalver-Cruz:** Conceptualization, Methodology, Investigation,
806 Writing - Review & Editing. **Pascale Satour:** Methodology, Investigation, Resources, Writing - Review

807 & Editing. **Sylvia Anton**: Conceptualization, Writing - Review & Editing. **Anne-Marie Cortesero**:
 808 Conceptualization, Writing - Review & Editing. **Blas Lavandero**: Conceptualization, Writing - Review &
 809 Editing. **Bruno Jaloux**: Conceptualization, Methodology, Investigation, Writing - Review & Editing,
 810 Project administration, Funding acquisition.

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812

813 Appendix A – Field geographic coordinates

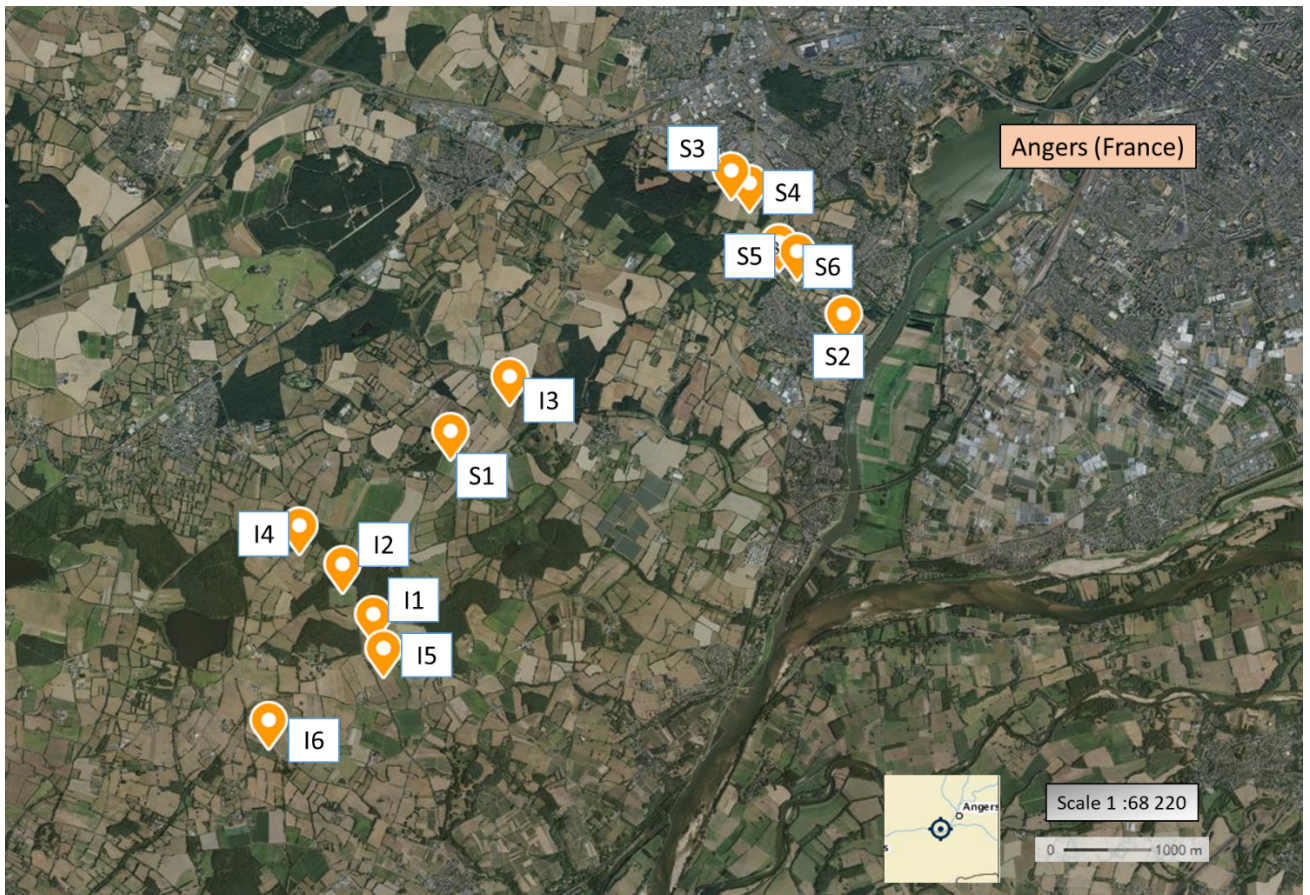
814

815 Table A.4 GPS coordinates, surface (ha) of the surveyed fields. “Faba bean” column indicates how faba bean was sown:
 816 broadcast seeding ('BS') or in rows.

Crop ID	Crop Type	Farmer ID	Municipality	Latitude	Longitude	Surface	Faba bean
S1	Single crop	A	Savennières	47.430616	-0.665451	7.35	/
S2	Single crop	A	Bouchemaine	47.441029	-0.605287	2.7	/
S3	Single crop	A	Beaucouzé	47.454512	-0.622562	8	/
S4	Single crop	A	Beaucouzé	47.453875	-0.619794	4	/
S5	Single crop	A	Bouchemaine	47.447716	-0.614654	0.5	/
S6	Single crop	A	Bouchemaine	47.44706	-0.612551	1	/
I1	Intercrop	C	Savennières	47.41185	-0.675935	9.3	BS
I2	Intercrop	C	Savennières	47.417542	-0.681085	5.6	BS
I3	Intercrop	B	Bouchemaine	47.437169	-0.654821	6.2	Rows
I4	Intercrop	C	Savennières	47.422769	-0.688466	5.5	BS
I5	Intercrop	C	Savennières	47.407436	-0.674819	6.2	BS
I6	Intercrop	C	La Possonnière	47.400988	-0.693273	1.95	BS

817

818



819

820 Figure A.5 Location of the different fields. From Geoportail (<https://www.geoportail.gouv.fr>). Last access on 04/11/2020.

821

822 Appendix B – Sugar metabolic dynamics

823

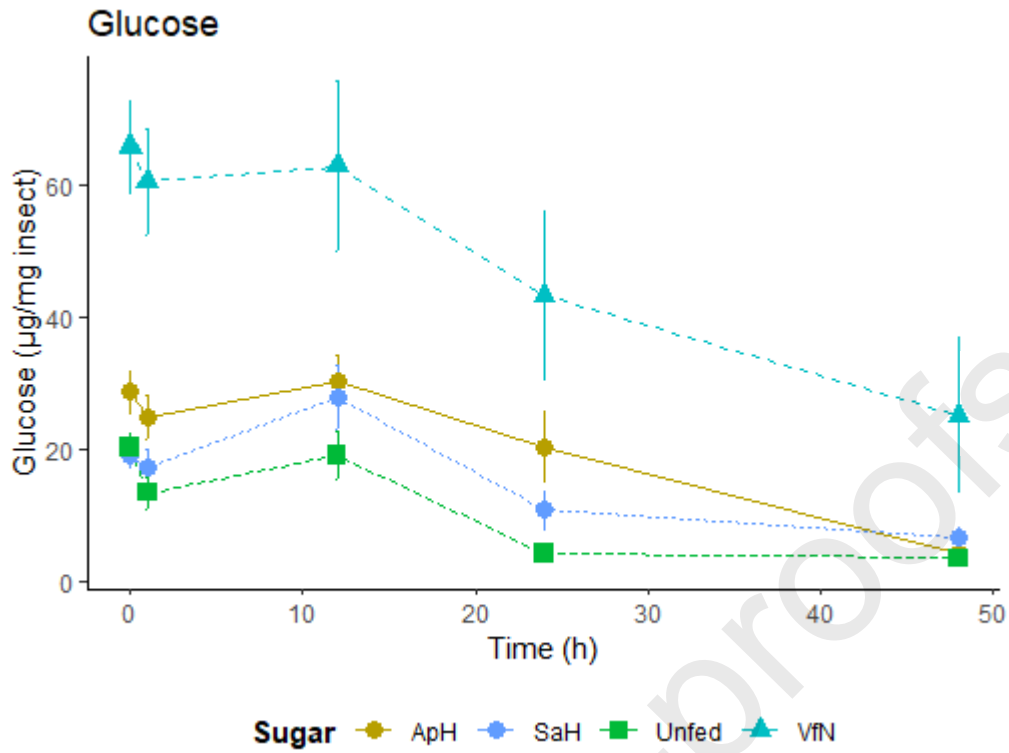
824 These figures describe the dynamics of the measured sugars in the different treatments, as
 825 well as ratios between these sugars. The treatments 'VsN' (fed on *Vicia sativa* nectar) and 'AfH' (fed
 826 on *Aphis fabae* nectar) are not included as we did not have the dynamics for these treatments (values
 827 for these treatments at 0h after feeding can be found in Table 1 and Figure 2 in the main manuscript).

828 Note that the colours are not the same as in Figure 2 in the main manuscript: here red circles
 829 are for individuals fed with *Acyrtosiphon pisum* honeydew (ApH), green triangles are for individuals
 830 fed with *Sitobion avenae* honeydew (SaH), blue squares are for unfed individuals and purple crosses
 831 are for individuals fed with *Vicia faba extrafloral* nectar (VfN).

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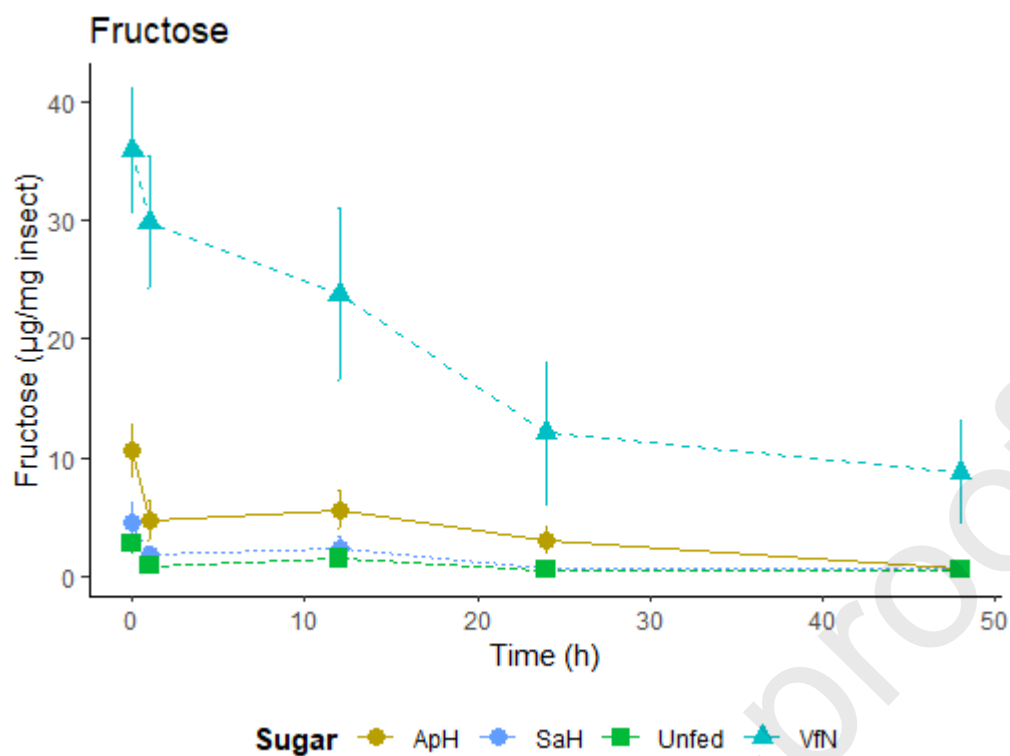
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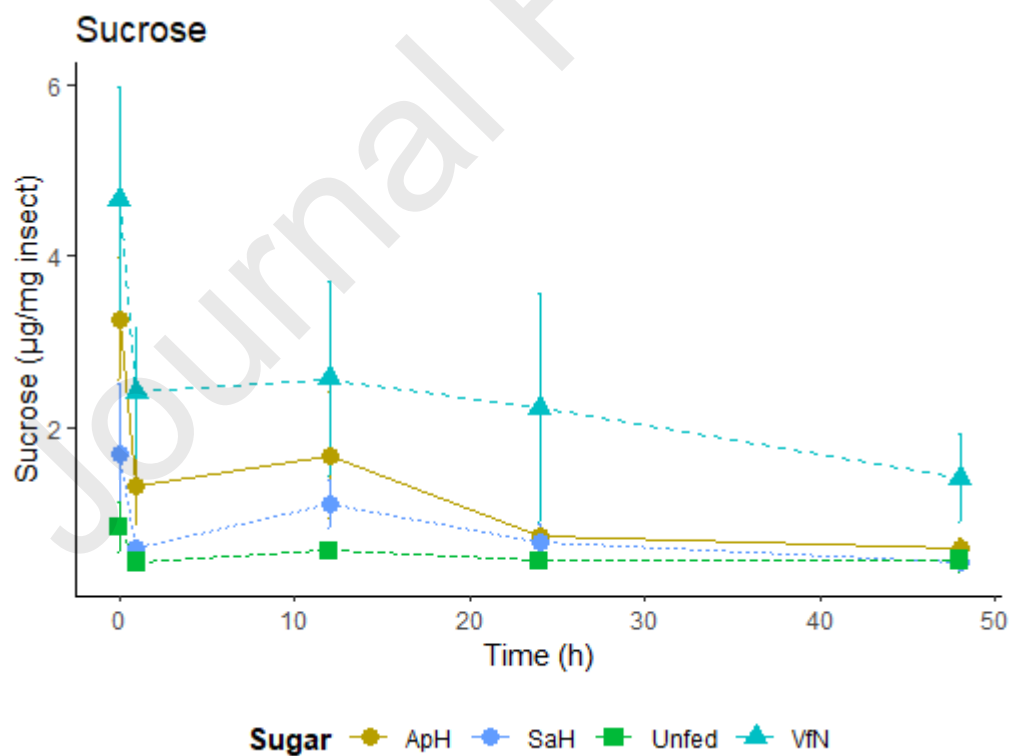
836 *Figure B.1 Glucose sugar metabolic dynamics of Aphis ervi females after receiving a feeding treatment (mean \pm SE at*
 837 *different times after feeding). ApH = Acyrthosiphon pisum honeydew, SaH = Sitobion avenae honeydew, VfN = Vicia faba*
 838 *extrafloral nectar.*

839

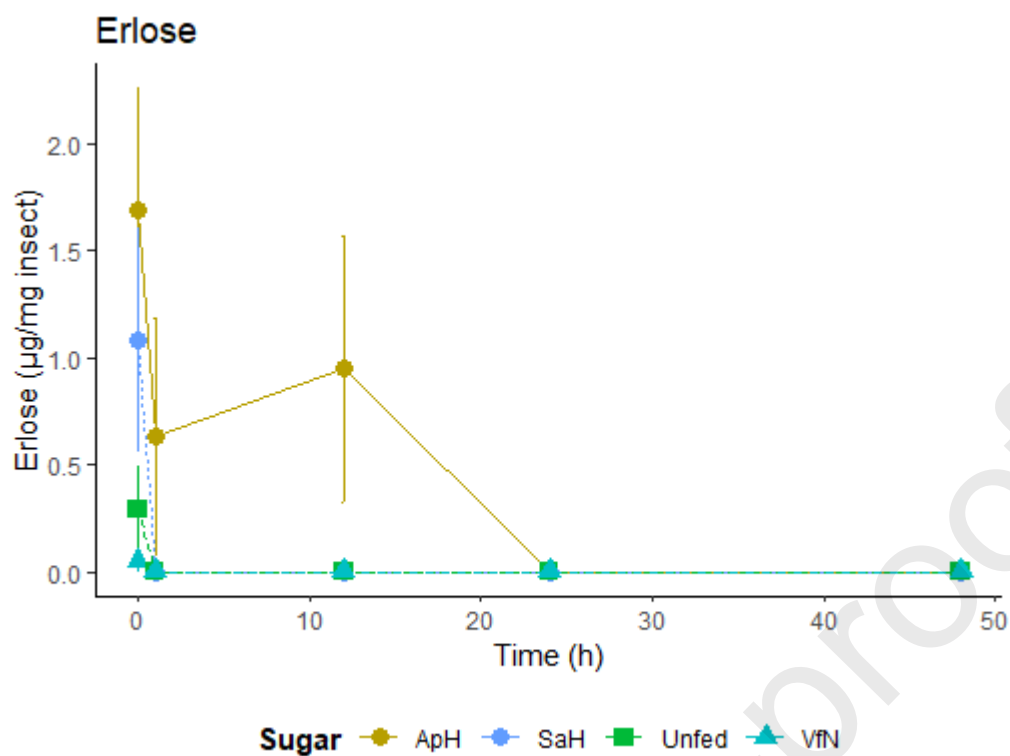


840
 841 *Figure B.2 Fructose sugar metabolic dynamics of *Aphidius ervi* females after receiving a feeding treatment (mean \pm SE at*
 842 *different times after feeding). ApH = *Acyrtosiphon pisum* honeydew, SaH = *Sitobion avenae* honeydew, VfN = *Vicia faba**
 843 *extrafloral nectar.*

844

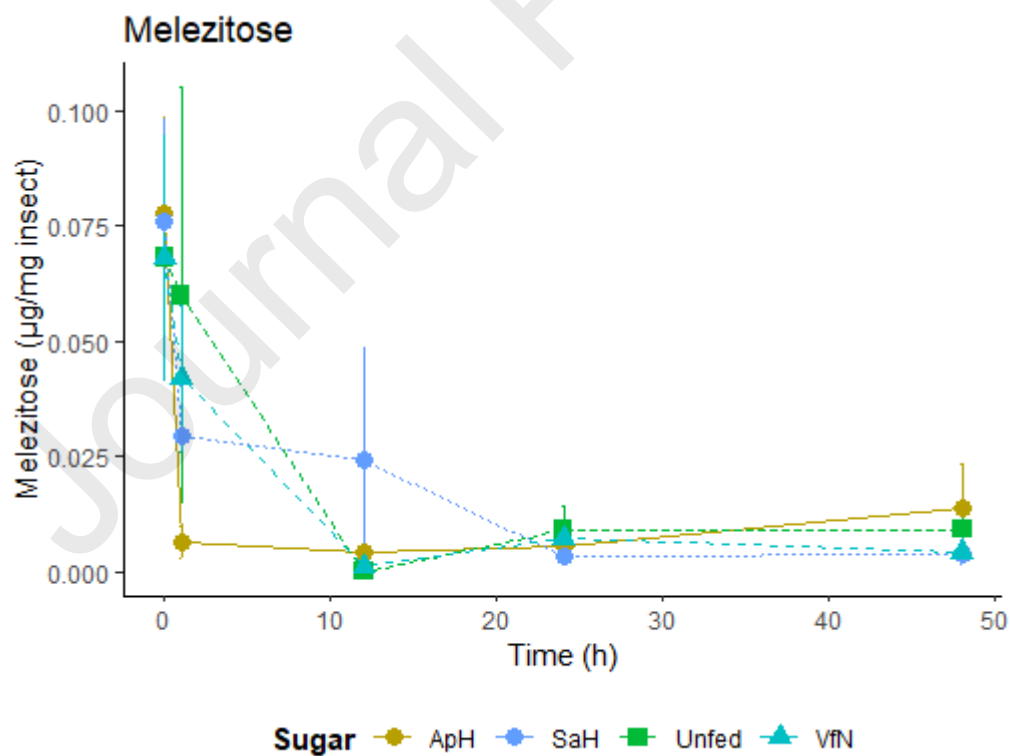


845
 846 *Figure B.3 Sucrose sugar metabolic dynamics of *Aphidius ervi* females after receiving a feeding treatment (mean \pm SE at*
 847 *different times after feeding). ApH = *Acyrtosiphon pisum* honeydew, SaH = *Sitobion avenae* honeydew, VfN = *Vicia faba**
 848 *extrafloral nectar.*

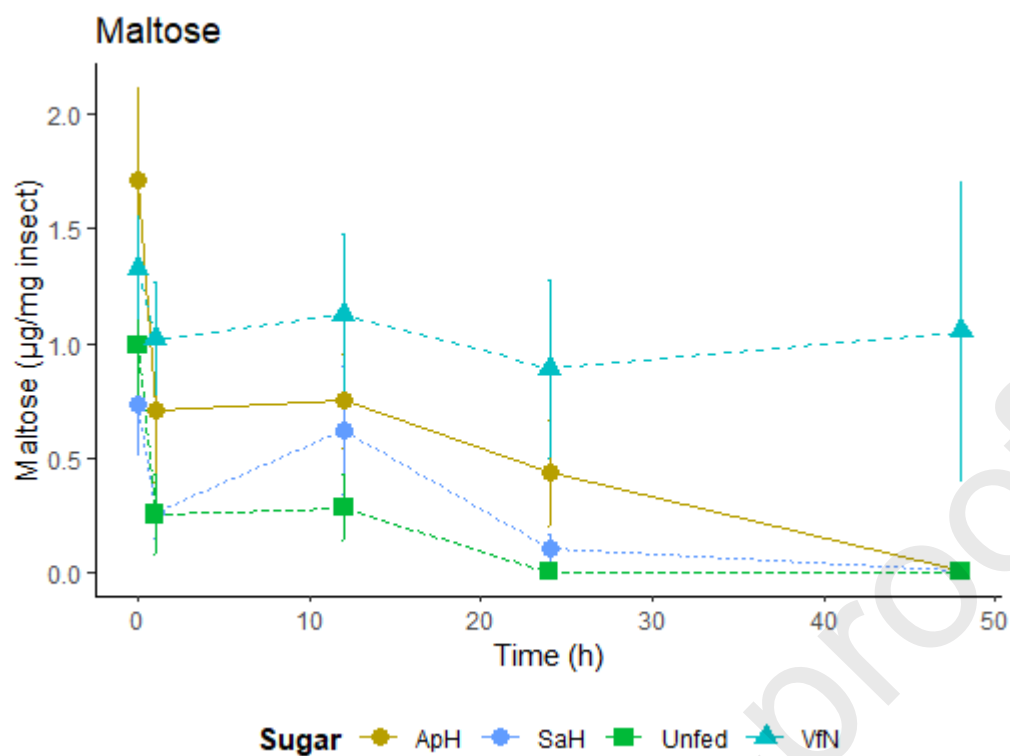


849
 850 *Figure B.4 Erlöse sugar metabolic dynamics of *Aphidius ervi* females after receiving a feeding treatment (mean \pm SE at*
 851 *different times after feeding). ApH = *Acyrtosiphon pisum* honeydew, SaH = *Sitobion avenae* honeydew, VfN = *Vicia faba**
 852 *extrafloral nectar.*

853

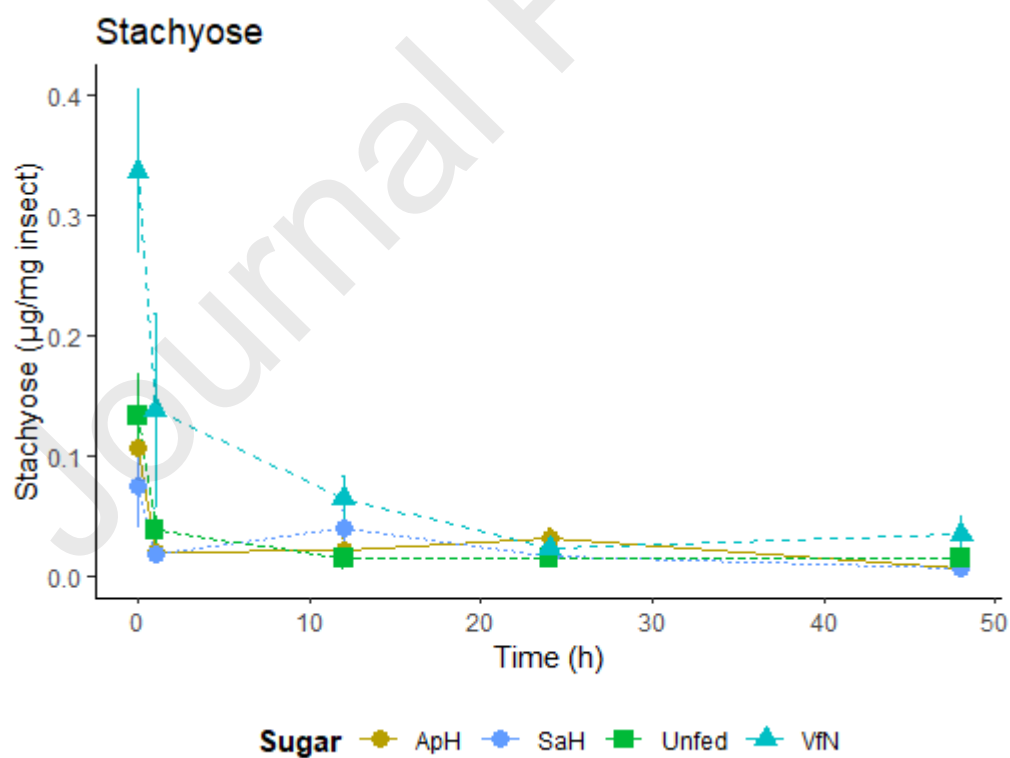


854
 855 *Figure B.5 Melezitose sugar metabolic dynamics of *Aphidius ervi* females after receiving a feeding treatment (mean \pm SE at*
 856 *different times after feeding). ApH = *Acyrtosiphon pisum* honeydew, SaH = *Sitobion avenae* honeydew, VfN = *Vicia faba**
 857 *extrafloral nectar.*

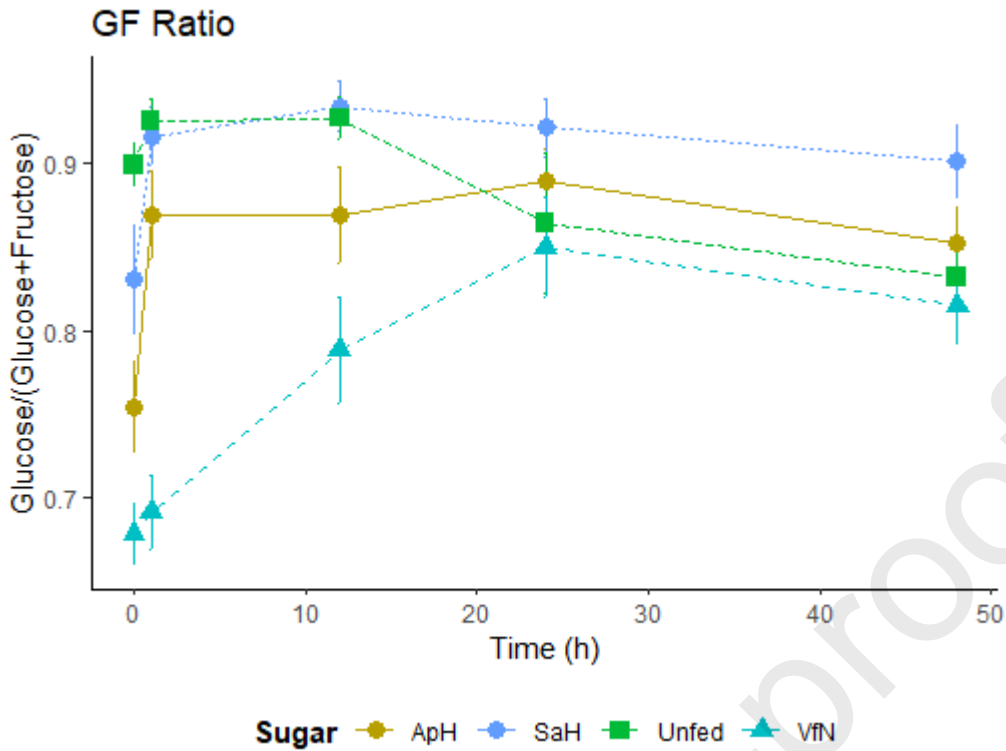


858
 859 *Figure B.6 Maltose sugar metabolic dynamics of *Aphidius ervi* females after receiving a feeding treatment (mean \pm SE at*
 860 *different times after feeding). ApH = *Acyrtosiphon pisum* honeydew, SaH = *Sitobion avenae* honeydew, VfN = *Vicia faba**
 861 *extrafloral nectar.*

862



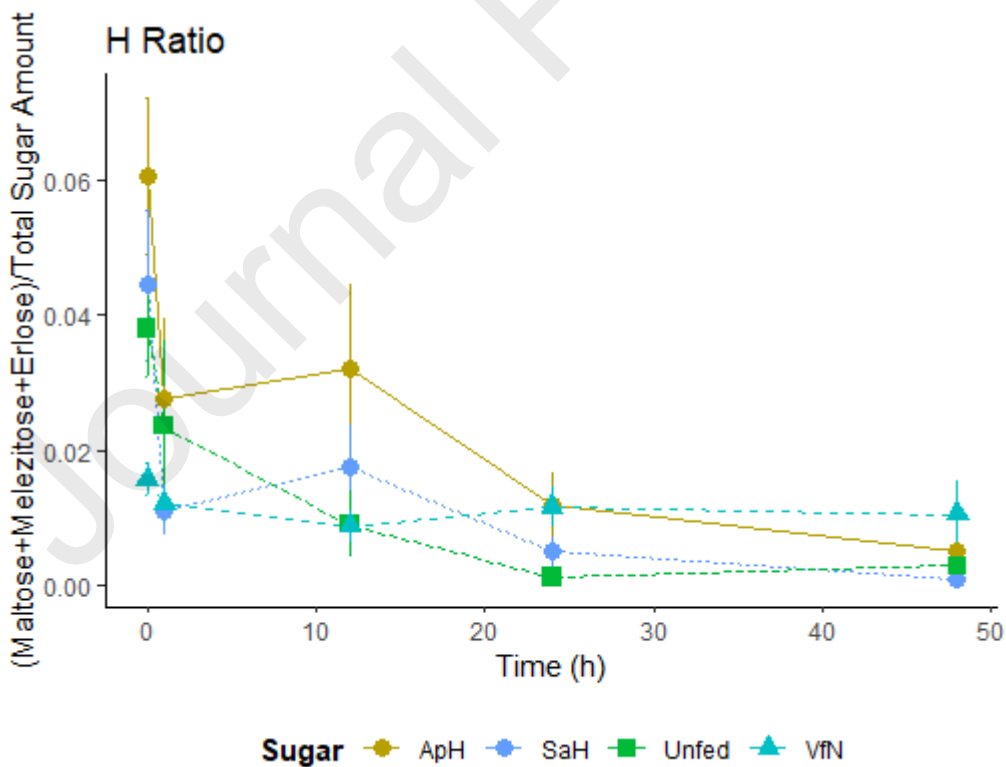
863
 864 *Figure B.7 Stachyose sugar metabolic dynamics of *Aphidius ervi* females after receiving a feeding treatment (mean \pm SE at*
 865 *different times after feeding). ApH = *Acyrtosiphon pisum* honeydew, SaH = *Sitobion avenae* honeydew, VfN = *Vicia faba**
 866 *extrafloral nectar.*



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Figure B.8 Dynamics of the GF Ratio (Glucose/Glucose+Fructose) of *Aphidius ervi* females after receiving a feeding treatment (mean \pm SE at different times after feeding). ApH = *Acyrtosiphon pisum* honeydew, SaH = *Sitobion avenae* honeydew, VfN = *Vicia faba* extrafloral nectar.

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Figure B.9 Dynamics of the GF Ratio (Maltose+Erlose+Melezitose/Total Sugar Amount) of *Aphidius ervi* females after receiving a feeding treatment (mean \pm SE at different times after feeding). ApH = *Acyrtosiphon pisum* honeydew, SaH = *Sitobion avenae* honeydew, VfN = *Vicia faba* extrafloral nectar.

876

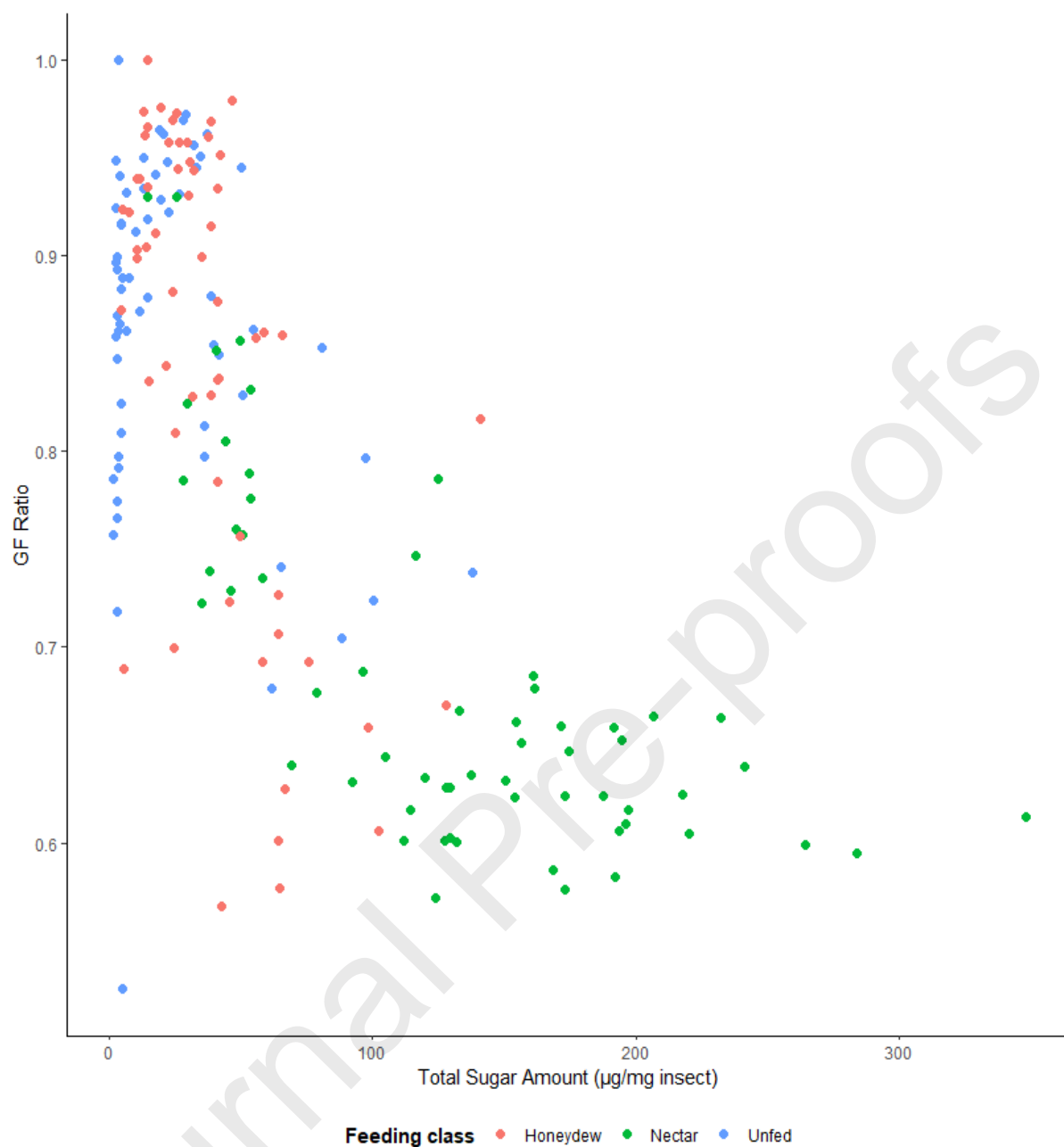
877 Appendix C – Lab data visualisation

878

879 Figure C.1 represents the GF Ratio of parasitoid females according to their total sugar amount
880 for each **feeding class** (reminder: feeding class \neq feeding treatment: insects that fed more than 12h
881 ago are in the « unfed » feeding class), complementary to Figure 3 in the main manuscript and as is
882 classically done in parasitoid nutritional ecology experiments (Steppuhn & Wäckers 2004, Hogervorst
883 et al. 2007a, Tena et al. 2013). Overall, the same pattern can be seen as in Figure 3. Note that Total
884 Sugar Amount was not chosen as a predictive variable to avoid information redundancy (all sugars
885 were already included as predictive variables when training the classifier).

886 Figures C.2 to C.5 show two-dimensional representations of the lab data, plotted against some
887 of the variables used for prediction and that were selected as most informative by the Random Forest
888 classifier. This time, colours represent the **feeding treatment** (*i. e.* what was actually eaten by insects,
889 even if more than 12h ago) and **not the feeding class**. Shapes represent time spent starving after
890 feeding (fed individuals) or after emergence (unfed individuals). Different patterns of variation can be
891 observed between and within treatments. It can notably be seen that insects from the “unfed” feeding
892 class with high sugar profiles are actually individuals that fed from nectar more than 12h ago (e.g.
893 comparing figure C.1 and C.2): however, setting a different detection time would have been no better
894 as some insects that fed from nectar more than 12h ago also have low profiles.

895

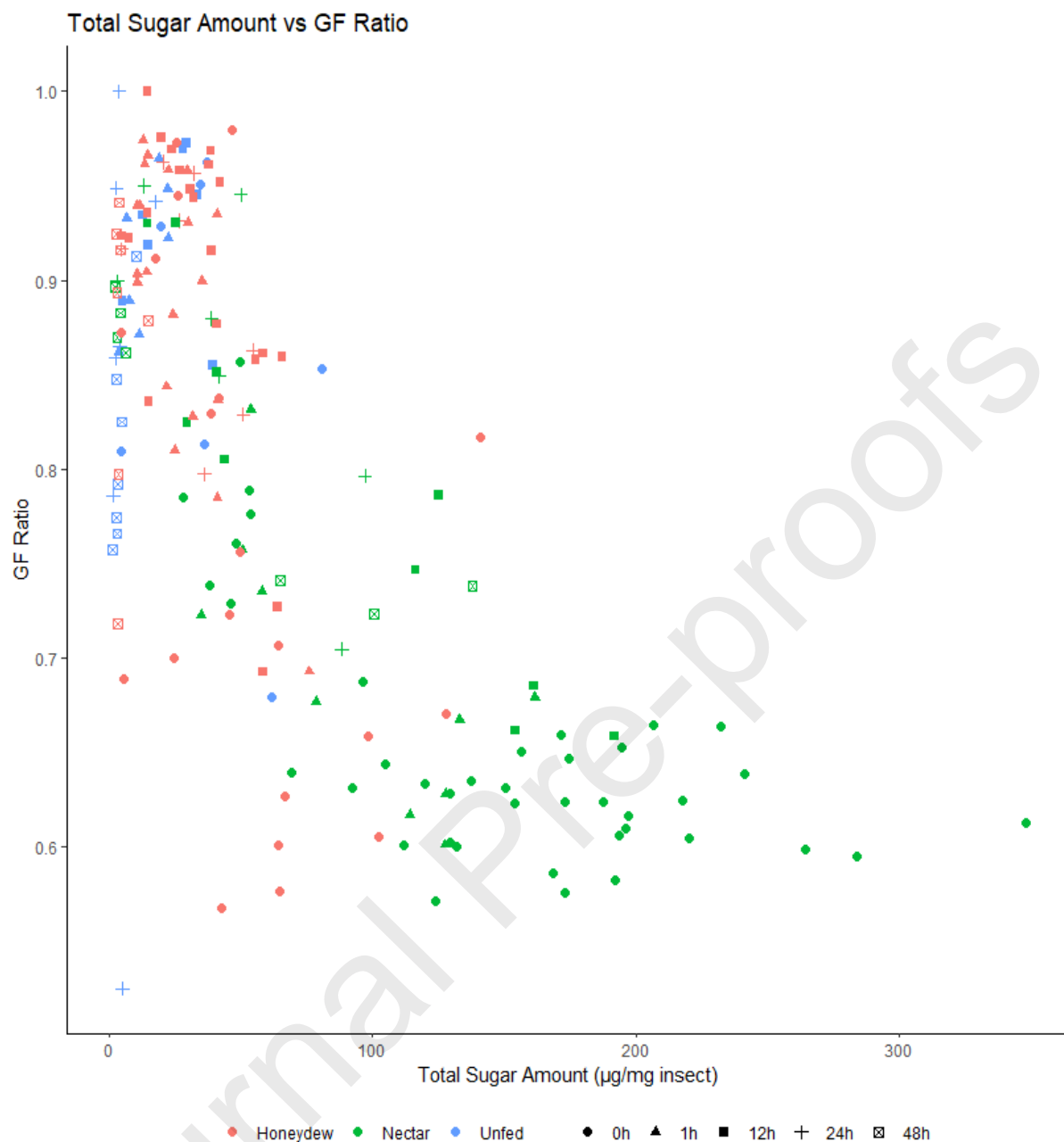


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897

898 *Figure C.1 Two-dimensional representation of the lab dataset, according to the total sugar amount of *Aphidius ervi* females*
899 *($\mu\text{g}/\text{mg}$ insect) vs the Glucose/(Glucose + Fructose) Ratio (GF Ratio). Colours refer to the different feeding classes. These classes*
900 *were assigned to insects after setting a 12h 'detection time': insects that have last fed for more than 12 hours are labelled as*
901 *'unfed'.*

902



903

904 *Figure C.2 Two-dimensional representation of the lab dataset, according to the total sugar amount of *Aphidius ervi* females*
 905 *($\mu\text{g}/\text{mg}$ insect) vs the Glucose/(Glucose + Fructose) Ratio (GF Ratio). Colours refer to the different feeding treatments. Shapes*
 906 *refer to the time spent starving after feeding (fed individuals) or after emergence (unfed individuals).*

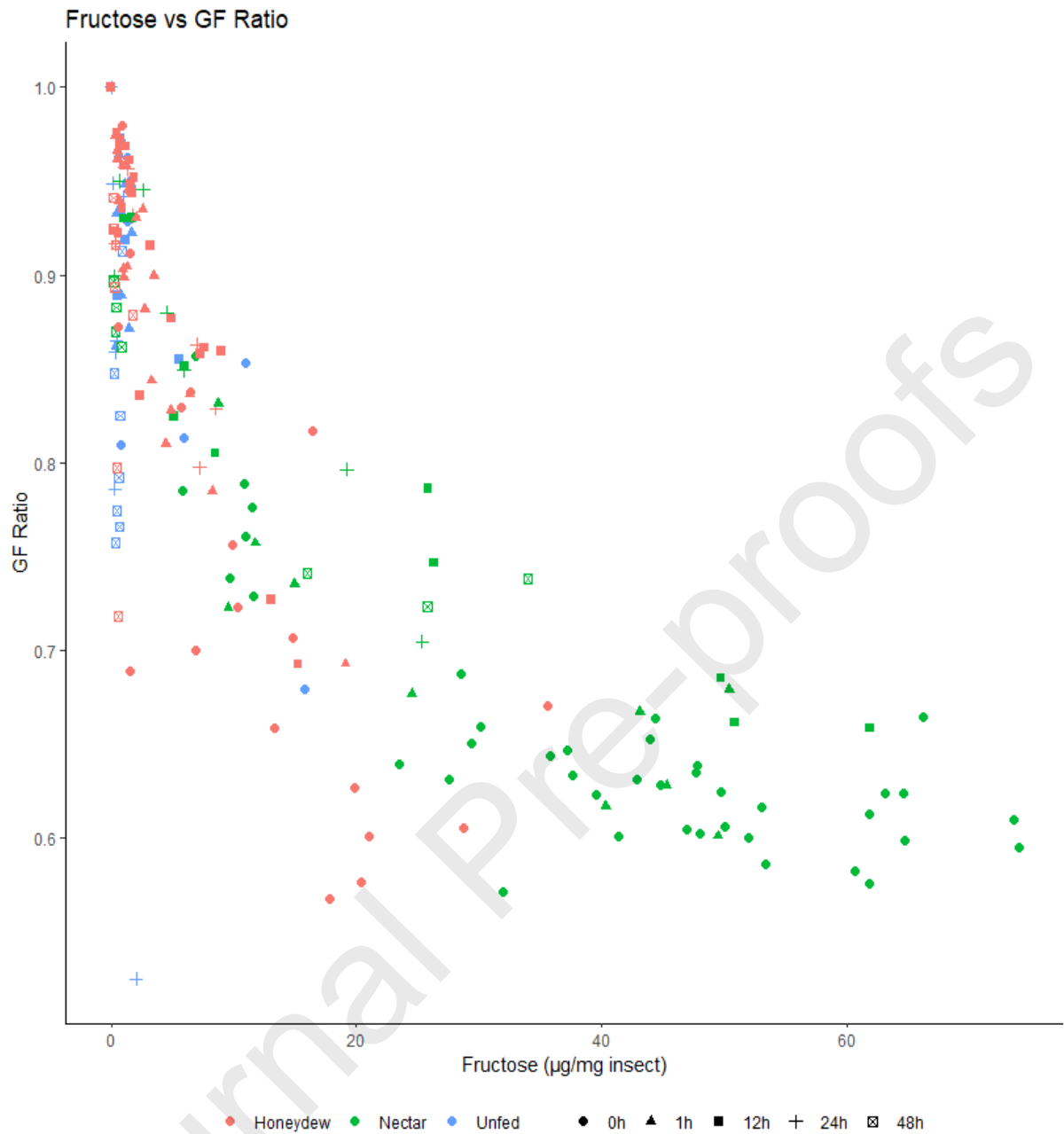
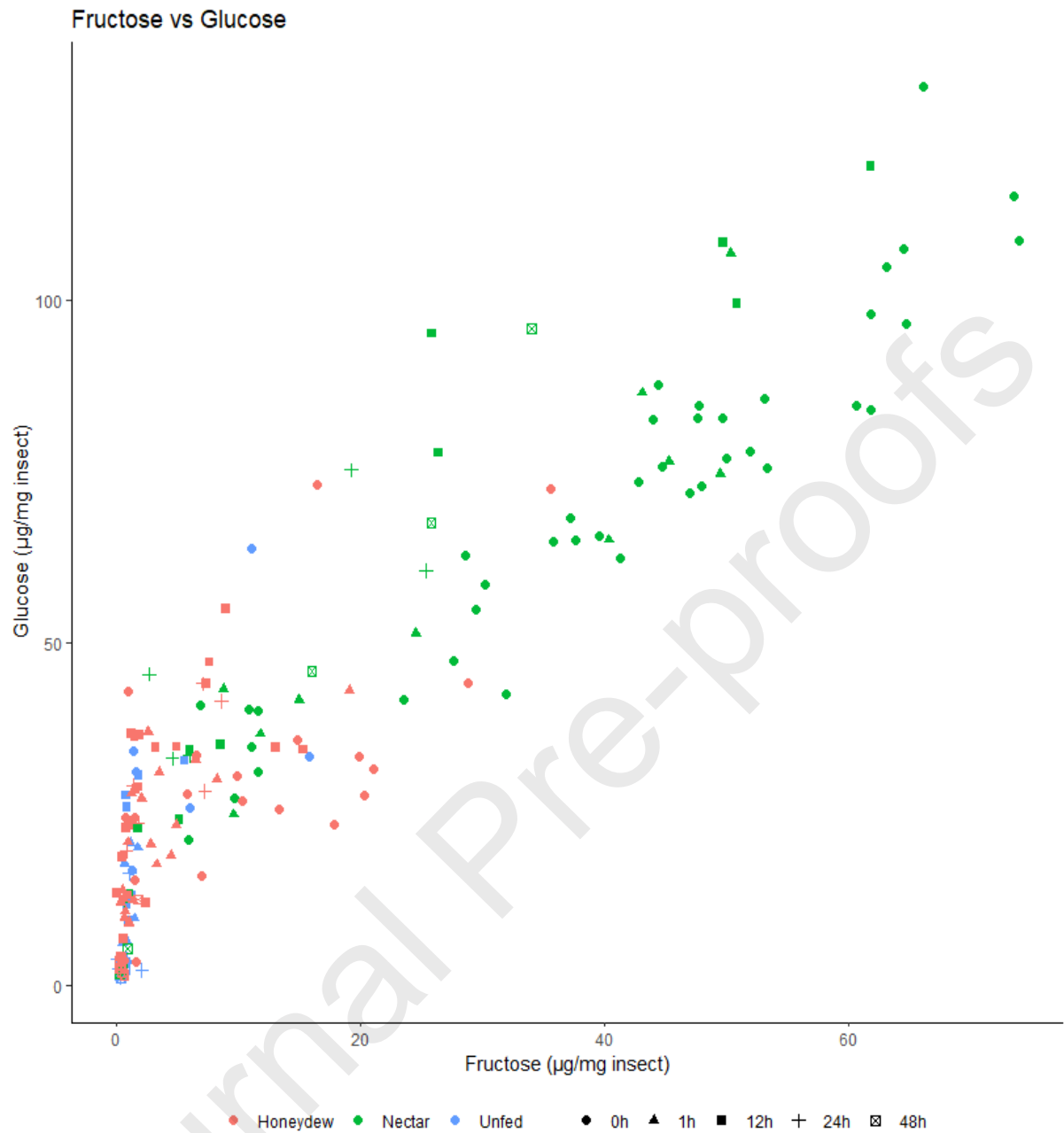


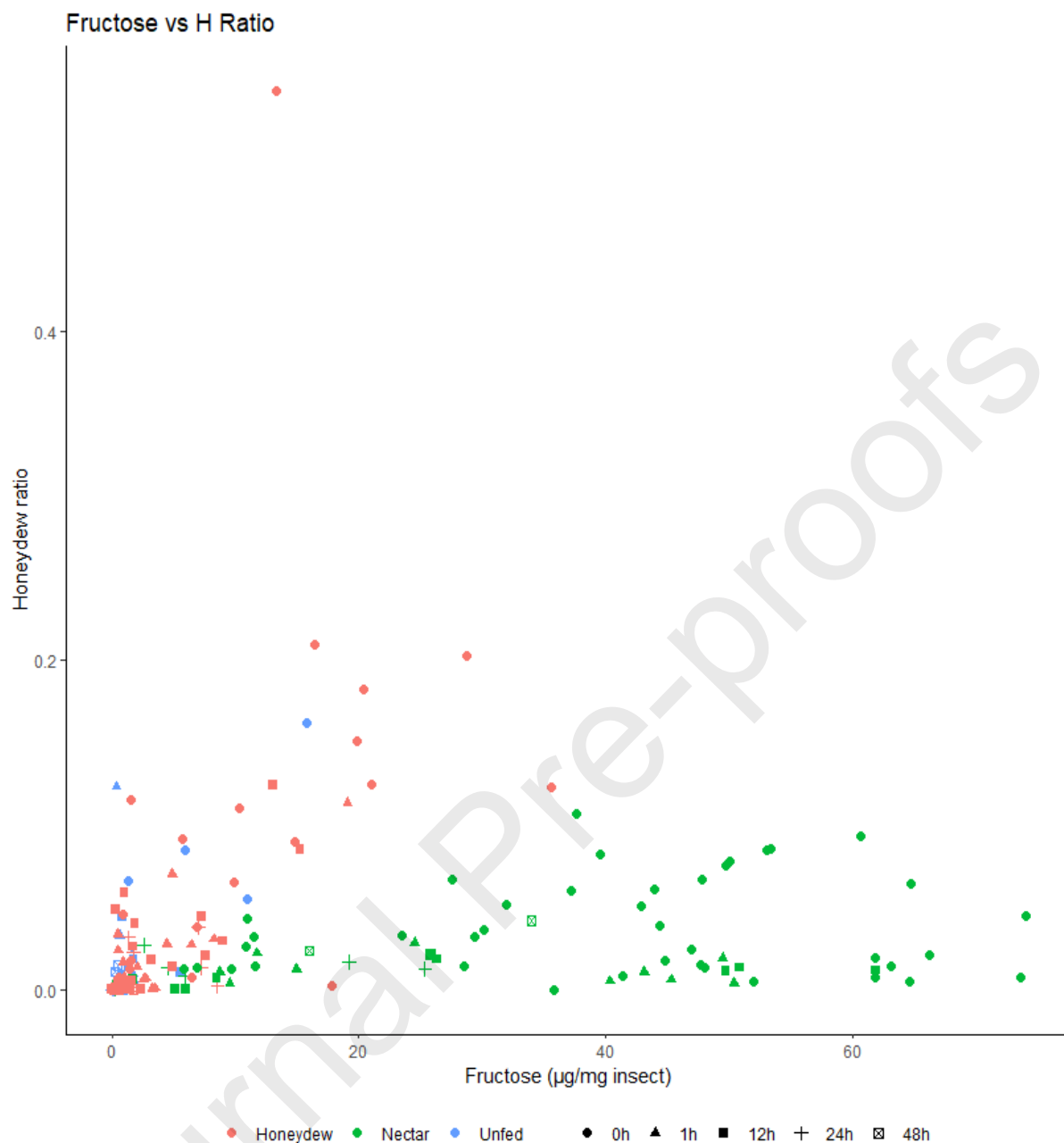
Figure C.3 Two-dimensional representation of the lab dataset, according to the Fructose content of *Aphidius ervi* females ($\mu\text{g}/\text{mg}$ insect) vs the Glucose/(Glucose + Fructose) Ratio (GF Ratio). Colours refer to the different feeding treatments. Shapes refer to the time spent starving after feeding (fed individuals) or after emergence (unfed individuals).



912

913 *Figure C.4 Two-dimensional representation of the lab dataset, according to the Fructose content of *Aphidius ervi* females*
 914 *($\mu\text{g}/\text{mg}$ insect) vs their Glucose content. Colours refer to the different feeding treatments. Shapes refer to the time spent*
 915 *starving after feeding (fed individuals) or after emergence (unfed individuals).*

916



917

918 *Figure C.5 Two-dimensional representation of the lab dataset, according to the Fructose content of *Aphidius ervi* females*
 919 *($\mu\text{g}/\text{mg}$ insect) vs the (Maltose + Erllose + Melezitose)/Total sugar amount Ratio (H Ratio). Colours refer to the different feeding*
 920 *treatments. Shapes refer to the time spent starving after feeding (fed individuals) or after emergence (unfed individuals).*

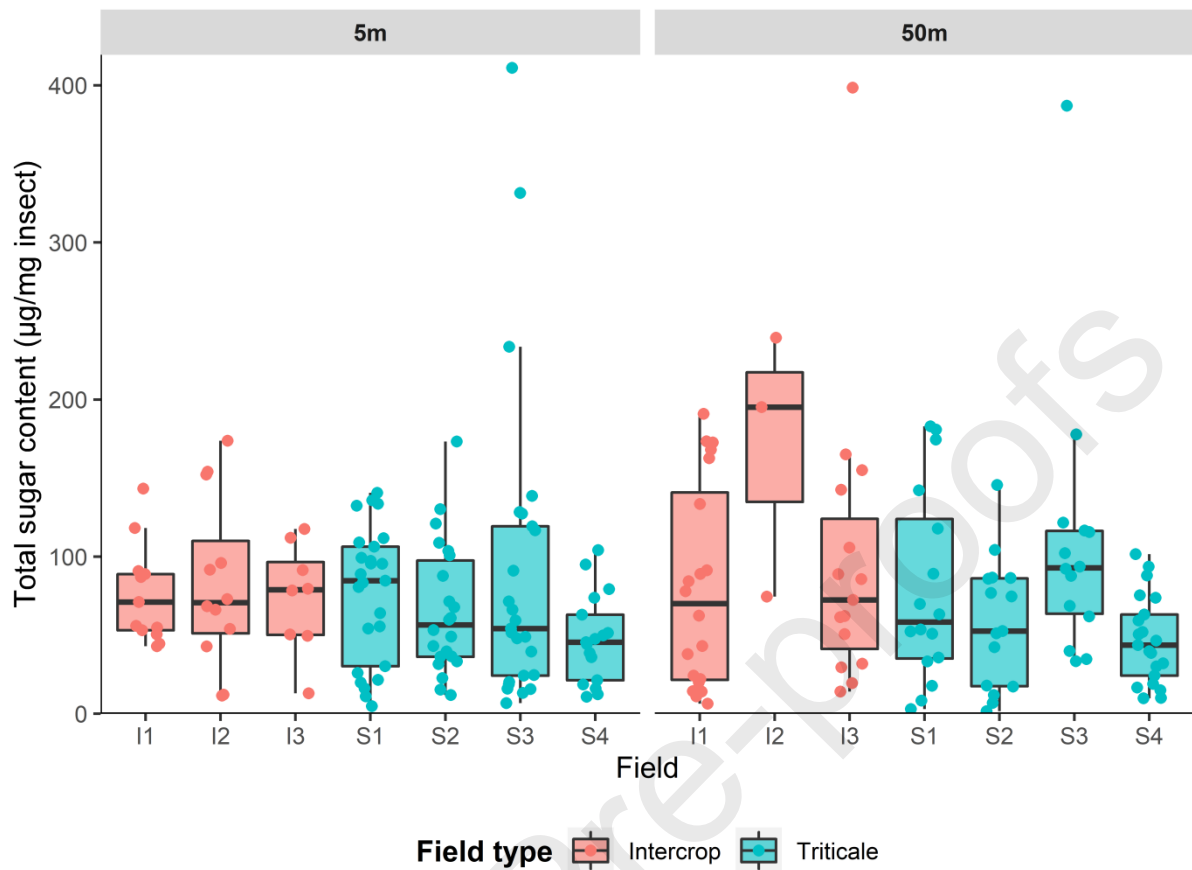
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923 [Appendix D – Additional field data](#)

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925 Sugar levels of field-caught insects



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927 *Figure D.1 Total sugar content (µg/mg insect) of Aphidius spp. females captured in each field, at 5m and 50m from the border.*
 928 *S1, S2, S3, S4 correspond to single crop triticales fields and I1, I2, I3 correspond to faba bean-triticales intercrop fields. Colours*
 929 *represent field type (see legend). Boxplot labels represent the different fields investigated.*

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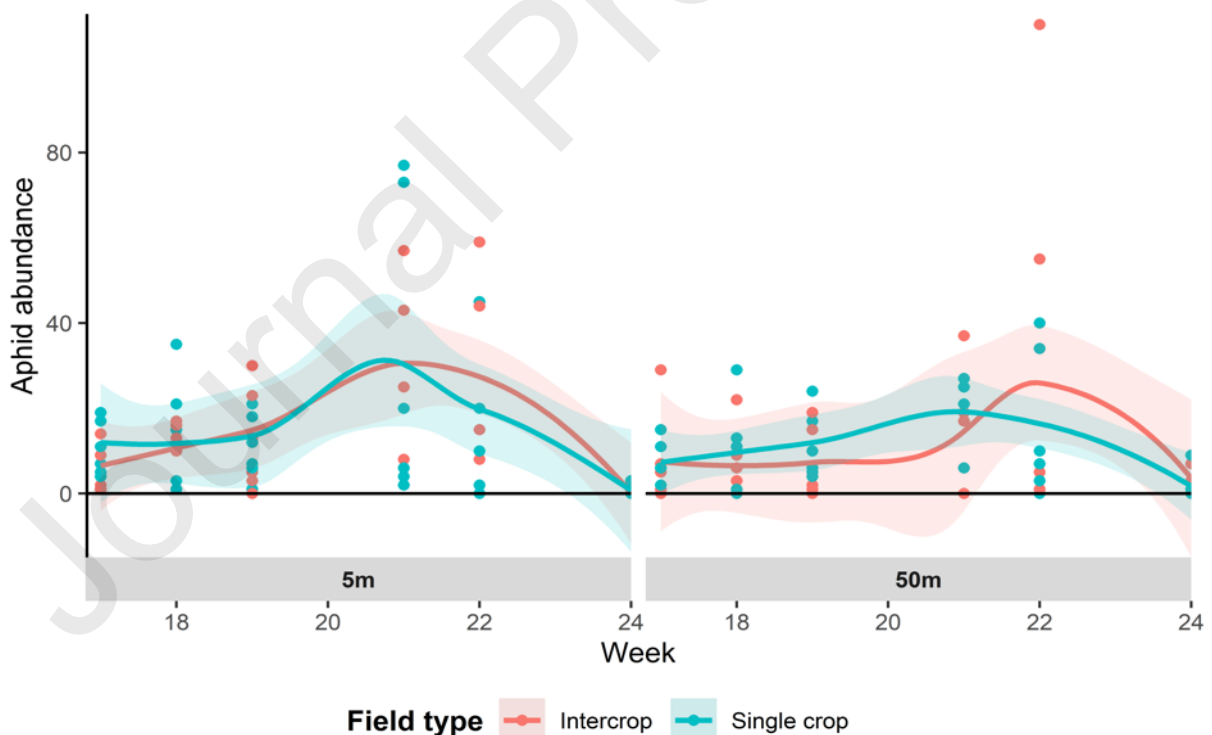
932 Field surveys: raw data

933

934 *Table D.5 Total number of aphids from each species counted during the season, on triticale (Sitobion avenae, Metopolophium*
 935 *dirhodum and Rhopalosiphum padi) and on faba bean (Acyrtosiphon pisum, Aphis spp.), in 6 triticale-faba bean intercrops*
 936 *and triticale single crops and at 5m or 50m from the field edge (total summed values for all fields and distances from the edge*
 937 *are also reported). Aphis spp. consisted mainly of Aphis fabae but may also include Aphis craccivora individuals. On triticale,*
 938 *aphids were counted at seven dates on 50 randomly chosen tillers. On faba bean, aphids were counted at seven dates on 50*
 939 *randomly chosen plants. For Aphis spp., aphids were individually counted up to 50 individuals. When more than 50 individuals*
 940 *were present, the length of the hemp occupied by 50 aphids (cm) was used to estimate the abundance of the colony.*

Culture type	Distance from the field edge	<i>Sitobion avenae</i>	<i>Metopolophium dirhodum</i>	<i>Rhopalosiphum padi</i>	<i>Acyrtosiphon pisum</i>	<i>Aphis spp.</i>
Intercrop	5m	433	40	7	77	31269
	50m	358	1	30	77	8462
Single crop	5m	446	45	4	0	0
	50m	379	12	3	0	0
Total		1616	98	44	154	39731

941

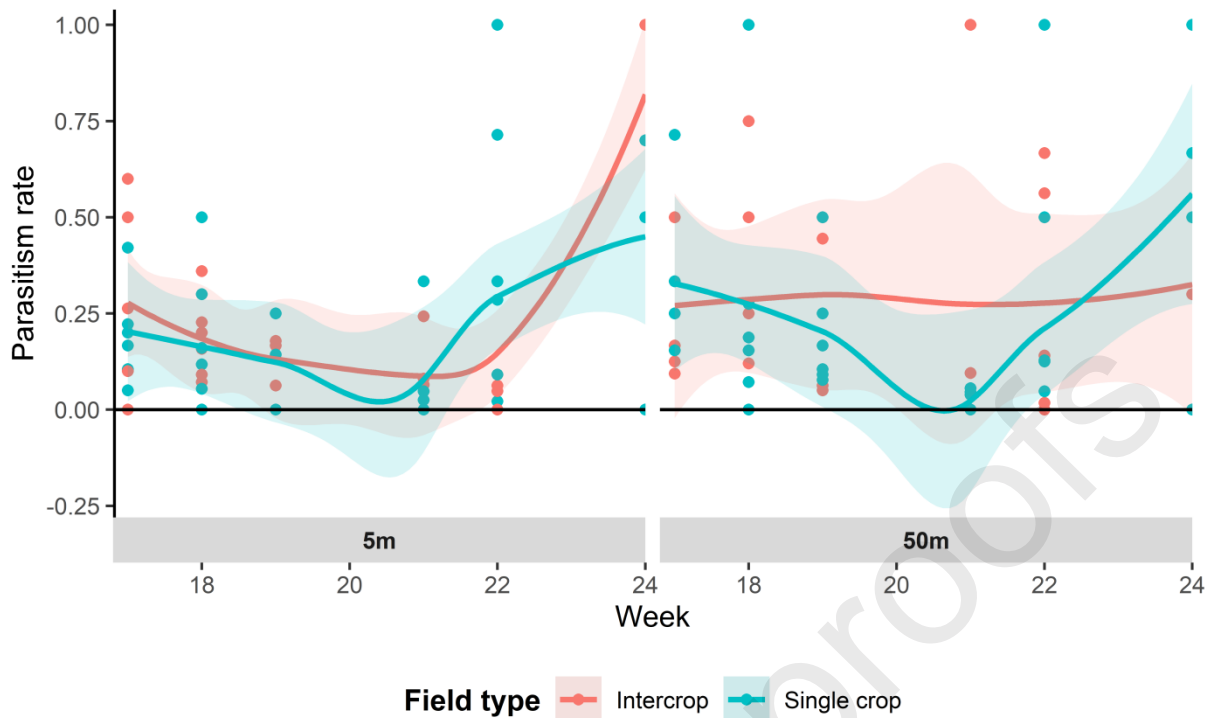


942

943 *Figure D.2 Aphid abundance estimated at different sampling dates in pure crops (blue) and intercrops (red). The curves*
 944 *correspond to LOESS smoothers ($\pm 1C95$), fitted to better visualise the general trend of each aphid population dynamics.*

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Figure D.3 Parasitism rate by *Aphidius* spp. parasitoids estimated at different sampling dates in pure crops (blue) and intercrops (red). The curves correspond to LOESS smoothers (\pm IC95), fitted to better visualise the general trend of each aphid population dynamics. Parasitism rate was computed as the number of mummies counted on 50 triticale tillers, divided by the sum of mummies and aphids found on the same tillers.