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

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## ABSTRACT

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

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

## 1. INTRODUCTION

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

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

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

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

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

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

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

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

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

## 2. MATERIAL AND METHODS

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

### 2.1 Lab experiment – Reference sugar profiles

#### 2.1.1 Biological material

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

### 2.1.2 Sugar food collection

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

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

### 2.1.3 Feeding experiment

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

### 2.1.4 HPLC Analysis of sugar profiles

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



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

## 2.2 Field experiment

### 2.2.1 Study sites

The study sites were located in the Maine-et-Loire department (West of France), in the municipalities of Beaucouzé, Bouchemaine, Savennières and La Possonnière. Six pure triticale fields and six triticale-faba bean intercrop fields from three farmers were investigated (see Appendix A for coordinates). Triticale was sown in rows in all fields. In intercrops, faba beans were sown using broadcast seeding, without a particular spatial arrangement, except for one field where they were sown in rows (Table A.1). Both plants were sown so that they occupied the same surface, although much variability was observed during the season and across fields (triticale:  $33 \pm 20$  % surface,  $65 \pm 53$  tillers/m<sup>2</sup> ; faba bean:  $29 \pm 19$  % surface,  $14 \pm 9$  plants/m<sup>2</sup>). All fields were organically managed and received no pesticide or biopesticide treatment. They were all bordered by woody field margins containing abundant spontaneous vegetation (grasses, flowers), including nectar-producing flowers (plants from the Apiaceae family were predominant). Experiments were carried out between weeks 17 (24<sup>th</sup> April) and 24 (14<sup>th</sup> June) in 2018. Temperature during the experiments ranged from 21.8 °C to 30.8 °C and humidity ranged from 47% to 81% RH.

### 2.2.2 Insect sampling and surveys

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

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

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

## 2.3 Data analysis

### 2.3.1 Feeding pattern predictions from HPLC data

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

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

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

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

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



### 2.3.2 Comparison between field treatments

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

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

## 3. RESULTS

### 3.1 Lab experiment

#### 3.1.1 Sugar spectrum of food sources

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

#### 3.1.2 Parasitoid sugar profiles

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

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

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

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

### 3.1.3 Model training

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

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

## 3.2 Field experiment

### 3.2.1 Feeding patterns in single crop and intercrop fields

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

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

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

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

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

## 4. DISCUSSION

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

### 4.1 Sugar profile variability: implications for feeding detection

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

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

#### 4.2 Sugar limitation in single crops

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

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

#### 4.3 Parasitoid feeding patterns in intercrops vs single crops

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

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

#### 4.4 Implications for biological control

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

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

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

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



## Declaration of interest

The authors declare no competing interest.

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## Data availability statement

All data presented and scripts needed to reproduce analyses can be found on the following repository: <https://github.com/MartinLuquetEcology/Parasitoid-sugar-feeding-and-parasitism-in-intercrops>.

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## Table legends

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

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

## Figure legends

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

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

**Figure 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 classes. These classes were assigned to insects after setting a 12h 'detection time': insects that have last fed for more than 12 hours are labelled as 'unfed'.

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

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

Table 2

	Food source	n	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)	<b>120.4<sup>b</sup></b> (20.8)
		ApH	15	52.4 (14.7)	22.8 (7.8)	23.2 (12.4)	0 (0)	0 (0)	0 (0)	0 (0)	<b>98.4<sup>a</sup></b> <sup>b</sup> (32.9)

<b>B. Parasitoids (<math>\mu\text{g}/\text{mg insect}</math>)</b>	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)	<b>36.4<sup>a</sup></b> (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)	<b>840.8<sup>bc</sup></b> (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)	<b>938.5<sup>c</sup></b> (118.4)
	Honeydew	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)	<b>25.4<sup>a</sup></b> (3.0)
		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)	<b>63.9<sup>c</sup></b> (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)	<b>46.1<sup>b</sup></b> (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)	<b>27.5<sup>a</sup></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)	<b>103.3<sup>d</sup></b> (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)	<b>194.9<sup>e</sup></b> (13.5)

Table 3

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

## Highlights

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

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

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

## Appendix A – Field geographic coordinates

Table A.4 GPS coordinates, surface (ha) of the surveyed fields. “Faba bean” column indicates how faba bean was sown: 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

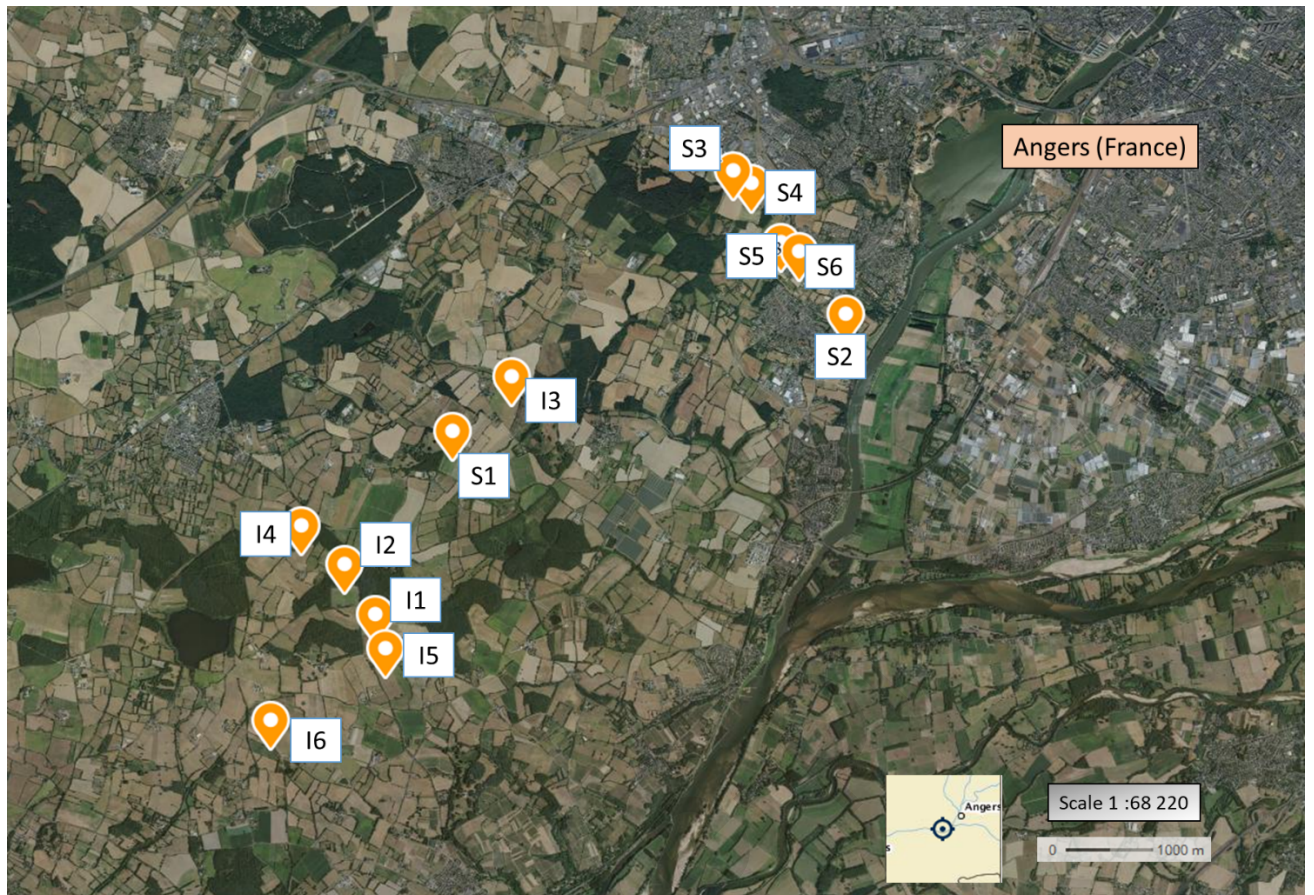


Figure A.5 Location of the different fields. From Geoportail (<https://www.geoportail.gouv.fr>). Last access on 04/11/2020.

## Appendix B – Sugar metabolic dynamics

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

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

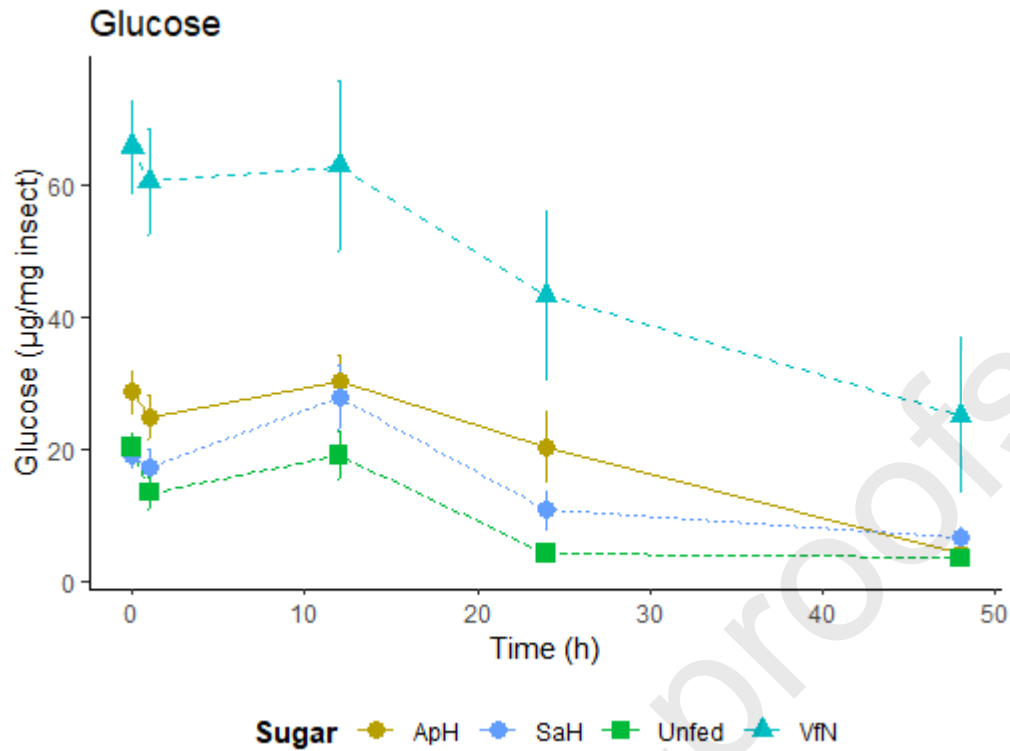


Figure B.1 Glucose sugar metabolic dynamics of *Aphidius ervi* females after receiving a feeding treatment (mean  $\pm$  SE at different times after feeding). ApH = *Acyrtosiphon pisum* honeydew, SaH = *Sitobion avenae* honeydew, VfN = *Vicia faba* extrafloral nectar.



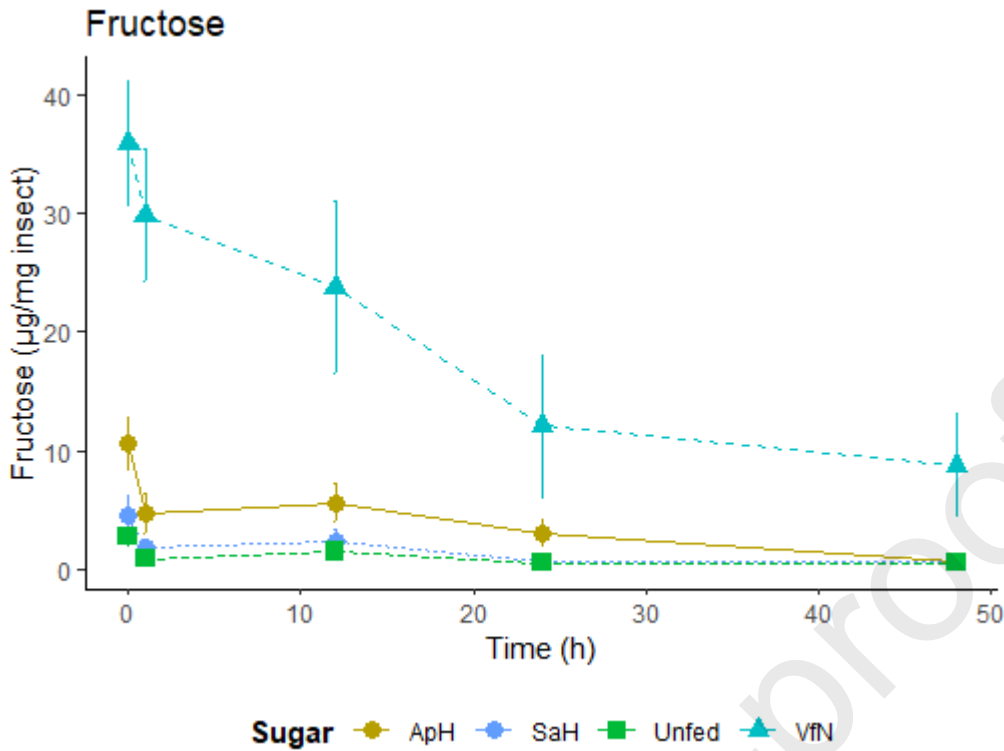


Figure B.2 Fructose sugar metabolic dynamics of *Aphis ervi* females after receiving a feeding treatment (mean  $\pm$  SE at different times after feeding). ApH = *Acyrtosiphon pisum* honeydew, SaH = *Sitobion avenae* honeydew, VfN = *Vicia faba* extrafloral nectar.

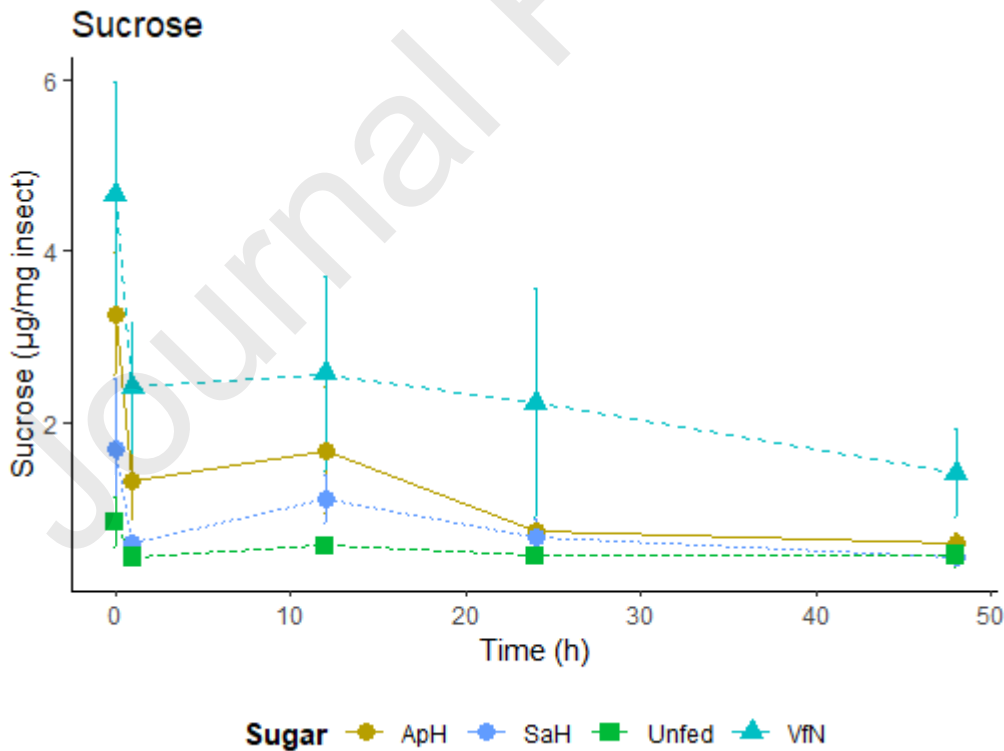


Figure B.3 Sucrose sugar metabolic dynamics of *Aphis ervi* females after receiving a feeding treatment (mean  $\pm$  SE at different times after feeding). ApH = *Acyrtosiphon pisum* honeydew, SaH = *Sitobion avenae* honeydew, VfN = *Vicia faba* extrafloral nectar.

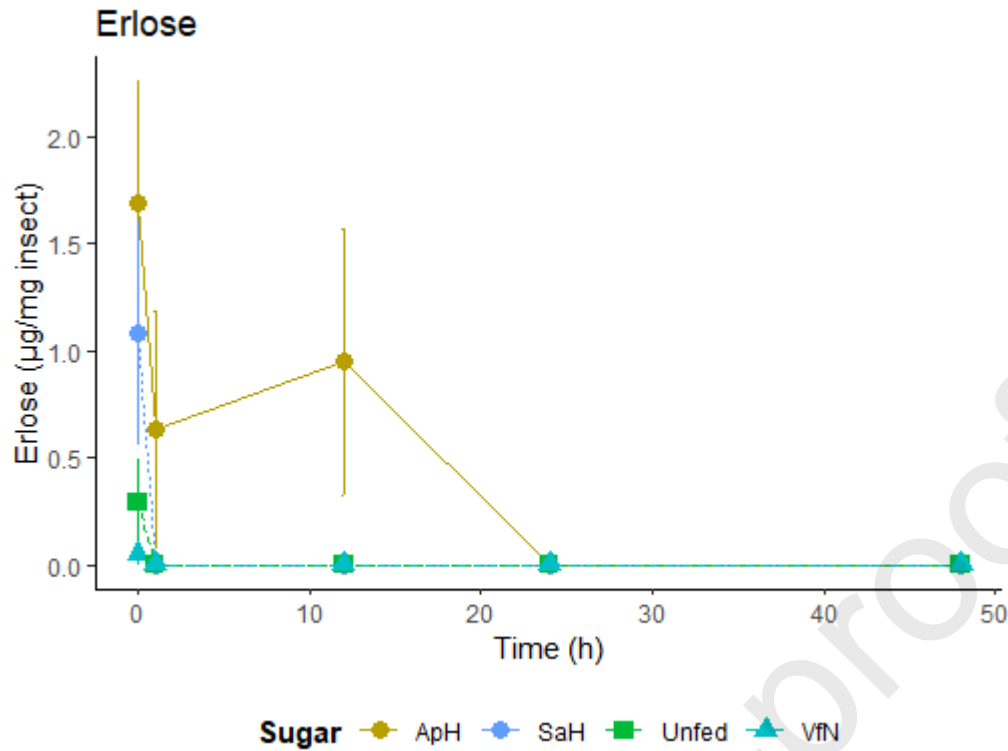


Figure B.4 Erllose sugar metabolic dynamics of *Aphis ervi* females after receiving a feeding treatment (mean  $\pm$  SE at different times after feeding). ApH = *Acyrtosiphon pisum* honeydew, SaH = *Sitobion avenae* honeydew, VfN = *Vicia faba* extrafloral nectar.

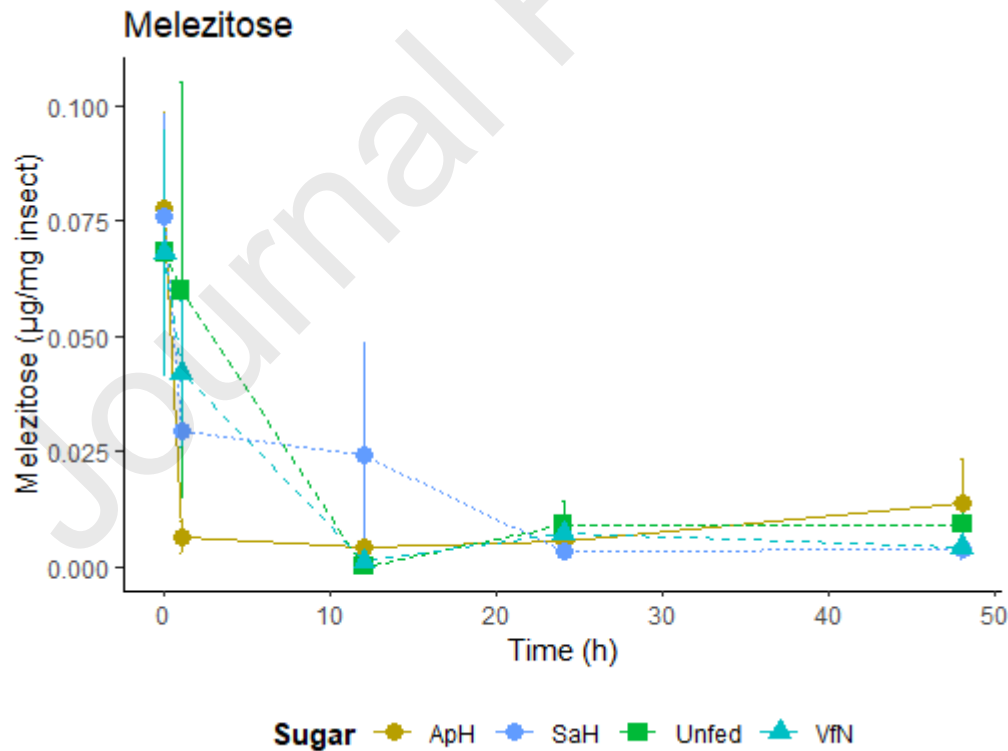


Figure B.5 Melezitose sugar metabolic dynamics of *Aphis ervi* females after receiving a feeding treatment (mean  $\pm$  SE at different times after feeding). ApH = *Acyrtosiphon pisum* honeydew, SaH = *Sitobion avenae* honeydew, VfN = *Vicia faba* extrafloral nectar.

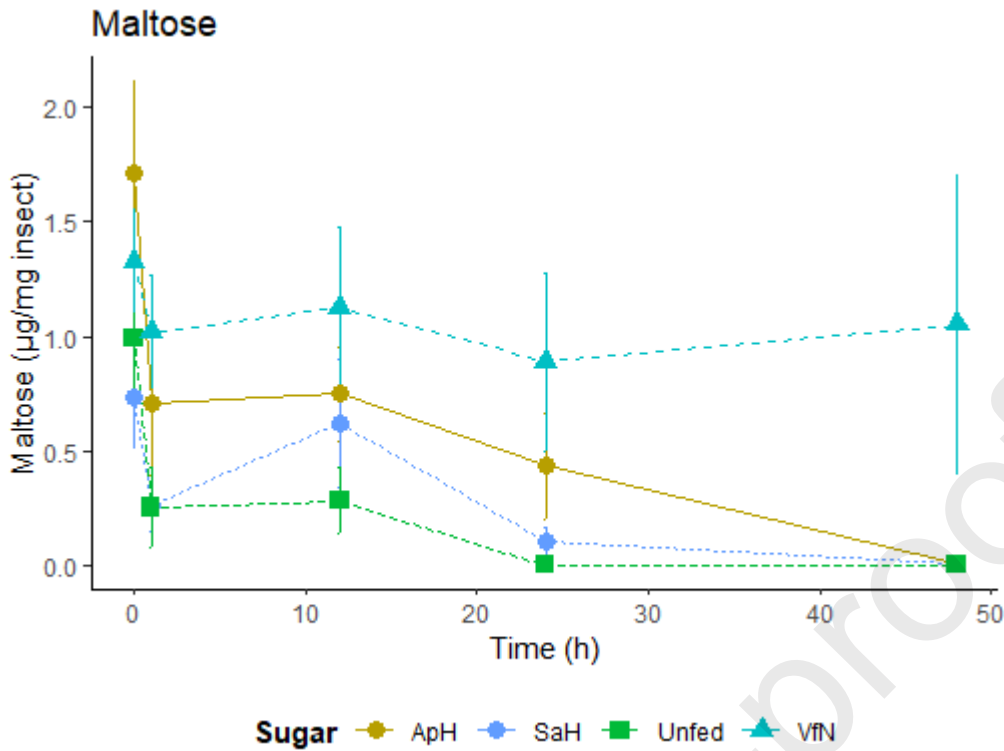


Figure B.6 Maltose sugar metabolic dynamics of *Aphis ervi* females after receiving a feeding treatment (mean  $\pm$  SE at different times after feeding). ApH = *Acyrtosiphon pisum* honeydew, SaH = *Sitobion avenae* honeydew, VfN = *Vicia faba* extrafloral nectar.

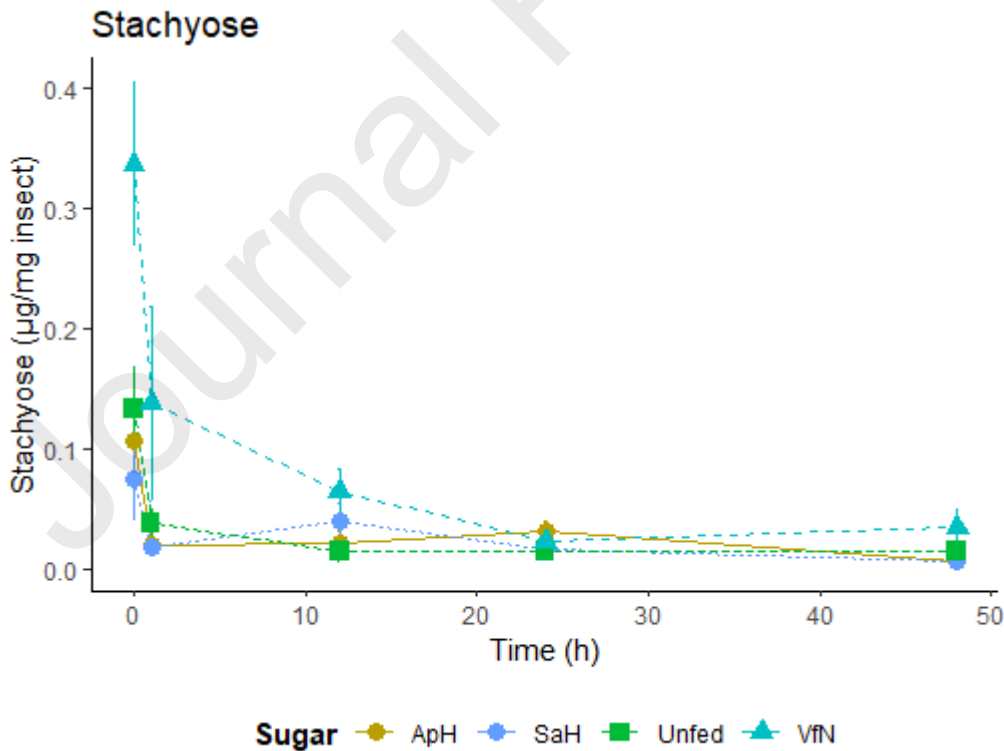


Figure B.7 Stachyose sugar metabolic dynamics of *Aphis ervi* females after receiving a feeding treatment (mean  $\pm$  SE at different times after feeding). ApH = *Acyrtosiphon pisum* honeydew, SaH = *Sitobion avenae* honeydew, VfN = *Vicia faba* extrafloral nectar.

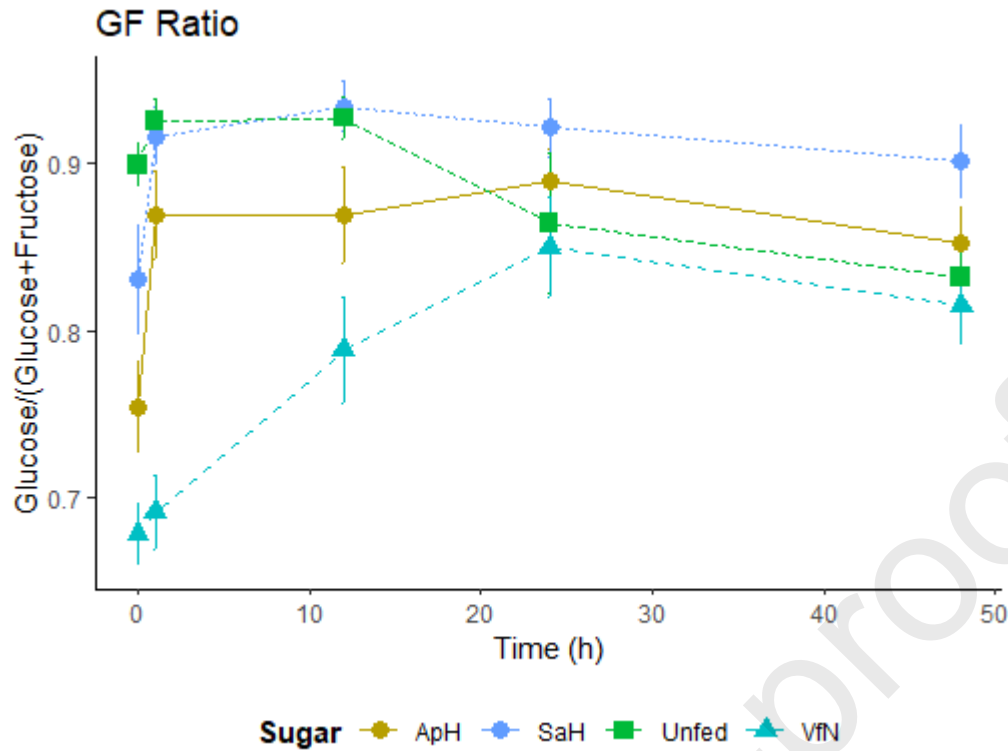


Figure B.8 Dynamics of the GF Ratio (Glucose/Glucose+Fructose) of *Aphidius ervi* females after receiving a feeding treatment (mean  $\pm$  SE at different times after feeding). ApH = *Acyrtosiphon pisum* honeydew, SaH = *Sitobion avenae* honeydew, VfN = *Vicia faba* extrafloral nectar.

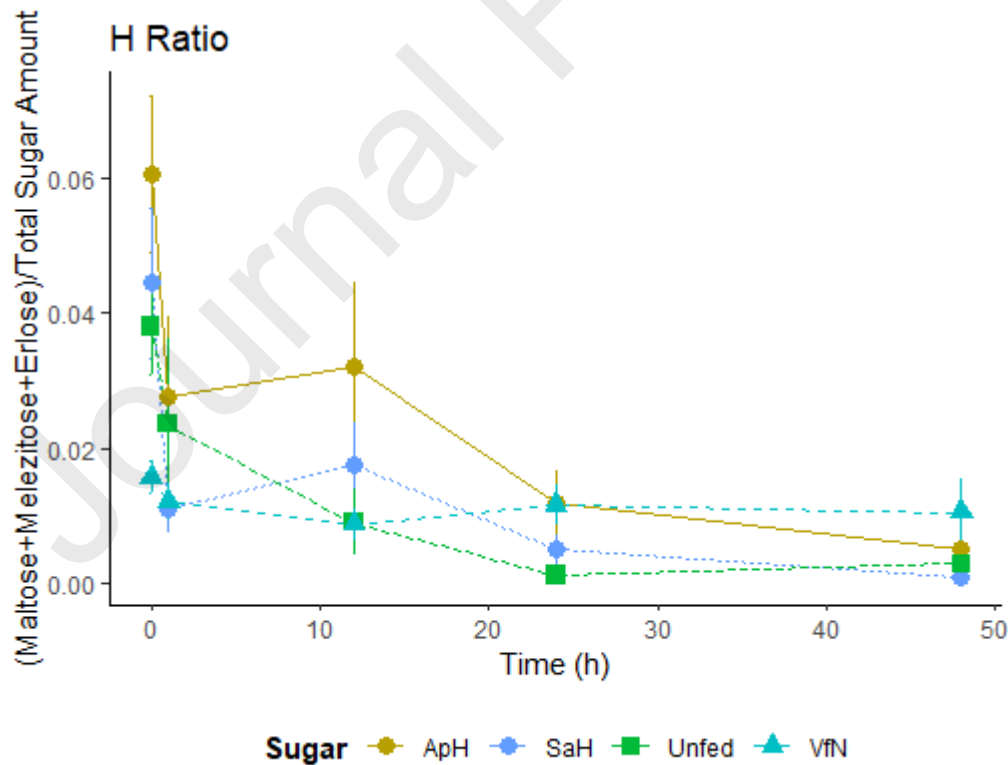


Figure B.9 Dynamics of the GF Ratio (Maltose+Erlose+Melezitose/Total Sugar Amount) of *Aphidius ervi* females after receiving a feeding treatment (mean  $\pm$  SE at different times after feeding). ApH = *Acyrtosiphon pisum* honeydew, SaH = *Sitobion avenae* honeydew, VfN = *Vicia faba* extrafloral nectar.

## Appendix C – Lab data visualisation

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

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

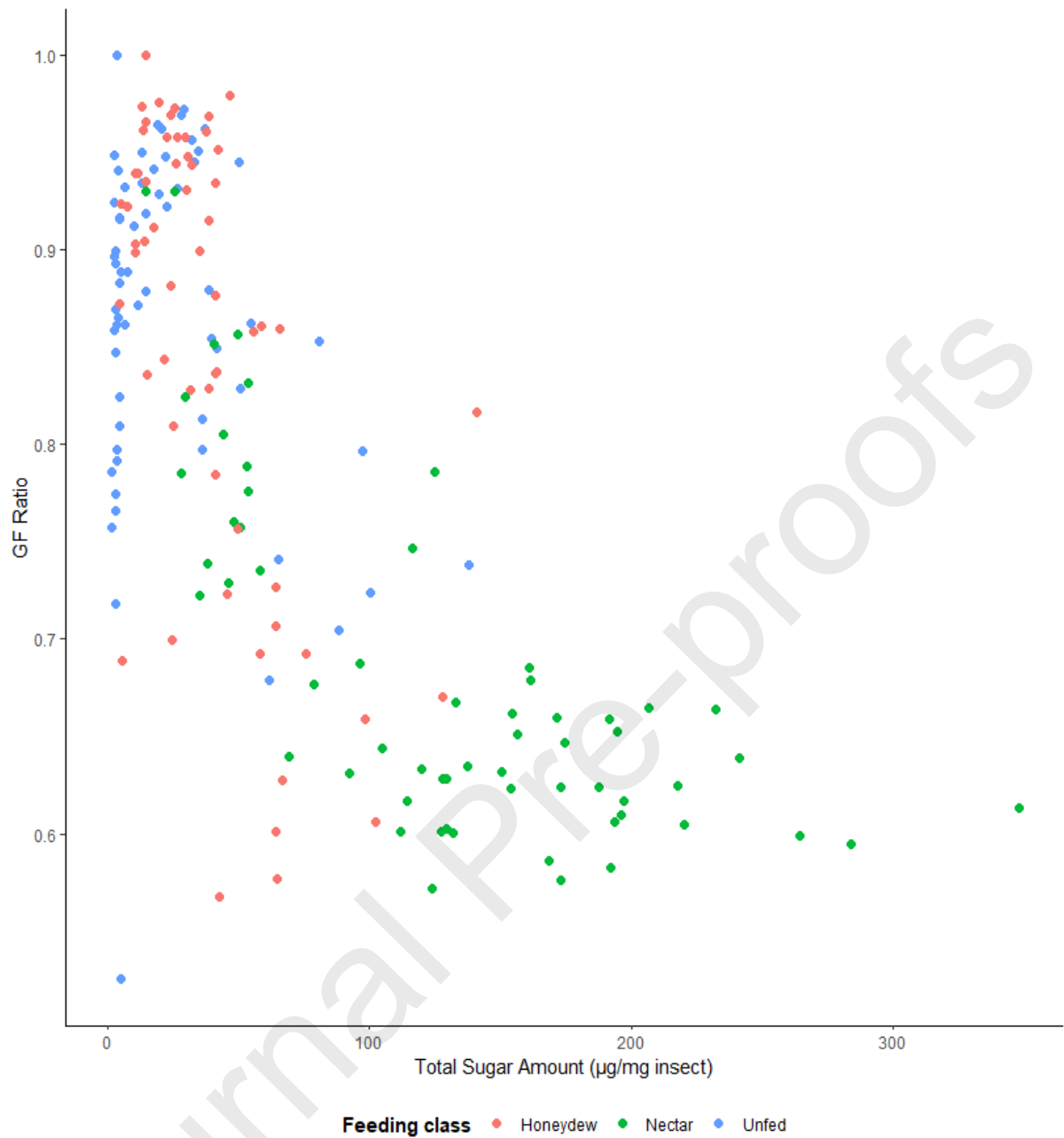


Figure C.1 Two-dimensional representation of the lab dataset, according to the total sugar amount of *Aphidius ervi* females ( $\mu\text{g}/\text{mg}$  insect) vs the Glucose/(Glucose + Fructose) Ratio (GF Ratio). Colours refer to the different feeding classes. These classes were assigned to insects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



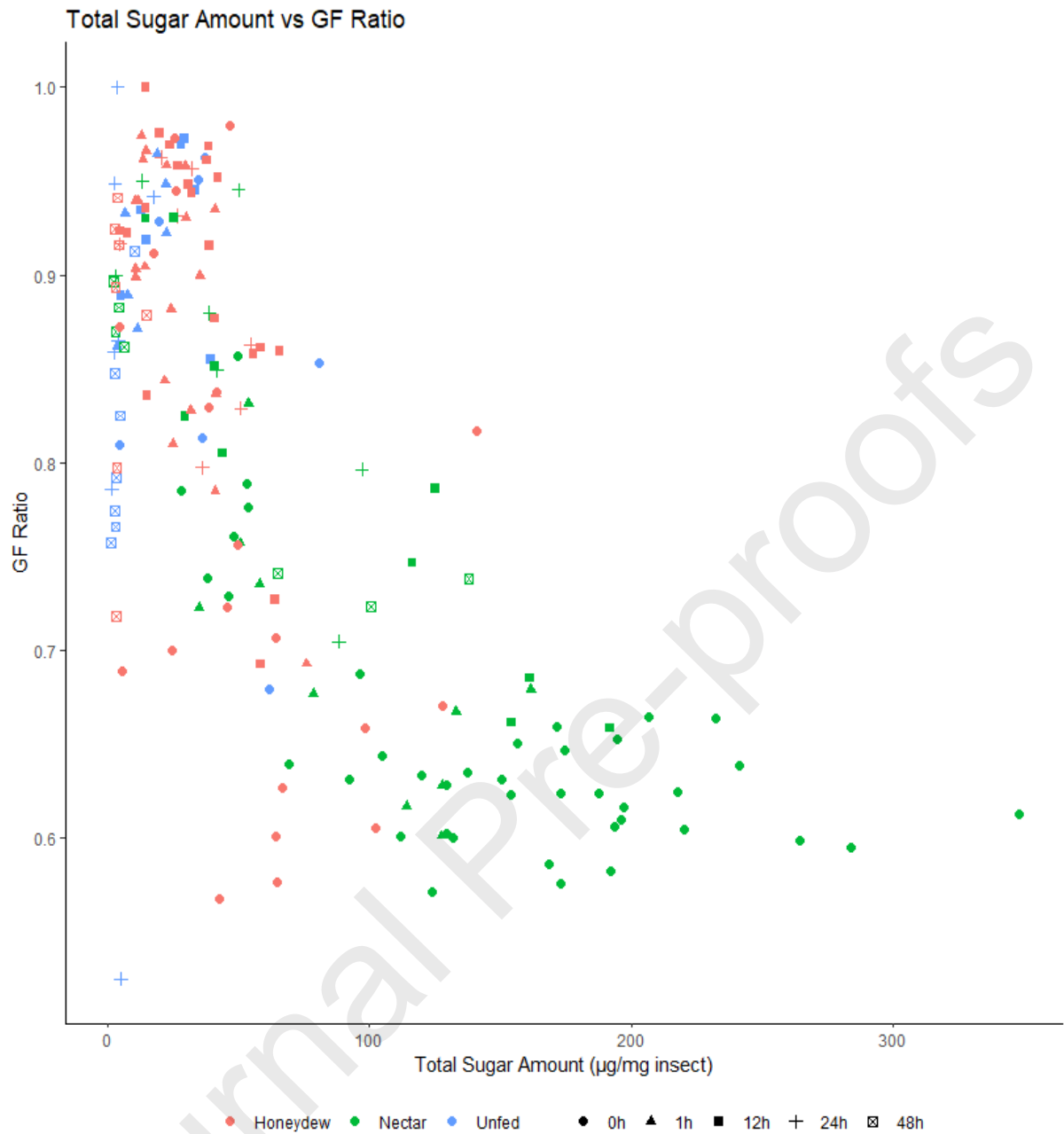


Figure C.2 Two-dimensional representation of the lab dataset, according to the total sugar amount of *Aphidius ervi* females ( $\mu\text{g}/\text{mg}$  insect) vs the Glucose/(Glucose + Fructose) Ratio (GF Ratio). Colours refer to the different feeding treatments. Shapes refer to the time spent starving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

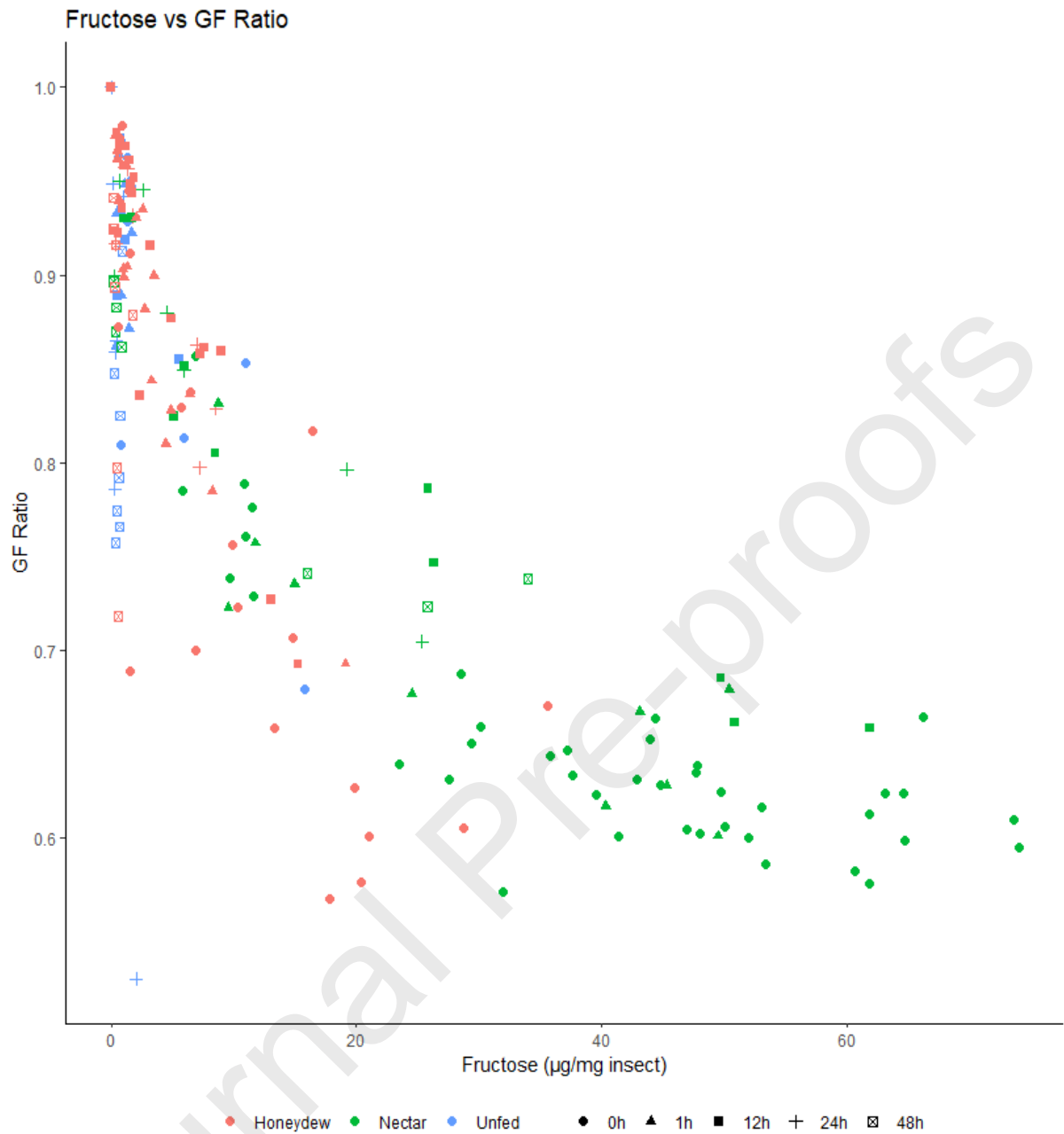


Figure C.3 Two-dimensional representation of the lab dataset, according to the Fructose content of *Aphidius ervi* females ( $\mu\text{g}/\text{mg}$  insect) vs the Glucose/(Glucose + Fructose) Ratio (GF Ratio). Colours refer to the different feeding treatments. Shapes refer to the time spent starving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

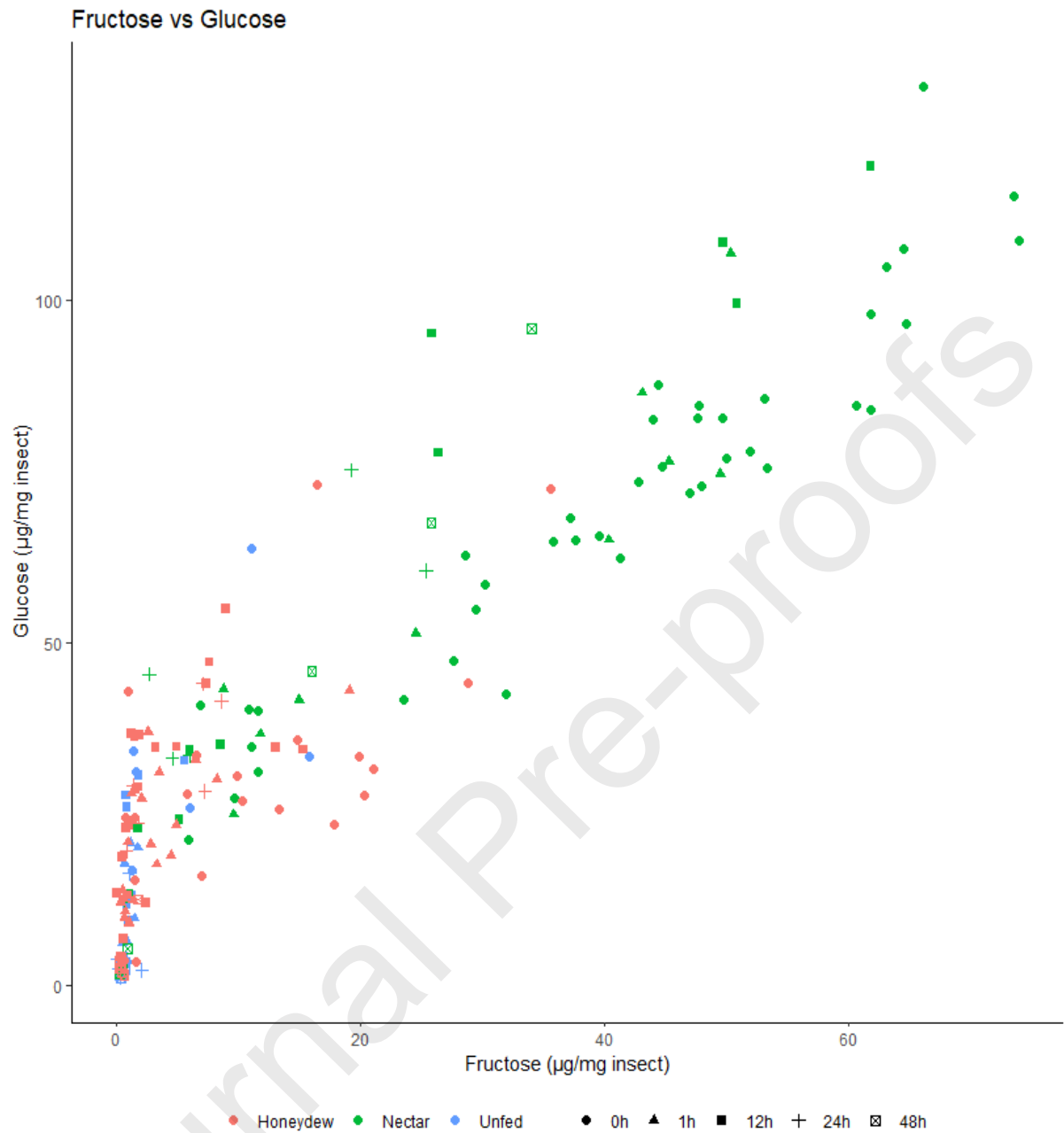


Figure C.4 Two-dimensional representation of the lab dataset, according to the Fructose content of *Aphidius ervi* females ( $\mu\text{g/mg insect}$ ) vs their Glucose content. Colours refer to the different feeding treatments. Shapes refer to the time spent starving after feeding (fed individuals) or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

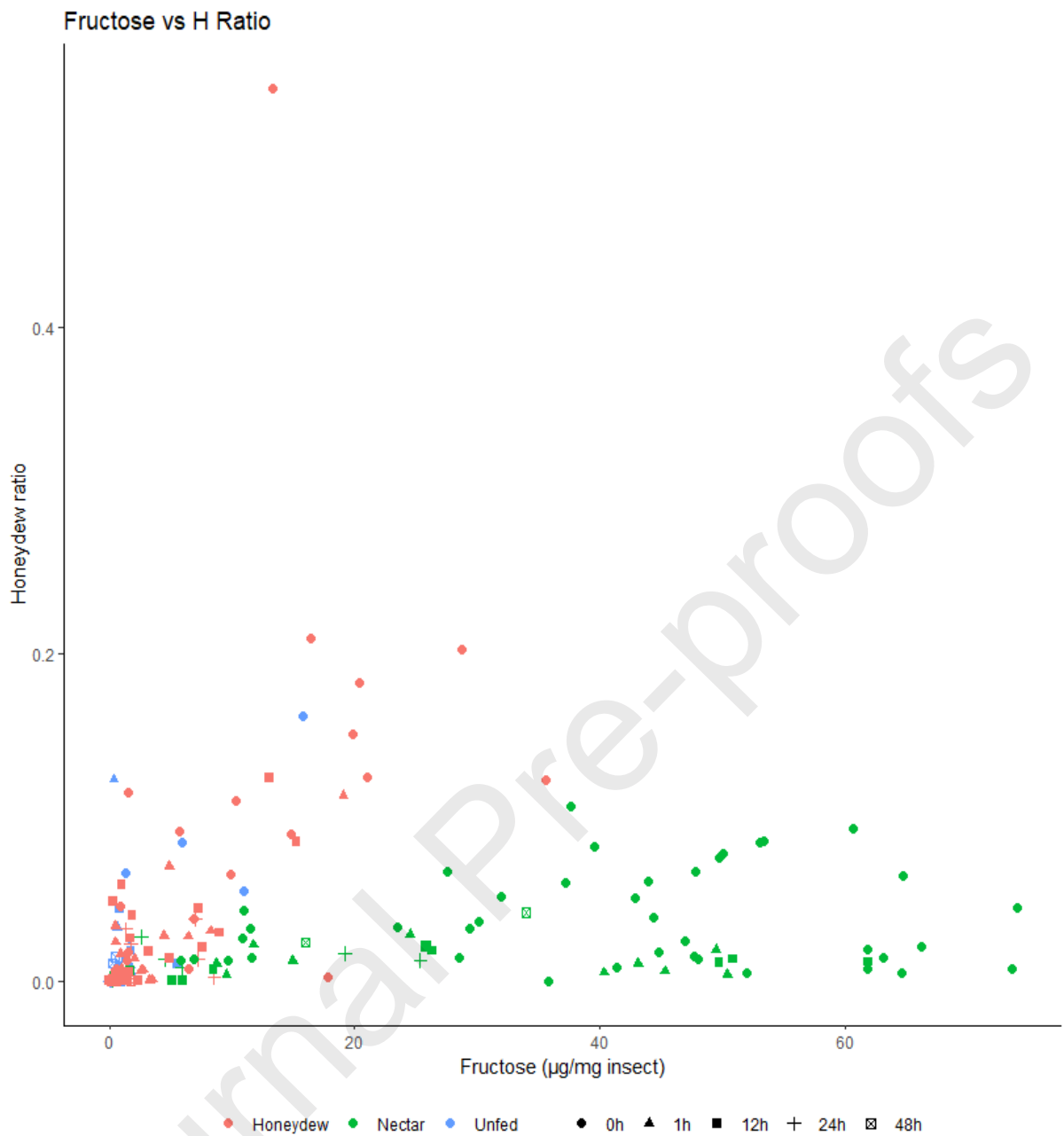
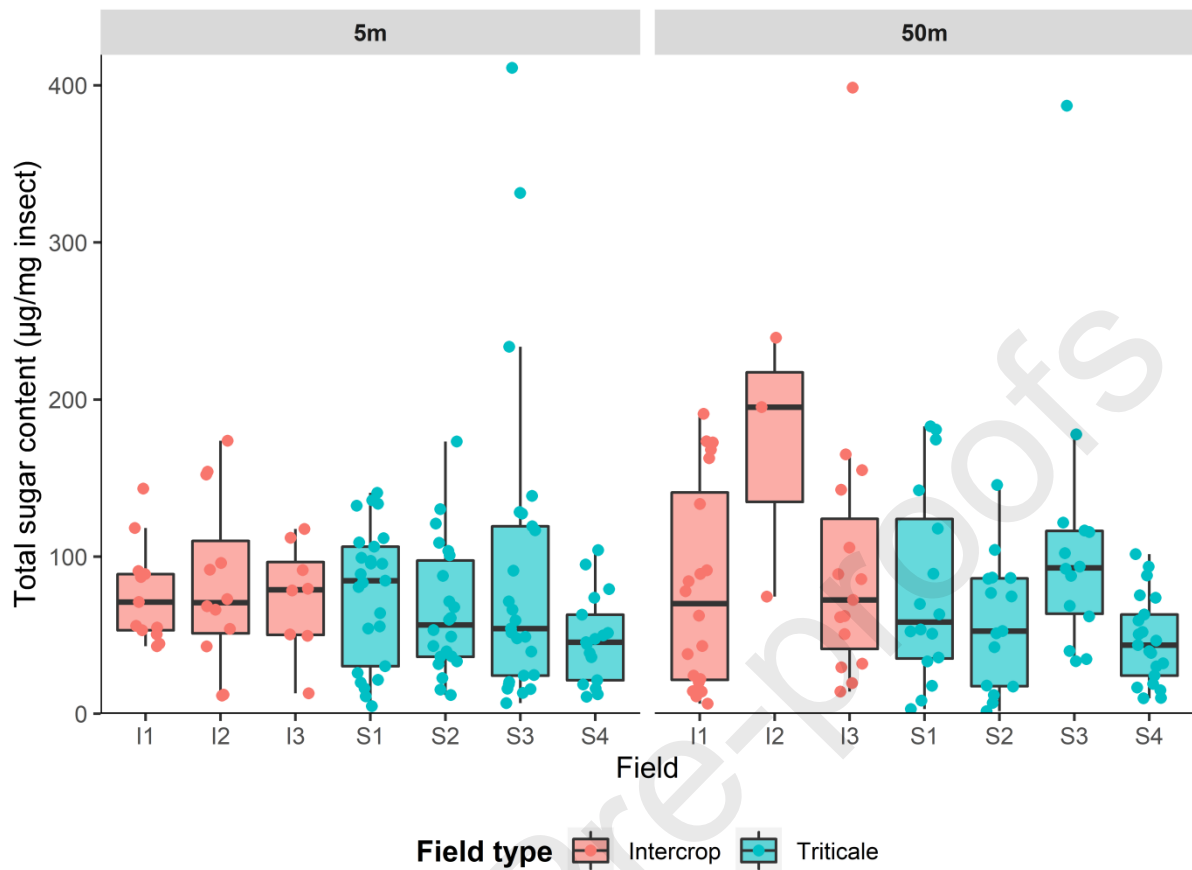


Figure C.5 Two-dimensional representation of the lab dataset, according to the Fructose content of *Aphidius ervi* females (µg/mg insect) vs the (Maltose + Erlucose + Melezitose)/Total sugar amount Ratio (H Ratio). Colours refer to the different feeding treatments. Shapes refer to the time spent starving after feeding (fed individuals) or after emergence (unfed individuals).

## Appendix D – Additional field data

## 925 Sugar levels of field-caught insects



926  
 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.*

## Field surveys: raw data

Table D.5 Total number of aphids from each species counted during the season, on triticale (*Sitobion avenae*, *Metopolophium dirhodum* and *Rhopalosiphum padi*) and on faba bean (*Acyrtosiphon pisum*, *Aphis* spp.), in 6 triticale-faba bean intercrops and triticale single crops and at 5m or 50m from the field edge (total summed values for all fields and distances from the edge are also reported). *Aphis* spp. consisted mainly of *Aphis fabae* but may also include *Aphis craccivora* individuals. On triticale, aphids were counted at seven dates on 50 randomly chosen tillers. On faba bean, aphids were counted at seven dates on 50 randomly chosen plants. For *Aphis* spp., aphids were individually counted up to 50 individuals. When more than 50 individuals 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</i> spp.
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

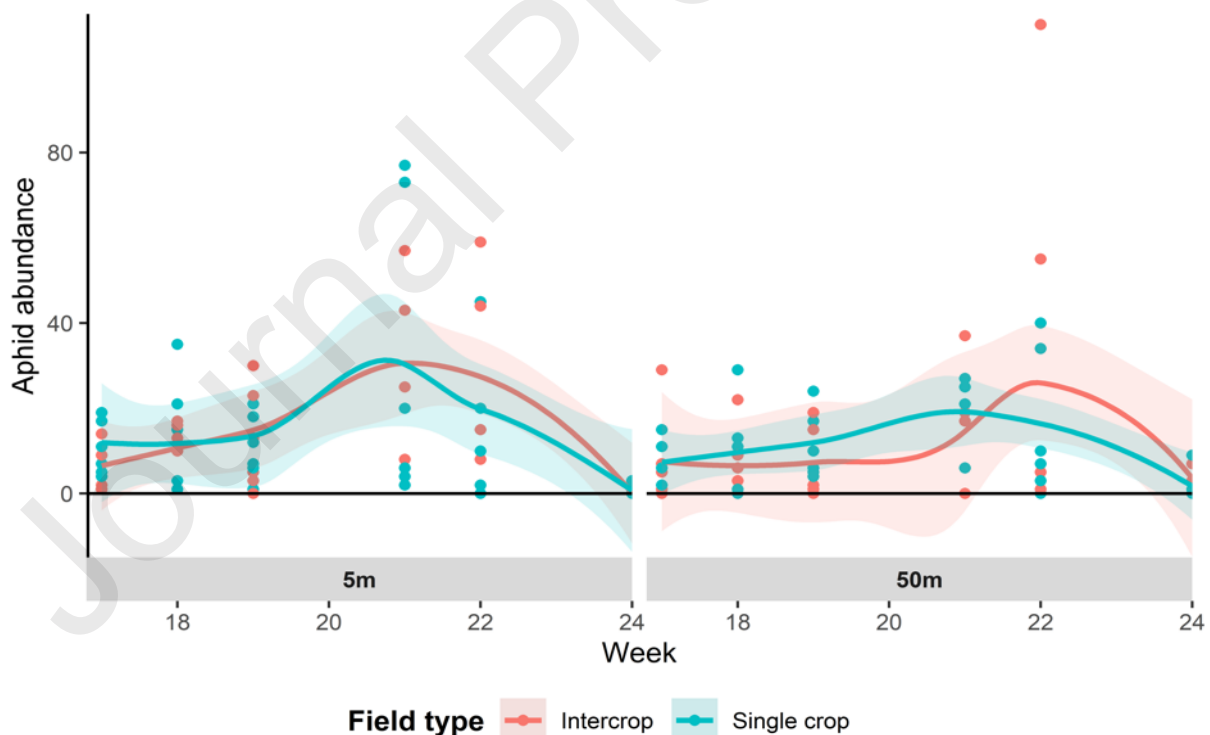


Figure D.2 Aphid abundance estimated at different sampling dates in pure crops (blue) and intercrops (red). The curves correspond to LOESS smoothers ( $\pm$  IC95), fitted to better visualise the general trend of each aphid population dynamics.



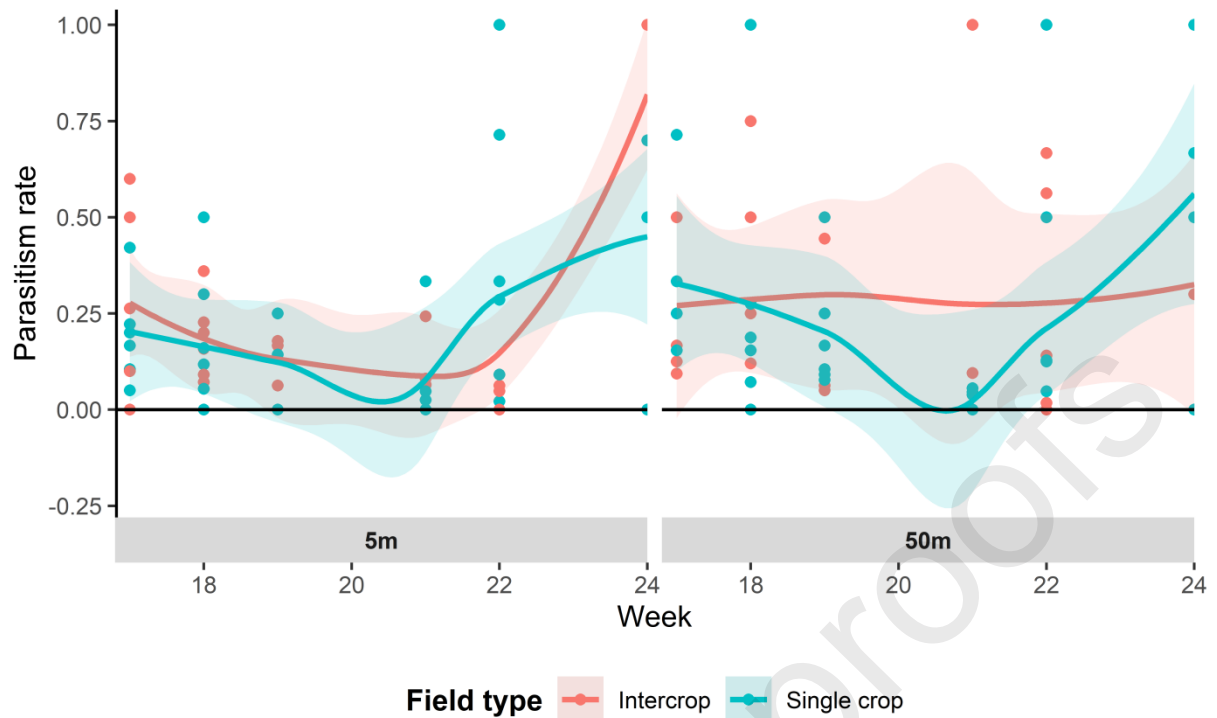


Figure D.3 Parasitism rate by *Aphidius* spp. parasitoids estimated at different sampling dates in pure crops (blue) and intercrops (red). The curves correspond to LOESS smoothers ( $\pm$  IC95), fitted to better visualise the general trend of each aphid population dynamics. Parasitism rate was computed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