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ADULT PARASITOIDS OF HONEYDEW-PRODUCING INSECTS PREFER
HONEYDEW SUGARS TO COVER THEIR ENERGETIC NEEDS

--Manuscript Draft--

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Abstract:	To meet their carbohydrate requirements, adult parasitoids exploit a broad range of sugar resources, including floral and extrafloral nectar and honeydew. Although honeydew might be the predominant sugar source, especially in agricultural systems, it is often nutritionally inferior to sugar sources like nectar. Given its broad availability, it may be expected that sugar-feeding insects have evolved specialized adaptations to deal with this often inferior sugar source. This would apply especially to organisms that have a close association with honeydew producers. Here we hypothesized that parasitoids of honeydew-producing insects show a particularly pronounced response to those sugars that predominantly occur in honeydew such as fructose, sucrose, melezitose and trehalose, and to a lesser extent glucose. To test this hypothesis, we investigated sugar consumption, feeding behavior and survival of the aphid parasitoid <i>Aphidius ervi</i> on several sugars. Our results show that <i>A. ervi</i> adults consumed typical honeydew sugars the most (sucrose, fructose, trehalose and melezitose), whereas intake of glucose or melibiose was considerably lower. Rhamnose, which does not occur in aphid honeydew, was not or only marginally consumed. When different sugars were provided at the same time, <i>A. ervi</i> adults preferred sucrose or fructose over glucose or melezitose. Furthermore, a pre-exposure to sucrose or fructose significantly reduced subsequent intake of glucose, suggesting an acquired distaste for glucose after being exposed to highly preferred sugars such as sucrose and fructose. Altogether, this study shows that <i>A. ervi</i> adults are well adapted to optimize the

exploitation of honeydew as a sugar source.

Sint-Katelijne-Waver, Belgium, May 12th, 2016

Dear editor,

Please find attached our manuscript 'Adult parasitoids of honeydew-producing insects prefer honeydew sugars to cover their energetic needs' that we would like to submit to Journal of Chemical Ecology. This study builds upon our expertise and findings gained in previous studies, such as those of co-author Prof. Felix Wäckers (some of which that were also published in Journal of Chemical Ecology).

Most adult parasitoids depend on carbohydrate-rich food such as nectar and honeydew to fulfil their carbohydrate requirements. Although honeydew might be the predominant sugar source, especially in agricultural systems, it is often nutritionally inferior to sugar sources like nectar. Given its broad availability, it may be expected that sugar-feeding insects have evolved specialized adaptations to deal with this often inferior sugar source. Here we hypothesized that parasitoids of honeydew-producing insects show a particularly pronounced response to those sugars that predominantly occur in honeydew such as fructose, sucrose, melezitose and trehalose, and to a lesser extent glucose. To test this hypothesis, we investigated sugar consumption, feeding behavior and survival of the aphid parasitoid *Aphidius ervi* on a range of different sugars. Our results show that *A. ervi* adults consumed typical honeydew sugars the most (sucrose, fructose, trehalose and melezitose), while glucose and the non-honeydew sugars melibiose and rhamnose were ingested at lower levels or only marginally consumed. When different sugars were provided at the same time, *A. ervi* adults preferred sucrose or fructose over glucose or melezitose. Furthermore, a pre-exposure to sucrose or fructose significantly reduced subsequent intake of glucose. Altogether, our study shows that *A. ervi* adults are well adapted to optimize the exploitation of honeydew as a sugar source.

We hope that this manuscript is meeting your quality standards and look forward to receiving comments in due time.

Sincerely,

Prof. Dr. Ir. Bart Lievens

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1 ADULT PARASITOIDS OF HONEYDEW-PRODUCING INSECTS
2 PREFER HONEYDEW SUGARS TO COVER THEIR ENERGETIC
3 NEEDS

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Abstract - To meet their carbohydrate requirements, adult parasitoids exploit a broad range of sugar resources, including floral and extrafloral nectar and honeydew. Although honeydew might be the predominant sugar source, especially in agricultural systems, it is often nutritionally inferior to sugar sources like nectar. Given its broad availability, it may be expected that sugar-feeding insects have evolved specialized adaptations to deal with this often inferior sugar source. This would apply especially to organisms that have a close association with honeydew producers. Here we hypothesized that parasitoids of honeydew-producing insects show a particularly pronounced response to those sugars that predominantly occur in honeydew such as fructose, sucrose, melezitose and trehalose, and to a lesser extent glucose. To test this hypothesis, we investigated sugar consumption, feeding behavior and survival of the aphid parasitoid *Aphidius ervi* on several sugars. Our results show that *A. ervi* adults consumed typical honeydew sugars the most (sucrose, fructose, trehalose and melezitose), whereas intake of glucose or melibiose was considerably lower. Rhamnose, which does not occur in aphid honeydew, was not or only marginally consumed. When different sugars were provided at the same time, *A. ervi* adults preferred sucrose or fructose over glucose or melezitose. Furthermore, a pre-exposure to sucrose or fructose significantly reduced subsequent intake of glucose, suggesting an acquired distaste for glucose after being exposed to highly preferred sugars such as sucrose and fructose. Altogether, this study shows that *A. ervi* adults are well adapted to optimize the exploitation of honeydew as a sugar source.

Key Words - *Aphidius ervi*, Honeydew, Sugar consumption, Sugar feeding, Survival.

52 INTRODUCTION

53 Due to their ability to regulate herbivorous insect populations, parasitoids play an
54 important role as keystone species in natural ecosystems and as biological control agents
55 of insect pests. While parasitoid larvae are carnivorous, developing in or on their
56 arthropod host, the majority of adult parasitoids depend on carbohydrates as an energy
57 source (Jervis et al. 1993; Wäckers 2004). As a result, feeding on sugar sources is
58 important for survival and reproduction of many adult parasitoids. It can increase
59 reproductive success by affecting host searching behavior (Wäckers and Swaans 1993,
60 Takasu and Lewis 1995; Olson et al. 2005), egg maturation (Olson et al. 2005), fecundity
61 (Schmale et al. 2001) and longevity (Azzouz et al. 2004; Wyckhuys et al. 2008).
62 Furthermore, it has been shown that sugar consumption is important to initiate and fuel
63 parasitoid flight, thereby increasing search area and host encounter rate (Hausmann et al.
64 2005, Takasu and Lewis 1995; Olson et al. 2005). To meet their carbohydrate
65 requirements, parasitoids exploit a broad range of sugar resources, including floral and
66 extrafloral nectar and honeydew, which is the sugar-rich excretion product of phloem-
67 feeding arthropods such as aphids and whiteflies (Wäckers 2005). Whereas floral and
68 extrafloral nectar may be readily available in natural systems, they are generally scarce
69 in agricultural systems due to low availability of flowering plants. As a result, honeydew
70 is often the most prevalent source of carbohydrates available in agricultural ecosystems
71 (Wäckers 2005). However, in comparison with nectar, honeydew may be relatively
72 unsuitable as a food source due to unfavourable sugar composition, the presence of hostile
73 plant-derived secondary metabolites and/or compounds synthesized by the honeydew-
74 producing insects (Wäckers 2000, Tena et al. 2016).

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75 Sugar composition of honeydew depends on both the plant species and honeydew-
76 excreting insects (Kloft et al. 1985; Fischer and Shingleton 2001; Hogervorst et al. 2003;
77 2007; Fischer et al. 2005; Wäckers 2005). Besides plant-derived sugars such as fructose,
78 glucose, maltose or sucrose, more complex insect-synthesized disaccharides such as
79 trehalose and trehalulose, or oligosaccharides like erlose, melezitose and raffinose can be
80 present in honeydew. Many honeydews are dominated by sucrose and its hexose
81 components glucose and fructose, although there are also honeydews that are low in these
82 sugars and are dominated by insect-synthesized oligosaccharides (Kloft et al. 1985;
83 Fischer and Shingleton 2001; Hogervorst et al. 2003; 2007; Fischer et al. 2005; Wäckers
84 2005). Notably, aphid honeydews contain in general more fructose than glucose (Kloft et
85 al. 1985; Fischer and Shingleton 2001; Hogervorst et al. 2003; 2007; Fischer et al. 2005),
86 probably because the glucose moiety of ingested sucrose is assimilated more efficiently
87 by the honeydew producer and/or is incorporated more often into oligosaccharides
88 (Thompson 2003; Karley et al. 2005).

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89 In contrast to nectar, which has a primary function in mutualistic interactions,
90 honeydew is primarily a waste product allowing phloem-feeding insects to excrete excess
91 carbohydrates (Wäckers 2005). In a number of cases honeydew has obtained a secondary
92 function in a protective mutualism with tending ants. As ants effectively monopolize
93 honeydew from tended sap feeders, it is typically the honeydew from sap feeders that are
94 not tended by ants that is available to other arthropods. Those honeydew sources are also
95 commonly exploited by parasitoids and predators of the sap feeders. As a consequence,
96 there is usually little benefit to the honeydew producer in having honeydew being
97 palatable or nutritionally suitable. Nevertheless, given the fact that honeydew is often the
98 predominant source of exogenous sugars, it may be expected that predators and

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99 parasitoids of honeydew producers would have evolved adaptations to effectively exploit
100 those sugar sources. Indeed, the short labrum found in most parasitoids is well-suited to
101 imbibe highly concentrated sugar solutions like honeydew (Wäckers et al. 2008).
102 Accordingly, it can be expected that they prefer sugars that are commonly available in
103 honeydew and that they would be able to survive better on honeydew sugars than
104 parasitoids that attack hosts that do not produce honeydew. To test this hypothesis we
105 used the solitary aphid parasitoid *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae) as
106 a model organism. *A. ervi* adults rarely engage in host feeding, and mostly feed on
107 honeydew from their aphid hosts (Quicke 2014). First, the feeding behavior and sugar
108 consumption was evaluated for parasitoids exposed to sugars that typically occur in aphid
109 honeydew and those that do not or rarely occur in honeydew. Next, we studied survival
110 of the parasitoids when fed with the different sugars. Finally, choice experiments were
111 conducted to determine sugar preferences and to investigate whether feeding responses
112 to sugars changed after prior feeding experience with the same or other sugars.

113 114 MATERIALS AND METHODS

115 *Study Species.* Experiments were performed using adults of *Aphidius ervi* (Haliday)
116 (Hymenoptera: Braconidae). *A. ervi* is a solitary endoparasitoid that attacks many aphid
117 species, including several species of economic importance. The larvae develop within the
118 host, and adults feed primarily on aphid honeydew to cover their energetic needs (Quicke
119 2014). *A. ervi* is widely distributed and is also commonly used for biological control of
120 aphids in greenhouses. To perform the experiments, *A. ervi* mummies were provided by
121 Biobest (Ervi-system[®], Westerlo, Belgium). Once received, *A. ervi* mummies were placed

122 in ventilated plexiglass boxes (12 x 12 x 1.5 cm) and kept under controlled conditions (22
123 °C, 70 % relative humidity and 16:8 h light:dark photoperiod) until adult emergence. Prior
124 to starting the experiments, insects were subjected to a dark period of eight hours. All
125 experiments were performed with feeding-inexperienced adults that were less than 24
126 hours old.

127

128 *Test Sugars.* The sugars used in the experiments represented a number of sugars that are
129 typically present or absent in aphid honeydew (Table 1). The honeydew sugars included
130 the plant-derived sugars fructose (overrepresented in aphid honeydew relatively to
131 nectar), glucose (underrepresented in aphid honeydew relatively to nectar) and sucrose,
132 as well as the aphid-synthesized sugars melezitose and trehalose. Rhamnose and
133 melibiose were included to represent sugars that are not typically known to occur in aphid
134 honeydew. All sugars were diluted with distilled water to obtain sugar solutions of equal
135 molecular weights, filter sterilized (syringe filter, pore size 0.2 µm, Pall Life Sciences,
136 Ann Arbor MI, USA) and stored in microcentrifuge tubes at -20 °C until required.

137

138 *Test Assay.* The test assay used was based on the Capillary Feeder (CAFE) described by
139 Ja et al. (2007), with some major adjustments (Fig. 1). Briefly, a plastic insect cage
140 (height: 12.5 cm; diameter: 10.0 cm) was provided with four calibrated glass
141 micropipettes (5.0 µl, Blaubrand Intramark, Wertheim, Germany) filled with 4.0 µl of the
142 tested sugar solution and a mineral oil overlay (1.0 µl) to minimize evaporation. These
143 capillaries were inserted through the lid (at the corners of a square of 4.5 cm x 4.5 cm in
144 the middle of the lid) via truncated 200 µl yellow pipette tips. To meet the water

145 requirements of the parasitoids, a filter paper imbibed with 500 µl sterile demineralized
146 water was put at the bottom of the cage at the start of the experiment, and supplemented
147 with another 500 µl water daily in the longevity experiments. To allow entry of air, the
148 lid of the cage was pierced and covered with a fine mesh (2.5 cm by 2.5 cm; mesh size
149 0.27 mm x 0.88 mm).

150

151 *Experiments.* A number of experiments were performed using the test assay described
152 above. For each experiment, after being subjected to a dark period of eight hours, 15 adult
153 parasitoids were released in each cage, and five replicates were included. Each
154 experiment also included an identical CAFE chamber without parasitoids to determine
155 evaporative losses which were subtracted from experimental readings. All experiments
156 were conducted under controlled conditions of 22 °C, 70 % relative humidity and with a
157 16:8 h light:dark cycle, starting with the light period. First, sugar consumption and effect
158 on parasitoid longevity was assessed for each tested sugar (Table 1). Sugar consumption
159 was measured every hour over a total of nine hours during the light period. To accurately
160 measure the amount of sugar consumed from each capillary, a digital caiper (Mitutoyo
161 Digimatic, resolution 0.01 mm) was used. Subsequently, parasitoid longevity was
162 assessed by counting and removing dead individuals daily. To avoid microbial
163 contamination of the sugars, capillaries were replaced daily. Next, a series of choice
164 experiments was conducted to investigate feeding behavior when insects are provided a
165 choice between different sugar solutions. More specifically, insects were presented with
166 two different sugar solutions through two pairs of truncated pipette tips arranged in an
167 alternating pattern, and sugar consumption was assessed over a period of nine hours as

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168 described above. Finally, the effect of previous sugar intake on the subsequent feeding
169 response to the same and other sugars was investigated by first allowing inexperienced
170 parasitoids to contact either fructose, glucose or sucrose for 5 s. Afterwards, insects were
171 subjected to the sugar consumption assay where either fructose, glucose or sucrose was
172 supplied ad libitum, and sugar consumption was assessed over a nine hour period as
173 described earlier.

174

175 *Statistical Analysis.* To test whether consumption differed between sugars, a repeated-
176 measures analysis of variance (ANOVA) was used with sugar as fixed factor and hourly
177 sugar consumption as dependent variable. A Tukey HSD post hoc test was performed to
178 investigate which sugars were more consumed than others. To test whether the time to
179 death differed between the different sugars provided, survival curves were generated and
180 compared using Kaplan-Meier estimates of the survival function. To determine whether
181 survival curves were significantly different, a log-rank statistic was performed followed
182 by Holm-Sidak correction to account for each of the pairwise comparisons. The effect of
183 short pre-exposure to specific sugars on the subsequent feeding response to the same and
184 other sugars was evaluated with a two-way ANOVA. We used first feeding (for 5 s) and
185 second sugar exposure as fixed factors, and sugar consumption after nine hours of sugar
186 exposure as dependent variable. All statistical analyses were conducted using SPSS (IBM
187 SPSS Statistics for Windows, Version 23.0).

188

189

RESULTS

190 *Sugar Intake.* Sugar consumption over a period of nine hours varied significantly between
191 the different sugars provided ($F_{6,28} = 9.39$; $P < 0.001$) (Fig. 2). The highest consumption
192 rates were observed for the sugars sucrose, trehalose, melezitose and fructose. The
193 glucose or melibiose intake was a mere 25% of that of sucrose, while rhamnose was
194 hardly ingested at all (Fig. 2).

195
196 *Effect Of Sugars On Parasitoid Longevity.* The various sugars tested differed
197 considerably with regard to their effect on parasitoid longevity ($\chi^2 = 329.56$; $df = 7$; $P <$
198 0.001) (Fig. 3). Parasitoids lived longest when provided with glucose, sucrose, melezitose
199 or fructose, followed by melibiose and trehalose. Compared to the control, rhamnose did
200 not significantly enhance parasitoid life span (Fig. 3) with no individuals surviving
201 beyond five days. In contrast, more than 50 % of the tested individuals were still alive
202 after seven days when provided with sucrose, fructose, glucose or melezitose. Compared
203 to individuals fed on water only, these sugars increased the average and maximum life
204 span by a factor 2.5-3.3 and 4.5-5, respectively, resulting in a maximal survival of 18 to
205 20 days compared to four days for the water control. Melibiose and trehalose had a less
206 pronounced effect, increasing the average parasitoid longevity by a factor 1.7 and 1.4 and
207 the maximum survival by a factor 3.5 and 3, respectively.

208
209 *Sugar Intake When Different Sugars Are Provided Simultaneously.* In order to further
210 investigate the feeding behavior of *A. ervi*, sugar intake was assessed over a nine-hour
211 period when individuals were given the choice between two sugars. Experiments were
212 performed for the four sugars that substantially prolonged parasitoid longevity (i.e.
213 fructose, glucose, melezitose and sucrose) (Fig. 4). The results showed that sucrose intake

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214 was always higher than intake of melezitose. This difference was even more pronounced
215 when comparing sucrose to glucose, but not significant when putting sucrose against
216 fructose. Fructose consumption exceeded feeding on melezitose. When a choice was
217 given between fructose and glucose, the former was consumed at almost double the
218 volume, albeit that this difference was only marginally significant ($P = 0.083$) (Fig. 4D).
219 When a choice was given between a 3:1 and a 1:3 fructose-glucose mixture (Fig. 4G), no
220 significant differences were observed.

221
222 *Effect Of Previous Sugar Experience On Subsequent Sugar Consumption.* Overall, the
223 intake of the second sugar differed between sugars in this experiment ($F_{2,36} = 11.33$; $P <$
224 0.001). Furthermore, the brief initial sugar experience affected consumption of the second
225 sugar (significant interaction in two-way ANOVA: $F_{4,36} = 3.96$; $P = 0.009$) (Fig. 5).
226 Glucose consumption was reduced relative to fructose or sucrose consumption in those
227 individuals that had been given a pre-exposure to one of the latter two sugars, but not
228 when the pre-exposure involved glucose (Fig. 5).

229

DISCUSSION

230
231 In many ecosystems honeydew represents the primary source of exogenous sugars and
232 therefore it constitutes a crucial food to a broad range of insects (Wäckers 2005).
233 However, in comparison to other sugar sources, such as nectar, honeydew is often
234 nutritionally inferior, although there is considerable variation in the quality of honeydew
235 depending on the honeydew producer and its host plant (Wäckers et al. 2008). Assuming
236 that sugar-feeding insects have evolved adaptations to those food sources that readily

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237 occur within their habitat, we hypothesized that aphid parasitoids are adapted to those
238 sugars that are overrepresented in aphid honeydew relative to open nectar sources
239 (fructose, melezitose, trehalose, and sucrose) and to a lesser extent to those sugars that
240 are underrepresented (glucose) (Kloft et al. 1985; Fischer and Shingleton 2001;
241 Hogervorst et al. 2003; 2007; Fischer et al. 2005).

242 To test this hypothesis, we investigated sugar consumption and feeding behavior
243 in the aphid parasitoid *A. ervi* when exposed to (choices of) individual sugars. Our results
244 show that *A. ervi* adults consumed typical honeydew sugars the most (sucrose, fructose,
245 trehalose and melezitose), whereas intake of glucose or melibiose (only sporadically
246 found in honeydew) was considerably lower when monitoring sugar consumption over a
247 period of nine hours. The monosaccharide rhamnose, which does not normally occur in
248 honeydew (Wäckers 2001), was not or only marginally consumed. When different sugars
249 were provided at the same time, *A. ervi* adults preferred sucrose or fructose over glucose
250 or melezitose. Interestingly, a pre-exposure to sucrose or fructose significantly reduced
251 subsequent intake of glucose (at least at the short term), suggesting an acquired distaste
252 for glucose after being exposed to highly preferred sugars such as sucrose and fructose.
253 Makatiani et al. (2014) first demonstrated this phenomenon, showing that the braconid
254 parasitoid *Microplitis croceipes* exhibits an acquired distaste for maltose or fructose
255 following a brief exposure to other sugars. The results in our study suggest that *A. ervi*
256 can discriminate between different sugars and exhibits a strong preference for sucrose
257 and fructose. These observations may reflect the fact that both sucrose and fructose are
258 often the most predominant sugars in many honeydews, whereas glucose is often
259 underrepresented (Kloft et al. 1985; Fischer and Shingleton 2001; Hogervorst et al. 2003;
260 2007; Fischer et al. 2005). For example, Hogervorst et al. (2007) reported that the sugar

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261 composition of honeydew samples from five aphid species feeding on potato or wheat
262 plants constituted on average of 35.4 % sucrose and 36.1 % fructose, while they only
263 contained 7.4 % glucose.

264 Previous studies have shown that innate gustatory sugar responses of parasitoids
265 are often positively correlated with the nutritional quality of the sugar in terms of
266 parasitoid longevity and realized reproductive fitness (Wäckers 1999, 2001; Luo et al.
267 2013; Makatiani et al. 2014). However, despite the weak innate gustatory response to
268 glucose and the acquired distaste to this sugar, *A. ervi* parasitoids lived as long on glucose
269 as on sucrose, fructose and melezitose. The reason for this apparent mismatch between
270 the gustatory response and nutritional suitability in the case of glucose remains to be
271 explained. The average lifespan on these sugars was 7.5 to 9.6 days compared to 2.9 days
272 for the food-deprived control. These results are somewhat lower than those reported for
273 some other hymenopteran parasitoids with mean longevity ranging from 3.7 to 4.5 days
274 for food-deprived wasps and 11.5 to 13.8 days for sugar-fed ones (Azzouz et al. 2004).
275 Sucrose, fructose and glucose tend to be nutritionally most suitable, whereas aphid-
276 synthesized honeydew sugars such as melezitose and trehalose tend to represent a lower
277 nutritional value (Wäckers 2005). However, in contrast to studies on other parasitoids
278 (Wäckers 2001; Zobelein 1955), we found that *A. ervi* can survive equally well on the
279 common honeydew-sugar melezitose as on dominant nectar sugars such as sucrose,
280 fructose and glucose. This could be seen as further evidence that this aphid parasitoid has
281 adapted to the exploitation of aphid produced honeydew. On the other hand, wasps only
282 survived an average of 4 days on another honeydew sugar, trehalose, despite showing a
283 strong gustatory response to this sugar (comparable to other typical honeydew sugars). A
284 similar poor performance on trehalose has also been reported for other braconid wasps

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285 like *Anaphes iole* and *Cotesia glomerata* and the ichneumon wasp *Diadegma*
286 *semiclausum* (Williams and Roane 2007; Wäckers 2001; Winkler et al. 2005). Although
287 *A. ervi* showed a strong feeding response to trehalose, the less pronounced survival on
288 this sugar may be explained by the lack of a suitable enzyme to digest trehalose, which is
289 an alpha-linked disaccharide of two glucose units. The fact that the parasitoids perform
290 well on other sugars with an α -glucosidic bond such as sucrose and melezitose indicates
291 the presence of an α -glucosidase, which may not act on trehalose. This is in accordance
292 with the specific α -glucosidases reported in honeybees, having a high activity in
293 hydrolyzing sucrose and maltose, without acting on trehalose (Huber and Mathison,
294 1976). The generally poor performance of parasitoids on trehalose, which is a major
295 haemolymph sugar in insects, may also explain why host feeding by parasitoids often
296 only has a marginal impact on longevity (Jervis and Kidd 1986). Gustatory response and
297 nutritional value were again aligned in the case of rhamnose, where we see no benefits in
298 terms of longevity, and an absence of a feeding response. Even when consumed, this
299 sugar does not serve as an energy source (Wäckers 2001). Also for other parasitoids such
300 as *A. iole* and *C. glomerata*, rhamnose has been shown to be nutritionally unsuitable
301 (Wäckers 1999, 2001; Beach et al. 2003; Williams and Roane 2007).

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302 Altogether, our study shows that *A. ervi* adults are well adapted to optimize the
303 exploitation of honeydew as a sugar source to cover their energetic needs. We have shown
304 that *A. ervi* adults prefer sugars that dominate aphid honeydew and when feeding on these
305 sugars can substantially increase their longevity. Whereas this work has broadened our
306 view on how *A. ervi* copes with honeydew as a sugar source, further work is needed using
307 other *Aphidius* species, or more generally other parasitoids of honeydew-producing

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308 insects to generalize our results. Additionally, it remains to be investigated whether the
309 established trends also hold for other important life history parameters such as fecundity.

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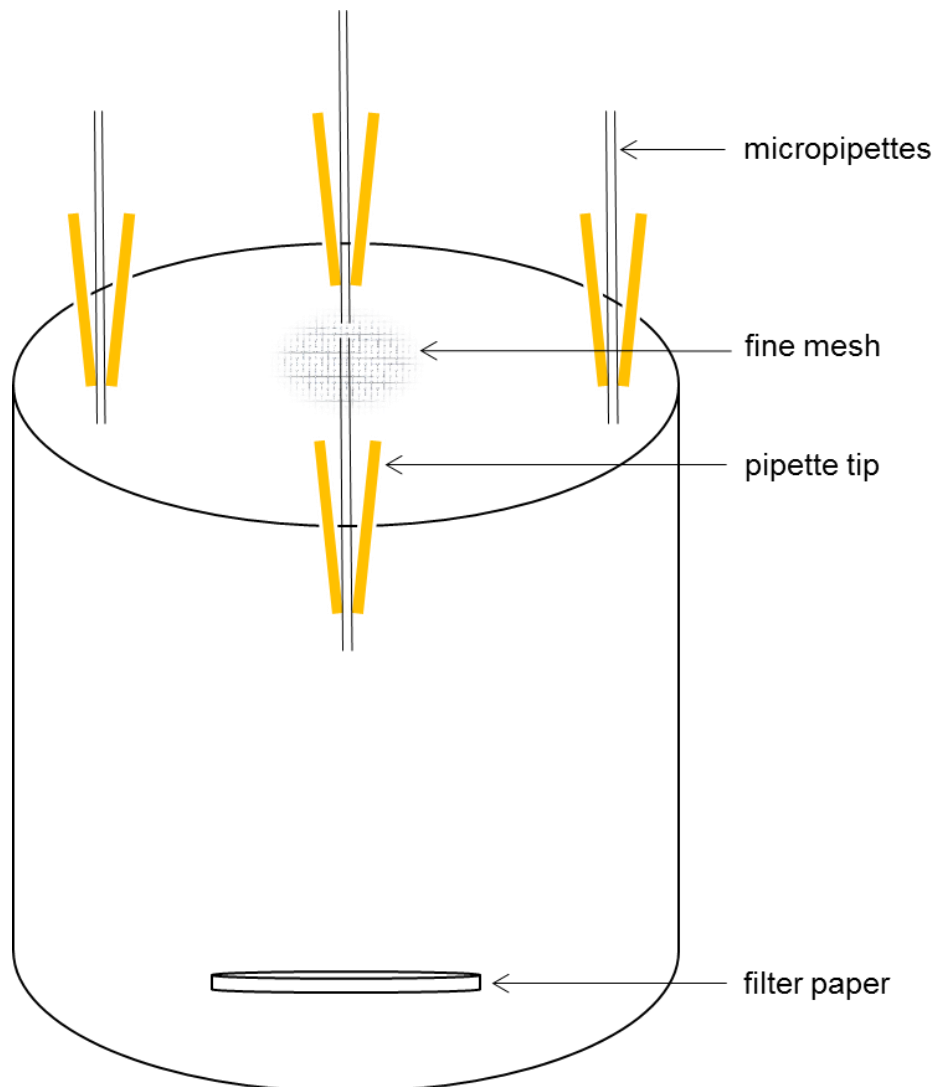
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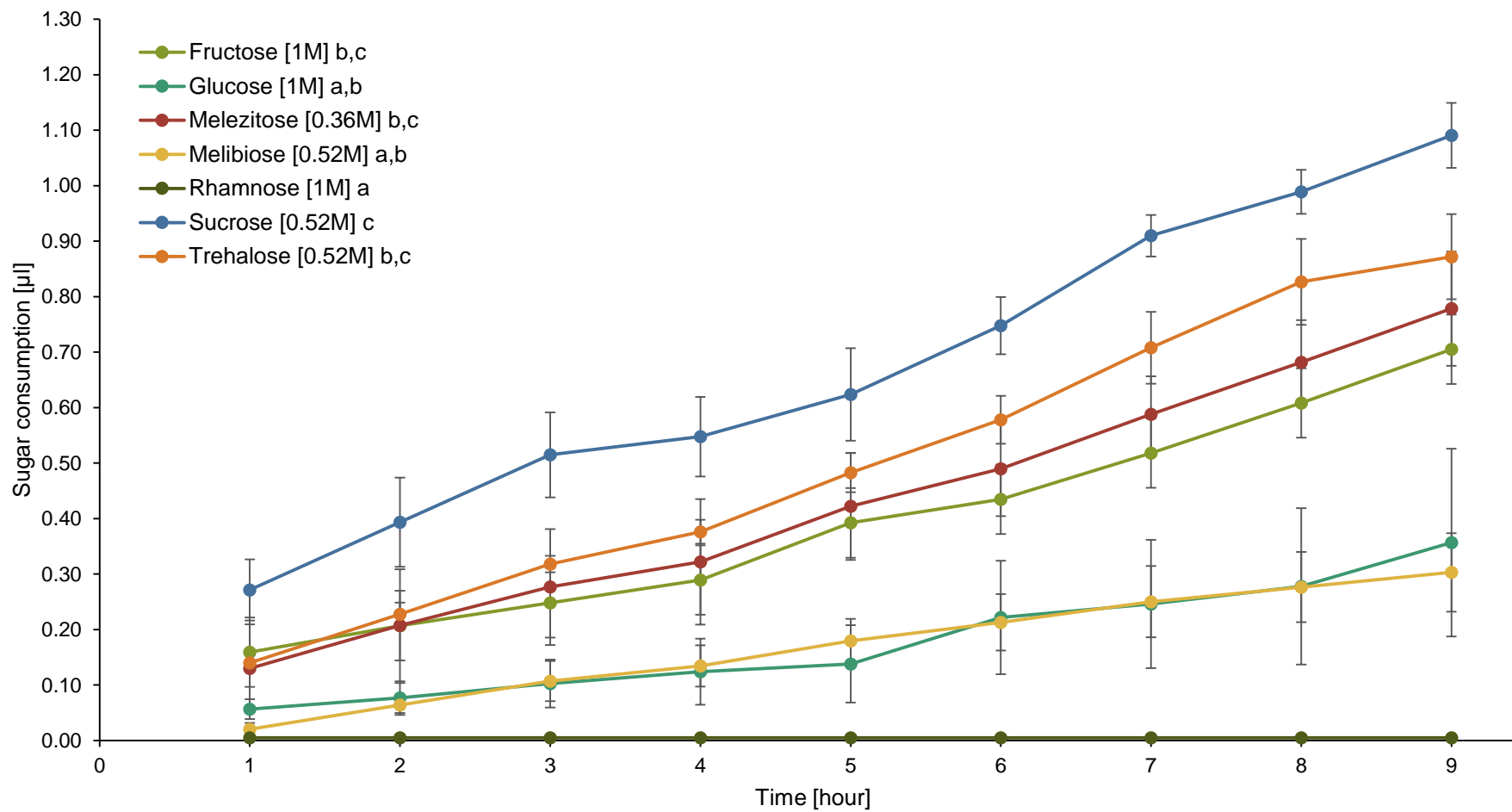
1 FIGURES

2 **Fig. 1** Schematic diagram of the Capillary Feeder (CAFE) assay. Test sugars (4.0 μ l)
3 were provided in four glass micropipettes, topped with a mineral oil overlay (1.0 μ l) to
4 minimize evaporation. These micropipettes were introduced through the lid via truncated
5 200 μ l yellow pipette tips. To allow entry of air in the insect cage, the lid of the chamber
6 was pierced and covered with a fine mesh (2.5 cm by 2.5 cm; mesh size 0.27 mm x 0.88
7 mm). A water imbibed filter paper at the bottom of the chamber ensured the availability
8 of water

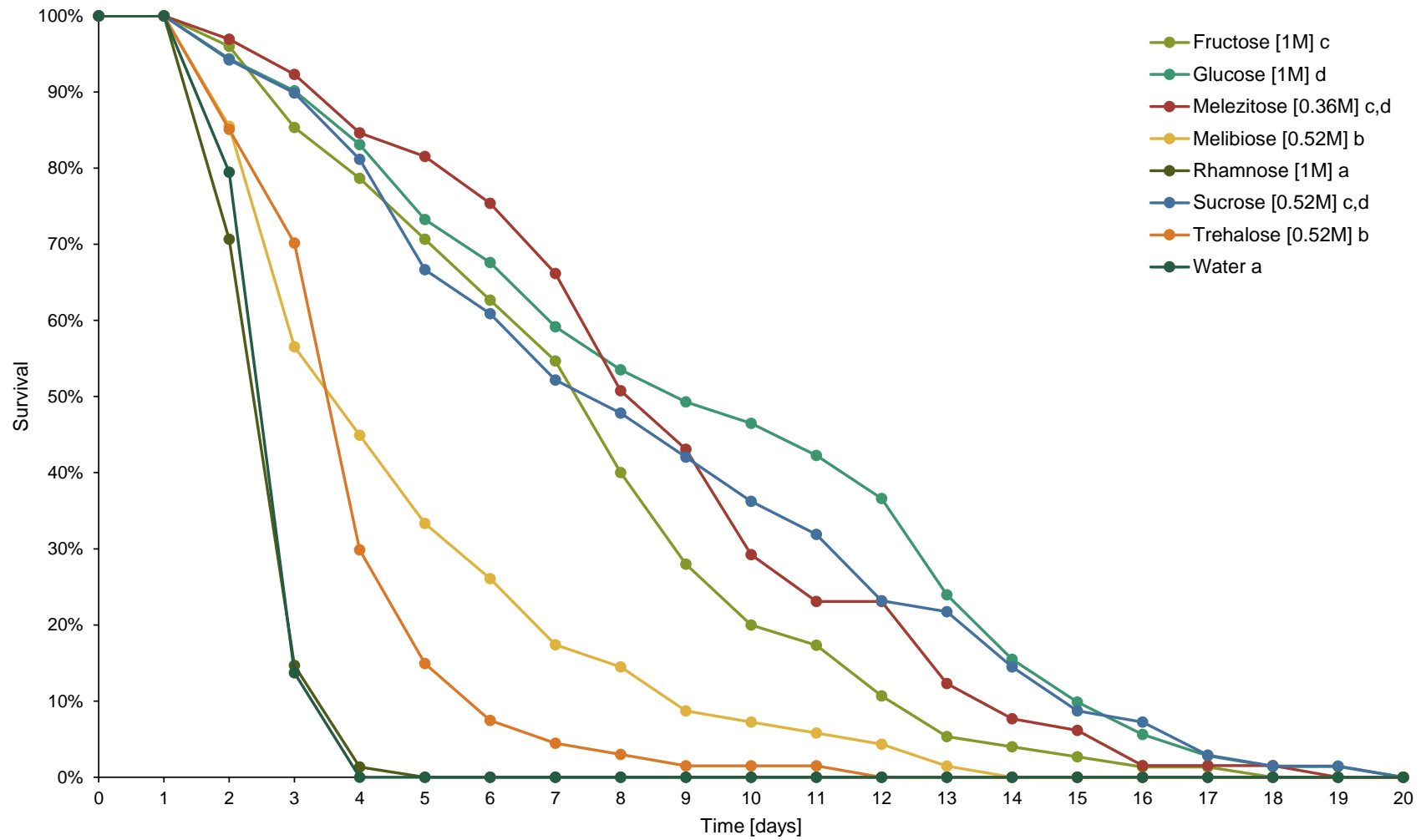


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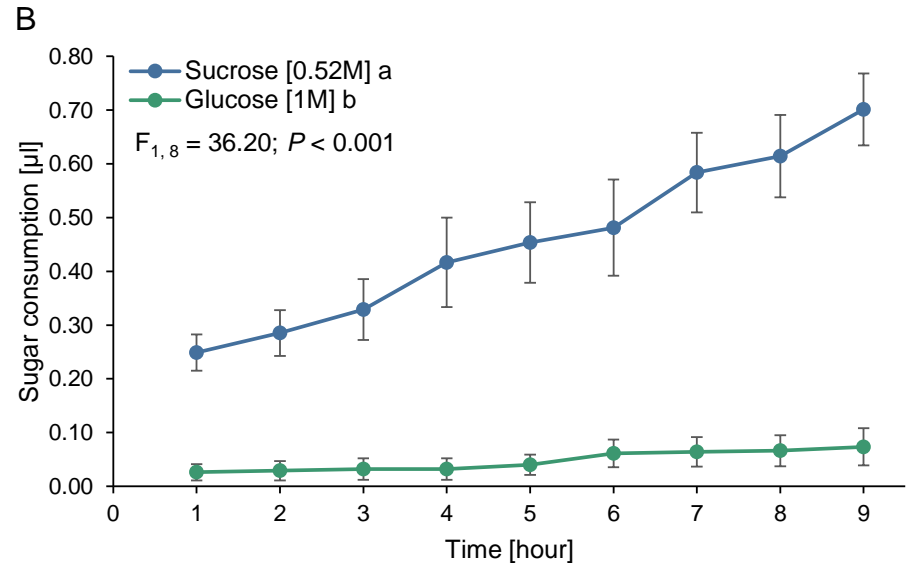
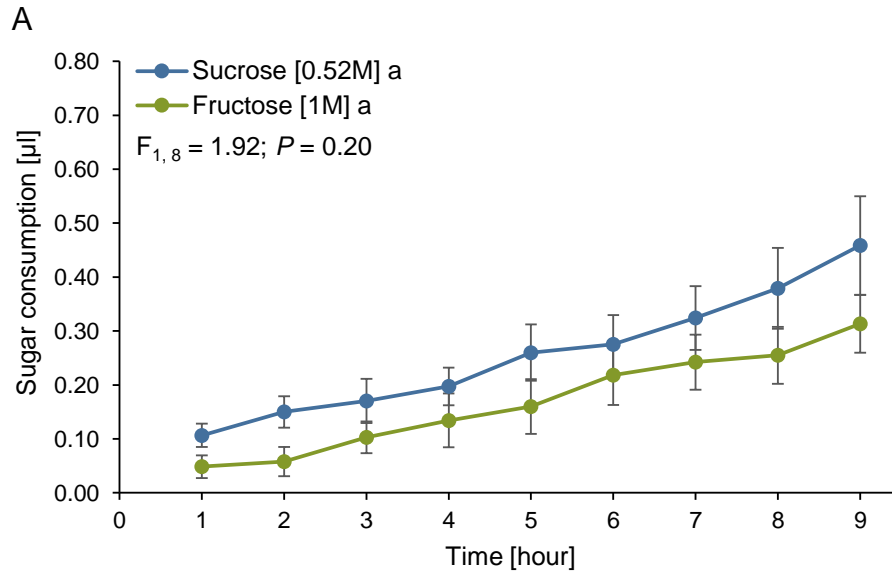
10 **Fig. 2** Mean sugar consumption (n = 5) of different test sugars by feeding-inexperienced adult *Aphidius ervi* parasitoids (n = 15) over a nine-hour
11 period. Sugars were supplied at equal weight concentrations. Error bars represent standard error of the mean. Different letters indicate that sugar
12 consumption was significantly different at the 95 % confidence level (repeated measures ANOVA; $F_{6,28} = 9.39$; $P < 0.001$)

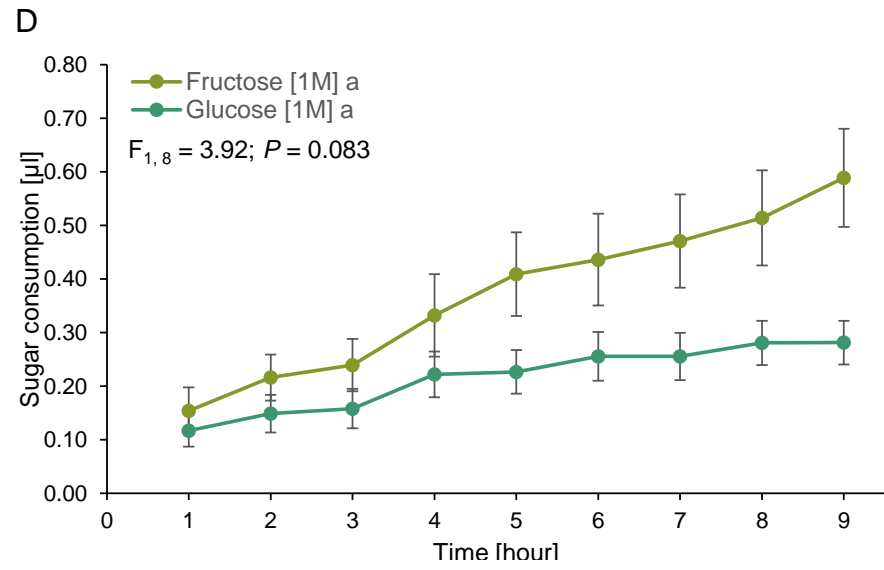
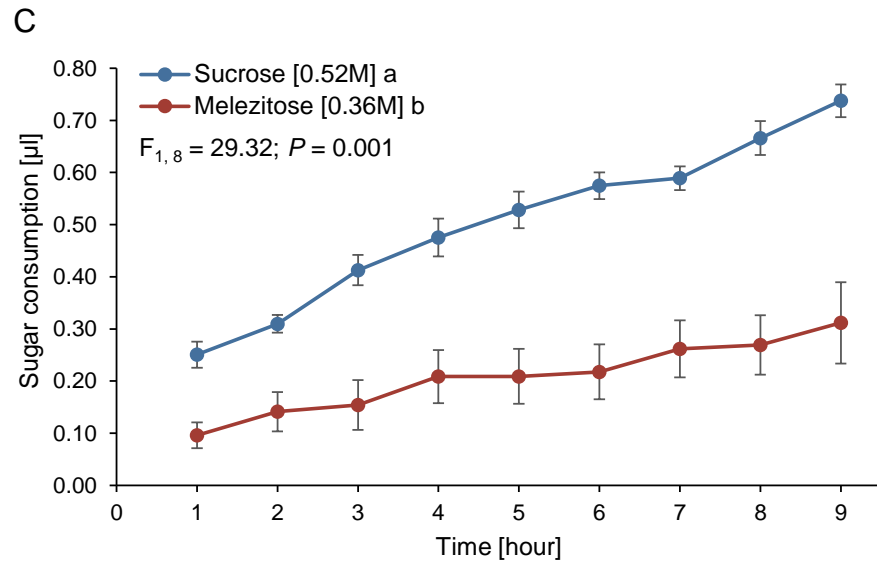


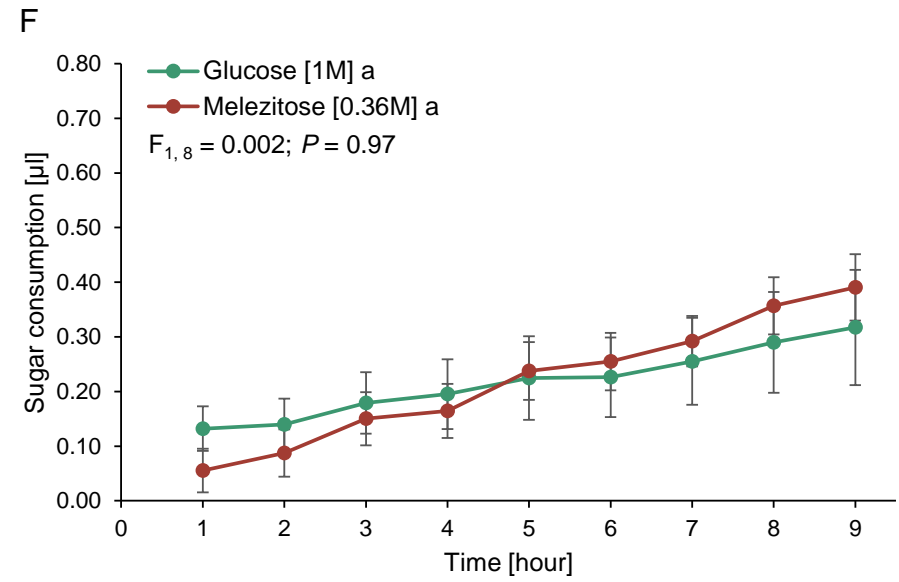
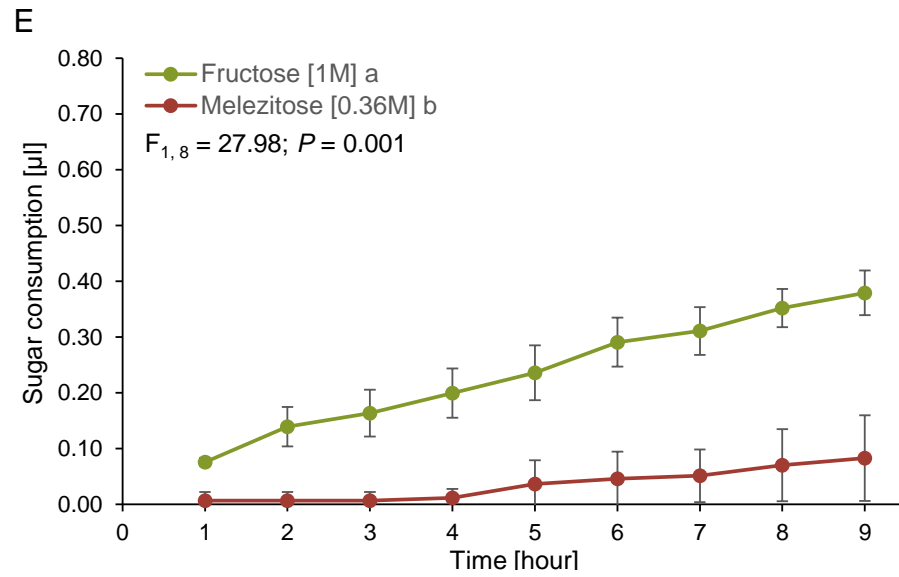
14 **Fig. 3** Survival time curves of adult *Aphidius ervi* parasitoids supplied with different sugars at equal weight concentrations. Capillaries with sugars
15 were replaced daily to avoid microbial contamination. Different letters indicate that treatments were significantly different at the 95 % confidence
16 level (df = 7; $\chi^2 = 329.56$; $P < 0.001$). 75 parasitoids (distributed over five Capillary Feeder assays) were examined for each treatment

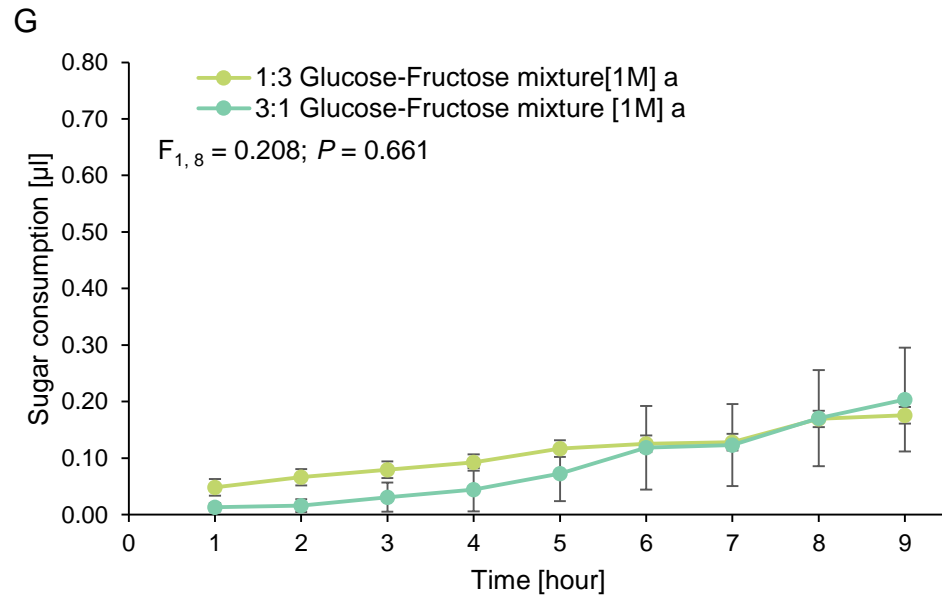


18 **Fig. 4** Mean sugar consumption (n = 5) by feeding-inexperienced adult *Aphidius ervi* parasitoids (n = 15) when different sugar solutions were
19 provided simultaneously over a nine-hour period. Sugars were supplied at equal weight concentrations. Tested combinations were (A) sucrose and
20 fructose, (B) sucrose and glucose, (C) sucrose and melezitose, (D) fructose and glucose, (E) fructose and melezitose, (F) glucose and melezitose,
21 and (G) a 1:3 glucose-fructose mixture and a 3:1 glucose-fructose mixture. Error bars represent standard error of the mean. Different letters indicate
22 that sugar consumption was significantly different at the 95 % confidence level (repeated measures ANOVA)

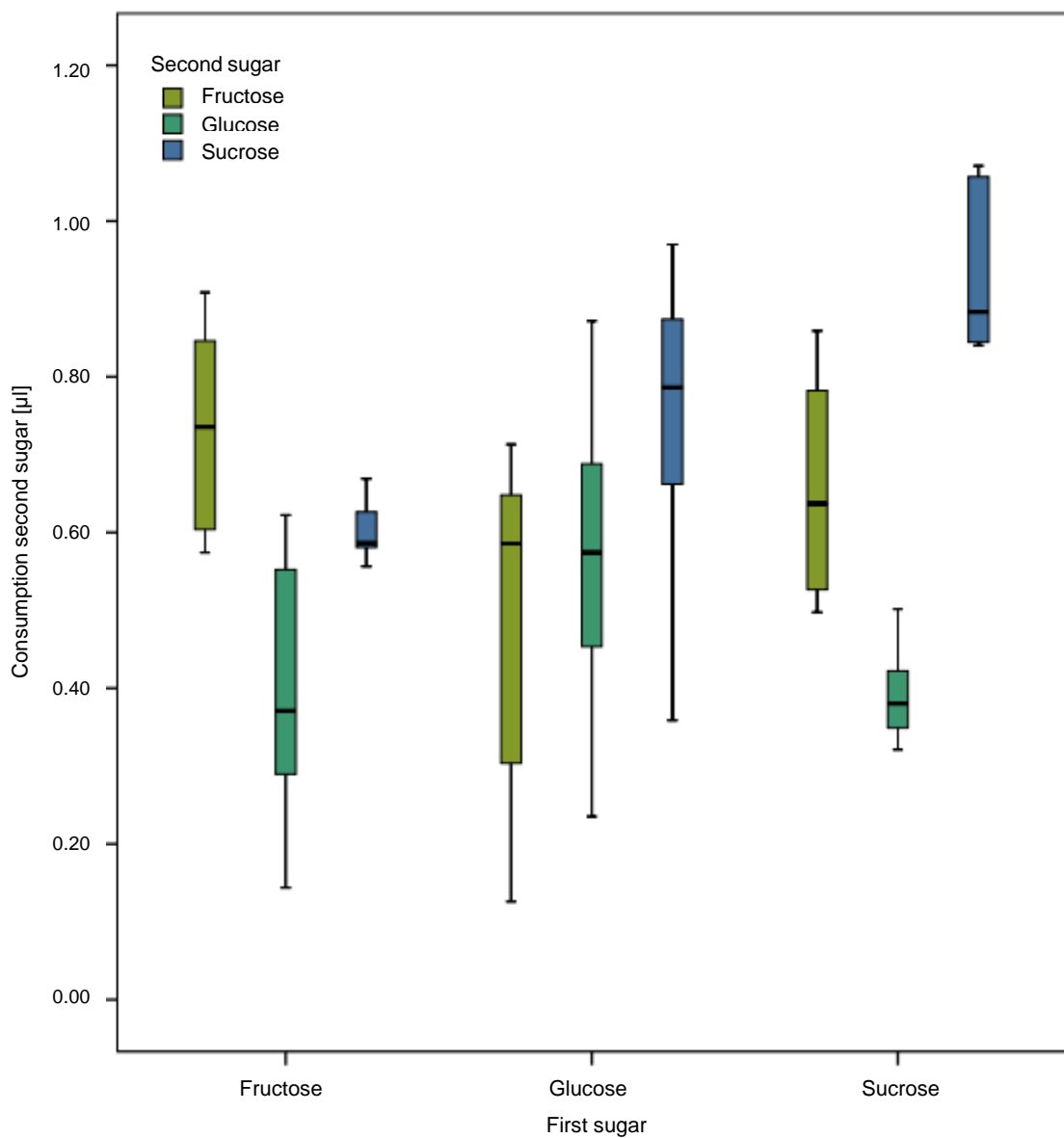








24 **Fig. 5** Effect of previous sugar feeding (x-axis) on subsequent sugar consumption (y-axis)
25 by adult *Aphidius ervi* parasitoids (represented by box plots). Measurements were made
26 nine hours after the start of the experiment. Each experiment was replicated five times
27 using 75 individuals per replicate. Box plots are a graphical representation of the five-
28 number summary, the bottom and top of the box are the 25th and 75th percentile, and the
29 band near the middle of the box is the 50th percentile (i.e. the median). The whiskers at
30 both ends of the box extend to the most extreme data point



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1 TABLES

2 **TABLE 1. SUGARS USED IN THIS STUDY**

Sugar	Glycolytic linkage	Molecular weight	Concentration	Natural source ^a	Supplier
D(-)-fructose	-	180.16 g/mol	1 M	Honeydew, floral and extrafloral nectar	Acros Organics
D(+)-glucose	-	180.16 g/mol	1 M	Honeydew, floral and extrafloral nectar	Sigma
D(+)-melibiose	Galactose- α (1,6)-Glucose	342.30 g/mol	0.53 M	Floral nectar	Sigma
D(+)-melezitose	Glucose- α (1,3)-Fructose- β (2,1) α -Glucose	504.44 g/mol	0.36 M	Honeydew, rare in floral and extrafloral nectar	Sigma
L(+)-rhamnose	-	164.16 g/mol	1.1 M	Extrafloral nectar	VWR
D(+)-sucrose	Glucose- α (1,2) β -Fructose	342.30 g/mol	0.53 M	Honeydew, floral and extrafloral nectar	Sigma
D(+)-trehalose	Glucose- α (1,1) α -Glucose	342.30 g/mol	0.53 M	Honeydew	Sigma

3 ^a Wäckers, 2001