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

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,

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

INTRODUCTION

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

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

In contrast to nectar, which has a primary function in mutualistic interactions, honeydew is primarily a waste product allowing phloem-feeding insects to excrete excess carbohydrates (Wäckers 2005). In a number of cases honeydew has obtained a secondary function in a protective mutualism with tending ants. As ants effectively monopolize honeydew from tended sap feeders, it is typically the honeydew from sap feeders that are not tended by ants that is available to other arthropods. Those honeydew sources are also commonly exploited by parasitoids and predators of the sap feeders. As a consequence, there is usually little benefit to the honeydew producer in having honeydew being palatable or nutritionally suitable. Nevertheless, given the fact that honeydew is often the predominant source of exogenous sugars, it may be expected that predators and parasitoids of honeydew producers would have evolved adaptations to effectively exploit those sugar sources. Indeed, the short labrum found in most parasitoids is well-suited to imbibe highly concentrated sugar solutions like honeydew (Wäckers et al. 2008). Accordingly, it can be expected that they prefer sugars that are commonly available in honeydew and that they would be able to survive better on honeydew sugars than parasitoids that attack hosts that do not produce honeydew. To test this hypothesis we used the solitary aphid parasitoid *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae) as a model organism. *A. ervi* adults rarely engage in host feeding, and mostly feed on honeydew from their aphid hosts (Quicke 2014). First, the feeding behavior and sugar consumption was evaluated for parasitoids exposed to sugars that typically occur in aphid honeydew and those that do not or rarely occur in honeydew. Next, we studied survival of the parasitoids when fed with the different sugars. Finally, choice experiments were conducted to determine sugar preferences and to investigate whether feeding responses to sugars changed after prior feeding experience with the same or other sugars.

MATERIALS AND METHODS

Study Species. Experiments were performed using adults of *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae). *A. ervi* is a solitary endoparasitoid that attacks many aphid species, including several species of economic importance. The larvae develop within the host, and adults feed primarily on aphid honeydew to cover their energetic needs (Quicke 2014). *A. ervi* is widely distributed and is also commonly used for biological control of aphids in greenhouses. To perform the experiments, *A. ervi* mummies were provided by Biobest (Ervi-system®, Westerlo, Belgium). Once received, *A. ervi* mummies were placed in ventilated plexiglass boxes (12 x 12 x 1.5 cm) and kept under controlled conditions (22 °C, 70 % relative humidity and 16:8 h light:dark photoperiod) until adult emergence. Prior to starting the experiments, insects were subjected to a dark period of eight hours. All experiments were performed with feeding-inexperienced adults that were less than 24 hours old.

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

Test Assay. The test assay used was based on the Capillary Feeder (CAFE) described by Ja et al. (2007), with some major adjustments (Fig. 1). Briefly, a plastic insect cage (height: 12.5 cm; diameter: 10.0 cm) was provided with four calibrated glass micropipettes (5.0 µl, Blaubrand Intramark, Wertheim, Germany) filled with 4.0 µl of the tested sugar solution and a mineral oil overlay (1.0 µl) to minimize evaporation. These capillaries were inserted through the lid (at the corners of a square of 4.5 cm x 4.5 cm in the middle of the lid) via truncated 200 µl yellow pipette tips. To meet the water requirements of the parasitoids, a filter paper imbibed with 500 µl sterile demineralized water was put at the bottom of the cage at the start of the experiment, and supplemented with another 500 µl water daily in the longevity experiments. To allow entry of air, the lid of the cage was pierced and covered with a fine mesh (2.5 cm by 2.5 cm; mesh size 0.27 mm x 0.88 mm).

Experiments. A number of experiments were performed using the test assay described above. For each experiment, after being subjected to a dark period of eight hours, 15 adult parasitoids were released in each cage, and five replicates were included. Each experiment also included an identical CAFE chamber without parasitoids to determine evaporative losses which were subtracted from experimental readings. All experiments were conducted under controlled conditions of 22 °C, 70 % relative humidity and with a 16:8 h light:dark cycle, starting with the light period. First, sugar consumption and effect on parasitoid longevity was assessed for each tested sugar (Table 1). Sugar consumption was measured every hour over a total of nine hours during the light period. To accurately measure the amount of sugar consumed from each capillary, a digital caiper (Mitutoyo Digimatic, resolution 0.01 mm) was used. Subsequently, parasitoid longevity was assessed by counting and removing dead individuals daily. To avoid microbial contamination of the sugars, capillaries were replaced daily. Next, a series of choice experiments was conducted to investigate feeding behavior when insects are provided a choice between different sugar solutions. More specifically, insects were presented with two different sugar solutions through two pairs of truncated pipette tips arranged in an alternating pattern, and sugar consumption was assessed over a period of nine hours as described above. Finally, the effect of previous sugar intake on the subsequent feeding response to the same and other sugars was investigated by first allowing inexperienced parasitoids to contact either fructose, glucose or sucrose for 5 s. Afterwards, insects were subjected to the sugar consumption assay where either fructose, glucose or sucrose was supplied ad libitum, and sugar consumption was assessed over a nine hour period as described earlier.

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

RESULTS

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

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

Sugar Intake When Different Sugars Are Provided Simultaneously. In order to further investigate the feeding behavior of *A. ervi*, sugar intake was assessed over a nine-hour period when individuals were given the choice between two sugars. Experiments were performed for the four sugars that substantially prolonged parasitoid longevity (i.e. fructose, glucose, melezitose and sucrose) (Fig. 4). The results showed that sucrose intake was always higher than intake of melezitose. This difference was even more pronounced when comparing sucrose to glucose, but not significant when putting sucrose against fructose. Fructose consumption exceeded feeding on melezitose. When a choice was given between fructose and glucose, the former was consumed at almost double the volume, albeit that this difference was only marginally significant ($P = 0.083$) (Fig. 4D). When a choice was given between a 3:1 and a 1:3 fructose-glucose mixture (Fig. 4G), no significant differences were observed.

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

DISCUSSION

In many ecosystems honeydew represents the primary source of exogenous sugars and therefore it constitutes a crucial food to a broad range of insects (Wäckers 2005). However, in comparison to other sugar sources, such as nectar, honeydew is often nutritionally inferior, although there is considerable variation in the quality of honeydew depending on the honeydew producer and its host plant (Wäckers et al. 2008). Assuming that sugar-feeding insects have evolved adaptations to those food sources that readily occur within their habitat, we hypothesized that aphid parasitoids are adapted to those sugars that are overrepresented in aphid honeydew relative to open nectar sources (fructose, melezitose, trehalose, and sucrose) and to a lesser extent to those sugars that are underrepresented (glucose) (Kloft et al. 1985; Fischer and Shingleton 2001; Hogervorst et al. 2003; 2007; Fischer et al. 2005).

To test this hypothesis, we investigated sugar consumption and feeding behavior in the aphid parasitoid *A. ervi* when exposed to (choices of) individual 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 (only sporadically found in honeydew) was considerably lower when monitoring sugar consumption over a period of nine hours. The monosaccharide rhamnose, which does not normally occur in honeydew (Wäckers 2001), 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. Interestingly, a pre-exposure to sucrose or fructose significantly reduced subsequent intake of glucose (at least at the short term), suggesting an acquired distaste for glucose after being exposed to highly preferred sugars such as sucrose and fructose. Makatiani et al. (2014) first demonstrated this phenomenon, showing that the braconid parasitoid *Microplitis croceipes* exhibits an acquired distaste for maltose or fructose following a brief exposure to other sugars. The results in our study suggest that *A. ervi* can discriminate between different sugars and exhibits a strong preference for sucrose and fructose. These observations may reflect the fact that both sucrose and fructose are often the most predominant sugars in many honeydews, whereas glucose is often underrepresented (Kloft et al. 1985; Fischer and Shingleton 2001; Hogervorst et al. 2003; 2007; Fischer et al. 2005). For example, Hogervorst et al. (2007) reported that the sugar composition of honeydew samples from five aphid species feeding on potato or wheat plants constituted on average of 35.4 % sucrose and 36.1 % fructose, while they only contained 7.4 % glucose.

Previous studies have shown that innate gustatory sugar responses of parasitoids are often positively correlated with the nutritional quality of the sugar in terms of parasitoid longevity and realized reproductive fitness (Wäckers 1999, 2001; Luo et al. 2013; Makatiani et al. 2014). However, despite the weak innate gustatory response to glucose and the acquired distaste to this sugar, *A. ervi* parasitoids lived as long on glucose as on sucrose, fructose and melezitose. The reason for this apparent mismatch between the gustatory response and nutritional suitability in the case of glucose remains to be explained. The average lifespan on these sugars was 7.5 to 9.6 days compared to 2.9 days for the food-deprived control. These results are somewhat lower than those reported for some other hymenopteran parasitoids with mean longevity ranging from 3.7 to 4.5 days for food-deprived wasps and 11.5 to 13.8 days for sugar-fed ones (Azzouz et al. 2004). Sucrose, fructose and glucose tend to be nutritionally most suitable, whereas aphid-synthesized honeydew sugars such as melezitose and trehalose tend to represent a lower nutritional value (Wäckers 2005). However, in contrast to studies on other parasitoids (Wäckers 2001; Zoebelein 1955), we found that *A. ervi* can survive equally well on the common honeydew-sugar melezitose as on dominant nectar sugars such as sucrose, fructose and glucose. This could be seen as further evidence that this aphid parasitoid has adapted to the exploitation of aphid produced honeydew. On the other hand, wasps only survived an average of 4 days on another honeydew sugar, trehalose, despite showing a strong gustatory response to this sugar (comparable to other typical honeydew sugars). A similar poor performance on trehalose has also been reported for other braconid wasps like *Anaphes iole* and *Cotesia glomerata* and the ichneumon wasp *Diadegma semiclausum* (Williams and Roane 2007; Wäckers 2001; Winkler et al. 2005). Although *A. ervi* showed a strong feeding response to trehalose, the less pronounced survival on this sugar may be explained by the lack of a suitable enzyme to digest trehalose, which is an alpha-linked disaccharide of two glucose units. The fact that the parasitoids perform well on other sugars with an α-glucosidic bond such as sucrose and melezitose indicates the presence of an α-glucosidase, which may not act on trehalose. This is in accordance with the specific α-glucosidases reported in honeybees, having a high activity in hydrolyzing sucrose and maltose, without acting on trehalose (Huber and Mathison, 1976). The generally poor performance of parasitoids on trehalose, which is a major haemolymph sugar in insects, may also explain why host feeding by parasitoids often only has a marginal impact on longevity (Jervis and Kidd 1986). Gustatory response and nutritional value were again aligned in the case of rhamnose, where we see no benefits in terms of longevity, and an absence of a feeding response. Even when consumed, this sugar does not serve as an energy source (Wäckers 2001). Also for other parasitoids such as *A. iole* and *C. glomerata*, rhamnose has been shown to be nutritionally unsuitable (Wäckers 1999, 2001; Beach et al. 2003; Williams and Roane 2007).

Altogether, our study shows that *A. ervi* adults are well adapted to optimize the exploitation of honeydew as a sugar source to cover their energetic needs. We have shown that *A. ervi* adults prefer sugars that dominate aphid honeydew and when feeding on these sugars can substantially increase their longevity. Whereas this work has broadened our view on how *A. ervi* copes with honeydew as a sugar source, further work is needed using other *Aphidius* species, or more generally other parasitoids of honeydew-producing insects to generalize our results. Additionally, it remains to be investigated whether the established trends also hold for other important life history parameters such as fecundity.

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FIGURES

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

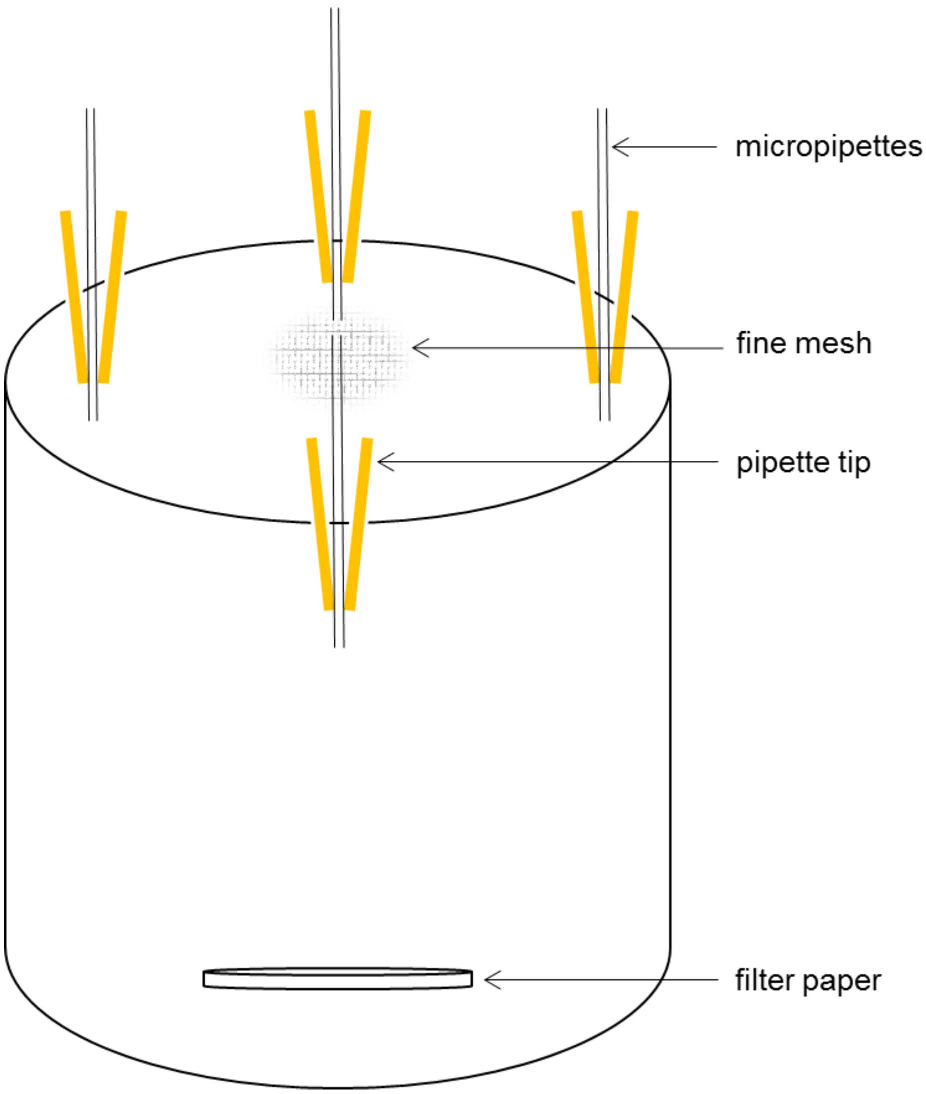
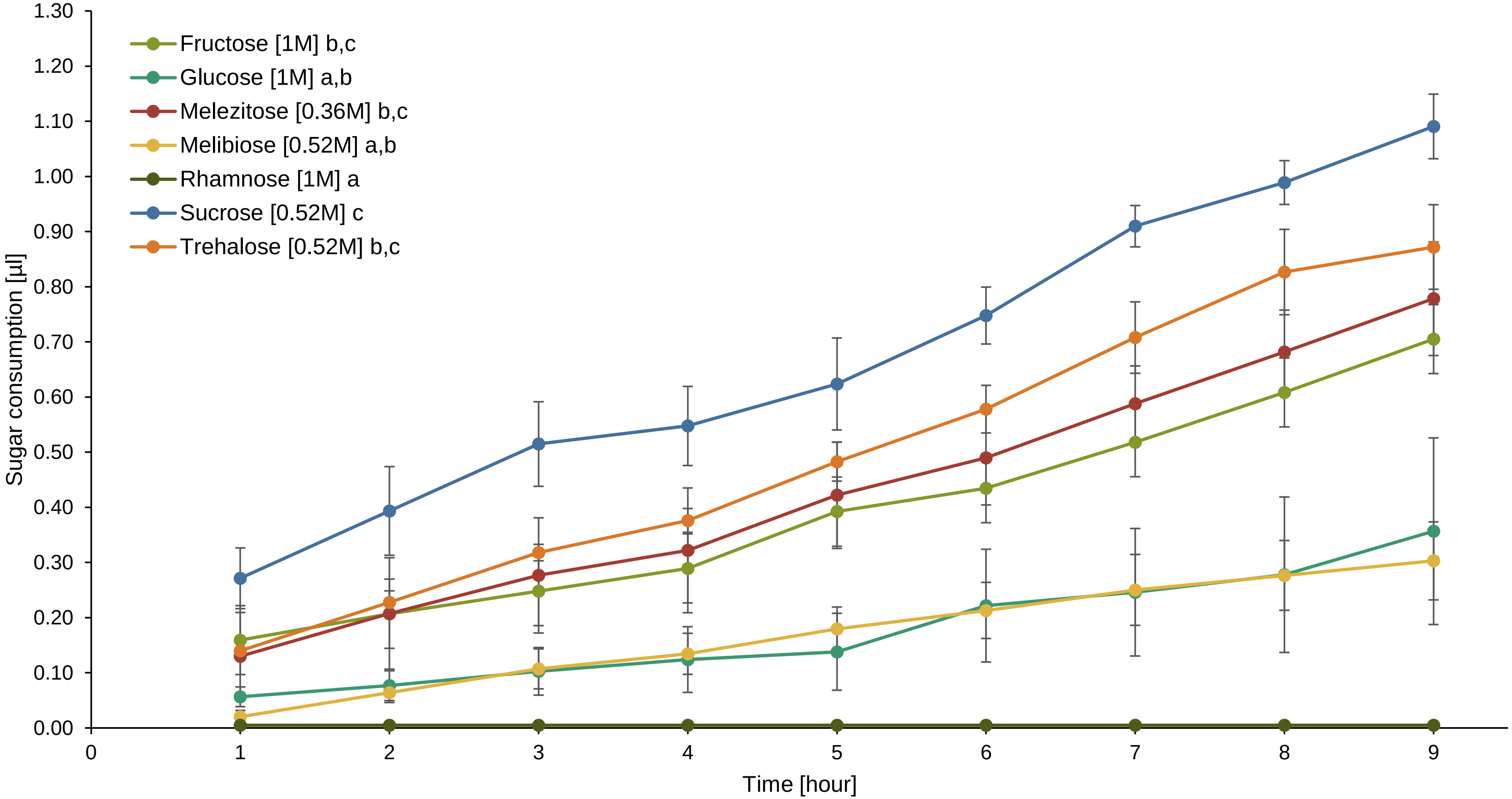


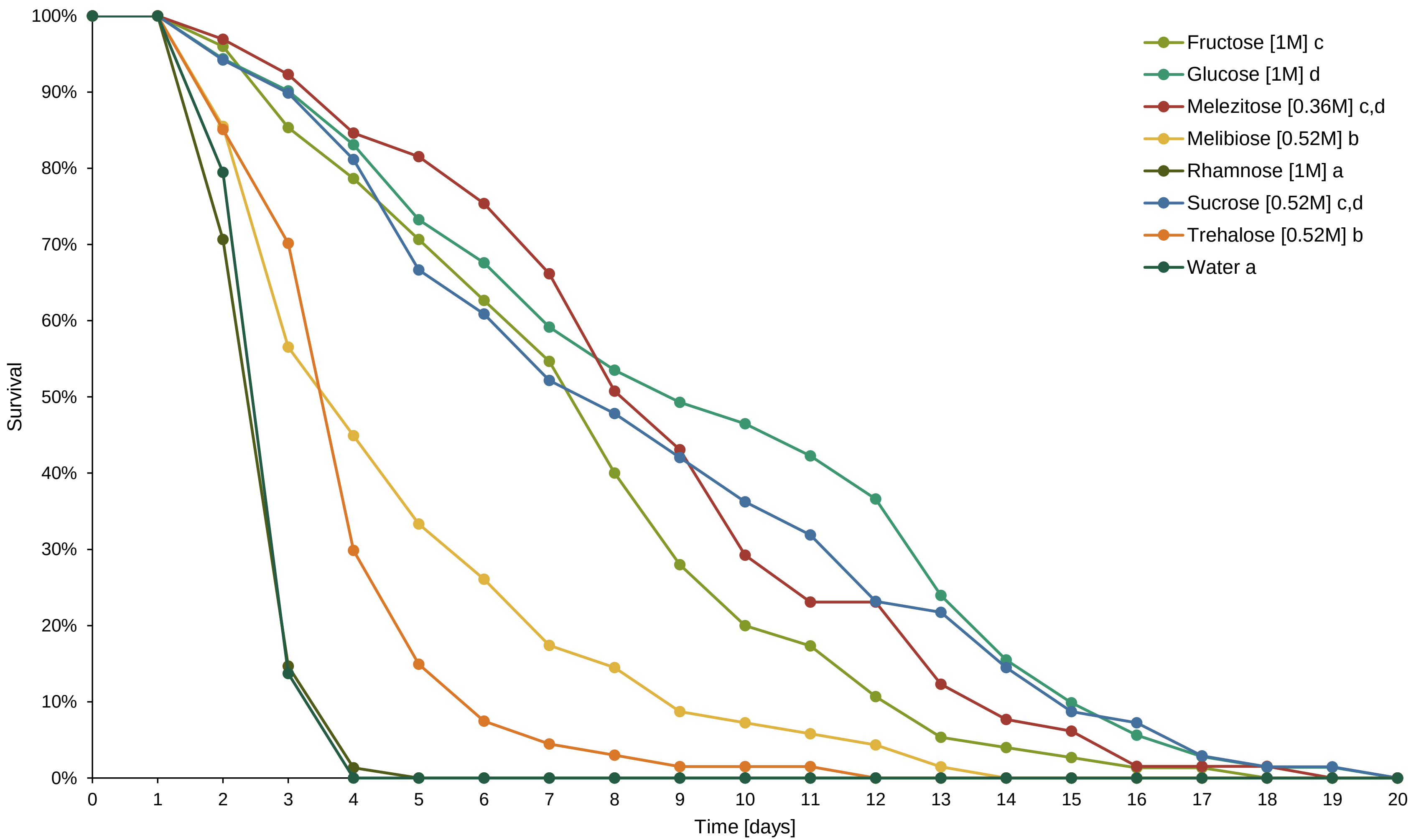
Fig. 2 Mean sugar consumption (n = 5) of different test sugars by feeding-inexperienced adult *Aphidius ervi* parasitoids (n = 15) over a nine-hour period. Sugars were supplied at equal weight concentrations. Error bars represent standard error of the mean. Different letters indicate that sugar consumption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



1.30
1.20
1.10
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Sugar consumption [µl]
Fructose [1M] b,c
Glucose [1M] a,b
Melezitose [0.36M] b,c
Melibiose [0.52M] a,b
Rhamnose [1M] a
Sucrose [0.52M] c
Trehalose [0.52M] b,c
0
1
2
3
4
5
6
7
8
9
Time [hour]

13

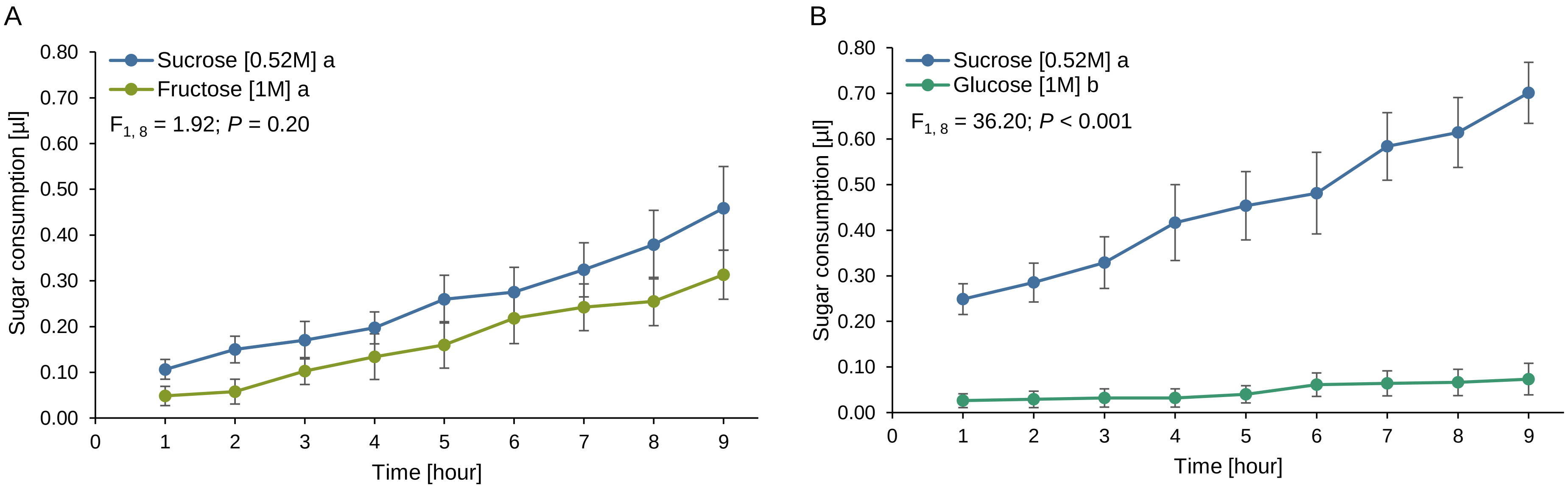
Fig. 3 Survival time curves of adult *Aphidius ervi* parasitoids supplied with different sugars at equal weight concentrations. Capillaries with sugars were replaced daily to avoid microbial contamination. Different letters indicate that treatments were significantly different at the 95 % confidence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



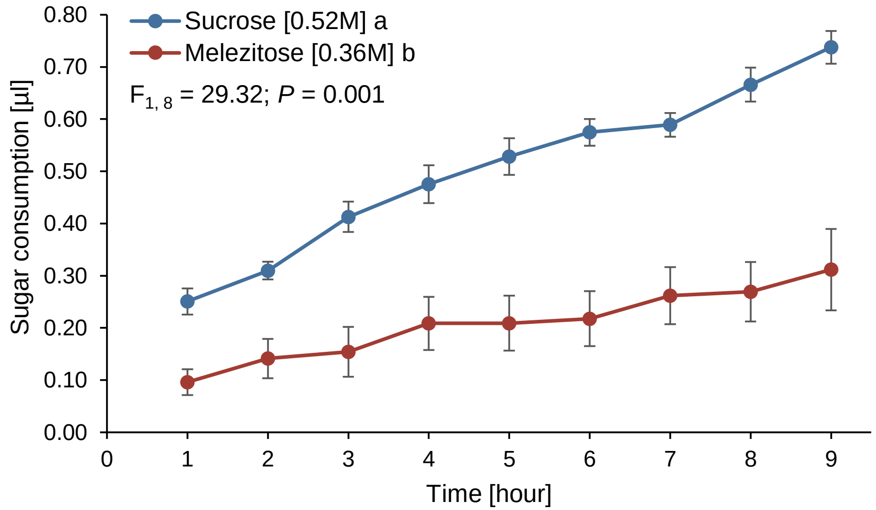
Survival
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
Time [days]
Fructose [1M] c
Glucose [1M] d
Melezitose [0.36M] c,d
Melibiose [0.52M] b
Rhamnose [1M] a
Sucrose [0.52M] c,d
Trehalose [0.52M] b
Water a

17

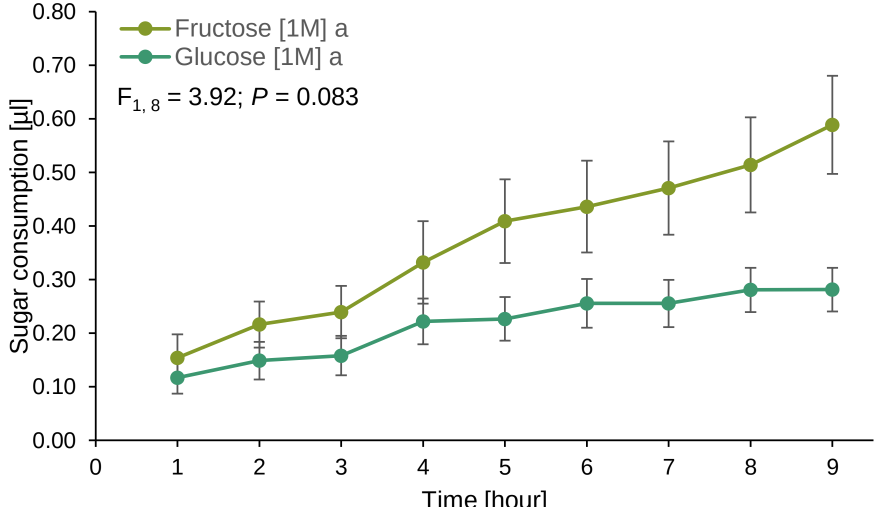
Fig. 4 Mean sugar consumption (n = 5) by feeding-inexperienced adult *Aphidius ervi* parasitoids (n = 15) when different sugar solutions were provided simultaneously over a nine-hour period. Sugars were supplied at equal weight concentrations. Tested combinations were (A) sucrose and fructose, (B) sucrose and glucose, (C) sucrose and melezitose, (D) fructose and glucose, (E) fructose and melezitose, (F) glucose and melezitose, 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 that sugar consumption was significantly different at the 95 % confidence level (repeated measures ANOVA)

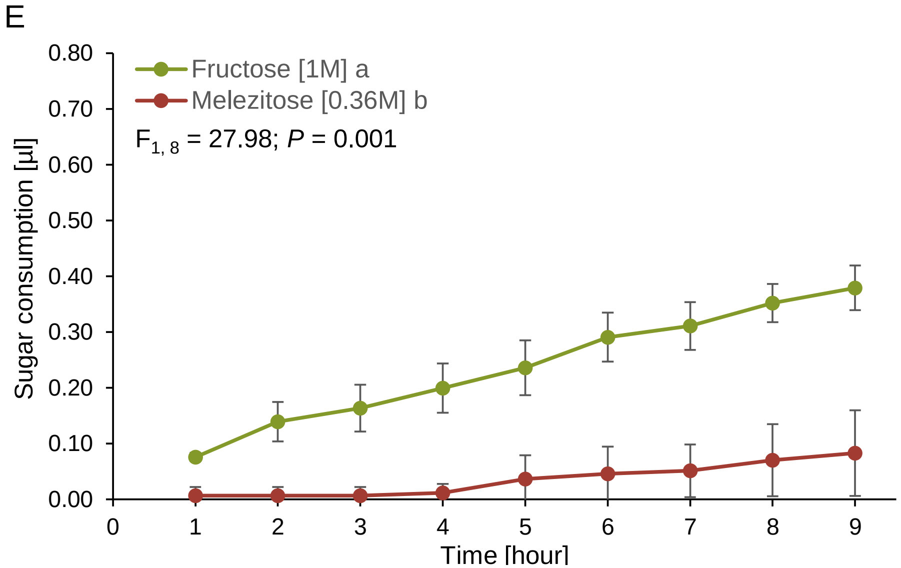


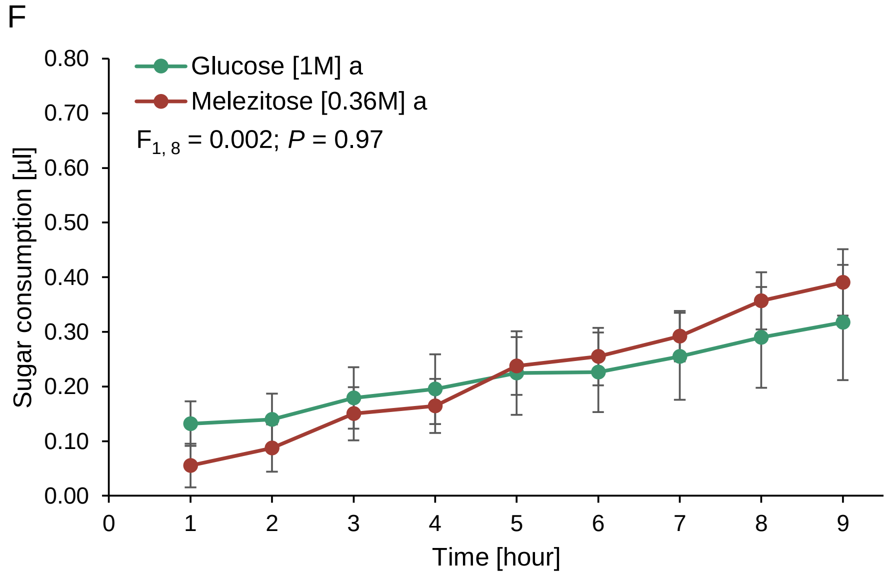
C


Sucrose [0.52M] a
Melezitose [0.36M] b
$F_{1, 8}$ = 29.32; P = 0.001
Sugar consumption [µl]
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0 1 2 3 4 5 6 7 8 9
Time [hour]

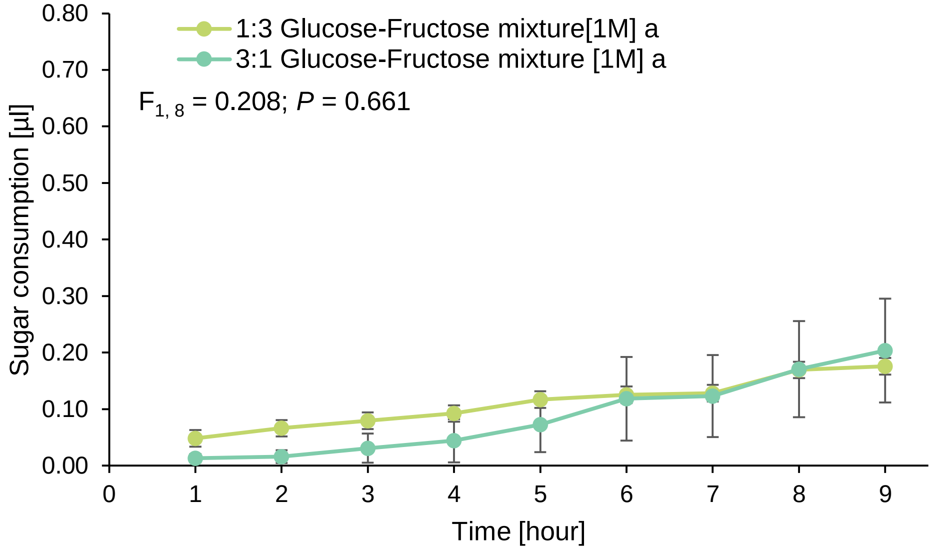
D


Fructose [1M] a
Glucose [1M] a
$F_{1, 8}$ = 3.92; P = 0.083
Sugar consumption [µl]
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0 1 2 3 4 5 6 7 8 9


E
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Sugar consumption [µl]
Fructose [1M] a
Melezitose [0.36M] b
F1, 8 = 27.98; P = 0.001
0 1 2 3 4 5 6 7 8 9
Time [hour]


F
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Sugar consumption [µl]
Glucose [1M] a
Melezitose [0.36M] a
F1, 8 = 0.002; P = 0.97
0 1 2 3 4 5 6 7 8 9
Time [hour]

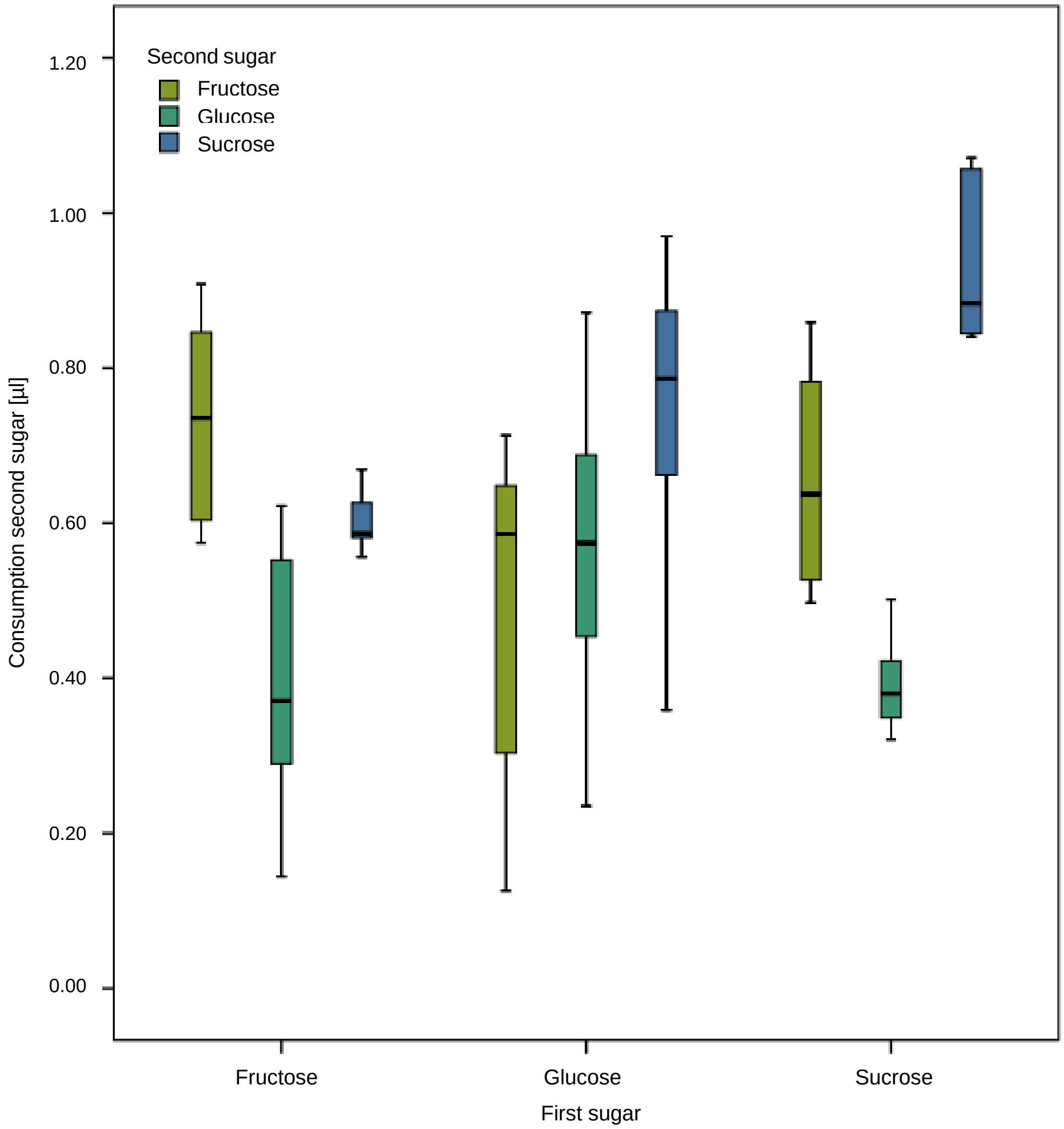
G



1:3 Glucose-Fructose mixture[1M] a
3:1 Glucose-Fructose mixture [1M] a
F1, 8 = 0.208; P = 0.661
Sugar consumption [µl]
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
1
2
3
4
5
6
7
8
9
Time [hour]

23

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



TABLES

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

[a] Wäckers, 2001