This is a pre-print of an article published in *Basic and Applied Ecology*. The final authenticated version is available online at: https://doi.org/10.1016/j.baae.2018.04.003

Cite as: Tena, A., Senft, M., Desneux, N., Dregni, J., & Heimpel, G. E. (2018). The influence of aphid-produced honeydew on parasitoid fitness and nutritional state: A comparative study. *Basic and Applied Ecology*, *29*, 55-68.

The influence of aphid-produced honeydew on parasitoid fitness and nutritional state: a comparative study

Alejandro Tena[a*], Matthias Senft[b], Nicolas Desneux[c], Jonathan Dregni[d], George E. Heimpel[d*]

[a]Instituto Valenciano de Investigaciones Agrarias (IVIA), Unidad Asociada de Entomología UJI–IVIA, Centro de Protección Vegetal y Biotecnología, Spain

[b]Terrestrial Ecology Research Group, Department of Ecology and Ecosystem Management, School of Life Sciences Weihenstephan, Technische Universität München, Hans-Carl-von-Carlowitz-Platz 2, 85354 Freising, Germany

[c]INRA (French National Institute for Agricultural Research), Univ. Nice Sophia Antipolis, CNRS, UMR 1355-7254, Institut Sophia Agrobiotech, 06903, Sophia Antipolis, France.

[d]Department of Entomology, University of Minnesota, St. Paul, Minnesota 55108.

* Corresponding authors: Dr. Alejandro Tena.

email: atena@ivia.es

Dr. George Heimpel

email: heimp001@umn.edu

Abstract

Honeydew is a sugar-rich resource excreted by many hemipteran species and is a key food source for other insect species such as ants and parasitoid wasps. Here, we evaluated the nutritional value of 14 honeydews excreted by 13 aphid species for the generalist aphid parasitoid *Lysiphlebus testaceipes* to test a series of hypotheses concerning variation in the nutritional value of honeydew. There was a positive correlation between the body sugar content of honeydew-fed parasitoids and their longevity. This information is valuable for biological control researchers because it demonstrates that the nutritional state of honeydew-fed parasitoids in the wild can indicate their fitness, independently of the honeydew source they have fed on.

Although the carbohydrate content and longevity of *L. testaceipes* differed greatly among the different honeydews, we did not find a significant effect of aphid or host plant phylogeny on these traits. This result suggests that honeydew is evolutionarily labile and may be particularly subject to ecological selection pressures. This becomes apparent when considering host aphid suitability: *Schizaphis graminum,* one of the most suitable and commonly used hosts of *L. testaceipes,* produced honeydew of the poorest quality for the parasitoid whereas *Uroleucon sonchi*, one of the few aphids tested that cannot be parasitized by *L. testaceipes*, excreted the honeydew with the highest nutritional value. These data are consistent with the hypothesis that hemipterans are subject to selection pressure to minimize honeydew quality for the parasitoids that attack them.

Keywords: Hymenoptera; Aphididae; Nutritional ecology; Biological control; carbohydrates; *Lysiphlebus testaceipes*

Highlights

- The carbohydrate content and longevity of *Lysiphlebus testaceipes* differed greatly when it fed on 14 different honeydews.
- There was not a significant effect of aphid or host plant phylogeny on the quality of honeydew for parasitoids.
- There was a positive correlation between the body sugar content of honeydew-fed parasitoids and their longevity.
- The nutritional state of honeydew-fed parasitoids can provide information on their fitness, independently of the sugar source they have fed on.

62 •

63 ## Introduction

64 Honeydew is a sugar-rich resource excreted by many hemipteran species, attracting insects
65 belonging at least to 49 families, including bees and natural enemies (Zöebelein, 1956a; b;
66 Lundgren, 2009). Many adults of dipteran and hymenopteran parasitoids depend on honeydew
67 as a food source, often the most common source of available sugar in agroecosytems (Wäckers,
68 van Rijn, & Heimpel, 2008; Lundgren, 2009; Tena, Wäckers, Heimpel, Urbaneja, & Pekas,
69 2016). A great number of ant species, which tend and protect hemipterans, also feed on and
70 would not survive without honeydew (Way, 1963; Styrsky & Eubanks, 2007; Pekas, Tena,

71 Aguilar, & Garcia-Marí, 2011). However, the quality of honeydew as a diet for insects is highly

72 variable (Avidov, Balshin, & Gerson, 1970; Völkl, Woodring, Fischer, Lorenz, & Hoffmann,
73 1999; Wäckers et al., 2008; Tena, Llácer, & Urbaneja, 2013; Tena et al., 2016).

74 Debate on the quality of honeydew as a diet for insects has been ongoing since before the
75 publications of Zöebelein (1956a; b). Honeydew is considered to be a mixture of plant-derived
76 compounds that vary with several biotic and abiotic factors (Maltais & Auclair, 1962; Fischer &
77 Shingleton, 2001; Wäckers et al., 2008). However, the composition of honeydew has a genetic
78 component with high heritability (Völkl et al. 1999; Wäckers, 2000). Consequently, it is
79 expected that evolution has also shaped the quality of honeydew as carbohydrate source for
80 insects. On the one hand, high-quality honeydew may be favored to attract and retain
81 mutualistic ant species that tend and protect hemipteran colonies, but on the other hand, it may
82 attract and retain natural enemies and increase their fitness with potential negative consequences
83 on hemipteran populations if ants are absent (Evans & England 1996; Wäckers et al., 2008;

84 Tena et al., 2016). In these cases, low-quality honeydew may be favored to limit honeydew
85 feeding by natural enemies (Wäckers, 2000; Wäckers et al., 2008). Here, we determine whether
86 aphid honeydew varies in quality for the generalist aphid parasitoid *Lysiphlebus testaceipes* and
87 also whether the quality exhibits a phylogenetic signal. To do this, we categorized the quality of
88 honeydews produced by 13 aphid species as food sources for *L. testaceipes* by measuring: i) the
89 content of fructose and total sugars of the honeydews and ii) their effect on the longevity and
90 nutritional state of *L. testaceipes* females fed on these honeydews.

91 *Lysiphlebus testaceipes* is a solitary koinobiont and pro-ovigenic (or with a very high ovigenic
92 index) endoparasitoid of aphids (van Steenis, 1994). It does host feed (Desneux, Barta,
93 Delebeque, & Heimpel 2009;) and parasitizes more than 100 aphid species in various genera,
94 tribes and subfamilies (Pike et al., 2000). It is native to North America where it is an effective
95 natural enemy of the greenbug, *Schizaphis graminum* (Royer, Pendleton, Elliott, & Giles, 2015).
96 The parasitoid was introduced to Europe (Starý, Lyon, & Leclant, 1988) where it has achieved
97 some success as a biological control agent (Costa & Starý, 1988; Starý, Lyon, & Leclant, 1988)
98 and also has been found to attack numerous aphid species in various ecosystems (Starý et
99 al.,1988; Starý, Lumbierres, & Pons, 2005; Mitrović et al., 2013; Zikic et al., 2015). Therefore,
100 this parasitoid likely feeds on honeydews from various aphid species that differ greatly in their
101 quality as a sugar source. Moreover, some of these aphids feed on different host plants
102 (Holman, 2009) and therefore may produce different honeydew quality for insects depending on
103 the plant. *L. testaceipes* is thus a highly suitable model for a comparison of the quality of aphid
104 honeydews on parasitoid fitness.

105 To determine whether parasitoids feed on honeydew in the field, as well as to evaluate their
106 nutritional state, researchers have used high-performance liquid chromatography (HPLC), or
107 anthrone tests over the last decade (Casas et al., 2003; Steppuhn & Wäckers, 2004; Heimpel et
108 al., 2004; Lee, Andow, & Heimpel, 2006; Hogervorst, Wäckers, & Romeis, 2007a; Winkler,
109 Wäckers, & Pinto, 2009; Tena, Pekas, Wäckers, & Urbaneja, 2013; Dieckhoff, Theobald,
110 Wäckers, & Heimpel, 2014; Tena, Pekas, Cano, Wäckers, & Urbaneja, 2015; Calabuig et al.,

111 2015). This information is valuable for biological control researchers, especially when
112 parasitoid carbohydrate contents are low; as this indicates that parasitoids are sugar-limited and
113 that providing a sugar source may increase their fitness and biological control potential
114 (Heimpel & Jervis, 2005; Tena et al., 2016). However, an observation of high energy reserves
115 may be misleading if low-quality carbohydrates for insects have been ingested. This is
116 especially important when multiple hemipteran species with different honeydew qualities are
117 present at a given field site (Pekas et al., 2011; Tena, Hoddle, & Hoddle, 2013; Tena, Llácer, &
118 Urbaneja, 2013; Tena et al., 2013).

119 Here, we used anthrone tests to quantify the carbohydrate levels (fructose, other sugars,
120 glycogen and total carbohydrates) of *L. testaceipes* females fed on different honeydews, and we
121 determined i) whether the sugar content of different honeydews is correlated with the nutritional
122 state and longevity of parasitoids fed on these honeydews; and ii) whether the nutritional state
123 of parasitoids fed on different honeydews is correlated with their longevity. Positive correlations
124 would indicate that carbohydrate level of honeydews and/or parasitoids reflect also the fitness of
125 honeydew-fed parasitoids.

126 ## Materials and methods

127 ### *Insect colonies*

128 Our culture of *L. testaceipes* was initiated with individuals collected from parasitized
129 soybean aphids, *Aphis glycines* in Minnesota, USA in the year preceding these studies and had
130 thus been in culture for approximately 25 generations on soybean aphid. Just prior to these
131 studies, the parasitoid population was transferred to *Schizaphis graminum* on barley at 25 °C,
132 65% relative humidity (RH) and 16:8 h light:dark (L:D). This *S. graminum* colony had been
133 held in the laboratory for 10 years and was collected from a rye field in St. Paul, Minnesota,
134 USA. The other aphid colonies were similarly collected from surrounding field settings and kept
135 for between 1 and 10 years. All were kept as asexually reproducing populations. Parasitized
136 aphids that had died and contained *L. testaceipes* pupae ('mummies') were removed from barley

137 leaves and kept in plastic tubes (8 cm height x 2.5 cm diameter) until the emergence of adult
138 parasitoids. Between 40 and 60 parasitoid mummies were introduced per tube, while no food or
139 water was supplied. Newly emerged parasitoids were collected at 9:00 and 17:00, but only the
140 former were used in the experiments. Therefore, females used for all experiments were less than
141 16 hours old, had been in contact with males, and had never been fed or been in contact with
142 living aphids. These females were placed individually into glass vials (3.0 cm height and 0.8 cm
143 diameter) sealed with a moistened cotton plug and randomly assigned to the different treatments
144 (see below).

145 Thirteen aphid species were tested, all of which were reared at the Entomology
146 Department of the University of Minnesota on their respective host plants at 25 °C, 65% relative
147 humidity (RH) and 16:8 h light:dark (L:D). Table 1 shows the aphid species, host plants and
148 sample sizes for each assay. *Aphis gossypii* was fed on two plants: cotton (*Gossypium hirsutum*)
149 and common milkweed (*Asclepias syriaca*). Thus, we obtained fourteen different honeydews
150 from 13 aphid species. These aphid species were chosen because they cover a broad
151 phylogenetic range of aphids within Aphididae. More information on the aphids used in this
152 study can be found in Blackman and Eastop (2000; 2008).

153 ***Honeydew collection***

154 To collect honeydew, we followed a protocol similar to the one described by Tena et al. (2013).
155 Pieces of Parafilm© were placed below aphid colonies on the different infested plants such that
156 honeydew excreted by the aphids accumulated on the Parafilm©. The next day, the Parafilm©
157 containing droplets of honeydew was collected, placed in a Petri dish (2.5 cm height and 9 cm
158 diameter) with a piece of moistened paper and frozen until further use (Hogervorst et al., 2007a;
159 Tena et al., 2013).

160 ***Adult survival***

161 To assess the influence of continuous access to honeydew on *L. testaceipes* longevity, a piece of
162 Parafilm© with droplets of honeydew prepared as described above was provided. Vials were

checked at 8:00 and 18:00 daily to determine parasitoid survivorship. Treatments with only water and with honey streaked lightly onto the wall of the vial plus water were used as negative and positive control diets, respectively. Sugar sources were replaced every two days to prevent crystallization and desiccation but honeydew excreted by *R. padi* was renewed daily because sooty mold was observed after 24 hours in preliminary assays. Cotton was moistened with water twice daily after checking the survival. Parasitoids that were lost or succumbed to accidental deaths were excluded from the analyses.

Honeydew analyses

Cold and hot anthrone tests were used to determine the content of fructose and total sugars present in the honeydews, respectively. For this, Parafilm© with accumulated honeydew was dried in an oven for 24 to 48 hours at 40 °C and dried honeydew was collected into 0.6 mL microcentrifuge tubes and dissolved into 25% ethanol at a concentration of 1 mg/mL. The cold anthrone test was done on 50 µL of each dissolved honeydew sample placed into separate 1.5 mL centrifuge tubes into which 950 µL anthrone solution was added (anthrone solution was prepared as described below). Anthrone was also added to the sucrose standard tubes so that each contained a final volume of 1000 µL. All sample and standard tubes were vortexed and then left to incubate at room temperature for 1.5 hours. The same procedure was followed for the hot anthrone test for sugars but samples and standards were incubated in a dry bath at 90 °C for 12 minutes after adding the anthrone solution. Samples and standards were then cooled on ice. Incubated samples and standards from both tests were vortexed and placed in a 96-well plate. Three 200 µL replicates of each sample were placed in separate wells. Absorbance of samples and standards were recorded at 620 nm. The absorbance was compared to absorbance values of known fructose or sucrose standards with two replicates per read.

In addition to the contents of fructose and total sugars obtained with the anthrone tests, we also used the fructose:total sugars ratio to estimate the relative fructose content of the honeydews.

189 ***Parasitoid nutritional analyses***

190 Living parasitoids were frozen (-80 °C) at 9:00 am two days after they were exposed to the
191 nutritional treatment. The hind tibia length was measured as a proxy for parasitoid size before
192 the carbohydrate content was analyzed since parasitoid size was likely to affect nutrient storage
193 capacity (Briegel, 1990; Olson, Fadamiro, Lundgren, & Heimpel, 2000). Parasitoids were rinsed
194 by vortexing individually for 10 sec in 1 mL warm (60 °C) autoclaved deionized water in a 1.5
195 mL centrifuge tube. Parasitoids were then carefully transferred to a new 1.5 mL centrifuge tube
196 and kept on ice.

197 The carbohydrate content of parasitoids, in particular fructose, total sugars and glycogen
198 were analyzed using a quantitative anthrone assay (modified after Olson et al., 2000). After
199 adding 5 µL sodium sulfate 2% (w/v) to the centrifuge tube, the parasitoids were crushed using
200 autoclaved glass pestles. Pestles were rinsed with 45 µL of methanol:chloroform (2:1), which
201 was added to the solution. After centrifuging at 13,000 rpm for 2 min, the supernatant,
202 containing all soluble sugars, was transferred to 100 mm glass test tubes. The white precipitate
203 containing insoluble high molecular sugars (e.g. glycogen) was kept on ice until used for the
204 glycogen assay.

205 A cold anthrone test was used to analyze the fructose content. The supernatant was
206 boiled at 90 °C for approximately 1.5 min in a dry bath incubator until all of the liquid was
207 gone. After cooling on ice for 15 min, 100 µL of anthrone reagent as prepared in Olson et al.
208 (2000) (375 mg anthrone dissolved in 70% concentrated sulfuric acid) was added and vortexed
209 for 10 sec. After incubating for 1.5 hours at room temperature, 75 µL were transferred to wells
210 in a 96-well plate and the absorbance at 620 nm was measured. Then, the same samples were
211 heated at 65 °C for 2 hours (hot anthrone test) to analyze the total sugar content of the parasitoid
212 and the absorbance was again read at 620 nm.

213 The white precipitate containing insoluble high molecular weight sugars (glycogen) was
214 analyzed by adding 200 µL of the anthrone reagent. The resulting solution was vortexed and

100 µL was transferred to a new 1.5 mL centrifuge tube while avoiding floating body parts. The solution was heated at 90 °C for 3 min in a dry bath incubator, after which the tubes were placed on crushed ice for 15 min, and 75 µL were transferred to a 96-well plate. The absorbance was again read at 620 nm. The absorbance of all three assays was compared to absorbance values of known fructose, sucrose standards dissolved in 25% ethanol or glycogen standards dissolved in dH_2O with two replicates per read.

The cold anthrone test is based upon the property of the anthrone reagent, which reacts with only fructose within 1 hour at room temperature (Heimpel et al. 2004). Since fructose is not present at detectable levels in the hemolymph or most or all insects, exposing insects to the anthrone reagent at room temperature for limited periods of time can be used to indicate the quantity of gut sugars present in a sample (Heimpel et al., 2004). The hot anthrone test, on the other hand detects all sugars and can therefore be interpreted to measure the sum of sugars present in the gut and hemolymph. The glycogen test uses a hot anthrone approach to measure the glycogen content. Glycogen represents a long-term energy storage for insect parasitoids. We report here these three measurements as well as a sum of all three that we refer to as 'total carbohydrates'.

The aphid phylogeny and phylogenetic signal

We determined whether the sugar content in the honeydews and the sugar content in the parasitoids that were offered the honeydews clustered on the aphid phylogeny. This was done using the analysis of traits mode in the software package Phylocom to test for a phylogenetic signal for the various measures of sugar composition on the aphid phylogeny (Webb, Ackerly, & Kembel, 2007; version 4.2). The phylogeny came from Desneux, Blahnik, Delebecque, and Heimpel (2012) with *Aphis fabae* inserted according to Coeur d'Acier, Jousselin, Martin, and Rasplus (2007). For simplicity, this analysis was done without branch lengths, utilizing only the topography of the tree. Phylogenetic signal is detected by Phylocom using randomization of trait values across the tips of the phylogeny to compare observed and randomized patterns for

241 signs of clustering. We conducted 10,000 randomizations for each trait and considered
242 clustering to be significant (indicating phylogenetic signal) if > 9,500 randomizations showed
243 lower levels of variance for the observed than the randomized values. Because the sugar
244 composition of the honeydews may be affected by the host plant of the aphid (Fischer &
245 Shingleton, 2001; Pringle, Novo, Ableson, Barbehenn, & Vannette, 2014) we also tested for
246 phylogenetic signal of the host plants (as in Desneux et al., 2012) using the same procedures in
247 Phylocom.

248 ***Statistical analysis***

249 Carbohydrate levels were compared using ANOVA and Tukey's HSD for multiple
250 comparisons. The normality assumption was assessed using Shapiro's test, and
251 homoscedasticity assumption was assessed with the Levene test. The fructose:total sugars ratio
252 was arcsine square root transformed in order to fulfill normality and homoscedasticity
253 assumptions. In addition, simple linear regressions were used to relate i) carbohydrate levels of
254 honeydew and parasitoids, ii) carbohydrate levels of honeydew and parasitoid longevity and iii)
255 carbohydrate levels of parasitoids and their longevity. Statistical analyses were run on absolute
256 amounts of nutrients instead of on absorbance values. In the case of the parasitoids, the absolute
257 amounts were divided by the tibia length of each parasitoid.

258 The longevity was analyzed using the survival functions of the "OIsurv" package (Diez,
259 2013) with standard specification in R, version 3.2.2 (R Core Team, 2015). We used the
260 "survfit" function to calculate estimates of the different survival curves using the Kaplan-Meier
261 method. A non-parametric cox proportional hazards model ("coxph" function) was used to
262 analyse the effect of the food source treatment on parasitoid survival. The assumption of
263 proportional hazards was tested by visual inspections using the "cox.zph" function. The Kaplan-
264 Meier survival curves of indiviudal treatments were compared with the log-rank test "survdiff"..
265 The latter uses the G-rho family of tests of Harrington and Fleming (1982) and weights S(t)^rho

on each death (S is the Kaplan-Meier estimate of survival). Rho was set to zero for the log-rank test.

Results

Carbohydrate content of 14 honeydews

M. asclepiadis, U. sonchi and *A. nerii* produced honeydews with the highest content of fructose (measured with the cold anthrone test), followed by that of *R. maidis* and a group of six species ($F_{13, 41}$ = 243.7; $P < 0.0001$) (Fig. 1A). *M. persicae, A. monardae, A. gossypii* (fed on cotton) and *R. padi* produced the honeydew with the lowest content of fructose. This analysis showed that the fructose content in honeydew produced by *A. gossypii* was affected by host plant. However, we found no evidence that fructose content is clustered either on the aphid phylogeny (phylogenetic signal analyses in Phylocom: $P = 0.64$; see Appendix A: Fig. 1) nor on the plant phylogeny ($P = 0.13$).

For total sugar content measured by the hot anthrone test, honeydews can be divided into three groups ($F_{13, 41}$ = 65.6; $P < 0.0001$) (Fig. 1B). The honeydew produced by *A. gossypii* fed on common milkweed, *M. asclepiadis* and *U. sonchi* contained the highest concentration of sugars, whereas *A. monardae*, *A. glycines*, *S. graminum* and *A. asclepiadis* produced the honeydew with the lowest sugar concentration. We found no evidence that the mean total sugar content is clustered either on the aphid phylogeny (phylogenetic signal analyses in Phylocom: $P = 0.38$; see Appendix A: Fig. 1) or on the plant phylogeny ($P = 0.21$).

The fructose:total sugars ratio also varied significantly among honeydews from 0.55 ± 0.01 in *M. asclepiadis* and *A. nerii* to 0.05 ± 0.002 in *M. persicae* ($F_{13, 41}$ = 142.3; $P < 0.0001$) (Fig. 1C). The graph represents a staircase from *M. asclepiadis* to *A. gossypii* fed on cotton (0.23 ± 0.01). The fructose:total sugars ratio was not significantly correlated with the total sugar content of the honeydews ($R^2 = 0.28$; $F_{1, 13} = 0.03$; $P = 0.86$).

Effect of honeydew produced by 13 aphid species on **L. testaceipes** *longevity*

We found significant differences among parasitoid longevities depending on the offered food source (Cox proportional hazards: likelihood ratio = 165.1; df = 15; $P < 0.0001$). Parasitoids fed on honeydew or honey lived significantly longer than parasitoids fed on water only (Table 2; Fig. 2; and see Appendix A: Fig. 2). However, only the survival of parasitoids fed on honeydew of *A. oestlundi* [4.7 ± 0.3 days (mean ± SE)] and *U. sonchi* (4.6 ± 0.4 days) was not significantly different from the survival of parasitoids fed on honey (4.8 ± 0.3 days). There were marginally significant differences between the survivorship of *L. testaceipes* fed on honeydew of the same aphid species, *A. gossypii*, reared on two plant species (cotton and common milkweed). The lifespan of parasitoids fed honeydew from *A. gossypii* reared on cotton or on common milkweed was of 3.3 ± 0.2 days and 3.9 ± 0.2 days, respectively (Fig.2, species; Table 2). When comparing the effect of honeydews from different aphid species (*A. nerii*, *A. asclepiadis* and *M. asclepiadis*) reared on the same host plant (swamp milkweed), *A. asclepiadis* (2.6 ± 0.2 days) produced honeydew of poorer quality (here and throughout the results section: we use "honeydew quality" as a measurement of the increment of parasitoid longevity) than *A. nerii* (3.5 ± 0.2 days) (Fig. 2, host plant; Table 2). *M. asclepiadis* produced honeydew of intermediate quality (2.9 ± 0.2 days). Similarly, when comparing the effect of honeydews from different aphid species (*R. maidis*, *R. padi* and *S. graminum*) reared on barley, *R. padi* (2.6 ± 0.2 days) and *S. graminum* (2.7 ± 0.3 days) produced honeydew of poorer quality than *R. maidis* (3.4 ± 0.2 days).When comparing the effect of honeydews from different aphid species within the same genus, there were significant differences between the honeydew of *R. maidi*s [3.4 ± 0.2 days (mean ± SE)] and *R. padi* (2.6 ± 0.2 days) ($\chi^2_1 = 7.96$, $P < 0.01$) (Fig. 2, genus; Table 2). There were also significant differences among the honeydews excreted by the aphid species of genus *Aphis* (Fig. 2, genus; Table 2). *A. oestlundi* produced the honeydew of highest quality and *A. asclepiadis* the poorest.

316 Finally, we found no evidence that mean survival of *L. testaceipes* fed on honeydew of
317 different aphid species is clustered either on the aphid phylogeny (phylogenetic signal analyses
318 in Phylocom: $P = 0.23$) or on the plant phylogeny ($P = 0.3$) (Appendix A: Fig. 3).

319 ***Effect of honeydew produced by 12 aphid species on* L. testaceipes *nutritional state***

320 The source of honeydew had a significant effect on the amount of fructose (cold anthrone test;
321 $F_{13, 188} = 8.13$; $P < 0.0001$), total sugars (hot anthrone test; $F_{13, 188} = 4.06$; $P < 0.0001$), glycogen
322 ($F_{13, 188} = 4.41$; $P < 0.0001$) and total carbohydrates ($F_{13, 188} = 6.51$; $P < 0.0001$) in *L. testaceipes*
323 females (Fig. 3). Fructose was not detected in unfed parasitoids and these parasitoids contained
324 the lowest levels of total carbohydrates.

325 Parasitoids fed on honeydew produced by *U. sonchi* had the highest content of fructose
326 (Fig. 3A); followed by those fed on honeydew from *A. fabae, A. glycines, A. monardae,* and *A.*
327 *nerii*; a third group was composed by *R. maidis, M. asclepiadis,* and *A. gossypii* (on common
328 milkweed and cotton); and finally honeydew from *M. persicae, R. padi* and *S. graminum* were
329 the poorest according to the fructose content of *L. testaceipes*. The content of fructose measured
330 with the cold anthrone test was ten times lower than the total sugar content of the insect.

331 The pattern was slightly different for the total sugar content (Fig. 3B). Parasitoids fed on
332 honeydew produced by *U. sonchi* again had the highest content of total sugars; followed by
333 those fed on honeydew excreted by *A. glycines, A. fabae, A. monardae, M. asclepiadis, M.*
334 *persicae* and *A. gossypii* (on cotton); the third group was composed of *A. nerii, R. maidis, R.*
335 *padi* and *A. gossypii* (on common milkweed); and finally honeydew produced by *S. graminum*
336 resulted in the lowest levels of total sugars within *L. testaceipes*.

337 Although the pattern was less clear for the content of glycogen, the consumption of
338 honeydew produced by *S. graminum* also resulted in the lowest levels of glycogen within *L.*
339 *testaceipes* (Fig. 3C). The amount of glycogen was generally lower than that of sugars.

According to the amount of total carbohydrates (total sugars + glycogen) that parasitoids contained, honeydews could be divided in four main groups (Fig. 3D). Parasitoids fed on honeydew produced by *U. sonchi, A. fabae, A. glycines* and *A. monardae* had the highest levels of carbohydrates, followed by those fed on honeydew excreted by *A. gossypii* (on cotton), *M. persicae*, *M. asclepiadis*, *A. nerii* and *R. maidis.* The third group was composed of *A. gossypii* (on common milkweed) and *R. padi*, and finally honeydew produced by *S. graminum* was the lowest according to the carbohydrate content of *L. testaceipes*.

Finally, we found no evidence for phylogenetic clustering of fructose (phylogenetic signal analyses in Phylocom: $P = 0.65$), total sugars ($P = 0.21$), total carbohydrates ($P = 0.45$) or glycogen ($P = 0.081$) in *L. testaceipes* females fed on honeydew of different aphid species (Appendix A: Fig. 4).

Relationship between the sugar content of honeydew produced by 12 aphid species and the nutritional state and longevity of* L. testaceipes *when fed on them

The fructose content of honeydew was not correlated with fructose content of parasitoids fed on honeydew ($P = 0.41$). Similarly, the total content of sugars in the honeydew was correlated with neither the total content of sugars ($P = 0.71$) nor total content of total carbohydrates (sugars + glycogen) ($P = 0.84$) of the parasitoids fed on honeydew.

Neither the content of fructose ($P = 0.88$) nor the content of total sugars ($P = 0.59$) were correlated with the longevity of honeydew fed parasitoids.

Relationship between the nutritional state of* L. testaceipes *and its longevity when fed on honeydew excreted by 12 aphid species

We detected a significant positive relation between the mean longevity and the mean content of the three carbohydrates in the parasitoids (fructose: $P < 0.0001$; total sugars: $P = 0.0042$; glycogen: $P = 0.001$), as well as with total content of carbohydrates ($P < 0.0001$) (Fig. 4). A stronger relationship was observed for fructose ($R^2 = 75.5\%$), followed by the total content of

carbohydrates (R^2 = 71.3%), glycogen (R^2 = 61.1%) and total sugars (R^2 = 50.9%). Since the correlations could be greatly amplified by the presence of negative and positive controls (water and honey), we re-ran them without the controls to evaluate the variance among honeydews only. The correlation remained positive and significant for fructose (P = 0.006; R^2 = 54.1%), and total content of carbohydrates (P = 0.0036; R^2 = 58.8%), and positive and marginally significant for total sugars (P = 0.0498; R^2 = 33.2%), and glycogen (P = 0.052; R^2 = 32.6%).

Discussion

We analyzed the sugar content of 14 honeydews produced by 13 species of aphids and evaluated their nutritional value for the generalist aphid parasitoid *L. testaceipes*. Although there was a high variation among the different honeydews, none of the honeydews appeared to be toxic (i.e. causing higher mortality of parasitoids than a water control) and none were of a higher quality for parasitoid performance than honey. The lack of phylogenetic signal for the nutritional value of honeydew suggests that this characteristic is evolutionarily labile and may be particularly subject to ecological selection pressures. Finally, for the first time, we provide evidence that the longevity of honeydew-fed parasitoids is positively correlated with their carbohydrate contents, especially their content of fructose. The latter result may be due to direct positive effects of fructose, or indirectly due to higher oligosaccharide levels in honeydews with lower fructose levels.

Honeydew composition

Other studies have shown that honeydew composition within the same aphid species can vary with host plant (as demonstrated herein and by Fischer & Shingleton, 2001; Pringle et al., 2014), phenological and physiological status of the plant (Maltais & Auclair, 1962), presence of ants (Fischer & Shingleton, 2001; Yao & Akimoto, 2002) and aphid age (Fischer,Völkl, Schopf, & Hoffmann, 2002). Although honeydew composition can vary with the host plant and honeydew reflects the host plant amino acid composition (Leroy et al., 2011), we did not

observe a significant effect of plant phylogeny on the sugar and fructose content of the honeydews produced by 13 aphid species.

Apart from host-plant-mediated factors, the presence of microorganisms in aphids and honeydew is another factor that may affect the quality of honeydew as carbohydrate source for insects and explain the lack of phylogenetic signals. For example, in a proteomic study, almost 30% of the 96 proteins identified in the honeydew of the pea aphid *Acyrthosiphon pisum* (Harris) were homologous to bacterial sequences (Sabri et al., 2013). This result highlights the importance of other organisms (i.e. the host aphid and its microbiota, including endosymbiotic bacteria and gut flora) on the composition of honeydew. Recently, it has been demonstrated that the facultative endosymbionts *Hamiltonella defensa* and *Regiella insecticola* modify the content of *A. fabae* honeydew, in this case, reducing the concentrations of amino acids (Schillewaert, Vantaux, Parmentier, Vorburger, & Wenseleers, 2016). Moreover, honeydew can be a nutrient-rich resource for insect pathogens, reducing the quality of honeydew as a diet for insects (Lundgren, 2009; Lievens et al., 2015).

Finally, it is worth mentioning that we used the content of fructose and total sugars to measure honeydew quality for *L. testaceipes*. Previous studies have used the sucrose:hexose ratio to measure the quality of several nectars for parasitoid fitness without finding a clear relation (Tompkins, Wratten, & Wäckers, 2010). HLPC (high-performance liquid chromatography) could be used in future studies because this technique allows determination of the presence of characteristic hemipteran-synthesized sugars, such as melezitose or erlose (Tena et al., 2013). Although it has been recently demonstrated that some parasitoids have specifically adapted to the consumption of specific sugars present in honeydew, these sugars tend to reduce the nutritional value of honeydew (Wäckers, 2001; Lenaerts et al., 2016; Goelen et al., 2017).

Effect of honeydew on **L. testaceipes** *nutritional state and longevity*

The fourteen aphid honeydews showed high variation in their effect on nutritional state and longevity of *L. testaceipes*. As expected, sugar and glycogen levels of honeydew-fed parasitoids

were higher compared to unfed ones, and the latter did not contain fructose, consistent with previous field and laboratory studies (Olson et al., 2000; Lee, Heimpel, & Leibee, 2004; Heimpel et al., 2004; Lee, Andow, & Heimpel, 2006; Wäckers, Lee, Heimpel, Winkler, & Wagenaar, 2006; Hogervorst et al., 2007a; Hogervorst, Wäckers, & Romeis2007b; Wyckhuys, Strange-George, Kulhanek, Wäckers, & Heimpel, 2007; Tena et al., 2013; 2015; Calabuig et al., 2015). *L. testaceipes* contained the highest sugar levels (both fructose and total) when females fed on honeydew produced by *U. sonchi.* This honeydew raised *L. testaceipes* fructose and total sugar content by a factor greater than 5 and 3, respectively, compared to the poorest honeydew. The high level of fructose, a sugar that is mostly present in the digestive system of insects (Heimpel et al. 2004), suggests that *L. testaceipes* fed on *U. sonchi* honeydew at a high rate. This hypothesis is also supported by the fact that this honeydew did not contain a high fructose-total sugar ratio. *L. testaceipes* also reached the greatest longevity when fed *U. sonchi* honeydew, which reinforces the high nutritional value of this honeydew for this parasitoid. It is interesting to highlight that *U. sonchi,* together with *M. asclepiadis,* are not parasitized by *L. testaceipes* (N. Desneux & G.E. Heimpel, unpublished data).

On the other hand, our data show that *S. graminum,* which is a highly suitable host for *L. testaceipes* (N. Desneux & G.E. Heimpel, unpublished data) and a commonly attacked in the native range of the parasitoid (Royer et al., 2015), produced the poorest honeydew for this parasitoid. These data support the hypothesis that hemipterans, and especially aphids, are subjected to strong selection pressure to minimize the quality of honeydew for insects, as any nutritional benefit to natural enemies has the potential to negatively impact fitness of the aphid species producing it (Wäckers, 2000; 2001; Wäckers et al., 2008). In the case of *S. graminum* honeydew, the effect of host plant cannot be disregarded because this aphid and *R. padi* were both reared on barley (*H. vulgare*) and both produced honeydew with relatively low nutritional value for *L. testaceipes.* The honeydew of these aphids might contain some of the secondary metabolites produced by *H. vulgare* and/or its endophytes (Schulz & Boyle, 2005), as it is known that aphids sequester secondary metabolites when feeding on toxic plants and also

443 excrete them in the honeydew (Malcolm, 1990; Zust & Agrawal, 2015). These compounds
444 could be detrimental to aphid natural enemies. Despite this caveat, there was no overall effect of
445 host-plant phylogeny on honeydew composition or quality for insects as noted above.

446 To better understand the role of toxic plants on honeydew quality, we compared
447 honeydew of three aphid species reared on swamp milkweed *A. incarnata,* which likely contains
448 high levels of cardenolide toxins (Agrawal & Malcolm, 2002; Agrawal, 2005; Agrawal,
449 Petschenka, Bingham, Weber, & Rasmann, 2012). In terms of parasitoid longevity, the
450 specialist aphid *A. asclepiadis* excreted honeydew of poorer nutritional value for *L. testaceipes*
451 compared to that excreted by the generalist aphid *A. nerii.* This result is in concordance with a
452 recent study, which showed that *M. asclepiadis* - despite producing the lowest amounts of
453 cardenolides in its honeydew – produces higher concentrations of an apolar cardenolide
454 compared to generalist aphids reared on the common milkweed *A. syriaca* (Züst & Agrawal,
455 2015). Apolar cardenolides are considered more toxic because contrary to polar ones they can
456 pass through cell membranes via passive diffusion (Frick & Wink, 1995; Agrawal et al., 2012;
457 Züst & Agrawal, 2015).

458 ***Relation between the nutritional state of* L. testaceipes *and its longevity when fed on***
459 ***honeydew***

460 Our results show that the nutritional state of honeydew-fed parasitoids when they were two days
461 old was positively correlated with their longevity; i.e. there was a positive correlation between
462 carbohydrate contents and parasitoid fitness. This information is valuable for biological control
463 researchers because it demonstrates that the nutritional state of honeydew-fed parasitoids in the
464 wild also provides information on their fitness. So far, HPLC or anthrone tests have been used
465 to determine the nutritional state of parasitoids that have fed on honeydew and nectar in the field
466 (Casas et al. 2003; Heimpel et al. 2004; Lavandero et al. 2005; Lee, Andow, & Heimpel, 2006;
467 Hogervorst et al. 2007a; Lee & Heimpel 2008; Winkler et al. 2009; Desouhant et al. 2010; Tena
468 et al. 2013, 2015; Dieckhoff et al. 2014; Calabuig et al., 2015). This information is useful when

carbohydrate contents of parasitoids are low; as this indicates that parasitoids are sugar-limited and it is necessary to provide a sugar source to increase their fitness and biocontrol potential (Tena et al., 2016). However, if energy reserves (sugar content) in field parasitoids are high, this might only show part of the picture, lacking information about the quality of the sugar source and eventually the impact on parasitoid fitness (Tena et al., 2016). In our data set, for example, parasitoids fed on *A. fabae* honeydew contained high sugar contents but their longevity was lower than expected given the overall relationship. A potential explanation is that *A. fabae* honeydew might be a phagostimulant for *L. testaceipes* as occurs in ants, even if its nutritional value is poor for the parasitoid (Völk, et al. 1999).

Interestingly, Wyckhuys et al.(2008) also observed that carbohydrate content was positively related with the longevity of the aphid parasitoid *Binodoxys communis* when it had access to three carbohydrate sources: honey, sucrose and honeydew excreted by the soybean aphid, *A. glycines*. Parasitoids fed on honey had the highest sugar content when they were 2-4-days old and lived the longest, whereas those fed on honeydew had the lowest sugar content and lived the shortest. Parasitoids fed on sucrose had an intermediate sugar content and lifespan. Lee et al. (2004) found similar results when comparing buckwheat nectar and soybean aphid honeydew in the parasitoid *Diadegma insulare* Cresson (Hymenoptera: Ichneumonidae). Therefore, the positive correlation between carbohydrate content and longevity found here may not be exclusive to honeydew-fed parasitoids, but also include those fed on other sugar sources. A potential explanation for this correlation is that the toxic metabolites of honeydew, such as cardenolides (Malcolm, 1990), also deter feeding as has been demonstrated in herbivores that feed on plants with high contents of these secondary metabolites. In fact, a wide range of animals show preingestive (gustatory) sensitivity to cardenolides (Agrawal et al., 2012).

Acknowledgements

We thank Leroy Bondhus, Lanfen Qiu, and Tony Charvoz for laboratory assistance. M. Senft, N. Desneux and A. Tena were supported by the FP7-PEOPLE-2013-IRSES program (project APHIWEB, grant no. 611810).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at XXXXX.

References

Agrawal, A.A. (2005). Natural selection on common milkweed (*Asclepias syriaca*) by a community of specialized insect herbivores. *Evolutionary Ecology Research,* 7, 651–667.

Agrawal, A.A., & Malcolm, S.B. (2002). Once upon a milkweed. *Natural History,* 111, 48–53.

Agrawal, A.A., Petschenka, G., Bingham, R.A., Weber, M.G., & Rasmann, S. (2012). Toxic cardenolides: chemical ecology and coevolution of specialized plant–herbivore interactions. *New Phytologist*, 194, 28-45.

Avidov, Z., Balshin, M., & Gerson, U. (1970). Studies on *Aphytis coheni,* a parasite of the California red scale, *Aonidiella aurantii* in Israel. *Entomophaga*, 15, 191-207.

Blackman, R.L., & Eastop V.F. (2000). *Aphids on the world's crops an identification and information guide*. John Wiley & Sons, New York.

Blackman, R.L., & Eastop, V.F. (2008). *Aphids on the World's Herbaceous Plants and Shrubs, 2 Volume Set*. John Wiley & Sons, New York.

Briegel, H. (1990). Metabolic relationship between female body size, reserves, and fecundity of *Aedes aegypti*. *Journal of Insect Physiology*, 36, 165-172.

Calabuig, A., Tena, A., Wäckers, F.L., Fernández-Arrojo, L., Plou, F.J., Garcia-Marí, F., et al. (2015). Ants impact the energy reserves of natural enemies through the shared honeydew exploitation. *Ecological Entomology*, 40, 687-695.

Casas, J., Driessen, G., Mandon, N., Wielaard, S., Desouhant, E., van Alphen, J.J.M., et al. (2003). Energy dynamics in a parasitoid foraging in the wild. *Journal of Animal Ecology,* 72, 691-697.

Coeur d'Acier, A., Jousselin, E., Martin, J.F., & Rasplus, J.Y. (2007). Phylogeny of the genus *Aphis* Linnaeus, 1758 (Homoptera: Aphididae) inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, 42, 598-611.

Desneux, N., Barta, R.J., Delebeque, C.J., & Heimpel. G.E. (2009). Transient host paralysis as a means of reducing self-superparasitism in koinobiont endoparasitoids. *Journal of Insect Physiology,* 55, 321-327.

Desneux, N., Blahnik, R., Delebecque, C.J., & Heimpel, G.E. (2012). Host phylogeny and specialisation in parasitoids. *Ecology Letters*, 15, 453-460.

Diez, D.M. (2013). OIsurv: Survival analysis supplement to OpenIntro guide. R package version 0.2. http://CRAN.R-project.org/package=Oisurv

Dieckhoff, C., Theobald, J.C., Wäckers, F.L., & Heimpel, G.E. (2014). Egg load dynamics and the risk of egg and time limitation experienced by an aphid parasitoid in the field. *Ecology and Evolution*, 4, 1739-1750.

Evans, E.W., & England, S. (1996). Indirect interactions in biological control of insects: pests and natural enemies in alfalfa. *Ecological Applications*, 6, 920-930.

Fischer, M.K., & Shingleton, A.W. (2001). Host plant and ants influence the honeydew sugar composition of aphids. *Functional Ecology*, 15, 544-550.

Fischer, M.K., Völkl, W., Schopf, R., & Hoffmann, K.H. (2002). Age-specific patterns in honeydew production and honeydew composition in the aphid *Metopeurum fuscoviride*: implications for ant-attendance. *Journal of Insect Physiology*, 48, 319-326.

Frick, C., & Wink, M. (1995). Uptake and sequestration of ouabain and other cardiac glycosides in *Danaus plexippus* (Lepidoptera: Danaidae): evidence for a carrier-mediated process. *Journal of Chemical Ecology*, 21, 557–575.

Goelen, T., Baets, D., Kos, M., Paulussen, C., Lenaerts, M., Rediers, H., et al. (2018). Gustatory response and longevity in *Aphidius* parasitoids and their hyperparasitoid *Dendrocerus aphidum*. *Journal of Pest Science*, 91, 1-10.

Harrington, D.P., & Fleming, T.R. (1982). A class of rank test procedures for censored survival data. *Biometrika*, 69, 553-566.

Heimpel, G.E., & Jervis, M.A. (2005). Does floral nectar improve biological control by parasitoids. *Plant-provided food for carnivorous insects: A protective mutualism and its applications* (eds F.L. Wäckers, P.C.J. van Rijn & J. Bruin) pp. 267-304. Cambridge University Press, Cambridge.

Heimpel, G.E., Lee, J.C., Wu, Z., Weiser, L., Wäckers, F., & Jervis, M.A. (2004). Gut sugar analysis in field-caught parasitoids: adapting methods originally developed for biting flies. *International Journal of Pest Management,* 50,193-198.

Hogervorst, P.A.M, Wäckers, F.L., & Romeis, J. (2007a). Detecting nutritional state and food source use in field-collected insects that synthesize honeydew oligosaccharides. *Functional Ecology*, 21, 936-946.

Hogervorst, P.A.M., Wäckers, F.L., & Romeis, J. (2007b). Effects of honeydew sugar composition on the longevity of *Aphidius ervi*. *Entomologia Experimentalis et Applicata,* 122, 223-232.

Holman, J. (2009). *Host plant catalog of aphids paleartic region.* Springer, Netherlands.

Lenaerts, M., Abid, L., Paulussen, C., Goelen, T., Wäckers, F., Jacquemyn, H., et al. (2016). Adult parasitoids of honeydew-producing insects prefer honeydew sugars to cover their energetic needs. *Journal of Chemical Ecology*, 42, 1028-1036.

Lee, J.C., & Heimpel, G.E. (2008). Floral resources impact longevity and oviposition rate of a parasitoid in the field. *Journal of Animal Ecology,* 77, 565-572.

Lee, J.C., Andow, D.A., & Heimpel, G.E. (2006). Influence of floral resources on sugar feeding and nutrient dynamics of a parasitoid in the field. *Ecological Entomology*, 31, 470-480.

Lee, J.C., Heimpel, G.E., & Leibee, G.L. (2004). Comparing floral nectar and aphid honeydew diets on the longevity and nutrient levels of a parasitoid wasp. *Entomologia Experimentalis et Applicata*, 111, 189-199.

Leroy, P.D., Wathelet, B., Sabri, A., Francis, F., Verheggen, F.J., Capella, Q., et al. (2011). Aphid-host plant interactions: does aphid honeydew exactly reflect the host plant amino acid composition? *Arthropod-Plant Interactions*, 5, 193-199.

Lievens, B., Hallsworth, J.E., Pozo, M.I., Belgacem, Z.B., Stevenson, A., Willems, K.A., et al. (2015). Microbiology of sugar-rich environments: diversity, ecology and system constraints. *Environmental Microbiology*, 17, 278-298.

Lundgren, J.G. (2009). *Relationships of Natural Enemies and Non-prey Foods*. Springer International, Dordrecht.

Malcolm, S.B. (1990). Chemical defence in chewing and sucking insect herbivores: plant-derived cardenolides in the monarch butterfly and oleander aphid. *Chemoecology*, 1, 12-21.

Maltais, J.B., & Auclair, J.L. (1962). Free amino acid and amide composition of pea leaf juice, pea aphid haemolymph, and honeydew, following the rearing of aphids on single pea leaves treated with amino compounds. *Journal of Insect Physiology*, 8, 391-399.

Mitrović, M., Petrović, A., Kavallieratos, N.G., Starý, P., Petrović-Obradović, O., Tomanović, Ž., et al. (2013). Geographic structure with no evidence for host-associated lineages in European populations of *Lysiphlebus testaceipes*, an introduced biological control agent. *Biological Control*, 66, 150-158.

Olson, D.M., Fadamiro, H., Lundgren, J.G., & Heimpel, G.E. (2000). Effects of sugar feeding on carbohydrate and lipid metabolism in a parasitoid wasp. *Physiological Entomology*, 25, 17-26.

591 Pekas, A., Tena, A., Aguilar, A., & Garcia-Marí, F. (2011). Spatio-temporal patterns and
592 interactions with honeydew-producing Hemiptera of ants in a Mediterranean citrus orchard.
593 *Agricultural and Forest Entomology*, 13, 89-97.
594 Pike, K.S., Starý, P., Miller, T., Graf, G., Allison, D., Boydston, L., et al. (2000). Aphid
595 parasitoids (Hymenoptera: Braconidae: Aphidiinae) of northwest USA. *Proceedings of the*
596 *Entomological Society of Washington,* 102, 688-740.
597 Pringle, E.G., Novo, A., Ableson, I., Barbehenn, R.V., & Vannette, R.L. (2014). Plant-derived
598 differences in the composition of aphid honeydew and their effects on colonies of aphid-tending
599 ants. *Ecology and Evolution*, 4, 4065-4079.
600 R Core Team (2015). R: A language and environment for statistical computing. R Foundation
601 for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
602 Royer, T.A., Pendleton, B.B., Elliott, N.C., & Giles, K.L. (2015). Greenbug (Hemiptera:
603 Aphididae) biology, ecology, and management in wheat and sorghum. *Journal of Integrated*
604 *Pest Management*, 6, 19.
605 Sabri, A., Vandermoten, S., Leroy, P.D., Haubruge, E., Hance, T., Thonart, et al. (2013).
606 Proteomic investigation of aphid honeydew reveals an unexpected diversity of proteins. *PloS*
607 *One*, 8, e74656.
608 Schillewaert, S., Vantaux, A., Parmentier, T., Vorburger, C., & Wenseleers, T. (2016). The
609 influence of facultative endosymbionts on honeydew carbohydrate and amino acid composition
610 of the black bean aphid *Aphis fabae*. *Physiological Entomology*. doi:10.1111/phen.12181
611 Schulz, B., & Boyle, C. (2005). The endophytic continuum. *Mycological Research*, 109, 661-
612 686.
613 Starý, P., Lumbierres, B., & Pons, X. (2004). Opportunistic changes in the host range of
614 *Lysiphlebus testaceipes* (Cr.), an exotic aphid parasitoid expanding in the Iberian Peninsula.
615 *Journal of Pest Science*, 77, 139-144.

Starý, P., Lyon, J.P., & Leclant, F. (1988). Biocontrol of aphids by the introduced *Lysiphlebus testaceipes* (Cress.) (Hym., Aphidiidae) in Mediterranean France. *Journal of Applied Entomology*, 105, 74–87.

Steppuhn, A., & Wäckers, F.L. (2004). HPLC sugar analysis reveals the nutritional state and the feeding history of parasitoids. *Functional Ecology*, 18, 812-819.

Styrsky, J.D., & Eubanks, M.D. (2007). Ecological consequences of interactions between ants and honeydew-producing insects. *Proceedings of the Royal Society of London B: Biological Sciences*, 274, 151-164.

Tena, A., Hoddle, C.D., & Hoddle, M.S. (2013). Competition between honeydew producers in an ant–hemipteran interaction may enhance biological control of an invasive pest. *Bulletin of Entomological Research*, 103, 714-723.

Tena, A., Llácer, E., & Urbaneja, A. (2013). Biological control of a non-honeydew producer mediated by a distinct hierarchy of honeydew quality. *Biological Control*, 67, 117-122.

Tena, A., Pekas, A., Cano, D., Wäckers, F.L., & Urbaneja, A. (2015). Sugar provisioning maximizes the biocontrol service of parasitoids. *Journal of Applied Ecology*, 52, 795-804.

Tena, A., Pekas, A., Wäckers, F.L., & Urbaneja, A. (2013). Energy reserves of parasitoids depend on honeydew from non-hosts. *Ecological Entomology*, 38, 278-289.

Tena, A., Wäckers, F.L., Heimpel, G.E., Urbaneja, A., & Pekas, A. (2016). Parasitoid nutritional ecology in a community context: the importance of honeydew and implications for biological control. *Current Opinion in Insect Science*, 14, 100-104.

Tompkins, J.M., Wratten, S. D., & Wäckers, F.L. (2010). Nectar to improve parasitoid fitness in biological control: Does the sucrose: hexose ratio matter?. *Basic and Applied Ecology*, 11, 264-271.

van Steenis M.J. (1994). Intrinsic rate of increase of *Lysiphlebus testaceipes* Cresson (Hym.: Braconidae), a parasitoid of *Aphis gossypii* Glover (Hom., Aphididae) at different temperatures. *Journal of Applied Entomology*, 118, 399-406.

Völkl, W., Woodring, J., Fischer, M., Lorenz, M.W., & Hoffmann, K.H. (1999). Ant-aphid mutualisms: the impact of honeydew production and honeydew sugar composition on ant preferences. *Oecologia*, 118, 483-491.

Wäckers, F.L. (2000). Do oligosaccharides reduce the suitability of honeydew for predators and parasitoids? A further facet to the function of insect-synthesized honeydew sugars. *Oikos*, 90, 197-201.

Wäckers, F.L. (2001). A comparison of nectar-and honeydew sugars with respect to their utilization by the hymenopteran parasitoid *Cotesia glomerata*. *Journal of Insect Physiology*, 47, 1077-1084.

Wäckers, F.L., Lee, J.C., Heimpel, G.E., Winkler, K., & Wagenaar, R. (2006). Hymenopteran parasitoids synthesize 'honeydew-specific'oligosaccharides. *Functional Ecology*, 20, 790-798.

Wäckers, F.L., van Rijn, P.C., & Heimpel, G.E. (2008). Honeydew as a food source for natural enemies: making the best of a bad meal? *Biological Control*, 45, 176-184.

Way, M.J. (1963). Mutualisms between ants and honeydew-producing homoptera. *Annual Review of Entomology*, 8, 307–344.

Webb, C., Ackerly, D., & Kembel, S. (2007). Software for the Analysis of Community Phylogenetic Structure and Character Evolution, with Phylogeny Tools. Version 3.41. In: Users Manual Version 3.41. http://www.phylodiversity.net/phylocom

Winkler, K., Wäckers, F., & Pinto, D.M. (2009). Nectar-providing plants enhance the energetic state of herbivores as well as their parasitoids under field conditions. *Ecological Entomology,* 34, 221-227.

Wyckhuys, K.A., Strange-George, J.E., Kulhanek, C.A., Wäckers, F.L., & Heimpel, G.E. (2008). Sugar feeding by the aphid parasitoid *Binodoxys communis*: how does honeydew compare with other sugar sources? *Journal of Insect Physiology*, 54, 481-491.

Yao, I., & Akimoto, S.I. (2002). Flexibility in the composition and concentration of amino acids in honeydew of the drepanosiphid aphid *Tuberculatus quercicola*. *Ecological Entomology*, 27, 745-752.

Zikic, V., Stankovic, S.S., Milosevic M., Petrovic-Obradovic, O., Petrovic, A., Starý, P., & Tomanovic, Z. (2015). First detection of *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Aphidiinae) in Serbia: an introduced species invading Europe? *North-Western Journal of Zoology*, 11, 97-101.

Zoebelein, G. (1956a). Der Honigtau als Nahrung der Insekten. Teil I. *Journal of Applied Entomology*, 38, 369-416.

Zoebelein, G. (1956b). Der Honigtau als Nahrung der Insekten: Teil II. *Journal of Applied Entomology,* 39*,* 129-167.

Züst, T., & Agrawal, A.A. (2015). Population growth and sequestration of plant toxins along a gradient of specialization in four aphid species on the common milkweed *Asclepias syriaca*. *Functional Ecology*, 30, 547-556.

680

681 **Figure legends**

682 **Fig. 1.** Sugar content (mean ± SE) of 14 honeydews produced by 13 aphid species. (A) Fructose
683 content (measured using the cold anthrone test). (B) Total sugar content (measured using the hot
684 anthrone test). (C) Fructose:total sugar ratio. Sugar contents and ratio for species subtended by
685 lines do not differ ($P > 0.05$, Tukey test).

686 **Fig. 2.** Survivorship of *Lysiphlebus testaceipes* females subjected to 16 diet treatments: water,
687 honey and 14 honeydews. Within the boxes, black dots represent the average and horizontal
688 lines the median. Horizontal lines are used to group aphids of the same species (species), genus
689 (genus), or reared on the same host plants (host plant). Statistical differences between treatments
690 are presented in Table 2.

691 **Fig. 3.** Mean content (± SE) of (A) fructose, (B) total sugars, (C) glycogen and (D) total
692 carbohydrates in *Lysiphlebus testaceipes* females that were two days old and had access to 14
693 diet treatments: water, honey and 12 honeydews. Carbohydrate contents for species subtended
694 by lines do not differ ($P > 0.05$, Tukey test).

695 **Fig. 4.** Correlation between the longevity of *Lysiphlebus testaceipes* females and the content of
696 carbohydrates [(A) fructose, (B) other sugars, (C) glycogen and (D) total carbohydrates] when
697 they fed on 14 diet treatments: water, honey and 12 honeydews. Each point represents the mean
698 ± SE longevity (Y-axis) and mean carbohydrate content (X-axis) of females fed with one of the
699 14 treatments: water = W; honey = H; *A. fabae =Af; A. glycines = Agl; A. gossypii* on cotton =
700 *Agc; A. gossypii* on common milkweed = *Agm; A. monardae =Am; A. nerii = An; M.*
701 *asclepiadis = Ma; M. persicae = Mp; R. maidis = Rm; R. padi = Rp; S. graminum = Sg; U.*
702 *sonchi =Us.*

703

Table 1. Aphid species, phylogenetic relationships, host plants (from which aphids were collected and on which they were cultured), and number of replicates in the assays of longevity and carbohydrates.

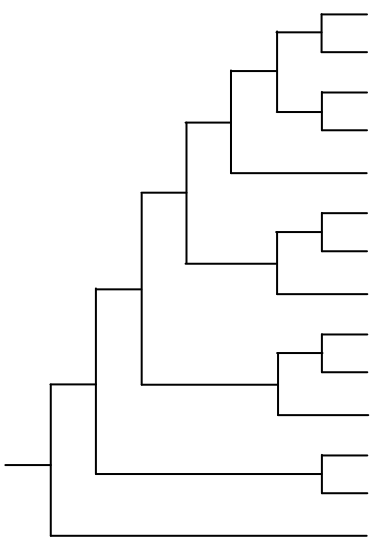
Phylogeny	Aphid species	Host plant species	Replicates	
			Longevity	Carbohydrates
	*Aphis oestlundi* Gillete	*Oenothera biennis*	10	-
	Aphis monardae Oestlund	*Monarda fistulosa*	19	8
	Aphis gossypii Glover	*Gossypium hirsutum*	23	18
	Aphis gossypii Glover	*Asclepias syriaca*	27	18
	Aphis glycines Matsumara	*Glycine max*	20	18
	Aphis asclepiadis F.	*Asclepias incarnata*	19	-
	Aphis nerii Boyer de Fonscolombe	*Asclepias incarnata*	16	9
	Aphis fabae Scopoli	*Vicia fabae*	27	17
	Rhopalosiphum maidis F.	*Hordeum vulgare*	16	18
	Rhopalosiphum padi L.	*Hordeum vulgare*	26	15
	Schizaphis graminum Rondani	*Hordeum vulgare*	25	18
	Myzus persicae Sulzer	*Brassica oleracea*	23	18
	Uroleucon sonchi (L.)	*Sonchus spp.*	20	9
	Myzocallis asclepiadis (Monell)	*Asclepias incarnata*	26	15
	Water control		40	7
	Honey control		41	18

Table 2. Pairwise differences between survival curves of *Lysiphlebus testaceipes* females subjected to 16 diet treatments: water, honey and 14 honeydews. Survival curves were estimated by using the Kaplan-Meier method and pairwise compared using a log-rank test using the "survdiff" function in R. Values are significance levels; bold indicates significance $P < 0.05$.

Treatment	Water	*A. fabae*	*A. glycines*	*A. monardae*	*A. neri*	*A. oestlundi*	*A.gossypii* (cotton)	*A.gossypii* (milkweed)	*A. asclepiadis*	*M. asclepiadis*	*M. persicae*	*R. maidis*	*R. padi*	*S. graminum*	*U. sonchi*	Honey
Water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. fabae	**<0.001**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. glycines	**<0.001**	0.1117	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. monardae	**<0.001**	0.2401	0.8128	-	-	-	-	-	-	-	-	-	-	-	-	-
A. nerii	**<0.001**	0.7319	0.1832	0.2908	-	-	-	-	-	-	-	-	-	-	-	-
A. oestlundi	**<0.001**	**<0.05**	0.1838	0.3524	**<0.05**	-	-	-	-	-	-	-	-	-	-	-
A. gossypii (cotton)	**<0.001**	0.9051	0.0653	0.1528	0.6646	**<0.01**	-	-	-	-	-	-	-	-	-	-
A.gossypii (milkweed)	**<0.001**	0.1469	0.8735	0.8908	0.1439	0.194	0.0616	-	-	-	-	-	-	-	-	-
A. asclepiadis	**<0.01**	0.054	**<0.001**	**<0.01**	**<0.05**	**<0.001**	**<0.05**	**<0.001**	-	-	-	-	-	-	-	-
M. asclepiadis	**<0.001**	0.3951	**<0.05**	**<0.05**	0.228	**<0.01**	0.4072	**<0.01**	0.2584	-	-	-	-	-	-	-
M. persicae	**<0.001**	0.7938	0.1589	0.3479	0.7681	**<0.05**	0.8215	0.1791	**<0.05**	0.3577	-	-	-	-	-	-
R. maidis	**<0.001**	0.7513	<0.05	0.1416	0.9082	**<0.01**	0.8418	0.0704	**<0.01**	0.2763	0.7334	-	-	-	-	-
R. padi	**<0.001**	**<0.05**	**<0.001**	**<0.001**	**<0.05**	**<0.001**	**<0.05**	**<0.001**	0.9711	0.2084	**<0.05**	**<0.01**	-	-	-	-
S. graminum	**<0.001**	0.0953	**<0.001**	**<0.01**	**<0.05**	**<0.001**	0.1083	**<0.001**	0.6289	0.4667	0.0711	**<0.05**	0.5684	-	-	-
U. sonchi	**<0.001**	**<0.05**	0.2479	0.1993	**<0.05**	0.8749	**<0.01**	0.1503	**<0.001**	**<0.001**	**<0.05**	**<0.01**	**<0.001**	**<0.001**	-	-
Honey	**<0.001**	**<0.001**	**<0.01**	**<0.05**	**<0.01**	0.2094	**<0.001**	**<0.01**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.5758	-

Fig. 1.

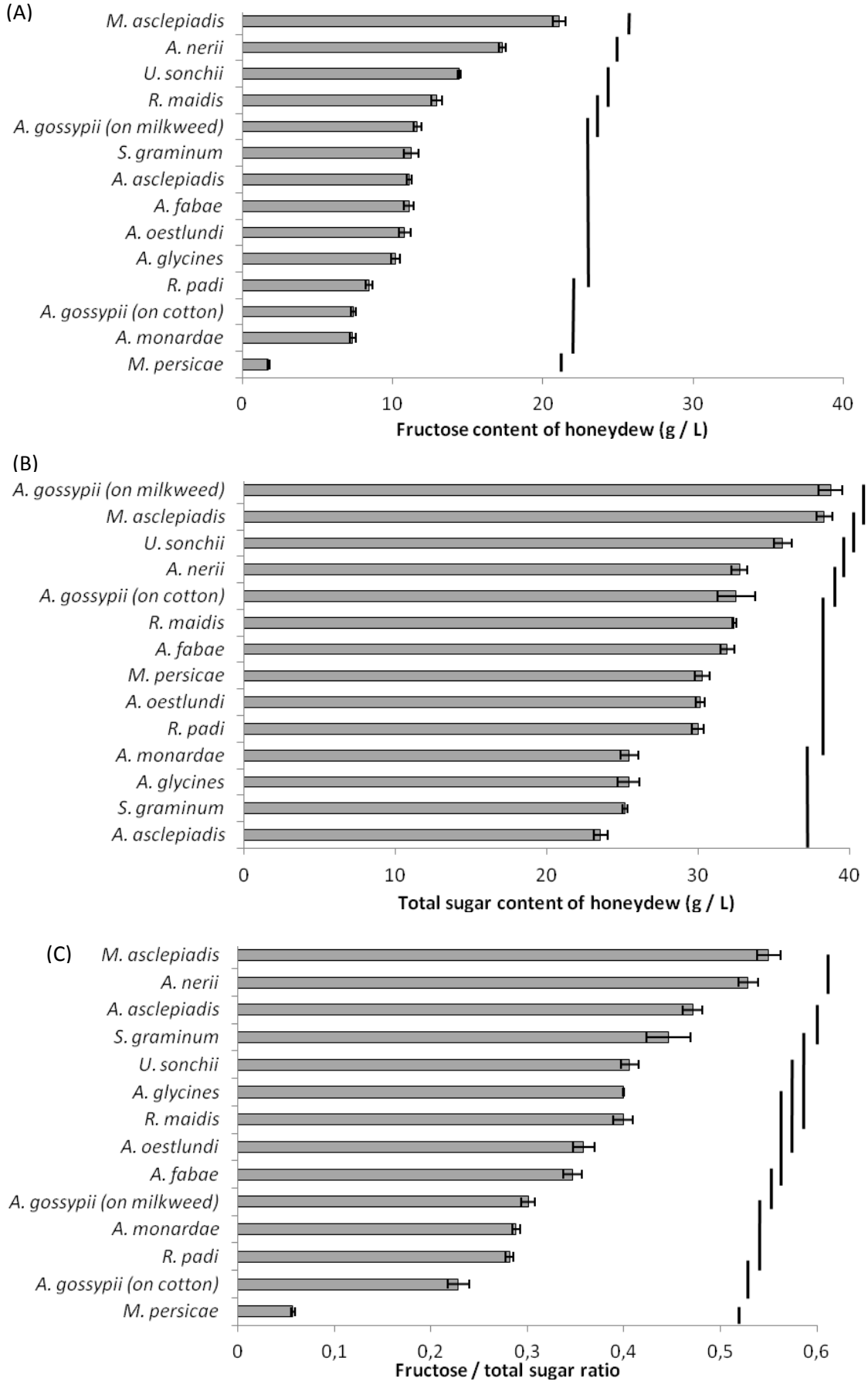

(A)
M. asclepiadis
A. nerii
U. sonchii
R. maidis
A. gossypii (on milkweed)
S. graminum
A. asclepiadis
A. fabae
A. oestlundi
A. glycines
R. padi
A. gossypii (on cotton)
A. monardae
M. persicae
0
10
20
30
40
Fructose content of honeydew (g / L)
(B)
A. gossypii (on milkweed)
M. asclepiadis
U. sonchii
A. nerii
A. gossypii (on cotton)
R. maidis
A. fabae
M. persicae
A. oestlundi
R. padi
A. monardae
A. glycines
S. graminum
A. asclepiadis
0
10
20
30
40
Total sugar content of honeydew (g / L)
(C)
M. asclepiadis
A. nerii
A. asclepiadis
S. graminum
U. sonchii
A. glycines
R. maidis
A. oestlundi
A. fabae
A. gossypii (on milkweed)
A. monardae
R. padi
A. gossypii (on cotton)
M. persicae
0
0,1
0,2
0,3
0,4
0,5
0,6
Fructose / total sugar ratio

Fig. 2.

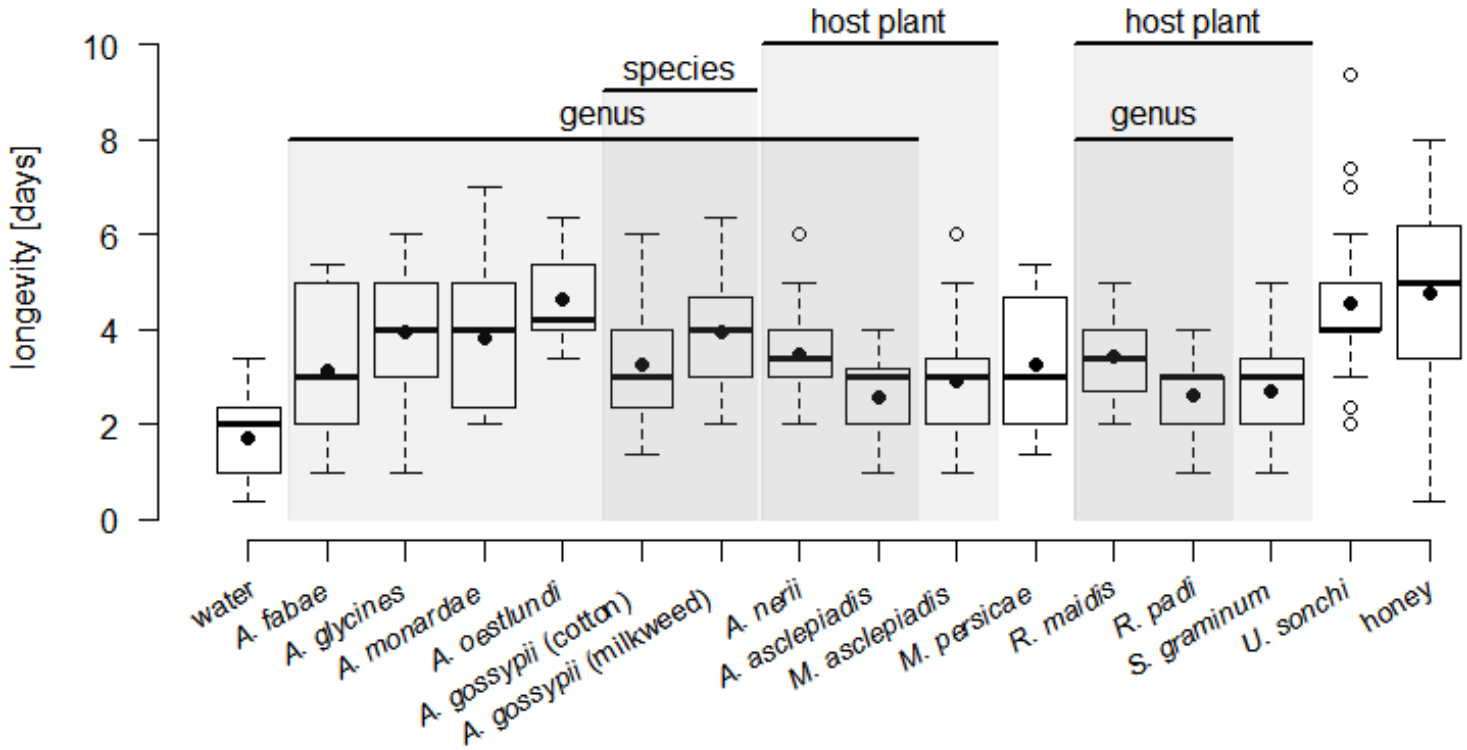
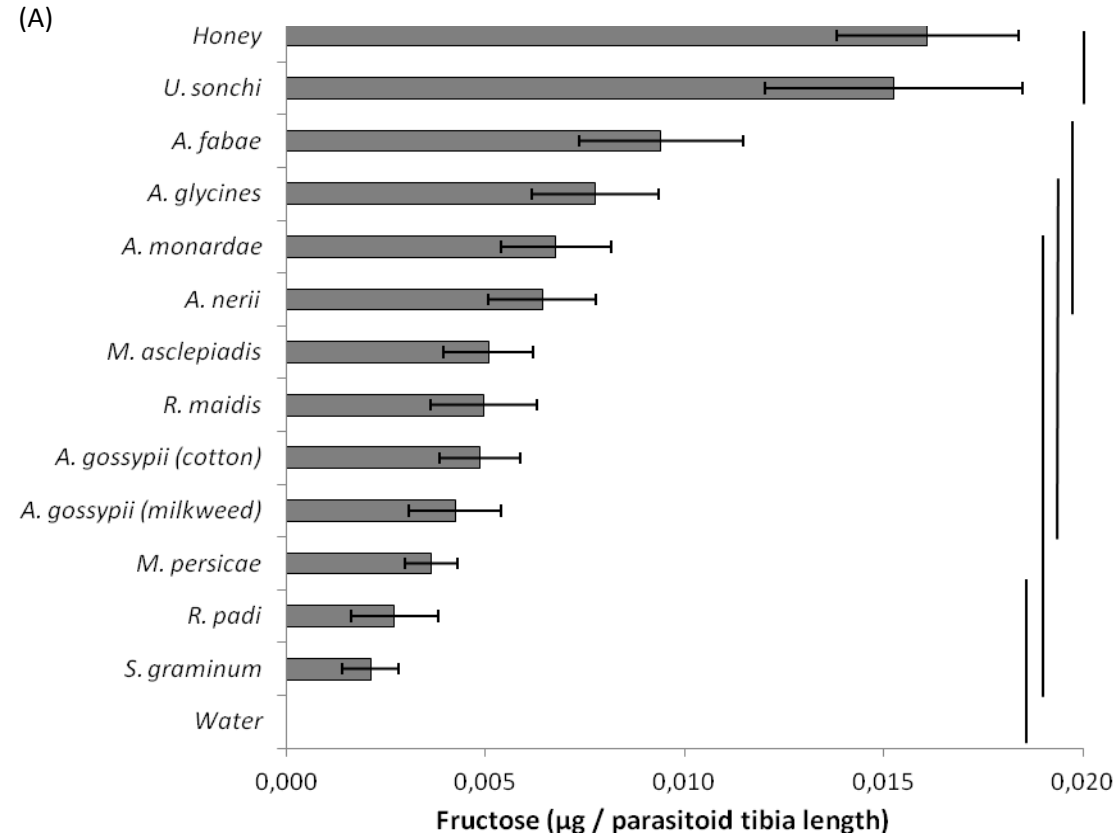
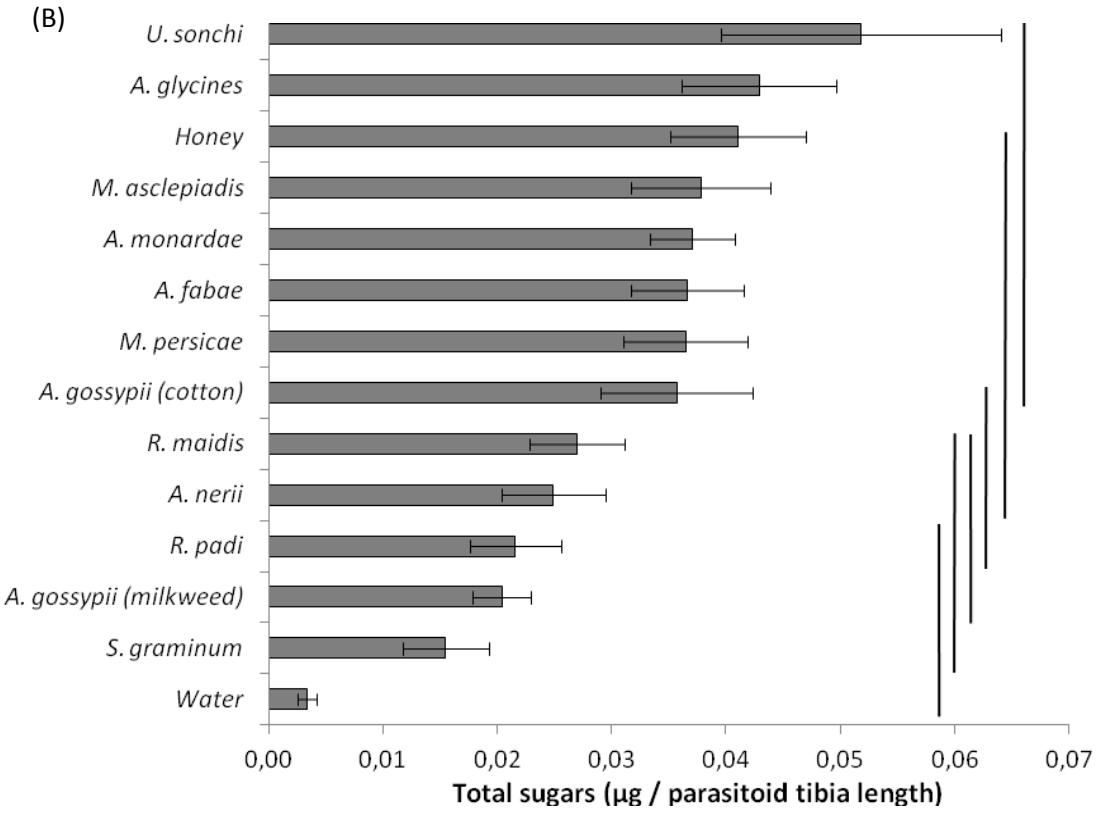
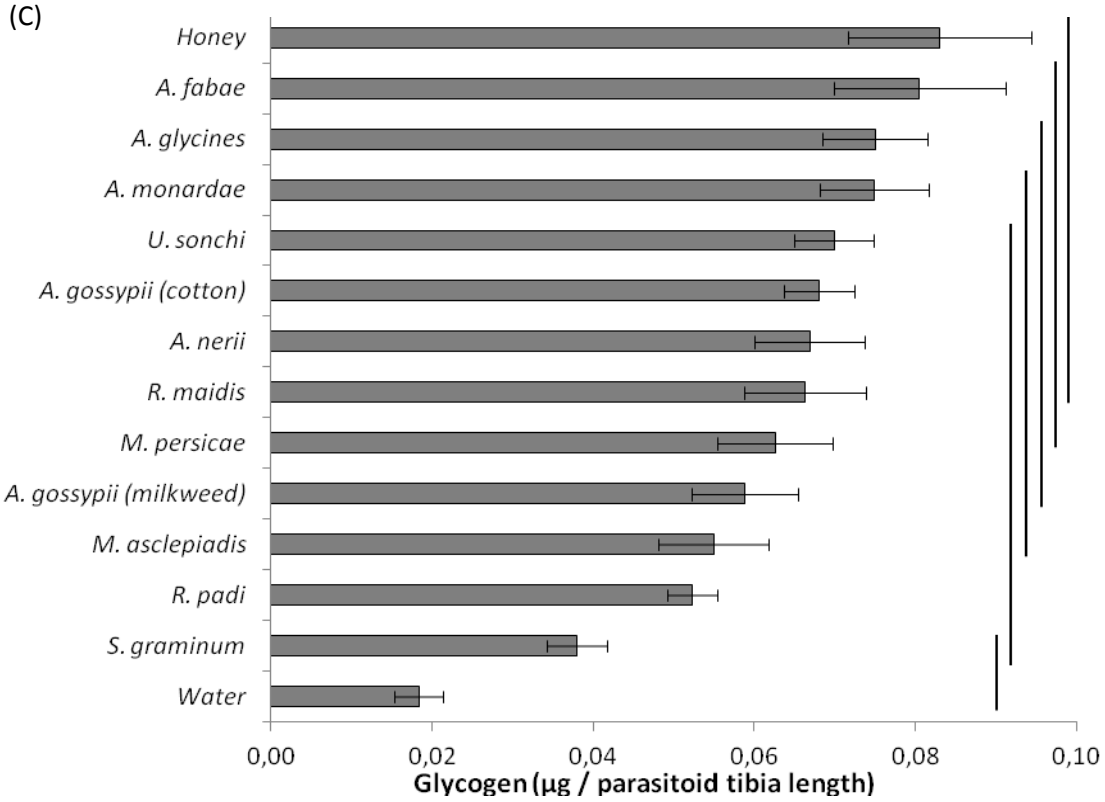

longevity [days]
0
2
4
6
8
10
genus
species
host plant
genus
host plant
water
A. fabae
A. glycines
A. monardae
A. oestlundi
A. gossypii (cotton)
A. gossypii (milkweed)
A. nerii
A. asclepiadis
M. asclepiadis
M. persicae
R. maidis
R. padi
S. graminum
U. sonchi
honey

Fig. 3.


(A)
Honey
U. sonchi
A. fabae
A. glycines
A. monardae
A. nerii
M. asclepiadis
R. maidis
A. gossypii (cotton)
A. gossypii (milkweed)
M. persicae
R. padi
S. graminum
Water
0,000
0,005
0,010
0,015
0,020
Fructose (µg / parasitoid tibia length)


(B)
U. sonchi
A. glycines
Honey
M. asclepiadis
A. monardae
A. fabae
M. persicae
A. gossypii (cotton)
R. maidis
A. nerii
R. padi
A. gossypii (milkweed)
S. graminum
Water
0,00
0,01
0,02
0,03
0,04
0,05
0,06
0,07
Total sugars (µg / parasitoid tibia length)

(C)


Honey
A. fabae
A. glycines
A. monardae
U. sonchi
A. gossypii (cotton)
A. nerii
R. maidis
M. persicae
A. gossypii (milkweed)
M. asclepiadis
R. padi
S. graminum
Water
0,00
0,02
0,04
0,06
0,08
0,10
Glycogen (µg / parasitoid tibia length)

(D)

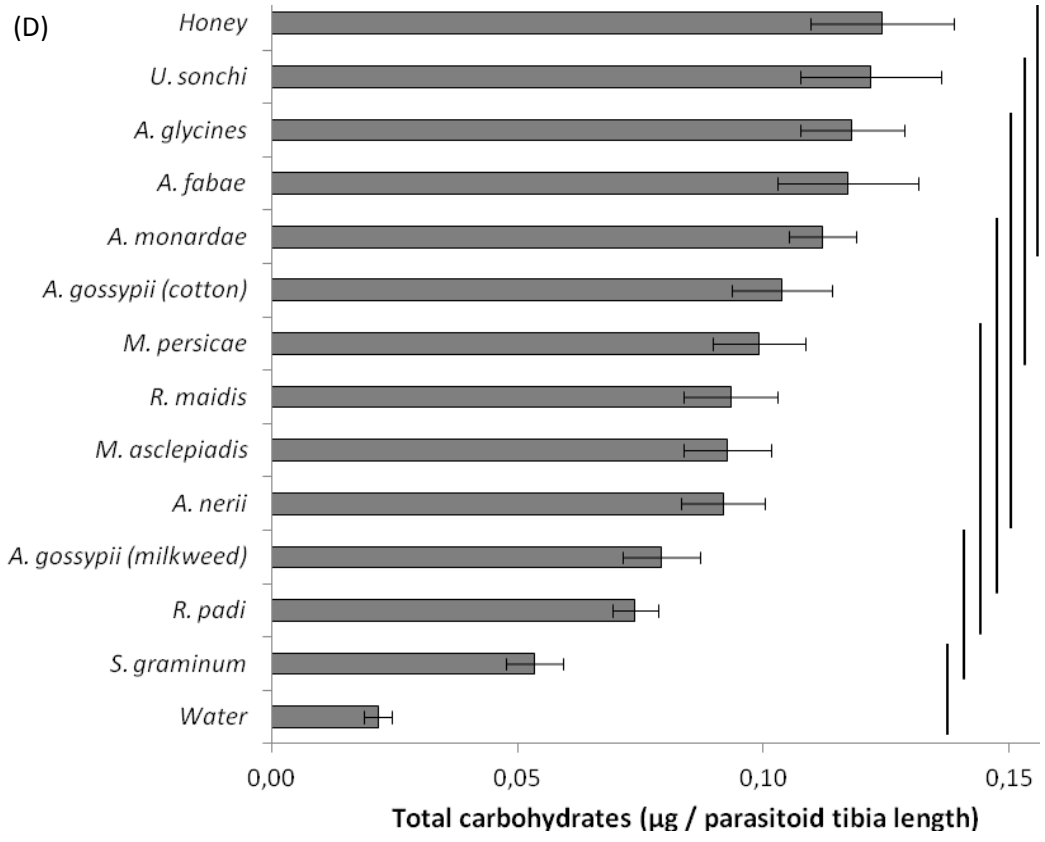
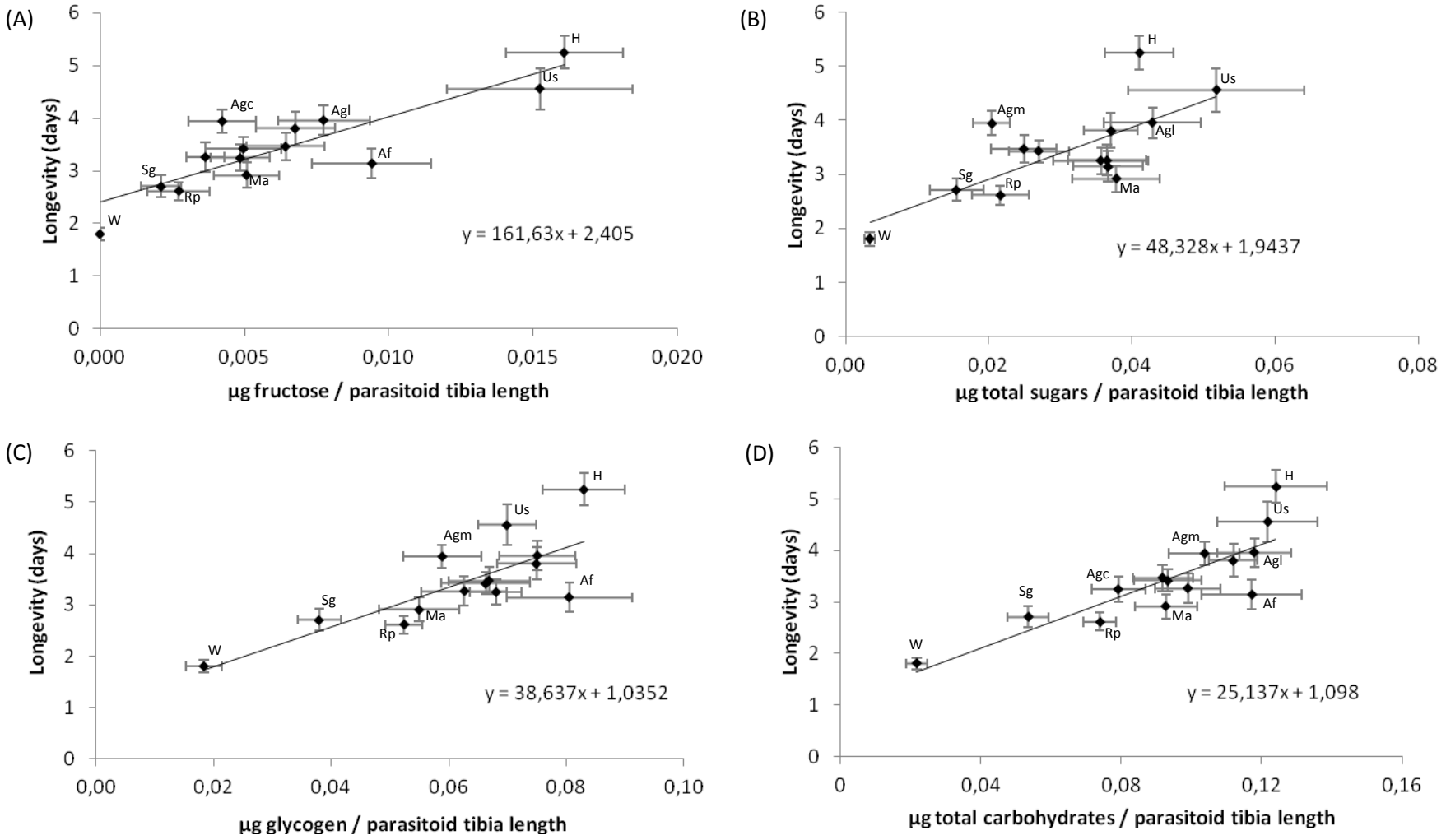

Honey
U. sonchi
A. glycines
A. fabae
A. monardae
A. gossypii (cotton)
M. persicae
R. maidis
M. asclepiadis
A. nerii
A. gossypii (milkweed)
R. padi
S. graminum
Water
0,00
0,05
0,10
0,15
Total carbohydrates (µg / parasitoid tibia length)

Fig. 4.


(A)
Longevity (days)
µg fructose / parasitoid tibia length
y = 161,63x + 2,405
(B)
Longevity (days)
µg total sugars / parasitoid tibia length
y = 48,328x + 1,9437
(C)
Longevity (days)
µg glycogen / parasitoid tibia length
y = 38,637x + 1,0352
(D)
Longevity (days)
µg total carbohydrates / parasitoid tibia length
y = 25,137x + 1,098
W
Rp
Sg
Ma
Agc
Agm
Agl
Af
Us
H

Appendix A. Supplementary data

Fig. 1. Effect of aphid phylogeny on sugar content (mean ± SE) of 14 honeydews excreted by aphids. A) Fructose content (measured with cold anthrone test). B) Total sugar content (measured with hot anthrone test). * on cotton; ** on common milkweed

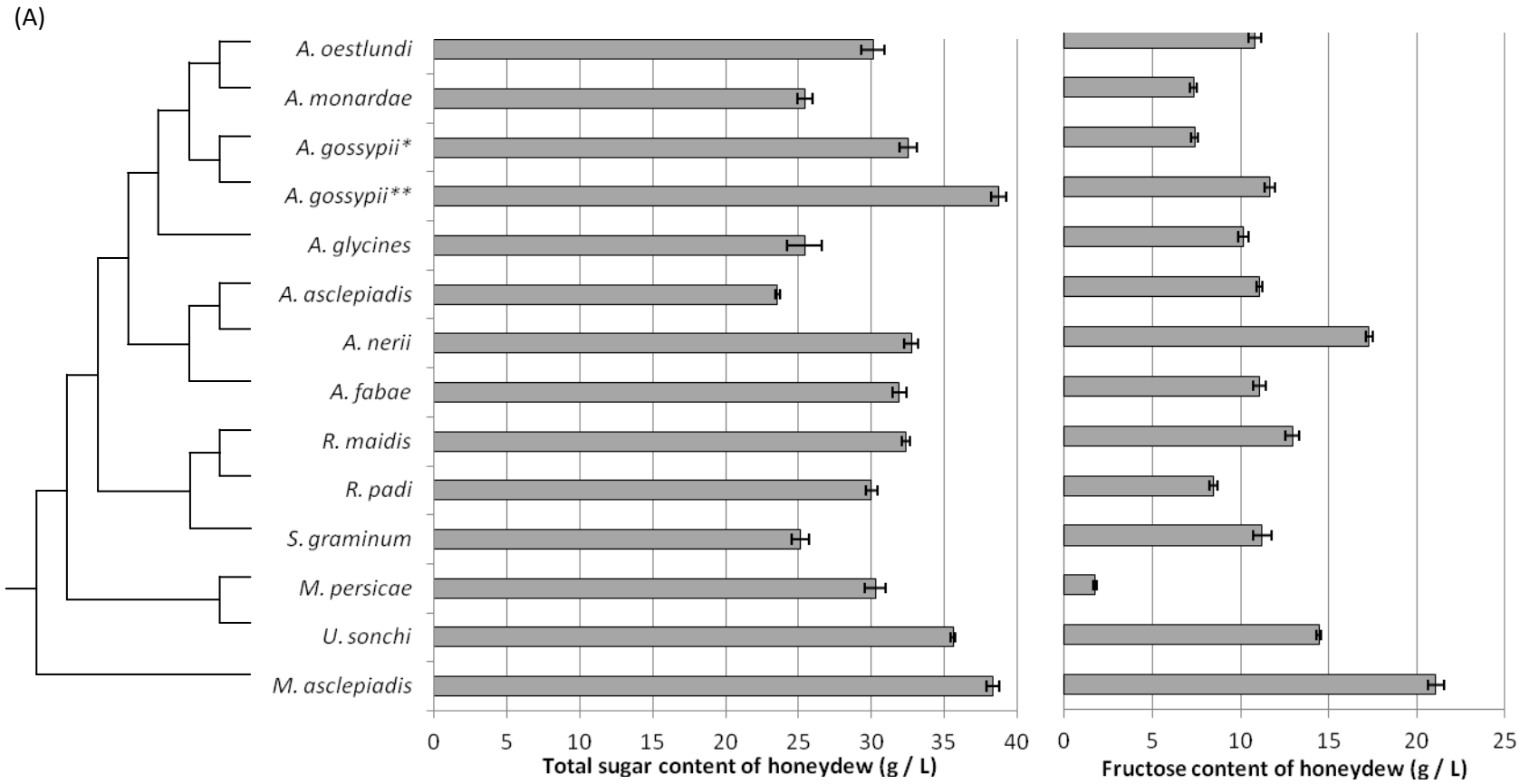


Fig. 2. Survivorship curves based on Kaplan-Meier estimates of female *Lysiphlebus testaceipes* subjected to 16 diet treatments: water, honey and 14 honeydews.

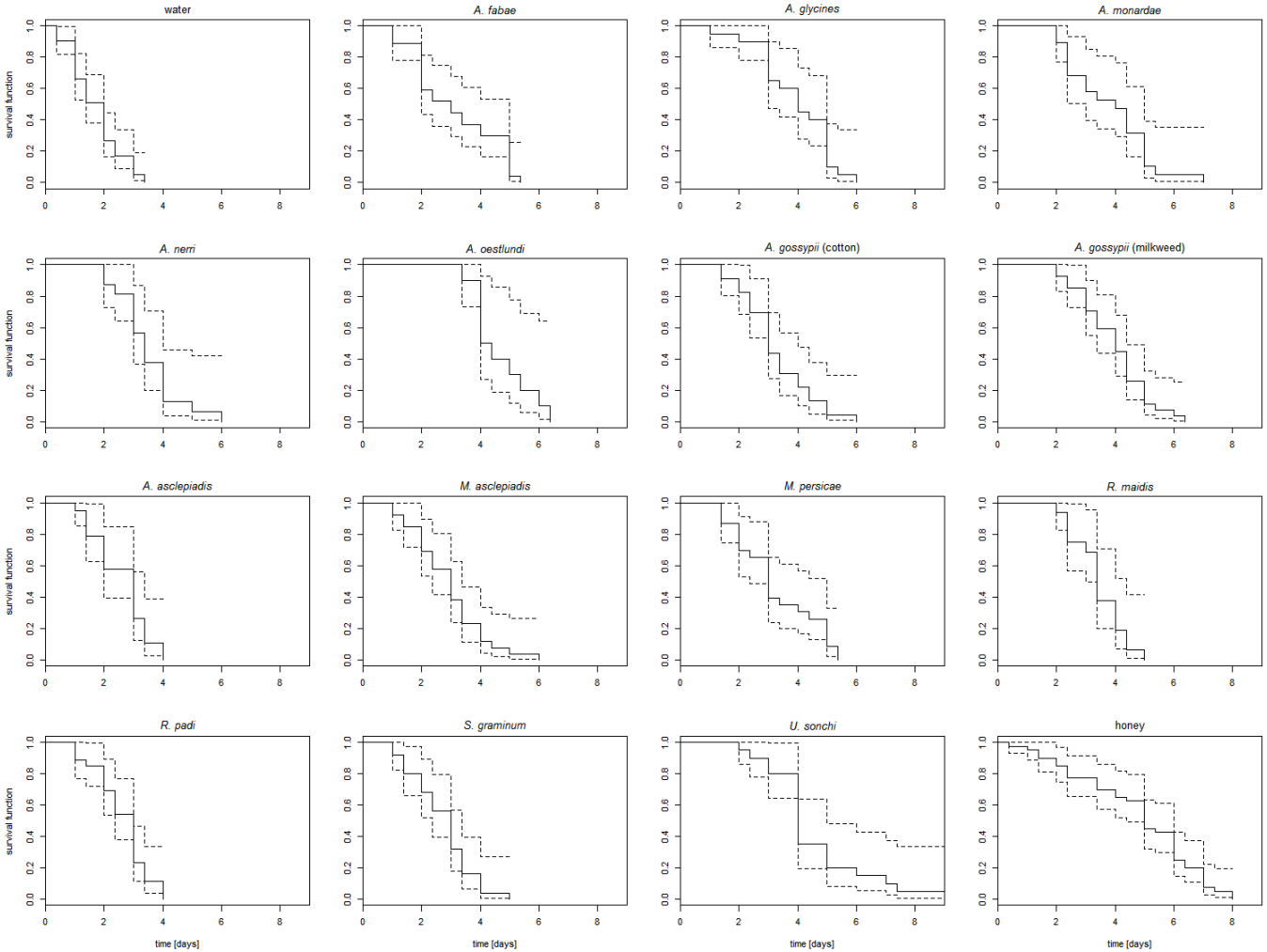


Fig. 3. Effect of aphid phylogeny on the survivorship of *Lysiphlebus testaceipes* females subjected to 14 honeydews excreted by 13 aphid species. Within the boxes, black dotes represent the average and horizontal lines the median. *on cotton; **on common milkweed

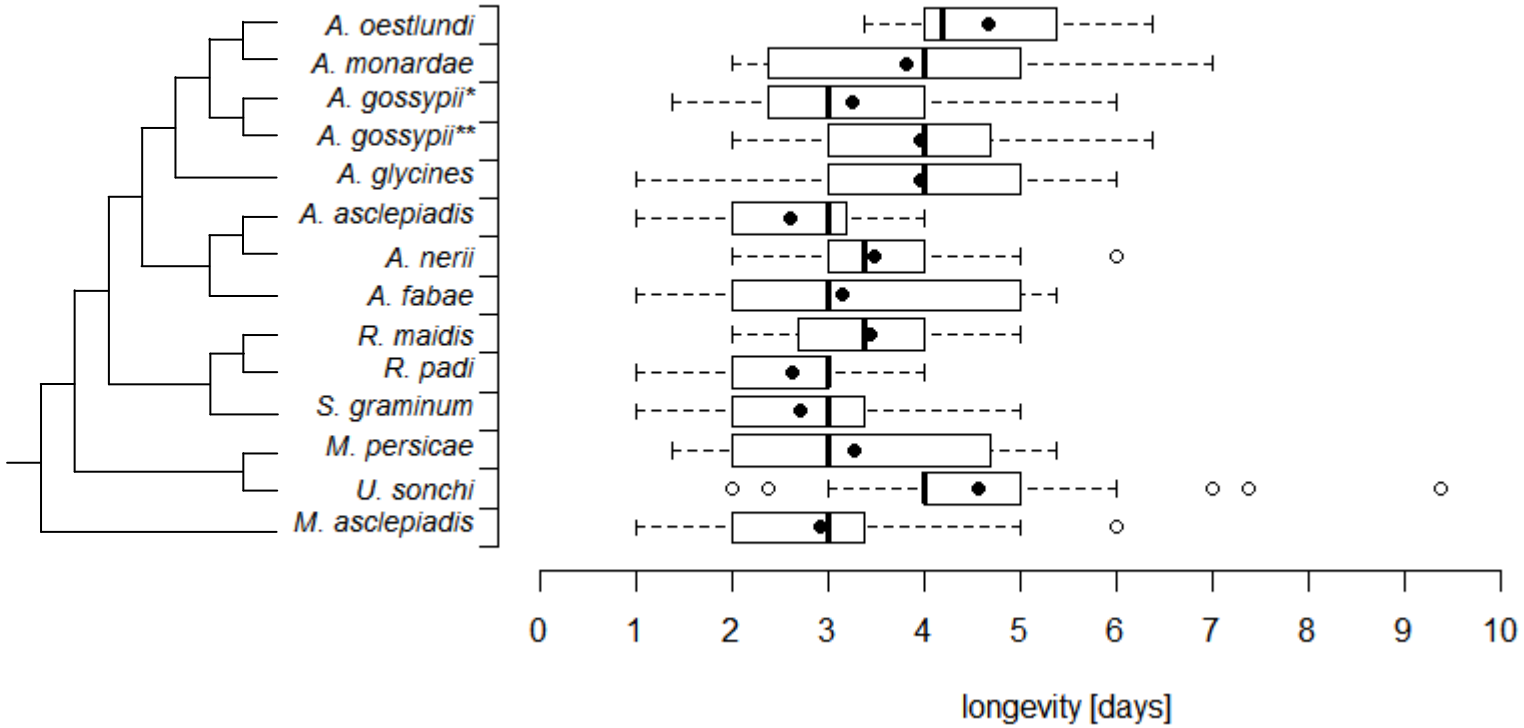


Fig. 4. Effect of aphid phylogeny on the carbohydrate content of *Lysiphlebus testaceipes* females subjected to 12 honeydews.