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[Monticelli, L. S., Tena, A., Idier, M., Amiens-Desneux, E., & Desneux, N. (2019). Quality of aphid honeydew for a parasitoid varies as a function of both aphid species and host plant. *Biological Control*, 104099.]

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The final publication is available at

[\[http://dx.doi.org/10.1016/j.biocontrol.2019.104099\]](http://dx.doi.org/10.1016/j.biocontrol.2019.104099)

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**1Quality of aphid honeydew for a parasitoid varies as a function of both aphid species
2and host plant.**

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9

10Author Contributions: AT, ND and LSM conceived and designed the experiments. LSM, MI
11and EA performed the experiments. LSM analyzed the data. LSM, AT and ND wrote the
12manuscript; other authors provided editorial advice.

13ABSTRACT

14Honeydew i.e., the sugar-rich excretion of phloem-sap feeders, is the most available sugar
15source in agroecosystems which enables parasitoids to increase their fitness. For the
16parasitoids of honeydew-producing insects, honeydew is associated with host presence,
17suggesting that parasitoids can find carbohydrate sources and hosts at the same place. Hence,
18these parasitoids may have evolved to efficiently use the honeydew produced by their main
19hosts as a carbohydrate source. In this context, we assessed the relationship between the adult
20fitness (in terms of longevity and fecundity) of the aphid parasitoid *Aphelinus abdominalis*
21(which prefer attack aphid species belonging to the Macrosiphini tribe), fed on honeydew
22produced by 19 different plant-aphid combinations, and parasitoid preference (i.e., the choice
23of female to oviposit) and ability to produce offspring in these combinations. Honeydew
24quality was modulated by both the plant and aphid host species. The honeydew produced by
25eight aphid species (e.g., *Macrosiphum euphorbiae* and *Aphis craccivora*) resulted in high
26parasitoid longevity (same longevity as the honey-fed positive control) and an increase in egg
27load between 24 and 48 hours, compared to the negative control (fed only water). In contrast,
28nine aphid species (e.g., *Brevicoryne brassicae* and *Aphis nerii*) excreted low-quality
29honeydew that did not increase parasitoid longevity or egg load (similar to the negative
30control water-fed parasitoids). There was no relationship between *A. abdominalis* adult fitness
31when fed various honeydews and its preference as well as its ability to produce offspring in
32the hosts producing these honeydews. Several hypotheses that may explain this lack of
33relationship are discussed.

34**Key words:** Longevity, egg load, *Aphelinus abdominalis*, Aphidini, Macrosiphini, Siphini,
35nutritional ecology.

36INTRODUCTION

37After adult emergence, female parasitoids generally need to search for food and hosts (Sirot
38and Bernstein 1996; Bernstein and Jervis 2008; Lightle et al. 2010). Indeed, adult parasitoids
39need to feed on carbohydrate sources, such as nectar or honeydew, to increase their survival
40and fecundity (Jervis et al. 1993; Wäckers 2001; Wäckers et al. 2008; Tena et al. 2016;
41Benelli et al. 2017). When carbohydrate sources and hosts are in different plant organs or
42even different plant species, female parasitoids have to spend time and energy and must also
43engage in dangerous trips between hosts and carbohydrate sources (Wäckers et al. 2008). In
44contrast, for parasitoids of phloem-sap feeders, such as aphids, whiteflies, scales or
45mealybugs, carbohydrate sources are directly associated with host presence because these
46insects excrete honeydew. This sugar-rich excretion of phloem-sap feeders is the most
47available sugar source in agroecosystems (Jervis 1998; Wäckers et al. 2008; Lundgren 2009;
48Tena et al. 2016).

49 Honeydew is largely consumed by parasitoids in nature (Lee et al. 2006; Hogervorst et
50al. 2007a; Tena et al. 2013a and 2016; Calabuig et al. 2015). Although it was previously
51considered a low nutritional source for parasitoids when compared to nectar, in reality,
52honeydew has a highly variable nutritional value (Wäckers et al. 2008; Vollhardt et al. 2010;
53Tena et al. 2018). In fact, it has been recently suggested that parasitoids whose hosts excrete
54honeydew may have evolved to efficiently locate and digest honeydew as a food source
55(Goelen et al. 2018). Honeydew has already been shown to be involved in host localization
56and acceptance by aphid parasitoids (e.g., Bouchard and Cloutier 1984; Hågvar & Hofsvang
571989; Budenberg 1990; Grasswitz and Paine 1993; Han and Chen 2002; Hatano et al. 2008;
58Lenaerts et al. 2016). For example, the aphid parasitoid *Diaeretiella rapae* is attracted by the
59kairomones emitted by the honeydew of its main host: *Brevicoryne brassicae* (Shaltiel and
60Ayal 1998). Thus, parasitoids can find carbohydrate sources and hosts in the same patch,

61reducing the energy wasted by female parasitoids for food foraging, thus leading to higher
62parasitism rates (Jervis and Kidd 1986; Wäckers 2005; Lee et al. 2006; Wäckers et al. 2008;
63Lightle et al. 2010; Rasekh et al. 2010; Tena et al. 2013a and 2015). Hence, we may
64hypothesize that honeydew-producer parasitoids have evolved to efficiently use the honeydew
65produced by their hosts as a carbohydrate source.

66 An important number of studies have assessed the role of honeydew excreted by
67aphids in parasitoid fitness (Wäckers et al. 2001; Hogervorst et al. 2007b; Vollhardt et al.
682010; Hopkinson et al. 2013; Tena et al. 2013b; Charles and Paine 2016; Lenaerts et al. 2016;
69Tena et al. 2018) but these studies tested a limited number of aphid genera (up to 6) and did
70not consider the honeydew-producer parasitoids preference (i.e., the choice of female to
71oviposit) and capability to produce offspring in these same species. However, this information
72is needed to evaluate the parasitoid nutritional state and to provide useful cues for biological
73control programs. In this context, we assessed, under laboratory conditions, the relationship
74between the preference and the ability to produce offspring of the aphid parasitoid *Aphelinus*
75*abdominalis* on 19 plant-aphid combinations (previous published results by our lab:
76Monticelli et al. 2019, experiment 3) and the impact of their honeydew as an adult food
77source on its longevity (experiment 1) and egg load (experiment 2). *A. abdominalis* is a
78solitary koinobiont endoparasitoid attacking preferentially aphids from the Macrosiphini tribe
79(Monticelli et al. 2019), able to live up to 30 days and classified as a synovigenic parasitoid
80i.e., the number of eggs produced and available for oviposition depends on carbohydrate
81sources (Olson et al. 2005).

82

83

84MATERIAL AND METHODS

85 *Biological materials*

86 All the plants used for aphid and parasitoid colonies were grown in climatic chambers (23 ± 2
87 $^{\circ}\text{C}$, RH $65 \pm 5\%$ and photoperiod 16:8 L: D h) and were between 1 and 4 week-old depending
88 on the plant species (e.g., wheat used to feed *Schizaphis graminum* was 1 week-old whereas
89 milkweed used to feed *Macrosiphum euphorbiae* was 4 week-old). Table 1 describes the
90 fourteen aphid species tested and their respective host plants, as well as the sample size for the
91 experiments 1 and 2. The aphid species used belong to the Aphidini, Macrosiphini and Siphini
92 tribes, have 10 different plant host species, and were maintained in climatic chambers at $23 \pm$
93 $^{\circ}\text{C}$, RH $65 \pm 5\%$ and photoperiod 16:8 L: D hours. Two non-infested plants were introduced
94 per week in a ventilated cage (40 x 30 x 30 cm) covered by mesh containing six infested
95 plants by the respective aphid species. Every week, two dead plants were removed from the
96 cage. *M. euphorbiae* and *Myzus persicae* both were reared on three different plant species:
97 *Solanum tuberosum* (potato), *S. lycopersicum* (tomato) and *Asclepias syriaca* (milkweed) for
98 *M. euphorbiae* and *Brassica oleracea* (cabbage), *Vicia fabae* (bean) and *Triticum aestivum*
99 (wheat) for *M. persicae*. *Metopolophium dirhodum* was maintained on 2 different plant
100 species: *Triticum aestivum* (wheat) and *Hordeum vulgare* (barley).

101 *Aphelinus abdominalis* colony was initiated from parasitoids naturally colonizing *M.*
102 *euphorbiae* on *S. lycopersicum* in the INRA Sophia Antipolis greenhouse complex and
103 maintained on their principal hosts (most frequent and suitable host): *Acyrtosiphon pisum*
104 (Hullé et al. 2006) in climatic chambers (23 ± 2 $^{\circ}\text{C}$, RH $65 \pm 5\%$ and photoperiod 16:8 L: D
105 h) for at least 5 generations before experiments. Two fully-infested plants by *A. pisum* were
106 introduced per week in a ventilated cage (60 x 60 x 60 cm) covered by mesh containing six
107 plants and on average 30 adult parasitoids. Every week, two dead plants were removed from
108 the cage. Before experiments, the parasitized aphid mummies were isolated in 0.6mL

109Eppendorf tubes and parasitoid emergence was checked daily at 8am and 6pm to obtain newly
110emerged, unfed and unmated parasitoid females.

111

112*Honeydew collection and analyses*

113The protocol used was similar to that described in Tena et al. (2013b, 2018). Pieces of
114Parafilm[®] were placed inside the aphid cages, directly underneath aphid colonies. After 24
115hours, the Parafilm[®] were retrieved, isolated in petri dishes (diameter: 9cm and height: 2.5cm)
116along with a moistened cotton to avoid crystallization, and then frozen until further use.

117 Fructose is one of the main sugars present on honeydew (Wäckers 2001; Hogervorst et
118al. 2007a, b; Wyckhuys et al. 2008; Faria et al. 2008; Tena et al. 2013a, 2018). To analyze the
119fructose content of the 19 different honeydews, we used the Anthrone test protocol as
120described by Tena et al. (2018). After drying honeydew in the oven for 48 hours at 40°C, it
121was then collected and dissolved in 25% ethanol, in order to obtain a honeydew solution with
122a concentration of 1mg/mL. 950µL of anthrone solution were added to 50µL of either the
123honeydew solution or a standard solution of fructose, contained in 1.5mL Eppendorf tubes.
124Then, the sample and standard tubes were incubated for 1.5 hours at room temperature,
125vortexed and placed (3*200µL) in a 96-well plate which was then read by a
126spectrophotometer at 620nm.

127

128*Experiment 1: Parasitoid longevity*

129Unfed and unmated female parasitoids less than 15 hours-old were placed individually into
130glass tubes (diameter: 1.2cm, height: 7.5cm) containing a honeydew-spotted piece of
131Parafilm[®] and sealed with a piece of moistened cotton. Parasitoids fed on honey or only water
132were used as controls. The Parafilm[®] was replaced every two days and the cotton was

133dampened with water daily. Parasitoid survival was checked twice daily, at 8am and 6pm, and
134tibia length was measured after death (to make sure there was no systematic bias in size of
135adults among the treatment), under the microscope (x40, Olympus CX40).

136

137*Experiment 2: Parasitoid egg load*

138Newly emerged unfed and unmated female parasitoids were placed in 2.0mL Eppendorf tubes
139containing a piece of honeydew-spotted Parafilm[®] and a moistened cotton. In order to monitor
140the egg maturation dynamic, the number of eggs were counted 24 and 48 hours later. After
141this time, parasitoids were frozen until their dissection under the microscope (x40) performed
142in order to determine their total egg count and their tibia length (to avoid systematic bias in
143size of adults among the treatment). As previously, newly emerged unfed and unmated female
144parasitoids, and fed on honey or only water, were used as controls.

145

146*Experiment 3: Parasitoid preference and ability to produce offspring*

147In a previous study (Monticelli et al. 2019), we evaluated behavioral and physiological host
148range of *A. abdominalis* i.e., the proportion of host attacked and enable producing offspring,
149on the same aphid and plant species tested in this study, with the exceptions of *A. glycines*, *M.*
150*eurphobiae* (milkweed), *M. dirhodum* (barley), *M. persicae* (bean and wheat) and *S. maydis*
151(data missing). One individual aphid from one of the 13 plant-aphid combinations tested was
152placed on a single plant leaf placed upside down under a binocular magnifier (8×). One naïve
153mated female (between 24 and 48 hour-old) was then introduced under a plastic dome
154(diameter: 1 cm, height: 0.65 cm) during 10 minutes or until the parasitoid exhibited
155oviposition behavior. The experiment was repeated 30 times to determine the proportion of
156host attacked by the parasitoid in each plant-aphid combination (preference). At least 20

157aphids having been attacked from each plant-aphid combination were then placed on their
158respective host plant species during 10 days and the proportion of parasitoid emerged from
159these aphids was monitored.

160

161*Statistical analyses*

162All the statistical analyses were performed using R. 3.3.3. Fructose contents as well as female
163parasitoid tibia length were compared among the different plant and aphid species tested using
164a linear model (normality of the data and of the model residuals were checked). The impact of
165the host plant and the aphid species producing the honeydew on the parasitoid survival when
166fed on these honeydews was compared using a cox model (Kaplan-Meyer Log rank) and the p
167values were adjusted following the Bonferroni adjustment method for multi-comparison.

168Parasitoid egg load was compared among the different plant and aphid species tested using a
169generalized linear model following a poisson distribution. Multi-comparison tests were
170completed using the ‘multcomp’ package with a Tukey adjustment, when necessary.

171Correlations between (i) fructose content and parasitoid longevity and (ii) fructose content
172and parasitoid egg production were analyzed using a correlation test (method = Pearson) to
173test whether parasitoid fitness increases with the content of fructose in honeydew. Finally,
174correlation between (i) parasitoid longevity and both parasitoid preference and ability to
175produce offspring as well as (ii) parasitoid egg production and both parasitoid preference and
176ability to produce offspring were performed to test the potential relationship between host
177suitability and honeydew quality as food source. In all the correlations, we used a comparative
178analysis of independent contrasts (calculation of phylogenetically independent variables, as
179described by Felsenstein (1985)) (CAIC, package ‘caper’).

180

181

182RESULTS

183Honeydew analyses

184The fructose content of honeydew varied depending upon both the plant and the aphid species
185producing the honeydew (Table 2, Figure 1 and Appendix A). The fructose concentration was
1864-fold higher in the honeydew excreted by *S. maydis* than in that excreted by *R. padi*, when
187both aphids were reared on wheat whereas the fructose content was similar among the
188honeydews produced by different aphid species feeding on bean and cabbage. Fructose
189concentration in the honeydew of *M. euphorbiae* was 2-fold higher on tomato than on potato
190whereas the fructose content did not vary among the honeydews produced by *M. persicae* and
191*M. dirhodum* feeding on different plant species. When considering honeydew from each
192plant-aphid combination as a unit, fructose content was highest in the honeydew excreted by
193*S. maydis* (not significantly different from the female fed with honey) and lowest in the
194honeydew produced by *A. nerii*, *R. padi*, *A. glycines* and *M. dirhodum* (on barley) (Appendix
195A).

196

197Experiment 1: Parasitoid longevity

198Female size, measured as tibia length, did not vary depending upon both the plant and the
199aphid species (Table 2, Appendix A). The diet provided, however, affected the longevity of *A.*
200*abdominalis* females (Table 2, Figure 2 and Appendix B). The longevity of females fed on *A.*
201*craccivora* honeydew was 2-fold higher than that of those fed on *A. pisum* honeydew when
202both aphid species were reared on bean as well as longevity of females fed on *M. persicae*
203honeydew was 1.6-fold higher than that of those fed on *S. graminum* honeydew when both
204aphid species were reared on wheat (Figure 2). Similarly, female longevity was 2-fold higher
205when fed on wheat-reared *M. persicae* honeydew than when fed on cabbage-reared *M.*

206 *persicae* honeydew as well as longevity of females fed on tomato-reared *M. euphorbiae*
207 honeydew was 3.3-fold higher than that of those fed on milkweed-reared *M. euphorbiae*.
208 When considering each plant-aphid combination as a unit, female parasitoids feeding on
209 honeydew excreted by three specific plant-aphid combinations, including *A. craccivora* (on
210 bean), *A. glycines* (on soybean) and *M. euphorbiae* (on tomato), had the highest longevity and
211 survived 16 days (not significantly different from the female fed with honey, Appendix B). In
212 comparison, females fed on honeydew of three other specific aphid combinations, including
213 *B. brassicae* (on cabbage), *M. euphorbiae* (on milkweed) and *M. persicae* (on cabbage),
214 survived only 5 days (not significantly different from the female fed with water). The diet
215 provides by the 13 other plant-aphid combinations enable parasitoids to survive on average 8
216 days (including *A. gossypii*, *M. dirhodum* (wheat), *M. persicae* (bean and wheat), *S. avenae*,
217 and *S. maydis* which enable parasitoid to significantly live longer than females fed water).
218 Female longevity was not correlated with honeydew fructose concentration ($R^2 = 0.13$, $df =$
219 19, $P = 0.108$).

220

221 *Experiment 2: Parasitoid egg load*

222 Female size did not vary depending on the host plant and the aphid species tested after 24 and
223 48 hours (Table 2, Appendix A) whereas the parasitoid eggs load after 24 and 48 hours of
224 feeding do (Table 2, Figure 3 and Appendix C). For example, the egg load of females fed on
225 *A. craccivora* honeydew during 48 hours was 1.5-fold higher than that of those fed on *A.*
226 *fabae* honeydew, when both aphid species were reared on bean as well as longevity of
227 females fed on *S. maydis* honeydew was 1.4-fold higher than that of those fed on *M. persicae*
228 honeydew when both aphid species were reared on wheat (Figure 3). Similarly, females egg
229 load was 1.3-fold higher when fed wheat-reared *M. persicae* honeydew than when fed that of
230 cabbage-reared *M. euphorbiae* as well as longevity of females fed on wheat-reared *M.*

231 *dirhodum* honeydew was 1.2-fold higher than that of those fed on barley-reared *M. dirhodum*.
 232 When considering each plant-aphid combination as a unit, female parasitoids produced the
 233 highest number of eggs (average of 16) when fed 24 hours on honey or honeydew excreted by
 234 *A. glycines* and *S. graminum* (Appendix C). In contrast, females fed honeydew produced by
 235 *A. gossypii* produced only 10 eggs in 24 hours, which was even lower than the number of eggs
 236 produced by the female fed only water (14 eggs). The honeydew produced by the 16 other
 237 plant-aphid combinations enable parasitoids to produce on average 13 eggs after 24 hours of
 238 feeding (not significantly different from the number of eggs produced by female fed water
 239 only). After feeding for 48 hours, female parasitoids produced the highest number of eggs (on
 240 average 21) when fed on honey or honeydew produced by *S. maydis* (Appendix C). In
 241 contrast, females fed water or honeydew of *A. fabae*, *M. euphorbiae* (on milkweed) and *M.*
 242 *persicae* (on cabbage) produced only 10 eggs. The honeydew produced by the 15 other plant-
 243 aphid combinations enable parasitoids to produce on average 15 eggs after 48 hours of
 244 feeding (not significantly higher compared to female fed water except for females fed
 245 honeydew excreted by *M. euphorbiae* (tomato) and *S. graminum* which produced a higher
 246 number of eggs).

247 The number of eggs per female produced between 24 and 48 hours increased when fed
 248 honey or honeydew excreted by *A. craccivora*, *A. gossypii*, *M. euphorbiae* (on tomato), *M.*
 249 *dirhodum* (on wheat) or *S. maydis* (all $P < 0.008$), whereas egg number decreased in females
 250 fed only water ($P = 0.023$) (Appendix C). When parasitoids fed on honeydew of the others
 251 aphid species tested, their egg number remained constant between 24 and 48 hours (all $P >$
 252 0.11). Finally, after 48 hours of feeding, female egg load was correlated with the fructose
 253 content of the honeydews on which they fed ($R^2 = 0.29$, $df = 19$, $P = 0.011$) whereas it was not
 254 correlated after only 24 hours of feeding ($R^2 = 0.03$, $df = 19$, $P = 0.455$).

Experiment 3: Relationship between parasitoid fitness and its preference/ability to produce offspring

The proportion of individual aphids stung by *A. abdominalis* varied between 0.05 (*A. craccivora*) and 0.91 (*M. euphorbiae* on tomato) (Figure 4A) whereas the proportion of parasitoid enable producing offspring varied between 0 (*A. craccivora*, *A. fabae*, *B. brassicae*, *R. padi* and *S. graminum*) and 0.9 (*M. euphorbiae* on tomato) (Figure 4B). There was no correlation between parasitoid preference or ability to produce offspring and either longevity ($t = 0.4$, $df = 7$, $P = 0.726$; $t = 0.4$, $df = 7$, $P = 0.735$, respectively) or egg load after 48 hours of parasitoids fed on honeydew ($t = -1.7$, $df = 7$, $P = 0.126$; $t = -0.7$, $df = 7$, $P = 0.528$, respectively) (Figure 4).

DISCUSSION

As honeydew is associated with host presence, we tested the relationship between the honeydew excreted by 19 different plant-aphid combinations as a food source for adult parasitoids and parasitoid preference as well as ability to produced offspring in these host-aphid complexes. Host plant and aphid species highly modulated the fructose content of honeydew as well as parasitoid longevity and egg load. Parasitoids lived longer and had a constant or increase number of eggs when they fed on honey or honeydew of eight different aphid species, including *Macrosiphum euphorbiae* and *Aphis craccivora*. In contrast, parasitoids fed only water or honeydew of nine aphid species (e.g., *Brevicoryne brassicae* and *Aphis nerii*) had a shortened longevity and a low egg load (similar to that of the starved parasitoids). Finally, there was no relationship between parasitoid fitness when fed on various honeydews and the ability to produce offspring in these same aphids excreting the honeydew.

281 *Host plant and aphid species highly modulates honeydew fructose content.*

282 Honeydew is composed of host plant phloem contents as well as the oligosaccharides
283 synthesized by sap-feeders. It has already been largely studied and is identified as being
284 species-specific (Wäckers et al. 2000, 2001; Fischer and Shingleton 2001; Hogervorst et al.
285 2007b). According to Hogervorst et al. (2007b), aphid species has the most important role in
286 shaping honeydew composition. However, in our study, we observed a strong variability
287 among the honeydew produced by the same aphid species reared on different host plants or
288 different aphid species reared on the same plant. The former study (Hogervorst et al. 2007b)
289 evaluated the quantity of 10 different sugars present in the honeydews (only 1 was evaluated
290 in this study), however they compared only 5 aphid species reared on 2 different plant species.
291 These results suggest that more studies evaluating the honeydew composition of a great
292 number of sap-feeding species are needed in order to more clearly identify the role of the host
293 plant and the plant-feeders in honeydew composition. Moreover, many other factors in the
294 field may impact honeydew composition, such as aphid age (Fischer et al. 2002), the presence
295 of symbionts (endosymbiotic or gut flora) or ants (Fischer and Shingleton 2001; Schillewaert
296 et al. 2017), and aphid colony size (Faria et al. 2008).

297

298 *Parasitoid fitness is modulated by honeydew quality*

299 The high longevity and egg load of parasitoid fed on honeydews of various plant-aphid
300 combinations confirms that (i) aphid parasitoids are well adapted to efficiently use honeydew
301 as carbohydrate source and (ii) honeydew is not a nutritionally inferior food source compared
302 to honey or nectar (Wäckers et al. 2008). The fructose concentration of honeydew must
303 positively impact both parasitoid longevity and fecundity (Wäckers et al. 2001; Hogervorst et

304al. 2007b; Vollhardt et al. 2010; Hopkinson et al. 2013; Zhang et al. 2014; Charles and Paine
3052016; Lenaerts et al. 2016; Tena et al. 2018). In our study, the egg load of parasitoids was
306positively correlated with fructose content; corroborating the implication of fructose in
307parasitoid egg maturation. By contrast, longevity was not correlated with fructose content,
308suggesting that various factors, such as plant-derived and aphid synthesized compounds as
309well as viscosity (Wink and Römer 1986; Wäckers 2000; 2005) may modulate the quality of
310honeydew. Indeed, in contrast with the other carbohydrate sources such as floral and
311extrafloral nectars, which have evolved to attract organisms feeding on sugar, honeydew is a
312waste product of sap-feeder organisms which tend to minimize its attraction for natural
313enemies (Wäckers et al. 2008). Therefore, honeydew composition is not optimized for
314parasitoid nutrition and toxic compounds may weaken the parasitoid efficiency as biological
315control agent (Wink and Römer 1986; Züst and Agrawal 2016). *Asclepias* spp plants and the
316species *Brassica oleracea* produce toxic secondary metabolites (cardenolides and
317glucosinolates, respectively; Mooney et al. 2008; Pratt et al. 2008), which may compromise
318honeydew quality; possibly explaining the low longevity of *A. abdominalis* when fed different
319honeydews produced by aphids reared on these plants.

320 Parasitoid egg load was not affected by honeydew quality after 24 hours of feeding.
321The egg load of parasitoids fed on honey or honeydews was the same as that of parasitoids fed
322only water. The female parasitoid energy reserves seem to be sufficient for the production of
323eggs during the first 24 hours (Wu and Heimpel 2007). After 48 hours of feeding, honeydew
324quality had a strong impact on parasitoid egg load and we observed the potential mechanisms
325that explain these differences: (i) reduction of the egg load when parasitoid females were fed
326only water, (ii) stabilization of the egg load between 24 and 48 hours and (iii) augmentation of
327the egg load when females were fed honey or five different honeydews (produced by e.g., *S.*
328*maydis*, wheat-reared *M. dirhodum*). In synovigenic parasitoids, starved individuals may

329resorb their eggs to retrieve energy and increase their longevity when no or only low-quality
330food is available (Roitberg 1989; Olson et al. 2005; Benelli et al. 2017), potentially explaining
331the observed egg number reduction in females fed only water. Parasitoid ability to increase
332the number of eggs produced between 24 and 48 hours indicates that the honeydews
333implicated are high-quality food sources.

334

335*Fitness-preference/ ability to produce offspring relationship*

336We hypothesized that parasitoids of honeydew-producers may have evolved to efficiently use
337the honeydew produced by their main hosts as a carbohydrate source; suggesting the existence
338of a positive correlation between both parasitoid preference and ability to produce offspring
339and parasitoid fitness when fed on the honeydew produced by their main hosts. However,
340there was no relationship between the fitness of *A. abdominalis* when fed various honeydews
341and its both preference and ability to produce offspring in these honeydew-producers. The
342degree of specialization in parasitoids may explain the lack of relationship as generalist
343species has been described as more flexible than specialist species in dealing with nutrient
344deficiency (Cicero et al. 2012; Benelli et al. 2017). As explained above, honeydew is a waste
345product of sap-feeder and the organisms producing non-detectable and non-palatable
346honeydews should be selected to minimize their attraction for natural enemies (Wäckers et al.
3472008). By contrast, parasitoids of honeydew producers might have evolved to detect and use
348efficiently honeydew produced by their main hosts. Despite the preference of *A. abdominalis*
349for the aphids belonging to the Macrosiphini tribe, it is a generalist aphid parasitoid able to
350produce offspring in many different aphid species (Monticelli et al. 2019) and is thus, by
351definition, not specialized on a particular host. Generalist parasitoids use to switch between
352the most abundant hosts (Vet and Dicke 1992; Bonsall and Hassell 1999) and did not develop
353adaptation to use efficiently honeydew excreted by their main host. For example, *Aphidius*

354 *ervi* is also a generalist aphid parasitoid (Monticelli et al. 2019) and its longevity did not vary
355 among the honeydews produced by three hosts varying in their suitability for offspring
356 development (Hogervorst et al. 2007b). In contrast, a specialist parasitoid, able to (i) use cues
357 from honeydew to detect their hosts and (ii) circumvent the defensive toxic compounds
358 excreted in aphid honeydews (Vet and Dicke 1992), may be adapted to one specific
359 honeydew. However, there is little evidence supporting this hypothesis as there is no studies
360 testing the relationship between parasitoid offspring production and fitness when fed on the
361 honeydew of their main hosts. Inversely, empirical data may contradict the hypothesis. For
362 example, the specialist parasitoid *Lysiphlebus testaceipes* able to produce offspring in *A. nerii*
363 (Kavallieratos et al. 2004), an aphid containing cardenolides (i.e., toxic cardiac glycosides;
364 Züst and Agrawal 2016), did not increase its longevity when fed honeydew excreted by this
365 species than honeydews produced by both poor and high host-quality (Tena et al. 2018).

366 Given the impact of the plant and aphid species on the parasitoid fitness, variation on
367 the parasitoid population dynamic and its efficiency as biological control agent are strongly
368 expected (Wäckers et al 2008; Benelli et al. 2017). Studies testing honeydew impact in field
369 experiment as well as involving specialist parasitoid are needed, as parasitoids able to find
370 food and hosts at the same location may minimize time wastage, therefore increasing their
371 parasitism rate and efficiency as biological control agents.

372

373

374ACKNOWLEDGEMENTS

375We thank Philippe Bearez and Christiane Metay-Merrien for technical assistance, and Tara
376Malanga for English polishing. This research was supported by a grant from the Marie-Curie
377F7-IRSES Action to ND, AT and LSM (APHIWEB project, grant number: 611810) and a
378PhD fellowship from the Doctoral School *Sciences de la Vie et de la Santé* ED85 to LSM.

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531 **TABLE** Table 1. Aphid species, their host plants, tribes and the sample size for the 2 different experiments as well as the fructose concentration
532 (μg of fructose / mL of honeydew) of the honeydew produced by the aphid. Concentrations followed by the same letter do not differ ($P > 0.05$)
533 and the concentration of the water and honey solutions were 0.00 ± 0.00 and 15.10 ± 0.67 , respectively.

534

Tribe	Subtribe	Aphid species	Host plant species	Experiments (replicates)
Aphidini	Aphidina	<i>Aphis craccivora</i>	<i>Vicia fabae</i>	1 (31), 2 (40)
		<i>Aphis fabae</i>	<i>Vicia fabae</i>	1 (27), 2 (39)
		<i>Aphis glycines</i>	<i>Glycine max</i>	1 (30), 2 (40)
		<i>Aphis gossypii</i>	<i>Cucurbita moschata</i>	1 (30), 2 (40)
		<i>Aphis nerii</i>	<i>Asclepias incarnata</i>	1 (29), 2 (40)
	Rhopalosiphina	<i>Rhopalosiphum padi</i>	<i>Triticum aestivum</i>	1 (30), 2 (40)
		<i>Schizaphis graminum</i>	<i>Triticum aestivum</i>	1 (27), 2 (41)
Macrosiphini	Dactynotinae	<i>Acyrtosiphon pisum</i>	<i>Vicia fabae</i>	1 (29), 2 (40)
			<i>Asclepias syriaca</i>	1 (29), 2 (40)
		<i>Macrosiphum euphorbiae</i>	<i>Solanum lycopersicum</i>	1 (29), 2 (40)
			<i>Solanum tuberosum</i>	1 (30), 2 (40)
		<i>Metopolophium dirhodum</i>	<i>Triticum aestivum</i>	1 (27), 2 (41)
			<i>Hordeum vulgare</i>	1 (29), 2 (40)
	Myzines	<i>Sitobion avenae</i>	<i>Triticum aestivum</i>	1 (32), 2 (40)
		<i>Brevicoryne brassicae</i>	<i>Brassica oleracea</i>	1 (32), 2 (40)
			<i>Vicia fabae</i>	1 (29), 2 (41)
		<i>Myzus persicae</i>	<i>Brassica oleracea</i>	1 (27), 2 (39)
Siphini			<i>Triticum aestivum</i>	1 (29), 2 (41)
		<i>Sipha maydis</i>	<i>Triticum aestivum</i>	1 (33), 2 (41)
		Water control		1 (30), 2 (62)
		Honey control		1 (28), 2 (62)

535 Aphid colonies were initiated from ≥ 200 collected individuals naturally colonizing fields in Europe during 2013-2015 and infesting their
536 respective host plant species. All aphids were maintained (for 35-45 generations) on their host plant in a ventilated cage (60 x 60 x 60 cm)
537 covered by mesh, under controlled conditions (23 ± 2 °C, RH $65 \pm 5\%$ and photoperiod 16:8h L:D).

538 Table 2. Effect of the host plant and aphid species on the honeydew quality, the parasitoid longevity and fecundity after 24 or 48 hours of
539 feeding. Significant effects of both the host plant and the aphid species on the honeydew quality and parameters of the experiment 1 and 2 are
540 indicated in bold text. The test value represents the F value for the fructose concentration as well as the tibia length, the score (logrank) for the
541 parasitoid longevity and the χ^2 for the egg load.

542

Parameters	Host plant species			Aphid species		
	Df	Test value	P value	Df	Test value	P value
Honeydew analyses						
Fructose concentration	5, 38	3.5	0.011	13, 38	3.7	< 0.001
Experiment 1						
Female tibia length	5, 526	1.3	0.276	13, 256	0.9	0.539
Female longevity	5	147.3	< 0.001	13	134.4	< 0.001
Experiment 2						
Female tibia length after 24h	5, 361	0.5	0.809	13, 361	0.9	0.555
Female tibia length after 48h	5, 364	0.1	0.981	13, 364	1.6	0.079
Female egg load after 24h	5	14.1	0.015	13	65.7	< 0.001
Female egg load after 48h	5	48.1	< 0.001	13	112.8	< 0.001

543FIGURES

544**Figure 1.** Mean fructose concentration (μg of fructose / mL of honeydew) ($\pm$ SEM) of
545different honeydews produced by different aphid species reared on the same host plant or a
546single aphid species reared on different host plants. For each condition, bars followed by the
547same letter are not significantly different (LM followed by multicomparison tests).

548**Figure 2.** Survival curve of female parasitoids fed different honeydews produced by different
549aphid species reared on the same host plant or a single aphid species reared on different host
550plants. For each condition, plant or/ aphid species followed by the same letter are not
551significantly different (Cox model (Kaplan-Meyer Log rank) and the p values were adjusted
552following the Bonferroni adjustment method for multi-comparison).

553**Figure 3.** Mean number ($\pm$ SEM) of egg produced by female parasitoids after 48 hours
554feeding on different honeydews produced by different aphid species reared on the same host
555plant or a single aphid species reared on different host plants. For each condition, bars
556followed by the same letter are not significantly different (GLM followed by multicomparison
557tests).

558**Figure 4.** (A) Longevity and egg load after 48 hours of feeding of *A. abdominalis* fed 13
559different honeydews in function of the parasitoid preference (i.e., proportion of individual
560aphids successfully stung; data from Monticelli et al. 2019). (B) Longevity and egg load after
56148 hours of feeding of *A. abdominalis* fed 13 different honeydews in function of the parasitoid
562ability to produce offspring (data from Monticelli et al. 2019). The order of the species when
563the proportion of emerged parasitoids is 0 corresponds to their order by longevity (highest to
564lowest) when the female was fed the honeydew of this species.

565Figure 1.

Different aphid species
growing on the same host
plant

567Figure 2.

568

569

Different aphid species
growing on the same
host plant

One aphid species
growing on different
host plants

570Figure 3.

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572

573Figure 4A.

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581Figure

4B.

582
 583tribes, the fructose concentration (μg of fructose / mL of honeydew) of the honeydew produced by the aphid and the tibia length of female
 584parasitoids used in the Experiment 1 and 2. The fructose content of honeydew varied depending upon the plant-aphid combinations producing the
 585honeydew ($F_{18,38} = 3.63$, $P < 0.001$). The fructose concentration was analyzed using a LM and considering each plant-aphid combination as a
 586unique treatment. Concentrations followed by the same letter do not differ ($P > 0.05$) and the concentration of the water and honey solutions were
 5870.00 $\pm$ 0.00 and 15.10 $\pm$ 0.67, respectively.

588

Appendix A. Aphid species, their host plants,

Tribe	Subtribe	Aphid species	Host plant species	[Fructose] ± SEM	Tibia length ± SEM Experiment 1	Tibia length ± SEM Experiment 2
Aphidini	Aphidina	<i>Aphis craccivora</i>	<i>Vicia fabae</i>	7.04 ± 0.74 ab	0.45 ± 0.01	0.44 ± 0.001
		<i>Aphis fabae</i>	<i>Vicia fabae</i>	6.6 ± 1.92 ab	0.45 ± 0.01	0.44 ± 0.001
		<i>Aphis glycines</i>	<i>Glycine max</i>	3.81 ± 0.39 b	0.46 ± 0.01	0.45 ± 0.001
		<i>Aphis gossypii</i>	<i>Cucurbita moschata</i>	4.99 ± 2.45 ab	0.45 ± 0.01	0.45 ± 0.001
		<i>Aphis nerii</i>	<i>Asclepias incarnata</i>	2.68 ± 1.43 b	0.47 ± 0.01	0.44 ± 0.01
	Rhopalosiphina	<i>Rhopalosiphum padi</i>	<i>Triticum aestivum</i>	3 ± 0.58 b	0.46 ± 0.01	0.45 ± 0.001
		<i>Schizaphis graminum</i>	<i>Triticum aestivum</i>	4.22 ± 0.30 ab	0.45 ± 0.01	0.45 ± 0.001
Macrosiphini	Dactynotine	<i>Acyrtosiphon pisum</i>	<i>Vicia fabae</i>	8.81 ± 0.42 ab	0.46 ± 0.01	0.45 ± 0.001
			<i>Asclepias syriaca</i>	8.29 ± 0.99 ab	0.48 ± 0.01	0.45 ± 0.001
		<i>Macrosiphum euphorbiae</i>	<i>Solanum lycopersicum</i>	8.76 ± 0.40 ab	0.46 ± 0.01	0.45 ± 0.001
			<i>Solanum tuberosum</i>	4.4 ± 0.71 ab	0.48 ± 0.01	0.45 ± 0.001
		<i>Metopolophium dirhodum</i>	<i>Triticum aestivum</i>	6.42 ± 1.06 ab	0.45 ± 0.01	0.45 ± 0.001
			<i>Hordeum vulgare</i>	3.85 ± 1.18 b	0.47 ± 0.01	0.45 ± 0.001
		<i>Sitobion avenae</i>	<i>Triticum aestivum</i>	8.24 ± 1.44 ab	0.47 ± 0.01	0.45 ± 0.001
	Myzines	<i>Brevicoryne brassicae</i>	<i>Brassica oleracea</i>	4.95 ± 1.60 ab	0.45 ± 0.01	0.45 ± 0.001
			<i>Vicia fabae</i>	9.21 ± 0.66 ab	0.45 ± 0.01	0.45 ± 0.001
		<i>Myzus persicae</i>	<i>Brassica oleracea</i>	7.96 ± 1.52 ab	0.46 ± 0.01	0.45 ± 0.001
Siphini			<i>Triticum aestivum</i>	4.43 ± 1.94 ab	0.47 ± 0.01	0.45 ± 0.001
		<i>Sipha maydis</i>	<i>Triticum aestivum</i>	10.6 ± 1.13 a	0.46 ± 0.01	0.45 ± 0.001

589**Appendix B.** Longevity (in hours $\pm$ SEM) of *Aphelinus abdominalis* females subjected to 21
590diet treatments: water, honey and 19 honeydews. The diet provided affected the longevity of
591*A. abdominalis* females (Score (logrank) = 225.5, $P < 0.001$). The impact of diet provided on
592the parasitoid longevity was analyzed using a cox model (Kaplan-Meyer Log rank)
593considering each plant-aphid combination as a unique treatment. Diet source subtended by
594lines do not differ ($P > 0.05$, Cox model (Kaplan-Meyer Log rank)). A= asclepias, B= barley,
595C= cabbage, P= potato, T= tomato and W= wheat.

596 **Appendix C. (a)** Number of eggs produced ($\pm$ SEM) by *Aphelinus abdominalis* females after
597 24 or 48 hours subjected to 21 diet treatments: water, honey and 19 honeydews. The diet
598 provided affected *A. abdominalis* egg load after 24 ($\chi^2_{20} = 83.7$, $P < 0.001$) and 48 hours ($\chi^2_{20} =$
599 255.3, $P < 0.001$) of feeding (the impact of diet provided on the parasitoid egg load was
600 analyzed using a GLM following a poisson distribution and considering each plant-aphid
601 combination as a unique treatment). Diet source subtended by lines do not differ in the
602 number of egg produce at 48 hours. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (different number
603 of eggs between 24 and 48 hours for this aphid species). **(b)** Mean number ($\pm$ SEM) of egg
604 produced by female parasitoids after 24 hours feeding on different honeydews produced by
605 different aphid species reared on the same host plant or a single aphid species reared on
606 different host plants. For each condition, bars followed by the same letter are not significantly
607 different (GLM followed by multicomparison tests). A= asclepias, B= barley, C= cabbage, P=
608 potato, T= tomato and W= wheat.

609

610(a)

611(b)

612

613

Different aphid species
growing on the same host
plant

One aphid species
growing on different host
plants

0 5 10 15 20 25
Parasitoid egg load after 24 hours feeding