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## Large interclone differences in melezitose secretion in the facultatively ant-tended black bean aphid *Aphis fabae*

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## ABSTRACT

Many aphids are known to engage in a trophic mutualism with ants, whereby the aphids secrete sugary-rich honeydew which is collected by the ants for food, and the ants, in exchange, protect the aphids against natural enemies. Previous results, however, suggest that the production of some of the honeydew sugars, such as the ant-attractant trisaccharide melezitose, may induce an indirect cost to the aphids. This led us to believe that large differences in the nature of the secreted honeydew might exist, due to some clones capitalizing more or less on their mutualistic interaction with ants, or due to some “cheater” clones foregoing the production of particular sugars, instead taking advantage of the ant-attracting effect of other non sugar-deficient clones, co-occurring on the same plant. Here we present data on clonal variation in the composition of honeydew of the black bean aphid *Aphis fabae* which confirm this prediction. In particular, our results show that there was large interclone variation in the amount of glucose, melezitose and total sugar produced. The variation in the production of melezitose, however, showed particularly large differences, with 54% (7 out of 13) of the clones screened being virtually deficient for the production of this sugar, irrespective of whether the aphid colonies were ant-tended or not. The consequences of this finding in the context of the evolution and maintenance of the ant–aphid mutualism, as well as the adaptive benefits of oligosaccharide synthesis in aphids and other insects are discussed.

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### 1. Introduction

From coral building algae to the insects that pollinate plants or the nitrogen fixing bacteria associated with plants: cooperative mutualisms are common in nature and ecologically very important (Boucher, 1988; Bronstein, 1994; Sachs et al., 2004). In insect societies, one of the best examples and well-studied cases of a mutualism occurs in the interaction between ants and aphids, whereby ants feed on sugary-rich honeydew excreted by the aphids which, in exchange, protect the aphids against natural enemies (Hölldobler and Wilson, 1990; Stadler and Dixon, 2005; Way, 1963). For the aphids, ant attendance usually entails various benefits, including better protection against predators and parasitoids (Banks, 1962; Dixon and Agarwala, 1999; Majerus et al., 2007; Yao et al., 2000), a reduced risk of contracting fungal infections, due to the grooming action of the ants and the removal of exuviae and infected individuals (Banks, 1962; Matsuura and Yashiro, 2006; Nielsen et al., 2010), and reduced indirect competition with untended aphids (Minarro et al., 2010). The ants, in turn, benefit

nutritionally from harvesting honeydew, as it is rich in mono-, di- and trisaccharides and amino acids (Detrain et al., 2010; Douglas, 2003; Fischer et al., 2005, 2002; Woodring et al., 2004; Yao and Akimoto, 2002). In some cases, ant attendance, however, may also have some costs, given that the production of an unusually large quantity or high quality of honeydew is likely to be energetically expensive (Fischer and Shingleton, 2001; Stadler and Dixon, 2005; Yao and Akimoto, 2002; Yao et al., 2000). Significant fitness costs, for example, have been demonstrated in *Aphis fabae cirsiacanthoidis* (Scopoli) (Stadler and Dixon, 1998), where ant attendance results in a prolonged developmental time, delayed offspring production, smaller gonads, fewer well-developed embryos and a reduced mean relative growth rate, and in *Tuberculatus quercicola* (Yao et al., 2000), where it resulted in the production of smaller and less fecund adults. The balance of these costs and benefits probably explains why only a third of the aphid species in Europe are obligate mutualists and another third are only facultative mutualists, with the remainder not being ant-tended (Bristow, 1991; Stadler, 1997).

Ant attendance appears to be modulated primarily by the total amount of honeydew secreted, the total sugar concentration that is present in the honeydew, as well as by the presence of one particular trisaccharide – melezitose, which appears to act as an ant attractant (Detrain et al., 2010; Duckett, 1974; Fischer et al.,

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2005; Kiss, 1981; Völkl et al., 1999; Woodring et al., 2004), and also appears to be one of the most nutritionally valuable sugars for them (Boevé and Wäckers, 2003). Indeed, the honeydew of ant-tended species typically contains relatively more melezitose than that of untended ones (Fischer and Shingleton, 2001; Woodring et al., 2004, 2007) and the concentration of melezitose and other ant-attracting sugars, as well as amino acid production, is in some species facultatively tuned in response to ant attendance (in *Chaitophorus populeti* and *Chaitophorus populialbae*, Fischer and Shingleton, 2001; and *T. quercicola*, Yao and Akimoto, 2001, 2002). The total amount of honeydew secreted, as well as its composition has also been shown to vary greatly among different homopteran species (Auclair, 1963; Fischer and Shingleton, 2001; Hendrix et al., 1992; Hogervorst et al., 2007a,b; Völkl et al., 1999; Woodring et al., 2004), although it is also sometimes dependent upon the instar that is sampled (Fischer et al., 2002; Henneberry et al., 2000), the host plant that is used by the homopteran (Fischer and Shingleton, 2001; Fischer et al., 2005) and seasonal or environmental variation in host plant sap composition (Wool et al., 2006). Given these large interspecific differences, the aphids have been shown to end up competing for the mutualistic services provided by the ants (Cushman and Addicott, 1989; Engel et al., 2001; Fischer et al., 2001; Völkl et al., 1999; Woodring et al., 2004).

Although the synthesis of oligosaccharides, and particularly of melezitose, in sap-feeding insects has often been explained as a strategy to cater for the gustatory preferences of ants (e.g. Detrain et al., 2010; Fischer et al., 2005; Kiss, 1981; Völkl et al., 1999; Woodring et al., 2004), it is clear that this cannot be the sole reason for its production. Indeed, although *Lasius niger* and *Myrmica rubra* both prefer melezitose (and to a lesser extent also sucrose) over monosaccharides such as glucose or fructose (Duckett, 1974; Völkl et al., 1999; Woodring et al., 2004; Fischer et al., 2005; Detrain et al., 2010), several other ants have been shown to not display such a preference (Blüthgen and Fiedler, 2004; Cornelius et al., 1996). In addition, melezitose is also produced in some insect species that are not tended by ants, e.g. in the honeydew producing whitefly *Bemisia tabaci* (Byrne and Miller, 1990) and in the rose-grain aphid *Metopolophium dirhodum* (Hogervorst et al., 2007b), as well as in a number of other non-honeydew excreting arthropods, including parasitic wasps, hoverflies and lacewings (Hogervorst et al., 2007b). This fact underlines that the synthesis of oligosaccharides likely has other functions than strictly in ant-recruitment.

One likely function for sap-feeding insects as well as other insects for which sucrose forms a major constituent of their diet is in regulating the osmotic pressure in the gut (e.g. in aphids, Douglas, 2003, 2006; Fisher et al., 1984; Rhodes et al., 1997; Wilkinson et al., 1997). Sucrose can only be ingested after hydrolysis to its constituent glucose and fructose units, as only monosaccharides can be absorbed through the gut wall (Ashford et al., 2000; Weil, 1978). However, since the osmotic pressure of insect haemolymph is usually lower than that of the phloem sap, this creates osmotic stress and therefore causes water loss through the gut wall (Douglas, 2003; Kennedy and Stroyan, 1959). One solution to reduce this osmotic pressure is to convert mono- and disaccharides to di- and trisaccharides (Douglas, 2006; Rhodes et al., 1997; Wilkinson et al., 1997). Consistent with this interpretation, aphids appear to switch to the production of oligosaccharides such as melezitose particularly when they feed on plant sap with a high sucrose content (Rhodes et al., 1997). The fact that ant-tended species typically produce more melezitose can also be explained by the fact that the ants often force the aphids to feed at higher rates than when they are untended (Woodring et al., 2004, 2007; Yao and Akimoto, 2001), and that this increases osmotic stress (Fischer and

Shingleton, 2001; Woodring et al., 2004, 2007). Direct evidence for a role in regulating gut osmolarity was also provided by Woodring et al. (2007), who showed that in *Metopeurum fuscoviride*, melezitose production reduced gut osmolarity by 25–35% and by Karley et al. (2005), who showed that pea aphids fail to osmoregulate on a 0.75 M sucrose diet after injecting them with the sucrase inhibitor acarbose. Recently, the sucrase (or technically,  $\alpha$ -glucosidase) *APS1* has also been cloned from the pea aphid, and the enzyme was shown to be expressed in the posterior midgut membrane (Price et al., 2007), consistent with the observed sucrase activity in that area (Ashford et al., 2000; Cristofolletti et al., 2003). The hydrolysis of sucrose makes available a unit of glucose as well as a unit of fructose, which is the monosaccharide that is most readily transported across the gut wall (Ashford et al., 2000), probably through the action of sugar transporter *Ap\_ST3* (Ashford et al., 2000; Price et al., 2010). Probably also via the action of *APS1*, the glucose unit can then further be linked to a sucrose unit via transglucosidation (Price et al., 2007), thereby forming melezitose, or – by the same principle – other oligosaccharides, which are then excreted into the honeydew (Ashford et al., 2000; Rhodes et al., 1997).

Aside from a role in ant attraction and osmoregulation, secreting melezitose has also been suggested to be beneficial in warding off predators and parasitoids. For example, in parasitoids the secretion of oligosaccharides has been shown to hinder their gustatory perception of other mono- and disaccharides (Wäckers, 1999, 2000), so that they will less likely go onto exploit honeydew as a food source and end up parasitizing aphids. In addition, in some parasitoids and aphid predators, melezitose has been shown to be nutritionally less valuable than other sugars (Ide et al., 2007; Wäckers, 2000, 2001, 2008; Wyckhuys et al., 2008). Nevertheless, this is not a universal pattern, since for the parasitoid *Cotesia glomerata*, melezitose was shown to be a highly suitable food source (Hausmann et al., 2005).

Despite this large number of hypotheses about the adaptive benefits of honeydew sugar biosynthesis in aphids, and the large number of studies that have documented clonal genetic variation in various of their characteristics (e.g. Braendle and Weisser, 2001; Douglas, 1997; Gorur et al., 2005; Kunert et al., 2005; Müller, 1983; Schwartzberg et al., 2008; Tosh et al., 2003), no detailed study has as yet been performed on clonal variation in honeydew secretion and composition. This is surprising, given that this is obviously a key driver in the evolution and maintenance of the ant–aphid mutualism, and that the discovery of clonal variation in the production of oligosaccharides would offer great perspectives for testing the various hypothesized benefits of their synthesis. Here we present the first such study, using the facultatively ant-tended black bean aphid *A. fabae fabae* as a model. Our expectation was that large differences in the nature of the secreted honeydew might exist, due to some clones capitalizing more or less on their interaction with ants, or due to some “cheater” clones foregoing the production of particular sugars attractive to ants, thereby limiting the cost of ant attendance, and instead allowing them to take advantage of the ant-attracting effect of other non-deficient clones, co-occurring on the same plant. The latter hypothesis seemed plausible, given that in an earlier study, we found that two thirds of all *A. f. fabae* colonies are polyclonal (Vantaux et al., 2011). In addition, the fact that some ant-attractant sugars, such as melezitose, tend to be produced mainly in species that are ant-tended (Fischer and Shingleton, 2001; Woodring et al., 2004, 2007), or only facultatively, in response to ant attendance (Fischer and Shingleton, 2001), suggests that an increased production induced by increased ant attendance is costly.

## 2. Materials and methods

### 2.1. Collection and maintenance of *A. fabae* clones

The black bean aphid *A. fabae* is a facultative ant mutualist which represents a complex of at least four subspecies which share the same primary hosts (primarily spindle, *Euonymus europaeus*, and to a lesser extent Guelder rose, *Viburnum opulus*, and sweet mock orange, *Philadelphus coronarius*), on which sexual reproduction and hibernation at the egg stage occur. This sexually produced generation alternates with several asexually produced generations in the summer, during which the aphids feed on a variety of secondary summer hosts, some of which are used to define subspecies, such as *A. fabae fabae*, which is found, among others, on the broad bean *Vicia faba* (Stroyan, 1984). In our study, we used 13 *A. fabae* clones, 10 of which were collected from *V. faba* bean plants grown in gardens in the surroundings of Leuven, Belgium. Another two clones came from the Agrocampus in Rennes, France (C6 and C13), and one clone was provided by the Rothamsted Research Institute, Hertfordshire, UK (C12). All collected clones collected in Belgium came from sites where the black garden ant *L. niger* was the dominant species, implying that most were probably ant-tended, although possibly to different extents. All clonal lineages were genotyped to make sure that they were all genetically distinct (for methods see Vantaux et al. (2011)). After collection, the identified clones were propagated asexually by placing them on *V. faba* bean plants (seeds provided by Somers Seeds NV, Mechelen, Belgium) under controlled laboratory conditions (L16:D8 photoperiod cycle and  $20 \pm 1$  °C).

### 2.2. Sampling of honeydew and extrafloral nectar (EFN)

Honeydew for chemical analysis was sampled in two ways. Following the first method, which we used to estimate the total quantity of sugars produced over a period of 24 h, honeydew was sampled by putting ten aphids (4th larval stage or adults) on a leaf enclosed in a plastic box (50 × 25 × 30 mm). After 24 h, the box and leaves were rinsed with 400 µL of a 0.02% sodium azide solution, boiled for 5 min and dried out overnight in an oven at 60 °C. Before HPLC analysis, these samples were then re-dissolved in 200 µL of 0.02% sodium azide. In this way, 2–6 (avg. 3.5) samples per clone were analyzed. According to the second method, which was used to accurately measure the concentration of all sugars present in the honeydew, we placed ten aphids on a leaf and a Petri dish with liquid paraffin underneath, which served to prevent evaporation. After 48 h, we then collected 0.5 µL of honeydew from the liquid paraffin, diluted it with 50 µL of 0.02% sodium azide, and boiled the samples for 5 min. In this way, 3–9 (avg. 6.5) samples per clone could be analyzed. All samples were stored at –20 °C before further chemical analysis. For comparison, we also sampled 0.5 µL of extrafloral nectar (EFN) from 5 *V. faba* plants using a microcapillary, which was diluted with 50 µL of 0.02% sodium azide, boiled for 5 min to stop enzymatic reactions and stored at –20 °C before further chemical analysis. EFN was sampled both before and 3 days after 10 aphids were introduced on a given plant.

### 2.3. Measuring honeydew composition in relation with ant-attendance

For five of the clones from which the concentration of honeydew sugars was measured (all except C1), and 2 of which were high-melezitose secreting and 3 of which were low-melezitose secreting clones, we checked whether honeydew composition was facultatively tuned in response to ant attendance. This was done by infesting four bean plants with 5 apterous adults per plant per clone and allowing *L. niger* ants (colonies containing a single

queen, some brood and a hundred workers) to tend two of the plants via a paper bridge, whilst leaving the other two untended. The plant pots were first wrapped in plastic film to prevent the ants from nesting inside the pots. After 6 days, 1–5 (avg. 4.5) apterous adults were used to measure the quantity of sugars produced in 24 h following the protocol described above. After these 24 h they were used to infest four new bean plants, after which the same procedure was repeated two times, for 4 and 3 weeks in a row, respectively.

### 2.4. HPLC carbohydrate analysis

HPLC was used to analyse the carbohydrates present in all honeydew and extrafloral nectar samples. Before chemical analysis, all samples were passed through a mixed-bed Dowex column to obtain a neutral fraction (cf. Van den Ende et al. (1996)). This was done by allowing 50 µL of sample solution followed by 1.2 mL of distilled water to pass through the column. The samples were then centrifuged for 5 min and stored at –20 °C until they could be chemically analysed. The latter was done using a Dionex ICS 3000 HPLC, equipped with pulsed amperometric detection (Dionex, Sunnyvale, CA, USA), and using an injection volume of 25 µL, a flow rate of  $1 \text{ mL} \times \text{min}^{-1}$  and a temperature of 32 °C. A CarboPac TM PA-100 guard (4 × 50 mm) and a CarboPac TM PA-100 (4 × 250 mm) in series were equilibrated with 90 mM NaOH for 9 min. At the time of injection, a Na-acetate gradient was applied as follows: 0–6 min, 0–10 mM; 6–16 min, 10–100 mM and 16–26 min, 100–175 mM. Regeneration of the columns was done with 500 mM Na-acetate for 5 min. Profiles were integrated using the software Chromeleon. The amounts of products were determined by comparing the peak areas with known amounts of standard compounds.

### 2.5. Statistical analyses

To satisfy parametric assumptions, the amount and concentration of the different sugars secreted were  $\log_2(x + 1)$  transformed prior to further statistical analysis. The total amount of the different sugars produced per aphid per 24 h in 13 clones was compared using mixed model ANOVAs and Tukey HSD post hoc tests, with clone being included as a random factor. The different sugar concentrations in the honeydew of six clones were also compared using a mixed model ANOVAs and Tukey post hoc tests, with clone again being included as a random factor. Correlation analyses were conducted to see if the relative concentrations of melezitose correlated with the relative concentrations of the other sugars across the six clones. The honeydew composition in relation to ant-attendance was compared using a general linear model (GLM) in which the absolute concentrations of the different sugars were coded as the dependent variable, ant-attendance and whether or not clones were melezitose deficient were coded as fixed factors, clone was included as a fixed factor nested within melezitose, and week was included as a continuous covariate. The concentration of the different sugars of EFN before and after aphids were introduced on a given plant were compared using a Wilcoxon matched paired test. All statistical tests reported were performed using the GLM module of *Statistica 9.1* (StatSoft, Inc.).

## 3. Results

### 3.1. Identity of the sugars present in honeydew

A total of eight sugars were present in *A. fabae* honeydew: trehalose, glucose, fructose, sucrose, melezitose, maltose, erlose and maltotriose. Other studies also found trace amounts of xylose, raffinose, melibiose and turanose in *A. fabae* honeydew (Detrain et al.,

2010; Fischer et al., 2005; Völkl et al., 1999). Nevertheless, in our samples, these could not be detected.

### 3.2. Clonal variation in total amount of sugars produced by the aphids

There was significant interclone variation in the total amount of sugar (ANOVA,  $F_{(12,32)} = 2.62$ ,  $P = 0.015$ ), glucose (ANOVA,  $F_{(12,32)} = 3.15$ ,  $P = 0.004$ ) and melezitose (ANOVA,  $F_{(12,32)} = 15.37$ ,  $P < 0.0001$ ) produced by individual aphids per day (Fig. 1). The melezitose quantity was significantly positively correlated with the total amount of sugars ( $r = 0.40$ ,  $P = 0.006$ ) and the erlose quantity ( $r = 0.39$ ,  $P = 0.008$ ). The difference in the amount of melezitose secreted was particularly spectacular, with 54% (7 out of 13) of the clones screened being virtually deficient for the production of this sugar (Fig. 1), and with there being a 33-fold difference in the amount of melezitose secreted by high- and low-melezitose secreting clones, respectively. Interestingly, 3

out of 5 of the bean plants from which we recovered our aphid colonies contained a mix of high- and low melezitose secreting clones (plants P2, P4 and P5, Fig. 1). In fact, all 3 plants which were infested by multiple clones of *A. fabae* had high- and low-melezitose secreting clones co-occurring on the same plant.

### 3.3. Clonal variation in the concentration of sugars present in honeydew

The comparison of the different sugar concentrations in the honeydew of six clones was significantly different for two sugars, trehalose (ANOVA,  $F_{(5,33)} = 3.12$ ,  $P = 0.02$ ) and melezitose (ANOVA,  $F_{(5,33)} = 53.34$ ,  $P < 0.0001$ ; Table 1; Fig. 2). However, the difference in melezitose production again proved to be most pronounced, with three of the six clones screened being virtually deficient in the production of melezitose (Fig. 2), and with there being a 104 fold difference in the average melezitose secretion by the three low-melezitose and the three high-melezitose secreting clones (Table 1). Interestingly, one clone (C1) which was inferred to secrete only low amounts of melezitose and total sugars after being screened using the method described above turned out to secrete honeydew with a high concentration of melezitose when screened using the current method. This might be explained, however, if this clone had an unusually low honeydew secretion rate. For the other five clones, however, there was a close correspondence in melezitose and total sugar production when measured using both methods. The relative melezitose concentration was significantly positively correlated with the total sugar concentration ( $r = 0.44$ ,  $P = 0.005$ ), and significantly negatively correlated with the relative trehalose, glucose, fructose, sucrose, erlose and maltotriose concentrations ( $r = -0.45$ ,  $r = -0.60$ ,  $r = -0.67$ ,  $r = -0.59$ ,  $r = -0.45$ ,  $r = -0.59$ , respectively, all  $P < 0.05$ ).

### 3.4. Comparison of the sugar profiles of honeydew and EFN

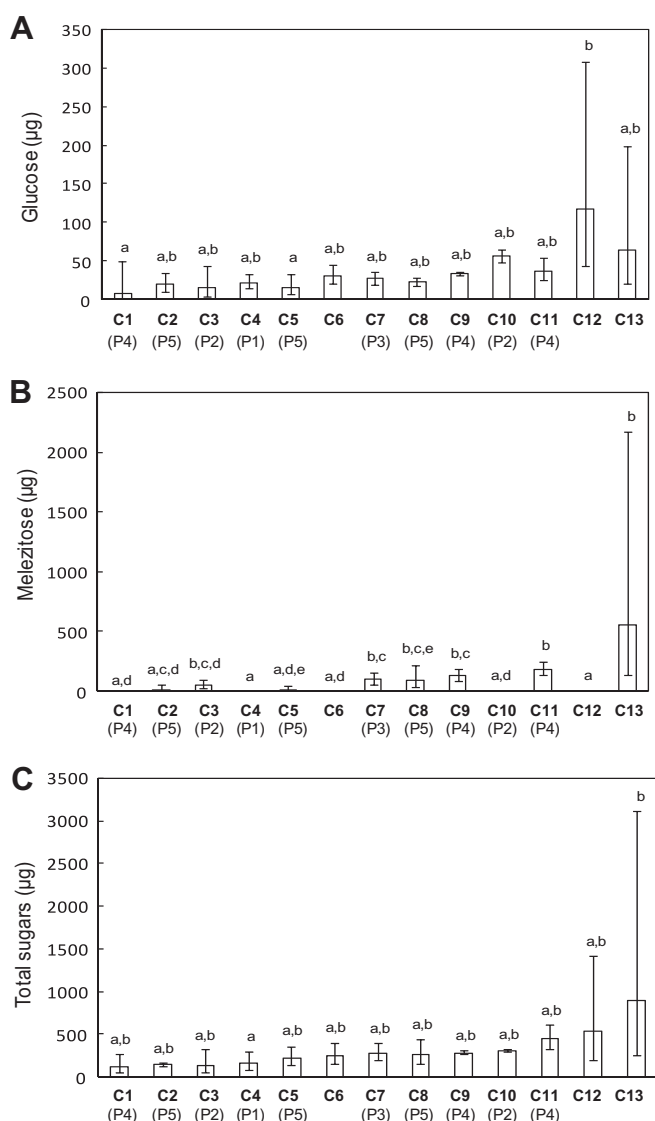
As expected, honeydew and EFN showed large differences in their sugar composition. In the EFN only glucose, fructose and sucrose were found and their concentration was a lot lower than that found in honeydew (Table 1). There was no significant difference in the concentration of any of the three sugars present in EFN before or after aphid infestation (Wilcoxon matched paired test:  $N = 4$ ,  $Z = 0.73$ ,  $P = 0.47$  for all three sugars).

### 3.5. Honeydew composition in relation to ant-attendance

The different high- and low-melezitose secreting clones differed in their maltotriose secretion only (Table 2), whereas significant differences were found between high- and low-melezitose secreting clones in all sugars except sucrose, erlose and the total sugar concentration. Unlike a previous study on other species (Fischer and Shingleton, 2001), ant-attendance had no significant effect on the amount of melezitose secreted, though it slightly increased maltotriose production (Table 2). Significant temporal changes in secretion over the course of the experiment were observed for the sugars trehalose, fructose, maltose, maltotriose, as well as for the total sugar concentration (Table 2). However, if a Bonferroni correction would be applied, none of these differences would actually remain significant. Overall, this means there was no evidence for the honeydew sugar composition being facultatively tuned in response to ant attendance.

## 4. Discussion

Overall, our results provide the first evidence that there is large interclone variation in the quantity of different sugars secreted in

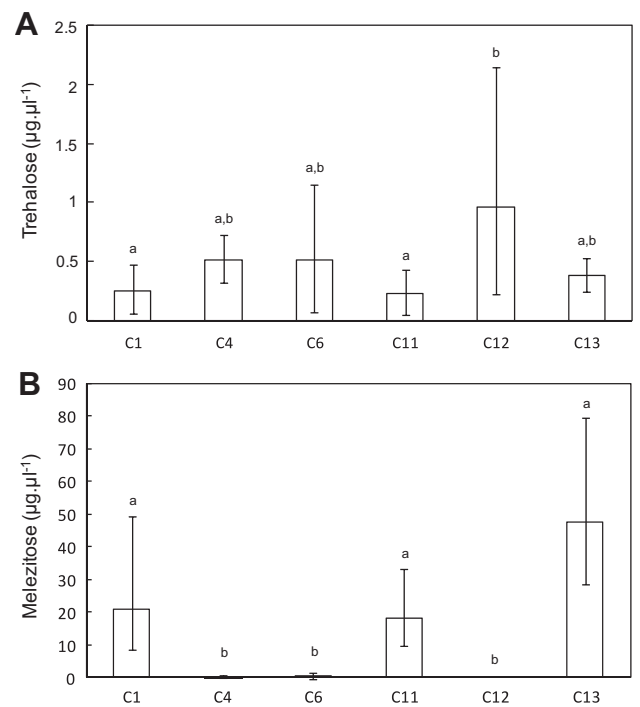


**Fig. 1.** Estimates of the total amount of different sugars (mean  $\pm$  SD) and total sugar produced in the honeydew of thirteen *Aphis fabae* clones feeding on *Vicia faba* ( $\mu\text{g}$  per individual per 24 h). Different letters indicate significant differences (based on Tukey HSD post hoc tests). (A) Glucose quantities ( $P = 0.004$ ), (B) melezitose quantities ( $P < 0.0001$ ), (C) total amount of sugar ( $P = 0.015$ ). For the 10 clones collected in Belgium we also mention which individual bean plants the clones were recovered from using the plant code shown in brackets.

**Table 1**  
Concentration ( $\mu\text{g}\cdot\mu\text{L}^{-1}$ ) and relative abundance of identified sugars in the honeydew of *Aphis fabae* individuals which were not tended by ants, as well as of extra floral nectar (EFN). Sample sizes (total number of individuals sampled) are given in brackets. H = High-melezitose secreting clones, L = low-melezitose secreting clones together. EFN A = extrafloral nectar after aphids had fed on the plant. EFN B = extrafloral nectar before aphids had fed on the plant.

|              | Absolute concentrations $\pm$ SE |                  |                   |                  |                  |                   | Mean absolute concentrations $\pm$ SE |                 |                  |                  |                  |                 |       |       |       |       |       |       | Relative concentrations (%) |       |       |     |  |  |
|--------------|----------------------------------|------------------|-------------------|------------------|------------------|-------------------|---------------------------------------|-----------------|------------------|------------------|------------------|-----------------|-------|-------|-------|-------|-------|-------|-----------------------------|-------|-------|-----|--|--|
|              | C1 (9)                           | C4 (6)           | C6 (5)            | C11 (9)          | C12 (7)          | C13 (3)           | EFN A (4)                             | EFN B (5)       | H (21)           | L (18)           | H+L (39)         | EFN (9)         | C1    | C4    | C6    | C11   | C12   | C13   | H                           | L     | H+L   |     |  |  |
| Trehalose    | 0.27 $\pm$ 0.07                  | 0.52 $\pm$ 0.08  | 0.60 $\pm$ 0.30   | 0.24 $\pm$ 0.06  | 1.21 $\pm$ 0.55  | 0.39 $\pm$ 0.08   | /                                     | /               | 0.27 $\pm$ 0.04  | 0.81 $\pm$ 0.23  | 0.52 $\pm$ 0.12  | /               | 0.52  | 2.27  | 1.9   | 0.61  | 3.44  | 0.54  | 0.56                        | 2.66  | 1.29  |     |  |  |
| Glucose      | 2.10 $\pm$ 0.38                  | 2.10 $\pm$ 0.73  | 2.74 $\pm$ 1.16   | 2.21 $\pm$ 0.23  | 2.69 $\pm$ 0.41  | 1.47 $\pm$ 0.61   | 1.23 $\pm$ 0.37                       | 1.43 $\pm$ 0.38 | 2.06 $\pm$ 0.21  | 2.51 $\pm$ 0.41  | 2.26 $\pm$ 0.22  | 1.34 $\pm$ 0.25 | 4.11  | 8.98  | 8.67  | 5.65  | 7.65  | 2.04  | 4.20                        | 8.24  | 5.61  |     |  |  |
| Fructose     | 10.08 $\pm$ 1.62                 | 9.89 $\pm$ 3.22  | 12.83 $\pm$ 4.70  | 8.93 $\pm$ 1.02  | 9.17 $\pm$ 1.53  | 7.25 $\pm$ 2.45   | 1.14 $\pm$ 0.35                       | 1.42 $\pm$ 0.40 | 9.18 $\pm$ 0.87  | 10.42 $\pm$ 1.71 | 9.75 $\pm$ 0.91  | 1.30 $\pm$ 0.26 | 19.71 | 41.35 | 40.38 | 22.79 | 26.07 | 10.07 | 18.74                       | 34.22 | 24.12 |     |  |  |
| Sucrose      | 7.00 $\pm$ 1.86                  | 7.18 $\pm$ 2.55  | 10.63 $\pm$ 5.97  | 4.42 $\pm$ 0.74  | 17.68 $\pm$ 5.97 | 4.37 $\pm$ 1.07   | 0.20 $\pm$ 0.07                       | 0.26 $\pm$ 0.08 | 5.52 $\pm$ 0.89  | 12.22 $\pm$ 3.01 | 8.61 $\pm$ 1.55  | 0.23 $\pm$ 0.05 | 13.69 | 29.44 | 33.63 | 11.27 | 50.29 | 6.08  | 11.26                       | 40.12 | 21.29 |     |  |  |
| Melezitose   | 27.53 $\pm$ 6.68                 | 0.20 $\pm$ 0.13  | 0.71 $\pm$ 0.71   | 20.86 $\pm$ 3.65 | 0                | 52.32 $\pm$ 16.78 | /                                     | /               | 28.21 $\pm$ 4.39 | 0.27 $\pm$ 0.20  | 15.31 $\pm$ 3.25 | /               | 53.86 | 0.84  | 2.25  | 53.24 | 0     | 72.68 | 57.60                       | 0.87  | 37.87 |     |  |  |
| Maltose      | 2.31 $\pm$ 0.59                  | 1.26 $\pm$ 0.39  | 1.00 $\pm$ 0.55   | 1.39 $\pm$ 0.28  | 0.79 $\pm$ 0.20  | 1.00 $\pm$ 0.98   | /                                     | /               | 1.74 $\pm$ 0.31  | 1.00 $\pm$ 0.21  | 1.40 $\pm$ 0.20  | /               | 4.51  | 5.33  | 3.18  | 3.55  | 2.25  | 1.52  | 3.55                        | 3.31  | 3.47  |     |  |  |
| Eriose       | 0.88 $\pm$ 0.24                  | 1.35 $\pm$ 0.48  | 1.56 $\pm$ 0.95   | 0.59 $\pm$ 0.12  | 1.97 $\pm$ 0.77  | 2.88 $\pm$ 1.03   | /                                     | /               | 1.04 $\pm$ 0.24  | 1.65 $\pm$ 0.41  | 1.32 $\pm$ 0.23  | /               | 1.72  | 5.54  | 4.92  | 1.51  | 5.60  | 4.00  | 2.13                        | 5.41  | 3.27  |     |  |  |
| Maltotriose  | 0.96 $\pm$ 0.23                  | 1.52 $\pm$ 0.46  | 1.54 $\pm$ 0.65   | 0.54 $\pm$ 0.12  | 1.65 $\pm$ 0.63  | 2.21 $\pm$ 0.64   | /                                     | /               | 0.96 $\pm$ 0.18  | 1.57 $\pm$ 0.32  | 1.24 $\pm$ 0.18  | /               | 1.88  | 6.25  | 4.86  | 1.39  | 4.69  | 3.08  | 1.96                        | 5.17  | 3.08  |     |  |  |
| Total sugars | 51.11 $\pm$ 10.82                | 24.04 $\pm$ 5.67 | 31.61 $\pm$ 12.96 | 39.19 $\pm$ 5.83 | 35.16 $\pm$ 8.87 | 71.98 $\pm$ 23.55 | 2.56 $\pm$ 0.78                       | 3.12 $\pm$ 0.82 | 48.98 $\pm$ 6.30 | 30.47 $\pm$ 5.13 | 40.44 $\pm$ 4.35 | 2.87 $\pm$ 0.54 | 100   | 100   | 100   | 100   | 100   | 100   | 100                         | 100   | 100   | 100 |  |  |

the honeydew of the black bean aphid *A. fabae*. Variation in the amount of melezitose secreted proved to be the largest, with ca. half of all clones being virtually deficient in producing this sugar (Fig. 1, Table 1), whereas for the other half, melezitose constituted over half of the total amount of sugar secreted (Table 1). This large variation may explain why melezitose was found to be absent or rare in *A. fabae* honeydew in some studies (Detrain et al., 2010; Völkl et al., 1999), but found to be a major component in others (Fischer et al., 2005; Woodring et al., 2004). These results are interesting, given that melezitose is known to act as an ant-attractant (Detrain et al., 2010; Duckett, 1974; Fischer et al., 2005; Kiss, 1981; Völkl et al., 1999; Woodring et al., 2004) and therefore plays a key role in the ant-aphid mutualism. The large interclone variation that we found in the production of this sugar may mean that the high-melezitose secreting clones would be more frequently visited by ants and that they would therefore capitalize more on their interaction with ants than the low-melezitose secreting ones. Alternatively perhaps, it could be that the melezitose-deficient clones are “cheaters”, which forego producing sugars that are attractive to ants and thereby avoid the cost of ant attendance, instead taking advantage of the ant-attracting effect of other non-deficient clones, co-occurring on the same plant. The latter hypothesis is also plausible, given that we found several instances of high- and low-melezitose secreting co-occurring on the same plant (Fig. 1) and that also in an earlier study, we found that two thirds of all *A. fabae fabae* colonies are polyclonal (Vantaux et al., 2011). In addition, the fact that melezitose tends to be produced mainly in species that are ant-tended (Fischer and Shingleton, 2001; Woodring et al., 2004, 2007), or is produced only facultatively, in response to ant attendance (Fischer and Shingleton, 2001), suggests that an increased production induced by increased ant attendance would indeed be costly. Measurement of the fitness and level of ant attendance of low- and high-melezitose secreting



**Fig. 2.** Sugar concentrations ( $\mu\text{g}/\mu\text{L}$ ) measured in the honeydew of six *Aphis fabae* clones feeding on *Vicia faba* (mean  $\pm$  SD). Different letters indicate significant differences (Tukey HSD post hoc tests). (A) Trehalose concentrations ( $P = 0.02$ ), (B) melezitose concentrations ( $P < 0.0001$ ).

**Table 2**  
GLM results for the eight sugars and the total sugar concentrations found in the honeydew of *A. fabae* colonies which were ant-attended or not over a period of 4 weeks (Bonferroni correction:  $P < 0.0055$ ).

|                    | d.f. | Trehalose |        |      | Glucose |        |       | Fructose |        |       | Sucrose |        |      | Melezitose |       |       | Maltose |       |       | Eritose |       |       | Maltotriose |        |       | Total |      |       |  |
|--------------------|------|-----------|--------|------|---------|--------|-------|----------|--------|-------|---------|--------|------|------------|-------|-------|---------|-------|-------|---------|-------|-------|-------------|--------|-------|-------|------|-------|--|
|                    |      | SS        | F      | P    | SS      | F      | P     | SS       | F      | P     | SS      | F      | P    | SS         | F     | P     | SS      | F     | P     | SS      | F     | P     | SS          | F      | P     | SS    | F    | P     |  |
| Intercept          | 1    | 164.05    | 364.18 | 0.00 | 816.06  | 366.53 | 0.00  | 1094.05  | 436.45 | 0.00  | 683.29  | 155.70 | 0.00 | 182.2      | 75.65 | 0.00  | 144.13  | 70.52 | 0.00  | 167.44  | 76.16 | 0.00  | 1826.621    | 945.22 | 0.00  |       |      |       |  |
| Clone (Melezitose) | 3    | 3.09      | 2.29   | 0.09 | 4.30    | 0.64   | 0.59  | 6.73     | 0.89   | 0.45  | 26.04   | 1.98   | 0.13 | 0.12       | 0.95  | 19.23 | 2.66    | 0.06  | 19.08 | 3.11    | 0.035 | 20.89 | 3.17        | 0.03   | 6.526 | 1.13  | 0.35 |       |  |
| Melezitose         | 1    | 1.97      | 4.38   | 0.04 | 16.61   | 7.46   | 0.009 | 18.65    | 7.44   | 0.009 | 2.04    | 0.46   | 0.50 | 331.38     | 52.68 | 0.00  | 19.12   | 7.94  | 0.007 | 2.71    | 1.33  | 0.26  | 12.77       | 5.81   | 0.30  | 0.571 | 0.30 | 0.59  |  |
| Ant-attendance     | 1    | 1.05      | 2.32   | 0.13 | 1.90    | 0.85   | 0.36  | 0.01     | 0.005  | 0.94  | 0.49    | 0.11   | 0.74 | 1.05       | 0.17  | 0.68  | 5.49    | 2.28  | 0.13  | 2.56    | 1.25  | 0.27  | 9.58        | 4.36   | 0.04  | 0.053 | 0.02 | 0.87  |  |
| Week               | 4    | 6.07      | 3.37   | 0.02 | 17.08   | 1.92   | 0.12  | 37.416   | 3.73   | 0.01  | 29.34   | 1.67   | 0.17 | 5.9        | 0.23  | 0.92  | 29.90   | 3.10  | 0.02  | 18.37   | 2.25  | 0.08  | 20.86       | 2.37   | 0.07  | 25.85 | 3.34 | 0.018 |  |
| Error              | 45   | 20.27     |        |      | 100.19  |        |       | 112.80   |        |       | 197.46  |        |      | 283.06     |       |       | 108.37  |       |       | 91.98   |       |       | 98.92       |        |       | 86.96 |      |       |  |

clones in a natural setting would be required to test these hypotheses.

An alternative hypothesis which could explain the variation in melezitose production is that the high- and low-melezitose secreting clones each target different sieve elements, and that this results in differences in feeding rate or in the feeding on sap containing different amounts of sucrose (Dinant, 2008; Fiehn, 2003). The clones that feed at the highest rate or on sap with the highest amount of sucrose would then experience the most osmotic stress, and would consequently benefit from producing melezitose (Douglas, 2003, 2006; Fisher et al., 1984; Rhodes et al., 1997; Wilkinson et al., 1997). Consistent with this interpretation, we found that the amount of melezitose secreted tended to correlate with the total amount of honeydew sugar produced, which is probably a good indicator of the total amount of honeydew produced, as well as with their feeding rate. In other studies, species that are ant-tended have been shown to typically feed at higher rates than untended ones (Woodring et al., 2004, 2007; Yao and Akimoto, 2001), and the higher osmotic stress that is induced by this might explain why some species facultatively adjust their melezitose secretion in response to ant attendance (Fischer and Shingleton, 2001). Interestingly though, there was no evidence that in our case, any of the clones modulated their melezitose production in response to ant attendance.

Another result from our study is that we found *V. faba* extra floral nectar (EFN) to contain only very low concentrations of sugars compared to *A. fabae* honeydew. In fact, the concentrations we found were much lower than those found in an earlier study by Engel et al. (2001). Nevertheless, this result could be explained by the fact that the composition of EFN is known to display large intraspecific variation (Blüthgen et al., 2004). As in our study, though, Engel et al. (2001) also did not find any differences in the EFN secreted by plants which were or were not infested by *A. fabae*. According to the ant distraction hypothesis, extra-floral nectaries may have evolved to defend plants against ant-homopteran mutualism by attracting ants to the extra-floral nectaries (Becerra and Venable, 1989). Given the low amount of sugars produced by the *V. faba* EFN in our study, this hypothesis is unlikely to apply though to our study system. In addition, contrary to the ant distraction hypothesis, it has also been shown that extra-floral nectaries sometimes result in the attraction of relatively more ants, and that if small aphid colonies happen to be present on these plants they end up being better protected against natural enemies compared to the situation when they had to attract all ants by themselves (Katayama and Suzuki, 2010).

In summary, our data demonstrate that there is large variation in the amount of the ant-attractant sugar melezitose secreted by different *A. fabae* clones. As pointed out, this could have significant implications in the context of the evolution and maintenance of the ant-aphid mutualism, and affect the osmoregulatory capacity of the different aphid clones. More generally, the high- and low-melezitose secreting clones that we identified will also form an excellent model to test the various suggested benefits of oligosaccharide synthesis in aphids and other insects. Further research would be required to measure the fitness and levels of ant attendance of the high- and low-melezitose secreting *A. fabae* in a natural setting to determine unambiguously how these two alternative reproductive phenotypes manage to stably coexist in the population.

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## References

- Ashford, D.A., Smith, W.A., Douglas, A.E., 2000. Living on a high sugar diet: the fate of sucrose ingested by a phloem-feeding insect, the pea aphid *Acyrtosiphon pisum*. *Journal of Insect Physiology* 46, 335–341.
- Auclair, J.L., 1963. Aphid feeding and nutrition. *Annual Review of Entomology* 8, 439–490.
- Banks, C.J., 1962. Effects of the ant *Lasius niger* (L.) on insects preying on small populations of *Aphis fabae* Scop on bean plants. *Annals of Applied Biology* 50, 669–679.
- Becerra, J.X.I., Venable, D.L., 1989. Extrafloral nectaries: a defense against ant–Homoptera mutualisms? *Oikos* 55, 276–280.
- Blüthgen, N., Fiedler, K., 2004. Preferences for sugars and amino acids and their conditionality in a diverse nectar-feeding ant community. *Journal of Animal Ecology* 73, 155–166.
- Blüthgen, N., Gottsberger, G., Fiedler, K., 2004. Sugar and amino acid composition of ant-attended nectar and honeydew sources from an Australian rainforest. *Austral Ecology* 29, 418–429.
- Boevé, J.L., Wäckers, F.L., 2003. Gustatory perception and metabolic utilization of sugars by *Myrmica rubra* ant workers. *Oecologia* 136, 508–514.
- Boucher, D.H., 1988. *The Biology of Mutualism: Ecology and Evolution*. Oxford University Press.
- Braendle, C., Weisser, W.W., 2001. Variation in escape behavior of red and green clones of the pea aphid. *Journal of Insect Behavior* 14, 497–509.
- Bristow, C.M., 1991. Why are so few aphids ant-tended? In: Huxley, C.R., Cutler, D.F. (Eds.), *Ant–Plant Interactions*. Oxford University Press.
- Bronstein, J.L., 1994. Our current understanding of mutualism. *Quarterly Review of Biology* 69, 31–51.
- Byrne, D.N., Miller, W.B., 1990. Carbohydrate and amino-acid composition of phloem sap and honeydew produced *Bemisia tabaci*. *Journal of Insect Physiology* 36, 433–439.
- Cornelius, M.L., Grace, J.K., Yates, J.R., 1996. Acceptability of different sugars and oils to three tropical ant species (Hymenoptera, Formicidae). *Anzeiger Fur Schadlingskunde Pflanzenschutz Umweltschutz* 69, 41–43.
- Cristofolletti, P.T., Ribeiro, A.F., Deraison, C., Rahbe, Y., Terra, W.R., 2003. Midgut adaptation and digestive enzyme distribution in a phloem feeding insect, the pea aphid *Acyrtosiphon pisum*. *Journal of Insect Physiology* 49, 11–24.
- Cushman, J.H., Addicott, J.F., 1989. Intra- and interspecific competition for mutualists: ants as a limited and limiting resource for aphids. *Oecologia* 79, 315–321.
- Detrain, C., Verheggen, F.J., Diez, L., Wathelet, B., Haubruge, E., 2010. Aphid–ant mutualism: how honeydew sugars influence the behaviour of ant scouts. *Physiological Entomology* 35, 168–174.
- Dinant, S., 2008. Phloem, transport between organs and long-distance signalling. *Comptes Rendus Biologies* 331, 334–346.
- Dixon, A.F.G., Agarwala, B.K., 1999. Ladybird-induced life-history changes in aphids. *Proceedings of the Royal Society of London Series B-Biological Sciences* 266, 1549–1553.
- Douglas, A.E., 1997. Provenance, experience and plant utilisation by the polyphagous aphid, *Aphis fabae*. *Entomologia Experimentalis et Applicata* 83, 161–170.
- Douglas, A.E., 2003. The nutritional physiology of aphids. *Advances in Insect Physiology* 31, 73–140.
- Douglas, A.E., 2006. Phloem–sap feeding by animals: problems and solutions. *Journal of Experimental Botany* 57, 747–754.
- Duckett, D.P., 1974. Further studies of ant–aphid interactions. Imperial College, University of London, London.
- Engel, V., Fischer, M.K., Wäckers, F.L., Völkl, W., 2001. Interactions between extrafloral nectaries, aphids and ants: are there competition effects between plant and homopteran sugar sources? *Oecologia* 129, 577–584.
- Fiehn, O., 2003. Metabolic networks of *Cucurbita maxima* phloem. *Phytochemistry* 62, 875–886.
- Fischer, M.K., Hoffmann, K.H., Völkl, W., 2001. Competition for mutualists in an ant–Homopteran interaction mediated by hierarchies of ant attendance. *Oikos* 92, 531–541.
- 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., Hoffmann, K.H., 2005. Honeydew production and honeydew sugar composition of polyphagous black bean aphid, *Aphis fabae* (Hemiptera: Aphididae) on various host plants and implications for ant-attendance. *European Journal of Entomology* 102, 155–160.
- 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.
- Fisher, D.B., Wright, J.P., Mittler, T.E., 1984. Osmoregulation by the aphid *Myzus persicae*: a physiological role for honeydew oligosaccharides. *Journal of Insect Physiology* 30, 387.
- Gorur, G., Lomonaco, C., Mackenzie, A., 2005. Phenotypic plasticity in host–plant specialisation in *Aphis fabae*. *Ecological Entomology* 30, 657–664.
- Hausmann, C., Wäckers, F.L., Dorn, S., 2005. Sugar convertibility in the parasitoid *Cotesia glomerata* (Hymenoptera: Braconidae). *Archives of Insect Biochemistry and Physiology* 60, 223–229.
- Hendrix, D.L., Wei, Y.A., Leggett, J.E., 1992. Homopteran honeydew sugar composition is determined by both the insect and plant species. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 101, 23–27.
- Henneberry, T.J., Jech, L.F., de la Torre, T., Hendrix, D.L., 2000. Cotton aphid (Homoptera: Aphididae) biology, honeydew production, sugar quality and quantity, and relationships to sticky cotton. *Southwestern Entomologist* 25, 161–174.
- Hogervorst, P.A.M., Wäckers, F.L., Romeis, J., 2007a. Effects of honeydew sugar composition on the longevity of *Aphidius ervi*. *Entomologia Experimentalis et Applicata* 122, 223–232.
- Hogervorst, P.A.M., Wäckers, F.L., Romeis, J., 2007b. Detecting nutritional state and food source use in field-collected insects that synthesize honeydew oligosaccharides. *Functional Ecology* 21, 936–946.
- Hölldobler, B., Wilson, E.O., 1990. *The Ants*. Springer-Verlag, Berlin.
- Ide, T., Suzuki, N., Katayama, N., 2007. The use of honeydew in foraging for aphids by larvae of the ladybird beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). *Ecological Entomology* 32, 455–460.
- Karley, A.J., Ashford, D.A., Minto, L.M., Pritchard, J., Douglas, A.E., 2005. The significance of gut sucrose activity for osmoregulation in the pea aphid, *Acyrtosiphon pisum*. *Journal of Insect Physiology* 51, 1313–1319.
- Katayama, N., Suzuki, N., 2010. Extrafloral nectaries indirectly protect small aphid colonies via ant-mediated interactions. *Applied Entomology and Zoology* 45, 505–511.
- Kennedy, J.S., Stroyan, H.L.G., 1959. Biology of aphids. *Annual Review of Entomology* 4, 139–160.
- Kiss, A., 1981. Melezitose, aphids and ants. *Oikos* 37, 382.
- Kunert, G., Otto, S., Rose, U.S.R., Gershenzon, J., Weisser, W.W., 2005. Alarm pheromone mediates production of winged dispersal morphs in aphids. *Ecology Letters* 8, 596–603.
- Majerus, M.E.N., Sloggett, J.J., Godeau, J.F., Hemptinne, J.L., 2007. Interactions between ants and aphidophagous and coccidophagous ladybirds. *Population Ecology* 49, 15–27.
- Matsuura, K., Yashiro, T., 2006. Aphid egg protection by ants: a novel aspect of the mutualism between the tree-feeding aphid *Stomaphis hirukawai* and its attendant ant *Lasius productus*. *Naturwissenschaften* 93, 506–510.
- Minarro, M., Fernandez-Mata, G., Medina, P., 2010. Role of ants in structuring the aphid community on apple. *Ecological Entomology* 35, 206–215.
- Müller, F.P., 1983. Differential alarm pheromone responses between strains of the aphid *Acyrtosiphon pisum*. *Entomologia Experimentalis et Applicata* 34, 347–348.
- Nielsen, C., Agrawal, A.A., Hajek, A.E., 2010. Ants defend aphids against lethal disease. *Biology Letters* 6, 205–208.
- Price, D.R.G., Karley, A.J., Ashford, D.A., Isaacs, H.V., Pownall, M.E., Wilkinson, H.S., Gatehouse, J.A., Douglas, A.E., 2007. Molecular characterisation of a candidate gut sucrose in the pea aphid, *Acyrtosiphon pisum*. *Insect Biochemistry and Molecular Biology* 37, 307–317.
- Price, D.R.G., Tibbles, K., Shigenobu, S., Smertenko, A., Russell, C.W., Douglas, A.E., Fitches, E., Gatehouse, A.M.R., Gatehouse, J.A., 2010. Sugar transporters of the major facilitator superfamily in aphids; from gene prediction to functional characterization. *Insect Molecular Biology* 19, 97–112.
- Rhodes, J.D., Croghan, P.C., Dixon, A.F.G., 1997. Dietary sucrose and oligosaccharide synthesis in relation to osmoregulation in the pea aphid, *Acyrtosiphon pisum*. *Physiological Entomology* 22, 373–379.
- Sachs, J.L., Mueller, U.G., Wilcox, T.P., Bull, J.J., 2004. The evolution of cooperation. *Quarterly Review of Biology* 79, 135–160.
- Schwartzberg, E.G., Kunert, G., Roese, U.S.R., Gershenzon, J., Weisser, W.W., 2008. Alarm pheromone emission by pea aphid, *Acyrtosiphon pisum*, clones under predation by lacewing larvae. *Entomologia Experimentalis et Applicata* 128, 403–409.
- Stadler, B., 1997. The relative importance of host plants, natural enemies and ants in the evolution of life-history characters in aphids. In: Dettner, K., Bauer, G., Völkl, W. (Eds.), *Vertical Food Web Interactions*. Springer Verlag, Berlin, pp. 241–256.
- Stadler, B., Dixon, A.F.G., 1998. Costs of ant attendance for aphids. *Journal of Animal Ecology* 67, 454–459.
- Stadler, B., Dixon, A.F.G., 2005. Ecology and evolution of aphid–ant interactions. *Annual Review of Ecology Evolution and Systematics* 36, 345–372.
- Stroyan, H.L.G., 1984. *Aphids-Pterocommatinae and Aphidinae (Aphidini)*, Homoptera, Aphididae. Royal Entomological Society of London, London.
- Tosh, C.R., Powell, G., Hardie, J., 2003. Decision making by generalist and specialist aphids with the same genotype. *Journal of Insect Physiology* 49, 659–669.
- Van den Ende, W., Mintiens, A., Speleers, H., Onouha, A.A., Van Laere, A., 1996. The metabolism of fructans in roots of *Cichorium intybus* during growth, storage and forcing. *New Phytologist* 132, 555–563.
- Vantaux, A., Billen, J., Wenseleers, T., 2011. Levels of clonal mixing in the black bean aphid *Aphis fabae*, a facultative ant mutualist. *Molecular Ecology* doi:10.1111/j.1365-294X.2011.05204.x.
- 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., 1999. Gustatory response by the hymenopteran parasitoid *Cotesia glomerata* to a range of nectar and honeydew sugars. *Journal of Chemical Ecology* 25, 2863–2877.
- 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., van Rijn, P.C.J., 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. Mutualism between ants and honeydew-producing Homoptera. *Annual Review of Entomology* 8, 307–344.
- Weil, J.-H., 1978. *Biochimie Générale*. Masson, Paris.
- Wilkinson, T.L., Ashford, D.A., Pritchard, J., Douglas, A.E., 1997. Honeydew sugars and osmoregulation in the pea aphid *Acyrtosiphon pisum*. *Journal of Experimental Biology* 200, 2137–2143.
- Woodring, J., Wiedemann, R., Fischer, M.K., Hoffmann, K.H., Völkl, W., 2004. Honeydew amino acids in relation to sugars and their role in the establishment of ant-attendance hierarchy in eight species of aphids feeding on tansy (*Tanacetum vulgare*). *Physiological Entomology* 29, 311–319.
- Woodring, J., Wiedemann, R., Völkl, W., Hoffmann, K.H., 2007. Oligosaccharide synthesis regulates gut osmolality in the ant-attended aphid *Metopeurum fuscoviride* but not in the unattended aphid *Macrosiphoniella tanacetaria*. *Journal of Applied Entomology* 131, 1–7.
- Wool, D., Hendrix, D.L., Shukry, O., 2006. Seasonal variation in honeydew sugar content of galling aphids (Aphidoidea: Pemphigidae: Fordinae) feeding on *Pistacia*: host ecology and aphid physiology. *Basic and Applied Ecology* 7, 141–151.
- Wyckhuys, K.A.G., 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., 2001. Ant attendance changes the sugar composition of the honeydew of the drepanosiphid aphid *Tuberculatus quercicola*. *Oecologia* 128, 36–43.
- 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.
- Yao, I., Shibao, H., Akimoto, S., 2000. Costs and benefits of ant attendance to the drepanosiphid aphid *Tuberculatus quercicola*. *Oikos* 89, 3–10.