

Plant cues for aphid navigation in vascular tissues

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SUMMARY

The ability of aphids to detect and find sieve tubes suggests that aphids receive cues for sieve-tube recognition by taking samples. Specific natural conditions such as pH value, sugar species and concentration, viscosity, and oxygen pressure may enable sieve-tube detection. We tested the preference of *Megoura viciae* and *Myzus persicae* for potential plant-borne orientation parameters in artificial choice-chamber systems. Both species preferred sucrose (in comparison with fructose, glucose, raffinose or sorbitol) at concentrations of 15–22.5% (over a tested range of 0–22.5%) and at approximately pH 7 (over a tested range of pH 5–8). This preference matches the composition of the sieve-tube sap of their host plants. Likewise, *Rhopalosiphum padi* (normally found on barley plants with sucrose in the phloem sap) and *Macrosiphum euphorbiae* (normally found on pumpkin plants with raffinose-family oligosaccharides in the phloem sap) showed a significant preference for sucrose. In the absence of sucrose, however, *M. euphorbiae* strongly preferred raffinose. No clear preference for any carbohydrate was observed for *Macrosiphum rosae* and *Aphis pomi* (both normally found on plants with various amounts of sorbitol in the phloem sap). Electrical penetration graph (EPG) measurements of *M. persicae* feeding on artificial diets confirmed that sieve tubes are recognized solely by a combination of carbohydrate abundance and a neutral to slightly alkaline pH.

Supplementary material available online at <http://jeb.biologists.org/cgi/content/full/213/23/4030/DC1>

Key words: aphid preference, choice chamber, electrical penetration graph, phloem, plant-borne orientation cues.

INTRODUCTION

Aphids ingest their food from sieve tubes (Auclair, 1963; Miles, 1987; Prado and Tjallingii, 1994) that contain a concentrate of nutrients (Hayashi and Chino, 1990; van Bel, 2003). Sieve-tube sap is withdrawn by specialized aphid mouth parts (stylets) (Auclair, 1963; Miyazaki, 1987). Prior to ingestion, aphids secrete watery saliva, probably to interfere with plant occlusion reactions induced by stylet damage (Will and van Bel, 2006; Will et al., 2007; Will et al., 2009; Carolan et al., 2009).

Transmission electron microscopy (TEM) reconstructions demonstrate relatively straightforward stylet progression through the extravascular tissue that occurs largely intercellularly. The stylet orientation becomes increasingly diffuse within the vascular bundle region, as the TEM reconstructions show several dead-end tracks of gel saliva, which is secreted to function as a lubrication and protection sheath for the fragile mouth parts (Tjallingii and Hogen Esch, 1993). Even punctured sieve tubes are frequently rejected as sources of nutrition for unknown reasons (Tjallingii and Hogen Esch, 1993). Once a sieve tube is selected for sap withdrawal, the aphid may stay there for several days (Prado and Tjallingii, 1994), during which sieve-tube sap is ingested passively (Prado and Tjallingii, 1994; Tjallingii and Cherqui, 1999; Miles, 1999) owing to the high turgour pressure of sieve tubes (Auclair, 1963).

According to a long-standing hypothesis (Auclair, 1969), aphids seem to employ information from punctured plant cells along the stylet pathway for sieve-tube recognition by sensing the high sugar content and slightly alkaline pH values characteristic of sieve-tube sap.

For instance, sucrose concentrations in the sieve-tube sap of grasses are extremely high [17–25% in *Oryza sativa* (Fukumorita and Chino, 1982; Hayashi and Chino, 1990), 9% in *Triticum aestivum* (Hayashi and Chino, 1986) and 38% in *Zea mays* (Ohshima et al., 1990)]. In many plant species (Zimmermann and Ziegler, 1975), sucrose is the predominant transport sugar [e.g. *Nicotiana tabacum* (Heineke et al., 1994) and *Spinacia oleracea* (Winter et al., 1994)]. It should be noted that sieve-tube sap in several plant groups contains significant amounts of raffinose-family oligosaccharides (RFOs) (e.g. Cucurbitaceae) or sugar alcohols (e.g. Rosaceae) (Zimmermann and Ziegler, 1975). Carbohydrate concentrations in sieve-tube sap are generally 5–50-fold higher than in parenchyma cells and several hundred times higher than in the apoplast (Minchin and Thorpe, 1984; Tetlow and Farrar, 1993; Lohaus et al., 1995; Nadwodnik and Lohaus, 2008). Vacuolar sucrose concentrations tend to be lower than in the cytosol of parenchyma cells (Winter et al., 1993; Winter et al., 1994).

Like cytosolic compartments, sieve tubes have a luminal pH of ~7.5 [e.g. *O. sativa* (Fukumorita and Chino, 1982); *Ricinus communis* (Vreugdenhil and Koot-Gronsveld, 1989); *Vicia faba*, *Hordeum vulgare*, *Cucurbita sativa*, *Cucurbita pepo* (Hafke et al., 2005)]. This contrasts sharply with the vacuolar pH values of 5.0–5.5 in parenchyma cells [(Taiz, 1992); e.g. *Z. mays* (Roberts et al., 1980); *Riccia fluitans* (Bertl et al., 1984)] and apoplast pH of 5–6 (Felle et al., 2005; Felle, 2006) but resembles the cytoplasmic pH of parenchyma cells (e.g. Felle and Bertl, 1986; Frohmeyer et al., 1998; Felle et al., 2000).

As reviewed previously (Auclair, 1963; Auclair, 1969), limited sucrose concentration series and pH series have been offered to

investigate the effects of diets on growth, reproduction and survival of diet-reared populations over several days. Mittler and Dadd tested sucrose concentrations to establish optimal aphid diets (Mittler and Dadd, 1963b). In other cases, two diets (e.g. sucrose vs water, sucrose vs sucrose with amino acids) were compared to investigate diet preference (Mittler and Dadd, 1964) or uptake rates of diet solutes (Mittler and Dadd, 1962; Mittler and Dadd, 1963a). Later, an extensive series of amino acid/sucrose diets was employed to determine optimal growth conditions (Simpson et al., 1995).

These experiments do not show, however, whether aphids recognize sieve tubes through optimal conditions for their growth. It would indeed be practical if aphids were to recognize sieve tubes through sucrose sensing, but no unequivocal proof for this obvious concept has been provided thus far. A number of questions remain to be answered. How do aphids identify sieve tubes in species translocating appreciable amounts of non-sucrose carbohydrates? Are aphids capable of recognizing different sucrose concentrations, as sucrose occurs to some level in all cells? Do aphids perceive cell turgour and viscosity, which are usually commensurate with the carbohydrate concentration? Can aphids sense cellular pH, as sieve tubes contain saps with pH values between 7.0 and 7.5?

In the present study, these issues were addressed by systematically offering a broad range of sugars, sugar concentrations, solute viscosities and pH values to the aphids *Megoura viciae* (monophagous) and *Myzus persicae* (polyphagous) to assess whether aphid preference in an artificial environment mirrors the composition of the phloem sap in the respective host plants. Furthermore, *Rhopalosiphum padi*, *Macrosiphum rosae*, *Aphis pomi* and *Macrosiphum euphorbiae*, which naturally colonize plants with sieve-tube saps of diverse sugar composition, were employed to test whether the carbohydrate composition of phloem sap acts as a universal cue for sieve-tube recognition.

MATERIALS AND METHODS

Cultivation of host plants and rearing of aphids

Host plants [*Vicia faba* L. cv. Witkiem major (Nunhem Zaden, Haalen, The Netherlands); *Capsicum* spp. L.; *Hordeum vulgare* L.

cv. Auriga (IPAZ, Justus-Liebig-University, Gießen, Germany); *Cucurbita pepo* L. cv. Maxima Duchesne (Enza Zaden GmbH & Co. KG, Dannstadt-Schauernheim, Germany)] were cultivated in a greenhouse under daylight plus artificial light sources (Philips SUNT, IP65 and SON-T Agro 400) at 20–22°C, a relative humidity of 65% and a 17h:7h light:dark photoperiod. Pot plants of *Rosa* spp. L. and *Malus* spp. Miller, both approximately three years old, were purchased from Obi, Gießen, Germany and Gartenbaumschule Engelhardt, Gießen, Germany, respectively.

Under the above-mentioned environmental conditions, aphid species *Megoura viciae* (Buckton), *Myzus persicae* (Sulzer) and *Rhopalosiphum padi* (L.; the olive-green apterous morph feeding on cereals) were reared in Perspex® boxes (approximately 50 cm×50 cm×60 cm), covered with a gauze cloth for better air circulation. *Macrosiphum euphorbiae* (Thomas), *Macrosiphum rosae* (L.) and *Aphis pomi* (De Geer) were reared without Perspex® boxes on the host plants listed in Table 1. In both cases, under these conditions, parthenogenetically generated, apterous aphid colonies developed.

Phloem-sap collection for HPLC carbohydrate analysis by means of EDTA-facilitated exudation and stylectomy

Sieve-tube samples for HPLC analysis were collected from leaves of *V. faba* (one youngest mature double leaf), *Capsicum* spp. (four youngest mature leaves), *H. vulgare* (group of 10 plants, cut just above the stem/root junction) and *C. pepo* (one mature leaf). The growth stages of the plants are given in Table 1. The respective plant parts were excised with a razor blade, immediately re-cut under 5 mmol l⁻¹ EDTA/1 mmol l⁻¹ MES (pH 7.0, adjusted with KOH) to ensure exudation of photoassimilates (cf. King and Zeevaart, 1974) and transferred into reaction cups with 2.5 mmol l⁻¹ EDTA/1 mmol l⁻¹ MES (pH 7.0, adjusted with KOH) (1.5 ml for *V. faba*, *Capsicum* spp. and *H. vulgare* or 2 ml for *C. pepo*). The collection media were replaced every hour during 6h of constant environmental conditions under artificial light (Philips IP65) at 20°C and a relative humidity of 65%, and the samples were frozen immediately. After sampling, the fresh mass of the plant material was determined. The samples were mixed with a spatula tip of

Table 1. Aphid species, their respective host plants and the respective translocated carbohydrates under the ambient conditions

Aphid species	Feeding type	Host plant	Dominant phloem transport sugar(s)	Analysis method
<i>Megoura viciae</i>	Monophagous	<i>Vicia faba</i> 3–4 weeks old	Sucrose	HPLC after EDTA-exudation and stylectomy (for results, see supplementary material Figs S1A, S2A) McDonald et al., 1995
<i>Myzus persicae</i>	Polyphagous	<i>Capsicum</i> spp. 7–8 weeks old	Sucrose	HPLC after EDTA exudation (for results, see supplementary material Fig. S1B) Turner and Wien, 1994
<i>Rhopalosiphum padi</i>	Oligophagous	<i>Hordeum vulgare</i> 10 days old	Sucrose	HPLC after EDTA exudation and stylectomy (for results, see supplementary material Figs S1C, S2B) Riens et al., 1994
<i>Macrosiphum rosae</i>	Monophagous	<i>Rosa</i> spp. Young shoots of ~3-year-old pot plants	Sucrose + sorbitol	Zimmermann and Ziegler, 1975
<i>Aphis pomi</i>	Monophagous, autoecious	<i>Malus</i> spp. Young shoots of ~3-year-old pot plants	Sorbitol + sucrose	Zimmermann and Ziegler, 1975
<i>Macrosiphum euphorbiae</i>	Polyphagous	<i>Cucurbita pepo</i> 3–4 weeks old	Raffinose-family oligosaccharides (RFOs) + sucrose	HPLC after EDTA exudation (for results, see Fig. S1D in supplementary material) Haritatos et al., 1996

Polyclar (Serva, Heidelberg, Germany) and boiled for 10 min. The samples were cooled and centrifuged at 9390 *g* at 4°C. 600 µl of each sample was transferred to HPLC-vials, and the sugar content was analysed by HPLC [HPLC-PAE installed in a DX 500 system (Dionex, Sunnyvale, CA, USA)] with a 250 mm (length) × 3 mm (diameter) analytical CarboPac™ PA20 column (Dionex) with a 50 mm guard column for isocratic separation (35 mmol l⁻¹ NaOH)].

For phloem-sap collection by stylectomy, *M. viciae* were placed onto the upper part of a horizontally fixed *V. faba* plant and left overnight for settling. After cutting the aphid stylets with a microcautery device (HF-microcautery unit CA-50; Syntech, Hilversum, The Netherlands) (Fisher and Frame, 1984), stylet exudate droplets solidified. After 5–10 min, solidified exudate droplets were collected using a borosilicate microcapillary with a tip diameter of 0.1 mm fitted onto a micromanipulator. The capillary tip was manipulated into a hook by gentle heating to simplify sampling. Ten exudate droplets were dissolved in 600 µl Millipore water and were stored at -20°C. Exudate droplets of phloem sap collected from *R. padi* on *H. vulgare* were sampled as described previously (Gaupels et al., 2008) after six hours of stylet exudation. Droplets were also dissolved to 600 µl with Millipore water and stored at -20°C. Stylectomy samples were transferred into HPLC-vials without further processing.

Choice-chamber systems

We designed a choice-chamber system (Fig. 1) that is more practical than previous versions (e.g. Mittler and Dadd, 1964) due to its ease of use, the higher number of testable conditions in one experimental set-up, and the use of numerous individuals. The choice-chamber system consists of a square, dark grey plastic plate [three different sizes depending on the aphid species and colony size: 24 cm × 24 cm, 2.5 cm in thickness (large); 15 cm × 15 cm, 2 cm in thickness (medium); and 7.5 cm × 7.5 cm, 2 cm in thickness (small)]. Eight square, shallow test baths (4 cm × 4 cm, 2.5 cm × 2.5 cm or 1.25 cm × 1.25 cm, respectively) were milled into the surface of the plate to a depth of 3 mm to contain the test solutions. In addition, shallow boundary grooves were milled to delimit the test areas and prevent potential diet mixture (Fig. 1A). The choice-chamber system was designed so that the starting distances of the aphids to the respective test baths were equal (Fig. 1A).

Test solutions

The test solutions consisted essentially of a modified diet (Cherqui and Tjallingii, 2000) containing 438 mmol l⁻¹ sucrose (15%) and the amino acids L-serine (100 mmol l⁻¹), L-methionine (100 mmol l⁻¹) and L-aspartic acid (100 mmol l⁻¹). The pH value was adjusted to pH 7.2 with KOH. After sterile filtering through Rotilabo® syringe filters (PVDF-membrane, pore size 0.45 µm; Carl-Roth GmbH, Karlsruhe, Germany), the test solutions were stored at 4°C. The composition was varied in terms of the amino acid and sucrose concentration, the sugar species (fructose, glucose, sucrose, raffinose, sorbitol, galactose or mannose), the pH (5.0–8.0; buffered with 10 mmol l⁻¹ citrate, MES or TRIS at the appropriate pH values) or the viscosity.

After being cleaned with 70% alcohol, the test baths were filled with test solutions, and the entire choice-chamber system was covered with a layer of stretched Parafilm® (Miles and Harrewijn, 1991; Madhusudhan and Miles, 1998) under sterile conditions. For each experiment, either two or four test solutions, which differed by a single parameter, were offered in eight test baths. Opposite test baths contained identical test solutions (see Fig. 3).

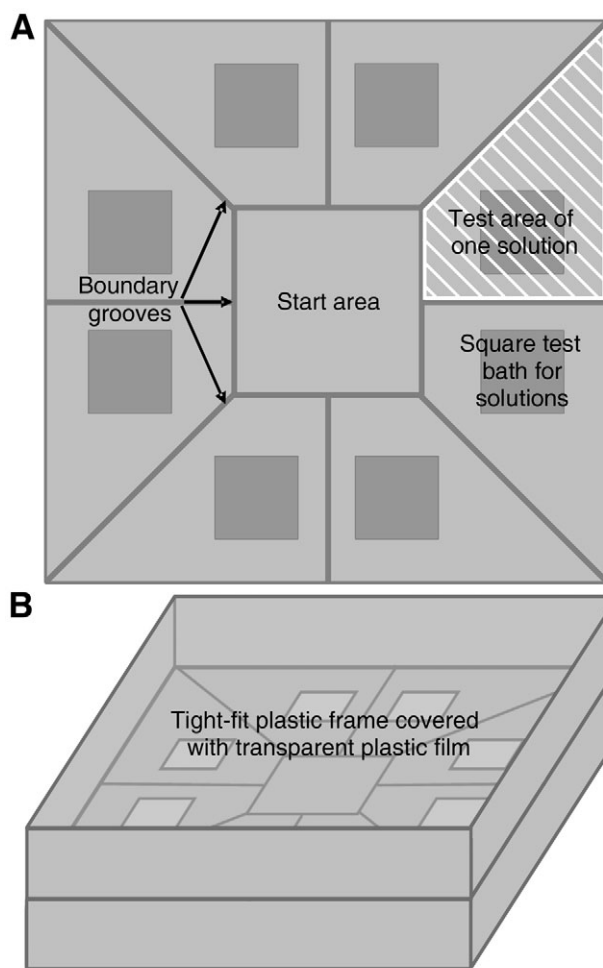


Fig. 1. Schematic design of the choice-chamber system. (A) The choice-chamber system consisted of a dark grey, square plastic plate. The test areas (hatched) were delineated by milled boundary grooves to avoid mixing of the test solutions. The test solutions were filled in flat, square test baths, milled in the test area surfaces and covered with stretched Parafilm®. (B) During the experiments, the aphids were trapped inside a tight-fit plastic frame covered with transparent plastic film.

Collection of aphids for choice experiments and experimental design

Megoura viciae, *M. persicae*, *R. padi* and *M. euphorbiae* were collected in plastic boxes by gently shaking colonized plants. Collected individuals were mainly reproducing adults of differing ages; earlier developmental stages were rare. For collection of *A. pomi*, a transparent plastic tube was fitted to a dark plastic box containing infested leaves, and aphids naturally congregated in the plastic tube. Individuals of *M. rosae* were collected in the same manner or directly from outdoor rose plants. The number of individual aphids per choice-chamber experiment (Table 2) was determined by density experiments, which assessed the lowest degree of mutual disturbance (T.W. and A.J.E.v.B., unpublished).

Aphids were placed in the centre of the start area and caged under a tight-fit plastic frame with transparent film (Fig. 1B) at the numbers given in Table 2. The choice-chamber system was incubated for 24 h (17 h:7 h light:dark photoperiod, light intensity ~3100 lx) under evenly distributed illumination (scattered light from neon lamps; Philips TLD 58W25, Amsterdam, The Netherlands). At the end of the foraging period, the aphid distribution was photographed

Table 2. Mean number of used individuals per choice-chamber system

	<i>M. viciae</i>	<i>M. persicae</i>	<i>R. padi</i>	<i>M. rosae</i>	<i>A. pomi</i>	<i>M. euphorbiae</i>
Large choice-chamber system	~500	–	–	–	–	–
Medium choice-chamber system	~300	~400	~400	~150	–	~350
Small choice-chamber system	–	–	–	~50	~150	–

The numbers have been assessed to impose a minimal degree of mutual disturbance regarding the size of the adult individuals and the colony size. The respective use of the choice-chamber systems in the trials of *M. viciae* and *M. rosae* is indicated in the respective results.

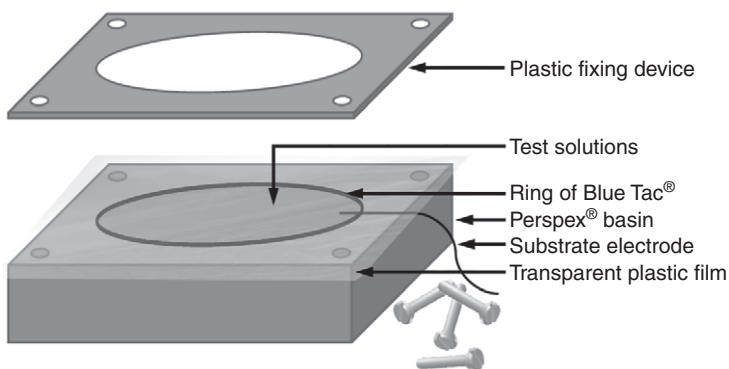
using digital cameras. The digital high-resolution colour pictures were treated with Corel® PhotoPaint (Ottawa, ON, Canada) to optimize brightness, contrast and colour in order to quantify aphid distribution. Manual counting of individuals from digital pictures of the choice-chamber system provided positional quantification. The very low numbers of dead aphids (appearing as brown-black coloured aphids or, in the case of *R. padi*, showing an unnatural posture in the digital colour pictures) were not scored for analysis.

To document behaviour of aphids during the foraging period and to ensure that the experimental duration was optimal, a series of images was taken in a separate experiment with a digital camera (Canon PowerShot S40 or A85; Tokyo, Japan) fixed above the choice chamber. Images were taken every 5 min for 24 h with the software Canon® Remote-Capture. These images were converted into a fast-motion video film (see Movies 1 and 2 in supplementary material).

Data handling and statistical analyses

Aphid distribution was analysed using two sets of data acquisition. Initially, the number of aphids was counted on the test areas enclosed by the boundary grooves (see Fig. 1A) and is referred to as 'raw data' (see supplementary material Figs S3–S23). To compensate for the effects of background random distributions, the number of aphids on the start area and boundary grooves was assumed to represent the random background distribution of aphids. The random background density (N_{back} ; number of aphids per cm^2 surface outside the test areas; Fig. 1A) was then subtracted from the aphid density on the test areas (N_{test}). The resultant net non-random density per test area (see Eqn 1, in which N is the number of aphids) was multiplied by the test area surface (a ; in cm^2) to determine the absolute number of aphids (referred to as 'corrected data' in supplementary material Figs S3–S23) on each test area (N_{abs}). Negative density values were set to zero. Corrected data are presented in the figures (Figs 4–8), and both datasets are presented in the supplementary material (supplementary material Figs S3–S23) to provide the highest degree of transparency in the data:

$$N_{\text{abs}} = (N_{\text{test}} - N_{\text{back}}) \times a. \quad (1)$$



Statistical analysis was performed using the software SigmaStat® 3.0 (SPSS Inc., Chicago, IL, USA), executing the Friedman repeated-measures analysis-of-variance on ranks test (Friedman-test), used in experimental series with four test solutions, and the *t*-test, used in experimental series with two test solutions, for both distribution data sets. The significance limit was $P=0.05$. If the statistical significance of the Friedman test was <0.05 , a *post hoc* pairwise comparison analysis (Dunn's method, Tukey test and Student-Newman-Keuls test) was automatically deployed by the statistical software to determine significant differences between groups (supplementary material Tables S1–S35).

Diet-EPGs of *Myzus persicae*

Feeding behaviour was assumed to be expressed by sustained E2 waveforms [waveform definition after Prado and Tjallingii (Prado and Tjallingii, 1994)] on various diets by electrical penetration graph (EPG) analysis, as used in previous studies (Tjallingii, 1978; Tjallingii, 1995). Test solutions were offered in a Perspex® basin (Fig. 2), composed of a Perspex® block (10 cm × 10 cm) with a milled circular bath (9 cm in diameter, 2 mm in depth), covered with transparent plastic film (Alpac® Ohler, Plettenberg-Ohle, Germany), previously used in EPG experiments (Will et al., 2008). The copper substrate electrode was coated with silver-conductive paint to avoid chemical reactions with the diets and was fixed with superglue (UHU Sekunden Alleskleber; UHU GmbH and Co. KG, Bühl, Germany) into the Perspex® basin. The basin was filled to its maximum in order to keep the plastic film stretched, which was sealed with a ring of BlueTac® and fixed with a screwable plastic fixing device onto the basin. Adult and apterous individuals of *M. persicae* were prepared for EPG as described previously (Will et al., 2008; Will et al., 2009). EPGs lasted 8 h, starting directly after putting aphids onto the plastic film, and were repeated eight times each. Waveforms were recorded using a DC EPG Giga-4 amplifier and PROBE software (W. F. Tjallingii, Wageningen Agricultural University, Wageningen, The Netherlands). Statistical analysis was performed by analysis of variance (significance limit $P=0.05$) for each waveform.

Fig. 2. Perspex® basin set-up for EPG analysis. The basin was filled with a test solution until the fluid surface bulged so as to keep a transparent plastic film stretched taut. The plastic film was sealed with a ring of BlueTac® and fixed with a plastic fixing device that was screwed tightly onto the Perspex® basin. The substrate electrode was placed into the basin and was fixed with superglue.

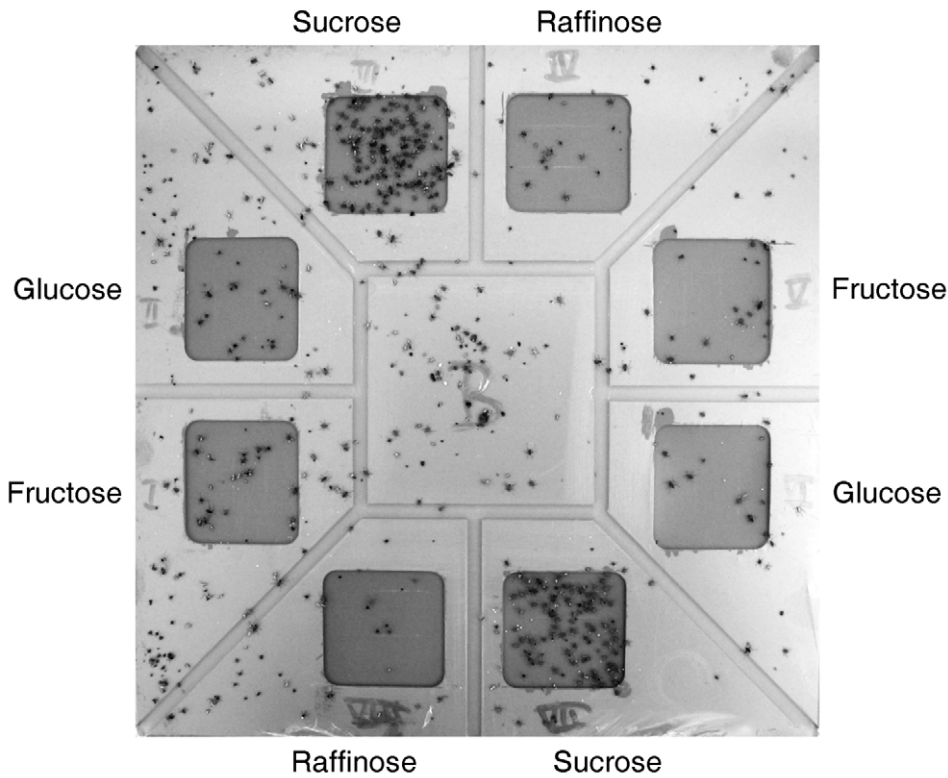


Fig. 3. Layout of the choice-chamber system and visualisation of *Megoura viciae* distribution after 24 h. This digital image shows the distribution of aphids having probed the diverse test solutions after puncturing a Parafilm® cover. The picture presents a typical distribution with an aggregation on the baths containing the favourite solution (here sucrose). The plastic cover frame (cf. Fig. 1B) that encaged the aphids was removed before taking the picture.

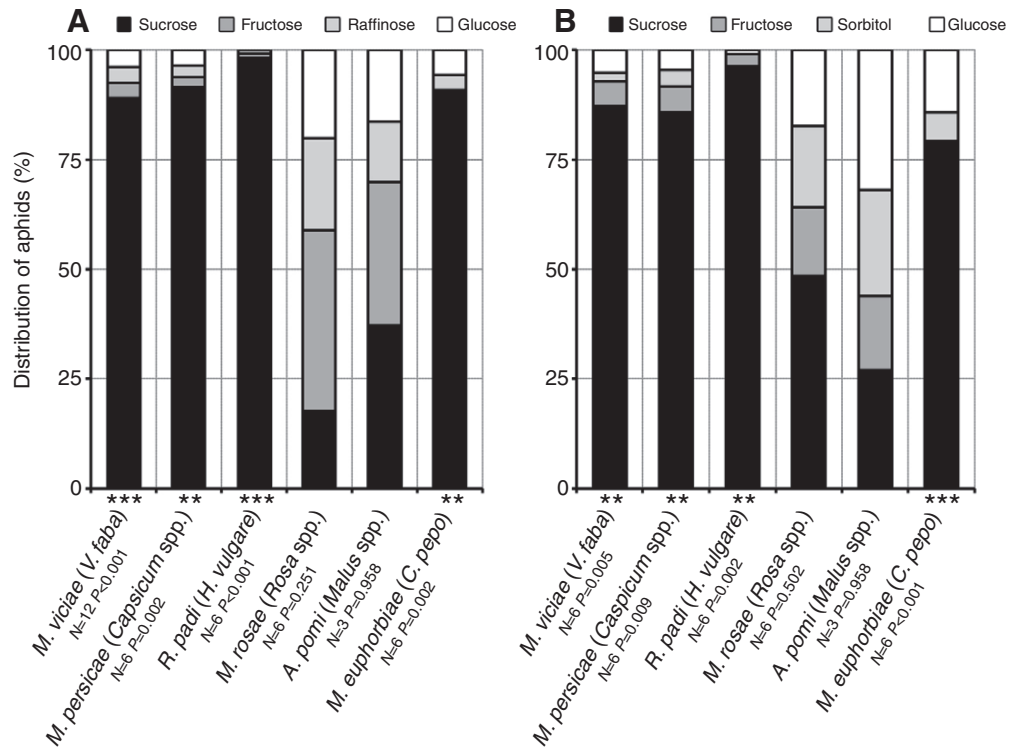


Fig. 4. Preference of six aphid species for various carbohydrates transported in sieve tubes (natural host plants of aphids given in parentheses). Aphid distribution between test baths filled with diverse carbohydrates present in the phloem sap: fructose, glucose, sucrose and raffinose (A) or fructose, glucose, sucrose and sorbitol (B). All test solutions were at a concentration of 219 mmol l⁻¹. Statistically significant differences for carbohydrate preference of an aphid species are marked with asterisks: ****P*<0.001, ***P*<0.01 (Stahel, 2008; Polasek, 1997). Experiments with *M. viciae* used large (A) and medium (B) choice-chamber systems (Table 2). Experiments with *M. rosae* used small (A) and medium (B) choice-chamber systems (Table 2). For detailed statistical results, see supplementary material Figs S5, S6 and Tables S3–S12.

RESULTS

General observations

Aphid preferences for various orientation parameters were assessed using a modification of classic choice-chamber systems (Fig. 1). The majority of aphids remained motionless in the centre of the system for the first 12 h of the experimental period (see Movies 1 and 2 in supplementary material). A few individuals acted as ‘pioneer colonizers’ (Pettersson, 1994) and explored different test solutions, and during the last 12 h the remaining majority of aphids settled and began feeding. The aphids often aggregated on one of the two preferred test baths, which become almost overcrowded, whereas the other solutions remained almost deserted. Aphid densities on the non-preferred solutions were strikingly even (Fig. 3).

Aphid preference for several orientation parameters was investigated using the species *M. viciae* and *M. persicae* (Fig. 8; supplementary material Figs S3, S4, S12–S23) whereas preferences for carbohydrates (Figs 4–7 and supplementary material Figs S5–S11) were tested with four other aphid species (*R. padi*, *M. rosae*, *A. pomi* and *M. euphorbiae*). Host plants, aphid species and predominant carbohydrates in the sieve tubes of the respective plant species are listed in Table 1.

Preference for the basic diet

In preliminary experiments, the suitability of the diet was tested by offering solutions with or without sucrose and different concentrations of amino acids to *M. viciae* (see supplementary material Figs S3, S4 and Tables S1, S2). In agreement with earlier reports (Mittler and Dadd, 1964), the aphids show a statistically significant preference ($P < 0.001$, $N = 12$) for diets containing a combination of sucrose and amino acids (supplementary material Fig. S3). The viscosity of the solution was of minor importance (supplementary material Fig. S4), as shown by the low preference for an equally viscous solution of mannitol as compared with the sucrose solution (supplementary material Fig. S4). Therefore, the slightly modified diet of Cherqui and Tjallingii (Cherqui and Tjallingii, 2000) was adopted as the basic medium in further experiments.

Preference for carbohydrate species found in host phloem translocation stream

In most plants, sieve-tube sap does not only contain sucrose but also other carbohydrates (e.g. Flora and Madore, 1996; van Bel and Hess, 2008). In *V. faba*, phloem sap contains sucrose as the predominant sugar (87% of the total sugars), but minor amounts of glucose and fructose also occur (McDonald et al., 1995). In pepper (Turner and Wien, 1994; Demir et al., 2008) and barley plants (Riems et al., 1994), sucrose is also the dominant carbohydrate. Approximately equimolar amounts of sucrose and carbohydrates of the raffinose-family oligosaccharides are translocated in sieve tubes of cucurbits (Turgeon and Webb, 1975; Haritatos et al., 1996). Furthermore, Rosoideae (Zimmermann and Ziegler, 1975) translocate high amounts of sucrose, but low amounts of sorbitol, in sieve tubes (e.g. *Rosa* spp.) whereas Pomoideae translocate high amounts of sorbitol (e.g. *Malus* spp.). Determination of the sieve-tube carbohydrate composition of the host plants used in this study by HPLC (see supplementary material Figs S1, S2) largely confirmed the data reported in literature. The presence of specific carbohydrates may provide aphids with a cue for detecting sieve tubes that may also correlate with host specificity. Therefore, the preference of aphids for carbohydrates known to be translocated in the sieve tubes of their host plants was tested.

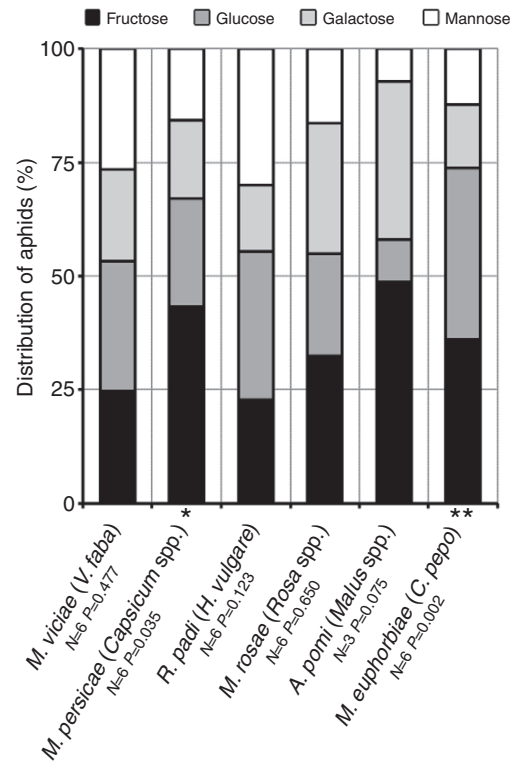


Fig. 5. Preference of six aphid species for various hexoses (natural host plants of aphids given in parentheses). Aphid distribution between test baths filled with diverse hexoses: fructose, glucose, galactose and mannose. All test solutions were at a concentration of 219 mmol l⁻¹. Statistically significant differences for carbohydrate preference of an aphid species are marked with asterisks: ** $P < 0.01$, and * $P < 0.05$ (Stahel, 2008; Polasek, 1997). Experiments with *M. viciae* used medium choice-chamber systems (Table 2). Experiments with *M. rosae* used small choice-chamber systems (Table 2). For detailed statistical results, see supplementary material Fig. S7 and Tables S13–S16.

All aphid species tested here, with the exception of *M. rosae* and *A. pomi*, showed a significant preference for sucrose (in comparison with glucose, fructose and raffinose) (Fig. 4 and supplementary material Movie 1). It thus appears that aphid species living on Rosaceae lack the capacity to distinguish between monosaccharides, disaccharides and sugar alcohols.

Preference for hexoses

Most aphids do not appear to discriminate between monosaccharides (Fig. 5), as individuals were mainly distributed at random over the arena when monosaccharides alone were offered. However, *M. persicae* and *M. euphorbiae* deviate from this random distribution (Fig. 5) without a clear preference for a particular hexose (see supplementary material Fig. S7). Most aphid species tended to prefer raffinose over monosaccharides (Fig. 6A), and this is particularly prevalent in *M. euphorbiae*, which naturally occurs on cucurbits that translocate oligosaccharides of the raffinose family. *Aphis pomi*, however, showed a significant preference for monosaccharides over raffinose (Fig. 6A; see supplementary material Fig. S8). With the exception of *R. padi*, aphids were not repelled significantly by sorbitol (Fig. 6B), although a preference for sorbitol in aphid species that naturally feed on plants from the Rosaceae was not evident (Fig. 6B).

Taken together, the data show that aphids that naturally occur on plants in which sucrose is a major translocate seem to prefer

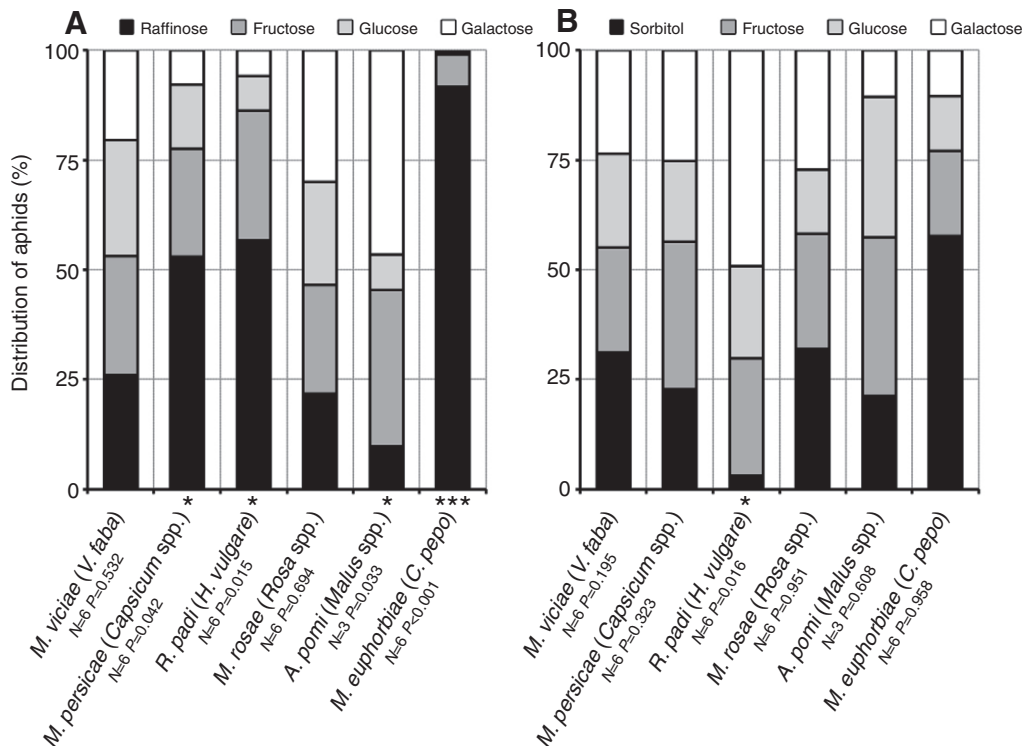


Fig. 6. Preference of six aphid species for raffinose (A) or sorbitol (B) vs hexoses (natural host plants of aphids given in parentheses). Aphid distribution between test baths filled with diverse carbohydrates: raffinose vs fructose, glucose, galactose (A) or sorbitol vs fructose, glucose, galactose (B). All test solutions were at a concentration of 219 mmol l⁻¹. Statistically significant differences for carbohydrate preference of an aphid species are marked with asterisks: ****P*<0.001, **P*<0.05 (Stahel, 2008; Polasek, 1997). Experiments with *M. viciae* used medium choice-chamber systems in both trials (Table 2). Experiments with *M. rosae* used small (A) and medium (B) choice-chamber systems (Table 2). For detailed statistical results, see supplementary material Figs S8, S9 and Tables S17–S23.

carbohydrates in the order of sucrose, raffinose, monosaccharides, sorbitol (Figs 4–6), while aphids that naturally occur on plants translocating larger amounts of sorbitol do not seem to be capable of recognizing a specific carbohydrate (Figs 4–6).

Preference for sucrose mixtures

In intact plants, aphids experience the presence of carbohydrate mixtures rather than single carbohydrates. Therefore, preference was compared between sucrose and a number of sucrose/carbohydrate combinations in choice tests (Fig. 7). Apart from *M. persicae*, which showed a preference for sucrose alone, aphid species did not discriminate between sucrose and the sucrose mixtures. Thus, the presence of sucrose seems to be of overriding importance and a decisive cue for all aphids except those that naturally occur on Rosaceae, which do not seem to be able to discriminate between carbohydrates (Fig. 4).

Preference for the sucrose concentration

Having demonstrated that sucrose is the preferred sugar for most aphid species, the question arises as to which concentration is optimal (Fig. 8A,B; supplementary material Figs S13, S15, Table S30). When sucrose was offered in concentration steps of 7.5%, aphids showed a statistically significant unequal distribution over the arena [*M. viciae*, *P*<0.001 (Fig. 8A); *M. persicae*, *P*=0.019 (supplementary material Fig. S13)]. After pairwise comparisons, a preference of 15% sucrose for *M. viciae* (Fig. 8A) and 22.5% sucrose for *M. persicae* (supplementary material Fig. S13) was detected. To increase the resolution, preference was tested using smaller increments in

concentration. The preference for a single concentration of sucrose was less evident when the steps offered were smaller [*M. viciae*, *P*=0.021 (Fig. 8B); *M. persicae*, *P*=0.457 (supplementary material Fig. S15)]. A significant difference between 10% and 25% sucrose was only evident after pairwise comparison for *M. viciae* (Fig. 8B).

Preference for viscosity

The optimum sucrose concentration of approximately 15% (see Fig. 8A,B) suggests that the gustatory attractiveness of increasing sucrose concentrations is counteracted by a negative sensory experience. It is suspected that the viscosity of the sucrose solution plays an important role when choosing a diet concentration. We offered test solutions with different mannitol concentrations of varying viscosity (Fig. 8D; mannitol is a sugar alcohol translocated in some plant families). Prior to the viscosity experiments, the absence of any preference for mannitol was demonstrated (Fig. 8C). The viscosity of sugars and sugar alcohols differs strongly (Lang, 1978; Turgeon, 1995); e.g. mannitol solutions are nearly half as viscous as sucrose solutions at the same concentration. When sucrose and mannitol solutions with an equal viscosity were offered (Fig. 8C), aphids significantly preferred sucrose (*P*<0.001) (supplementary material Movie 2). When comparing mannitol solutions in concentrations of up to 657 mmol l⁻¹, aphids showed a tendency to prefer lower viscosities (Fig. 8D), but only the preference for the lowest and highest mannitol concentration was significantly different (*P*=0.043) (Fig. 8D). The observed preference for 15% sucrose (Fig. 8A) thus seems to result from a trade-off between the positive effect of increasing sucrose concentrations (Fig. 8A) and

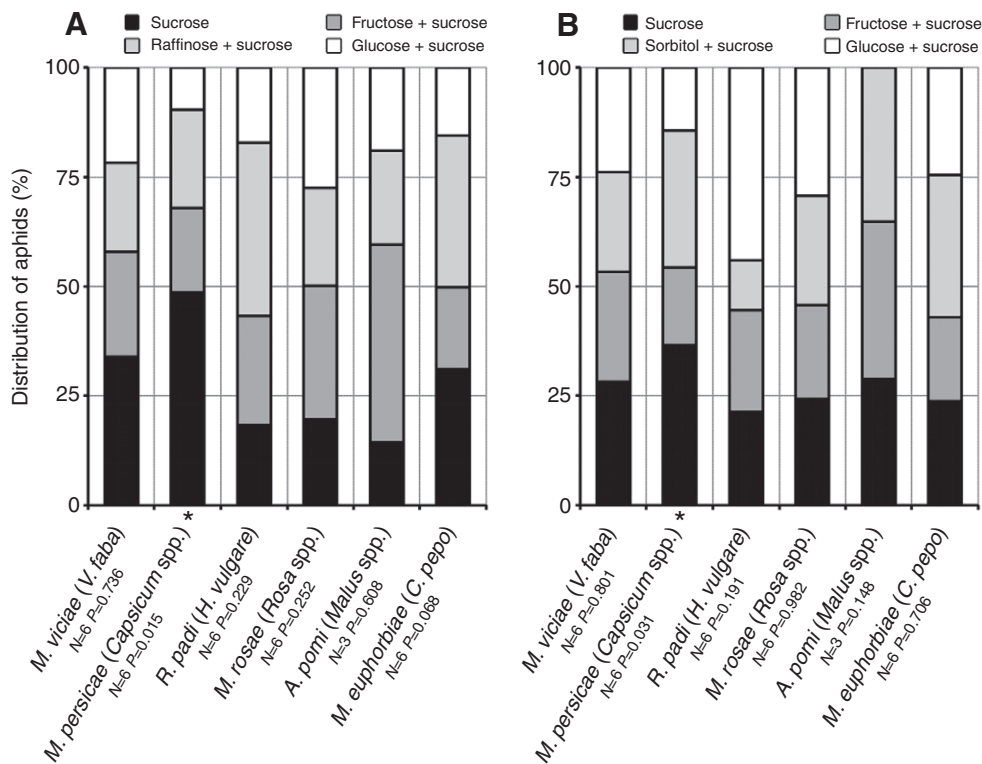


Fig. 7. Preference of six aphid species for various sucrose mixtures (natural host plants of aphids given in parentheses). Aphid distribution between test baths filled with diverse sucrose mixtures present in the phloem sap: sucrose ($109.5 \text{ mmol l}^{-1}$) vs sucrose with fructose, glucose or raffinose (A) or sucrose ($109.5 \text{ mmol l}^{-1}$) vs sucrose with fructose, glucose or sorbitol (B). All sucrose mixtures were at a total concentration of 219 mmol l^{-1} . Statistically significant differences for carbohydrate preference of an aphid species were marked with asterisks: $*P < 0.05$ (Stahel, 2008; Polasek, 1997). Experiments with *M. viciae* used medium choice-chamber systems in both trials (Table 2). Experiments with *M. rosae* used small choice-chamber systems in both trials (Table 2). For detailed statistical results, see supplementary material Figs S10, S11 and Tables S24–S28.

the negative impact of the correspondingly increasing viscosities (Fig. 8D).

Preference for pH

In choice chambers, pH values were offered in steps of 1.0 pH units (Fig. 8E; supplementary material Fig. S19) in the range between pH 5 and pH 8, but including pH 7.2, which approximates the pH of the phloem sap. Differences between diet pH at the start and the end of the experiment were negligible (~ 0.05 pH units). Individuals of *M. viciae* showed a statistically significant unequal distribution between the test solutions ($P < 0.001$) with a preference for pH 7.2 over pH 5 and pH 8, but not when compared with pH 6 (Fig. 8E). By contrast, *M. persicae* showed no significant difference in its distribution patterns but a trend to prefer pH 6 ($P = 0.572$) (supplementary material Fig. S19).

To improve the resolution of pH discrimination, pH preference was tested using smaller (pH 0.5) increments in the concentration range (pH 6.0 to pH 7.5 in Fig. 8F). For *M. viciae*, a non-significant trend ($P = 0.662$) in preference for pH 7.5 was observed (Fig. 8F) whereas *M. persicae* showed a significant preference for pH 6 ($P = 0.020$) (supplementary material Fig. S2, Table S34). In a second series of linear pH experiments (pH 5 to pH 8), *M. viciae* showed a statistically significant uneven distribution ($P = 0.035$) with a preference for pH 7 and pH 6 over pH 5 after pairwise comparison (Fig. 8G). No significant difference was found between pH 7.0 and pH 8.0 (Fig. 8G). Again, for *M. persicae*, a non-significant trend ($P = 0.284$) in preference for pH 6 was observed (Fig. S23 in

supplementary material). Thus, aphids seem to favour pH values that are similar to the pH of sieve-tube sap.

Diet-EPGs with *M. persicae*

The preference of aphids for neutral pHs and sucrose was further tested by EPG recordings of *M. persicae* feeding on solutions of hexoses (a mixture of fructose and glucose in equal portions) or sucrose alone at a pH of 5.0 or 7.2 (Fig. 9). After statistical analysis of the respective EPG waveforms, the aggregate 'ingestion' phases [according to Prado and Tjallingii (Prado and Tjallingii, 1994), waveform E2], which reflect sieve-tube feeding, were significantly longer for the sucrose-containing diet of pH 7.2 than for all other diets offered (Fig. 9; degree of significance for 'non-probing', $P = 0.039$; degree of significance for 'ingestion', $P = 0.008$). Moreover, the number of sustained ingestion phases was highest on diets containing sucrose with a pH of 7.2 (Table 3). These results confirm the preference of *M. persicae* for diets that are similar to the composition of sieve-tube sap and suggest that it is highly probable that aphids recognize sieve elements using a combination of carbohydrate and pH information. Defining E2 waveforms longer than 15 min as 'sustained ingestion', 15 events of sustained E2 were observed with 'sucrose diet pH 7.2' whereas a maximum of three sustained E2 events occurred with all other diets (Table 3). The first E2- and the first sustained E2 event occurred earlier in both sucrose diets and at higher rates than in the hexose or sugar-free diets (Table 3). In addition, the first sustained E2 events occurred after approximately 100 min in seven of the eight EPG

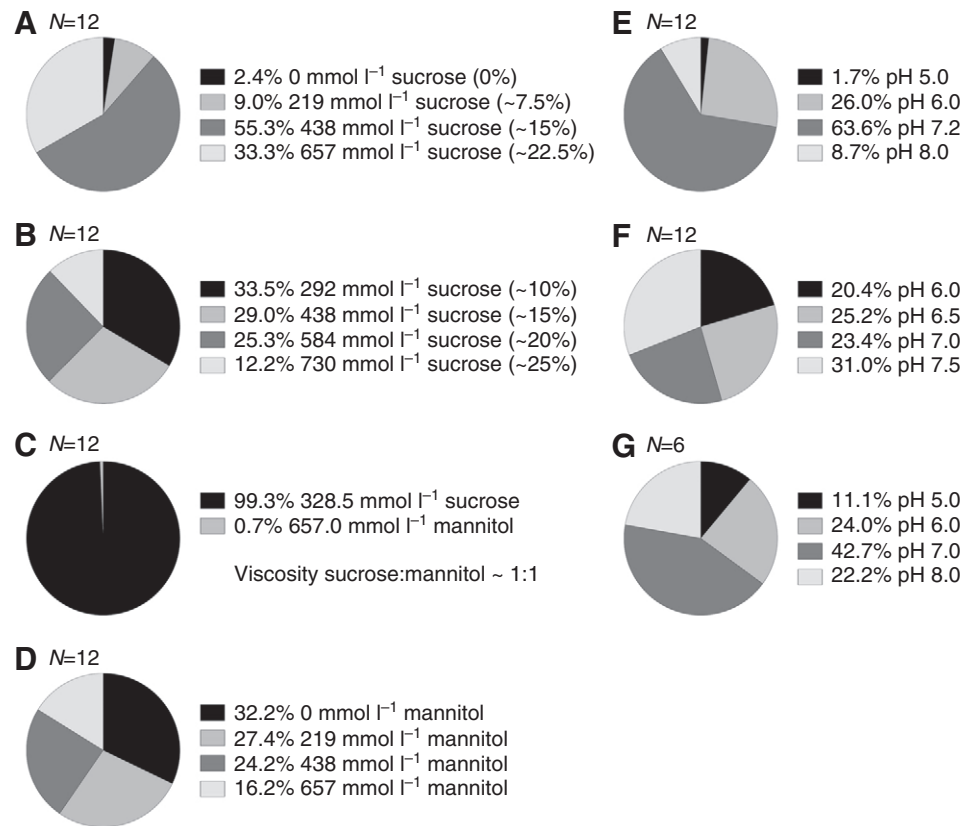


Fig. 8. Preference of *M. viciae* for various plant parameters. (A) Percentage of aphid distribution between test baths filled with solutions of different sucrose concentration (7.5% steps). Significance of the data, corrected for background distribution, $P < 0.001$. For detailed statistical results, see supplementary material Fig. S12 and Table S29. (B) Percentage of aphid distribution between test baths filled with solutions of different sucrose concentration (5% steps). Significance of the data, corrected for background distribution, $P = 0.021$. For detailed statistical results, see supplementary material Fig. S14 and Table S31. (C) Percentage of aphid distribution between test baths filled with sucrose and mannitol solutions at equal viscosity. Significance of the data, corrected for background distribution, $P < 0.001$. For detailed statistical results, see supplementary material Fig. S16. (D) Percentage of aphid distribution between test baths filled with solutions of different mannitol osmolarities. Significance of the data, corrected for background distribution, $P = 0.043$. For detailed statistical results, see supplementary material Fig. S17 and Table S32. (E) Percentage of aphid distribution between test baths filled with solutions of different pH values (0.8–1.2 pH unit steps). Significance of the data, corrected for background distribution, $P < 0.001$. For detailed statistical results, see supplementary material Fig. S18 and Table S33. (F) Percentage of aphid distribution between test baths filled with solutions of different pH values (0.5 pH unit steps). Significance of the data, corrected for background distribution, $P = 0.662$. For detailed statistical results, see supplementary material Fig. S20. (G) Percentage of aphid distribution between test baths filled with solutions of different pH values (1.0 pH unit steps). Significance of the data, corrected for background distribution, $P = 0.035$. For detailed statistical results, see supplementary material Fig. S22 and Table S35. Experiments in A–F used large choice-chamber systems (Table 2). Experiments in G used medium choice-chamber systems (Table 2).

experiments (sustained E2 did not occur in one EPG recording) using ‘sucrose diet pH 7.2’ whereas lower numbers of sustained E2 events occurred later in the EPG period for all other diets (Table 3).

DISCUSSION

The quest for orientation cues by use of aphid-adapted choice chambers

The fact that, in previous experiments, sucrose was the most suitable carbohydrate substrate for aphid growth and propagation suggested that sucrose is an important cue for aphid orientation (Auclair, 1963; Auclair, 1969; Mittler and Dadd, 1964; Kimmins, 1982). Previous choice-chamber experiments (Mittler and Dadd, 1964) using *M. persicae* showed a distinct preference for sucrose solutions in comparison with amino acid or control solutions. Furthermore, adaxial application of sucrose to *Sorghum bicolor* leaves resulted in a rapid search for the sucrose-containing drop by abaxially feeding aphids of the species *Rhopalosiphum maidis* (Kimmins, 1982).

These studies identified sucrose as the optimal carbohydrate substrate for aphid growth, but the role of sucrose as the sole cue for sieve-tube recognition remained inconclusive. The optimal carbohydrate diet for growth and propagation does not necessarily indicate cues for sieve-tube recognition, particularly in species where sucrose is not the predominant C-translocate. Hence, we have systematically and unequivocally determined cues for sieve-tube recognition using choice-chamber systems and EPG recording.

The choice-chamber systems are technically simple but effective experimental tools. Parafilm® covers (Fig. 2) have been employed in a variety of experiments (e.g. Tjallingii, 1978; Tjallingii, 1985; Tjallingii, 1995; Cherqui and Tjallingii, 2000) and are apparently interpreted by aphids as a plant cuticle. After stylet penetration through the Parafilm®, aphids sample and probe test solutions, as evidenced by EPG (Will et al., 2007), and either decide to leave the test solution or stay on it and ingest for longer periods (Fig. 3). This behaviour resembles the foraging behaviour of aphids on intact plants and is therefore taken as recognition of preferential nutritional conditions.

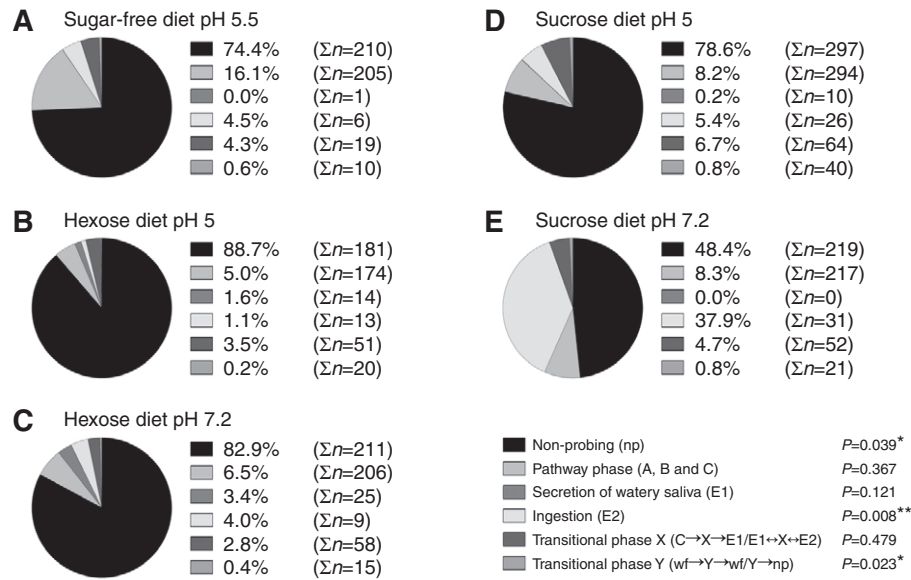


Fig. 9. Percentage duration of waveforms in electrical penetration graph (EPG) runs of *M. persicae* on different diets. EPGs lasted 8 h and were repeated eight times each with adult, apterous individuals of *M. persicae*. The waveforms (wf) were defined according to Prado and Tjallingii (Prado and Tjallingii, 1994): A=cuticle penetration, B=sheath salivation, C=pathway activities, E1=secretion of watery saliva, E2=ingestion and salivation of watery saliva. The transitional phases X and Y describe the short phases between two clear waveforms. Σn expresses the total number of events of the respective waveform in eight EPG experiments. Only the durations of the phases ‘non-probing’ (np) and ‘ingestion’ (E2) show visual and statistical significant differences between sucrose diet pH 7.2 and all other offered diets. The statistical test was performed with the duration results of each waveform (in eight experiments each) acquired with the respective diets. Significance of the differences between ‘sucrose diet pH 7.2’ and other diets is marked with asterisks: ** $P < 0.01$, and * $P < 0.05$ (Stahel, 2008; Polasek, 1997). For detailed statistical results, see supplementary material Tables S36–S39.

Fast-motion movies

Fast-motion movies (supplementary material Movies 1, 2) illustrate that it took more than 12 h before a distinct aphid distribution was established. Only a few aphids explored the immediate surroundings during the initial experimental period. The majority of the aphids

left the start area during the second half of the trial period and aggregated relatively rapidly on certain test solutions. Aphid aggregation has been described by several authors (Kennedy et al., 1967; Way and Cammel, 1970; Dixon and Logan, 1972; Way, 1973; Kay, 1976) and it has been speculated that this behaviour is due to the secretion of aggregation pheromones (Kay, 1976) by pioneer colonizers (Pettersson, 1994). Other investigators (reviewed by Prokopy and Roitberg, 2001) have claimed that a combination of tactile and visual cues is responsible for the aggregation. At first sight, the aggregation behaviour in our experiments seems to weaken the significance of the preference experiments, as most aphids seem to simply follow the pioneers, but it should be noted that every single aphid appears to make an individual choice. It punctures the Parafilm[®], feeds from the diet, as demonstrated by the production of honeydew (results not shown), and makes a decision to stay.

Preference for sucrose

In our choice-chamber tests, *M. viciae*, *M. persicae*, *R. padi* and *M. euphorbiae* exhibited a strong preference for sucrose (Fig. 4). The overriding preference for sucrose is confirmed by experiments in which sucrose is offered in combination with other carbohydrates (Fig. 7). In the absence of sucrose as a choice medium, these aphid species favour raffinose, in particular *M. euphorbiae*, which occurs naturally on *Cucurbita* (Fig. 6A). No preference was observed when only monosaccharides were available (Fig. 5). In conclusion, this group of aphids has the ability to discriminate between sucrose and other carbohydrates and a limited capacity to distinguish raffinose among other carbohydrates (Figs 4–6). *Macrosiphum rosae* and *A. pomi* appear to be unable to discriminate between carbohydrates including sucrose (Figs 4–6), so the carbohydrate cue for these aphids remains elusive.

Table 3. Occurrence of first E2-waveforms in EPG-runs of *M. persicae* on different diets

		Mean time of first occurrence (min)	Number of events
Sugar free diet pH 5.5	First E2	200	4
	First sustained E2	244	3
	Total sustained E2		3
Hexose diet pH 5	First E2	130	4
	First sustained E2	113	1
	Total sustained E2		1
Hexose diet pH 7.2	First E2	268	1
	First sustained E2	302	1
	Total sustained E2		3
Sucrose diet pH 5	First E2	44	6
	First sustained E2	33	1
	Total sustained E2		2
Sucrose diet pH 7.2	First E2	78	7
	First sustained E2	106	7
	Total sustained E2		15

The mean times of occurrence of the first E2, the first sustained E2 waveform per diet (eight experiments), as well as the number of events are shown. In some cases, the first sustained E2 waveform was followed by others, such that the total number of sustained events exceeds that of the first sustained E2 waveforms. For detailed results, see supplementary material Table S40.

Preference for sucrose concentration and viscosity

In choice-chamber experiments, a strong preference for sucrose concentrations ranging between 15% (Fig. 8A) and 20% (supplementary material Fig. S13) (cf. Mittler and Dadd, 1964) was observed. This optimum is remarkable because it is unlikely that aphids avoid sucrose concentrations higher than 15% due to gustatory preferences (Fig. 8A; supplementary material Fig. S13). More likely, aphids might not opt for sucrose concentrations higher than 15% under choice-chamber conditions because the viscosity of >20% sucrose hinders ingestion, as shown for other viscous fluids (Fig. 8D) (cf. Mittler, 1967). The optimum may reflect the natural ability of aphids to counteract osmotic stress caused by high sucrose concentrations. Perception of osmotic stress may stimulate xylem-sap drinking by aphids (Prado and Tjallingii, 1994). When the experimental conditions prevent aphids from drinking, further nutrient uptake may be restricted, which explains the reduced preference for high sucrose concentrations in choice chambers (Fig. 8A). Alternatively, a reduced preference for high sucrose concentrations may be explained by a limited capacity to enzymatically transform monosaccharides to oligosaccharides (Wilkinson et al., 1997).

Aphids settling on intact plants experience very different pressure conditions, which may provide an alternative explanation for the preference optimum in choice chambers. The high turgour pressure in sieve tubes [$\sim 1\text{--}2$ MPa; $\sim 0.7\text{--}2$ MPa in *Quercus rubrum* (Hammel, 1968); $\sim 1.3\text{--}1.5$ MPa in *O. sativa* (Fukumorita and Chino, 1982)] presumably drives the viscous transport sap through the narrow stylet channels into the aphid body. For instance, ingestion of sucrose concentrations up to 38% has been reported for phloem sap of *Z. mays* collected *via* aphid stylets (Ohshima et al., 1990). In the absence of turgour pressure – as in choice-chamber experiments – aphids must actively ingest their nutrients. The sucking capacities of the cibarial pump (Dixon, 1998) may not be sufficient to ingest syrup-like substances through stylet tips with diameters of between $0.7\ \mu\text{m}$ (Tjallingii and Hogen Esch, 1993) and $2\ \mu\text{m}$ (Klingauf, 1987) and through the food channel, which has a mean diameter of $0.5\ \mu\text{m}$ in *M. persicae* (Auclair, 1963). In this context, the preference for low concentrations of mannitol (Fig. 8D) might be interpreted as a mechanism to acquire nutrients with the lowest muscular effort.

Preference for pH

Our study indicates a high preference of *M. viciae* for pH 7.0 to 7.2 (Fig. 8E,G), which is close to the pH values (~ 7.5) in the vacuole-less sieve tubes (Fukumorita and Chino, 1982; Vreugdenhil and Koot-Gronsveld, 1989; Hafke et al., 2005). Similar results were obtained for *M. euphorbiae* (Cartier, 1968). For orientation inside vascular bundles, pH sensing may assist aphids to discriminate between xylem vessels (pH 4.5–6.5) (Richardson et al., 1982; Kosegarten et al., 1999; Buchanan et al., 2000; Lopez-Millan et al., 2000) and sieve tubes (pH 7.0–7.5). The pH trials (Fig. 8E–G) indicate that aphids can distinguish between pH values provided that the steps are large enough. This suggests that aphids only react to major pH differences such as those between the translocation conduits in vascular bundles.

According to our experiments, aphids have difficulty discriminating between pH values when the increments are small (Fig. 8F). The same pattern is evident for sucrose concentrations in the middle of the tested range (Fig. 8B). The importance of the size of the increment is puzzling at first sight but it may indicate a good strategy for detecting nutritional sources. Small variations between the contents of successive cells may not influence the aphids whereas

abrupt changes in optimal sucrose and pH conditions (as provided by sieve tubes) could inform aphids about a successful penetration.

Diet-EPGs with *M. persicae*

The diets with either sucrose at pH 5 (Fig. 9D) or hexose at pH 7.2 (Fig. 9C) provoke short ingestion phases equal to those of control diets without the favourable cues (Fig. 9A,B). Apparently, neither pH value nor the presence of concentrated sucrose alone is sufficient for sieve-tube recognition. It thus appears obligatory for sieve-tube recognition and corresponding secretion of aqueous saliva that both pH and sucrose concentration meet the required sensory threshold. Hence, preference experiments using choice chambers were feasible, because varying amounts of sucrose were offered at pH 7.2 or varying pHs in solutes containing 15% sucrose. On the sugar-free diet with pH 5.5 (Fig. 9A), EPG waveforms that reflect secretion of gel saliva occur more frequently than in other diets. As this diet closely resembles the apoplast fluid, aphids may sense the pH and low viscosity as a stimulus to produce gel saliva, which facilitates stylet progression between cell walls.

Concluding remarks

In summary, aphids settling on plants in which sucrose is a major translocate seem to prefer carbohydrates in the order: sucrose \geq raffinose \geq monosaccharides \geq sorbitol (Figs 4–6). This order of preference suggests that the sensory perception of sucrose has an important role in the detection of sieve elements. Both sucrose and RFO-carbohydrates contain a glucose/fructose configuration that may be perceived by the gustatory organs of aphids. Gene families for receptors with presumptive perception abilities for sugars have recently been annotated (Smadja et al., 2009). *Macrosiphum rosae* and *A. pomi*, both occurring naturally on sorbitol-translocating plant species, seem to lack the ability to discriminate sucrose from other carbohydrates (Fig. 4). How such aphids identify the sieve tubes remains a mystery.

Megoura viciae has a preference for 15% sucrose (Fig. 8A) and pH 7.2 (Fig. 8E). The sieve-tube content of *V. faba* has a pH of 7.5 and contains sucrose as the principal transport sugar (McDonald et al., 1995; Hafke et al., 2005). EPG recordings (Fig. 9) convincingly demonstrate that both conditions are required for sieve-tube recognition. The preference matches with the conditions in sieve tubes and seems to explain why this aphid can recognize the sieve tubes as the source of nutrition.

It is questionable whether sucrose is the principal factor for aphid orientation. As discussed before, aphids seem to prefer the highest sucrose concentrations offered, until feedback control on osmotically damaging amounts comes into play (Fig. 8A,D). The search for optimal conditions may explain the ‘wavy behaviour’ (i.e. frequent rejection of sieve tubes by the stylet tips) of the stylet in the vascular region (Tjallingii and Hogen Esch, 1993). Aphids appear to search for the sieve tube containing the most attractive sucrose concentration, since an absolute set-point for sucrose acceptability is lacking. As the pH value is more reliable as a fixed set-point for orientation, we believe that pH is the prime orientation cue for aphids despite the fact that EPGs indicate sucrose being the more effective cue (Fig. 9).

This concept assumes that aphids can discriminate between parenchyma and sieve tubes, although cytosolic compartments of the most diverse cells have a pH similar to that of sieve-tube sap (Felle, 1988) (cytosolic pH ranges between 7.0 and 7.5). However, when the stylet tip enters the vacuole after vigorous piercing of the cell wall, the final probe would be of an acidic nature (pH 5.0). Such mechanical stylet behaviour is inferred from pictures showing

maxillary tips residing against the wall of the sieve element opposite to the site of entry in *Lactuca sativa* phloem (W. F. Tjallingii, unpublished results) or halfway across sieve elements in *V. faba* (Tjallingii and Hogen Esch, 1993). Despite the apparent mechanical logic, the penetration of stylets into vacuoles is far from certain. The absence of gel-saliva plugs on the tonoplast (Tjallingii and Hogen Esch, 1993) is taken as circumstantial evidence against tonoplast penetration. Moreover, events associated with virus dissemination by aphids most likely occur in the cytoplasm (Powell et al., 2006). In conclusion, vacuolar insertion of stylets may explain the rejection of parenchyma cells, but it remains to be proven by microscopy of punctured parenchyma cells.

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HPLC-results after EDTA-exudation and Stylectomy

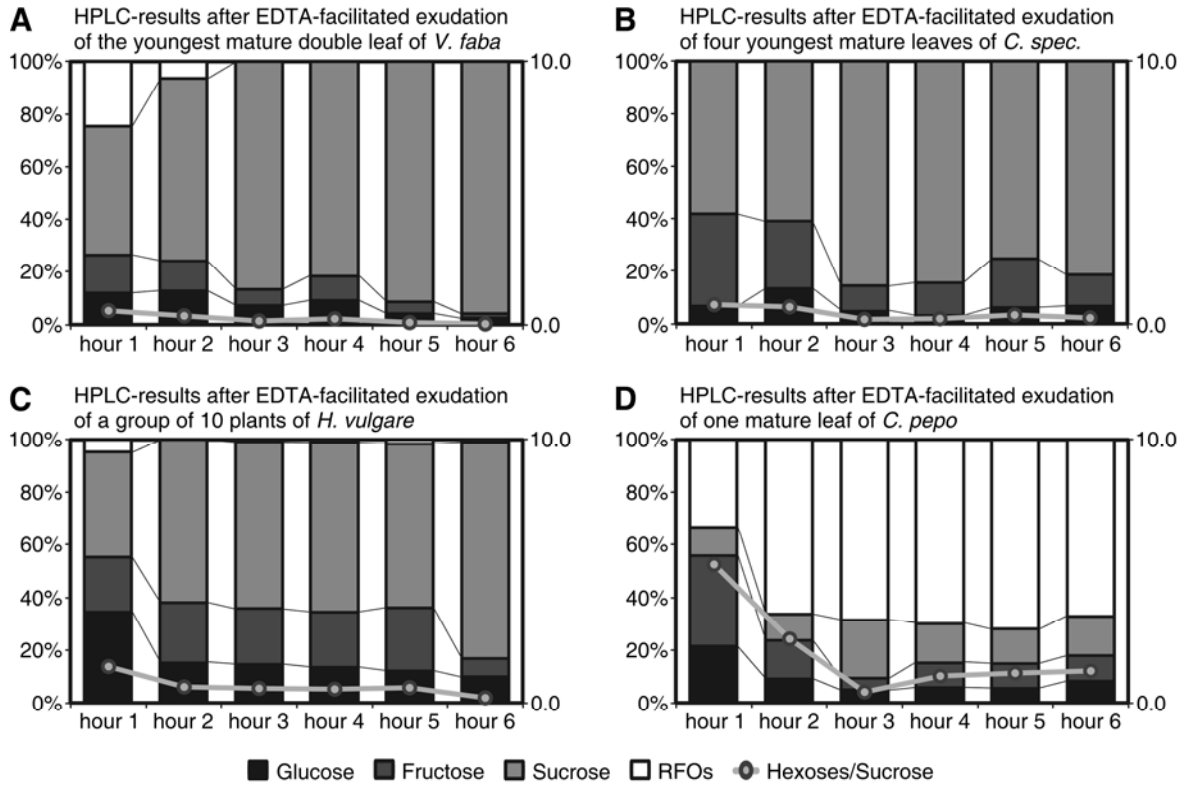


Fig. S1 HPLC-results after EDTA-facilitated exudation of the aphid host plants *V. faba* (A), *C. spec.* (B), *H. vulgare* (C) and *C. pepo* (D).

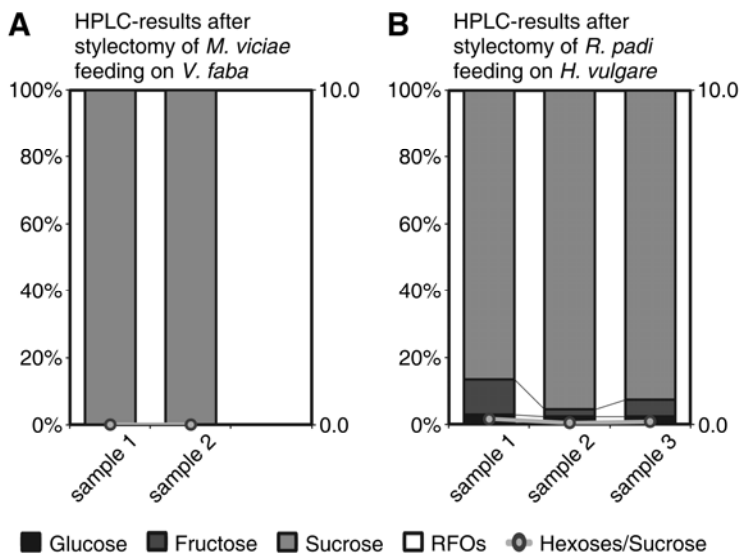


Fig. S2 HPLC-results after stylectomy of *M. viciae* on *V. faba* (A) and *R. padi* on *H. vulgare* (B).

Preference for basic diet

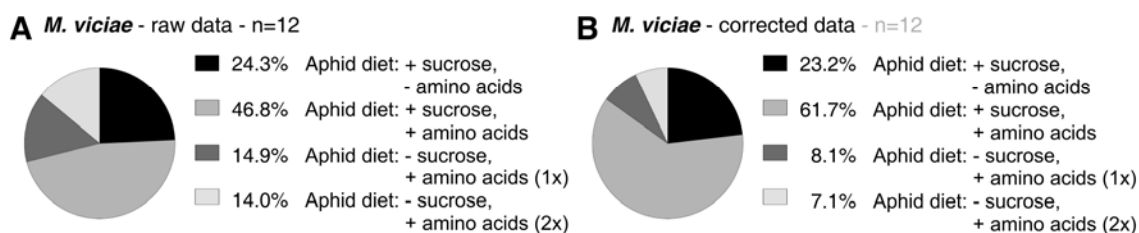


Fig. S3 Percentage of aphid distribution (*M. viciae*) between test baths filled with solutions of different sucrose (438 mM) and amino acid composition (100 mM or 200 mM, each) (amino acid series A). (A) Significance of the raw data: $p_{raw\ data} = < 0.001$; $X^2 = 22.000$ with 3 degrees of freedom. (B) Significance of the data, corrected for background distribution: $p_{corrected\ data} = < 0.001$; $X^2 = 22.000$ with 3 degrees of freedom. Experiments were done with medium choice chamber systems.

Tab. S1 Results of All-Pairwise-Multiple-Comparison-Procedures for amino acid series A of *M. viciae* (Fig. S3). If the Friedman-test resulted in a p -value of $p = < 0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = < 0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:

Dunn's Method / Tukey Test / Student-Newman-Keuls Method

$p = < 0.05$: yes (y), no (n), do not test (dnt, d)

$p = < 0.05?$	Diet +SUC -AS ¹		Diet +SUC +AS ²		Diet -SUC +AS 1x ³		Diet -SUC +AS 2x ⁴	
	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$
	Diet +SUC -AS ¹	---	---	---	---	---	---	---
Diet +SUC +AS ²	no (n/n/y)	no (n/n/y)	---	---	---	---	---	---
Diet -SUC +AS 1x ³	no (n/n/y)	no (n/n/y)	yes (y/y/y)	yes (y/y/y)	---	---	---	---
Diet -SUC +AS 2x ⁴	yes (y/y/y)	yes (y/y/y)	yes (y/y/y)	yes (y/y/y)	no (n/n/n)	no (n/n/n)	---	---

¹ Diet with sucrose but without amino acids; ²Diet with sucrose and amino acids; ³Diet without sucrose but with amino acids (single concentration); ⁴Diet without sucrose but with amino acids (double concentration)

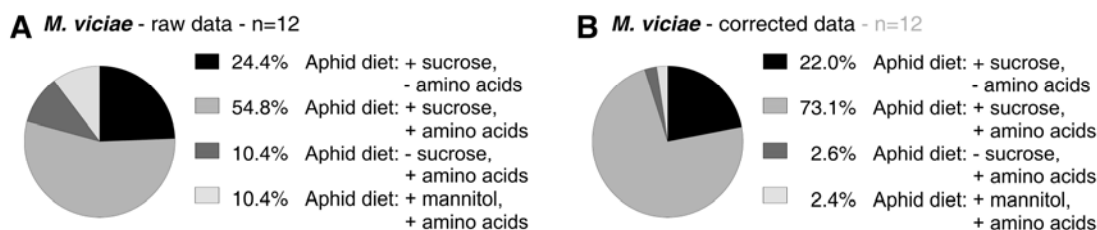


Fig. S4 Percentage of aphid distribution (*M. viciae*) between test baths filled with solutions of different sucrose (438 mM) and amino acid composition (100 mM each), and mannitol (823 mM) (amino acid series B). (A) Significance of the raw data: $p_{raw\ data} = < 0.001$; $X^2 = 31.400$ with 3 degrees of freedom. (B) Significance of the data, corrected for background distribution: $p_{corrected\ data} = < 0.001$; $X^2 = 30.000$ with 3 degrees of freedom. Experiments were done with medium choice chamber systems.

Tab. S2 Results of All-Pairwise-Multiple-Comparison-Procedures for amino acid series B of *M. viciae* (Fig. S4). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0,05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	Diet +SUC -AS ¹		Diet +SUC +AS ²		Diet -SUC +AS ³		Diet +MAN +AS ⁴	
	$P_{raw\ data}$	$P_{corrected\ data}$	$P_{raw\ data}$	$P_{corrected\ data}$	$P_{raw\ data}$	$P_{corrected\ data}$	$P_{raw\ data}$	$P_{corrected\ data}$
	Diet +SUC -AS ¹	---	---	---	---	---	---	---
Diet +SUC +AS ²	no (n/n/y)	no (n/n/y)	---	---	---	---	---	---
Diet -SUC +AS ³	yes (y/y/y)	yes (y/y/y)	yes (y/y/y)	yes (y/y/y)	---	---	---	---
Diet +MAN +AS ⁴	yes (y/y/y)	yes (y/y/y)	yes (y/y/y)	yes (y/y/y)	no (n/n/n)	no (n/n/n)	---	---

¹ Diet with sucrose but without amino acids; ²Diet with sucrose and amino acids; ³Diet without sucrose but with amino acids; ⁴Diet with mannitol and amino acids

Preference for carbohydrate species translocated in sieve tubes

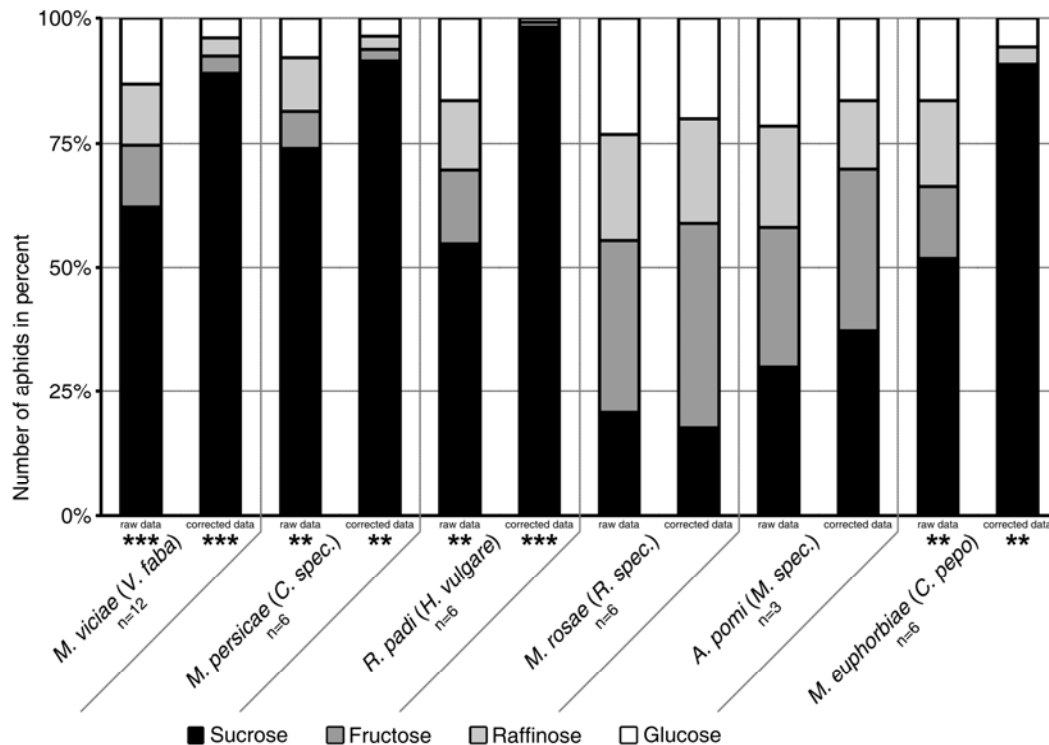


Fig. S5 Preference of six aphid species for various carbohydrates transported in sieve tubes (sugar species series A). Aphid distribution between test baths filled with diverse carbohydrates present in the phloem sap (fructose, glucose, sucrose and raffinose). All test solutions were at a concentration of 219 mM. For a better overview, the statistically significant differences for carbohydrate preference of an aphid species were marked with asterisks (*) indicating the following significance classes: *** $p \leq 0.001$, ** $p \leq 0.01$, and * $p \leq 0.05$

(Stahel, 2008; Polasek, 1997). Experiments using *M. viciae* were done with large choice chamber systems. Experiments using *M. rosae* were done with small choice chamber systems. For exact statistical results see Tab. S3.

Tab. S3 Statistical results of sugar species series A.

	statistical results of raw data	statistical results corrected data
<i>M. viciae</i>	$P_{raw\ data} = < 0.001$ $X^2 = 23.218$ with 3 degrees of freedom	$P_{corrected\ data} = < 0.001$ $X^2 = 25.618$ with 3 degrees of freedom
<i>M. persicae</i>	$P_{raw\ data} = 0.006$ $X^2 = 12.600$ with 3 degrees of freedom	$P_{corrected\ data} = 0.002$ $X^2 = 14.362$ with 3 degrees of freedom
<i>R. padi</i>	$P_{raw\ data} = 0.009$ $X^2 = 11.534$ with 3 degrees of freedom	$P_{corrected\ data} = < 0.001$ $X^2 = 16.846$ with 3 degrees of freedom
<i>M. rosae</i>	$P_{raw\ data} = 0.138$ $X^2 = 5.518$ with 3 degrees of freedom	$P_{corrected\ data} = 0.251$ $X^2 = 4.096$ with 3 degrees of freedom
<i>A. pomi</i>	$P_{raw\ data}(\text{estimated}) = 0.972$ $P_{raw\ data}(\text{exact}) = 1.000$ $X^2 = 0.231$ with 3 degrees of freedom	$P_{corrected\ data}(\text{estimated}) = 0.956$ $P_{corrected\ data}(\text{exact}) = 0.958$ $X^2 = 0.321$ with 3 degrees of freedom
<i>M. euphorbiae</i>	$P_{raw\ data} = 0.006$ $X^2 = 12.559$ with 3 degrees of freedom	$P_{corrected\ data} = 0.002$ $X^2 = 14.935$ with 3 degrees of freedom

Tab. S4 Results of All-P airwise-Multiple-Comparison-Procedures for sugar species series A of *M. viciae* (Fig. S5). If the Friedman-test resulted in a p -value of $p = < 0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = < 0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:

Dunn's Method / Tukey Test / Student-Newman-Keuls Method

$p = < 0,05$: yes (y), no (n), do not test (dnt, d)

$p = < 0.05?$	Sucrose ¹		Fructose ¹		Raffinose ¹		Glucose ¹	
	$P_{raw\ data}$	$P_{corrected\ data}$	$P_{raw\ data}$	$P_{corrected\ data}$	$P_{raw\ data}$	$P_{corrected\ data}$	$P_{raw\ data}$	$P_{corrected\ data}$
Sucrose ¹	---	---	---	---	---	---	---	---
Fructose ¹	yes (y/y/y)	yes (y/y/y)	---	---	---	---	---	---
Raffinose ¹	yes (y/y/y)	yes (y/y/y)	dnt (d/d/d)	dnt (d/d/d)	---	---	---	---
Glucose ¹	yes (y/y/y)	yes (y/y/y)	dnt (d/d/d)	no (n/n/n)	no (n/n/n)	dnt (d/d/d)	---	---

¹ 219 mM

Tab. S5 Results of All-Pairwise-Multiple-Comparison-Procedures for sugar species series A of *M. persicae* (Fig. S 5). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
 Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0,05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	Sucrose ¹		Fructose ¹		Raffinose ¹		Glucose ¹	
	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$
Sucrose ¹	---	---	---	---	---	---	---	---
Fructose ¹	yes (y/y/y)	yes (y/y/y)	---	---	---	---	---	---
Raffinose ¹	no (n/n/y)	yes (y/y/y)	no (n/n/n)	dnt (d/d/d)	---	---	---	---
Glucose ¹	yes (y/y/y)	no (n/n/y)	dnt (d/d/d)	no (n/n/n)	dnt (d/d/d)	dnt (d/d/d)	---	---

¹ 219 mM

Tab. S6 Results of All-Pairwise-Multiple-Comparison-Procedures for sugar species series A of *R. padi* (Fig. S 5). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
 Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0,05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	Sucrose ¹		Fructose ¹		Raffinose ¹		Glucose ¹	
	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$
Sucrose ¹	---	---	---	---	---	---	---	---
Fructose ¹	yes (y/y/y)	yes (d/y/y)	---	---	---	---	---	---
Raffinose ¹	yes (y/y/y)	yes (y/y/y)	dnt (d/d/d)	no (n/n/n)	---	---	---	---
Glucose ¹	no (n/n/y)	yes (n/y/y)	no (n/n/n)	dnt (d/d/d)	dnt (d/d/d)	dnt (d/d/d)	---	---

¹ 219 mM

Tab. S7 Results of All-Pairwise-Multiple-Comparison-Procedures for sugar species series A of *M. euphorbiae* (Fig. S5). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
 Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0,05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	Sucrose ¹		Fructose ¹		Raffinose ¹		Glucose ¹	
	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$
	Sucrose ¹	---	---	---	---	---	---	---
Fructose ¹	yes (y/y/y)	yes (y/y/y)	---	---	---	---	---	---
Raffinose ¹	no (n/n/y)	yes (n/y/y)	no (n/n/n)	dnt (d/d/d)	---	---	---	---
Glucose ¹	yes (y/y/y)	? (d/n/y)	dnt (d/d/d)	no (n/n/n)	dnt (d/d/d)	dnt (d/d/d)	---	---

¹ 219 mM

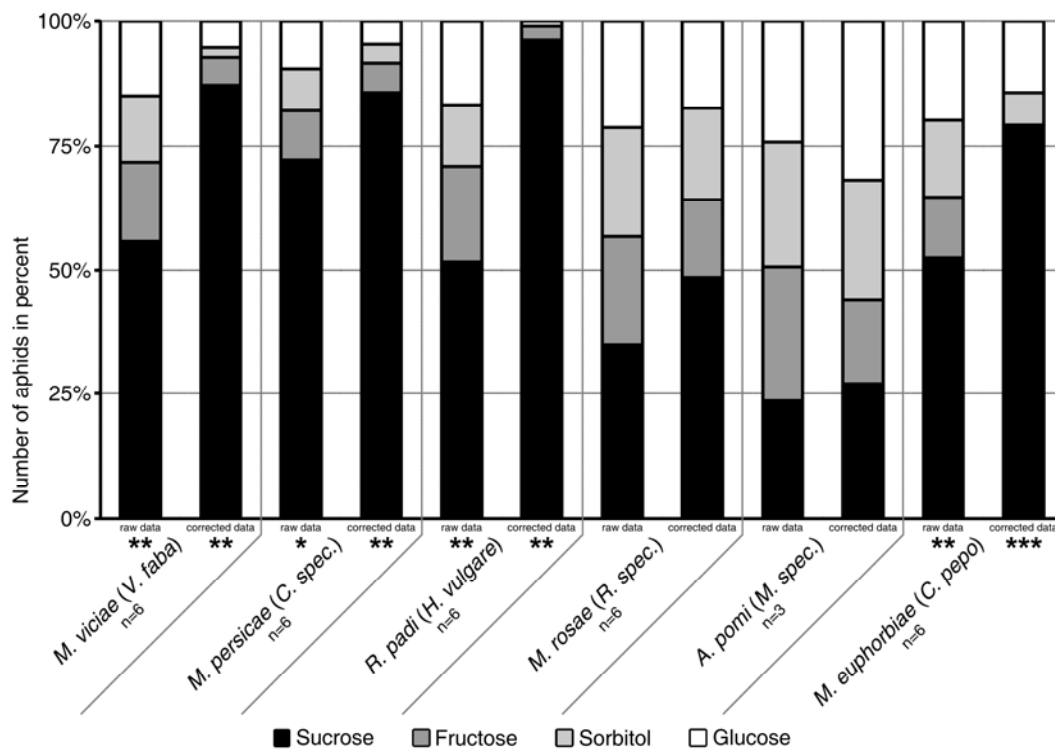


Fig. S6 Preference of six aphid species for various carbohydrates transported in sieve tubes (sugar species series B). Aphid distribution between test baths filled with diverse carbohydrates present in the phloem sap (fructose, glucose, sucrose and sorbitol). All test solutions were at a concentration of 219 mM. For a better overview, the statistically significant differences for carbohydrate preference of an aphid species were marked with asterisks (*) indicating the following significance classes: *** $p \leq 0.001$, ** $p \leq 0.01$, and * $p \leq 0.05$ (Stahel, 2008; Polasek, 1997). Experiments using *M. viciae* were done with medium choice chamber systems. Experiments using *M. rosae* were done with and medium choice chamber systems. For exact statistical results see Tab. S8.

Tab. S8 Statistical results of sugar species series B.

	statistical results of raw data	statistical results corrected data
<i>M. viciae</i>	$p_{raw\ data} = 0.009$ $X^2 = 11.600$ with 3 degrees of freedom	$p_{corrected\ data} = 0.005$ $X^2 = 12.776$ with 3 degrees of freedom
<i>M. persicae</i>	$p_{raw\ data} = 0.010$ $X^2 = 11.441$ with 3 degrees of freedom	$p_{corrected\ data} = 0.009$ $X^2 = 11.534$ with 3 degrees of freedom
<i>R. padi</i>	$p_{raw\ data} = 0.002$ $X^2 = 14.600$ with 3 degrees of freedom	$p_{corrected\ data} = 0.002$ $X^2 = 15.000$ with 3 degrees of freedom
<i>M. rosae</i>	$p_{raw\ data} = 0.515$ $X^2 = 2.288$ with 3 degrees of freedom	$p_{corrected\ data} = 0.502$ $X^2 = 2.357$ with 3 degrees of freedom
<i>A. pomi</i>	$p_{raw\ data}(\text{estimated}) = 0.855$ $p_{raw\ data}(\text{exact}) = 0.910$ $X^2 = 0.778$ with 3 degrees of freedom	$p_{corrected\ data}(\text{estimated}) = 0.934$ $p_{corrected\ data}(\text{exact}) = 0.958$ $X^2 = 0.429$ with 3 degrees of freedom
<i>M. euphorbiae</i>	$p_{raw\ data} = 0.002$ $X^2 = 15.000$ with 3 degrees of freedom	$p_{corrected\ data} = < 0.001$ $X^2 = 16.345$ with 3 degrees of freedom

Tab. S9 Results of All-Pairwise-Multiple-Comparison-Procedures for sugar species series B of *M. viciae* (Fig. S6). If the Friedman-test resulted in a p -value of $p = < 0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement, if there is a significance between the compared groups ($p = < 0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = < 0.05$: yes (y), no (n), do not test (dnt, d)

$p = < 0.05?$	Sucrose ¹		Fructose ¹		Sorbitol ¹		Glucose ¹	
	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$
Sucrose ¹	---	---	---	---	---	---	---	---
Fructose ¹	no (n/n/y)	? (d/n/y)	---	---	---	---	---	---
Sorbitol ¹	yes (y/y/y)	yes (y/y/y)	no (n/n/n)	no (n/n/n)	---	---	---	---
Glucose ¹	yes (y/y/y)	yes (n/y/y)	dnt (d/d/d)	dnt (d/d/d)	dnt (d/d/d)	dnt (d/d/d)	---	---

¹ 219 mM

Tab. S1 0 Results of All-Pairwise-Multiple-Comparison-Procedures for sugar species series B of *M. persicae* (Fig. S6). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement, if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0.05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	Sucrose ¹		Fructose ¹		Sorbitol ¹		Glucose ¹	
	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$
Sucrose ¹	---	---	---	---	---	---	---	---
Fructose ¹	no (n/n/y)	no (n/n/y)	---	---	---	---	---	---
Sorbitol ¹	yes (y/y/y)	yes (y/y/y)	no (n/n/n)	no (n/n/n)	---	---	---	---
Glucose ¹	yes (y/y/y)	yes (y/y/y)	dnt (d/d/d)	dnt (d/d/d)	dnt (d/d/d)	dnt (d/d/d)	---	---

¹ 219 mM

Tab. S1 1 Results of All-Pairwise-Multiple-Comparison-Procedures for sugar species series B of *R. padi* (Fig. S6). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement, if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0.05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	Sucrose ¹		Fructose ¹		Sorbitol ¹		Glucose ¹	
	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$
Sucrose ¹	---	---	---	---	---	---	---	---
Fructose ¹	dnt (d/d/y)	no (n/n/y)	---	---	---	---	---	---
Sorbitol ¹	yes (y/y/y)	yes (y/y/y)	no (n/n/n)	no (n/n/n)	---	---	---	---
Glucose ¹	no (n/n/y)	yes (y/y/y)	dnt (d/d/d)	dnt (d/d/d)	dnt (d/d/d)	dnt (d/d/d)	---	---

¹ 219 mM

Tab. S1 2 Results of All-Pairwise-Multiple-Comparison-Procedures for sugar species series B of *M. euphorbiae* (Fig. S 6). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement, if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
 Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0.05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	Sucrose ¹		Fructose ¹		Sorbitol ¹		Glucose ¹	
	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$
	Sucrose ¹	---	---	---	---	---	---	---
Fructose ¹	yes (y/y/y)	yes (y/y/y)	---	---	---	---	---	---
Sorbitol ¹	yes (y/y/y)	yes (y/y/y)	dnt	dnt	---	---	---	---
Glucose ¹	no (n/n/y)	no (n/n/y)	no (n/n/y)	no (n/n/y)	dnt	dnt	---	---

¹ 219 mM

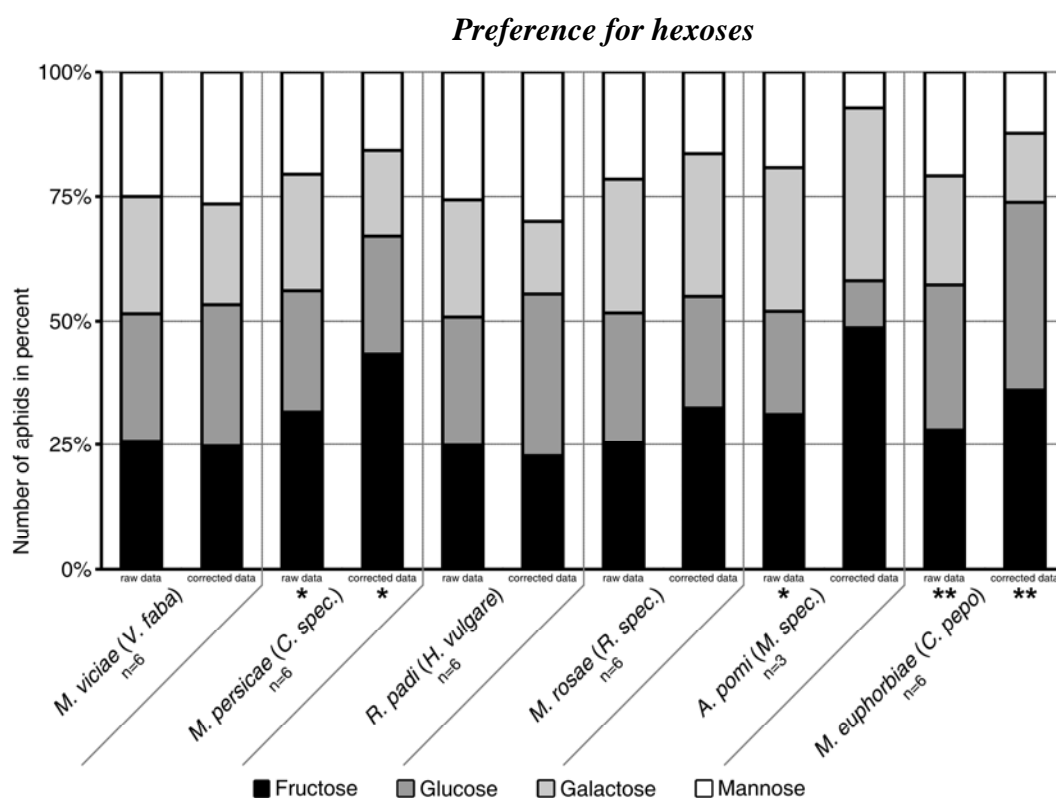


Fig. S7 Preference of six aphid species for various hexoses. Aphid distribution between test baths filled with diverse hexoses (fructose, glucose, galactose and mannose): All test solutions were at a concentration of 219 mM. For a better overview, the statistically significant differences for carbohydrate preference of an aphid species were marked with asterisks (*) indicating the following significance classes: *** $p \leq 0.001$, ** $p \leq 0.01$, and * $p \leq 0.05$ (Stahel, 2008; Polasek, 1997). Experiments using *M. viciae* were done with medium choice chamber systems. Experiments using *M. rosae* were done with small choice chamber systems. For exact statistical results see Tab. S13.

Tab. S13 Statistical results of hexoses series.

	statistical results of raw data	statistical results corrected data
<i>M. viciae</i>	$p_{raw\ data} = 0.535$ $X^2 = 2.186$ with 3 degrees of freedom	$p_{corrected\ data} = 0.477$ $X^2 = 2.492$ with 3 degrees of freedom
<i>M. persicae</i>	$p_{raw\ data} = 0.035$ $X^2 = 8.600$ with 3 degrees of freedom	$p_{corrected\ data} = 0.035$ $X^2 = 8.600$ with 3 degrees of freedom
<i>R. padi</i>	$p_{raw\ data} = 0.457$ $X^2 = 2.600$ with 3 degrees of freedom	$p_{corrected\ data} = 0.123$ $X^2 = 5.769$ with 3 degrees of freedom
<i>M. rosae</i>	$p_{raw\ data} = 0.535$ $X^2 = 2.186$ with 3 degrees of freedom	$p_{corrected\ data} = 0.650$ $X^2 = 1.642$ with 3 degrees of freedom
<i>A. pomi</i>	$p_{raw\ data}(\text{estimated}) = 0.042$ $p_{raw\ data}(\text{exact}) = 0.017$ $X^2 = 8.200$ with 3 degrees of freedom	$p_{corrected\ data}(\text{estimated}) = 0.089$ $p_{corrected\ data}(\text{exact}) = 0.075$ $X^2 = 6.517$ with 3 degrees of freedom
<i>M. euphorbiae</i>	$p_{raw\ data} = 0.005$ $X^2 = 12.724$ with 3 degrees of freedom	$p_{corrected\ data} = 0.002$ $X^2 = 14.898$ with 3 degrees of freedom

Tab. S14 Results of All-Pairwise-Multiple-Comparison-Procedures for hexoses series of *M. persicae* (Fig. S7). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0,05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	Fructose ¹		Glucose ¹		Galactose ¹		Mannose ¹	
	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$
Fructose ¹	---	---	---	---	---	---	---	---
Glucose ¹	dnt (d/d/d)	dnt (d/d/d)	---	---	---	---	---	---
Galactose ¹	no (n/n/n)	no (n/n/n)	dnt (d/d/d)	dnt (d/d/d)	---	---	---	---
Mannose ¹	yes (y/y/y)	yes (y/y/y)	no (n/n/n)	no (n/n/n)	dnt (d/d/d)	dnt (d/d/d)	---	---

¹ 219 mM

Tab. S15 Results of All-Pairwise-Multiple-Comparison-Procedures for hexoses series of *A. pomi* (Fig. S7). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0,05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	Fructose ¹		Glucose ¹		Galactose ¹		Mannose ¹	
	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$
Fructose ¹	---	---	---	---	---	---	---	---
Glucose ¹	no (n/n/n)	---	---	---	---	---	---	---
Galactose ¹	dnt (d/d/d)	---	dnt (d/d/d)	---	---	---	---	---
Mannose ¹	dnt (d/d/d)	---	dnt (d/d/d)	---	dnt (d/d/d)	---	---	---

¹ 219 mM

Tab. S16 Results of All-Pairwise-Multiple-Comparison-Procedures for hexoses series of *M. euphorbiae* (Fig. S7). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0,05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	Fructose ¹		Glucose ¹		Galactose ¹		Mannose ¹	
	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$
Fructose ¹	---	---	---	---	---	---	---	---
Glucose ¹	no (n/n/n)	no (n/n/n)	---	---	---	---	---	---
Galactose ¹	dnt (d/d/y)	yes (n/y/y)	yes (y/y/y)	yes (y/y/y)	---	---	---	---
Mannose ¹	no (n/n/y)	? (d/n/y)	yes (y/y/y)	yes (y/y/y)	dnt (d/d/n)	no (d/n/n)	---	---

¹ 219 mM

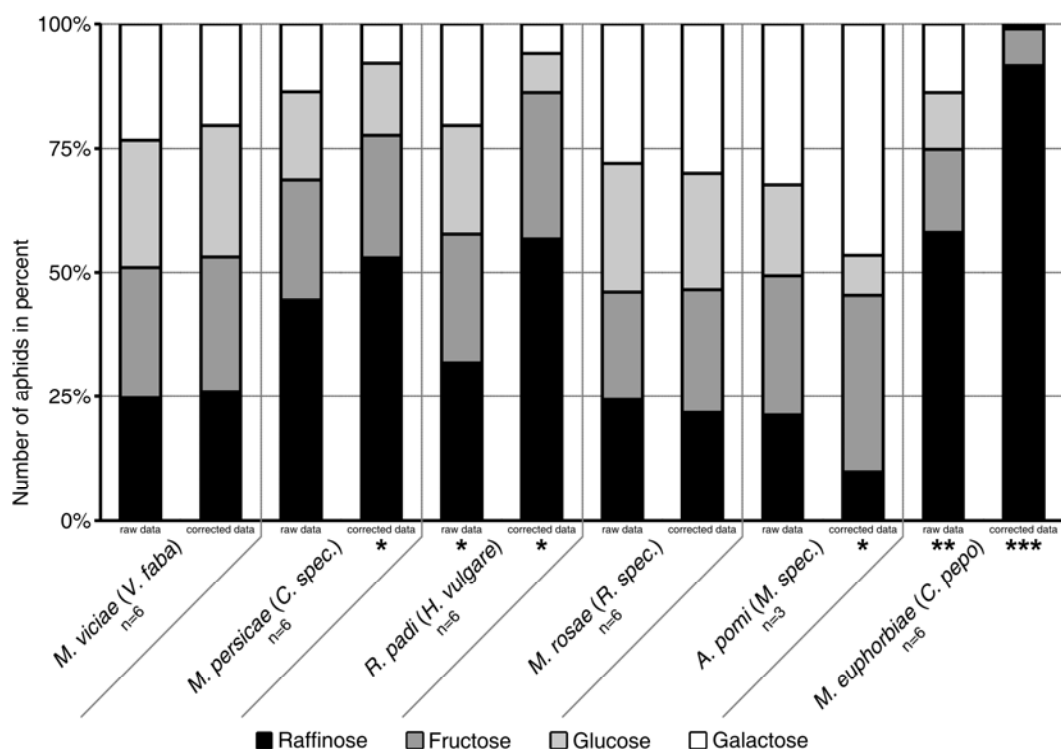


Fig. S8 Preference of six aphid species for raffinose vs. hexoses. Aphid distribution between test baths filled with diverse carbohydrates (raffinose vs. fructose, glucose, galactose). All test solutions were at a concentration of 219 mM. For a better overview, the statistically significant differences for carbohydrate preference of an aphid species were marked with asterisks (*) indicating the following significance classes: *** $p \leq 0.001$, ** $p \leq 0.01$, and * $p \leq 0.05$ (Stahel, 2008; Polasek, 1997). Experiments using *M. viciae* were done with medium choice chamber systems. Experiments using *M. rosae* were done with small choice chamber systems. For exact statistical results see Tab. S17.

Tab. S17 Statistical results of raffinose vs. hexoses series.

	statistical results of raw data	statistical results corrected data
<i>M. viciae</i>	$p_{raw\ data} = 0.284$ $X^2 = 3.800$ with 3 degrees of freedom	$p_{corrected\ data} = 0.532$ $X^2 = 2.200$ with 3 degrees of freedom
<i>M. persicae</i>	$p_{raw\ data} = 0.050$ $X^2 = 7.800$ with 3 degrees of freedom	$p_{corrected\ data} = 0.042$ $X^2 = 8.186$ with 3 degrees of freedom
<i>R. padi</i>	$p_{raw\ data} = 0.013$ $X^2 = 10.800$ with 3 degrees of freedom	$p_{corrected\ data} = 0.015$ $X^2 = 10.400$ with 3 degrees of freedom
<i>M. rosae</i>	$p_{raw\ data} = 0.694$ $X^2 = 1.448$ with 3 degrees of freedom	$p_{corrected\ data} = 0.694$ $X^2 = 1.448$ with 3 degrees of freedom
<i>A. pomi</i>	$p_{raw\ data}(estimated) = 0.081$ $p_{raw\ data}(exact) = 0.075$ $X^2 = 6.724$ with 3 degrees of freedom	$p_{corrected\ data}(estimated) = 0.056$ $p_{corrected\ data}(exact) = 0.033$ $X^2 = 7.552$ with 3 degrees of freedom
<i>M. euphorbiae</i>	$p_{raw\ data} = 0.007$ $X^2 = 12.259$ with 3 degrees of freedom	$p_{corrected\ data} = < 0.001$ $X^2 = 16.286$ with 3 degrees of freedom

Tab. S18 Results of All-Pairwise-Multiple-Comparison-Procedures for raffinose vs. hexoses series of *M. persicae* (Fig. S8). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0,05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	Raffinose ¹		Fructose ¹		Glucose ¹		Galactose ¹	
	$p_{\text{raw data}}$	$p_{\text{corrected data}}$	$p_{\text{raw data}}$	$p_{\text{corrected data}}$	$p_{\text{raw data}}$	$p_{\text{corrected data}}$	$p_{\text{raw data}}$	$p_{\text{corrected data}}$
Raffinose ¹	---	---	---	---	---	---	---	---
Fructose ¹	---	dnt (d/d/y)	---	---	---	---	---	---
Glucose ¹	---	no (n/n/y)	---	dnt (d/d/d)	---	---	---	---
Galactose ¹	---	yes (y/y/y)	---	no (n/n/n)	---	dnt (d/d/d)	---	---

¹ 219 mM

Tab. S19 Results of All-Pairwise-Multiple-Comparison-Procedures for raffinose vs. hexoses series of *R. padi* (Fig. S8). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0,05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	Raffinose ¹		Fructose ¹		Glucose ¹		Galactose ¹	
	$p_{\text{raw data}}$	$p_{\text{corrected data}}$	$p_{\text{raw data}}$	$p_{\text{corrected data}}$	$p_{\text{raw data}}$	$p_{\text{corrected data}}$	$p_{\text{raw data}}$	$p_{\text{corrected data}}$
Raffinose ¹	---	---	---	---	---	---	---	---
Fructose ¹	no (n/n/n)	dnt (d/d/n)	---	---	---	---	---	---
Glucose ¹	yes (y/y/y)	no (n/n/y)	dnt (d/d/d)	dnt (d/d/y)	---	---	---	---
Galactose ¹	yes (y/y/y)	yes (y/y/y)	no (n/n/n)	no (n/n/y)	dnt (d/d/d)	dnt (d/d/n)	---	---

¹ 219 mM

Tab. S20 Results of All-Pairwise-Multiple-Comparison-Procedures for raffinose vs. hexoses series of *A. pomi* (Fig. S8). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
 Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0,05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	Raffinose ¹		Fructose ¹		Glucose ¹		Galactose ¹	
	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$
Raffinose ¹	---	---	---	---	---	---	---	---
Fructose ¹	---	dnt (d/d/d)	---	---	---	---	---	---
Glucose ¹	---	dnt (d/d/d)	---	dnt (d/d/d)	---	---	---	---
Galactose ¹	---	dnt (d/d/d)	---	dnt (d/d/d)	---	no (n/n/n)	---	---

¹ 219 mM

Tab. S21 Results of All-Pairwise-Multiple-Comparison-Procedures for raffinose vs. hexoses series of *M. euphorbiae* (Fig. S8). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
 Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0,05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	Raffinose ¹		Fructose ¹		Glucose ¹		Galactose ¹	
	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$
Raffinose ¹	---	---	---	---	---	---	---	---
Fructose ¹	no (n/n/y)	no (n/n/y)	---	---	---	---	---	---
Glucose ¹	yes (y/y/y)	yes (y/y/y)	no (n/n/n)	dnt (d/d/d)	---	---	---	---
Galactose ¹	yes (y/y/y)	yes (y/y/y)	dnt (d/d/d)	no (n/n/n)	dnt (d/d/d)	dnt (d/d/d)	---	---

¹ 219 mM

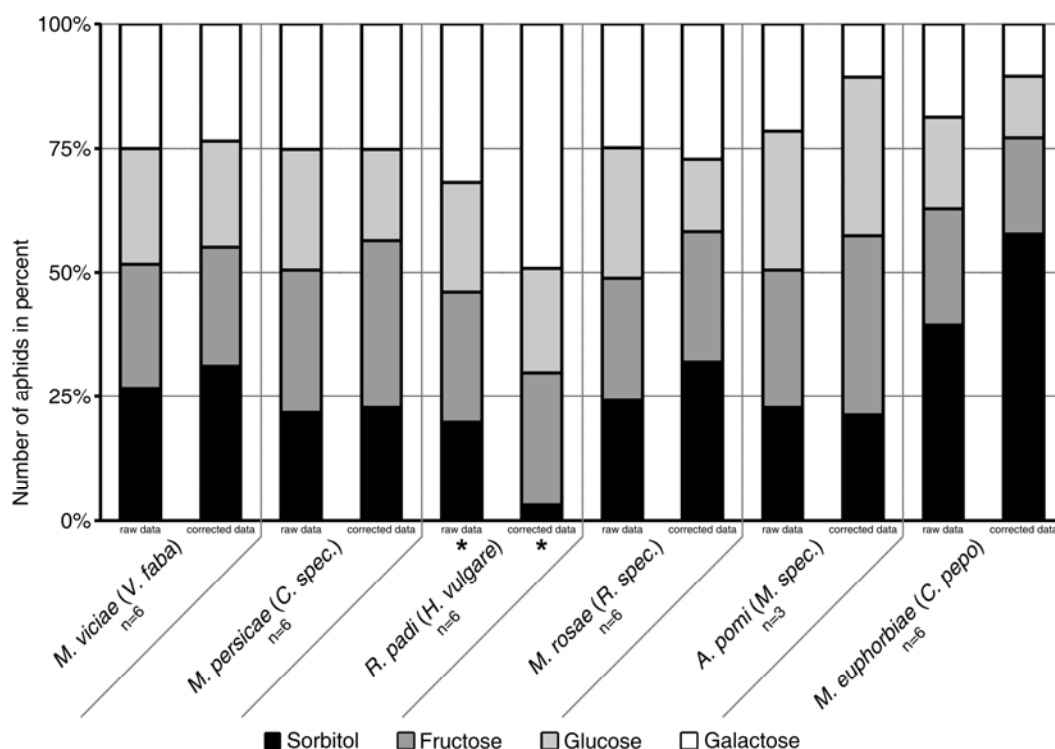


Fig. S9 Preference of six aphid species for sorbitol vs. hexoses. Aphid distribution between test baths filled with diverse carbohydrates (sorbitol vs. fructose, glucose, galactose). All test solutions were at a concentration of 219 mM. For a better overview, the statistically significant differences for carbohydrate preference of an aphid species were marked with asterisks (*) indicating the following significance classes: *** $p \leq 0.001$, ** $p \leq 0.01$, and * $p \leq 0.05$ (Stahel, 2008; Polasek, 1997). Experiments using *M. viciae* were done with medium choice chamber systems. Experiments using *M. rosae* were done with medium choice chamber systems. For exact statistical results see Tab. S22.

Tab. S22 Statistical results of sorbitol vs. hexoses series.

	statistical results of raw data	statistical results corrected data
<i>M. viciae</i>	$p_{raw\ data} = 0.441$ $X^2 = 2.695$ with 3 degrees of freedom	$p_{corrected\ data} = 0.195$ $X^2 = 4.707$ with 3 degrees of freedom
<i>M. persicae</i>	$p_{raw\ data} = 0.271$ $X^2 = 3.915$ with 3 degrees of freedom	$p_{corrected\ data} = 0.323$ $X^2 = 3.482$ with 3 degrees of freedom
<i>R. padi</i>	$p_{raw\ data} = 0.013$ $X^2 = 10.729$ with 3 degrees of freedom	$p_{corrected\ data} = 0.016$ $X^2 = 10.309$ with 3 degrees of freedom
<i>M. rosae</i>	$p_{raw\ data} = 0.978$ $X^2 = 0.200$ with 3 degrees of freedom	$p_{corrected\ data} = 0.951$ $X^2 = 0.349$ with 3 degrees of freedom
<i>A. pomi</i>	$p_{raw\ data}(estimated) = 0.670$ $p_{raw\ data}(exact) = 0.727$ $X^2 = 1.552$ with 3 degrees of freedom	$p_{corrected\ data}(estimated) = 0.565$ $p_{corrected\ data}(exact) = 0.608$ $X^2 = 2.036$ with 3 degrees of freedom
<i>M. euphorbiae</i>	$p_{raw\ data} = 0.949$ $X^2 = 0.356$ with 3 degrees of freedom	$p_{corrected\ data} = 0.958$ $X^2 = 0.310$ with 3 degrees of freedom

Tab. S23 Results of All-Pairwise-Multiple-Comparison-Procedures for sorbitol vs. hexoses series of *R. padi* (Fig. S9). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
 Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0,05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	Sorbitol ¹		Fructose ¹		Glucose ¹		Galactose ¹	
	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$
Sorbitol ¹	---	---	---	---	---	---	---	---
Fructose ¹	dnt (d/d/d)	no (n/n/n)	---	---	---	---	---	---
Glucose ¹	dnt (d/d/d)	dnt (d/d/d)	no (n/n/n)	dnt (d/d/d)	---	---	---	---
Galactose ¹	yes (y/y/y)	yes (y/y/y)	no (n/n/y)	dnt (d/d/y)	yes (y/y/y)	no (n/n/y)	---	---

¹ 219 mM

Preference for sucrose mixtures

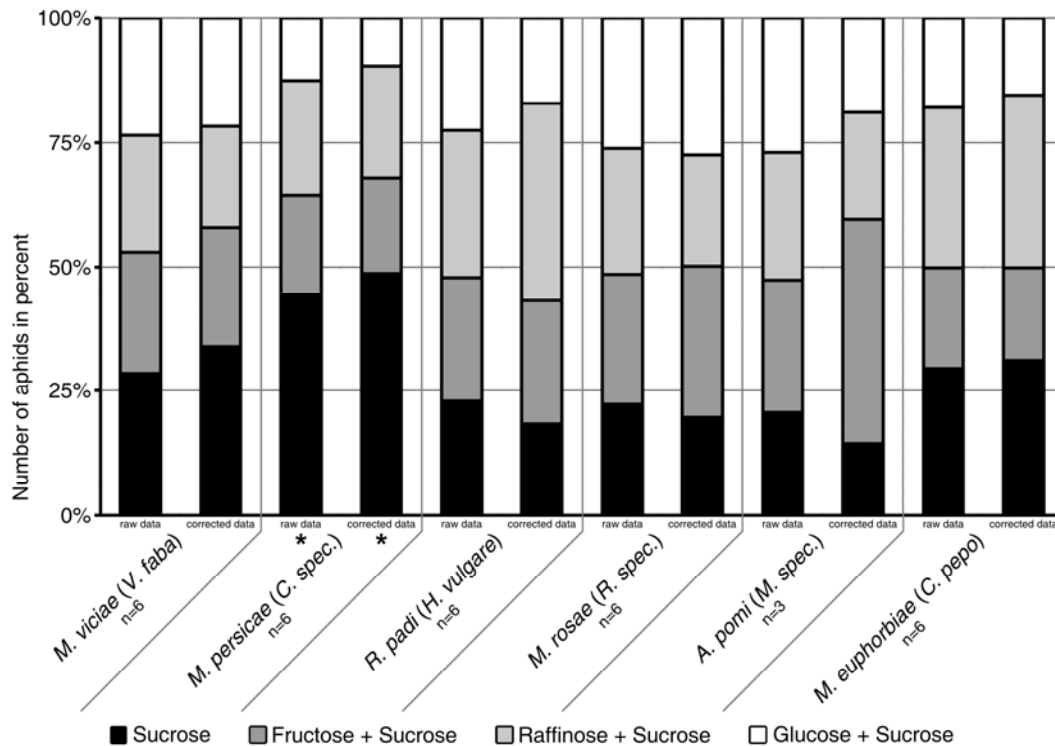


Fig. S10 Preference of six aphid species for various sucrose mixtures (sucrose mixtures series A). Aphid distribution between test baths filled with diverse sucrose mixtures present in the phloem sap (sucrose (109.5 mM) vs. sucrose with fructose, glucose or raffinose). All sucrose mixtures were at a total concentration of 219 mM. For a better overview, the statistically significant differences for carbohydrate preference of an aphid species were marked with asterisks (*) indicating the following significance classes: *** $p \leq 0.001$, ** $p \leq 0.01$, and * $p \leq 0.05$ (Stahel, 2008; Polasek, 1997). Experiments using *M. viciae* were done with medium choice chamber systems. Experiments using *M. rosae* were done with small choice chamber systems. For exact statistical results see Tab. S24.

Tab. S24 Statistical results of sucrose mixtures series A.

	statistical results of raw data	statistical results corrected data
<i>M. viciae</i>	$p_{raw\ data} = 0.624$ $X^2 = 1.759$ with 3 degrees of freedom	$p_{corrected\ data} = 0.736$ $X^2 = 1.271$ with 3 degrees of freedom
<i>M. persicae</i>	$p_{raw\ data} = 0.015$ $X^2 = 10.525$ with 3 degrees of freedom	$p_{corrected\ data} = 0.015$ $X^2 = 10.525$ with 3 degrees of freedom
<i>R. padi</i>	$p_{raw\ data} = 0.229$ $X^2 = 4.322$ with 3 degrees of freedom	$p_{corrected\ data} = 0.229$ $X^2 = 4.322$ with 3 degrees of freedom
<i>M. rosae</i>	$p_{raw\ data} = 0.562$ $X^2 = 2.053$ with 3 degrees of freedom	$p_{corrected\ data} = 0.252$ $X^2 = 4.091$ with 3 degrees of freedom
<i>A. pomi</i>	$p_{raw\ data}(estimated) = 0.887$ $p_{raw\ data}(exact) = 0.958$ $X^2 = 0.643$ with 3 degrees of freedom	$p_{corrected\ data}(estimated) = 0.516$ $p_{corrected\ data}(exact) = 0.608$ $X^2 = 2.280$ with 3 degrees of freedom
<i>M. euphorbiae</i>	$p_{raw\ data} = 0.068$ $X^2 = 7.138$ with 3 degrees of freedom	$p_{corrected\ data} = 0.068$ $X^2 = 7.138$ with 3 degrees of freedom

Tab. S25 Results of All-Pairwise-Multiple-Comparison-Procedures for sucrose mixtures series A of *M. persicae* (Fig. S10). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement, if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:

Dunn's Method / Tukey Test / Student-Newman-Keuls Method

$p = <0.05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	Sucrose ¹		Fructose ¹ + Sucrose ¹		Raffinose ¹ + Sucrose ¹		Glucose ¹ + Sucrose ¹	
	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$
	Sucrose ¹	---	---	---	---	---	---	---
Fructose ¹ + Sucrose ¹	no (n/n/y)	no (n/n/y)	---	---	---	---	---	---
Raffinose ¹ + Sucrose ¹	dnt (d/d/y)	dnt (d/d/y)	dnt (d/d/d)	dnt (d/d/d)	---	---	---	---
Glucose ¹ + Sucrose ¹	yes (y/y/y)	yes (y/y/y)	dnt (d/d/d)	dnt (d/d/d)	no (n/n/n)	no (n/n/n)	---	---

¹ 109.5 mM

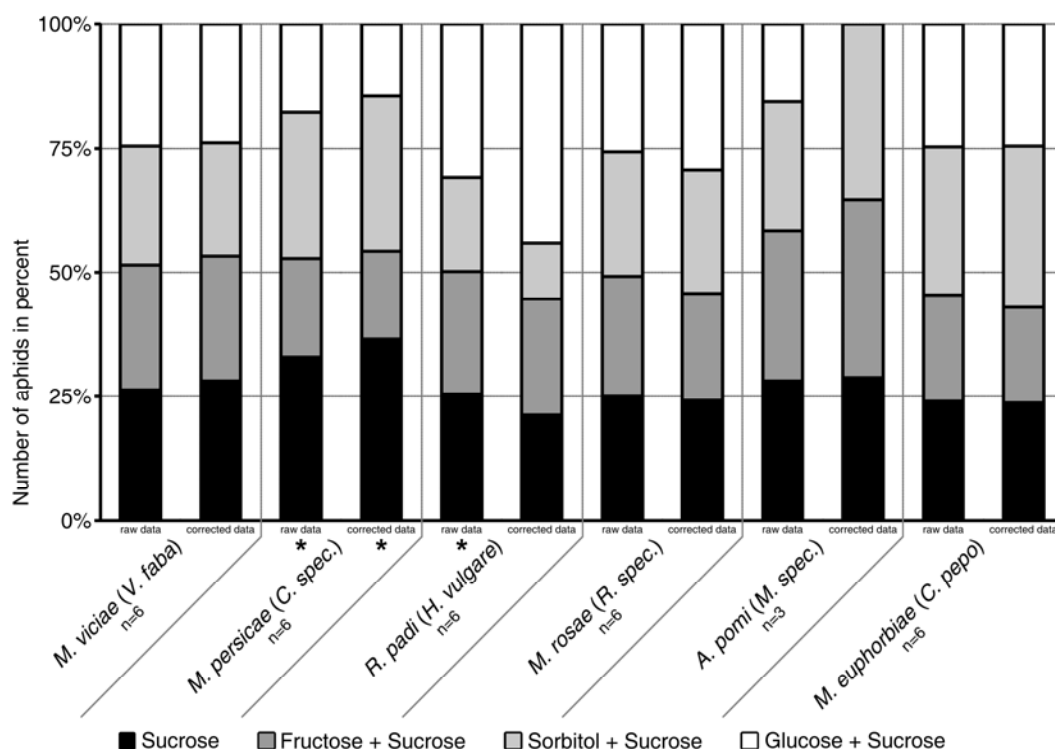


Fig. S11 Preference of six aphid species for various sucrose mixtures (sucrose mixtures series B). Aphid distribution between test baths filled with diverse sucrose mixtures present in the phloem sap (sucrose (109.5 mM) vs. sucrose with fructose, glucose or sorbitol). All sucrose mixtures were at a total concentration of 219 mM. For a better overview, the statistically significant differences for carbohydrate preference of an aphid species were marked with asterisks (*) indicating the following significance classes: *** $p \leq 0.001$, ** $p \leq 0.01$, and * $p \leq 0.05$ (Stahel, 2008; Polasek, 1997). Experiments using *M. viciae* were done with medium choice chamber systems. Experiments using *M. rosae* were done with small choice chamber systems. For exact statistical results see Tab. S26.

Tab. S26 Statistical results of sucrose mixtures series B.

	statistical results of raw data	statistical results corrected data
<i>M. viciae</i>	$p_{raw\ data} = 0.801$ $X^2 = 1.000$ with 3 degrees of freedom	$p_{corrected\ data} = 0.801$ $X^2 = 1.000$ with 3 degrees of freedom
<i>M. persicae</i>	$p_{raw\ data} = 0.024$ $X^2 = 9.400$ with 3 degrees of freedom	$p_{corrected\ data} = 0.031$ $X^2 = 8.898$ with 3 degrees of freedom
<i>R. padi</i>	$p_{raw\ data} = 0.032$ $X^2 = 8.800$ with 3 degrees of freedom	$p_{corrected\ data} = 0.191$ $X^2 = 4.750$ with 3 degrees of freedom
<i>M. rosae</i>	$p_{raw\ data} = 0.921$ $X^2 = 0.491$ with 3 degrees of freedom	$p_{corrected\ data} = 0.982$ $X^2 = 0.170$ with 3 degrees of freedom
<i>A. pomi</i>	$p_{raw\ data}(estimated) = 0.122$ $p_{raw\ data}(exact) = 0.148$ $X^2 = 5.800$ with 3 degrees of freedom	$p_{corrected\ data}(estimated) = 0.128$ $p_{corrected\ data}(exact) = 0.148$ $X^2 = 5.690$ with 3 degrees of freedom
<i>M. euphorbiae</i>	$p_{raw\ data} = 0.706$ $X^2 = 1.400$ with 3 degrees of freedom	$p_{corrected\ data} = 0.706$ $X^2 = 1.400$ with 3 degrees of freedom

Tab. S27 Results of All-Pairwise-Multiple-Comparison-Procedures for sucrose mixtures series B of *M. persicae* (Fig. S11). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement, if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0.05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	Sucrose ¹		Fructose ¹ + Sucrose ¹		Sorbitol ¹ + Sucrose ¹		Glucose ¹ + Sucrose ¹	
	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$
Sucrose ¹	---	---	---	---	---	---	---	---
Fructose ¹ + Sucrose ¹	no (n/n/y)	? (d/n/y)	---	---	---	---	---	---
Sorbitol ¹ + Sucrose ¹	dnt (d/d/y)	dnt (d/d/y)	dnt (d/d/d)	dnt (d/d/d)	---	---	---	---
Glucose ¹ + Sucrose ¹	yes (y/y/y)	yes (n/y/y)	dnt (d/d/d)	dnt (d/d/d)	no (n/n/n)	no (d/n/n)	---	---

¹ 109.5 mM

Tab. S28 Results of All-Pairwise-Multiple-Comparison-Procedures for sucrose mixtures series B of *R. padi* (Fig. S11). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement, if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0.05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	Sucrose ¹		Fructose ¹ + Sucrose ¹		Sorbitol ¹ + Sucrose ¹		Glucose ¹ + Sucrose ¹	
	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$
Sucrose ¹	---	---	---	---	---	---	---	---
Fructose ¹ + Sucrose ¹	dnt (d/d/d)	---	---	---	---	---	---	---
Sorbitol ¹ + Sucrose ¹	no (n/n/y)	---	dnt (d/d/y)	---	---	---	---	---
Glucose ¹ + Sucrose ¹	dnt (d/d/d)	---	no (n/n/n)	---	yes (y/y/y)	---	---	---

¹ 109.5 mM

Preference for the sucrose concentration

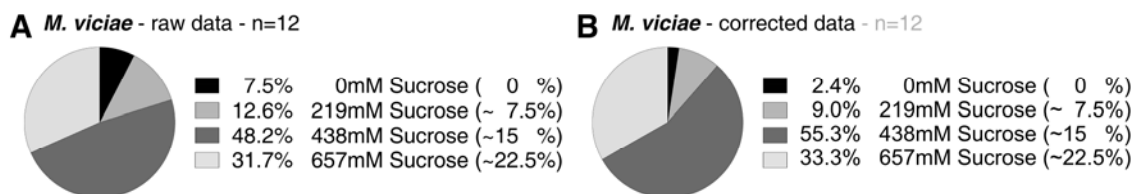


Fig. S12 Percentage of aphid distribution (*M. viciae*) between test baths filled with solutions of different sucrose concentration (7.5% steps) (sucrose concentration series A). (A) Significance of the raw data: $p_{raw\ data} = < 0.001$; $X^2 = 24.500$ with 3 degrees of freedom. (B) Significance of the data, corrected for background distribution: $p_{corrected\ data} = < 0.001$; $X^2 = 25.300$ with 3 degrees of freedom. Experiments were done with large choice chamber systems.

Tab. S29 Results of All-Pairwise-Multiple-Comparison-Procedures for sucrose concentration series A of *M. viciae* (Fig. S12). If the Friedman-test resulted in a p -value of $p = < 0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = < 0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:

Dunn's Method / Tukey Test / Student-Newman-Keuls Method

$p = < 0.05$: yes (y), no (n), do not test (dnt, d)

$p = < 0.05?$	0 mM Sucrose (~0% (w/v))		219 mM Sucrose (~7.5% (w/v))		438 mM Sucrose (~15% (w/v))		657 mM Sucrose (~22.5% (w/v))	
	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$
	0 mM Sucrose (~0% (w/v))	---	---	---	---	---	---	---
219 mM Sucrose (~7.5% (w/v))	no (n/n/y)	no (n/n/y)	---	---	---	---	---	---
438 mM Sucrose (~15% (w/v))	yes (y/y/y)	yes (y/y/y)	yes (y/y/y)	yes (y/y/y)	---	---	---	---
657 mM Sucrose (~22.5% (w/v))	yes (y/y/y)	yes (y/y/y)	no (n/n/y)	no (n/n/y)	no (n/n/y)	no (n/n/y)	---	---

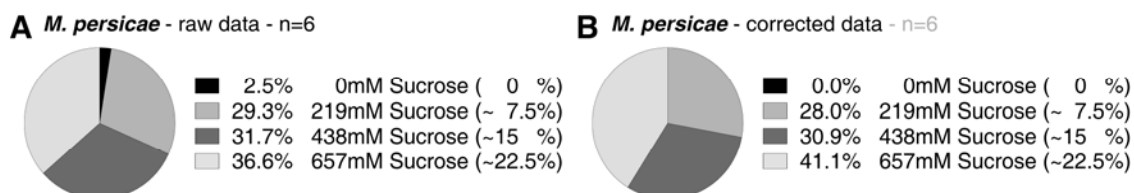


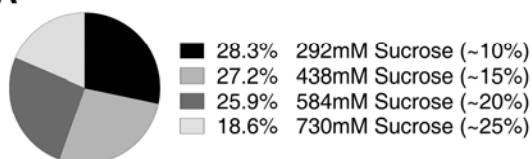
Fig. S13 Percentage of aphid distribution (*M. persicae*) between test baths filled with solutions of different sucrose concentration (7.5% steps) (sucrose concentration series A). (A) Significance of the raw data: $p_{raw\ data} = 0.012$; $X^2 = 11.000$ with 3 degrees of freedom. (B) Significance of the data, corrected for background distribution: $p_{corrected\ data} = 0.019$; $X^2 = 9.915$ with 3 degrees of freedom.

Tab. S30 Results of All-Pairwise-Multiple-Comparison-Procedures for sucrose concentration series A of *M. persicae* (Fig. S13). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
 Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0,05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	0 mM Sucrose (~0% (w/v))		219 mM Sucrose (~7.5% (w/v))		438 mM Sucrose (~15% (w/v))		657 mM Sucrose (~22.5% (w/v))	
	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$
0 mM Sucrose (~0% (w/v))	---	---	---	---	---	---	---	---
219 mM Sucrose (~7.5% (w/v))	no (n/n/y)	? (d/n/y)	---	---	---	---	---	---
438 mM Sucrose (~15% (w/v))	yes (y/y/y)	yes (n/y/y)	dnt (d/d/d)	dnt (d/d/d)	---	---	---	---
657 mM Sucrose (~22.5% (w/v))	yes (y/y/y)	yes (y/y/y)	no (n/n/n)	no (n/n/n)	dnt (d/d/d)	dnt (d/d/d)	---	---

A *M. viciae* - raw data - n=12



B *M. viciae* - corrected data - n=12

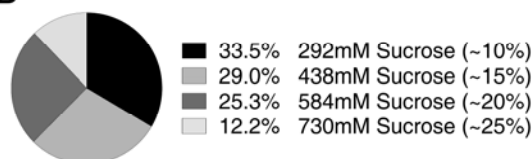


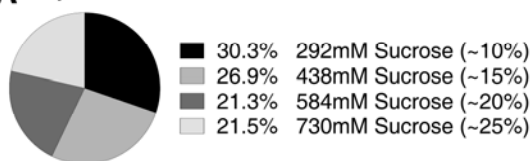
Fig. S14 Percentage of aphid distribution (*M. viciae*) between test baths filled with solutions of different sucrose concentration (5% steps) (sucrose concentration series B). (A) Significance of the raw data: $p_{raw\ data} = 0.017$; $\chi^2 = 10.210$ with 3 degrees of freedom. (B) Significance of the data, corrected for background distribution: $p_{corrected\ data} = 0.021$; $\chi^2 = 9.783$ with 3 degrees of freedom. Experiments were done with large choice chamber systems.

Tab. S31 Results of All-Pairwise-Multiple-Comparison-Procedures for sucrose concentration series B of *M. viciae* (Fig. S14). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0,05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	292 mM Sucrose (~10%(w/v))		438 mM Sucrose (~15% (w/v))		584 mM Sucrose (~20% (w/v))		730 mM Sucrose (~25% (w/v))	
	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$
292 mM Sucrose (~10%(w/v))	---	---	---	---	---	---	---	---
438 mM Sucrose (~15% (w/v))	dnt (d/d/d)	dnt (d/d/d)	---	---	---	---	---	---
584 mM Sucrose (~20% (w/v))	no (n/n/n)	no (n/n/n)	dnt (d/d/d)	dnt (d/d/d)	---	---	---	---
730 mM Sucrose (~25% (w/v))	yes (y/y/y)	yes (y/y/y)	no (n/n/y)	no (n/n/y)	dnt (d/d/y)	dnt (d/d/y)	---	---

A *M. persicae* - raw data - n=6



B *M. persicae* - corrected data - n=6

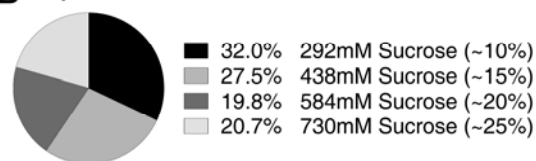
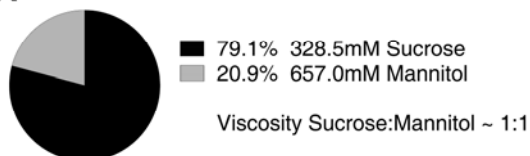


Fig. S15 Percentage of aphid distribution (*M. persicae*) between test baths filled with solutions of different sucrose concentration (5% steps) (sucrose concentration series B). (A) Significance of the raw data: $p_{raw\ data} = 0.457$; $X^2 = 2.600$ with 3 degrees of freedom. (B) Significance of the data, corrected for background distribution: $p_{corrected\ data} = 0.457$; $X^2 = 2.600$ with 3 degrees of freedom.

Preference for viscosity

A *M. viciae* - raw data - n=12



B *M. viciae* - corrected data - n=12

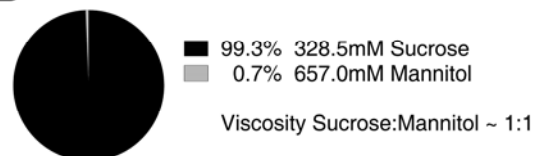


Fig. S16 Percentage of aphid distribution (*M. viciae*) between test baths filled with sucrose and mannitol solutions at equal viscosity. (A) Significance of the raw data: $p_{raw\ data} = < 0.001$, $t = 22.090$ with 22 degrees of freedom. (B) Significance of the data, corrected for background distribution: $p_{corrected\ data} = < 0.001$, $t = 10.322$ with 22 degrees of freedom. Experiments were done with large choice chamber systems.

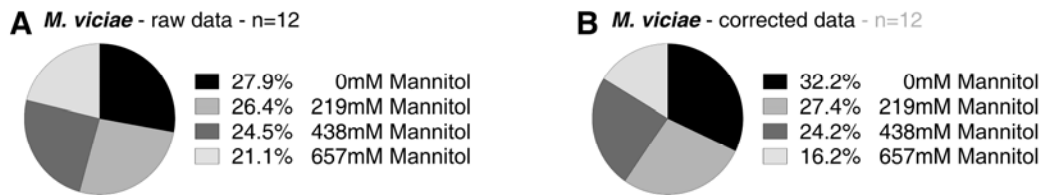


Fig. S17 Percentage of aphid distribution (*M. viciae*) between test baths filled with solutions of different mannitol osmolarities (osmolarity series). (A) Significance of the raw data: $p_{raw\ data} = 0.004$, $X^2 = 13.256$ with 3 degrees of freedom. (B) Significance of the data, corrected for background distribution: $p_{corrected\ data} = 0.043$, $X^2 = 8.172$ with 3 degrees of freedom. Experiments were done with large choice chamber systems.

Tab. S32 Results of All-Pairwise-Multiple-Comparison-Procedures for osmolarity series of *M. viciae* (Fig. S17). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
 Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0,05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	0 mM Mannitol		219 mM Mannitol		438 mM Mannitol		657 mM Mannitol	
	$P_{raw\ data}$	$P_{corrected\ data}$	$P_{raw\ data}$	$P_{corrected\ data}$	$P_{raw\ data}$	$P_{corrected\ data}$	$P_{raw\ data}$	$P_{corrected\ data}$
0 mM Mannitol	---	---	---	---	---	---	---	---
219 mM Mannitol	dnt (d/d/d)	dnt (d/d/d)	---	---	---	---	---	---
438 mM Mannitol	no (n/n/n)	dnt (d/d/d)	dnt (d/d/d)	dnt (d/d/d)	---	---	---	---
657 mM Mannitol	yes (y/y/y)	dnt (d/d/d)	yes (y/y/y)	no (n/n/n)	no (n/n/y)	dnt (d/d/d)	---	---

Preference for pH



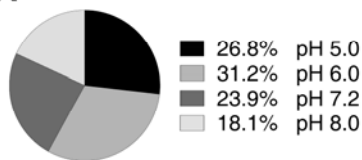
Fig. S18 Percentage of aphid distribution (*M. viciae*) between test baths filled with solutions of different pH values (0.8 to 1.2 pH unit steps) (pH series A). (A) Significance of the raw data: $p_{raw\ data} = < 0.001$, $X^2 = 26.110$ with 3 degrees of freedom. (B) Significance of the data, corrected for background distribution: $p_{corrected\ data} = < 0.001$, $X^2 = 24.700$ with 3 degrees of freedom. Experiments were done with large choice chamber systems.

Tab. S33 Results of All-Pairwise-Multiple-Comparison-Procedures for pH series A of *M. viciae* (Fig. S18). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
 Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0,05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	pH 5		pH 6		pH 7.2		pH 8	
	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$	$p_{raw\ data}$	$p_{corrected\ data}$
pH 5	---	---	---	---	---	---	---	---
pH 6	yes (y/y/y)	yes (y/y/y)	---	---	---	---	---	---
pH 7.2	yes (y/y/y)	yes (y/y/y)	no (n/n/y)	no (n/n/y)	---	---	---	---
pH 8	no (n/n/y)	no (n/n/y)	no (n/n/n)	no (n/n/n)	yes (y/y/y)	yes (y/y/y)	---	---

A *M. persicae* - raw data - n=6



B *M. persicae* - corrected data - n=6

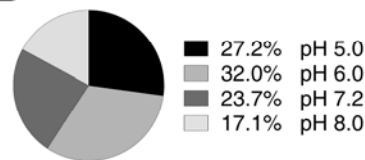
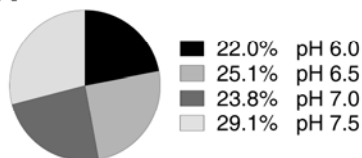


Fig. S19 Percentage of aphid distribution (*M. persicae*) between test baths filled with solutions of different pH values (0.8 to 1.2 pH unit steps) (pH series A). (A) Significance of the raw data: $p_{raw\ data} = 0.572$, $X^2 = 2.000$ with 3 degrees of freedom. (B) Significance of the data, corrected for background distribution: $p_{corrected\ data} = 0.572$, $X^2 = 2.000$ with 3 degrees of freedom.

A *M. viciae* - raw data - n=12



B *M. viciae* - corrected data - n=12

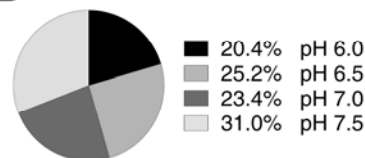
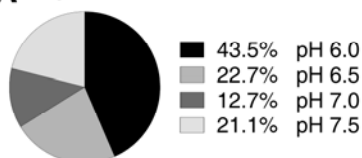


Fig. S20 Percentage of aphid distribution (*M. viciae*) between test baths filled with solutions of different pH values (0.5 pH unit steps) (pH series B). (A) Significance of the raw data: $p_{raw\ data} = 0.637$, $X^2 = 1.700$ with 3 degrees of freedom. (B) Significance of the data, corrected for background distribution: $p_{corrected\ data} = 0.662$, $X^2 = 1.588$ with 3 degrees of freedom. Experiments were done with large choice chamber systems.

A *M. persicae* - raw data - n=6



B *M. persicae* - corrected data - n=6

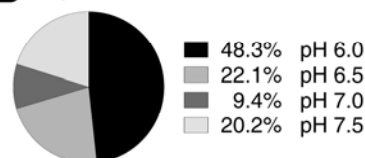


Fig. S21 Percentage of aphid distribution (*M. persicae*) between test baths filled with solutions of different pH values (0.5 pH unit steps) (pH series B). (A) Significance of the raw data: $p_{raw\ data} = 0.024$, $X^2 = 9.400$ with 3 degrees of freedom. (B) Significance of the data, corrected for background distribution: $p_{corrected\ data} = 0.020$, $X^2 = 9.800$ with 3 degrees of freedom.

Tab. S34 Results of All-Pair wise-Multiple-Comparison-Procedures for pH series B of *M. persicae* (Fig. S21). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0,05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	pH 6.0		pH 6.5		pH 7.0		pH 7.5	
	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$
pH 6.0	---	---	---	---	---	---	---	---
pH 6.5	dnt (d/d/y)	dnt (d/d/y)	---	---	---	---	---	---
pH 7.0	yes (y/y/y)	yes (y/y/y)	no (n/n/n)	no (n/n/n)	---	---	---	---
pH 7.5	no (n/n/y)	no (n/n/y)	dnt (d/d/d)	dnt (d/d/d)	dnt (d/d/d)	dnt (d/d/d)	---	---



Fig. S22 Percentage of aphid distribution (*M. viciae*) between test baths filled with solutions of different pH values (1.0 pH unit steps) (pH series C). (A) Significance of the raw data: $p_{raw\ data} = 0.035$, $X^2 = 8.600$ with 3 degrees of freedom. (B) Significance of the data, corrected for background distribution: $p_{corrected\ data} = 0.035$, $X^2 = 8.600$ with 3 degrees of freedom. Experiments of were done with medium choice chamber systems.

Tab. S35 Results of All-Pairwise-Multiple-Comparison-Procedures for pH series C of *M. viciae* (Fig. S22). If the Friedman-test resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0,05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	pH 5		pH 6		pH 7		pH 8	
	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$	$p_{raw\ data}$	$P_{corrected\ data}$
pH 5	---	---	---	---	---	---	---	---
pH 6	no (n/n/n)	no (n/n/n)	---	---	---	---	---	---
pH 7	yes (y/y/y)	yes (y/y/y)	dnt (d/d/d)	dnt (d/d/d)	---	---	---	---
pH 8	dnt (d/d/d)	dnt (d/d/d)	dnt (d/d/d)	dnt (d/d/d)	no (n/n/n)	no (n/n/n)	---	---



Fig. S23 Percentage of aphid distribution (*M. persicae*) between test baths filled with solutions of different pH values (1.0 pH unit steps) (pH series C). (A) Significance of the raw data: $p_{raw\ data} = 0.145$, $X^2 = 5.400$ with 3 degrees of freedom. (B) Significance of the data, corrected for background distribution: $p_{corrected\ data} = 0.284$, $X^2 = 3.800$ with 3 degrees of freedom.

Diet-EPGs with M. persicae

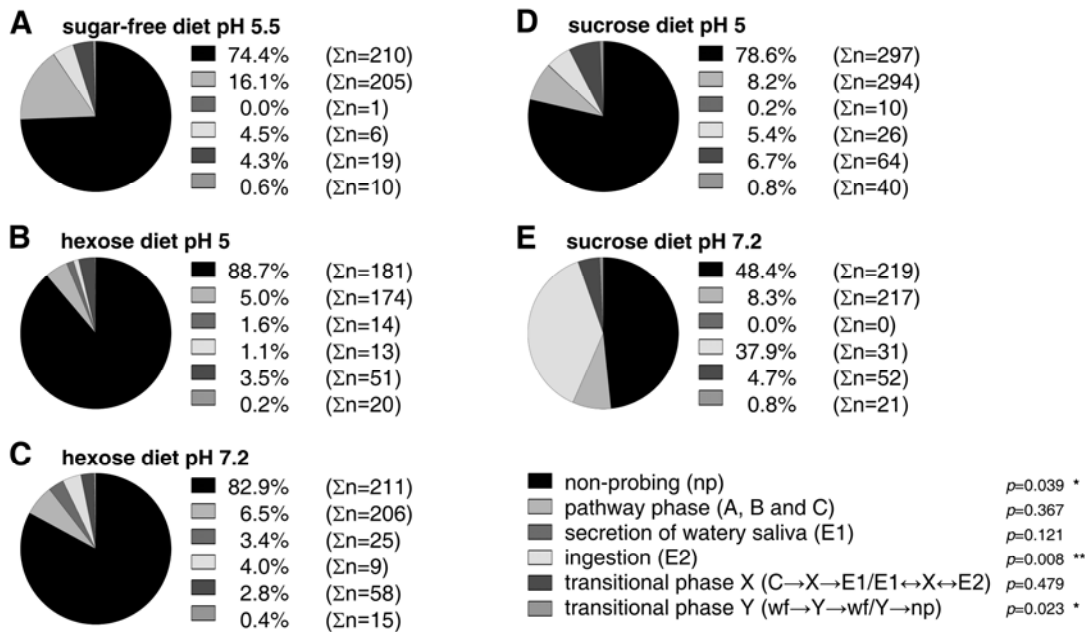


Fig. S24 Percentage duration of waveforms in electrical penetration graph (EPG) runs of *M. persicae* on different diets. EPGs lasted 8h and were repeated eight times each with adult, apterous individuals of *M. persicae*. The waveforms (wf) were defined according to Tjallingii (Tjallingii, 1996): A=cuticle penetration, B=sheath salivation, C=pathway activities, E1=secretion of watery saliva, E2=ingestion and salivation of watery saliva. The transitional phases X and Y describe the short phases between two clear waveforms. Σn expresses the total number of events of the respective waveform in eight EPG experiments. Only the durations of the phases 'non-probing' (np) and 'ingestion' (E2) show visual and statistical significant differences between sucrose diet pH 7.2 and all other offered diets. The statistical test was performed with the duration results of each waveform (in eight experiments each) acquired with the respective diets. Significance of the differences between 'sucrose diet pH 7.2' and other diets is marked with asterisks: *** $p \leq 0.001$, ** $p \leq 0.01$, and * $p \leq 0.05$ (Stahel, 2008; Polasek, 1997). For exact statistical results see Tab. S36.

Tab. S36 Statistical results of percentage duration of waveforms in EPG-runs of *M. persicae* on different diets. The statistical test was performed with the duration results of each waveform (with 8 experiments each) in the respective diets.

	statistical results
non-probing	$p = 0.039$ $X^2 = 10.100$ with 4 degrees of freedom
A, B and C	$p = 0.367$ $X^2 = 4.300$ with 4 degrees of freedom
secretion of watery saliva	$p = 0.121$ $X^2 = 7.299$ with 4 degrees of freedom
ingestion	$p = 0.008$ $X^2 = 13.702$ with 4 degrees of freedom
transitional phase X (C→X→E1/E1↔X↔E2)	$p = 0.479$ $X^2 = 3.490$ with 4 degrees of freedom
transitional phase Y (waveform→Y→waveform/Y→np)	$p = 0.023$ $X^2 = 11.356$ with 4 degrees of freedom

Tab. S37 Results of All-Pairwise-Multiple-Comparison-Procedures for the waveform “non-probing” (Fig. S24). If the ANOVA resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0,05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	sugar-free diet pH5.5	hexose diet pH5	hexose diet pH7.2	sucrose diet pH5	sucrose diet pH7.2
	p	p	p	p	p
sugar-free diet pH5.5	---	---	---	---	---
hexose diet pH5	no (n/n/n)	---	---	---	---
hexose diet pH7.2	dnt (d/d/d)	dnt (d/d/d)	---	---	---
sucrose diet pH5	dnt (d/d/d)	dnt (d/d/d)	dnt (d/d/d)	---	---
sucrose diet pH7.2	dnt (d/d/y)	yes (y/y/y)	no (n/n/y)	dnt (d/d/y)	---

Tab. S38 Results of All-Pairwise-Multiple-Comparison-Procedures for the wave form “ingestion” (Fig. S24). If the ANOVA resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0,05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	sugar-free diet pH5.5	hexose diet pH5	hexose diet pH7.2	sucrose diet pH5	sucrose diet pH7.2
	p	p	p	p	p
sugar-free diet pH5.5	---	---	---	---	---
hexose diet pH5	dnt (d/d/d)	---	---	---	---
hexose diet pH7.2	dnt (d/d/d)	dnt (d/d/d)	---	---	---
sucrose diet pH5	dnt (d/d/d)	dnt (d/d/d)	no (n/n/n)	---	---
sucrose diet pH7.2	dnt (d/d/y)	no (n/n/y)	yes (y/y/y)	dnt (d/d/y)	---

Tab. S39 Results of All-Pairwise-Multiple-Comparison-Procedures for the waveform “diffus b” (Fig. S24). If the ANOVA resulted in a p -value of $p = <0.05$, three All-Pairwise-Multiple-Comparison-Procedures were carried out: Dunn's-Method, Tukey-Test and Student-Newman-Keuls-Method. These comparison procedures gave a statement if there is a significance between the compared groups ($p = <0.05$: yes (y), no (n), do not test (dnt, d)). If all three comparison procedures gave a positive statement (yes (bolded)), the statistical significant difference of the respective two groups was certified.

All-Pairwise-Multiple-Comparison-Procedures:
Dunn's Method / Tukey Test / Student-Newman-Keuls Method
 $p = <0,05$: yes (y), no (n), do not test (dnt, d)

$p = <0.05?$	sugar-free diet pH5.5	hexose diet pH5	hexose diet pH7.2	sucrose diet pH5	sucrose diet pH7.2
	p	p	p	p	p
sugar-free diet pH5.5	---	---	---	---	---
hexose diet pH5	dnt (d/d/d)	---	---	---	---
hexose diet pH7.2	dnt (d/d/d)	dnt (d/d/d)	---	---	---
sucrose diet pH5	no (n/n/n)	dnt (d/d/d)	dnt (d/d/d)	---	---
sucrose diet pH7.2	dnt (d/d/d)	dnt (d/d/d)	dnt (d/d/d)	dnt (d/d/d)	---