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HONEYDEW SUGARS AND OSMOREGULATION IN THE PEA APHID *ACYRTHOSIPHON PISUM*

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Summary

Pea aphids, *Acyrthosiphon pisum*, containing their symbiotic bacteria (untreated aphids) and experimentally deprived of their bacteria by treatment with the antibiotic rifampicin (antibiotic-treated aphids) were reared on the plant *Vicia faba*. The sugars in the honeydew produced by untreated aphids comprised predominantly the monosaccharides glucose and fructose, while the honeydew of antibiotic-treated aphids contained considerable amounts of oligosaccharides of up to 16 hexose units. The honeydew and haemolymph of the aphids were iso-osmotic, and their osmotic pressure was significantly lower in untreated aphids (0.91–0.95 MPa) than in antibiotic-treated aphids (1.01–1.05 MPa) ($P<0.05$). For insects reared on chemically defined diets containing 0.15–1.0 mol l^{-1} sucrose (osmotic pressure 1.1–4.0 MPa), the osmotic pressure of the aphid haemolymph did not vary with dietary osmotic pressure, but was regulated to approximately 1.0 MPa in untreated and 1.3 MPa in antibiotic-treated aphids. The sugars in the aphid honeydew varied with dietary sucrose concentration; with monosaccharides dominant at low concentrations and oligosaccharides dominant at high concentrations of dietary sucrose. The lowest dietary sucrose concentration at which honeydew oligosaccharides were detected was 0.2 mol l^{-1} for the antibiotic-treated aphids and 0.3 mol l^{-1} for untreated aphids. These data indicate that the aphid, and not its associated microbiota, mediates the synthesis of oligosaccharides when the osmotic pressure of the ingesta is high.

Key words: *Acyrthosiphon pisum*, aphid, osmoregulation, sucrose, transglycosylation, oligosaccharide.

Introduction

Many insects of the order Homoptera, including most aphids, feed on the phloem sap of plants, a diet rich in sugars and usually dominated by sucrose. The insects ingest the phloem sugars at rates in excess of their requirement for carbon, and high concentrations of unassimilated sugars are voided in their honeydew (Klingauf, 1987).

The ingestion of phloem sap has implications for the osmotic relationships of insects. As a direct consequence of its high sugar content, the osmotic pressure of phloem sap can be substantially greater than that of the body fluids of the insect, and the feeding insect would therefore be expected to lose water to the gut contents (Kennedy and Stroyan, 1959). It has been shown, however, that aphids feeding on either plants or chemically defined diets of osmotic pressure up to three times that of their haemolymph reduce the osmotic pressure of the ingested fluids, such that the osmotic pressure of the honeydew is comparable to that of their haemolymph (Downing, 1978; Fisher *et al.* 1984). Two processes are involved: the assimilation of ingested sugars (Mittler and Meikle, 1991; Rhodes *et al.* 1996) and the enzymatic transformation of ingested disaccharide sugars to oligosaccharides (Fisher *et al.* 1984; Walters and Mullin, 1988). The importance of oligosaccharide formation to the osmotic relationships of phloem-feeding insects is indicated by the positive relationship between dietary sucrose concentration and oligosaccharide content of the honeydew (expressed as a percentage of total honeydew carbohydrate) for both *Myzus persicae* (Fisher *et al.* 1984) and *Macrosiphum euphorbiae* (Walters and Mullin, 1988). Oligosaccharides have also been identified in the honeydew of phloem-feeding whitefly *Bemisia tabaci* (Hendrix *et al.* 1992; Davidson *et al.* 1994).

The enzymology of sugar transformations in phloem-feeding insects is only partially understood. It is widely accepted that the dietary sucrose in aphids is hydrolysed to its constituent monosaccharides, glucose and fructose, and these are assimilated. What is not known is the identity of the

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enzyme catalysing the hydrolysis. The enzyme has been termed an α-glucosidase (Srivastava and Auclair, 1963; Walters and Mullin, 1988), but whether it can be classified as α-D-glucosidase (EC 3.2.1.20, maltase) or as sucrose α-D-glucohydrolase (EC 3.2.1.48, sucrase) is as yet unclear. Oligosaccharide formation may also be mediated by this α-glucosidase, which catalyses transglycosylation, as described for extracts of the aphid *Macrosiphum euphorbiae* at sucrose concentrations greater than 0.3 mol l^{-1} (Walters and Mullin, 1988). Multiple enzyme systems may, however, contribute to the metabolism of ingested sucrose, because a variety of sugars, including the trisaccharide melezitose and the disaccharide trehalulose, have been described in the honeydew of some phloem-feeding insects (e.g. Bacon and Dickinson, 1957; Byrne and Miller, 1990). The uncertainty about the enzymology of sugar transformations is compounded by the possibility that bacteria associated with the insects may mediate certain of the sugar transformations in the insect. In particular, the honeydew of the whitefly *Bemisia argentifolii* (=*B. tabaci*) contains the disaccharide trehalulose and oligosaccharides; and Davidson *et al.* (1994) implicated bacteria of the genus *Bacillus* in the insect gut in oligosaccharide synthesis, and intracellular bacteria (in insect cells called mycetocytes) in trehalulose production. Early studies of Srivastava and Auclair (1963) also suggested that gut bacteria in the pea aphid *Acyrthosiphon pisum* may contribute to the hydrolysis of ingested sucrose. Resolution of the contribution of bacteria to the sugar composition of insect honeydew is of general physiological importance because if the enzymes are microbial, then the activity or population size of the microbiota would influence the osmotic responses of insects to phloem sap.

The present study arose from experiments on the impact of antibiotics on honeydew production by the pea aphid *Acyrthosiphon pisum* (Prosser *et al.* 1992; Wilkinson and Douglas, 1995*a*), during which it was routinely observed that the honeydew of antibiotic-treated aphids was very ‘sticky’ and viscous. There is a substantial body of data indicating that, at appropriate dosage, an antibiotic selectively disrupts all bacteria associated with aphids (Houk and Griffiths, 1980; Douglas, 1989; Rahbe *et al.* 1994), raising the possibility that bacteria associated with the insects may influence the sugar composition or content of aphid honeydew. The microbiota of most aphids is dominated by bacteria of the genus *Buchnera*, which have been implicated in the nutrition of aphids, especially through the provision of essential amino acids (Douglas, 1988*a*; Douglas and Prosser, 1992; Febvay *et al.* 1995). These bacteria are not located in the insect gut, but in specialised insect cells, mycetocytes, in the haemocoel (Buchner, 1965; Douglas, 1989; Baumann *et al.* 1995).

The purpose of this study was to establish the impact of eliminating the symbiotic bacteria by antibiotic treatment on the honeydew sugars and osmotic pressure of the haemolymph and honeydew of *Acyrthosiphon pisum*. The design and interpretation of experiments were aided by several published studies on the sugar relationships of *A. pisum*. In particular, Auclair (1963) reported that the honeydew of plant-reared *A. pisum* contains sucrose, glucose and fructose, and Rhodes (1992) identified oligosaccharides in the honeydew of *A. pisum* reared on diets containing more than 0.29 mol l^{-1} sucrose. The assimilation of dietary sucrose by *A. pisum* has been investigated by Mittler and Meikle (1991) and Rhodes *et al.* (1996). In addition, the microbiology of the clone of *A. pisum*, OX2, used here has been studied intensively. This aphid bears both *Buchnera* sp. and secondary symbionts (Houk and Griffiths, 1980), but detailed microscopical and molecular analyses (C. T. Davies, L. H. Birkle and A. E. Douglas, unpublished results) have indicated that clone OX2 does not contain other bacteria, including rickettsias and various gut microrganisms present in some other *A. pisum* clones (Chen *et al.* 1996; Grenier *et al.* 1994; Harada *et al.* 1996).

Materials and methods

The aphids

The experimental aphids were derived from a parthenogenetic culture of *Acyrthosiphon pisum* Harris clone OX2 maintained on *Vicia faba* (broad bean) cultivar The Sutton, 3–4 weeks post sowing. Larvae deposited by apterous aphids over 24 h (described as 0-day-old aphids) were reared for 2 days on a chemically defined diet, with or without the antibiotic rifampicin at 50 μg ml^{-1} (Rahbe *et al.* 1994). The diet was formulation A of Prosser and Douglas (1992) containing 0.5 mol l^{-1} sucrose and 0.15 mol l^{-1} amino acids, and was prepared as described in Douglas (1988*b*). The 2-day-old aphids were transferred to *V. faba* or to a diet containing 0.15–1.0 mol l^{-1} sucrose (but otherwise as above). All experimental aphids were maintained in a plant growth cabinet at 18 °C with a 18 h:6 h light:dark photoperiod and a relative humidity of 75 %, conditions that minimised the evaporation of honeydew (T. L. Wilkinson, unpublished results).

The phloem sap of Vicia faba

The phloem sap of *V. faba* was obtained from the severed stylets of aphids, as generated by high-frequency radiomicrocautery (Unwin, 1978), according to the procedure of Pritchard (1996). Immediately after successful cautery of the stylets of a feeding aphid, a drop of immersion oil was applied to the stylets, and droplets of phloem sap exuded into the oil were collected using silanised microcapilliaries.

In preliminary experiments with *A. pisum*, very few severed stylets exuded phloem sap, and phloem sap was therefore collected from the stylets of a different aphid species, *Megoura viciae*, which also utilises *V. faba*. All samples were collected from aphids feeding from the abaxial surface of fully expanded leaves.

The honeydew and haemolymph of aphids

Honeydew produced over 48 h by 7- to 9-day-old aphids was collected on aluminium foil strips positioned below aphids feeding on plants or diets. The sugars were identified by high-

performance liquid chromatography (HPLC), gel-filtration chromatography and matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI/TOF-MS) (Stahl *et al.* 1991). Mono-, di- and trisaccharides were separated isocratically in 70 % acetonitrile by HPLC using a System Gold delivery system (Beckman, UK) equipped with a Spherisorb S5NH column (Hichrom, UK) and Beckman 156 refractive index detector. The complete honeydew glycan profile was determined by gel-filtration chromatography on an Oxford GlycoSystems RAAM 2000 GlycoSequencer with refractive index detection. Mass profiles of honeydew oligosaccharides were obtained from 0.5 µl samples by MALDI/TOF-MS (Finnigan MAT Ltd, UK) with 2,5-dihydroxybenzoic acid as the matrix. Honeydew droplets for osmotic pressure determinations were collected over 12 h in paraffin oil (Merck) positioned below feeding 8-day-old aphids. Individual droplets were recovered from the oil using silanised microcapilliaries. To obtain aphid haemolymph, 8-day-old aphids were immersed in paraffin oil and punctured with a pulse of radio waves from the radiomicrocautery apparatus (see above). Exuding haemolymph was collected from the oil using silanised microcapilliaries.

Osmotic pressure determinations

The osmotic pressure of 0.05–0.1 nl samples of phloem sap and the haemolymph and honeydew of aphids was determined by freezing-point depression (Malone and Tomos, 1992), calibrated against 0–0.6 mol l^{-1} NaCl standard.

Results

Plant-reared aphids

The 2-day-old experimental aphids settled readily onto *V. faba* and fed, producing honeydew. The antibiotic-treated aphids grew more slowly than the untreated aphids, and at 8 days, they weighed 0.75±0.057 mg and 2.22±0.069 mg (mean ± S.E.M., *N*=20), respectively. Insect mortality was less than 5 %. These data are consistent with previous studies on the effect of antibiotic treatment on the performance of *A. pisum* (Douglas, 1992; Wilkinson and Douglas, 1995*b*).

The honeydew sugars of untreated aphids were dominated by monosaccharides with small amounts of sucrose and larger oligosaccharides, as revealed by gel filtration analysis (Fig. 1A). The monosaccharides were identified by HPLC as glucose and fructose, in equimolar proportions (data not shown). The gel filtration profile of the honeydew of antibiotic-treated aphids (Fig. 1B) consisted of a regular series of components, which were identified by MALDI/TOF-MS as oligosaccharides of up to 16 hexose units, plus one compound (peak 4 in Fig. 1B) of intermediate size between 3 and 4 hexose units. This peak was removed by mixed-bed ion-exchange chromatography (data not shown), suggesting that it was not a neutral oligosaccharide. The chemical identity of peak 4 has not been investigated further.

The osmotic pressures of the honeydew and haemolymph of 8-day-old aphids feeding from *V. faba* are shown in Table 1. Both untreated and antibiotic-treated aphids produced honeydew that was broadly iso-osmotic with their haemolymph (see statistical analysis in the legend to Table 1). The osmotic pressure values were, however, significantly greater in antibiotic-treated aphids than in untreated aphids.

The experiments described below were designed to explore the processes underlying the two principal results of this study on plant-reared aphids: first, the difference between the sugar composition of honeydew produced by untreated and antibiotic-treated aphids; and second, the elevated osmotic pressure of haemolymph and honeydew of antibiotic-treated aphids. Two approaches were used. One was an analysis of the

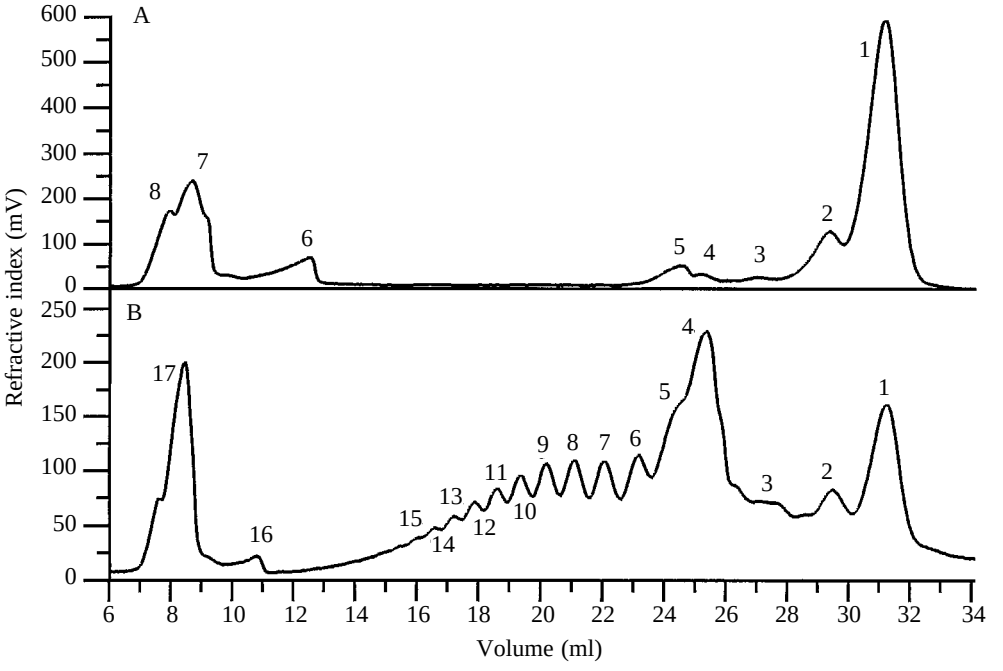

Fig. 1. Gel filtration profiles of honeydew from 7- to 9-day-old *Acyrthosiphon pisum* feeding from *Vicia faba*. (A) Untreated aphids. Peak 1, monosaccharides; 2, disaccharides; 3, trisaccharides; 4 and 5, unknown; 6, baseline shift; 7 and 8, void volume of column. (B) Antibiotic-treated aphids. Peak 1, monosaccharides; 2, disaccharides; 3–5, unknown; 6–15, hexoses of 7–16 units; 16, baseline shift; 17, void volume of column.

Table 1. *The osmotic pressure of haemolymph and honeydew of 8-day-old* Acyrthosiphon pisum *feeding from* Vicia faba

	Osmotic pressure (MPa)	
	Haemolymph	Honeydew
Untreated aphids	0.92±0.026 (18)	0.95±0.033 (8)
Antibiotic-treated aphids	1.07±0.026 (18)	1.05±0.029 (9)

Values are means ± S.E.M. The number of replicates is given in parentheses.

Results from ANOVA: antibiotic treatment $F_{1,49}$=22.3, P<0.001; haemolymph/honeydew, $F_{1,49}$=0.02, P>0.05; interaction, $F_{1,49}$=0.71, P>0.05.

osmotic pressure of the phloem sap of *V. faba*. The other was a study of osmoregulation and honeydew sugars of aphids reared on chemically defined diets containing different sucrose concentrations.

The osmotic pressure of phloem sap utilised by aphids

The secondary veins on the abaxial surface of *V. faba* leaves are the feeding site most commonly used by both untreated and antibiotic-treated *A. pisum* (A. E. Douglas, unpublished results). The mean osmotic pressure of the phloem sap from these veins, as obtained from the severed stylets of the aphid *Megoura viciae*, was 1.91 MPa. A small proportion of antibiotic-treated *A. pisum* fed from tertiary veins and, as the data in Table 2 show, the mean osmotic pressure of phloem sap from these veins is 11–13 % higher than that of the primary and secondary veins, although the difference was not statistically significant ($F_{2,22}$=0.76, P>0.05).

Diet-reared aphids

The detailed analyses of honeydew production by diet-reared apids was restricted to diets containing 0.15–1.0 mol l^{-1} sucrose, which supported aphid growth with very low mortality (data not shown).

The composition of sugars in the honeydew of aphids varied with dietary sucrose concentration. When reared on diets containing more than 0.5 mol l^{-1} sucrose, both the antibiotic-treated and untreated aphids produced honeydew dominated by oligosaccharides (Fig. 2), but the contribution of oligosaccharides to the total sugar content of the honeydew declined progressively with a reduction in the dietary sucrose concentration until, at 0.15 mol l^{-1} dietary sucrose, all the sugars in the honeydew were monosaccharides and sucrose.

Table 2. *The osmotic pressure of phloem sap from* Vicia faba

Leaf vein	Osmotic pressure (MPa)
Primary	1.93±0.175 (5)
Secondary	1.91±0.086 (16)
Tertiary	2.16±0.209 (4)

Values are means ± S.E.M.
The number of replicates is given in parentheses.

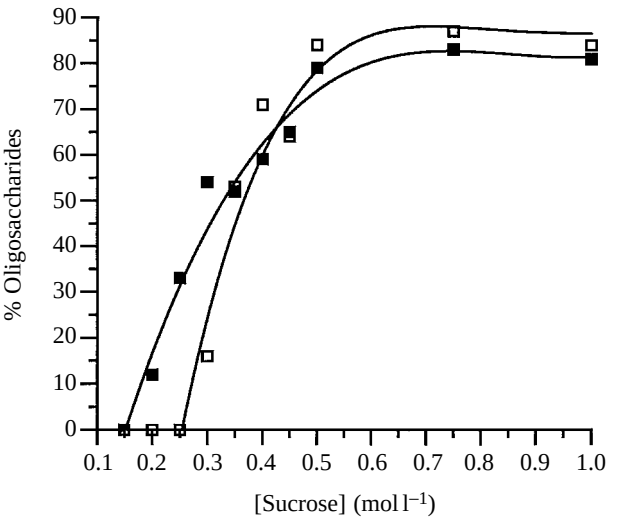

Fig. 2. Contribution of oligosaccharides (as percentage peak area in gel-filtration profiles) to the total honeydew glycan profile of 7- to 9-day-old *Acyrthosiphon pisum* feeding from chemically defined diets containing 0.15–1.0 mol l^{-1} sucrose. □, untreated aphids; ■, antibiotic-treated aphids.

However, as illustrated in Fig. 2, the lowest dietary concentration at which honeydew oligosaccharides were detected differed between the untreated aphids (at 0.3 mol l^{-1}) and antibiotic-treated aphids (0.2 mol l^{-1}). Between 0.2 and 0.3 mol l^{-1} dietary sucrose, only the antibiotic-treated aphids produced honeydew that contained oligosaccharides. We note that the composition of sugars in the honeydew produced by both untreated and antibiotic-treated aphids feeding on diet containing 0.25 mol l^{-1} dietary sucrose paralleled that of plant-reared aphids.

The osmotic pressure of the diets containing 0.15–1.0 mol l^{-1} sucrose was 1.0–4.0 MPa. The osmotic pressure of the haemolymph of aphids reared on these diets was very uniform: approximately 1.3 MPa for the antibiotic-treated aphids and 1.0 MPa for untreated aphids (Fig. 3). This difference between the two groups of aphids was statistically significant (statistical analysis in legend to Fig. 3) and was maintained across the full range of dietary sucrose concentrations. The statistically significant effect of dietary sucrose concentration and the interaction term may be attributable to fluctuations in the osmotic pressure of untreated aphids on diets containing 0.25–0.3 mol l^{-1} sucrose. This fluctuation was consistently obtained and may reflect the shift between monosaccharides and oligosaccharides in the honeydew over this range of dietary sucrose concentrations (see Fig. 2).

Discussion

The key conclusions from this study are twofold. First, antibiotic-treated *A. pisum* produce honeydew containing oligosaccharides (Figs 1, 2). This indicates that the insect, and not its associated bacteria, is responsible for oligosaccharide production in the aphid *A. pisum*, in contrast to the conclusion

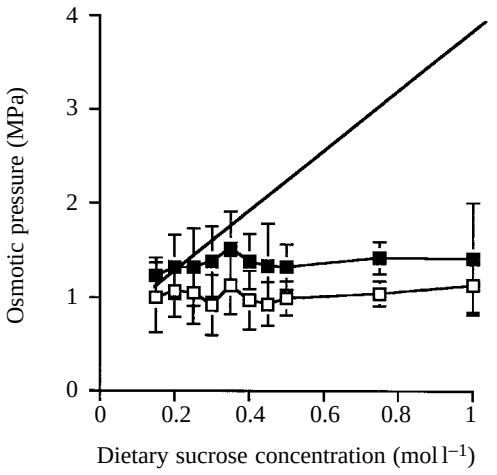

Fig. 3. Osmotic pressure of the haemolymph of *Acyrthosiphon pisum* feeding from chemically defined diets containing 0.15–1.0 mol l^{-1} sucrose. □, untreated aphids; ■, antibiotic-treated aphids (values are means ± 95 % confidence limits for 10 replicates, or five replicates for antibiotic-treated aphids on 0.15 mol l^{-1} sucrose diet). The fitted regression line for osmotic pressure of the diet, measured empirically, is shown. ANOVA: antibiotic treatment, $F_{1,175}$=464.2, P<0.001; dietary sucrose concentration $F_{9,175}$=8.2, P<0.001; interaction, $F_{9,175}$=2.51, 0.05>P>0.01.

of Davidson *et al.* (1994) that the gut microbiota contribute to oligosaccharide synthesis in the whitefly *Bemisia argentifolii*. Second, both untreated and antibiotic-treated *A. pisum* reduce the osmotic pressure of ingested food to values broadly iso-osmotic with their haemolymph (Table 1; Fig. 3). The implication is that the osmotic responses of *A. pisum* are mediated by the aphid and not by its microbiota. More generally, osmoregulation by *A. pisum* is not impaired by antibiotic treatment, a result consistent with the metabolic and behavioural evidence that antibiotics, administered at appropriate levels, do not have a direct deleterious effect on aphids (Douglas, 1988*a*; Wilkinson and Douglas, 1995*a*).

Full interpretation of the results obtained here requires consideration of two further issues: why the osmotic pressure of haemolymph is elevated in antibiotic-treated aphids, and why antibiotic-treated and untreated aphids do not generate honeydew oligosaccharides over the same range of dietary sucrose concentrations. These topics are addressed, in turn, below.

The osmotic pressure of haemolymph in antibiotic-treated aphids

The antibiotic-treated aphids used in this study weighed approximately one-third as much as the untreated aphids. If the osmotic pressure of the haemolymph varied inversely with aphid body size, this difference could account for the elevated osmotic pressure in the haemolymph of antibiotic-treated aphids. As yet, there is no direct information on this issue. Size-related effects are not, however, important in inter-species comparisons of haemolymph osmotic pressure. For example, the osmotic pressure of *Macrosiphum albifrons* (body mass >5 mg) haemolymph, at 1.13 MPa (=476 mosmol l^{-1}) (Pelletier and Clark, 1995), lies within the range of values obtained for *Myzus persicae* (body mass <0.5 mg), at 1.15–1.30 MPa (=485–545 mosmol l^{-1}) (Fisher *et al.* 1984) and 0.83–1.19 MPa (=348–500 mosmol l^{-1}) (Downing, 1978).

An alternative explanation for the elevated osmotic pressure of the haemolymph of antibiotic-treated aphids is that it is a consequence of the elevated amino acid titres in these insects. For 8-day-old *A. pisum* reared on *V. faba*, the amino acid titres of untreated and antibiotic-treated aphids are 34 mmol l^{-1} and 96 mmol l^{-1}, respectively (Adams *et al.* 1996); the discrepancy of 62 mmol l^{-1} corresponds to 0.15 MPa, a value in remarkable agreement with the difference between the osmotic pressure of haemolymph of antibiotic-treated and untreated aphids (Table 1). The elevated amino acid titres in antibiotic-treated aphids have been linked directly to the principal function of the symbiotic bacteria in aphids, the provision of essential amino acids to the aphid tissues (Douglas, 1988*a*; Douglas and Prosser, 1992; Febvay *et al.* 1995; Sasaki and Ishikawa, 1995; Wilkinson and Douglas, 1996). Specifically, it has been proposed that protein synthesis by aphids, whose bacteria are eliminated by antibiotics, is limited by the shortfall of one or a few essential amino acids, and that other non-limiting amino acids accumulate in the aphid tissues (Prosser and Douglas, 1991). If this interpretation is correct, then the elevated osmotic pressure of the haemolymph of antibiotic-treated aphids is a direct consequence of the absence of essential amino acid provisioning by the symbiotic bacteria.

Variation in the oligosaccharide content of aphid honeydew

The demonstration in this study that the oligosaccharide content of the honeydew of *A. pisum* increases progressively with dietary sucrose concentration (Fig. 2) is consistent with previous studies of Fisher *et al.* (1984) on *Myzus persicae*, Walters and Mullin (1988) on *Macrosiphum euphorbiae* and Rhodes (1992) on *A. pisum*. In particular, the detection here of honeydew oligosaccharides in untreated *A. pisum* feeding from diets containing 0.3 mol l^{-1}, but not 0.25 mol l^{-1}, sucrose is in excellent agreement with the conclusion of Rhodes (1992) that honeydew oligosaccharides are generated by *A. pisum* on diets containing more than 10 % (0.29 mol l^{-1}) sucrose. The sugar content of diet-reared aphids (Fig. 2) also provides the basis to interpret the difference between the sugar composition of honeydew produced by untreated and antibiotic-treated *A. pisum* feeding on plants (Fig. 1). On both phloem sap and diet containing 0.25 mol l^{-1} sucrose, antibiotic-treated aphids produced honeydew that contained oligosaccharides, while the honeydew of untreated aphids on these diets lacked oligosaccharides. We predict that both untreated and antibiotic-treated aphids feeding on plants with phloem sap of higher osmotic pressure than *V. faba* would produce honeydew containing oligosaccharides.

The data obtained in this study, however, pose one major problem: that the osmotic pressure of the haemolymph of antibiotic-treated aphids is higher than that of untreated aphids and, therefore, if oligosaccharides have an osmoregulatory

function, one would anticipate oligosaccharide production to be initiated at a higher concentration of dietary sucrose by antibiotic-treated aphids than by untreated aphids. This is the reverse of the observation that the lowest dietary sucrose concentration at which aphid honeydew contains oligosaccharides is $0.2\,mol\,l^{-1}$ for antibiotic-treated aphids and $0.3\,mol\,l^{-1}$ for untreated aphids.

This apparent anomaly may reflect the properties of the enzyme(s) mediating monosaccharide and oligosaccharide synthesis in the aphid gut. The α-glucosidase detected in isolated guts (Srivastava and Auclair, 1963) of aphids is believed to mediate both the hydrolysis of sucrose to glucose and fructose, and, at high sucrose concentrations, transglycosidase activity, i.e. oligosaccharide synthesis (Walters and Mullin, 1988). For other enzymes with similar activity, oligosaccharide production is promoted by the monosaccharide products of sucrose hydrolysis (e.g. Somiari and Bielecki, 1995). If the aphid enzyme has these characteristics, then the accumulation of glucose and fructose in the gut lumen would enhance oligosaccharide synthesis. Antibiotic-treated *A. pisum* assimilate sucrose at lower rates than untreated aphids (Y. Rahbe, personal communication). Monosaccharides are, therefore, expected to accumulate in the gut lumen of the antibiotic-treated aphids, with the resultant promotion of oligosaccharide synthesis at lower dietary concentrations than in the guts of untreated aphids. Research on the biochemical properties of the enzymes in aphid guts is required to assess the validity of this hypothesis.

In conclusion, this study has demonstrated, first, that the aphid, and not its associated microbiota, mediates the enzymatic modification of ingested sugars that is central to the osmotic response of these insects to the high osmotic pressure of plant phloem sap; and, second, that *A. pisum* produces honeydew that is iso-osmotic with the insect haemolymph over a wide range of dietary osmotic pressures. The extent to which the kinetic properties of the aphid α-glucosidase contribute to this remarkable capacity for osmoregulation remains to be established. Other processes (e.g. the rate of monosaccharide uptake across the gut wall, which could vary with the osmotic pressure of the haemolymph) may also play a crucial role in the osmoregulation of aphids.

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