The Journal of Experimental Biology 205, 30090–3018 (2002)
Printed in Great Britain © The Company of Biologists Limited 2002
JEB4395

Amino acid composition and nutritional quality of potato leaf phloem sap for aphids

A. J. Karley[1,*], A. E. Douglas[1] and W. E. Parker[2]

[1]*Department of Biology, University of York, Heslington, York, YO10 5YW, UK* and [2]*ADAS Woodthorne, Wergs Road, Wolverhampton, WV6 8TQ, UK*

*Author for correspondence (e-mail: ajk9@york.ac.uk)

Accepted 24 June 2002

Summary

To define plant 'nutritional quality' for aphids, the causal basis of the variation in aphid performance between host plants of different developmental ages was explored using the aphids *Myzus persicae* and *Macrosiphum euphorbiae* on potato plants (*Solanum tuberosum*). Both aphid species performed better on developmentally young ('pre-tuber-filling') plants than on mature ('tuber-filling') plants. Aphid performance did not vary with leaf phloem sucrose:amino acid ratio but could be related to changes in the amino acid composition of the phloem, which included a developmental shift from high glutamine levels in pre-tuber-filling plants to low glutamine levels in tuber-filling plants. Aphid performance on chemically defined 'young' and 'old' diets, with amino acid composition corresponding to that of phloem amino acid composition in pre-tuber-filling and tuber-filling plants, respectively, confirmed that phloem amino acid composition contributed to low aphid performance on tuber-filling plants. The relatively poor performance on 'old' diets could be accounted for, at least in part, by depressed feeding rates. These data suggest that amino acid composition of the phloem is one factor shaping the nutritional quality of plants for aphids.

Key words: aphid, amino acid, *Myzus persicae*, *Macrosiphum euphorbiae*, plant quality, phloem sap, potato.

Introduction

Variation in the performance and abundance of herbivorous insects has frequently been attributed to variation in host plant quality. Host plant allelochemicals, specific anatomical features and nutrients have all been invoked as determinants of host plant quality (Agrawal, 2001; Baldwin et al., 2001; Mattson, 1980). However, the term 'host plant quality' generates two linked problems. The first problem is circularity. For example, a difference in aphid performance on two host plant treatments is often interpreted as evidence that the plants differ in quality; this difference in quality is used, in turn, to explain the variation in insect performance, without consideration of the plant factors underlying the differences in their quality (e.g. Müller et al., 2001; De Barro, 1992). The second problem is the lack of distinction between correlation and cause. For example, if the two plant treatments above differ consistently in one character (e.g. nitrogen content or the level of an allelochemical), it is often concluded that this character is responsible for the difference in insect performance without any attempt to demonstrate the causal basis for this correlation (e.g. Cisneros and Godfrey, 2001; Docherty et al., 1997; Güntner et al., 1997). The large amount of literature describing multiple plant factors that have been suggested, but not demonstrated, to influence the performance of herbivorous insects does little to promote understanding of the mechanistic basis of insect–plant interactions.

Here, we investigate the causal basis of the reduction in aphid abundance on developmentally mature potato plants relative to young plants (Taylor, 1955; Mackauer and Way, 1976; Parker et al., 2000). Field experiments have previously demonstrated that plant factors contribute to differences in aphid performance for the two principal aphid species infesting potato plants in the UK, *Macrosiphum euphorbiae* and *Myzus persicae* (A. J. Karley, A. E. Douglas, W. E. Parker and J. J. Howard, manuscript in preparation). The present study aimed to establish the suitability (i.e. 'quality') for aphids of young and mature potato plants in terms of their phloem nutrient composition, focusing on the principal phloem nutrients: sucrose and amino acids (Fisher, 2000). Extensive studies of aphid physiology, mainly using chemically defined diets, have revealed the central role of sucrose concentration, amino acid concentration and composition, and sucrose:amino acid ratio in shaping aphid performance (Auclair, 1963; Dadd, 1985; Douglas, 1998).

The specific objectives were: (1) to quantify the nutritional characteristics of, and aphid performance on, potato plants of different developmental age under controlled conditions; (2) to correlate aphid performance with the principal plant nutrient factors of phloem sap carbon and nitrogen; and (3) to identify the causal basis of the link between phloem nutrient composition and aphid performance using chemically defined diets that mimic phloem nutrient profiles of plants at different

developmental ages. By informing us of the plant nutritional factors that influence aphid performance, this approach might lead to the development of crop-management practices that exploit the 'natural' variation in plant suitability for aphids, and thus aid aphid pest management.

Materials and methods

Insects

Clonal cultures of *Myzus persicae* Sulzer (ADAS 99/12, ADAS 99/13) and *Macrosiphum euphorbiae* Thomas (ADAS 99/5, ADAS 99/10, ADAS 99/11) were derived from single parthenogenetic females collected in July 1999 from a commercial potato crop of *Solanum tuberosum* cv Wilja near Wolverhampton, UK (Grid reference OS 813 026); *M. persicae* clone RB/4158 was donated by Professor R. Blackman (Natural History Museum, London, UK). Routine cultures of the aphids were maintained on caged plants or on excised leaves of young (4–8 week-old) plants of *S. tuberosum* cv Wilja (during summer months) or cv Swift (during winter months) at 20°C with 18 h:6 h L:D cycle.

Nutritional indices of plants

The analyses were conducted on potato plants (*Solanum tuberosum*) cv Wilja (Edwin Tucker and Sons Ltd, Devon, UK) grown from tubers in 10 l pots of John Innes No. 3 compost, under glass (15–24°C) between June and August 1999. The plants were watered daily and sprayed weekly with nicotine as insect control. Sampling commenced when the plants reached the emergence and shoot-expansion stage (Jefferies and Lawson, 1991), at 3 weeks after planting, and continued until tuber filling had become the principal dry matter sink at 10.5 weeks after planting.

There were three elements to each harvest: (1) Phloem sap analysis by the EDTA exudation technique of King and Zeevart (1974). Briefly, the terminal leaflet was excised from a compound leaf midway along the shoot axis and inserted immediately into 0.2 ml 5 mmol l^{-1} EDTA solution, pH 7.5. The samples were incubated for 90 min in the dark in a sealed chamber equilibrated at 25°C with a dish of saturated KH_2PO_4 to maintain high humidity. The EDTA samples were frozen at –20°C until ready for analysis of sugar and amino acid content (see chemical analyses below). Parallel experiments confirmed that the same array of amino acids detected in the EDTA phloem exudates was also detected in phloem exudates obtained from severed stylets of aphids feeding from potato plants (K. V. Pescod, A. J. Karley and A. E. Douglas, unpublished data). (2) Leaf carbon:nitrogen (C:N) content. The leaflet used for phloem exudation was weighed, dried at 60–75°C for 48 h and re-weighed, then ball-milled to a fine powder. The carbon and nitrogen contents were determined by gas chromatography using a C,H,N NA2100 Brewanalyser (CE Instruments, Wigan, UK) with urea standards. (3) Dry matter accumulation. The above-ground (shoots, leaves and flowers/berries) and below-ground (roots, stolons and tubers) parts of each plant were cleaned of soil, dried at 60–75°C for 48 h and weighed to an accuracy of 0.01 g.

Aphid performance

The performance of *M. persicae* ADAS 99/12 and *M. euphorbiae* ADAS 99/11 on potato plants cv. Wilja was quantified under glasshouse conditions (described above). Newborn nymphs were confined in mesh-covered clip-on leaf cages of 2.5 cm internal diameter (clip-cages) attached to the abaxial surface of a leaf midway along the shoot axis of 'pre-tuber-filling' (3–5 weeks after planting) or 'tuber-filling' (9–11 weeks after planting) plants. Aphids were transferred to fresh plants (3-weeks-old and 9-weeks-old) at fortnightly intervals throughout the experiment. All six aphid clones were used for the performance analysis on chemically defined diets because of considerable interclonal variation in the performance of various aphid species on diets (Srivastava et al., 1985; Sandström and Pettersson, 1994). 2-day-old nymphs from aphid cultures on plants were transferred to diet sachets, prepared according to Prosser and Douglas (1992). Diets contained 0.5 mol l^{-1} sucrose, 0.15 mol l^{-1} amino acids and vitamins, mineral ions and organic acids (as described by Prosser and Douglas, 1992). The diet solution was buffered with KH_2PO_4 to give a final pH of 7.5. The diet amino acid compositions representative of the phloem sap of pre-tuber-filling and tuber-filling potato plants are shown in Table 1, and the diets containing these amino acids are described as 'young' and 'old', respectively. The relative composition of the amino acids of phloem exudates sampled from plants at five and 10.5 weeks after planting was used as the basis for diet construction (Table 1). These mol% values were adjusted to allow inclusion of cysteine and proline, which were not detected by our high-performance liquid chromatography (hplc) method. Additionally, the percentage of essential amino acids was increased by 55% in both diets because aphids do not settle readily or thrive on diets that mimic the low essential amino acid contents of phloem sap (A. E. Douglas, unpublished data). Taking into account these two adjustments, the mol% and mmol l^{-1} values for each amino acid used in the diets are shown in Table 1.

For aphids on plants or diets, the experiments were monitored daily. The dates when they reached adulthood, initiated reproduction and died were scored, and any offspring produced were counted each day and removed. Daily fecundity was quantified as (total number of offspring/adult lifespan in days). R_m (estimated intrinsic rate of population increase) was calculated as $0.738(\ln N)/T_d$, where N is the number of offspring produced by an aphid in the time period equivalent to the pre-reproductive development period (T_d) (Wyatt and White, 1977). For the diet-reared aphids, the relative growth rate (RGR) was also determined as $\ln(f/i)/t$, where i and f are the fresh masses of 2-day-old nymphs and teneral adults, respectively, and t is the development time. Aphids were weighed to the nearest μg on a Mettler MT5 microbalance. Any alate adults generated were excluded from performance analyses.

Table 1. *Amino acid composition of diets formulated to mimic the phloem sap composition of developmentally young and mature potato plants*

Amino acid[a]	Week 5 plants[b] mol%	'Young' diet mol% (mmol l^{-1})	Week 10.5 plants[c] mol%	'Old' diet mol% (mmol l^{-1})
Alanine (Ala)	4.1	3.3 (4.9)	4.0	2.8 (4.2)
Arginine[d] (Arg)	2.6	3.8 (5.7)	3.0	4.4 (6.6)
Asparagine (Asn)	6.1	4.9 (7.4)	4.7	3.3 (4.9)
Aspartate (Asp)	12.8	10.4 (15.6)	14.1	9.8 (14.7)
Cysteine (Cys)	–	1.5 (2.2)	–	1.3 (1.9)
Glutamine (Gln)	30.1	24.4 (36.6)	11.0	7.6 (11.5)
Glutamate (Glu)	16.7	13.5 (20.3)	23.9	16.6 (24.9)
Glycine (Gly)	1.8	1.4 (2.1)	1.5	1.0 (1.5)
Histidine[d] (His)	0.7	1.0 (1.4)	1.2	1.8 (2.7)
Isoleucine[d] (Ile)	1.6	2.3 (3.4)	3.7	5.4 (8.0)
Leucine[d] (Leu)	1.6	2.3 (3.4)	4.2	6.2 (9.3)
Lysine[d] (Lys)	2.4	3.5 (5.2)	3.6	5.2 (7.8)
Methionine[d] (Met)	1.0	1.5 (2.2)	1.3	1.9 (2.9)
Phenylalanine[d] (Phe)	2.1	3.0 (4.6)	4.0	5.8 (8.7)
Proline (Pro)	–	3.3 (4.9)	–	2.8 (4.2)
Serine (Ser)	6.7	5.4 (8.1)	5.5	3.8 (5.7)
Threonine[d] (Thr)	4.3	6.4 (9.6)	4.1	6.0 (9.1)
Tryptophan[d] (Trp)	2.4	3.5 (5.3)	4.1	6.0 (8.9)
Tyrosine (Tyr)	0.5	0.4 (0.6)	1.3	0.9 (1.4)
Valine[d] (Val)	2.8	4.1 (6.2)	4.9	7.1 (2.9)
Essential amino acids (% of total)	22.4	31.4	34.0	50.0

[a]Abbreviated amino acid names are shown in parentheses.
[b]Pre-tuber-filling plants.
[c]Tuber-filling plants.
[d]Essential amino acids as defined by Morris (1991).

Nutrient uptake and assimilation

The radiolabelled inulin technique (Wright et al., 1985; Wilkinson and Ishikawa, 1999; Douglas et al., 2001) was used to quantify aphid feeding and the uptake and assimilation of dietary glutamate and glutamine by diet-reared aphids. The purity of radiochemicals was confirmed by thin-layer chromatography (tlc) and hplc. 2-day-old nymphs were reared to the final instar (6-day-old nymphs for *M. persicae*; 8-day-old nymphs for *M. euphorbiae*) on the 'young' and 'old' diets. Ten replicate aphid pairs were then allowed to feed for 48 h from the same diet formulation but supplemented with [^{3}H]inulin (Sigma, 1 mCi ml^{-1}) at 0.3 MBq ml^{-1} diet solution and either L-[U-^{14}C]glutamate or L-[U-^{14}C]glutamine (Amersham Pharmacia Biotech UK Ltd, 50 μCi ml^{-1}) at 0.3 MBq ml^{-1} diet. The radioactive diet sachets were administered on a Perspex ring (2.5 cm diameter, 0.7 cm height) above a GF/C glass-fibre filter (2.5 cm diameter, Whatman), so that honeydew produced by the aphids accumulated on the filter. Aphids feeding from replicate non-radioactive sachets were included as controls. Each aphid pair was homogenised in 0.2 ml ice-cold 0.05 mol l^{-1} Tris-HCl, pH 7.5. To quantify the radioactivity, the filters and samples of the aphid homogenates were shaken with Ultima-Gold™ XR scintillation fluid (Packard Bioscience B.V., Gröningen, The Netherlands) and counted in a Packard Tri-Carb Liquid Scintillation Analyzer using pre-set ^{3}H/^{14}C dual windows. The volume of diet ingested was calculated from the recovery of [^{3}H]inulin in honeydew; inulin is not transported across aphid guts nor metabolised by these aphids (A. E. Douglas, unpublished data). The amount of dietary [^{14}C]amino acid assimilated by the aphids was calculated from the difference between the amount ingested (as determined from feeding rate) and the amount recovered as ^{14}C in the honeydew. The respiratory loss was estimated from the difference between the amount assimilated (obtained as described above) and the ^{14}C content of the aphid tissues (see Wilkinson et al., 2001).

Chemical analyses

The sucrose content of phloem exudates was quantifed by the method of Dahlqvist (1984). Each 10 μl exudate sample was hydrolysed to completion with 10 U invertase (Cat. no. I-4504, Sigma-Aldrich, Gillingham, Dorset) per ml in 50 mmol l^{-1} sodium acetate buffer, pH 4.5 at 37°C for 30 min, and the glucose produced was determined by the Sigma Diagnostics glucose assay kit (GAGO-20) using glucose standards, following manufacturer's instructions but with *o*-dianisidine concentration increased to 100 μg ml^{-1}.

Amino acids in phloem exudates and aphid honeydew were separated by reverse-phase hplc, following derivatization with *o*-phthaldialdehyde (Jones et al., 1981), using a Hewlett-

Packard HP1100 Series autosampling LC system with C_{18} ZORBAX™ Eclipse XDB-C8 column and fluorescence detection. Amino acids were quantified by comparison with the AA-S-18 (Sigma) reference amino acid mixture, supplemented with asparagine, glutamine and tryptophan. All protein amino acids, except proline and cysteine, could be detected using this method, with a detection limit of approximately 0.5 pmol.

The protein content of aphid homogenates was quantified by the microassay method of BioRad, following manufacturer's instructions, with bovine serum albumin as standard.

Statistical analyses

Parametric statistical tests were applied to datasets confirmed to be normally distributed (Ryan–Joiner one-sample test) with homogeneous variances (Bartlett's test), which required logarithmic transformation where indicated. Analysis of variance (ANOVA) or *t*-tests were applied to test the impact of diet composition on aphid RGR, R_m and feeding rate. Where appropriate, 'clone' was nested as a random factor within 'species' in the ANOVA model, and aphid protein content was included as a covariate. In the multivariate analysis of variance (MANOVA) analyses of phloem exudate amino acids, normality or homogeneity of datasets could not be achieved for all variates (even following exclusion of outlier values identified using critical values for Dixon's test) and, therefore, replicate MANOVA tests were used with and without the data for these variates. Principal components analysis (PCA) with a correlation matrix to standardise variables (Randerath, 1996) was used to explore the impact of plant age on the amino acid composition of phloem exudates. The non-parametric tests used were Kruskall–Wallis and Spearman Rank tests.

Results

Plant characteristics

The total dry mass of the potato plants increased from shoot emergence through to tuber development, both for below-ground (roots and tubers) and above-ground (stems, leaves, flowers/berries) parts of the plant (Fig. 1A). Dry matter was predominantly partitioned into the shoot until 7–9 weeks after planting (the 'pre-tuber-filling' period). A switch in partitioning occurred at 9.5 weeks after planting, when dry matter accumulation in the roots and tubers exceeded shoot dry-matter accumulation, and tuber filling became the predominant dry-matter sink. Flower buds started to appear at 5 weeks after planting, and flowers started opening at 7 weeks after planting; flowers that developed into berries did so from 9–10 weeks after planting (data not shown).

The C:N ratio in oven-dried leaf tissue increased steadily throughout the first 9 weeks of growth, then increased rapidly over the remainder of the experimental period (Fig. 1B). The C:N ratio data did not conform to the criteria of normality of error distribution and homogeneity of variance required for parametric statistical testing. The ratio of sucrose:amino acid (mol:mol) in phloem exudates varied significantly during the experiment (Kruskal–Wallis: H_6= 21.65, P<0.01). Spearman rank correlation analysis demonstrated no significant correlation between leaf C:N and exudate sucrose:amino acid over time nor for individual sampling dates (Table 2).

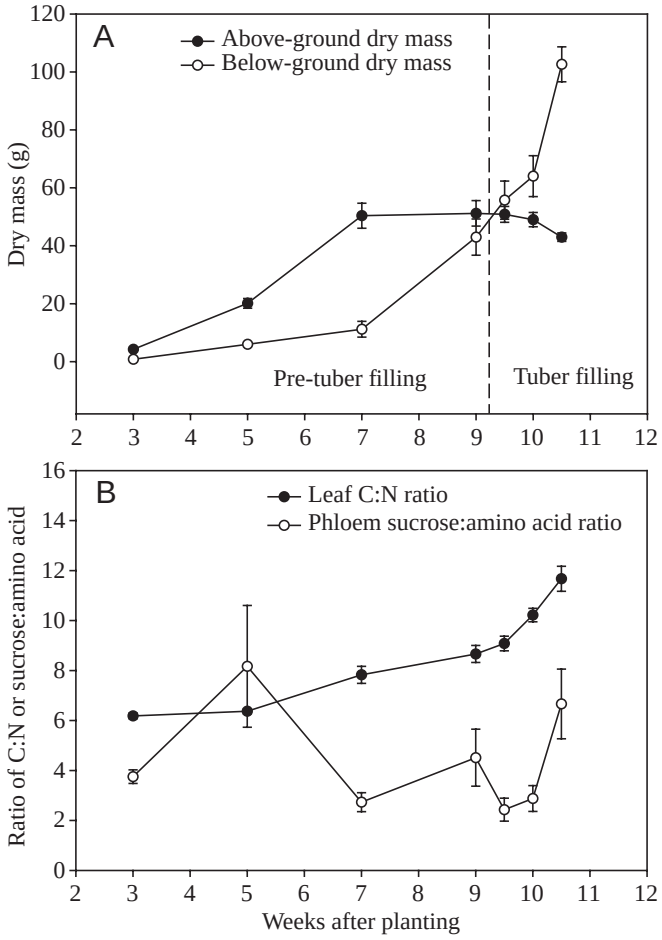

Fig. 1. (A) Mean dry mass of above-ground (stems, leaves, flowers/berries) and below-ground (roots, tubers, stolons) plant parts measured in potato plants grown under glass. The broken line indicates the switch in dry-matter partitioning (see text for explanation). Values are the means ± S.E.M. of ten plants. (B) Ratio of carbon to nitrogen (C:N) (as percentage of dry mass) in dried leaf tissue and the molar ratio of sucrose to amino acid (sucrose:amino acid) in leaflet phloem exudates sampled from potato plants grown under glass. Values are the means ± S.E.M., N=9–10.

The amino acids in phloem exudates were dominated by the non-essential amino acids glutamate and aspartate and their amides, accounting for 28–56% of the total amino acids. The amino acid composition of exudates varied significantly with time (MANOVA of log-transformed nmol exuded data, Wilk's test: $F_{108,225}$=4.549, P<0.01). To explore the variation in phloem amino acid composition, PCA was applied to compare data for 'pre-tuber-filling' dates and 'tuber-filling' dates, i.e. the dates before and after the switch in dry matter allocation at 9–9.5 weeks after planting (see Fig. 1A).

The first two principal components (PC 1 and PC 2) accounted for 51% of the variation in the dataset (Fig. 2). A

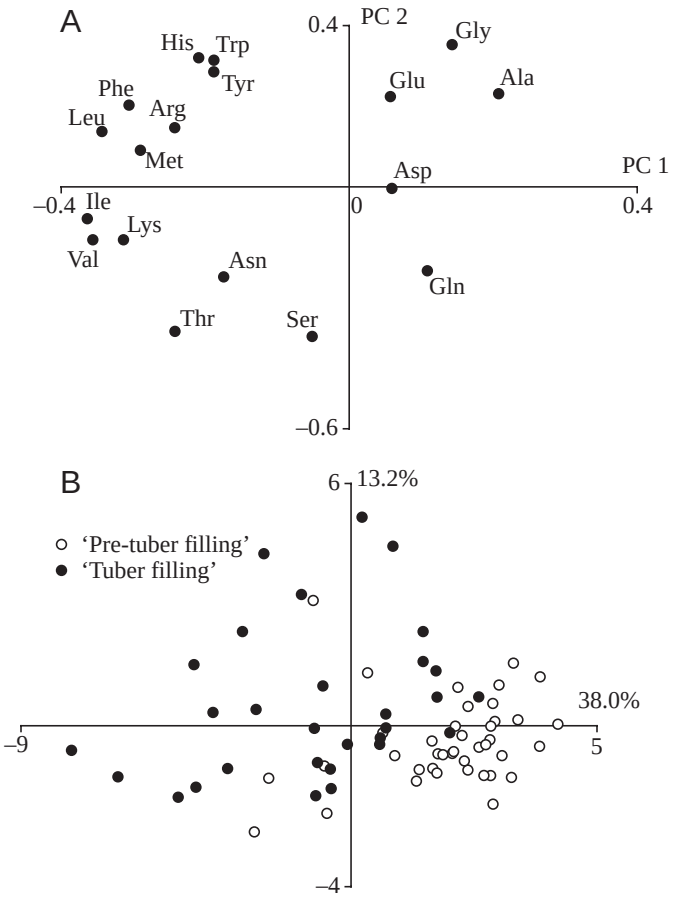

Fig. 2. Principal component analysis of amino acid mol% data in leaf phloem exudates sampled from potato plants grown under glass during sequential stages of plant development: ‘pre-tuber filling’ and ‘tuber filling’ (see text and Fig. 1 for explanation). (A) Attribute loadings on the first two components PC 1 and PC 2. Amino acid abbreviations are denoted in Table 1. (B) Plot of the sample scores on PC 1 and PC 2 which explain, respectively, 38.0% and 13.2% of the variation in the dataset.

plot of the amino acid attributes revealed that PC 1 tended to separate essential from non-essential amino acids (see Table 1 for list of essential and non-essential amino acids), while the amino acids glutamate and aspartate were separated from their amides, glutamine and asparagine, by PC 2 (Fig. 2A). Other non-essential amino acids (e.g. glycine, serine and alanine) were also separated along PC 2 (Fig. 2A). These two axes achieved good, but not perfect, separation of the ‘pre-tuber-filling’ and ‘tuber-filling’ samples (Fig. 2B), indicating a shift during plant development in the amino acid composition of the exudates. The key shift was from pre-tuber-filling plants with exudates dominated by non-essential amino acids, particularly glutamine, asparagine, serine and threonine (with an average of 22.4% essential amino acids in week 5), to tuber-filling plants with exudates dominated by the essential amino acids (with an average of 34% essential amino acids in week 10.5) and by variation in the non-essential amino acids glutamate, glycine and alanine.

Table 2. *Spearman rank correlation coefficients for leaf carbon:nitrogen ratio and exudate sucrose:amino acid ratio on each sampling date*

Weeks after planting	Correlation coefficient	Probability value
3	0.576	0.082
5	0.317	0.406
7	0.164	0.651
9	0.297	0.405
9.5	0.139	0.701
10	0.91	0.803
10.5	0.576	0.082
All time points	–0.085	0.487

Similar results were obtained in a separate experiment on the amino acid content of phloem exudates of potato plants reared in June–August 2001 under glasshouse conditions and infested with aphids (data not shown). This indicated that the time-dependent shift in phloem amino acid composition of potato plants was linked to plant development and occurred independently of aphid infestation.

Aphid performance

Aphids of *M. persicae* ADAS 99/12 and *M. euphorbiae* ADAS 99/11 performed less well on tuber-filling plants (9–11 weeks) than on pre-tuber-filling plants (3–5 weeks) by the indices of development time and survival to adulthood, mean daily fecundity and R_m (Table 3).

The chemically defined diets were acceptable to all clones of both *M. persicae* and *M. euphorbiae*, as indicated by the ready settling of the 2-day-old nymphs onto the diet sachets, by low pre-adult mortality (<20% for *M. persicae* clones and <27% for *M. euphorbiae* clones) and by a low incidence of stillborn offspring (0.1–4.5% of offspring produced by each clone). The mean age at death did not vary significantly between those feeding on ‘young’ and ‘old’ diets for any clone (data not shown), with the exception of *M. persicae* clone ADAS 99/12, for which the aphids feeding on the ‘old’ diet formulation died before those on ‘young’ diets (mean age at death of 34.5 days and 50 days, respectively).

The mean RGR of nymphs varied from 0.23 g^{-1} g^{-1} d to 0.32 g^{-1} g^{-1} d for *M. euphorbiae* clones and from 0.32 g^{-1} g^{-1} d to 0.37 g^{-1} g^{-1} d for *M. persicae* clones and, for all clones except *M. euphorbiae* clone ADAS 99/11, the mean RGR was greater on the ‘young’ diet than on the ‘old’ diet (Fig. 3A). ANOVA revealed that the effects of both diet formulation and aphid species, but not the interaction term, were statistically significant; the term ‘aphid clone’ was also significant (Fig. 3A). R_m displayed a similar pattern to RGR (Fig. 3B). The mean R_m was significantly higher for *M. persicae* than for *M. euphorbiae* and also for aphids on ‘young’ diet rather than on ‘old’ diet, with significant interclonal variation but non-significant interaction term (Fig. 3B). Inspection of the data revealed that the higher R_m of aphids on the ‘young’ diet compared with the ‘old’ diet mainly resulted from their greater

Table 3. *Aphid performance on developmentally pre-tuber-filling and tuber-filling plants*

Species	Plant age	% surviving to adulthood	Median development time in days	Mean fecundity (nymphs adult^{-1} day^{-1})[a]	R_m[a]
Macrosiphum euphorbiae (ADAS 99/11)	Pre-tuber-filling	90.0 (40)	9 (34)	1.28 (±0.10)	0.254 (±0.007)
	Tuber-filling	82.5 (40)	10 (33)	0.70 (±0.11)	0.193 (±0.009)
		Fisher's exact $P>0.05$	$H_1=18.93$ $P<0.01$	$t_{17}=3.80$ $P<0.01$	$t_{14}=5.43$ $P<0.01$
Myzus persicae (ADAS 99/12)	Pre-tuber-filling	92.2 (77)	8 (72)	0.91 (±0.10)	0.245 (±0.006)
	Tuber-filling	63.8 (69)	12 (44)	0.41 (±0.04)	0.152 (±0.011)
		$\chi^2_1=17.427$ $P<0.01$	$H_1=75.09$ $P<0.01$	$t_{18}=4.92$ $P<0.01$	$t_{17}=6.91$ $P<0.01$

[a]Values are the means ± S.E.M., N=7–10 clip-cages.

fecundity rather than a difference in the pre-reproductive development period (data not shown).

The R_m values for *M. persicae* ADAS 99/12 and *M. euphorbiae* ADAS 99/11 on pre-tuber-filling and tuber-filling plants are included on Fig. 3B for comparison. With the exception of *M. euphorbiae* ADAS 99/11 on pre-tuber-filling plants, values of R_m on plants were lower than those on diets.

Aphid feeding and nutrient assimilation

Aphid feeding rate of final instar *M. persicae* aphids on the 'young' and 'old' diet formulations is shown in Fig. 4. ANCOVA (shown in Fig. 4) revealed that diet uptake by *M. persicae* was significantly depressed on the 'old' diet, relative to the 'young' diet, even after the effect of body size (measured as aphid protein content, which also varied significantly) on feeding rate was taken into account as the covariate; i.e. the low feeding rates on 'old' diet could not be contributed entirely to small aphid size. The feeding rate of *M. persicae* also varied significantly between clones, but this variation could not be related to interclonal differences in size (aphid protein content) or any other aspect of aphid performance, and its basis was not investigated here.

Radiolabelled glutamate or glutamine was included in the diet sachets used for analysis of aphid feeding rates displayed in Fig. 4, so that aphid metabolism of dietary glutamate and glutamine, the dominant amino acids in the diets (and in potato phloem sap) could be monitored. Less than 10% of the radioactivity from both [^{14}C]glutamine and [^{14}C]glutamate was recovered from the honeydew of aphids (*M. persicae* ADAS 99/12, ADAS 99/13 and RB/4158, *M. euphorbiae* ADAS 99/11) feeding from both diet formulations, and, consistent with previous studies on other aphid species (Febvay et al., 1995; Wilkinson et al., 2001), <80% of the assimilated radioactivity was lost from the aphids under all treatments, presumably by respiration. No substantial difference was identified in the metabolism of these amino acids by aphids on the two diets.

Discussion

Aphid performance and developmental changes in plant nutritional quality

A key result of this study is that the superior performance of aphids on pre-tuber-filling potato plants, compared with tuber-filling plants (Table 3), correlated with changes in the phloem amino acid composition and with the increase in total leaf C:N ratio; there were no clear trends in phloem sucrose:amino acid ratio that could predict the decline in aphid performance. Poor aphid performance on developmentally mature plants is consistent with data for several aphid species (van Emden and Bashford, 1971; Williams, 1995; Kazemi and van Emden, 1992), and our data reflect those of other studies that have identified a correlation between aphid performance and phloem amino acid composition (Weibull, 1988; Kazemi and van Emden, 1992; Sandström and Pettersson, 1994).

We have evidence that the correlation between aphid performance and potato phloem amino acid composition is robust because consistent developmental shifts in amino acid composition have been demonstrated for potato plants over two consecutive years under glass (this study) and across three consecutive years in the field (A. J. Karley et al., manuscript in preparation). In addition, studies involving a number of plant species have confirmed that the amino acid composition of phloem sap derived from EDTA-induced exudation reflects that of stylectomy-derived phloem sap (Weibull et al., 1990; Sandström et al., 2000), including potato (K. V. Pescod, A. J. Karley and A. E. Douglas, unpublished data).

A striking aspect to the phloem amino acid composition data is that the dominant amino acid in the phloem sap of pre-tuber-filling plants is glutamine, which declines dramatically as the

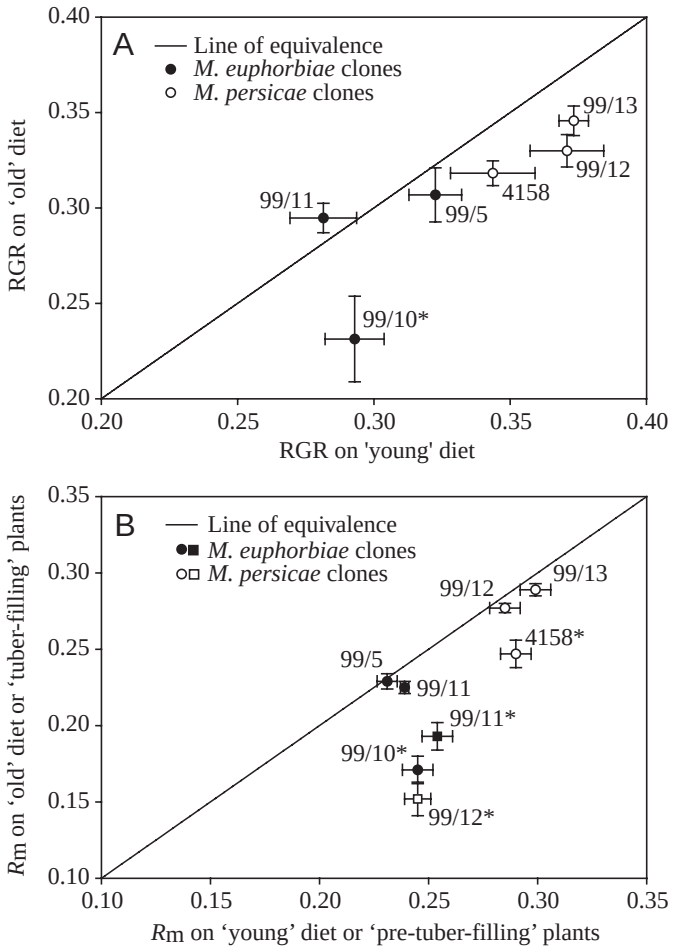

Fig. 3. Variation in (A) the relative growth rate (RGR) and (B) the estimated intrinsic rate of population increase (R_m) of aphids developing from offspring raised on 'young' and 'old' diets (circles) and for 'pre-tuber-filling' and 'tuber-filling' plants (squares). Values are either the mean RGR ± S.E.M. (N=8–15) or the mean R_m ± S.E.M.. (N=7–14) for clones of *Myzus persicae* (open symbols) or *Macrosiphum euphorbiae* (closed symbols). Points falling below the line of equivalence indicate clones that perform better on the 'young' diet. *Clones showing a significant difference, in post-hoc analysis, between 'young' and 'old' diets. ANOVA for RGR: diet: $F_{1,129}$=13.57, P<0.001; species: $F_{1,129}$=10.72, P<0.05; interaction: $F_{1,129}$=0.37, P>0.05; clone (subspecies): $F_{4,129}$=5.60, P<0.001. ANOVA for R_m: diet: $F_{1,120}$=31.95, P<0.001; species: $F_{1,120}$=37.87, P<0.001; interaction: $F_{1,120}$=1.43, P>0.05; clone (subspecies): $F_{4,120}$=4.75, P<0.01.

plants develop, allowing glutamate and aspartate to predominate in the phloem of tuber-filling plants. High relative levels of glutamate have been implicated in reduced nutritional quality of phloem sap for aphids (Douglas, 1993) and in reduced aphid performance on 'resistant' cultivars of some plant species (Chen et al., 1997; Weibull, 1988). A second feature is that the phloem of tuber-filling plants is relatively enriched in essential amino acids, nutrients that animals cannot synthesise *de novo*. Thus, the phloem of pre-tuber-filling plants could be regarded as less nutritious than that of tuber-filling plants, in apparent contradiction with the poor performance of aphid clones on the latter (and on 'old' diet composition). However, aphids are nutritionally 'buffered' from variation in the dietary supply of these nutrients because they obtain supplementary amino acids from their symbiotic bacteria, *Buchnera aphidicola* (Douglas, 1998; Shigenobu et al., 2000), and the rate of bacterial synthesis of amino acids increases in response to low dietary supply (Febvay et al., 1999; Douglas et al., 2001). Thus, the impact of the non-essential amino acid component of the diet on aphid performance must outweigh any effects of the essential amino acids.

The nitrogen content of the diet is known to influence aphid performance (Prosser et al., 1992; Abisgold et al., 1994; Girousse and Bournoville, 1994; Febvay et al., 1988) but it was not determined in this study because the EDTA exudation technique does not allow quantification of the amount of phloem exuded nor, therefore, of phloem nutrient concentrations (Weibull et al., 1990). However, the absence of a clear developmental trend in phloem sucrose:amino acid ratio (Fig. 1B), which can be quantified reliably by the EDTA method, precluded diet construction to examine the impact of developmental shifts in total nitrogen availability on aphid performance.

In the absence of phloem analysis, the poor aphid performance on tuber-filling plants might have been attributed to elevated leaf C:N ratio (Fig. 1B), which is widely recognised as an indicator of low plant nutritional quality. However, the total C:N ratio of plant tissue did not covary with the sucrose:amino acid ratio of the phloem sap on which the aphids feed, suggesting that plant indices based on the elemental composition (e.g. C:N, N content per unit dry mass) of the total plant tissue are not necessarily an accurate index of the nutritional quality of the plant for phloem-feeding insects.

We reasoned that if the correlation between phloem amino acid composition and aphid performance could be reproduced with chemically defined diets, then phloem amino acid composition is a likely factor contributing to the difference in aphid performance between the pre-tuber-filling and tuber-filling plants. The results obtained across multiple clones provide general qualitative support and contribute to a sparse literature that attempts to translate correlative observations into dietary studies of the causal basis for nutritional effects on aphid performance (Sandström, 1994, 2000; Bolsinger and Flückiger, 1989). Exact replication of performance between plant and diets would not be expected because the diets, by definition, lack the mechanical and olfactory cues of a plant. The performance of *M. persicae* clone ADAS 99/12 on both diet formulations was superior to its performance on potato plants (Fig. 3B), indicating that potato is not an optimal host for this aphid species. In addition, the R_m for *M. euphorbiae* clone ADAS 99/11 on tuber-filling plants was lower than that on 'old' diet (Fig. 3B). These two observations indicate that additional plant factors (allelochemical or physical) not monitored in this study also contribute to the reduction in aphid performance on tuber-filling plants. The performance of both aphid species on the 'young' diet was comparable with that on

favoured plants such as spring cabbage (*M. persicae*; Jenkins, 2001) and immature potato plants (*M. euphorbiae*; Fig. 3B) and, consequently, the results obtained with diet-reared aphids cannot be attributed to a non-specific malaise on diets.

Although the physiological basis of the difference between aphid performance on 'young' and 'old' diets remains to be resolved fully, the results summarised in Fig. 4 indicate that it involves reduced feeding rate on the 'old' diet. This is despite the higher concentration of the amino acid methionine, a known phagostimulant for aphids (Mittler, 1967; Srivastava and Auclair, 1974), in the 'old' diet than in the 'young' diet. [However, dietary glutamate inhibits feeding by the pea aphid *Acyrthosiphon pisum* (Srivastava et al., 1983) and its effects might obscure those of methionine].

A potentially rewarding line of future investigation relates to the reduced amide:acid ratio of the 'old' diet. Much of the glutamine and glutamate ingested by aphids is respired (Febvay et al., 1995; Wilkinson et al., 2001), and the release of the carbon skeleton (α-ketoglutarate) of these amino acids for entry into the tricarboxylic acid (TCA) cycle involves removal of amino groups. De-amination releases two amino groups per proton for glutamine but only one amino group per proton for glutamate (this will also be true for asparagine and aspartate, respectively, if these are de-aminated and transaminated to form glutamate); high relative levels of glutamate and aspartate could increase the acid load, which would have implications for pH homeostasis in the aphid tissues. The resultant metabolic stress might contribute to the difference in aphid performance between 'young' and 'old' diets and pre-tuber-filling and tuber-filling plants. It might, however, be simplistic to attribute variation in aphid performance on diets and plants of different amino acid composition to a change in the concentration of single amino acids. The impact of dietary amino acid composition on aphid nutrition and general physiology might be driven by complex interactions between the various amino acids.

Why does the phloem nutrient profile vary with plant developmental age?

When considering variation in phloem amino acid composition, it is important to distinguish between the developmental age of plant parts and of the entire plant. This study sampled from the fully expanded 'source' leaf at the node midway along the shoot axis and, thus, was considered to be developmentally equivalent across the different plant ages studied. Consequently, the variation in phloem amino acid composition obtained in Fig. 2 relates exclusively to developmental age of the plant. Complementary study of field-grown potato plants has revealed that the shift in amino acid composition with plant developmental age is broadly uniform across leaves from the apex to basal position along the shoot axis (A. J. Karley, A. E. Douglas, W. E. Parker and J. J. Howard, manuscript in preparation). In other words, the developmental effects obtained are systemic, at least with respect to the shoot system. Developmental changes in phloem amino acid composition have not been studied widely; but,

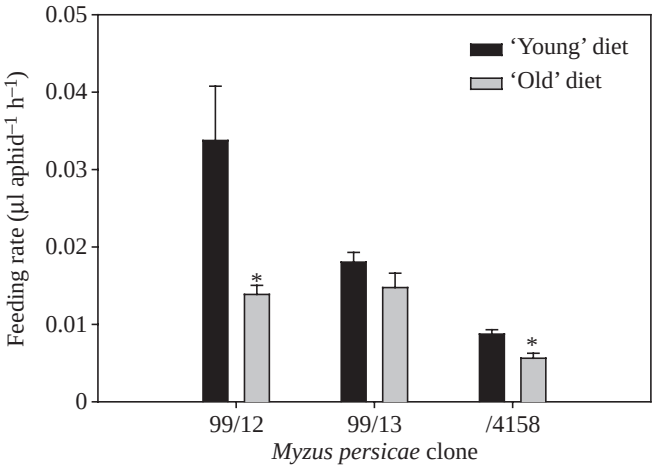

Fig. 4. Feeding rate on radiolabelled 'young' and 'old' diets by three clones of *Myzus persicae*. Values are the means ± S.E.M. (N=15–20). Analysis of covariance (ANCOVA) after log-transformation of feeding rate: aphid protein content (covariate): $F_{1,111}$=9.31, P<0.05; diet: $F_{1,111}$=33.86, P<0.05; clone: $F_{2,111}$=52.90, P<0.05; interaction: $F_{2,111}$=1.09, P>0.1. *Significant difference between diets (two-sample t-test of log-transformed data: ADAS 99/13, RB/4158; or Mann–Whitney U-test of untransformed data: ADAS 99/12) using Bonferroni correction for multiple tests.

Corbesier et al. (2001) reported high phloem glutamine levels exported from leaves of white mustard *Sinapis alba* and thale cress *Arabidopsis thaliana* at flowering induction (which is broadly equivalent to the pre-tuber-filling plants of potato), and Boggio et al. (2000) linked high glutamate levels in the pericarp of the tomato *Lycopersicon esculentum* to phloem import of glutamate during fruit ripening (which is equivalent to the tuber-filling stage in potato). These results raise the possibility that the developmental shifts in phloem amino acid composition described in this study commonly occur in other plant species and might not be specific to potato.

The processes underlying developmental shifts in phloem amino acid composition are anticipated to include developmental regulation of transporter expression for phloem loading of amino acids in leaf vascular tissue, for which there is sound evidence (Kwart et al., 1993; Fischer et al., 1995). Other processes that might be involved include metabolism, unloading, retrieval and xylem–phloem transfer of amino acids along the length of the translocation pathway (Fischer et al., 1995; Rentsch and Frommer, 1996; Hirner et al., 1998). The relative abundance of glutamate, glutamine and aspartate, the dominant amino acids in potato phloem sap, is probably shaped mostly by the activities of glutamine synthetase (GS) and aspartate aminotransferase in the source tissues and/or sieve elements (McGrath and Coruzzi, 1991; Lam et al., 1996; Vincent et al., 1997; Finnemann and Schjoerring, 2000). GS activity is regulated by 14-3-3 proteins (Finnemann and Schjoerring 2000), and certain 14-3-3 isoforms are known to be under developmental regulation in potato (Wilczynski et al., 1998).

Although the data are fragmentary, there are clear indications in the literature that the developmental shift in phloem amino acids may be general among plants. The reasons for this variation are probably related to fundamental aspects of plant physiology, i.e. the shifts reflect the role of amino acids in both the form of nitrogen transported and in the partitioning of nitrogen during plant development. In addition, there are indications that glutamine may act as a signal in relation to nitrogen acquisition (Coruzzi and Zhou, 2001), flowering (Vincent et al., 1997) and senescence (Markiewicz et al., 1996).

This study suggests that one consequence of the developmental changes in phloem amino acid composition may be a changing acceptability to phloem-feeding herbivores. This change in 'plant nutritional quality' for herbivores can be defined predominantly in terms of the relative amounts of the amino acid amides glutamine and asparagine and their acids (glutamate and aspartate) in the phloem. While there is no evidence that the phloem amino acid profiles have evolved purely as an 'anti-herbivore defence', the possibility cannot be excluded that certain profiles that would be compatible with plant function have been selected against because they would promote insect attack and population increase. Future research on the interplay between phloem sieve-element function and the nutritional physiology of phloem-feeding insects should aim to (1) resolve why phloem amino acid profiles vary with plant age and (2) establish a quantitative measure of phloem nutrient changes and the consequences for insect herbivory.

We thank Kim Simpson and Margaret Pasquini, both at the University of York, for technical support. C, H, N analyses were conducted at the Centre for Biomedical Accelerator Mass Spectrometry Ltd, York, UK. This research was funded by the UK Department for Environment, Food and Rural Affairs under programme HP0131.

References

Abisgold, J. D., Simpson, S. J. and Douglas, A. E. (l994). Nutrient regulation in the pea aphid *Acyrthosiphon pisum*: application of a novel geometric framework to sugar and amino acid consumption. *Physiol. Entomol*. **19**, 95-102.

Agrawal, A. A. (2001). Mechanisms, ecological consequences and agricultural implications of tri-trophic interactions. *Curr. Opin. Plant Biol.* **3**, 329-335.

Auclair, J. L. (1963). Aphid feeding and nutrition. *Annu. Rev. Entomol.* **8**, 439-490.

Baldwin, I. T., Halitschke, R., Kessler, A. and Schittko, U. (2001). Merging molecular and ecological approaches in plant–insect interactions. *Curr. Opin. Plant Biol.* **4**, 351-358.

Boggio, S. B., Palatnik, J. F., Heldt, H. W. and Valle, E. M. (2000). Changes in amino acid composition and nitrogen metabolizing enzymes in ripening fruits of *Lycopersicon esculentum*. *Plant Sci.* **159**, 125-133.

Bolsinger, M. and Flückiger, W. (1989). Ambient air pollution induced changes in amino acid pattern of phloem sap in host plants – relevance to aphid infestation. *Environ. Pollut.* **56**, 209-216.

Chen, J. Q., Rahbé, Y., Delobel, B., Sauvion, N., Guillard, J. and Febvay, G. (1997). Melon resistance to the aphid *Aphis gossypii*: behavioural analysis and chemical correlations with nitrogenous compounds. *Ent. Exp. Appl.* **85**, 33-44.

Cisneros, J. J. and Godfrey, L. D. (2001). Midseason pest status of the cotton aphid (Homoptera: Aphididae) in California cotton: is nitrogen a key factor? *Environ. Entomol.* **30**, 501-510.

Corbesier, L., Havelange, A., Lejeune, P., Bernier, G. and Périlleux, C. (2001). N content of phloem and xylem exudates during the transition to flowering in *Sinapis alba* and *Arabidopsis thaliana*. *Plant Cell Environ.* **24**, 367-375.

Coruzzi, G. M. and Zhou, L. (2001). Carbon and nitrogen sensing and signalling in plants: emerging 'matrix effects'. *Curr. Opin. Plant Biol.* **4**, 247-253.

Dadd, R. H. (1985). Nutrition: Organisms. In *Comprehensive Insect Physiology, Biochemistry, and Pharmacology*, vol. 4 (ed. G. A. Kerkut and L. I. Gilbert), pp. 313-391. Oxford, UK: Pergamon Press.

Dahlqvist, A. (1984). α-Glucosidases (disaccharidases). In *Methods of Enzymatic Analysis*, vol. 4 (ed. H. U. Bergmeyer), pp. 208-217. Weinheim: Verlag Chemie.

De Barro, P. J. (1992). The role of temperature, photoperiod, crowding and plant quality on the production of alate viviparous females of the bird cherry-oat aphid, *Rhopalosiphum padi*. *Ent. Exp. Appl.* **65**, 205-214.

Docherty, M., Wade, F. A., Hurst, D. K., Whittaker, J. B. and Lea, P. J. (1997). Responses of tree-sap feeding herbivores to elevated CO_2. *Global Change Biol.* **3**, 51-59.

Douglas, A. E. (1993). The nutritional quality of phloem sap utilised by natural aphid populations. *Ecol. Entomol.* **18**, 31-38.

Douglas, A. E. (1998). Nutritional interactions in insect–microbial symbioses: aphids and their symbiotic bacteria *Buchnera*. *Annu. Rev. Entomol.* **43**, 17-37.

Douglas, A. E., Minto, L. B. and Wilkinson, T. L. (2001). Quantifying nutrient production by the microbial symbionts in an aphid. *J. Exp. Biol.* **204**, 349-358.

Febvay, G., Bonnin, J., Rahbé, Y., Bournoville R., Delrot, S. and Bonnemain, J. L. (1988). Resistance of different lucerne cultivars to the pea aphid *Acyrthosiphon pisum*: influence of phloem composition on aphid fecundity. *Ent. Exp. Appl.* **48**: 127-134.

Febvay, G., Liaddouze, I., Guillard, J. and Bonnot, G. (1995). Analysis of energetic amino acid metabolism in *Acyrthosiphon pisum*: a multidimensional approach to amino acid metabolism in aphids. *Arch. Insect Biochem. Physiol.* **29**, 45-69.

Febvay, G., Rahbé, Y., Rynkiewiecz, M., Guillard, M. and Bonnot, G. (1999). Fate of dietary sucrose and neosynthesis of amino acids in the pea aphid, *Acyrthosiphon pisum*, reared on different diets. *J. Exp. Biol.* **202**, 2639-2652.

Finnemann, J. and Schjoerring, J. K. (2000). Post-translational regulation of cytosolic glutamine synthetase by reversible phosphorylation and 14-3-3 protein interaction. *Plant J.* **24**, 171-181.

Fischer, W.-N., Kwart, M., Hummel, S. and Frommer, W. B. (1995). Substrate specificity and expression profile of amino acid transporters (AAPs) in *Arabidopsis*. *J. Biol. Chem.* **270**, 16315-16320.

Fisher, D. B. (2000). Long distance transport. In *Biochemistry and Molecular Biology of Plants* (ed. B. B. Buchanan, W. Gruissem and R. L. Jones), pp. 730-784. Rockville, MD, USA: American Society of Plant Physiologists.

Girousse, C. and Bournoville, R. (1994). Role of phloem sap quality and exudation characteristics on performance of pea aphid grown on lucerne genotypes. *Ent. Exp. Appl.* **70**, 227-235.

Güntner, C., González, A., Dos Reis, R., González, G., Vázquez, A., Ferreira, F. and Moyna, P. (1997). Effect of *Solanum* glycoalkaloids on potato aphid, *Macrosiphum euphorbiae*. *J. Chem. Ecol.* **23**, 1651-1659.

Hirner, B., Fischer, W. N., Rentsch, D., Kwart, M. and Frommer, W. B. (1998). Developmental control of H^+/amino acid permease gene expression during seed development of *Arabidopsis*. *Plant J.* **14**, 535-544.

Jefferies, R. A. and Lawson, H. M. (1991). A key for the stages of development of potato (*Solanum tuberosum*). *Ann. Appl. Biol.* **119**, 387-389.

Jenkins, E. V. (2001). Colonisation by phytophagous insects of herbs introduced into young farm woodlands. PhD Thesis, University of York, UK.

Jones, B. N., Pääbo, S. and Stein, S. (1981). Amino acid analysis and enzymatic sequence determination of peptides by an improved *o*-phthaldialdehyde precolumn labelling procedure. *J. Liq. Chrom.* **4**, 565-586.

Kazemi, M. H. and van Emden, H. F. (1992). Partial antibiosis to *Rhopalosiphum padi* in wheat and some phytochemical correlations. *Ann. Appl. Biol.* **121**, 1-9.

King, R. W. and Zeevart, J. A. D. (1974). Enhancement of phloem exudation from cut petioles by chelating agents. *Plant Physiol.* **53**, 96-103.

Kwart, M., Hirner, B., Hummel, S. and Frommer, W. B. (1993). Differential expression of two related transporters with differing substrate specificity in *Arabidopsis thaliana*. *Plant J.* **4**, 993-1002.

Lam, H.-M., Coschigano, K. T., Oliviera, I. C., Melo-Oliviera, R. and Coruzzi, G. M. (1996). The molecular-genetics of nitrogen assimilation into amino acids in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 569-593.

Mackauer, R. H. and Way, M. J. (1976). *Myzus persicae* Sulz. an aphid of world importance. In *Studies in Biological Control* (ed. V. L. Delucchi), pp. 51-119. Cambridge, UK: Cambridge University Press.

Markiewicz, E., Wilczynski, G., Rzepecki, R., Kulma, A. and Szopa, J. (1996). The 14-3-3 protein binds to the nuclear matrix endonuclease and has a possible function in the control of plant senescence. *Cell. Mol. Biol. Lett.* **1**, 391-415.

Mattson, W. J. (1980). Herbivory in relation to plant nitrogen content. *Annu. Rev. Ecol. System.* **11**, 119-161.

McGrath, R. B. and Coruzzi, G. M. (1991). A gene network controlling glutamine and asparagine biosynthesis in plants. *Plant J.* **1**, 275-280.

Morris, J. G. (1991). Nutrition. In *Environmental and Metabolic Animal Physiology* (ed. C. L. Prosser), pp. 231-276. New York, USA: John Wiley & Sons.

Mittler, T. E. (1967). Effect on aphid feeding of dietary methionine. *Nature* **214**, 386.

Müller, C. B., Williams, I. S. and Hardie, J. (2001). The role of nutrition, crowding and interspecific interactions in the development of winged aphids. *Ecol. Entomol.* **26**, 330-340.

Parker, W. E., Howard J. J., Karley A. J. and Douglas A. E. (2000). Crop growth stage and the phenology of aphid populations on potato. In Proceedings of *The Brighton Crop and Pests Conference 2000*, pp. 955-960, Farnham, Surrey, UK: The British Crop Protection Council.

Prosser, W. A., Simpson, S. J. and Douglas, A. E. (l992). How an aphid (*Acyrthosiphon pisum*) symbiosis responds to variation in dietary nitrogen. *J. Insect Physiol.* **38**, 301-307.

Prosser, W. A. and Douglas, A. E. (1992). A test of the hypotheses that nitrogen is upgraded and recycled in an aphid (*Acyrthosiphon pisum*) symbiosis. *J. Insect Physiol.* **38**, 93-99.

Randerath, P. F. (1996). Ordination. In *Biological Data Analysis: A Practical Approach* (ed. J. C. Fry), pp. 251-287. Oxford, UK: IRL Press.

Rentsch, D. and Frommer, W. B. (1996). Molecular approaches towards an understanding of loading and unloading of assimilates in higher plants. *J. Exp. Bot.* **47**, 1199-1204.

Sandström, J. (1994). Performance of pea aphid (*Acyrthosiphon pisum*) clones on host plants and synthetic diets mimicking the same plants phloem amino acid composition. *J. Insect Physiol.* **40**, 1051-1057.

Sandström, J. and Pettersson, J. (1994). Amino acid composition of phloem sap and the relation to intraspecific variation in pea aphid (*Acyrthosiphon pisum*) performance. *J. Insect Physiol.* **40**, 947-955.

Sandström, J., Telang, A. and Moran, N. A. (2000). Nutritional enhancement of host plants by aphids – a comparison of three aphid species on grasses. *J. Insect Physiol.* **46**, 33-40.

Shigenobu, S., Watanabe, H., Hattori, M., Sakaki, Y. and Ishikawa, H. (2000). Genome sequence of the endocellular bacterial symbiont of aphids *Buchnera* sp. APS. *Nature* **407**, 81-86.

Srivastava, P. N. and Auclair, J. L. (1974). Effect of amino acid concentration on diet uptake and performance by the pea aphid, *Acyrthosiphon pisum* (Homoptera: Aphididae). *Can. Ent.* **106**, 149-156.

Srivastava, P. N., Auclair, J. L. and Srivastava, U. (1983). Effect of non-essential amino acids on phagostimulation and maintenance of the pea aphid, *Acyrthosiphon pisum*. *Can. J. Zool.* **61**, 2224-2229.

Srivastava, P. N., Gao, Y., Levesque, J. and Auclair, J. L. (1985). Differences in amino acid requirements between two biotypes of the pea aphid, *Acyrthosiphon pisum*. *Can. J. Zool.* **63**, 603-606.

Taylor, C. E. (1955). Growth of the potato plant and aphid colonization. *Ann. Appl. Biol.* **43**, 151-156.

van Emden, H. F. and Bashford, M. A. (1971). The performance of *Brevicoryne brassicae* and *Myzus persicae* in relation to plant age and leaf amino acids. *Ent. Exp. Appl.* **14**, 349-360.

Vincent, R., Fraisier, V., Chaillou, S., Limami, M. A., Deleens, E., Phillipson, B., Douat, C., Boutin, J.-P. and Hirel, B. (1997). Overexpression of a soybean gene encoding cytosolic glutamine synthetase in shoots of transgenic *Lotus corniculatus* L. plants triggers changes in ammonium assimilation and plant development. *Planta* **201**, 424-433.

Weibull, J. H. W. (1988). Free amino acids in the phloem sap from oats and barley resistant to *Rhopalosiphum padi*. *Phytochem.* **27**, 2069-2072.

Weibull, J., Ronquist, F. and Brishammar, S. (1990). Free amino acid composition of leaf exudates and phloem sap. A comparative study in oats and barley. *Plant Physiol.* **92**, 222-226.

Wilczynski, G., Kulma, A. and Szopa, J. (1998). The expression of 14-3-3 isoforms in potato is developmentally regulated. *J. Plant Physiol.* **153**, 118-126.

Wilkinson, T. L. and Ishikawa, H. (1999). The assimilation and allocation of nutrients by symbiotic and aposymbiotic pea aphids, *Acyrthosiphon pisum*. *Ent. Exp. Appl.* **91**, 195-201.

Wilkinson, T. L., Minto, L. B. and Douglas, A. E. (2001). Amino acids as respiratory substrates in aphids: an analysis of *Aphis fabae* reared on plants and diets. *Physiol. Entomol.* **26**, 225-228.

Williams, C. T. (1995). Effects of plant age, leaf age and virus yellows infection on the population dynamics of *Myzus persicae* (Homoptera: Aphididae) on sugarbeet in field plots. *Bull. Ent. Res.* **85**, 557-567.

Wright, J. P., Fisher, D. B. and Mittler, T. E. (1985). Measurement of aphid feeding rates on artificial diets using H-3-inulin. *Ent. Exp. Appl.* **37**, 9-11.

Wyatt, I. J. and White, P. F. (1977). Simple estimation of intrinsic increase rates for aphids and tetranychid mites. *J. Appl. Ecol.* **14**, 757-766.