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Feeding behaviour of the aphid *Rhopalosiphum padi* (Hemiptera: Aphididae) on nitrogen and water-stressed barley (*Hordeum vulgare*) seedlings

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Abstract

Electrical penetration graphs (EPGs) were used to examine the probing behaviour of adult apterous *Rhopalosiphum padi* (Linnaeus) on barley seedlings grown under conditions of nitrogen or water stress. Aphids took significantly longer to reach and ingest from sieve elements of nitrogen-deficient seedlings than from nitrogen-sufficient seedlings but there were no such differences between water-stressed or well-watered seedlings. On both nitrogen and water-stressed seedlings the average length of each individual period of salivation into the sieve element was significantly greater compared with their respective unstressed controls.

Introduction

The performance of an aphid on a particular plant depends not only on the nutritive quality of the phloem sap, but also on the aphid's ability to avoid or overcome plant defences and establish a sustained feeding site within a sieve element. Sieve element acceptance (sustained phloem ingestion) may take several hours and is often preceded by many brief punctures of the sieve elements and by short periods of ingestion from one or several sieve elements (Tjallingii & Mayoral, 1992; Tjallingii & Hogen Esch, 1993). Ingestion of phloem sap is always preceded by a period of salivation into the sieve element (Prado & Tjallingii, 1994) and it has been proposed that salivary secretions may function to improve the physical or chemical quality of phloem sap (Miles, 1999). Increased periods of salivation into sieve elements have been recorded on more resistant plant varieties (Caillaud *et al.*, 1995; Ramirez & Niemeyer, 1999) and on less suitable host plant species (Girma *et al.*, 1992), indicating that this behaviour may be particularly important on poor quality hosts.

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Electrical penetration graphs (EPGs) have been used for comparison of aphid probing behaviour on resistant and susceptible plant cultivars, primarily in an effort to resolve locations of plant resistance (e.g. Caillaud *et al.*, 1995; Cole, 1997; Klingler *et al.*, 1998). Such analyses allow factors interfering with probing activities to be inferred, for example at the epidermis, mesophyll or phloem level (Tjallingii, 1990). In combination with chemical analyses of phloem sap, investigations have shown relationships between probing behaviour, phloem biochemistry and aphid performance (e.g. Cole, 1997; Klingler *et al.*, 1998). However, most of these studies have compared different plant varieties or species exhibiting varying degrees of aphid resistance. Examination of the probing and feeding behaviour of an aphid on a single plant species grown under different environmental conditions has been largely overlooked.

Ponder *et al*. (2000) found that nitrogen deficiency in barley resulted in lowered intrinsic rate of increase of the bird cherry–oat aphid *Rhopalosiphum padi* (Linnaeus) (Hemiptera: Aphididae). This was attributed in part to a lowered concentration of phloem amino acids and in part to difficulties in achieving phloem ingestion. In the present paper, feeding behaviour was compared on barley seedlings grown under water stress and nitrogen stress to investigate the effects of plant environmental stress on the phloem

feeding behaviour of *R. padi*. Under both stress treatments, aphid intrinsic rate of increase (R_m) was reduced compared with the unstressed controls (Ponder *et al.*, 2000; K.L. Ponder, unpublished)

Materials and methods

Aphids

A clone of *R. padi* was started from a single individual taken from a long-term culture at the University of Birmingham. The clone was maintained on well-watered barley seedlings (*Hordeum vulgare* Linnaeus var. Igri (Poaceae)) grown in compost in a controlled environment room maintained at 18:6 L:D cycle at 18–20°C. Apterous adults were used for experiments.

Different plant watering treatments

Five germinated barley seeds (the same cultivar as used for the stock aphid culture) were planted in pots 10 cm diameter and 10 cm deep containing a 50:50 compost and vermiculite mixture, and watered daily with tap water. When shoots were 2–3 cm long, the pots were divided into two treatments and either watered daily (control) or weekly (water-stressed) with 30 ml tap water. Plants were grown in a 18:6 L:D cycle at 18–20°C. Experiments were performed on seedlings at the 3–5 leaf stage. At this stage, water-stressed plants were visibly wilted and smaller (leaf length and dry weight) than the well-watered control plants.

Different plant nitrogen treatments

Germinated barley seedlings were transferred to a mesh over an aerated, nitrogen-free hydroponic nutrient solution (table 1) and kept in the dark for a further 24 h before being exposed to a 18:6 L:D cycle at 18–20°C. When the shoots were 2–3 cm long, half the seedlings were transferred to a nutrient solution containing an additional 8 mol m^{-3} nitrogen as ammonium nitrate (nitrogen-sufficient) and the other half were kept in the original nitrogen free solution (table 1). Seedlings were thinned to three per pot (capacity 900 ml) and held in place with foam bungs. Nutrient solution was replaced approximately every three days. Experiments were performed on seedlings at the 2–4 leaf stage.

Electrical penetration graphs

Electrical penetration graphs (EPGs) were obtained using a 4-channel DC system (Tjallingii, 1990). A DAS-800 card (Keithley Metrabyte Corporation) was used to convert the analogue signal to a digital output. Experiments took place in an electrically earthed Faraday cage, under constant room illumination. Aphids were starved for an hour before being attached, via their dorsum, to a 2 cm length of 25 μm gold wire (Goodfellow) with silver conductive paint (RS Components). Four aphids were monitored simultaneously – either two on nitrogen-sufficient seedlings and two on nitrogen-deficient seedlings; or two on well-watered seedlings and two on water-stressed seedlings. Aphids were checked regularly for the first hour of recording and those that had fallen from their plant replaced. After this, insects were left for the remainder of the 300 min recording and those that failed to remain on the plant were discarded from analyses. 'Stylet 2.5®' software (supplied by W.F. Tjallingii, Wageningen, The Netherlands) was used for data acquisition. Data were collected from between 21 and 27 insects in each experimental group (table 2).

Table 1. Chemical composition of hydroponic nutrient solutions A (nitrogen-deficient) and B (8 mol m^{-3} nitrogen) used in experiments.

	Concentration in solution (mol m^{-3})	
	Solution A	Solution B
$CaCl_2$	2	2
$MgSO_4$	1	1
KH_2PO_4	4	4
NaFeEDTA	0.015	0.015
HBO_3	0.25	0.25
$MnSO_4$	0.002	0.002
$CuSO_4$	0.002	0.002
$NaMoO_4$	0.00025	0.00025
NH_4NO_3	–	4
Total nitrogen	0	8

Data analysis

Detailed descriptions of waveforms are given in Tjallingii (1990). Penetration activities were divided into pathway phases (waveforms A, B, C), xylem ingestion (waveform G), phloem salivation (waveform E1) and phloem ingestion (waveform E2) and their durations and frequencies recorded. The time taken to initiate sieve element salivation and the time to sustained phloem ingestion (>10 min) were recorded from the start of the first penetration and from the start of the probe that contained them. Aphids that failed to show sieve element-associated behaviours during the length of the recording were considered to take the full length of the recording period (i.e. 300 min) to start phloem activities. Where data was normally distributed, a t-test was performed to compare treatments; otherwise a Mann Whitney test with Bonferroni correction was used.

Results

Different watering treatments

The duration of each recorded activity was similar in the two watering treatments, as was time taken to show sieve element salivation (E1) or phloem sap ingestion (E2). However, there was a trend of increased time to reach the sieve elements on water-stressed seedlings (table 2) and many *R. padi* failed to show any phloem-related behaviours during the 5 h recordings on these plants. There was no statistical difference in number of E1 and E2 events in both treatments, but a smaller proportion of probes contained a period of E2 on water-stressed compared with well-watered seedlings ($U = 451$; $P = 0.05$) (fig. 1a). The length of each individual E1 period was longer on water-stressed seedlings ($t_{(56)} = 2.29$; $P = 0.03$) (fig. 1b). There were no differences in length of E2 periods ($t_{(32)} = 0.25$; $P = 0.81$) (fig. 1c). Xylem ingestion was infrequent on both treatments.

Different nitrogen treatments

The duration of phloem ingestion (pattern E2) was increased on nitrogen-sufficient seedlings compared with

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Table 2. Summary of electrical penetration graph results of *Rhopalosiphum padi* feeding on barley (*Hordeum vulgare*) seedlings grown with or without nitrogen (N-sufficient and N-deficient) or well-watered or under water stress.

Plant treatment	N-sufficient n = 21	N-deficient n = 21	Well-watered n = 27	Water-stressed n = 22
Duration of (min)				
Non-penetration (np)	22.3 a	95.9 b	44.3	105.7
Pathway (A,B,C)	132.1	155.4	156.7	134.2
Salivation in sieve tube (E1)	4.0	5.2	3.3	0
Phloem ingestion (E2)	91.5 a	31.4 b	21.2	0
Time taken to (min)				
First E1 from start of expt.	48.6 a	151.7 b	87.4	300
First E2 from start of expt.	76.4 a	154.3 b	121.1	300
Sustained E2 from start of expt.	130.3 a	214.9 b	262.2	300
First E1 from start of probe	17.5	26.4	52.7	300
First E2 from start of probe	28.5	44.0	73.9	300
Sustained E2 from start of probe	28.5 a	83.6 b	144.0	300
Number of				
Aphids reaching phloem	15	19	21	9
E1 events	5.1 (0.9)	4.5 (0.9)	3.0	0
E2 events	2.5 (0.5)	1.9 (0.4)	1.0	0

Values are medians. Letters indicate significant differences (Mann Whitney, $P < 0.05$) between nitrogen-sufficient and deficient plants or between water-stressed and well-watered plants.

nitrogen-deficient seedlings (table 2; U = 536; $P = 0.03$). On the latter, there was a significant increase in time spent not probing (U = 357; $P = 0.02$). There were no significant differences in total duration of other recorded activities. Time taken to initiate phloem salivation (first E1 pattern) from the start of the recording was reduced on nitrogen-sufficient compared with nitrogen-deficient seedlings (U = 347; $P = 0.01$).

There were no differences in the numbers of probes made, or in number of phloem feeding periods, but the proportion of probes that exhibited phloem feeding behaviour was significantly higher on nitrogen-sufficient seedlings (U = 395; $P = 0.04$) (fig. 1a). In addition, on nitrogen-deficient seedlings, a higher proportion of aphids reached a sieve element and salivated (showed pattern E1) but did not achieve phloem ingestion (U = 317; $P = 0.02$). The average lengths of individual E1 periods were significantly greater on nitrogen-deficient seedlings ($t_{(85)} = 2.35$; $P = 0.02$) (fig. 1b), but there were no significant differences in the average length of each individual E2 period ($t_{(69)} = 0.75$; $P = 0.47$). Xylem ingestion was infrequent in both treatments.

Discussion

Different watering treatments

Analysis of aphid feeding patterns using electrical penetration graphs on water-stressed compared with well-watered plants has not been reported before. Nutrient concentrations in the phloem sap increase under conditions of water stress (Tully & Hanson, 1979; Girousse *et al.*, 1996), but the effect this has on aphids is varied, with water stress both increasing (Wearing & van Emden, 1967) and reducing (Kennedy & Booth, 1959; Sumner *et al.*, 1986; Pons & Tatchell, 1995) aspects of performance. Analysis of phloem sap amino acids from the barley seedlings used in the present study indicated that water-stressed plants had higher concentrations (mean = 149.8 mM) of amino acids than well-watered controls (mean = 96 mM) (K.L. Ponder, unpublished). However, sample sizes were too small (3 and 7) to allow more detailed analysis of individual amino acid concentrations. The similarity in time taken to reach the sieve elements and duration of phloem ingestion between the two watering treatments suggests phloem amino acids were not limiting aphid performance on water-stressed seedlings. Isaacs *et al.* (1998) calculated that whitefly had a lower ingestion rate (inferred from reduced volume of honeydew produced) on water-stressed plants. It could be expected that aphids would not need to ingest as much phloem sap from water-stressed plants with more concentrated phloem sap, in order to take in the same quantity of amino acids as aphids on well-watered controls. If intake rate was lower (due to reduced phloem turgor, for example) then the actual time spent feeding may need to increase relative to controls in order to ingest the same quantity of amino acids. Differences in ingestion rates cannot be determined from EPG analyses alone; data from honeydew excretion are also required.

Different nitrogen treatments

Feeding behaviour of *R. padi* on nitrogen-deficient barley was discussed in more detail by Ponder *et al*. (2000). Only behaviours relating to sieve element activities will be considered here. The time taken for the aphid to show sieve element activities from the start of the experiment was greater on nitrogen-deficient seedlings. However, EPG analysis cannot distinguish a brief penetration into a sieve element from penetration of other cells, which occurs frequently (Tjallingii & Hogen Esch, 1993) and, as a result, the time taken for aphids to locate and subsequently reject sieve elements may have been overestimated. Compared with aphids on nitrogen-sufficient seedlings, on nitrogen-deficient seedlings more aphids that reached the sieve elements (showed E1) failed to follow this with phloem ingestion (E2), and it took longer within a probe to show sustained (>10 min) ingestion. This indicates that once reached and sampled, the sieve element was more frequently rejected on nitrogen-deficient seedlings.

The average length of each sieve element salivation period was significantly longer on nitrogen-deficient seedlings. Caillaud *et al*. (1995) and Prado (1997) working on

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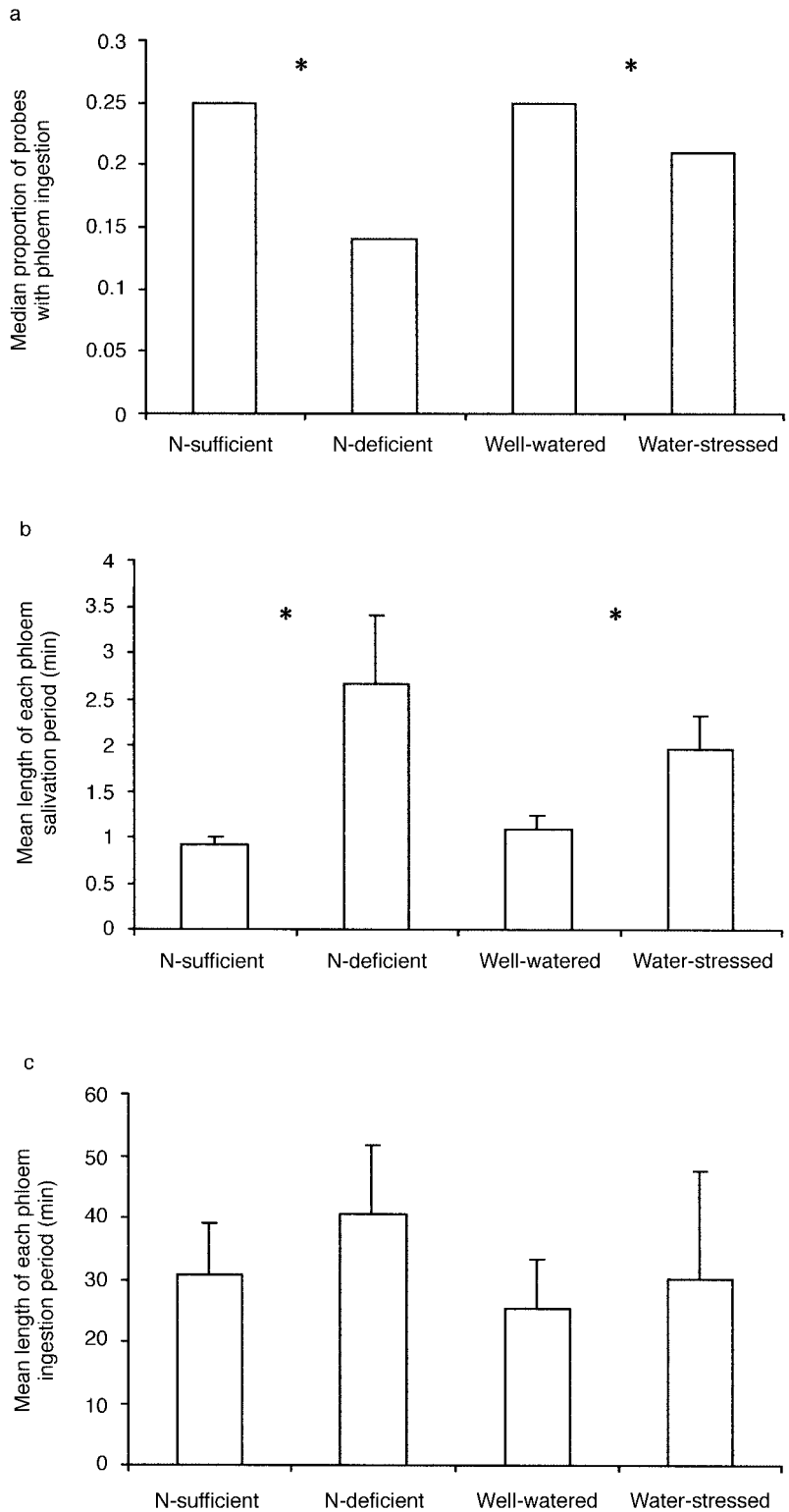

Fig. 1. Phloem-related behaviours of *Rhopalosiphum padi* feeding on barley seedlings. (a) Proportion of probes into plant that contained a period of phloem ingestion; (b) mean duration of each period of salivation into a sieve element and (c) mean duration of each period of phloem sap ingestion. Error bars are standard errors. Asterisks significant differences ($P < 0.05$)

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resistant and susceptible wheat lines also showed these trends in occurrence and duration of phloem associated behaviours and Girma *et al*. (1992) found that the Russian wheat aphid *Diuraphis noxia* (Mordvilko) (Hemiptera: Aphididae) salivated more and ingested less on a less acceptable host (sorghum) than its preferred host (wheat). This implies that there may be some factor in the sieve elements that deters or delays the aphids from ingesting phloem on lower quality (in this case nitrogen-deficient) seedlings.

Analysis of phloem sap amino acids showed a reduction in the concentration of non-essential amino acids from nitrogen-deficient seedlings (Ponder *et al.*, 2000). Reduced total nitrogen content could have been responsible for the lowered performance of *R. padi* on the nitrogen-deficient plants but it might be expected that the aphids would show a compensatory response and feed for longer periods. Longer recordings, coupled with collection of excreted honeydew, are required to investigate this. Work with artificial diets has shown that certain amino acids can also have phagostimulatory roles in diet acceptance (Srivastava & Auclair, 1975); it is possible that lack of an appropriate feeding stimulant in the phloem sap of nitrogen-deficient seedlings may have delayed or prevented ingestion from these plants.

Sieve element salivation

The length of individual phloem salivation periods (E1) were longer on both water-stressed and nitrogen-stressed seedlings compared with their respective controls. This supports the view that increased periods of E1 occur on poor quality host plants. However, whilst the phloem sap from water-stressed plants was more concentrated than well-watered plants, that from nitrogen-deficient seedlings was similar in concentration to nitrogen-sufficient plants but had a reduced amino acid concentration. Therefore a single explanation that can satisfy both stress treatments is difficult.

Several reasons for salivation into sieve elements have been reported. It may improve the chemical quality of the phloem, for example by detoxifying harmful compounds (Miles & Oertli, 1993; Urbanska *et al.*, 1998) and there is evidence that aphid feeding can result in elevated levels of phloem amino acids (Sandström *et al*., 2000), possibly due to some salivary component. It may also improve the 'physical' quality of the phloem, for example by suppressing the wounding response of the plant, thereby facilitating sustained ingestion of sap by the aphid (Miles, 1999). Salivation is evidently a key event in aphid acceptance of a sieve element and the composition of aphid saliva is likely to be crucial in allowing exploitation of new host plants. Characterization of both behavioural and chemical aspects of aphid salivation are important for a better understanding of the mechanisms involved in aphid–plant interactions.

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