Eur. J. Entomol. **91**: 47–52, 1994
ISSN 1210–5759

Sieve element acceptance by aphids

W. FREDDY TJALLINGII

Agricultural University, Department of Entomology,
Binnenhaven 7, 6709 PD Wageningen, The Netherlands

Aphids, host plant, phloem, intracellular, feeding behavior, probing, stylets

Abstract. *Aphis fabae* took 3 to 4 hours, after access to a plant, to show the first puncture of a sieve element. A further hour was needed before sustained ingestion started. Early probes did not show sieve element punctures, in general. Within later probes, showing these punctures, the average time needed to reach them was 30 min. Early sieve element punctures were mainly accompanied by the secretion of a watery saliva. The composition of this saliva and the function of its secretion is unknown. It is speculated that this saliva secretion makes the phloem sap more readily available and/or improves its quality for aphids. Further research is needed to test this hypothesis.

INTRODUCTION

Food plant selection by aphids involves stylet penetration (or probing) by which they reach the sieve elements on which they feed. The response to plant semiochemicals by aphids is initiated via the internal gustatory organ, as they lack external taste organs (Wensler, 1977). In addition to host plant recognition, aphids have to locate the phloem sieve elements, which are inside the vascular bundle encased in many layers of cells. Transmission electron microscopy (TEM) has shown that the stylets puncture many, if not all the plant cells along the stylet path (Tjallingii & Hogen Esch, 1993). Notwithstanding these intracellular punctures, the cells generally appear to survive and the stylet path remains completely extracellular. Using the electrical penetration graph (EPG) technique this was confirmed and clear evidence was provided that the intracellular punctures, which are represented by potential drops (pd's), do not last longer than 5–10 seconds (Tjallingii, 1985). All aphid species tested behaved similarly in this respect.

Micrographs (TEM) of EPG recorded probes indicate that when sieve elements are reached and punctured they are not immediately accepted for feeding. The aphid stylets can penetrate from the epidermis to the phloem in about 15 minutes. However, phloem sap ingestion is often delayed and started during a later sieve element puncture, either in the same, or a subsequent probe (Tjallingii, 1990b).

In an EPG a typical sieve element puncture is represented by waveform E. These punctures have a longer duration (> 10 s) than the pathway punctures (pd's) into other cells, and have a different waveform. There is evidence, however, that pathway punctures into sieve elements may occur (Tjallingii & Hogen Esch, 1993). Two sieve element waveforms, E1 and E2 (Tjallingii, 1990a) have been distinguished. The E1 waveform has recently been correlated (Prado & Tjallingii, 1993) with salivation into a sieve element without any ingestion, whereas E2 reflects passive ingestion and concurrent continuous secretion of saliva. From earlier work (Tjallingii, 1988) it appears that the saliva secreted

during E2 presumably does not enter the plant but is immediately mixed with the ingested sap and may probably operate for digestion. The E1 saliva, on the other hand, must enter the sieve elements since there is no indication of sap ingestion during E1. It is unclear why E1 salivation waveforms differ from E2 salivation signals and there is no experimental indication for the function of these two types of sieve element salivation so far. Waveform E1 is always the first activity recorded when a sieve element is punctured and it is often the only activity recorded during short (10 s – some mins) sieve element punctures.

This study uses the E waveforms in EPGs to investigate the occurrence of sieve element punctures by *Aphis fabae*, probing leaves and stems of its host plant, *Vicia faba.*

MATERIALS AND METHODS

A. fabae was reared on broad beans (Dutch cv. 'drie maal wit') at a photoperiod of 16 h light per day and a temperature of about 20°C. Apterous adults were wired and connected to EPG amplifiers (DC system; Tjallingii, 1988). EPG's of 8 h, from about 15 aphids probing on leaves and stems were stored on a computer (PC) hard disk using STYLET 2.0 software. Starting time and duration of waveforms were retrieved, and separate probes (PEN) and E waveforms (E), referred to as sieve element punctures, were distinguished. E waveforms were divided into those with durations of less than 10 min, longer than or 10 min (sus E), and longer than one hour (E >1h). Within E, E1 and E2 waveforms were distinguished. Also,

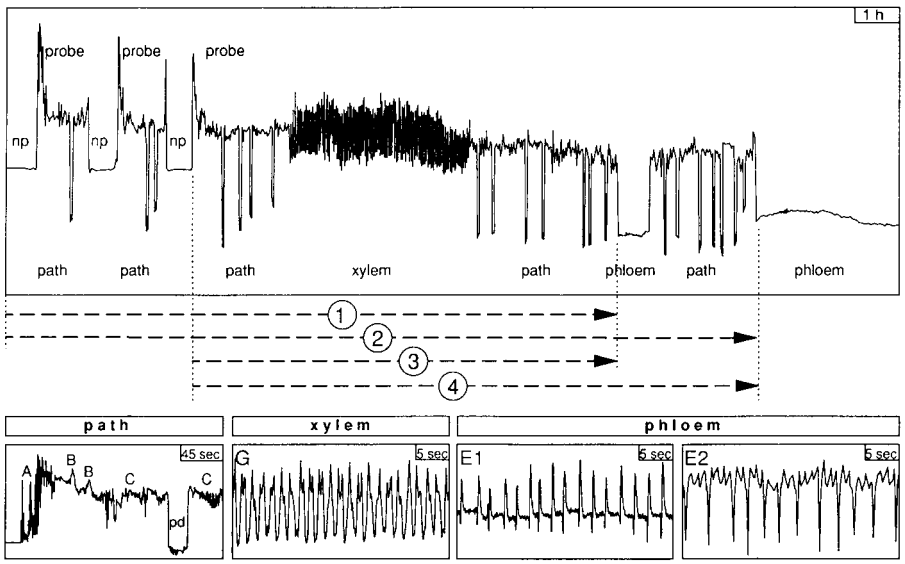

Fig. 1. Electrical penetration graph (EPG) of an aphid. Top trace: overview of the main features. np – non-probing; probe – period of stylet penetration; path – pathway phase; xylem – xylem phase; phloem – phloem sieve element phase. Arrows, phloem parameters: (1) – time from the access to a plant to the first sieve element puncture; (2) – time from plant access to sustained sieve element puncture with ingestion; (3) and (4) – as (1) and (2) but from the beginning of the probe instead of plant access. Bottom traces: detailed waveforms of each phase in the top trace. Path: A – first stylet-plant contact; B – sheath material secretion; C – other 'pathway activities'; pd – potential drop, intracellular pathway puncture. Xylem: G – active xylem ingestion, 'drinking'. Phloem: E1 – sieve element salivation; E2 – sieve element ingestion.

the time to a sieve element puncture, measured from the beginning of the experiment (plant access) and from the start of the probe in which the puncture occurred, were used as parameters (Fig. 1).

RESULTS

Durations of successive probes or penetrations increased and were separated by decreasing periods of non-penetration. This is reflected in the changing number of penetrations and the durations of penetration (PEN) and non-penetration (NP) (Table 1.). With time the percentage of time spent in sieve element punctures increased (E/PEN). The number of new sieve element punctures (new E) increased up to the 4th hour and then decreased gradually due to the increasing duration of each puncture. Occurring over a period of several hours, a puncture contributes only to this parameter in the hour of its appearance.

TABLE 1. Probing by *A. fabae* on beans. Mean number (#) of probes and duration (h) of stylet penetration (probing) (PEN) and non-penetration (NP), sieve element puncture as a percentage of probing time E/PEN, and number of newly initiated punctures in sieve elements (new E) per hour.

hour			1	2	3	4	5	6	7	8
leaf	PEN	(#)	4.6	2.3	2.4	1.2	1.5	0.6	0.3	0.5
	PEN	(h)	0.75	0.87	0.87	0.91	0.93	0.95	0.97	0.96
	NP	(h)	0.25	0.13	0.13	0.09	0.07	0.05	0.03	0.04
	E/PEN	(%)	0.5	1.9	4.5	18.2	43.5	65.1	64.6	78.5
	new E	(#)	0.1	0.3	0.2	0.8	0.5	0.5	0.5	0.5
stem	PEN	(#)	3.9	2.3	1.3	1.1	0.8	1.9	1.6	1.2
	PEN	(h)	0.67	0.88	0.89	0.89	0.88	0.85	0.85	0.95
	NP	(h)	0.33	0.12	0.11	0.11	0.12	0.15	0.15	0.05
	E/PEN	(%)	1.4	20.4	35.9	34.7	45.8	51.2	46.5	53.3
	new E	(#)	0.1	0.9	0.5	0.4	0.6	0.1	0.3	0.6

Sieve element punctures were always associated with the salivation waveform (E1). A few punctures that lasted for less than 10 minutes showed the ingestion waveform (E2) additionally. This was significantly more common on stems than on leaves (Table 2, < 10 min). Sieve element punctures lasting longer than 10 minutes nearly always showed E2 (Table 2, > 10 min). Alternation between E1 and E2 patterns was rather common during sustained periods of E but these two waveforms were not mutually exclusive, i.e. they often occurred together with the tendency for E2 to become more marked. As many long sieve element punctures started late, this tendency was not always very clear.

TABLE 2. Total number of sieve element punctures (periods with E waveforms) on leaf and stem, all showing salivation (E1), and the number (fraction) that showed ingestion (E2) additionally.

	punctures with	leaf	stem
< 10 min	E1	27	28
	E1 and E2	3	17
> 10 min	E1	15	21
	E1 and E2	15	19

On average, *A. fabae* needed 3.5 hours to puncture a sieve element (1st E) when probing on leaves, and 5.2 hours to achieve a sustained puncture (sus E). On stems it was 3.6 and 4.3 h respectively (Table 3), which is not statistically significantly different from the values for leaves. Before achieving a sustained sieve element puncture, the aphids made significantly more (12.3, $p < 0.1$) separate probes on leaves than on stems (8.5). As is common in behaviour, these parameters showed a large individual variation (Table 3 and Fig. 2).

TABLE 3. Time needed to achieve the first observable puncture of a sieve element (1st E) and the first sustained sieve element puncture (sus E) which generally includes ingestion. Time measured from access to the plant and from the start of the probe in which the phenomenon occurred. Significance, $p < 0.01$ (Man-Whitney U-test). Parameter numbers (1–4) refer to Fig. 1.

	parameter	plant part	time to (hours)		sign.
			AVG	SD	
plant access	(1) 1st E	leaf	3.4	1.7	a
		stem	3.6	2.9	a
	(2) sus E	leaf	5.2	1.8	b
		stem	4.3	2.8	b
probe	(3) 1st E	leaf	0.7	0.4	c
		stem	0.8	0.4	c
	(4) sus E	leaf	1.0	0.5	d
		stem	3.7	3.4	e

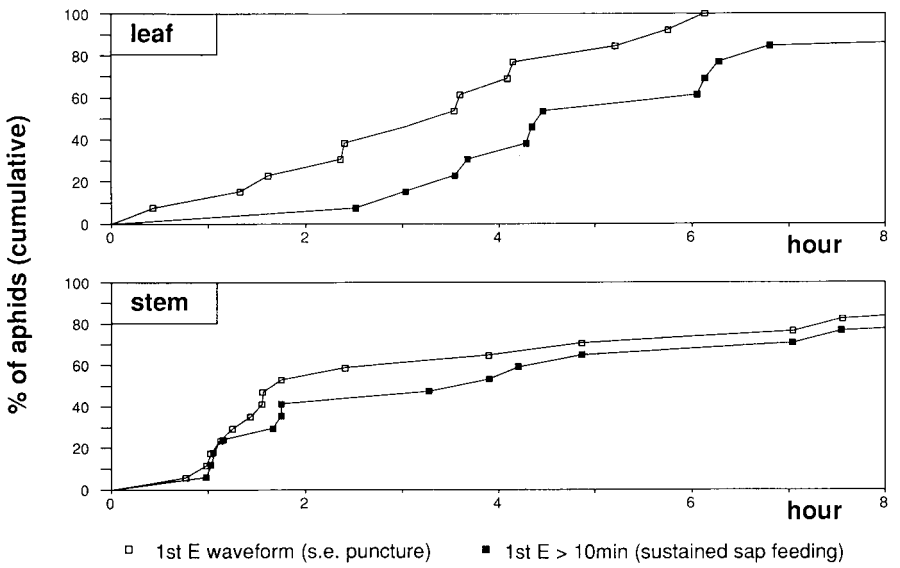

Fig. 2. Cumulative percentage with time of aphids that achieved first sieve element puncture (1st E) and first sustained (> 10 min) sieve element puncture with ingestion when probing on bean leaves and stems.

The first sieve element puncture occurred after 26 min, but other aphids, three on stems, generated no E waveforms within the 8 hours of the experiment. Similarly, the earliest sustained E occurred after 59 minutes, but 6 aphids, 2 on leaves and 4 on stems, did not generate a sustained E waveform. E waveforms lasting longer than 10 min mostly lasted longer than 1 hour as well. In only 6 cases, 2 on leaves and 4 on stems, were the sieve element punctures ended between 10 minutes and 1 hour. Nevertheless, sieve element punctures of more than 1 hour were not always sustained for much longer. Ten aphids, 4 on leaves and 6 on stems, withdrew their stylets before the end of the experiment and 14 aphids continued with E waveforms up to the end of the experiment.

Within a probe, the mean time needed to achieve the first E waveform (sieve element puncture) was 46 (± 25) minutes with no significant difference between leaf and stem (Table 3). However, the time needed within a probe to achieve a sustained E waveform (ingestion) was significantly ($p < 0.1$) longer on stems. Probes that achieved sustained ingestion (sus E) had a mean of 2 to 3 preceding shorter sieve element punctures on leaves and about 6 on stems.

DISCUSSION AND CONCLUSIONS

Although the average time needed for the stylets of aphids to penetrate from the epidermis to a sieve element was not more than 45 minutes (average within a probe), it took the aphids 3 to 4 hours to achieve this after gaining access to a plant. Moreover, another hour or more was needed to achieve a sieve element puncture with sustained sap ingestion (feeding). Before the onset of feeding aphids made several probes and short sieve element punctures. Thus, reaching a sieve element is not the only prerequisite for sustained ingestion by aphids.

On the basis of these results there is no reason to consider the stem as a less or more suitable feeding site than the leaf. Aphids tended to start phloem feeding on stems more quickly, but on the other hand, the percentage of time spent in sieve elements stops at about 50% on stems and increases up to 78% on leaves (Table 1). It is difficult to explain these differences and the number of replicates does not allow much to be concluded.

During early sieve element punctures secretion of saliva (E1) is a major activity. In their ultrastructural study of stylet pathways, Tjallingii & Hogen Esch (1993) did not find any salivary sheath material inside punctured sieve elements. Probably, the saliva secreted into sieve elements is of a watery type, as suggested by Miles (1972). There is nothing known about the composition of the E1 saliva or of its function. The composition of saliva secreted during E2 and other waveforms is also not known.

Earlier Tjallingii & Mayoral (1992), speculated on the role of the E1 saliva suppressing the defence or wound reactions of sieve elements, or in inducing changes in plant metabolism that make the phloem sap more acceptable. Though the above results provide no further support for this hypothesis, they pose questions about the function of salivation during both sieve element activities, represented by waveforms E1 and E2. It is clear however, that we need to know the chemical composition of the saliva if we are to understand the aphid-plant interactions.

REFERENCES

MILES P.W. 1972: The saliva of Hemiptera. *Adv. Insect Physiol.* **9**: 183–255.

PRADO E. & TJALLINGII W.F. 1993: Aphid activities during sieve element punctures. In Kindlmann P. & Dixon A.F.G. (eds): *Critical Issues in Aphid Biology. Proc. 4th Int. Symp. Aphids.* České Budějovice, pp. 109–112.

TJALLINGII W.F. 1985: Membrane potentials as an indication for plant cell penetration by aphid stylets. *Entomol. Exp. Appl.* **38**: 187–193.

TJALLINGII W.F. 1988: Electrical recording of stylet penetration activities. In Harrewijn P. & Minks A.K. (eds): *Aphids, their Biology, Natural Enemies and Control. Vol. 2B.* Elsevier, Amsterdam, pp. 95–108.

TJALLINGII W.F. 1990a: Continuous recording of stylet penetration activities by aphids. In Campbell R.K. & Eikenbary R.D. (eds): *Aphid-plant Genotype Interactions.* Elsevier, Amsterdam, pp. 89–99.

TJALLINGII W.F. 1990b: Stylet penetration parameters from aphids in relation to host-plant resistance. *Symp. Biol. Hung.* **39**: 411–419.

TJALLINGII W.F. & HOGEN ESCH T. 1993: Fine structure of aphid stylets routes in plant tissues in correlation with EPG signals. *Physiol. Entomol.* **18**: 317–328.

TJALLINGII W.F. & MAYORAL A. 1992: *Criteria for Host-Plant Selection by Aphids.* Proc. 8th Int. symp. Insect-plant relationships, Kluwer Acad. Publ., Dordrecht, pp. 280–282.

WENSLER R.J. 1977: The fine structure of distal receptors on the labium of the aphid Brevicoryne brassicae L. (Homoptera). *Cell Tiss. Res.* **181**: 409–421.