

## Pattern of cell punctures by the aphid *Sitobion fragariae* (Sternorrhyncha: Aphididae) and cell arrangement in the leaf tissues of wheat, *Triticum aestivum*

CLAUDIO C. RAMÍREZ, NANCY OLEA and HERMANN M. NIEMEYER

Laboratorio de Química Ecológica, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile; e-mail: claudio@abulafia.ciencias.uchile.cl

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**Abstract.** The pattern of cell punctures in the electrical penetration graph was studied from the start of a probe leading to the first phloem phase by the aphid *Sitobion fragariae* (Walker) (Sternorrhyncha: Aphididae) on two cultivars of the wheat, *Triticum aestivum* L., differing in hydroxamic acid concentration. In addition, the spatial arrangement of cells in the leaf of the host plant was histologically observed and the number of cells between the epidermis and phloem counted. The results suggest a feeding deterrent effect of hydroxamic acids and a constant stylet puncturing activity into plant cells.

### INTRODUCTION

Aphids are a group of specialised phloem-feeding insects (Dixon, 1985). During penetration into plant tissues before achieving a phloem phase, the aphid's stylets produce a variable number of cell punctures (Pollard, 1977). Light and electron microscopy studies coupled with electrical penetration graphs (EPG) show that nearly every cell in the epidermis, parenchyma and vascular bundles encountered by the stylet before a phloem phase occurs, is punctured (Spiller et al. 1985; Kimmins, 1986; Tjallingii & Hogen Esch, 1993). The phloem phase starts with a short period of salivation into a sieve element (E1 waveform in EPG records), which is usually followed by phloem ingestion (Prado & Tjallingii, 1994).

The aim of this work is to explore a possible relationship between the pattern of EPG-recorded cell punctures in the first probe ending in a phloem phase and the histologically-estimated numbers of cells from the epidermis to a sieve element, and the eventual effect of the plant chemistry on the parameters studied. The system studied included the aphid *Sitobion fragariae* (Walker) on two cultivars of the wheat *Triticum aestivum* L. differing in hydroxamic acids (Hx).

### MATERIAL AND METHODS

#### Insects

Apterous virginoparous individuals of a single clone of the aphid *S. fragariae* (Walker) were kept on oat (*Avena sativa* L. cv. Nahuén) under laboratory conditions, at  $20 \pm 2^\circ\text{C}$  and a 16L:8D photoperiod. Probing behaviour was monitored through electrical penetration graphs, or EPG's (Tjallingii, 1978). Waveforms have been correlated with the position of the stylet in the plant tissue (Tjallingii, 1978); consequently, stylet activities can be assessed in detail. In our study, it was assumed that each potential drop (pd) in the signal recorded revealed a cell puncture (penetration through the plasmalemma), whether it occurred in cells of the epidermis, mesophyll or sieve elements (Tjallingii, 1985). Aphids were withdrawn from the stock culture and hooked to the EPG system. Activities were followed until a phloem phase was observed. This period included a variable number of probes, comprising the last one which ended in a phloem phase, as judged from the observation of the E1 waveform. Probes in which xylem ingestion occurred were excluded from the analysis.

#### Plants

The first leaf of 7-day old seedlings of wheat *T. aestivum* cvs. Millaleu and Naofén, were used for aphid stylet monitoring and histological preparations. Chemical analysis showed that Hx concentration was significantly higher in cv. Naofén than in cv. Millaleu ( $1.94 \pm 0.31$  mmol/kg fresh weight,  $n = 8$ , and  $1.09 \pm 0.30$  mmol/kg fresh weight,  $n = 8$ , respectively;  $F(1,14) = 29.21$ ,  $n = 16$ ,  $p = 0.00009$ ).

In order to estimate the number of cells from the epidermis to the sieve elements, histological preparations of unharmed seedlings of both cultivars were produced. Segments corresponding to the upper third of the leaf ( $n = 22$  leaves of each cultivar), were fixed in 3–4% formaldehyde in 0.1 M sodium phosphate pH 7.2. To improve infiltration, the segment was kept in the fixative under vacuum for 24 h. After rinsing and dehydration with ethanol, samples were infiltrated under vacuum with EPON 812 resin, and 2  $\mu\text{m}$  thick transversal sections of the embedded segments were stained with 1% toluidine blue in 1% sodium borate. The sections were mounted on glass slides for light microscopy observations. One section was then chosen for further studies which showed sufficient incorporation of staining agent. The number of cells intercepted by an imaginary transect (Fig. 1B) drawn from a point between two epidermal cells to the closest sieve element in each leaf section of the upper surface was counted. The central and the two adjacent vascular bundles were used for countings in each section.

#### Pattern of cell punctures and number of cells in the plant tissue

In order to describe the pattern of cell punctures, each probe ending with a phloem phase was split into two parts of equal duration after subtracting the duration of potential drops, and the number of cell punctures in each period was determined. Similarly, the transect used to count the number of cells in the leaf tissue was divided into two segments of equal length, allowing the characterisation of the "external part of the mesophyll", which included cells from epidermis and mesophyll, and the "internal part of the mesophyll", which included mesophyll cells of the outer and inner bundle sheath and vascular cells until the sieve element (Fig. 1B).

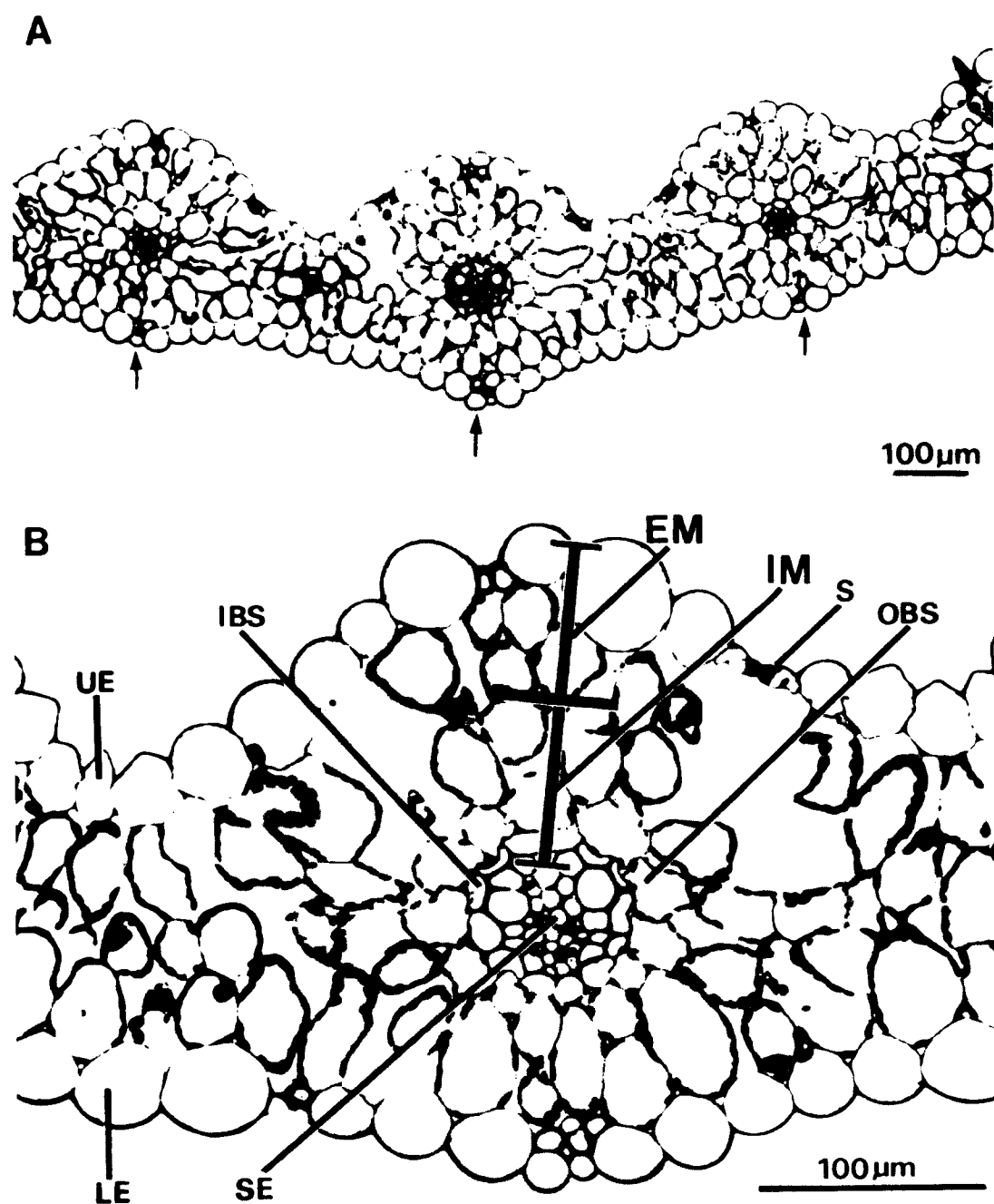


Fig. 1. Light microscopy preparations of a transversal section of the leaf of a wheat *T. aestivum* seedling. Since no differences between cultivars were apparent, only cv. Millaleu is shown. A: central and two lateral veins; arrows indicate the veins sampled. B: detail of central vein. Transects studied included the external mesophyll (EM) and internal mesophyll (IM) of the upper surface of the leaf (adaxial surface). UE – upper epidermis, LE – lower epidermis, OBS – outer bundle sheath, IBS – inner bundle sheath, S – stoma, SE – sieve element.

## RESULTS AND DISCUSSION

Mean duration of cell punctures did not differ significantly between the two parts of the recording in the low-Hx cultivar

(Table 1), while in the high-Hx cultivar cell punctures were significantly shorter during the first part of the recording (Table 1). Moreover, during the first part of the probes, a significantly lower number of cell punctures was observed in the low-Hx cul-

TABLE 1. Pattern of cell punctures from EPG recording of the first probe ending in a phloem phase (a) and number of cells estimated histologically along a defined transect (b). Mean and standard errors are shown. p-level calculated with t-test for dependent samples or independent samples (duration of the probe).

Parameter	Low Hx (n = 19)			High Hx (n = 16)		
a) EPG recordings						
	First part of recording	Second part of recording	p-level	First part of recording	Second part of recording	p-level
Number of cell punctures	5.9 ± 1.1	9.3 ± 1.4	0.04	8.3 ± 1.6	12.0 ± 2.7	0.03
Duration of cell punctures (sec)	5.1 ± 0.14	5.0 ± 0.08	0.63	4.6 ± 0.08	5.0 ± 0.11	0.04
Duration of the probe (min)	22.6 ± 3.6			23.8 ± 3.1		0.34
b) Histological analysis						
	External part of transect	Internal part of transect	p-level	External part of transect	Internal part of transect	p-level
Number of cells	1.6 ± 0.03	2.3 ± 0.04	0.0001	1.7 ± 0.03	2.4 ± 0.04	0.0001
c) Ratios						
Estimated number of punctures per cell	3.6 ± 0.8	4.0 ± 0.9	0.94	4.9 ± 1.2	5.0 ± 1.8	0.98

tivar than in the same period in the high-Hx cultivar ( $p = 0.03$ ,  $t$  test for independent samples; not shown in Table 1). These results suggest a deterrent effect of Hx at the level of epidermis and/or mesophyll as has been suggested earlier (Givovich & Niemeyer, 1991; Mayoral et al., 1996). In support of this suggestion of feeding deterrence by Hx, the number of probes before the 1st probe that showed a phloem phase was significantly higher in the high-Hx than in the low-Hx cultivar ( $4.70 \pm 0.58$  and  $7.22 \pm 0.91$  in low- and high-Hx cultivars respectively;  $p = 0.02$ ,  $t$  test for independent samples), and the total accumulated probing time before the probe ending in a phloem phase ( $16.0 \pm 3.2$  and  $41.8 \pm 7.8$  min in low- and high-Hx cultivars respectively;  $p = 0.004$ ,  $t$ -test for independent samples). It is important to note that the comparisons discussed are not distorted by the duration of the probe before the phloem phase, since these did not differ between cultivars (Table 1).

The number of cell punctures increased significantly as penetration progressed within the probe with a phloem phase (Table 1), and the number of cells estimated histologically was higher in the internal part of the transect than in its external part in both cultivars (Table 1). Moreover, the estimated number of punctures per cell did not differ significantly along the transect/probe studied in both cultivars (ratios in Table 1). Given that aphids seem to follow a trial-and error procedure in which nearly every cell encountered is punctured (Tjallingii & Hogen Esch, 1993), this suggests a constant rate of stylet puncturing activity within any given cell. We do not at present have an explanation to this; however, not quite unexpectedly, different aphid-plant combinations yield different quantitative results. For example, in *Aphis fabae/Vicia faba*, Tjallingii & Hogen Esch (1993) report ca. 1.4 punctures per cell in the vascular bundle.

The results obtained rest on one important constraint, which is that the stylet penetration was associated with a unidimensionally-evaluated pattern of inner structure of the leaf (number of cells encountered along a straight transect), whereas stylets penetrate plant tissue tridimensionally (Pollard, 1977). This parsimonious approach serves as an initial point of reference for further increases of complexity and realism. Comparison between the pattern of cell punctures and plant tissues with different cell arrangements (i.e. stem, leaf) in other aphid-plant

systems would shed further light into the mechanism of aphid stylet penetration into plants.

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