Salivation into sieve elements in relation to plant chemistry: the case of the aphid *Sitobion fragariae* and the wheat, *Triticum aestivum*

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**Abstract**

Extended sieve element salivation (E1 waveform in the electrical penetration graph) is a characteristic activity during early sieve element punctures, particularly in resistant plants. In order to explore a chemically-mediated mechanism of resistance associated with sieve element salivation, we compared the pattern of feeding behaviour of the aphid, *Sitobion fragariae* (Walker), on two cultivars of the wheat *Triticum aestivum* L., with different concentrations of hydroxamic acids (Hx). During 24 h of electronic monitoring, aphids dedicated over 50% of the total time to phloem ingestion from the sieve elements. Total time allocated to E1 in the experiment, time to first E1 within the experiment, time allocated to E1 before a sustained phloem ingestion (E2) and the contribution of sieve element salivation to the phloem phase (E1/[E1+E2]) were significantly higher in the high-Hx cultivar. The increased salivation in plants with higher contents of Hx suggests the existence, at least in this system, of a chemically-mediated sieve element constraint.

**Introduction**

Feeding behaviour of cereal aphids has been intensively studied in relation to plant resistance. Comparison of feeding behaviour in susceptible/resistant plants as well as in host/non-host plants has been used to explore the plant factors accounting for the resistance (Spiller, 1988; Girma et al., 1992; Caillaud et al., 1995; Mayoral et al., 1996). Recently, studies using the electrical penetration graph (EPG) technique have reported increased salivation into sieve elements (E1 pattern) (see review in Prado, 1997), as well as delay in sieve element ingestion (Givovich & Niemeyer, 1995; Caillaud et al., 1995) of aphids feeding on resistant plants. In order to explore a chemically-mediated mechanism associated with sieve element salivation, we studied the feeding behaviour of the aphid *Sitobion fragariae* (Walker) on two cultivars of wheat, *T. aestivum*, varying in their content of hydroxamic acids (Hx), with special emphasis on EPG parameters related to salivation in sieve elements. Several investigations on Hx have addressed their role in the resistance of wheat against aphids (Niemeyer, 1990; Nicol et al., 1992; Givovich et al., 1994; Mayoral et al., 1996, Nicol & Wratten, 1997).

**Materials and methods**

**Insects.** A clonal colony of *S. fragariae*, derived from an individual collected in central Chile, was established in the laboratory on oat (*Avena sativa* L. cv. Nahuén) at 20 ± 2°C and L16:D8 photoperiod.

**Plants.** The first leaves of 7-day-old seedlings were used from cultivars Naofén and Millaleu of the wheat *T. aestivum*. Chemical analysis showed that the concentration of Hx (determined as the glucoside of DIMBOA following the procedure described by Weibull & Niemeyer, 1995) was significantly higher in cv. Naofén than in cv. Millaleu (1.94±0.31 mmol/kg fresh weight, n = 8, and 1.09±0.30 mmol/kg fresh weight, n = 8, respectively; F(1, 14) = 29.21, n = 16, P < 0.001).
Experimental set-up. Each experimental aphid was monitored over 24 h using the EPG technique (Tjallingii, 1978). All signals were recorded on a PC hard disk and analysed with the EPGview software (Flores et al., 1998, unpubl.) Detailed assessment of aphid activities could be achieved as during aphid probing the location of the stylet tips and their activities produce recognisable patterns of voltage changes in the recorded signal (Tjallingii & Hogen Esch, 1993; Prado & Tjallingii, 1994).

Parameters used to study probing behaviour. Aphid feeding behaviour was continuously monitored for 24 h on cv. Millaleu (low-Hx) and cv. Naofén (high-Hx) seedlings. A total of 23 recordings were performed for each cultivar. The following wave forms were recorded and recognised: non-probing (NP), pathways activities (C), salivation into a sieve element (E1), ingestion from the sieve elements (E2), xylem ingestion (G), and difficulties during stylet penetration (F). Timing the occurrence of each pattern also allowed the evaluation of the following parameters related to activities in sieve elements: (1) proportion of the time allocated to each activity, (2) time to first E1 within the experiment, (3) total duration of E1 periods within the experiment, (4) number of E1 periods, (5) mean duration of E1 periods, (6) duration of E1 periods before the first sustained E2 (i.e., E2 for more than 8 minutes) in the experiment, (7) duration of E1 periods before the first sustained E2 within a probe (a probe, here, is a continuous period without withdrawal of the stylets from the plant), (8) mean duration of the E1 period preceding the first sustained E2, (9) contribution of total E1 to total phloem phase (E1/[(E1+E2)], and (10) time to the first sustained E2 in the experiment.

Results and discussion

The major aphid activity during 24 h of electronic monitoring was phloem ingestion, to which aphids dedicated over 50% of the total time (Figure 1). The number of aphids showing phloem phase was not significantly different between cultivars (20 out of 23 and 21 out of 23 in low and high-Hx cultivar, respectively; \( Z = 0.94, P = 0.34, \) test for two proportions [Zar, 1996]). Similarly, the proportion of time devoted to phloem ingestion did not show differences associated with Hx variation, a result comparable with those reported for other cereal aphid species (Girma et al., 1992; Givovich & Niemeyer, 1996; Mayoral et al., 1996). Surprisingly, xylem ingestion (G pattern) was significantly higher in the low-Hx cultivar, although the number of aphids showing G pattern was not significantly different (12 out of 20 and 8 out of 21 in low and high-Hx cultivar, respectively; \( Z = 1.09, P = 0.27, \) test for two proportions [Zar, 1996]). These results are in contradiction with other studies in other cereal aphid species (Givovich & Niemeyer, 1995). It is likely that in the present system, xylem ingestion was reduced simply as a consequence of the higher proportion time devoted to E1 in high-Hx cultivar (Figure 1).

Focusing on sieve element salivation, the high-Hx cultivar showed significantly different results to the low-Hx cultivar in the following E1-related parameters (see Table 1): time to first E1 within the experiment, total duration of E1 periods, duration of the E1 periods before the first sustained E2 and contribution of sieve element salivation to the phloem phase. Clearly, Hx affect the process of salivation into the sieve elements.

Since Hx are mainly present in meristematic, epidermal, mesophyll and vascular tissue (Epstein et al., 1986, Argandoña et al., 1987), it is reasonable to speculate that Hx are perceived by the aphid during cell punctures during the pathway to sieve elements. The deterrent effect of Hx would cause a delay in the time needed to attain a first sieve element salivation (Niemeyer & Pérez, 1995; Ramírez et al., 1999). Brief cell punctures in the sieve elements may also occur (Tjallingii & Hogen Esch, 1993) and can contribute in such a delay. On the other hand, the increased duration of salivation periods seems more likely to be the result
Table 1. EPG parameters of feeding behaviour related to salivation and ingestion in the sieve elements over the 24-h recording period. Probabilities were calculated using a Mann–Whitney test

<table>
<thead>
<tr>
<th>EPG parameter</th>
<th>Low Hx</th>
<th>High Hx</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 20</td>
<td>n = 21</td>
<td></td>
</tr>
<tr>
<td>2. Time to first E1 (min)</td>
<td>(X ± SE)</td>
<td>(X ± SE)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>69.8 ± 10.4</td>
<td>140.5 ± 36.2</td>
<td>0.033</td>
</tr>
<tr>
<td>3. Total duration of E1 periods (min per 24 h)*</td>
<td>44.5 ± 10.2</td>
<td>102.2 ± 16.0</td>
<td>0.002</td>
</tr>
<tr>
<td>4. Number of E1 periods (#)</td>
<td>5.1 ± 0.9</td>
<td>8.4 ± 1.6</td>
<td>0.260</td>
</tr>
<tr>
<td>5. Mean duration of E1 periods (min)</td>
<td>9.9 ± 1.1</td>
<td>28.4 ± 9.1</td>
<td>0.170</td>
</tr>
<tr>
<td>6. Duration of E1 before first sustained E2 (min)</td>
<td>30.3 ± 7.1</td>
<td>54.7 ± 9.7</td>
<td>0.041</td>
</tr>
<tr>
<td>7. Duration of E1 before first sustained E2 within a probe (min)</td>
<td>25.7 ± 5.8</td>
<td>46.6 ± 9.3</td>
<td>0.077</td>
</tr>
<tr>
<td>8. Mean duration of the E1 preceding first sustained E2 (min)</td>
<td>11.1 ± 1.6</td>
<td>30.2 ± 9.0</td>
<td>0.123</td>
</tr>
<tr>
<td>9. Contribution of E1 to phloem phase (%)</td>
<td>11.2 ± 4.4</td>
<td>19.3 ± 4.9</td>
<td>0.018</td>
</tr>
<tr>
<td>10. Time to first sustained E2 (min)</td>
<td>228.6 ± 46.5</td>
<td>291.2 ± 54.5</td>
<td>0.251</td>
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</table>

*Figure 1 shows these values as the proportion of total time allocated to different probing activities.

of Hx in the phloem sap (Givovich et al., 1994; but see Caillaud & Niemeyer, 1996). However, a direct causal link between the Hx content and E1 duration must be made with caution because the Hx content of extracts of macerated whole leaves does not correlate strongly with the content in the phloem sap (Givovich et al., 1994).

In conclusion we have shown that Hx are associated with a delay in the time to start the process of salivation in the sieve elements and with a increase in the process of salivation itself, suggesting that these compounds may be acting both at the level of the epidermis/mesophyll and the phloem.

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