Host plant changes produced by the aphid *Sipha flava*: consequences for aphid feeding behaviour and growth

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**Abstract**

Induced plant responses may affect the behaviour and growth of the attacking herbivore insect. The aphid *Sipha flava* (Forbes) produces reddish spots on the infested leaf of its host plant *Sorghum halepense* (L.). In order to assess the consequences on the aphid of this presumptive induced plant response, we studied the feeding behaviour and growth of *S. flava* on previously infested and non-infested leaves of *S. halepense*. Considering that the reddish pigment could play a defensive role, its effect on aphid survival was determined in artificial diets. In addition, changes in the histology of the leaf and the chemical nature of the induced pigment were also studied. Aphids devoted a significantly shorter total time to non-penetration activities in infested than in non-infested leaves. Time before the first phloem ingestion tended to be shorter in infested leaves. The mean relative growth rate of *S. flava* nymphs was significantly higher on infested than on non-infested leaves. Survival of aphids on diet containing the reddish extract was not significantly different from that on the control diet. Infestation of *S. halepense* by *S. flava* produced a reddish coloration in the leaf, which was identified as an anthocyanin by UV-visible spectrometry. Light microscopy showed that only mesophyll cells of previously infested plants presented swelled, dispersed, and heterogeneously stained chloroplasts with a higher accumulation of starch granules, no grana arranged in stacks, and reduction in the amount of inner membranes (thylakoids), relatively to chloroplasts of non-infested leaves. Scanning electron micrographs of leaf surface revealed reduced presence of crystalline epicuticular waxes of epidermal cells in infested leaves as compared to non-infested ones. The main conclusion is that the attack of *S. flava* to *S. halepense* leaves induced plant susceptibility where aphid feeding behaviour and growth were both enhanced on previously infested leaves.

**Introduction**

Accumulated evidence shows that chewing insect attack may induce changes in host plant quality. These changes may affect insect behaviour (Jongsma & Bolter, 1997; Havill & Raffa, 1999), and insect performance (Karban & Baldwin, 1997 and reference therein). Performance may be reduced (induced defense; Agrawal, 2000; Agrawal & Karban, 2000) or increased (induced susceptibility; Underwood, 1998; Karban & Kittelson, 1999). The impact of induced plant responses on both behavioural patterns and growth of chewing insects (McAuslane & Alborn, 2000; Van Dam et al., 2000) have been frequently addressed. However, few studies have investigated the consequences of induced plant response on phloem-feeding insects (Dorschner et al., 1987; Formusoh et al., 1992). Herein we address whether the feeding behaviour and growth of a phloem-feeding insect are affected by host plant changes due to previous insect attack.
Sipa flavă (Forbes) (Hemiptera: Aphididae) is a cereal aphid that uses different cultivated and wild Poaceae as hosts (Blackman & Eastop, 1984). This aphid is frequently found on older leaves of its host plants (Holman, 1974). It shows low mobility and persists on heavily attacked leaves, and its attack is generally associated with local appearance of reddish spots on infested leaves, which has been suggested to be an induced response (Breen & Teetes, 1986; Webster, 1990; Costa-Arbulú et al., 2001). The spot usually spreads with aphid feeding eventually to cover the entire leaf, accelerating its senescence (Breen & Teetes, 1986; Webster, 1990).

In the present work, the consequences on S. flavă of induced plant response were studied. The following question was addressed: what are the effects of the induced plant response on the feeding behaviour and growth of S. flavă? In addition, in order to characterize the host plant changes, tissue morphology between infested and non-infested leaves was compared. Considering that the reddish pigment could play a defensive role, its chemical nature and its effect on aphid survival were determined.

Materials and methods

Insects and plants. Sipa flavă individuals were collected from S. halepense growing close to wheat fields in Santiago, Chile. This aphid was recently reported in Chile on various grasses, most frequently on Sorghum halepense L. (Gonzales et al., 1998), a common perennial weed associated to crop areas (McWhorter, 1989). Aphids were maintained in a greenhouse for several generations on seedlings of Hordeum vulgare L. cv. F. Union. Seedlings of S. halepense were obtained from seed collected in the field and also reared in a greenhouse. Conditions in the greenhouse were 20 ± 2 °C, 14:10D, 5.7 Klux. Six-leaf plants were used to perform all experiments.

Feeding behaviour of S. flavă on non-infested and infested leaves. Feeding behaviour was electronically monitored using electrical penetration graphs (EPG). Two groups of ten wingless adults of S. flavă were individually subjected during 8 h to continuous EPG-monitoring. One group was monitored on S. halepense leaves (the third leaf of each plant) that were previously infested during 72 h with 15 aphids confined into a clip cage. The other group was monitored on control leaves which were not previously infested but were clipped in the same way as the infested leaves. EPG trials were run from around 9:00 a.m. until 17:00 with at least one replicate of both treatment and control per trial. A gold wire electrode (25 μm diameter) was fixed to the dorsum of aphids with conductive silver paint. Another electrode was inserted in the soil of the potted plant. Both electrodes were connected to a DC electric circuit (Tjallingii, 1978). When the aphid stylectes penetrate into the plant tissues, the electrical circuit is closed and the voltage changes are amplified and continuously monitored. All signals were recorded on a PC hard disk for detailed analysis with the EPGview software (A. Corvalán, N. Flores, C. C. Ramírez & H. M. Niemeyer, unpubl.). Different stylectes activities and the location of the stylectes' tip produce specific waveforms in the recorded signal, so the stylectes' activity and stylectes' tip position can be judged from the displayed signal (Tjallingii & Hogen Esch, 1993).

Growth of S. flavă on non-infested and infested leaves. This experiment evaluated the effect of changes in leaf quality on aphid growth. Synchronized second-instar nymphs from a stock culture were placed on the third leaf of S. halepense seedlings and confined with clip-cages. The control involved non-infested plants; the treatment involved plants infested on the third leaf with 15 aphids during four days prior to the experiment. After this time, the treatment leaves were carefully cleaned and immediately used. Mean relative growth rate of aphids, MRGR (Adams & van Emden, 1972), was determined as a measure of the performance of individual aphids. Nymphs were individually weighed (initial weight: 20–40 μg). Aphids were removed from the clip cages four days later and weighed. MRGRs [(ln final weight – ln initial weight) / number of days] between treatments were compared using t-test (n = ten replicates per treatment).

Light and electron microscopy observations of non-infested and infested leaves. Segments of an infested and a non-infested third leaf of experimental plants were fixed during 4 h in 3–4% glutaraldehyde in 0.1M sodium phosphate, pH 7.2, and post-fixed in 1% osmium tetroxide. Fixation and infiltration in EPON 812 resin were performed under vacuum at room temperature. For light microscopy, 2 μm transversal sections were stained with 1% toluidine blue in 1% sodium boroate. For electron microscopy, double-stained sections were viewed in a Zeiss EM 109 electron microscope.
Chemical characterisation of the reddish pigment.
Three-cm sections were excised from non-infested and infested leaves, and extracted for 24 h at 4 °C in 5 ml of 1% hydrochloric acid in methanol (v/v). Spectra were evaluated in the wavelength region from 400 to 700 nm using a Shimadzu UV-Visible recording spectrophotometer model UV-240.

Examination of the colours of the reddish extract under different conditions was undertaken, using as guidance the general classification of flavonoid pigments suggested by Harborne (1973). Spectra were recorded of the pigment alone and with ammonia under UV light, and of its alkalised methanolic solution. The changes of colour were registered.

Aphid survival on diet containing reddish pigment.
Aphid survival was evaluated on artificial diets containing the reddish pigment (Costa-Arbulu et al., 2001). Ten aphids were confined in a vertically placed Plexiglass cylinder (3.0 cm high, 2.5 cm ID). A plastic net covered one end of the cylinder, while the other end was closed with a sachet made from two Parafilm M ® membranes. The sachet contained either 200 μl of a 25% w/v sucrose solution (prepared in phosphate buffer pH 5.5) (control), or the same solution containing 8 mg of the reddish extract giving a concentration comparable to that found in infested leaves (treatment). The cylinders containing the aphids were kept at 20 °C in the dark for 48 h. At the end of this period aphid survival was recorded (n = 16 replicates).

Results

Feeding behaviour. EPG monitoring showed that, in comparison with aphids on non-infested plants, aphids that probed on previously infested leaves devoted a significantly shorter total time to non-probing activities (Table 1). Time in non-penetration was also longer when only time before the first phloem ingestion was considered. Activities related with pathway activities, xylem ingestion, phloem ingestion, sieve element salivation, and intracellular penetrations, did not differ between treatments (Table 1).

Growth of S. flava on non-infested and infested leaves.
Mean relative growth rate of S. flava nymphs was significantly higher (0.282 ± 0.021) on previously infested leaves than on non-infested ones (0.179±0.023) (t-test, t(1,18) = 3.26, P < 0.005).

Figure 1. Light microscopy micrographs of a transversal section of a non-infested (A) and previously infested (B) S. halepense leaf (590×). Scale bar: 17 μm.

Light and electron microscopy observations of non-infested and infested leaves. Light microscopy revealed that, mesophyll cells of infected leaves presented dispersed and heterogeneously stained chloroplasts (Figure 1B). In contrast, in non-infested leaves these organelles appeared healthy, in the periphery of the cytoplasmatic space, with intense homogeneous staining, particularly in cells of the bundle sheath (Figure 1A), and no reddish areas or conglomerates were found. Electron micrographs showed that chloroplasts of infested leaves presented a higher accumulation of starch granules, no grana arranged in stacks, and reduction in the amount of inner membranes (thylakoids) relative to chloroplasts of non-infested leaves (Figure 2A and B). Scanning electron micrographs showed that crystalline epicuticular waxes on the leaf surface were less abundant and more heterogeneously distributed on infested leaves compared to non-infested ones (Figure 3A and B).
Table 1. Electropenetration graph parameters (Mean ± SE) obtained from 8h recordings (N=10 per treatment) of the probing behaviour of the aphid S. flava feeding on previously infested and non-infested leaves of S. halepense. P values from non-parametric Mann-Whitney U test.

<table>
<thead>
<tr>
<th>EPG Parameter</th>
<th>Infested leaves</th>
<th>Non-infested leaves</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time devoted to non-penetration before the first phloem phase (min)</td>
<td>16.5 ± 5.0</td>
<td>39.7 ± 10.4</td>
<td>0.03</td>
</tr>
<tr>
<td>Total time devoted to non-penetration (min)</td>
<td>26.4 ± 5.8</td>
<td>85.0 ± 24.0</td>
<td>0.04</td>
</tr>
<tr>
<td>Time devoted to pathway activities before the first phloem phase (min)</td>
<td>16.4 ± 5.6</td>
<td>25.3 ± 8.4</td>
<td>0.45</td>
</tr>
<tr>
<td>Total time devoted to pathway activities (min)</td>
<td>94.1 ± 29.8</td>
<td>135.4 ± 30.2</td>
<td>0.33</td>
</tr>
<tr>
<td>Duration of intracellular penetrations (s)</td>
<td>4.2 ± 0.1</td>
<td>4.2 ± 0.1</td>
<td>0.91</td>
</tr>
<tr>
<td>Total time devoted to intracellular penetrations (min)</td>
<td>1.9 ± 0.9</td>
<td>2.1 ± 0.4</td>
<td>0.36</td>
</tr>
<tr>
<td>Total time devoted to xylem ingestion (min)</td>
<td>136.3 ± 33.1</td>
<td>119.0 ± 34.6</td>
<td>0.80</td>
</tr>
<tr>
<td>Duration of each salivation into sieve elements (min)</td>
<td>18.7 ± 3.8</td>
<td>56.0 ± 17.7</td>
<td>0.80</td>
</tr>
<tr>
<td>Time to first phloem phase (min)</td>
<td>58.0 ± 18.0</td>
<td>99.1 ± 14.0</td>
<td>0.08</td>
</tr>
<tr>
<td>Duration of phloem ingestion periods (min)</td>
<td>27.8 ± 9.2</td>
<td>13.0 ± 5.1</td>
<td>0.13</td>
</tr>
<tr>
<td>Total time devoted to phloem ingestion (min)</td>
<td>212.6 ± 54.7</td>
<td>132.0 ± 44.6</td>
<td>0.26</td>
</tr>
</tbody>
</table>

A

B

Figure 2. Electron microscopy micrographs of chloroplast of a non-infested (A) and a previously infested (B) leaf (2000×). Note that the chloroplast of the infested leaf showed a higher accumulation of starch granules, no grana arranged in stacks, and reduction in the amount of inner membranes (thylakoids, T), relative to the chloroplasts of the non-infested leaf. Scale bar: 0.5 μm.

Chemical characterisation of the reddish pigment. Absorbance spectra of extracts from infected and non-infected leaves differed considerably in the 400–600 nm region. The extract from infected leaves showed a peak at 525–530 nm, appeared red under the UV light (alone), blue under the UV light after ammoniacal exposure, and green in alkaline solution. These patterns are characteristic of anthocyanins (Harborne, 1973).

Aphid survival on diet containing reddish pigment. Percentage aphid survival in the treatment containing reddish pigment (67 ± 4.0, mean ± SE) was not significantly different from the control (76 ± 4.0, mean ± SE) (t-test, t(1,30) = 1.7, P = 0.09).

Discussion

EPG results showed that on previously infested S. halepense leaves aphids devoted less time to non-penetration activities, suggesting that mainly epidermal factors affected the plant's susceptibility to the aphid. Similar reduced non-probing periods after previous infestation have been reported (Prado & Tjallingii, 1997). Interestingly, lower time in non-probing on infested leaves was associated with decreased amounts of crystalline epidermal waxes, as revealed by scanning electron micrographs (Figure 3). The role of epicuticular waxes in aphid-plant interactions varies depending on the interacting species (Thompson, 1963; Way & Murdie, 1965; Stoner, 1990; Ni et al., 1998). In the present case, our results suggest that suppression of epicuticular waxes increases plant susceptibility.

The trends to achieve more rapidly a phloem phase and also to spend more time feeding on infested leaves than on non-infested ones (Table 1) suggest that
phloem factors cannot be totally neglected in relation to plant susceptibility. Higher mean relative growth rate (MRGR) of nymphs feeding on the reddish spotted leaf suggests that previous infestation enhanced phloem quality. This could arise from the rapid leaf senescence produced by the typical feeding pattern of *S. flavia*, which would accelerate nutrient translocation via the phloem vessels and act like a sink of assimilates (Hawkins et al., 1987), in particular for the translocation of nitrogen, a frequently limiting resource for strictly phytophagous insects (Mattson, 1980). Even though this report did not evaluate directly host nutritional quality, previous studies have shown that aphid feeding may increase the soluble nitrogen available in phloem vessels (Dorschner et al., 1987; Formusoh et al., 1992; Telang et al., 1999; Sandström et al., 2000), enhancing the nutritional value of the host. Interestingly, studies with the aphid *Schizaphis graminum* Rondani, which produces a pattern of leaf damage similar to that produced by *S. flavia*, have shown that senescent tissues represent good quality resources, which increase aphid reproduction (Ryan et al., 1990; Dorschner et al., 1987; Formusoh et al., 1992).

The reddish pigment has the chemical characteristics of an anthocyanin (Harborne, 1973). Previous work has shown that abiotic factors such as irradiation, temperature and water stress (Chalker-Scott, 1999; Shichijo & Hashimoto, 1997), and some biotic factors such as fungal and bacterial infection, induce their production. The present study shows that aphids also lead to an increase of their production. Previous work shows that anthocyanins could play a defensive role against infection (Coley & Aide, 1989; Snyder & Nicholson, 1990; Schutt & Netzly, 1991). However, this is not supported by the increased aphid growth and the feeding patterns in reddish leaves with respect to non-infested leaves, and the lack of effect of the pigment on aphid survival in artificial diets. In contrast, Costa-Arbulú et al. (2001) reported decreased performance of *S. flavia* on previously infested relative to non-infested *S. halepense*. This apparent contradiction may be explained by considering the different methodologies employed in determining performance. Thus, MRGR, which has been suggested to reflect nutritional quality better than other fitness components (Wellings et al., 1980), was determined in the present study for each aphid exposed to the experimental plant for four days, while the determination of reproductive success by Costa-Arbulú et al. (2001) exposed aphids (approximately 20 nymphs produced into a clip cage on each experimental plant) for eight days. This latter time period is sufficiently long for the aphid to feed on low-quality dying tissue. Visible necrotic damage is apparent 5 to 7 days after the first infestation of *S. halepense* by *S. flavia* and aphids tend to move to other leaves (W. L. González, unpubl.).

The main ultrastructural differences between infected and non-infected leaves were related to changes in chloroplast structure such as its swelling, loss of grana structure, and increase of the number of starch granules. Generally, when aphids penetrate plant tissues the styles’ route is predominantly intercellular-intramural; however, almost every cell along the styles’ pathway suffers from punctures (Tjallingii & Hogen Esch, 1993; Ramírez et al., 1999), which may eventually lead to destruction of the cells (Brzezina et al., 1986). Other consequences of aphid damage reported, including disruption of chloroplast and cellular membranes and enlargement of the plastoglobuli within chloroplasts (Ryan et al., 1990; Morgham et al., 1994), are consistent with our results. In spite of these cellular and tissular histological differences between

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**Figure 3.** Scanning electron microscopy micrographs of abaxial surface of a non-infested (A) and previously infested (B) *S. halepense* leaf (1000×). Crystalline epicuticular waxes are visible on non-infested leaves, while they are absent on the infested ones. Scale bar: 10 μm.
infested and non-infested plants, no differences were found in the time devoted by the aphid to intracellular incursions or in duration of each cellular penetration, suggesting that, at least at the cell membrane level, cells were not dramatically altered.

In conclusion, the attack of *S. flavum* to *S. halepense* induced plant susceptibility. Thus, aphid feeding was facilitated and growth was enhanced on previously infested leaves. Further work should also consider the role of induced plant responses on behaviour and performance of heterospecific aphids feeding on the shared host plant (*S. halepense*), on within plant aphid distribution (Gianoli, 1999; González et al., 2001), and on interspecific aphid competition (Gianoli, 2000; González et al., 2002).

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