Abstract

1 Colonies of the aphid *Rhopalosiphum maidis* on Johnsongrass, *Sorghum halepense*, usually occur inside the whorl. The present work assessed the role of two biotic factors, plant quality and parasitism by *Lysiphlebus testaceipes*, in determining within-plant distribution and performance of *R. maidis*. The mean relative growth rate of aphids inside the whorl and on a mature leaf was compared, and the concentration of hydroxamic acids in those tissues determined as an indicator of plant quality. Parasitism effectiveness and parasitoid behaviour were evaluated using three treatments: aphid colonies placed (1) wholly inside the whorl, (2) on the inner and outer surfaces of the whorl, and (3) on a mature leaf.

2 The mean relative growth rate of aphids was lower on the whorl than on the mature leaf, and hydroxamic acid concentration in the whorl was higher than in the mature leaf.

3 The number of parasitized dead aphids (mummies) inside the whorl was considerably lower than in the other two treatments. Mummies were present in 80% of the replicates in the whorl, whereas mummies were present in all replicates of the other treatments.

4 Host location time of the parasitoid was increased on the mature leaf compared with the other treatments. No significant differences in the residence time of the parasitoid in the aphid colony occurred between treatments.

5 Host location time showed a negative correlation, and residence time of the parasitoid a positive correlation with the number of aphids on the outer surface of the whorl.

Keywords Aphids, hydroxamic acids, *Lysiphlebus testaceipes*, parasitoids, plant quality, *Rhopalosiphum maidis*, *Sorghum halepense*.

Introduction

The nutritional composition of plants, e.g. soluble nitrogen and carbohydrates, and their levels of defensive secondary metabolites vary substantially from one tissue to another (Raupp & Denno, 1983; Rosenthal & Berenbaum, 1991). The parts of plants on which aphids feed may therefore differ in their quality as substrates for the development of aphid populations (Whitham, 1980). On the other hand, specific structures of the plant may enable aphids to escape the attack of predators (Kareiva & Sahakian, 1990; Grevstad & Klepetka, 1992) and parasitoids (Gardner & Dixon, 1985; Reed et al., 1992), thereby providing refuges for these phytophagous insects (Price, 1980, 1988). Thus, plant traits as well as interactions between biotic and abiotic factors may affect aphid performance (Morris, 1992) and within-plant aphid distribution (Jansson & Smilowitz, 1986; Lampert, 1989).

The corn leaf aphid, *Rhopalosiphum maidis* (Fitch), is a common pest of cereals such as maize, sorghum and barley (Ortega et al., 1980). This aphid also colonizes wild Gramineae (Poaceae) in the surroundings of crops (Blackman & Eastop, 1984). Colonies of *R. maidis* in maize are usually found inside the whorl formed by the youngest leaves of the plant, occupying outer positions only when the size of the colony increases beyond a threshold level or after the plant grows and the tassel becomes exposed (Foott, 1977). In sorghum, *R. maidis* colonies have also been found in the whorl (Gonzáles, personal observation). *Rhopalosiphum maidis* is attacked in the field by predacious Coccinellidae (Coleoptera) and Syrphidae (Diptera) (Frazer,
and parasitized by Braconidae (Hymenoptera) (Starý, 1987; Feng et al., 1992).

In preliminary field observations, colonies of *R. maidis* were found on mature plants of the common weed Johnsongrass, *Sorghum halepense* (L.) Pers., in the vicinity of wheat crops in Santiago, Chile (late spring, November 1996). All infested plants (n = 47) had aphid colonies in the whorl, and only four of these plants had an additional colony outside of the whorl. Most aphids, both inside and outside the whorl, were found on the abaxial surface of leaves. The percentage of mummies was lower in the colonies inside the whorl (1%) than in those outside it (12%). The braconid *Lysiphlebus testaceipes* (Cresson) was the only parasitoid species emerging from the collected mummies.

In the present work, the role of plant quality and the risk of parasitism in shaping the within-plant distribution pattern of *R. maidis* were investigated. The performance of aphids in the whorl and on a mature leaf of Johnsongrass were evaluated. In addition, the concentration of hydroxamic acids, defensive secondary metabolites of Poaceae (Niemeyer, 1988; Niemeyer & Pérez, 1995), within those plant tissues was determined. Finally, the effect of feeding site of aphids on the risk of parasitism by *L. testaceipes*, a generalist parasitoid of aphids (Starý et al., 1988), was addressed.

**Materials and Methods**

**Stock cultures and plant material**

Cultures of *R. maidis* and *L. testaceipes* originally collected in 1996 from Johnsongrass in Santiago, Chile, were maintained on barley (*Hordeum vulgare* L. cultivar F. Union). Barley lacks hydroxamic acids (Niemeyer, 1988). Cultures were maintained in a room at 25 ± 2°C and a photoperiod of LD 14 : 10 h. All experiments were performed using Johnsongrass (5–7-week-old plants) grown from seed outdoors during the austral summer and autumn months (24 ± 8°C and 11–14 h of light per day).

**Aphid performance**

Second-instar nymphs born within a 12-h period were placed inside plastic clip cages (2 cm diameter × 2 cm height) attached to the following parts of each plant: (1) abaxial surface of the youngest leaf (within the whorl), and (2) abaxial surface of a mature leaf (fourth or fifth oldest leaf in a plant with seven or eight leaves). The youngest leaf was carefully extended to allow the clip cage to be attached. Neither chlorosis nor symptoms of damage were observed on both young and mature leaves after the 4 days of experimentation. Mean relative growth rate of aphids (MRGR; Adams & Van Emden, 1972) was determined as a measure of the performance of individual aphids. Thirty nymphs were each weighed (initial weight: 15–20 μg) and then individually caged on both plant sites studied (one cage per plant site). Aphids were removed from the clip cages after 4 days and weighed. MRGRs [(ln final weight – ln initial weight)/number of days] for each site were compared using a Wilcoxon matched pairs test, as the data did not fit a normal distribution and both sites belonged to the same plant in each replicate (n = 15 plants).

**Analysis of secondary metabolites**

Hydroxamic acids were quantified in the youngest leaf and in a mature leaf (fourth or fifth oldest leaf in a plant with seven or eight leaves) using a modification of the HPLC method developed by Niemeyer et al. (1989). A sample (150–250 mg) of each plant part (n = 16 plants) was macerated with 1 mL distilled water, using a mortar and pestle. The aqueous extract was left at room temperature for 15 min and then taken to pH 3 with 0.1 N H3PO4. The extract was centrifuged at 13000 g for 15 min and a 50 μL aliquot of the supernatant directly injected into a high performance liquid chromatograph. A RP-100 Lichrospher-C18 column was used with a constant solvent flow of 2 mL/min and the following linear gradients between solvents A (MeOH) and B (0.5 mL H3PO4 in 1 L H2O): 0–1.5 min, 5% A; 1.5–5 min, 30% A; and then constant 30% A to 9 min. Detection of DIBOA, the main hydroxamic acid aglucone present in the extracts, was performed at 263 nm, its retention time being 7.5 min with a 0.05 min range. Results were compared using a Wilcoxon matched pairs test for the reasons given in the preceding section.

**Evaluation of parasitism**

The experiment consisted of three treatments using Johnsongrass plants (initial n = 15 in each treatment), and was performed in a room at 25 ± 2°C, photoperiod LD 14 : 10 h, and 48 W/m² total radiation from incandescent and fluorescent white lamps. Ninety adult aphids of similar size were placed with a fine camelhair brush on each of the following plant sites: (1) inside the whorl, (2) distributed between the inner and the outer surface of the whorl (between 10 and 40 aphids being located on the outer surface of the whorl), and (3) on the mature leaf. Aphids had to be confined in clip cages for the first 30 min to assure settlement in the treatments. After approximately 3 h no further aphid movements occurred between sites. At this point, approximately 80 aphids had settled at each plant site (Table 2). Each infested plant was then isolated using a plastic tube (40 cm diameter × 75 cm height) and a mated female parasitoid was released next to the base of the plant. Parasitoid behaviour was then observed continuously for 5 h, noting when its location in the plant changed. ‘Host location time’ was defined as the time elapsed from the release of the parasitoid to the first observation of the parasitoid amongst the aphid colony, and ‘residence time’ as the accumulated time the parasitoid was observed within the aphid colony. At the end of the observation period, the parasitoid was removed and each group of aphids was transferred to isolated barley seedlings located in the same room. The resulting number of mummies was counted 10–12 days later. A colony was labelled as ‘parasitised’ when at least one mummy was found in it. Three replicates from the treatment wholly inside the whorl were accidentally lost, therefore the sample size for this treatment was n = 12. Results were compared using a Kruskal–Wallis *ANOVA* because data did not fit a normal distribution. Correlations (Spearman Rank Order correlations) between both host location and residence times and the number of aphids outside the whorl were determined for the treatment distributed between the inner and the outer part of the whorl. This was done just for this treatment because it was the only one
encompassing variation in the number of aphids available for parasitization (i.e. in the size of the aphid patch).

**Results**

**Aphid performance and analysis of secondary metabolites**

MRGR of *R. maidis* on the youngest leaf was significantly lower (Wilcoxon matched pairs test, $Z = 2.31, P < 0.05$) than on the mature leaf of the same plant (Table 1). The leaves also differed in hydroxamic acid concentration, the youngest leaf having a higher concentration (Wilcoxon matched pairs test, $Z = 4.73, P < 0.01$) than did the mature leaf (Table 1).

**Evaluation of parasitism**

Parasitoids showed an initial tendency to move upwards, irrespective of the position of aphids on the plant. Thus, in both treatments where aphids were located on the top of the plant (either wholly or partially inside the whorl) the sequence of leaves contacted by the parasitoid, which was released next to the base of the plant, indicated a straightforward movement to the upper leaves, with no backward movements. This was observed for 14 out of 15 parasitoids in the treatment with aphids partially inside the whorl, and all 12 parasitoids in the treatment with aphids wholly inside it. In the case of the treatment on the mature leaf (a lower leaf), 13 out of 15 parasitoids initially ‘overlooked’ the aphids while in the other treatments where aphids were located on the top of the plant, aphids were found to be inside the whorl by more than half of the parasitoids in both treatments (Kruskal–Wallis ANOVA, $H(2,42) = 27.05, P < 0.001$) than in the other two treatments (Table 2). Host location time by the parasitoid showed a negative correlation ($n = 15, r = -0.761, P < 0.001$) and residence time in the aphid colony a positive correlation ($n = 15, r = 0.713, P < 0.01$) with the number of aphids established outside of the whorl.

**Discussion**

Plant suitability for aphids normally changes with plant and tissue age (Leather & Dixon, 1981; Wiktelius, 1987), the changes affecting aphid feeding and nutrition (Klingauf, 1987). Younger leaves usually have a higher concentration of primary metabolites, e.g. soluble nitrogen, than mature ones (Merritt, 1996; Dixon, 1998; p. 38). Therefore, assuming this pattern to hold in the present system, an enhanced aphid performance on younger leaves would be expected. However, aphid growth rate was lower on the whorl (youngest leaves) than on a mature leaf. This suggests the involvement of non-nutritional factors in the leaves. The concentration of hydroxamic acids was higher in the whorl. Previous work has reported negative correlations between performance of *R. maidis* and concentration of hydroxamic acids in Poaceae (Long et al., 1977; Givovich & Niemeyer, 1994). The same pattern has been found for other cereal aphids in laboratory bioassays (Bohidar et al., 1986; Thackray et al., 1990; Givovich & Niemeyer, 1995) and in the field (Gianoli et al., 1996). The concentration of hydroxamic acids depends on the plant structure assayed and its age (Argandoña et al., 1981), being higher in younger leaves than in mature ones. Consequently, these results suggest that hydroxamic acid concentration in Johnsongrass leaves could be a factor accounting for the MRGR values determined for *R. maidis*. It may be hypothesized that the effect of chemical defences might have overcome the presumably richer nutrient composition of the whorl (Langer, 1979; Dixon, 1998), resulting in a negative effect on aphid performance.

Results of the present study further suggest that the whorl may be regarded as a sheltered environment for aphids. Previous studies have indicated that natural enemies of aphids, such as coccinellids and parasitoids, are inefficient in finding prey in concealed environments such as the leaf-rolling caused by the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Reed et al., 1992; Kauffman & Laroche, 1994). In the present case, the level of parasitization was significantly higher (Kruskal–Wallis ANOVA, $H(2,42) = 4.25, P = 0.12$; Table 2). The number of mummies found in the parasitized colonies inside the whorl was significantly lower (Kruskal–Wallis ANOVA, $H(2,42) = 27.05, P < 0.001$) than in the other two treatments (Table 2). There was no significant difference in residence time among treatments (Kruskal–Wallis ANOVA, $H(2,42) = 0.12$, $P = 0.71$).

### Table 1

Mean Relative Growth Rate (MRGR) of individuals of *R. maidis* on the youngest leaf and on a mature leaf of Johnsongrass, and hydroxamic acid levels (Hx) in those tissues.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>MRGR (mg/g day$^{-1}$) ($n = 15$)</th>
<th>Hx (mmol/kg fresh weight) ($n = 16$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young leaf</td>
<td>0.397 ± 0.015a</td>
<td>3.88 ± 0.54a</td>
</tr>
<tr>
<td>Mature leaf</td>
<td>0.438 ± 0.012b</td>
<td>2.17 ± 0.35b</td>
</tr>
</tbody>
</table>

*a Means followed by a different letter within a column are significantly different, Wilcoxon matched pairs test (P < 0.05). Mean ± SE shown.

### Table 2

Evaluations of parasitism of *R. maidis* by *L. testaceipes*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of plants (colonies)</th>
<th>Number of aphids per colony</th>
<th>Colonies parasitized</th>
<th>Host location time (min)</th>
<th>Residence time (min)</th>
<th>Number of mummies in parasitized colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>WI</td>
<td>15</td>
<td>82 ± 1a</td>
<td>12</td>
<td>38.8 ± 4.0a</td>
<td>51.3 ± 4.2a</td>
<td>3 ± 1a</td>
</tr>
<tr>
<td>WIO$^b$</td>
<td>15</td>
<td>81 ± 1a</td>
<td>15</td>
<td>31.4 ± 4.1a</td>
<td>68.1 ± 7.4a</td>
<td>17 ± 2b</td>
</tr>
<tr>
<td>ML</td>
<td>15</td>
<td>79 ± 1a</td>
<td>15</td>
<td>73.3 ± 7.1b</td>
<td>66.1 ± 4.4a</td>
<td>22 ± 1b</td>
</tr>
</tbody>
</table>

*Means followed by a different letter within a column are significantly different (Kruskal–Wallis ANOVA, $P < 0.001$).

$^b$The mean number of aphids (± SE) inside the whorl was 58 ± 3 and outside the whorl was 22 ± 3.
of parasitism was lowest inside the whorl. This advantage could counterbalance the deleterious effect for aphids settling in a plant site where development is hindered, and could explain the distribution pattern of R. maidis colonies found in the field. The most disadvantageous location for colonies of R. maidis on Johnsongrass is therefore the outer surface of the whorl, where intrinsic plant factors limit aphid growth and the aphids are easily discovered and attacked by parasitoids.

In support of a previous report on the behaviour of L. testaceipes (Städdler & Völkli, 1991), parasitoids in the treatment with all aphids inside the whorl foraged principally at the edge of the aphid colony and spent little time in secluded plant areas. Consequently, the number of mummies inside the whorl were lower than in the other treatments, despite the fact that residence times were not different. On the other hand, even though the parasitoids were released next to the base of the plant, they showed a marked orientation towards the apex, therefore finding the aphid colonies located on the whorl before those located on the mature leaf. Thus, parasitoid behaviour would explain why the large mature leaf, a presumably more ‘apparent’ site (sensu Feeny, 1976), was not associated with a shorter host location time.

The results indicate that the whorl plays two important but contrasting roles for the corn leaf aphid: (1) as a structural refuge against natural enemies, and (2) as a low quality substrate. The system is a dynamic one. It can be expected that as aphid number increases, the limited space inside the whorl and the growth of the plant will expose them to parasitoid attacks, as indicated by the correlations obtained between both host location and residence times and number of aphids outside of the whorl. This would exert a pressure on aphids to reach the newly formed whorl, where, in spite of the fact that their growth will be reduced, they would gain protection from parasitoids, i.e. they would reach an ‘enemy-free space’ (Jeffries & Lawton, 1984; Hopkins & Dixon, 1997).

This work provides some insights into the pattern of within-plant distribution of R. maidis and the potential trade-offs between plant quality and protection against parasitoids. Further work should also consider other factors, such as the presence of predators (e.g. coccinellids) or mutualists (e.g. attendant ants), and possible roles of the whorl as protection against extreme environmental conditions.

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References


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