Use of Electrical Penetration Graphs and Phloem Sap Chemical Analysis in Studies of the Effects of Hydroxamic Acids in Cereals on Aphid (Homoptera: Aphididae) Feeding Behavior

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Phloem sap from wheat seedlings differing in hydroxamic acid (Hx) concentrations was collected using aphid stylets excised by microcautery. DIMBOA glucoside, the major Hx in wheat seedlings, was found in phloem sap in concentrations which did not differ between cultivars. Using electrical penetration graphs, the feeding behavior of the cereal aphid Rhopalosiphum padi *(L.) was studied in wheat seedlings and also in artificial diets differing in Hx concentrations. Linear correlations were found between: i) Hx concentrations in whole seedlings and time taken by aphids to engage in a committed phloem ingestion, and ii) DIMBOA concentration in diets and ingestion times in them. Mean committed phloem ingestion times were not significantly different between the cultivars studied. These results point to a feeding deterrency by DIMBOA in the plant's mesophyll tissue.*

Hydroxamic acids (Hx) are a family of secondary metabolites occurring in cereals as ß-O-D-glucopyranosides which are hydrolyzed by endo-ß-glucosidases when the plant is injured (Hofman and Hofmanova 1969). Both glucosides and aglucones have been shown to be major mechanisms of defense of cereals against aphids (Niemeyer 1991). The most abundant Hx in wheat is the 2-ß-O-D-glucopyranoside of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA-Glc) (Fig. 1) (Niemeyer 1988).

Hydroxamic acids affect cereal aphids through antibiosis and antixenosis.

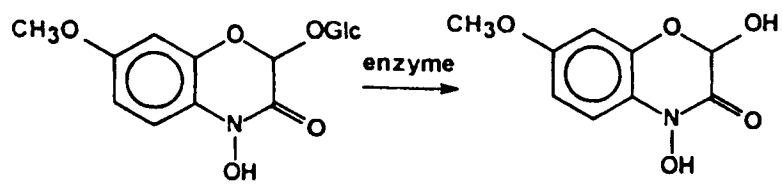

Fig. 1. Enzymatic conversion of DIMBOA glucoside to its aglucone, DIMBOA.

Antibiosis is manifested as a decrease in aphid survival on diets to which Hx are added (Long et al. 1977; Argandoña et al. 1980, 1981, 1983; Corcuera et al. 1982). On whole seedlings with high levels of Hx relative to seedlings with low levels of Hx, there is a decrease in aphid survival and a lower intrinsic rate of increase of aphid populations (Argandoña et al. 1980, Bohidar et al. 1986, Leszczynski et al. 1989, Thackray et al. 1990a). Additionally, there is an increase in mean relative growth rate of aphids on seedlings with low levels of Hx relative to seedlings with high levels of Hx (Thackray et al. 1990b). Antixenosis by Hx is manifested by aphids selecting plants containing lower Hx concentrations when they are given a choice between plants with different levels of Hx. This antixenotic effect has been described for winged morphs (Nicol et al. 1992) and for wingless morphs (Givovich and Niemeyer 1991).

Hydroxamic acids have been shown to occur in the mesophyll and also in vascular bundles of wheat seedlings (Argandoña et al. 1987). However, because aphids ingest mainly from the phloem of their host plants (Pollard 1973), the presence of Hx in the sieve elements is of greater interest (Molyneux et al. 1990). Herein, we describe the collection of phloem sap from wheat seedlings through excised aphid stylets and its chemical analysis.

In this research project, we also used electronic monitoring to study the feeding behavior of *Rhopalosiphum padi* (L.) during its search for sieve elements in 6 cultivars of wheat seedlings with different levels of Hx, and also while on artificial diets containing 4 concentrations of Hx. Data on feeding parameters provide insights into host plant acceptance in relation to Hx concentration by this aphid species.

Materials and Methods

Biological Material. A multiclonal stock of *R. padi* was started from aphids collected in wheat fields near Santiago, Chile, and reared on oat seedlings, *Avena sativa* L. cv. Nahuén, in a growth room at 22 ± 3°C and a photoperiod of 16:8 (L:D) h. Alate adults of similar age were used in all experiments. Aphids were starved for 2 h before they were used in EPG experiments.

Six cultivars of wheat (*Triticum aestivum* L.)–Platifén, Millaleu, Mexifén, Nobo, Anza, and Maitén, were used for the electronic monitoring study; they were chosen on the basis of differences in their Hx concentrations at decimal growth stage 10 (Zadoks et al. 1974). Cultivars Millaleu, Nobo, and Maitén, representing low, medium, and high Hx concentrations in whole seedlings,

respectively, also were used for phloem sap collection. Wheat cultivars were obtained from the Instituto de Investigaciones Agropecuarias (INIA). Seedlings were grown in a growth room as indicated above for aphids.

Electrical Penetration Graphs. Electrical penetration graphs (EPGs) were recorded with a DC electronic feeding monitor (Tjallingii 2000). EPGs may be used for monitoring aphid stylet penetration behavior because different waveforms in the EPGs have been correlated with different probing behaviors during host plant acceptance (Tjallingii 1990). Among the parameters which may be determined from EPGs, the time an aphid requires to engage in committed phloem ingestion (time to develop a potential drop that is longer than 8 min and contains E1 waveforms followed by transition waveforms and E2 waveforms thereafter) is important in the assessment of substrate acceptance (McLean and Kinsey 1968, Dreyer et al. 1984).

EPG recordings were performed with seedlings in the 1-leaf stage (decimal growth stage 10) (Zadoks et al. 1974). EPGs of 20 individuals per wheat cultivar were recorded; each recording lasted 10 h. EPGs also were recorded in artifical diets for a period of 3 h, and ingestion times, judged by the presence of an E waveform (Tjallingii 1990), were determined for 20 aphid individuals at each DIMBOA concentration in the diet. The artificial diets consisted of 25% sucrose in 0.1 M phosphate buffer, pH 6.5, to which DIMBOA was added to final concentrations of 1, 3, 5, and 7 mM. These concentrations cover the range found in seedlings of a world collection of wheats (Nicol et al. 1992).

Phloem Sap Collection. The stylets of *R. padi* were amputated by microcautery using techniques similar to those described by Unwin (1978). Experiments were performed in a climatized chamber room at 21–27°C and 70–90% RH. Only those aphids were used which showed a posture suggesting effective feeding (short proboscis, antennae pointing backward, and honeydew production) and were positioned on the abaxial leaf surface ≈2 cm from the apex of the leaf and within 2 mm of the central vein. Phloem sap flowing from the excised stylets was collected in a 0.5-μl micropipette for 3 h following stylet excision. An individual plant was used only once for phloem sap collection. After collection, the micropipette was rinsed with 30 μl of distilled water, and the resulting solution was analyzed by high-performance liquid chromatography (HPLC) essentially as described by Niemeyer et al. (1989a).

The nature of the chromatographic peak suspected to correspond to DIMBOA-Glc in the phloem sap was ascertained in the following ways: (1) comparison of its retention time with that of an authentic sample of DIMBOA-Glc obtained from maize, *Zea mays* L., cv. Tracy T129, as described by Lyons et al. (1988), (2) comparison of the chromatogram of the product of enzymatic hydrolysis in the sample by cell-free wheat extracts (Cuevas et al. 1992) with the product of enzymatic hydrolysis of authentic DIMBOA-Glc subjected to the same conditions, and (3) comparison of the chromatogram of the decomposition products of the enzymatic hydrolysate with authentic DIMBOA-Glc hydrolysate subjected to the same decomposition conditions (Bravo and

Niemeyer 1986). Hx concentrations in the seedlings were determined as described by Niemeyer et al. (1989a).

Results and Discussion

DIMBOA-Glucoside in the Phloem Sap of Wheat Seedlings. The only Hx-related compound detected in the phloem sap of the 3 wheat cultivars studied was DIMBOA-Glc (no aglucone was found). The presence of DIMBOA-Glc in the feeding site of aphids provides a mechanistic support for the correlations previously described between aphid performance and Hx levels in whole seedlings, and is consistent with the presence of DIMBOA-Glc in the honeydew of aphids feeding on wheat seedlings (Leszczynski and Dixon 1990, Givovich et al. 1992) and also with the presence of Hx in aphid bodies (Niemeyer et al. 1989b).

Feeding Behavior of *R. padi* on Wheat Seedlings and Artificial Diets. The time to engage in committed phloem ingestion was correlated positively and linearly with whole-seedling Hx concentration (Fig. 2), indicating feeding deterrency by Hx toward *R. padi*. Feeding deterrency by Hx in the plant could be located at the sieve elements, in which case aphids would assess levels of DIMBOA-Glc in sieve elements until one with a low concentration was found, a process which would take longer when the mean DIMBOA-Glc concentration in the sap was higher. However, 2 lines of evidence contradict this hypothesis. First, a measure of feeding deterrency in the phloem is the average time an aphid remains in committed phloem ingestion, yet these times did not differ significantly among the 6 cultivars studied (Table 1). Second, mean DIMBOA-Glc sap concentrations did not differ among the 3 cultivars whose phloem sap

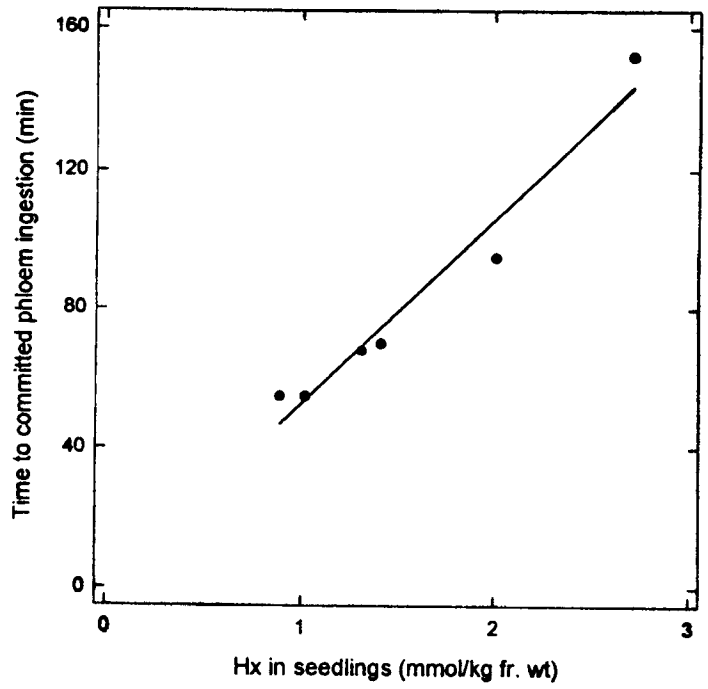

Fig. 2. Effect of concentration of hydroxamic acids in wheat seedlings on mean time taken by *R. padi* to engage in committed phloem ingestion. The regression equation and probability level for the line is $y = (53.7 \pm 5.5)\,x + (0.07 \pm 9.3)$ ($P = 0.00068$).

Table 1. Time in committed phloem ingestion of *R. padi* in 6 wheat cultivars

Wheat cultivar	Hydroxamic acids, mmol/kg fresh wt[a]	Mean time in committed phloem ingestion, min
Platifén	0.89a	353a
Millaleu	1.02b	345a
Mexifén	1.31c	342a
Nobo	1.41c	355a
Anza	2.01d	343a
Maitén	2.71e	354a

Values in the same column followed by the same letter are not significantly different at $P < 0.05$ (ANOVA followed by the Duncan multiple comparisons test).

[a]In whole seedlings.

Table 2. Concentration of hydroxamic acids (Hx) in phloem sap and in aerial parts of seedlings of 3 wheat cultivars

	Hydroxamic acid concentration[a]		
Sample	Millaleu	Nobo	Maitén
Phloem sap	0.69a	1.09a	1.19a
Seedling	0.79a	1.53b	2.72c

Values in the same row followed by the same letter are not significantly different at $P < 0.05$ (ANOVA followed by the Duncan multiple comparisons test).

[a]Concentrations are expressed in mM (phloem sap) or in mmol/kg fresh weight (seedlings).

was analyzed (Table 2), and hence should not lead to different feeding deterrencies in these cultivars. Alternatively, the positive linear correlation shown in Fig. 2 could indicate feeding deterrency by Hx in the mesophyll. This is plausible because the Hx levels in whole seedlings (a quantity expected to reflect concentrations in the mesophyll, the tissue contributing most importantly to the fraction of the plant analyzed) of the 3 cultivars analyzed do differ significantly from each other (Table 2).

The hypothesis that feeding deterrency was located in mesophyll also was tested by studying the ingestion of DIMBOA in artificial diets (DIMBOA was used because this is the compound expected to be encountered by aphids when they penetrate mesophyll cells and produce the hydrolysis of DIMBOA glucoside). Fig. 3 shows that the time ingesting from diets correlated negatively and linearly with DIMBOA concentration in the diet, supporting the feeding deterrency effect of DIMBOA in this system.

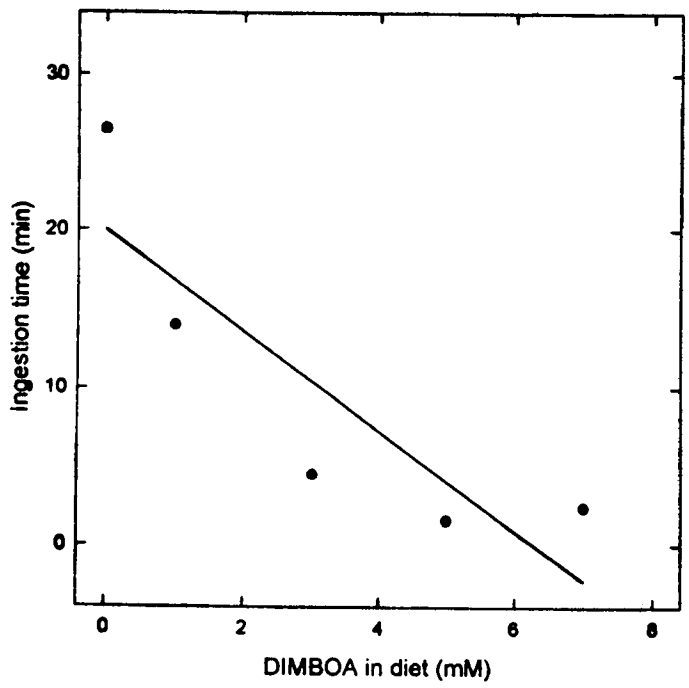

Fig. 3. Effect of concentration of DIMBOA in artificial diets on mean time spent by *R. padi* in diet ingestion. The regression equation and probability level for the line is y = (-3.3 ± 1.04) x + (19.7 ± 4.3) ($P = 0.057$).

In conclusion, chemical analysis of wheat phloem sap obtained by microcautery of aphid stylets, in combination with electrical monitoring of the feeding behavior of the aphid *R. padi* on wheat seedlings differing in Hx levels, provide support for the previous proposal of Hx as the causative factor of negative correlations between aphid performance and Hx concentration in cereals, and suggest that mesophyll Hx concentration has a strong effect on feeding behavior of *R. padi* on wheat seedlings.

Acknowledgments

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