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PRESENCE OF A HYDROXAMIC ACID GLUCOSIDE IN WHEAT PHLOEM SAP, AND ITS CONSEQUENCES FOR PERFORMANCE OF *Rhopalosiphum padi* (L.) (HOMOPTERA: APHIDIDAE)

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Abstract—Phloem sap of wheat seedlings differing in whole leaf hydroxamic acid (Hx) concentrations was collected by cutting stylets of feeding aphids. DIMBOA-glucoside was the only Hx-related product found. Concentration of DIMBOA-glucoside in phloem sap showed a tendency to be negatively correlated with aphid performance.

Key Words—Hydroxamic acids, phloem sap analysis, aphid resistance, cereals, wheat, *Rhopalosiphum padi*, Homoptera, Aphididae.

INTRODUCTION

Several investigations have pointed out the importance of hydroxamic acids (Hx) in the resistance of wheat and other cereals against aphids (Niemeyer, 1988). These compounds are present in the intact plant as glucosides, which are hydrolyzed by *endo*-β-glucosidases when the tissue is injured (Hofman and Hofmanova, 1969). The main aglucone in wheat extracts is DIMBOA (Figure 1).

Hx affect aphid feeding behavior and performance through their antibiotic and antixenotic properties (Givovich and Niemeyer, 1991; Niemeyer, 1991).

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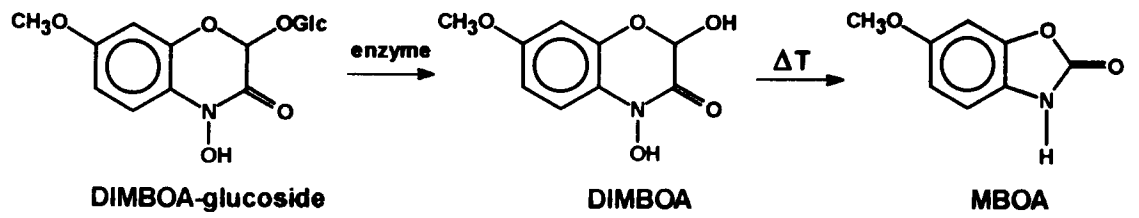

FIG. 1. Biochemical and chemical transformations of hydroxamic acid glucosides present in wheat extracts.

The exact role of Hx in host plant discrimination by aphids is still unknown. Important issues are the localization of Hx in the plant and the stages in the assessment of the plant during which an aphid encounters them. Hx have been found both in the mesophyll and in the vascular bundles of wheat (Argandoña et al., 1987). Moreover, honeydew from aphids feeding on wheat contained Hx-glucosides (Leszczynski and Dixon, 1990; Givovich et al., 1992), suggesting the presence of these compounds in the plant sieve elements.

Here, we present the results of collection and analysis of wheat phloem sap of seedlings of wheat cultivars known to differ in total Hx concentrations and data on the performance of the aphid *Rhopalosiphum padi* (L.) reared on seedlings of these cultivars.

METHODS AND MATERIALS

Insect Material. Individuals of *R. padi* were collected in wheat fields near Santiago and reared on oat seedlings in a growth chamber at 19–25°C, and 16L:8D photoperiod.

Plant Material. Wheat seeds were obtained from Instituto de Investigaciones Agropecuarias (INIA). Three wheat cultivars (*Triticum aestivum* L. cvs. Millaleu, Nobo, and Maitén) were chosen on the basis of their Hx concentrations at the seedling stage (Givovich and Niemeyer, 1991). Experiments were carried out with seedlings in the one-leaf stage (decimal growth stage 10) (Zadoks et al., 1974), which contain the highest concentrations of Hx (Argandoña et al. 1980). Oat seedlings (*Avena sativa L.*, cv. Nahuen) lack Hx and were used as the control. Hx concentrations in plants was determined by HPLC according to Niemeyer et al. (1989a).

Phloem Analysis. Phloem sap collection was carried out by aphid stylet microcautery (Unwin, 1978). Six-day-old wheat seedlings grown under similar conditions in a plant growth chamber at 25°C were each infested with 5–10 adult aphids of similar weights. Aphids were allowed to remain on the plants for *ca.* 12 hr. The aphid whose stylet was to be cut was chosen among those feeding in a given position on the abaxial leaf surface (2 cm from the top of

the leaf, and within 2 mm of the central vein). Honeydew production was taken as an indication that the aphid chosen was indeed feeding. Phloem sap was collected for 3 hr following stylet excision. Only one sap collection was made per day, starting almost at the same time of the day. Sap was collected with a 0.5-μl micropipet in an environment of 20–25°C and 95% relative humidity. After collection, the micropipet was rinsed with 30 μl of distilled water. Hx concentrations in these solutions were determined by injecting them directly into a high-performance liquid chromatograph and analyzing them under conditions similar to those described by Niemeyer et al. (1989a).

The presence of DIMBOA-glucoside in the phloem sap of wheat cultivars was determined by comparison of its chromatogram with that of an authentic sample obtained from maize (*Zea mays* L. cv. Tracy T129) (Lyons et al., 1988). Confirmation of the identity of the compound was obtained by: (1) subjecting the sample to enzymatic glucoside hydrolysis by cell-free wheat extracts and detecting DIMBOA in the product, and (2) subjecting the product of enzymatic hydrolysis to heating under basic conditions (pH 9, 80°C, 1 hr) (Bravo and Niemeyer, 1986) and detecting MBOA—the main decomposition product of DIMBOA—in the mixture (Figure 1). Retention times for DIMBOA-glucoside, DIMBOA, and MBOA were 2.43, 3.47, and 6.17 min, respectively (Figure 2). Cell-free extracts of wheat seedlings were prepared by macerating leaf tissue in

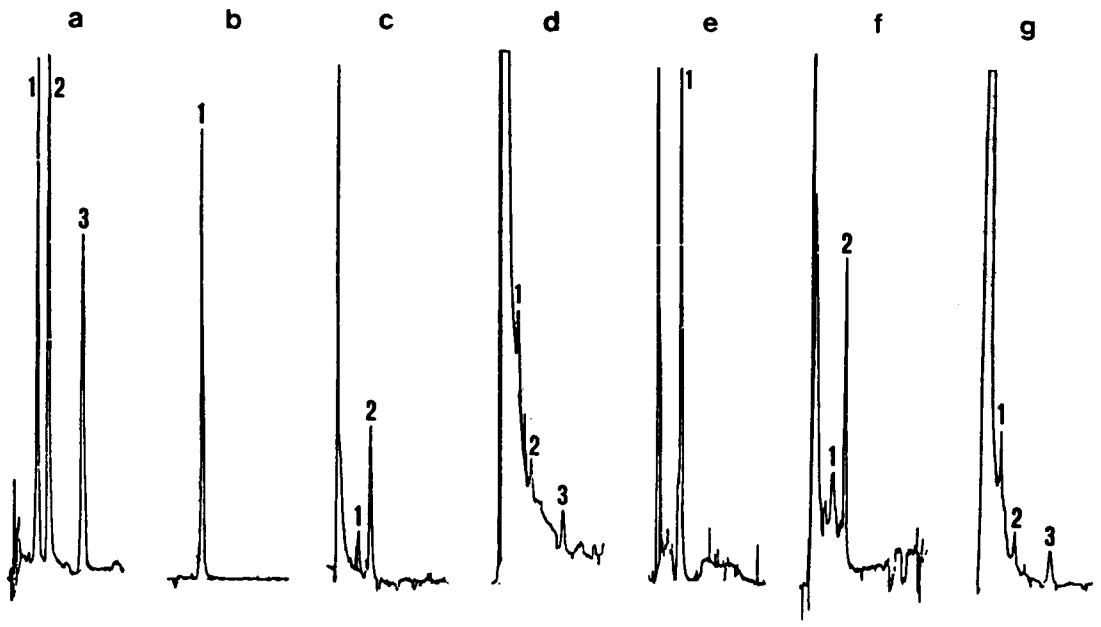

FIG. 2. Confirmation of the presence of DIMBOA-glucoside in the phloem sap of a wheat seedling: (a) standards of DIMBOA-glucoside **(1)**, DIMBOA **(2)**, and MBOA **(3)**; (b) DIMBOA-glucoside standard; (c) product of the enzymatic transformation of (b); (d) product of the decomposition of (c); (e) phloem sap sample; (f) enzymatic transformation of (e); (g) decomposition of (f).

aqueous buffered solution, centrifuging at 20,000*g*, and filtering the resulting suspension through Sephadex G-25 M (Cuevas et al., 1992).

Determination of Aphid Performance. Mean relative growth rate (MRGR), a parameter highly correlated with the intrinsic rate of increase (r_m; Leather and Dixon, 1984), was determined (Adams and van Emdem, 1972) in order to evaluate aphid performance on the seedlings studied. First- or second-instar nymphs were weighed, and those with very similar weights were caged individually onto the abaxial leaf surface of a test plant and removed for weighing 96 hr later. Twenty replicated measurements were made for each wheat cultivar. MRGRs determined in different wheat cultivars were compared using ANOVA/ Duncan's test.

RESULTS AND DISCUSSION

If a plant secondary metabolite is to be important for plant resistance to aphids through antibiosis, it must be present in the sieve elements, since that is the tissue fed on by aphids. Hence, the presence of Hx in the sieve elements is of importance for establishing Hx as the causative factor of the negative correlations described between aphid performance and Hx content of cereals (Niemeyer, 1991). DIMBOA-glucoside had previously been found in the honeydew of aphids feeding on Hx-containing wheat seedlings (Leszczynski and Dixon, 1990; Givovich et al., 1992), suggesting its ingestion from sieve elements. The present study is the first unambiguous demonstration that Hx are present in the phloem sap of wheat seedlings. Our results show the presence of DIMBOA-glucoside as the only hydroxamic acid or related compound detected in the phloem sap of wheat seedlings (Figure 2). Other compounds derived from DIMBOA-glucoside were found earlier in whole aphids (Niemeyer et al., 1989b) and in aphid honeydew (Leszczynski and Dixon, 1990). It is likely that the presence of these compounds was the result of metabolization within the aphid (Leszczynski and Dixon, 1992; Leszczynski et al., 1992) or of chemical transformation of the honeydew samples (Bravo and Niemeyer, 1986).

Hx have been found in high concentrations in meristematic tissue (Epstein et al., 1986). Their translocation from undifferentiated tissues to differentiated ones has been proposed to occur via the apoplastic fluid (Zúñiga and Massardo, 1991). The present results suggest the phloem as an alternative translocation route.

Individual determinations of the concentration of DIMBOA-glucoside in the phloem were not correlated with Hx concentrations in the whole leaf extracts (Figure 3). However, mean Hx content of whole leaf extracts showed very large and clear differences between wheat cultivars (Figure 4), and DIMBOA-glucoside concentrations in the phloem sap showed no significant differences among

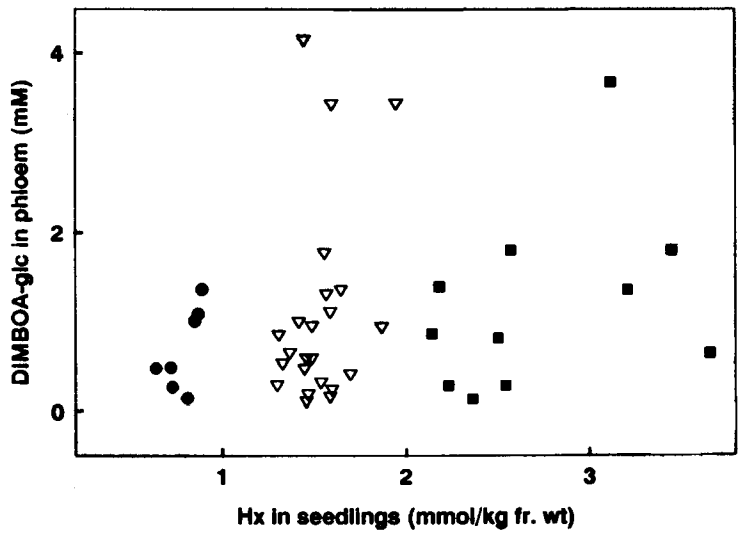

FIG. 3. Concentration of hydroxamic acids in whole leaf and of DIMBOA-glucoside in the phloem sap of wheat seedlings of equivalent developmental stages. Phloem sap was collected through excised aphid stylets. Wheat cultivars used were: Millaleu (●), Nobo (▽) and Maitén (■).

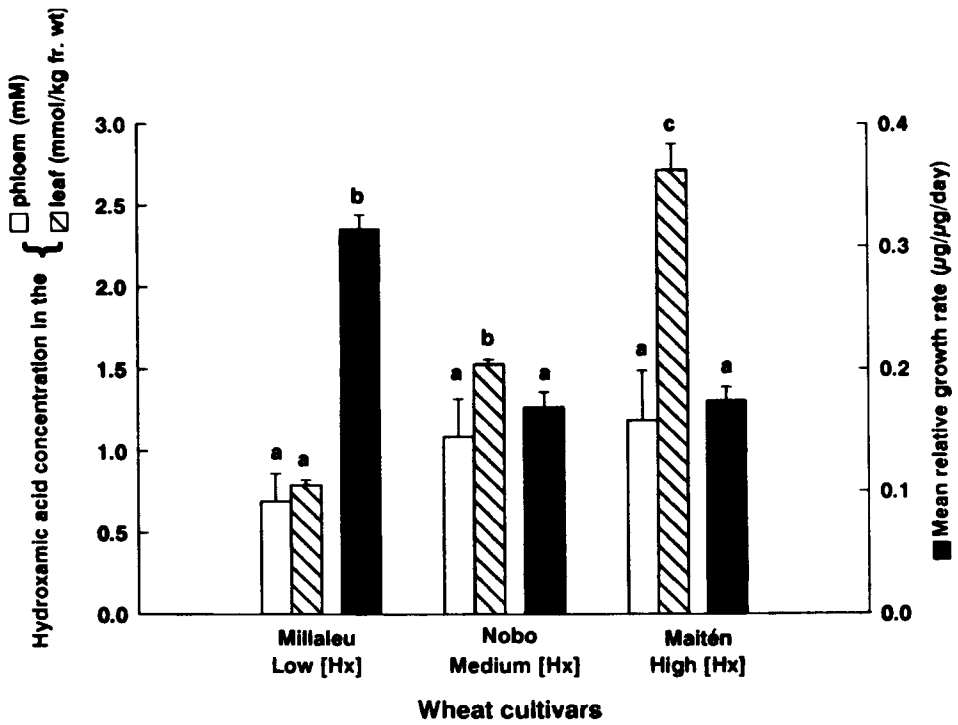

FIG. 4. Mean relative growth rates of first instars of the aphid *Rhopalosiphum padi* reared in seedlings of three cultivars of wheat (■), and concentration of DIMBOA-glucoside in the phloem sap (□) and Hx concentration in the aerial part (▨) of the seedlings.

cultivars (Figure 4). This could indicate that the turnover of Hx outside the phloem differs between wheat cultivars. It is noteworthy that in each of the three cultivars studied, the variability of the glucoside concentration in the phloem sap is considerably larger than in the whole leaf extracts. High variations in concentrations of other compounds, such as amino acids and sugars, have been reported in phloem collected from severed aphid stylets (Girousee et al., 1991). The variation may be explained by the fact that aphids feed from a single sieve tube and concentrations may differ between sieve tubes, possibly reflecting the biosynthetic activity of the area where the sieve tube is loaded. This variation could also be a result of unknown differences in the physiological condition of the plants or the presence of gradients of Hx along the phloem vessels. Our results thus indicate that aphids feeding on wheat seedlings are exposed to considerable differences in the concentration of DIMBOA glucoside in the phloem sap.

Aphids feeding on the wheat cultivars studied showed significant differences in mean relative growth rates, which tended to be negatively correlated to the mean concentration of DIMBOA glucoside in the phloem sap and also to the mean Hx concentration in plant extracts (Figure 4). Interestingly, however, the duration of committed phloem ingestion from sieve elements in the wheat cultivars chosen is independent of the Hx concentration in the aerial parts of a seedling (Givovich and Niemeyer, 1991). This may be interpreted as a combination of several factors: (1) nutrients, as well as other secondary metabolites in the sieve elements, may be masking the known feeding deterrent effect of Hx; (2) the concentrations of Hx in the sap samples may be below the threshold necessary to elicit feeding deterrency in an aphid; or, in view of the present results, (3) aphids may select sieve elements with low concentrations of Hx in plants with relatively high whole leaf Hx concentrations. Figure 4 shows that in spite of an almost 100% increase in whole leaf Hx concentration in going from the cultivar Nobo to Maitén, mean phloem Hx level remains unchanged and, interestingly, mean relative growth rate does not change either. This may be interpreted as the aphid needing to make a selection of sieve tube when it encounters high concentrations of Hx in the plant.

Hx fulfill a double role in host plant discrimination by aphids. At the mesophyll, they act as antixenotics (feeding deterrents), which lead to the selection of plants with lower content of Hx by wingless aphids (Givovich and Niemeyer, 1991) and also by winged ones (Nicol et al., 1992). At the sieve elements, they may act as antixenotics as suggested above and also as antibiotics, which decrease aphid performance, measured either as mean relative growth rate (Thackray et al., 1990a), survival (Argandoña et al., 1980), reproductive rate (Corcuera et al., 1982), or intrinsic rate of increase (Bohidar et al., 1986; Thackray et al., 1990b). Hence, Hx constitute a double barrier towards aphids. They display, within the same molecule, the characteristics of a behavioral pest

control agent and a physiological pest control agent, thus providing for the possibility of efficient and stable resistance (Rice, 1993). Breeding wheat cultivars with high Hx concentrations appears to be a desirable component of integrated pest management of aphids (Escobar and Niemeyer, 1993).

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