Journal of Chemical Ecology, Vol. 18, No. 6, 1992

HYDROXAMIC ACID GLUCOSIDES IN HONEYDEW OF APHIDS FEEDING ON WHEAT

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(Received October 25, 1991; accepted January 23, 1992)

Abstract—DIMBOA glucoside (2-O-β-D-glucopyranosyl-4-hydroxy-7-methoxy-1,4-benzoxazin-3-one), the main hydroxamic acid (Hx) in intact wheat plants, was detected in the honeydew of *Rhopalosiphum padi* feeding on seedlings of six wheat cultivars that differed in their concentration of Hx, suggesting that the chemical circulates in the phloem. Neither the aglucone (DIMBOA) nor its main breakdown product were found in any of the honeydew samples. Honeydew production by aphids caged on seedlings of the wheat cultivars and DIMBOA glucoside concentrations in the honeydew followed biphasic curves when plotted against Hx concentration, suggesting passive ingestion of the chemical from the phloem at low Hx concentrations and limited ingestion due to feeding deterrency by Hx in mesophyll cells at high Hx concentrations. The presence of plant toxins such as Hx glucosides in the phloem sap, the main ingesta of aphids, and in the mesophyll cells, has major implications for plant defense, through a feeding deterrent effect during stylet penetration, and deterrency (antixenosis) along with antibiosis during feeding.

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0098-0331/92/0600-0841$06.50/0

Key Words—*Rhopalosiphum padi*, Homoptera, Aphididae, wheat, maize, DIMBOA, hydroxamic acids, aphid honeydew.

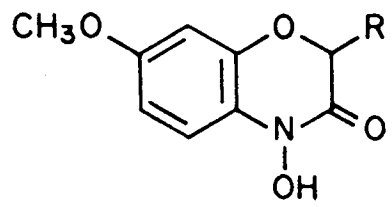
INTRODUCTION

Hydroxamic acids (Hx) are present in a number of Gramineae (Niemeyer, 1988a,b; Zuñiga et al., 1983; Copaja et al., 1991a,b; Barría et al., 1991; Niemeyer et al., 1991) including wheat. Hx exist in the intact plant as glucosides from which the more toxic aglucone is released upon disruption of the plant tissue and contact with a β-glucosidase (Hofman and Hofmanova, 1969; Cuevas et al., 1992). The most common Hx in maize and wheat tissue is 2-O-β-D-glucopyranosyl-4-hydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA glucoside) (Niemeyer, 1988a) (Scheme 1).

Hydroxamic acids are believed to play a role in the resistance of the plants to pests and diseases (Niemeyer, 1988a). Thus, a number of workers have reported negative correlations between the Hx content of maize and wheat and the performance of insect herbivores feeding on the plants. Most of the work has been concerned with the effects of Hx on the European corn borer (Klun and Robinson, 1969; Campos et al., 1989), the western corn rootworm (Xie et al., 1990), and cereal aphids (Long et al., 1977; Argandoña et al., 1980; Beck et al., 1983; Bohidar et al., 1986; Thackray et al., 1990; Givovich and Niemeyer, 1991).

The concentration of hydroxamic acids is higher in the vascular bundles than in the mesophyll (Argandoña and Corcuera, 1985; Argandoña et al., 1987). However, there is no clear evidence that Hx circulate in the phloem. Thus, although Niemeyer et al. (1989b) found DIMBOA in the bodies of aphids feeding on wheat, Molyneux et al. (1990) failed to find 6-methoxybenzoxazolin-2-one (MBOA, the main breakdown product of DIMBOA) in the honeydew of *Schizaphis graminum* feeding on wheat.

The aim of the experiments reported here was to determine whether DIMBOA and/or its glucoside could be detected in the honeydew of aphids feeding on wheat.



DIMBOA R = OH

DIMBOA glucoside R = O-β-D-glucopyranosyl

SCHEME 1.

METHODS AND MATERIALS

Determination of Hx and DIMBOA in Plant Material. High-performance liquid chromatography (HPLC) was employed for DIMBOA analysis in wheat seedlings. The method was essentially that described by Niemeyer et al. (1989a).

Determination of DIMBOA and Its Glucoside in Aphid Honeydew. Aphid honeydew was analyzed for DIMBOA glucoside, DIMBOA, and MBOA using HPLC. A standard of DIMBOA glucoside was obtained as described by Lyons et al. (1988).

Insect Material. Aphids were reared on oats (cv. Nahuen), a plant lacking Hx, in a chamber at 22°C with a 6° range.

Presence of DIMBOA Glucoside in Honeydew of R. padi. The wheat cultivars employed were chosen to contain a wide range of Hx concentrations. Experiments were carried out with seedlings at the one-leaf stage, when Hx levels are highest (Argandoña et al., 1980). Oats (cv. Nahuen) were used as a control without Hx. Twenty adult aphids were placed in a clip cage (2 cm diameter) fitted with a disk of aluminum foil at the bottom and clipped to the abaxial surface of a leaf of a wheat or oat seedling. After 36 hr, the cage was detached from the plant and the aluminum disk weighed. After washing successively with methanol and water and drying, the disk was weighed again. The difference between these measurements was attributed to honeydew produced. The methanol–water washings were concentrated to dryness, redissolved in 200 μl of water, and analyzed by HPLC. Three sets of experiments, each consisting of seven clip cages were performed.

RESULTS

Presence of DIMBOA Glucoside in Honeydew of R. padi *Feeding on Wheat.* DIMBOA glucoside was found in the honeydew of aphids caged on all six wheat cultivars. Neither aglucone nor MBOA were found in the honeydew samples analyzed (detection limit = 0.1 mmol/kg fresh weight).

DIMBOA glucoside concentrations in the honeydew of aphids feeding on wheat are shown in the lower curve of Figure 1. Rates of honeydew production are shown in relation to whole-leaf DIMBOA concentration in the upper curve of Figure 1. Honeydew production in oats, a plant lacking Hx, was 5.35 $\pm$ 0.16 μg/aphid/36 hr.

DISCUSSION

R. padi is mainly a phloem feeder (Pollard, 1973). Hydroxamic acids are known to occur in mesophyll cells of wheat plants (Argandoña et al., 1987). The present finding of DIMBOA glucoside in aphid honeydew suggests that the

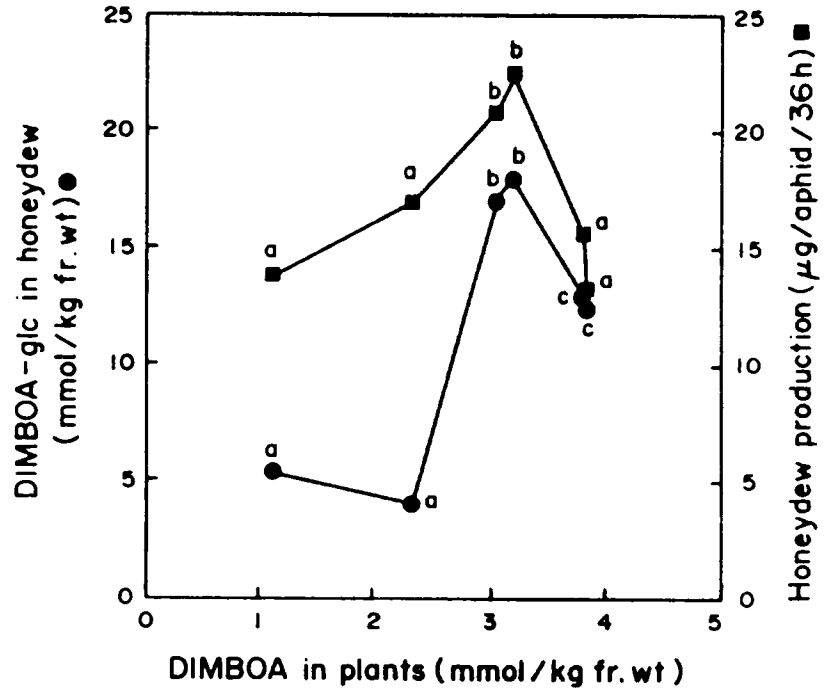


FIG. 1. Mean concentration of DIMBOA glucoside in honeydew (●) and mean honeydew production (■) of *R. padi*, as a function of DIMBOA concentration in wheat seedlings where they fed for 36 hr. Points in each curve followed by different letters are significantly different ($P < 0.01$).

chemicals are also present in wheat phloem. Further evidence for the presence of DIMBOA glucoside in phloem is provided by the failure to detect the aglucone in any of the honeydew samples: if prolonged ingestion had occurred from mesophyll cells ruptured during stylet penetration, then contact with β-glucosidase would be expected and DIMBOA should have been found in the honeydew. The lack of any DIMBOA in the samples also suggests the absence of a suitable β-glucosidase in the digestive tract of *R. padi* or the presence of efficient detoxifying glucosyltransferases. The failure of Molyneux et al. (1990) to find MBOA in the honeydew of *Schizaphis graminum* feeding on a DIMBOA-containing wheat is consistent with these results, since the glucoside does not produce the aglucone breakdown product MBOA under the conditions they used. The results are also consistent with electronic monitoring studies of aphid feeding behavior, which suggest the presence of hydroxamic acids in the phloem sap of the same wheat cultivars employed in this study, albeit in concentrations below those producing feeding deterrency (Givovich and Niemeyer, 1991).

The relationship between glucoside concentration present in honeydew and whole-leaf DIMBOA concentration in wheat followed a biphasic pattern (lower curve in Figure 1). This suggests passive ingestion from phloem sap at low leaf concentrations, and a combination of feeding deterrency, possibly during stylet penetration of the mesophyll, and ingestion from tissues with lower or non-existent Hx glucoside concentrations at high whole-leaf concentrations. Elec-

tronic monitoring of aphid feeding behavior showed that ingestion from xylem, a tissue where Hx have not been detected (Argandoña and Corcuera, 1985), increased as whole-leaf Hx concentration increased (Givovich and Niemeyer, 1991).

A biphasic curve can also be seen in the relationship between honeydew production and whole-leaf concentration of DIMBOA in the wheat cultivars (upper curve in Figure 1). The negative correlation between honeydew production and DIMBOA concentration at the high DIMBOA range may be attributed to feeding deterrency by DIMBOA. The positive correlation obtained at the low DIMBOA range may be attributed to a higher ingestion rate (and consequently, higher excretion rate) in order to compensate for the toxic effects of increasing Hx concentration of the ingesta. These effects may be related to the capacity of Hx to inhibit mitochondrial energy-linked reactions (Niemeyer et al., 1986) and digestive enzymes (Cuevas et al., 1990). Interestingly, honeydew production in oats, a plant lacking Hx, is comparable to that in the lower-DIMBOA-containing wheats.

These results suggest that Hx may be playing different roles in resistance to aphids depending on where the toxin is located in the plant. A high mesophyll concentration of Hx may provide a level of feeding deterrency during stylet penetration, while a high phloem sap concentration may also provide deterrency combined with antibiosis. Further research should determine if DIMBOA glucoside can be found directly in phloem sap.

Acknowledgments—The authors wish to express their gratitude to Miss Lucía Muñoz for able technical assistance. Financial support by International Program in the Chemical Sciences (IPICS), Agency for International Development, International Foundation for Science, FONDECYT, DTI-UCh, and the Science and Engineering Research Council (U.K.) is gratefully acknowledged. H.C. is indebted to IPICS for a fellowship to visit the laboratory in Chile.

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