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INGESTION OF THE BENZOXAZINONE DIMBOA FROM WHEAT PLANTS BY APHIDS

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Key Word Index—*Triticum*; Gramineae; *Metopolophium dirhodum*; *Sitobion avenae*; aphid resistance; hydroxamic acids; DIMBOA; feeding deterrency.

Abstract—DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) was found in nymphs of the aphids *Metopolophium dirhodum* and *Sitobion avenae* which had fed on DIMBOA-containing wheat seedlings and artificial diets. DIMBOA levels found in aphids were lower in those plants and diets with higher DIMBOA levels, suggesting an aphid-feeding deterrency effect of DIMBOA. The results are discussed in relation to the involvement of DIMBOA in aphid resistance of wheat.

INTRODUCTION

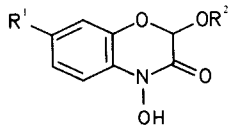
Hydroxamic acids derived from 1,4-benzoxazin-3-one (Hx) have been isolated from extracts of cereals such as wheat, maize and rye [1]. They have been claimed to play an important role in the defence of the plant against aphids. This claim has been based on inverse relationships found between Hx levels and aphid performance in infestation experiments using different cereals [2], different cultivars of the same cereal [3–5] and different parts within the same plant [6]. The correlations have included the aphids *Rhopalosiphum maidis* [3], *Metopolophium dirhodum* [2], *Schizaphis graminum* [6] and *Sitobion avenae* [5]. In addition, Hx in artificial diets exhibit antibiotic and antifeedant properties towards *S. graminum* at concentrations comparable to those found in the plant [7].

A necessary condition for associating a plant secondary compound with host plant defence against an insect is that the insect must ingest or come into contact with the compound during its interaction with the plant. In this paper we describe a method for quantifying Hx in aphids and use it to show that Hx are indeed ingested by aphids when feeding on Hx-containing diets and wheat seedlings. Implications of this finding in relation to wheat resistance to aphids are discussed.

RESULTS AND DISCUSSION

Analytical procedure

A method was developed for the quantitation of Hx in insect extracts based on the different relative solubilities in water and ether of the dissociated and undissociated forms of Hx. This allowed the recovery of Hx from the insect fat and also its further purification by re-extracting it into an organic solvent. DIMBOA and DIBOA were recovered from the extracts to the extent of 82.0 and 99.7% respectively (Fig. 1). These values most likely reflect the decomposition of Hx, which occurs faster in the case of DIMBOA [8]. Losses due to decomposition were nevertheless minimized by using aqueous extracts of pH values (3 and 11) corresponding to minima in the decomposition rate-pH profile of DIMBOA [9].



DIMBOA : $R^1 = CH_3O$, $R^2 = H$
DIBOA : $R^1 = H$, $R^2 = H$

The only hydroxamic acid found in the wheat plants analysed was DIMBOA. However, the described method should be applicable to studies of aphids feeding in DIBOA-containing plants, such as rye [10].

Aphids feeding on diets

Aphids (*M. dirhodum*) were allowed to feed on DIMBOA-containing diets. At concentrations below 0.5 mM, the DIMBOA levels found in the aphids increased with increasing levels in the diet (Fig. 2A). The trend was reversed at higher concentrations of DIMBOA in the diet.

These results point to a feeding deterrent effect of DIMBOA towards *M. dirhodum* at concentrations above *ca* 1 mM. The feeding deterrency of DIMBOA towards the aphid *S. graminum* has been documented based on results from electronic monitoring of feeding behaviour [7].

The change in weight of aphids as a function of DIMBOA concentration in the diet (Fig. 2B) was consistent with a feeding deterrent effect. Thus, aphids experienced a net weight gain in the control diet or in that containing the lowest DIMBOA concentration. However, as the DIMBOA concentration was further increased beyond 0.5 mM, aphids suffered a net weight loss which tended to plateau.

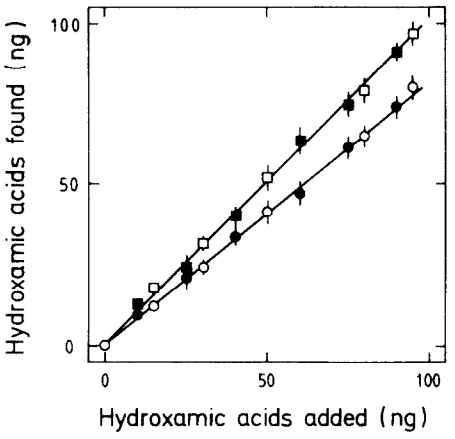


Fig. 1. Recovery of DIBOA and DIMBOA from insect extracts. Constant amounts (equivalent to 50 mg of aphids from the colony growing on barley) of the alkaline aphid extract were added to tubes containing varying amounts of hydroxamic acids. Samples were processed as in Experimental. Vertical lines represent standard deviations from the mean of four replicate analyses. Empty symbols: *M. dirhodum*; filled symbols: *S. avenae*; circles: DIMBOA; squares: DIBOA. Slopes determined were 0.997 ± 0.03 for DIBOA and 0.820 ± 0.04 for DIMBOA.

Part of the above effect may however be attributed to the antibiotic effects of ingested DIMBOA [11] upon aphid symbiotes providing or supplementing the aphid's nutritional requirements [12]; or to DIMBOA impairment of food digestibility, caused by inhibition of digestive enzymes in the aphid [8, 13].

Aphids feeding on plants

Aphids (*M. dirhodum*) were allowed to feed for 40 hr on wheat plants with different DIMBOA levels. The results (Fig. 3) were similar to those in the diet experiments (Fig. 2A), i.e. as the DIMBOA levels in the plants increased, the DIMBOA levels in the aphids first increased and then decreased. DIMBOA would thus behave as feeding deterrent also in the plant.

Experiments were also performed with *S. avenae* feeding for 20 hr on different wheat cultivars (Fig. 3). The DIMBOA levels in the aphids were consistently smaller than in the experiment with *M. dirhodum*, a probable reflection of shorter feeding time; the pattern observed between DIMBOA in the plant and in the insect, was similar to that of *M. dirhodum*.

The range of DIMBOA concentrations used in the diets (0–4 mM) is comparable with the range in plant leaves (0–4 mmol/kg fr. wt). Additionally, the levels of DIMBOA found in the aphids feeding in diets (Fig. 2A) are comparable with those found in aphids feeding on plants (Fig. 3). As aphids normally feed at greater rates in plants than in artificial diets [14], these results suggest that DIMBOA is more concentrated in the site of uptake by the aphid than in the leaf as a whole. Interestingly, the highest levels of hydroxamic acids in wheat leaves were found in the vascular bundles [15].

The stage of the aphid feeding process at which Hx are ingested is, however, not clear at this moment. Hx may also be ingested when the aphid probes in search of

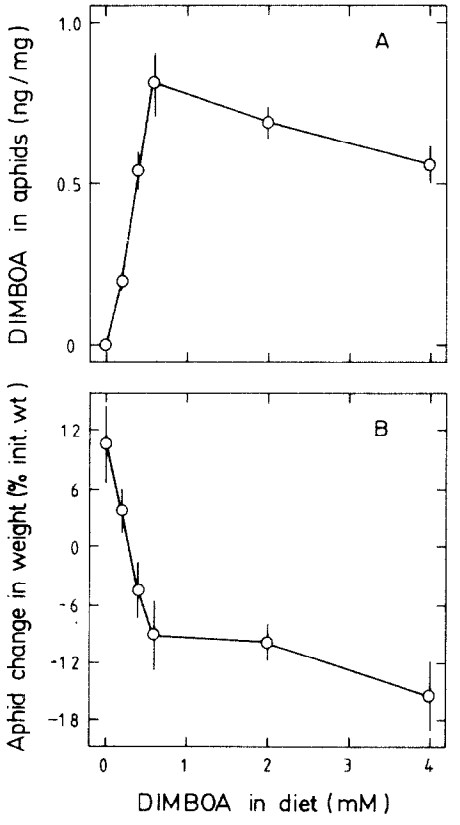


Fig. 2. Performance of *M. dirhodum* in artificial diets containing different concentrations of DIMBOA. A: DIMBOA in the aphids and in the diets. DIMBOA in the aphids was determined as described in Experimental. B: Change in aphid weight. Results are expressed as percent of the initial weight of aphids. Initial weight were determined just before aphids were transferred to the diets. Vertical bars represent standard deviations from the mean.

phloem vessels. It was recently demonstrated that *M. dirhodum* feeding on wheat seedlings cause damage to mesophyll cells involving destruction of the cell contents, vacuole membrane damage or rupture of the cell wall [16]. It was suggested that the loss of cytoplasmic material in the damaged cells may be attributed to its ingestion by the aphid [16]. These results are consistent with evidence from electron microscopy showing intracellular penetration by aphid stylets in the case of *S. avenae* [17] and of *S. graminum* [18] feeding on wheat and also with evidence from electrical penetration graphs showing stylet puncture of mesophyll cell membranes by different aphid species feeding on host and non-host plants, both on susceptible and resistant cultivars [19], as well as non-phloem ingestion by *S. avenae* on oats [20].

Several conditions should be fulfilled in order to associate a secondary plant compound with host plant resistance to aphids, for instance, correlations between the concentration of the compound in the plant and degree of plant resistance, encounter or ingestion of the compound by the aphid, a relationship between biological activity of the compound in the plant and in a synthetic diet.

The first of these conditions is well documented in the case of hydroxamic acids from cereals, as discussed

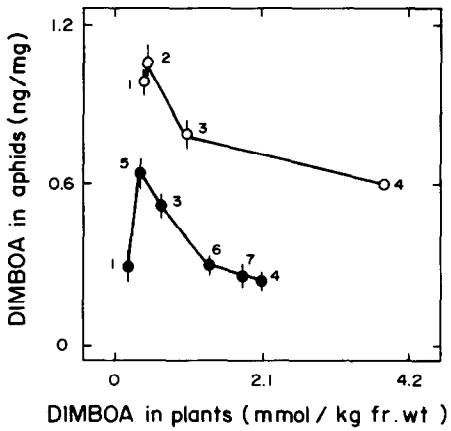


Fig. 3. Effect of DIMBOA levels in wheat plants on DIMBOA levels in aphids feeding on them. Nymphs of *M. dirhodum* (empty circles) or *S. avenae* (filled circles) were allowed to feed on wheat seedlings for 40 and 20 hr, respectively. For these experiments, the 10 cohorts were randomly grouped in two sets to provide sufficient sensitivity, and each set analysed separately. Cultivars used were: Huenufen (1), Sonka (2), Naofen (3), Quilafen (4), SNA-2 (5), Likay (6) and SNA-3 (7). Vertical lines represent standard deviations from the mean. Standard deviations of the determinations of DIMBOA in the plants varied between 3 and 9% and were omitted for simplicity.

above. In this paper we have provided evidence for the ingestion of the compound by aphids from plants and diets, and for feeding deterrency by DIMBOA both in plants and in diets. This strongly argues in favour of the involvement of hydroxamic acids in the resistance of wheat to aphids.

EXPERIMENTAL

Aphids. Two species were used: *Metopolophium dirhodum* (Walk). and *Sitobion avenae* F. Colonies were reared on barley seedlings, a plant lacking hydroxamic acids [2], grown in a greenhouse at $20 \pm 3°$ with 16:8 (light:dark) light regime. Aphids used in each experiment differed in age by at most 24 hr and were third or fourth instar nymphs.

Plants. Seeds of *Triticum durum* cvs SNA-2, SNA-3 and Quilafen, and *T. aestivum* cvs Huenufen, Naofen, Sonka and Likay, were obtained from INIA, Chile. Plants were grown in a greenhouse under the conditions described above, and were in decimal growth stage 10 [21] when used.

Artificial diets. Consisted of a mixture of amino acids, minerals, vitamins and sucrose, as described elsewhere [22].

Reference compounds. 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), the main Hx in wheat and maize, was isolated from extracts of *Zea mays* cv. T129S, as described [23]. Its demethoxylated analogue, DIBOA, the main Hx in rye, was synthesized as described [24].

Analytical methods. Both insect and plant extracts were analysed by high performance liquid chromatography (HPLC).

Insects (30 to 100 mg) were macerated successively with 3 × 0.33 ml of 10 mM pH 11 Pi buffer. The extract was centrifuged 10 min at 6000 *g*. The supernatant was taken to pH 3 with 0.1 N H_2PO_4 and the resulting suspension centrifuged 10 min at 6000 *g*. The new supernatant was extracted with 3 × 2 ml diethylether. The pooled ethereal phases were concd to dryness under red. pres. and the residue taken to vol. (*ca* 150–300 μl) with water. Aliquots of this solution (100 μl) were injected into the column in a Merck–Hitachi L-6200 liquid chromatograph coupled to a Merck–Hitachi L-4000 variable wavelength detector.

A 125 × 4 mm RP-18 column was used with a constant solvent flow of 1.5 ml/min and the following linear gradients between solvents A (MeOH) and B (0.5 ml H_2PO_4 in 1 l water): 0–4.5 min, 25–45% A; 4.5–5 min, 45–100% A; 5–10 min, constant at 100% A; 10–10.1 min, 100–25% A; 10.1–13 min, constant at 25% A. Detection was carried out at 263 nm. Retention times were 2.7 ± 0.03 min for DIBOA and 3.65 ± 0.03 min for DIMBOA. Crude aqueous extracts of plant material were analysed as described elsewhere [25].

Aphids feeding on diets. Nymphs of *M. dirhodum* from the stock colony (four cohorts of 40 to 60 aphids each) were starved for 2 hr before being transferred to diets, and were kept on the diets for 40 hr. DIMBOA content and weight change of aphids were determined as a function of concentration of DIMBOA in the diet.

Aphids feeding on wheat plants. Aphids from the stock colony were placed in 3 cm diameter leaf clip cages attached 2–2.5 cm below the tip of 8–9 cm tall seedlings. Ten cohorts of 40 aphids each were set up. DIMBOA content of the aphids was determined in plants with different DIMBOA levels.

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REFERENCES

1. Niemeyer, H. M. (1988) *Phytochemistry* **27**, 3349.
2. Argandoña, V. H., Luza, J. G., Niemeyer, H. M. and Corcuera, L. J. (1980) *Phytochemistry* **19**, 1665.
3. Long, B. J., Dunn, G. M., Bowman, J. S. and Routley, D. G. (1977) *Crop Sci.* **17**, 55.
4. Corcuera, L. J., Argandoña, V. H., Peña, G. F., Pérez, F. J. and Niemeyer, H. M. (1982) *Proceedings of the Fifth International Symposium on Insect-Plant Relationships* (Visser, J. H. and Minks, A. K., eds), p. 33. Pudoc, Wageningen.
5. Bohidar, K., Wratten, S. D. and Niemeyer, H. M. (1986) *Ann. Appl. Biol.* **109**, 193.
6. Argandoña, V. H., Niemeyer, H. M. and Corcuera, L. J. (1981) *Phytochemistry* **20**, 673.
7. Argandoña, V. H., Corcuera, L. J., Niemeyer, H. M. and Campbell, B. C. (1983) *Ent. Exp. Appl.* **34**, 134.
8. Pérez, F. J. and Niemeyer, H. M. (1985) *Phytochemistry* **24**, 2963.
9. Niemeyer, H. M., Bravo, H. R., Peña, G. F. and Corcuera, L. J. (1982) *Chemistry and Biology of Hydroxamic Acids* (Kehl, H., ed.), p. 22. Karger A. G., Basel.
10. Zúñiga, G. E., Argandoña, V. H., Niemeyer, H. M. and Corcuera, L. J. (1983) *Phytochemistry* **22**, 2665.
11. Niemeyer, H. M., Calcaterra, N. B. and Roveri, O. A. (1986) *Biochem. Pharmacol.* **35**, 3909.
12. Houk, E. J. and Griffiths, G. W. (1980) *Ann. Rev. Entomol.* **25**, 161.
13. Niemeyer, H. M. and Pérez, F. J. (1987) *Proceedings of the Sixth International Symposium on Insect Plant Relationships* (Labeyrie, V., Fabres, G. and Lachaise, D. eds), p. 49. Dr W. Junk, Amsterdam.
14. Dadd, R. H. and Mittler, T. E. (1965) *J. Insect Physiol.* **11**, 717.
15. Argandoña, V. H., Zúñiga, G. E. and Corcuera, L. J. (1987) *Phytochemistry* **26**, 1917.

16. Brzezina, A. S., Spiller, N. J. and Llewellyn, M. (1986) *Ent. Exp. Appl.* **42**, 195.
17. Spiller, N. J., Kimmins, F. M. and Llewellyn, M. (1985) *Ent. Exp. Appl.* **38**, 585.
18. Al-Mousawi, A. H., Richardson, P. E. and Burton, R. L. (1983) *Ann. Entomol. Soc. Am.* **76**, 964.
19. Tjallingii, W. F. (1985) *Ent. Exp. Appl.* **38**, 187.
20. Scheller, H. V. and Shukle, R. H. (1986) *Ent. Exp. Appl.* **40**, 189.
21. Tottman, D. R. and Makepeace, R. J. (1979) *Ann. Appl. Biol.* **93**, 221.
22. Argandoña, V. H., Peña, G. F., Niemeyer, H. M. and Corcuera, L. J. (1982) *Phytochemistry* **21**, 1573.
23. Queirolo, C. B., Andreo, C. S., Niemeyer, H. M. and Corcuera, L. J. (1983) *Phytochemistry* **22**, 2455.
24. Jernow, J. L. and Rosen, P. (1975) *U.S. Patent* 3, 862, 180.
25. Niemeyer, H. M., Pesel, E., Copaja, S. V., Bravo, H. R., Franke, S. and Francke, W. (1989) *Phytochemistry* **28**, 447.