13. Effect of Cyclic Hydroxamic Acids from Cereals on Aphids

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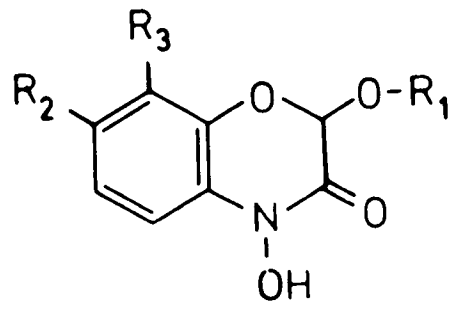
Abstract. The resistance of barley, several cultivars of wheat, triticale and maize to aphids and hydroxamic acid content of plant extracts were correlated. Aphids such as *Metopolophium dirhodum*, *Schizaphis graminum* and *Rhopalosiphum maidis* were inhibited by DIMBOA, the main hydroxamic acid present in maize and wheat extracts. DIMBOA decreased survival and reproduction rate of aphids fed on artificial diets. The effect on aphids of the naturally-present glucoside of DIMBOA was substantially less than that of DIMBOA. Therefore, hydrolysis of the glucoside on infection may be required for resistance to aphids.

Introduction

Several closely related cyclic hydroxamic acids have been isolated from maize, wheat, rye, and *Coix* [6,13,14] (fig. 13.1). These molecules inhibit bacterial growth [5,10], spore germination [9], and insect development [8]. It has been proposed that these compounds act as defense chemicals against insects [8,11] and fungi [9]. Although the mode of action of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA)—the main hydroxamic acid in maize and wheat extracts—on these pathogens remains unknown, it has been reported recently that DIMBOA inhibits energy transfer reactions in spinach chloroplasts and in bovine mitochondria [12].

Since aphids cause important damage to cereal crops, it is of interest to study the possible role of cyclic hydroxamic acids in the resistance of cereals to aphids. In previous papers we proposed that DIMBOA and related compounds play a role in resistance to *Metopolophium dirhodum* (Walker) [2] and in determining distribution of *Schizaphis graminum* (Rondani) on the leaves [3]. In this paper we report on the effects of DIMBOA on several cereal aphids.



	R_1	R_2	R_3
I	H	H	H
II	Glc	H	H
III	H	OMe	H
IV	Glc	OMe	H
V	H	OMe	OMe
VI	Glc	OMe	OMe

Fig. 13.1. Cyclic hydroxamic acids from *Gramineae*. III, DIMBOA; IV, DIMBOA-Glc,β-D-glucosyl.

Material and Methods

Plant Material. Seeds were obtained from Instituto Nacional de Investigaciones Agropecuarias, Departamento de Sanidad Vegetal, Universidad de Chile, and Sociedad Nacional de Agricultura.

Preparation of Extracts and Purification of Compounds. Plant tissue was macerated in water, filtered through cheesecloth, and left 15 minutes at room temperature. The extract was adjusted to pH 3 with 1 M HCl and centrifuged at 8500*g* for 15 minutes. The supernatant was extracted into ethyl ether (2 vol × 3) and the organic phases were evaporated to dryness. These extracts were used to quantitate hydroxamic acids (see below) and the isolate DIMBOA by a procedure previously described [15]. Coleoptiles of 6-day-old seedlings of *Zea mays* L. cv LH Rinconada were used to isolate DIMBOA.

The 2-O-β-D-glucoside of DIMBOA (DIMBOA-Glc) was obtained from aqueous extracts of boiled maize seedlings that were passed through SP-Sephadex-Fe (4) and Sephadex G-10 columns (3) as described (12). UV, IR, and NMR spectra of DIMBOA and DIMBOA-Clc were obtained.

Quantitation of Hydroxamic Acids. Hydroxamic acids form a blue complex upon the addition of $FeCl_3$ reagent (50 g $FeCl_3$-$6H_20$, 500 ml 95% ethanol and 5 ml 14 M HC1). The concentration of hydroxamic acids in the tissues was determined by comparing the absorbance of extracts with a standard curve made with DIMBOA (λ max = 590 nm, $\epsilon_{590} = 1315$). Thus, the values reported represent DIMBOA equivalents [2,14].

Aphids and Diet. Aphids were collected from fields near Santiago and allowed to reproduce on barley plants kept inside a nylon cage in the labora-

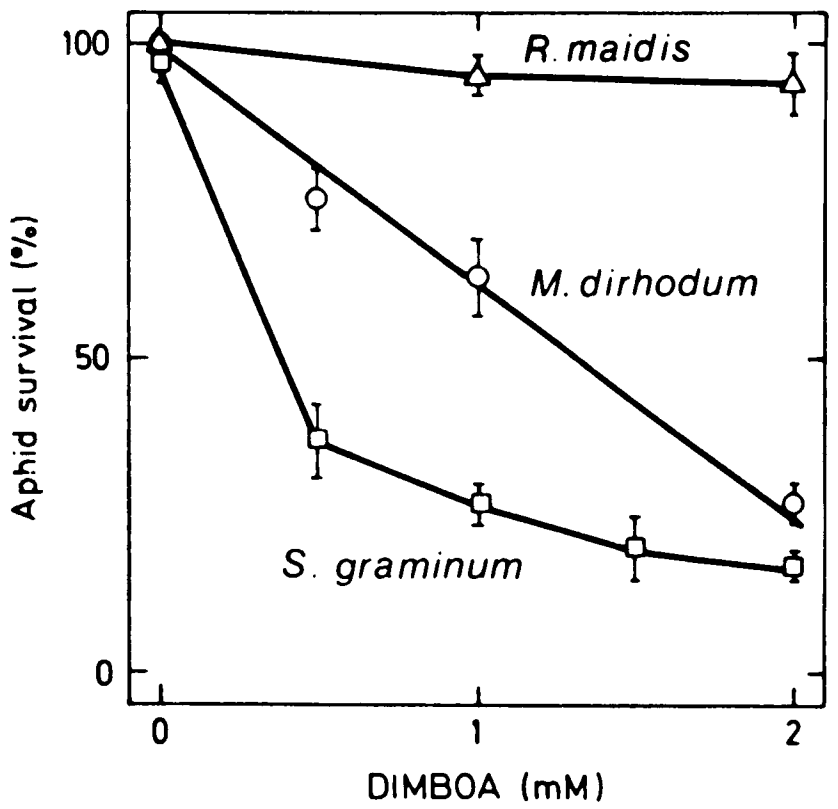


Fig. 13.2 Effect of DIMBOA on *Rhopalosiphum maidis*, *Metopolophium dirhodum* and *Schizaphis graminum*. Survival was measured after feeding aphid nymphs with an artificial diet for 24 hours. Each point is the mean of three samples of 10 individuals each. Vertical bars are standard errors of the mean.

tory. For feeding experiments, a pH 5.5 aqueous solution of 30% sucrose, amino acids, vitamins, and mineral salts placed between two layers of parafilm M were used [1].

Results

Effect of Hydroxamic Acids on Resistance of Gramineae to Aphids. The growth rate of population of *M. dirhodum* (table 13.1) and of *S. graminum* (table 13.2) on several *Gramineae*, as well as the hydroxamic acid content of the plant extracts were measured. The highest growth rate were obtained in barley, a plant that lacks hydroxamic acids. Furthermore, growth rates decreased as the content of hydroxamic acid increased. Young plants of *Triticale* and *Zea mays*, the two species with the highest content of these compounds, were resistant to *M. dirhodum*

Table 13.1. Hydroxamic acid content and susceptibility of different Gramineae to *Metopolophium dirhodum*

Species	Hydroxamic acid in leaf extracts (mmole/kg fr. wt)	Aphids/sample		Population [b] growth rate (per day)
		Initial [a]	Final	
Hordeum distichum				
cv Fola Union	ND[c]	12	94 ± 3	0.41
Triticum aestivum				
cv Huenufen	0.52	12	70 ± 7	0.35
Naofen	1.16	12	58 ± 6	0.31
Triticum durum				
cv SNA-3	3.11	12	39 ± 3	0.23
Triticale				
cv 951-1978	4.48	12	6 ± 3	−0.14
Zea mays				
cv T125 L22	7.98	12	0	—

[a] The infestation was carried out on 7-day-old greenhouse-grown plants. The experiment lasted 5 days. Values are average of three samples of 12 plants each.
[b] Growth rate = (ln Nf/Ni) / Δt.
[c] Not detected. The detection limit was 8×10^{-4} mmole/kg fr. wt.

Table 13.2. Hydroxamic acid content and susceptibility of several cultivars of wheat to *Schizaphis graminum*.

Wheat cultivar	Hydroxamic acids in leaf extracts (mmole/kg fr. wt)	Aphids/sample		Population growth rate [b] (per day)
		Initial [a]	Final	
T. aestivum				
cv Sonka	0.61	6	86	0.44
Likay	0.89	6	83	0.43
Cajeme	1.37	6	52	0.36
Naofen	1.50	6	25	0.24
T. durum				
cv SNA-1	1.85	6	21	0.21

[a] The infestation was carried out in 10-day-old greenhouse-grown plants. The experiment lasted 6 days. Each sample consisted of six plants.
[b] Growth rate = (ln Nf/Ni) / Δt.

Effect of DIMBOA on Survival of Several Species of Aphids. Aphid nymphs of *Rhopalosiphum maidis* (Fitchii), *M. dirhodum*, and *S. graminum* were fed DIMBOA in an artificial diet. After 48 hours, survival was measured (fig. 13.2). While survival of *S. graminum* and *M. dirhodum* was substantially decreased, survival of *R. maidis* was not greatly affected up to 2 mM DIMBOA.

Effect of DIMBOA on Reproduction of S. graminum. Aphid nymphs were diet-fed DIMBOA at concentrations lower than those in the previous experiment, to measure the effect of this compound on aphid reproduction. An inverse linear correlation was found between the logarithm of the reproductive index and the concentration of DIMBOA (fig. 13.3). Thus, DIMBOA decreased reproduction rate at low concentrations and became lethal at high concentrations.

Effect of DIMBOA and DIMBOA-Glc on Schizaphis graminum. DIMBOA is present in intact tissue as 2-β-D-glucoside [6]. Therefore, it is of interest to compare the activities of DIMBOA and of DIMBOA-Glc on aphids. These

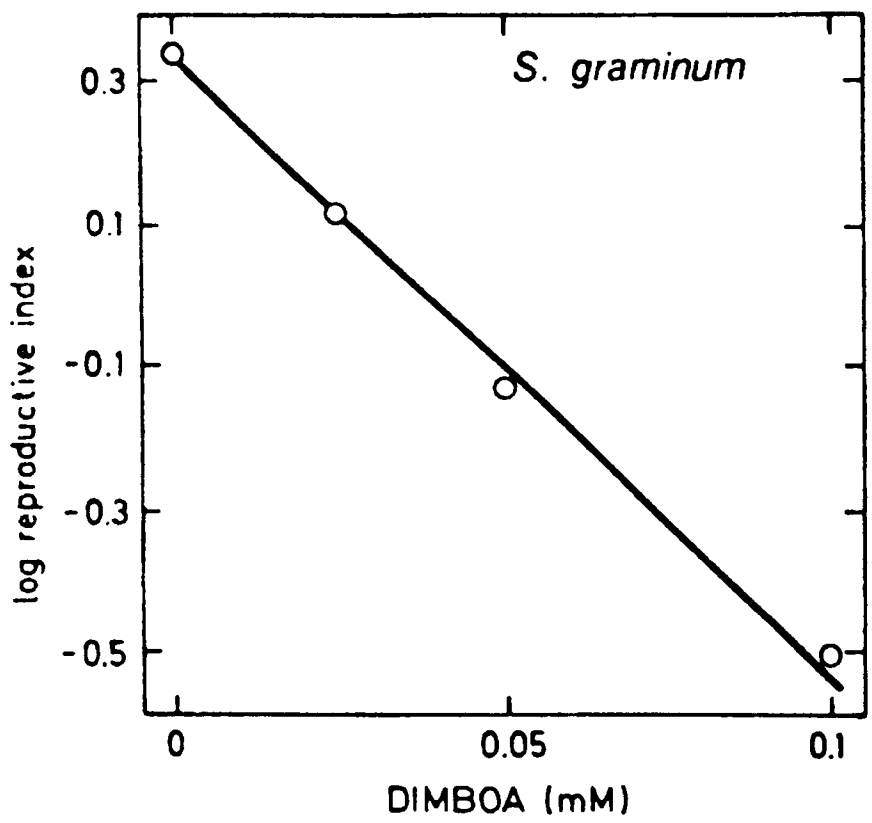


Fig. 13.3. Effect of DIMBOA on *Schizaphis graminum* fed with artificial diets. The reproductive index (ri = number of nymphs/average number of adults) was measured after feeding aphid adults for 72 hours.

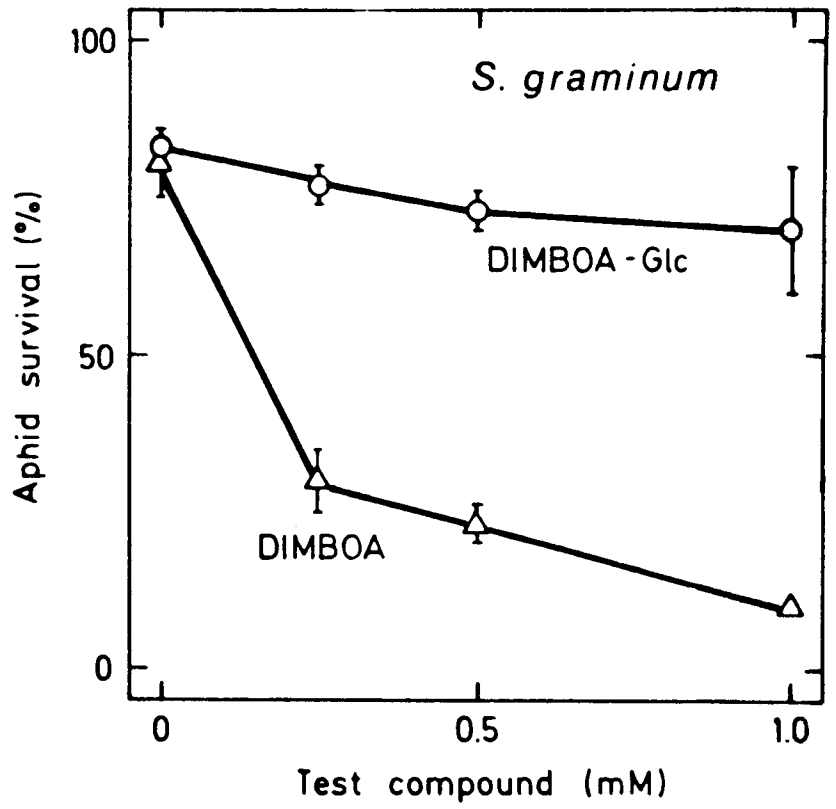


Fig. 13.4. Effect of DIMBOA and DIMBOA-Glc on *Schizaphis graminum* fed on artificial diets. Survival was measured after feeding the aphids for 48 hours. Each point is the mean of three samples consisting of 10 aphids each. Vertical bars are standard errors of the mean.

compounds were fed in diets to aphid nymphs. Survival was measured 48 hours later (fig. 13.4). While 1 mM DIMBOA reduces the number of aphids to 10%, DIMBOA-Glc reduces it to only 70%.

Discussion

The content of hydroxamic acids of several species of Gramineae and cultivars of wheat correlated inversely with resistance to the aphids *M. dirhodum* and *S. graminum*. DIMBOA, the major hydroxamic acid in wheat and maize cultivars, affected survival of these aphids in artificial diets, confirming earlier results [2,3,11]. Therefore, it is likely that hydroxamic acids constitute a chemical defense of Gramineae against aphids.

DIMBOA also affected reproduction rates of aphids fed with artificial diets (figs. 13.2 and 13.3) at concentrations that may be lower than those found in

the plant. The hydroxamic acid concentration may reach up to 6 mmole/kg of fresh weight in some varieties. However, their concentration within the plant cell is not known, since they may be compartmentized at cellular level. Hydroxamic acids could affect the resistance of cereals to aphids by decreasing reproduction, by increasing mortality, and/or by acting as feeding deterrents.

The hydroxamic acid concentration in wheat, rye, and maize varies with plant age, reaching a maximum during the first week and then continously decreasing [3]. Increased susceptibility of older plants to aphids appears to be related to the lower hydroxamic acid content of these plants [2]. On the other hand, aphid susceptibility to hydroxamic acid may determine its host preference. For example, maize, the Gramineae with the highest content of hydroxamic acid, is more susceptible to *R. maidis* than to other aphid species. *R. maidis* is more resistant to DIMBOA fed in artificial diets than *S. graminum* and *M. dirhodum*.

The naturally present DIMBOA-Glc shows little activity compared to DIMBOA. It appears that the relatively inactive glucoside is hydrolyzed to the more active DIMBOA upon infestation of the plant by aphids or other pathogens.

Acknowledgment

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