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Effect of a benzoxazinone from wheat on aphids

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Abstract

2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), the main benzoxazinone isolated from wheat extracts, decreases aphid survival and reproduction rates in artificial diets. The effect of the naturally-present 2-0-β-D-glucoside was less than that of DIMBOA. Therefore, hydrolysis of the glucoside upon infestation may be required for resistance of cereals to aphids. The biological activity of DIMBOA is decreased by addition of cysteine to the diets. DIMBOA reacts with thiols with rates proportional to reduction potentials of thiols. With ethanethiol, DIMBOA gives addition and/or reduction products. These properties of DIMBOA may be related to its mode of action.

Key-words: wheat, *Metopolophium dirhodum, Schizaphis graminum, Rhopalosiphum maidis*, hydroxamic acids, DIMBOA, plant resistance, survival, reproduction

Wheat extracts contain benzoxazinones (Willard & Penner, 1976), the most abundant of which is 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA, Figure 1), a cyclic hydroxamic acid. This and related hydroxamic acids are important in resistance of several Gramineae to insects (Klun et al., 1967; Argandoña et al., 1980, 1981). In addition DIMBOA inhibits bacterial growth in culture and spore germination (Elnaghy & Linko, 1962; Corcuera et al., 1978). In this paper we describe the effects of DIMBOA on aphids fed with artificial diets and explore chemical properties of DIMBOA that may be related to its mode of action.

Materials and methods

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

Before extraction and purification of the compounds involved, the plant tissue was macerated in water and filtered through cheese-cloth. The extract was adjusted to pH 3 with aqueous HCl (1 mol/l) and centrifuged in a force field of 8500 g_n (83.3 kN) for 15 min. The supernatant was extracted three times with 2 volumes of ethyl ether and the organic phases were evaporated to dryness. These extracts were

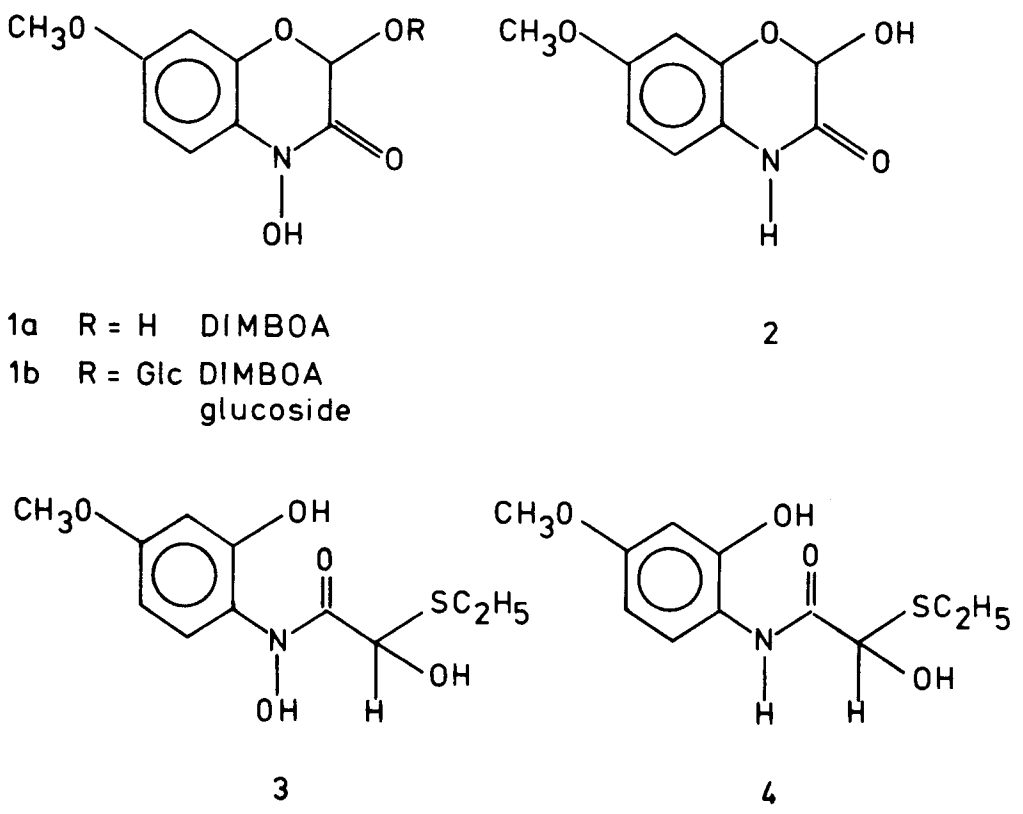

Fig. 1. Products (2,3,4) isolated from the reaction of DIMBOA (1a) with ethanethiol.

used for quantitation of hydroxamic acids, and to isolate DIMBOA by a procedure previously described (Woodward et al., 1978b). Coleoptiles of 6-day old seedlings of *Zea mays* L. cultivar LH Rinconada were used to isolate DIMBOA.

The 2-0-β-D-glucoside of DIMBOA was obtained from aqueous extracts of boiled maize seedlings which were passed through SP-Sephadex-Fe (Corbett & Chipko, 1978) and Sephadex G-10 columns (Hofman & Hofmanova, 1969). UV, IR and NMR spectra of DIMBOA and its glucoside were obtained.

Hydroxamic acids form a blue complex upon addition of $FeCl_3$ reagent (50 g of $FeCl_3.6H_2O$, 500 ml aqueous ethanol (volume fraction 95%) and 5 ml of HCl (concentration 14 mol/l)). The concentration of hydroxamic acids in the tissues was determined by comparing the absorbance of the extracts with a standard curve made with DIMBOA ($\lambda_{max} = 590$ nm, $\varepsilon_{590} = 131.5\ m^2.mol^{-1}$). Thus, the values reported represent DIMBOA equivalents (Woodward et al., 1978a; Argandoña et al., 1980). Rates of disappearance of hydroxamic acids from solutions of DIMBOA were followed by withdrawing aliquots, adding them to $FeCl_3$ reagent and measuring the absorbance at 590 nm.

Aphids were collected from fields near Santiago and allowed to reproduce on barley plants kept inside a nylon cage in the laboratory. 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 was used (Auclair, 1965; Argandoña et al., 1980).

Table 1. Hydroxamic acid content and susceptibility of several cultivars of wheat to *Schizaphis graminum*.

Wheat	Hydroxamic acids in leaf extracts (mmol/kg fresh weight)	Aphids/sample		Population growth rate[2] (per day)
		initial[1]	final	
Triticum durum				
cv. SNA-1	1.85	6	21	0.21
Tricitum aestivum				
cv. Naofen	1.50	6	25	0.24
cv. Cajeme	1.37	6	32	0.36
cv. Likay	0.89	6	83	0.43
cv. Sonka	0.61	6	86	0.44

1. The infestation was carried out in 10-day old greenhouse-grown plants. The experiment lasted 6 days. Each sample consisted of 6 plants.
2. Growth rate = $(\ln n_f/n_i)/\Delta t$.

Results

Several varieties of wheat were infested with *Schizaphis graminum*. Hydroxamic acids in the leaves as well as aphid population growth rate were measured (Table 1). A negative correlation was found between hydroxamic acid content and aphid population growth rate on the same leaves, suggesting a possible role of these compounds in resistance of the plants to the *S. graminum*.

Three species of aphids which normally attack Gramineae were fed with artificial diets with or without DIMBOA (Figure 2). A major decrease in survival of *S. graminum* and *Metopolophium dirhodum* was observed while *Rhopalosiphum maidis* was not greatly affected. At lower concentrations DIMBOA decreased the reproduction rate of *S. graminum* (Figure 3).

Survival of *S. graminum* in diets was greater when aphids were fed DIMBOA-glucoside than DIMBOA (Figure 4), suggesting that the naturally-present glucoside is not the active compound.

The half-life of DIMBOA in solutions containing cysteine was shorter than in solutions without the aminoacid (Table 2). The products of the reaction of DIMBOA with cysteine were not toxic to *S. graminum* (Table 3). The toxicity of DIMBOA to *S. graminum* was decreased by the presence of cysteine in the diets (Table 3). Since most media used to assay biological activity of DIMBOA contain cysteine, it may be concluded that the activity of DIMBOA is greater than previously reported.

Products isolated from the reaction of DIMBOA with ethanethiol are shown in Figure 1, marked as 2, 3 and 4 (Niemeyer et al., 1982). They arose from hemiacetal-hemithioacetal exchange and/or from reduction of the hydroxamic acid to amide. Second-order rate constants for the reaction of DIMBOA with cysteine, mercaptoethanol and dithiothreitol were determined (Table 4). The logarithms of these rate constants were linearly correlated to the reduction potentials of the thiols (Table 4), suggesting that the rates measured corresponded to the reduction step. This is sup-

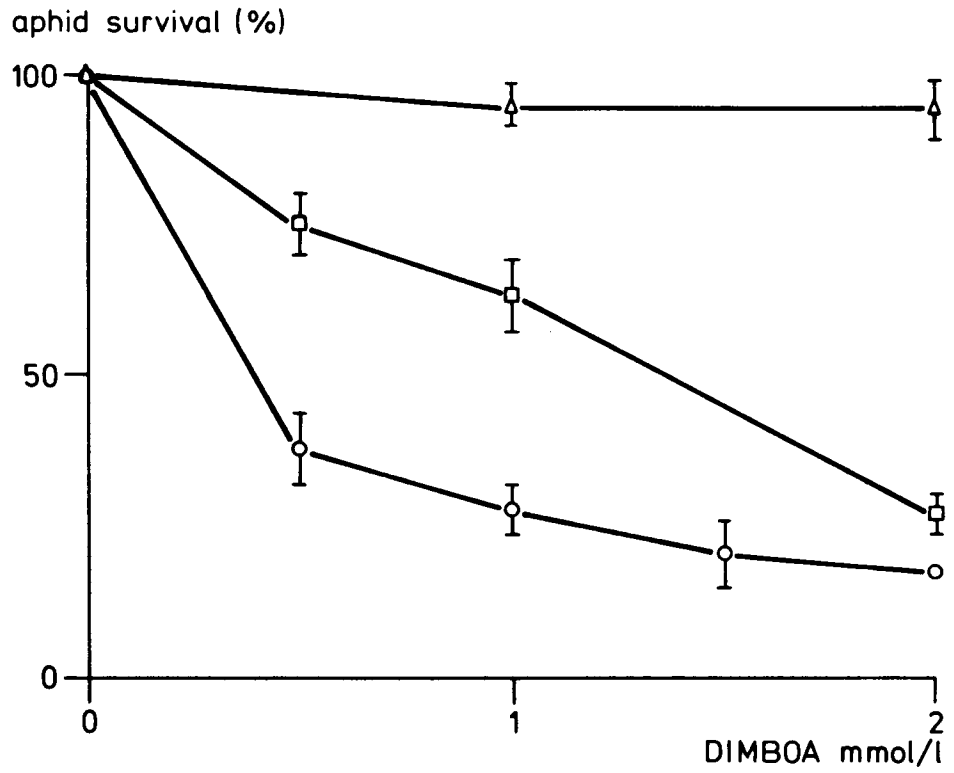

Fig. 2. Effect of DIMBOA on *Rhopalosiphum maidis* (△), *Metopolophium dirhodum* (□) and *Schizaphis graminum* (○). Survival was measured after feeding aphid nymphs with an artificial diet for 24 h. Each point is the mean of three samples of ten individuals each. Vertical lines indicate standard errors.

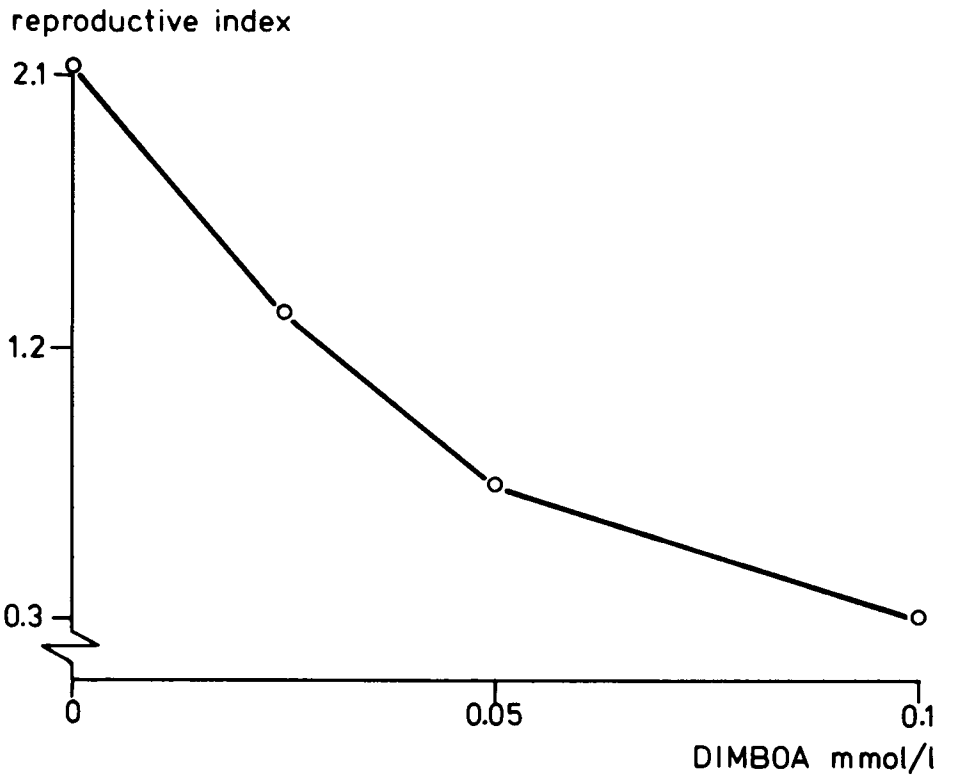

Fig. 3. Effect of DIMBOA on *Schizaphis graminum* fed with artificial diets. The reproductive index (number of nymphs/average number of adults) was measured after feeding aphid adults for 72 h.

Table 2. Effect of cysteine on the rate of decomposition of DIMBOA at substance concentration 4 mmol/l in insect diet and in pH 5.5 potassium hydrogen phthalate at 28°C.

Solution	Cysteine (mmol/l)	Half-life of DIMBOA (h)
Buffer	0.0	50.8
Buffer	2.8	34.1
Insect diet	0.0	48.2
Insect diet	2.8	31.9

Table 3. Effect of cysteine, DIMBOA and DIMBOA decomposition products on survival of *Schizaphis graminum*.

Concentration in diet (mmol/l)		Aphid survival after 24 h (%)
cysteine	DIMBOA	
0.0	0.0	100
2.8	0.0	100
0.0	4.0 (decomposed)[1]	100
2.8	4.0 (decomposed)[1]	100
0.0	4.0	20
2.8	4.0	40

1. Prior to feeding, DIMBOA (4 mmol/l) was decomposed for a period of 14 half-lives (see Table 2) in insect diets with or without cysteine.

Table 4. Rate constants (k_2) for the reactions of DIMBOA with thiols.

Thiol	k_2 (1/mol · min)[1]	$-E_0$ (V)[2]
Dithiothreitol	0.98	0.37
Mercaptoethanol	0.73	0.28
Cysteine	0.54	0.21

1. Per thiol group in the molecule. Reactions were carried out at 31°C in pH 8 phosphate buffer.
2. Reduction potential.

ported by rate data on the addition of water (Cliffe & Waley, 1961) and of thiols (Lienhard & Jencks, 1966) to aldehydes. These are substantially faster than our measured rates.

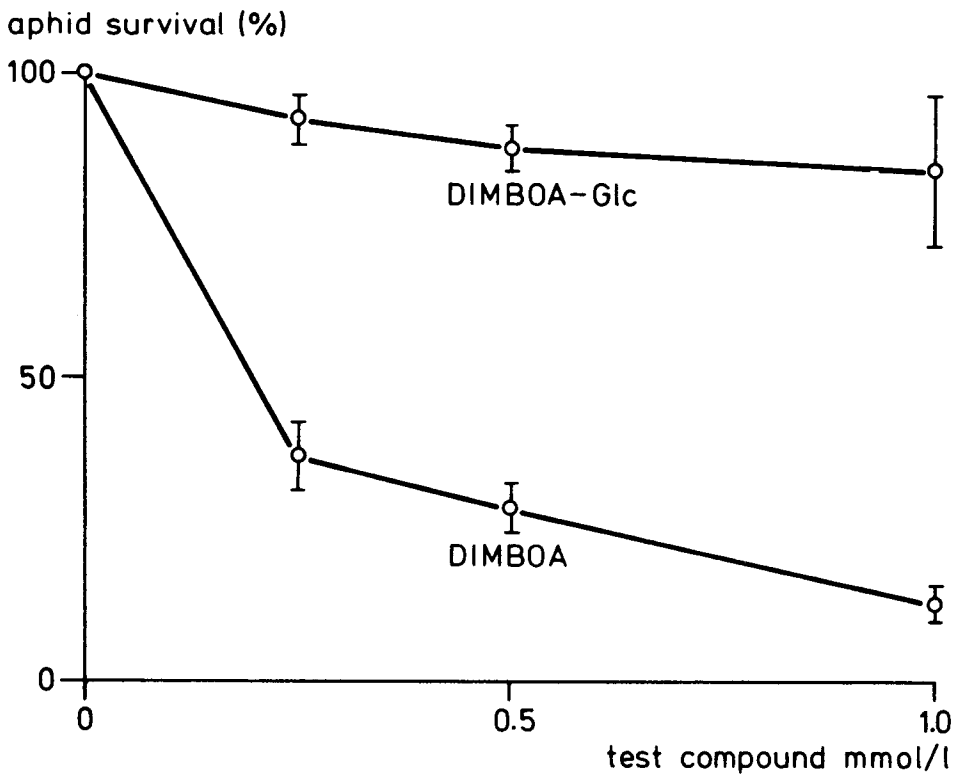

Fig. 4. Effect of DIMBOA and DIMBOA-glucoside on *Schizaphis graminum* fed with artificial diets. Survival was measured after feeding the aphids for 48 h. Each point is the mean of three samples consisting of ten aphids each. Vertical lines indicate standard errors.

Discussion

DIMBOA decreased survival and reproduction rate of *S. graminum* fed with artificial diets. The content of this compound in leaves correlated with resistance to the aphid. It is likely then that these hydroxamic acids constitute a chemical defense of wheat against these aphids. The effects in artificial diets were observed using similar or lower concentrations of hydroxamic acids than those found in plant extracts.

The mode of action of these compounds on aphids is presently unknown. Hydroxamic acids could affect aphids by decreasing reproduction, increasing mortality and/or acting as feeding deterrents.

The reactivity of DIMBOA with thiols suggests that DIMBOA may be an inhibitor of enzymes whose activity depends on the presence of cysteine residues. DIMBOA has been described as an energy transfer inhibitor in chloroplasts and mitochondria (Queirolo et al., 1981). Although it is not known how DIMBOA decreases ATP synthesis in these organelles, it is possible that its reactivity with cysteine is responsible for this action.

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