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EFFECT OF CYSTEINE ON STABILITY AND TOXICITY TO APHIDS OF A CYCLIC HYDROXAMIC ACID FROM GRAMINEAE

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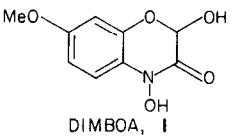
Key Word Index—*Zea mays*; Gramineae; 1, 4-benzoxazin-3-ones; *Schizaphis graminum*; aphid.

Abstract—Toxicity of DIMBOA, the major cyclic hydroxamic acid in maize extracts, to the aphid *Schizaphis graminum*, was decreased by addition of cysteine to the insect diet. The LD_{50} for DIMBOA on aphids was, after 24 hr, 2.1 and 0.9 mM in diets with and without added cysteine, respectively. DIMBOA decomposed 1.5 times faster in diets or buffer with added cysteine. Decomposition products of DIMBOA (4 mM) in insect diets with or without added cysteine were not toxic. It is suggested that the observed variations in toxicity of DIMBOA are a consequence of differences in its rate of disappearance from the diet.

INTRODUCTION

Extracts of several Gramineae contain cyclic hydroxamic acids [1], 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA, 1) being the most abundant in maize extracts [2]. This compound inhibits bacterial and fungal growth [3, 4] and insect development [5]. DIMBOA and other related compounds appear to play a role in resistance of maize to the European corn borer [5] and of wheat and maize to aphids [6–8].

The inhibitory activity of DIMBOA has been measured in complex culture media [3] or in insect diets [5–7] whose main constituents are amino acids, vitamins, sucrose and inorganic salts. In this paper we report on the effect of amino acids in the diet, and in particular of cysteine, on the stability of DIMBOA and its toxicity towards the aphid *Schizaphis graminum* (Rondani).



DIMBOA, I

RESULTS

Effect of DIMBOA and cysteine on aphid survival

Nymphs of *S. graminum* were fed DIMBOA in diets with or without cysteine. The presence of cysteine had no effect on survival of aphids fed for 24 hr (Fig. 1). However, when DIMBOA was added, survival was greater in diets with cysteine. The LD_{50} for DIMBOA was 2.1 and 0.9 mM in the presence or absence of cysteine, respectively.

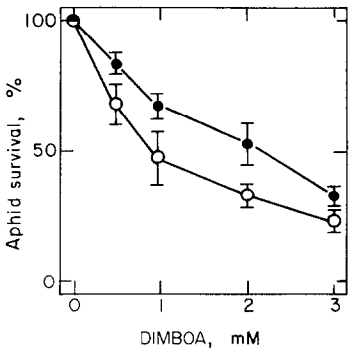


Fig. 1. Effect of DIMBOA on *Schizaphis graminum* (Rondani) feeding in diets with (●) or without (○) 2.8 mM added cysteine. Survival, expressed as per cent of initial individuals, was measured after feeding the aphids for 24 hr. Each point represents the average of three samples of 10 individuals each. Vertical bars are s.e.m.

Interaction of DIMBOA with cysteine

DIMBOA is unstable in aqueous solutions [9, 10]. Its decomposition was studied in insect diets and buffer solutions with or without added cysteine. The half-life for disappearance of DIMBOA in buffer solution is similar to that in diet without cysteine (Table 1). Addition of cysteine both to diet and buffer accelerates the reaction by a factor of 1.5. These results indicate that cysteine was the only compound in the solution causing a major effect on the decomposition of DIMBOA.

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Table 1. Effect of cysteine on rate of decomposition of DIMBOA in insect diet and in buffer*

Solution	Cysteine (mM)	DIMBOA, half-life (hr)
Insect diet	0.0	48.2
Insect diet	2.8	31.9
Buffer	0.0	50.8
Buffer	2.8	34.1

*Reactions were performed with 4.0 mM DIMBOA in insect diet (see Experimental) or in 50 mM, pH 5.5, potassium hydrogen phthalate at 28°.

Table 2. Effect of cysteine, DIMBOA, and DIMBOA decomposition products on survival of *Schizaphis graminum* (Rondani)

Concn in diet (mM)*		Aphid survival (%)†	
Cysteine	DIMBOA	24 hr	48 hr
0.0	0.0	100	100
2.8	0.0	100	100
0.0	4.0	20 ± 3	0
2.8	4.0	40 ± 6	10 ± 3
0.0	4.0 (decomposed)‡	100	100
2.8	4.0 (decomposed)‡	100	100

*Insect diet composition is described in the Experimental.

†Measured as per cent of individuals initially present. Each point is the average of three samples of 10 aphids each.

‡Prior to feeding, DIMBOA (4mM) was decomposed for 14 half-lives in insect diet solutions with or without cysteine.

Biological activity of decomposition products of DIMBOA

DIMBOA was allowed to decompose in diet solutions with or without cysteine. The toxicity on aphids of the decomposition products of DIMBOA in these solutions was measured (Table 2). While DIMBOA decreased aphid survival, its decomposition products did not affect it up to at least 48 hr of feeding. Hence, the toxicity of DIMBOA is not due to its decomposition products.

DISCUSSION

Cysteine decreased the toxicity of DIMBOA by reacting with it to give non-toxic products. The activity of DIMBOA is usually assayed in complex bacterial or fungal growth media or in insect diets, supplemented with amino acids. Since cysteine is added to these diets, it would be expected that the toxicity of DIMBOA is greater than previously reported.

DIMBOA is an energy transfer inhibitor in chloroplasts and mitochondria [11]. The ATPase activity of CF_1 is inhibited by DIMBOA. The activation of CF_1 depends, among other factors, on the presence of SH groups in the proper amounts and locations in the enzyme [12]. Cysteine, but not alanine, affects the decomposition of DIMBOA, suggesting that the presence of a sulfhydryl group is a determinant for the reaction of cysteine with DIMBOA. It will be of interest to study the nature of the reaction between DIMBOA and thiols. This knowledge may indicate the mechanism of inhibition of ATPases by DIMBOA, as well as other inhibitory properties of this compound.

EXPERIMENTAL

Aphids. Individuals of *Schizaphis graminum* (Rondani) were collected from naturally infested barley and allowed to reproduce on barley plants kept inside a nylon net under continuous light in the laboratory.

Feeding and diet composition. Aphids fed from a diet solution placed between two layers of Parafilm M [13]. The diet contained 100 mg alanine, 400 mg arginine, 50 mg aspartic acid, 200 mg glutamic acid, 100 mg serine, 150 mg threonine, 100 mg histidine, 200 mg leucine, 100 mg methionine, 10 mg *i*-inositol, 250 mg choline chloride, 200 mg KH_2PO_4, 200 mg $MgSO_4$, 30 g sucrose and 100 ml water. The pH was adjusted to 5.5 with 0.1 N HCl. Cysteine was added when indicated (35 mg). Aphid survival was 100% until at least the fourth day of feeding.

Kinetic studies. DIMBOA was isolated from extracts of *Zea mays* as described [10]. Rates of disappearance of hydroxamic acids from solutions of DIMBOA and added compounds were followed by withdrawing aliquots, adding them to $FeCl_3$ reagent (50 g $FeCl_3 \cdot 6H_2O$, 500 ml 95% ethanol and 5 ml of 12 M HCl) and measuring the absorbance of the resulting blue complex at 590 nm.

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