TOXICITY AND FEEDING DETERRENCY OF HYDROXAMIC ACIDS FROM GRAMINEAE IN SYNTHETIC DIETS AGAINST THE GREENBUG, *SCHIZAPHIS GRAMINUM*

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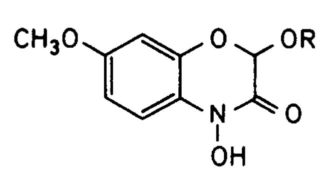
2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), the main hydroxamic acid isolated from maize extracts, increased the mortality of *Schizaphis graminum* when fed in artificial diets. Electrically-monitored feeding assays showed that DIMBOA acted as a feeding deterrent at concentrations as low as 1 mM. On 12 mM DIMBOA diets, feeding by aphids was completely inhibited. Additional feeding experiments showed that when DIMBOA was ingested there was an increase in aphid mortality relative to that of aphids which did not ingest the compound. Thus, the deleterious effects of DIMBOA on aphids are due to feeding deterrency and toxicity. The 2-ß-0 glucoside of DIMBOA (DIMBOA-Glc), the form in which DIMBOA naturally occurs in Gramineae, had a slight effect on lowering aphid survival and an appreciable feeding-deterrent effect on diet-fed aphids. The relevance of the effects of DIMBOA and DIMBOA-Glc on aphids to resistance of certain graminaceous crops against aphids is discussed.

KEY WORDS: Maize extract — Hydroxamic acid — Feeding deterrent — Greenbug — *Schizaphis graminum*.

The presence of secondary metabolites in plants may be of importance in protecting them against herbivores. For example, naturally occurring flavonoids and related compounds may be toxic and/or act as feeding-deterrents towards the greenbug, *Schizaphis graminum* (Rondani) (Dreyer & Jones, 1981; Todd *et al.*, 1971), a worldwide aphid pest of a number of graminaceous crops. Hydroxamic acids isolated from cereal extracts (Fig. 1) (Willard & Penner, 1976) have been reported to play a role in resistance of certain cereals to aphids (Argandoña *et al.*, 1980, 1981). These compounds, when offered to aphids in artificial diets at concentrations up to 1 mM, decrease the insect reproductive rate and at higher concentrations increase their mortality (Corcuera *et al.*, 1982). In this paper we report that the effects of hydroxamic acids from Gramineae on aphids may be due to both toxicity and feeding deterrency.



R	Compound
H	DIMBOA
glucosyl	DIMBOA-Glc

Fig. 1. Hydroxamic acids in maize extracts.

MATERIALS AND METHODS

Aphids. Individuals of *S. graminum* (Rondani) were collected from naturally infested barley near Santiago and allowed to reproduce on plants of *Hordeum distichum* L. cv Fola Union kept under continuous light at room temperature. For electrically-monitored assays, individuals of *S. graminum* biotype C, obtained from a colony maintained on *Sorghum bicolor* (L.) Moench cv BOK-8, in Berkeley, CA, were used.

Feeding and diet composition. Aphid feeding assays were made by using holidic diets placed between two layers of Parafilm M (Argandoña *et al.*, 1982). All feeding experiments were performed under permanent light. Usually 3 samples of 10 aphids were used. Standard errors varied between 3 and 9% and were omitted for simplicity.

Electrically-monitored assays. The procedures and specifications used for electrically monitoring aphid-probing behavior were previously outlined (Campbell *et al.*, 1982). Diets of selected DIMBOA concentrations were prepared just prior to the recording assays. The diet was injected into plastic vial-caps (300 µl) over which was stretched a Parafilm Membrane. A platinum wire, inserted through the plastic cap into the diet, served as the voltage input electrode. A gold wire affixed to the dorsum of the test aphid with silver conductive paint served as a lead to the input of the amplifier. Interpretations of the wave-forms recorded during electric monitoring of aphid-feeding behavior on the diets was based upon similar wave-forms observed by McLean and Kinsey (1964) and McLean (1970) for the pea aphid, *Acyrthosiphon pisum* (Harris), feeding on artificial diets. The analysis was based upon duration of I-waves (i.e. ingestion), S-waves (i.e. salivation) and non-probing over 2 hr assay periods.

Isolation of compounds. Compounds were isolated from 7-day old seedlings of *Zea mays* L. grown under continuous light at 28 ± 2°. DIMBOA was isolated from ethereal extracts of plants macerated at room temperature by a procedure described previously (Argandoña *et al.*, 1980). DIMBOA-Glc was isolated from aqueous extracts of boiled leaves, by passing the extracts through an SP-Sephadex-Fe column and further through a Sephadex G-10 column (Queirolo *et al.*, 1981). The UV, NMR and mass spectra were as reported previously (Gahagan & Mumma, 1967; Tipton *et al.*, 1967).

RESULTS

Effects of DIMBOA on aphids. Incorporation of DIMBOA into artificial diets resulted in significant increases in mortality of aphids at all concentrations tested in comparison with those aphids fed on control diets (Fig. 2). Mortality of nymphs reared on 6 and 8 mM DIMBOA diets increased sharply, beginning 12 hr after exposure to the diets and reaching 100% within 24 hr. Aphids which were starved (i.e. "no diet", Fig. 2) showed similar levels of mortality to those aphids reared on the 6 and 8 mM DIMBOA diets. This suggests that in these three cases, starvation was the main cause of death. To determine if DIMBOA acted as a feeding deterrent, the feeding behavior of aphid nymphs was examined in a series of tests where the aphids were given a choice between two diets placed in petri dishes fitted with two horizontal layers of Parafilm which enclosed two different diets (2 cm apart), one without

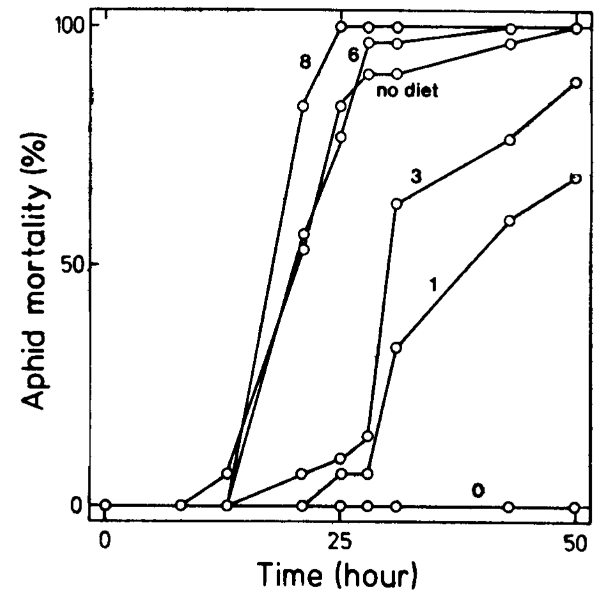


Fig. 2. Mortality of aphids feeding on diets with different concentrations of DIMBOA. Numbers on the lines represent concentrations of DIMBOA (mM) in the diets.

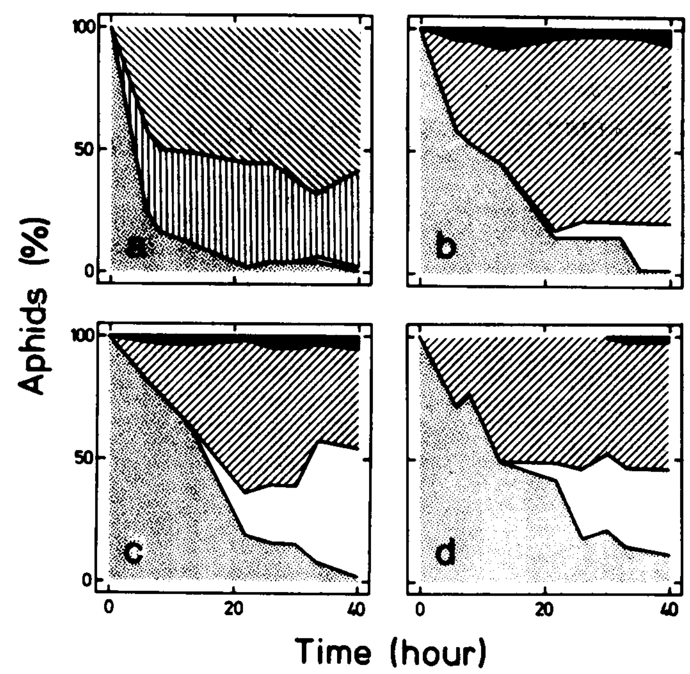


Fig. 3. Distribution and mortality of aphids exposed to the alternative of diets with and without DIMBOA. a = control diet *vs.* control diet; b = 1 mM DIMBOA *vs.* control diet; c = 3 mM DIMBOA *vs.* control diet; d = 6 mM DIMBOA *vs.* control diet. Aphid distribution and mortality was followed for 40 hr (dotted = wandering aphids; striped = aphids in control diets; black = aphids in the diet with DIMBOA; blank = dead aphids).

DIMBOA and the other with or without (control experiment). When diets were controls (*viz.*, without DIMBOA) most aphids were stationed on the diets (Fig. 3). When the concentration of DIMBOA was increased in one of the diets, the number of wandering aphids increased. Those aphids which did not wander were mainly settled on the control diets. Aphids settled on the 1 and 3 mM DIMBOA diets, but rarely settled on the diet containing 6 mM DIMBOA. From these observations it appears that DIMBOA can act as a feeding deterrent. Since aphids on diets with DIMBOA started to die earlier than aphids fed with control diets only, it is possible DIMBOA also had a toxic effect that led to early mortality.

Electrically-monitored feeding behavior. Aphids ingested from diets containing 1 mM DIMBOA (Fig. 4A) while there was virtually no ingestion observed when aphids probed the 8 mM DIMBOA diets. On diets containing 8 mM Dimboa, aphids typically exhibited short, brief periods of salivation followed by withdrawal of stylets from the diet (Fig. 4B). Furthermore, there was a negative correlation between mean duration of ingestion by aphids and DIMBOA concentrations in diets (Fig. 5).

Toxicity and feeding deterrency of DIMBOA. Cohorts of aphid nymphs were fed on diets containing 0 to 8 mM DIMBOA for 12 hours

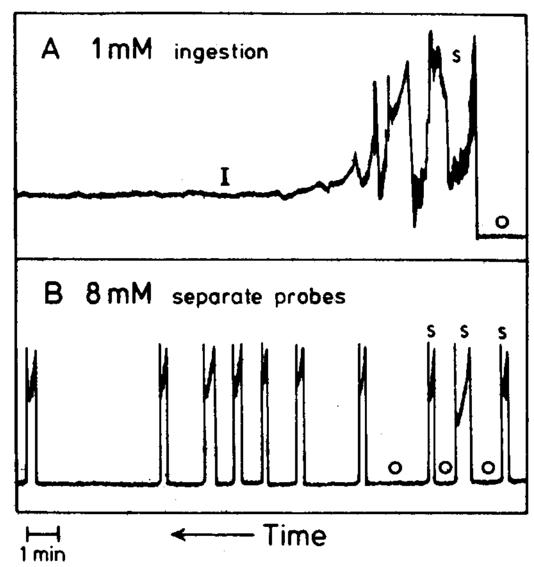


Fig. 4. Representative wave-forms in electrically recorded responses of aphids (adult *S. graminum*) feeding on artificial diets. A = 1 mM DIMBOA and B = 8 mM DIMBOA. O = baseline (*viz.* stylets withdrawn from the diet); S = salivation (*viz.* stylets inserted into diet accompanied by injection of saliva and tasting of dietary compounds); I = ingestion.

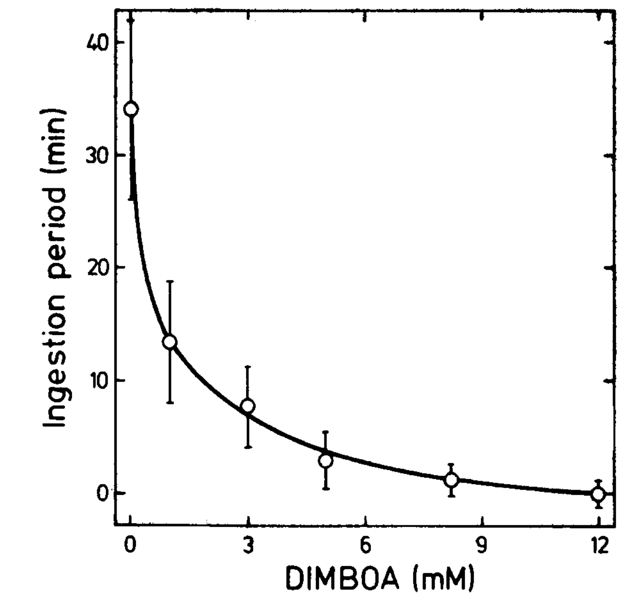


Fig. 5. Response of aphids to DIMBOA in artificial diets. Response of aphids was based upon the mean total duration of ingestion (*i.e.* I wave-form) as electrically recorded. Each mean represents the results from 10 recordings of 10 separate aphids. Lines around means are 95% confidence limits.

and then transferred to diets without DIMBOA. Aphid mortality was measured as a function of time and of DIMBOA concentration in the initial diet (Fig. 6). Aphid mortality of aphids feeding on 0 and 8 mM were the lowest. Since aphids exhibit limited or no ingestion from diets containing 8 mM or higher

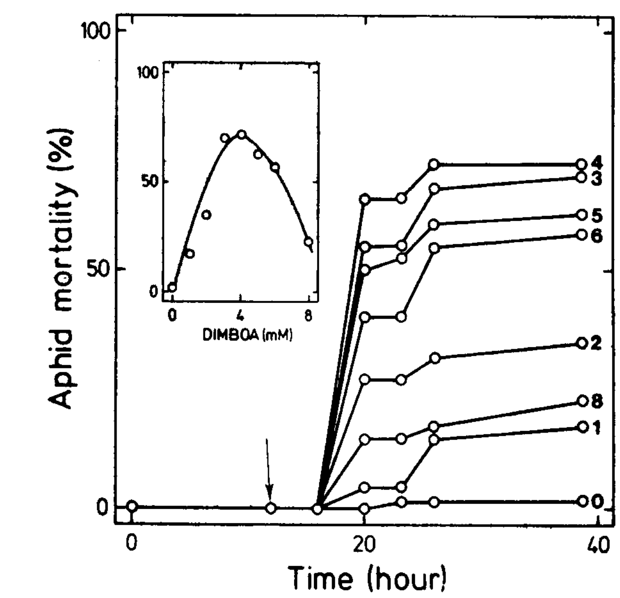


Fig. 6. Effect on aphid mortality of exposure for a limited time to different concentrations of DIMBOA. Nymphs of *S. graminum* were fed on diets containing 0 to 8 mM DIMBOA for 12 hr and then transferred (arrow) to control diets. Numbers at far right represent concentrations of DIMBOA in original diets. Inset: cross-section of curves at 39 hr.

DIMBOA, but do show ingestion from diets containing less DIMBOA (Fig. 5), the effect of DIMBOA in promoting aphid mortality in the 3 mM and 4 mM cohorts may be the result of a physiologically toxic effect after the uptake of DIMBOA by the aphids. Hence, lower mortality of aphids which were exposed to the 8 mM DIMBOA diets was probably the result of a lower toxic dose of DIMBOA due to non-feeding by the aphids.

Effect of DIMBOA glucoside. DIMBOA is present in intact tissues of certain graminaceous plants as the 2-ß-0-glucoside (DIMBOA-Glc) (Wahlroos & Virtanen, 1959). Therefore, it was of interest to compare the activities of DIMBOA with that of DIMBOA-Glc against aphids. These compounds were fed separately in diets to aphid nymphs. Survival and number of aphids stationed on the diets were measured after 21 hours of feeding. Survival of aphids fed with diets containing no hydroxamic acid, or 4 mM DIMBOA-Glc or DIMBOA was 100, 87 and 40%, respectively. Additionally, the proportion of aphids feeding was 90% in the diet containing no hydroxamic acid and 15 and 8% in diets containing 4 nM DIMBOA-Glc or DIMBOA, respectively. Thus, both compounds caused deleterious effects on aphids, DIMBOA-Glc being less active than DIMBOA.

DISCUSSION

It has been proposed that cyclic hydroxamic acids are the main factors of resistance in wheat and rye to aphid attack (Argandoña *et al.*, 1980). The hydroxamic acid content in leaves of wheat seedlings is about 6 mmoles/kg fresh weight and gradually decreases as the plant matures to about 0.1 mmoles/kg fresh weight (Argandoña *et al.*, 1981). These natural concentrations of hydroxamic acids fall within our experimental range in which DIMBOA and its glucoside acted as feeding deterrents against aphids and decrease their survival and at which DIMBOA was toxic after its uptake during limited periods of ingestion.

Toxicity of DIMBOA against several organisms is well documented. DIMBOA inhibits bacterial and fungal growth and insect development (Corcuera *et al.*, 1978; Couture *et al.*, 1971; Klun *et al.*, 1967). DIMBOA is an inhibitor of energy transduction in mitochondria and in chloroplasts reacts with coupling factor one (CF_1), thus inhibiting photophosphorylation (Queirolo *et al.*, 1981). Additionally, DIMBOA reacts with cysteine in artificial diets of aphids (Argandoña *et al.*, 1982) and reacts with other thiols *in vitro* (Niemeyer *et al.*, 1982). Hence, it is possible that the toxicity of DIMBOA to aphids may result from the interference with the acquisition of certain essential amino acids from the diet and/or inactivation of digestive enzymes. Furthermore, the activity of the prokaryotic symbiotes of aphids to provide or supplement nutritional requirements (*cf.* Houk & Griffiths, 1980) may be suppressed as a result of the antibiotic effects of DIMBOA.

Although hydroxamic acids are present in the leaves of wheat, rye and maize, their precise location in tissues of the plant are currently unknown. *S. graminum* feeds predominantly from the phloem and secondarily from mesophyll tissue of its host plants (Campbell *et al.*, 1982). Hence, it would be of interest to determine whether hydroxamic acids are present in these tissues. Moreover, DIMBOA occurs in plant tissues as the glucoside. Although DIMBOA-Glc exhibits feeding deterrency against *S. graminum*, it shows little toxicity. However, DIMBOA-Glc is rapidly converted to the aglycone by glucosidases, also present in the plant. Hence, if there is disruption of plant tissues where DIMBOA-Glc and glucosidases are compartmentized by aphid probing, there is a likelihood of the formation of the more toxic aglycone. In conclusion, we concur with the viewpoint of Long *et al.* (1977) that resistance against aphids in certain graminaceous crop plants could be achieved by breeding for cultivars having elevated levels of hydroxamic acids. However, resistance of certain sorghum and barley cultivars against greenbugs must result from other factors since extracts of seedlings and mature plants of these cultivars lack detectable levels of DIMBOA (Todd *et al.*, 1971; Argandoña *et al.*, 1982; C. A. Elliger & B. C. Campbell, unpubl.)

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RÉSUMÉ

Toxicité et répulsion provoquées par des acides hydroxamiques de Gramineae dans des régimes synthétiques sur le puceron Schizaphis graminum

La 2,4-dihydroxy-6-metoxy-1,4-benzoxazin-3-one (DIMBOA), acide hydroxamique le plus abondant dans des extraits de maïs, accroît la mortalité du puceron *Schizaphis graminum* nourri artificiellement. Des essais d'alimentation contrôlés par des moyens électriques démontrent que DIMBOA agit comme un répulsif, même à la concentration de 1 mM; dans des régimes à 12 mM, l'alimentation des pucerons est supprimée. Des expériences complémentaires indiquent une augmentation de la mortalité des individus nourris avec DIMBOA par rapport aux témoins. Par conséquent, les effets nuisibles de DIMBOA sur les pucerons sont dus à sa répulsion. Le 2-ß-0-glucoside de DIMBOA (DIMBOA-Glc) a un effet important de répulsion sur les pucerons nourris artificiellement, mais affecte peu leur survie. L'importance des effets des DIMBOA et DIMBOA-Glc sur les pucerons par rapport à la resistance des céréales contre ces insectes est discutée.

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