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HYDROXAMIC ACID CONTENT IN WILD AND CULTIVATED GRAMINEAE

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Key Word Index—*Zea mays*; *Triticum durum*; Gramineae; benzoxazin-3-ones; *Schizaphis graminum*; greenbug; aphid.

Abstract—The content of two hydroxamic acids, 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) and 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), in cultivated and wild species of Gramineae was determined. *Zea mays* and *Triticum durum* contained both DIBOA and DIMBOA, the latter being in greater concentrations. *Secale cereale* and *Arundo donax* contained only DIBOA, while *Elymus gayanus* and *Chusquea cumingii* contained only DIMBOA. *Poa annua*, *Bromus unioloides*, *Dactylis glomerata*, *Phalaris canariense*, *Lolium perenne*, *Hordeum* species, *Setaria verticilata*, *Cynodon dactylon* and a *Sorghum* hybrid lacked these hydroxamic acids. The maximum concentration of hydroxamic acid in *A. donax* was found at the end of summer, and the minimum at the beginning of winter. In annual plants, such as wheat, while neither acid was found in the fruits, their concentrations in coleoptiles and leaves increased rapidly reaching a maximum 4 days after germination and decreasing gradually afterwards. DIBOA and DIMBOA had toxic and feeding deterrent effects on the greenbug *Schizaphis graminum* at concentrations similar to those found in both cultivated and wild Gramineae.

INTRODUCTION

Cyclic hydroxamic acids [1, 2] have been isolated from *Zea mays*, *Triticum* species, *Secale cereale* and *Coix lachryma* (Jobi) [1]. It has been suggested that these molecules play a role in plant resistance against the insects *Ostrinia nubilalis* [2], *Rhopalosiphum maidis* [3, 4], *Metopolophium dirhodum* [5] and *Schizaphis graminum* [6]. Also these hydroxamic acids inhibit population growth or development of several plant pathogens such as *Erwinia carotovora* [7], *Puccinia graminis* [8] and *Helminthosporium turcicum* [9]. In addition, since these acids are found in concentrations up to 6.3 mM in Gramineae [6], and they show high complexation constants for several cations [10, 11], it has been suggested that they may participate in transport or metabolism of minerals.

We report here the presence of hydroxamic acids in cultivated and wild Gramineae and their variation with respect to age and time of year, and the effects of DIBOA and DIMBOA on the aphid *S. graminum*.

RESULTS

Quantitation of hydroxamic acids in Gramineae

Hydroxamic acids were determined in several species of Gramineae by the $FeCl_3$ and TLC–UV procedures (see Experimental). DIMBOA and/or DIBOA were detected in seven of the seventeen species analysed (Table 1). Moreover, these compounds are present in wild species in similar concentrations to those found in cultivated cereals. The concentration determined by the $FeCl_3$ procedure was always slightly higher than that determined by the TLC–UV method. This difference may be due in part to the presence of other hydroxamic acids in the extract [12].

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Variation in content of hydroxamic acids during plant development

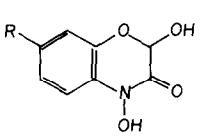
Variations in the concentration of hydroxamic acids during the year in *Arundo donax* and *Chusquea cumingii*, two perennial Gramineae, were determined by the $FeCl_3$ method (Fig. 1). In both cases the highest concentration was found at the end of the summer and the lowest during the winter months.

In the case of an annual plant such as *Triticum durum* (cv SNA-3), the highest concentration was reached in the leaves 4 days after germination (Fig. 2). Both DIMBOA and DIBOA determined by the TLC–UV method followed the same pattern as total acid determined by the $FeCl_3$ method.

In *Elymus gayanus*, a wild species, the concentration of DIMBOA (TLC–UV method) was 3.6, 2.9 and 2.6 mmol/kg fr. wt in the fourth (youngest), third and second leaves, respectively. We had previously shown that the same phenomenon occurs in cultivated plants [6]. The concentration of DIMBOA in internodes was 0.45 mmol/kg fr. wt.

Biological activity of DIMBOA and DIBOA

The activity of DIMBOA on aphids has been described [4]. We have now compared the activity of DIMBOA with that of DIBOA on *Schizaphis graminum* aphids. Both compounds had a similar effect on survival (Fig. 3) of



1 R=H , DIBOA
2 R=MeO, DIMBOA

Table 1. Hydroxamic acid content of several Gramineae*

Tribes and species†	TLC–UV method DIMBOA (mmol/kg fr. wt)	TLC–UV method DIBOA (mmol/kg fr. wt)	$FeCl_3$ method Hydroxamic acids (mmol/kg fr. wt)
Bambuseae			
Chusquea cumingii	0.40 ± 0.03	n.d.	0.45 ± 0.05
Arundineae			
Arundo donax	n.d.	0.67 ± 0.04	0.73 ± 0.08
Festuceae			
Bromus unioloides	n.d.	n.d.	n.d.
Poa annua	n.d.	n.d.	n.d.
Dactylis glomerata‡	n.d.	n.d.	n.d.
Phalarideae			
Phalaris canariense‡	n.d.	n.d.	n.d.
Hordeae			
Lolium perenne	n.d.	n.d.	n.d.
Elymus gayanus	3.60 ± 0.013	n.d.	4.02 ± 0.02
Secale cereale‡	n.d.	4.25 ± 0.01	4.76 ± 0.02
Triticum durum‡			
cv SNA-3	3.16 ± 0.06	0.10 ± 0.03	3.52 ± 0.09
cv Huenufen	0.74 ± 0.02	n.d.	0.79 ± 0.06
Hordeum chilensis	n.d.	n.d.	n.d.
H. distichum	n.d.	n.d.	n.d.
Chlorideae			
Cynodon dactylon	n.d.	n.d.	n.d.
Andropogoneae			
Sorghum sudánense × *S. vulgare*	n.d.	n.d.	n.d.
Paniceae			
Setaria verticillata	n.d.	n.d.	n.d.
Maydeae			
Zea mays‡			
cv T129s	4.83 ± 0.03	n.d.	5.27 ± 0.03
cv BxBx	2.86 ± 0.01	0.61 ± 0.06	3.89 ± 0.09

*Leaves (3 g) of each species were analysed by both the TLC–UV and $FeCl_3$ methods. Values represent the average of three samples ± the standard error of the mean. In cultivated Gramineae (‡), samples were taken of 6-day-old seedlings. In wild Gramineae, samples were taken of the youngest leaf. n.d. = Not detected.

†According to refs. [18, 19].

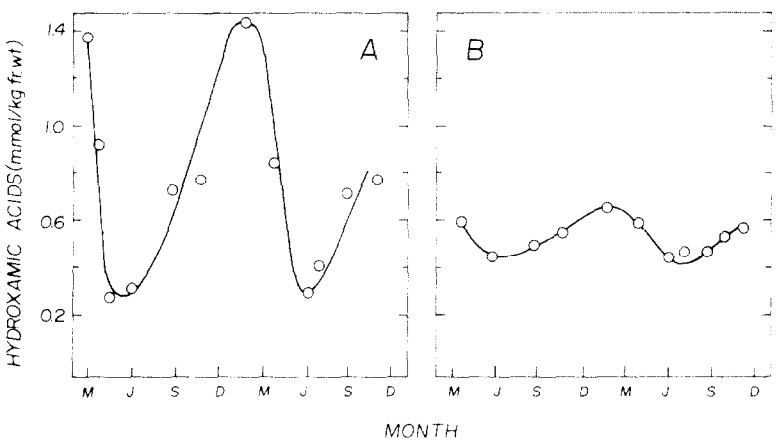


Fig. 1. Variation of hydroxamic acid content with time of year in *Arundo donax* (A) and *Chusquea cumingii* (B) leaves. Hydroxamic acids were measured by the $FeCl_3$ method. Samples were taken from March 1980 until November 1981. M,J,S,D correspond to March, June, September and December, respectively.

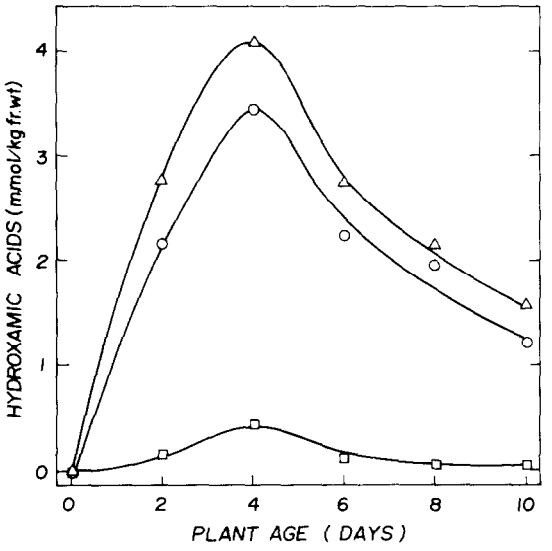


Fig. 2. Variation of hydroxamic acid content with age in seedlings of *Triticum durum* cv SNA-3. Seedlings were grown under permanent light at 28°. Symbols represent total hydroxamic acid determined by the $FeCl_3$ method (△), and DIMBOA (○) and DIBOA (□) determined by the TLC–UV method.

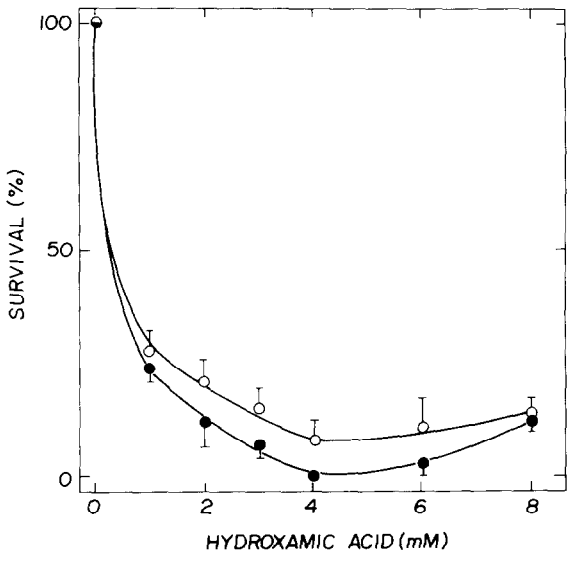


Fig. 3. Effect of DIMBOA (●) and DIBOA (○) upon survival of *Schizaphis graminum* reared on artificial diets. Survival, expressed as per cent of initial individuals, was measured after feeding the aphids for 48 hr. Each point is the average of three samples of ten aphids each. Vertical bars are standard errors of the mean. The experiments were performed at 25 ± 2°.

aphids at concentrations of hydroxamic acids normally found in plants. Both compounds also appeared to cause feeding deterrency since 63% of the aphids fed with diet alone were stationed on the diet after 12 hr, while only 22 and 15% of the aphids fed with diet containing 6 mM DIMBOA or DIBOA, respectively, were stationed on the diet.

DISCUSSION

Hydroxamic acids appear to protect several cultivated cereals against aphids [6]. Their presence in wild Gramineae in concentrations similar to those found in cultivated plants suggests that this insect resistance mechanism may also be operating in wild plants. For example, the highest concentration of these acids in *Arundo donax* is found at the end of summer when insects are abundant, and when due to the dry conditions of the area studied plants are scarce. The presence of these acids in wild Gramineae may affect their role as alternate host for aphids. It is possible that by controlling those Gramineae that lack hydroxamic acid, a reduction in the population of hibernating aphids may be obtained in areas dedicated to cereal crops.

Hydroxamic acids are present in higher concentrations in younger leaves of both wild and cultivated species. In addition, the highest concentration in annual species is reached at the fourth day after germination [6]. Thus, it is likely that they are important in the protection of younger organs which are tender and usually lack structural defences.

EXPERIMENTAL

Plant materials. Seeds of *Zea mays* were donated by Tracy Seed Co. Seeds of other cultivated plants were donated by Departamento de Sanidad Vegetal, Facultad de Ciencias Agrarias, Forestales y Medicina Veterinaria, Universidad de Chile. Leaves of wild Gramineae were collected at several places in the Santiago valley. Seeds of cultivated plants were germinated in a greenhouse at 28 ± 2° under permanent light.

Isolation of DIBOA and DIMBOA. DIMBOA and DIBOA were isolated from maize and rye, respectively, by described procedures [13, 14]. These compounds were characterized by their UV, NMR and IR spectra, by their reaction with $FeCl_3$ reagent, and by their chromatographic properties, as compared with authentic standards.

Preparation of extracts. The youngest leaves of every species were macerated in H_2O and filtered through cheesecloth at ambient temp. The extract was adjusted to pH 3 with HCl and centrifuged at 6000 *g* for 15 min. The supernatant was extracted into Et_2O (2 vols. × 3) and the organic phases were evapd to dryness. These extracts were used for quantitation.

Quantitation of hydroxamic acids. The acids were quantitated by the $FeCl_3$ method and by a combined TLC–UV method. Hydroxamic acids form with $FeCl_3$ reagent (50 g of $FeCl_3 \cdot 6H_2O$; 500 ml 95% EtOH and 5 ml 1.5 M HCl) a blue complex whose absorbance is measured at 590 nm (ε_{590} = 1315 A/mmol per ml of DIMBOA). The concn in the extracts was determined by comparing the absorbance of the extract with a standard curve made with DIMBOA. The validity of this method has been discussed [12].

For the TLC–UV method, the organic residue was dissolved in 0.2 ml EtOH, spotted on a TLC silica gel GF 254 plate (5 × 20 cm) and developed with benzene–Et_2O (1:4). Compounds with the same R_f as DIMBOA (0.22) or DIBOA (0.29) were extracted with EtOH and after determining their UV spectra were quantitated by their absorbance at 263 nm for DIMBOA and 252 nm for DIBOA. The limit of detection by this procedure was 50 nmol/g fr. wt. Since DIMBOA and DIBOA are unstable [13, 15], the amounts determined in the extracts were corrected by a recovery factor (50 and 70%, respectively) of the corresponding standards subjected to the same procedures.

Aphids. Aphid nymphs 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.

Diet composition. The diet was a pH 5.5 aq. soln of 30% sucrose, amino acids, vitamins and mineral salts placed between two layers of Parafilm M [16, 17].

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