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EFFECT OF CONTENT AND DISTRIBUTION OF HYDROXAMIC ACIDS IN WHEAT ON INFESTATION BY THE APHID *SCHIZAPHIS GRAMINUM*

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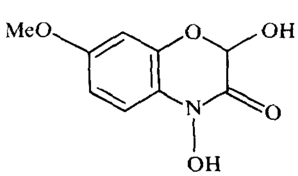
Key Word Index—*Triticum durum*; *T. aestivum*; *Hordeum distichum*; Gramineae; 1,4-benzoxazin-3-ones; resistance factor; *Schizaphis graminum*; greenbug; aphids.

Abstract—The content of hydroxamic acids in wheat plants shows substantial variations between different species and cultivars (1.0–6.3 mmol/kg fr. wt). It also varies with the age of the plant and the organ assayed. The maximum concentration is reached by the fourth day in epicotyls and roots. The amount in leaves at different plant ages is always higher in the younger leaves. Based on feeding and infestation experiments, it is proposed that the distribution of aphids on leaves of different ages is regulated by the hydroxamic acid content.

INTRODUCTION

Cyclic hydroxamic acids have been isolated from several species of Gramineae [1–7]. These molecules have attracted attention because they seem to play a role in the defense of the plant against the insects *Ostrinia nubilalis* [8], *Rhopalosiphum maidis* [9] and *Metopolophium dirhodum* [10] and against several plant pathogens such as *Puccinia graminis* [11–13] and *Helminthosporium turcicum* [14]. These compounds also appear to be responsible for the detoxification of s-triazine-derived herbicides [15, 16]. In addition, due to their high complexation constants for iron [17], their participation in mineral metabolism has been suggested.

In a previous paper [10] we showed that hydroxamic acid concentration in several species of Gramineae, both natural and incorporated, correlates with resistance to the aphid *Metopolophium dirhodum* and that 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA, **1**), the major hydroxamic acid isolated from wheat extracts, is deleterious to aphids fed on artificial diets. Based on these and other results, it was proposed that hydroxamic acids are naturally occurring protective factors of Gramineae against *M. dirhodum*. We now report on the distribution of hydroxamic acids in different organs of a wheat plant and its variation with age, and on the relationship between hydroxamic acid content in leaves and distribution of aphids among them.



1 DIMBOA

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RESULTS AND DISCUSSION

Hydroxamic acid concentration in wheat extracts

Tops (coleoptile plus leaves) of 8-day-old plants of several wheat varieties germinated in the dark at 28° were analysed for their hydroxamic acid content. All varieties tested contained hydroxamic acids, and a substantial variation was found between them. Thus, concentrations ranging from 3.2 to 6.3 mmol/kg fr. wt were found in the 4 cultivars of *Triticum durum* tested and from 1.0 to 2.5 in 15 cultivars of *T. aestivum* The *T. durum* cv SNA-3 was chosen for further studies due to its high content of hydroxamic acids.

Variation of hydroxamic acid content with plant age.

Hydroxamic acids were found to be absent from seeds of *T. durum* cv SNA-3 (detection limit = 8×10^{-4} mmol/kg fr. wt) and became detectable around the second day after germination (Fig. 1a). Thereafter the concentration in the extracts increased abruptly reaching a maximum by the fourth day (5.0 mmol/kg fr. wt in tops and 4.0 in roots) and then decreased progressively. Similar behaviour was observed with *T. aestivum* cv Huenufen, *Secale cereale* cv Emerald and *Zea mays* cv LH Rinconada. In these cases the maximum hydroxamic acid concentration in tops was also reached by the fourth day (15.2 mmol/kg fr. wt in corn, 7.4 in rye, 5.0 in wheat SNA-3 and 3.2 in wheat Huenufen). In contra-distinction, the total content of the whole plant continued to increase with age after the fourth day (Fig. 1b). It is not clear, at present, how the changes in the plant fr. wt and the rates of biosynthesis and/or degradation of the acids interact to increase and subsequently decrease the hydroxamic acid concentration.

Hydroxamic acids were present in coleoptiles, leaves and roots, the highest concentration being found in leaves and the lowest in roots (Table 1). As expected, the amount in leaves and roots decreases with the plant age. In addition, the leaves of 11, 18 and 30-day-old wheat plants

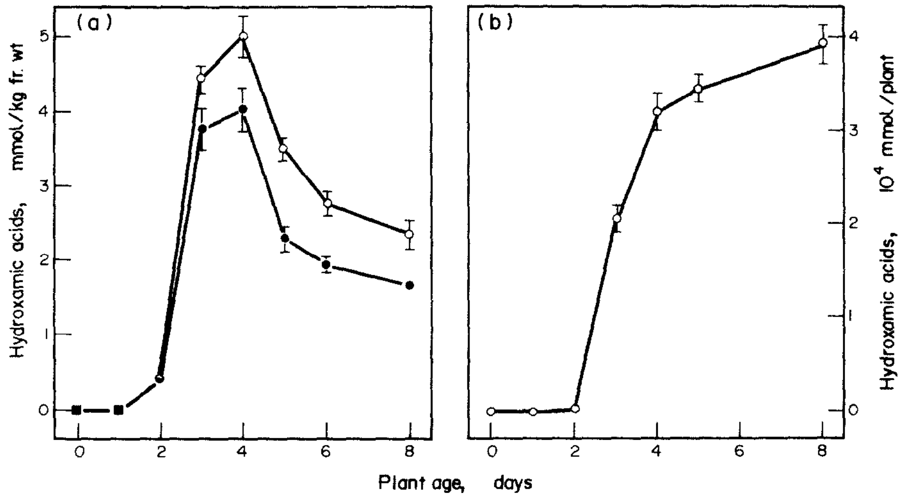

Fig. 1. Variation of hydroxamic acid content with age in seedlings of *Triticum durum* cv SNA-3. (a) Content of hydroxamic acids in (■) embryos, (○) tops (coleoptile plus leaves) and (●) roots. (b) Total content per plant. Plants were grown in vermiculite at 28 ± 3° under continuous light. Vertical bars indicate standard errors of the mean of three samples.

were analysed for hydroxamic acids. At any given plant age, older leaves have a lower hydroxamic acid content than younger ones (Table 2). Each leaf shows the highest content when it emerges.

In spite of hydroxamic acid content in Gramineae reaching values up to 5% of the plant dry wt, their physiological role has not yet been determined. In contrast, their role in plant resistance to pests and pathogens has received considerable attention. Quantitative correlations have been described between the logarithm of DIMBOA concentration in different corn cultivars and their resistance to the European corn borer, *Ostrinia nubilalis* [18–20]. In addition, an inverse linear correlation was described between the hydroxamic acid content of different Gramineae and the growth rate of the population of the aphid *Metopolophium dirhodum* living on them [10].

To test whether the distribution of aphids on the leaves of a wheat plant is influenced by their hydroxamic acid content, the following experiment was performed.

Hydroxamic acid content and distribution of Schizaphis graminum *on the leaves of wheat and barley*

Seeds of wheat and barley (*Hordeum distichum* L. cv Fola Union, a plant lacking hydroxamic acids) were independently sown in soil-filled pots kept in a greenhouse. After 63 days the plants in each pot were thinned to five individuals. A barley leaf infested with ten adults of the greenbug *Schizaphis graminum* (Rondani) was introduced and the pot was isolated by a nylon net. Six days later, the aphids present on each of the three youngest leaves were counted and the hydroxamic acid content of these leaves was determined. In barley, where hydroxamic acids are absent, the number of aphids was similar in the three leaves tested. On the other hand, the leaves of wheat plants differed both in number of aphids and in hydroxamic acid content. An inverse linear correlation which includes the leaves of wheat and of barley was found between the logarithm of the final number of aphids on each of the first three leaves and their hydroxamic acid content (Fig. 2).

In experiments where nymphs of *S. graminum* were fed an artificial diet, DIMBOA caused a decrease in survival. The deleterious effects are apparent at concentrations comparable to hydroxamic acid concentrations found in plant extracts (Fig. 3). However, it is not known whether the amount of hydroxamic acids ingested by aphids feeding on artificial diets or on plants are similar.

Since aphid populations on leaves correlate inversely with hydroxamic acid content in leaves, and these acids

Table 1. Distribution of hydroxamic acids in *Triticum durum* cv SNA-3*

Plant age (days)	Hydroxamic acids† (mmol/kg fr. wt)		
	Coleoptile	Leaves	Roots
5	4.01 ± 0.20	4.45 ± 0.33	2.73 ± 0.15
8	—	3.28 ± 0.11	1.87 ± 0.42

* Seedlings were grown in a greenhouse under continuous light at 28 ± 3° and were irrigated with distilled water.
† Values represent mean ± s.e. of three samples.

Table 2. Hydroxamic acid concentration in leaves of *Triticum durum* cv SNA-3 at different plant ages*

	Hydroxamic acids‡ (mmol/kg fr. wt)		
Leaf†	11-day-old plants	18-day-old plants	30-day-old plants
1st	2.06 ± 0.04	0.63 ± 0.02	0.13 ± 0.01
2nd	2.38 ± 0.03	0.85 ± 0.03	0.19 ± 0.01
3rd	—	1.62 ± 0.04	0.29 ± 0.02
4th	—	1.57 ± 0.17	0.74 ± 0.02
5th	—	2.09 ± 0.19	0.65 ± 0.08
6th	—	—	0.80 ± 0.01

* Plants were grown in a greenhouse under continuous light at 28 ± 3° and were irrigated with distilled water.
† Leaves are ordered from the oldest (the first to appear) to the youngest.
‡ Values represent mean ± s.e. of three samples.

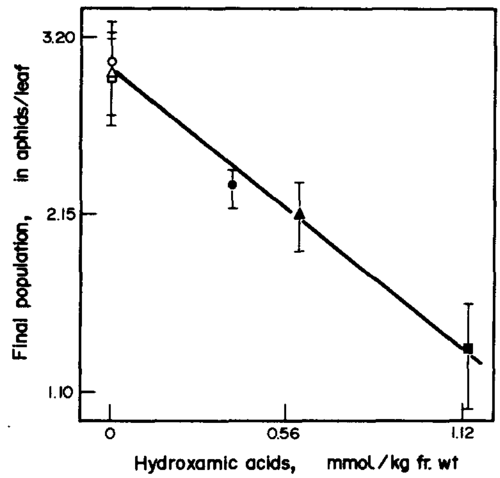

Fig. 2. Effect of hydroxamic acid content of leaves of 63-day-old plants of barley (*Hordeum distichum* cv Fola Union) and wheat (*Triticum durum* cv SNA-3) on the distribution of the aphid *Schizaphis graminum* (Rondani) among them. Empty symbols: barley; filled symbols: wheat; ○, ●: first (older) leaf; △, ▲: second leaf; □, ■ : third leaf. Plants were grown in a greenhouse under continuous light at 28 ± 3°. Ordinate values represent the mean of ten samples of five leaves each.

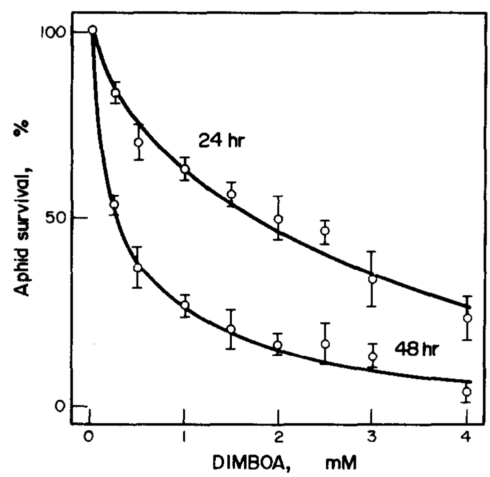

Fig. 3. Effect of DIMBOA upon *Schizaphis graminum* (Rondani) fed with artificial diets. Survival, expressed as per cent of initial individuals, was measured after feeding the aphids for 24 or 48 hr. Each point is the mean of three samples of ten aphids each. Vertical bars are standard errors of the mean. The experiments were performed at 25 ± 2° under continuous light.

produce deleterious effects on the aphids, it is proposed that the distribution of hydroxamic acids in wheat regulates the distribution of aphids among the different organs of the plant. The decrease with age of the concentration of hydroxamic acids may be, in addition to climatic conditions, a factor in the heavy aphid infestation that occurs when the plant is 2 months old.

EXPERIMENTAL

Plant materials. Seeds of barley (*Hordeum distichum* L. cv Fola Union) and of different cvs of *Triticum durum* L. and *T. aestivum* L. were obtained from Instituto de Investigaciones Agropecuarias, Sociedad Nacional de Agricultura and Departamento de Sanidad Vegetal, Facultad de Agronomía, Universidad de Chile.

Preparation of extracts. Plant tissue was macerated in H_2O, filtered through cheesecloth and left 15 min at room temp. The extract was adjusted to pH 3 with M HCl and centrifuged at 8500 *g* for 15 min. The supernatant was extracted into Et_2O (2 vol. × 3) and the organic phases were evapd to dryness. These extracts were used for quantification of hydroxamic acids.

Quantification of hydroxamic acids. Hydroxamic acids form, with ferric chloride reagent (50 g of $FeCl_3 \cdot 6H_2O$, 500 ml 95% EtOH and 5 ml 1.5 M HCl), a blue-coloured complex whose absorbance was measured at 590 nm. The ε_{590} was 1315 *A*/mmol of DIMBOA. The concn of hydroxamic acids in the tissues was determined by comparing the *A* of the extract with a standard curve made with DIMBOA. Thus, the values reported represent DIMBOA equivalents. The validity of this analytical method has been discussed [7]. Quantitative hydrolysis of the DIMBOA glycoside and related molecules present in the plant to the corresponding aglycones was assumed. DIMBOA was isolated from maize seedlings by a procedure described previously [21].

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.

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 [22].

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REFERENCES

1. Hofman, J. (1974) *Ochr. Rostlin* 156.
2. Willard, J. I. and Penner, D. (1976) *Residue Rev.* **64,** 67.
3. Koyama, T. (1955) *J. Pharm. Soc. Jpn* **75,** 702.
4. Virtanen, A. I. and Hietala, P. K. (1959) *Suom. Kemistil. Sect. B* **32,** 138.
5. Wahlroos, O. and Virtanen, A. I. (1959) *Acta Chem. Scand.* **13,** 1906.
6. Hofman, J. and Hofmanova, O. (1969) *Eur. J. Biochem.* **8,** 109.
7. Woodward, M. D., Corcuera, L. J., Helgeson, J. P., Kelman, A. and Upper, C. D. (1979) *Plant Physiol.* **63,** 14.
8. Klun, J. A., Tipton, C. L. and Brindley, T. A. (1967) *J. Econ. Entomol.* **60,** 1529.
9. Long, B. J., Dunn, G. M., Bowman, J. S. and Routley, D. G. (1977) *Crop Sci.* **17,** 55.
10. Argandoña, V. H., Luza, J. G., Niemeyer, H. M. and Corcuera, L. J. (1980) *Phytochemistry* **19,** 1665.
11. El Naghy, M. A. and Linko, P. (1962) *Physiol. Plant.* **15,** 764.
12. El Naghy, M. A. and Shaw, M. (1966) *Nature* **210,** 417.
13. Knott, D. R. and Kumar, J. (1972) *Physiol. Plant Pathol.* **2,** 393.
14. Long, B. J., Dunn, G. M. and Routley, D. G. (1978) *Crop Sci.* **18,** 573.
15. Hamilton, R. H. (1964) *J. Agric. Food Chem.* **12,** 14.
16. Tipton, C. L., Husted, R. R. and Tsao, F. H.-C. (1971) *J. Agric. Food Chem.* **19,** 484.
17. Tipton, C. L. and Buell, E. L. (1970) *Phytochemistry* **9,** 1215.
18. Klun, J. A. and Brindley, T. A. (1966) *J. Econ. Entomol.* **59,** 711.
19. Klun, J. A. and Robinson, J. F. (1969) *J. Econ. Entomol.* **62,** 214.
20. Klun, J. A., Guthrie, W. D., Hallauer, A. R. and Russell, W. A. (1970) *Crop Sci.* **10,** 87.
21. Woodward, M. D., Corcuera, L. J., Helgeson, J. P. and Upper, C. D. (1978) *Plant Physiol.* **61,** 796.
22. Auclair, J. L. (1965) *Ann. Entomol. Soc. Am.* **58,** 855.