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CHANGES IN HYDROXAMIC ACID LEVELS OF WHEAT PLANTS INDUCED BY APHID FEEDING

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Key Word Index—*Triticum aestivum*; *T. durum*; *Secale cereale*; Gramineae; *Metopolophium dirhodum*; aphid; induced plant defences; hydroxamic acids; DIMBOA; 1,4-benzoxazin-3-ones.

Abstract—Seedlings of four wheat cultivars were infested with *Metopolophium dirhodum* nymphs. After aphids had fed for 40 hr on the plants, the levels of the defense metabolite 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one had changed. The changes depended on the cultivar and the portion of the leaf examined. The increase was greater in cultivars Naofen (45.0% at the tip of the leaf, 96.2% at the base where aphids were feeding) and Quilafen (14.7 and 35.8% respectively). The increase was not significant in cultivars Huenufen and Sonka. A simple and sensitive high performance liquid chromatographic method is described for the quantitation of the above benzoxazinone and its demethoxylated analogue using small amounts of plant tissue.

INTRODUCTION

Hydroxamic acids (Hx) are constitutive secondary metabolites in several Gramineae [1], including the cereals wheat, maize and rye [2]. The main acid in wheat and maize is 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one, DIMBOA [1, 3], while in rye it is the demethoxylated analogue DIBOA [4]. These compounds have been associated with the resistance of the plant to pathogenic fungi [5–8] and bacteria [9], and to insects such as the European corn borer *Ostrinia nubilalis* [10] and cereal aphids [11–14].

The level of hydroxamic acids in a plant varies with the age of the plant [12], and, within a plant, with the age of the plant part concerned [15]. It is also affected by growth conditions such as light intensity [16], photoperiod [17], temperature [17], nitrogen fertilization [18, 19] and availability of iron in the soil [20]. We now report that Hx levels may also vary upon infestation by aphids. Hx were quantified by a new high performance liquid chromatographic (HPLC) method suitable for fast analysis using small amounts of plant tissue. Using this technique, we have studied the effects of aphid feeding on the concentration of hydroxamic acids locally, using an empty aphid cage as a control.

RESULTS AND DISCUSSION

Analysis of hydroxamic acids

Recovery of hydroxamic acids by the method described is quantitative (Fig. 1). This, coupled with the reproducibility of retention times (see Experimental), shows it to be a reliable method for quantifying individual hydroxamic acids.

Several methods have been described for the quantitation of hydroxamic acids in cereal extracts. Some of them rely on the quantitation of benzoxazolinones which arise from the decomposition of hydroxamic acids [21–24]. It

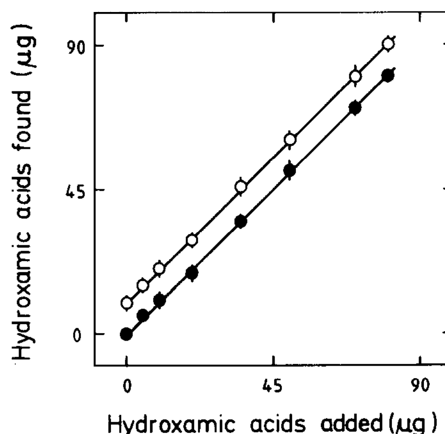


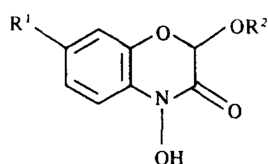
Fig. 1. Recovery of hydroxamic acids from a wheat extract. Eight-day-old seedlings of *T. aestivum* cv. Huenufen (0.55 g fr. wt) were macerated with 3 × 3 ml of water. Fifteen min later, the solution was taken to pH 3 with 0.1 N H_3PO_4. Aliquots of this solution (1 ml) were added to tubes containing different amounts of DIBOA and DIMBOA and were analysed as in the Experimental. Vertical lines represent standard deviations from the mean of four replicated experiments. O = DIMBOA; ● = DIBOA. The intersections of the lines drawn with the *y*-axis represent hydroxamic acids originally present in the wheat extract.

has been shown however, that this reaction is not quantitative, its yield depends on pH, temperature and composition of the reaction medium [25, 26]. Other methods described using gas chromatography [3], thin layer chromatography [1] or high performance liquid chromatography [27] require extensive manipulation of the sample. While this work was in progress [28], another method employing HPLC was reported [29]. While glucosides of hydroxamic acids as well as aglucones could be analysed, this method still involved considerable sample manipulation. Colorimetic methods based on the formation of blue complexes between hydroxamic acids and ferric chloride [30, 31] has been extensively used, but distinction is not possible between different hydroxamic acids in an extract. The method described herein allows a fast and reliable quantitation of different hydroxamic acids in small amounts of plant tissue.

In agreement with earlier reports [1, 32], only DIMBOA could be found in wheat plants. Additionally, two rye cultivars were analysed. As expected [33], only DIBOA was found, the levels differing markedly for the two cultivars: 6.8 mmol/kg fr. wt in Permontra and 0.5 in Merkator. It would be interesting to obtain data for the relative pest resistance of these cultivars.

Hydroxamic acid levels and aphid infestation

The results from the analysis of four different cultivars under non-infestation and infestation conditions are given in Table 1. Two cultivars, Huenufen and Sonka, did not show statistically significant changes in DIMBOA levels upon caging or aphid infestation. Cultivar Naofen, although unaffected by caging, showed substantial increases in DIMBOA levels upon aphid infestation. The increases differed between the infested area (base of the leaf, 96.2% increase) and the area above it, which remained uninfested (tip of the leaf, 45.0% increase). Cultivar Quilafen showed a behaviour similar to Naofen, the increases in DIMBOA levels being in this case smaller (35.8% at the base and 14.7% at the tip of the leaf). Additionally, all cultivars had substantially higher DIMBOA levels in the tip of the leaf than in the base, the differences between tip and base depending on the cultivar.



R^1 = H, MeO

R^2 = H, glucosyl

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

DIBOA R^1 = H, R^2 = H

Nitrogen translocation towards the site of aphid feeding has been demonstrated in the cases of *Sitobion avenae* [34] and *Rhopalosiphum padi* [35] in wheat. Furthermore, when colonies of the aphid *Aphis fabae* fed on *Vicia faba*, aminoacids including tryptophan accumulated in the phloem exudates [36]. The process resulted mainly from enhanced synthesis, although at later stages induced protein breakdown was demonstrated [36]. This effect may be related to our observation of accumulation of hydroxamic acids upon aphid feeding, since hydroxamic acids and tryptophan share a considerable portion of their biosynthetic pathway [37, 38].

Abnormal growth of plant tissue associated with aphid feeding has been related to the plant hormone indoleacetic acid [39], originating either from the plant or from the insect [40]. It is interesting to note that this phenomenon may also be associated with the capacity of aphids to divert the plant enzyme system to produce tryptophan and tryptophan-related metabolites such as hydroxamic acids and indoleacetic acid. The effect may also be related to the known capacity of hydroxamic acids to bind to auxin receptors [41]. Further work will be needed to unravel the nature of these plausible relationships.

EXPERIMENTAL

Plants. Seeds of *Triticum durum* cv. *Quilafen* and *T. aestivum* cvs Huenufen, Naofen and Sonka were obtained from INIA, Chile, and germinated in a greenhouse at $20 \pm 3°$ with a 16 light: 8

Table 1. DIMBOA levels in infested and uninfested wheat seedlings

Cultivar	Leaf portion*	DIMBOA (mmol/kg fr. wt)†		
		Control	Cage only	Cage with aphids
Huenufen	Tip	0.31 ± 0.07	0.74 ± 0.05	0.71 ± 0.06
	Base	0.43 ± 0.04	0.45 ± 0.04	0.43 ± 0.03
Sonka	Tip	0.74 ± 0.14	0.74 ± 0.05	0.68 ± 0.07
	Base	0.49 ± 0.03	0.45 ± 0.04	0.44 ± 0.05
Naofen	Tip	1.23 ± 0.08	1.31 ± 0.12	1.90 ± 0.11
	Base	0.68 ± 0.06	0.53 ± 0.05	1.04 ± 0.07
Quilafen	Tip	3.80 ± 0.10	3.88 ± 0.15	4.45 ± 0.13
	Base	3.01 ± 0.26	2.88 ± 0.11	3.91 ± 0.12

*Tip refers to the uppermost portion (2 to 2.5 cm long) of the leaf. Base refers to the following 3 cm sector of the same leaf, where the cage was clipped. The aerial parts of the seedlings were 8 to 9 cm long.

†Value reported are the mean of 5 replicates. Standard deviations are given.

dark photoperiod. 8-day-old seedlings at growth stage 10 [42] were used. They were between 8 and 9 cm tall.

Aphids. Colonies of *Metopolophium dirhodum* (Walk.) were kept on barley plants, a Hx-lacking cereal [12]. Adult females were allowed to deliver nymphs on barley plants for a period of 24 hr. After this period, adults were withdrawn and nymphs left to develop until the third or fourth instar.

Infestation experiments. Plants were subjected to three different treatments: (a) undisturbed, (b) with an empty clip cage [43] attached to it 2 to 2.5 cm below the tip of the leaf; and (c) with a clip cage in the position mentioned containing a cohort of 40 aphids.

Reference compounds. DIMBOA was isolated from extracts of *Zea mays* L. cv. T129, as described [44]. DIBOA was synthesized essentially as described [45].

Analytical method. Plant material (20–50 mg fr. wt) was macerated successively with 3×0.33 ml H_2O using mortar and pestle. The aq. extract was left at room temp. for 15 min and was then taken to pH 3 with 0.1 N H_3PO_4. The extract was then centrifuged at 6000 *g* for 10 min. and a 100 μl aliquot of the supernatant directly injected into a high performance liquid chromatograph (Merck-Hitachi L-6200). A 125-4 mm RP-18 column was used with a constant solvent flow of 1.5 ml/min and the following linear gradients between solvents A (MeOH) and B (0.5 ml H_3PO_4 in 1 l H_2O): 0 to 4.5 min—25 to 45% A; 4.5 to 5 min–45 to 25% A; 5 to 7 min—constant at 25% A. Detection was carried out at 263 nm. Retention times were 2.65 ± 0.04 min for DIBOA and 3.60 ± 0.05 min for DIMBOA.

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REFERENCES

1. Zûniga, G. E., Argandoña, V. H., Niemeyer, H. M. and Corcuera, L. J. (1983) *Phytochemistry* **22**, 2665.
2. Willard, J. I. and Penner, D. (1976) *Residue Rev.* **64**, 67.
3. Woodward, M. D., Corcuera, L. J., Helgeson, J. P., Kelman, A. and Upper, C. D. (1979) *Plant Physiol.* **63**, 14.
4. Virtanen, A. I. and Hietala, P. K. (1969) *Acta Chem. Scand.* **14**, 499.
5. ElNaghy, M. A. and Linko, P. (1962) *Physiol. Plant.* **15**, 764.
6. BeMiller, J. N. and Pappelis, A. J. (1965) *Phytopathology* **55**, 1237.
7. Long, B. J., Dunn, G. M. and Routley, D. G. (1975) *Crop Sci.* **15**, 333.
8. Toth Toldi, E. (1984) *Novenytermeles* **33**, 213.
9. Corcuera, L. J., Woodward, M. D., Helgeson, J. P., Kelman, A. and Upper, C. D. (1978) *Plant Physiol.* **61**, 791.
10. Klun, J. A., Guthrie, W. D., Hallauer, A. R. and Russell, W. A. (1970) *Crop Sci.* **10**, 87.
11. Long, B. J., Dunn, G. M., Bowman, J. S. and Routley, D. G. (1977) *Crop Sci.* **17**, 55.
12. Argandoña, V. H., Luza, J. G., Niemeyer, H. M. and Corcuera, L. J. (1980) *Phytochemistry* **19**, 1665.
13. Beck, S. D., Dunn, G. M., Routley, D. G. and Bowman, J. S. (1983) *Crop Sci.* **23**, 995.
14. Bohidar, K., Wratten, S. D. and Niemeyer, H. M. (1986) *Ann. Appl. Biol.* **109**, 193.
15. Argandoña, V. H., Niemeyer, H. M. and Corcuera, L. J. (1981) *Phytochemistry* **20**, 673.
16. Manuwoto, S. and Scriber, J. M. (1985) *Agric. Ecosyst. Environ.* **14**, 221.
17. Epstein, W. W., Rowsemitt, C. N., Berger, P. J. and Negus, N. C. (1986) *J. Chem. Ecol.* **12**, 2011.
18. Sosnavaya, O. N. and Merezhinskii, Y. G. (1979). *Soviet Agric. Sci.* **14**.
19. Tang, D. Z. and Ge, Y. H. (1981) *Plant Protection (Zhiwu Baohu)* **7**, 2.
20. Manuwoto, S. and Scriber, J. M. (1985) *J. Chem. Ecol.* **11**, 1469.
21. Scism, A. J., BeMiller, J. N. and Caskey, A. L. (1974) *Anal. Biochem.* **58**, 1.
22. Tang, C. S., Chang, S. H., Hoo, D. and Yanagihara, K. H. (1975) *Phytochemistry* **14**, 2077.
23. Robinson, J. F., Klun, J. A., Guthrie, W. D. and Brindley, T. A. (1982) *J. Kansas Entomol. Soc.* **55**, 297.
24. Malan, C., Visser, J. H. and van de Venter, H. A. (1986) *S. Afr. J. Plant Soil* **3**, 82.
25. Woodward, M. D., Corcuera, L. J., Helgeson, J. P. and Upper, C. D. (1978) *Plant Physiol.* **61**, 796.
26. Bravo, H. R. and Niemeyer, H. M. (1986) *Heterocycles* **24**, 335.
27. Gutiérrez, C., Guerrero, A., Castañera, P. and Torres, J. V. (1982) *A. Agric. Food Chem.* **30**, 1258.
28. Copaja, S. V., Bravo, H. R. and Niemeyer, H. M. (1987) *Primera Reunión de la Sociedad Latinoamericana de Fitoquimica*, Montevideo, Uruguay, November 15–20.
29. Lyons, P. C., Hipskind, J. D., Wood, K. V. and Nicholson, R. L. (1988) *J. Agric. Food Chem.* **36**, 57.
30. Hamilton, R. H. (1964) *J. Agric. Food Chem.* **12**, 14.
31. Long, B. J., Dunn, G. M. and Routley, D. G. (1974) *Crop Sci.* **14**, 601.
32. Wahlroos, O. and Virtanen, A. I. (1959) *Acta Chem. Scand.* **13**, 1906.
33. Virtanen, A. I. and Hietala, P. K. (1960) *Acta Chem. Scand.* **14**, 499.
34. Jahn, B. and Merbach, W. (1984) *Tag.-Ber. Akad. Landwirtsch.-Wiss. DDR, Berlin* **224**, 437.
35. Jahn, B., Merbach, W., Freier, B., Köllner, V. and Wetzel, Th. (1987) *J. Appl. Entomol.* **103**, 107.
36. Poehling, H. M. (1985) *Mitt. Dtsch. Ges. All. Angew. Ent.* **4**, 366.
37. Reimann, J. E. and Byerrum, R. U. (1964) *Biochemistry* **3**, 847.
38. Tipton, C. L., Wang, M.-C., Tsao, F. H.-C., Lin Tu, C.-C. and Husted, R. R. (1973) *Phytochemistry* **12**, 347.
39. Forrest, J. M. S. (1987) in *Aphids. Their Biology, Natural Enemies and Control* (Minks, A. K. and Harrewijn, P., eds), p. 341. Elsevier, Amsterdam.
40. Miles, P. W. (1987) in *Aphids. Their Biology, Natural Enemies and Control* (Minks, A. K. and Harrewijn, P., eds), p. 321. Elsevier, Amsterdam.
41. Venis, M. A. (1979) in *Plant Growth Substances* 1979, (Skoog, F., ed.), p. 61. Springer, Berlin.
42. Tottman, D. R. and Makepeace, R. J. (1979) *Ann. Appl. Biol.* **93**, 221.
43. Noble, M. D. (1958) *Can. Entomol.* **90**, 760.
44. Queirolo, C. B., Andreo, C. S., Niemeyer, H. M. and Corcuera, L. J. (1983) *Phytochemistry* **22**, 2455.
45. Jernow, J. L. and Rosen, P. (1975) *U. S. Patent* **3**: 862, 180.