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Accumulation of hydroxamic acids during wheat germination

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Abstract

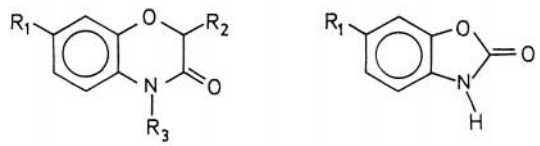
Hydroxamic acids, quantified by HPLC, varied in concentration between three cultivars of wheat. Seeds, roots, leaves and the entire plants were analysed separately. No hydroxamic acids were present in seeds throughout the 7 days of germination studied. Leaves accumulated relatively high concentrations of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and lower concentrations of its demethoxylated analogue (DIBOA). Higher concentrations of DIBOA were recorded in roots than in leaves of two of the cultivars. Maximal concentrations of DIBOA in the entire plant occurred prior to those of DIMBOA. Although the concentrations of the two hydroxamic acids decreased in all parts of the plant at the latter stages of germination, the absolute quantity of these compounds remained stable, indicating a growth dilution effect. The results show that formation of hydroxamic acids is initiated in the early stages of germination and support the idea that DIBOA is a precursor of DIMBOA. In view of the relatively high concentrations of hydroxamic acids in roots, the possibility of allelopathic control of weeds and root-feeding pests is discussed.

Keywords: *Triticum*; Hydroxamic acids; Germination; Leaves; Roots; Host-plant resistance

1. Introduction

Hydroxamic acids (Hx) are cyclic 4-hydroxy-1,4-benzoxazin-3-ones which have been isolated from wheat, maize and rye (Niemeyer, 1988). The main Hx in wheat and maize is 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) (**1**) (Zúñiga, Varanda, & Corcuera, 1988; Woodward, Corcuera, Helgeson, Kelman, & Upper, 1979), while in rye it is the demethoxylated analogue, DIBOA (**2**) (Virtanen & Hietala, 1960). It has been proposed that DIMBOA and DIBOA are present in plants as glucosides (Corcuera, Woodward, Helgeson, Kelman, & Upper, 1978). These compounds are rapidly converted to aglucones by β-glucosidases upon crushing the tissues (Hofman & Hofmanová, 1971). This family of compounds has been associated with the resistance of plants to bacteria, fungi and insects (Tipton, Klun, Husted, & Pierson, 1967; Klenke, Russell, Gunthrie, Martinson, & Pedersen, 1987).

Hx have not been detected in cereal seeds (Argandoña, Luza, Niemeyer, & Corcuera, 1980), but appear upon germination of wheat, maize and rye (Klun & Robinson, 1969; Argandoña et al., 1980). The relative levels of Hx vary between species and cultivars (Klun & Robinson, 1969; Virtanen, Hietala, & Wahlroos, 1957; Argandoña, Niemeyer, & Corcuera, 1981; Copaja, Niemeyer, & Wratten, 1991) and are higher in stem than in leaf tissue of maize (Long, Dum, & Routley, 1978). Previous studies analysing the concentrations of Hx in seedlings of cereals showed that maximal concentrations in roots and aerial parts occurred between four and six days after germination (Argandoña et al., 1981; Copaja et al., 1991; Nicol,



DIMBOA : R_1 = MeO ; R_2 = R_3 = OH
DIBOA : R_1 = H ; R_2 = R_3 = OH

MBOA : R_1 = MeO
BOA : R_1 = H

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Table 1. The maximal concentrations of DIBOA and DIMBOA in three *Triticum* cultivars during the first 7 days of germination

Cultivar	Leaf		Root		Entire plant		Total content in seedling (10^{-4} mmol/plant)	
	DIBOA	DIMBOA	DIBOA	DIMBOA	DIBOA	DIMBOA	DIBOA	DIMBOA
Quilafén	4.12 ± 0.67 (66 h)	8.12 ± 1.83 (66 h)	6.08 ± 1.14 (48 h)	2.20 ± 0.90 (48 h)	1.75 ± 0.41 (54 h)	1.47 ± 0.26 (96 h)	1.41 ± 0.32 (168 h)	2.31 ± 0.53 (168 h)
Astron	0.68 ± 0.20 (66 h)	4.33 ± 0.60 (66 h)	2.69 ± 0.65 (66 h)	3.88 ± 1.36 (96 h)	1.49 ± 0.22 (42 h)	0.94 ± 0.16 (78 h)	1.30 ± 0.25 (42 h)	1.82 ± 0.50 (168 h)
Hartog	0.51 ± 0.29 (66 h)	4.03 ± 0.56 (66 h)	0.31 ± 0.15 (66 h)	4.95 ± 1.31 (78 h)	0.42 ± 0.27 (48 h)	1.71 ± 0.44 (78 h)	0.25 ± 0.11 (54 h)	1.33 ± 0.43 (168 h)

In mmol/kg fr. wt. unless stated. Each value is the mean of six samples with 95% confidence limits. Detection limit = 1 μmol/kg fr. wt.

Copaja, Wratten, & Niemeyer, 1992). The highest concentrations occurred in the leaves of seedlings. Despite the subsequent decline in concentrations of Hx, the total amount within the whole seedling continued to increase, albeit at a slower rate. This increase continued up to 8 days after germination (Argandoña et al., 1981).

Further research was conducted using HPLC analysis to determine the concentrations of the specific Hx, DIMBOA and DIBOA in varying ages of callus cultures of radical apices and leaves of 7-day-old wheat seedlings. DIBOA was found only in leaves of *Triticum durum* cv. SNA-3, which had higher concentrations of DIMBOA than leaves of the other cultivars studied. It was suggested that DIMBOA originates from DIBOA and that the accumulation of Hx may be dependent on nutrient availability (Zúñiga, Copaja, Bravo, & Argandoña, 1990).

The aim of this study was to investigate how changes in seedling age and in subsequent rates of biosynthesis are related to the formation and degradation of specific Hx. Previous research has mainly focused on analysing Hx in either leaves or roots separately (Niemeyer, 1988). The research reported here examines levels of Hx simultaneously in seeds, roots and leaves of wheat seedlings previously investigated for maximal concentrations of Hx in leaves (Nicol et al., 1992). This analysis of Hx in different parts of the plant allows a more comprehensive insight into possible relationships between them. Furthermore, recording the total content of Hx in the plant, enables a measurement of any growth dilution effect on previously reported concentrations of Hx in wheat seedlings (Nicol et al., 1992).

2. Results and discussion

The maximal levels of DIBOA and DIMBOA in the three cultivars were recorded during the first week of germination (Table 1). No Hx were detected in samples of seeds of the three cultivars over the course of the experiment (data not recorded in Table 1).

The three cultivars, Quilafén, Astron and Hartog were selected from an earlier worldwide screen of cultivars (Nicol et al., 1992) for their relatively high, medium and low concentrations of DIMBOA in leaves, respectively. These concentrations were similar in this work except for those of DIMBOA in cv. Hartog which were much higher than previously reported (Table 1). This may be explained by the fact that these high concentrations occurred at 66 h and rapidly declined to a concentration close to earlier concentrations of DIMBOA recorded (Nicol et al., 1992). In the earlier work, seedlings were not analysed until 3 days after germination, so earlier concentrations of Hx

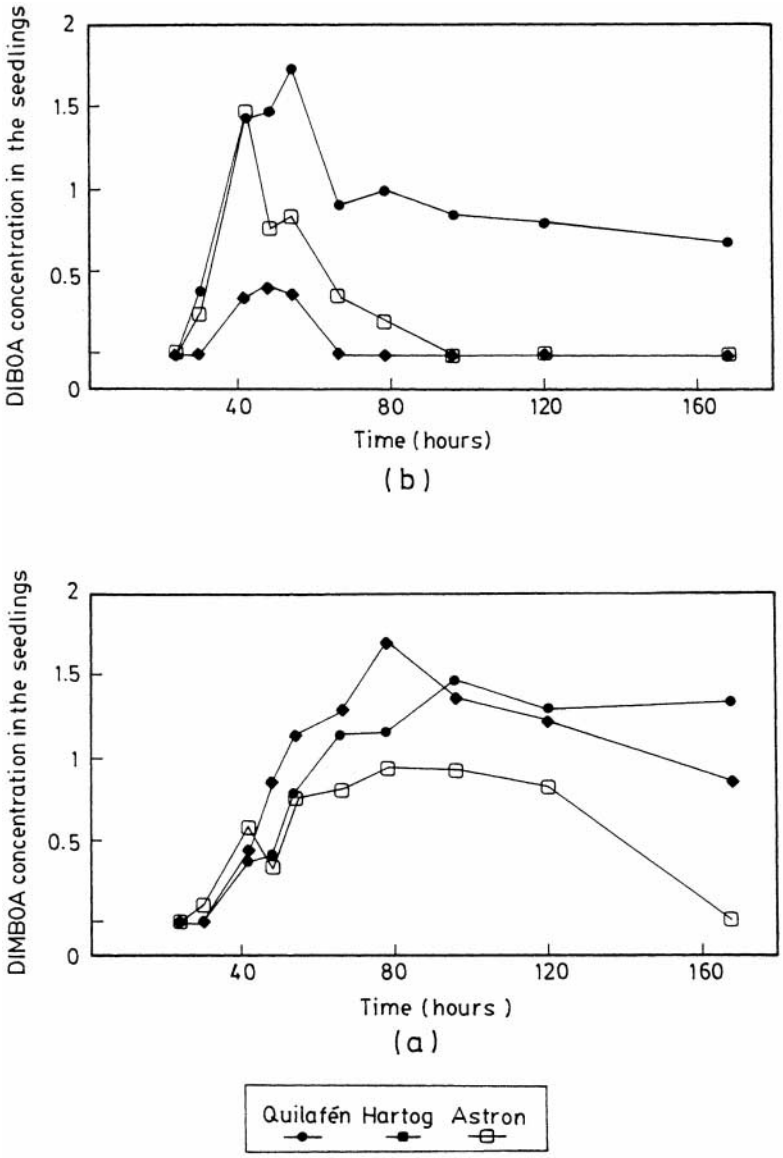

Fig. 1. Concentration of Hx (mmol/kg fr. wt.) in the seedling during 7 days of germination.

were not available. The inability to detect any Hx in the seeds of the three cultivars confirms previous findings that no Hx occur within wheat seeds (Argandoña et al., 1980). This is important as the seed is the only part of the plant that is used for human consumption. The desirable effects of high levels of Hx in the plant would not directly affect the quality of the final food product.

The relative concentrations of DIMBOA in the seedling, for the first 48 h of the experiment, were similar for all three cultivars (Fig. 1(a)). After this, maximal concentrations in the three cultivars were reached after 78 to 96 h (Table 1), followed by a decrease in DIMBOA concentration (Fig. 1(a)).

These concentrations differed significantly between cultivars. DIBOA levels in the seedling followed a similar pattern (Fig. 1(b)). However, maximal concentrations occurred earlier, between 42 and 56 h (Table 1).

Maximal concentrations of DIMBOA in leaves of the three cultivars occurred within the first 66 h of germination (Table 1) and then gradually declined. Concentrations of DIBOA in the leaves followed the same pattern (Fig. 2), but were relatively lower. Hx in roots of the three cultivars were expressed at different concentrations and different times, with all concentrations decreasing in the later stages of germination (Fig. 3). Much higher concentrations of DIBOA were recorded in roots than in leaves for two of the cultivars.

Over time the concentrations of Hx in all parts of the plant decreased in the later stages of germination (Figs. 2 and 3). However, the total content of Hx did

not decrease up to 168 h, the duration of this experiment (Fig. 4).

The maximal levels of DIBOA occurred before or at the same time as those of DIMBOA in leaf, root or entire plant tissue (Table 1). Similar results were obtained for the concentration of Hx in callus tissue of *Triticum durum* cv. SNA-3 and led to the suggestion that DIMBOA originates from DIBOA (Zúñiga et al., 1990).

In the leaves of the three cultivars, maximal concentrations of DIBOA seem to be quantitatively positively related to those of DIMBOA. The concentrations of

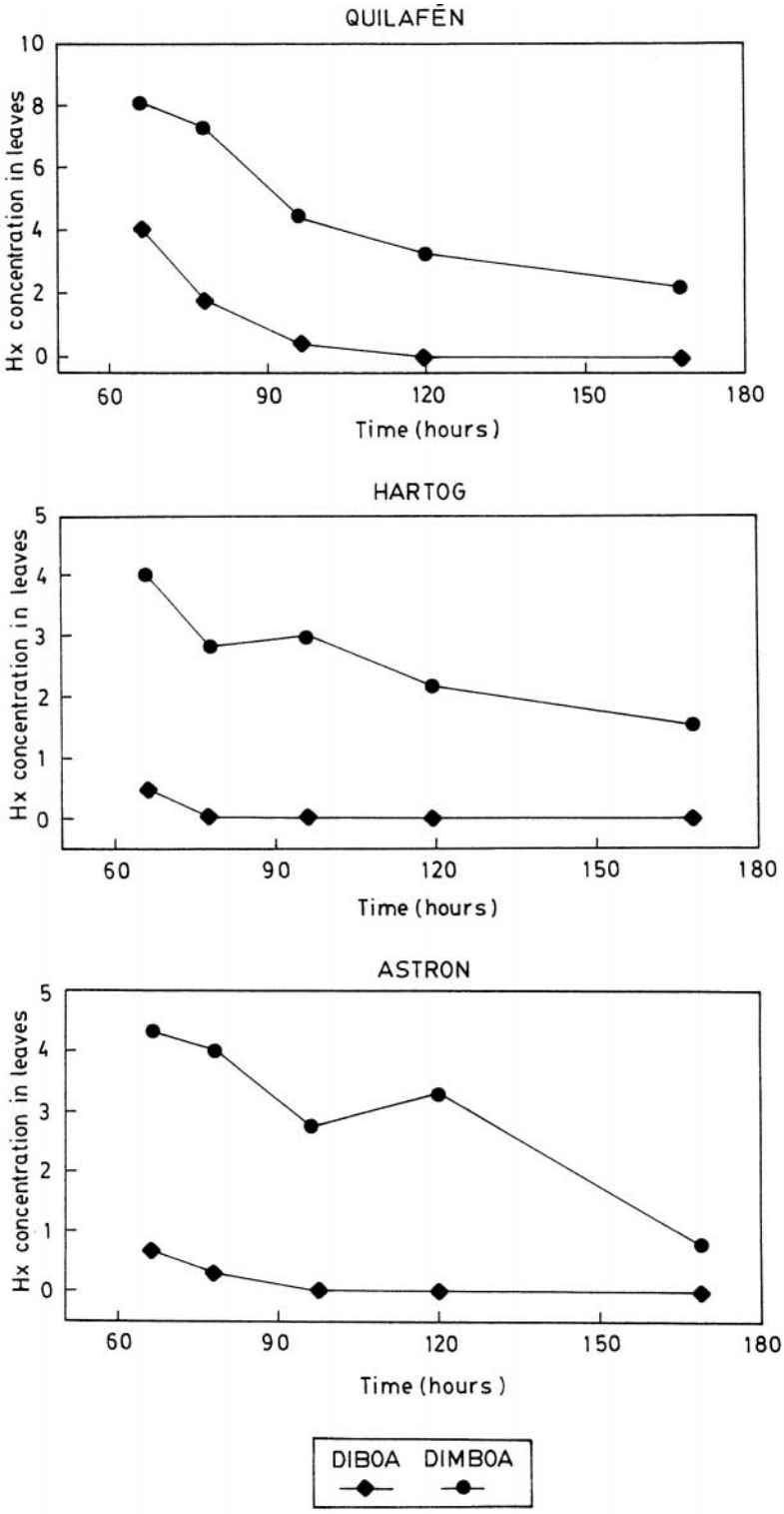

Fig. 2. Concentration of Hx (mmol/kg fr. wt.) in leaves of three cultivars during 7 days of germination.

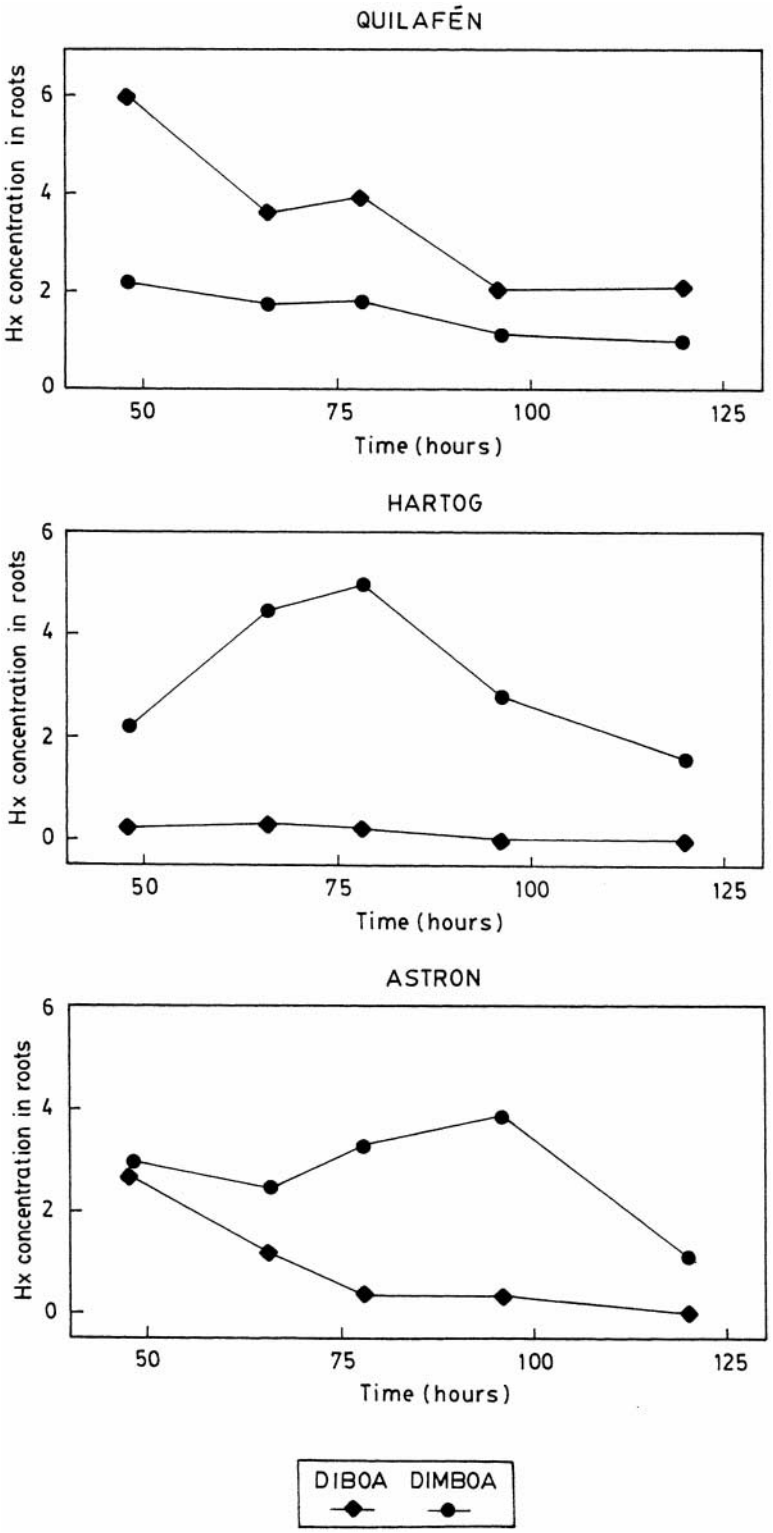

Fig. 3. Concentration of Hx (mmol/kg fr. wt.) in roots of three cultivars during 7 days of germination.

Hx in roots had no such obvious relationship. More cultivars would have to be analysed to determine the validity of these trends and of any relationship between concentrations of Hx in the leaves and those in the roots of the same cultivar. If such a relationship exists this may alleviate the need for conducting a separate screen analysing root tissue in evaluating cultivars with potentially useful levels of Hx.

The relatively high levels of Hx in the roots of all three cultivars tested could prove effective in the con-

trol of pest feeding directly upon this part of cereal crops. Such pests include the corn root aphid *Anuraphis maidiradicis* (Forb.) and nematodes like the cereal root eelworm *Heterodera avenae* (Wollenw), which infest the following cereals in decreasing order of importance, winter or spring oats, spring wheat and barley, winter wheat and rye, with maize seemingly not acting as a host (Bonnemaison, 1980). This seems to correlate positively with recorded levels of Hx in these crops. Of course, this may only be due to environmen-

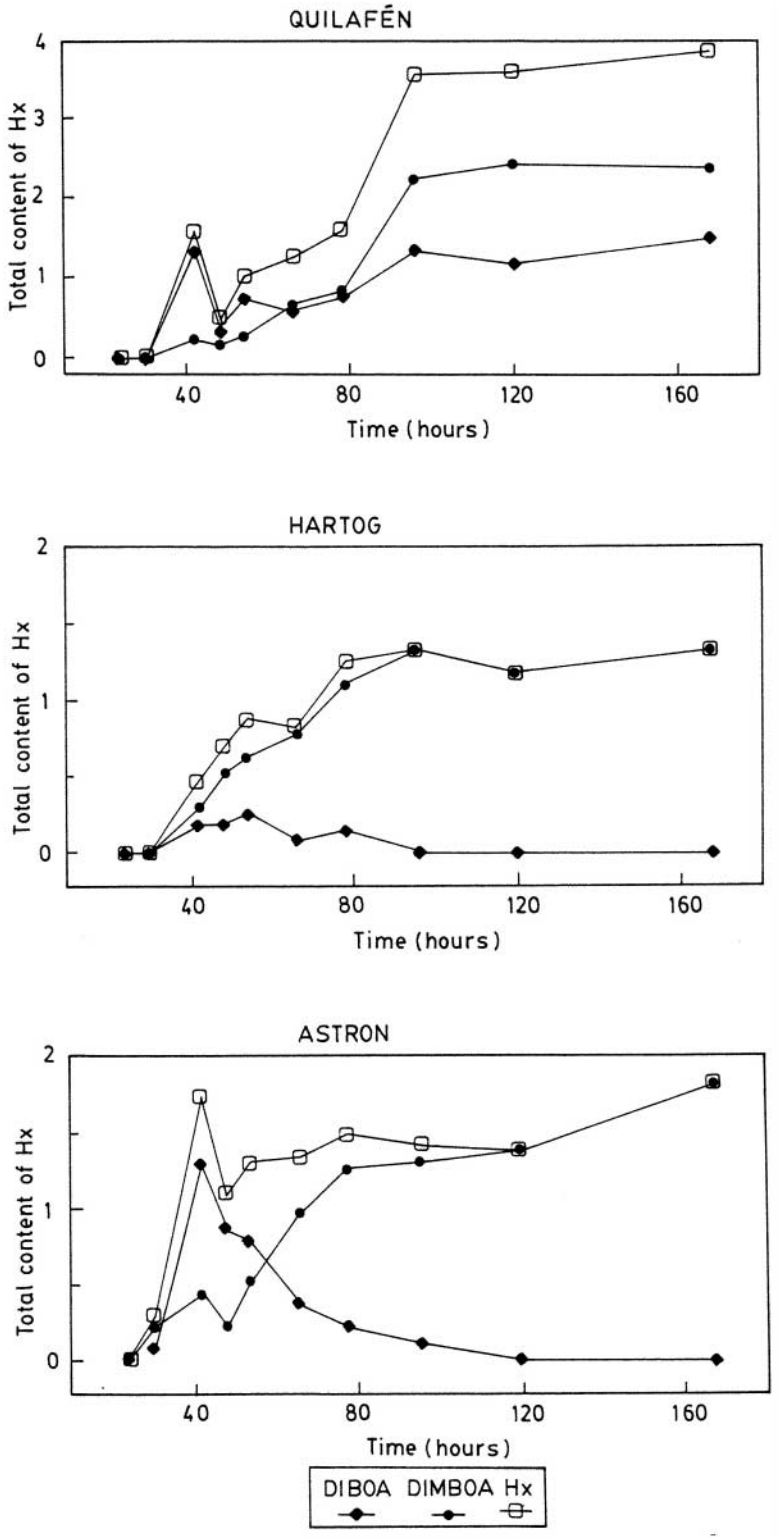

Fig. 4. The total plant content of DIMBOA, DIBOA and two Hx combined (mmol/kg fr. wt.) during 7 days of germination.

tal or other factors and an understanding of the plant and insect physiology would help assess the potential of Hx in the control of root-feeding pests. However, simple antibiotic and antixenotic tests could prove rewarding. Recently, it has been observed that Hx concentration in the root system of maize plants plays a role in resistance to the western corn root-worm larvae *Diabrotica virgifera* (*virgifera*) (Le Conte), under field conditions (Assabugui, Arnason, & Hamilton, 1995; Assabugui, Hamilton, & Arnason, 1995).

Additionally, the high concentration of Hx found in roots may prove valuable in the allelopathic control of weeds, DIBOA and BOA (**1**), its decomposition products, are involved in the well documented allelopathic effects of rye (Fuerst & Putnam, 1983; Barnes & Putnam, 1987; Barnes, Putnam, Burke, & Aasen, 1987; Perez, 1990). On the other hand DIMBOA and its decomposition product MBOA (**1**), inhibit root growth of the wild oats *Avena sativa* and *Avena fatua* (Perez, 1990), which normally infest summer wheat fields (Bell & Nalewaja, 1968; Cudvey, Jordan, Holt, & Reints, 1989). Wild oats can cause great economic damage with a significant reduction in yield (Hanf, 1968) and are particularly prevalent in North America and Australia (Sharma & Van den Born, 1978).

One of the main drawbacks of using Hx as an effective means of control of cereal pests is the rapid decline of concentrations, being low in flag leaves and very low in ears of wheat (Nicol & Wratten, 1997). Results investigating the effect of Hx on cereal pests have concentrated on young leaves of plants, in which maximal concentrations occur (Virtanen et al., 1957; Argandoña et al., 1981; Molot & Anglade, 1968; Guthrie et al., 1981; Trackray, Wratten, Edwards, & Niemeyer, 1990). Further research should be conducted to see if the total content of Hx remains stable at all growth stages and if this level is related to maximal concentrations in young seedlings. As no Hx are found in wheat seeds or seeds of other cereals (Argandoña et al., 1980; Hanf, 1968) and the total level of Hx increase during early growth, Hx production must be initiated during germination. More information on the biosynthesis of Hx may provide the opportunity of enhancing these levels through biochemical techniques. Of course, other techniques may also become available to adapt plants genetically, such as the use of molecular biology or intercrossing cultivars with high levels of Hx. If high concentrations of Hx can be readily sustained within the plant, the control of pests of cereal plants would become more feasible.

3. Experimental

3.1. Plant material

Five seeds of *Triticum aestivum* cv. Hartog were planted into each of 24 6 cm diameter plastic pots containing vermiculite and allowed to germinate in a plant growth room. The temperature was $20 \pm 2^{\circ}C$; relative humidity ranged from 55–65%; photoperiod was 12 h. At specific intervals, up to 168 h, one whole seedling, including roots, was taken from each pot. These were then sorted into six replicates of each of four plant part samples. These were leaf only, roots only, seed only or entire seedling. For each plant part sample the appropriate plant material was cut, weighted and frozen at -20° ready for subsequent Hx analysis.

3.2. Analysis of hydroxamic acids

Each plant part sample was macerated successively with 3×0.33 ml of water, using a pestle and mortar. The aq. extract was left at room temp. for 15 min, adjusted to pH 3 with 0.1 N H_3PO_4 and centrifuged at 10,000 rpm for 15 min. Aliquots of the supernatant were filtered (0.45 μm) and then analysed using a Gilson 712 HPLC with a Lichrospher 100 RP-18 (5 μm) column (125×4 mm). The gradient profile of solvent A (MeOH) and solvent B (0.5 ml H_3PO_4 in 1 l H_2O) was 0–7 min, 30% A; 7–7.5 min, 30 to 100% A; 7.5–9 min, constant at 100% A; 9–13 min, 100 to 30% A. The flow rate was 1 ml/min and detection was carried out at 263 nm. The injection volume was 50 μl.

The compounds were identified by comparison of the R_t's with previously isolated standards (DIBOA, R_t 2.7 ± 0.2 min; DIMBOA, R_t 3.5 ± 0.2 min). For these, DIMBOA was isolated from extracts of *Zea mays* (L.) cv. T129, as described before (Queirolo, Andreo, Niemeyer, & Corcuera, 1983) and DIBOA was synthesised according to a previously described method (Jernow & Rosen, 1975).

The experiment was repeated for *T. durum* cv. Quilafén and *T. aestivum* cv. Astron. The three cultivars were selected because their DIMBOA concentrations covered the full range found in a worldwide screen of wheat cultivars (Nicol et al., 1992).

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