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OCCURRENCE OF DIBOA IN WILD *HORDEUM* SPECIES AND ITS RELATION TO APHID RESISTANCE

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Key Word Index—*Hordeum*; Gramineae; aphid resistance; *Rhopalosiphum padi*, hydroxamic acids; DIBOA.

Abstract—The secondary metabolite 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) was found in seedlings of wild *Hordeum* species, but not of cultivated barley (*Hordeum vulgare*). DIBOA levels correlated negatively with performance of the aphid *Rhopalosiphum padi* on seedlings.

INTRODUCTION

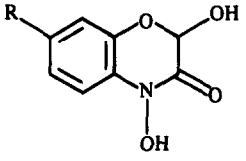
In wheat and maize, the hydroxamic acid DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) [1] plays a major role in aphid resistance. The growth of aphids and their populations on wheat and maize plants correlates negatively with DIMBOA levels in the plants [2–7], the compound is toxic and alters feeding behaviour of aphids on artificial diets and on wheat plants [2, 4, 5, 8–11]. Hydroxamic acids have not been found in cultivated barley and aphid performance has been related to the presence of the indole alkaloid gramine [12]. We describe the finding of DIBOA (7-demethoxylated analogue of DIMBOA) in seedlings of wild barley (*Hordeum*) species, demonstrate that DIBOA is toxic to the aphid *Rhopalosiphum padi* in artificial diets, and show a correlation between DIBOA levels and performance of *R. padi* on plants of wild *Hordeum* species.

RESULTS AND DISCUSSION

Quantitation of DIBOA in Hordeum *spp.*

A survey of barley cultivars (*Hordeum vulgare* L.) in search of hydroxamic acids using the $FeCl_3$ method of analysis [13] did not yield positive results [14] so, several sensitive methods were used to ascertain the presence of DIBOA in wild barley. The initial criterion was the coincidence of retention time on HPLC with that of a synthetic standard [15] and with a standard isolated from rye seedlings [16]. As DIBOA decomposes in aqueous solutions to benzoxazolin-2-one as the main product [17] and retention time of the latter compound can be easily distinguished from that of DIBOA on HPLC, aqueous extracts to be analysed were heated and aliquots withdrawn after different time intervals were analysed by HPLC. The peak attributed to DIBOA progressively disappeared and a peak with the retention time of synthetic BOA [18] appeared in parallel.

Lastly, a GLC method was developed that allowed the separation of different hydroxamic acids and their corresponding benzoxazolinones. Extracts from wild barley produced, after derivatization, a peak with the same retention time as synthetic DIBOA. Quantification of DIBOA in extracts was carried out by HPLC.



	R
DIBOA	H
DIMBOA	MeO

DIBOA levels in wild barley and aphid performance

The role of hydroxamic acids in cereals in host plant resistance to aphids has been shown to involve antibiosis [4, 6, 7] and feeding deterrency [9–11]. Studies have been carried out mainly with maize and wheat, which have DIMBOA as the main Hx [8, 13]. DIBOA has been found to be toxic against the aphid *Schizaphis graminum*, when tested in artificial diets [8]. DIBOA is also toxic to *R. padi* when incorporated into artificial diets (Fig. 1).

A screening for resistance against *R. padi* has been carried out in *Hordeum* species [19]. DIBOA levels determined in the same accessions screened for aphid resistance correlated strongly with the published resistance ratings (Fig. 2). Thus, it is likely that in *Hordeum*, as in other Gramineae, hydroxamic acids play a role in aphid resistance, although this does not preclude the role of other factors such as amino acids [20].

Hydroxamic acids have not been found in cultivated barley. In these plants, aphid resistance appears to be related to the presence of indole alkaloids, such as gramine [12]. The absence of hydroxamic acids in cultivated barley and the corresponding loss of potential for resistance to aphids and to diseases towards which DIBOA is active [21], may reflect the loss of genetic variability and resistance mechanisms in modern crops [22].

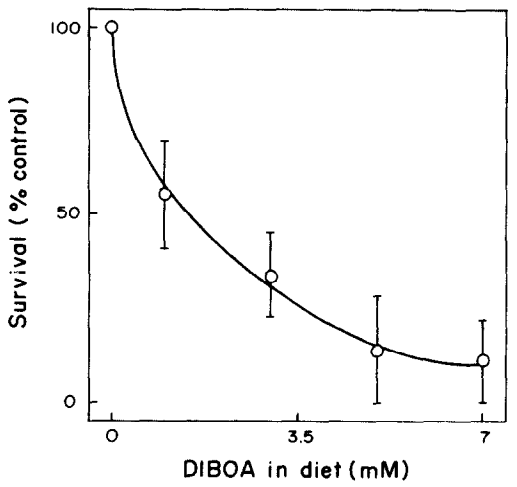


Fig. 1. Effect of DIBOA in artificial diets on survival of nymphs of *Rhopalosiphum padi*. Survival was determined after 24 hr in the minimum diet (see Experimental). For each concentration, eight samples with 10 initial aphids were followed. Mean survival of aphids fed with a diet without DIBOA was 92.5%. Vertical bars are standard errors of the mean.

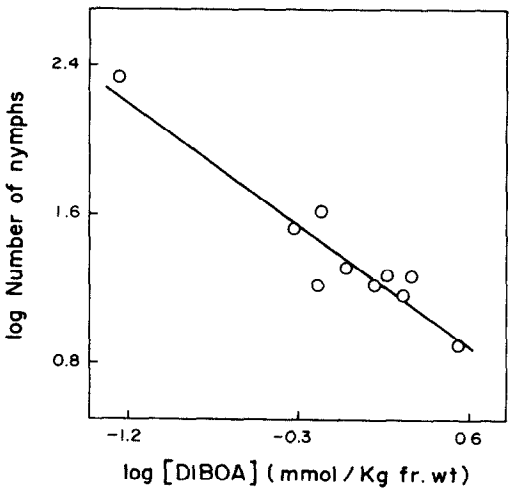


Fig. 2. Correlation of DIBOA in *Hordeum* seedlings with performance of *R. padi*. The latter variable corresponds to the number of aphids produced per new-born nymph living on a plant of a given species over a period of 16 days. Species of *Hordeum* (cultivar or accession in parentheses) used were, in order of increasing DIBOA concentrations: *vulgare* cv. Tellus, *roshevitzii* (H-179), *flexuosum* (H-1110), *lechleri* (*H*-1277), *jubatum* (H-2030), *lechleri* (H-1324), *brachyantherum* (H-1969), *comosum* (H-1333), *lechleri* (H-1205) and *brachyantherum* (H-1972). Aphid performance was taken from ref. [19]. The correlation coefficient for the line shown was 0.907.

EXPERIMENTAL

Plants. Seedlings were grown under a 12L:12D photoperiod at 22° with a 3° range, and harvested when they were 7–8 cm tall.

Aphids. A colony originating from individuals collected in wheat fields near Santiago was kept on oat plants (*Avena sativa* L.) in a culture room with 12L:12D photoperiod and 22° with a 3° range.

Reference compounds. DIBOA and BOA were synthesized as described previously [15, 18].

HPLC analysis. Aerial tissue from seedlings (20–50 mg fr. wt) was macerated successively with 3 × 0.33 ml H_2O using a mortar and pestle. The aq. extract was left at room temp. for 15 min and then taken to pH 3 with 0.1 M H_3PO_4, centrifuged at 10 000 *g* for 15 min and filtered (0.45 μm). Aliquots of the supernatant (50 μl) were examined by HPLC. The analysis was carried out with a Lichrosfer RP-18 column (100 × 4 mm) with constant solvent flow of 1.5 ml min^{-1} and the following linear gradients between solvents A (MeOH) and B (0.5 ml H_3PO_4 in 1 l, H_2O): 0–9.5 min, 30–50% A; 9.5–10 min, 50–30% A; 10–13 min, constant at 30% A. Detection was carried out at 263 nm. Retention times for DIBOA and BOA were 4.5 ± 0.3 min and 6.0 ± 0.3 min, respectively.

GC analysis. Samples were prepared in the same form as for HPLC, and were then lyophilized. Dry samples (20–50 mg) were dissolved in 50 μl of N,O-bis(trimethylsilyl)acetamide (BTSA) and the mixture heated at 52° for 15 min. A 5 μl sample was injected into a CBPS capillary column. The temp. gradient was 170° for 3 min followed by increases of 3° min^{-1} for 12 min. The injection port and detector were kept at 300°. The R_t for derivatized DIBOA was 9.33 ± 0.03 min.

Decomposition of DIBOA. Aq. extracts were taken to pH 8 and heated at 52°. Aliquots were taken every 2 hr and injected into the HPLC. DIBOA and BOA were quantified simultaneously.

Effect of DIBOA in artificial diet on R. padi. Aphid nymphs were withdrawn from the colony and placed on a sachet containing a full diet for 24 hr. They were then transferred to a minimum diet containing DIBOA at concentrations 0, 1, 3, 5 and 7 mM. Survival was determined after different time intervals. The full diet consisted of a mixt. of aminoacids, minerals and vitamins as described [23]. The minimum diet contained 20% sucrose in pH 6.5 Pi buffer.

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