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A screen of worldwide wheat cultivars for hydroxamic acid levels and aphid antixenosis

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Summary

In a screen of seedlings of a worldwide range of 47 cultivars of *Triticum* (mainly *T. aestivum*) the concentration of the hydroxamic acid DIMBOA ranged between 1 and 8 mmol/kg fresh wt. In a bioassay in which alatae of the aphid *Sitobion avenae* were released among replicated test seedlings, there were highly significant correlations between aphid 'preference' and DIMBOA levels in the seedlings. The value of these results in work leading to the production of aphid-resistant cultivars is discussed.

Key words: *Triticum*, aphids, hydroxamic acids, DIMBOA, screening, antixenosis

Introduction

Hydroxamic acids (Hx) are secondary plant chemicals present in Gramineae (Niemeyer, 1991; Copaja, Barría & Niemeyer, 1991; Barría, Copaja & Niemeyer, 1992; Copaja, Niemeyer & Wratten, 1991) showing significant deleterious effects on organisms such as fungi, bacteria and insects on cereals (Niemeyer, 1988*a*; Xie, Arnason, Philogène & Lambert, 1990). Information from experiments in which aphids are reared on artificial diets containing DIMBOA, the main Hx in wheat (Niemeyer, Pesel, Franke & Francke, 1989; Niemeyer *et al.*, 1988), and from electronic monitoring of aphid feeding (Argandoña, Corcuera, Niemeyer & Campbell, 1983; Givovich & Niemeyer, 1991), suggests that DIMBOA exerts both toxic and antifeedant effects, with complete inhibition of feeding by the aphid *Schizaphis graminum* (Rondani) at a concentration of 12 mM DIMBOA (Argandoña *et al.*, 1983). In addition, negative relationships have been recorded between various levels of aphid performance and Hx levels in plant tissues of widely different Gramineae, involving *Metopolophium dirhodum* (Walker), *S. graminum, Rhopalosiphum padi* (L.) and *Sitobion avenae* (Fabricius), (Argandoña, Luza, Niemeyer & Corcuera, 1980; Corcuera, Argandoña & Niemeyer, 1982; Bohidar, Wratten & Niemeyer, 1986; Thackray, Wratten, Edwards & Niemeyer, 1990; Wratten, Martin, Rhind & Niemeyer, 1990).

The maximum recorded levels of Hx in cultivated wheat range from 1.4 to 10.9 mmol/kg fresh weight (Niemeyer, 1988*b*; Thackray *et al.*, 1990; Copaja, Niemeyer & Wratten, 1991). Higher levels are found in some wild Triticeae (Copaja, Barría & Niemeyer, 1991; Barría *et al.*, 1992; Niemeyer, Copaja & Barría, 1991), with wild *Secale* having a maximum

concentration of nearly 40 mmol/kg fresh wt (Barría *et al.*, 1992). There is therefore germplasm potentially available for breeding programmes aimed at producing cultivars with higher Hx levels.

Due to differences between some wild Gramineae and modern wheats, in agronomical traits such as grain quality, yield, growth habit and adaptation to different environments, it may be advantageous to demonstrate usefully high levels of Hx in modern hexaploid wheat cvs. To investigate this potential, Copaja, Niemeyer & Wratten (1991) screened seedlings of 52 Chilean cvs of *Triticum* and demonstrated a range in Hx levels from 1.4 to 10.9 mmol/kg fresh weight. This was a wider range of concentrations than that demonstrated in artificial diet studies to cause marked negative effects on aphid performance. Copaja, Niemeyer & Wratten (1991) also analysed the Hx levels in the wheat cvs which had been involved in the pedigree of a modern cv. Maris Freeman. They showed that there was no overall decline in Hx levels between the generations leading to Maris Freeman, confirming that modern cvs may indeed be a useful source of Hx for plant breeding.

The aim of this paper is to extend the screen of wheat cvs beyond the Chilean collection screened by Copaja, Niemeyer & Wratten (1991) and to assess the same cvs for their suitability for aphids via an antixenosis bioassay using the pest aphid species *Sitob.* *avenae*.

Materials and Methods

Choice of plant material

A range of seed material from currently grown cvs of *Triticum aestivum* (L.) and *Triticum durum* (L.) was obtained from various international sources, representing a large geographical and climatic spectrum (Table 1).

Aphid culturing

A non-clonal culture of *S. avenae* was maintained on barley seedlings (cv. Strauss) inside a culture box based on the design of Scopes, Randall & Biggerstaff (1975). Day length was 16 h and temperature was 20°C with a 2°C range.

Quantification of DIMBOA content in wheat seedlings

The method of extraction and quantification closely followed that of Copaja, Niemeyer & Wratten (1991). The shoot of a seedling, cut at its junction with the seed, was macerated progressively with three batches of 0.33 ml distilled water using a pestle and mortar. After 15 min, 1–2 drops of 0.1 N H_3PO_4 were added to the extract to bring it to pH 3. The sample was then centrifuged at 13 000 rpm for 15 min and the supernatant filtered (0.45 μm). Aliquots of 50 or 100 μl were then injected into a Gilson 712 HPLC using a Lichrospher 100RP-18 (5 μm) column (125 mm × 4 mm). The gradient profile of solvent A (MeOH) and solvent B (0.5 ml H_3PO_4 in 1 litre H_2O) was 0–9 min: 30% A to 50% A; 9–9.5 min: 50% A to 30% A; 9.5–10 min: constant at 30% A. Flow rate was 1.5 ml/min and the detection was carried out at 263 nm. The retention time of DIMBOA at 263 nm was 3.5 ± 0.2 min. The reference compound DIMBOA was obtained from ethereal extracts of wheat seedlings, as described by Queirolo, Andreo, Niemeyer & Corcuera (1983).

Screening of cvs for DIMBOA content

Approximately 15 seeds of each cultivar were planted in each of ten 6-cm-diameter plastic pots containing vermiculite and allowed to germinate in a plant growth room.

Table 1. *Maximum DIMBOA content in wheat cultivars (±95% confidence limits)*

Cultivar	DIMBOA level (mmol/kg fresh wt)	Replicates	Age (days)	Origin (country)
Dollarbird	0.99 ± 0.25	9	4	Australia
Hartog	1.25 ± 0.28	9	4	Australia
Perquenco	1.25 ± 0.14	9	5	Chile
Betta	1.29 ± 0.38	10	4	S. Africa
Letaba	1.46 ± 0.20	10	4	S. Africa
Osprey	1.47 ± 0.32	10	5	Australia
Molopo	1.64 ± 0.52	10	4	S. Africa
Opata	1.78 ± 0.20	8	4	Mexico
Seri	1.82 ± 0.32	10	4	Mexico
Huenufén	1.92 ± 0.31	10	5	Chile
Banks	1.95 ± 0.24	10	4	Australia
Tugela	2.00 ± 0.41	10	4	S. Africa
SA2199	2.15 ± 0.71	9	5	Russia
Ommid	2.24 ± 0.67	10	4	Iran
Malifén	2.32 ± 0.33	8	4	Chile
Mogham 2	2.45 ± 0.52	10	4	Iran
Cumpas	2.46 ± 0.18	9	4	Mexico
Caldwell	2.49 ± 0.46	10	4	U.S.A.
Vulcan	2.52 ± 0.30	10	5	Australia
Tonic	2.58 ± 0.25	9	4	England
Genesis	2.66 ± 0.35	9	4	England
Naofén	2.83 ± 0.50	8	4	Chile
Mogham 1	2.90 ± 0.80	10	4	Iran
Mogham 1 rp.	2.92 ± 0.44	10	4	Iran
Talon	3.03 ± 0.40	8	4	England
Pagode	3.06 ± 0.15	8	5	Germany
Rosella	3.13 ± 0.37	8	4	Australia
Madsen	3.22 ± 0.50	9	4	U.S.A.
Apollo	3.25 ± 0.54	10	4	Germany
Bacanora	3.56 ± 0.40	8	4	Mexico
Konini	3.60 ± 0.56	9	6	New Zealand
Ocoruni	3.73 ± 0.32	8	4	Mexico
W.R. Springs	3.84 ± 0.51	10	4	Canada
HYS20W	3.88 ± 0.41	9	4	France
Sperber	3.96 ± 0.47	9	4	Germany
Balpus	3.98 ± 0.66	7	4	England
Likafén	3.99 ± 0.43	7	5	Chile
Astron	4.11 ± 0.39	10	4	Germany
Charwal	4.21 ± 0.81	8	4	Pakistan
Stephens	4.31 ± 0.58	9	5	U.S.A.
Altar	4.60 ± 0.52	8	4	Mexico
Wadanak	5.28 ± 0.45	9	5	Pakistan
Malcolm	5.68 ± 0.68	8	4	U.S.A.
Rawal	5.78 ± 0.87	9	4	Pakistan
Belikh	6.21 ± 0.72	8	5	Pakistan
Faisacabad	6.63 ± 0.38	9	4	Pakistan
Quilafén	8.07 ± 0.45	7	5	Chile

The temperature was 20°C with a range of 2°C; relative humidity ranged from 45–65%; photoperiod was 12 h and light intensity was 400 μE m^{-2} s^{-1}. Four, five, six and seven days after planting, one healthy seedling of representative size from each pot was cut at the junction with the seed, its length was measured and the sample was weighed and frozen at −20°C ready for subsequent Hx analysis.

Aphid antixenosis experiments

Ten wheat cvs previously analysed for DIMBOA were selected to include DIMBOA concentrations representing the full range found during screening. Within this range, cvs were selected which were at the same height at four days; also as wide a range of countries of origin as possible was included.

For each selected cv. four seeds were planted in each of 27 plastic spittle pots of 4 cm diameter and 4 cm depth containing vermiculite. The pots were placed in 27 randomised blocks, each block containing a full replicate of the ten cvs, in the growth room described above. On the morning of the fourth day after planting, seedlings were thinned to one seedling per pot, providing plants of similar size in each of ten pots per cv. These pots were re-arranged in a randomised block design of 10 distinct blocks, each block containing a full replicate of the 10 cvs, within a metal arena measuring 0.5 m × 0.5 m × 0.15 m. The arena was then filled to just above pot level with vermiculite; PTFE suspension had previously been painted on the inside edge of the arena to prevent the escape of crawling aphids. Four hundred alate *S. avenae* collected from a culture 24 h previously and stored without food in polystyrene specimen tubes were then dispersed from the tubes evenly over 25 equal-spaced sites in areas between the plants. Three counts were made at two, four and six hours after infestation, respectively, of the number of alatae settled on each seedling. Immediately after the last count, aphids were removed gently with a fine brush and the seedlings cut, measured, weighed and frozen ready for Hx analysis, as described above.

Results

The DIMBOA concentrations of the cvs studied ranged from 0.99 to 8.07 mmol/kg fresh wt (Table 1). The majority of seedlings had maximal levels of DIMBOA at four days after planting. In every cv. the amount of DIMBOA decreased rapidly two days after the maximal level and it was assumed that whole plant concentrations would not rise to this level again (Argandoña, Niemeyer & Corcuera, 1981).

The relationship between the mean number of alate aphids per cv. and mean DIMBOA concentration per cv. is shown in Fig. 1. There was a significant negative relationship between the mean number of alate *S. avenae* present on each cv. after each of the assessment periods 2, 4 and 6 h and the mean DIMBOA level per cv. However, the relationship did not differ significantly (intercept or slope) so that data for the three periods were pooled The relationship for the pooled data was: $\log y = 0.84 - 0.22x$, $r = -0.81$, $P < 0.001$. There was no significant relationship between the mean number of alate *S. avenae* present on each cv. and the mean seedling height per cv. (pooled assessment periods: $\log y = -0.46 + 0.22x$, $r = 0.31$, $P > 0.05$). The ranking of the DIMBOA content of the 10 cvs selected for the bioassay, analysed under non-bioassay and bioassay conditions (Table 2) was strongly positively correlated (Spearman's rank correlation; $r = 0.77$, $P < 0.02$).

Discussion

Table 1 shows the wide range of DIMBOA concentrations found in the cvs screened. The range is very similar to that demonstrated by Copaja, Niemeyer & Wratten (1991) for a screen of cvs from within one country (Chile), implying that the DIMBOA levels in currently-grown wheat seedlings may not extend far beyond those in the earlier Chilean screen.

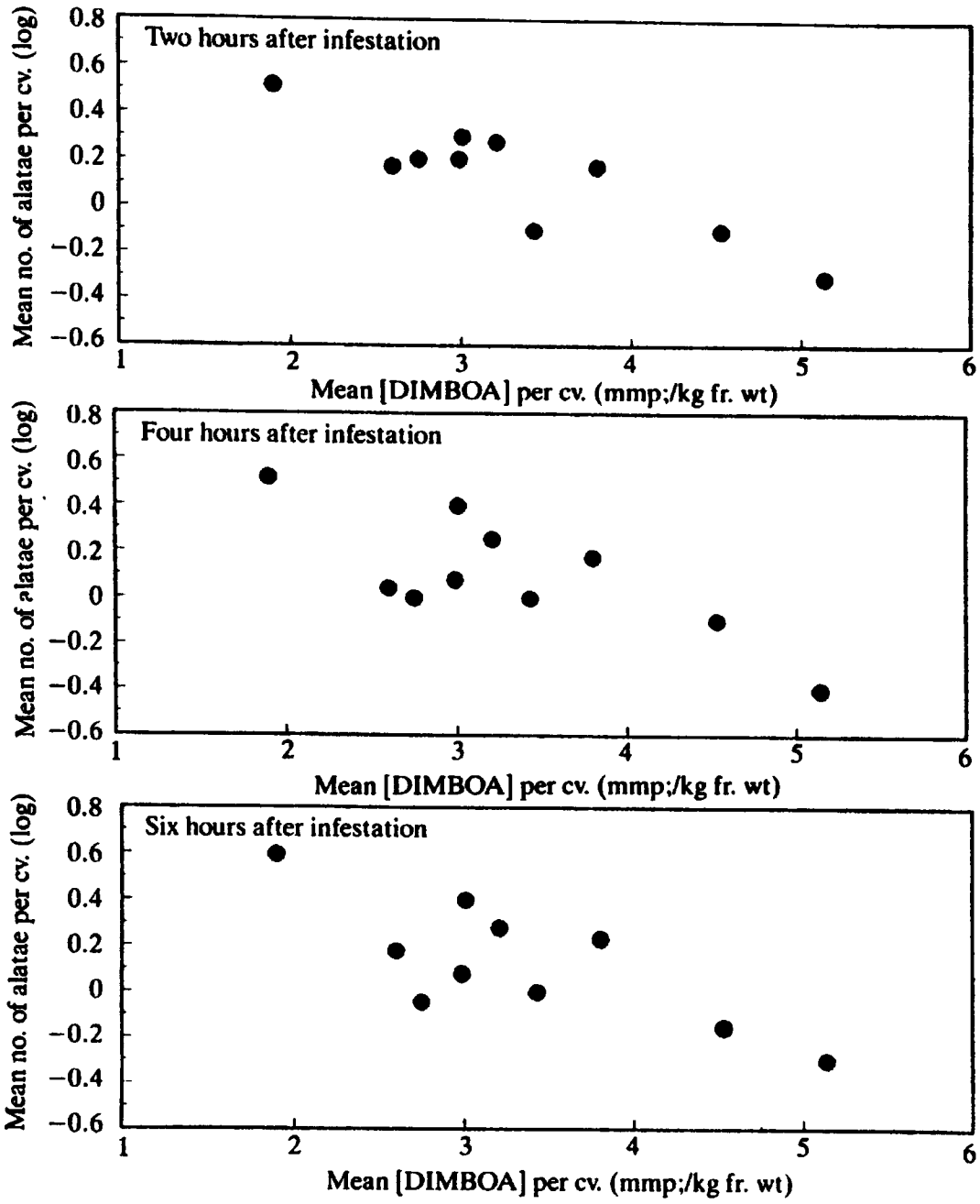

Fig. 1. The relationship between the mean number of alate *Sitobion avenae* settling per cultivar and the mean DIMBOA concentration at 2, 4 and 6 h after initial infestation.

Table 2. *Comparison of the mean DIMBOA concentrations of selected cultivars in non-bioassay and bioassay material and the mean number of* S.avenae *counted per seedling over the duration of the bioassay (± 95% confidence limits)*

Cultivar	Mean [DIMBOA] mmol/kg fresh wt non-bioassay	bioassay	Mean alates per seedling
Dollarbird	0.99 ± 0.25	1.90 ± 0.52	3.50 ± 0.66
Betta	1.29 ± 0.38	2.99 ± 0.96	1.33 ± 0.42
Opata	1.78 ± 0.20	2.60 ± 0.63	1.87 ± 0.73
Mogham 2	2.45 ± 0.52	3.21 ± 0.66	1.37 ± 0.43
Genesis	2.66 ± 0.35	3.80 ± 0.83	1.57 ± 0.65
Mogham 1	2.90 ± 0.80	3.01 ± 0.47	2.33 ± 0.58
W.R.S.	3.84 ± 0.51	2.75 ± 0.98	1.17 ± 0.37
Astron	4.11 ± 0.39	3.43 ± 0.91	0.93 ± 0.34
Altar	4.60 ± 0.52	5.14 ± 0.87	0.47 ± 0.29
Malcolm	5.68 ± 0.68	4.53 ± 1.07	0.77 ± 0.44

The fact that the range of DIMBOA concentrations of the 10 selected cvs under non-bioassay and bioassay conditions was strongly positively correlated, suggests that despite some individual variation, the relative difference in DIMBOA content between the cvs remains similar under both sets of conditions. Any individual variation recorded may be due to the effects of induction or the different conditions experienced by the wheat seedling, such as the lower density of planting under bioassay conditions. Most published work on Hx levels and aphid resistance has concerned antibiosis, in which aphids have usually been confined to a plant of a particular cultivar (Argandoña *et al.*, 1980; Bohidar *et al.*, 1986; Thackray *et al.*, 1990), or dual choice tests between two cvs. This work is the most complete antixenosis bioassay to date concerning this aphid/biochemical interaction.

The present seedling antixenosis bioassays confirm the potential of Hx as aphid resistance factors in modern cultivars. In the case of *R. padi*, which is largely a seedling pest of small-grain cereals in W. Europe (Vickerman & Wratten, 1979) and Chile, (Prado 1991 and personal communication), this conclusion is supported by the results of Givovich & Niemeyer (1991), who showed that probing behaviour and consequently virus transmission by this aphid were influenced by DIMBOA levels in wheat cultivars at the seedling stage. For *S. avenae*, Hx levels in seedlings are less relevant in W. Europe and elsewhere as the species is mainly a mature-plant pest, although it is implicated in virus transmission to wheat seedlings in Northern England (McGrath & Bale, 1989). Potentially useful levels of Hx have been identified in wheat flag levels by Leszynski, Wright & Bakowski (1990) and by Thackray *et al.* (1990), but the analyses used were based on a colorimetric method involving a complex formation with ferric chloride. These results need to be confirmed using HPLC since, although data obtained with the ferric chloride method correlates well with HPLC data for seedlings (Thackray *et al.*, 1990), they may be distorted by other chemicals present in the mature plant.

Although there are apparent patterns in the DIMBOA levels among the 47 cultivars screened in this work, the range of cultivars used is a very small proportion of those available worldwide. Although cultivars from Pakistan and Germany, for example, occupy the higher ranges of DIMBOA concentrations, the inclusion of more cultivars from these sources may confound the pattern; hence, no conclusions concerning any genetic pattern should be drawn at this stage. It is of interest, however, that the range of DIMBOA levels demonstrated here does not exceed that shown for a single-country screen (Copaja, Niemeyer & Wratten, 1991). The five Chilean cultivars re-screened here, under different plant growth conditions from those of Copaja, Niemeyer & Wratten (1991), mainly gave slightly lower levels. If the data from the present screen are corrected approximately to take this into account, the DIMBOA level in no cultivar exceeds the maximum level found in the earlier screen Chilean cultivars.

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