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Resistance to the aphids *Sitobion avenae* and *Rhopalosiphum padi* in Gramineae in relation to hydroxamic acid levels

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Summary

Antibiotic resistance to the aphid *Sitobion avenae* was assessed in relation to levels of hydroxamic acids (Hx) in a wide genetic range of cultivars and species of *Triticum*. Within hexaploid and tetraploid *Triticum* material, total plant concentrations of Hx explained a significant proportion of the variation in intrinsic rate of increase (r_m) of *S. avenae*. Significant correlations were also found between resistance to *Rhopalosiphum padi* and Hx levels. Although the concentrations of Hx in whole plants declined during seedling growth, concentrations of Hx in newly-emerging leaves remained high in plants of all ages, including in the emerging flag leaves of mature plants. When the mean relative growth rate of *S. avenae* over three days was used instead of r_m and the control of environmental conditions was improved, a higher proportion of the variation in aphid performance was explained by Hx concentrations in six cultivars.

Key words: Resistance, Gramineae, aphids, hydroxamic acids

Introduction

Partial host-plant resistance can make a substantial contribution to reducing the damaging effects of cereal aphids and other pests and therefore to reducing insecticide use. However, aphid resistance has not been deliberately bred into any UK wheat variety (Vickerman & Wratten, 1979). The possibility of exploiting inherited, low-level resistance in wheat has prompted the screening of many British cultivars and breeding lines of hexaploid wheat, *Triticum aestivum* L., but resistance levels have not varied markedly (Lowe, 1981, 1982). An exception has been the demonstration of the role of awns in wheat in conferring aphid resistance (Acreman, 1984), although this has also not been exploited by plant breeders. Recently interest has moved towards wheat species other than *T. aestivum*, notably the diploid Einkorn wheat, *Triticum monococcum* L., an ancestor of *T. aestivum*, which shows resistance to the aphids *Metopolophium dirhodum* Wlk. and *Sitobion avenae* F. (Spiller & Llewellyn, 1986; Sotherton & Lee, 1988) and the tetraploid *Triticum durum* Desf., cultivars of which show resistance

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to the aphid *S. avenae* (Bohidar, Wratten & Niemeyer, 1986). The progress of plant breeders in the search for resistant genes is however still restrained by the absence of a reliable, rapid and convenient assay for resistance, and by the lack of information on the mechanisms of resistance when it is found and on its genetic basis.

Hydroxamic acids (Hx) occurring in cereal extracts, in particular the compound 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), have been shown to be involved in the resistance of cereals to bacteria (Corcuera *et al.*, 1978), fungi (ElNaghy & Linko, 1962) and several insects including the aphid species *M. dirhodum, Schizaphis graminum* Rond. and *Rhopalosiphum maidis* Fitch (Corcuera, Argandoña & Niemeyer, 1982). *S. avenae* has been investigated in this context only in preliminary work by Bohidar *et al.* (1986) who found that 96% of the variance in the resistance of seedlings of six cultivars was explained by concentrations of Hx.

Hx exist in the plant as glucosides and are enzymically hydrolysed to release the active aglucone upon injury (Hofman & Hofmanova, 1969). Antifeedant properties of Hx towards aphids have been shown in diets (Argandoña, Corcuera, Niemeyer & Campbell, 1983) and in plants (Niemeyer, Pesel, Franke & Francke, 1989). Antibiosis of Hx has also been shown in diets (Corcuera, Argandoña & Niemeyer, 1982). Whilst Hx are found only at low levels in whole plant analyses of most modern hexaploid wheats, higher concentrations have been associated with lines of some tetraploid wheats such as *T. durum* cultivars SNA3 and SNA2 (Bohidar *et al.*, 1986). Niemeyer (1988) examined Hx levels in other tetraploid *Triticum* species.

The objectives of the present investigation were 1) to assess antibiotic resistance to the cereal aphids *S. avenae* and *Rhopalosiphum padi* L. in relation to levels of Hx in a wide genetic range of cultivars and species of the genus *Triticum* and 2) to attempt to explain the residual variation by analysis of the temporal and spatial distribution of Hx within the plant. Young plants were used for most of the work although some analyses were carried out on plants at a range of growth stages including plants at the grain development stage. There were two reasons why mainly seedlings were used. Firstly, the concentration of Hx for the total plant is highest at seedling emergence and declines with age in most wheats (so that quantification of lower concentrations becomes increasingly less accurate). Secondly, whilst *S. avenae* can be particularly damaging to mature plants during grain-ripening it and *R. padi* can be important pests of young seedlings in the autumn, as vectors of BYDV (barley yellow dwarf virus).

Materials and Methods

Choice of plant material

The seed material used in this study was chosen using criteria based on the phylogeny of *Triticum aestivum* (Riley, 1965). A total of 20 lines were investigated including representatives of both hexaploid and tetraploid *Triticum* species (Fig. 1). There were seven Chilean cultivars: Naofen, Likay, Sonka, Huenufen, SNA3, SNA2, Quilafen. *Triticum durum* cv. SNA3, *T. aestivum* cvs Avalon and Jerico were used to examine temporal variation of concentrations of Hx. These lines represented high, medium and low concentrations of Hx respectively. *T. durum* cv. SNA3, and *T. aestivum* cv. Likay were used to study the spatial variation in concentrations of Hx in young seedlings. These lines were chosen for their high and low concentrations of Hx respectively. The examination of Hx levels in four different growth stages of mature wheat utilised *T. aestivum* cv. Mission, which was grown on a field site at Chilworth, Hampshire. For investigating the relationship between concentrations of Hx and the mean relative growth rate of *Sitobion avenae* on a small group of taxa grown under carefully controlled conditions, six lines were selected: *T. aestivum* cvs Avalon, Armada, Apostle, Naofen, Likay and *T. durum* cv. SNA3. They were again chosen to represent a range of hydroxamic acid concentrations and were also readily available in sufficient quantities of seed.

Assessment of the relationship between the intrinsic rate of increase of aphids and concentrations of hydroxamic acids

The methods used for producing test insects and plants for assessment of Hx concentration and aphid performance were similar to those used by Bohidar *et al.* (1986), with the exception that fecundity was recorded over five days only (when nymph production was highest), rather than over 10 days; the shorter period was found to be sufficient to give consistent and reliable differences in the values for the intrinsic rate of natural increase (r_m; Birch, 1948). The r_m values for *Sitobion avenae* on each cultivar were calculated using a computer program incorporating the 'Jack-knife' technique to give standard errors (Miller, 1974; Bissel, 1977; Birch & Wratten, 1984). Aphids were introduced onto the plants at the early two-leaf stage (G. S. 11-12, Zadoks, Chang & Konzak, 1974), about 7 days after seedling emergence; at the same time, concentrations of Hx were determined in uninfested plants of the same cohort.

The method of Hx extraction followed that of Bohidar *et al.* (1986) and was based on the colorimetric absorption of a hydroxamic acid-ferric chloride complex. This procedure does not differentiate between the different hydroxamates present in extracts; however, Niemeyer *et al.* (1989*b*) using high performance liquid chromatography (HPLC) found that DIMBOA was the main or only Hx in the Chilean wheats. Zuñiga, Argandoña, Niemeyer & Corcuera (1983) determined concentrations of Hx in a wide variety of wild and cultivated Gramineae using both the ferric chloride method and a TLC-UV procedure. The latter technique differentiates between individual hydroxamic acids. The total Hx concentration determined by the ferric chloride method was consistently slightly higher than that determined by the TLC-UV method (on average 10% higher) and the authors suggested that this might be due to the presence of other hydroxamic acids in the extracts. There is a potential danger that ferric chloride will react with phenolic substances present in the extract since it is not a specific reagent for Hx. Extracting samples into ether attempts to avoid this problem. However, in those species where no DIMBOA or DIBOA were detected by TLC-UV methods, the ferric chloride procedure also failed to detect any reactive substances, suggesting that in this *Triticum* system there is no problem arising from the non-specificity of the reagent. A comparison of ferric chloride and GLC methods was made in maize by Woodward *et al.* (1979) which showed a direct relationship between DIMBOA determined by GLC and total hydroxamates determined by the ferric chloride method.

The relationship between r_m of *S. avenae* and concentrations of Hx in 7-day-old seedlings for each variety was tested using regression analysis. Similar analyses were performed on the data for *Rhopalosiphum padi* feeding on a selected group of plant taxa.

Temporal and spatial variation in hydroxamic acid concentrations

A study of temporal and spatial changes in concentrations of Hx in selected taxa was made. Hx were assessed in whole plants of a variety of ages and individual assessments were made of leaves of different ages in two-, three-, four- and five-leaved plants.

For the examination of the temporal variation of concentrations of Hx, seeds of each variety were sown on the same day and grown under identical conditions to those used in Bohidar *et al.* (1986). Within each cohort, seedlings were harvested at daily intervals from two days after germination up to the eleventh day. Hx are absent in the seed itself (Argandoña, Niemeyer & Corcuera, 1981). Four 1 g samples of leaf and stem material (including the leaf sheath) of each cultivar were required each day for establishing the concentrations of Hx in whole plants. Differences in temporal variation between lines were analysed using a two-way analysis of variance followed by Tukey's multiple range test, having established the comparisons to be made before the test was carried out (Sokal & Rolf, 1981). In a subsidiary experiment flag leaves were collected from field-grown *T. aestivum* plants (cv. Mission) of known growth stages. Four 1 g samples taken from a mixture of dissected flag leaves from different plants were used in the analysis of flag leaves at each growth stage.

In order to examine variation in concentrations of Hx between the different leaves of young seedlings, similar procedures were used. A proportion of the seedlings within each cohort were harvested as each new leaf emerged, at the two-, three-, four- and five-leaf stages, enabling leaf blade samples at each growth stage to be analysed for temporal variation in concentrations.

Assessment of the relationship between aphid mean relative growth rate and hydroxamic acid levels in six cultivars

Since the measurement of aphid performance used in the major screening of 20 lines was made over a considerable period of time (at least 14 days), fluctuations in Hx levels could have contributed to the variation found in the relationship between r_m and concentrations of Hx. Variation may also have been attributable to small changes in environmental conditions over the period of assessment. Leather & Dixon (1981) showed a strong correlation between r_m and the mean relative growth rate (mrgr) of *R. padi*. For the re-assessment of six lines under carefully controlled environmental conditions, mrgr was used so as to considerably shorten the length of the assessment period required to give a reliable measure of aphid performance on each line.

The seeds of all six cultivars were germinated on damp filter paper in complete darkness at 15 ± 1°C. After four days, groups of six seedlings were planted in 9-cm diameter plastic pots containing John Innes No. 2 seed compost, given a drench of the fungicide ethyrimol (as Milstem) to limit mildew infection and kept on trays in culture rooms as described in Bohidar *et al.* (1986). Transparent plastic tubes 8.5 cm in diameter and 24 cm high with Terylene mesh tops, were then placed over each pot so as to mimic the conditions described below for the mrgr experiments. These pots were arranged in a six by six Latin square arrangement and the seedlings harvested for Hx analysis seven days after germination, at growth stage 11-12, separated into individual leaves, weighed into four 1 g samples and stored at −20°C for later analysis.

For examining aphid performance on the different cultivars, seeds were germinated as above and 25 seedlings of each variety planted individually in 7-cm diameter plastic pots as above. Seven days after germination, one previously weighed second-instar aphid was gently introduced onto the oldest leaf of each seedling and a transparent plastic tube 6.5 cm in diameter with a Terylene mesh top placed over the plant to restrict aphid movement. The pots were positioned randomly on trays in culture rooms as described in Bohidar *et al.* (1986). All pots were watered with Hewitt's Long Ashton Solution (Hewitt, 1966) every two days to prevent depletion in essential nutrients or water supply.

The methods for culturing and selecting aphids were as above. Aphids were monitored daily, with their position on the plant noted, and then re-weighed three days (72 hours) after being placed on the plant. The mean relative growth rate for each aphid species on each cultivar was calculated following the method of van Emden (1969) as:

$$\frac{\log_e \text{final weight} - \log_e \text{initial weight}}{3}$$

One-way analysis of variance was used to test for differences in Hx concentrations between different pots. The relationship between mrgr and concentrations of Hx in 7-day old seedlings of each variety was tested using regression analysis.

Results

The relationship between concentrations of Hx and r_m of *Sitobion avenae* for hexaploid and tetraploid *Triticum* material is shown in Fig. 1. Values for concentrations of Hx ranged from 1.98 to 27.15 m mole/kg dry weight and were particularly high in some of the tetraploid wheats such as *Triticum durum* cv. SNA3, *Triticum turgidum* L. and *Triticum dicoccum* (Shrank.) Schulb. Values for r_m ranged from 0.140 to 0.309, the highest values (indicating the least resistance) being associated predominantly with the hexaploid wheats and the lowest values with some of the tetraploid wheats. The total concentration of Hx in the plant explained a significant proportion of the variation in intrinsic rate of increase of *S. avenae* on hexaploid and tetraploid *Triticum* material ($\log y = 0.61 - 0.15 \log x$; $r = -0.59$, $P < 0.01$). For *Rhopalosiphum padi* on hexaploid and tetraploid wheat, the relationship between r_m concentrations of Hx was also significant ($\log y = 0.51 - 0.04 \log x$; $r = -0.89$, $P < 0.05$), but the range of the r_m values obtained was small within the limited group of material studied.

The temporal variation in concentrations of Hx seen in three cultivars is shown in Fig. 2. Although total plant concentrations of Hx declined rapidly during the early seedling growth period, they did not fall to zero and the results from Tukey's multiple range test showed that the rate of decline varied significantly between each line. In all three lines studied the rate of decline was greatest during the first 4 days following germination, after which levels declined very slowly or not at all (Fig. 2).

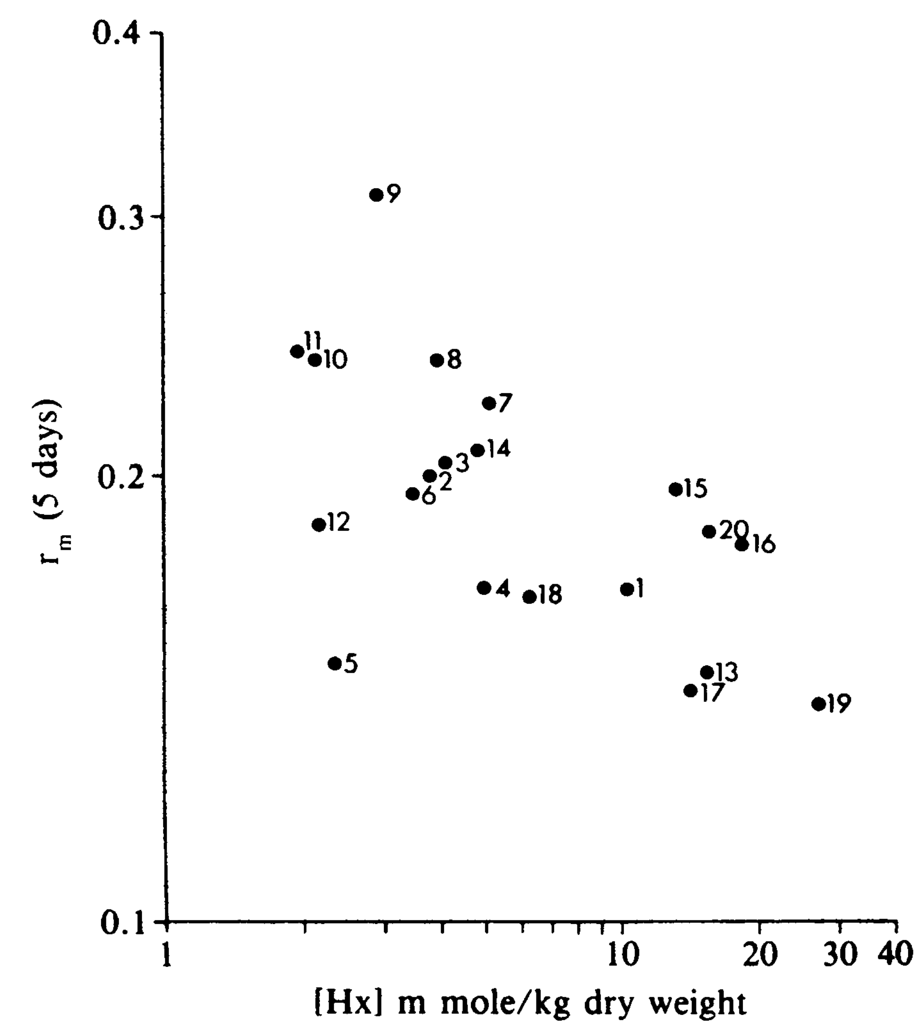

Fig. 1 The relationship between intrinsic rate of natural increase (r_m) of *Sitobion avenae* and levels of total hydroxamic acids [Hx] in 7-day-old seedlings of hexaploid and tetraploid *Triticum* ($\log y = 0.61 - 0.15 \log x$; $r = -0.59$; $P < 0.01$). Numbers denote lines. The species are: 1-12 = *Triticum aestivum*, 13-16 = *Triticum durum*, 17-18 = *Triticum polonicum*, 19 = *Triticum turgidum*, 20 = *Triticum dicoccum*. (1) Avalon, (2) Armada, (3) Hobbit, (4) Musket, (5) Jerico, (6) Naofen, (7) Likay, (8) Sonka, (9) Huenufen, (10) Bezostaya, (11) Cappelle, (12) 15189, (13) SNA3, (14) SNA2, (15) Quilafen, (16) 435016, (17) 384345, (18) 384338, (19) 352542, (20) 319869.

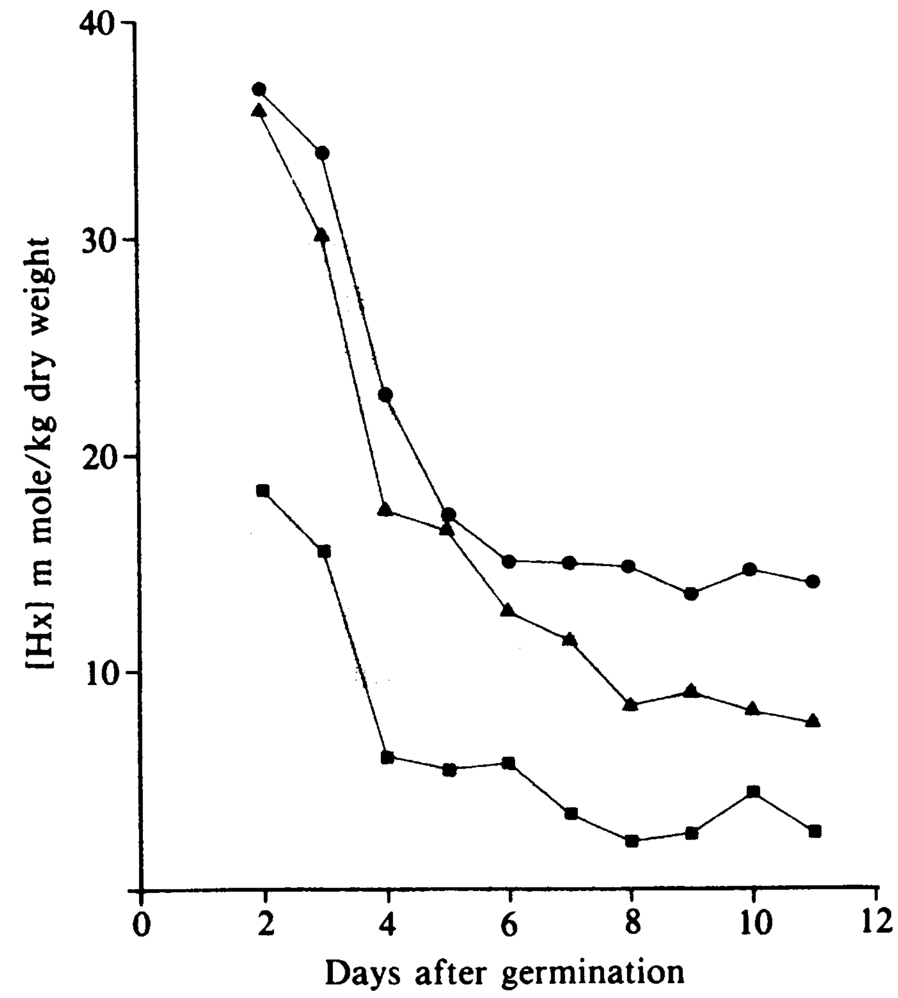

Fig. 2 Variation of concentrations of hydroxamic acids in whole plants in relation to age in three seedling cultivars. Key: ● *Triticum durum* cv. SNA3; ▲ *Triticum aestivum* cv. Avalon; ■ *Triticum aestivum* cv. Jerico.

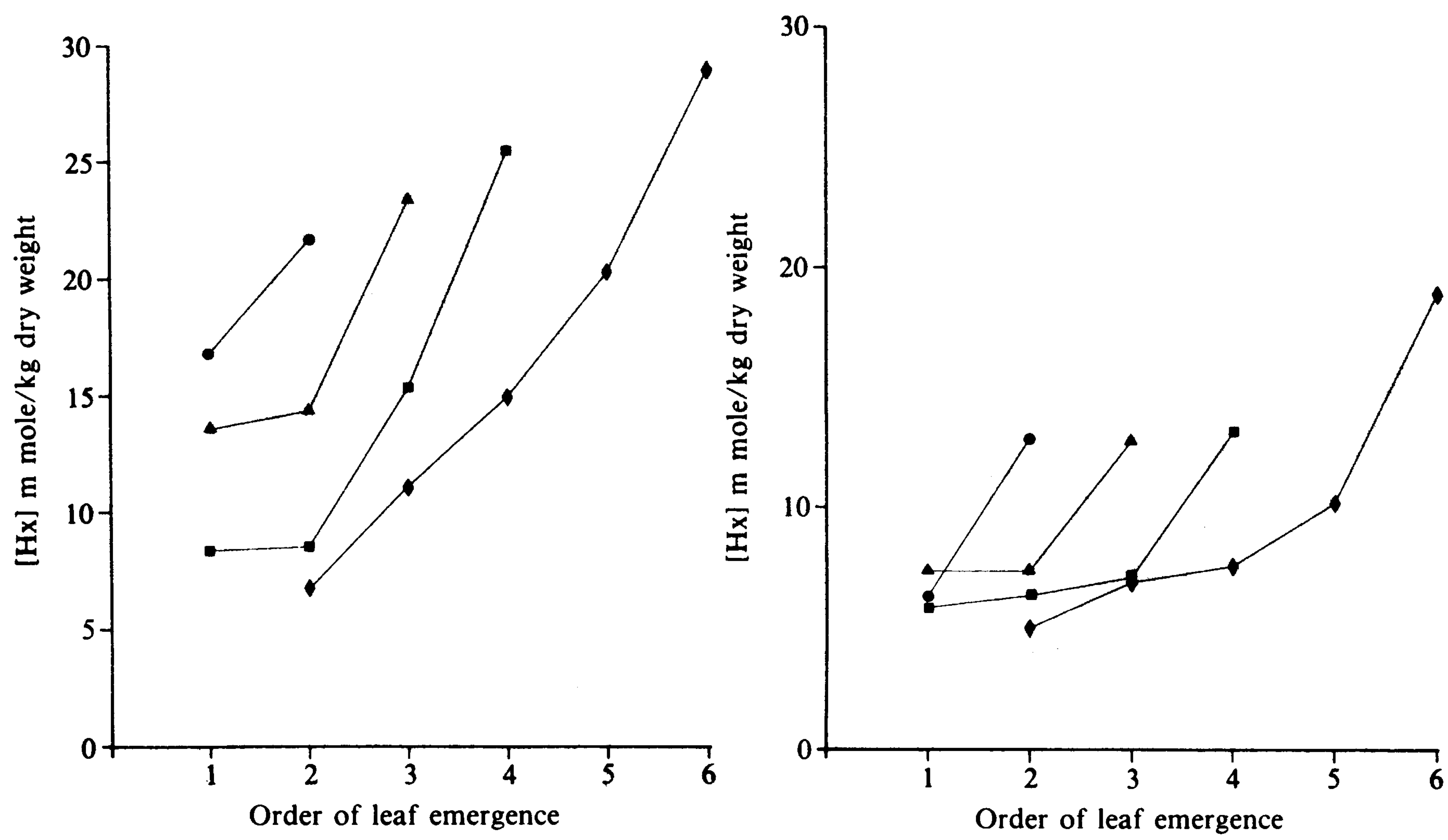

Fig. 3 The concentration of hydroxamic acids [Hx] in individual leaves of a. *Triticum durum* cv. SNA3 and b. *Triticum aestivum* cv. Likay, of plants at the ● 2-, ▲ 3-, ■ 4- and ◆ 6-leaved stages.

Concentrations of Hx were highest in the emerging leaves of seedlings of *T. durum* cv. SNA 3 and *T. aestivum* cv. Likay but declined sharply as the leaves aged (Fig. 3). At all seedling ages, the last leaf to emerge had the highest concentrations of Hx. Concentrations of Hx in the maturing leaves of a four-leaved plant of SNA3 ranged from 8.4 to 15.3 m mole/kg dry weight but a much higher concentration of 25.5 m mole/kg dry weight was found in the

Table 1. *Hydroxamic acid concentration in the flag leaves of mature plants of* Triticum aestivum *cv. Mission*

Crop stage	[Hx] m mole/kg dry weight	95% confidence limit lower	upper
Half inflorescence emerged	10.8	10.4	11.2
Emergence complete	9.2	8.9	9.6
Antithesis complete	5.1	4.7	5.4
Early milk	1.1	0.8	1.5

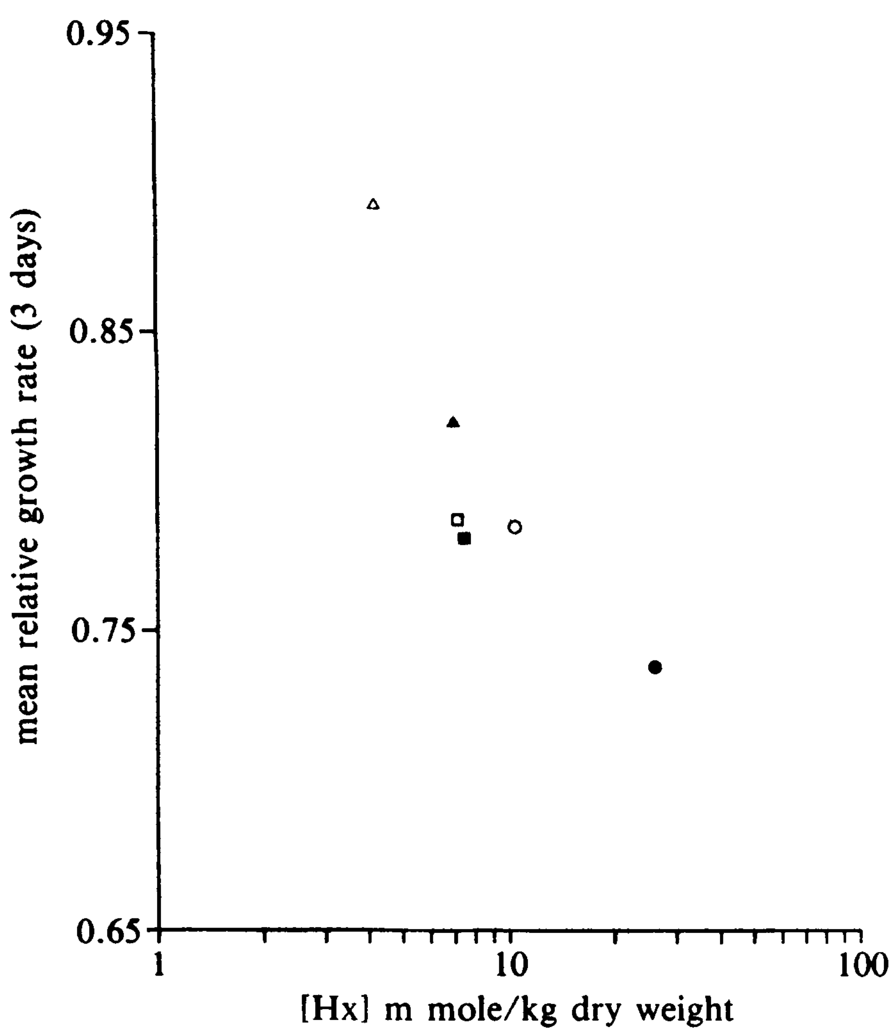

Fig. 4 The relationship between mean relative growth rate of *Sitobion avenae* and hydroxamic acid levels [Hx] in the first leaf of 7-day-old seedlings of six cultivars. Key: ● SNA3, ■ Naofen ▲ Likay, ○ Avalon, □ Apostle, △ Armada.

emerging fourth leaf. Similarly in mature plants of *Triticum aestivum* cv. Mission, the newly emerging flag leaf had relatively high concentrations of Hx (10.8 m mole/kg dry weight), which declined rapidly during anthesis to 1.1 m mole/kg dry weight (Table 1).

When mean relative growth rate was used as a measure of performance for *S. avenae* on six selected cultivars under controlled environmental conditions, the correlation between aphid performance and Hx levels in the oldest leaf was very strong (r = -0.69, $P < 0.05$) (Fig. 4).

Discussion

Although resistance to aphids in modern wheat is generally low, a wide range of resistance can be seen when the range of genotypes screened is increased. Hydroxamic acids (Hx) explained 35% of the resistance to *Sitobion avenae* in a selection of 20 seedling tetraploid and hexaploid wheats when r_m was the measure of aphid performance. When environmental conditions were more strictly controlled and mean relative growth rate over three days used, 47% of the resistance to *S. avenae* was explained by Hx in the oldest leaf of six seedling wheats. It is unlikely that any single plant characteristic could account for all variation in plant resistance; however, with a greater understanding of the behaviour of Hx in cereals and their effects on aphid feeding behaviour it may be possible to explain some of the residual variation in the relationship between concentrations of Hx and r_m.

It is possible that the effect of fluctuating environmental conditions such as water availability and light intensity could have contributed to the high variation found in the relationship between r_m and concentrations of Hx. Manuwoto & Scriber (1985) found that low-intensity light produced higher levels of Hx in maize plants than in controls. Hx levels are also affected by photoperiod (Epstein, Rowsemitt, Berger & Negus, 1986). In the first major screening of 20 cultivars in the present work, not all lines could be grown and assessed on the same date due to limitations on space and time. Consequently there may have been small variations in the environmental conditions under which different cultivars were grown. When the period of assessment of aphid performance was reduced from around 13-18 days (for r_m assessments) to three days (for mrgr assessments) and the plant growing conditions kept constant by careful attention to water and nutrient supply for a group of cultivars all grown over the same time period, a stronger relationship was established between aphid performance and levels of Hx (47%).

It remains to be established whether and, if so how *S. avenae* on wheat ingests Hx and at what concentrations in the plant they become an important resistance mechanism. This study has shown that concentrations of Hx vary between taxa and are always highest in newly emerging leaves. Thus, the correlation between the aphids' feeding site on the plant and the local concentrations of Hx may greatly affect the probing and settling behaviour of the aphids, given that antifeedant properties of Hx have been demonstrated. Inverse relationships between DIMBOA levels in aphids feeding on wheat plants and DIMBOA levels of the plants themselves have been found (Niemeyer *et al.*, 1989*b*), indicating possible feeding deterrency by DIMBOA. In addition, the relationship between aphid performance and concentrations of Hx should be re-examined in the light of recent evidence that localised aphid damage to wheat can induce increases in concentrations of Hx (Niemeyer *et al.*, 1989) and also in aphid nymphal mortality (Thackray, Morse & Leech, 1988). Inducibility of Hx in different cultivars may vary greatly.

It was previously thought (Argandoña *et al.*, 1981) that concentrations of Hx decline rapidly during seedling growth, reaching very low levels in maturing plants; however, the present study has shown that concentrations may be relatively high in newly emerging leaves, including emerging flag leaves. For example, concentrations of Hx in the newly emerged flag leaves of *Triticum aestivum* cv. Mission were 10.8 m mole/kg dry weight, compared with values of 12.9 and 21.7 in the new leaves of 9-day old seedlings of *T. aestivum* cv. Likay and *Triticum durum* cv. SNA3 respectively. In a tillering plant, a fairly high proportion of leaf material might therefore be high in concentrations of Hx, thus giving a greater degree of protection to the young tissues of the plant than was previously supposed. The localisation of Hx in areas of sensitive new growth has important implications for the plant's resistance to aphid damage. Until recently Hx were not thought to be of importance in the resistance of mature wheat plants to insects, but the evidence now suggests that they may play a role in wheat plants of all ages against aphid attack. Leszczynski, Wright & Bakowski (1989), for instance, demonstrated a highly significant negative correlation (r = 0.905) between *S. avenae* r_m and Hx for flag leaves.

Further studies of aphid behaviour on resistant and susceptible cultivars, incorporating a range of concentrations of Hx, are required together with studies of the relationship between concentrations of Hx and survival of other cereal pests. A negative relationship between concentrations of Hx and r_m for *Rhopalosiphum padi* has already been shown in the present study and future investigations will consider this and other cereal aphids with Hx levels determined in particular plant parts in relation to feeding behaviour, such as the leaf sheaths where *R. padi* mainly feeds.

From this study, a useful matrix of data relating to the taxonomic pattern of resistance within *Triticum* has emerged and it is hoped that the increasing knowledge of the behaviour of Hx within the plant might lead to the use of hydroxamic acid analysis in plant breeding programmes, both as a standard when selecting promising lines for resistance to cereal aphids and also in the isolation of resistant genes.

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