HYDROXAMIC ACIDS - POTENTIAL RESISTANCE FACTORS IN WHEAT AGAINST THE CEREAL APHIDS *SITOBION AVENAE* AND *RHOPALOSIPHUM PADI*

D.J. THACKRAY, S.D. WRATTEN, P.J. EDWARDS

Department of Biology, The University, Southampton, SO9 5NH, U.K.

H.M. NIEMEYER

Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile.

ABSTRACT

A significant negative correlation had previously been found between the intrinsic rate of increase (r_m) of *Sitobion avenae* and concentrations of hydroxamic acid ([Hx]) of 20 lines of tetraploid and hexaploid *Triticum*. The effects of aphid feeding, artificial damage and of varying environmental conditions on subsequent [Hx] and mean relative growth rates (RGR) of aphids were examined in an attempt to explain the residual variation in the above relationship. Re-assessment of six cultivars under carefully controlled conditions showed a strong correlation between *S. avenae* mean RGR and [Hx] in the youngest leaf (leaf one) of seedlings. A significant correlation was also found when cultivars were assessed as mature plants.

INTRODUCTION

The progress of plant breeders in their search for host-plant resistance in wheat to aphids is restrained by a number of factors. These include the absence of a reliable, rapid and convenient assay for resistance and the lack of information on the mechanisms of resistance when it is found. Possibly as a result, aphid resistance has not been deliberately bred into any UK wheat variety. This is despite the substantial contribution which partial resistance could make in the control of aphids (Acreman, 1984).

DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) is the most abundant compound in a group of cyclic hydroxamic acids (Hx) which have been isolated in wheat, maize and several wild Gramineae. These compounds are found in the plant as glucosides which are enzymically hydrolysed to the corresponding aglucones when the plant tissue is injured (Hofman & Hofmanova, 1969). Hydroxamic acids have been shown to be involved in the resistance of cereals against bacteria, fungi and several insects including aphids (Niemeyer, 1988). Bohidar *et al.*(1986) showed that 96% of the variation in the resistance of seedlings of six wheat cultivars to *S. avenae* was explained by [Hx]. Thackray *et al.* (1990) found Hx explained 35% of the resistance to *S. avenae* (measured as r_m) when the genetic range was increased to 20 seedling tetraploid and hexaploid wheats. Leszczynski *et al.* (1989) have recently shown strong correlations between r_m of *S. avenae* and [Hx] in flag leaves of *Triticum* during anthesis (GS 60 - 69).

The objectives of the present investigation were to attempt to explain the residual variation in the relationship between resistance to *S. avenae* and [Hx] found in the previous study of a wide genetic range of *Triticum* material (Thackray *et al.*, 1990). The effects of artificial damage and aphid feeding and of manipulating water and nutrient availability on [Hx] were studied and a small group of *Triticum* taxa were re-assessed under very carefully controlled conditions using a method based on a shorter period of assessment of aphid performance (mean relative growth rate) than that needed for r_m. This assessment examined both seedling and mature plants.

MATERIALS AND METHODS

Plant and insect material

Triticum aestivum cv. Avalon was used in experiments examining the effects of aphid infestations, of artificial damage and of nutrient availability on [Hx]. This is a modern cultivar grown commercially in the UK, with relatively high [Hx] in the seedling and available in large quantities of seed material. Six cultivars were selected for further assessment of the relationship between aphid performance and [Hx]. These were *T. aestivum* cvs. Avalon, Armada, Apostle, Naofen and Likay and *Triticum durum* cv. SNA3. As seedlings, these cultivars had previously been shown to represent Hx concentrations ranging from 3.52 to 15.41 m mole/kg dry weight (Thackray *et al.*, 1990). Seeds were sown in John Innes No. 2 compost, given a drench of the fungicide Milstem (ethirimol) to discourage mildew and kept in a culture box as described in Bohidar *et al.* (1986).

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 and an appraisal of the technique may be found in Thackray *et al.* (1990).

Stock cultures of *S. avenae* and *Rhopalosiphum padi* were clonal, originating from single parthenogenetic females and were maintained on barley (cv. Golden Promise), which is Hx lacking, in the culture room as above. All experiments were conducted in the culture room, unless otherwise indicated, at 20 °C with a 2 °C range, 60-70% relative humidity and a 16 hour photoperiod.

Effects of aphid infestation and of artificial damage on [Hx]

9-day-old plants were infested with about 50 3rd-instar *S. avenae* per seedling and measurements of [Hx] made after 24, 48 and 96 hours of infestation. P.V.C. cylinders with terylene mesh tops were placed over seedlings to restrict aphid movement. They were also placed over control plants which were kept free of aphids. Plants were grown in groups of seven to a pot with ten replicates per treatment. All aphids were removed by gentle brushing before plants were dissected into leaf one and leaf two (leaving leaf sheaths intact with each leaf) and weighed into four 1g samples for each treatment which were stored at -20 °C for later anlysis.

80 grit carborundum powder was gently rubbed over a 4cm-long area of the lower surface of leaf one of ten-day-old plants. Control plants received no damage. Plants were grown in groups of seven to a pot with twenty replicates per treatment. Plant material was harvested 48 and 96 hours after damage and four 1g pooled samples were taken from each of leaf one, leaf two and leafsheaths for storage.

Effects of manipulating nutrient availability on [Hx] and mean RGR

Plastic flower pots containing John Innes No. 2 and planted with groups of 10 seedlings for Hx analysis or with single seedlings for mean RGR study, were placed in individual Petri dishes to avoid cross contamination of water sources and then put on trays in the culturing facilities previously mentioned. Treatments were arranged alternately:
Control - Plants watered as usual with no added fertiliser.
Fertilised - Seeds watered upon sowing with 25 ml/seedling of a solution of I.C.I. Liquid Garden Plus (14 ml/4.5 l water; contains 7% nitrogen, 6% soluble phosphoric acid, 5% potash), and watered with the same solution (25 ml/seedling) when five days old.

When the plants were 6 days old (GS 11) 3rd-instar nymphs of approximately the same weight (180 - 230 g) were removed from the stock culture of *S. avenae*, weighed on a micro balance and placed individually on the centre leaf section of leaf one of each of 20 replicate plants for each treatment. A P.V.C. tube placed over each plant prevented movement onto neighbouring plants. Clip cages were not used since these might cause damage to the plant and thus interfere with the results.

Aphids were re-weighed after two days and the mean relative growth rate (RGR) calculated as:

$$\frac{\log_e \text{final weight} - \log_e \text{initial weight}}{\text{no. of days over which weight increase measured}} + 0.5$$

Occasionally an aphid showed a decrease in weight over the measurement period and the weighting of 0.5 was used in all calculations to avoid negative values which complicate statistical analyses.

Relationship between *S. avenae* mean RGR and [Hx] for six selected cultivars examined at GS 11 and GS 47

Seedlings

Seedlings were harvested for Hx analysis seven days after germination, at growth stage 11/12, dissected into leaf one and two and weighed into 1g samples for storage at -20 °C. Mean RGR was measured over 72 hours, 2nd-instar aphids having been placed on 7-day-old plants (GS 11) and confined with P.V.C. tubes. Most aphids remained on leaf one, where they had been placed, throughout the experiment. Mean RGR for both *S. avenae* and the bird cherry oat aphid *R. padi* were measured, with 25 replicates each. All pots were watered with Hewitt's Long Ashton Nutrient Solution every two days to ensure no depletion in essential nutrients.

Mature plants

Previously vernalised seeds of all cultivars were sown and maintained in a glasshouse at a temperature of 20 °C with a 5 °C range and a minimum 16 hour photoperiod. All pots were regularly watered with Hewitt's solution until the plants were at growth stage 21, sprayed with the fungicide Tilt Turbo (propiconazole + tridemorph) and transferred to a field site at Chilworth, Hampshire. 32 plants of each cultivar were placed in each of six 2m x 2m x 2m field cages which were covered in Tygan (1mm mesh) to prevent natural infestations of aphids and also as a barrier against rabbit grazing. The plants were watered daily until roots were well established and then twice a week during the following dry period. Flag leaves were analysed for [Hx] at GS 47 and mean RGR (with 24 replicates) of *S. avenae* measured over 72 hours as before. Individual aphids were confined on the flag leaf with clip cages.

RESULTS

Figure 1 shows the effects of aphid infestation on the Hx concentrations of different leaves of the plant. Over the 96-hour period of infestation, Hx concentrations in the leaves of all seedlings declined but were always highest in leaf two (the youngest leaf) of the infested plants. There were no significant differences in Hx concentrations between treatments for leaf one, but Hx concentrations were significantly higher in leaf two of plants subjected to 48 and 96 hours of infestation. Differences in the letter notation within pairs of columns indicates significant differences in [Hx] between control and infested plants.

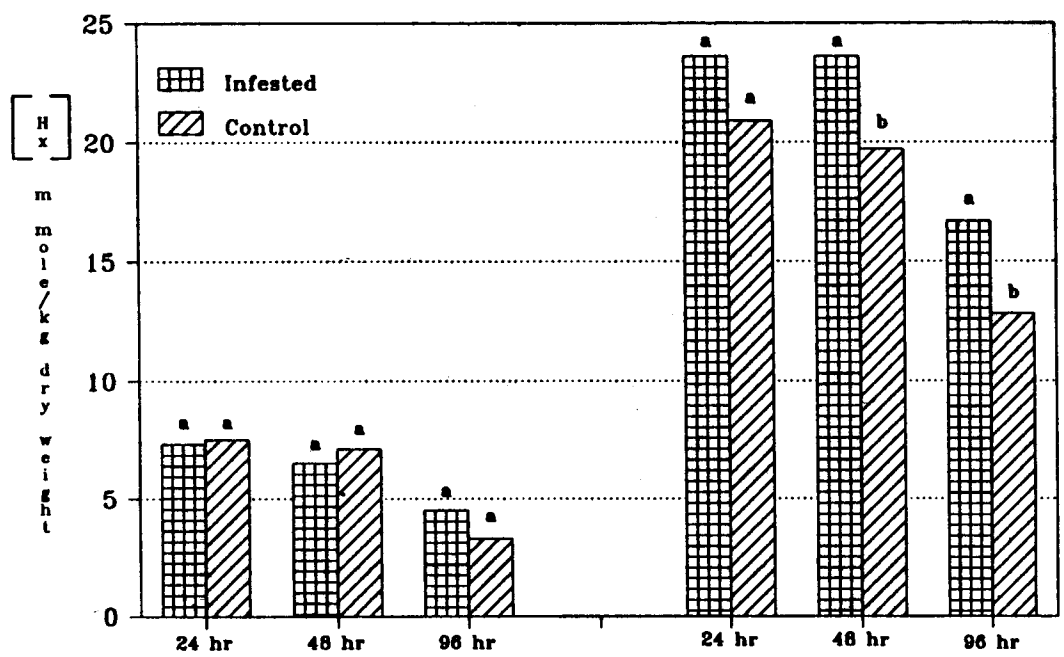
Carborundum damage also affected [Hx]. At 48 hours after damage the Hx levels in sheaths, leaf one and leaf two of damaged plants were

significantly higher than in those of undamaged plants ($F = 25.56$; d.f. = 1,36; $P < 0.001$; $F = 20.77$; $P < 0.001$; $F = 211.6$; $P < 0.001$; respectively). At 96 hours there were no significant differences in Hx concentrations between treatments.

The investigation into the effects of nutrient availability on [Hx] showed no significant difference between treatments for total plant [Hx] in 6-day-old plants but the mean [Hx] in 8-day-old plants was slightly higher in fertilised plants than in control plants ($F = 24.32$; d.f. = 1,12; $P < 0.005$). The mean RGR for aphids on fertilised plants was significantly lower than on control plants ($F = 9.8$; d.f. = 1,31; $P < 0.05$).

FIGURE ONE

The [Hx] of aphid infested and un-infested seedlings of Avalon after 24, 48 and 96 hours of infestation



Differences in letter notation within pairs of columns indicates significant differences between treatments.

Values for mean relative growth rate (RGR) of *S. avenae* and *R. padi*, and [Hx] obtained in the screening of plant material at GS 11 and GS 47 are given in Table 1.

TABLE 1 Mean RGR of 2nd instar aphids and [Hx] for six Chilean and UK cultivars at GS 11 and GS 47.

Cultivar	[Hx] m mole/kg dry wt			Mean RGR		
	Leaf 1	Leaf 2	Flag leaf	*S. avenae* GS 11	*S. avenae* GS 47	*R. padi* GS 11
SNA3	25.07	39.51	4.77	0.74	0.732	0.89
Avalon	10.01	31.70	1.51	0.79	0.792	0.91
Naofen	7.21	21.14	2.79	0.78	0.752	0.86
Apostle	7.13	18.40	1.61	0.79	0.849	0.88
Likay	6.92	17.79	2.78	0.82	0.766	0.92
Armada	4.16	11.06	1.82	0.89	0.786	0.92

The relationship between mean RGR of *S. avenae* and [Hx] in leaf two of seedlings of the six cultivars studied is shown in Figure 2. The strongest correlation (69%) was found between mean RGR for *S. avenae* and [Hx] in leaf two ($r = -0.83$; $P < 0.05$); 55% of the variation between mean RGR for *S. avenae* was explained by Hx concentrations in leaf one but this relationship was not significant ($r = -0.74$). The correlation between mean RGR of *R. padi* and [Hx] in either leaf was not significant (leaf one, $r = -0.17$; leaf two, $r = -0.20$). The variation in mean RGR was very small for *R. padi* within the six cultivars studied.

A significant relationship was also shown between mean RGR of *S. avenae* and [Hx] of the flag leaf of six cultivars at GS 47. 63% of the variation in mean RGR between cultivars was explained by [Hx] ($r = -0.80$; $P < 0.05$). The relationship between mean RGR of *S. avenae* on flag leaves and the [Hx] of leaf one of seedlings at GS 11 of the same cultivar was not significant ($r = -0.54$; $r^2 = 30\%$).

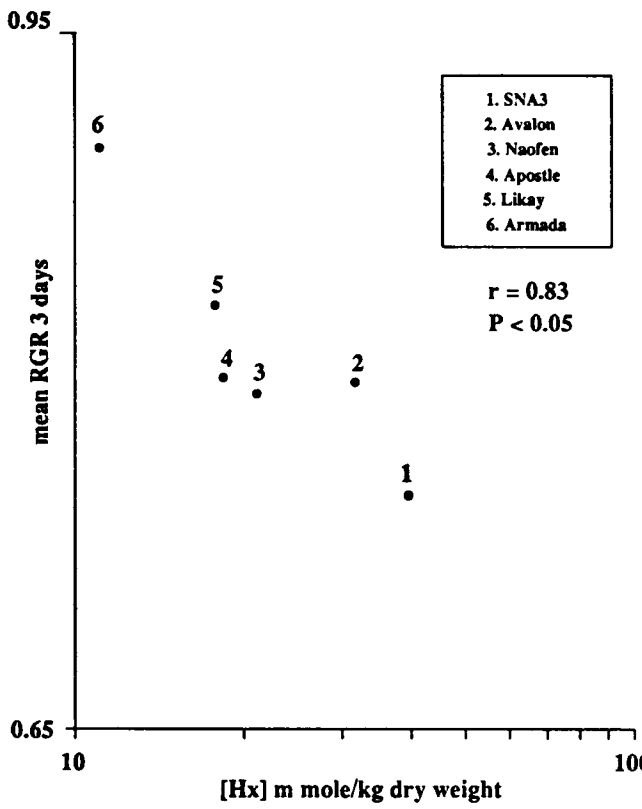


FIGURE TWO

The relationship between mean RGR of *Sitobion avenae* and the [Hx] in leaf two of seedlings of six cultivars at growth stage 11.

DISCUSSION

The aims of this study were to take an applied approach in attempting to elucidate and validate the possible role of hydroxamic acids as resistance factors in wheat and to establish the viability of using [Hx] as an indicator of resistance in wheat to aphids. The experimental work reported here along with similar studies conducted at Southampton have demonstrated the existence of an induced response to aphid feeding and to artificial damage although changes in [Hx] were not very large and appeared short-lived. Changes in external environmental factors such as water and nutrient availability have been shown to affect [Hx] and aphid mean RGR and thereby emphasise the need for carefully controlled and constant working conditions. Hx concentrations appear to be readily affected by many factors, in particular the age and growth stage of plant material and concentrations within certain tissues may be constantly changing, possibly influenced by translocation of the glucoside within the phloem, though there is as yet no proof for this hypothesis. A greater knowledge of the behaviour of Hx in localised areas of the plant within the different tissues would be a considerable aid to interpretation of effects on aphids.

The conclusions from this study and those of others working on resistance would appear to encourage further assessments of the potential of these compounds, particularly DIMBOA, as resistance factors in modern wheat to aphids. The use of high performance liquid chromatography (HPLC) would greatly facilitate further study by offering a technique which requires only small amounts of material and is specific for individual hydroxamic acids. The significant relationships found between the performance of *S. avenae* (measured as r_m or mean RGR) and [Hx] of seedling plants should encourage the continued screening of seedling and mature plant taxa for suitable germplasm with high levels of DIMBOA in particular, for use in wheat breeding programmes. Whilst a significant relationship was not demonstrated between *R. padi* mean RGR and seedling [Hx] this may be attributable to the feeding sites of this aphid which are confined mainly to the leaf sheaths. Total leaf analysis may have masked the [Hx] which these aphids were actually encountering. Recent work by Wratten *et al.* (1990) using HPLC has indicated a strong negative correlation ($r^2 = 0.75$) between the mean RGR of *S. avenae* and [Hx] of a selection of cultivars previously examined by Lowe (eg. Lowe, 1981) for mature plant resistance. Furthermore, a strong negative relationship ($r^2 = 0.80$) between mean RGR of *R. padi* and [Hx] of leaf sheaths of a number of *Triticum* seedlings has also been demonstrated (Wratten *et al.*, 1990). Given these encouraging developments, the use of HPLC by plant breeders to assess for [Hx] as a measure of resistance in new lines looks a promising technique for the future.

ACKNOWLEDGEMENTS

We wish to thank Dr C. Law of the former Plant Breeding Institute, UK and Plant Breeding International for provision of plant material. This work was supported by grants from the U.K. Agricultural and Food Research Council and from the Southampton University Research Fund. Finance for the poster was provided by Hampshire Arable Systems Ltd.

REFERENCES

Acreman, T.M. (1984) The contribution of resistance to cereal aphid control. *Proceedings of 1984 British Crop Protection Conference - Pests and Diseases*, 31-37.

Bohidar, K.; Wratten, S.D.; Niemeyer, H.M. (1986) Effects of hydroxamic acids on the resistance of wheat to the aphid *Sitobion avenae*. *Annals of Applied Biology* **109**, 193-198.

Hofman, J.; Hofmanova, O. (1969) 1,4-Benzoxazine derivatives in plants. Sephadex fractionation and identification of a new glucoside. *European Journal of Biochemistry* **8**, 109-112.

Leszczynski, B.; Wright, L.C.; Bakowski, T. (1989) Effect of secondary substances on winter wheat resistance to grain aphid. *Entomologia Experimentalis et Applicata* **52**, 135-139.

Lowe, H.J.B. (1981) Resistance and susceptibility to colour forms of the aphid *Sitobion avenae* in spring and winter wheats (*Triticum aestivum*). *Annals of Applied Biology* **99**, 87-98.

Niemeyer, H.M. (1988) Hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-ones), defense chemicals in the Gramineae. *Phytochemistry* **27**, 3349-3359.

Thackray, D.J.; Wratten, S.D.; Edwards, P.J.; Niemeyer, H.M. (1990) Resistance to the aphids *Sitobion avenae* and *Rhopalosiphum padi* in Gramineae in relation to hydroxamic acid levels. *Annals of Applied Biology* **116**, 573-582.

Wratten, S.D.; Martin, J.J.; Rhind, J.J.; Niemeyer, H.M. (1990) Mechanisms of resistance in cereals to pests. *Proceedings of 1990 Brighton Crop Protection Conference - Pests and Diseases* (in press).