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Hydroxamic acids affecting barley yellow dwarf virus transmission by the aphid *Rhopalosiphum padi*

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Abstract

2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), a hydroxamic acid (Hx) occurring in wheat, was shown to deter feeding by the aphid *Rhopalosiphum padi* (L.), and to reduce BYDV transmission to the plant. Dual choice tests with wheat leaves showed the preferential settlement of aphids on leaves with lower levels of DIMBOA. Electric monitoring of aphid feeding behaviour showed that in seedlings with higher DIMBOA levels fewer aphids reached the phloem and they needed longer times to contact a phloem vessel than in those with lower levels. When aphids carrying BYDV were allowed to feed on wheat cultivars with different DIMBOA levels, fewer plants were infected with BYDV in the higher DIMBOA cultivars than in the lower ones. Preliminary field experiments showed a tendency for wheat cultivars with higher Hx levels to be more tolerant to infection by BYDV than lower Hx level ones.

Introduction

The barley yellow dwarf viruses (BYDV) are members of the luteoviruses group affecting cereals (Oswald & Houston, 1953). As with other luteoviruses, they can only be transmitted by aphid vectors. These viruses are circulative within the vector, and are restricted to the phloem of the plant (Rochow & Duffus, 1981). Studies on BYDV transmission by *Sitobion avenae* (F.) to oat (*Avena Sativa* L.) showed that the vector needed plant access periods longer than a certain threshold in order to transmit the virus (Scheller & Shukle, 1986). The direct feeding damage by aphids has been diminished in Chile mainly via biological control (Zuñiga, 1985), but BYDV still remains a problem since virus transmission does not require a high population of the vector (Herrera & Quiroz, 1988). The principal BYDV strain present in Chilean crops is PAV (Herrera, 1984), which is transmitted by the aphids *S. avenae*, *Rhopalosiphum padi* (L.) and *Metopolophium dirhodum* (Wlk.) (Plumb, 1983).

The presence in the plant of resistance factors to the vectors might diminish the probability of virus transmission (Gibson & Plumb, 1977), in particular if such factors shorten the period during which virus may be inoculated. Hydroxamic acids (Hx) in cereals, and in particular 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) (Fig. 1) have been shown to play an important role in the resistance of the plant to pests and diseases (Niemeyer, 1988). The following results suggest that Hx may also be important in the prevention of virus transmission.

i) aphid survival on diets decreased as

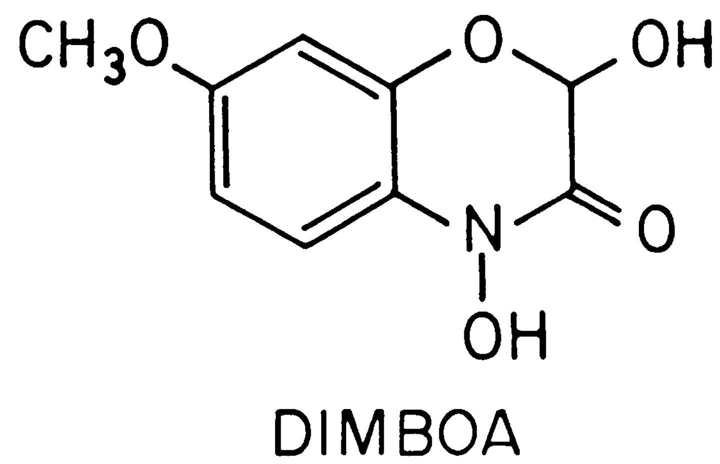

Fig. 1. Structure of DIMBOA.

DIMBOA concentration increased up to 4 mM; at higher concentrations survival increased eventually reaching the levels obtained with starved aphids. The interpretation of this phenomenon may be that at low DIMBOA concentrations aphids fed normally and ingested the compound, which produced its toxic effects decreasing aphid survival. As the DIMBOA concentration further increased, aphids were repelled by the compound, feeding was arrested and toxic effects were absent (Argandoña *et al.*, 1983).

ii) when aphids fed on diets with DIMBOA concentrations higher than 1 mM, the levels of DIMBOA in the aphids correlated negatively with the levels of the compound in the diets. A similar observation was made in aphids feeding on plants containing different DIMBOA levels (Niemeyer *et al.*, 1989b).

iii) studies on feeding behaviour of *Schizaphis graminum* (Rond.) showed that the average ingestion period for aphids feeding on diets with different DIMBOA concentrations, was inversely correlated with the levels of this compound (Argandoña *et al.*, 1983).

The purpose of this work is to examine the relationship between feeding deterrence by DIMBOA and BYDV transmission by aphids to wheat.

Materials and methods

Plant material. The cultivars employed were kindly supplied by Dr. Ignacio Ramírez and Ms. Mireya Zerené of the Instituto de Investigaciones Agropecuarias (INIA). Lines were classified by them as tolerant or susceptible to BYDV, as described (Herrera & Quiroz, 1988). In all the experiments carried out with the six wheat lines chosen (Platifén, Millaleu, Mexifén, Nobo, Anza and Maitén), plants were in the 1-leaf stage (G.S. 10, Zadoks *et al.*, 1974; Tottman & Makepeace, 1979). Seedlings show the highest levels of DIMBOA at this stage (Argandoña *et al.*, 1981).

Aphids. *R. padi* was collected in wheat fields near Santiago and kept in a rearing chamber at 22 ± 3 °C and a L16:D8 photoregime. Aphids used in the experiments were apterous and differed in age by at most 24 h.

Choice experiments. Leaves of two cultivars grown on different pots were placed in a clip cage and 4 apterous aphid adults were introduced into the cage. Six hours later, the distribution of aphids in the cage was recorded. Each experiment consisted of 22 clip cages. Duplicate or triplicate experiments were performed.

Electrical penetration graphs (EPG). The system used has been described in detail (Tjallingii, 1988). The EPG technique consists of forming a closed circuit between plant and aphid by gluing one electrode to the aphid and placing another in the soil where the plant is growing. The circuit is closed when the aphid inserts its stylets into the plant. The signals produced are amplified and registered. For each cultivar studied, 20 apterous aphid adults were wired and their feeding behaviour recorded for 6 h. The mean time to reach the phloem and mean time ingesting from the phloem or from the xylem were determined. For 6 aphid adults on each line the recording was prolonged to 12 h and the mean duration of phloem sap ingestion was determined. Phloem sap ingestion was considered to occur when the characteristic EPG pattern developed for more than 8 min (Kimmins & Tjallingii, 1985).

Infection experiments. An aphid was allowed to feed for 6 h on wheat plants in the 1-leaf stage.

After that, the infectivity of each individual aphid was tested by transferring it to a single oat plant and killing it by spraying with insecticide 24 h later. Virus were allowed to develop for 1 month both in wheat and in oat. This experiment was carried out with twenty aphids for each of the six wheat accessions chosen. The presence of BYDV in wheat and oat plants was detected by means of an ELISA test (Lister & Rochow, 1979). Each set of experiments was repeated twice for each cultivar.

Controls. Oat was used as the control without hydroxamic acids in all the experiments described above. Results did not show significant differences with those obtained with wheat cultivar Platifén. Hence, they are not commented further.

Hx analysis. They were performed on wheat and oat plants at the 1-leaf stage, by high performance liquid chromatography (Niemeyer *et al.*, 1989a).

Results

DIMBOA levels in INIA lines. The accessions studied were classified by INIA as tolerant or susceptible to BYDV mainly on the basis of differences in yield of artificially infested vs. uninfested plants (Herrera & Quiroz, 1988). Figure 2 shows that most accessions classified as tolerant contained higher DIMBOA concentrations and most susceptible ones contained lower DIMBOA concentrations. Two exceptions occurred (lines 4 and 7), which probably present direct intrinsic tolerance towards the virus.

Choice tests. Choice tests with plants containing the same or different levels of DIMBOA showed that aphid distribution among high and medium or low Hx containing plants differed significantly, i.e. given the choice, aphids preferred to feed on plants with lower levels of DIMBOA (Table 1).

EPG's. Different signals in EPG's provided a means of recording the path of the stylet inside the plant leaf. Thus, it was possible to distinguish, among other variables, whether an aphid is probing or feeding in the phloem or in the xylem. The feeding time periods obtained in this way are shown in Table 2.

EPG's showed that i) aphids took longer to reach the phloem when the DIMBOA level in the plant was higher than when it was lower; ii) the higher the DIMBOA concentrations the lesser the number of aphids reaching the phloem; iii) once an aphid reached the phloem, its ingestion period did not vary significantly with Hx content.

Table 1. Effect of DIMBOA levels on aphids in feeding choice tests ⁺

	Low Hx		Medium Hx		High Hx	
	(0.9)# Platifen	(1.0) Millaleu	(1.3) Mexifen	(1.4) Nobo	(2.0) Anza	(2.7) Maiten
Platifen	39 : *38*	34 : *28*	43 : *36*	33 : *28*	53 : *27**	42 : *21**
Millaleu		35 : *40*	37 : *31*	38 : *32*	56 : *24**	42 : *21**
Mexifen			36 : *36*	38 : *38*	39 : *28*	57 : *18**
Nobo				36 : *31*	44 : *28**	44 : *31**
Anza					26 : *26*	37 : *40*
Maiten						21 : *23*

⁺ Paired values corresponds to cv at left: cv above.
mmol DIMBOA/Kg fr. wt.
* Differences in distribution are significant ($p(X^2) < 0.1$).

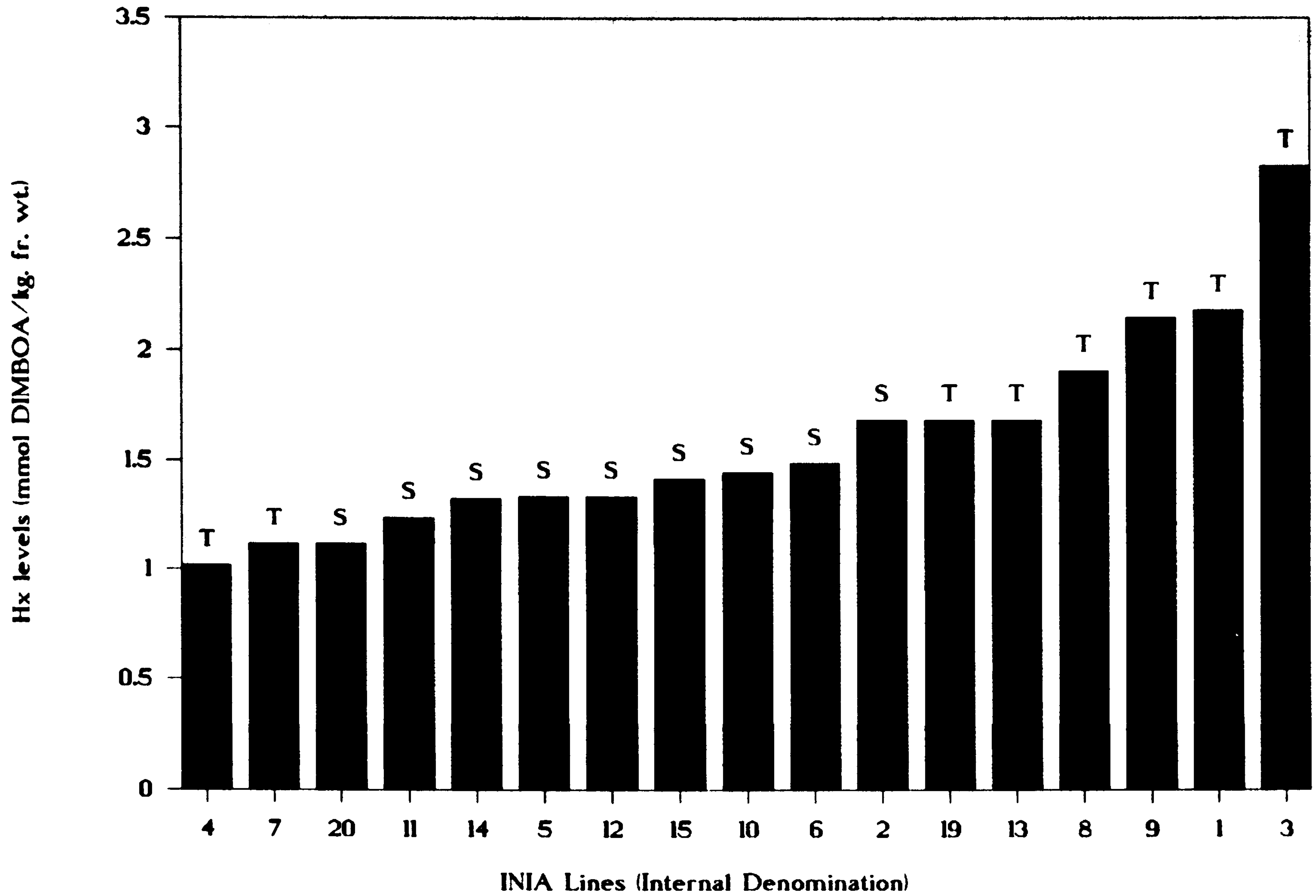

Fig. 2. Reaction to BYDV transmission on different wheat lines, and their hydroxamic acids levels (T = tolerant, S = susceptible).

Table 2. Effect of DIMBOA in wheat on aphid feeding

	Platifen	Millaleu	Mexifen	Nobo	Anza	Maiten
DIMBOA (mmol/kg fr. wt.)	0.9	1.0	1.3	1.4	2.0	2.7
% aphids arriving to phloem in 6 h	95	85	50	50	30	50
Time to phloem ingestion (min)	55a*	55a	67b	71b	95c	153d
Time in phloem (min)	353a	345a	342a	355a	343a	354a
Time in xylem ingestion (min)	34a	35a	50b	51b	77c	83c

* Values in the same row followed by the same letter are not significantly different at $p < 0.05$ (ANOVA/Duncan's test).

Table 3. Effect of DIMBOA on BYDV transmission by *R. padi*

	Platifen	Millaleu	Mexifen	Nobo	Anza	Maiten
DIMBOA (mmol/kg fr. wt.)	0.9	1.0	1.3	1.4	2.0	2.7
Nr of infected wheat plants	16	20	13	13	11	5
Nr of infected oat plants	18	20	20	18	20	16

Infection test. The infection results of Table 3 show a negative correlation between Hx content and BYDV transmission. Virus titres showed no significant differences among infected plants of the cultivars studied.

Discussion

The results obtained in choice tests (Table 1) supported the idea that aphids prefer those plants that contain lower levels of DIMBOA, suggesting that DIMBOA acts as a feeding deterrent in the plant. EPG's (Table 2) showed that this effect is perceived by the aphid during the period of stylet penetration, before reaching the phloem tissue.

On the other hand, the mean time of phloem sap ingestion did not differ among the cultivars studied, suggesting that Hx are not present in the phloem sap or that they occur at concentrations below the feeding deterrent level (Niemeyer *et al.*, 1989b). These facts would imply that the threshold period of plant access needed for BYDV transmission, as postulated by Scheller and Shukle (1986), might be related to Hx levels, in the sense that these compounds would be a barrier for aphids before reaching a phloem vessel. Phloem sap analysis, for instance by means of a stylet excision technique (Weibull *et al.*, 1986), would give quantitative evidence of Hx presence or absence.

The results in Table 3 suggest that higher DIMBOA levels in wheat would interfere with BYDV transmission. Hence, Hx could be considered a resistance factor in wheat plants increasing the proportion of aphids searching for suitable feeding tissue by probing but not feeding, and dying or leaving the plant without transmitting virus, as described in general by Gibson and Plumb (1977).

It is known that alate aphids are important in the fitness of an aphid population due to their capability to migrate long distances and to decide whether a plant is or is not a suitable host for further colonization (Robert, 1987). Young apterous adults such as those used in our tests are less able to migrate long distances, but they also tend to move from plant o plant when the host suitability decreases (Klingauf, 1987). Although our choice tests (Table 1) would corroborate that apterous aphids are able to detect plant unsuitability, it seems desirable to develop similar experiments with alate *R. padi* in order to compare the behaviour of these two morphs. Since *Prunus padus* L., does not exist in Chile, this aphid could use wild Gramineae as host to overwinter (Carter *et al.*, 1980). Apterous morphs coming from an overwintering host and those coming from wheat crops may show differences in their behaviour. It would prove interesting to carry out comparative studies to test this idea.

Our results have been obtained with only one cereal aphid species. It would be desirable to carry out similar studies on other species such as *M. dirhodum* and *S. avenae* because they are able to transmit BYDV and show a differential sensitivity towards Hx (Corcuera *et al.*, 1982; Niemeyer *et al.*, 1989b).

These results were obtained with plants in the 1-leaf stage. As the plant ages, Hx content decreases (Argandoña *et al.*, 1981; Thackray *et al.*, 1990). However, they remain concentrated in the flag leaf (Thackray *et al.*, 1990; Leszczynski *et al.*, 1989). On the other hand, in Chilean crops it has been observed that the most important peak in aphid population occurs when the second node in wheat is noticeable (Herrera & Quiroz, 1988). To project our results to field conditions it would be desirable to develop cultivars possessing high Hx levels throughout the life of the plant or at least at growth stages when aphid populations are high. A possible role of Hx preventing BYDV transmission by aphids in older plants, at the time when natural populations are high under field conditions can be deduced from the results reported in this work (Fig. 2). Thus, with two exceptions, plants classified as tolerant to BYDV showed higher levels of Hx than those which were classified as susceptible to the virus.

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