
Hydroxamic Acids: Chemical Defences in Wheat Against Aphids

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Introduction

Aphids (Homoptera : Aphididae) damage plants by withdrawing nutrients from them, by inoculating toxins in their saliva, by transmitting viral diseases, and by promoting the growth of saprophytic fungi on the honeydew they produce (Wellings *et al.*, 1990). Control of aphid populations using synthetic pesticides is expensive and requires careful implementation to be cost effective in many production systems. Classical biological control of aphids is possible and has been achieved on a number of occasions in the field (Hughes, 1990). However, aphid life-history traits, such as short development times and high rates of increase, often result in poor population regulation and frequent outbreaks. Host plant resistance, produced either through traditional breeding or transgenic methods, offers a robust alternative.

Plant secondary compounds play a key role in the relationship of an aphid with its host plant. Knowledge of their mechanism of action and their biosynthesis may lead eventually to their manipulation in the plant and their use as substitutes for synthetic pesticides, with considerable environmental and economic advantages.

This paper will describe some plant chemicals active at the plant-aphid interface, with particular emphasis on hydroxamic acids from cereals which are active against aphids.

Chemicals Involved in Host Finding by Aphids

Host finding by an aphid depends on its being carried by the wind to an area with potential host plants and, at close range, on visual and olfactory cues arising from the plant. After aphid alightment on a potential host, chemical cues predominate. These may be surface chemicals in the epicuticular lipid layer of the plant or in plant trichomes, chemicals encountered while the aphid searches for its final feeding site, the phloem, or chemicals circulating in the phloem itself.

Numerous plant secondary compounds have been shown to play a role in aphid-plant interactions. For example, cyanogenic glucosides in the genus *Prunus* (Pettersson, 1973) and glucosinolates in the genus *Brassica*

(Wensler, 1962; Gill & Bakhetia, 1985; Weber *et al.*, 1986) generate volatiles which are attractive to aphids at close range; alkanes in the epicuticular waxes of *Vicia* are aphid attractants (Klingauf, 1978), while E- β -farnesene in the trichomes of some *Solanum* species are aphid repellents (Lapointe & Tingey, 1986; Avé *et al.*, 1987); internal plant chemicals such as phenolics stimulate colonisation by aphids (Miles, 1985) while hydroxamic acids are deleterious to aphids (Niemeyer, 1988) and phloem constituents, such as quinolizidine alkaloids, may act as positive or negative stimuli towards aphid colonisation, depending on the specific aphid-plant combination (Smith, 1966; Wegorek & Krzymanska, 1971; Wink & Witte, 1985; Wink & Romer, 1986).

Hydroxamic acids (Hx) are one of the best described examples of plant secondary compounds involved in plant-aphid interactions (Figure 1). They derive from 2,4-dihydroxy-1,4-benzoxazin-3-one and are present in the intact plant as 2- β -O-D-glucopyranosides (Hx-Glc), which are hydrolysed by endo-glucosidases when the tissue is injured (Niemeyer, 1988a).

Hydroxamic acids were discovered in the 1950's during studies on fusariosis-resistant cultivars of rye (Virtanen & Hietala, 1960). Since the late 1970s the possible involvement of Hx in aphid resistance of cereals has attracted increased interest. Inverse correlations have been described between Hx concentration in cereal plants and the size of populations of *Rhopalosiphum maidis* developing on maize plants (Long *et al.*, 1977; Beck *et al.*, 1983), population growth rate of *Metopolophium dirhodum* (Argandoña *et al.*, 1980) and *Schizaphis graminum* (Argandoña *et al.*, 1983) on wheat and rye seedlings, intrinsic rate of natural increase of *Sitobion avenae* on wheat seedlings (Bohidar *et al.*, 1986; Thackray *et al.*, 1990a) and mean relative growth rate of *Rhopalosiphum padi* individuals on wheat seedlings (Thackray *et al.*, 1990b).

Aphid survival decreased when increasing concentrations of both Hx glucosides and aglucones were incorporated into artificial diets (Corcuera *et al.*, 1982; Zúñiga *et al.*, 1983; Barría *et al.*, 1992), pointing to Hx as the cause of the antibiosis shown by the previously described correlations.

Hx also proved to be feeding deterrents to aphids (Argandoña *et al.*, 1983; Niemeyer *et al.*, 1989; Givovich & Niemeyer, 1991). Apterous aphids settled preferentially on leaves with lower levels of Hx when presented with wheat seedlings differing in Hx content in dual choice tests. Similarly, alate aphids released among wheat seedlings settled preferentially on seedlings with lower Hx levels (Nicol *et al.*, 1992). Electric monitoring of aphid feeding behaviour showed that, on seedlings with higher Hx levels, fewer aphids reached the phloem within a given time period, and they needed longer times to contact a phloem vessel than in those with lower levels. When aphids carrying the barley yellow dwarf virus (BYDV) were allowed to feed on wheat cultivars with different Hx levels, fewer plants were infected with BYDV in high Hx concentration cultivars compared to low concentration ones. Hence, Hx show a potential to control viral infection of wheat seedlings through feeding deterrence towards aphid vectors (Givovich & Niemeyer, 1991).

Chemical Nature of the Wheat-Aphid Interface

Analysis of whole bodies of aphids feeding on wheat seedlings containing Hx showed the presence of DIMBOA (Niemeyer *et al.*, 1989), but aphid honeydew contained DIMBOA-Glc, suggesting that this chemical circulates in the phloem (Givovich *et al.*, 1992). Neither the aglucone DIMBOA nor its main breakdown product were found in the honeydew samples. Both the production of honeydew within a limited time period, and the concentration of DIMBOA-Glc in the honeydew samples, followed biphasic curves when plotted against Hx concentration in the wheat seedlings; as the seedling Hx concentration increased, each dependent variable first increased and then decreased. These curves suggested active uptake of phloem at low seedling Hx concentrations and limited ingestion of Hx at high seedling Hx concentrations. The following model can account for these facts: Aphids take up the aglucone while piercing mesophyll cells in search of the phloem, but ingest the glucoside mainly when feeding from the phloem. The feeding deterrent effect of the aglucone increases the time taken by an aphid to reach the phloem, and diminishes the rate of virus transmission. Once the aphid is feeding in the phloem it encounters a glucoside concentration which is too low to deter feeding, but which contributes to the reduction of its fitness.

Effect of Hx in Wheat Seedlings on the Aphid Predator *Eriopsis connexa* (Coleoptera : Coccinellidae)

Aphid populations are partially controlled by natural enemies. Coccinellids, such as *E. connexa*, are one of the most common aphid predators. Aphids which had been feeding on wheat seedlings of cultivars differing in Hx levels were offered *ad libitum* as food to *E. connexa* larvae. Aphids which had fed on cultivars containing higher Hx levels were associated with higher survival rates and shorter development times of the predator. This suggests that higher Hx levels in wheat may promote aphid control by this predator, as well as conferring a level of resistance to the plant (Martos *et al.*, 1992).

Availability of Germplasm for Breeding Programs to Increase Hx Levels in Wheat

Given that increased levels of Hx in wheat seems to be a desirable goal, germplasm which might lead to the accumulation of high levels of Hx in seedlings was sought. Analysis of a family of related British wheat cultivars showed that the production of agronomically improved cultivars had not resulted in a significant change in Hx concentration (Copaja *et al.*, 1991b). However, within the world's currently used wheat cultivars, a wide range of Hx levels (varying about eight-fold) was found, indicating the potential for Hx increase in agronomically suitable cultivars (Nicol *et al.*, 1992). Moreover, even higher Hx levels were found in wild relatives of wheat, opening the possibility for obtaining high-Hx wheats through wide hybridisation (Niemeyer 1988b; Thackray *et al.*, 1990a; Copaja *et al.*, 1991a).

Hx are absent in the seed of cereals, peak at the young seedling stage and decrease thereafter (Niemeyer, 1988a); young tissue in a given plant may accumulate Hx levels higher than the average concentration in the plant as a whole (Thackray *et al.*, 1990a). Hence, it also seems desirable to maintain sufficient Hx levels throughout the development of cereal plants, while ensuring their absence from the seed. The study of enzymes involved in the biosynthesis (Bailey & Larson, 1989) and degradation (Bailey & Larson, 1991; Cuevas *et al.*, 1992) of Hx may provide information needed for achieving this goal when combined with the aid of the methods of molecular biology. However, techniques which avoid the use of *Agrobacterium tumefaciens* as a vector may have to be implemented since its growth is inhibited by DIMBOA (Sahi *et al.*, 1990).

A related hydroxamic acid, DIBOA, is present in extracts of wild *Hordeum* species, but is absent from barley cultivars (Barria *et al.*, 1992). This observation quantitatively accounted for the reported performance of

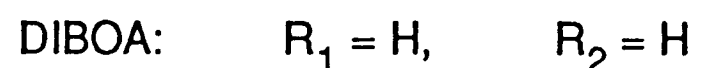
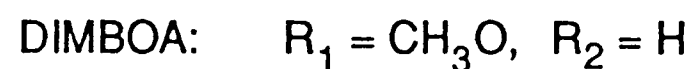
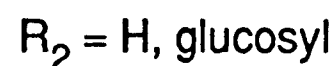
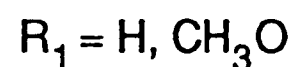
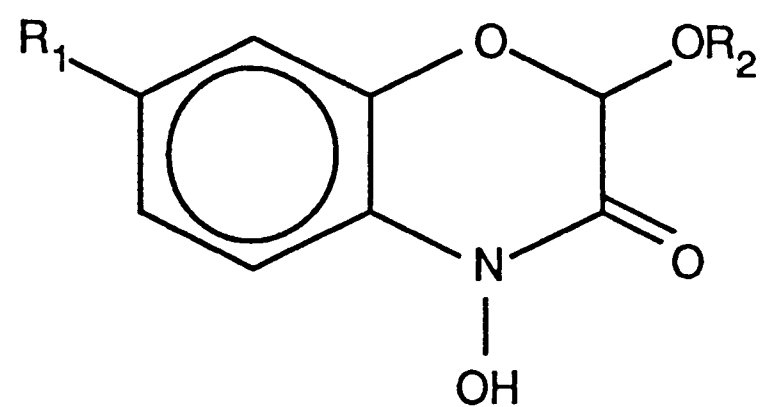


Figure 1 Main hydroxamic acids in the Gramineae

R. padi on wild and cultivated barley (Weibull, 1987). This result also suggests that it might be desirable to reinstate the complete biosynthetic pathway into commercial barleys.

Interestingly, Hx concentrations have been successfully used in the elucidation of the phylogeny of cultivated wheats (Figure 2). While there is agreement with the association of genome G to *T. speltoides*, genome A with *T. urartu*, and genome D with *T. tauschii*, the origin of genome B remains undetermined. Different lines of evidence have supported the association of this genome with each of the species constituting the Sitopsis section of the genus *Triticum*. Figure 2 shows that when two genomes are added to form a new higher ploidy species, an averaging out of Hx concentration occurs. Consequently, genome B may be associated with the accumulation of high concentrations of Hx. Analysis of accessions of each of the five members of the Sitopsis section showed that *T. bicorne*, *T. longistimum* and *T. searsii* accumulated low levels of DIBOA (mean values of 0.25, 0.48 and 1.27 mmol/kg fr. wt, respectively), *T. sharonense* accumulated low levels of DIMBOA (0.17) and only *T. speltoides* accumulated high levels of DIMBOA (5.59). Hence, the most likely donor of genome B of wheat is *T. speltoides* (Niemeyer *et al.*, 1992).

Conclusions

In this paper we have concentrated on one family of secondary chemicals in wheat and indicated how they can confer resistance on pest aphids. Our studies have shown that the chemical nature of the plant-insect interaction is very complex and involves a range of mechanisms which are still poorly understood. These

interactions vary with the species of aphid involved, the genetic origin of the host-plant, and ontological changes in the plant which may also interact with environmental conditions. The performance of natural enemies may also be a function of the particular mechanisms involved in these insect-plant interactions.

In the particular case of hydroxamic acids, the apparent utility of these chemicals in modifying the fitness of a broad range of organisms, and the observed level of variation in plant chemistry between lines of hosts indicate the importance of developing a clear understanding of the mechanisms of interaction. In more general terms, this is also true of the entire field of host plant resistance. Use of resistant plants is likely to become a core tactic in sustainable agriculture strategies and their full potential will only be achieved if greater attention is paid to basic studies in insect-plant interactions. To do this we need to develop interactive research teams, with strength in agronomy, insect behaviour, chemistry and ecology.

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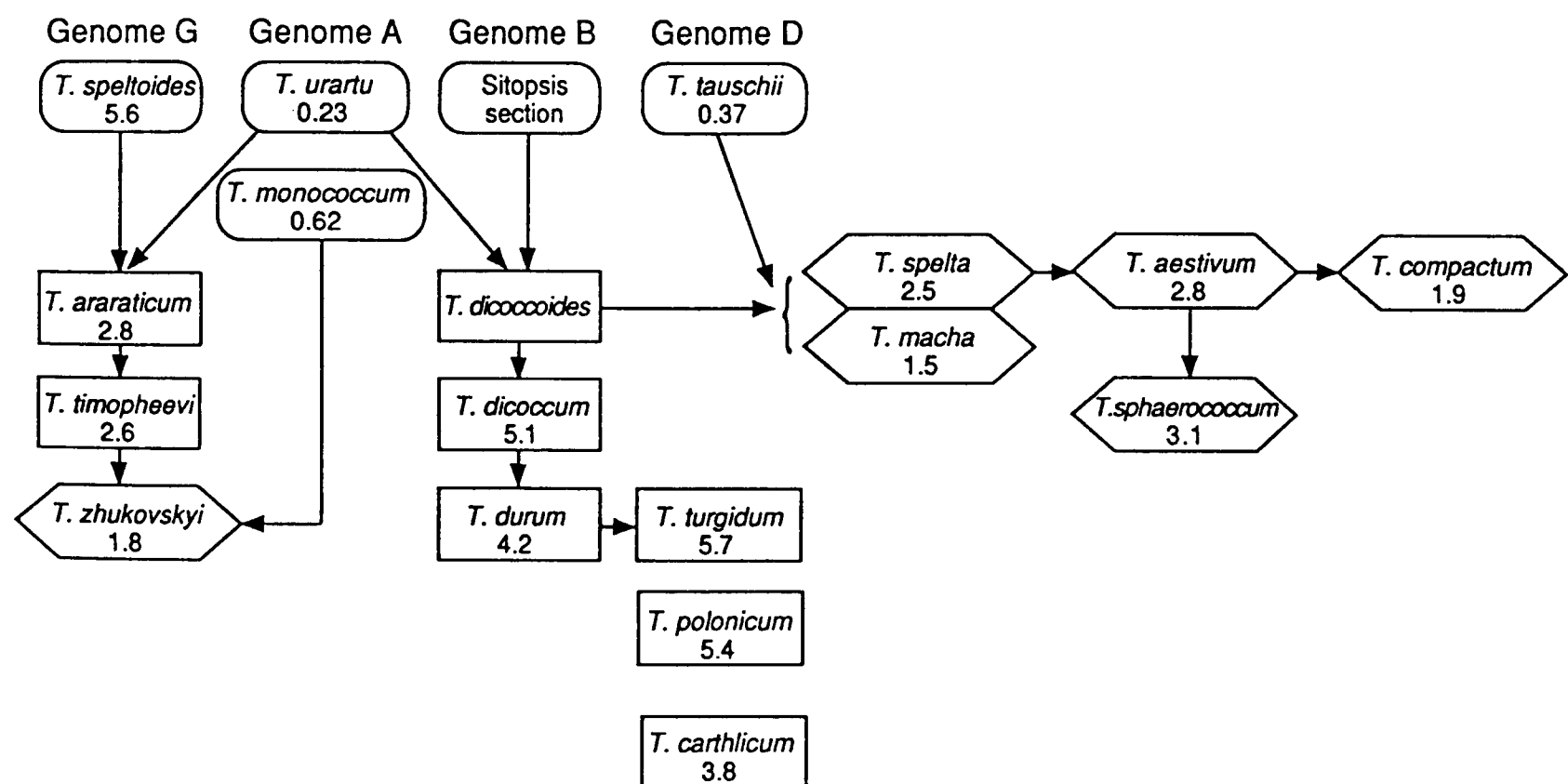


Figure 2 Phylogeny of cultivated wheats and mean hydroxamic acid levels (mmol/kg fr. wt)

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