

Possible involvement of the phloem sealing system in the acceptance of a plant as host by an aphid

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Received 28 September 1995; received after revision 12 April 1996; accepted 23 April 1996

Abstract. Possible reasons for the rejection of some lines of *Triticum monococcum* (Tm44 and Tm46) by the aphid *Sitobion avenae* were explored. In all *T. monococcum* lines studied, whether unfavourable (non-host/resistant plant) or favourable (host/susceptible plant), the concentrations of hydroxamic acids, a family of aphid-resistance factors in cereals, were significantly lower than the levels in the favourable host-plant *Triticum aestivum* cv. Therefore, hydroxamic acids did not account for the host/non-host patterns observed. Phloem sap was collected by stylectomy from young seedlings of favourable and unfavourable plants. In non-aphid-resistant genotypes, the success in stylectomy, the proportion of amputated stylets resulting in long (>1 min) exudations, the average duration of exudation, and the final volume of sap exuded, were higher than in the aphid-resistant genotypes. It is concluded that aphid interference with the phloem sealing system of the plant is a likely mechanism of rejection of *T. monococcum* lines Tm44 and Tm46 as hosts by *S. avenae*.

Key words. Aphids (*Sitobion avenae*); *Triticum* spp; phloem sap; secondary compounds (hydroxamic acids); stylectomy; aphid-plant interactions; plant resistance.

Although aphid-plant interactions have been extensively studied, the basic determinism underlying these interactions remains largely unknown, mainly owing to the complex nature of aphid feeding behaviour in contrast to that of other phytophagous insects. Aphid feeding involves not only the phloem sap, but also other plant tissues overlying the vascular tissues, which may interact with the insect mouth parts and therefore play an important role in the final acceptance of plants as suitable hosts [1, 2].

Until now, most research has concentrated on correlations between plant chemicals and aphid fitness, and have usually been performed using artificial diets, whole-plant extracts or whole-leaf extracts [3]. However, these substrates may not accurately reflect those encountered by aphids, either along their route to the sieve elements, or when ingesting sieve sap. Useful recently developed methods of studying the feeding behaviour of aphids and the chemical constitution of their food source include: 1) electronic feeding monitors which give information about the activities of aphids while their stylets are inside plant tissue [4, 5], and 2) phloem sap collection methods. These are being increasingly used in the evaluation of which compounds (mostly amino acids or/and secondary plant metabolites) might make certain plants suitable or unsuitable as hosts for aphids [6–8], and involve facilitation of exu-

dation by ethylene diamine tetraacetate chelation (EDTA method [9]) and the amputation of aphid stylets (stylectomy [10]).

An additional reason for the unsuitability of certain plants as aphid hosts may be mechanical interference when the aphid inserts its stylets into the plant [3]. Using transmission electron microscopy, Tjallingii and Hogen Esch showed recently that both the stylet canal of the aphid and most sieve elements of the plant studied contained an electron-dense mass of fibrous structure similar to phloem proteins (P-proteins) after gelation [11]. These proteins are known to play an important role in the phloem sealing system in dicotyledons: they are usually activated when plants are injured and, along with callose, result in sieve tubes being plugged [12, 13].

In this paper, we investigated the basis of rejection of some lines of an ancient diploid wheat by a cereal aphid. A high level of resistance to the cereal aphid *Sitobion avenae* Fabr. has been described in some lines of *Triticum monococcum* L. [14]. Resistance factors were shown to be located in the sieve elements and to inhibit the ingestion of sap by the aphid [15]. Either a chemical or a mechanical process was hypothesised. Possible chemical deterrence mechanisms in the phloem could include 1) the occurrence of a feeding deterrent or 2) the absence of a feeding stimulant, both of which would reduce the ingestion of nutrients by the aphid. A mechanical mechanism could involve the activation of the phloem sealing system of the plant, which would result in the blocking of sieve elements or/and in the plugging

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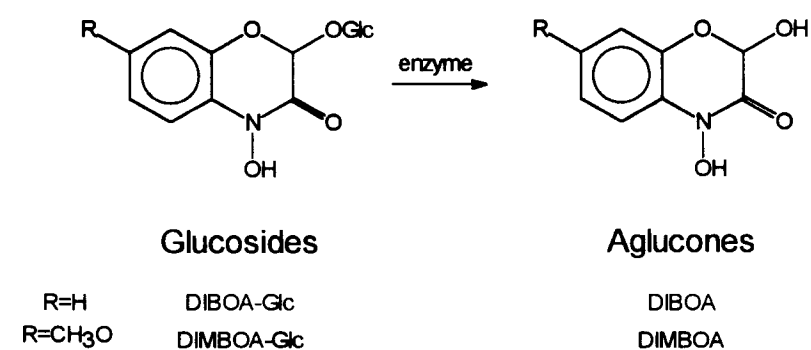


Figure 1. General structure of hydroxamic acids (Hx) from Gramineae. When the plant tissue is injured, the glucosides are hydrolysed to aglucones by endo- β -glucosidases.

of the aphids' stylets, thus reducing sap availability for ingestion. Considering the chemical mechanism, we studied a class of secondary compounds called hydroxamic acids (Hx) (fig. 1) since convincing relationships have been established between the concentration of these compounds and the resistance of wheat varieties to *S. avenae* [16–19]; these compounds have also been shown to occur in the phloem of *Triticum aestivum* [7]. With respect to the possible involvement of the phloem sealing system, we studied several characteristics of plant sap exudation by means of stylectomy of feeding aphids [8, 20].

Materials and methods

Four wheat genotypes were chosen on the basis of their host/non-host (i.e. susceptible/resistant) status, assessed in previous experiments [14, 21]: Arminda is a very favourable *T. aestivum* cultivar (susceptible; S1); Tm47 is the only favourable line of *T. monococcum* that has been described (S2); Tm46 and Tm44 are two unfavourable *T. monococcum* lines in which resistance mechanisms are different (resistant; respectively R1 and R2) [22]. Seedlings were used in the one- or two-leaf stage, i.e. when they were 8 to 10 days old and concentrations of Hx were close to the maximum attainable levels [23]. This growth stage is also close to that used in experiments designed to evaluate resistance levels in plants [14]. Aphids used were virginoparous apterae of *S. avenae* (clone Sar2, collected in 1987 in Brittany, France). Aphids were cultured on oat seedlings (*Avena sativa* L., cv. Nahuen) since this species lacks hydroxamic acids.

For the evaluation of Hx levels in susceptible and resistant wheat genotypes, three collection methods were considered. The first method, referred to as plant maceration (whole-leaf extracts), does not exclusively sample the phloem but was used for comparison with results attained by other authors. The first fully expanded leaf was cut and quickly weighed and macerated for 3 min in 1 ml of HCl-glycine buffer at pH 2. The extract was centrifuged at 12,000 g for 15 min and the supernatant was kept for analysis. The second tech-

nique, referred to as EDTA chelation [9], is known to produce large samples of phloem sap, and although some contaminants from tissues other than the sieve tubes may be included in the exudate, this method has been widely used to assay a wide range of other chemical compounds in the phloem sap of many plant species. The tops of four leaves (3–4 cm) were cut and quickly weighed and placed in 1.5 ml vials containing 200 μ l of a 5 mM EDTA solution. All vials were sealed with Parafilm and placed in a climatized room (85–90% relative humidity, 25 °C) for 20 h in darkness. Then the leaves were removed and the solutions kept for analysis. The third technique, referred to as stylectomy, allows for the collection of very pure phloem sap samples. Aphid stylets were amputated using an HF micro-cautery apparatus [10] in a climatized chamber room (20–26 °C; 65 to 90% relative humidity). Five aphids were allowed to settle on wheat seedlings for ca. 15 h. Stylets were cut from aphids that were feeding at the abaxial leaf surface, 4–5 cm from the top of the leaf, and on major veins. Only those aphids which showed a posture suggesting penetration of stylets in the plant tissues and effective feeding were used (this was attested by a very short proboscis, associated with antennae pointing backwards and honeydew production). The feeding aphid was approached frontally with an electrolytically sharpened needle prepared from 0.4 mm tungsten wire. Stylets were cut using a 0.2-s-pulse. A 0.5- μ l micropipet was then placed over the exuding stylets and the volume exuded was estimated every 15 min by measuring the length of the fluid column precisely, using a measuring eyepiece. The duration, final volume exuded and rate of exudation could therefore be evaluated. When exudation stopped, the micropipet was rinsed with 30 μ l of distilled water and the exudate collected in a small vial. For all methods, collections always began between 09.00 and 12.00 h and vials were kept at –20 °C until Hx analyses were performed. Hx concentrations in either macerated plants, EDTA extracts or pure phloem sap samples were determined using a high-performance liquid chromatograph (Shimadzu LC-9A). A 125-4 mm RP-18 column (Merek) was used, with a constant solvent flow (1.5 ml/min) and a constant proportion of solvents A (MeOH, 85%) and B (0.5 ml H₃PO₄ in 1 l H₂O, 15%) for 16 min. Detection of Hx was carried out at 263 nm. Retention times for DIMBOA-Glc, DIBOA and DIMBOA were 6.3, 10.8, and 12.4 min, respectively. Reference DIMBOA-Glc and DIMBOA were obtained from maize (*Zeamays* L. cv. Tracy T129) [24, 25]. DIBOA was synthesized as described elsewhere [26]. A peak appearing at 4.8 min was taken to correspond to DIBOA-Glc, on the basis of its position in the chromatogram relative to DIBOA. The results were subjected to a one-way analysis of variance (ANOVA) followed by the multiple mean comparison of Student-Newmans-Keuls test using the

SAS general linear procedures (SAS Institute). Data normality was checked prior to every ANOVA.

Results and discussion

Table 1 shows the mean hydroxamic acid content of extracts of macerated plants and table 2 the mean hydroxamic acid content of EDTA-exudates, both quantities expressed per kg of fresh weight of original plant tissue. Significant interspecific differences were found for all Hx studied, with the exception of DIBOA. The total concentration of Hx in *T. aestivum* cv. Arminda was two to three times higher than that in any of the *T. monococcum* lines. Previous work undertaken with other chemical methods for Hx analysis showed the same difference in Hx concentration between these two species [27, 28]. Susceptible and resistant lines of *T. monococcum* did not differ significantly in Hx levels. Some clear differences were found between the results from each collection method. First, no Hx were found in pure phloem sap samples collected by stylectomy from either susceptible or resistant plants (results not shown). This result contrasts with recent work in which Hx were found in the sieve sap collected using another cereal aphid, *Rhopalosiphum padi* L., feeding on *T. aestivum* [7]. The main difference between the *R. padi* experiment and this one is the size of the phloem sap samples, as most of the earlier work was carried out on 300 to 400 nl samples (Niemeyer, pers. commun.) while the present collections were between 9 and 124 nl. The

apparent contradiction may be explained by the failure to detect low levels of Hx in the small volumes of sap collected, or/and by intrinsically lower Hx levels in the cultivar Arminda. Second, lower concentrations of Hx in the glucosidic form and no Hx in the aglucone form were found in EDTA-exudates when compared with macerated plants. This is consistent with the presence of only Hx glucosides in phloem sap collected by stylectomy [7].

In spite of the differences in the results from the three collection methods employed, the major conclusion of this Hx analysis is that these secondary compounds fail to explain the host/non-host status of plants and consequently the resistance mechanisms present in Tm44 and Tm46. In contrast, stylectomy parameters from aphids feeding on each of the wheat genotypes showed a clear distinction between susceptible and resistant plants (fig. 2). The overall success ratio for amputation was higher for susceptible Arminda and Tm47 (80% and 69%, 31 and 37 attempts, respectively) than for resistant Tm46 and Tm44 (57% and 59%, 63 and 71 attempts, respectively) ($p = 0.0012$, $df = 59$). In the latter case, amputations often failed because aphids were more easily disturbed and tended to retract their stylets rapidly. A higher proportion of amputated stylets produced long (>1 min) exudations in the susceptible plants (48% and 36%, respectively) than in the resistant ones (22% and 19%, respectively) (fig. 2A) ($p = 0.0014$, $df = 59$). Amputation performed during behavioural events other than sap ingestion has been claimed not to

Table 1. Levels of hydroxamic acids in whole plant extracts of host (susceptible) and non-host (resistant) wheat genotypes. Mean \pm SE (mmol/kg fresh weight of plant tissue).^a

	Arminda (S1)	Tm47 (S2)	Tm46 (R1)	Tm44 (R2)	p (ANOVA) ^b
DIMBOA-Glc	1.21 \pm 0.21 ^a	0.29 \pm 0.51 ^b	0.22 \pm 0.35 ^b	0.25 \pm 0.29 ^b	0.01
DIMBOA	0.34 \pm 0.21 ^a	0.09 \pm 0.11 ^b	0.04 \pm 0.05 ^b	0.06 \pm 0.09 ^b	0.011
DIBOA-Glc	0.25 \pm 0.16	0.32 \pm 0.21	0.31 \pm 0.18	0.41 \pm 0.21	0.12
DIBOA	0.11 \pm 0.05 ^a	0.05 \pm 0.11 ^b	0.01 \pm 0.05 ^b	nd ^c	0.043
Total Hx	1.91 \pm 0.18 ^a	0.75 \pm 0.23 ^b	0.58 \pm 0.16 ^b	0.72 \pm 0.18 ^b	0.006

^aNumber of replicates per genotype = 16.

^bMeans within a row followed by the same letter are not significantly different at $\alpha = 0.05$.

^cnd = not detectable.

Table 2. Hydroxamic acids content of EDTA exudates from host (susceptible) and non-host (resistant) wheat genotypes. Mean \pm SE (mmol of hydroxamic acid exuded/kg fresh weight of plant tissue).^a

	Arminda (S1)	Tm47 (S2)	Tm46 (R1)	Tm44 (R2)	p (ANOVA) ^b
DIMBOA-Glc	0.17 \pm 0.15 ^a	0.04 \pm 0.04 ^b	0.04 \pm 0.05 ^b	0.02 \pm 0.05 ^b	0.03
DIMBOA	nd ^c	nd	nd	nd	-
DIBOA-Glc	0.03 \pm 0.04	0.04 \pm 0.06	0.04 \pm 0.05	0.05 \pm 0.06	0.4
DIBOA	nd	nd	nd	nd	-
Total Hx	0.21 \pm 0.18 ^a	0.09 \pm 0.11 ^b	0.08 \pm 0.1 ^b	0.08 \pm 0.1 ^b	0.04

^aNumber of replicates per genotype = 18 to 23.

^bMeans within a row followed by the same letter are not significantly different at $\alpha = 0.05$.

^cnd = not detectable.

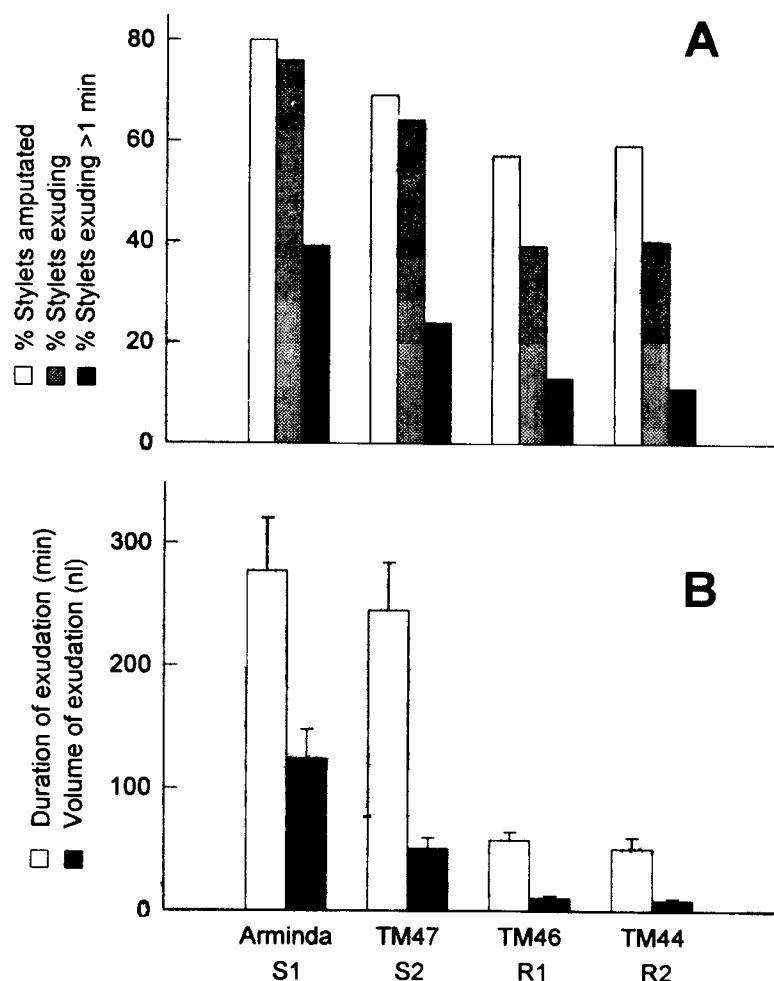


Figure 2. Stylectomy parameters obtained from *Sitobion avenae* feeding on host (susceptible, S1 and S2) and non-host (resistant, R1 and R2) wheat genotypes. Number of attempts to sever stylets were 31, 37, 69 and 71 respectively.

lead to exudation [8]. Hence, one explanation for the poor exuding ratio observed on Tm44 and Tm46 could be that aphids were not actually ingesting sieve element sap at the time of stylet amputation. This is possible since although the aphids did exhibit a general behaviour indicative of sap ingestion, feeding was not monitored accurately by methods such as electrical penetration graphs (DC-EPG) [4, 5]. A second explanation could be the occurrence of blocking reactions in sieve elements of resistant genotypes, which hinder phloem sap uptake flow. Some evidence supporting this latter suggestion is provided by the mean duration of all exudations that lasted longer than one minute: a highly significant difference occurred between susceptible plants, with a duration over 4 h, and resistant ones with a duration of less than 1 h (fig. 2B) ($p = 0.00025$, $df = 37$). As a result, the final volume of phloem sap exuded from Tm44 and Tm46 was consistently reduced when compared with that from susceptible plants ($p = 0.0016$, $df = 37$). It is worth noting that although Tm47 exhibited exudation periods comparable to those of Arminda, the final sap volume produced was less than half, presumably because of the lower rate of exudation generally observed on *T. monococcum* (less than 19 nl/h) as compared to *T. aestivum* (up to 34 nl/h).

In the light of these results, we conclude that host acceptance or rejection by *S. avenae* in the taxa studied is associated with the phloem sealing system of the

plant. In this context, it is of interest to consider the specificity of this aphid-plant interaction, that is, why cereal aphids other than *S. avenae* are not affected by Tm44 and Tm46 resistance and thus accept these genotypes as hosts (Di Piet pers. commun.), and what distinguishes the *T. monococcum* line Tm47 from Tm44 and Tm46 in terms of suitability for *S. avenae* feeding. Sealing systems in the phloem are assumed to prevent excessive bleeding when the sieve elements are severed and to regulate the flow of sap when necessary [12]. In dicotyledons, callose and P-proteins have been shown to accumulate rapidly in the sieve plate pores as a result of both the pressure release and the changes in the redox potential caused by injury to the sieve tubes [13], while in monocotyledons sealing of the sieve elements is achieved by callose and inclusions originating from plastids [29–31]. Given these plant defenses, the ability of aphids to ingest sieve sap is remarkable. Other authors have hypothesized that salivary components secreted by the aphid during feeding on a favourable plant may prevent P-protein gelation, thus enabling sieve-sap ingestion for long periods [11]. The protein subunits in sieve-tube exudates clearly differ from species to species [13, 32]. Aphid saliva characteristics also vary from one species to another [33], and even within biotypes (activity of pectinases [34]). Thus, some specificity in aphid-plant interactions could arise from compatible relationships between the phloem sealing system and the aphid saliva composition.

In the case of *S. avenae* feeding on resistant Tm44 and Tm46, the aphid might be unable to ingest sieve element sap because it does not affect 1) the redox potential resulting in plastid disruption and the release of proteinaceous inclusions or/and 2) the depolymerization of the callose lining the sieve plate. Cereal aphid species other than *S. avenae*, for example *R. padi*, whose performances and feeding behaviour are hardly affected on Tm44 and Tm46, might be able to prevent both reactions and thus are able to feed on these plants because of differences in their saliva. Indeed, we did verify that stylectomy parameters obtained from *R. padi* on resistant Tm44 resembled those found for *S. avenae* on susceptible Tm47 (Niemeyer, unpubl.). Finally, Arminda and Tm47 might be favourable to *S. avenae* because this aphid is able to overcome the phloem sealing system of these plants.

Recent work suggests that our general hypothesis is feasible. First, a β -1,3-glucanase, the enzyme involved in callose depolymerization, was found in aphids [35]. Second, oxidizing enzymes that could affect the redox system in plants are secreted by aphids [36]. Third, in interactions between plants and pathogens, resistance in some barley lines to the ml-o powdery mildew was associated with an enhanced callose deposition [37], and two different reports suggest that pathogenicity of the parasite is due to its ability to produce a β -1,3-glucanase

[38, 39]. Further investigations involving electron microscopy should allow an accurate study of the response of sieve elements to aphid feeding, and will help to assess the involvement of the plant sealing system in *T. monococcum* rejection or resistance to *S. avenae*.

Acknowledgements. The authors thank Drs Y. Rahbé and W. F. Tjallingii for their valuable criticisms on the manuscript and Amy Bouck for general English revisions. This work was supported by the ECOS committee (collaboration between France and Chile) (Action no. B9302) and the International Program in the Chemical Sciences at Uppsala University.

- 1 Klingauf F. A. (1988) Host-plant finding and acceptance. In: Aphids, Their Biology, Natural Enemies and Control, vol. 2B, pp. 209–223, Minks A. K. and Harrewijn P. (eds), Elsevier Science Publishers, Amsterdam
- 2 Klingauf F. A. (1988) Feeding, adaptation and excretion. In: Aphids, Their Biology, Natural Enemies and Control, vol. 2B, pp. 225–253, Minks A. K. and Harrewijn P. (eds), Elsevier Science Publishers, Amsterdam
- 3 Montllor C. B. (1993) Aphid feeding and nutrition. In: Insect-plant Interactions III, pp. 173–196, Bernays E. A. (ed.), CRC, Boca Raton, USA
- 4 Tjallingii W. F. (1988) Electronic recording of plant penetration behaviour by aphids. *Entomologia Exp. Appl.* **24**: 521–530
- 5 Tjallingii W. F. (1988) Electrical recording of stylet penetration activities. In: Aphids, Their Biology, Natural Enemies and Control, vol. 2B, pp. 95–108, Minks A. K. and Harrewijn P. (eds), Elsevier Science Publishers, Amsterdam
- 6 Grousse C., Bonnemain J. L., Delrot S. and Bournoville R. (1991) Sugar and amino acid composition of phloem sap of *Medicago sativa* – A comparative study of two collecting methods. *Plant Physiol. Biochem.* **29**: 41–48
- 7 Givovich A., Sandström J., Niemeyer H. M. and Pettersson, J. (1994) Presence of a hydroxamic acid glucoside in wheat phloem sap, and its consequences for performance of *Rhopalosiphum padi* (L.) (Homoptera: Aphididae). *J. Chem. Ecol.* **20**: 1923–1930
- 8 van Helden M., Tjallingii W. F. and van Beek T. A. (1994) Phloem sap collection from lettuce (*Lactuca sativa* L.): methodology and yield. *J. Chem. Ecol.* **20**: 3173–3190
- 9 King R. W. and Zeevaart J. A. D. (1974) Enhancement of phloem exudation from cut petioles by chelating agents. *Plant Physiol.* **53**: 96–103
- 10 Unwin F. M. (1978) A versatile high frequency microcautery. *Physiol. Entomol.* **3**: 71–73
- 11 Tjallingii W. F. and Hogen Esch Th. (1993) Fine structure of the stylet route in plant tissue by some aphid species. *Physiol. Entomol.* **18**: 317–328
- 12 Eschrich W. (1975) Sealing systems in phloem. In: Encyclopedia of Plant Physiology, vol. 1. Transport in Plants I. Phloem Transport, pp. 39–54, Zimmermann M. H. and Milburn J. A. (eds), Springer Verlag, Berlin
- 13 Evert R. F. (1990) Dicotyledons. In: Sieve Elements: Comparative Structure, Induction and Development, pp. 103–137, Behnke H. D. and Sjölund R. D. (eds), Springer, Berlin
- 14 Di Pietro J. P., Soster C., Chaubet B. and Caillaud C. M. (1993) The resistance of different lines of *Triticum* species to the aphid *Sitobion avenae*. *Bull. International Organisation for Biological Control* **16**: 110–116
- 15 Caillaud C. M., Pierre J. S., Chaubet J. P. and Di Pietro J. P. (1995) Analysis of wheat resistance to the cereal aphid *Sitobion avenae* using electrical penetration graphs and flowcharts combined with correspondence analysis. *Entomologia Exp. Appl.* **75**: 9–18
- 16 Bohidar K., Wratten S. D. and Niemeyer H. M. (1986) Effects of hydroxamic acids on the resistance of wheat to the aphid *Sitobion avenae*. *Ann. Appl. Biol.* **109**: 193–198
- 17 Niemeyer H. M. (1988) Hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-ones), defence chemicals in the gramineae. *Phytochemistry* **27**: 3349–3358
- 18 Givovich A. and Niemeyer H. M. (1995) Effect of hydroxamic acids on feeding behaviour and performance of cereal aphids on wheat. *Eur. J. Entomol.* **91**: 371–374
- 19 Givovich A. and Niemeyer H. M. (1995) Comparison of the effect of hydroxamic acids from wheat on five species of cereal aphids. *Entomologia Exp. Appl.* **74**: 115–119
- 20 Grousse C. and Bournoville R. (1994) Role of phloem sap quality and exudation characteristics on performance of pea aphid grown on lucerne genotypes. *Entomologia Exp. Appl.* **70**: 227–235
- 21 Caillaud C. M., Dedryver C. A. and Simon J. C. (1994) Development and reproductive potential of the cereal aphid *Sitobion avenae* on resistant wheat lines (*Triticum monococcum*). *Ann. Appl. Biol.* **125**: 219–232
- 22 Caillaud C. M., Di Pietro J. P., Chaubert B. and Pierre J. S. (1995) Application of discriminant analysis to electrical penetration graphs of the aphid *Sitobion avenae* feeding on resistant and susceptible wheat. *J. Appl. Entomol.* **119**: 103–106
- 23 Argandoña V. H., Luza J. G., Niemeyer H. M. and Corcuera L. J. (1980) Role of hydroxamic acids in the resistance of cereals to aphids. *Phytochemistry* **19**: 1665–1668
- 24 Lyons P. C., Hipskind J. D., Wood K. V. and Nicholson R. L. (1988) Separation and quantification of cyclic hydroxamic acids and related compounds by high-pressure liquid chromatography. *J. Agric. Food Chem.* **36**: 57–60
- 25 Queirolo C. B., Andreo C. S., Niemeyer H. M. and Corcuera L. J. (1983) Inhibition of ATPase from chloroplasts by an hydroxamic acid from the gramineae. *Phytochemistry* **22**: 2455–2458
- 26 Jernow J. L. and Rosen P. (1975) 2H-1,4-benzoxazin-3(4H)-ones. U.S. Patent 3,862,180; 8 pp
- 27 Niemeyer H. M. (1988) Hydroxamic acids content of *Triticum* species. *Euphytica* **37**: 289–293
- 28 Niemeyer, H. M., Copaja, S. V. and Barria, B. N. (1992) The triticae as sources of hydroxamic acids, secondary metabolites in wheat conferring resistance against aphids. *Hereditas* **116**: 295–299
- 29 Walsh M. A. and Melaragano J. E. (1981). Structural evidence for plastid inclusions as a possible 'sealing' mechanism in the phloem of monocotyledons. *J. Exp. Bot.* **32**: 311–320
- 30 Eleftheriou E. P. (1984) Sieve-element plastids of *Triticum* and *Aegilops* (Poaceae). *Plant Syst. Evol.* **145**: 119–133
- 31 Eleftheriou E. P. (1990) Monocotyledons. In: Sieve Elements: Comparative Structure, Induction and Development, pp. 139–159, Behnke H. D. and Sjölund R. D. (eds), Springer Verlag, Berlin
- 32 Toth K. F. and Sjölund R. D. (1994) Monoclonal antibodies against phloem-P-protein from plant tissue cultures. *Am. J. Bot.* **8**: 1378–1383
- 33 Miles P. W. (1987) Feeding processes of Aphidoidea in relation to effects on their food plants. In: Aphids, Their Biology, Natural Enemies and Control, vol. 2C, pp. 321–339, Minks A. K. and Harrewijn P. (eds), Elsevier Science Publishers, Amsterdam
- 34 Campbell B. C. and Dryer D. L. (1985) Host-plant resistance of sorghum: differential hydrolysis of sorghum pectic substances by polysaccharases of greenbug *biotypes* (*Schizaphis graminum*, Homoptera Aphididae). *Arch. Insect Biochem. Physiol.* **2**: 203–215
- 35 Dreyer D. L. and Campbell B. C. (1987) Chemical basis of host-plant resistance to aphids. *Plant Cell Environ.* **10**: 353–361
- 36 Peng Z. and Miles P. W. (1988) Studies on the salivary physiology of plant bugs: function of the catechol oxidase of the rose aphid. *J. Insect Physiol.* **34**: 1027–1033
- 37 Skou J. P. (1985) On the enhanced callose deposition in barley with ml-o powdery mildew resistance genes. *Phytopathol. Z.* **112**: 207–216
- 38 van Hoof A., Leykam J., Schaeffer H. J. and Walton J. D. (1991) A single beta 1,3-glucanase secreted by the maize pathogen *Cochliobolus carbonum* acts by an exolytic mechanism. *Physiol. Molec. Plant Pathol.* **39**: 259–267
- 39 Brockmann B., Smit R. and Tudizynski P. (1992) Characterization of an extracellular beta 1,3-glucanase of *Claviceps purpurea*. *Physiol. Molec. Plant Pathol.* **40**: 191–201