Acta Agric. Scand., Sect. B, Soil and Plant Sci. 1993: 43, 163–167
Printed in Denmark . All rights reserved

ACTA AGRICULTURÆ SCANDINAVICA
ISSN 0906-4710

Potential of Hydroxamic Acids in Breeding for Aphid Resistance in Wheat

Escobar, C. A. and Niemeyer, H. M. (Departamento de Química, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile). Potential of hydroxamic acids in breeding for aphid resistance in wheat. Accepted January 20, 1993. Acta Agric. Scand., Sect. B, Soil and Plant Sci. 43: 163–167, 1993. © Acta Agric. Scand. 1993.

Swedish wheats were analysed at the seedling stage for hydroxamic acids (Hx), a family of natural aphid resistance factors. Analysis comprised the historical development from old landraces to modern cultivars, and included cultivars in the Swedish National List of Cultivars for 1992–93. Spring and winter wheats contained similar average Hx concentrations, 2.12 ± 0.801 ($n=26$) and 2.37 ± 0.816 ($n=49$) mmol/kg fr. wt, respectively. Cultivars recommended for 1992–93 showed lower levels of Hx, 1.14 ± 0.574 ($n=7$) and 1.97 ± 0.751 ($n=8$), respectively. Breeding wheat for higher Hx levels as a means of obtaining increased aphid resistance is discussed.

Carlos A. Escobar and Hermann M. Niemeyer
Departamento de Química, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile

Key word: DIMBOA.

Introduction

Hydroxamic acids (Hx) are a family of secondary metabolites of cereals discovered over three decades ago in relation to fungal diseases of rye (Virtanen & Hietala, 1960). They are present in wheat and maize, as well as in a wide range of wild Gramineae (Niemeyer, 1988a; 1988b; Zúñiga et al., 1983; Copaja et al., 1991a; Barría et al., 1991; Copaja et al., 1991b; Niemeyer et al., 1992). Hx exist in the intact plant as glucosides which, upon injury of the plant tissue, are transformed to the more toxic aglucones by an *endo*-β-glucosidase (Hofman & Hofmanova, 1969; Cuevas et al., 1992). The most abundant aglucone in wheat and maize extracts is 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) (Niemeyer, 1988a).

Hx in maize have been associated with resistance to the European corn borer, *Ostrinia nubilalis* (Hübner). This resistance has been enhanced through breeding for high Hx levels (Grombacher et al., 1986; Guthrie et al., 1986).

Hx have been shown to be a major biochemical mechanism of resistance of wheat to aphids, acting through antibiosis and feeding deterrency. Thus, negative correlations have been described between Hx levels in the plants and growth rate and intrinsic rate of natural increase of cereal aphid populations (Argandoña et al., 1980; Corcuera et all, 1982; Bohidar et al., 1986; Thackray et al., 1990a), and mean relative growth rate of aphids (Thackray et al., 1990b). Population increase of aphids feeding on excised barley leaves (originally lacking Hx) into which different levels of DIMBOA had been incorporated produced similar negative correlations (Argandoña et al., 1980). Survival of cereal aphids in artificial diets decreased with increasing DIMBOA concentrations in them (Corcuera et al., 1983; Argandoña et al., 1983).

In choice tests, both winged and wingless *Rhopalosiphum padi* L. preferentially settled on seedlings with lower Hx levels (Nicol et al., 1992; Givovich & Niemeyer, 1991). Electronic monitoring of aphid feeding behavior showed that in seedlings with higher Hx levels, fewer aphids reached the phloem within a given time, and they required longer times to contact a phloem vessel (Givovich & Niemeyer, 1991). This feeding deterrency decreased transmission of barley yellow dwarf virus to wheat seedlings (Givovich & Niemeyer, 1991).

Aphids are important pests of wheat in Sweden, causing losses in the neighbourhood of 10 million Swedish crowns per year (Mörner & Sigvald, 1989). This paper presents a screening of Swedish wheats for Hx concentrations in relation to prospects for breeding for aphid resistance.

Material and methods

Seeds were obtained from Svalöf AB, Nordiska Genbanken and W. Weibull AB. Twenty seeds of each accession were sown in batches of ten in two 6 cm diameter pots containing vermiculite and kept in a plant growth chamber at 25°C, 70% RH, with a 12 h photophase. Six days after planting, seedlings in decimal growth stage 10 (Zadoks et al., 1974) and measuring between 5 and 8 cm, were harvested. The fresh material was weighed (the aerial part of one seedling constituted one pseudo-replicate; four pseudo-replicates were analysed of each accession), and an aqueous extract produced which was submitted to high performance liquid chromatography on a reversed-phase column. Hx were quantified in the eluent with a UV-VIS detector (Niemeyer et al., 1989). DIMBOA was the only Hx detected under the conditions employed. Standard errors of the mean calculated for each set of pseudo-replicates were always lower than 15%, and were omitted from the figures for clarity.

Results and discussion

Concentrations of DIMBOA in extracts of a set of Swedish spring and winter wheats starting from the Scandinavian local varieties (old landraces) and ending with modern cultivars, are shown in Figs. 1 and 2. Mean DIMBOA concentrations were 2.12 ± 0.801 mmol/kg fr. wt for spring wheats ($n = 26$) and 2.37 ± 0.816 mmol/kg fr. wt for winter wheats ($n = 49$). These values compare poorly with those found within a set of wheat cultivars currently used around the world (mean DIMBOA concentration 3.20 ± 1.540, $n = 47$), which included cultivars with up to 8 mmol/kg fr. wt (Nicol et al., 1992).

Mean DIMBOA concentrations in the Swedish National List of Cultivars for 1992–93 were 1.14 ± 0.574 mmol/kg fr. wt for spring wheats ($n = 7$) and 1.97 ± 0.751 mmol/kg fr. wt for winter wheats ($n = 8$). The value for currently listed spring wheats is

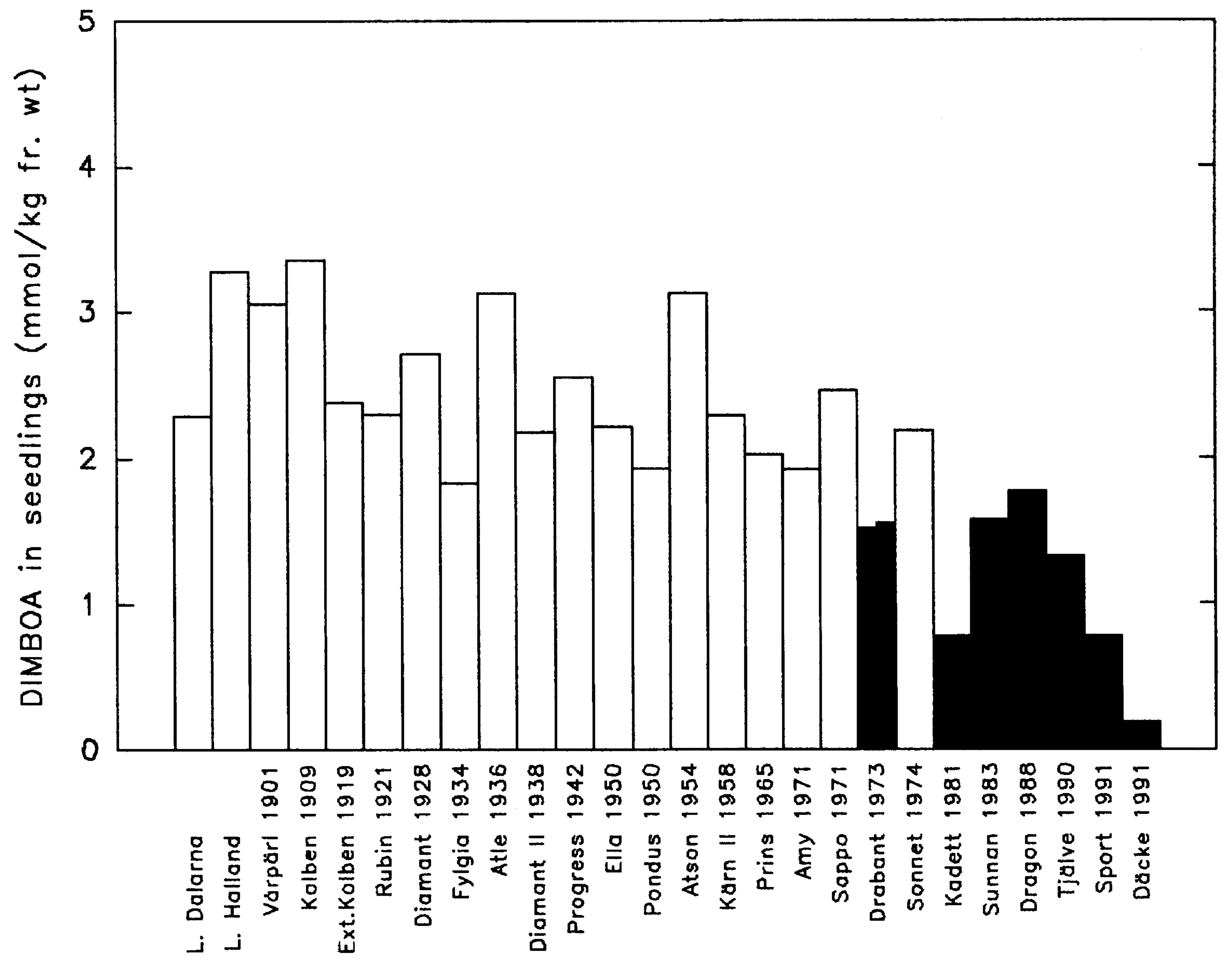

Fig. 1. DIMBOA concentrations in 6-day-old seedlings of Swedish spring wheats; ■ = cultivars in the Swedish National List of Cultivars for 1992–93. The year of release of the cultivars is indicated.

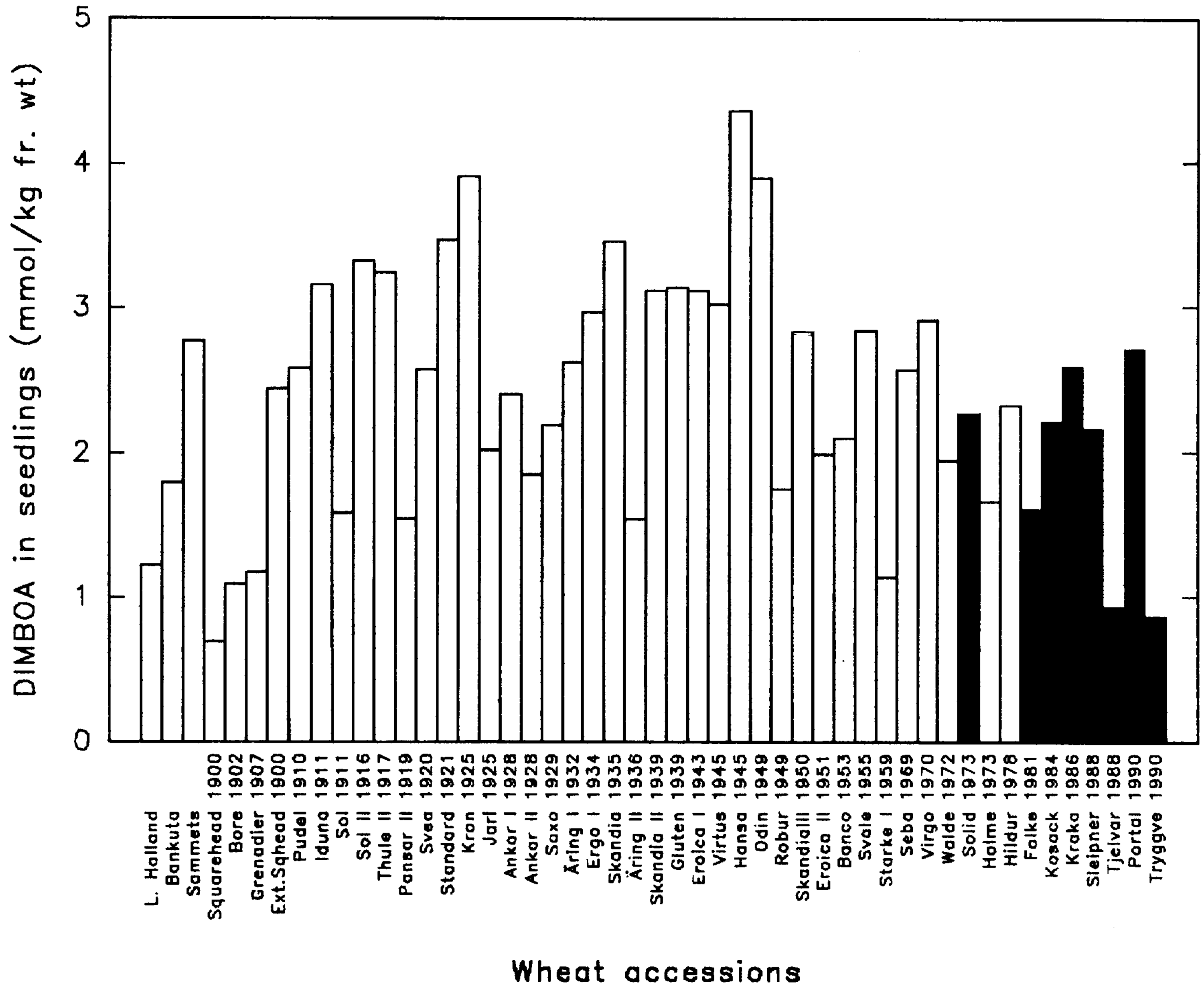

Fig. 2. DIMBOA concentrations in 6-day-old seedlings of Swedish winter wheats; ■ = cultivars in the Swedish National List of Cultivars for 1992–93. The year of release of the cultivars is indicated.

considerably lower than the overall average for spring wheats (Fig. 1). While the leading cultivars for the 1992–93 season are the spring wheat Dragon and the winter wheat Kosack, which are among the highest in DIMBOA levels within the Swedish National List of Cultivars for 1992–93, the two new high-protein spring wheat varieties, Dacke and Sport, have exceptionally low concentrations of DIMBOA.

The values reported show that there is potential for increase in Hx concentrations in Swedish wheats through standard breeding practices using old Swedish cultivars or cultivars from elsewhere in the world. Even higher Hx levels were found among wild relatives of wheat, thus providing the possibility of increasing Hx in wheat through wide hybridisation (Niemeyer, 1988b; Thackray et al., 1990a; Copaja et al., 1991a).

Several lines of argument point to the usefulness of hydroxamic acids in aphid resistance: (i) Hx are capable of reducing aphid populations through antibiosis (Bohidar et al., 1986; Thackray et al., 1990a; 1990b) and antixenosis (Nicol et al. 1992), and of decreasing infection of wheat by barley yellow dwarf virus through feeding deterrency (Givovich & Niemeyer, 1991); (ii) the development time of the predatory ladybird *Eriopis connexa* (Germar.) is shorter and the number of aphids ingested higher when the beetle feeds on aphids from a high-Hx wheat cultivar as compared with an intermediate-Hx cultivar (Martos et al., 1992), suggesting that higher levels in wheat could potentiate the beneficial effects of the predator; (iii) Hx have been shown to be involved in allelopathic effects, and are thus of potential benefit for weed control (Barnes et al., 1987; Pérez, 1990; Pérez & Ormeño-Núñez, 1991); (iv) sub-lethal doses of insecticide were more effective on aphids feeding on a high-Hx wheat cultivar than on a low one (Nicol et al., 1993); (v) non-volatile chemical defences naturally existing in a plant are more friendly to the environment, since they need not be sprayed and are normally retained within the plant. In the case of Hx in wheat, they are not present in the seed (Niemeyer, 1988a) and hence do not represent a hazard to human health, and their incorporation into the soil through crop residues is unlikely to be of significance owing to the decrease of Hx levels in older plant tissue.

Thus, the increase of Hx levels in wheat might bring about a number of beneficial effects within an integrated pest management strategy. A relatively small increase in host plant resistance can reduce the probability of a pest outbreak, especially in combination with the action of natural enemies, even without pesticide use (van Emden & Wratten, 1991).

The economic losses associated with the possible yield penalty for producing higher levels of defence chemicals in an annual crop may be compensated for by a reduction in the input of agrochemicals (Lovett, 1980a; 1980b).

Hydroxamic acids are absent from the seed, increase upon germination (peaking at the young seedling stage) and decrease thereafter (Niemeyer, 1988a). This decrease has been used as an argument against the usefulness of Hx in aphid resistance. However, although concentrations in the plant as a whole do decrease, the youngest tissue in a wheat plant retains a high concentration of Hx (Argandoña et al., 1980; Argandoña et al., 1981; Zuñiga et al., 1983; Niemeyer, 1988b; Thackray et al., 1990a; Copaja et al., 1991a), and thus Hx may have important consequences for defence against aphids feeding on young plant tissue. For example, a negative correlation has been found between mean relatively growth rate of *Sitobion avenae* (Fabr.) and Hx concentration in flag leaves of wheat plants (Leszczynski et al., 1989).

Emergence of resistant biotypes of aphids is a common feature of aphid–host plant interactions (Puterka & Burton, 1991). Intense selective pressure on aphid populations may be avoided by incorporating into the breeding program other resistance mechanisms such as non-glaucousness and phenols, both of which have been suggested to play a role in wheat resistance against *S. avenae* (Lowe et al., 1985; Leszczynski et al., 1989).

The establishment of breeding programs aimed at increasing Hx levels in wheat seems justified. Alternatively, if techniques of genetic engineering are chosen which involve the use of *Agrobacterium tumefaciens* as a vector, care should be exercised, since DIMBOA shows adverse effects on populations of *A. tumefaciens* and on the induction of virulence genes in the presence of acetosyringone (Sahi et al., 1990).

Acknowledgements

Grants from the International Program in the Chemical Sciences, Uppsala University (IPICS), the Swedish Agency for Research Cooperation with Developing Countries (SAREC), and Fondo Nacional de Ciencia y Tecnología (FONDECYT) are gratefully acknowledged. The authors are indebted to Dr. Thomas Jonasson (formerly at Svalöf AB), Ms. Birgitte Lund (Nordiska Genbanken) and Dr. Gunnar Svensson (W. Weibull AB) for providing the seeds, to Drs. Jens Weibull, S.D. Wratten and Inger Ahman for helpful criticism of the manuscript, and to Dr. Arnulf Merker for his interest in this work.

References

Argandoña, V. H,., Corcuera, L. J., Niemeyer, H. M. & Campbell, B. C. 1983. Toxicity and feeding deterrency of hydroxamic acids from Gramineae in synthetic diets against the greenbug, *Schizaphis graminum.* Entomol. Exp. Appl. 34, 134–138.

Argandoña, V. H., Luza, J. G., Niemeyer, H. M. & Corcuera, L. J. 1980. Role of hydroxamic acids in the resistance of cereals to aphids. Phytochemistry 19, 1665–1668.

Argandoña, V. H., Niemeyer, H. M. & Corcuera, L. J. 1981. Effect of content and distribution of hydroxamic acids in wheat on infestation by *Schizaphis graminum.* Phytochemistry 20, 673–676.

Barnes, J. P., Putnam, A. R., Burke, B. A. & Aasen, A. J. 1987. Isolation and characterization of allelochemicals in rye herbage. Phytochemistry 26, 1385–1390.

Barría, B. N., Copaja, S. V. & Niemeyer, H. M. 1991. Occurrence of DIMBOA in wild *Hordeum* species and relation to aphid resistance. Phytochemistry 31, 89–91.

Bohidar, K., Wratten, S. D. & Niemeyer, H. M. 1986. Effects of hydroxamic acids on the resistance of wheat to the aphid *Sitobion avenae.* Ann. Appl. Biol. 109, 193–198.

Copaja, S. V., Barría, B. N. & Niemeyer, H. M. 1991a. Hydroxamic acid content of perennial Triticeae. Phytochemistry 30, 1531–1534.

Copaja, S. V., Niemeyer, H. M. & Wratten, S. D. 1991b. Hydroxamic acid levels in Chilean and British wheat seedlings. Ann. Appl. Biol. 118, 223–237.

Corcuera, L. J., Argandoña, V. H. & Niemeyer, H. M. 1982. Effect of cyclic hydroxamic acids from cereals on aphids. In: Kehl, H. (ed). Chemistry and Biology of Hydroxamic Acids, Karger AG, Basel, pp. 111–118.

Cuevas, L., Niemeyer, H. M. & Jonsson, L. M. V. 1992. Partial purification and characterization of a hydroxamic acid glucoside β-D-glucosidase from maize. Phytochemistry 31, 2609–2612.

Van Emden, H. F. & Wratten, S.D. 1991. Tri-trophic interactions involving plants in the biological control of aphids. In: Peters, D. C., Webster, J. A. & Chlouber, C. S. (eds.) Aphid–Plant Interactions: Populations to Molecules. MP-132, USDA Agricultural Research Service, Oklahoma State University, pp. 29–43.

Givovich, A. & Niemeyer, H. M. 1991. Hydroxamic acids affecting barley yellow dwarf virus transmission by the aphid *Rhopalosiphum padi.* Entomol. Exp. Appl. 59, 79–85.

Grombacher, A. W., Russell, W. A. & Guthrie, W. D. 1986. Resistance to first-generation European corn borer (Lepidoptera: Pyralidae) and DIMBOA concentration in midwhorl leaves of the BS9 maize synthetic. J. Kansas Ent. Soc. 62, 103–107.

Guthrie, W. D., Tseng, C. T., Russell, W. A., Coats, J. R., Robbins, J. C. & Tollefson, J. J. 1986. DIMBOA content at seven stages of plant development in a maize synthetic cultivar. J Kansas Ent. Soc. 59, 356–360.

Hofman, J. & Hofmanova, O. 1969. 1,4-Benzoxazine derivatives in plants. Sephadex fractionation and identification of a new glucoside. Eur. J. Biochem. 8, 109–112.

Leszczynski, B., Wright, L. C. & Bakowski, T. 1989. Effect of plant secondary substances on winter wheat resistance to the grain aphid. Entomol. Exp. Appl. 52, 135–139.

Lovett, J. V. 1980a. Rational use of agro-chemicals. In: Lovett, J. V. & So, H. B. (eds.) Soil Management, Energy Use and Crop Production, University of New England Printing Unit, Australia, pp. 181–208.

Lovett, J. V. 1980b. Annual field crops. In: Perspectives in World Agriculture, compiled by Commonwealth Agricultural Bureaux, Farnham Royal, pp. 91–122.

Lowe, H. J. B., Murphy, G. J. P. & Parker, M. L. 1985. Non-glaucousness, a probable aphid-resistance character of wheat. Ann. Appl. Biol. 106, 555–560.

Martos, A., Givovich, A. & Niemeyer, H. M. 1992. Effect of DIMBOA, an aphid resistance factor in wheat, on the aphid predator *Eriopis connexa* Germar (Coleoptera: Coccinellidae). J. Chem. Ecol. 18, 469–479.

Mörner, J. & Sigvald, R. 1989. Estimation of crop losses due to pests and diseases in Sweden. Nordic Plant Protection Conference (Copenhagen), pp. 111–118.

Nicol, D., Copaja, S. V., Wratten, S. D. & Niemeyer, H. M. 1992. A screen of worldwide wheat cultivars for hydroxamic acid levels and aphid antixenosis. Ann. Appl. Biol. 121, 11–18.

Nicol, D., Eaton, N., Wratten, S. D. & Copaja, S. V. 1993. Effects of DIMBOA levels in wheat on the susceptibility of the grain aphid (*Sitobion avenae*) to deltamethrin. Ann. Appl. Biol., in press.

Niemeyer, H. M. 1988a. Hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-ones), defence chemicals in the Gramineae. Phytochemistry 27, 3349–3358.

Niemeyer, H. M. 1988b. Hydroxamic acid content of *Triticum* species. Euphytica 37, 289–293.

Niemeyer, H. M., Copaja, S. V. & Barría, B. N. 1992. The Triticeae as sources of hydroxamic acids, secondary metabolites in wheat conferring resistance against aphids. Hereditas 116, 295–299.

Niemeyer, H. M., Pesel, E., Copaja, S. V., Bravo, H. R., Franke, S. and Francke, W. 1989. Changes in hydroxamic acid levels of wheat plants induced by aphid feeding. Phytochemistry 28, 447–449.

Pérez, F. J. 1990. Allelopathic effects of hydroxamic acids on *Avena sativa* and *A. fatua.* Phytochemistry 29, 773–776.

Pérez, F. J. & Ormeño-Núñez, J. (1991) Difference in hydroxamic acid content in roots and root exudates of wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.): possible role in allelopathy. J. Chem. Ecol. 17, 1037–1043.

Puterka, G. J. & Burton, R. L. 1991. Aphid genetics in relation to host plant resistance. In: Peters, D. C., Webster, J. A. & Chlouber, C. S. (eds.) Aphid-Plant Interactions: Populations to Molecules. MP-132, USDA Agricultural Research Service, Oklahoma State University, pp. 59–69.

Sahi, S. V., Chilton, M.-D. & Chilton, W. S. 1990. Corn metabolites affect the growth and virulence of *Agrobacterium tumefaciens.* Proc. Natl. Acad. Sci. USA 87, 3879–3883.

Thackray, D. J., Wratten, S. D., Edwards, P. J. & Niemeyer, H. M. 1990a. Resistance to the aphids *Sitobion avenae* and *Rhopalosiphum padi* in Gramineae in relation to hydroxamic acid levels. Ann. Appl. Biol. 116, 573–582.

Thackray, D. J., Wratten, S. D., Edwards, P. J. & Niemeyer, H. M. 1990b. Hydroxamic acids – potential resistance factors in wheat against the cereal aphids *Sitobion avenae* and *Rhopalosiphum padi.* Proceedings of the 1990 Brighton Crop Protection Conference, Pests and Diseases, pp. 215–220.

Virtanen, A. I. & Hietala, P. K. 1960. Precursors of benzoxazolinone in rye plants. I. Precursor II, the aglucone. Acta Chem. Scand. 14, 499–502.

Zadoks, J. C., Chang, T. T. & Konsak, C. F. 1974. A decimal code for the growth stage of cereals. Weed Res. 14, 415–421.

Zúñiga, G. E., Argandoña, V. H., Niemeyer, H. M. & Corcuera, L. J. 1983. Hydroxamic acid content in wild and cultivated Gramineae. Phytochemistry 22, 2665–2668.