Hydroxamic Acids in *Secale cereale* L. and the Relationship with their Antifeedant and Allelopathic Properties

Sylvia V. Copaja[a,*], Elisa Villarroel[a], Héctor R. Bravo[a], Lorena Pizarro[b], and Víctor H. Argandoña[b]

[a] Departamento de Química, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile. Fax: 56(2)2713888. E-mail: scopaja@uchile.cl
[b] Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile

* Author for correspondence and reprint requests

Z. Naturforsch. **61 c**, 670–676 (2006); received December 27, 2005/March 14, 2006

Contents of the hydroxamic acids 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA), and 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) in leaves and roots of 14 cultivars of rye, *Secale cereale* L., were determined. Dynamics of accumulation in three cultivars were evaluated. DIBOA was the main cyclic hydroxamic acid in leaves but the contents differed significantly between the cultivars. Both DIBOA and DIMBOA were present in the roots. Maximum concentration of DIBOA in leaves and DIMBOA in roots was reached between 48–54 h and 54–72 h after germination, respectively. Antifeedant activity of DIBOA towards the aphid *Rhopalosiphum padi* and the feeding behavior were studied by electronic recording in barley leaves treated with different contents of DIBOA. The deleterious activity of DIBOA could arise by starvation and/or a toxic effect. Additionally, allelopathic potential of pure DIBOA and aqueous extracts of leaves and roots of rye (Tetra-Baer) on the germination of lettuce (*Lactuca sativa*) and rye (Tetra-Baer) seeds was evaluated. A high percentage of germination inhibition of pure DIBOA and the extracts of leaves and roots was observed. The activity is in agreement with the contents of hydroxamic acids in the plants. The substrates had no allelopathic effect on rye seeds.

Key words: Hydroxamic Acids, Antifeedant, Allelopathy

Introduction

Cereals of great agricultural importance, like wheat, maize and rye, produce hydroxamic acids (Hx), a family of secondary metabolites discovered over three past decades as inhibitors of fungal diseases of rye (Virtanen and Hietala, 1960). They are also present in a wide range of wild Gramineae (Zúñiga *et al*., 1983; Barría *et al*., 1991; Copaja *et al*., 1991; Niemeyer *et al*., 1992) and other higher plants (Pratt *et al*., 1995; Bravo and Copaja 2002; Alipieva *et al.,* 2003; Bravo *et al*., 2004a, 2005). Hx exist in the intact plant as glucosides which, upon injury of plant tissue, are transformed to toxic aglucones by glucosidase (Hofman and Hofmanová, 1969; Cuevas *et al*., 1992). The main Hx found in rye extracts is DIBOA (Fig. 1), while DIMBOA is the main Hx present in wheat and maize. The concentration of Hx within cultivated Gramineae is variable. The man-made wheat/rye hybrid triticale contains both 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) and 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) (Niemeyer *et al*., 1992). Breeding maize for increased concentrations of DIMBOA led to enhanced resistance against the European corn borer (Grombacher *et al.*, 1989). Inverse relationships between performance of cereal aphids on wheat and DIMBOA concentration have been reported (Givovich and Niemeyer, 1996; Thackray *et al*., 1990; Niemeyer *et al*., 1992) supporting the proposal that increasing DIMBOA levels in wheat plants afford increased resistance against aphids (Bohidar *et al*., 1986; Thackray *et al*., 1990; Gianoli and Niemeyer, 1996; Nicol *et al*., 1991, 1993). DIBOA toxicity against the cereal aphid *Schizaphis graminum* (Rondani) is similar to that of DIMBOA (Zúñiga *et al*., 1983). DIBOA is absent in cultivated barley, however, it is present in wild *Hordeum* L. species and its concentration correlates negatively with fecundity of the cereal aphid *Rhopalosiphum padi* L. (Barría *et al*., 1991).

The dynamics of Hx accumulation, particularly of DIMBOA, has been well studied in wheat and

maize. Hx have not been detected in cereal seeds but start accumulating during the first stages of seedling development. The pattern of accumulation in aerial parts and roots varies among species and also among cultivars. Hx concentration in younger leaves is higher than that in older ones (Argandoña *et al*., 1981; Copaja *et al*., 1999), and young tissues of mature plants still retain Hx levels significant for insect resistance (Thackray *et al*., 1990; Nicol and Wratten, 1997).

The highest recorded levels of Hx in cultivated wheat range from 1.4 to 10.8 mmol/kg fresh weight (Copaja *et al*., 1991; Thackray *et al*., 1990). Higher contents are found in some wild Triticeae (Copaja *et al*., 1991; Barría *et al*., 1991; Niemeyer *et al*., 1992), with wild *Secale* having a maximum content of nearly 40 mmol/kg fresh weight (Barría *et al*., 1991).

The allelopathic potential of Hx has been rather extensively studied only with rye (Sicker and Schulz, 2002; Huang *et al*., 2003; Burgos *et al*., 2004). An inhibitory effect of rye on growth of wheat was already observed many years ago. Rye mulch reduces the biomass of dicotyledonous weeds dramatically up to 90%. Living rye showed a similar effect like rye mulch, the weeds biomass was reduced by 84%. DIBOA and 2-benzoxazolinone (BOA), the main product of decomposition of DIBOA, were identified as allelochemicals mainly responsible for phytotoxicity. Young plants have a higher allelopathic potential due to DIBOA than mature ones. Fresh rye mulch contained 20–50 mmol/ha hydroxamic acids but have only half of this content after 12 days and after 121–168 days the compounds are not detectable anymore. Allelochemicals such as benzoxazinoids can influence germination, growth and development of neighboring plants, but the inhibition or stimulation produced by allelochemicals is determined by the concentration of the metabolites in the plants. Resistance can reduce the probability of a pest outbreak, specially in combination with natural enemies, even without pesticide use (Van Emden and Wratten, 1990).

In this work, more evidence about the dynamics of Hx accumulation in rye was investigated. Antifeedant activity and feeding behavior of *Rhopalosiphum padi* were evaluated. In addition, allelopathic potential of pure and aqueous extracts was determined.

Experimental

Chemicals

DIMBOA standard was isolated from extracts of maize (*Zea mays* L. cv. T-129). DIBOA standard was synthesized as previously described (Matlin *et al*., 1979).

Plant materials

Seeds of each rye (*Secale cereale*) cultivar were planted in plastic pots containing vermiculite and allowed to germinate in a plant growth room. The temperature in the growth room was (20 ± 2) °C, relative humidity ranged from 55–65%, and the photoperiod was 12 h. At 96 h seeds, whole seedling, roots and leaves were analyzed for Hx. Tetra-Baer cultivar was obtained from CAMBEX-Baer, Temuco, Chile and the other cultivars were obtained from Botanical Garden of the Polish Academy of Sciences, Warsaw, Poland.

Chemical analyses

0.1 g of plant material, either leaves or roots of rye, were macerated 3 times in succession with 0.5 mL of water using a pestle and mortar. The aqueous extract was left at room temperature for 30 min, adjusted to pH 3 with 0.1 N H_3PO_4 and centrifuged at $7000 \times g$ for 15 min. Aliquots of the supernatant were filtered (0.45 μm) and then analyzed using a Shimadzu LC-6A HPLC instrument with a Lichrospher 100 RP-18 (5 μm) column (125×4 mm). The gradient profile of solvent A (MeOH) and solvent B (0.5 mL H_3PO_4 in 1 L H_2O) was: 0–7 min, 30% A; 7–7.5 min, 30% A to 100% A; 7.5–9 min, constant at 100% A; 9–13 min, 100% A to 30% A. Flow rate was 1 mL/min and detection was carried out at 263 nm. Injection volume was 50 μL. The detection limit was 1.0 μmol kg^{-1} fr. wt. All experiments were done with five replicates. The compounds contents were obtained by linear regression from calibration curves. The compounds were identified by comparison of retention times with previously isolated standards [DIBOA retention time was (2.7 ± 0.2) min and DIMBOA retention time was (3.5 ± 0.2) min].

Insect bioassays

Rhopalosiphum padi aphids were collected from naturally infested barley and allowed to reproduce on barley plants kept under a light/dark photope-

riod of 12 h/12 h at (20 ± 5) °C. Third-instar individuals were used to facilitate manipulation.

Feeding choice assays

Each assay consisted of 10 plates with 10 individuals in each plate. Aphid feeding deterrence assays were conducted using two pieces of a young barley leaf (1.0 cm^2) over a thin layer of agar to diminish dehydration (Hx is not present naturally in barley). One piece of leaf was sprayed previously with 10 μL of a 10% solution of the compound in acetone (w/v) and the other was sprayed only with the solvent (control). Once the solvent had evaporated, 10 individuals were distributed at random on each plate.

After 24 h the number of aphids on each leaf piece was counted. A total of 20 trials was done under the same conditions. The repellence index (RI) was calculated as: $RI = [1 - (T/C) \times 100]$, where T is the number of aphids on the treated piece and C is the number of aphids on the control piece.

Electronic recording of the probing behavior

Aphid probing behavior was recorded using a discontinuous current instrument (DC system) (Tjallingii, 1985). *R. padi* starved for 1 h were tethered to a gold wire electrode. This electrode was fixed to the dorsum of the thorax with silver paint and then connected to the amplifier before placing the insect on a barley leaf. The amplifier used was a four-channel DC system (with an input resistance of 1 GΩ and a gain of 50×). Leaves, aphids and amplifier were placed in a Faraday cage. Aphid probing behavior on treated and control plants was recorded simultaneously for 2 h. A total of three different wave forms per treatment was analyzed using EPG patterns described by Jansen *et al.* (1989) and the following probing behavior variables were calculated: total ingestion time, total non-ingestion time and salivation time. The data were analyzed by one-way ANOVA.

Allelopathic activity assays

Extracts of leaves and roots of 8-day-old rye (Tetra-Baer) were prepared from fresh tissue. Solutions used for the test were prepared by weighing 50 g and 10 g of leaves and roots tissues and macerating with 20 mL of water. The aqueous extracts were left at room temperature for 1 h and used directly. 50 lettuce seeds (*Lactuca sativa*) were uniformly placed in each petri dish covered with a cotton film. A group of 10 dishes was coated by a 1.7 mM solution of DIBOA. Four groups of 10 dishes were coated with the respective extracts solutions. The dishes were sealed and incubated at (25 ± 2) °C in a light-dark cycle for 7 d.

A similar procedure was carried out with 45 seeds of rye (Tetra-Baer) in each dish. Germination was expressed as percentage of the control (seeds coated only with water).

Results and Discussion

The content of Hx in cereal plants is related to their toxic activity against pathogens and allelopathic potential. Both characteristics might bring about a number of beneficial effects within an integrated pest management strategy.

Several lines of argument point to the usefulness of Hx in aphid resistance:

1.) Hx are capable of reducing aphid populations through a toxic effect (Bohidar *et al.*, 1986; Thackray *et al.*, 1990) and antixenosis (Nicol *et al.*, 1991) and of decreasing infection of wheat by barley yellow dwarf virus through feeding deterrent (Givovich and Niemeyer, 1991).

2.) The development time of the predatory lady bird *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 potentially beneficial effects of the predator.

3.) Sub-lethal doses of an insecticide are more effective on aphids feeding on a high-Hx wheat cultivar than on a low one (Nicol *et al.*, 1993).

4.) Non-volatile chemical defenses naturally existing in a plant are more friendly to the environment, since they do not need to be sprayed and normally retained within the plant.

5.) Hx in wheat (Copaja *et al.*, 1999) are not present in the seed and hence do not represent a hazard to human health.

Hx were not detected in samples of seeds of the fourteen cultivars of rye during the course of the experiments. DIBOA and DIMBOA (Fig. 1) contents in leaves and roots at 96 h after planting are reported in Table I.

DIBOA was the main Hx in leaves but the contents differed significantly between the cultivars.

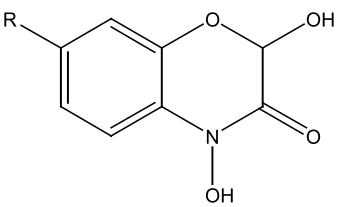
Table I. Content of DIBOA and DIMBOA in 14 rye cultivars at 96 h after germination.

Cultivar[a]	Leaves		Roots	
	DIBOA	DIMBOA	DIBOA	DIMBOA
	[mmol/kg fr. wt.]			
Motto	19.22 ± 1.58	nd[b]	nd	nd
Otello	15.51 ± 1.20	nd	0.79 ± 0.30	1.49 ± 0.63
Charkovskaja	13.82 ± 1.71	nd	1.99 ± 0.09	0.92 ± 0.12
Vjatka	10.08 ± 1.67	nd	0.83 ± 0.13	1.69 ± 0.51
Petka	8.22 ± 0.73	nd	1.29 ± 0.75	0.48 ± 0.07
Zelder	8.04 ± 0.83	nd	0.37 ± 0.08	0.39 ± 0.15
Tetra-Baer	6.85 ± 0.70	nd	0.98 ± 0.35	0.50 ± 0.13
Toivo	6.64 ± 1.01	nd	0.33 ± 0.20	0.35 ± 0.19
Sarakovskaja	3.51 ± 0.66	nd	0.67 ± 0.19	0.45 ± 0.13
Kartner	2.83 ± 0.44	nd	0.56 ± 0.09	0.26 ± 0.04
Silnilkovskaja	1.41 ± 0.42	nd	0.26 ± 0.07	0.61 ± 0.08
Barroso	1.32 ± 0.28	0.60 ± 0.24	0.21 ± 0.15	0.29 ± 0.09
Breno	0.85 ± 0.20	nd	2.48 ± 0.28	0.78 ± 0.38
Dankovskie	0.73 ± 0.48	nd	0.87 ± 0.12	0.76 ± 0.27

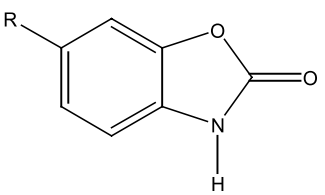
Each value is the mean of six samples with 95% confidence limit, detection limit 0.1 mmol/kg fr. wt.

[a] Cultivars were obtained from CAMBEX-Temuco, Chile and Botanical Garden of the Polish Academy of Sciences, Warsaw, Poland.

[b] nd, not detected.



DIBOA: R = H
DIMBOA: R = MeO-



BOA: R = H
MBOA: R = MeO-

Fig. 1. Structure of 1,4-benzoxazin-3-ones and 2-benzoxazolinones.

DIMBOA was not detected in rye leaves with the exception of cv. Barroso. Both Hx are present in root tissues. In cv. Motto only DIBOA is present in the leaves. This is the first time that DIMBOA is detected in high content in rye roots.

The dynamics of DIBOA and DIMBOA accumulation in seedlings was evaluated in Tetra-Baer, Petka and Sarakovskaja cultivars. The accumulation of DIBOA in leaves at four days after germination was similar in these three cultivars. Maximum content was reached between 48–54 h after germination followed by a gradual decline (Fig. 2). Dynamic accumulation of DIMBOA in roots followed a similar pattern and the maximum content was reached between 54–72 h.

The different contents of DIBOA and DIMBOA in leaves and roots tissues could be related to the biosynthesis pathway of both aglycones. A proposed biosynthetic route (Glawischning *et al.*, 1997; Desai *et al.*, 1996) suggests that DIBOA should be precursor of DIMBOA. The enzymes that catalyze the hydroxylation and methoxylation of carbon atom C7 in the aromatic ring of DIBOA should be expressed in the tissues. If these enzymes are not present, only DIBOA is produced. When they are partially expressed, as in the root tissues, a mixture of DIBOA and DIMBOA is produced.

Insecticidal activity of Hx has been studied mainly against cereal aphids. These insects obtain their food from phloem by penetrating the plant tissues through epidermal and mesophyll cell layers using their stylet-like mouth parts to feed on photoassimilates translocated in the phloem sieve elements (Pollard, 1973). The deleterious effect of Hx can arise from toxicity or starvation effects. In artificial diets, electronically monitored feeding assays have shown that DIMBOA has antifeedant effects at concentrations as low as 1mM, but when the concentration reached 12 mM feeding is completely inhibitet (Argandoña *et al.*, 1983). DIMBOA and DIBOA have similar toxicity activity on *Schizapis graminum* aphid (Zúñiga *et al.*, 1983).

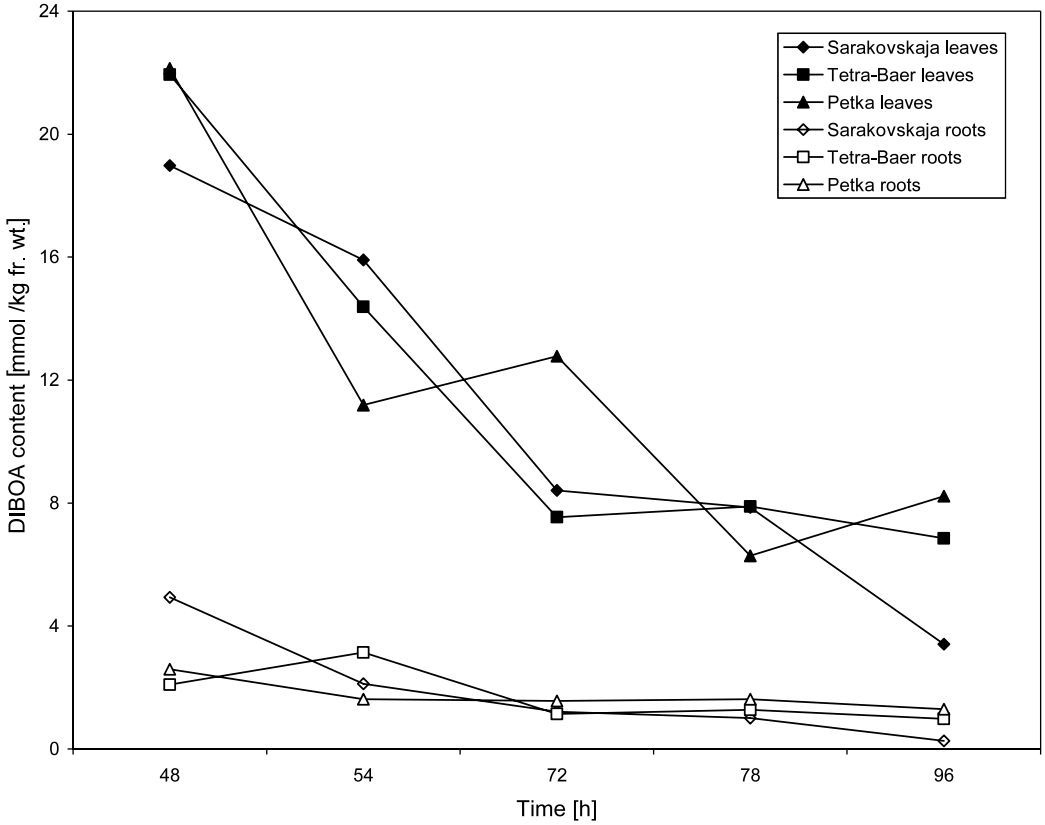


Fig. 2. Decline of DIBOA from leaves and roots of Petka, Sarakovskaja and Tetra-Baer.

As reported previously, the antifeedant activity of chemicals might be mediated by the sensitive hairs present on the proboscis, antennae and legs of the aphids (Levin, 1973). This activity depends on the concentration of the compounds deposited on the contact surface and the diffusion into the leaf tissues which are determined by lipophilicity. DIBOA shows a stronger antifeedant effect on *Rhopalosiphum padi* than DIMBOA (Bravo *et al*., 2004b). More clarity about the effect of DIBOA on the feeding behavior of *R. padi* was obtained from electronic recording of the probing behavior on barley leaves treated with different concentrations of pure DIBOA. Results are shown in Table II.

Repellence activity (RI) is in agreement with the DIBOA concentration. Feeding behavior shows that DIBOA reduced the ingestion time of *R. padi* at all doses tested and increased the non-ingestion time. At 2.8 and 5.5 mM concentration of DIBOA the feeding of the insects decreased by more than 50% with respect to control. The time of salivation was not affected significantly. These results suggest that part of the deleterious activity of DIBOA might arise from a starvation effect.

Lettuce (*Lactuca sativa*) and rye (*Secale cereale*) cv. Tetra-Baer seeds were used to study the allelopathic potential of pure DIBOA and extracts of leaves and roots of rye (Tetra-Baer). The percentage of germination inhibition of lettuce seeds is shown in Table III. 100% of germination inhibition was produced by a 1.7 mM solution of pure DIBOA. Inhibition by leaf extracts (extracts 1 and 2) is in agreement with the concentration of DIBOA contained. Similarly, germination was inhibited in a high percentage by roots extracts (extracts 3 and 4). In these cases, the inhibition should arise from the concentration of the mixture DIBOA-DIMBOA present in the roots.

DIBOA and DIMBOA decompose to the main product BOA and MBOA, respectively. These compounds also have allelopathic activity; so part

Table II. Repellence index (RI) and feeding behavior of *R. padi* on barley leaves treated with DIBOA.

DIBOA [mM]	RI (%)	Ingestion time (%)	Salivation time (%)	Non-ingestion time (%)
Control	0	44.2 ± 6.1	2.5 ± 0.5	53.3 ± 6.1
0.5	13 ± 1.8	27.0 ± 7.0	3.1 ± 0.7	69.9 ± 6.8
2.8	27 ± 5.6	17.0 ± 5.4	1.9 ± 0.8	80.9 ± 5.4
5.5	51 ± 6.6	18.8 ± 4.7	3.3 ± 0.7	78.0 ± 5.2

Each value is the mean of 10 samples ± s.e.
The aphids were controlled during 120 min; that represents 100% of the time.

Table III. Percentage of germination inhibition on lettuce seeds of pure DIBOA and extracts of leaves and roots of rye (Tetra-Baer).

Substrates	(%)
DIBOA (1.7 mM)	100
Extract 1 (16.1 mM DIBOA)	100
Extract 2 (3.5 mM DIBOA)	100
Extract 3 (2.3 mM DIBOA; 1.1 mM DIMBOA)	100
Extract 4 (0.5 mM DIBOA; 0.25 mM DIMBOA)	45

Extracts 1 (leaves) and 3 (roots): 50 g of macerated tissues in 20 mL of water; extracts 2 (leaves) and 4 (roots): 10 g of macerated tissues in 20 mL of water. All extracts were prepared with 8-day-old plants.

of the effect could be due to these compounds. In these cases the decomposition of DIBOA and DIMBOA was less than 10%. According to these results, the allelopathic potential of Hx in the aqueous extracts is preserved.

Pure DIBOA and extracts of leaves and roots did not inhibit the germination of rye seeds. The only effect seen was a little delay in the germination.

Further research should be considered to see if the total contents of Hx remain stable at all growth stages and whether this level is related to maximal concentrations in young seedlings of rye cultivars. More understanding of the molecular aspects of allelopathic interaction is a prerequisite for the application of these allelochemicals as modern pest control strategies.

Acknowledgements

This study was supported by the Departamento de Química, Facultad de Ciencias, Universidad de Chile. We thank Dr. Rybezynski and Campex Baer for provision of seeds.

Alipieva K. I., Taskova R. M., Evstatieva L. N., Handjieva N. V., and Popov S. S. (2003), Benzoxazinoids and iridoid glucosides from four *Lamium* species. Phytochemistry **64**, 1413–1417.

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

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

Barría B. N., Copaja S. V., and 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., and Niemeyer H. M. (1986), Effects of hydroxamic acids on the resistance of wheat to the aphid *Sitobion avenae*. Ann. Appl. Biol. **109**, 195–198.

Bravo H. R. and Copaja S. V. (2002), Contents and morphological distribution of 2,4-dihydroxy-1,4-benzoxazin-3-one and 2-benzoxazolinone in *Acanthus mollisi* in relation to protection from larvae of *Pseudaletia impuncta*. Ann. Appl. Biol. **140**, 129–132.

Bravo H. R., Copaja S. V., and San Martín J. (2004a), Contents of 1,4-benzoxazin-3-ones and 2-benzoxazolinone from *Stenandrium dulce* (Nees). Z. Naturforsch. **59c**, 177–180.

Bravo H. R., Copaja S. V., and Argandoña V. H. (2004b), Chemical basis for the antifeedant activity of natural hydroxamic acids and related compounds. J. Agric. Food Chem. **52**, 2598–2601.

Bravo H. R., Copaja S. V., Figueroa-Duarte S., Lamborot M., and San Martín J. (2005), 1,4-Benzoxazin-3-one, 2-benzoxazolinone and gallic acid from *Calceolaria thyrsiflora* Graham, and their antimicrobial activity. Z. Naturforsch. **60c**, 389–393.

Burgos N. R., Talbert R. E., Kim K. S., and Kuk Y. I. (2004), Growth inhibition and root ultrastructure of *Cucumber* seedling exposed to allelochemical from rye (*Secale cereale*). J. Chem. Ecol. **30**, 671–689.

Copaja S. V., Barría B. N., and Niemeyer H. M. (1991), Hydroxamic acid content of perennial Triticeae. Phytochemistry **30**, 1531–1534.

Copaja S. V., Nicol D., and Wratten S. D. (1999), Accumulation of hydroxamic acids during wheat germination. Phytochemistry **50**, 17–24.

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

Desai S. R., Kumar P., and Chilton W. S. (1996), Indole is an intermediate in the biosynthesis of cyclic hydroxamic acids in maize. J. Chem. Soc. Chem. Commun., 1321–1325.

Gianoli A. and Niemeyer H. M. (1996), Cost and benefits of hydroxamic acids-related resistance in winter wheat against the bird cherry oat aphid, *Rhopalosiphum padi* (L.). Ann. Appl. Biol. **129**, 83–90.

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

Givovich A. and Niemeyer H. M. (1996), Role of hydroxamic acids in the resistance of wheat to the Russian wheat aphid *Diuraphis noxia* (Mordvilko) (Hom. Aphidae). J. Appl. Ent. **120**, 537–539.

Glawischning E., Eisenrich W., Backer A., and Gierl A. (1997), Biosynthesis origin of oxygen atoms in DIMBOA from maize: NMR studies with $^{18}O_2$. Phytochemistry **45**, 715–718.

Grombacher A. W., Russell W. A., and Gunthrie W. D. (1989), Resistance to first-generation European corn borer (Lepidoptera: *Pyralidae*) and DIMBOA concentration in midwhorl leaves of the BS9 maize synthetic. J. Kansas Entomol. Soc. **62**, 103–107.

Hofman J. and Hofmanová O. (1969), 1,4-Benzoxazine derivatives in plants. Sephadex fraction and identification of a new glucoside. Eur. J. Biochem. **8**, 109–112.

Huang Z., Haig T., Wu H., Ann M., and Pratley J. (2003), Correlation between phytotoxicity on annual ryegrass (*Lolium rigidum*) and production dynamics of allelochemicals within root exudates of an allelophatic wheat. J. Chem. Ecol. **29**, 2263–2279.

Jansen J. A., Tjallingii W. F., and van Lenteren J. C. (1989), Electrical recording and ultrastructure of stylet penetration by the green-house whitefly. Entomol. Exp. Appl. **52**, 69–81.

Levin D. A. (1973), The role of trichomes in plant defense. Quart. Rev. Biol. **48**, 3–15.

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

Matlin S. A., Sammes P. G., and Upton R. M. (1979), The oxidation of trimethyl silylated amides to hydroxamic acids. J. Chem. Soc. Perkin Trans. I, 2481–2487.

Nicol D. and Wratten S. D. (1997), The effect of hydroxamic acid concentration at late growth stages of wheat on the performance of the aphid *Sitobion avenae*. Ann. Appl. Biol. **130**, 387–396.

Nicol D., Copaja S. V., Wratten S. D., and Niemeyer H. M. (1991), A screen of worldwide wheat cultivars for hydroxamic acid levels and aphid antixenosis. Ann. Appl. Biol. **121**, 11–18.

Nicol D., Wratten S. D., Eaton N., and Copaja S. V. (1993), Effects of DIMBOA levels in wheat on the susceptibility of the grain aphid (*Sitobion avenae*) to deltamethrin. Ann. Appl. Biol. **122**, 427–433.

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

Pollard D. G. (1973), Plant penetration by feeding aphids (Hemiptera, Aphidoidea). A review. Bull. Entomol. Res. **62**, 631–714.

Pratt K., Kumar P., and Chilton W. S. (1995) Cyclic hydroxamic acids in dicotyledoneous plants. Biochem. Syst. Ecol. **23**, 781–785.

Sicker D. and Schulz M. (2002), Benzoxazinones in plants. Occurrence, synthetic access and biological activity. In: Studies in Natural Products Chemistry (Atta-ur-Rahman, ed.). Elsevier, New York, pp. 185–232.

Thackray D. J., Wratten S. D., Edwards P. J., and Niemeyer H. M. (1990), Resistance to the aphids *Sitobion avenae* and *Rhopalosiphum padi* in Gramineae in relation to hydroxamic acid levels. Ann. Appl. Biol. **126**, 573–582.

Tjallingii W. F. (1985), Electrical nature of recorded signals during stylet penetration by aphids. Entomol. Exp. Appl. **38**, 177–186.

Van Emden H. F. and Wratten S. D. (1990), Tri-trophic interactions involving plants in the biological control of aphids. In: Aphid-Plant Interactions: Populations to Molecules (Peters D. C., Webster J. A., and Chlouber C. S., eds.). Oklahoma State University and USDA/Agricultural Research Service, Stillwater, OK, pp. 29–43.

Virtanen A. I. and Hietala P. K. (1960), Precursors of benzoxazolin-one in rye plants I Precursor II, The aglucone. Acta Chem. Scand. **14**, 499–502.

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