Chemoecology 8 (1998) 19–23
0937–7409/98/010019–05 $1.50 + 0.20

CHEMOECOLOGY

Allocation of herbivory-induced hydroxamic acids in the wild wheat *Triticum uniaristatum*

Ernesto Gianoli and Hermann M. Niemeyer

Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile,
e-mail: E.G.:aletheia@abulafia.ciencias.uchile.cl

Summary. We characterized the induction of hydroxamic acids (Hx) by aphid infestation in the wild wheat *Triticum uniaristatum* by addressing the following questions: i) Do different leaves have similar responses to aphid damage?, ii) Is the Hx induction localized or systemic?, iii) How long does the induction last?, and iv) Is the degree of damage related to the magnitude of induced Hx? Based on earlier results on this wheat/aphid system (lack of costs of Hx induction) we expected to find the plant exhibiting cost-saving patterns of response to herbivory. Aphid infestation in the primary leaf led to induced levels of Hx, but no differences in Hx levels were found after infestation of the secondary leaf. Induction of Hx was restricted to the infested leaf (primary leaf). Induced Hx levels exhibited by the primary leaf at the end of aphid infestation were not observed 2 days later. Finally, different aphid densities (between 10 and 40 aphids per leaf) did not produce significant differences in Hx levels in infested primary leaves. Characteristics of Hx induction by aphid infestation in *T. uniaristatum* partially support the expected cost-saving patterns in the allocation of induced defenses.

Key words. defense – induced defense – herbivory – aphids – *Rhopalosiphum padi* – wild wheat – *Triticum uniaristatum* – Poaceae – hydroxamic acids – costs of defense

Introduction

Induced phytochemical responses following damage have been reported for a wide range of plant-herbivore systems (Tallamy & Raupp 1991). However, seldom have these induced responses been thoroughly characterized in terms of underlying mechanisms, spatial and temporal dynamics, biotic and abiotic constraints to their expression, and associated costs imposed to the plant. Baldwin (1991) and Zangerl & Berenbaum (1995) provide partial coverage of such topics. The documentation of costs of defenses is a central issue in the discussion addressing the evolution of chemical defenses in plants (Simms 1992). Hence, a better knowledge of the costs of induced defenses and their relation with the above listed topics is necessary to achieve a proper understanding of the observed patterns of defense allocation in plants.

Costs of plant defenses have been defined at two levels. Direct costs (see Gershenzon 1994) are taken as those resources devoted to synthesis, storage and maintenance of the defensive metabolites, while indirect costs, expressed in terms of plant fitness, arise from allocating to defense resources that would otherwise be invested in growth and/or reproduction (Zangerl & Bazzaz 1992). The expected positive correlation of both components of costs of defense (Bazzaz *et al.* 1987) has been recently tested and shown to hold in an induced defense system. Thus, on one hand, indirect costs were not detected for aphid infestation-induced hydroxamic acids in a wild wheat, and, on the other hand, induction at the infested tissue resulted from a spatial reallocation of hydroxamic acids from non-infested tissue, a process claimed not to involve direct costs (Gianoli & Niemeyer 1997a).

The aim of the present study is to characterize the phenomenon of induced phytochemical responses in the biological system already mentioned: the wild wheat *Triticum uniaristatum* L. and its main secondary metabolites, the hydroxamic acids (Niemeyer *et al.* 1992), which are induced following a short-term infestation by the cereal aphid *Rhopalosiphum padi* (L.) (Gianoli & Niemeyer 1997a). Hydroxamic acids (Hx) are a family of secondary metabolites typical of Gramineae (Poaceae) (Niemeyer 1988) that have been shown to decrease aphid survival and reproduction (Argandoña *et al.* 1980, Thackray *et al.* 1990). Hx may also be induced by aphid feeding on bread wheat *Triticum aestivum* L., induction being affected by plant genotype (Niemeyer *et al.* 1989), environmental conditions (Gianoli & Niemeyer 1996) and aphid genotype (Gianoli *et al.* 1997). In addition, aphid mortality was higher in wheat plants exhibiting increased Hx levels induced by previous aphid infestation (Thackray *et al.* 1988). Finally, constitutive accumulation of Hx did not impose a cost in terms of grain yield in several wheat cultivars (Gianoli *et al.* 1996).

In this work, we address the following questions: i) Do different leaves of *T. uniaristatum* seedlings re-

Correspondence to: E. Gianoli

spond in the same way to aphid damage?, ii) Is the induction of Hx localized or systemic?, iii) How long does this induction persist?, and iv) Is the magnitude of induced Hx related to the degree of damage? In accordance with previous work on this system, i.e. lack of costs of Hx induction, we expect to find cost-saving patterns in the response of this wild wheat to herbivory.

Materials and methods

Basic experiment of induction of Hx

The experiment was performed as previously described (Gianoli & Niemeyer 1997a). Seeds of *T. uniaristatum* were germinated in individual plastic pots (25 ml) filled with potting soil (Anasac, Santiago, Chile) and then developed in a growth chamber at 15°C and L12:D12 photoperiod. When seedlings attained growth stage 12 (primary leaf fully unfolded, secondary leaf visible, Zadoks *et al.* 1974), each one was infested with 20 individuals of the cereal aphid *R. padi* (second or third instar apterae) confined in a cylindrical plastic clip-cage (2 cm diameter × 2 cm height) attached to the primary leaf. Empty clip-cages were placed on control plants. Treatments were assigned randomly. After 48 h of infestation, clip-cages were removed and aphids were withdrawn from the infested seedlings with a fine brush. Aphid feeding was verified by observing honeydew production. Aphid survival was always about 100%. Immediately after aphid removal, primary leaves of both control and treated plants were analyzed for DIBOA (2,4-dihydroxy-1,4-benzoxazin-3-one), the main Hx aglucone in extracts of *T. uniaristatum* (Niemeyer *et al.* 1992). Plant material was macerated with 1 ml H_2O, using mortar and pestle. The aqueous extract was left at room temperature for 15 min and then taken to pH 3 with 0.1 N H_3PO_4. The extract was centrifuged at 13000 g for 15 min and a 50 µl aliquot of the supernatant directly injected into a high performance liquid chromatograph (Shimadzu, Kyoto, Japan). An RP-100 Lichrospher-C18 column (Merck, Darmstadt, Germany) was used with a constant solvent flow of 1.5 ml/min and the following linear gradients between solvents A (MeOH) and B (0.5 ml H_3PO_4 in 1 l H_2O): 0 to 7 min, 30% A; 7 to 9 min, 100% A; 7 to 13 min, 30% A. DIBOA was detected at 263 nm. Chromatographic standards of DIBOA were obtained by extraction from rye seedlings.

One-way ANOVA was used to compare control and infested treatments (n = 8). No difference in fresh weight between control and infested tissue was found by the time of analysis of Hx in all of the experiments performed (data not shown), hence allowing comparisons of concentrations of Hx (mmol/kg fresh weight). All statistical analyses were performed using the STATISTICA statistical package (StatSoft, Inc 1993).

Responsiveness to aphid infestation of other plant module

Given that the primary leaf showed induced responses following aphid infestation (Gianoli & Niemeyer 1997a), we tested whether the secondary leaf was also capable of such responses. Hence, the protocol of the basic experiment was applied to a group of seedlings at growth stage 13 (secondary leaf unfolded, Zadoks *et al.* 1974), with the infestation site being the secondary leaf. Comparisons of control and infested seedlings were performed by a one-way ANOVA (n = 8).

Spatial extent of the Hx induction

This experiment was performed in order to assess whether the increase in Hx levels was restricted to the infested leaf (*i.e.*, localized) or whether it was extended to non-infested organs (*i.e.*, systemic). The basic experiment of Hx induction was repeated, this time extending the Hx analysis to all plant organs (primary leaf, secondary leaf, stem, root) in both treatments (control and infested). Hx levels in each organ were compared between treatments by one-way ANOVAs (n = 8).

Table 1 Concentration of Hx (mmol/kg fresh weight, Mean ± SE) in organs of *T. uniaristatum* seedlings at growth stage 13. INF = secondary leaf infested with aphids for 48 h, CON = control seedlings. P-values refer to one-way ANOVAs (n = 8)

	CON	INF	P
Primary leaf	0.163 ± 0.025	0.221 ± 0.044	0.29
Secondary leaf	1.313 ± 0.051	1.217 ± 0.046	0.32
Stem	3.382 ± 0.353	3.087 ± 0.312	0.56
Root	0.561 ± 0.079	0.585 ± 0.093	0.89

Temporal persistence of the induction of Hx

To determine the duration of the induced state in the infested leaf (primary leaf), the basic experiment was repeated, varying the time between the end of aphid infestation and the analysis of Hx. The treatments were: 0, 2, 4, and 8 days after the end of infestation. Comparisons of control and infested seedlings were performed for each treatment by one-way ANOVAs (n = 8).

Relationship between degree of damage and magnitude of Hx induction

This experiment assessed whether the magnitude of Hx induction obtained following infestation by 20 aphids (Hx increase of 70% above control levels, Gianoli & Niemeyer 1997a) could be increased by still greater infestation densities. Densities of 10, 20, 30 and 40 aphids per clip-cage were used in replicated basic experiments (n = 8). A LSD-test following an ANOVA was performed to examine differences of means among treatments.

Results

As previously reported (Gianoli & Niemeyer 1997a), the basic experiment of Hx induction showed higher levels of Hx in the infested primary leaf ($P < 0.02$, one-way ANOVA). In contrast, infestation of the secondary leaf resulted in no differences in Hx levels between treatments ($P < 0.05$, one-way ANOVA) in the infested leaf as well as in the non-infested organs (Table 1).

Induction of Hx by aphid infestation was restricted to the infested primary leaf (Table 2). Differences in Hx levels of both primary and secondary leaves listed in Tables 1 and 2 are due to the natural dynamics of Hx accumulation: higher levels for younger tissues and a steady decrease following an early peak within each leaf (Argandoña *et al.* 1981).

With regard to duration of the induced response, although the infested primary leaves consistently showed average levels of Hx above than those of the control leaves, this difference was only statistically sig-

Table 2 Concentration of Hx (mmol/kg fresh weight, Mean ± SE) in organs of *T. uniaristatum* seedlings at growth stage 12. INF = primary leaf infested with aphids for 48 h, CON = control seedlings. P-values refer to one-way ANOVAs (n = 8)

	CON	INF	P
Primary leaf	0.178 ± 0.011	0.263 ± 0.033	0.04
Secondary leaf	3.689 ± 0.536	4.027 ± 0.283	0.59
Stem	3.551 ± 0.369	2.666 ± 0.318	0.11
Root	0.642 ± 0.086	0.631 ± 0.098	0.93

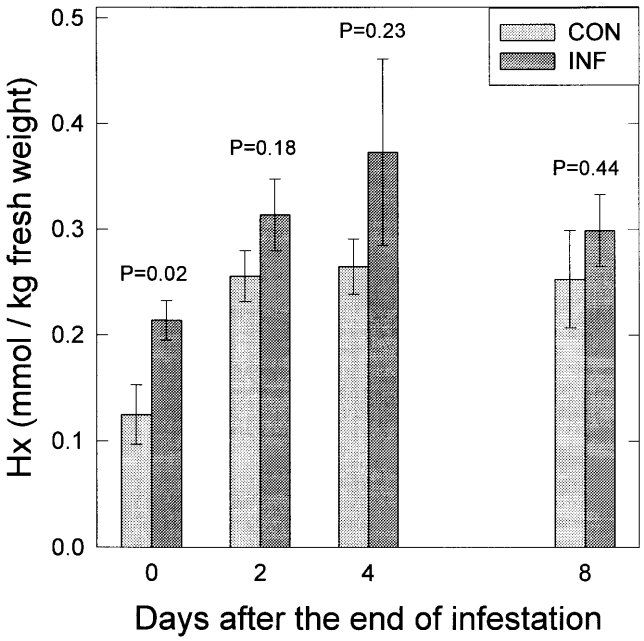

Fig. 1 Hydroxamic acid (Hx) content (Mean ± SE, 8 replicates) of the primary leaf of aphid-infested (20 individuals, 48 h) (INF) and non infested control (CON) seedlings of *T. uniaristatum* at different days after the end of aphid infestation. P-values (one-way ANOVA) for comparisons between control and infested plants are shown

nificant ($P < 0.05$, one-way ANOVA) at day 0 after the end of aphid infestation (Fig. 1).

Finally, different degrees of damage (aphid densities in the clip-cage) did not lead to significant differences in Hx levels in the primary leaf of infested *T. uniaristatum* seedlings (LSD-test following a one-way ANOVA) (Fig. 2).

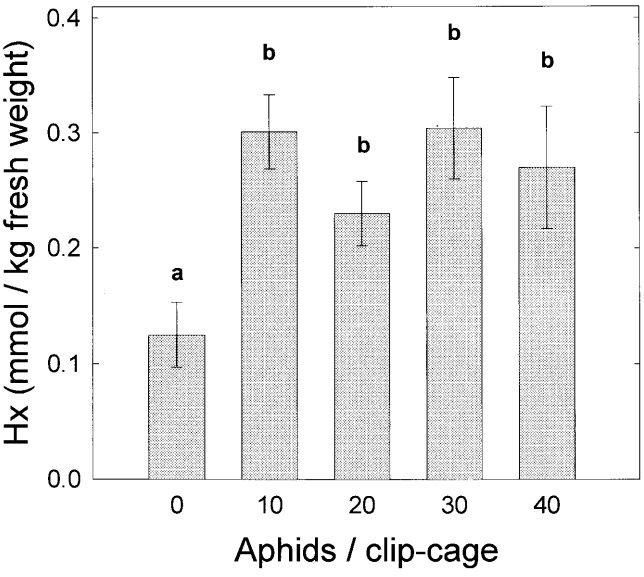

Fig. 2 Hydroxamic acid (Hx) content (Mean ± SE, 8 replicates) of the primary leaf of seedlings of *T. uniaristatum* after 48 h of infestation by different levels of aphid infestation. Means in columns sharing lowercase letters are not significantly different (LSD-test following one-way ANOVA)

Discussion

Plants must allocate to growth, reproduction, and defense a budget of limited resources (Bazzaz *et al.* 1987; Herms & Matson 1992). Within the framework of the Optimal Defense theory (McKey 1974; Rhoades 1979), the production of chemical defenses is considered to impose a cost to the plant. Hence, the theory predicts that plants invest in these defenses only when, where, and in the amounts required. Inducible defenses represent an efficient way to achieve such a result, since they divert resources only when required, *i.e.*, when herbivore damage occurrs. However, production of induced defenses may exact a cost to plants (Baldwin *et al.* 1990).

Aphid-induced increase of Hx in the wild wheat *T. uniaristatum* was not found to impose an indirect cost to the plant (Gianoli & Niemeyer 1997a). This was partially explained by a preliminary assessment of the underlying mechanism of such induction, i.e., induction could be attributed to non-costly translocation of Hx from non-infested organs (Gianoli & Niemeyer 1997a). In this report we characterized such Hx induction to gain insights into the observed lack of costs to the plant.

Unlike the primary leaf, the secondary leaf of *T. uniaristatum* seedlings failed to exhibit induced levels of Hx. This is surprising given that expanding leaves (as were the secondary leaves in this experiment) are normally expected to exhibit greater biochemical responses following damage than expanded leaves (as were the primary leaves in the basic experiment) (Coleman & Jones 1991). Such a pattern has been reported earlier (Frischknecht *et al.* 1987, Hartley & Firn 1989, but see Zangerl & Berenbaum 1995). The results obtained here may be explained by taking into account the endowment of Hx of either leaf at the time of the corresponding experiments. Thus, in accordance with the described dynamics of Hx accumulation in cereal seedlings (Argandoña *et al.* 1981), the secondary leaf showed substantially higher constitutive levels of Hx than the primary leaf (mean values: 1.313 and 0.178, respectively; Tables 1 and 2). Therefore, it is conceivable that, since the secondary leaf is sufficiently protected by constitutive Hx, it will not receive added Hx produced by induction mechanisms. Conversely, the primary leaf, limited in its constitutive levels of defense by the natural dynamics of accumulation of Hx, achieves a higher degree of protection with induced levels of such defense. This explanation is based on the idea of tradeoffs between constitutive and induced pools of defense (see Mole 1994) and may be viewed as a way to limit the cost of defense.

Induction of Hx in the primary leaf of *T. uniaristatum* seedlings was not systemic; it was restricted to the damaged leaf. Since systemic responses (*e.g.*, Baldwin 1988) imply the allocation of defenses to sites other than the one under attack, this localized induction of Hx suggests a cost-saving response. On the other hand, Hx induction in the primary leaf lasted less than 48 h. Although this may also be viewed as a cost-saving

feature, the benefits to the plant, in terms of protection against herbivores, may be non-significant. Similar short-term induced responses have been found for tropane alkaloids (Khan & Harborne 1990), cucurbitacins (Tallamy & McCloud 1991), cardenolides (Malcolm & Zalucki 1996) and furanocoumarins (Zangerl & Berenbaum 1995), the latter being the only work focusing on cost issues.

The intensity of initial damage may affect the magnitude of induced responses (Karban 1991). Seedlings of *T. uniaristatum* with heavier loads of aphids than that those in the basic experiment (20 aphids/clip-cage) did not show higher levels of Hx induction. Interestingly, 10 aphids per clip-cage were sufficient to elicit Hx induction; a result different from that obtained for cultivated wheat (Gianoli & Niemeyer, 1997b). The phenomenon of Hx induction in this wild wheat appears to possess a discrete nature. Thus, a certain degree of damage triggers Hx induction but greater damage does not lead to increased induction. This may be explained by analogy to the so-called "constraints of design" in animal ecological physiology (Calow 1987), *i.e.*, greater induced responses are not observed because the metabolic machinery of the tissue involved is unable to accumulate (or translocate) Hx above a given level. It has been stated earlier that plants bear constraints to the expression of their phenotypic plasticity (Schlichting 1986).

In seedlings of *T. uniaristatum*, induced levels of Hx after aphid infestation were only observed in the primary leaf. This induction exhibited restrictions in duration, localization and magnitude. This pattern of allocation of induced defenses led us to consider induction of Hx in this system as a low-cost response, a result in accordance with previous work that failed to find an indirect cost of such Hx induction (Gianoli & Niemeyer 1997a). However, at this point we cannot rule out the existence of other kinds of costs.

Finally, in order to gain a better understanding of the patterns of allocation of induced defenses – and their assumed costs – it is also necessary to evaluate the benefits associated with them. In the case of Hx, the benefits may be related to their known role in plant resistance against pests and diseases, in mineral nutrition and hormonal activity (reviewed in Niemeyer 1988 and Niemeyer & Pérez 1995).

Acknowledgements

We thank L. Kirchgessner for technical assistance. We also thank two anonymous referees for helpful criticism of the manuscript. This work was supported by the International Program in the Chemical Sciences at Uppsala University, the Presidential Chair in Sciences awarded to HMN, and a grant from FONDECYT (1961035). EG was supported by a Latin American Plant Sciences Network fellowship (93–M8) during part of the development of this work. Seeds of *T. uniaristatum* were kindly provided by The John Innes Institute (UK), INRA – Le Rheu (France) and USDA, ARS (USA).

References

Argandoña VH, Luza JG, Niemeyer HM, Corcuera LJ (1980) Role of hydroxamic acids in the resistance of cereals to aphids. Phytochemistry 19:1665–1668

Argandoña VH, Niemeyer HM, Corcuera LJ (1981) Effect of content and distribution of hydroxamic acids in wheat on infestation by *Schizaphis graminum*. Phytochemistry 20:673–676

Baldwin IT (1988) The alkaloidal responses of wild tobacco to real and simulated herbivory. Oecologia 77:378–381

Baldwin IT (1991) Damage-induced alkaloids in wild tobacco. Pp 71–84 *in* Tallamy DW, Raupp MJ (eds) Phytochemical Induction by Herbivores. New York: John Wiley & Sons

Baldwin IT, Sims CL, Kean SE (1990) The reproductive consequences associated with inducible alkaloidal responses in wild tobacco. Ecology 71:252–262

Bazzaz FA, Chiariello NR, Coley PD, Pitelka LF (1987) Allocating resources to reproduction and defense. Bioscience 37:58–67

Calow P (1987) Evolutionary Physiological Ecology. GB-Cambridge: Cambridge University Press

Coleman JS, Jones CG (1991) A phytocentric perspective of phytochemical induction by herbivores. Pp 3–46 *in* Tallamy DW, Raupp MJ (eds) Phytochemical Induction by Herbivores. New York: John Wiley & Sons

Frischknecht, PM, Bättig M, Baumann TW (1987) Effect of drought and wounding stress on indole alkaloid formation in *Catharanthus roseus*. Phytochemistry 26:707–710

Gershenzon J (1994) Metabolic costs of terpenoid accumulation in higher plants. J Chem Ecol 20:1281–1328

Gianoli E, Niemeyer HM (1996) Environmental effects on the induction of wheat chemical defences by aphid infestation. Oecologia 107:549–552

Gianoli E, Niemeyer HM (1997a) Lack of costs of herbivory-induced defenses in a wild wheat. Integration of physiological and ecological approaches. Oikos 80:269–275

Gianoli E, Niemeyer HM (1997b) Characteristics of hydroxamic acid induction in wheat triggered by aphid infestation. J Chem Ecol 23:2695–2705

Gianoli E, Caillaud CM, Chaubet B, Di Pietro JP, Niemeyer HM (1997) Variability in grain aphid *Sitobion avenae* (F.) (Homoptera: Aphididae) performance and aphid-induced phytochemical responses in wheat. Environ Entomol 26:638–641

Gianoli E, Papp M, Niemeyer HM (1996) Costs and benefits of hydroxamic acids-related resistance in winter wheat against the bird cherry-oat aphid, *Rhopalosiphum padi* L. Ann Appl Biol 129:183–190

Hartley SE, Firn RD (1989) Phenolic biosynthesis, leaf damage and insect herbivory in birch (*Betula pendula*). J Chem Ecol 15:275–283

Herms DA, Mattson WJ (1992) The dilemma of plants: to grow or defend. Q Rev Biol 67:283–335

Karban R (1991) Inducible resistance in agricultural systems. Pp 402–420 *in* Tallamy DW, Raupp MJ (eds) Phytochemical Induction by Herbivores. New York: John Wiley & Sons

Khan MB, Harborne JB (1990) Induced alkaloid defence in *Atropa acuminata* in response to mechanical and herbivore leaf damage. Chemoecology 1:77–80

Malcolm SB, Zalucki MP (1996) Milkweed latex and cardenolide induction may resolve the lethal plant defence paradox. Entomol exp appl 80:193–196

McKey D (1974) Adaptive patterns in alkaloid physiology. Am Nat 108:305–320

Mole S (1994) Trade-offs and constraints in plant-herbivore defense theory: a life-history perspective. Oikos 71:3–12

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

Niemeyer HM, Copaja SV, Barría BN (1992) The Triticeae as sources

of hydroxamic acids, secondary metabolites in wheat conferring resistance against aphids. Hereditas 116:295–299

Niemeyer HM, Pérez FJ (1995) Potential of hydroxamic acids in the control of cereal pests, diseases and weeds. Pp 260–269 *in* Inderjit, Dakshini KMM, Einhellig FA (eds) Allelopathy. Organisms, Processes, and Aplications. Washington/DC: ACS Symp Ser 582

Niemeyer HM, Pesel E, Copaja SV, Bravo HR, Franke S, Francke W (1989) Changes in hydroxamic acids levels of wheat plants induced by aphid feeding. Phytochemistry 28:447–449

Rhoades DF (1979) Evolution of plant chemical defense against herbivores. Pp 3–54 *in* Rosenthal GA, Janzen DH (eds) Herbivores: Their Interaction with Secondary Plant Metabolites. Orlando/FL: Academic Press

Schlichting CD (1986) The evolution of phenotypic plasticity in plants. Ann Rev Ecol & Syst 17:667–693

Simms EL (1992) Costs of plant resistance to herbivory. Pp 392–425 *in* Fritz RS, Simms EL (eds) Plant Resistance to Herbivores and Pathogens. Ecology, Evolution and Genetics. Chicago/IL: University of Chicago Press

Tallamy DW, McCloud E (1991) Squash beetles, cucumber beetles and inducible cucurbit responses. Pp 155–182 *in* Tallamy DW, Raupp MJ (eds) Phytochemical Induction by Herbivores. New York : John Wiley & Sons

Tallamy DW, Raupp MJ (1991) Phytochemical Induction by Herbivores. New York: John Wiley & Sons

Thackray DJ, Morse S, Leech C (1988) The role of hydroxamic acids in wheat and maize in conferring resistance to aphids. Asp Appl Biol 17:22–27

Thackray DJ, Wratten SD, Edwards PJ, Niemeyer HM (1990) Resistance to the aphids *Sitobion avenae and Rhopalosiphum padi* in Gramineae in relation to hydroxamic acid levels. Ann Appl Biol 116:573–582

Zadoks JC, Chang TT, Konsak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421

Zangerl AR, Bazzaz FA (1992) Theory and pattern in plant defense allocation. Pp 363–391 *in* Fritz RS, Simms EL (eds) Plant Resistance to Herbivores and Pathogens. Ecology, Evolution and Genetics. Chicago/IL: University of Chicago Press

Zangerl AR, Berenbaum MR (1995) Spatial, temporal and environmental limits on xanthotoxin induction in wild parsnip foliage. Chemoecology 5/6:37–42

Received 15 January 1997; accepted 7 July 1997.