

Costs and benefits of hydroxamic acids-related resistance in winter wheat against the bird cherry-oat aphid, *Rhopalosiphum padi* L.

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Summary

Estimations of infestation by the bird cherry-oat aphid (*Rhopalosiphum padi*) as well as measurements of grain yield in 26 Hungarian winter wheat cultivars under field conditions were correlated with the concentration of hydroxamic acids (Hx) in seedlings of those cultivars. The significant inverse relationship between infestation ratings and Hx levels in wheat showed that Hx, despite their decreased accumulation at later plant phenological stages, may be able to confer resistance against aphid infestation in the field. Since no significant relationship was found between grain yield and Hx levels in plants it is suggested that Hx accumulation does not impose a cost to the plant in terms of yield. These findings support earlier claims stressing the potential of Hx as breeding targets for aphid resistance in wheat.

Key words: Hydroxamic acids, *Triticum aestivum*, *Rhopalosiphum padi*, host plant resistance, cost of resistance, plant breeding, winter wheat

Introduction

Plant secondary metabolites have been considered to be involved in plant resistance to insects since the last century and have become part of modern pest control strategies as externally applied pesticides since the late sixties. The next logical step is the development of resistant plant varieties possessing increased concentrations of such allelochemicals. Two important conditions a plant secondary metabolite must fulfil in order to be considered as a target in breeding programmes are: i) to provide effective resistance against the target pest in the field, and ii) to result in low or no penalties on crop yield due to higher levels of the compound in the plant.

Hydroxamic acids (Hx) are a family of plant secondary metabolites present in both cultivated and wild Gramineae (Niemeyer, 1988a; Copaja, Niemeyer & Wratten, 1991; Niemeyer, Copaja & Barría, 1992) playing a role in the resistance of cereal crops to pests and diseases (Niemeyer, 1988a). Hx occur in the plant as 2- β -O-D-glucopyranosides which are hydrolysed by plant glucosidases when the tissue is injured (Hofman & Hofmanová, 1971). Hx are absent from the seed, increase upon germination (peaking at the young seedling stage) and decrease thereafter (Argandoña, Niemeyer & Corcuera, 1981); in mature plants the youngest tissue still retains a high concentration of Hx (Thackray, Wratten, Edwards & Niemeyer, 1990).

Hx have been identified as a resistance factor against the European corn borer, *Ostrinia nubilalis* Hübner. Breeding maize for increased concentrations of Hx led to increased resistance to the insect (Grombacher, Russell & Guthrie, 1989). Likewise, Hx levels in wheat and maize seedlings were negatively correlated with the survival, growth rate and adult weight of cereal aphids and the intrinsic rate of increase of aphid populations (Argandoña *et al.*, 1981; Thackray *et al.*, 1990). Survival of cereal aphids in artificial diets decreased with increasing Hx concentrations in them (Corcuera, Argandoña & Niemeier, 1982). In addition, in laboratory trials, cereal aphids chose wheat seedlings with lower Hx levels in dual-choice tests (Givovich & Niemeier, 1991) and required more time to attain a sustained phloem ingestion in wheat seedlings with higher Hx levels (Givovich & Niemeier, 1995). Finally, effectiveness of aphid control by a predator and by insecticide treatment was improved in higher Hx wheat cultivars (Martos, Givovich & Niemeier, 1992; Nicol, Eaton, Wratten & Copaja, 1993). Although these are strong arguments for the inclusion of Hx in strategies for the integrated control of aphid pests in cereals, field experiments are needed to validate the laboratory results.

Continuous selection for pest resistance in crops may involve a cost in fitness components for the plants (reviewed in Simms, 1992a). Nonetheless, these costs should be contrasted with the benefits of increased resistance. The economic costs associated with such yield losses may be compensated for by a reduction in the input of agrochemicals.

This paper reports on the existence of a relationship between Hx and resistance of wheat cultivars against the bird cherry-oat aphid, *Rhopalosiphum padi* L., under field conditions, and estimates the yield costs of such resistance factor.

Materials and Methods

Plant material

Twenty-six Hungarian winter wheat genotypes provided by the Cereal Research Institute, Szeged, Hungary, were used for both infestation and Hx quantification experiments.

Infestation experiments

Experiments were conducted at the Ságvári Station of the Cereal Research Institute, Szeged, Hungary. Infestation experiments were performed as described by Papp & Mesterházy (1993). Four control and four naturally infested plots were used, each plot consisting of two rows (165 cm long, 20 cm apart) with about 70 wheat plants per row. Aphid infestation was visually estimated as a percentage of surface covered by aphids related to the total surface of the plant. Infestation was assessed at 27 (early infestation) and 35 (late infestation) days after average heading. Insect infestation was prevented in control plots by applying three times in the season Karate 5 EC (5% lambda-cyhalotrin active agent) at a dosage of 0.2 litre ha⁻¹ in 300 litre water. Fungal diseases were controlled in all plots by Bayleton 25 WP (25% triadimefon active agent) at a dosage of 0.5 kg ha⁻¹ in 300 litre water. At harvest, yield was determined as g/20 heads and thousand-kernel mass (Papp & Mesterházy, 1993). Averages of infestation ratings and yields as well as mean Hx concentrations were included in linear regression analysis.

Quantification of Hx in wheat seedlings

Approximately 15 seeds of each cultivar were planted individually in six-cm-diameter plastic pots containing vermiculite, and allowed to germinate in a plant growth room. The

temperature was 20°C with a 4°C range; relative humidity ranged from 45% to 65%; photoperiod was 12 h, and light intensity was 200 $\mu\text{E m}^{-2} \text{s}^{-1}$. Seven days after planting, six to 10 healthy seedlings of representative size (8–10 cm) were cut at the junction with the seed. Plant material (20–50 mg fresh weight) was macerated successively with 3 \times 0.33 ml H_2O , using mortar and pestle. The aqueous extract was left at room temperature for 15 min and was then taken to pH 3 with 0.1 N H_3PO_4 . The extract was then centrifuged at 10 000 g for 10 min and a 50 μl aliquot of the supernatant directly injected into a high performance liquid chromatograph. An RP-100 Lichrospher-C18 column was used with a constant solvent flow of 1.5 ml min^{-1} and the following linear gradients between solvents A (MeOH) and B (0.5 ml H_3PO_4 in 1 litre H_2O): 0 to 4.5 min, 30 to 50% A; 4.5 to 10 min, 50 to 30% A; 10 to 13 min, constant at 30% A. Detection was carried out at 263 nm. Retention time was 4.5 \pm 0.3 min for DIMBOA, the major Hx in wheat (Niemeyer, 1988a).

Results

The Hx levels recorded in the cvs examined ranged from 1.46 to 3.33 $\text{mmol}^{-1} \text{kg fr. wt}$ (Table 1), corresponding to medium levels in cultivated wheat (Nicol, Copaja, Wratten & Niemeyer, 1992). Average aphid infestations corresponded to 24% and 63% of the plant surface area for early and late infestation, respectively (Table 1). Significant inverse relationships between Hx levels in wheat seedlings and both early ($r = -0.50$, $P < 0.01$) and late ($r = -0.39$, $P < 0.05$) aphid infestation ratings were obtained (Fig. 1). There were non-

Table 1. *Hydroxamic acids (Hx) in seedlings of wheat cultivars and percentage of infestation by Rhopalosiphum padi (INF)*

Cultivar	Hx (mmol/kg fr. wt)	INF-early (27 days after heading)	INF-late (35 days after heading)
GK Lili	1.46	51.30	95.00
GK Mini Manó	1.50	31.30	80.80
Baranjka	1.54	31.70	71.70
GK István	1.64	40.40	79.20
GK Csilla-817	1.70	22.10	55.80
GK Örzse	1.70	32.90	76.70
Mv 8	1.70	20.80	45.80
Jkm-Rna2xGm-D1	1.76	30.00	71.70
Mv 13	1.79	26.70	71.70
GK Szöke	1.85	20.40	56.70
Mv 10	1.91	23.30	66.70
GK Réka	1.99	20.00	62.50
Jubilejnaja 50	2.08	20.00	47.50
GK Kinscö	2.14	24.60	65.00
Mv 4	2.16	15.80	44.20
GK Ságvári	2.27	20.80	53.30
Downy	2.49	12.50	51.70
GK Boglár	2.63	15.00	57.50
Bucsányi 20	2.65	19.20	53.30
GK Korány	2.71	17.10	58.30
GK Szemes	2.76	16.30	59.20
Sz-1500x8001-Sv	2.83	15.40	63.30
GK Koppány	3.03	35.00	72.50
GK Zombor	3.05	17.50	39.20
GK Laborc	3.20	30.00	70.80
GK Bence	3.33	14.20	60.00

significant relationships between the level of Hx and the weight of either 20 heads ($r = 0.20$, $P > 0.3$) or 1000 kernels ($r = 0.08$, $P > 0.6$) (Fig. 2).

Discussion

The yield losses caused by *Rhopalosiphum padi* infestation (average of 26 cvs: 43% in g/20 heads and 36% in 1000 kernel weight (Papp & Mesterházy, 1993) were in accord with previous reports on its economic impact in cereal crops in Europe (Leather, Walters & Dixon, 1989). The weak but significant inverse relationship between Hx levels at seedling stage and aphid infestation ratings at later stages suggests that Hx in wheat, despite its decreased accumulation at later plant phenological stages (Thackray *et al.*, 1990) may confer partial resistance against aphid infestation in the field. This reasoning assumes that: i) the percentual decrease of Hx levels with plant age is similar for different wheat cultivars, as has been shown to be the case for different *Triticum* species (Niemeyer, 1988b), and ii) young tissues in older

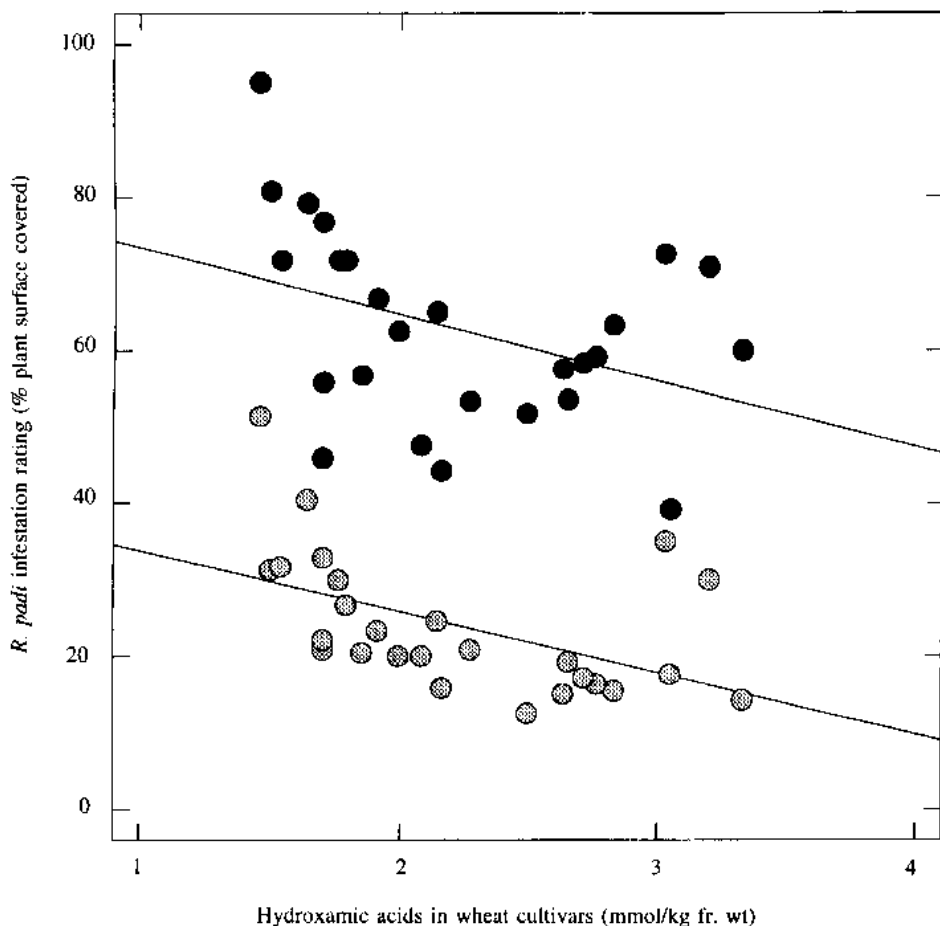


Fig. 1. The relationship between Hx levels in wheat seedlings of 26 cvs and early (27 days after heading) and late (35 days after heading) aphid infestation ratings. There were significant negative linear regressions for both early ($r = -0.50$, $P < 0.01$) and late ($r = -0.39$, $P < 0.05$) infestation ratings with Hx levels. ● Early infestation; ● Late infestation.

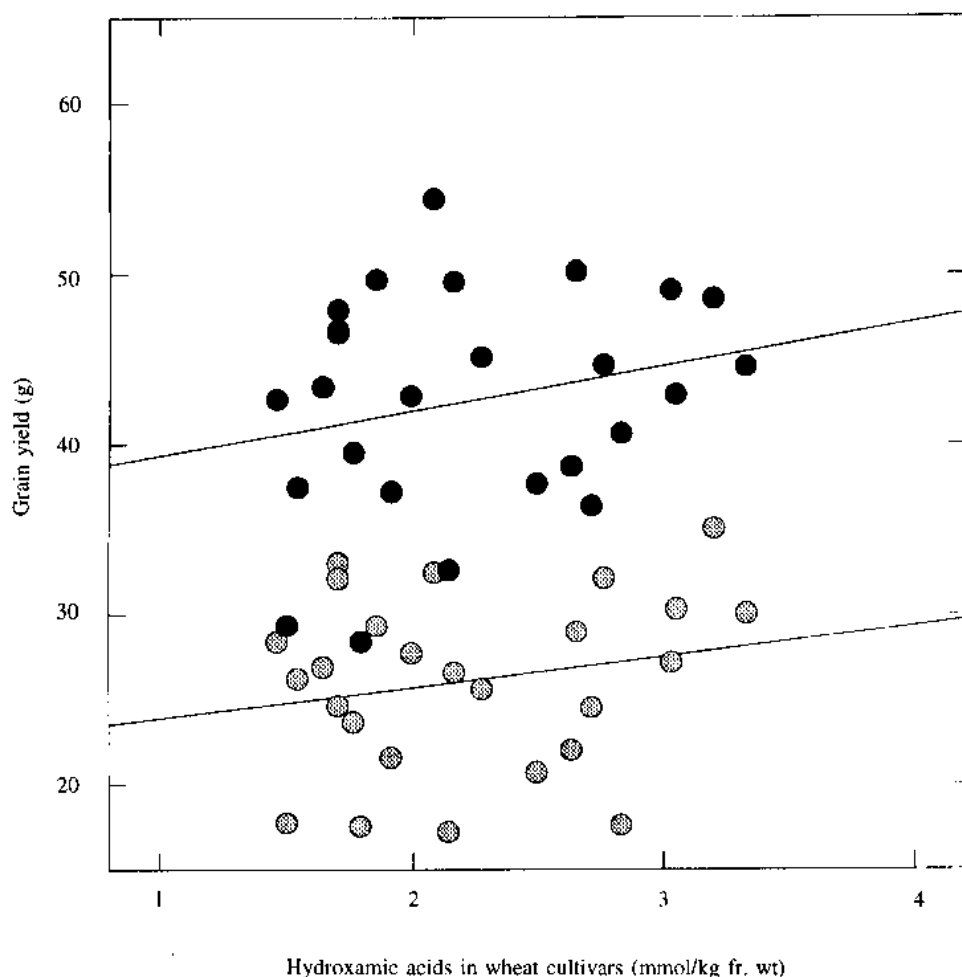


Fig. 2. The relationship between Hx levels in wheat seedlings of 26 cvs and yield. There were non significant linear regressions for both grain yield of 20 heads ($r = 0.20$, $P > 0.3$) and 1000 kernel weight ($r = 0.08$, $P > 0.6$). ● 1000 kernel; ● 20 heads.

wheat plants (up to stage Z69 (Zadoks, Chang & Konzak, 1974)) retain Hx at concentrations which are toxic to aphids (e.g. Corcuera *et al.*, 1982; Thackray *et al.*, 1990). The fact that early infestations showed higher significance levels and explained more residual variation may be accounted for by the decrease of Hx levels with plant age.

Since no significant relationship was found between grain yield and Hx levels in plants it is suggested that Hx accumulation does not impose a cost to the plant in terms of this fitness parameter. Although not uncommon (Simms & Rausher, 1987; Steward & Keeler, 1988), the lack of fitness costs for defence is opposed to what can be expected from the optimal-defence theory (Rhoades, 1979), which states that resources allocated to defence cannot simultaneously be allocated to other functions such as growth (see Herms & Mattson, 1992) or reproduction (Bazzaz, Chiariello, Coley & Pitelka, 1987). In this work, the yield cost of an early investment in secondary metabolites (Hx) was measured. Thus, *sensu stricto* a simultaneous evaluation of the allocation process was not performed. Nonetheless, as argued

above, Hx concentrations at seedling stage are indicators of Hx concentrations in plants at older phenological stages, and hence of the investment the plant makes in defence.

Simms (1992*b*) has proposed two plausible explanations for observed costless resistance: i) resistance traits may have been costly when they first arose, but costs were reduced by subsequent selection. This is unlikely in the case of Hx, given that cereal crops have been subjected to artificial selection directed to certain commercial-value traits not necessarily related to the fitness of the plant. Alternatively, Simms proposes that ii) traits involved in resistance may have benefits other than those associated with reducing herbivory. In the case of Hx, besides their effect on insects, fungi and bacteria and their allelopathic properties (Niemeyer & Pérez, 1995) little is known about their physiological roles. An interaction with the auxine receptor (Venis & Watson, 1978), a cytokinin-like activity (Pethó & Dinya, 1992) and a possible involvement in iron uptake (Pethó, 1992) have been described. Any of these mechanisms may account for the observed pattern.

The present work confirms earlier claims stressing the potential of Hx as breeding targets for aphid resistance in wheat (Nicol *et al.*, 1992; Escobar & Niemeyer, 1993). In addition, it strongly suggests that Hx in wheat seedlings can be reliable chemical markers for scoring aphid resistance in wheat cvs in a rapid and precise way.

Although other resistance mechanisms against aphids in wheat have been reported (Dreyer & Jones, 1981; Roberts & Foster, 1983; Lowe, Murphy & Parker, 1985; Jones & Clocke, 1987; Leszczynski, Wright & Bakowski, 1989) and should not be neglected as potential targets for breeding programmes, none of them has been as thoroughly studied as Hx. Nonetheless, further field experiments are needed to validate all the knowledge about the ecological role of Hx obtained in laboratory experiments.

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References

- Argandoña V H, Niemeyer H M, Corcuera I J. 1981. Effect of content and distribution of hydroxamic acids in wheat on infestation by *Schizaphis graminum*. *Phytochemistry* 20:673-676.
- Bazzaz F A, Chiariello N R, Coley P D, Pitelka L F. 1987. Allocating resources to reproduction and defense. *Bioscience* 37:58-67
- Copaja S V, Niemeyer H M, Wratten S D. 1991. Hydroxamic acid levels in Chilean and British wheat seedlings. *Annals of Applied Biology* 118:223-227.
- Corcuera I J, Argandoña V H, Niemeyer H M. 1982. Effect of cyclic hydroxamic acids from cereals on aphids. In *Chemistry and Biology of Hydroxamic Acids*, pp. 111-118. Ed. H Kehl. Basel: Karger AG.
- Dreyer D L, Jones K C. 1981. Feeding deterrence of flavonoids and related phenolics towards *Schizaphis graminum* and *Myzus persicae*: aphid feeding deterrents in wheat. *Phytochemistry* 20:2489-2493.

- Escobar C A, Niemeyer H M. 1993. Potential of hydroxamic acids in breeding for aphid resistance in wheat. *Acta Agriculturae Scandinavica* **43**:163–167.
- Givovich A, Niemeyer H M. 1991. Hydroxamic acids affecting barley yellow dwarf virus transmission by the aphid *Rhopalosiphum padi*. *Entomologia experimentalis et Applicata* **59**:79–85.
- Givovich A, Niemeyer H M. 1995. Comparison of the effect of hydroxamic acids from wheat on five species of cereal aphids. *Entomologia experimentalis et Applicata* **74**:115–119.
- Grombacher A W, Russell W A, Guthrie W D. 1989. Resistance to first-generation European corn borer (Lepidoptera: Pyralidae) and DIMBOA concentration in midwhorl leaves of the BS9 maize synthetic. *Journal of the Kansas Entomological Society* **62**:103–107.
- Hermes D A, Mattson W J. 1992. The dilemma of plants: to grow or defend. *The Quarterly Review of Biology* **67**:283–335.
- Hofman J, Hofmanová O. 1971. 1,4-Benzoxazine derivatives in plants: absence of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one from uninjured *Zea mays* plants. *Phytochemistry* **10**:1441–1444.
- Jones K C, Klocke J A. 1987. Aphid feeding deterrence of ellagitannins, their phenolic hydrolysis products and related phenolic derivatives. *Entomologia experimentalis et Applicata* **44**:229–234.
- Leather S R, Walters K F A, Dixon A F G. 1989. Factors determining the pest status of the bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae), in Europe: a study and review. *Bulletin of Entomological Research* **79**:345–360.
- Leszczynski B, Wright L C, Bakowski T. 1989. Effect of secondary plant substances on winter wheat resistance to grain aphid. *Entomologia experimentalis et Applicata* **52**:135–139.
- Lowe H J B, Murphy G J P, Parker M L. 1985. Non-glaucousness, a probable aphid resistance character of wheat. *Annals of Applied Biology* **106**:555–560.
- Martos A, Givovich A, Niemeyer H M. 1992. Effect of DIMBOA, an aphid resistance factor in wheat, on the aphid predator *Eriopsis connexa* Germar (Coleoptera: Coccinellidae). *Journal of Chemical Ecology* **18**:469–479.
- 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. *Annals of Applied Biology* **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. *Annals of Applied Biology* **122**:427–433.
- 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 acids content of *Triticum* species. *Euphytica* **37**:289–293.
- Niemeyer H M, Pérez F J. 1995. Potential of hydroxamic acids in the control of cereal pests, diseases and weeds. In *Allelopathy. Organisms, Processes, and Applications*, pp. 260–269. Eds K M M Dakshini and F A Einhellig. ACS Symposium Series 582. Washington, D.C.: Inderjit.
- Niemeyer H M, Copaja S V, Barria B N. 1992. The Triticeae as sources of hydroxamic acids, secondary metabolites in wheat conferring resistance against aphids. *Hereditas* **116**:295–299.
- Papp M, Mesterházy A. 1993. Resistance to bird cherry-oat aphid (*Rhopalosiphum padi* L.) in winter wheat varieties. *Euphytica* **67**:49–57.
- Pethó M. 1992. Occurrence and physiological role of benzoxazinone and their derivatives. III. Possible role of 7-methoxy benzoxazinone in the iron uptake of maize. *Acta Agronomica Hungarica* **41**:57–64.
- Pethó M, Dinya Z. 1992. Occurrence and physiological role of benzoxazinone and their derivatives. I. Cytokinin activity of 6-methoxy-benzoxazinone. *Acta Agronomica Hungarica* **44**:57–64.
- Rhoades D F. 1979. Evolution of plant chemical defense against herbivores. In *Herbivores: Their Interaction with Secondary Plant Metabolites*, pp. 3–54. Eds G A Rosenthal and D H Janzen. Orlando: Academic Press.
- Roberts J J, Foster J E. 1983. Effect of leaf pubescence in wheat on the bird cherry-oat aphid (Homoptera: Aphididae). *Journal of Economic Entomology* **76**:1320–1322.
- Simms E L. 1992a. Costs of plant resistance to herbivory. In *Plant Resistance to Herbivores and Pathogens. Ecology, Evolution and Genetics*, pp. 392–425. Eds R S Fritz and E L Simms. Chicago: University of Chicago Press.

- Simms E L. 1992b.** The evolution of plant resistance and correlated characters. In *Proceedings of the 8th International Symposium on Insect-Plant Relationships*, pp. 15–25. Eds S B Menken, J H Visser and P Harrewijn. Dordrecht: Kluwer Press.
- Simms E L, Rausher M D. 1987.** Costs and benefits of plant defense to herbivory. *American Naturalist* **130**:570–581.
- Steward J L, Keeler K H. 1988.** Are there trade-offs among antiherbivore defenses in *Ipomoea* (Convolvulaceae)? *Oikos* **53**:79–86.
- Thackray D J, Wratten S D, Edwards P J, Niemeyer H M. 1990.** Resistance to the aphids *Sitobion avenae* and *Rhopalosiphum padi* in Gramineae in relation to hydroxamic acid levels. *Annals of Applied Biology* **116**:573–582.
- Venis M A, Watson P J. 1978.** Naturally occurring modifiers of auxin-receptor interaction in corn: identification as benzoxazolinones. *Planta* **142**:103–107.
- Zadoks J C, Chang T T, Konzak C F. 1974.** A decimal code for the growth stages of cereals. *Weed Research* **14**:415–421.

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