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Lack of costs of herbivory-induced defenses in a wild wheat: integration of physiological and ecological approaches

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Aphid infestation triggered a significant induction of hydroxamic acids (Hx) in wild wheat *Triticum uniaristatum* seedlings in comparison to control seedlings. We hypothesized that indirect costs of Hx induction (expressed in plant fitness parameters) would be found if such induction was a consequence of enhanced local synthesis of Hx at the infested tissue and would not if the significantly less costly translocation of Hx was established to be the process underlying the induction. Results obtained following whole-plant analysis after aphid infestation suggested translocation of Hx from the stem as the process underlying the observed Hx induction in the infested leaf. Then, as expected, non-significant differences in growth, size and survival between control and infested plants were found. The present work stresses the importance of unraveling the sources of induced defenses in the understanding of the observed patterns of defense allocation in plants. The consistency of the results obtained with the predictions of the Optimal Defense theory was also discussed.

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Most discussions addressing the evolution of chemical defenses in plants within the framework of the Optimal Defense theory (McKey 1974, Rhoades 1979) have assumed that such defenses are costly (Feeny 1976, Mooney and Gulmon 1982, Rosenthal 1986, Fagerström 1989, Simms and Fritz 1990, Simms 1992a). The direct cost of defense is taken as those resources devoted to synthesis, storage and maintenance of secondary metabolites. The indirect cost of defense, mostly expressed in fitness terms, appears as a consequence of diverting to defense resources otherwise invested in growth and/or reproduction (Zangerl and Bazzaz 1992). Both components of cost, envisioned as physiological and ecological tradeoffs, respectively, are thought to be correlated (Bazzaz et al. 1987).

The existence of damage-inducible defenses in plants has been considered as a strategy that probably evolved to reduce the cost of defense by allocating resources to it only in case of necessity (Haukioja 1980, Herms and Mattson 1992). However, production of induced defenses may exact a cost to the plant. This has been experimentally tested (Brown 1988, Baldwin et al. 1990, Karban 1993), but only the study of Baldwin et al. (1990) detected a phenotypic cost (indirect cost). Although induced defenses have been documented in a large number of plants and plant-herbivore systems (Tallamy and Raupp 1991) seldom have they been sufficiently well characterized in order to attempt a mechanistic explanation (i.e. how is induction produced). Thus, the mechanisms of production of only four induced defenses have been at least partially unveiled: proteinase inhibitors in tomato (Ryan 1990, Pearce et al. 1991), quinolizidine alkaloids in lupine (Wink 1983), nicotine in wild tobacco (Baldwin 1989,

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Baldwin et al. 1994) and furanocoumarins in wild parsnip (Zangerl 1990, Zangerl and Berembaum 1995). Knowledge of the mechanisms of production of induced defenses has been considered of paramount importance in understanding patterns of plant defense and the responsiveness of these mechanisms to selection (Baldwin 1991).

Hydroxamic acids (Hx) are plant secondary metabolites found in wild and cultivated Gramineae (Niemeyer 1988) that play a major role in the resistance of cereals to insects (reviewed in Niemeyer and Pérez 1995). Negative correlations between the performance of cereal aphids and Hx levels in wheat seedlings have been described (Thackray et al. 1990, Nicol et al. 1992, Givovich and Niemeyer 1995). Constitutive Hx accumulation in cultivated wheat protected plants against aphid infestation in the field, and showed no cost in terms of grain yield (Gianoli et al. 1996). On the other hand, induced levels of Hx following insect damage have been detected in seedlings of some wheat and maize cultivars (Gutiérrez et al. 1988, Niemeyer et al. 1989, Leszczynski and Dixon 1990, Gianoli and Niemeyer 1996, Gianoli and Niemeyer 1996).

We herein report the occurrence of Hx induction triggered by aphid infestation on the wild wheat *Triticum uniaristatum* and consider whether such induction is a consequence of enhanced local synthesis of Hx at the infested tissue or the result of the significantly less costly translocation of Hx from uninfested plant parts, and whether the induction imposes an indirect cost to the plant. The aim of this paper is to establish the relationship between a physiological approach (i.e. direct costs) and an ecological one (i.e. indirect costs) to the phenomenon of induced defense. We hypothesize that indirect costs of Hx induction will be found if such induction is a consequence of enhanced local synthesis and will not if translocation of Hx is established to be the process underlying the induction. The consistency of the results obtained with the predictions of the Optimal Defense theory is also discussed.

Materials and methods

Plants

The wild wheat *Triticum uniaristatum* (*Aegilops uniaristata*) is an annual Triticeae that grows in edges of sclerophyllous forests, disturbed habitats and edges of cultivations (Kimber and Feldman 1987). This diploid species belongs to the same tribe of those diploid ancestral wheats that originated the cultivated hexaploid wheat *Triticum aestivum* (Feldman and Sears 1981). Presence of Hx in *T. uniaristatum* seedlings has been described (Niemeyer et al. 1992).

Induction of Hx

Seeds were germinated in individual plastic pots filled with soil (Anasac) and then developed in a growth chamber at 15°C and L:D 12:12 photoperiod. When seedlings attained growth stage 12 (primary leaf fully unfolded, secondary leaf visible; Zadoks et al. 1974) each was infested with 20 individuals of the cereal aphid *Rhopalosiphum padi* (second or third instar apterae) confined in a clip cage attached to the primary leaf. Aphids came from a laboratory polyclonal colony kept on oat seedlings. Empty clip cages were placed on control plants. Treatments were assigned randomly. After 48 h of infestation, aphids were removed from the infested seedlings and primary leaves of both control and treated plants were analyzed for DIBOA (2,4-dihydroxy-1,4-benzoxazin-3-one), the main Hx in *T. uniaristatum* (Niemeyer et al. 1992), by HPLC as previously described (Weibull and Niemeyer 1995). A one-way ANOVA was used to compare treatments ($n = 8$).

Evidence of the origin of induced Hx

In order to assess whether increases in Hx resulted from enhanced local synthesis in the infested leaf or was a consequence of translocation from uninfested organs, the experiment of Hx induction was repeated, this time extending the analysis to all plant organs (primary leaf, secondary leaf, stem, root) in both treatments (control and infested). First, it was determined whether the total amount (μmoles) of Hx per plant differed between treatments. Then, a comparison of relative allocation of Hx to plant organs within treatments was done. This relative allocation of Hx, expressed as [Hx-organ]/[Hx-plant], was used to provide evidence of translocation of Hx to the infested leaf from other plant organs. Total Hx per plant and relative allocations (arcsine-transformed) were compared between treatments by one-way ANOVAs.

Indirect costs of Hx induction

This experiment was designed to evaluate the existence of indirect costs of Hx induction by contrasting fitness components in control and induced plants. Costs should appear as a greater performance of the control plants. First, the infestation protocol was applied to seedlings ($n = 31$) growing individually in 2.5-l plastic pots filled with soil (Anasac). Control and infested treatments were assigned randomly. Once infestation ended (48 h), pots were transferred to a carbon fiber greenhouse where they were interspersed by a systematic design in accordance to Hurlbert (1984). In order to assess parameters related to plant fitness we noted in both treatments the following: 1) date of appearance of

new leaves, 2) leaf growth (elongation), 3) time to tillering, and 4) mortality. Four months after pots were transferred to the greenhouse the experiment ended, the plants were cut, and the aerial biomass oven-dried at 70°C for 48 h and weighed. Environmental conditions within the greenhouse were as follows: temperature min. 12–20°C, max. 29–36°C; light intensity max. 30–50 Klux; photoperiod 14–16 h light (summer time). Procedures and statistics applied to each set of data are described in the Results section.

Results

Induction of Hx

Aphid controlled infestation triggered an increased Hx accumulation (Hx induction) in *T. uniaristatum* seedlings of 71% in comparison to control seedlings (mean ± SE: 0.214 ± 0.019 and 0.125 ± 0.028 mmol/kg fresh wt, respectively) (Fig. 1). This difference was statistically significant: $F_{1,14} = 6.99$, $P = 0.019$, one-way ANOVA.

Evidence of the origin of induced Hx

Total amounts of Hx per plant (μmoles, mean ± SE) were similar ($F_{1,14} = 4.75$, $P = 0.845$, one-way ANOVA) between control (0.221 ± 0.011) and infested (0.215 ± 0.029) seedlings. This rendered de novo synthesis of Hx in infested seedlings as unlikely.

By extending the analysis of Hx to all plant organs and calculating the ratio between the relative allocation of Hx to the primary leaf (the induced, "sink", tissue) and each one of the other plant organs (potential "source" tissues) it was shown that the only significant difference between treatments was found for the primary leaf/stem relationship (Table 1). This suggested translocation of Hx from the stem to the infested leaf as the process underlying the observed induction.

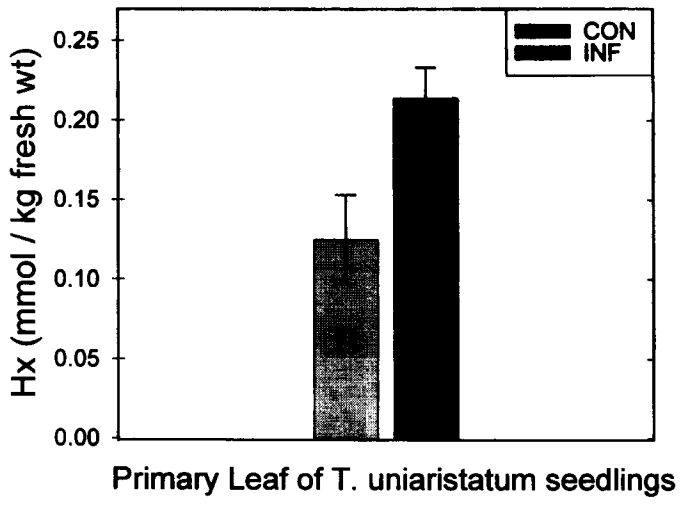

Fig. 1. Mean (±SE) concentration of hydroxamic acids (Hx) in the primary leaf of control (CON) and aphid infested (INF) seedlings of *T. uniaristatum* at the two-leaves stage. Aphid infestation (20 *R. padi* individuals confined to the primary leaf) lasted for 48 h, then both control and infested primary leaves were analyzed for Hx content ($n = 8$).

Table 1. Relative allocation of Hx (mean ± SE) between primary leaf and non-infested organs in *T. uniaristatum* seedlings. INF = infested with aphids, CON = control seedlings. Leaf 1 = primary leaf, Leaf 2 = secondary leaf. *P*-value refers to a one-way ANOVA ($n = 8$).

	CON	INF	P-value
Stem/Leaf 1	10.208 ± 1.173	5.713 ± 0.761	0.007
Leaf 2/Leaf 1	16.320 ± 2.242	13.915 ± 3.505	0.574
Root/Leaf 1	5.284 ± 0.618	3.769 ± 0.739	0.142

Indirect costs of Hx induction

Growth of primary and secondary leaves 3 and 7 d after the end of infestation did not differ between treatments (Table 2). Large differences between mean values were found for the primary leaf but the very high SE values rendered these differences non-significant. Since differences were less significant (Kruskal-Wallis one-way ANOVA) for the observation at 7 d for both leaves, further evaluations were dismissed.

The number of leaves produced by plants (mean ± SE) before they reached the tillering stage was similar ($F_{1,36} = 0.35$, $P = 0.557$, one-way ANOVA) for control (3.76 ± 0.20) and infested (3.90 ± 0.14) plants. In order to obtain a more precise assessment of the dynamics of leaf production, leaves within each treatment were considered as a population and the intrinsic rate of population growth of leaf number for control and infested plants was calculated from the logistic equation of population increase, following Bazzaz and Harper (1977). Values of r_m were obtained from the slope of linear regressions of the plot ln $(K - N)/N$ vs t for each treatment; where K = mean total number of leaves produced, N = mean number of leaves produced at day t, t = days after infestation. Values of r_m were 0.1056 for control plants and 0.1210 for infested ones (Fig. 2). An F test of homogeneity of slopes showed that such values did not differ significantly ($F_{1,6} = 5.99$, $P > 0.95$).

In order to determine whether Hx induction in infested seedlings affected their developmental program,

Table 2. Growth (mm, mean ± SE) of primary (Leaf 1) and secondary (Leaf 2) leaves of *T. uniaristatum* seedlings after infestation. INF = infested with aphids, CON = control seedlings ($n = 30$). *P*-value refers to a Kruskal-Wallis ANOVA.

	3 d after infestation		7 d after infestation	
	Leaf 1	Leaf 2	Leaf 1	Leaf 2
CON	1.43 ± 0.96	13.90 ± 1.59	1.73 ± 0.69	37.95 ± 5.30
INF	0.04 ± 0.04	16.72 ± 1.75	0.76 ± 0.22	42.35 ± 4.23
P	0.23	0.30	0.79	0.48

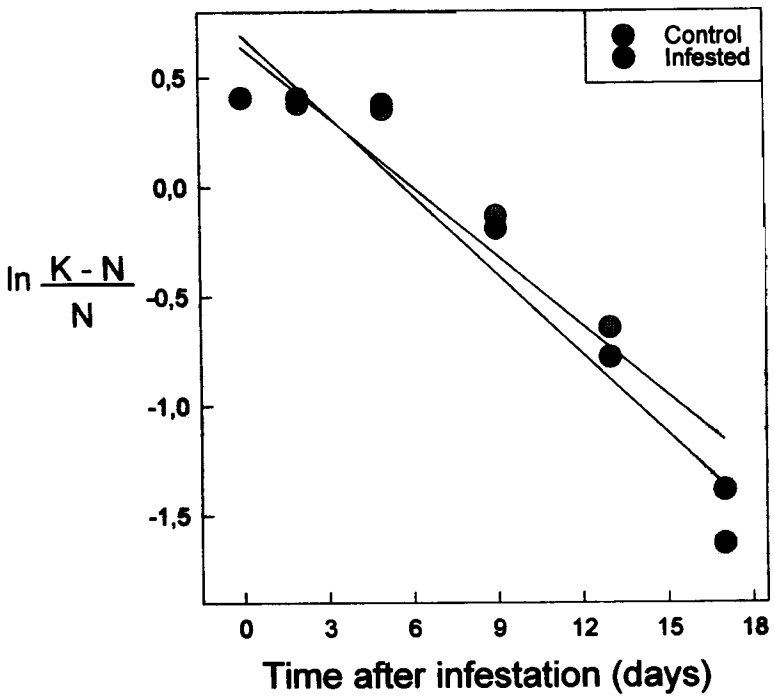

Fig. 2. Linear regressions between t = days after aphid infestation and ln $(K-N)/N$ for control and aphid infested plants of *T. uniaristatum*. K = mean total number of leaves produced; N = mean number of leaves produced at day t. The slope of each regression line equals the intrinsic rate of increase (r_m) of the population of leaves within that treatment. Aphid infestation lasted for 48 h (seedlings at the two-leaves stage). Each point represents an average of 16–30 plants.

the time that plants took to attain the tillering stage was evaluated. A frequency distribution of such parameter (Fig. 3) showed no significant differences among treatments (two-tailed Kolmogorov-Smirnov two-samples test, $\alpha = 0.05$).

Finally, no significant differences ($F_{1,30} = 0.22$, $P = 0.643$, one-way ANOVA) were found for final shoot biomass (Fig. 4) or mortality along the whole experiment (50% vs 45%) for control and infested plants.

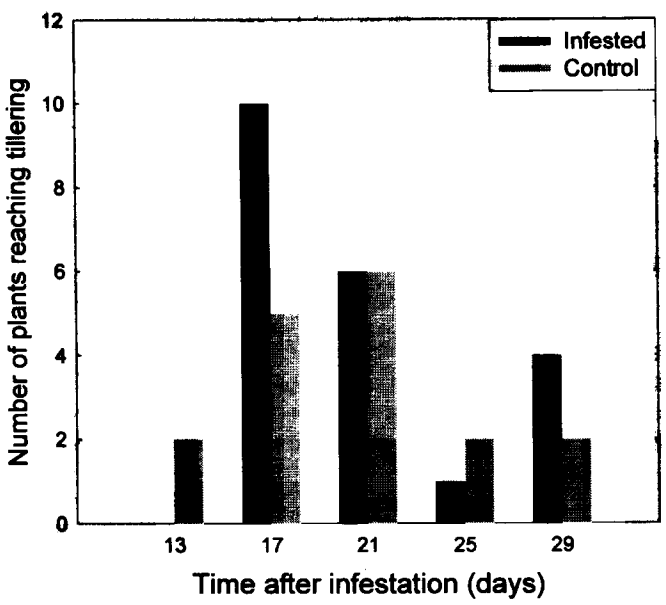

Fig. 3. Frequency distribution of the time to attain the stage of tillering for control and aphid infested plants of *T. uniaristatum*. Aphid infestation lasted for 48 h (seedlings at the two-leaves stage).

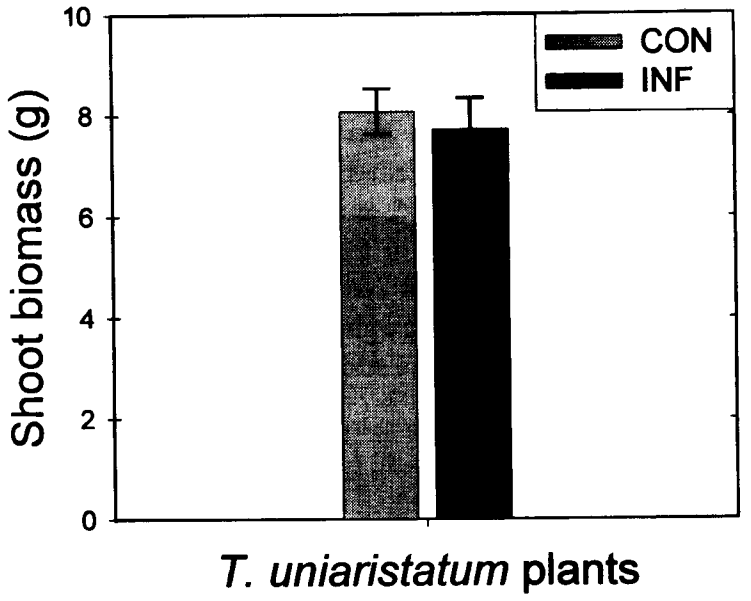

Fig. 4. Mean (±SE) dry weight of the shoot of control (CON) and aphid infested (INF) plants of *T. uniaristatum* four months after infestation ($n = 16$). Aphid infestation lasted for 48 h (seedlings at the two-leaves stage).

Discussion

Induction of Hx

The amount of Hx induction in this experiment (71%) was relatively large in comparison with reported Hx increases following aphid infestation in wheat (range of 15–96%, Niemeyer et al. 1989, Leszczynski and Dixon 1990) in spite of the magnitude of the stimulus applied to trigger the induced response in the present work (20 aphids, 48 h) being less than those applied in the cited reports. No symptoms of damage could be observed by the end of the infestation period. Likewise, no significant metabolic changes in barley seedlings were found for similar aphid loads (Cabrera et al. 1994). Therefore, it seems that the stimulus applied was sufficient to represent a cue of attack to the plant without inflicting a significant damage (a well-desired condition for studies addressing costs of induced defenses, see below). A study performed in bread wheat (*Triticum aestivum*) showed that aphid densities lower than 20 per seedling were not able to induce Hx accumulation, 48 h later, in the infested leaf (Gianoli and Niemeyer unpubl.). This suggests that 20 aphids per seedling is a probable threshold of infestation to observe Hx induction in these systems.

Evidence of the origin of induced Hx

The aim of this experiment was to determine whether Hx induction was a consequence of enhanced local synthesis in the infested tissue or the result of the significantly less costly translocation from uninfested plant parts. Evidence found suggested translocation from the stem to the primary leaf as the process underlying the observed Hx induction. It must be stressed that these results are not conclusive since the most

appropriate experimental tool (utilization of labeled isotopes, e.g. Baldwin et al. 1994) was not used. Nevertheless, although unable to determine precisely the rate and net direction of transport, this coarser approach allowed us to distinguish between the two general processes that could be accounting for the Hx induction.

The suggested translocation is likely to occur via phloem, where Hx have been found (Givovich et al. 1994), although this should be tested experimentally (e.g. Gowan et al. 1995). At first sight the pattern of translocation found might appear unusual, given that phloem translocation of metabolites occurs generally from mature leaves to actively growing areas (Richardson 1975). However, it has been stated that general phloem translocation patterns may be changed by interference from wounding (Taiz and Zeiger 1991) and, more pertinent to the present case, aphids have been shown to be capable of changing source-sink relations within the plant (Dixon 1975). With regard to the energetic demand of translocation of compounds such as secondary metabolites, it is considered that their loading and unloading to and from the sieve elements occurs passively and, once in the sieve elements, their transport along the phloem is driven by the bulk flow generated by the active load of sugars and amino acids (Taiz and Zeiger 1991).

Results obtained suggest that *T. uniaristatum* seedlings react to aphid infestation by increasing the concentration of their main defensive metabolites at the attacked tissue through non-costly translocation from non attacked tissues. This spatial reallocation of defenses following a localized insect attack (Table 1) leads to consider that induction of Hx in this system and under these conditions does not entail a direct cost to the plant. In accordance with earlier suggestions concerning constitutive defenses (Bazzaz et al. 1987), the lack of direct costs of Hx induction should be reflected in a lack of indirect costs (i.e. absence of differences in fitness components between infested and control plants). Testing of this expected correspondence is by no means superfluous. Whereas the confirmation of lack of costs at the ecological level would validate the integration of approaches pointed out above and hence the predictive value of discerning the mechanism responsible for induction, finding indirect costs would challenge the assumptions of either non-costly translocation or Hx as the "silver bullet" (cf. Karban 1993) utilized by plants for defense.

With regard to previous work on well-characterized induced defenses systems, Baldwin (1989) found evidence of a costly mechanism of alkaloid induction in leaves of wild tobacco arising from increased synthesis of alkaloids in roots and further translocation to leaves. A later report determined the existence of phenotypic costs of these damage-induced responses (Baldwin 1991). In their turn, Zangerl and Berembaum (1995) found that damage-induced xanthotoxin (a furanocoumarin) production in wild parsnip despite being rapid, localized and of short duration (features tending to minimize the cost of an induced defense, as the authors pointed out) was a consequence of in situ biosynthesis. This induced response is expected to impose an indirect cost to the plant, although this remains to be tested in such system. However, it should be kept in mind that the nature of plant responses following mechanical damage and herbivore attack may differ (Hartley and Lawton 1987) and hence the consequences of mechanical damage may not be ecologically significant.

Indirect costs of Hx induction

An inherent complication of the experiments designed to measure costs of induced defenses is the difficulty to separate the costs of the induced response from the costs of the damage applied to trigger such responses. Attempts to overcome this difficulty have 1) used inducer chemicals (Brown 1988), 2) used chemicals able to inhibit the induced response (Baldwin 1991), or 3) simply assumed such coupling of effects as a realistic representation of what occurs in nature (Karban 1993). All three approaches have shortcomings since 1) chemical triggering may not produce the whole range of responses affected by natural infestation, 2) chemical inhibitors may in turn affect plant response in non-measurable ways, or 3) if costs are found no conclusive interpretation may be produced, respectively. The wheat–Hx system provides a way to cope with this problem based on the fact that increasing aphid infestations above the threshold of 20 aphids/seedling (up to 50 aphids/seedling) produced the same levels of Hx induction (Gianoli and Niemeyer 1997) but, presumably, increased damage levels. The effect of damage in fitness (experiment of indirect costs) could have been determined in treatments differing in level of damage yet producing the same level of Hx induction. However, this experimental protocol was not followed since the former experiment yielded no indirect costs for Hx induction in *T. uniaristatum*.

Non-significant differences in fitness parameters between control and infested plants were found. Parameters measured were related to 1) growth, a good proximal measure of plant fitness (Silvertown 1982), 2) survival, and 3) size, which is an accurate predictor of reproductive output in plants (Samson and Werk 1986, Aarsen and Taylor 1992). Regarding size, Baldwin (1991) described similar direct relationships between allocation to vegetative and reproductive structures for induced and non-induced wild tobacco plants, and hence concluded that the costs associated with such induced responses (decreased reproductive output) could be seen simply as a decrement in plant size.

At least three explanatory hypotheses to the lack of indirect costs of induced defenses may be put forward. First, costs do exist but were undetectable under the prevailing experimental conditions (e.g. Brown 1988). Second, defensive metabolites analyzed possess other physiological roles related with growth or phenology of plants that counterbalanced the costs incurred in their production (after Simms 1992b). Third, fitness parameters measured were not appropriate or were evaluated at the wrong time (e.g. Karban 1993). All of these hypotheses assume or suggest the occurrence of some events or processes whose evaluation is not always feasible. Although the validity of these hypotheses cannot be neglected a priori, the pattern found in the present work (lack of indirect costs of Hx induction) may be satisfactorily explained by an initial physiological approach that addressed the origin of such induced Hx, whereby a lack of direct costs of such phenomena was established.

The fact that induced accumulation of Hx did not entail a fitness cost to plants of *T. uniaristatum* may be viewed at first glance as a challenge to the validity of the Optimal Defense theory. However, the postulate of the Optimal Defense theory dealing with costs of defense assumes, in order to justify such cost, an exclusive use of resources i.e. the cost arises from the diversion to defense of resources otherwise allocated to growth or reproduction. In this work, such possibility was ruled out by determining that induced Hx arose from a spatial reallocation of resources already committed to defense. Therefore, the results obtained can be considered as consistent with predictions based on the Optimal Defense theory.

It should be noted that the phenomenon of induced defenses, although not originally considered in the Optimal Defense theory, does fit into it if one considers that a leading thought of the theory is the allocation of defenses where they are most needed. In other words, while in the realm of constitutive defenses the theory states a larger allocation of defenses to plant tissues with a higher probability of being attacked, in the realm of induced defenses this may be interpreted as a higher allocation of defenses where an actual attack has occurred. Empirical tests of the validity of the Optimal Defense theory to patterns of induced defenses have been undertaken only recently (Baldwin and Ohnmeiss 1994, Baldwin et al. 1994, Ohnmeiss and Baldwin 1994, Baldwin and Karb 1995, Zangerl and Rutledge 1996).

The present work showed experimentally that the integration of direct and indirect costs of induced defenses in plants is feasible, as follows from an earlier theoretical suggestion concerning constitutive defenses (Bazzaz et al. 1987). In addition, this work stresses the importance of unraveling the sources of induced defenses in the understanding of the observed patterns of defense allocation in plants.

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References

Aarsen, L. W. and Taylor, D. R. 1992. Fecundity allocation in herbaceous plants. – Oikos 65: 225–232.

Baldwin, I. T. 1989. The mechanism of damage-induced alkaloids in wild tobacco. – J. Chem. Ecol. 15: 1661–1680.

– 1991. Damage-induced alkaloids in wild tobacco. – In: Tallamy, D. W. and Raupp, M. J. (eds), Phytochemical induction by herbivores. Wiley, New York, pp. 71–84.

– and Ohnmeiss, T. E. 1994. Coordination of photosynthetic and alkaloidal responses to damage in uninducible and inducible *Nicotiana sylvestris*. – Ecology 75: 1003–1014.

– and Karb, M. J. 1995. Plasticity in allocation of nicotine to reproductive parts in *Nicotiana attenuata*. – J. Chem. Ecol. 21: 897–909.

– Sims, C. L. and Kean, S. E. 1990. The reproductive consequences associated with inducible alkaloidal responses in wild tobacco. – Ecology 71: 252–262.

– Karb, M. J. and Ohnmeiss, T. E. 1994. Allocation of ^{15}N from nitrate to nicotine: production and turnover of a damage-induced mobile defense. – Ecology 75: 1703–1713.

Bazzaz, F. A. and Harper, J. L. 1977. Demographic analysis of the growth of *Linum usitatissimum*. – New Phytol. 78: 193–208.

– Chiariello, N. R., Coley, P. D. and Pitelka, L.F. 1987. Allocating resources to reproduction and defense. – Bioscience 37: 58–67.

Brown, D. G. 1988. The cost of plant defense: an experimental analysis with inducible proteinase inhibitors in tomato. – Oecologia 76: 467–470.

Cabrera, H. M., Argandoña, V. H. and Corcuera, L. J. 1994. Metabolic changes in barley seedlings at different aphid infestation levels. – Phytochemistry 35: 317–319.

Dixon, A. F. G. 1975. Aphids and translocation. – In: Zimmermann, M. L. and Milburn, J. A. (eds), Transport in plants. I. Phloem transport. Springer-Verlag, Berlin, pp. 154–170.

Fagerström, T. 1989. Anti-herbivory chemical defense in plants: a note on the concept of cost. – Am. Nat. 133: 281–287.

Feeny, P. P. 1976. Plant apparency and chemical defense. Rec. Adv. Phytochem. 10: 1–40.

Feldman, M. and Sears, E. R. 1981. The wild gene resources of wheat. – Sci. Am. 244: 98–109.

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

– Papp, M. and Niemeyer, H. M. 1996. Costs and benefits of hydroxamic acids-related resistance in winter wheat against the bird cherry-oat aphid, *Rhopalosiphum padi*. – Ann. Appl. Biol. 129: 83–90.

Givovich, A. and Niemeyer, H. M. 1995. Comparison of the effect of hydroxamic acids from wheat on five species of cereal aphids. – Entomol. Exp. Appl. 74: 115–119.

– Sandström, J., Niemeyer, H. M. and Pettersson, J. 1994. Presence of a hydroxamic acid glucoside in wheat phloem sap, and its consequences for the performance of *Rhopalosiphum padi* (L.) (Homoptera: Aphididae). - J. Chem. Ecol. 20: 1923–1930.

Gowan, E., Lewis, B. A. and Turgeon, R. 1995. Phloem transport of antirrhinoside, an iridoid glycoside, in *Asarina scandens* (Scrophulariaceae). – J. Chem. Ecol. 21: 1781–1788.

Gutiérrez, C., Castañera, P. and Torres, V. 1988. Wound-induced changes in DIMBOA (2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-one) concentration in maize plants by *Sesamia nonagrioides* Lef. (Lepidoptera, Noctuidae). – Ann. Appl. Biol. 113: 447–454.
Hartley, S. E. and Lawton, J. H. 1987. Effects of different types of damage on the chemistry of birch foliage, and the response of birch feeding insects. – Oecologia 74: 432–437.
Haukioja, E. 1980. On the role of plant defences in the fluctuation of herbivore populations. – Oikos 35: 202–213.
Herms, D. A. and Mattson, W. J. 1992. The dilemma of plants: to grow or defend. – Q. Rev. Biol. 67: 283–335.
Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. – Ecol. Monogr. 54: 187–211.
Karban, R. 1993. Costs and benefits of induced resistance and plant density for a native shrub, *Gossypium thurberi*. – Ecology 74: 9–19.
Kimber, G. and Feldman, M. 1987. Wild wheat: an introduction. – Sp. Rep. 353. Coll. Agric., U. Miss-Col.
Leszczynski, B. and Dixon, A. F. G. 1990. Resistance of cereals to aphids: interaction between hydroxamic acids and the aphid *Sitobion avenae* (Homoptera: Aphididae). – Ann. Appl. Biol. 117: 21–30.
McKey, D. 1974. Adaptive patterns in alkaloid physiology. – Am. Nat. 108: 305–320.
Mooney, H. A. and Gulmon, S. L. 1982. Constraints on leaf structure and function in reference to herbivory. – Bioscience 32: 198–206.
Nicol, D., Copaja, S. V., Wratten, S. D. and Niemeyer, H. M. 1992. Screen of worldwide wheat cultivars for hydroxamic acids levels and aphid antixenosis. – Ann. Appl. Biol. 121: 11–18.
Niemeyer, H. M. 1988. Hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-ones), defence chemicals in the Gramineae. – Phytochemistry 27: 3349–3358.
– and Pérez, F. J. 1995. Potential of hydroxamic acids in the control of cereal pests, diseases and weeds. – In: Inderjit, Dakshini, K. M. M. and Einhellig, F. A. (eds), Allelopathy. Organisms, processes, and applications. ACS Symp Ser 582, Washington, pp. 260–269.
– , Pesel, E., Copaja, S. V., Bravo, H. R., Franke, S. and Francke, W. 1989. Changes in hydroxamic acids levels of wheat plants induced by aphid feeding. – Phytochemistry 28: 447–449.
– , 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.
Ohnmeiss, T. E. and Baldwin, I. T. 1994. The allometry of nitrogen allocation to growth and an inducible defense under nitrogen-limited growth. – Ecology 75: 995–1002.
Pearce, G., Strydom, D., Johnson, S. and Ryan, C. A. 1991. A polypeptide from tomato leaves induces wound-inducible proteinase inhibitor proteins. – Science 253: 895–898.
Rhoades, D. F. 1979. Evolution of plant chemical defense against herbivores. – In: Rosenthal, G. A. and Janzen, D. H. (eds), Herbivores: their interaction with secondary plant metabolites. Academic Press, Orlando, FL, pp. 3–54.
Richardson, M. 1975. Translocation in plants. – Camelot Press, Southampton.
Rosenthal, G. A. 1986. The chemical defenses of higher plants. – Sci. Am. 254: 76–81.
Ryan, C. A. 1990. Protease inhibitors in plants. Genes for improving defenses against insects and pathogens. – Annu. Rev. Phytopatol. 28: 425–449.
Samson, D. A. and Werk, K. S. 1986. Size-dependent effects in the analysis of reproductive effort in plants. – Am. Nat. 127: 667–680.
Silvertown, J. W. 1982. Introduction to plant population biology. – Longman, New York.
Simms, E. L. 1992a. Costs of plant resistance to herbivory. – In: Fritz, R. S. and Simms, E. L. (eds), Plant resistance to herbivores and pathogens. Ecology, evolution and genetics. Univ. of Chicago Press, Chicago, pp. 392–425.
– 1992b. The evolution of plant resistance and correlated characters. – In: Menken, S. B., Visser, J. H., Harrewijn, P. (eds), Proceedings of the 8th international symposium on insect-plant relationships. Kluwer, Dordrecht, pp. 15–25.
– and Fritz, R. F. 1990. The ecology and evolution of host-plant resistance to insects. – Trends Ecol. Evol. 5: 356–360.
Taiz, L. and Zeiger, E. 1991. Plant physiology. – Benjamin Cummings, California.
Tallamy, D. W. and Raupp, M. J. 1991. Phytochemical induction by herbivores. – Wiley, New York.
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.
Weibull, J. and Niemeyer, H. M. 1995. Changes in dihydroxymethoxybenzoxazinone glucoside content in wheat plants infected by three plant pathogenic fungi. – Physiol. Mol. Plant Pathol. 47: 201–209.
Wink, M. 1983. Wounding-induced increase of quinolizidine alkaloid accumulation in lupin leaves. Z. Naturforsch. 38: 905–909.
Zadoks, J. C., Chang, T. T. and Konsak, C. F. 1974. A decimal code for the growth stages of cereals. – Weed Res. 14: 415–421.
Zangerl, A. R. 1990. Furanocoumarin induction in wild parsnip: evidence for an induced defense against herbivores. – Ecology 71: 1926–1932.
– and Bazzaz, F. A. 1992. Theory and pattern in plant defense allocation. – In: Fritz, R. S. and Simms, E. L. (eds), Plant resistance to herbivores and pathogens. Ecology, evolution and genetics. Univ. of Chicago Press, Chicago, pp. 363–391.
– and Berembaum, M. R. 1995. Spatial, temporal and environmental limits on xanthotoxin induction in wild parsnip foliage. – Chemoecology 5/6: 37–42.
– and Rutledge, C. E. 1996. The probability of attack and patterns of constitutive and induced defense. A test of optimal defense theory. – Am. Nat. 147: 599–608.