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Environmental effects on the induction of wheat chemical defences by aphid infestation

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Abstract The effects of temperature and photoperiod on the ability of wheat (*Triticum aestivum* L.) seedlings to show induced responses (increased accumulation of hydroxamic acids, Hx) upon infestation by the bird cherry-oat aphid, *Rhopalosiphum padi* L. were evaluated under laboratory conditions. Induction of Hx was significantly higher at lower temperatures. No such clear trend was found for the photoperiod effect. The significant effect of environmental conditions on growth rate of seedlings and the significant negative correlation between growth rate prior to infestation and induction of Hx suggested that environmental effects on induced responses were at least partially mediated through their effect on plant growth rate. After statistically uncoupling the effect of environmental conditions from the effect of plant growth rate, the effect of temperature on induction of Hx was no longer significant. Therefore, the temperature effect was mediated by plant growth rate.

Key words Temperature · Growth · Hydroxamic acids · Herbivory · Induced defences

Introduction

There is considerable evidence that shows that damage to plant tissues by herbivorous insects may induce changes in the secondary metabolism of the plant (Tallamy and Raupp 1991 and references therein; Baldwin et al. 1994; Redak and Capinera 1994). On the other hand, plants under different environmental conditions may show changes in resource allocation to

main biological functions such as growth, reproduction and defence (Bazzaz et al. 1987; Chapin et al. 1987; Herms and Mattson 1992). Hence it could be expected that the way plants respond to herbivory should be somehow tuned to the environmental conditions under which the plant-herbivore interaction occurs. The effects of both temperature and photoperiod on induced resistance to insects, measured as insect performance parameters (Karban 1987) as well as herbivory-induced levels of plant secondary metabolites (Haukioja and Niemelä 1979; Neuvonen and Danell 1987; Wolfson and Murdoch 1990) have been described. However, the latter reports used experimental designs unable to distinguish between environmental and plant phenology effects on the observed induced defences.

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 (Niemeyer and Pérez 1995). Negative correlations between the performance of cereal aphids and Hx levels in wheat seedlings have been reported (Thackray et al. 1990; Nicol et al. 1992; Givovich and Niemeyer 1995). In addition, increases in Hx concentration 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).

We herein describe the effects of temperature and photoperiod on the ability of wheat (*Triticum aestivum* L.) seedlings to show induced responses (changes in accumulation of Hx) upon controlled infestation by the bird cherry-oat aphid, *Rhopalosiphum padi* L., under laboratory conditions. We also looked at the involvement of seedling growth rate – which differed among treatments – on the patterns found. The experimental design used (infested seedlings were at the same phenological stage) allowed us to distinguish between environmental and plant phenology effects. Implications of our findings in issues of plant defence strategies are also discussed.

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Materials and methods

Seeds of *T. aestivum* cv. Paleta were obtained from INIA, Chile, and germinated in individual plastic pots (25 ml) filled with soil (Anasac). Seedlings grew in a growth chamber at the temperatures and photoperiods described below. Colonies of *R. padi* were kept on oat plants (an Hx-lacking cereal) in a greenhouse at $20 \pm 3^\circ\text{C}$ and L12:D12 photoperiod.

For infestation 20 aphids (second or third instar apterae) were confined in a clip cage attached to the primary leaf of a seedling at growth stage 12 (Zadoks et al. 1974). Empty clip cages were placed on control plants. Each treatment included eight seedlings. The basic infestation procedure was repeated in the growth chamber under the nine different environmental conditions arising from a factorial array of three temperatures (15, 20 and 25°C) and three photoperiods (8, 12 and 16 h light). The infestation of seedlings of the same phenological stage – but different chronological age between treatments – allowed the distinction between environmental and plant phenology effects.

After 48 h of infestation, aphids were removed from the infested seedlings and both control and treated plants were analysed for total Hx by HPLC as previously described (Weibull and Niemeyer 1995). An induction index defined as $\text{INDX} = [\text{Hx}]$ of infested plant/mean[Hx] of control plants was determined at each environmental condition and a two-way ANOVA was performed, with temperature and photoperiod as fixed effects. In addition, growth rate (mm/day) prior to infestation was estimated for each individual seedling and correlated with its induction index. Indexes were transformed [$Y = \log(\text{INDX} + 1)$] prior to analysis in order to achieve the statistical assumptions of the test.

Results

Induction of Hx was significantly affected by temperature ($P < 0.002$), lower temperatures leading to increased induced responses. The highest INDX values were obtained at 15°C for any photoperiod. However, INDX values at 25°C were higher (although not significantly, LSD test) than at 20°C for the 16 and 12 h light treatments. No clear trend was found for the effect of photoperiod ($P > 0.79$). In addition, the interaction of effects was significant ($P < 0.05$) (Fig. 1). As expected, seedling growth rate prior to infestation was significantly affected by both temperature and photoperiod as well as by their interaction, higher temperatures and longer daylengths enabling plants to grow faster (Table 1).

In the context of varying Hx accumulation with plant phenological stage (Argandoña et al. 1981) it was deemed interesting to study the relationship between seedling growth rate and the strength of induced responses. Seedling growth rate (mm/day) prior to infestation was significantly negatively correlated with INDX ($r = -0.28$, $P < 0.02$, $n = 72$) (Fig. 2).

Since both temperature and light regime significantly affected seedling growth rate prior to infestation (Table 1), it was determined whether the described effect of temperature on INDX (Fig. 1) reflected merely the effect of growth rate on INDX. The two-way ANOVA was repeated including “growth rate prior to infesta-

Table 1 Growth rate (mm/day) prior to infestation of wheat seedlings under different temperature and light regimes. Means of 8 replicates \pm SE. Different lower case letters indicate statistically significant differences within rows and columns ($P < 0.05$, LSD test)

Temperature	Daylength		
	16	12	8
25	$18.41 \pm 0.31\text{a}$	$16.39 \pm 0.39\text{c}$	$15.62 \pm 0.29\text{c}$
20	$13.65 \pm 0.20\text{d}$	$17.32 \pm 0.32\text{b}$	$15.76 \pm 0.25\text{c}$
15	$12.94 \pm 0.33\text{de}$	$12.11 \pm 0.36\text{e}$	$11.09 \pm 0.16\text{f}$

Two-way ANOVA P -values for temperature (T), photoperiod (P) and $T \times P$ were all < 0.0001

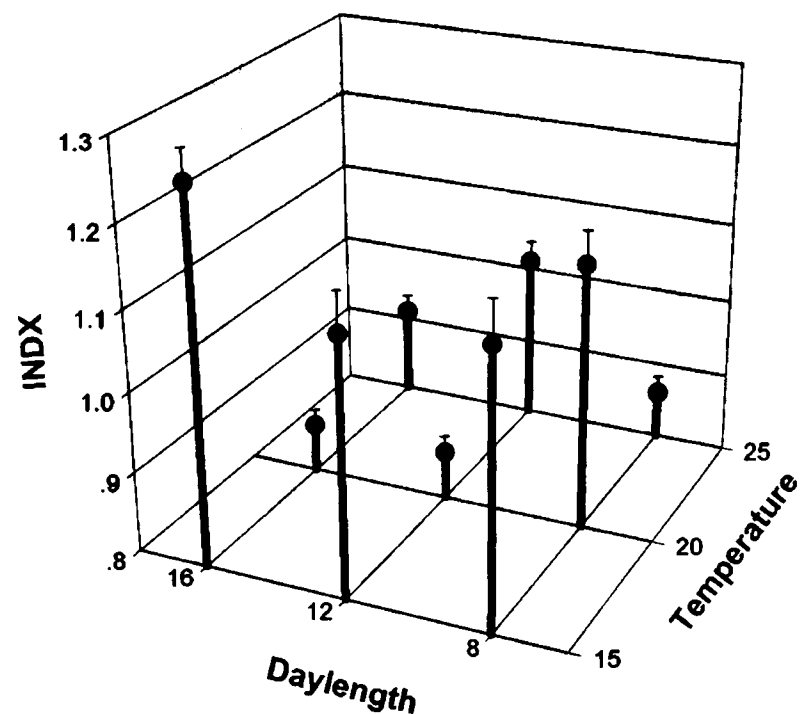


Fig. 1 Effect of temperature and photoperiod on induced levels of hydroxamic acids (Hx) in wheat seedlings upon aphid infestation. $\text{INDX} = [\text{Hx}]$ of infested plant/mean [Hx] of control plants under each treatment. See Table 2 for statistical analysis results

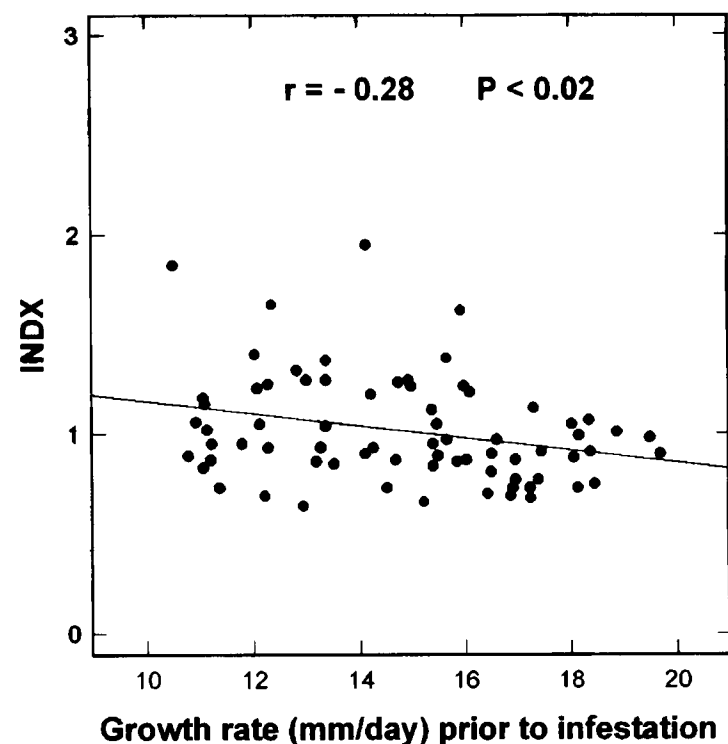


Fig. 2 The relationship between wheat seedling growth rate prior to infestation by aphids and induced levels of Hx upon aphid infestation. $\text{INDX} = [\text{Hx}]$ of infested plant/mean [Hx] of control plants under each treatment

Table 2 Analysis of variance of the induction of Hx index (INDX) in wheat seedlings grown under nine environmental treatments arising from three temperature and three photoperiod combinations.

Between brackets: values obtained including "seedling growth rate prior to infestation" as a covariate

Source	<i>df</i>	Mean square	<i>F</i> -ratio	<i>P</i> -value
Temperature (T)	2 [2]	0.095 [0.024]	7.508 [1.855]	0.001 [0.165]
Photoperiod (P)	2 [2]	0.003 [0.004]	0.228 [0.316]	0.797 [0.730]
T × P	4 [4]	0.335 [0.031]	2.641 [2.391]	0.042 [0.060]
Error	63 [62]	0.013 [0.013]		

tion" as a covariate, i.e., removing its effect. In this new analysis the former strongly significant effect of temperature on INDX disappeared. Likewise, the interaction effect became non-significant (Table 2).

Discussion

In view of the significant effect of temperature on induced levels of Hx by aphid feeding it may be asked whether this pattern results from effects on the insect, on the plant or on both. It is unlikely that the observed pattern is a consequence of differences in aphid feeding. Lower temperatures should decrease aphid feeding and hence damage and Hx induction given that optimal performance of aphids occurs in the 20–25 °C range (Dixon 1985). This range is near the highest temperature used in our experiments. Therefore, differential aphid feeding cannot account for the greater induced levels of Hx observed at lower temperatures. With regard to the effect on plants, it was clear that seedlings growing under higher temperatures or longer daylengths reached the phenological stage suitable for infestation earlier. Consequently, a major evident effect of environment on plants is variation of growth rate. Given that seedling growth rate prior to infestation was significantly negatively correlated with induction of Hx it is possible that the temperature effect on Hx induction was mediated by its effect on plant growth rate. Statistical analysis showed that after removing the effect of plant growth rate the effect of temperature on Hx induction was no longer significant. Therefore, the effect of temperature on induction of Hx was mediated by plant growth rate. This suggests an intrinsic effect of plant growth rate rather than the existence of environmental cues that trigger in plants an enhanced capacity to show induced responses after aphid feeding. Since the former significant effect of the interaction of factors also became non-significant following inclusion of the covariate, it seems that a synergistic effect of temperature and photoperiod on growth rate – a previously described phenomenon (Langer 1979) – was responsible for the initially significant interaction of factors on induction of Hx. Physiological and biochemical insights will help clarify the nature of the mechanisms underlying the observed pattern.

Earlier work on plant growth rate and induction has emphasised differences in the expression of inducible resistance (not always confirmed with phytochemical changes) depending on growth habits of plant species (Wallner and Walton 1979; Braga et al. 1991), rapidly growing species being more likely to exhibit induced responses. However, genotype, phenology or site differences may be responsible for such patterns. On the other hand, young, rapidly growing, leaves have been shown to respond more actively to damage than mature, slowly growing, leaves (Wellings and Dixon 1987; Edwards et al. 1992; Hanhimäki and Senn 1992), but, again, leaf ontogeny was not taken into account in the analysis.

Unlike the above studies, the present work deals with differences in plant growth rate and phytochemical induction with fixed plant phenology and leaf ontogeny. Here it is suggested that plants under environments that to a certain extent limit growth, are able to allocate more resources to inducible, less-costly defences (Zangerl and Bazzaz 1992) than plants under growth-enhancing environments. This pattern is consistent with earlier claims considering the higher energetic cost of replacement of biomass or resources loss to herbivory for slow-growing plants in comparison with fast-growing ones. Although this was originally proposed and evaluated at the interspecific level, considering constitutive defences and dealing with an evolutionary rather than ecological time scale (growth-rate theory, Coley et al. 1985), later work confirms its validity beyond its original scope (Coley 1988; Jing and Coley 1990; Sagers and Coley 1995). In the present work, the fact that slow-growing plants showed greater induced responses points to a shift in resource allocation whose causes and consequences in relation to herbivores cannot be neglected. Further experiments broadening the range of environmental conditions evaluated, adding other growth-modifier variables, and including herbivore as well as plant performance measurements should lead to a more definitive test of the stated proposition on cost-saving strategies. Furthermore, before assuming that the phenotypic plasticity in secondary metabolism is adaptive, it is essential to undertake studies dealing with the degree of genetic control over induction and hence its suitability for selection (e.g. Zangerl and Berenbaum 1990).

Plant-herbivore interactions are complex and dynamic phenomena affected by factors intrinsic to

each participant as well as by abiotic and biotic external factors. This paper points to the usefulness of considering the effect of environmental conditions on a putative mechanism of plant defence against insects, since the efficiency of such mechanism – and therefore its value as a plant resistance tool – could be significantly affected by the environmental conditions under which the plant-herbivore interaction occurs.

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