

# A phenotypic trade-off between constitutive defenses and induced responses in wheat seedlings<sup>1</sup>

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**Abstract:** In plant defense theory, a trade-off between constitutive and induced defenses in plants is expected on the basis of cost-saving arguments. Thus, assuming that investment in defense is costly to the plant in terms of reduced growth and/or reproduction, induced responses would be a less expensive alternative when constitutive defenses are low. Conversely, plants with high levels of constitutive defenses would gain little from inducible defense systems. Despite this rationale, such a trade-off has rarely been detected. The present study reports the occurrence of a phenotypic trade-off between constitutive and aphid-induced levels of secondary metabolites in wheat seedlings. The trade-off was detected after examining the variation in induced responses as a function of the variation in constitutive defenses generated by plant ontogeny (8 ontogenetic stages, from 5 to 26 days after germination) and the environment (different regimes of temperature, light, and nutrient availability). Plant growth rate and the rate of change of constitutive levels of defense were identified as correlated traits potentially mediating the negative association between induced responses and constitutive defenses. The possible generalization of this result is discussed. Results are also discussed invoking efficacy issues as well as a hypothesis that variability in plant quality may function per se as a resistant factor against herbivores. It is suggested that both induced responses and increased rates of change of constitutive defenses may provide such beneficial variability.

**Keywords:** trade-off, induced responses, cost of defense, induced defense, ontogeny, phenotypic plasticity, plant defense, secondary metabolites.

**Résumé :** La théorie sur les moyens de défense des végétaux prédit un échange entre les défenses constitutives et induites dans le but de réduire les coûts associés à la croissance des plantes. Si l'on admet qu'investir pour se défendre est onéreux pour une plante (chute de croissance, faible reproduction), on peut supposer qu'une défense induite constitue une alternative moins coûteuse chez les espèces qui possèdent peu de moyens de défenses constitutifs. À l'inverse, les végétaux qui possèdent beaucoup de défenses constitutives auraient peu d'avantages à se doter de systèmes de défense induits. Quoique logique, cette hypothèse a rarement été testée. Cette étude montre un échange phénotypique entre les métabolites secondaires constitutifs et ceux induits par la présence de pucerons chez des plantules de blé. L'échange fut détecté après avoir examiné la variation des réponses induites en fonction de la variation dans les défenses constitutives générées par l'ontogenèse (huit stades ontogéniques, de 5 à 26 jours après la germination) et l'environnement (différents régimes de température, de lumière ou d'éléments nutritifs). Le taux de croissance des plantes et le taux de changement des niveaux de défense constitutifs sont corrélés et amenuisent potentiellement l'association négative qui existe entre les réponses induites et les défenses constitutives. Nous discutons de la possible généralisation de ce résultat, notamment à la lumière du débat sur l'efficacité chez les plantes et sur l'hypothèse voulant que la variabilité des caractéristiques d'une plante puisse constituer une stratégie de défense contre les herbivores. Nous suggérons que les défenses induites tout comme les défenses constitutives sont des éléments clés d'une variabilité bénéfique chez les plantes.

**Mots-clés :** échange, réponses induites, coût de la défense, défense induite, ontogenèse, plasticité phénotypique, défenses des végétaux, métabolites secondaires.

## Introduction

Induced responses of plants to herbivory are widespread and have received theoretical and empirical attention (Tallamy & Raupp, 1991; Karban & Baldwin, 1997; Agrawal, Tuzun & Bent, 1999). A traditional topic in the discussion of the evolution of induced defenses is the hypothetical trade-off between constitutive and induced defenses (Herms & Mattson, 1992; Karban & Baldwin, 1997). Plants with high constitutive defenses should not need an increase of those defenses after damage to afford protection against further herbivory. Conversely, high levels of induced responses are expected in plants where constitutive defenses are low. It is assumed that defense allocation entails a cost to the plant due to the diversion of resources otherwise

invested in growth or reproduction (Herms & Mattson, 1992; Zangerl & Bazzaz, 1992). Thus, induced defenses have been considered less costly than constitutive defenses since they involve the investment of resources only when necessary (Karbon & Myers, 1989). However, experimental evidence of the existence of allocation costs of defense is not conclusive (Simms, 1992; Bergelson & Purrington, 1996; Agrawal, 2000; and references therein). Explanations for the evolution of induced defenses not based on cost-saving arguments have been put forward. The "moving target" hypothesis postulates that herbivory-induced variability in plant quality itself hinders herbivore performance, regardless of whether or not it involves an increased expression of a defensive trait (Karbon, Agrawal & Mangel, 1997).

A negative association, or trade-off, between constitutive defenses and induced responses has rarely been detected (Brody & Karban, 1992; Karban & Baldwin, 1997;

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English-Loeb, Karban & Walker, 1998; Agrawal, Gorski & Tallamy, 1999; Havill & Raffa, 1999; Keinänen *et al.*, 1999; Underwood *et al.*, 2000). These evaluations have been performed across a variety of taxonomic scales: Phenotypic and/or genotypic comparisons within species and among species, varieties, families, and clones are found. An implicit assumption in most of this work is that, for a given plant species or variety, both the constitutive and inducible endowments of defense can be represented by relatively fixed values. However, both types of defense are dynamic, and their expressions are affected by intrinsic and extrinsic factors (reviewed in Raupp & Denno, 1983; Waterman & Mole, 1989; Coleman & Jones, 1991; Karban & Baldwin, 1997). The present study addressed the association between constitutive defenses and induced responses taking into account the effect of intrinsic and extrinsic factors on both types of defense in an individual plant. Results are discussed based on efficacy and constraints issues rather than on cost-saving arguments.

The main objective of this study was to test for a trade-off between constitutive defenses and induced responses, as a function of the variation in constitutive defenses generated by *i*) plant ontogeny and *ii*) the environment (different regimes of temperature, light, and nutrients). I further investigated the involvement of other plant traits in the configuration of such a hypothetical trade-off. Thus, plant growth rate and the rate of change of constitutive defenses were evaluated for their association with the trading-off traits. The model system consisted of wheat seedlings (*Triticum aestivum* L.), a cereal aphid (*Rhopalosiphum padi* [L.]) as the challenging herbivore, and hydroxamic acids, secondary metabolites typical of Poaceae (Niemeyer, 1988; Gianoli & Niemeyer, 1998). Hydroxamic acids are associated with constitutive (Givovich & Niemeyer, 1995; Gianoli, Papp & Niemeyer, 1996) and induced (Gianoli & Niemeyer, 1997b; Gianoli, 1999) resistance of wheat against aphids. These secondary metabolites are sensitive to environmental variation (Gianoli & Niemeyer, 1997c) and show a distinct pattern of accumulation in early seedlings: rapid increase upon germination, peaking at an early stage, and decreasing asymptotically thereafter (Argandoña, Niemeyer & Corcuera, 1981).

## Methods

### STANDARD DETERMINATION OF CONSTITUTIVE AND INDUCED LEVELS OF HYDROXAMIC ACIDS

The experiment of induction of hydroxamic acids (Hx) consisted of placing 20 young adult *Rhopalosiphum padi* into a clip cage attached to the first leaf of two-leaf seedlings of wheat (*Triticum aestivum*, variety Paleta). Control seedlings bore empty clip cages on the first leaf. Aphids were removed 48 hours later, and the first leaf of both treatments was analyzed for concentration of Hx using HPLC (High Performance Liquid Chromatography) (details in Gianoli & Niemeyer, 1997c). The procedure was performed in a growth chamber, at 20°C, 12-hour photoperiod, 103  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR (Photosynthetically Active Radiation), unless otherwise stated. Plants were grown in potting soil and watered with tap water ( $N = 8$  plants treatment<sup>-1</sup>). Constitutive levels of Hx were obtained from the concentra-

tion ( $\text{mmol kg fresh weight}^{-1}$ ) of control plants. Induction of Hx by aphid infestation (INDX) was defined as the ratio of increase of Hx in infested plants relative to controls:  $\text{INDX} = (\text{mean [Hx] of infested plants} / \text{mean [Hx] of control plants})$ . Given that chemical analyses involved destructive harvest, the evaluation of the association between constitutive defenses and induced responses could not be carried out on an individual plant basis as is traditionally done for most trade-offs (Mole, 1994). Mean values instead of raw data for each experimental treatment were used throughout the statistical analyses.

### EVALUATIONS OF THE ASSOCIATION BETWEEN CONSTITUTIVE HYDROXAMIC ACIDS AND INDUCED RESPONSES

The first experiment addressed the relationship between the variation in constitutive Hx and the magnitude of aphid-induced responses with plant ontogeny. Wheat seedlings were evaluated for Hx and INDX (see above) during early development. Evaluations were done at 5, 8, 11, 15, 18, 21, 24, and 26 days after germination. Eight plants were assigned to each treatment, totaling 128 plants (two treatments  $\times$  8 ontogenetic stages). Plant height growth rate was also determined ( $\text{mm day}^{-1}$ ).

The second experiment addressed the relationship between the variation in constitutive Hx and the magnitude of aphid-induced responses across environmental regimes. Wheat seedlings at the two-leaf stage were evaluated for Hx and INDX through a range of environmental conditions ( $N = 8$  treatment<sup>-1</sup>). Seedlings were of the same phenological stage, although chronological age differed between treatments. The manipulated variables were temperature (15, 20, 25°C), photoperiod (8, 12, 16 hours), light intensity (77, 199  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR), and nutrient availability (watering a vermiculite substrate with two different NPK [30:20:15 g L<sup>-1</sup>] aqueous solutions differing ten-fold in concentration). Temperature and photoperiod were varied according to a factorial array, totaling nine treatments. Likewise, the effects of nutrients and light were evaluated under a factorial array (four treatments). Plant growth rate was determined. Data on the effect of temperature and photoperiod were modified (log-transformed) from Gianoli & Niemeyer (1996, 1997c). Half of the data on the effect of nutrient availability was modified (INDX values calculated and then log-transformed) from Gianoli & Niemeyer (1997b).

### STATISTICAL ANALYSES

The evaluation of the relationship between constitutive levels of secondary metabolites and the magnitude of their induction after herbivory entails an intrinsic methodological drawback. Plotting a ratio (Y-axis, induction) against a denominator (X-axis, constitutive levels) produces a mathematical bias towards a negative relationship. Nevertheless, most studies have failed to find such a trade-off (Karbon & Baldwin, 1997) despite this problem (Brody & Karban, 1992; Gianoli, unpubl. data on interspecific evaluations). In other words, if the strength of induction remains constant across different genotypes, growth stages, or environments that affect the constitutive levels of secondary metabolites, then the negative association would not be detected. In contrast, it would be verified if the relative induction (expressed, for instance, as percentage of increase) is greater when constitutive levels are lower. Taking those caveats into account,

two statistical analyses were carried out. First, linear regression analyses were performed to assess the relationship between the magnitude of induced responses and constitutive levels of Hx across ontogenetic stages and across environments. Regression lines, coefficients, and  $P$ -values were included in the plots, but significance was also tested using a bias-free analysis. This was done by checking whether the regression of the hydroxamic acid concentration of aphid-infested seedlings against those of the controls had a slope different from 1 (two-tailed  $t$ -test, Zar, 1996). Slopes significantly lower than 1 would indicate that the relative induction of Hx decreases as constitutive levels increase. Data were normalized by log-transformation ( $Y' = \text{LOG}[Y + 1]$ ) before analysis. Statistical analyses were performed using Statistica®.

## Results

A regression analysis ( $N = 8$ ) showed that the relationship between constitutive levels of hydroxamic acids (Hx) and induced responses (INDX) across ontogenetic stages of wheat seedlings was negative ( $r^2 = 0.48$ ,  $F_{1,6} = 5.57$ ,  $P = 0.056$ , Figure 1a). The regression of hydroxamic acid levels of aphid-infested seedlings against those of the controls had a slope significantly lower than 1 ( $m = 0.71 \pm 0.02$ ,  $t_{(2),7} = 16.28$ ,  $P < 0.001$ ). A further inspection of the identity of each point in Figure 1a suggested that a plot of the dynamics of constitutive Hx accumulation with time would shed light on the former result. Statistical testing using least squares demonstrated that the plot of Hx dynamics (Figure 2) fitted a reciprocal quadratic function ( $y = 1/a + bx + cx^2$ ) ( $r^2 = 0.95$ ,  $P < 0.001$ ) better than a linear decreasing function ( $r^2 = 0.74$ ,  $P < 0.004$ ). Moreover, previous work has described the dynamics of Hx accumulation in wheat seedlings as a rapid initial increase, early peak, and asymptotic decrease (Argandoña, Niemeyer & Corcuera, 1981). Therefore, the reciprocal quadratic function was deemed the best representative of Hx dynamics. It showed that the points located in regions of smooth change of Hx were those with the highest INDX values in figure 1a. Conversely, the points located in the vicinity of the inflexion points of the function were those with the lowest INDX values in figure 1a. This led to an evaluation of the relationship between the rate of change of Hx (RCH) and INDX. RCH was calculated as the derivative of the reciprocal quadratic function ( $y' = -[b + 2cx] / [a + bx + cx^2]^2$ ) evaluated at each point (Figure 2). The relationship between INDX and RCH (absolute value) was evaluated by a linear regression analysis, which showed a negative association ( $r^2 = 0.71$ ,  $F_{1,6} = 14.7$ ,  $P < 0.009$ , Figure 3a). Plant growth rate was positively associated with Hx ( $r^2 = 0.52$ ,  $F_{1,6} = 8.64$ ,  $P < 0.03$ ) and negatively associated with INDX ( $r^2 = 0.44$ ,  $F_{1,6} = 6.42$ ,  $P < 0.05$ ). Plant growth rate was positively (regression coefficient = 0.96) associated with RCH ( $r^2 = 0.93$ ,  $F_{1,6} = 81.01$ ,  $P < 0.0002$ ). Given that these two variables were highly correlated, a forward stepwise multiple regression analysis was performed to determine which of the two variables (RCH or plant growth rate) had the strongest effect on INDX. This analysis, which also included Hx and plant age as independent variables, showed that RCH was the more robust variable ( $\beta = -0.84$ ,  $r^2 = 0.71$ ,

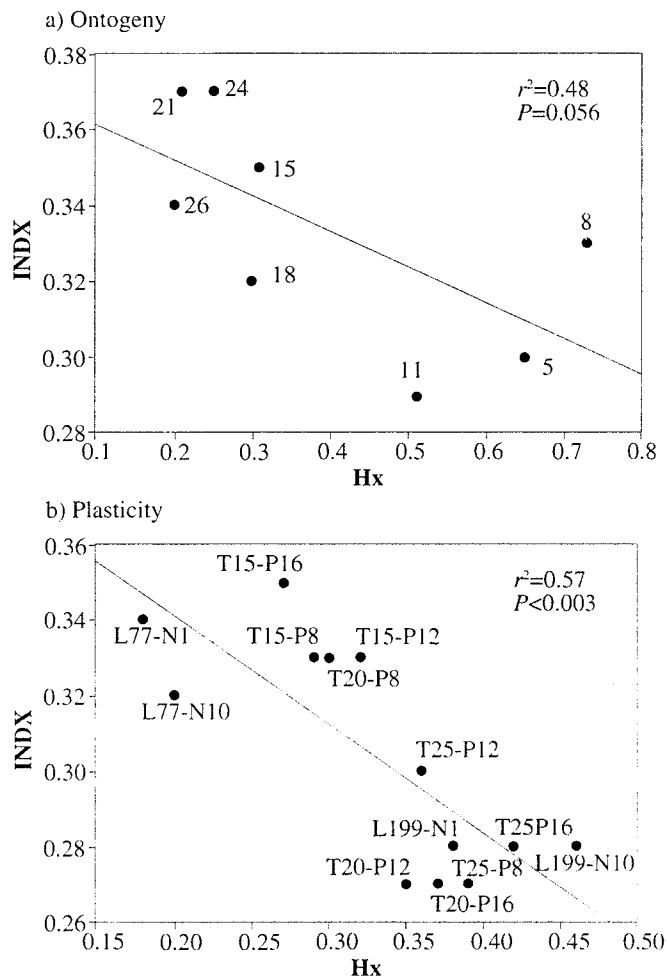


FIGURE 1. Linear regression between constitutive levels of hydroxamic acids (Hx,  $\text{mmol kg fresh weight}^{-1}$ ) and induced responses (INDX) following aphid infestation ( $\text{INDX} = \text{mean [Hx]} \text{ of infested plants} / \text{mean [Hx]} \text{ of control plants}$ ) in wheat seedlings. Values were log-transformed to meet normality. Transformed values are plotted. a) Ontogeny (wheat seedlings of different ages). Evaluations were taken at 5, 8, 11, 15, 18, 21, 24, and 26 days after germination, in independent samples. Each point is the mean of eight observations per axis (total number of experimental plants: 128). b) Plasticity (wheat seedlings grown under different environmental regimes). Nine points arising from a factorial array of 3 temperatures ( $T = 15, 20, 25^\circ\text{C}$ )  $\times$  3 photoperiods ( $P = 8, 12, 16$  hours); 4 points arising from a factorial array of 2 light intensities ( $L = 77, 199 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR)  $\times$  2 nutrient supply treatments ( $N = 2$  NPK aqueous solutions at a concentration ratio of 1:10). Each point is the mean of eight observations per axis (total number of experimental plants: 208). Letters and numbers close to the points indicate the environmental variable and its level.

$F_{1,6} = 14.7$ ,  $P < 0.009$ ). The rest of the variables were removed by the statistical procedure because their tolerance ( $1 - \text{redundancy}$ ) values were lower than the specified acceptable minimum (0.10). In particular, results indicated that plant growth rate was a redundant variable (redundancy = 92%), and hence RCH was determined as a potential causal factor of the negative association between Hx and INDX.

Wheat seedlings exhibited plasticity in the relative allocation to constitutive hydroxamic acids (Hx) and aphid-induced responses (INDX) after exposure to different environments. A regression analysis ( $N = 13$ ) showed that the relationship between Hx and INDX across environmental regimes was

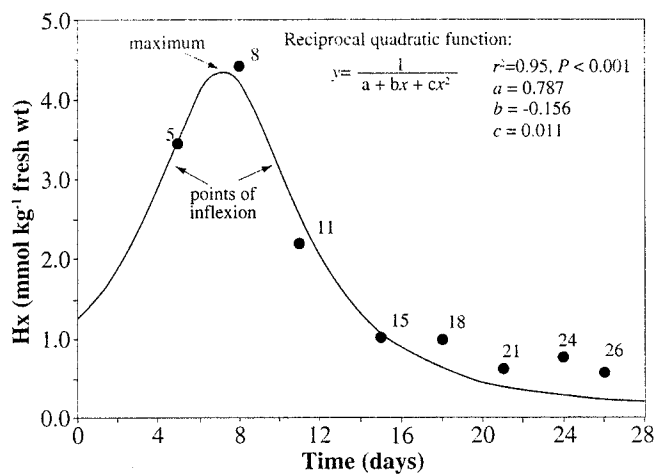


FIGURE 2. The accumulation of hydroxamic acids (Hx) with time in seedlings of wheat variety Paleta. The plot fits a reciprocal quadratic function ( $y = 1/[a + bx + cx^2]$ ), on which the maximum (at  $x = 7.16$ ) and the inflexion points (at  $x = 4.42, 9.76$ ) are indicated. Following the dynamics of the function, points located in the vicinity of inflexion points (*i.e.*, 5 and 11) have the highest instantaneous rate of change of the Y-variable (Hx), as evidenced by the computation of the derivative of the reciprocal quadratic function ( $y' = -[b + 2cx]/[a + bx + cx^2]^2$ ). Points located on the right side of the plot (*i.e.*, 15, 18, 20, 21, 24, and 26) approach an asymptote and hence have the lowest instantaneous rate of change of Hx. The point close to the maximum (*i.e.*, 8), where the derivative of the function equals zero, has a low instantaneous rate of change

negative ( $r^2 = 0.57$ ,  $F_{1,11} = 15.22$ ,  $P < 0.003$ , Figure 1b). The regression of hydroxamic acid levels of aphid-infested seedlings against those of the controls had a slope significantly lower than 1 ( $m = 0.79 \pm 0.02$ ,  $t_{(2),12} = 9.01$ ,  $P < 0.001$ ). Plant growth rate was positively associated with Hx ( $r^2 = 0.37$ ,  $F_{1,11} = 4.96$ ,  $P < 0.05$ ) and negatively associated with INDX ( $r^2 = 0.40$ ,  $F_{1,11} = 7.27$ ,  $P < 0.025$ , Figure 3b). Although both associations were only moderate, this indicated that plant growth rate might play a role in the negative association between Hx and INDX. However, in this set of experiments RCH could not be calculated as was done in the previous section because experimental plants were of the same phenological stage. Hence, the importance of plant growth rate vis-à-vis RCH in the Hx-INDX relationship could not be assessed.

## Discussion

In its broadest sense, a trade-off is recognized as a negative association between two phenotypic traits (Roff, 1992). Within plant defense theory, a trade-off between constitutive and induced defenses is expected on the basis of allocation costs of defense. However, positive demonstrations of a trade-off between constitutive and induced defenses are rarely found in the literature (Brody & Karban, 1992; Karban & Baldwin, 1997; English-Loeb, Karban & Walker, 1998; Agrawal, Gorski & Tallamy, 1999; Havill & Raffa, 1999; Keinänen *et al.*, 1999; Underwood *et al.*, 2000). The present study found a phenotypic trade-off between constitutive defenses (Hx) and induced responses (INDX) in wheat seedlings as a function of the variation in constitutive defenses with plant ontogeny and the environment.

Trade-offs at the phenotypic level may be based on *i*) negative genetic correlations, *ii*) resource-competition of

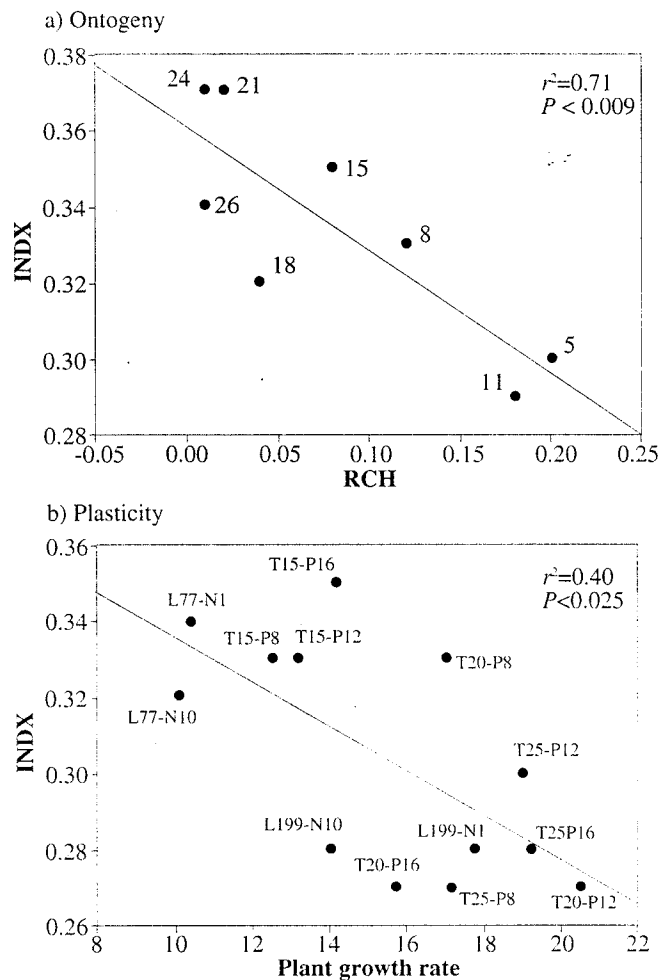


FIGURE 3. a) Linear regression between the rate of change (RCH,  $\text{mmol day}^{-1}$ ) of hydroxamic acids (Hx) and the magnitude of induced responses following aphid infestation (INDX = mean [Hx] of infested plants mean [Hx] of control plants $^{-1}$ ) in wheat seedlings of different ages (see Figure 1a for experimental treatments and number of replicates). Values were log-transformed to meet normality. Transformed values are plotted. b) Linear regression between plant growth rate (mm  $\text{day}^{-1}$ ) and induced responses following aphid infestation (INDX = mean [Hx] of infested plants mean [Hx] of control plants $^{-1}$ ) in wheat seedlings growing under different environmental regimes (see Figure 1b for experimental treatments and number of replicates). Values were log-transformed to meet normality.

traits that have a common resource base, *i.e.*, a physiological trade-off, or *iii*) environmental mediation, via differential phenotypic plasticity of the traits (Mole, 1994). The genetic basis of hydroxamic acid accumulation has been described (Frey *et al.*, 1997), but evidence of a genetic control of its induction after herbivory is still very limited (Gianoli *et al.*, 1997). A genetically grounded trade-off cannot be ruled out, but its evaluation is beyond the scope of the present study. From the perspective of a physiological trade-off, it may be asked which is the limiting factor that the two traits could be trading-off. Given that Hx and INDX are, metabolically, the same trait, competition for internal resources in the form of competing branches in a metabolic pathway (Gershenson, 1994) is difficult to conceive. On the other hand, in the present system both constitutive hydroxamic acids and aphid-induced responses had been previously evaluated for allocation costs, and these costs were not detected (Gianoli, Papp & Niemyer,

1996; Gianoli & Niemeyer, 1997a). Consequently, evidence is insufficient to infer that the negative association between Hx and INDX in wheat seedlings is based on a genetic or physiological trade-off.

Phenotypic trade-offs may arise after environmentally induced changes in resource allocation to the trading-off traits, hence reflecting phenotypic plasticity instead of a genetic or physiological trade-off. Thus, environmental constraints in resource supply (Mole & Zera, 1994) or environmental cues (Donohue & Schmitt, 1999) may generate inverse patterns of expression of phenotypic traits. Whereas a favorable environment (or a cue of it) would trigger the expression of a given plant trait, an unfavorable environment (or a cue of it) would cause the plant to channel resources into another trait, thereby configuring a trade-off between these traits. The trade-off between Hx and INDX in wheat found across environmental regimes may be considered a case of an environmentally driven trade-off. It may be explained in terms of environmental constraints as well as of environmental cues, as is discussed below.

Firstly, within a single genotype, plant growth rate is a good indicator of environmental quality. Consequently, the positive association of plant growth rate with Hx (higher constitutive defenses in better environments) and the negative association with INDX (higher induced responses in worse environments) give support to the hypothesis of an environmentally driven trade-off. Even though both associations are rather moderate, they suggest that plant growth rate is a correlated trait that may mediate the occurrence of the trade-off, which is ultimately determined by the quality of the environment. It remains to be seen why induced defenses increase and constitutive defenses decrease when the environment constrains plant growth. This pattern is opposite to theoretical predictions and early experimental work (Herms & Mattson, 1992; Karban & Baldwin, 1997).

Secondly, it might be speculated that the observed trade-off between Hx and INDX across environments is generated by plastic responses of wheat seedlings to environmental cues of herbivore abundance, a phenomenon akin to the induction of spines in bryozoans in response to predator cues (Harvell, 1998). *Rhopalosiphum padi*, the inducer aphid, arrives in cereal fields in early summer, when plants are at the seedling stage (Wikteliuss, Weibull & Pettersson, 1990). It has been shown that temperature is a reliable predictor of the date of arrival (Rautapää, 1976) and abundance (Honek, 1985) of *R. padi* in cereal fields. Wheat seedlings showed higher constitutive hydroxamic acids and lower induction after aphid infestation at higher temperatures and light intensities, "summer cues" associated with presence and/or abundance of *R. padi*. This agrees with theoretical and empirical observations that plants at high risk of herbivore attack should rely on high levels of constitutive defenses rather than on inducible defenses, and vice versa (Zangerl & Bazzaz, 1992; Zangerl & Rutledge, 1996).

Finally, plants may benefit from presenting a moving target to herbivores (Karbon, Agrawal & Mangel, 1997; Agrawal & Karban, 1999). Thus, it has been proposed that phenotypic changes elicited in plants by herbivory may reduce herbivore performance solely on the basis of increased variability and not necessarily because of the

escalation of defensive traits. In accordance with the moving target hypothesis, and considering that both Hx and the rate of change of Hx (RCH) were negatively associated with INDX, short-term variation in constitutive defenses (RCH) may be considered analogous to induced responses with regard to variability and its potential benefits for the plant. Hence, an induced response would be an alternative that enters into play when two conditions are present: *i*) constitutive levels of defense are low, and *ii*) the temporal variation (rate of change) of constitutive defenses is low. When either the natural dynamics of accumulation of hydroxamic acids (an intrinsic factor) or the environment under which the plant grows (an extrinsic factor) constrain both the constitutive expression of high levels of these secondary metabolites and their rate of change, then induced responses are expected to be increased. High variation (rate of change) of constitutive defenses is likely to be temporally confined to early stages of plant development, when secondary metabolism is more active (McKey, 1974; Frischknecht, Ulmer-Dufek & Baumann, 1986; Bowers & Stamp, 1993). In this way, as plants approach maturity, and both the mean and the temporal variation of constitutive defenses decrease, it is expected that induced responses will become more pronounced (Baldwin & Schultz, 1984, but see Ohnmeiss & Baldwin, 2000). Interestingly, a recent review found that induced responses tended to be stronger for young leaves. However, all but one of the species listed were woody plants (Karbon & Baldwin, 1997: Table 4). This raises the question of whether woody plants and herbaceous plants (as exemplified in the present study) differ in ontogenetic patterns of expression of induced responses.

The present work illustrates a broad approach to the study of the trade-off between constitutive defenses and induced responses. Further research on the mechanistic basis of a more narrowly defined trade-off, including experimental manipulation of constitutive and induced defenses, will confirm or negate the preliminary conclusions drawn from this phenotypic trade-off.

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### Literature cited

- Agrawal, A. A., 2000. Benefits and costs of induced plant defense for *Lepidium virginicum* (Brassicaceae). *Ecology*, 81: 1804-1813.
- Agrawal, A. A., P. M. Gorski & D. W. Tallamy, 1999. Polymorphism in plant defense against herbivory: Constitutive and induced resistance in *Cucumis sativus*. *Journal of Chemical Ecology*, 25: 2285-2304.
- Agrawal A. A. & R. Karban, 1999. Why induced defenses may be favored over constitutive strategies in plants. Pages 45-61 in R. Tollrian & C. D. Harvell (ed.). *The Ecology and Evolution of Inducible Defenses*. Princeton University Press, Princeton, New Jersey.

- Agrawal, A. A., S. Tuzun & E. Bent, 1999. Inducible Plant Defenses Against Pathogens and Herbivores: Biochemistry, Ecology, and Agriculture. American Phytopathological Society Press, St. Paul, Minnesota.
- Argandoña, V. H., H. M. Niemeyer & L. J. Corcuera, 1981. Effect of content and distribution of hydroxamic acids in wheat on infestation by *Schizaphis graminum*. *Phytochemistry*, 20: 673-676.
- Baldwin, I. T. & P. Schultz, 1984. Damage and communication induced changes in yellow birch leaf phenolics. Pages 25-33 in *Proceedings of the Eighth Annual Forest Biology Workshop*. Utah State University Press, Logan, Utah.
- Bergelson, J. & C. B. Purrington, 1996. Surveying patterns in the cost of resistance in plants. *American Naturalist*, 148: 536-558.
- Bowers, M. D. & N. E. Stamp, 1993. Effects of plant age, genotype, and herbivory on *Plantago* performance and chemistry. *Ecology*, 74: 1778-1791.
- Brody, A. K. & R. Karban, 1992. Lack of a tradeoff between constitutive and induced defenses among varieties of cotton. *Oikos*, 65: 301-306.
- Coleman, J. S. & C. G. Jones, 1991. A phytochemical perspective of phytochemical induction by herbivores. Pages 3-46 in D. W. Tallamy & M. J. Raupp (ed.). *Phytochemical Induction by Herbivores*. John Wiley & Sons, New York.
- Donohue, K. & J. Schmitt, 1999. The genetic architecture of plasticity to density in *Impatiens capensis*. *Evolution*, 53: 1377-1386.
- English-Loeb, G., R. Karban & M. A. Walker, 1998. Genotypic variation in constitutive and induced resistance in grapes against spider mite herbivores. *Environmental Entomology*, 27: 297-304.
- Frey, M., P. Chomet, E. Glawischnig, C. Stettner, S. Gruen, A. Winklmaier, W. Eisenreich, A. Bacher, R. B. Meeley, S. P. Briggs, K. Simcox & A. Gierl, 1997. Analysis of a chemical plant defense mechanism in grasses. *Science*, 277: 696-699.
- Frischknecht, P. M., J. Ulmer-Dufek & T. W. Baumann, 1986. Purine alkaloid formation in buds and developing leaflets of *Coffea arabica*: Expression of an optimal defence strategy? *Phytochemistry*, 25: 613-616.
- Gershenson, J., 1994. Metabolic costs of terpenoid accumulation in higher plants. *Journal of Chemical Ecology*, 20: 1281-1328.
- Gianoli, E., 1999. Within-plant distribution of *Rhopalosiphum padi* on wheat seedlings is affected by induced responses. *Entomologia Experimentalis et Applicata*, 93: 227-230.
- Gianoli, E., C. M. Caillaud, B. Chaubet, J. P. Di Pietro & H. M. Niemeyer, 1997. Variability in grain aphid *Sitobion avenae* (F.) (Homoptera:Aphididae) performance and aphid-induced phytochemical responses in wheat. *Environmental Entomology*, 26: 638-641.
- Gianoli, E. & H. M. Niemeyer, 1996. Environmental effects on the induction of wheat chemical defences by aphid infestation. *Oecologia*, 107: 549-552.
- Gianoli, E. & H. M. Niemeyer, 1997a. Lack of costs of herbivory-induced defenses in a wild wheat: Integration of physiological and ecological approaches. *Oikos*, 80: 269-275.
- Gianoli, E. & H. M. Niemeyer, 1997b. Characteristics of hydroxamic acid induction in wheat triggered by aphid infestation. *Journal of Chemical Ecology*, 23: 2695-2705.
- Gianoli, E. & H. M. Niemeyer, 1997c. Environmental effects on the accumulation of hydroxamic acids in wheat seedlings: The importance of plant growth rate. *Journal of Chemical Ecology*, 23: 543-551.
- Gianoli, E. & H. M. Niemeyer, 1998. DIBOA in wild Poaceae: Sources of resistance to the Russian wheat aphid (*Diuraphis noxia*) and the greenbug (*Schizaphis graminum*). *Euphytica*, 102: 317-321.
- Gianoli, E., M. Papp & H. M. Niemeyer, 1996. Costs and benefits of hydroxamic acids-related resistance in winter wheat against the bird cherry-oat aphid, *Rhopalosiphum padi* (L.). *Annals of Applied Biology*, 129: 83-90.
- Givovich, A. & H. M. Niemeyer, 1995. Comparison of the effect of hydroxamic acids from wheat on five species of cereal aphids. *Entomologia Experimentalis et Applicata*, 74: 115-119.
- Harvell, C. D., 1998. Genetic variation and polymorphism in the inducible spines of a marine bryozoan. *Evolution*, 52: 80-86.
- Havill, N. P. & K. F. Raffa, 1999. Effects of elicitation treatment and genotypic variation on induced resistance in *Populus*: Impacts on gypsy moth (Lepidoptera: Lymantriidae) development and feeding behavior. *Oecologia*, 120: 295-303.
- Hermes, D. A. & W. J. Mattson, 1992. The dilemma of plants: To grow or defend. *Quarterly Review of Biology*, 67: 283-335.
- Honek, A., 1985. Temperature and plant vigour influence annual variation of abundance in cereal aphids (Homoptera, Aphididae). *Journal of Plant Disease Protection*, 92: 588-593.
- Karban, R. & I. T. Baldwin, 1997. *Induced Responses to Herbivory*. University of Chicago Press, Chicago, Illinois.
- Karban, R. & J. H. Myers, 1989. Induced plant responses to herbivory. *Annual Review of Ecology & Systematics*, 20: 331-348.
- Karban, R., A. A. Agrawal & M. Mangel, 1997. The benefits of induced defenses against herbivores. *Ecology*, 78: 1351-1355.
- Keinänen, M., R. Julkunen-Tiitto, P. Mutikainen, M. Walls, J. Ovaska & E. Vapaavuori, 1999. Trade-offs in phenolic metabolism of silver birch: Effects of fertilization, defoliation, and genotype. *Ecology*, 80: 1970-1986.
- McKey, D., 1974. Adaptive patterns in alkaloid physiology. *American Naturalist*, 108: 305-320.
- Mole, S., 1994. Trade-offs and constraints in plant-herbivore defense theory: A life history perspective. *Oikos*, 71: 3-12.
- Mole, S. & A. Zera, 1994. Differential resource consumption obviates a potential flight-fecundity trade-off in the sand cricket *Gryllus firmus*. *Functional Ecology*, 8: 573-580.
- Niemeyer, H. M., 1988. Hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-ones), defence chemicals in the Gramineae. *Phytochemistry*, 27: 3349-3358.
- Ohmmeiss, T. E. & I. T. Baldwin, 2000. Optimal defense theory predicts the ontogeny of an induced nicotine defense. *Ecology*, 81: 1765-1783.
- Raupp, M. J. & R. F. Denno, 1983. Leaf age as a predictor of herbivore distribution and abundance. Pages 91-124 in R. F. Denno & M. S. McClure (ed.). *Variable Plants and Herbivores in Natural and Managed Systems*. Academic Press, New York.
- Rautapää, J., 1976. Population dynamics of cereal aphids and method of predicting population trends. *Annales Agriculturae Fenniae*, 15: 272-293.
- Roff, D. A., 1992. *The Evolution of Life Histories. Theory and Analysis*. Chapman & Hall, New York.
- Simms, E. L., 1992. Costs of plant resistance to herbivory. Pages 392-425 in R. S. Fritz & E. L. Simms (ed.). *Plant Resistance to Herbivores and Pathogens. Ecology, Evolution and Genetics*. University of Chicago Press, Chicago, Illinois.
- Tallamy, D. W. & M. J. Raupp, 1991. *Phytochemical Induction by Herbivores*. John Wiley & Sons, New York.
- Underwood, N., W. Morris, K. Gross & J. R. Lockwood, 2000. Induced resistance to Mexican bean beetles in soybean: Variation among genotypes and lack of correlation with constitutive resistance. *Oecologia*, 122: 83-89.

- Waterman, P. G. & S. Mole, 1989. Extrinsic factors influencing production of secondary metabolites in plants. Pages 107-134 in E. A. Bernays (ed.). *Insect-Plant Interactions*, Vol. 1. CRC Press, Boca Raton, Florida.
- Wiktelius, S., J. Weibull & J. Pettersson, 1990. Aphid host plant ecology: The bird cherry-oat aphid as a model. Pages 21-36 in R. K. Campbell & R. D. Eikenbary (ed.). *Aphid-Plant Genotype Interactions*. Elsevier, Amsterdam.
- Zangerl, A. R. & F. A. Bazzaz, 1992. Theory and pattern in plant defense allocation. Pages 363-391 in R. S. Fritz & E. L. Simms (ed.). *Plant Resistance to Herbivores and Pathogens. Ecology, Evolution and Genetics*. University of Chicago Press, Chicago, Illinois.
- Zangerl, A. R. & C. E. Rutledge, 1996. The probability of attack and patterns of constitutive and induced defense: A test of optimal defense theory. *American Naturalist*, 147: 599-608.
- Zar, J. H., 1996. *Biostatistical Analysis*. Prentice Hall, New Jersey.