

## ENVIRONMENTAL EFFECTS ON THE ACCUMULATION OF HYDROXAMIC ACIDS IN WHEAT SEEDLINGS: THE IMPORTANCE OF PLANT GROWTH RATE

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**Abstract**—The effects of temperature and photoperiod on accumulation of hydroxamic acids (Hx) in wheat (*Triticum aestivum* L.) seedlings were evaluated under laboratory conditions. Hx concentrations were significantly higher at higher temperatures. No such clear trend was found for the photoperiod effect. The significant effect of temperature and photoperiod on growth rate of seedlings and the significant positive correlation between growth rate prior to analysis and levels of Hx, suggested that environmental effects on Hx accumulation were at least partially mediated through their effect on plant growth rate. After uncoupling the effect of environmental conditions from the effect of plant growth rate by statistical means the effect of temperature on Hx was no longer significant. Therefore, temperature effect was fully mediated by plant growth rate. Implications of the patterns found are discussed in issues of plant-defense general theories.

**Key Words**—Temperature, photoperiod, hydroxamic acids, growth, defense, secondary metabolites, wheat, Gramineae.

### INTRODUCTION

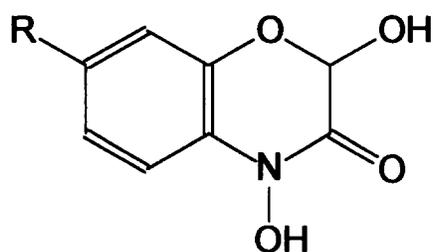
Plants growing under different environmental conditions may show changes in resource allocation to main biological functions such as growth, reproduction, and defense (Bazzaz et al., 1987; Chapin et al., 1987; Herms and Mattson, 1992). Concerning defense, numerous experimental studies have described the effect of environmental factors such as temperature, water, relative humidity,

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light, nitrogen, CO<sub>2</sub>, etc. on plant resistance traits (reviewed in Smith, 1989 and Waterman and Mole, 1989). The effects of both temperature and photoperiod on plant resistance to insects, measured as effects on insect performance parameters (Khan et al., 1986; Salas and Corcuera, 1991), as well as on constitutive levels of plant secondary metabolites (Hanson et al., 1983; Anderson et al., 1991) have been described. However, these reports have often overlooked phenological differences between plants arising from being subjected to different environmental conditions. These phenological differences may account for the resistance pattern observed, in view of the known relationship between plant or plant tissue age and levels of secondary metabolites (Bazzaz et al. 1987). Hence, since environmental conditions affect growth rate of plants, this must be also considered when attempting to distinguish the effect of environmental variables on defenses in plants.

Hydroxamic acids (Hx) (4-hydroxy-1,4-benzoxazin-3-ones, Figure 1) are plant secondary metabolites present in both cultivated and wild Gramineae (Niemeyer, 1988) related to the resistance of cereals to insects, fungi, and bacteria (reviewed in Niemeyer and Pérez, 1995). Hx occur naturally as 2-β-O-D-glucopyranosides which are hydrolyzed by plant glucosidases released following tissue disruption (Hofman and Hofmanová, 1971). Hx are absent from the seed, increase upon germination, peak at the young seedling stage and decrease thereafter (Argandoña et al., 1981) yet retaining high levels of Hx in the youngest tissue of mature plants (Thackray et al., 1990).

We herein describe the effects of temperature and photoperiod on the accumulation of Hx on wheat (*Triticum aestivum* L.) seedlings under laboratory



R=H            DIBOA

R=CH<sub>3</sub>O        DIMBOA

FIG. 1. Chemical structures of DIMBOA and DIBOA, the main hydroxamic acid aglucones in wheat extracts.

conditions and the involvement of seedling growth rate—which differed among treatments—on the patterns found. The experimental design used (seedlings analyzed were of the same phenological stage) allowed us to distinguish between environmental and plant phenology effects. Implications of our findings in issues of plant-defense general theories are discussed.

#### METHODS AND MATERIALS

*Plants.* Seeds of *T. aestivum* cv. Paleta were obtained from INIA, Chile, and germinated in a growth chamber in individual plastic pots (25 ml) filled with soil. Seedlings grew in a 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 light-hours). Light intensity, expressed as P.A.R., was  $117 \mu\text{mol photons m}^{-2} \cdot \text{s}^{-1}$  for all treatments. When seedlings attained growth stage 12 (first leaf completely unfolded, second leaf visible; Zadoks et al., 1974) the aerial biomass was analyzed for total Hx. Eight seedlings (height:  $170 \pm 5$  mm) were used for each treatment. The utilization of seedlings of the same phenological stage—but different chronological age between treatments—allowed the distinction between environmental and plant phenology effects. Growth rate of all seedlings was determined as follows: height at growth stage 12 to the nearest mm/age at such stage, determined to the nearest hour.

*Chemical Analysis.* Plant material was macerated with 1 ml  $\text{H}_2\text{O}$ , using mortar and pestle. The aqueous extract was left at room temperature for 15 min and then adjusted to pH 3 with 0.1 N  $\text{H}_3\text{PO}_4$ . The extract was centrifuged at 13 000 g for 15 min and a 50  $\mu\text{l}$  aliquot of the supernatant was 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 and the following linear gradients between solvents A (MeOH) and B (0.5 ml  $\text{H}_3\text{PO}_4$  in 11  $\text{H}_2\text{O}$ ): 0 to 7 min, 30% A; 7 to 9 min, 100% A; 7 to 13 min, 30% A. Compounds eluting from the column were detected at 263 nm.

*Statistical Analysis.* Hx concentrations (mmol/kg fresh weight) were analyzed with a two-way ANOVA with temperature and photoperiod as main effects and with a two-way ANCOVA with growth-rate as a covariate. Growth rate (mm/day) was determined for each individual seedling and correlated with its Hx level. All Hx concentrations were square-root transformed prior to analysis in order to normalize their distributions.

#### RESULTS

Accumulation of Hx was significantly affected by temperature ( $P < 0.001$ ), higher temperatures leading to increased levels of Hx (Table 1). No clear trend

TABLE 1. LEVELS OF HYDROXAMIC ACIDS (Hx) (MMOL/KG FR. WT) IN WHEAT SEEDLINGS GROWN UNDER DIFFERENT TEMPERATURES AND PHOTOPERIODS. MEAN  $\pm$  SE<sup>a</sup>

Temperature	Daylength		
	16	12	8
25	1.62 $\pm$ 0.24aA	1.31 $\pm$ 0.13aA	1.48 $\pm$ 0.14aA
20	1.35 $\pm$ 0.11aA	1.24 $\pm$ 0.09aAB	0.99 $\pm$ 0.07bB
15	0.84 $\pm$ 0.05bA	1.11 $\pm$ 0.08aA	0.95 $\pm$ 0.07bA

<sup>a</sup>Means sharing a lowercase letter within columns or an uppercase letter within rows are not significantly different ( $P < 0.05$ , LSD test).

was found for the effect of photoperiod ( $P > 0.40$ ). In addition, the interaction of effects was marginally significant ( $P < 0.05$ ) (Table 1). As expected, seedling growth rate prior to analysis was significantly affected by both temperature and photoperiod as well as by their interaction ( $P < 0.001$  in all cases), higher temperatures and longer daylengths enabling plants to grow faster (Table 2). On the other hand, seedling growth rate (mm/day) prior to analysis for Hx was significantly positively correlated with Hx accumulation ( $r = 0.44$ ,  $P < 0.001$ ,  $n = 72$ ) (Figure 2).

Since, as described above, both temperature and light regimes significantly affected seedling growth rate (Table 2), it may be questioned whether the described effect of temperature on Hx levels (Table 2) reflected merely the effect of growth rate on Hx. This situation was elucidated by performing the corresponding two-way ANOVA this time including "growth rate prior to analysis"

TABLE 2. GROWTH RATE (MM/DAY) PRIOR TO ANALYSIS FOR HYDROXAMIC ACIDS CONTENT OF WHEAT SEEDLINGS GROWN UNDER DIFFERENT TEMPERATURES AND PHOTOPERIODS. MEAN  $\pm$  SE<sup>a</sup>

Temperature	Daylength		
	16	12	8
25	18.86 $\pm$ 0.59aA	16.84 $\pm$ 0.29aB	15.29 $\pm$ 0.35aC
20	14.14 $\pm$ 0.20bC	17.25 $\pm$ 0.31aA	15.69 $\pm$ 0.36aB
15	13.23 $\pm$ 0.48bA	11.80 $\pm$ 0.22bB	11.32 $\pm$ 0.25bB

<sup>a</sup>Means sharing a lowercase letter within columns or an uppercase letter within rows are not significantly different ( $P < 0.05$ , LSD test).

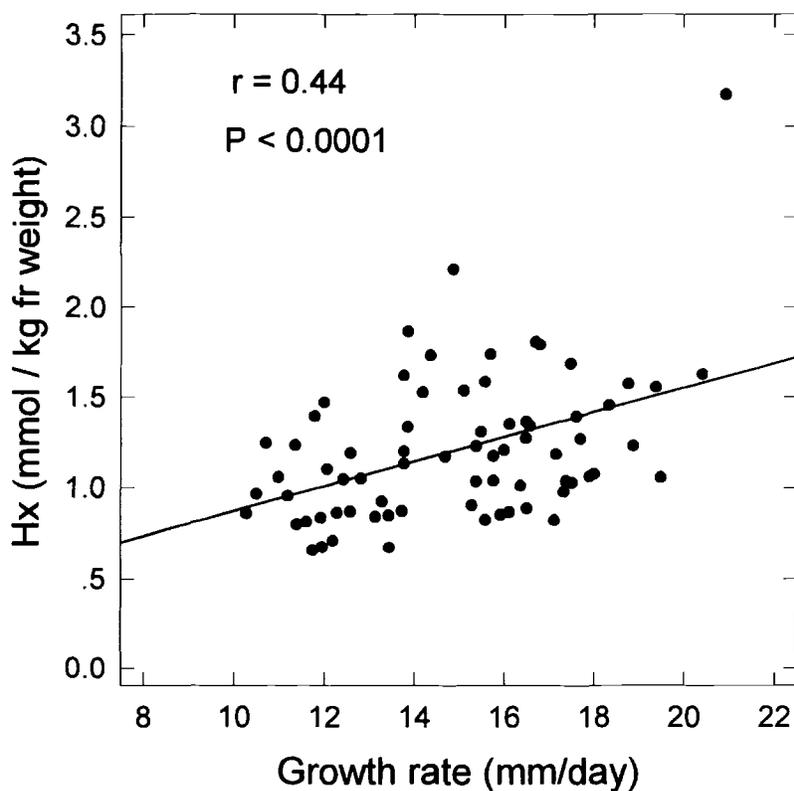


FIG. 2. The relationship between seedling growth rate prior to analysis and the accumulation of hydroxamic acids ( $n = 72$ ).

as a covariate, i.e., removing its effect. In this new analysis the former strongly significant effect of temperature on accumulation of Hx disappeared upon the inclusion of the covariate ( $P > 0.20$ ). No qualitative difference for the other sources of variance arose after the analysis (Table 3).

#### DISCUSSION

Accumulation of Hx in wheat seedlings under different temperatures showed a similar pattern to that found earlier (P. W. Wellings, pers. comm.). However, our findings are somehow in conflict with those of Epstein et al. (1986) regarding the effect of both temperature and photoperiod. These authors reported that shorter days and lower temperatures increased constitutive levels of 6-methoxy-

TABLE 3. ANALYSIS OF VARIANCE OF THE ACCUMULATION OF HYDROXAMIC ACIDS IN WHEAT SEEDLINGS GROWN UNDER DIFFERENT TEMPERATURE AND PHOTOPERIOD TREATMENTS. IN BRACKETS: VALUES OBTAINED INCLUDING "SEEDLING GROWTH RATE PRIOR TO ANALYSIS" AS A COVARIATE.

Source	df	Mean square	F-ratio	P-value
Temperature, T	2 (2)	0.292 (0.031)	14.808 (1.606)	0.000 (0.209)
Photoperiod, P	2 (2)	0.018 (0.002)		<i>n.s.</i> <sup>a</sup>
T × P	4 (4)	0.051 (0.061)	2.588 (3.138)	0.045 (0.020)
Error	63 (62)	0.0197 (0.019)		

<sup>a</sup>*n.s.* = not significant.

benzoxazolinone, a decomposition product of naturally occurring Hx (Niemeyer, 1988), in wheat plant extracts. However, these authors did not consider the fact that plants grown under low temperature and/or short photoperiod regimes were shorter and hence, contained a higher proportion of younger tissue, which in turn contains higher levels of Hx (Argandoña et al., 1981).

The results presented here show that temperature and photoperiod affect both primary and secondary metabolism of wheat seedlings, although their effects may be interwoven and, thereby, appear difficult to distinguish. It was clear that seedlings growing under higher temperatures and/or longer daylengths reached earlier the phenological stage considered for analysis. Consequently, a major evident effect on seedlings is variation of growth rate. Given that seedling growth rate prior to analysis was significantly positively correlated with accumulation of Hx it could be considered that the formerly stated temperature effect on accumulation of Hx was mediated by its effect on plant growth rate. An adequate statistical analysis showed that after removing the effect of plant growth rate the effect of temperature on accumulation of Hx was no longer significant. Therefore, the temperature effect was fully mediated through plant growth rate. This suggests the occurrence of an intrinsic effect of plant growth rate rather than a direct effect of the environment on metabolic processes along the Hx biosynthetic pathway.

Given that the effect of temperature (the factor most importantly affecting Hx accumulation) "vanished" after removing the effect of plant growth rate we will focus the discussion on the relationship of plant growth rate and the anti-herbivore defenses status of a given plant, population or species. General theories of plant defenses predict an inverse relationship between plant growth rate and defense investment (Zangerl and Bazzaz, 1992). This pattern is rationalized by the higher energetic cost of replacement of biomass loss by herbivory for

slow-growing plants in comparison with fast-growing ones. Although this was originally proposed and evaluated at interspecific level and dealing with an evolutionary rather than ecological time scale (growth-rate theory, Coley et al., 1985), some later reports support a broadening of its scope to comprise patterns at intraspecific level (e.g., Coley, 1986; Jing and Coley, 1990; Sagers and Coley, 1995).

The present work shows a direct relationship between plant growth rate and defense investment within an intraspecific context. Since this result cannot be explained within the former theoretical framework, it seems meaningful to seek proximal explanations for the pattern found e.g., a linkage between Hx and plant growth.

Although Hx have been thought to occur in the plant only as glucosides (Hofman and Hofmanová, 1971), the presence in wheat extracts of Hx aglucones and of the benzoxazolinones arising from the decomposition of the aglucones has been reported (Leszczynski and Dixon, 1990). Therefore, in order to discuss the physiological roles of Hx in the plant all three types of molecules should be considered.

A cytokinin activity (Petho and Dinya, 1992) and an inhibitory effect on auxin-induced coleoptile growth (Venis and Watson, 1978) have been described for benzoazolinones. In addition, both stimulant (Ray et al., 1977) and inhibitory (Venis, 1979) effects on the binding affinity of auxin analogues have been reported for benzoxazolinones and aglucones, respectively; hence, in view of such opposing evidence, this putative physiological role of Hx remains to be elucidated. On the other hand, a possible role of root-exuded Hx in iron uptake in grasses has been reported (Petho, 1993). This suggests at first glance a possible coupling of Hx accumulation with mineral nutrition and hence with growth. However, since the relative distribution of Hx in the shoot and in root exudates is unknown, this phenomenon cannot yet be considered to account for the pattern found (Figure 2). Further research is needed to determine how and to what extent Hx are related to the plant growth. The importance of the identification of the "primary" functions of a particular plant secondary metabolite has been stressed recently in the context of the expected physiological and ecological costs of chemically-based plant resistance to herbivores (Simms, 1992).

Further experiments broadening the range of environmental conditions evaluated or adding other growth-modifying variables and including plant performance measures should lead to a more definitive ascertainment of the pattern found as well as to a more clear notion of the ecological significance of the observed phenotypic plasticity in secondary metabolism coupled to the growth process. In addition, in view of the role of Hx in resistance against herbivores of crop plants, here it is suggested the usefulness of considering the effect of environmental conditions on such mechanism of plant defense against herbivores in order to assess accurately its efficiency as a tool in pest management programs.

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