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## Effect of defoliation on the patterns of allocation of a hydroxamic acid in rye (*Secale cereale*)

Hugo G. Collantes, Ernesto Gianoli\*, Hermann M. Niemeyer

*Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile*

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### Abstract

Hydroxamic acids are a class of secondary metabolites typical of grasses which play a role in resistance to herbivores. The effects of defoliation (simulated herbivory) on the allocation patterns of a hydroxamic acid (DIBOA) in rye seedlings were evaluated. Defoliated plants were compared with undefoliated plants 5, 7, 9 and 11 days after defoliation and germination, respectively. Shoot DIBOA of defoliated and undefoliated seedlings decreased with increasing age, but DIBOA concentration in defoliated seedlings was generally higher than in undefoliated ones. On the other hand, DIBOA concentration of the primary leaf was not affected by defoliation. The contribution of the primary leaf to the DIBOA content of the whole shoot as well as to the biomass of the whole shoot decreased with time for both defoliation treatments. As age increased the primary leaf of undefoliated seedlings decreased in relative DIBOA content at a greater rate than in relative biomass. The primary leaf of defoliated seedlings showed a similar rate of decrease for both variables. This pattern as well as that for DIBOA concentration in defoliated vs. undefoliated seedlings was interpreted as consistent with predictions of the Optimal Defense theory. In addition, the increased level of DIBOA in the shoot and, to a lesser extent, the unchanged DIBOA level in primary leaf of defoliated seedlings supported an extension of the 'reversion to juvenile phase' phenomenon described for woody plant–mammal interactions. © 1997 Elsevier Science B.V.

*Keywords:* Defoliation; Hydroxamic acids; DIBOA; Herbivory; Secondary metabolites; Rye; *Secale cereale*

### 1. Introduction

Hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-ones) (Hx) are a family of secondary metabolites present in wild and cultivated Gramineae [1, 2]. These compounds have been related to plant resistance against herbivores such as aphids, caterpillars

and rootworms, as well as against fungal and bacterial diseases (reviewed in [3]). In addition, allelopathic properties of Hx have been described [4, 5]. Hx are not present in seeds; they appear upon germination and thereafter their concentration increases in plant shoots, reaching a maximum a few days after and then decreasing progressively [6]. In addition, Hx concentration of younger leaves is higher than that of older ones [6], and young tissues of mature plants still retain Hx levels significant for insect resistance [7]. The main Hx present in rye,

\*Corresponding author. Fax: +56 2 2717503; e-mail: aletheia@abulafia.ciencias.uchile.cl

*Secale cereale*, is 2- $\beta$ -O-D-glucopyranosyl-4-hydroxy-1,4-benzoxazin-3-one (DIBOA-glc). The aglucone DIBOA is produced by enzymatic hydrolysis upon tissue damage [1].

Considerable evidence indicates that plants may show changes in their secondary metabolism upon damage by herbivores or by artificial damage [8]. These induced responses may confer enhanced resistance against herbivores to the attacked plants [9, 10]. Two kinds of damage may be distinguished in relation to the amount of plant biomass or resources lost: (i) that produced by chewing or sucking insects [11], which is localized and of relatively small magnitude, and (ii) that produced by browsing mammals [12] or by defoliation in crop systems devoted to livestock foraging [13], which is less localized and of relatively large magnitude. Induction of Hx as a consequence of insect attack or of artificial damage simulating it has been widely described [14–17], while the effect upon Hx metabolism of damage by defoliation remains unknown.

In this work we describe the effect of defoliation upon DIBOA allocation patterns in rye seedlings. In comparisons of defoliated and non-defoliated seedlings, growth after defoliation is considered analogous to growth after germination since both processes involve the production of new leaf tissue by the plant. Two possible outcomes are: (i) regrowth following defoliation will show similar or higher DIBOA levels than the corresponding undefoliated seedlings, an effect that could be viewed as a 'reversion to the juvenile phase' after defoliation *sensu* Kozłowski [18]; (ii) previously defoliated seedlings will show lower DIBOA levels than corresponding undefoliated seedlings, i.e. a chronological program of accumulation of Hx would prevail.

## 2. Materials and methods

### 2.1. Plants

Seeds of *Secale cereale* cv. Tetra were obtained from Campex Baer, Chile, and germinated in a growth chamber in individual plastic pots (25 ml) filled with soil (Anasac). Seedlings were grown in a growth chamber at 20 °C, 12 h photoperiod, and

117  $\mu\text{E m}^{-2} \text{s}^{-1}$ . Eight treatments arose from an array of two groups (undefoliated and defoliated seedlings) and four ages (5, 7, 9 and 11 days after germination or defoliation, respectively). The defoliated seedlings were cut just above the coleoptile five days after germination. Eleven seedlings were used for each treatment. At each age the leaves of the seedlings were cut and analyzed separately for DIBOA concentration. A two-way ANOVA was performed to analyze this variable. In addition, two parameters for DIBOA allocation patterns were assessed and analyzed: relative DIBOA allocation to leaves (percentage of shoot DIBOA content) and relative biomass of leaves (percentage of shoot biomass).

### 2.2. Chemical analysis

Leaves (40–70 mg) were macerated using mortar and pestle with ca. 300 mg sea sand in 1 ml H<sub>2</sub>O. The aqueous extract was left at room temperature for 15 min and then adjusted to pH 3 with 0.1 N H<sub>3</sub>PO<sub>4</sub>. The extract was centrifuged at 10 400 g for 15 min and a 100  $\mu\text{l}$  aliquot of the supernatant was directly injected into an HPLC (High Performance Liquid Chromatograph). An RP-100 Lichrospher-C18 column was used with a constant solvent flow of 1.5 ml min<sup>-1</sup> and the following linear gradients between solvents A (MeOH) and B (0.5 ml 85% H<sub>3</sub>PO<sub>4</sub> in 1 l H<sub>2</sub>O): 0 to 9 min 30% A, 9 to 11 min 100% A, 11 to 15 min 30% A. Detection of compounds eluting from the column was performed at 263 nm. DIBOA showed a retention time of  $3.3 \pm 0.1$  min.

## 3. Results

Observation of the growth pattern of defoliated rye seedlings showed that the primary leaf had a lopped tip while the secondary and tertiary leaves grew normally. This indicated that the primary leaf was the only one suffering direct morphological damage by defoliation at such time. Hence, the effect of defoliation upon DIBOA accumulation in the shoot as well as in the primary leaf of rye seedlings was studied.

DIBOA concentration of the shoot was affected

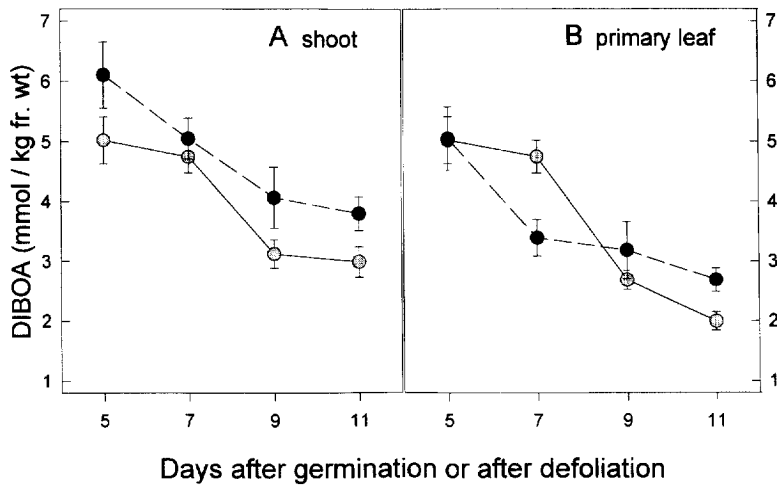


Fig. 1. A: variation of DIBOA concentration through time in the shoot of defoliated and undefoliated rye seedlings; B: variation of DIBOA concentration through time in the primary leaf of defoliated and undefoliated rye seedlings. ○ undefoliated, ● defoliated.

by defoliation and age but not by their interaction ( $P$ -values: 0.007, 0.000 and 0.735 for effects of defoliation ( $D$ ), age ( $A$ ) and  $D \times A$ , respectively; two-way ANOVA). DIBOA concentration decreased with increasing age and was greater in defoliated seedlings (Figure 1A). On the other hand, DIBOA concentration of the primary leaf was not affected by  $D$ , but was affected by  $A$  and the  $D \times A$  interaction ( $P$ -values: 0.678, 0.000 and 0.008 for effects of  $D$ ,  $A$  and  $D \times A$ , respectively; two-way ANOVA). DIBOA concentration declined with increasing age for both defoliation treatments. Interaction of factors occurred because primary leaves of undefoliated seedlings had higher DIBOA levels on day 7, but by day 11 concentrations were greater for defoliated plants (Figure 1B).

The number of leaves that had developed at each age differed between defoliated and undefoliated seedlings. Defoliated seedlings showed primary and secondary leaves even at day 5; the tertiary leaf appeared thereafter. Undefoliated seedlings showed only primary leaf at day 5 and day 7; the secondary leaf appeared thereafter and the tertiary leaf never became visible (Figure 2). Figure 2A shows that the contribution of the primary leaf to the DIBOA content of the whole shoot decreased with increasing age for both defoliated and undefoliated seedlings. Likewise, Figure 2B shows that the contribution of the primary leaf to the biomass

of the whole shoot decreased with time for both defoliated and undefoliated seedlings. For the undefoliated treatment, the rate of decline in the proportion of total shoot DIBOA that was in the primary leaf was greater than the rate of decline in the proportion of total shoot biomass in the primary leaf. In contrast, both proportions declined at a similar rate for defoliated seedlings.

#### 4. Discussion

Argandoña et al. [6] found that DIBOA concentration in the shoot of rye seedlings increased following germination, reaching a maximum a few days later, and thereafter decreased. They found that in each leaf DIBOA concentration was highest at emergence and followed the normal decrease thereafter. The dynamics of DIBOA concentration in shoot (Figure 1A) and primary leaf (Figure 1B) of undefoliated seedlings shows that they were both in the decreasing phase of Hx accumulation. In this regard, the higher DIBOA levels found in the shoot of defoliated, older seedlings as compared with undefoliated ones appear as a reversion to the juvenile phase upon defoliation *sensu* Kozłowski [18], as has been described for woody plant–mammal interactions [12].

Overall differences in DIBOA concentration in

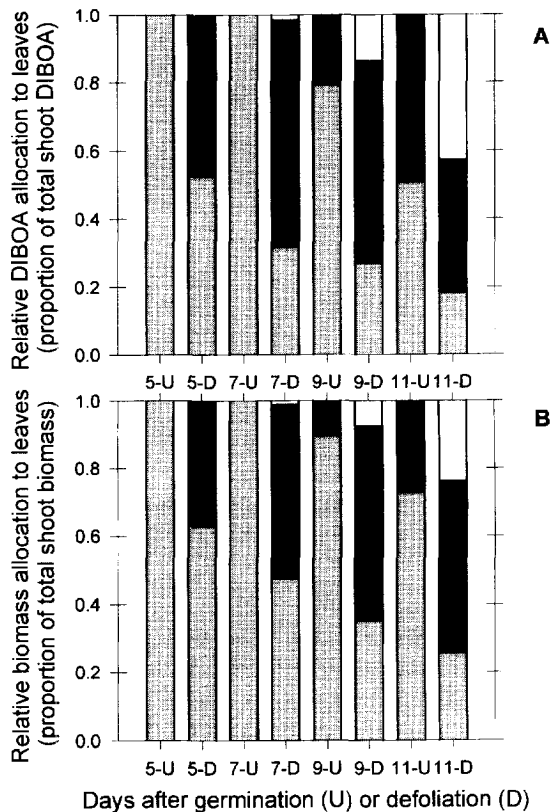


Fig. 2. A: comparison of the relative allocation of DIBOA to leaves (proportion of total shoot DIBOA) in defoliated and undefoliated rye seedlings; B: comparison of the relative biomass allocation to leaves (proportion of total shoot biomass) in defoliated and undefoliated rye seedlings. □ primary leaf, ■ secondary leaf, ▨ tertiary leaf.

the primary leaf of defoliated and undefoliated seedlings were non-significant. However, statistical interaction of factors (defoliation  $\times$  age) arose due to greater DIBOA levels in undefoliated plants at day 7 and lower at day 11 (Figure 1B). We lack a biological explanation of this result and hence keep the overall pattern: no effect of defoliation along the experiment. Since the undefoliated seedlings were in the decreasing phase of Hx accumulation, the fact that the primary leaf of undefoliated and of older defoliated seedlings did not differ in DIBOA levels may also be considered as a reversion to the juvenile phase. In the primary leaf of undefoliated seedlings the decrease with age in proportion of total shoot DIBOA was greater than the decrease in allocated biomass, whereas in the primary leaf of defoliated

seedlings allocations to defense and to biomass decreased similarly with time. These results suggest that following traumatic damage rye seedlings allocate differentially their defensive metabolites (Hx) to the attacked, re-growing leaf.

The results of this work are in accordance with one of the main predictions of the Optimal Defense theory [19, 20]: allocation patterns of plant defenses should be proportional to the probability of damage by herbivores (or, in this case, to the actual damage). Defoliation is a damage of relatively large magnitude since it involves the loss of almost all the aerial biomass of the plant. Our results showed higher Hx levels in the shoot of defoliated seedlings and a more lasting allocation of Hx to the primary leaf of these seedlings, hence they support the prediction of the Optimal Defense theory.

Undefoliated and defoliated seedlings differed in the growth of a lopped primary leaf after defoliation, and also in the number of leaves that had developed at each evaluation. This latter effect was due to growth of defoliated seedlings occurring later than that of undefoliated seedlings, hence showing late emerging leaves. The expression of higher Hx levels in new leaves would explain the higher Hx concentration found in the shoot of defoliated seedlings, which had leaves younger than those of undefoliated seedlings. As already mentioned, we considered growth after defoliation analogous to growth after germination since both processes involve new leaf tissue production by the plant and, moreover, since this is the scenario that herbivores would face under natural circumstances.

The explanation given for higher Hx concentration found in defoliated seedlings is analogous to that given by Bryant et al. [12] with regard to the reversion to the juvenile phase in mature woody plants upon browsing by mammals. The reversion to the juvenile phase found in both shoot and primary leaf of rye seedlings upon defoliation, in contrast to the maintenance of a chronological program for the accumulation of Hx, may confer on the plant an enhanced level of defense against herbivores. These responses are specially interesting in fodder crop systems, where plants better protected against insect pests could be obtained as a result of harvesting practices. In addition, this work has shown that rye seedlings react to a traumatic

damage in a way consistent with predictions of a current general theory of plant defense. Further experiments on the effect of defoliation on Hx allocation patterns to both above-ground and below-ground biomass should lead to a more definitive characterization of the pattern found, as well as to a clearer notion of the physiological processes involved in the phenomenon.

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