

# Allocation of a Hydroxamic Acid and Biomass During Vegetative Development in Rye

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The allocation of a hydroxamic acid (DIBOA) and biomass was evaluated in vegetative growth stages (GS) of rye. Allocation to the shoot, root/shoot ratio of allocation, and the effect of reduced pot volume on such patterns of allocation were evaluated. Higher allocation of biomass to the shoot relative to the root as the plant aged was observed. No significant differences in allocation of DIBOA were found at different plant GS. Reducing pot volume decreased final shoot biomass. The effect at an early GS was marginally non-significant, while it was highly significant at a late GS. The effect of reduced pot volume on DIBOA accumulation differed between the GS. At the early stage the DIBOA content per unit of biomass in the shoot increased significantly with reduced pot volume, whereas at the late stage it was not significantly different. The effects of reduced pot volume on the shoot were not a consequence of a significant shift in the corresponding root/shoot ratios. While the total content of DIBOA (nmol) and the total biomass (g) in the shoot varied at similar rates in the first GS evaluated, a substantially greater relative increase in biomass occurred in the last two GS.

## Introduction

Phytochemicals play a major role in plant resistance against herbivores. A detailed knowledge of the relationship between chemistry and biological activity, as well as of the effects of phytochemicals on the target herbivores is widely found in the literature (Rosenthal & Berenbaum, 1991). The dynamics of resource allocation in the plant with regard to the production of chemical defences has received less attention, however. This knowledge is of key importance from an ecological approach, since the particular within-plant distribution of chemical defences (and biomass) may

determine the success of any general strategy of resource allocation (Zangerl & Bazzaz, 1992; Herms & Mattson, 1992).

Hydroxamic acids (Hx) (1,4-benzoxazin-3-ones) are a well-studied family of phytochemicals, present mainly in Poaceae (Niemeyer, 1988; Niemeyer et al., 1992; Gianoli & Niemeyer, 1998). Hx normally occur in the plant as 2- $\beta$ -O-D-glucopyranosides which are hydrolysed by  $\beta$ -endoglucosidases when the tissue is injured (Hofman & Hofmanová, 1971). The released aglucones have more biological activity against insects (Corcuera et al., 1985), the heterocyclic ring of the aglucone accounting for much of such activity (Escobar et al., 1997). Hx have been identified as a resistance factor against herbivores such as cereal

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aphids, stemborers and rootworms, as well as against fungal and bacterial diseases (Niemeyer & Pérez, 1995). These compounds are absent from the seed and increase upon germination, and their concentrations peak at the young seedling stage and decrease thereafter (Argandoña et al., 1981); in mature plants the youngest tissue still retains a high concentration of Hx (Thackray et al., 1990).

The present paper addresses the allocation of DIBOA (2,4-dihydroxy-1,4-benzoxazin-3-one), the major hydroxamic acid in rye extracts (Niemeyer, 1988), and of biomass in rye, *Secale cereale* L. (Poaceae), along the vegetative stages of the plant. The study looked at the allocation to the shoot as well as at the root/shoot ratio of allocation. In addition, the effect of reduced availability of nutrients (reduced pot volume) on the allocation to biomass and DIBOA in the shoot and the root was evaluated at two growth stages of rye plants. Whereas biomass allocation was expressed as fresh weight (g), the evaluation of DIBOA allocation was performed considering defence gross content ( $\mu\text{mol}$  of hydroxamic acid) as the currency.

## Material and methods

### Plants

Seeds of rye, *S. cereale* L. cv. Tetra, were obtained from CAMPEX Baer, Chile, and germinated in a room at  $25 \pm 1^\circ\text{C}$ , with a 16 h photoperiod, and  $84 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR in individual plastic pots filled with potting soil (ANASAC). Rye plants were analysed for DIBOA content of the root and the shoot at five growth stages (GS) according to the decimal code of cereal growth stages (Zadoks et al., 1974). Growth stages evaluated were: GS 10 (seedling with first leaf through coleoptile), GS 12 (seedling with two leaves unfolded), GS 14 (seedling with four leaves unfolded), GS 21 (plant with a main shoot and one tiller) and GS 25 (plant with a main shoot and five tillers). GS 30 (leaf sheath erection) was only evaluated in the shoot. The following pot volumes were used for plants to be analysed at different growth stages. 25 ml: GS 10, GS 12; 120 ml: GS 14; 1 l: GS 21, GS 25; 2.5 l: GS 30. Plants to be analysed at GS 14, GS 21, GS 25 and GS 30 were transferred from the growth room to a carbon-fibre greenhouse when they attained GS 12. Two groups of seedlings at GS 14 and two groups of plants at GS 30 were used for the experiment on the effect of pot volume on the accumulation of DIBOA and biomass. Seedlings (GS 14) to be compared were raised in pots of 120 ml (normal volume) and 20 ml (reduced volume). Plants (GS 30) to be compared were transferred to pots of 2.5 l (normal volume) and 1 l

(reduced volume) when they attained GS 12. Environmental conditions within the greenhouse were as follows: temperature min.  $0\text{--}8^\circ\text{C}$ , max.  $18\text{--}33^\circ\text{C}$ ; light intensity range  $120\text{--}688 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR; photoperiod 10–12 h light (winter to spring time). At each growth stage evaluated, the root and the shoot were cut, weighed and analysed for DIBOA content. All treatments consisted of eight replicates. Ratio values were arcsine-transformed prior to statistical analysis. Given that plants at GS 12 and GS 14 were raised under environmental conditions different to those of older experimental plants, separate analyses were performed for each “environment”.

### Chemical analysis

Plant tissue was macerated using a mortar and pestle with approx. 300 mg sea sand in distilled  $\text{H}_2\text{O}$ . The volume of  $\text{H}_2\text{O}$  used was proportional to tissue biomass (fresh weight), starting with 1 ml of  $\text{H}_2\text{O}$  for tissue up to 100 mg. In all cases an aliquot (1 ml) of 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 10 400 g for 15 min and a 100  $\mu\text{l}$  aliquot of the supernatant was directly injected into a high-performance liquid chromatograph (HPLC). An RP-100 Lichrospher-C18 column was used with a constant solvent flow of  $1.5 \text{ ml min}^{-1}$  and the following linear gradients between solvents A (MeOH) and B (0.5 ml 85%  $\text{H}_3\text{PO}_4$  in 1 l  $\text{H}_2\text{O}$ ): 0–7 min 20% A, 7–9 min 100% A, 9–15 min 20% A. Detection of DIBOA was performed at 263 nm. DIBOA showed a retention time of  $4.2 \pm 0.2$  min.

### Currency of allocation

Content was used as the currency of DIBOA allocation because the comparison of concentrations prevents a total-budget perspective which, employing absolute content, would show the relative contribution of plant parts to the total pool of the plant. However, inasmuch as concentration is a ratio it is affected by both variations in the numerator (content) and the denominator (biomass). When using concentrations to compare plant parts it is unclear whether differences are a consequence of changes in biomass or in content. Furthermore, although the use of concentrations is widely seen as a means to standardize one variable for the effect of another (i.e. to eliminate the effect of biomass on content), it is often overlooked that this practice requires that content and biomass are isometrically related, i.e. that they are related linearly and the function passes through the origin (Raubenheimer & Simpson, 1992). Notwithstanding that concentration is a more meaningful currency when considering the effect of plant

Table 1. Mean root/shoot ratio (R/S) for biomass and DIBOA content of rye plants through time

R/S	GS 10 <sup>1</sup>	GS 12 <sup>1</sup>	GS 14 <sup>2</sup>	GS 21 <sup>2</sup>	GS 25 <sup>2</sup>
Biomass	0.49a	0.48a	0.29A	0.30A	0.19B
DIBOA	0.03a	0.03a	0.08A	0.12A	0.08A

GS: growth stage (Zadoks et al., 1974).

Means sharing letter and case within a row are not significantly different ( $P > 0.05$ , Tukey HSD test following a one-way ANOVA).

<sup>1</sup> Seedlings raised in a room with controlled environment.

<sup>2</sup> Plants raised in a greenhouse

defence on herbivores, recent work has suggested that studies focusing on plant allocation should use content as the currency (Gianoli et al., 1999; Koricheva, 1999).

## Results

### *Allocation of biomass and DIBOA to root and shoot*

No significant differences ( $P > 0.05$ , one-way ANOVA) in the root/shoot ratio for either biomass or DIBOA were found for the first two evaluated GS, raised in a room with controlled environmental conditions (Table 1). For the other three GS, raised in a greenhouse, it was found that the root/shoot ratio for biomass was significantly lower ( $P < 0.01$ , Tukey HSD test after one-way ANOVA) in the oldest GS (Table 1). The root/shoot ratio of DIBOA was not significantly different ( $P > 0.05$ , one-way ANOVA) among these GS (Table 1).

While the total content of DIBOA (nmol) and the total biomass (g) in the shoot varied at similar rates in the first four GS evaluated, a substantially greater relative increase in biomass occurred in the last two GS (Fig. 1).

### *Effect of reduced pot volume in shoot biomass and DIBOA*

Reducing pot volume decreased shoot final biomass. At GS 14 (short-term evaluation) the effect was marginally non-significant, whereas at GS 30 (long-term evaluation) the effect was highly significant (Table 2).

The effect of reduced pot volume on DIBOA accumulation, expressed in terms of the resultant  $\mu\text{mol DIBOA g}^{-1}$  biomass ratio—an appropriate parameter to compare plants of different size—differed between growth stages. Thus, at GS 14 the DIBOA content per unit of shoot biomass increased significantly with reduced pot size, whereas at GS 30 the ratios were not significantly different (Table 2). It is important to point out that the effects of reduced pot volume on the shoot at GS 14 were not a consequence of a significant shift in the corresponding

root/shoot ratios, as shown in Table 2. Owing to experimental shortcomings (plants were accidentally lost before root evaluations) the data on root of plants at GS 30 are lacking. Hence it was not possible to determine whether the root/shoot ratios varied with pot volume at this growth stage.

## Discussion

Patterns of allocation of biomass and DIBOA in rye were modified by the age of the plant as well as by the availability of nutrients. A similar flexibility in such allocation patterns had been shown previously in this species regarding the effect of defoliation (Collantes et al., 1997). In general, the accumulation of hydroxamic acids in cereals is sensitive to changes in environmental variables (Manuwoto & Scriber, 1985; Åhman & Johansson, 1994; Gianoli & Niemeyer, 1997).

The results of the present paper showed that the root/shoot ratios of biomass and DIBOA do not change during the early seedling stages. However, the results (in particular those performed in the greenhouse) also showed that the expected pattern of increasing biomass allocation to the shoot relative to

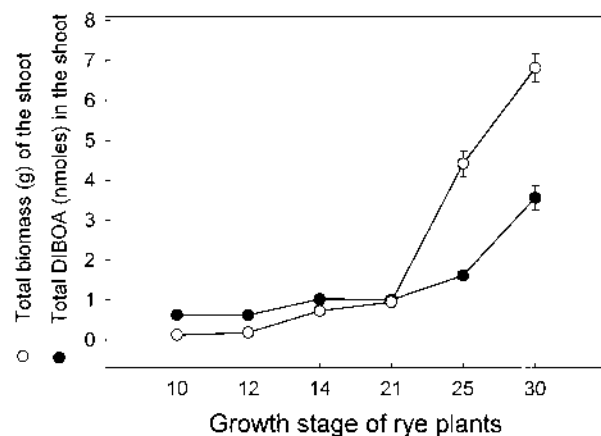


Fig. 1. Growth stage of rye plants and (○) total biomass (g) and (●) total DIBOA (nmoles) contained in the shoot. Bars represent SEM ( $n = 8$ ).

Table 2. Effect of pot volume on biomass and DIBOA (mean  $\pm$  SE) in rye shoots and in the corresponding root/shoot ratios (R/S) at two growth stages: GS 14 = seedling with four leaves unfolded; GS 30 = leaf sheath erection ( $n = 8$ )

	GS 14			GS 30 <sup>2</sup>		
	Pot volume		<i>P</i> -value <sup>1</sup>	Pot volume		<i>P</i> -value <sup>1</sup>
	120 ml	20 ml		2.5 l	1.0 l	
Biomass (g)	0.84 $\pm$ 0.06	0.71 $\pm$ 0.03	0.062	6.82 $\pm$ 0.36	2.56 $\pm$ 0.16	<0.001
DIBOA ( $\mu$ mol)	0.96 $\pm$ 0.08	1.29 $\pm$ 0.09	0.014	3.56 $\pm$ 0.35	0.99 $\pm$ 0.14	<0.001
Ratio $\mu$ mol g <sup>-1</sup>	1.18 $\pm$ 0.09	1.83 $\pm$ 0.13	0.001	0.53 $\pm$ 0.08	0.39 $\pm$ 0.05	0.115
R/S biomass	0.29 $\pm$ 0.02	0.32 $\pm$ 0.02	0.349	–	–	–
R/S DIBOA	0.08 $\pm$ 0.01	0.06 $\pm$ 0.01	0.502	–	–	–

Pot volumes listed first are those used for the analysis of biomass and DIBOA accumulation described in Fig. 1.

<sup>1</sup> One-way ANOVA.

<sup>2</sup> Root data are missing owing to experimental shortcomings (see text).

the root as the seedling turns into a plant was not accompanied by a significant change in the relative allocation of DIBOA to the shoot. None the less, the comparison of the dynamics of accumulation of shoot biomass and DIBOA content indicates that defence allocation does change with age. Thus, while the total content of DIBOA (nmol) and the total biomass (g) in the shoot varied at similar rates in the first GS evaluated, a substantially greater relative increase in biomass occurred in the last two GS (Fig. 1). This may reflect a lower relative allocation of resources to defence in the shoot at later GS. Similar patterns have been described for other annual species (Bazzaz et al., 1987), probably reflecting a switch in resource allocation from defence to processes related to upcoming reproduction.

Thus, it is likely that in rye plants at later stages, resources are mainly devoted to maximize reproductive issues, hence relaxing the investment in defensive metabolites such as DIBOA. The latter may be rationalized from two different standpoints. First, the cost of defence when the consumed resources could be otherwise directly utilized in seed formation (a process closely related to plant fitness) should be higher than that of an early defensive investment. Secondly, it may be a consequence of a higher tolerance of rye plants to herbivore attack in comparison with that of rye seedlings. This has been reported for another cereal crop (Gellner et al., 1990).

Reducing pot volume produced a decrease in shoot final biomass. This effect was more noticeable for plants evaluated at a late growth stage. This was not surprising because seedlings at GS 14 have recently terminated their dependence on the energetic supply of the endosperm, whereas older plants (GS 30) have largely depended on the resource availability of the substrate provided.

The results of the overall effect of reduced pot volume (and hence availability of nutrients) on shoot allocation of biomass and DIBOA are roughly in agreement with the explanations outlined above. Thus, at an early growth stage (GS 14) when defence allocation still consumes an important fraction of total resources, the resulting reduction in shoot biomass due to a lower availability of nutrients is accompanied by an increase in the relative defensive investment of the shoot. Given that this effect cannot be attributed to a significant shift in the corresponding root/shoot ratios, it seems that there is an active enhanced allocation of resources to defence under conditions that somehow announce a shortage of nutrients. The enhanced protection of plants growing in a comparatively “poor” habitat has been rationalized in terms of the higher energetic cost of replacement of biomass lost to herbivory for slow-growing plants in comparison with fast-growing ones (Coley et al., 1985).

The experimental decrease in biomass caused no differences in investments in DIBOA defences when plants attained an older phenological stage (GS 30). This agrees with the notion of a reduction in the relative importance of defence at the latest vegetative stages of rye plants, when resources should be mostly allocated to the preparation of the reproductive event.

The present work has described coarse patterns of resource allocation to biomass and defence during the vegetative development in rye. Further studies addressing finer patterns of allocation comprising roots, leaves and reproductive organs, together with experimental assessments of the actual benefit to the plant (expressed in terms of fitness) of either allocation strategy under different environments, would corroborate the preliminary conclusions drawn herein.

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