DEFOLIATION AFFECTS CHEMICAL DEFENSES IN ALL PLANT PARTS OF RYE SEEDLINGS

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Abstract—The effect of defoliation and consecutive defoliation (condefoliation) of rye seedlings on the allocation patterns of biomass and hydroxamic acids (Hx) was evaluated five days after treatment. Growth of condefoliated seedlings was lower than that of defoliated and nondefoliated ones. Concentration of Hx decreased in shoots of condefoliated seedlings compared to nondefoliated ones, while concentration of Hx in root exudates increased. Allocation of Hx to roots and root exudates increased at the expense of allocation to the shoots in condefoliated seedlings. The ratio of Hx-aglucone to Hx-glucoside was higher in shoots of defoliated and condefoliated seedlings. The decrease in quantity of defense in shoots was accompanied by an increase in its quality, given that aglucones are more toxic than glucosides. The increase in concentration of Hx—an allelopathic compound also involved in mineral uptake by roots—in root exudates of condefoliated seedlings was suggested to lead to an advantage in the acquisition of resources for the regeneration of lost biomass.

INTRODUCTION

Hydroxamic acids (Hx) are plant secondary metabolites present in wild (Niemeyer et al., 1992; Gianoli and Niemeyer, 1998a) and cultivated (Niemeyer, 1988) Poaceae and are involved in plant resistance to insect herbivores and pathogens (Bergvinson et al., 1995; Niemeyer and Pérez, 1995; Weibull and

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Niemeyer, 1995; Gianoli et al., 1996). In addition, allelopathic properties of Hx have been described in laboratory and field experiments (Barnes and Putnam, 1987; Pérez and Ormeño-Núñez, 1993; Mwaja et al., 1995). Hx are present in all plant parts, and their concentration in shoot, root, and root exudate differs between species and even between cultivars (Argandoña et al., 1981; Pérez and Ormeño-Núñez, 1991; Xie et al., 1991). These compounds occur mainly as glucosides that are transformed to the more biologically active aglucones (Corcuera et al., 1985) by enzymatic hydrolysis upon tissue damage (Hofman and Hofmanová, 1971) . The most abundant Hx in rye (*Secale cereale* L.) is 2-β-O-D-glucopyranosyl-4-hydroxy-1,4-benzoxazin-3-one (DIBOA-Glc).

Changes in Hx concentrations as a consequence of insect attack or localized artificial damage simulating it have been widely described (Gutiérrez et al., 1988; Niemeyer et al., 1989; Morse et al., 1991; Gianoli and Niemeyer, 1997, 1998b), while the effect on Hx metabolism of damage by defoliation, a process analogous to grazing or harvesting, has been reported recently in rye (Collantes et al., 1997) and in maize (Collantes et al., 1998). The latter work focused on the effect of defoliation on Hx levels and allocation patterns within the directly affected plant part, i.e., the shoot. The present work addresses the effect of defoliation on Hx levels and allocation patterns between shoots, roots, and root exudates of rye seedlings by determining the concentration of Hx the relative content of Hx (percentage of whole plant content of Hx), and the ratio of Hx-aglucone to Hx-glucoside. These parameters were also determined in seedlings subjected to a consecutive defoliation (hereafter called condefoliation) in order to evaluate the effect of extended damage on the induction of plant responses. In comparisons of nondefoliated and defoliated seedlings, growth after defoliation is considered analogous to growth after germination since both processes involve the production of new leaf tissue.

METHODS AND MATERIALS

Plants. Seeds of *S. cereale* cv. Tetra were obtained from CAMPEX-Baer, Chile, and germinated in individual plastic pots (100 ml) filled with sterilized sand (Anasac). Seedlings were grown in a room at $25 \pm 3°C$, 16L:8D photoperiod and were irrigated with a nutrient solution (Anasac) containing N, P, and K (6:4:3 ratio) and micronutrients. Defoliated seedlings were obtained by cutting the plant just above the coleoptile (removing all shoot material) five days after sowing; condefoliated seedlings by cutting again five days after the first defoliation. Nondefoliated, defoliated, and condefoliated seedlings (seven seedlings per treatment) were evaluated five days after sowing, defoliation, and condefoliation, respectively. The defoliation procedure mimics natural browsing damage in grasses (Belsky, 1992).

Extracts. Seedlings were carefully drawn from the sand to avoid root damage. Roots were washed with distilled water directly onto the pots to remove sand particles. Seedlings were weighed and then shoots and roots were separated and immediately macerated by using a mortar and pestle with ca. 300 mg sea sand in 1 ml 0.1 M glycine HCl buffer, pH 2. Plant parts were macerated immediately in order to avoid spurious conversion of Hx glucosides into aglucones due to the scission of the plant. The weight of roots and shoots of experimental seedlings was not directly determined but rather estimated from weight data from additional groups of seedlings ($N = 7$ each) subjected to the same conditions within each treatment. These seedlings were drawn from the sand, weighed, cut into root and shoot, and finally both roots and shoots were weighed. Linear relationships were established between total plant weight and shoot weight ($R^2 = 0.95$ for nondefoliated; $R^2 = 0.98$ for defoliated; and $R^2 = 0.86$ for condefoliated) and between total plant weight and root weight ($R^2 = 0.96$ for nondefoliated; $R^2 = 0.95$ for defoliated, and $R^2 = 0.73$ for condefoliated). From these relationships and the total weight of experimental seedlings, the independent weights of roots and shoots were estimated. Root–shoot ratios of biomass as well as Hx concentrations were calculated using these values. The sand in the pots was washed with 100 ml distilled water to obtain root exudates. The washing solution was evaporated to dryness under vacuum at 45° C and the dry residue extracted with 1 ml n-butanol. All extracts were centrifuged at 10,400g for 15 min and the supernatant stored in a freezer until analysis.

Chemical Analysis. A 100-ml aliquot of extracts was directly injected into an HPLC. An RP-100 LiChrospher-C18 column (5 mm ID; Merck) 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 85% H_3PO_4 in 1 liter H_2O): 0–9 min 30% A, 9–11 min 100% A, and 11–15 min 30% A. Detection of compounds eluting from the column was performed at 263 nm. Both DIBOA-glucoside and its aglucone were analyzed. Retention times for aqueous extracts were 2.7 ± 0.1 and 3.7 ± 0.1 min for DIBOA-glucoside and DIBOA-aglucone, respectively, and 1.8 ± 0.1 min for the DIBOA-aglucone in n-butanol. Identity of peaks was confirmed by coinjection of standards dissolved in water and n-butanol, respectively.

Statistical Analysis. One-way ANOVA was performed to analyze separately: shoot biomass, concentration of Hx, relative content of Hx and Hx aglucone–glucoside ratio. In order to comply with ANOVA assumptions, the following data transformations were performed: square-root for concentration of Hx in root exudates; logarithmic for shoot biomass and for relative content of Hx; and arc-sin for the Hx-aglucone to Hx-glucoside ratio. The concentration of Hx in root exudates was estimated by dividing the content of Hx in root exudates by the fresh weight of roots. A Newman-Keuls test was used to evaluate differences between treatments.

RESULTS

Shoot biomass of seedlings was affected by treatment (P < 0.001). Non-defoliated and defoliated seedlings showed no significant differences between them (P = 0.141), but both were significantly different from condefoliated ones (P < 0.001 for both comparisons) (Table 1). Root biomass of seedlings was not affected by treatment (P = 0.567), and root–shoot ratios differed between treatments (Table 1). The statistical significance of the latter differences could not be determined because those were single values, obtained after analyses of regression (see Methods and Materials).

Treatment affected the concentration of Hx in shoots (P = 0.021) and root exudates (P = 0.036), but not in roots (P = 0.280). No significant differences were found in shoots between nondefoliated and defoliated seedlings, nor between defoliated and condefoliated ones. However, nondefoliated and condefoliated seedlings were different (Table 2). Similar relations between nondefoliated, defoliated, and condefoliated seedlings were found in root exudates. However, the concentration of Hx in shoots of condefoliated seedlings was lower than nondefoliated ones, while in root exudates it was higher (Table 2).

On the other hand, the effect of treatment on the relative content of Hx was significant in shoots (P < 0.001), roots (P < 0.001), and root exudates (P < 0.001). No differences were found between nondefoliated and defoliated seedlings, while condefoliated ones were different from these two. These relations between nondefoliated, defoliated, and condefoliated seedlings were the same in shoots, roots, and root exudates. However, the relative content of Hx in condefoliated seedlings was lowest in shoots, and highest in roots and root exudates (Table 3).

The ratio of Hx-aglucone to Hx-glucoside was affected by treatment in shoots (P = 0.005) but not in roots (P = 0.828). Differences were found in shoots between nondefoliated seedlings and both defoliated and condefoliated ones, the latter two being not significantly different from each other and higher

TABLE 1. BIOMASS OF SHOOT AND ROOT OF RYE SEEDLINGS SUBJECTED TO
DEFOLIATION AND CONSECUTIVE DEFOLIATION

	Biomass (mg, mean ± SE)[a]		
	Nondefoliated	Defoliated	Condefoliated
Shoot	103.37 ± 8.66 a	129.18 ± 14.77 a	42.13 ± 4.53 b
Root	155.05 ± 12.99 a	139.95 ± 16.00 a	133.42 ± 14.36 a
R : R[b]	1.50	1.08	3.17

[a]Means followed by the same lowercase letter within rows are not significantly different (P > 0.05, Newman-Keuls test).
[b]Ratio of root–shoot biomass.

TABLE 2. CONCENTRATION OF HYDROXAMIC ACIDS IN RYE SEEDLINGS SUBJECTED TO
DEFOLIATION AND CONSECUTIVE DEFOLIATION

	Hx conc (mmol/kg fresh wt, mean ± SE)[a]		
	Nondefoliated	Defoliated	Condefoliated
Shoot	19.31 ± 1.48 a	17.13 ± 1.96 ab	12.78 ± 1.11 b
Root	3.93 ± 0.32 a	3.23 ± 0.32 a	3.44 ± 0.28 a
Exudates[b]	0.26 ± 0.03 a	0.64 ± 0.17 ab	0.47 ± 0.04 b

[a]Means followed by the same lowercase letter within rows are not significantly different ($P > 0.05$, Newman-Keuls test).
[b]The concentration of Hx was estimated by dividing the content of Hx in root exudates by the fresh weight of roots.

TABLE 3. RELATIVE CONTENT OF HYDROXAMIC ACIDS (PERCENTAGE OF
WHOLE PLANT CONTENT) IN RYE SEEDLINGS SUBJECTED TO
DEFOLIATION AND CONSECUTIVE DEFOLIATION[a]

	Nondefoliated	Defoliated	Condefoliated
Shoot	72.41 ± 2.90 a	80.05 ± 2.87 a	50.86 ± 1.78 b
Root	25.98 ± 2.92 a	17.11 ± 3.01 a	43.61 ± 1.89 b
Exudates	1.61 ± 0.12 a	2.84 ± 0.76 a	5.53 ± 0.74 b

[a]Values are means ± SE. Means followed by the same lowercase letter within rows are not significantly different ($P > 0.05$, Newman-Keuls test).

TABLE 4. RATIO OF HYDROXAMIC ACID-AGLUCONE TO HYDROXAMIC ACID-GLUCOSIDE
IN RYE SEEDLINGS SUBJECTED TO DEFOLIATION AND CONSECUTIVE DEFOLIATION[a]

	Nondefoliated	Defoliated	Condefoliated
Shoot	0.11 ± 0.01 a	0.18 ± 0.02 b	0.16 ± 0.01 b
Root	0.72 ± 0.12 a	0.69 ± 0.07 a	0.68 ± 0.04 a

[a]Values are means ± SE. Means followed by the same lowercase letter within rows are not significantly different ($P > 0.05$, Newman-Keuls test).

than nondefoliated ones (Table 4). Only the aglucone of DIBOA was found in root exudates.

DISCUSSION

Comparisons between nondefoliated, defoliated, and condefoliated plants are inevitably complicated by the fact that the seedlings under these treatments

differ in chronological age. This might be thought to be critical given that the dynamics of accumulation of Hx in cereals is sensitive to plant age (Argandoña et al., 1981). Nonetheless, previous results on rye seedlings with identical experimental procedures showed that the dynamics of defoliated plants does not follow the typical chronological pattern of Hx accumulation (Collantes et al., 1997). This indicates that the confounding effects of seedling age are overwhelmed by the treatment effects. Therefore, considering that there is no way to uncouple age and regrowth after defoliation, and for the sake of allowing comparisons, growth after defoliation is considered here as analogous to growth after germination, since both processes involve the production of new leaf tissue.

Research on plant responses induced by insect feeding or wounding has focused mainly on the increase in concentration of defensive compounds (Tallamy and Raupp, 1991). However, plants may respond to herbivore attack or artificial damage in a variety of ways. We considered that defoliation of rye seedlings may trigger responses at three different levels: (1) an increase in the concentration of Hx in any plant part (including root exudates); (2) changes in the allocation patterns of Hx among shoot, roots, and root exudates; and (3) qualitative changes in chemical defense by transformation of Hx-glucosides into the more toxic aglucones.

The concentration of Hx in plant parts was only affected in shoots and root exudates of condefoliated seedlings. However, while the Hx concentration of shoots decreased in condefoliated seedlings, that of root exudates increased, and hence constitutes an induced response (*sensu* Karban and Myers, 1989) upon defoliation. In this case, the plant compensated for loss of photosynthetic biomass—and hence a likely decrease in fitness—through the increased exudation of an allelopathic compound that could enhance its competitiveness vis-à-vis neighboring plants. The role of rye-exuded Hx in allelopathy has been widely substantiated (Barnes and Putnam, 1987; Pérez and Ormeño-Núñez, 1991, 1993; Mwaja et al., 1995). The fact that condefoliated rye seedlings show this effect, but defoliated ones do not, suggests that this response positively correlates with intensity of damage, as would be expected for an induced response (Karban, 1991; Zangerl and Bazzaz, 1992). However, given that Hx data of defoliated seedlings showed an unexpectedly high dispersion, leading to a lack of significant differences with the other treatments, it cannot be ruled out that rye seedlings also could respond to an initial defoliation by increasing Hx exudation.

The decrease in the shoot of Hx relative content observed in condefoliated seedlings could be a consequence of the decrease in relative shoot biomass in that treatment, as is evident from the root–shoot ratio and the shoot data. This might be envisioned as a match between biomass allocation and the defensive endowment of a plant part. However, data of root biomass and relative content of Hx challenge the generality of such an assertion. While the root biomass did not differ between treatments, roots of condefoliated seedlings had a higher relative content of Hx.

The higher allocation of Hx to roots and root exudates in condefoliated seedlings may be attributed to the role of below-ground parts in resource acquisition for regeneration of lost tissue. Interestingly, a role in iron uptake of root-exuded Hx has been reported (Pethó, 1993). Moreover, the increase in Hx exudation by condefoliated seedlings apparently occurred at the expense of Hx allocation to the shoot. This quantitative loss of defense in shoots may be compensated for by the production of qualitatively enhanced defenses through the transformation of DIBOA-Glc into its aglucone. Aglucones have been reported to have enhanced toxicity to herbivorous insects with respect to Hx glucosides (Corcuera et al., 1985). There is, indeed, a higher aglucone–glucoside ratio in the shoots of condefoliated seedlings, in which Hx concentration decreased, but not in the roots, where Hx concentration remained constant.

The present work emphasizes that induced plant responses to defoliation are not restricted to shoots. Condefoliation brought an increase in the allocation of Hx to roots and root exudates, probably due to an increase in their relative importance for plant fitness due to the initial loss of aerial biomass and the subsequent subcompensatory growth. Moreover, the increase in the exudation of Hx, compounds implicated in allelopathy, could lead to an advantage in the acquisition of resources for the regeneration of lost biomass if it negatively affects neighboring plants of other species. Autotoxicity by Hx does not occur (Niemeyer and Pérez, 1995). In addition, defoliation improved the quality of the chemical defense through the transformation of Hx-glucosides into more toxic aglucones. This response is somewhat analogous to cyanogenesis induced by herbivory in plants containing cyanogenic glycosides (Dirzo and Harper, 1982; Seigler, 1991).

Provided that mature wild Poaceae show responses to defoliation similar to those of rye seedlings, and given that Hx inhibits germination and seedling growth of several species (Wolf et al., 1985; Barnes and Putnam, 1987; Pérez, 1990; Chase et al., 1991), it is tempting to speculate that the increase in root exudation of Hx after natural defoliation [e.g., browsing by goats (Klansek et al., 1995) or hares (Wolfe et al., 1996)] could affect the distribution of plant species in grazed grassland communities (see Belsky, 1992). Further evaluation in natural systems of the actual benefits of these induced responses is needed to clarify the role of both natural (mammal grazing) and artificial (harvesting practices) defoliation on the ecology of rye plants. Hypothetical benefits should be evaluated mainly in terms of resistance to herbivory and plant–plant interactions.

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