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Changes in growth parameters and content of N-storage compounds in roots and rhizomes of *Calamagrostis epigejos* after repeated defoliation

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Young plants of rhizomatous grass *Calamagrostis epigejos* (L.) Roth, cultivated in controlled conditions at two levels of nitrogen availability (2 mM and 0.4 mM NH_4NO_3) were repeatedly defoliated in 7 days intervals. Most of the newly formed or stored organic compounds were used for shoot regeneration and rhizome expansion, while growth of roots was completely stopped. Morphogenetic processes, namely leaf and rhizome initiation, were affected by defoliation much less than biomass allocation. The defoliated plants had lower content of amino acids and more abundant soluble proteins in roots and rhizomes than intact plants. The response of *C. epigejos* plants to defoliation was similar at both levels of nitrogen availability. Our results indicate, that the rate of regrowth of experimental plants was hardly limited by depletion of internal nitrogen reserves, but some regulative effect of qualitative changes in stored nitrogen compounds on biomass allocation and morphogenetic processes cannot be excluded.

Key words: defoliation, roots, rhizomes, nitrogen storage, nitrogen availability.

Introduction

Calamagrostis epigejos is a perennial rhizomatous grass widely distributed over the whole central and northern Europe (for review see: Rebele & Lehman, 2001). It grows successfully in a wide variety of habitats but it is particularly abundant in thinned lowland forests and nutrient rich forest clearings. Rapid spreading of this grass has been observed recently in many other disturbed sites, and even in some infrequently mowed meadows.

The causes of successful spreading of *C. epigejos* are not sufficiently known. It is explained that this grass posses several traits of an invasive species (e.g., easy dissemination, vegetative spreading by long rhizomes). It is also well known that its ecological amplitude is remarkably broad, from water-logged littoral of fishponds to dry steppic grasslands, from deep shaded understory of coniferous forests to fully insolated clearings, and from oligotrophic sandy soils to eutrophic humus-rich habitats. The physiological characteristics and mechanisms underlying the observed adaptability and competitive ability are less known. Fast acclimation of *C. epigejos* to changes in radiation environment was analyzed in our previous paper (Gloser & Gloser, 1996), as well as some interac-

tions with mineral nutrition (GLOSER et al., 1996).

In most cases, *C. epigejos* is regarded as a serious weed, because its tall and dense stands in colonized sites, after outcompeting of lower or more palatable grassland species, persist for many years. In forest clearings, accumulated thick layer of slowly decomposing standing dead and litter of *C. epigejos* prevent spontaneous forest regeneration, and also the artificially planted tree seedling may be endangered.

Regular mowing is a frequently used management measure to prevent unwanted spreading of *C. epigejos* in dry grasslands, and also to suppress its dominance in forest clearings. An efficient application of mowing should be based on detail knowledge of physiological responses of this species to defoliation. From experiments done with some fodder grasses we know, that regeneration of removed leaves is a rather complex process, much dependent on translocation of mobile carbon and nitrogen reserves from undisturbed below-ground organs, as well as on some other internal and external factors (OURRY et al., 1988; THORNTON et al., 1994; LOUHLIA et al., 1999). However, for majority of wild grasses the necessary data are still lacking.

The aim of this work was to determine basic growth response of young plants of *C. epigejos* to repeated defoliation. We were also interested, to what extent may be the regrowth ability modified by different nitrogen availability in substrate and by the associated changes in content of nitrogen storage compounds in roots and rhizomes.

Material and methods

Plant cultivation

Plants of *Calamagrostis epigejos* (L.) ROTH, were grown from seeds collected in a forest clearing near Brno, Czech Republic. Seeds were sown in pots with washed quartz sand and seedlings were transferred into well stirred and aerated hydroponic culture five weeks after germination. The modified HOAGLAND solution contained nitrogen in the form of NH_4NO_3 and plants were divided into two groups according to nitrogen concentration in the solution. One group received complete nutrient solution with nitrogen concentration 2 mmol L^{-1} (HN-treatment) whereas the N concentration in solution was set at 0.4 mmol L^{-1} in the second group (LN-treatment). Nutrient solution in plastic containers was renewed every 4 to 5 days and its pH was adjusted daily to the value of 5.5 with H_2SO_4 or NaOH. The experiment was conducted in growth chamber with a 16 h photoperiod, irradiance of 340 $\pm$ 15 μmol m^{-2} s^{-1} PAR, and temperature of 20 °C/15 °C (day/night). Plants were rotated within the chamber weekly. Eight weeks after sowing, one group of plants in each N treatment was defoliated at 5 cm stubble height and the other group was left intact. Defoliated plants were then regrowing for 7 days before next defoliation. Altogether three defoliations followed by 7-day regrowth period took place during the experiment.

Growth analysis

Six plants from each treatment were repeatedly used for non-destructive determination of fresh mass, leaf area, number of leaves and rhizomes before each defoliation event and at the end of the last regrowth period. In case of defoliated plants, the amount of the removed biomass was also recorded. Destructive harvest of all plants was done at the end of the last regrowth period. Plants were separated into roots, rhizomes, and shoots (composed mainly of leaf sheaths and leaf blades). Samples were frozen in liquid nitrogen, freeze-dried for 48 h, ground and stored at −20 °C till chemical analyses. Leaf area of the whole plant was calculated as the sum of areas of leaf blades on digitalized images.

Chemical analyses

The extract of 40 mg of plant material in 2 × 1 mL of 0.05 M phosphate buffer (pH 7.5) was used for all chemical analyses of nitrogen storage compounds. Nitrate was determined after reduction to nitrite (CATALDO et al., 1975) and the content of soluble proteins by the staining with the Coomassie Brilliant Blue (BRADFORD, 1976) using bovine serum albumin as a standard. Content of total free amino acids was estimated with ninhydrin (ROSEN, 1957) using leucine as a standard. The proteins were removed from extract prior the analysis of amino acids by precipitation with 5% (final concentration) sulfosalicylic acid followed by centrifugation (14 000 × g for 20 min) because of the possible interference with assay.

Statistics

The significance of the effects of experimental factors was calculated by the multifactorial analysis of variance in STATISTICA v. 6 (StatSoft Inc., Tulsa, USA). The multiple comparison of means was based on the method of LSD contrasts. The homogeneity of variances was checked by BARTLET's and COCHRAN's tests, and heterogeneous sets of data were log-transformed before calculation.

Results

Removing of all green aboveground parts (leaf blades and sheaths) from experimental plants, denoted here as defoliation, seriously inhibited the rate of production of new biomass (Fig. 1). It should be mentioned, that even during rather short intervals between the two consecutive defoliations, the plants were able to produce some new

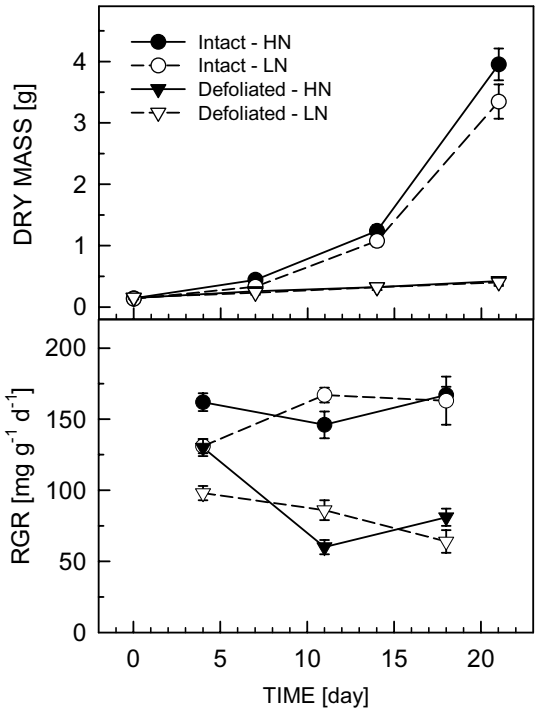

Fig. 1. Time course of changes of whole-plant dry mass and relative growth rate (RGR, mean values for the pertinent interval) during cultivation. Plants were grown in nutrient solution with either high or low nitrogen availability (HN and LN, respectively), and were defoliated three times during cultivation or left intact. Cumulative biomass production (including biomass of removed leaves in case of defoliated plants) is shown. Means ± standard error (if greater than symbol).

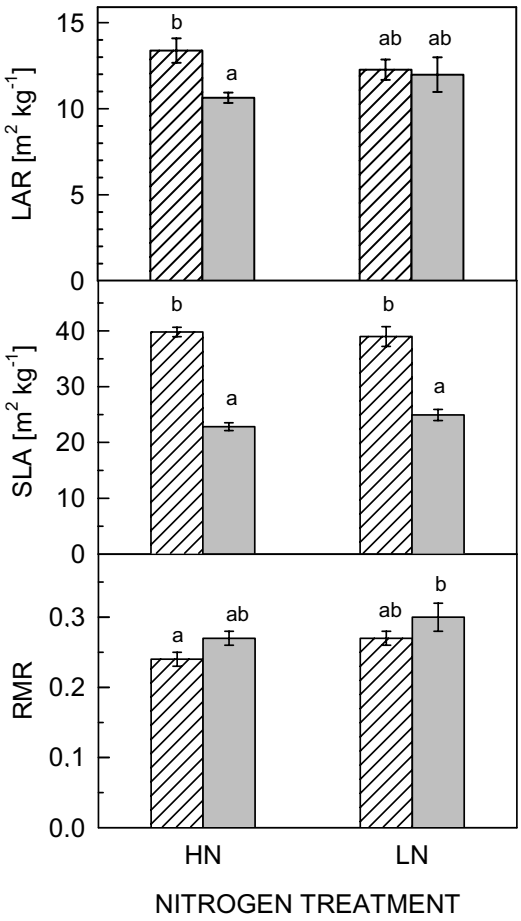

Fig. 2. Leaf area ratio (LAR), specific leaf area (SLA) and root mass ratio (RMR) of plants at the end of cultivation. Plants were grown in nutrient solution with either high or low nitrogen availability (HN and LN, respectively), and were defoliated three times during cultivation (dashed bars) or left intact (filled bars). Dissimilar letters denote significant difference ($p < 0.05$).

leaves which, eventually, contributed to the positive carbon balance and to some increments in cumulative values of newly produced biomass. Nevertheless, the mean values of RGR of defoliated plants were much lower than in the case of intact plants and remarkably stable after any of the three defoliations. Only very small ontogenetic drift in values of RGR was observed in control (intact) plants during the most important time period of the experiment. Nitrogen availability in nutrient solution had no significant effect on biomass accumulation and values of RGR in both defoliated and intact plants during the advanced phase of cultivation.

Some other growth-analytical parameters are presented in Figure 2. Defoliated plants had substantailly elevated values of SLA at both nitrogen treatments, because the newly regrowing leaves were always thinner than the leaves of intact plants. The mean values of SLA were derived from the leaves of all age categories actually present on the plants. No significant differences in LAR were found as a consequence of defoliation or nitrogen supply treatments. In case of RMR, only some small but significant increment was detected in intact plants cultivated at low nitrogen supply.

It is obvious from data presented in Table 1, that repeated defoliation inhibited relatively more biomass allocation to roots than to rhizomes, and even less affected was the number of rhizomes. The negative effect of defoliation was more serious on leaf number than on rhizomes, but not directly

Table 1. Dry mass (g) of whole plants, roots and rhizomes, leaf area (cm^2) and number of rhizomes and leaves per plant at the end of cultivation. Plants were grown in nutrient solution with either high or low nitrogen availability (HN and LN, respectively), and were defoliated three times during cultivation or left intact. Dissimilar letters denote significant difference ($p < 0.05$). The values are means ± standard error ($n = 6$).

		Dry mass			Number		
		Plant	Root	Rhizomes	Rhizomes	Leaves	Leaf area
Defoliated	HN	0.23 ± 0.01 a	0.05 ± 0.01 a	0.02 ± 0.01 a	5.0 ± 0.7 a	18.3 ± 1.6 a	30.97 ± 2.50 a
	LN	0.22 ± 0.02 a	0.06 ± 0.01 a	0.02 ± 0.01 a	4.0 ± 0.5 a	17.2 ± 2.5 a	27.47 ± 3.60 a
Intact	HN	3.95 ± 0.28 b	1.05 ± 0.09 b	0.16 ± 0.01 b	10.0 ± 1.5 b	65.8 ± 4.4 b	417.16 ± 21.30 b
	LN	3.35 ± 0.31 b	1.01 ± 0.10 b	0.14 ± 0.01 b	8.1 ± 1.2 b	54.0 ± 6.8 b	403.46 ± 51.90 b

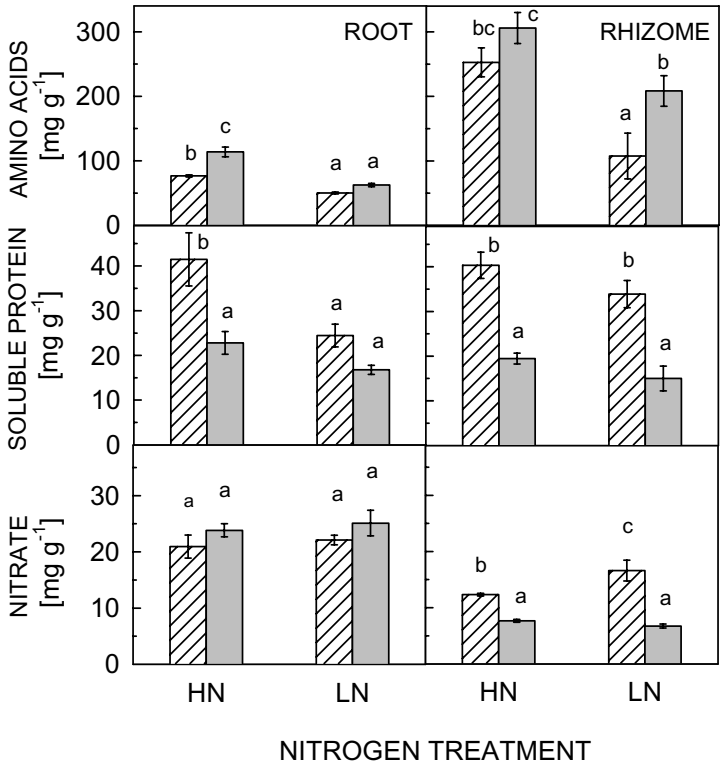

Fig. 3. The content of free amino acids, soluble protein and nitrate ions in roots and rhizomes of experimental plants at the end of cultivation. Plants were grown in nutrient solution with either high or low nitrogen availability (HN and LN, respectively), and were defoliated three times during cultivation (dashed bars) or left intact (filled bars). Dissimilar letters denote significant difference ($p < 0.05$).

related to the extremely large decrease of total leaf area. This indicates substantial differences in an average leaf size (in area units) of plants of the two treatments.

The changes in content of nitrogen storage compounds in roots and rhizomes after repeated defoliation are presented in Figure 3. The defoliated plants from both nitrogen treatments had lower content of amino acids in their roots and rhizomes, and surprisingly higher content of soluble proteins than the intact plants. Remarkable differences between roots and rhizomes were observed in case of amino acids, which were more abundant in rhizomes, while nitrate content was permanently higher in roots than in rhizomes.

Discussion

The effects of defoliation on growth and some other physiological processes have already been studied in numerous experiments with agriculturally important plants. The rate of regrowth, determining the overall productivity of grazed pastures or mowed meadows is of key importance for

any farmer. Wild and agriculturally unimportant grasses, however, have rarely been used for ecophysiological study of regrowth dynamics.

The use of internal reserves of carbon and nitrogen for supporting the newly growing organs, namely leaves is one of the most important factors affecting the regrowth of plants after defoliation (Ourry et al., 1990). Although carbon reserves are also utilized during regrowth, the supply of nitrogen may be more limiting for the regrowing plants (see Volenec et al., 1996 for review). The nitrogen incorporated in the emerging leaf area during several days after defoliation was predominantly derived from the remaining organs of *Lolium perenne* (Thornton & Millard, 1993; Louahlia et al., 2000). Moreover, a detailed studies of carbon and nitrogen use in leaf growth zone of *L. perenne* demonstrated that the predominant dependency of growing tissue on N from reserves after defoliation lasts for 3 to 6 days, whereas in case of carbon reserves is this period only 1 to 3 days (De Visser et al., 1997; Schnyder & De Visser, 1999).

Our previous experiments revealed, that the basic pattern and time-course of physiological and biochemical changes in *C. epigejos* after single defoliation was very similar to that found in some fodder grasses, e.g., *L. perenne*. In addition to strong inhibition of root growth, the uptake of nitrate ions was practically stopped for the first five days after defoliation and the plants had to use nitrogen only from their internal N-storage compounds. After this critical period, the nitrogen uptake, assimilation and net biomass production were restored. Taking into account the regrowth rate it was possible to estimate, that the internal reserve nitrogen pool was able to support growth processes of *C. epigejos* for about 11 days (Kavanová & Gloser, 2005).

In a subsequent experiment, the results of which are described in this paper, we tried to report on more critical depletion of N-storage substances by repeated defoliation. Our results indicate, that the defoliated plants were able to maintain permanently high amount of nitrates in roots, and also the amino acids pool was not much depleted even after the third defoliation. Unfortunately, we have not enough data on changes in the uptake rate of mineral nitrogen and its control by the physiological state of defoliated plants. However, it is obvious, that the relatively small depletion of nitrogen reserves could be influenced by not fully restricted nitrogen uptake, but also by very slow growth rate of repeatedly defoliated plants and, therefore, by slow incorporation of nitrogen stored in roots and rhizomes into regrowing shoots. Thus, it seems unlikely that the recovery of *C. epigejos* plants from severe defoliation in the present experiment was affected by the nitrogen deficiency. This conclusion is also supported by the fact, that variation in nitrogen availability in substrate had only negligible effect on the plant response after defoliation.

Our present results are in contrast with the previous experiments with *C. epigejos* where both positive plant growth response to N level supplied (Gloser & Gloser, 2000; Gloser V., unpubl.) and significant depletion of N storage compounds in plant organs after single defoliation (Kavanová & Gloser, 2005) were found. We suggest that the differences in cultivation media and/or available N form among experiments could, at least partly, explain these contradictory results. Plants in previous experiments were grown either in solid substrate or only in presence of nitrate as a nitrogen source whereas plants in the present experiment grew in hydroponics with ammonium and nitrate available in equimolar ratio. Uptake rate is usually considerably higher for ammonium ions than that for nitrate in grasses (Lycklama 1963; Koppisch et al., 1993; Bailey, 1998). Although ammonium uptake is, similarly to nitrate, down regulated after defoliation (Bailey, 1998) we can assume, that plants with combined NO_3^- + NH_4^+ supply have better potential to supply nitrogen to regrowing shoot from current uptake than plants under the same nitrogen availability but only in form of nitrate.

Moreover, N uptake rates in hydroponics with solution circulation are usually independent of mobility of particular N form and the effect of mass flow on N uptake is also minimized. Therefore, we could assume that total N uptake of plants in our experiment was significantly higher and, hence, depletion of internal N stores smaller than in the previous defoliation experiments with sole nitrate nutrition (Kavanová & Gloser, 2005).

The beneficial effects of combined nitrate and ammonium nutrition for growth of grasses have also been previously recognized. Higher biomass production was observed in plants supplied by both nitrate and ammonium in comparison with a supply of each form separately (Griffith & Streeter 1994; Gloser et al. 2002). This effect along with possibly smaller reduction of N uptake rates under smaller N concentration in nutrient solution may have resulted in a small plant response to different N treatments in the present experiment.

Our results indicate, that the regrowth rate

of the tested *C. epigejos* plants was hardly limited by depletion of internal nitrogen reserves, but some regulative effect of qualitative changes in the stored nitrogen compounds, particularly changes in types of dominant amino acids, on biomass allocation and on morphogenetic processes cannot be excluded. This problem will be subject of our next work.

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