

**SEASONAL CHANGES IN ALLOCATION OF NITROGEN-RICH
COMPOUNDS WITHIN A RHIZOMATOUS GRASS
*CALAMAGROSTIS EPIGEJOS***

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Summary

There is an evidence that use of internal nitrogen stores is important for survival and competitive ability of perennial plants. The aim of this work was to show changes in concentrations of nitrate, free amino acids and soluble protein in organs of perennial grass *C. epigejos* during re-growth after overwintering. Nitrogen storage in *C. epigejos* seems to occur preferentially in rhizomes, predominantly in form of amino acids. The contribution of storage proteins to whole-plant N storage should not be ignored but its relative importance for plant N budget must be carefully re-evaluated.

Keywords: nitrogen storage, N mobilization, nitrate, free amino acids, vegetative storage proteins

Introduction

The persistence and spreading of perennial grass *Calamagrostis epigejos* L. Roth. have been attributed to several factors. Its remarkable ability to survive extremely low light conditions and its fast growth response to improved light conditions was investigated previously (Gloser and Glaser 1996). However, the processes underlying the competitive ability of *C. epigejos* are still not completely understood. One of its potentially important traits could be the accumulation of storage compounds and their effective use for fast re-growth in early spring. A role of storage compounds in plant survival and re-growth after cutting was extensively studied with forage species (see Volenec et al. 1996 for

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review), but the information on wild species is very limited. The role of carbohydrates, which are usually the prevailing storage compounds in plants, was originally considered as predominant for plant re-growth. However, several later experiments showed only weak or no relationship between the amount of storage carbohydrates and the rate of re-growth (Volenec et al. 1996 and references therein). On the other hand, there was found close positive correlation between N concentration in remaining organs and the re-growth rate (Ourry et al. 1994, Volenec et al. 1996). The ability of plants to take up and assimilate inorganic nitrogen is lowered or stopped both in early spring and after defoliation (Clement et al. 1978, Ourry et al. 1990, Thomton and Millard 1993) and the plant demand for nitrogen is met by the N uptake from soil after the restoring of positive carbon balance of the whole plant (Clement et al. 1978). Thus, the availability and mobilization of nitrogen reserves must be considered as an important factor in survival and competitive ability of perennial plants.

Nitrate

Nitrogen is primarily stored in the form of storage proteins, amino acids and nitrate (Millard 1988, Chapin et al. 1990). All types of N storage compounds often vary seasonally in their concentrations. The seasonal variation of nitrate content in roots of two perennials *Taraxacum officinale* and *Cichorium intybus* showed a distinct maximum in late autumn and then declined to minimal concentrations in the beginning of new vegetation season (Cyr et al. 1990). Different pattern was found in below-ground organs of *Urtica dioica* where the concentration of nitrate increased in the autumn and remained stable until early spring (Rosnitschek-Schimmel 1985).

Nitrate accumulation can have several physiological consequences. The high concentration of nitrate in some tissues make a significant contribution to the osmotic potential, which could be used in driving cell expansion (Sprent and Thomas 1984). Osmotic function of nitrate is probably also employed in re-

growing leaves shortly after defoliation, when majority of osmotically active soluble carbohydrates are consumed by re-growing tissue. Despite of ability of many plants to store nitrate in large quantities in vacuoles, long term storage of N in form of nitrate usually does not occur. High energy costs for NO_3^- reduction make the nitrate unfavourable source of nitrogen for re-growing plants which are dependent on limited reserves of carbohydrates. Thus, it seems that nitrate plays only a transient role in N storage, oscillating in response to supply and demand (Heilmeyer and Monson 1994).

Amino acids

Many plants accumulate large quantities of amino acids and amides in their vegetative tissues (Millard 1988). The content of these compounds in perenniating organs usually rise in late autumn and remains high until late spring (Volenec et al. 1996). The marked increase in amino acid content in autumn is closely connected with remobilization of N from senescing plant parts (Millard, 1988) but may also reflect the temporary higher availability of N in soil.

The composition of free amino acid pool varies considerably during the growing season. High levels of glutamine, asparagine, arginine and alanine usually accumulate in below-ground organs before the winter dormancy (Rosnitschek-Schimmel, 1985; Nordin and Näsholm, 1997). These compounds possess low C to N ratio and thus minimizes the requirement of carbon skeletons for N storage. Nitrogen stored in form of free amino acids may contribute up to 20 % to present total N in overwintering organs (Nordin and Näsholm, 1997). High amplitude in amino acid content during vegetation compare to proteins and close correlation between the depletion rate of amino acids and plant regrowth rate lead some authors to conclusion that amino acids are the main N storage compounds (Sagisaka, 1987; Rosnitschek-Schimmel, 1985; Lähdesmäki et al., 1990). In comparison to storage proteins, amino acids are readily accessible for growth processes (no enzymatic decomposition is necessary) and their good

solubility facilitates rapid mobilization of N in spring as well as after defoliation.

Proteins

The role of proteins in N storage was underestimated for a long time. The identification and functions of vegetative storage proteins (VSPs) were recently summarised in two reviews focused on woody plants (Stepien et al., 1994) and herbaceous species (Staswick, 1994). Cyr and Bewley (1990a), provided the first evidence of utilization of VSPs in herbaceous plants and defined the following criteria for recognising of VSPs : (1) proteins that display preferential synthesis during development of storage organs, (2) proteins that are depleted from storage organs during reactivation of meristems and (3) proteins whose abundance greatly exceeds that of other proteins in perenniating organs. Since that, VSPs were identified in several dicot species (Table 1) but to our knowledge, the identification of VSPs in roots or rhizomes of grasses has not been yet reported.

Table 1 List of herbaceous species in which were recently identified specialized vegetative storage proteins (VSPs)

Species	Tissue	Size of VSPs	Source of data
<i>Taraxacum officinale</i>	Root	18 kDa	Cyr & Bewly (1990a)
<i>Cichorium intybus</i>	Root	18 kDa	Cyr & Bewly (1990a)
<i>Euphorbia esula</i>	Root	26 kDa	Cyr & Bewly (1990b)
<i>Medicago sativa</i>	Root	32, 19, 15 kDa	Hendershot & Volenec (1992)
<i>Glycine max</i>	Leaf	25 kDa	Staswick (1994)
<i>Trifolium repens</i>	Root	17.3 kDa	Bouchart et al. (1998)

Storage proteins may serve as an example of difficulties in classifying storage compounds. The same protein may have in some cases both the storage and metabolic role, especially as an enzyme (Chapin et al., 1990). Moreover, vegetative storage proteins are usually not located in specific storage

compartments. The most abundant protein in leaves of C₃ plants ribulose-1,5-bisphosphate carboxylase-oxygenase (*Rubisco*) may be decomposed by 30 to 50% without any effect on the rate of photosynthesis. The decreased amount of *Rubisco* was compensated for by its increased activity (Quick et al., 1991).

Storage of nitrogen in form of catalytically active proteins requires high energy costs in comparison to other types of N stores but it has several advantages. Firstly, with a help of increased level enzymes contributing to its carbon gain plant could recover effectively at least part of the energy costs for the protein synthesis. Secondly, storage of N as a protein avoids the potential osmotic embarrassment of nitrate reutilization. Thirdly, if plant was storing N in proteins, nitrate reduction and protein synthesis would occur in the part of vegetation season when demands of these highly energy consuming processes can be met easily by photosynthesis. In contrast, mobilization of N predominantly occurs when energy resources of plant are limited. Decomposition of storage proteins needs then less energy than reduction of nitrate. (Millard, 1988).

The aim of this contribution is to show changes in concentrations of N compounds in organs of *C. epigejos* during regrowth after overwintering. Identification of the most important type of N storage compound as well as localization of main N-reserves within the plant will be done in our next work.

Material and methods

Samples of *C. epigejos* were collected monthly in a forest clearing west of Brno approximately 5 h after the sunrise. In each of the 7 experimental plots 10 to 20 plants were collected to get sufficient amount of biomass for chemical analysis. Immediately after the sampling the plants were divided in roots, rhizomes and the rest of above-ground parts. Samples were then frozen in -80°C, lyophilized for 48 h and ground to 20-mesh. The extract of plant material

in 0.05 M phosphate buffer (pH 7.5) was used for all chemical analysis. Nitrate was assayed by colorimetric method of Cataldo et al. (1975) and the content of soluble proteins was determined by the Bradford's (1976) method using bovine serum albumin as a standard. Content of total free amino acids was estimated with ninhydrin (Rosen, 1957) with leucine as a standard. The protein was removed from extract prior the analysis of amino acids by precipitation with 5% (final concentration) sulfosalicylic acid and followed by centrifugation (14 000 x g for 20 minutes) because of the possible interference with assay.

Results and discussion

The content of nitrate (Figure 1A) was the highest in early spring in rhizomes (about 5 mg g⁻¹ DW) and in above-ground parts (4 mg g⁻¹ DW). The decrease in nitrate content by 20 to 50% from March to July is consistent with data observed with *Urtica dioica* (Rosnitschek-Schimmel, 1985). Roots of *C. epigejos* contained considerably less nitrate (2 mg g⁻¹ DW) and its variation during sampling period was minor. Changes in nitrate content in roots of herbaceous plants in spring were indeed much smaller than in other types of N compounds. Therefore, it seems that the reserves of nitrate have only minor importance for spring regrowth of *C. epigejos*, which is in agreement with the results of Rosnitschek-Schimmel (1985) and Cyr et al. (1990).

The content of free amino acids in rhizomes declined dramatically in April by about 40% (Figure 1B). Subsequent decrease was slower but steady and lead to final amino acid content 10 mg g⁻¹ (DW) which represents approximately 15 % of the content recorded in March. Similar changes were also found in above-ground plant parts. The content of amino acids in roots was three times lower than that in rhizomes and its decline from March to June was slower. These results are in line with those previously obtained for other herbaceous plants (Rosnitschek-Schimmel, 1985; Cyr et al., 1990) and indicate that the major

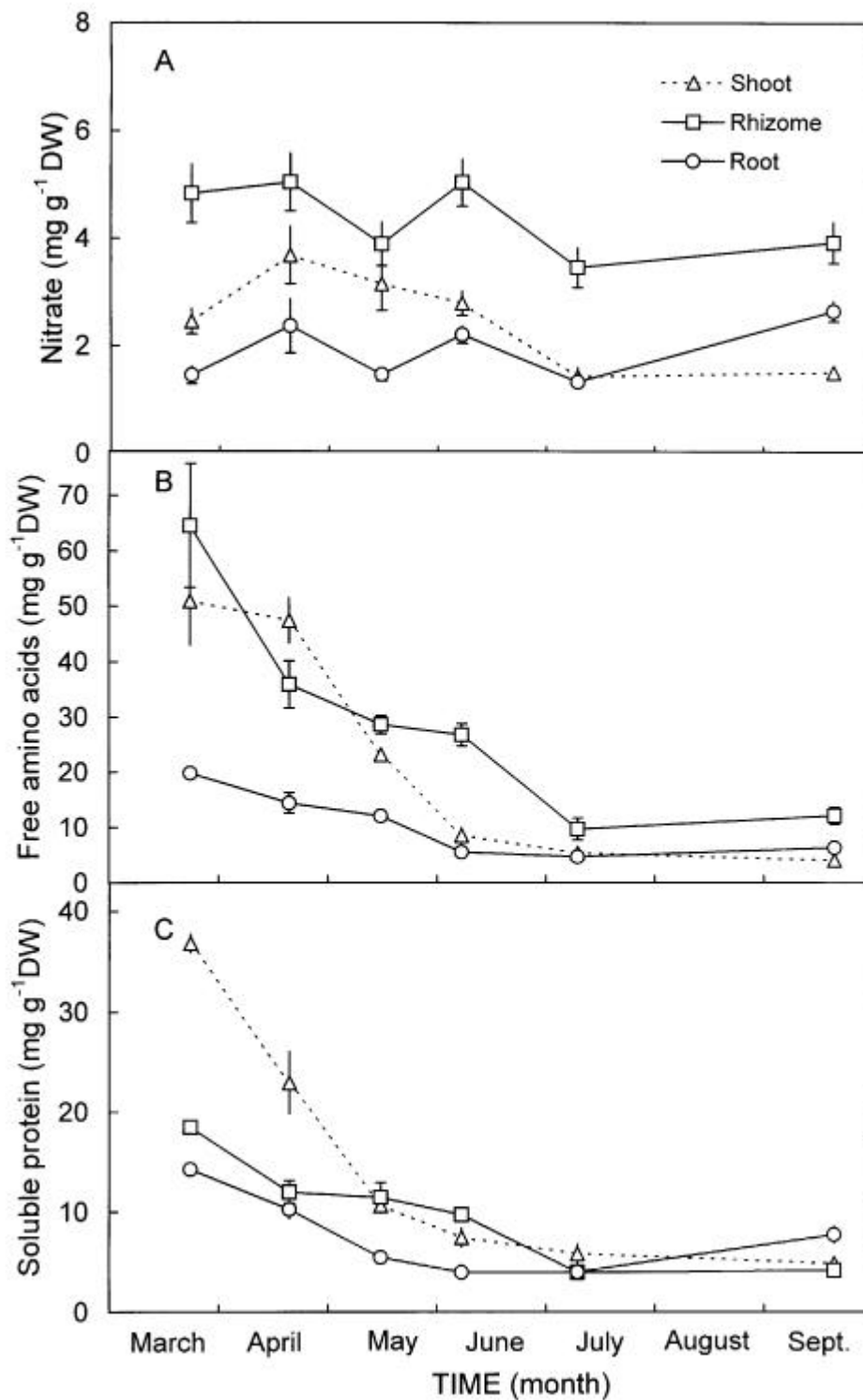


Figure 1 Monthly variation of (A) nitrate, (B) free amino acids and (C) soluble protein in various parts of *Calamagrostis epigejos* plants. Values represent means \pm SE of 7 replicates.

portion of nitrogen mobilized from internal sources for spring regrowth of *C. epigejos* was in form of amino acids. It suggests that amino acids are in this species the most important compound for nitrogen storage similarly to other herbaceous plants (Sagisaka, 1987; Rosnitschek-Schimmel, 1985; Lähdesmäki et al., 1990).

The content of soluble proteins (Figure 1C) was about three times smaller than in case of amino acids. The only exception was high protein content in young above-ground parts. Steep decline of protein content in above-ground organs during April and May was probably connected with rapid accumulation of structural biomass in these parts. The soluble protein content in below-ground organs also declined over the whole sampling period to 25 % of the content observed in March. The contribution of soluble protein to total amount of mobilized N was smaller than that of amino acids. However, the amplitude of relative changes in soluble protein pool during the sampling period and their seasonal pattern were comparable with those in amino acid pool. Thus, the involvement of soluble proteins in N storage in *C. epigejos* should not be omitted. Similar results obtained Cyr et al. (1990) with *Taraxaccum officinale* and *Cichorium intybus* plants. Moreover, the highest content of soluble protein in both species was found in winter month and then declined prior to resumption of growth. This suggests that relative importance of soluble proteins for N storage in *C. epigejos* may be greater than that indicated by presented data which do not include the whole seasonal pattern.

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