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# Effects of rhizome age and nutrient availability on carbohydrate reserves in *Rumex alpinus* rhizomes

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The allocation of carbon to non-structural carbohydrates was studied at two levels of nutrient availability in *Rumex alpinus*, a clonal plant with large carbohydrate reserves in its rhizomes. The studied plant was cultivated in sand and loam. Two other treatments included an interface between sand and loam: the rhizome segment initiated in the current year was planted in sand and the older rhizome segments in loam, and *vice versa*.
The plants growing on sand and the plants grown from the current year's rhizome placed in sand produced a smaller leaf area, less above-ground biomass and relatively smaller rhizome segments than plants from the other treatments. The concentration of total non-structural carbohydrates (TNC) in rhizome segments of the same age showed no differences between the treatments. However, significant differences in TNC concentration between rhizome segments of different ages were found. The rhizome segment produced in the last growing season showed the lowest concentration of TNC in dry mass (42.5%). In the next segment the highest value (54.4%) was found. Older segments showed TNC concentrations ranging between 53.3 and 48.6% and a decreasing tendency with segment age. These results are discussed in relation to plant regeneration in the field.

Key words: alpine dock, rhizome, total non-structural carbohydrates, nutrient level.

## Introduction

Storage carbohydrates in plants are essential to support the non-overlapping processes of assimilation, seasonal regrowth and recovery after disturbance (Fonda, Bliss 1966; Čížková-Končalová et al., 1992; Landa et al. 1992). Non-catastrophic carbohydrate demands are usually low (Shaver, Billings, 1976) but surplus of carbohydrates make plants more resistant to unpredictable catastrophic events (Baas, 1989; Chapin et al. 1990).

The accumulation of carbon in storage organs is affected by nutrient availability. Plants under nutrient shortage accumulate carbohydrates due to a surplus of photosynthates that cannot be used for growth. If nutrients are added, photosynthates are now utilised in growth, and correspondingly the level of carbohydrate reserves falls (Loomis, 1932; Baas, 1989; Čížková-Končalová, Bauer 1993; Ulrich 1955; Loomis, Wolker, 1963).

Carbohydrate concentrations in storage of plants growing in alpine habitats is often very high: 40% TNC was found in the dry mass of *Oxyria digyna* rhizomes (Scott, Russell, 1940), up to 64% in rhizomes of *Polygonum bistortoides*, up to 39% in roots of *Saxifraga rhomboidea*, 34% in roots of a *Calyptridium umbellatum* plant (MOONEY, BILLINGS, 1965) and 68% in rhizomes of *R. alpinus* (KLIMEŠ et al., 1993). The plants colonizing habitats above tree line may benefit from extensive storage as fast spring growth and regeneration based on storage carbohydrates are often crucial for their survival. Slow growth in severe environments may be enhanced if large storage is available.

We try to test a hypothesis that under nutrient shortage growth of aboveground organs is reduced while concentration of total nonstructural carbohydrates is enhanced. For our study we chose *Rumex alpinus* – an alpine plant with very high storage and a rigid architecture of rhizomes. A rhizome consists of annual increments. Their age can be easily estimated (KLIMEŠ, 1992). Young roots which are major absorbents of nutrients (ESAU, 1965) are concentrated on the youngest segment of rhizomes. Therefore, we expected that placing old and young parts of a rhizome in nutrient-poor substrate will have different effects on growth of the main shoot. It may play an important role in heterogeneous environments (STUEFER et al., 1994; WIJESINGHNE, HANDEL 1994). To evaluate the differences in the role of young and old rhizome segments, in experiment following treatments were established: (1) whole plant growing in loam (nutrient rich environment); (2) whole plant growing in sand (nutrient poor environment); (3) the rhizome segment initiated in the current year was planted in sand and the rest of the rhizome in loam and (4) *vice versa*.

The concentration of storage carbohydrates tends to decrease with age along the rhizomes (SHAVER, BILLINGS, 1976; FIALA, 1976; MASUZAWA, HOGETSU, 1977). In contrast to the plants in which the relationship between age and carbohydrate reserves has been studied *R. alpinus* has epigeotropic monopodial rhizome (sensu SEREBRYAKOVA, SEREBRYAKOV, 1965). It means that newly growing rhizome tip, which is often an important sink of carbohydrates, is missing in this species. Basipetal translocation of carbohydrates and a simple relationship between TNC concentrations and age, i.e. distance from the apical shoot, can be expected. As annual increments can easily be separated in *R. alpinus*, the carbohydrate concentrations of individual segments were recorded.

The following questions were formulated:
1) Is the carbohydrate concentration of a rhizome segment related to its age?
2) What is the effect of nutrient shortage on carbohydrate concentration?
3) Is storage accumulation and main shoot growth supported or suppressed if only the proximal part of a rhizome is situated in a nutrient rich environment?

## Material and methods

*R. alpinus* L. is a perennial herb with a monopodial epigeotropic rhizome. Rhizome is formed by a shortened base of a shoot which is pulled into soil by contraction of roots down to about 5 cm of soil depth. Apical bud is vegetative and indefinitely growing (KLIMEŠ, 1992). The rhizome is clearly segmented, each segment being the product of a single growing season. Length of rhizome segments is about 4 cm, minimal width about 2 cm, and maximal width about 3 cm. They persist for up to 20 years. After that the segments at the rhizome base decay (KLIMEŠ, 1992). The main storage compound accumulated in *R. alpinus* rhizome is starch. The concentration of total non-structural carbohydrates (TNC) in the dry mass of segments produced in the current year may be as great as 68% at the end of the growing season (KLIMEŠ et al., 1993).

*R. alpinus* plants were collected in the Krkonoše Mts, on a crest between the villages of Špindlerův Mlýn and Pec pod Sněžkou (1150 m a.s.l.) at the beginning of June 1990, when first 3 to 4 small leaves of *R. alpinus* plants were developed. The sixteen selected rhizomes were 5 years old and lacked lateral branches. All roots and leaves were removed to minimize differences between individual rhizomes. The plants were planted in a container in Třeboň (433 m a.s.l.), South Bohemia. *R. alpinus* rhizomes were cultivated on (1) loam, (2) sand, (3) a mixture of these: the rhizome segment which was initiated just before transplantation, early in spring 1990, was planted in sand and the rest of the rhizome in loam, (4) the rhizome segment initiated early in spring 1990 was planted in loam and the rest of the rhizome in sand (Fig. 1). Each treatment was replicated four times.

At the stage of the highest above-ground biomass, on August 14, 1990, the following parameters were recorded for each plant: number of shoots, number of leaves and leaf blade length of individual leaves.

Maximum leaf area (LA) [$cm^2$] per plant and dry mass of the leaves (LW) [g] were estimated from leaf blade length (LL, cm) in August using the following relationships: $LA = 0.174 * LL^{2.436}$, $R^2 = 0.869$, d.f. = 196 (based on data from several months and localities in Czechia and Slovakia), and dry mass of the leaves $LW = 0.09 * LL^{1.941}$, $R^2 = 0.985$, d.f. = 36 (KLIMEŠ, 1992).

Eight months after the experiment was started, on March 13, 1991, before spring growth was initiated, the plants were excavated, their rhizomes cleaned of soil and the number of lateral shoots counted. The rhizomes were then washed, divided into segments and their width measured. After that the surface layer with the vascular system was removed. Individual segments were dried at 60°C until constant weight. Samples were boiled in 80% ethanol and TNC contents were estimated after hydrolysis of starch with 4% HCl on a boiling water bath for 3 hrs. The resulting free sugars were determined photometrically using the Nelson reagent, with glucose as standard (NELSON, 1944; FIALA, 1978).

The results were tested using standard statistical methods (ANCOVA, LSD test, angular transformation of percentage values before testing – ZAR 1974).

Table 1. Plant components and biomass of *Rumex alpinus*. All variables are calculated per plant ($n = 4$ for each treatment). The differences within a row that are not significant at $P = 0.05$ are labelled with the same letter (LSD test).

| | Loam | Sand | Loam-to-Sand | Sand-to-Loam |
|---|---|---|---|---|
| Max. segment width in 1990 [mm] (=SW91)[2] | $30.0^a$ | $27.0^a$ | $26.9^a$ | $27.9^a$ |
| Max. segment width in 1989 [mm] (=SW90)[2] | $31.75^a$ | $33.0^a$ | $31.0^a$ | $30.0^a$ |
| SW90/SW89[2] | $0.94^b$ | $0.82^a$ | $0.87^{ab}$ | 0.93b |
| Number of shoots[1] | $2.75^a$ | $3.5^a$ | $3.75^a$ | $4.25^a$ |
| Number of shoots[2] | $5.75^a$ | $6.4^a$ | $9.25^a$ | $7.25^a$ |
| Number of leaves[1] | $9.5^a$ | $7.8^a$ | $8.3^a$ | $11.5^a$ |
| Leaf area [$cm^2$][1] | $1940.5^b$ | $958.6^a$ | $638.4^a$ | $1759.3^b$ |
| Above-ground dry mass [g][1] | $230.8^b$ | $120.8^a$ | $87.3^a$ | $217.1^b$ |

1 – August 14, 1990; 2 – March 13, 1991

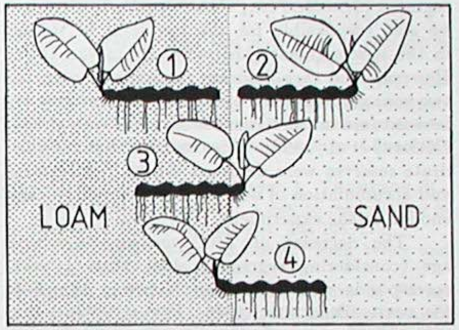


Fig. 1. Four treatments with *Rumex alpinus* included plants cultivated on (1) loam, (2) sand, and two mixed treatments: (3) the rhizome segment produced in the current year was planted in sand and the rest of the rhizome in loam, and (4) the rhizome segment produced in the current year was planted in loam and the rest of the rhizome in sand.

## Results

Plant growth The plants in the experiment regenerated within two weeks of planting. All rhizome apices remained alive. The plants growing wholly on loam and in the sand-to-loam treatment produced a larger leaf area and higher above-ground biomass than plants in the other treatments (Tab. 1). There was no difference between plants on loam and plants from the sand-to-loam treatment, or between plants on sand and plants from the loam-to-sand treatments. The number of lateral shoots per plant and the number of leaves per shoot did not differ between the treatments (Tab. 1). The number of lateral shoots recorded in March 1991 was however much higher than in August. This ranged between 5.8 and 9.3 per plant and was independent of substrate.

The segments produced during the 1990 season were smaller than the preceding ones. There was no effect of the treatments on the width of the segments produced in 1990, however, the reduction in segment size calculated as width of the rhizome segment produced in the last year/width of the preceding segment differed between the treatments; the plants growing on sand and from loam to sand showed a greater reduction in segment size than plants in the other treatments.

### Carbohydrate reserves

TNC concentration in *R. alpinus* rhizomes was independent of substrate but was affected by segment age ($F = 0.82$, $P > 0.05$ and $F = 16.14$, $P < 0.001$, respectively; the interaction between substrate and age was non-significant – $F = 0.574$, $P > 0.05$; the effect of a covariate – segment size – was non-significant: $F = 0.175$; ANCOVA). The highest TNC concentrations were found in the second segment (produced in 1989) (Tab. 2 and Fig. 2). The lowest value was found in the youngest rhizome segment formed in 1990. The carbohydrate concentration in all older segments was higher than in the youngest rhizome segment, and the concentration tended to decrease with segment age.

Table 2. Total non-structural carbohydrates [g • 100 g$^{-1}$] in *Rumex alpinus* rhizomes planted on different substrates. Rhizome segments were grouped into three age categories: 1. segment produced in 1990, 2. in 1989, 3. between 1985 and 1988. Note that in the treatments with rhizomes placed across the soil interface the segments of Age 1 were in a different environment than the rest of the rhizome. Standard deviations are in parentheses. No differences between means within rows were found (LSD test, $P = 0.05$).

| | Loam | Sand | Loam-to-Sand | Sand-to-Loam |
|---|---|---|---|---|
| Age1 | 39.63 | 40.03 | 45.75 | 44.40 |
| | (8.99) | (12.41) | (5.57) | (11.4) |
| Age2 | 53.18 | 56.38 | 51.90 | 56.18 |
| | (3.63) | (5.40) | (4.34) | (9.06) |
| Age3 | 48.90 | 52.09 | 53.20 | 51.24 |
| | (3.43) | (4.74) | (1.37) | (3.97) |

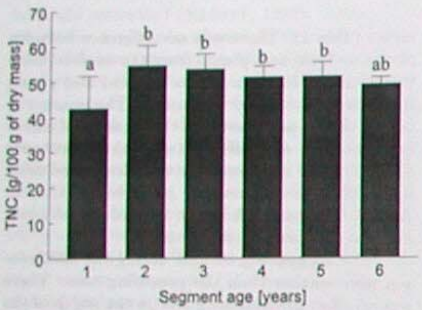


Fig. 2. Relation between total non-structural carbohydrates in *Rumex alpinus* rhizome segments and segment age. The data are pooled for all treatments. The error bar indicates standard error. The means which are not statistically different are labelled with the same letter (LSD, $P = 0.05$).

## Discussion

Plant growth was determined by the placement of the previous year rhizome segment. If it was located in nutrient-rich loam, the plants produced larger leaves and more above-ground biomass than those where it was placed in sand. In this study in March, we found that highest carbohydrate content occurred in the penultimate segment and TNC accumulation in the last segment was lower than in the preceding years. The low concentration of carbohydrates in the new segment suggests that regeneration of transplanted plants was too expensive in terms of carbohydrate utilization and/or available time for carbohydrate accumulation in new rhizome segments was too short. Besides, respiration enhanced by transplantation of the plants to lower elevation and export to older segment (JÓNSDÓTTIR, CALLAGHAN, 1988) might be also responsible for the low level of storage in the new segment (MOONEY, BILLINGS, 1965). However, the carbohydrate concentration in the two youngest segments of *R. alpinus* determined in late autumn in the Krkonoše Mts was very similar (KLIMEŠ et al., 1993). This confirms that carbohydrate reserves were depleted during regeneration and accumulation of new storage was not efficient enough in the experiment.

The decrease of TNC concentration with age (considering segments older than 1 year) corresponds well with data found in literature (SHAVER, BILLINGS, 1976; FIALA, 1976; MASUZAWA, HOGETSU, 1977). It is caused by carbon export to green parts of the plant (SHAVER, BILLINGS, 1976; LANDA et al., 1992), reduced import of carbon from green shoots (JÓNSDÓTTIR, CALLAGHAN, 1988) and continuous losses by respiration (STUDER, BRÄNDLE, 1994).

There was no difference in the carbohydrate content of rhizomes of plants grown on substrates differing in nutrient availability. This result does not correspond to the assumption that growth of aboveground parts is reduced under nutrient shortage while concentration of total nonstructural carbohydrates is enhanced. An alternative explanation that the plant possesses reserves of nitrogen compounds that are able to buffer differences in nutrient availability with time (JONASSON, CHAPIN, 1985) is not probable as a reduction in growth would not be expected in this case. Due to the low number of replications ($n = 4$) the power of the test was rather low, however, the low F value (0.82) indicates that accumulation of 'excess' carbon in secondary metabolites (LOOMIS, 1932; BAAS, 1989) or in roots (BLOOM et al., 1985), should also be considered.



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