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SEASONAL VARIATION IN THE NONSTRUCTURAL CARBOHYDRATE COMPOSITION OF RHIZOMES OF FOREST UNDERSTORY SPECIES

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Rhizomes of eight forest understory species were analyzed in spring, summer and autumn for total nonstructural carbohydrate (TNC) content partitioned as sucrose, free reducing sugars (FRS), fructosans and starch. Species studied were: *Maianthemum canadense* Desf., *Pteridium aquilinum* (L.) Kuhn, var *latiusculum* (Desv.) Underw., *Cornus canadensis* L., *Kalmia angustifolia* L., *Vaccinium angustifolium* Ait., *Gaultheria procumbens* L., *Chamaedaphne calyculata* (L.), Moench, Var. *angustifolia* (Ait.) Rehd., and *Rhododendron canadense* (L.) Torr.

TNC concentrations were highest in *M. Canadense* (56%-70% over the seasons) compared to 7% - 30% in the other species. They were highest in autumn for all species except for *P. aquilinum*. The most abundant single component of TNC was starch for all species except *M. canadense*, in which the fructosan concentration was highest. The storage polymers formed at least 95% of TNC in all cases and showed distinct seasonal changes. The concentrations of FRS and sucrose were always low (4% and 1.5% of TNC respectively), and showed no direct correlation with seasonality.

Les rhizomes de huit espèces de plantes du sous-bois forestier furent analysés au printemps, en été et en automne dans le but de déterminer leur contenu total en carbohydrates non structuraux (TNC); ces carbohydrates non structuraux se divisaient en sucrose, en sucres réducteurs libres, en fructosans et en amidon. Les espèces suivants furent étudiées : *Maianthemum canadense* Desf., *Pteridium aquilinum* (L.) Kuhn, var. *latisculum* (Desv.) Underw., *Cornus canadensis* L., *Kalmia angustifolia* L., *Vaccinium angustifolium* Ait., *Gaultheria procumbens* L., *Chamaedaphne calyculata* (L.), Moench, Var. *angustifolia* (Ait.) Rehd., and *Rhododendron canadense* (L.) Torr.

Les concentrations en TNC furent les plus élevées chez *M. canadense* (56%-70% sur toutes les saisons) comparativement a 7%-30% chez les autres espèces. Les concentrations les plus fortes furent enregistries en automne chez toutes les espèces excepté *P. aquilinum*. L'amidon représentait la fraction des TNC la plus importante chez toutes les espèces excepté *M. canadense*. La concentration en fructosan était la plus élévee chez cette espèce. Les polymères composant les réserves réprésentaient au moins 95 % des TNC chez toutes les espèces et montraient des variations saisonnières distinctes. Les concentrations eu FRS et en sucrose furent toujours faibles (4 % et 1.5 % respectivement), et n'ont montré aucune corrélation avec les saisons.

Introduction

Rhizomes are important as storage organs for energy reserves (Menke & Trlica 1981, Gallagher 1983). Stored reserves of starch (Townsend et al 1968, Smith 1969, Garrison 1971, Sturges & Trlica 1978) or fructosan (Smith 1969, Meier & Reid 1982) will influence the ability to regenerate following disturbance (Trilica & Cook 1971). Defoliation through disturbances such as fire, cutting and herbicide spray will result in changes in the source-sink relationship in plants because of loss of leaf photosynthesis (Watson & Caspar 1984). Rhizome role and clonal growth have been reviewed by Ashmun et al 1982 but confusion has arisen in the literature with respect to whether vegetative propagation is simply part of the growth and perennation strategy of a single genetic individual (the genet) as suggested by Kays and Harper (1974) or whether asexual reproduction serves a role of greater significance when it results in

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the production of physiologically independent ramets (Cook 1979). Production of numerous independent ramets can increase the survivorship of a locally adapted genet (Williams 1975; Abrahamson 1980). It was, therefore, important in this research to excavate rhizome material that was independent, so as to eliminate the possibility of genetic similarity.

There has been considerable interest in recent years in the control of forest understory species using a variety of disturbing influences such as cutting, burning, and treatment with herbicides. A significant part of this interest relates to the ability of plants to regenerate after such treatment and some evidence has been obtained linking carbohydrate reserves with this ability (Kausch et al 1981). However, little effort has been channelled into examining the nature of carbohydrate reserves in the underground parts of wild plants. We therefore wished to examine the quantity and type of carbohydrate storage products present in the rhizomes of some forest understory species. We also wanted to determine how this storage varied seasonally and how the quantity of nonstructural carbohydrates for each species relates to regrowth data obtained previously (Flinn 1980). We feel that answers to these questions will assist in deciding when defoliation treatments should be carried out to effect minimum regrowth of certain species (e.g. *Kalmia angustifolia*) that are competitive in the silvicultural management of the Acadian forest or maximum regrowth of commercially viable species such as *Vaccinium angustifolium*. We therefore chose these species along with six others for this study. All are common and prevalent species (number/m^2) beneath the hardwood and spruce-fir overstories of the Acadian forest (Flinn 1980) and all regenerate from surviving below-ground rhizomes.

Materials and Methods

Field Collection

The eight species studied were: *Gaultheria procumbens, Maianthemum canadense, Vaccinium angustifolium, Cornus canadensis, Pteridium aquilinum, Kalmia angustifolia, Chamaedaphne calyculata* and *Rhododendron canadense*. Taxonomic identification and nomenclature of species were based on Roland and Smith (1969). The field sites were described by Flinn and Pringle (1983). Rhizomes (25-50 cm long) were randomly collected from a range of habitats, i.e. spruce-fir, spruce and hardwood overstory, in the Nova Scotia Acadian forest in spring (June 1), summer (August 1) and Autumn (October 1) as described by Flinn and Pringle (1983). Seasonal collection for each species came from the same habitat, i.e. the summer collection of a particular species was taken from the same location as the spring habitat for that species. An attempt was made to ensure that, although the species came from the same habitat, they were from different populations. This was done by randomly selecting different locations in the same habitats separated by at least 10 m. More material had to be collected for rhizomes of smaller diameter such as *M. canadense* in order to have enough material for the analysis.

All samples were transported to the laboratory within one hour of excavation. They were washed, killed by fast oven drying, and dried completely at 70°C (up to 12 h depending on rhizome diameter). Dried rhizomes were ground in a Wiley Mill (1 mm mesh) for 30 sec and stored at room temperature. There was no significant change in the TNC after 6 months storage.

Variation in the total non-structural carbohydrate (TNC) content between rhizomes of a single species was determined for both *Chamaedaphne calyculata* and *P. aquilinum*. Individual rhizomes (50 cm long) from randomly chosen sites were dried, weighed, washed, ground, and then analyzed separately. Pooled samples were also prepared by grinding together rhizomes from at least 10 different plants for each of the eight species to give a sample of at least 4 g of rhizome tissue for analysis.

Extraction and Chemical Analysis

Pulverized samples were extracted with 80% ethyl alcohol at 70°C for 3 h (Heinze & Murneek 1940) and filtered through Whatman No 1 filter paper. Free reducing sugars in the 80% ethanol-soluble fraction were determined by a modification of the method of Shaeffer and Somogyi (Smith 1969; Ku et al 1978). The amount of sucrose in this fraction was determined by difference in reducing sugars before and after hydrolysis with 0.1 N H_2SO_4 for 15 m in a boiling water bath.

The starch in the 80% ethanol-insoluble material was determined by analysis of reducing sugars after complete hydrolysis of starch with crude alpha-amylase that contained maltase activity ("Takediastase" from *Aspergillus oryzae*, Sigma Chemical Co.). After drying at room temperature, the ethanol-insoluble materials were treated with 0.14% (w/v) alpha-amylase in buffer, pH 4.45, at 37°C for 48 h. The incubation mixture was filtered through Whatman No 1 filter paper. The filtrate was deproteinized by addition of saturated lead acetate, de-leaded with potassion oxalate and assayed for reducing sugars.

Fructosan in the deproteinized filtrate was determined by difference in reducing sugars before and after hydrolysis of the filtrate with 0.1N H_2SO_4 in a boiling water bath for 15 min. Hydrolysis of fructosans was complete (Smith 1969) and thin layer chromatography was used to confirm that fructose was the only sugar released during hydrolysis of the filtrate (Hay et al 1963). Calculations (per cent of total dry weight of sample) for each of the carbohydrate components were based on those described by Smith (1969) for determining TNC.

Statistical Analysis

Calculations of means, standard error of means, one- and two-factor analysis of variance were done using the statistical package SAS on a VAX 11/750 computer.

Table I Rhizome nonstructural carbohydrate components contributing to total carbohydrate reserves.

Nonstructural carbohydrate component		*Chamaedaphne calyculata*	*Pteridium aquilinum*
Mean FRS (%)	spring	0.28 ± 0.04 a	3.60 ± 0.25 a
	summer	0.42 ± 0.04 a	2.31 ± 0.30 b
	autumn	0.41 ± 0.06 a	1.78 ± 0.26 b
Mean sucrose (%)	spring	0.12 ± 0.03 a	0.10 ± 0.04 a
	summer	0.05 ± 0.01 a	0.02 ± 0.01 a
	autumn	0.12 ± 0.05 a	0.14 ± 0.07 a
Mean starch (%)	spring	7.28 ± 0.48 b	23.45 ± 1.45 a
	summer	9.08 ± 0.47 a	21.26 ± 3.53 a
	autumn	8.73 ± 0.36 a	8.46 ± 4.00 b
Mean fructosans (%)	spring	1.92 ± 0.44 a	0.99 ± 0.27 b
	summer	0.64 ± 0.26 b	2.14 ± 0.52 a
	autumn	0.59 ± 0.15 b	0.50 ± 0.32 b
Mean TNC (%)	spring	9.60 ± 0.52 a	28.16 ± 1.38 a
	summer	10.18 ± 0.51 a	25.73 ± 3.74 a
	autumn	9.85 ± 0.34 a	10.89 ± 4.30 b

For each species and variable, seasonal means labelled with different letters are statistically significantly ($p<.05$) distinct from each other, as determined by the Student-Newman-Keuls test, an *a posteriori* multiple range test. (± standard error of the mean for all cases, n = 10).

Results and Discussions

Variability in TNC content between individual rhizomes of *Chamaedaphne calyculata* was within 5% of the mean for all three seasons but *Pteridium aquilinum* showed much greater variability from one season to another (Table I). In spring TNC content in individual rhizomes of *P. aquilinum* was within 5% of the mean, but the range increased to over 10% in summer and to almost 40% in autumn. This high variation among rhizomes in autumn TNC content may be attributed, at least in part, to a depletion of reserves during rhizome elongation. Furthermore, many of the older segments of *P. aquilinum* rhizomes die off in autumn resulting in loss of TNC. *Vaccinium angustifolium* and *Kalmia angustifolia* have also been analyzed for TNC in individual rhizomes (Flinn et al unpublished) and show variability (<5% of the mean) comparable to that reported here for *Chamaedaphne calyculata*. These trends, similar to those observed by Townsend et al (1968) for *V. angustifolium* indicate that in general, variation in TNC is low among rhizomes of physiologically independent ramets. Thus, we are confident that data obtained using pooled samples of rhizomes from a single species (Fig. 1) reflect the TNC content of individual rhizomes of that species.

The FRS, sucrose, starch, and fructosan fraction differed significantly ($p<.05$) between *Chamaedaphne calyculata* and *P. aquilinum* (Table 1). There were significant differences within each species between mean seasonal levels of FRS, starch, and fructosans, but no significant difference between mean seasonal levels for sucrose in either species. The mean seasonal levels of TNC were significantly different from *P. aquilinum* but not for *Chamaedaphne calyculata* which indicated that the seasonal pattern of accumulation of carbohydrate reserves differed greatly for the two species (Table 1, Fig. 1). *P. aquilinum* showed a much higher concentration of TNC (especially starch) in spring and summer, followed by a dramatic decrease in autumn when the two species showed similar concentrations. The elevated starch levels in *P. aquilinum* during spring and summer were accompanied by relatively high FRS content during the same periods. It has been observed over a five year period that this is a time of rapid rhizome elongation in this species and the associated metabolic activity may require elevated levels of reducing sugars as precursors for anabolic growth pathways. Gallagher et al (1984) found that rhizomes of salt marsh plants showed a similar distinct concentration cycle for nonstructural carbohydrates.

There were clear similarities in TNC content for pooled rhizome samples of the five ericaceous species (Fig. 1), with spring values forming 11-15% (of total dry weight), summer 10-17%, and autumn 15-20%. The TNC was highest in autumn for this family and was most often lowest at the spring sampling. The slight deviation from this pattern for *Chamaedaphne calyculata* and the more pronounced effect in *K. angustifolia* may be a reflection of phenological differences for these evergreen species.

There was less TNC in *Cornus canadensis* during the spring than in any other species studied. This may be due to depletion of reserves during flowering and fruiting (Ting 1982). Fruiting was in progress in this species during the time of spring sampling.

The quantities of TNC were markedly greater in *Maianthemum canadense* (Fig. 1) due to the high accumulation of fructosans in this species. This observation is consistent with well documented reports that fructosans (fructans) are the major reserve carbohydrates of monocotyledons (Ting 1982, Smith 1969, Meier & Reid 1982). There were small amounts of fructosan in the spring or summer analyses of the 6 dicotyledon species examined (Fig. 1). The role of these fructosans is not clear. Starch content for *M. canadense* was comparable to that of other species.

All species except *P. aquilinum* showed the greatest starch or fructosan content in the autumn. This confirms results from studies on other plant parts in other species

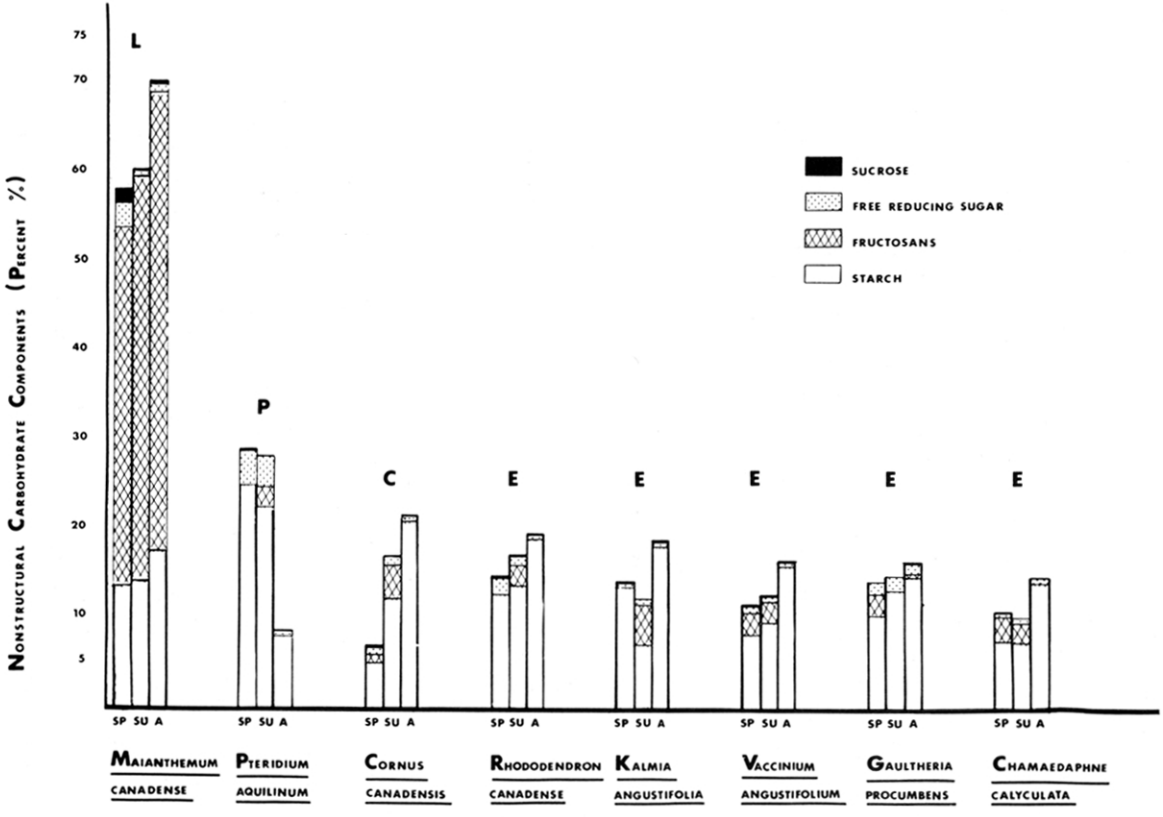

Fig. 1 Per cent of total dry weight of sucrose, free reducing sugar, fructosans and starch making up total nonstructural carbohydrate in pooled rhizome samples of eight forest understory species collected in early June (Sp = spring), August (Su = summer), October (A = autumn), grouped according to families: L = Liliaceae, P = Polypodiaceae, C = Cornaceae and E = Ericaceae.

(Lindahl et al 1949, Mooney & Billings 1960, Edelman & Jefford 1968, Townsend et al 1968, Smith 1969, Chubey & Dorell 1977, Roseff & Bernard 1978, Gallagher 1983, Gallagher et al 1984). The marked seasonal differences in TNC for very different species (Fig 1) such as *M. canadense* and *P. aquilinum* and the similarities observed for more related species (ericaceous family) strongly support the notion that each individual species has a distinct cycle for concentrating reserves in the rhizomes (Gallagher et al 1984, Ashmun et al 1982).

Free reducing sugars formed less than 4% of TNC in all species for all seasons. The data was significantly different ($P<0.05$) between species but not between seasons. This indicates that monosaccharides such as glucose function as precursors for biosynthesis (Preiss 1982).

The results presented here clearly indicate characteristically different patterns of carbohydrate storage in the rhizomes of different understory plants in the Acadian forest. On the other hand, more closely related species show similar patterns of carbohydrate reserve accumulation. A common feature for most species studied was the peak accumulation of nonstructural carbohydrate in the autumn. Since available reserves would be expected to influence the ability of these plants to regenerate following stress (Kausch et al 1981), this helps to explain the earlier observation (Flinn 1980) that such regrowth after fire was greatest if burning was carried out in autumn. Therefore, from this research, the most effective control of forest understory species in silvicultural management might be anticipated if defoliation were carried out in spring when rhizome carbohydrate reserves are low.

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