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Seasonal patterns of carbohydrate reserves in red spruce seedlings

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Summary We studied seasonal dynamics of carbohydrate storage in red spruce (*Picea rubens* Sarg.) seedlings by measuring starch and sugar concentrations of old needles (≥ one year old), new needles (< one year old), stems, and roots in two stands in the Green Mountains of Vermont. Although the two stands differed in many site characteristics including percent slope, aspect, soil type, drainage, and 564 m in elevation, concentrations and seasonal patterns of carbohydrates were similar for the two stands. For all tissues, starch concentrations peaked in late spring, declined through summer, and reached a minimum in winter. Sugar concentrations were greater than starch concentrations in all months except May and June. Sugar concentrations peaked in winter, and old needles showed a significant increase in sugar concentration between February and March. This increase in sugar concentration occurred without any reduction in localized starch concentrations or reductions in sugar or starch concentrations in new needles, stems or roots. Because March measurements were made toward the end of a prolonged thaw, a time when increases in photosynthesis have been documented for red spruce, it is likely that the March increase in sugar concentrations resulted from photosynthesis during the thaw. Compared with stems and roots, needles generally contained the highest concentration of carbohydrates and exhibited the greatest seasonal change in carbohydrate concentration. Needles were also the largest reservoir of carbohydrates throughout the year, especially during winter. Because of the critical roles of needles in photosynthesis and storage of carbohydrates, we conclude that any factors that disrupt the accumulation or availability of carbohydrates in red spruce needles will greatly alter plant carbon relations.

Keywords: needles, Picea rubens, roots, sugars, starch, stems.

Introduction

Long-lived evergreen foliage may provide important year-round storage of carbohydrates (Kimura 1969, Dickmann and Kozlowski 1970, Reader 1978). Foliar carbon stores could be of particular benefit in supporting early spring shoot growth when low soil temperatures limit the translocation of carbon reserves from roots (Chabot and Hicks 1982). Evergreen foliage also allows for a temporal extension of carbon fixation (Larcher 1980, Schulze 1982, Matyssek 1986). Photosynthetic measurements of conifers growing in mild winter climates have shown that significant carbon fixation can occur year-round (Fry and Phillips 1977, Webb 1977, Harrington et al. 1994). Emmingham and Waring (1977) estimated that, for Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) growing in western Oregon where summers are dry and winters are mild and wet, dormant season (late October through late April) photosynthesis accounted for as much as 55% of total annual carbon assimilation. However, field studies of conifers growing in cold northern regions suggest that carbon fixation during the cold season (fall through winter) may be intermittent, and limited to periods when temperatures are predominantly above 0 °C (Parker 1961, Jurik et al. 1988, Schaberg et al. 1995, Schaberg et al. 1998).

Carbon gains during the cold season could benefit evergreen trees by compensating for respiratory carbon losses of overwintering foliage (Sprugel 1989), contributing to the accumulation of cryoprotective sugars (Parker 1963) and providing additional resources for spring bud break and growth (Kimura 1969). However, the true impact of carbon gains during the cold season on annual carbon budgets of northern conifers remains uncertain (Schulze et al. 1977, Matyssek 1986).

Since the mid-1960s, growth rates of montane red spruce have declined in many parts of the eastern USA (Johnson et al. 1988, Cook and Zedaker 1992). Pollution-induced disruptions in carbon relations (McLaughlin et al. 1991, McLaughlin and Kohut 1992) and cold tolerance (DeHayes 1992, Johnson et al. 1996) have both been linked to this decline. Because carbohydrate concentrations, especially sugars, may play an important role in the development and maintenance of cold tolerance (Parker 1959, 1963, Sakai and Larcher 1987), these two pollution-induced disruptions in physiology may be related.

Because of suspected linkages between red spruce carbon relations and decline, a knowledge of the seasonal pattern of carbon storage in healthy red spruce (*Picea rubens* Sarg.) may help elucidate the mechanisms underlying pollution-induced disruptions in the physiology of this species. Assessment of carbon storage during the cold season is particularly important because injury during this period directly contributes to decline (Wilkinson 1990, DeHayes 1992, Tobi et al. 1995). We measured concentrations of starch and sugar reserves in needles, stems, and roots of red spruce seedlings collected approximately monthly from two stands in the Green Mountains of Vermont, to determine how carbon reserves change over time.

Methods

Study sites

Two red spruce stands from the Green Mountains of Vermont were selected. Physiographic and vegetative characteristics of these stands have been described (Donnelly et al. 1985). One stand is located on the upper portion of a 60% slope with a south-west exposure at 945 m on the west side of Mt. Abraham, about 1 km north of Lincoln Gap in Lincoln, Vermont. Mean age of dominant and co-dominant trees in 1987 was 57 years with a mean crown cover of 75%. Soils are a well-drained Lyman-Berkshire rocky complex (Griggs 1971). The second stand is in the Texas Falls Recreation Area, near Hancock, VT, at an elevation of 381 m. It is at the bottom of a 19% slope with a northeast aspect. Mean age of the overstory in 1987 was 84 years, with a crown cover of 95%. Soils are moderately well drained Berkshire-Marlow stony loams (Griggs 1971). Air temperatures were obtained from two nearby National Oceanic and Atmospheric, National Weather Service (NOAA-NWS), meteorological stations; one at South Lincoln, Vermont (elevation 616 m) about 5 km southwest of Mt. Abraham, and the other at Rochester, Vermont (elevation 253 m) about 4 km southeast of Texas Falls (NOAA-NWS records). An adiabatic lapse of –0.98 °C per 100 m increase in elevation was used to adjust weather station data and estimate air temperatures at the stands (Hemond and Fechner 1994).

Seedling collection

Ninety red spruce seedlings from each stand were randomly chosen, flagged, and numbered. Beginning June 1987, and repeated in eight of the next 11 months (July, August, October, December, February, March, April, and May), 10 flagged seedlings were randomly selected and excavated from each stand between 0900 and 1100 h. At both sites, new foliage was fully expanded at the time of the June 1987 collections, and buds were swollen but not broken when sampling ended in May 1998. Mean seedling age was 10 years at Mt. Abraham and 13 years at Texas Falls. Mean dry weight of seedlings was 18.5 g at Mt. Abraham and 32.9 g at Texas Falls.

For each month, seedlings were collected on two successive days, one stand per day. Seedlings were excavated carefully to preserve intact root systems, wrapped in plastic, placed in ice, and transported to a laboratory for same-day processing (Hinesley et al. 1992, Ashworth et al. 1993, Billow et al. 1994).

Root sampling was incomplete in February, March and April 1988 because frozen soils prevented excavation of entire root systems. Root samples for measurement of carbohydrate concentrations were collected for some seedlings during these months. However, the dry weights of root systems were estimated for February, March, and April, based on allometric relations among tissue dry weights from other months. Comparison of estimated and measured root weights for months when intact root systems were collected indicated that the allometric relationships used closely approximated root weights.

Tissue preparation and carbohydrate analysis

Frozen seedlings were separated into four tissue components: new needles (< 1 year old), old needles (≥ 1 year old), stems, and roots. Tissues were washed with distilled water and subsamples were placed in labeled vials, freeze-dried, and stored in a freezer (–40 °C) until analyzed. Starch and soluble sugar concentrations were determined as described by Haissig and Dickson (1979), except that dinitrosalicylic acid was used instead of glucose oxidase as the color-producing reagent. Tissue samples not analyzed for carbohydrates were oven-dried (70 °C; 96 h) and weighed. Oven-dried weights, freeze-dried weights, and carbohydrate concentrations of subsamples were used to calculate the total amounts of sugar and starch in tissues.

Statistical analysis

Analyses of variance were used to compare means and to determine the variability associated with stand, tissue type, and month of sampling. Because of differences in stand elevation, we expected that there would be differences in phenology, growth cessation, and other seasonally related physiology between stands. Therefore, we analyzed the data by means of a nested design, with calendar date nested within stand, even though this prevented the computation of a stand × date interaction. Changes in carbohydrate concentrations within specific tissues over time were assessed by mutually exclusive orthogonal contrasts, which compared concentrations from one month with concentrations from the previous sampling date. Analyses of variance were also conducted for individual months and tissue types to test for stand differences in carbohydrate storage. Data were transformed when necessary to meet statistical assumptions, and statistical analyses of starch and sugar data were performed separately. Differences were considered statistically significant if $P \leq 0.05$.

Results

Stand comparisons

Although the Mt. Abraham and Texas Falls sites are located on opposite slopes of the Green Mountains and differ in elevation by 564 m, air temperatures for the two stands were similar (Figure 1). Seedlings from the two stands differed in both age and size. However, overall seedling and tissue-specific concentrations of starch and sugar for the study as a whole did not differ between stands. Monthly trends in carbohydrate concentration were also remarkably similar between the two stands, with only subtle differences in concentration evident during early spring and mid- to late-summer. Sugar and starch concentrations of new needles were typical of this trend (Figure 1). Monthly mean carbohydrate concentrations were slightly but significantly different in July (sugar and starch),

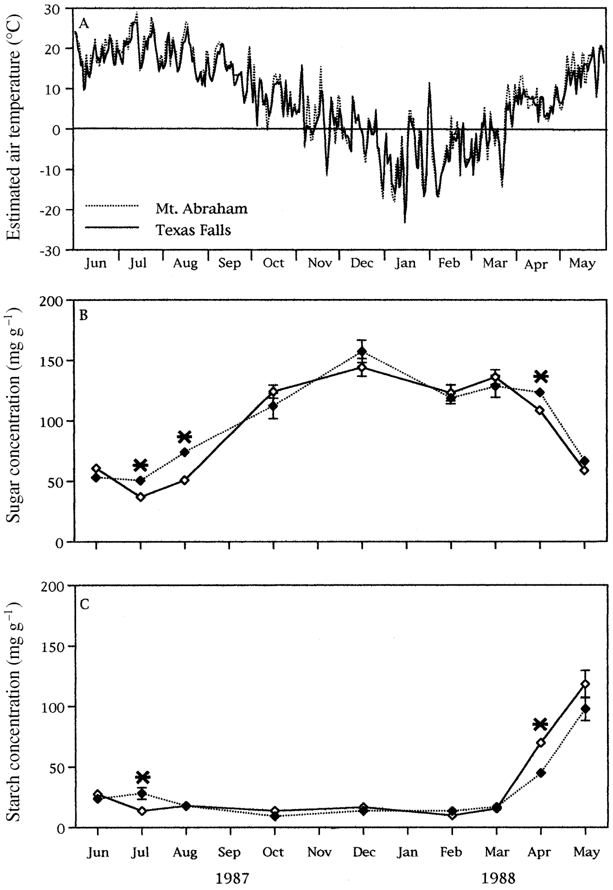

Figure 1. Temporal patterns in (A) daily estimated mean air temperature, (B) new needle sugar concentrations, and (C) new needle starch concentrations for red spruce seedlings from the Mount Abraham and Texas Falls study sites. All carbohydrate values are means of 10 observations and error bars are ± 1 SE of monthly means. In some cases, error bars are obscured by symbols representing means. Significant differences ($P \leq 0.05$; based on analyses of variance) between stands in monthly sugar and starch concentrations are identified with an asterisk.

August (sugar only), and April (sugar and starch). However, differences between stands were minimal compared with seasonal changes (Figure 1). Because concentrations and seasonal patterns of carbohydrate storage were similar for the two stands, data were combined for subsequent evaluations.

Seasonal patterns

In all red spruce tissues, sugar concentrations were higher than starch concentrations during every month except June 1987 and May 1988 (Figure 2). Sugar concentrations peaked during winter, whereas starch concentrations peaked in late spring, declined during summer, and reached minima in all tissues during late fall or winter.

Old needles Sugar concentrations were relatively low in June (91.2 ± 3.1 mg g^{-1}), increased to a maximum of 188.8 ± 6.3 mg g^{-1} in December, and then fell to 70.1 ± 3.0 mg g^{-1} by May. Starch concentrations showed a contrasting pattern, decreasing from a maximum of 130.3 ± 6.8 mg g^{-1} in June, to a minimum of 10.7 ± 0.8 mg g^{-1} by October, remaining low throughout winter, then increasing to 55.5 ± 4.2 mg g^{-1} by April and 88.1 ± 4.7 mg g^{-1} by May.

New needles With the exception of June and July, seasonal patterns of starch and sugar concentrations in new needles were comparable with those of old needles. Sugar concentrations of new needles were at a minimum of 44.0 ± 2.1 mg g^{-1} in July, increased to a maximum of 150.9 ± 5.9 mg g^{-1} in December, and then decreased to 62.8 ± 2.4 mg g^{-1} by May. Starch concentrations were low (25.6 ± 0.9 mg g^{-1}) when first sampled in June, decreased slightly to a minimum of 11.5 ± 0.7 mg g^{-1} in October, then increased to 57.2 ± 3.5 mg g^{-1} in April and 108.0 ± 7.6 mg g^{-1} by May.

Stems Sugar concentrations in stem tissue were more consistent throughout the year than those in needle tissue, increasing from 61.6 ± 2.8 mg g^{-1} in June to a maximum of 86.4 ± 5.6 mg g^{-1} in December and then decreasing to a minimum of 47.2 ± 3.3 mg g^{-1} in May. Stem starch concentrations were more variable than stem sugar concentrations. They were relatively high in June (57.5 ± 7.6 mg g^{-1}), fell to a minimum of 10.8 ± 1.3 mg g^{-1} in December, and then increased to a maximum of 67.0 ± 5.1 mg g^{-1} in May.

Roots Roots also exhibited less seasonal variation in carbohydrate concentration than foliage. However, root carbohydrate patterns were irregular. In part, this may have resulted from variability caused by the difficulty of sampling roots in winter. Sugar concentrations oscillated from June through December, increased to a maximum of 101.4 ± 10.0 mg g^{-1} in February and then fell to a minimum of 56.3 ± 4.2 mg g^{-1} in April. Starch concentrations averaged 55.9 ± 2.6 mg g^{-1}, decreased gradually through fall to 27.0 ± 2.6 mg g^{-1} in December, and increased to a maximum of 68.3 ± 2.9 mg g^{-1} in May.

Discussion

Starch and sugar concentrations in new and old needles, stems, and roots of red spruce seedlings did not differ between the two stands, despite dissimilarities in physiography, which suggests that seasonal carbohydrate dynamics in this species are relatively consistent. Concentrations and seasonal patterns of carbohydrates over the 12-month study period corresponded closely with tissue-specific data for red spruce and other conifers obtained over more limited time periods. For example, similar concentrations and seasonal pattern of foliar sugars and starch have been observed for potted (Alscher et al. 1989) and mature (Amundson et al. 1992) red spruce from June through December. Our carbohydrate data are consistent with reports of the foliar carbohydrate dynamics (e.g., Gholz and Cropper 1991, Webb and Kilpatrick 1993, Billow et al. 1994) and stem sugar and starch concentrations (e.g., Oren et al. 1988, Gholz and Cropper 1991) in other conifers, and sugar concentrations in red spruce roots (Alscher et al. 1989).

Seasonal patterns in root starch over the 12-month period

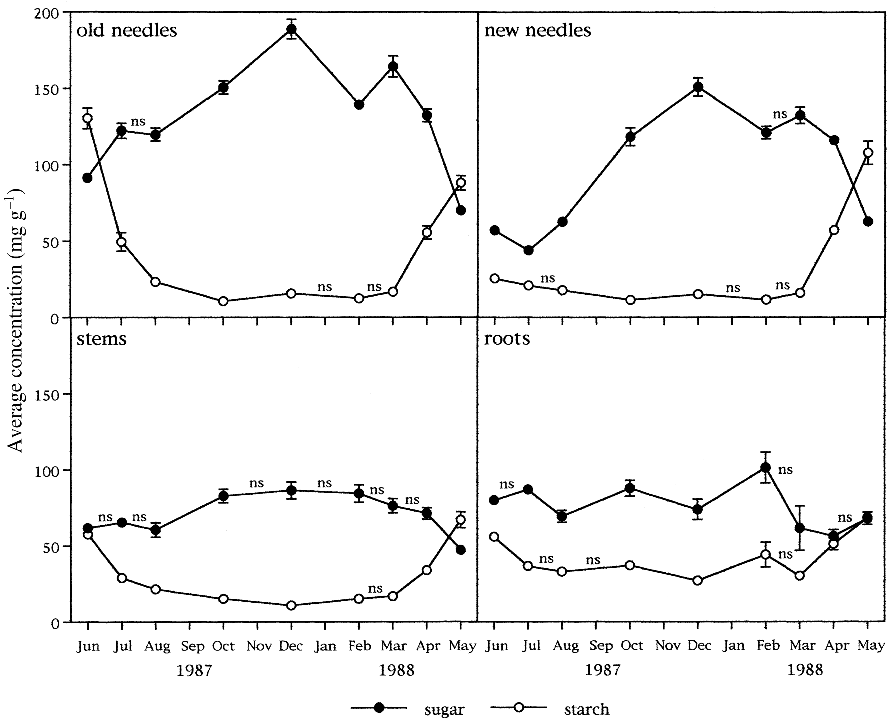

Figure 2. Seasonal patterns of starch and sugar concentrations in four plant tissues of red spruce seedlings collected from two stands in the Green Mountains of Vermont. All points are means of 20 observations, except for root means in February (*n* = 3), March (*n* = 6) and April (*n* = 18). Temporal changes among means were assessed by orthogonal contrasts which compared concentrations for one month with those for the next sampling date. Based on these analyses, all sequential means were statistically different from one another except those separated by the designation ns. Error bars are ± 1 SE of monthly means. In some cases error bars are obscured by symbols representing means.

mirrored those obtained for more limited time periods (Ericsson and Persson 1980, Adams et al. 1986, Cranswick et al. 1987, Oren et al. 1988, Gholz and Cropper 1991). However, root starch concentrations differ greatly among studies. Although root starch concentrations similar to those in our study have been reported (Cranswick et al. 1987, Gholz and Cropper 1991), values approximately double the highest concentrations that we observed have also been found (Ericsson and Persson 1980, Oren et al. 1988). Root starch concentration appears to be highly variable among species and is subject to variation caused by methodological differences (Gholz and Cropper 1991) and soil factors (Adams et al. 1986). In addition, differences in the proportion of coarse to fine roots sampled could influence reported starch concentrations. Some data indicate that coarse and fine roots have comparable starch concentrations (Cranswick et al. 1987, Oren et al. 1988), whereas other data indicate that starch concentrations are considerably higher in coarse roots than in fine roots (Ford and Deans 1977, Ericsson and Persson 1980, Gholz and Cropper 1991). For example, spring starch concentrations were more than twice as high in coarse (> 2 cm) roots of slash pine as in fine (< 1 mm) roots (Gholz and Cropper 1991). If this relationship holds true for red spruce, the moderate root starch concentrations that we observed could be the result of the careful harvest of entire root systems including most fine roots.

Unlike most deciduous hardwoods, our red spruce seedlings stored a significant proportion of their carbohydrates as sugar. Sugar concentrations were greater than starch concentrations during every season except for a brief period in spring (Figure 2). Seasonal patterns of sugar and starch concentrations tended to be inversely related. Sugar concentrations peaked in winter and were at their lowest values in spring, whereas starch concentrations peaked in late spring and declined through summer, reaching minima in all plant tissues during winter. Although synchronous and inverse changes in sugar and starch concentrations may partly reflect conversions between these compounds, seasonal changes in carbohydrate production, transport and utilization would also influence the observed patterns. For example, reductions in starch during the growing season probably reflected carbohydrate use associated with the construction and maintenance of new tissues (Olofinboba and Kozlowski 1973, Deans and Ford 1986).

From June to December, sugar concentrations exhibited a general increase in all tissues, except roots. This increase was particularly evident for foliage, and was most pronounced from August through December. Low temperatures reduce root and shoot growth in conifers (Andersen et al. 1986, Vapaavuori et al. 1992), and reductions in the strength of distal carbohydrate sinks limit phloem transport away from foliage and result in a buildup of foliar carbon reserves. Cold-induced reductions in phloem transport itself (Wardlaw and Bagnall 1981, Grusak and Minchin 1989) may also contribute to the accumulation of foliar sugars.

Autumnal increases in sugar concentrations can enhance plant cold hardiness (Parker 1959, 1963, Aronsson et al. 1976, Levitt 1980), and increases in particular sugars appear to be especially important in the development of winter cold tolerance of red spruce. Schaberg et al. (2000) found winter cold tolerance of montane red spruce was significantly correlated with foliar raffinose and sucrose concentrations. Raffinose and sucrose have been shown to help protect protein (Carpenter and Crowe 1988) and membrane (Anchordughy et al. 1987) systems from freezing injury *in vitro*.

Foliar sugar concentrations were generally high in winter, although they decreased in midwinter (Figure 2). Similar reductions in foliar sugar concentrations of cold-stored seedlings have been attributed to the conversion of sugars to starch (Ritchie 1982, Jiang et al. 1994). Although no changes in foliar starch concentrations accompanied the observed midwinter reductions in foliar sugars, there were increases in stem starch, root starch and sugar concentrations. Respiration has also been implicated in the loss of foliar sugars during winter (Ögren 1997, Ögren et al. 1997). Red spruce consistently experiences low rates of net respiration when temperatures fall below 0 °C (Schaberg et al. 1995, Schaberg et al. 1998), but respiration rates increase rapidly at temperatures above freezing (Schaberg et al. 1996).

Sugar concentrations in old needles increased slightly but significantly between February and March. This increase was not accompanied by any reductions in starch concentrations in old needles or significant reductions in sugar or starch concentrations in new needles, stems or roots. Although increased sugar concentrations in old needles could have occurred through conversions from a non-carbohydrate fraction, it seems more likely that it resulted from photosynthesis, because the timing of the increase in foliar sugar concentrations toward the end of a protracted thaw is precisely when winter photosynthesis has been observed in red spruce (Schaberg et al. 1995, Schaberg et al. 1998).

The relative importance of tissues for carbohydrate storage depended somewhat on season, but foliage was always the single largest reservoir of total carbohydrates (Figure 3). Although new needles stored less carbohydrate in the early part of their first growing season, they were comparable to old needles in other seasons. Old needles contained the highest concentrations of starch during late spring and early summer and the highest concentrations of sugar during every month. The large contribution of foliage to seedling carbohydrate stores, particularly of sugars during winter and starch in spring, differs substantially from the carbohydrate storage pattern commonly found in deciduous species. In addition to the contribution of foliar carbohydrates to the development and maintenance of cold tolerance and winter survival, they may also contribute energy for budbreak and shoot growth in spring when the availability carbohydrates in the stem and roots may be limited because of low temperatures (Kimura 1969, Dickmann and Kozlowski 1970, Reader 1978, Chabot and Hicks 1982).

We postulate that the function of red spruce foliage as a storage reservoir for carbohydrates is related to the reported decline of this species. The principal symptom of red spruce decline is a progressive deterioration of the crown. Regardless of the cause(s) of foliar damage, needle mortality reduces the capacity for both the production and storage of carbohydrates needed for sustaining growth and vigor. Thus extensive foliar loss could account for the declines in growth and increases in mortality reported for red spruce in recent decades (Johnson et al. 1988, Wilkinson 1990, Tobi et al. 1995).

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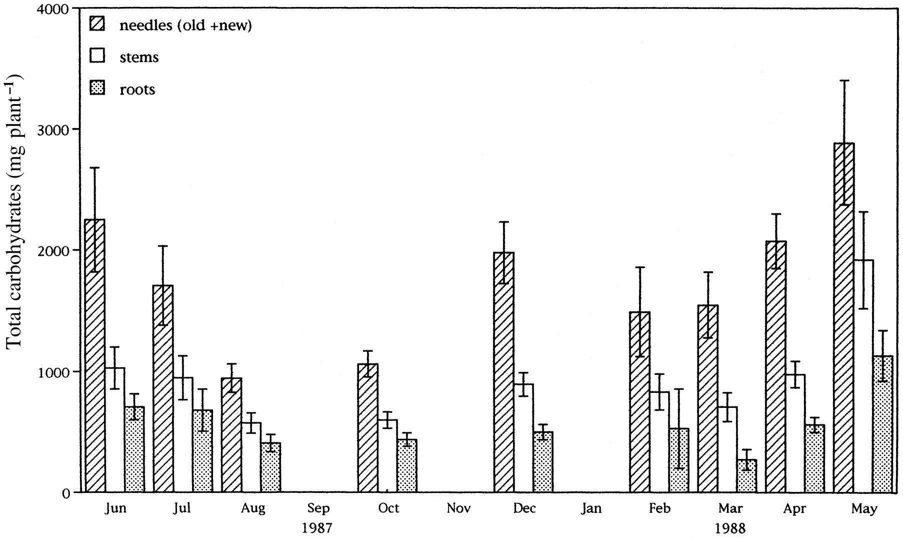

Figure 3. Seasonal patterns of the mass of total carbohydrates (starch plus sugar, mg plant^{-1}) in new and old needles, stems and roots of red spruce seedlings collected from two stands in the Green Mountains of Vermont. All bars are means of 20 observations, except for root means in February (n = 3), March (n = 6) and April (n = 18). Error bars are ± 1 SE of monthly means.

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