OVERWINTER STORAGE OF CARBOHYDRATE IN ASPEN

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ABSTRACT. Total nonstructural carbohydrate (TNC percent dry weight) of various tissues was monitored overwinter in four aspen (*Populus tremuloides* Michx.) clones in central Colorado. Midwinter TNC was highest in root phloem, followed by bark, small roots, root xylem and stem sapwood respectively. Photosynthates were not immediately translocated to roots with the onset of dormancy, but persisted in bark, possibly due to photosynthetic activity, throughout the fall before being transferred to root phloem tissue in mid to late winter. Small roots and woody tissue do not appear to play a significant role in carbohydrate storage. These TNC allocation patterns may help explain seasonal aspen herbivory, particularly the stripping of bark by deer, elk and moose in winter.

Key words: aspen, *Populus tremuloides,* browsing, carbohydrate reserves, silviculture

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INTRODUCTION

Aspen (*Populus tremuloides* Michx.) is the preeminent hardwood species in many western forested ecosystems. Aspen regenerates primarily by vegetative root suckering following fire, avalanches or other large-scale disturbances. Root suckering initiated by the death or injury of parent stems produces stands of genetically identical stems (clones) up to several hectares in size. The number and growth rate of new suckers is dependent upon the vigor of the parent clone and the carbohydrate content of its roots (Tew 1970).

Aspen is important to wildlife in western ecosystems as both habitat and forage. It is highly-preferred as a food source by beavers (*Castor canadensis*) having the highest energy and crude protein content among species utilized by beaver (Doucet et al. 1993). Aspen leaves and suckers are utilized by large ungulates and can be desirable part of the animal's diet, especially in winter when other forage is limited, or of low nutritional value (Hobbs et al. 1981, Miquelle and Van Ballenberghe, 1989). Aspen bark is also stripped from trees in winter by elk and moose (Debyle 1985). Specific genotypic preferences are exhibited by browsing animals. Some clones will be heavily browsed or barked while other nearby clones will be left untouched (Shepperd and Fairweather 1994).

Aspen's reliance on vegetative reproduction and its utilization by animals raise questions about the role that stored carbohydrates play in both processes. It is known that carbohydrates stored in roots play an important role in the sucker growth (but not initiation; Fey et al. 2003) and that carbohydrates accumulate over the summer reaching a maximum at the end of the active growing season (Schier and Zasada 1973, Tew 1970). It has also been shown that carbohydrate content varies by tissue type and developmental stage in the life cycle of aspen clones (Shepperd and Smith 1993). We know that carbohydrate content is high in the fall and low in the spring. Schier and Zasada (1973) reported that root carbohydrate content of six aspen clones in Utah and Alaska decreased slightly from 15-20% in September, to about 15% at their last measurement in October. However, no specific information has been published about the pattern of carbohydrate depletion in aspen over the course of the winter. Previous studies have reported conflicting patterns of overwinter carbohydrate depletion in other species. Nguyen et al. (1990) found that as poplar seedlings developed cold hardiness from August to November, starch concentrations declined and sugar concentrations increased in stems and branches. Roots >1 mm diameter were major repositories of starch and sugar late in the season. By the end of September, 80% of total tree TNC was in the roots. Even fine roots <0.5 mm showed substantial carbohydrate loading in late season. Brown et al. (1985) reported that above and below-ground starch content of young apple trees peaked at leaf fall then steadily declined until the following April. However, soluble sugars increased from leaf fall to February, then declined until April. Similar winter patterns have been observed in plane (*Platanus acerifolia*) (Haddad et al. 1995) and in red osier dogwood (Ashworth et al. 1993). Determining where carbohydrates are stored during the dormant season in mature aspen clones and the specific pattern of overwinter carbohydrate depletion might help explain seasonal herbivory and improve management strategies to regenerate aspen clones.

The study reported here was designed to answer several questions about the in-situ storage and depletion of carbohydrates in aspen clones: 1) How are non-

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structural carbohydrates apportioned among aspen xylem, phloem and root tissue during the dormant season? 2) Is there evidence of translocation of stored carbohydrates from stems to roots at the end of the growing season? 3) What is the pattern of non-structural carbohydrate content depletion during the dormant season (e.g. Does the carbohydrate content drop abruptly with the cessation of photosynthesis at the end of the growing season, or is it depleted gradually during the winter)?

METHODS

Four mature aspen clones representing a target population of aspen in the southern Rocky Mountains were selected for this study. Two clones from low and medium quality sites were chosen on the Fraser Experimental Forest in Central Colorado. The other two clones were selected in high-quality commercial aspen forests on Rabbit Ears Pass near Steamboat Springs in northwestern Colorado and on Kebler Pass near Gunnison, in central Colorado. Clones were selected without a particular bias in mind, but were chosen in areas that could be safely accessed by snowmobile in winter.

Tissue samples were collected from each of these clones prior to leaf-fall in August 1990, in November 1990, February, 1991, and prior to leaf-out in May 1991. For each collection, three dominant or co-dominant trees were randomly selected at each aspen clone. Bark samples were collected from each tree by using a wood chisel to remove a 10 x 25 cm rectangle of bark from the stem about 1.5 m from the ground. A sapwood sample was collected by chiseling 2 cm into the stem beneath the bark sample.

Root samples were collected within 3m of each sample tree at each visit. Beginning at a randomly selected point, snow, litter, and soil were excavated until sufficient roots were encountered to collect a sample. Collected roots were separated into two different size classes, those < 4 mm in diameter and those 4-20 mm in diameter. Roots > 20 mm (those near tree trunks) and root nodes (swelled sections containing aspen suckers) were not collected. No wood, bark or root material was collected that contained rotten or discolored tissue. Different trees were sampled at each visit to a clone to avoid any effect due to injury and under the presumption that individual tree variability is reduced for clonal species. Samples were collected for each of the five tissue types (bark, sapwood, large-root phloem, large-root xylem , and small-roots) from three trees in each of the four clones during each of the four visits, or 240 total samples.

Bark, sapwood, and root samples were separated by type and size, at each sample point within a clone, put into individual freezer bags, placed on ice in a cooler and returned to the lab where they were transferred to paper bags and oven-dried for 48 hr. at 65°C. Phloem tissue was peeled from large root samples and dried separately. Dried samples were then milled through a 0.5 mm screen and stored in sealed plastic vials.

Total nonstructural carbohydrate (TNC percent dry weight) contents were determined by acid extraction in 0.2N H_2SO_4 for 60 minutes, followed by analysis for reducing power using the Shaeffer-Somogyi copper-idometric titration procedure described by Smith (1981). Acid extraction was chosen to avoid long digestions and facilitate processing of the large number of samples collected. Duplicate extractions and titrations were performed in random order on all samples with fructose and blank standards in each run. A third determination was done in some cases to resolve discrepancies.

The target population for this study is aspen clones in the southern Rocky Mountains. Therefore, the four clones were considered the basic sampling units for analysis, each with four samples in time. TNC sampled for the five tissue types represents commensurable measurements, so the appropriate statistical analysis of TNC is a repeated measures analysis with tissue type nested within time. Degrees of freedom from four clones were too few to treat both time and tissue type as multivariate repeated measures, so time was included as the design factor in an analysis of variance approximation with tissue type included as a multivariate repeated measure. The sphericity assumption was not rejected ($p = 0.345$), which is required for this approach (Crowder and Hand 1990). The normality assumption was also not rejected for any tissue type (Shapiro-Wilk test, $p > 0.4$). Means were compared with 95% Tukey confidence intervals using Alpha = 0.01 as a Bonferroni adjustment to maintain Alpha = 0.05 across all five tissue types.

RESULTS

The 558 extractions that were processed contained from 0.63-28.1 % TNC. The random processing of samples and calibration procedures seemed to work well, with no difference in average TNC apparent between runs nor any dectable drift in TNC determinations from run to run. Consistent differences were observed among both tissue types and dates of collection ($p < 0.001$), but the time-by-tissue-type interaction was also significant ($p < 0.001$). Change in TNC across time was inconsistent among tissue types (Fig. 1).

Average TNC content of bark was 16.5% in August, fluctuated slightly to 17.7% in November and 15.8% in February, before dropping to 11.8% in May, which could be detected as different from the previous August value. Conversely, average root phloem TNC rose from 13.7% in August to 18.9% in February, then dropped to 15.4% in May. Small root TNC content did not vary over the dormant season, averaging about 10% from August to Feb-

ruary, then dropping to 8.6% in May, which was dectable from the fall values. Stem sapwood tissue contained little TNC and exhibited a dormant season pattern similar to that of small roots, averaging about 3.0% TNC until February, then dropping to 2.0% in May. Large root xylem tissue increased in average TNC from 5% in August to 8% in November, before dropping to about 6% in February and May.

DISCUSSION

The variation in TNC content among root tissues collected in this study exhibited a pattern similar to that observed by Shepperd and Smith (1993) in aspen tissue collected during the summer. Large-root phloem tissue contained more TNC than large-root xylem tissue or small root tissue, regardless of the time of collection. We observed similar differences between TNC content of bark and stem sapwood in this study. These differences most likely reflect the physiological functions of xylem and phloem tissue respectively.

A consistent pattern of TNC change occurred over the course of the winter among tissue types in the four clones that we studied. Bark TNC of aspen in our study remained high and did not significantly decline until after the February collection (Fig. 1), in contrast to the increase in bark TNC content from August to November that was previously reported in hybrid poplar (Nguyen et al. 1990) and in pecan (Worley 1979).

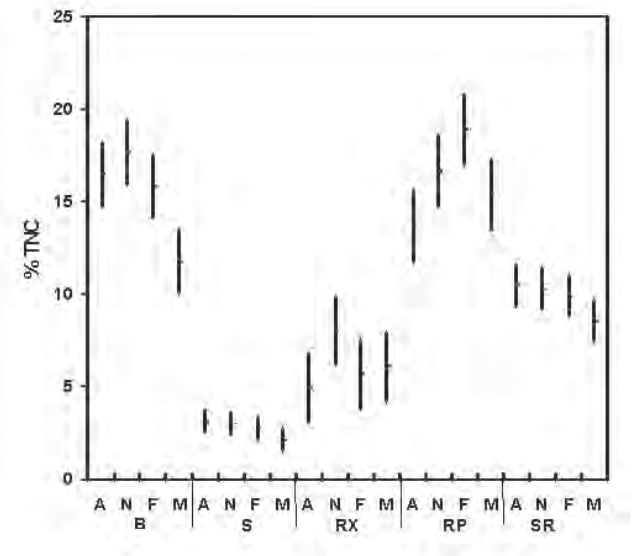

Figure 1. Average total nonstructural carbohydrate (TNC percent dry weight) of aspen tissue by month of collection (A = August, N = November, F = February, M = May) and tissue type (B = bark, S = sapwood, RX = root xylem, RP = root phloem, SR = small roots) with 95% confidence intervals.

Carbohydrates needed to reinitiate growth in the spring apparently accumulate in large aspen roots over the winter. TNC content of root phloem increased at each measurement following the onset of dormancy until February then dropped abruptly by the May collection. Even so, root phloem tissue contained considerably more carbohydrate in May than other tissues. Stem sapwood and small roots apparently do not play a major role in carbohydrate storage in aspen, since TNC content declined only slightly over winter in these tissues.

Root phloem contained more TNC in mid-winter than bark, indicating that the translocation of stored carbohydrate from the stem to the roots is not abrupt, but occurs gradually over winter. However, the maintenance of TNC in bark tissue until February seems contradictory. How could TNC levels not change in bark, yet increase in root phloem during the dormant season following leaf fall? A plausible explanation might be that aspen trees really aren't dormant all winter. Continuing carbon fixation from photosynthetic activity in the bark that is alive and does contain chlorophyll (Covington 1975, Strain and Johnson 1963) might be responsible. The high levels of carbohydrate available in aspen bark during the fall and mid winter also explain why aspen clones in deer and elk winter range are often repeatedly stripped of bark by the animals. A food source with over 15% available carbohydrate content would look very inviting when more nutritious forage is unavailable.

Management Implications

The results of this study underscore the importance of large roots in maintaining the health, vigor and vegetative reproductive capability of Rocky Mountain aspen clones. Carbohydrates that will be needed to reinitiate growth or suckering in the spring build up over the course of the winter in the phloem tissue of large roots. Although experience has shown that aspen clones often produce adequate numbers of suckers when they are harvested during the growing season, harvesting, burning, or otherwise killing aspen stems during or immediately after the growing season will certainly interrupt this pattern of carbohydrate translocation and may weaken the ability of the roots to produce new aspen suckers. This study indicates that prudent managers might chose to regenerate weakened or stressed clones during late fall or early winter to maximize root carbohydrate content and insure the best possible suckering response.

Similarly, managers must realize that aspen bark represents a viable source of food to browsing animals during late fall and mid winter and take appropriate precautions to avoid overuse of aspen during this period. Many aspen clones show evidence of animal barking without apparent damage. However, managers should be concerned when the majority of barked stems are infected or

dying and no new suckers are present in the stand. Such clones should be protected from animals to see if spontaneous suckering will occur, or regenerated by harvest, mechanical treatment (Shepperd 1996), or burning to stimulate suckering. New suckers in these clones will also require protection from browsing animals (Shepperd and Fairweather 1994) to insure adequate numbers survive to successfully regenerate the clone.

CONCLUSIONS

The aspen clones in this study exhibited a consistent pattern of dormant-season allocation of total nonstructural Carbohydrates. Midwinter carbohydrate content of aspen tissue was highest in root phloem, followed by bark, small roots, root xylem and stem sapwood respectively. Photosynthates were not immediately translocated to roots with the onset of dormancy, but persisted in bark tissue, possibly due to photosynthetic activity, throughout the fall before being transferred to root phloem tissue in mid- to late-winter. Small roots and woody tissue did not appear to play a significant role in carbohydrate storage. The patterns of carbohydrate allocation among aspen tissues that we found in this study help explain observed patterns of aspen herbivory, particularly the barking of mature stems and suggest management techniques that could be used to improve aspen regeneration success.

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