

The accretion of nonstructural carbohydrates changes seasonally in *Alnus incana* ssp. *rugosa* in accord with tissue type, growth, N allocation, and root hypoxia

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(Received November 23, 2004; Accepted March 14, 2005)

Abstract

Nonstructural carbohydrates afford immediate or reserve capacity in plants to support growth and metabolism, including that associated with symbiotic function. Seasonal patterns in the allocation and accumulation of nonstructural carbohydrates are complex in temperate deciduous trees and profoundly influence their growth, function, reproduction and ultimately their survival. Such patterns have been little studied in actinorhizal (*Frankia*-nodulated) plants. Our goal was to measure the seasonal accretion of nonstructural carbohydrates in leaves, stems, roots and nodules of *Alnus incana* ssp. *rugosa* in relation to temporal regimes of imposed root hypoxia induced by artificial flooding. Mortality was complete for early and late summer flooded seedlings while unflooded seedlings and those flooded in the fall survived. Nonstructural carbohydrate accretion was greater in foliage than in other tissues during early summer (May 10 – July 10) and late summer (July 10 – September 10), shifting to stems, roots and nodules in the fall (September 10 – November 8) in unflooded seedlings. Nearly three fourths of the seasonal nodule growth and nearly two thirds of the seasonal root growth occurred in the fall. The accumulation of nonstructural carbohydrates relative to growth rates increased continuously from summer to fall in all tissues except nodules, which grew mostly in the fall while maintaining overall plant levels of N fixation similar to those of the summer season. Contents of nonstructural carbohydrates declined from November to the following July consistent with the depletion of winter reserves to support rapid early leaf expansion and growth in the spring. Fall-flooded seedlings, however, had lower levels of nonstructural carbohydrates than unflooded plants both in November and the following July. The ratio of nonstructural carbohydrate accumulation to the accretion of fixed nitrogen increased from spring to fall in all tissues except nodules. In unflooded *A. incana* subsp. *rugosa* seedlings, foliar nonstructural carbohydrate contents were reduced by 76% with a concurrent increase in stem nonstructural carbohydrate contents, indicating efficient resorption of foliar nonstructural carbohydrates. Fall flooding arrested root and nodule growth and diminished foliar nonstructural carbohydrate resorption by 45%, further reducing seedling capacity to support subsequent spring growth. Sugar and starch accumulation vary seasonally in *A. incana* subsp. *rugosa* seedlings and hypoxic stress differentially inhibits survival, growth and symbiotic function according to season of occurrence.

Keywords: Actinorhiza, alder, nitrogen fixation, nonstructural carbohydrates, root hypoxia, symbiosis

1. Introduction

Total nonstructural carbohydrates (starch and soluble sugars, referred to as TNC) are biologically important because they provide immediate and reserve capacity for survival, growth, and metabolism of woody plants and their symbionts (Kobe, 1997; Canham et al., 1999). TNC reserves are essential for maintaining vigor during all stages of woody plant growth. The specific patterns of TNC

allocation and accumulation critically influence the survival, growth, physiological function (including symbiotic function), and reproduction of actinorhizal plants (nodulated by the diazotrophic actinomycete *Frankia*) (reviewed by Baker and Schwintzer, 1990) and other trees and shrubs (Kozłowski, 1992; Kozłowski and Pallardy, 1997). In fact, carbohydrate reserves, not biomass, have been closely correlated with survival of tree and shrub seedlings after overwintering or stress (Kobe, 1997; Canham et al., 1999).

In actinorhizal trees such as alders (*Alnus* spp.), the complex seasonal patterns of photosynthate allocation to various tissues and processes have not been elucidated. Yet

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these patterns are clearly important as a basis for understanding actinorhizal plant competitiveness and survival in nature (i.e. symbiotic function, vegetative growth, stump sprouting, response to herbivory, disease resistance, wound response, reproduction, chemical defense and recovery from drought or flood stress).

A previous report (Kaelke and Dawson, 2003) described seasonal patterns of N fixation as well as N and biomass allocation and accumulation in seedlings of speckled alder [*Alnus incana* ssp. *rugosa* (DuRoi) Clausen]. This small tree is an important component of the N economy of extensive wetlands of eastern North America (Furlow, 1979; Hurd, 1999; Hurd et al., 2001). This study includes results from subsequent analysis of the same tissue samples of Kaelke and Dawson (2003) to determine interrelated patterns of allocation and accumulation of TNC in speckled alder. Our objectives were, first, to ascertain the effects of seasonality together with early summer, late summer, and fall flooding (root hypoxia) on TNC accretion in leaves, stems, roots, and nodules in speckled alder; and secondly, to determine how experimental hypoxia treatments influence the accumulation of TNC relative to the previously reported accumulation of biomass and fixed nitrogen (Kaelke and Dawson, 2003).

We hypothesized that starch and soluble sugars increase throughout the growing season in speckled alder seedlings after spring depletion of carbohydrate reserves and that the relative magnitude of seasonal TNC accumulation and flux varies with tissue type and seasonality of flood stress (root hypoxia). Although speckled alder communities more commonly occur above the water table at the edges of wetlands (Hurd, 1999), we imposed seasonal flooding treatments in order to elucidate the differential effects of stress seasonality on plant processes such as TNC accumulation and allocation versus accumulation and allocation of biomass and fixed nitrogen. The results of our study indicate that there is interaction between the patterns of TNC accumulation and the seasonality of flood stress.

2. Materials and Methods

Study location, growing conditions, concentrations of the modified N-free nutrient solution, redox potential measurements, and sampling procedures were previously described (Kaelke and Dawson, 2003). To summarize, six replicates of bare-root, two-year-old, nodulated speckled alder seedlings were cultured beginning April 20 outdoors in central Illinois in 11.3-l plastic containers in a 1:1:1 (v/v/v) vermiculite: calcined clay: sand mixture. On May 10 alders were assigned to treatments randomly, with a subset destructively sampled to estimate initial dry mass of seedling tissues by regression with fresh weights. Alder seedling heights were determined but, because of variable heights and numbers of multiple stems per seedling, height measurements did not correlate well with seedling

phytomass and could not be used as a growth index. Seedlings were arranged in a randomized complete block design and assigned to one of five treatments in which seedlings were either unflooded or were flooded above the root collar in 500-l plastic tanks for one of the following periods: 1) early summer: May 10 – July 10, 2000; 2) late summer: July 10 – September 8, 2000; or 3) fall: September 8 – November 8, 2000. An additional group of alders were root-flooded continuously from May 10 – November 8, 2000. Unflooded alders were placed on saucers adjacent to the tanks. Alders were flooded in a modified N-free Hoagland's solution (Hoagland and Arnon, 1950; Kaelke and Dawson, 2003) while unflooded seedlings were leached weekly with the same solution. Four whole-plant destructive samplings occurred on May 10, July 10, September 8, and November 8, 2000. Surviving seedlings were over wintered in a cool room at 4°C, and placed outdoors on April 20, 2001 to resume growth until they were sampled on July 20, 2001.

Total nonstructural carbohydrates (starch and soluble sugars) were determined separately for all dried seedling tissues (leaves, stems, roots, and nodules) via an enzymatic process using the procedure of Smith (1969) as modified by Canham et al. (1999). Approximately 100 g of tissue sample containing starch and soluble sugars were digested and reduced to glucose equivalents. Digested samples were measured colorimetrically for glucose units on a spectrophotometer at 487 nm by the phenol-sulfuric acid method (DuBois et al., 1956).

Data derived from Kaelke (2003) and Kaelke and Dawson (2003) are employed for the calculation of ratios with TNC values and to profile the interrelated dynamics of structural carbohydrates and fixed nitrogen.

Data were log transformed and analyzed by one-way analysis of variance for each sample time (SYSTAT 1999). If significance ($P \leq 0.05$) was detected by ANOVA, treatment means were subsequently compared by a Fisher's protected LSD and reported as significantly different if $P \leq 0.05$.

3. Results

Total nonstructural carbohydrate content

Unflooded and fall-flooded alders had 100 percent survival through the fall and subsequent growing season until final sampling on July 20, 2001, while early summer- and late summer-flooded seedlings suffered complete mortality soon after the initiation of treatments. Unflooded seedlings from all treatments had similar TNC accumulation and tissue growth patterns and did not differ significantly from one another until the flooding treatments were imposed, hence data from the summer flooding treatments are not included in this report. Whole-seedling total nonstructural carbohydrate accretion and content were significantly reduced by fall root flooding relative to the unflooded treatment

(Tables 1 and 2). From November 8, 2000 to July, 2001 TNC accretion and content of leaves and nodules were significantly greater in unflooded seedlings than in the fall-flooded alders (Tables 1 and 2).

Seasonal TNC accretion and allocation

In the late summer period of 2000 and the summer growth season of 2001, most of the net TNC accretion in unflooded alders was partitioned into the leaves (Table 1). During the summer period, only about half of the new TNC in unflooded alders was allocated to perennial tissues while in the fall, about three-fourths of net, new TNC was partitioned into these tissues. TNC accretion in unflooded seedlings declined overall from November, 2000 to July, 2001. Fall-flooded seedlings had less net positive TNC accretion during fall and after over wintering during the subsequent summer growing season than unflooded

seedlings (Tables 1 and 2). In the fall, net TNC accretion of fall-flooded seedlings was in stems while, after over wintering the majority of summer TNC accretion occurred in the leaves.

In unflooded alders, the accretion of TNC relative to growth increased continuously from early summer to fall in all tissues except for nodules. In nodules this ratio declined greatly from summer to fall (Table 3). The ratio of TNC accumulation to growth for whole-seedlings was nearly identical for the summer of 2000 and the same period during 2001 after reaching a peak in the fall (Table 3). The accumulation of TNC relative to accretion of fixed N increased from early summer to fall in all tissues except nodules, in which it remained mostly steady from summer to fall (Tables 4 and 5). For entire seedlings, the ratio of TNC accretion to fixed N accretion increased greatly from early summer to fall and then decreased almost three-fold between fall and the subsequent July (Table 5).

Table 1. Initial total nonstructural carbohydrate (TNC) and net total nonstructural carbohydrate accretion (change in mean TNC content between adjacent harvests) of unflooded (A) and fall-flooded (B) *Alnus incana* ssp. *rugosa* seedlings and their components for early summer (May 10 – July 10, 2000), late summer (July 10 – September 8, 2000), fall (September 8 – November 8, 2000) growth periods, and through the following summer (November 8, 2000 – July 20, 2001) (n=6).

Time	Seedling TNC accretion (mg)	Leaf TNC accretion (mg)	Stem TNC accretion (mg)	Root TNC accretion (mg)	Nodule TNC accretion (mg)
A. Unflooded seedlings					
Initial, 2000	1037	517	323	173	25
Early summer	830	573	181	46	29
Late summer	6150	2899	1833	1194	223
Fall	14481*	3496*	5507*	4904*	573*
November – July 2001	4018*	6995*	-340*	-2185*	-452*
B. Fall-flooded seedlings					
Initial, 2000	1037	517	323	173	25
Early summer	792	516	219	24	32
Late summer	6858	2920	2160	1432	346
Fall	-30*	-2223*	2718*	-224*	-301*
November – July 2001	2368*	1610*	-116*	952*	-78*

*Denotes significant difference between corresponding mean values of unflooded and fall flooded alder seedlings using the F-test for ANOVA (P=0.05) and protected LSD test for means of log-transformed data (P=0.05)

Table 2. Biomass of unflooded (A) and fall-flooded (B) *Alnus incana* ssp. *rugosa* seedlings and their components (n=6). (Derived from Kaelke, 2003 and Kaelke and Dawson, 2003).

Date	Seedling (g)	Leaves (g)	Stems (g)	Roots (g)	Nodules (g)
A. Unflooded seedlings					
Initial – May 10, 2000	11.63	4.43	4.30	2.59	0.31
November 8	174.49*	42.25*	75.22*	49.42*	7.61*
Final – July 20, 2001	209.37*	56.59*	97.94*	51.18*	3.66*
B. Fall-flooded seedlings					
Initial – May 10, 2000	11.63	4.43	4.30	2.59	0.31
November 8	89.95*	10.25*	56.29*	20.74*	2.67*
Final – July, 20, 2001	96.09*	19.85*	53.45*	22.46*	0.33*

*Denotes significant difference between corresponding mean values of unflooded and fall flooded alder seedlings using the F-test for ANOVA (P=0.05) and protected LSD test for means of log-transformed data (P=0.05).

Table 3. Initial ratio and ratios of net total nonstructural carbohydrate (TNC) accretion [change in mean TNC content (mg) between adjacent harvests] versus absolute growth [dry biomass increase (g) between adjacent harvests] of unflooded (A) and fall-flooded (B) *Alnus incana* ssp. *rugosa* seedlings and their components for early summer (May 10 – July 10, 2000), late summer (July 10 – September 8, 2000), fall (September 8 – November 8, 2000) growth periods, and through the following summer (November 8, 2000 – July 20, 2001) (n=6). Negative ratios are not included. (Derived in part from Kaelke and Dawson, 2003).

Time	Seedling (mg:g)	Leaves (mg:g)	Stems (mg:g)	Roots (mg:g)	Nodules (mg:g)
A. Unflooded seedlings					
Initial, 2000	89	117	75	67	79
Early summer	48	65	29	26	60
Late summer	89	129	61	79	135
Fall	189	536	158	164	11
November – July 2001	52	124	–	–	–
B. Fall-flooded seedlings					
Initial, 2000	89	117	75	67	79
Early summer	49	61	42	13	53
Late summer	94	114	64	118	202
Fall	–	–	211	–	–
November – July 2001	386	168	–	553	–

Table 4. Total N Values of unflooded (A) and fall-flooded (B) *Alnus incana* ssp. *rugosa* seedlings and their components (n=6). (Derived from Kaelke, 2003 and Kaelke and Dawson, 2003).

Date	Seedling (mg)	Leaves (mg)	Stems (mg)	Roots (mg)	Nodules (mg)
A. Unflooded seedlings					
Initial – May 10, 2000	212	120	45	39	9
November 8	2383*	615*	926*	698*	143*
Final – July, 20, 2001	2576*	1242*	821*	441*	72*
B. Fall-flooded seedlings					
Initial – May 10, 2000	212	120	45	39	9
November 8	1087*	127*	675*	244*	41*
Final – July, 20, 2001	750*	279*	289*	172*	10*

*Denotes significant difference between corresponding mean values of unflooded and fall flooded alder seedlings using the F-test for ANOVA (P=0.05) and protected LSD test for means of log-transformed data (P=0.05)

Table 5. Initial ratio and ratios of net total nonstructural carbohydrate (TNC) accretion [change in mean TNC content (mg) between adjacent harvests] versus accretion of fixed N [change in mean N content (mg) between adjacent harvests] of unflooded (A) and fall-flooded (B) *Alnus incana* ssp. *rugosa* seedlings and their components for early summer (May 10 – July 10, 2000), late summer (July 10 – September 8, 2000), fall (September 8 – November 8, 2000) growth periods, and through the following summer (November 8, 2000 – July 20, 2001). Negative ratios are not included. (Derived in part from Kaelke and Dawson, 2003).

Time	Seedling (mg:g)	Leaves (mg:g)	Stems (mg:g)	Roots (mg:g)	Nodules (mg:g)
A. Unflooded seedlings					
Initial, 2000	5	4	7	4	3
Early summer	3	3	4	5	2
Late summer	7	6	8	8	8
Fall	14	–	9	10	6
November – July 2001	5	6	–	–	–
B. Fall-flooded seedlings					
Initial, 2000	5	4	7	4	3
Early summer	4	3	7	4	2
Late summer	7	5	8	12	13
Fall	–	–	8	–	–
November – July 2001	–	11	–	–	–

Table 6. Net foliar resorption of total nonstructural carbohydrate (TNC) in unflooded and fall-flooded *Alnus incana* ssp. *rugosa* seedlings from September 8 to November 8, 2000. Changes in means of leaf, woody tissue, and whole-seedling TNC content (mg TNC) between adjacent harvests were used to calculate the net relative proportions of leaf TNC retained in leaves, resorbed or lost with leaf drop.

Treatment	Net retention in leaves	Net resorption	Loss with leaf drop
Unflooded	24%	76%	0%
Fall-flooded	44%	55%	1%

Leaf TNC flux

Net translocation of TNC to woody tissues accounted for half of net leaf TNC flux in fall-flooded plants (Table 6). In unflooded controls, three-fourths of net seedling TNC content in the fall was translocated to woody tissues while one-fourth remained in the leaves (Table 6).

4. Discussion

In this study, the accretion and allocation of TNC in seedling tissues varied with season in unflooded speckled alder in general accord with patterns for other winter deciduous trees (Kozlowski, 1992; Larcher, 1995; Kozlowski and Pallardy, 1997). These patterns often include a spring and summer decline in TNC as buds break and leaves expand for production of photosynthate that will fuel the tree throughout the growing season. Typically, following the spring decline, there is a buildup of TNC reserves in perennial tissues affording woody plants a reserve of TNC for over wintering and depletion with vigorous growth the following spring.

In contrast with the general trend in other tissues, the ratios of TNC accretion to growth and TNC accretion to N fixed for nodules of unflooded speckled alder declined in the fall (Tables 3 and 5). Nodules of unflooded alders increased mass primarily in the fall when nearly three-fourths of the seasonal nodule growth and two-thirds of the seasonal root growth occurred (Table 2). Moreover, in the fall, unflooded nodules maintained rates of N fixation similar to those of the summer (Kaelke and Dawson, 2003). The roots continued to accumulate a large reserve of TNC through the end of the growing season while nodules were apparently expending carbohydrates for rapid fall growth and late-season N fixation. This trend underscores the importance of fall as a period of nodule development and activity in speckled alder.

All seedlings flooded in the fall survived while all alders flooded earlier in the growing season died. Perhaps this difference was owing to greater stress tolerance in the fall with lowered temperatures and respiratory rates as well as an

accumulation of TNC greater than that of the early summer- and late summer-flooded alders. The fall-flooded seedlings grew less and accreted less TNC than unflooded alder seedlings after over wintering and early-season growth the following year (Tables 2 and 4). The lack of vigor in the fall-flooded alders during the spring after flooding may have resulted in part from their reduced accretion and resorption of TNC in perennial tissues relative to unflooded seedlings (Tables 1, 3, and 6). Nodule growth through July 10 of the season subsequent to fall flooding was only 10% of that of unflooded seedlings (Table 2), which was a greater reduction by far than that occurring in other tissues of fall-flooded seedlings (~50%). This reflects the importance of fall for nodule growth and development in speckled alder seedlings. Overall seedling growth was halved while nitrogen content was reduced by two-thirds in fall-flooded alders relative to unflooded alders during the summer growing season following treatments (Tables 2 and 4). This may indicate that seedling growth during the season after fall flooding was reduced not only by a decrease in reserve carbohydrates, but also a severe inhibition of nodule growth resulting in reduced nitrogen fixation in the N-free substrate following fall hypoxia.

It is well known that carbohydrate reserves are important for survival during winter and for rapid spring growth in temperate deciduous woody angiosperms (Kozlowski, 1992; Larcher, 1995; Kozlowski and Pallardy, 1997). Yet it was previously determined that fall flooding greatly increased resorption of foliar nitrogen relative to unflooded speckled alder (Kaelke and Dawson, 2003), black alder, and other actinorhizal species (Dawson and Funk, 1981; Cote et al., 1986; Cote et al., 1989) which typically resorb a lower net percentage of foliar N than non-actinorhizal temperate deciduous trees. The increased resorption of N, but decreased net TNC resorption in fall-flooded speckled alder (Table 6), represents a puzzling differential effect of a seasonal environmental stress on two plant processes. Could the cause of this effect lie in a differential regulation by the plant hormone ABA, which is produced by plants under stress and underlies fall leaf senescence processes in temperate deciduous trees (Kozlowski and Pallardy, 1997)? Perhaps the reduced resorption of foliar TNC is a consequence of high energy demands for increased protein hydrolysis and N resorption in leaves of flooded plants? Also, flooded woody plants have reduced photosynthetic rates and would produce less photosynthate for TNC reserves (Kozlowski, 1997). The precise physiological mechanisms that account for this differential effect of fall hypoxia on plant processes and symbiotic functioning during the subsequent growing season remain to be elucidated.

Speckled alder seedlings are wetland plants that are not tolerant of flooding and waterlogged soils. Their niche is near small, cold streams and lakes with stable water levels (Hurd, 1999). Apparently, they avoid root hypoxia via a shallow rooting pattern and perhaps reduction of respiration

by low temperatures. Their absence from large river floodplains, which can be inundated for long periods during the growing season, may be the result of physiological limitations described in this and a previous study (Kaelke and Dawson, 2003). The seasonal patterns of TNC accretion in speckled alder differed according to the season of root hypoxia and may interact similarly with other stresses. The interaction of environmental stress with carbon and nitrogen allocation processes is likely an important determinant of speckled alder seedling survival, growth, symbiotic function, and distribution in nature.

Acknowledgements

We thank Kristin Pink for assistance with digestion and analysis of tissue samples and Jing-Shu Wang for providing technical assistance. This project was supported in part by USDA McIntyre-Stennis Project 1-6-56561 administered by the Illinois Agricultural Experiment Station.

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