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Seasonal carbohydrate dynamics and growth in Douglas-fir trees experiencing chronic, fungal-mediated reduction in functional leaf area

Research paper

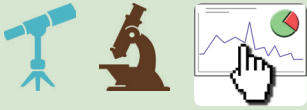# Seasonal carbohydrate dynamics and growth in Douglas-fir trees experiencing chronic, fungal-mediated reduction in functional leaf area

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Stored non-structural carbohydrates (NSCs) could play an important role in tree survival in the face of a changing climate and associated stress-related mortality. We explored the effects of the stomata-blocking and defoliating fungal disease called Swiss needle cast on Douglas-fir carbohydrate reserves and growth to evaluate the extent to which NSCs can be mobilized under natural conditions of low water stress and restricted carbon supply in relation to potential demands for growth. We analyzed the concentrations of starch, sucrose, glucose and fructose in foliage, twig wood and trunk sapwood of 15 co-occurring Douglas-fir trees expressing a gradient of Swiss needle cast symptom severity quantified as previous-year functional foliage mass. Growth (mean basal area increment, BAI) decreased by ~80% and trunk NSC concentration decreased by 60% with decreasing functional foliage mass. The ratio of relative changes in NSC concentration and BAI, an index of the relative priority of storage versus growth, more than doubled with increasing disease severity. In contrast, twig and foliage NSC concentrations remained nearly constant with decreasing functional foliage mass. These results suggest that under disease-induced reductions in carbon supply, Douglas-fir trees retain NSCs (either actively or due to sequestration) at the expense of trunk radial growth. The crown retains the highest concentrations of NSC, presumably to maintain foliage growth and shoot extension in the spring, partially compensating for rapid foliage loss in the summer and fall.

Introduction

Recent trends in drought-related tree mortality on a global scale have led to many questions regarding the role of non-structural carbohydrate (NSC) content as an indicator of overall tree vigor and demand for photosynthate (Sala et al. 2010, McDowell 2011, Ryan 2011, Johnson et al. 2012, Sala et al. 2012, Wiley and Helliker 2012). A first step toward resolving the uncertainty surrounding these issues could be achieved by developing a greater understanding of the different functions that may be performed by carbohydrate reserves stored within trees.

Traditionally, NSC accumulation has been viewed as a purely passive process signifying that the supply of photosynthate exceeds the demand from carbon sinks such as growth and respiratory metabolism. We are now aware that tree carbohydrate storage is multi-faceted, exhibiting patterns that suggest storage is partly passive and partly active (Chapin et al. 1990, Hoch et al. 2003, Körner 2003, Würth et al. 2005). Active

storage is defined as a prioritization of carbon allocation to storage over other carbon-dependent processes when resources are limited (Sala et al. 2012). Research by Bustan et al. (2011) has suggested that there is an active component to storage, such that in years of heavy fruit production the NSC content was not reduced in olive trees. In addition, Silpi et al. (2007) found that tapping rubber trees for carbon-rich latex actually caused NSC concentrations to increase at the apparent expense of growth.

Large carbohydrate reserves have been reported in mature trees across different climate types (Hoch et al. 2003, Würth et al. 2005), as well as different ecosystems and seasons (Hoch et al. 2002, Körner 2003), suggesting that trees allocate a substantial amount of assimilated carbon to storage (Hoch et al. 2003). Furthermore, it has been observed that trees have higher than average NSC concentrations and reduced growth during prolonged periods of mild-to-moderate stress such as long-term water deficit (Galvez et al. 2011, Muller et al. 2011, Woodruff and Meinzer 2011a) and low temperatures (Hoch and Körner 2009). Based on current knowledge, there are a few explanations for why trees do not mobilize their carbohydrate reserves for growth (Sala et al. 2012). First, during periods of environmental stress, such as water deficit, reduced cell turgor in expanding tissues would result in a constraint on sink strength resulting in reduced growth (Woodruff and Meinzer 2011b, Nikinmaa et al. 2013). Because growth is more sensitive to water deficit than photosynthesis is, photosynthesis would continue despite sharply reduced allocation to growth, resulting in an accumulation of NSCs (Muller et al. 2011). Second, carbohydrate reserves may become sequestered and no longer available for export due to compartmentalization that restricts their propensity to be loaded into the phloem (Quick et al. 1992) or impedes the access of enzymes to starch (Srichuwong and Jane 2007). Third, there may be an evolutionary advantage for trees to actively maintain a minimum level of carbohydrate reserves to support metabolism or to provide solutes for maintaining cell turgor and vascular integrity in an often stochastic and unpredictable environment, where water availability and growing conditions are not always optimal for plant functioning (Sala et al. 2012, Wiley and Helliker 2012).

Under conditions of water stress, it is difficult to determine whether the build up of NSCs is due to passive accumulation resulting from reduced sink demands, sequestration or active storage. To better understand the role of carbon storage in tree physiology, we must determine the extent to which storage is prioritized over growth in the absence of potentially confounding factors such as those resulting from water stress. The Douglas-fir (*Pseudotsuga menziessi* (Mirb.) Franco) disease called Swiss needle cast provides a unique natural experiment to examine the relative priorities of growth versus carbon storage in the absence of drought-related reductions in carbon assimilation, and water and phloem transport capacity. The disease is most prominent along the Oregon coast, a region with high rainfall and a maritime climate with a low risk of drought. Its prevalence and severity decrease sharply with increasing distance from the coast along a gradient of decreasing humidity and precipitation (http://sncc.forestry.oregonstate.edu/survey-maps). The pathogen that causes Swiss needle cast is an ascomycete (*Phaeocryptopus gaeumannii* (T. Rohde)) that colonizes Douglas-fir foliage. The spores are released in May and June, near the time of bud break for Douglas-fir, when they land and germinate on current-year needles (Stone et al. 2008). After about a year of incubation (in the most extreme cases of disease) during which the mycelia colonize the surface and inside of the needle, the mycelia produce fruiting bodies (pseudothecia) that block stomata. This blockage causes a significant decrease in stomatal conductance and photosynthesis (Manter et al. 2000). Once ~25–30% of stomata are blocked, the needle reaches a negative carbon balance, becomes chlorotic and abscises from the tree (Manter et al. 2000). There is a strong relationship between needle loss and the amount of pseudothecia present on the foliage of trees with Swiss needle cast (Manter et al. 2003), as well as between needle loss and reduced growth in diseased trees (Maguire et al. 2002, 2011). Growth can be reduced by as much as 20–50% in diseased stands (Maguire et al. 2002, Johnson et al. 2003, 2005). The fungus does not release any toxins, or cause any sort of known structural damage to the needle besides limiting the diffusion of carbon dioxide through the stomata and inside the needle. The disease has not yet been shown to result in tree mortality, and affected trees live for decades under a chronic reduction in carbon supply (Maguire et al. 2011).

The impact of Swiss needle cast on tree carbohydrate reserves is unknown, but if NSC accumulation in Douglas-fir has an active component, then this competing sink for photosynthate would exacerbate the impact of disease on growth and other carbon-dependent processes of lower or shared priority with storage. Swiss needle cast affords the opportunity to test for the relationship between growth, carbohydrate storage and disease symptoms because the disease involves a substantial reduction in carbon assimilation under conditions of low water stress at sites where the disease is prevalent. If NSC reserves remain largely undepleted under severe disease symptom severity, this result would provide evidence for prioritization of storage over growth, or that NSC has been sequestered. Additionally, if Swiss needle cast causes a greater relative decline in growth compared with NSC reserves, this would also provide evidence for prioritization of storage or storage sequestration as carbon assimilation is reduced. Finally, if NSC reserves become substantially depleted, this provides evidence for the ability of stored NSC to be mobilized when needed to maintain the physiological functioning and survival of trees.

The goals of this research were to: (i) determine how Swiss needle cast influences the partitioning of assimilated carbon between growth and carbohydrate reserves and to determine whether differences in disease severity influence the relative partitioning of carbon between these two sinks; (ii) identify any seasonal differences in the impact of the disease on the partitioning of carbon between growth and reserves; and (iii) establish the extent to which carbohydrate reserves are mobilized under natural conditions of high demand for carbon and low water stress. Based on previous research, we hypothesized that relative reductions in the growth of diseased trees would be greater than relative reductions in NSC reserves, and that seasonal fluctuations in NSC would be smallest in the most heavily diseased trees.

Materials and methods

Field site and sampling

A single Douglas-fir stand containing trees with a broad range of Swiss needle cast symptom expression was selected from an Oregon Department of Forestry unit (named Prairie Hill, 45.5°N 123.8°W) located on the Oregon coast near Tillamook at ~134 m above sea level. The region has a Pacific maritime climate with most of the mean annual precipitation of ~2800 mm falling between October and May. The mean annual temperature is ~10.1 °C, ranging from a mean of 5.7 °C in January to 15.3 °C in July. During the summer period from June to September, the mean precipitation is ~278 mm, the mean relative humidity hovers around 80% and the mean vapor pressure deficit generally remains <0.5 kPa (PRISM Climate Group, http://prism.oregonstate.edu, data summarized from 1980 to 2011). Previous studies along a coastal to interior transect have shown that Douglas-fir and other tree species growing in coastal sites similar to the one we selected do not

experience significant variation in their predawn water potential during the growing season (Runyon et al. 1994). The development of Swiss needle cast is favored by conditions that include high humidity and precipitation and relatively mild temperatures (Rosso and Hansen 2003, Latta et al. 2009, Zhao et al. 2011). Although *P. gaeumannii* spores have been detected in more interior stands that experience substantial summer drought, Douglas-fir trees in these stands do not show Swiss needle cast symptoms. Thus, selecting different stands along a climatic gradient to obtain a range of disease symptom expressions would have inevitably introduced several potentially confounding environmental variables into our study. Instead, we regressed tree response variables against a two-component index of symptom severity for the co-occurring trees at our study site (see below).

The stand was initially planted in 1990, with trees successively inter-planted through 1997. Trees were selected that ranged in planting date from 1990 to 1995, so the largest age difference between trees would be 5 years. The stand resides in a region of the Oregon coast where disease had been present for ~20 years before our study took place in 2012, so it is likely that the trees exhibited disease symptoms since planting. We non-randomly selected 15 trees (Table 1), with five trees roughly fitting into each of the three categories of overall needle retention (0–1.0, >1.0–2.0 and >2.0 years). Needle retention for each tree was estimated using a standard protocol in the field where we visually divided the crown into thirds and averaged the most common needle retention among branches in each third, and finally averaged the mean needle retentions of each third (e.g., Maguire et al. 2002).

In June of 2012, we collected two 5 mm cores to pith from opposite sides of each tree with an increment borer for annual growth measurement. We marked the sapwood boundary on each core to determine the average sapwood width. Cores

Table 1. Individual characteristics of the sampled trees in ascending order by basal area increment (BAI).

Tree ID	Mean BAI (cm^2)	DBH (cm)	Height (m)	Age (years)	Pseudothecia count[1] (%)	Mean foliage mass[1] (g)
7	3.4 (1.2)	9.1	14.0	19	36 (9)	0.05 (0.06)
1	4.8 (1.3)	10.2	17.9	20	25 (2)	0.23 (0.09)
3	4.9 (3.0)	9.9	20.1	15	27 (5)	0.25 (0.02)
5	5.0 (1.8)	11.5	14.5	20	22 (3)	0.24 (0.03)
8	5.7 (4.1)	10.5	8.7	17	17 (6)	0.19 (0.02)
13	6.7 (3.4)	12.1	15.9	14	29 (3)	0.36 (0.14)
14	6.8 (2.9)	12.7	14.7	18	23 (2)	0.18 (0.12)
10	7.6 (1.6)	14.0	15.4	20	35 (2)	0.06 (0.02)
9	9.1 (4.7)	13.5	11.0	16	22 (5)	0.36 (0.04)
2	14.1 (2.2)	18.5	23.1	20	5 (1)	0.36 (0.06)
15	16.8 (4.5)	21.5	20.6	19	22 (3)	0.66 (0.10)
4	16.8 (1.9)	18.3	23.0	17	16 (3)	0.34 (0.04)
12	17.5 (2.9)	19.1	24.1	17	13 (3)	0.50 (0.09)
11	23.3 (9.1)	21.2	18.4	21	5 (2)	0.62 (0.17)
6	26.3 (5.4)	25.8	17.9	20	9 (1)	0.36 (0.05)

Parentheses contain one standard deviation from the mean. DBH, diameter at breast height.
[1]One-year-old foliage.

were stored in paper straws for transport to the laboratory. One-year-old foliage was collected from three branches located in the sun-exposed lower-mid crown of each tree to assess the presence and abundance of pseudothecia on the needles (described below).

For NSC analyses, trunk sapwood tissue was sampled with an increment borer to a depth of 2 cm at 1.3 m height on opposite sides of each tree. Foliage and twig wood were sampled from two branches located in the sun-exposed lower-mid crown of each tree. The branches were divided immediately into segments representing growth from Years 2011, 2010 and 2009 (i.e., 1-, 2- and 3-year-old tissues). Foliage was removed from the twigs, and the total foliage mass (at field moisture content) was recorded for each segment from each branch and averaged for each branch, and then each tree (mean foliage mass, g). All samples (two trunk samples, and foliage and twig samples from 2011, 2010 and 2009) were immediately placed into sealed plastic bags and onto dry ice in a cooler. Sampling was always conducted between late morning and early afternoon. We did not attempt to sample roots because it would not have been possible to associate a given sample with a specific individual without conducting extensive, destructive excavations. In view of our previous observations showing substantial seasonal variation in NSC content in Douglas-fir trees (Woodruff and Meinzer 2011a), sampling took place three times over a seasonal cycle in 2012: around bud break (June 18), mid-summer (July 31) and late summer (September 4).

Disease severity assessment

The presence and abundance of fungus on 1-year-old foliage was determined by visual estimates of pseudothecia emerging from stomata using standard techniques (e.g., Manter et al. 2005). Estimates of the percentage of stomata that were occluded with pseudothecia (i.e., pseudothecia counts) for each of the three branches collected in the field were calculated by averaging pseudothecia counts from three positions of 10 randomly selected needles per branch. At each position, one on each longitudinal third of the needle, pseudothecia counts were conducted by selecting a region within each position with a random number table and visually counting the number of pseudothecia emerging from 50 consecutive stomata above the needle midrib and 50 consecutive stomata below the needle midrib (100 stomata total in each position). The percentage of pseudothecia-occluded stomata of each needle was averaged across 10 needles per branch, and averaged again across the three branches per tree to calculate the tree pseudothecia count.

To account for the amount of functional 1-year-old foliage remaining on a tree relative to its pseudothecia count, we created an index for disease symptoms by multiplying the percentage of non-occluded stomata (100 − % occluded stomata) by the average 1-year-old foliage dry mass per growth increment for each tree (i.e., functional foliage mass, g). We intentionally used the functional foliage mass as a measure of disease severity instead of the needle retention technique (described above) due to its advantages of being an integrated measure of two symptoms (pseudothecia abundance and foliage mass) and therefore more objective and precise in quantifying disease severity.

Chemical analyses

Trunk sapwood, twig wood and foliage samples were stored in a −20 °C freezer before being microwaved for 90 s to stop all enzymatic activity, oven dried for 72 h at 65 °C and ground to a fine powder with a ball mill. The dried and ground tissue samples were analyzed for the content of four components: starch, sucrose, glucose and fructose together, and total NSC following the procedure described by Woodruff and Meinzer (2011a). Deionized water was added to the samples, which were then heated in covered vials over steam for 90 min to extract NSC. After enzymatic conversion of glucose and fructose to gluconate-6-phosphate, the concentration of free glucose was determined photometrically on a 96-well microplate photometer (Multiscan FC, Thermo Scientific, Waltham, MA, USA). Samples were analyzed before and after enzymatic treatments of sucrose digestion by invertase (45 min reaction) and starch and sucrose digestion by amyloglucosidase (15-h reaction). Photometric analysis was based on the absorbance of samples at 340 nm in the solution with reference to the absorbance of a glucose reference solution. The combination of glucose and fructose content was determined from the photometric analysis of sample solutions without invertase or amyloglucosidase enzymatic treatment. Sucrose content of the samples was determined by subtracting the combination of glucose and fructose content from the glucose concentration of sample solutions following invertase enzyme treatment. Total NSC was determined from the amyloglucosidase reaction mixture, which contained the original concentrations of free glucose and fructose, plus glucose and fructose liberated from starch and sucrose. Inclusion of sucrose standards in each set of samples subjected to amyloglucosidase treatment indicated that amyloglucosidase hydrolyzed sucrose as well as starch. Starch content of the samples was determined by subtracting the glucose content of the sample solution following invertase enzyme treatment from the total NSC content. All NSC, starch, sucrose, and glucose and fructose values for each tissue of each tree are averages of the two sampled branches collected in the field at each sampling date and are presented in units of percentage of dry weight (% dry weight).

Growth analyses

Two increment cores per tree were collected for growth analyses. These were air-dried, glued to wooden mounts with the transverse face upward and sanded with progressively finer

sandpaper until annual rings were distinguishable. We measured ring widths to a precision of 0.01 mm using a stereo microscope interfaced with a Velmex rotating measuring table and Measure J2X® software. We used the cross-dating program COFECHA (Holmes 1983, Grissino-Mayer 2001) to identify possible missing and false rings. The ring widths from each tree were averaged for each year and basal area increment (BAI) was calculated assuming circularity of consecutive growth increments. We used the average BAI from 2000 to 2011 for each tree to represent tree growth (mean BAI, cm^2).

Statistical analyses

To determine the effect of disease symptom severity on the relative priority of storage versus growth, we created an index by taking the ratio of the relative values of NSC to those of BAI for each tree (NSC/BAI; values were normalized with respect to their highest values). Because the NSC/BAI metric combines different units of measurement for NSC and BAI, we normalized the two measures to better demonstrate changes in the relative priorities of storage and growth as symptom severity varied among trees. All analyses exploring the relationship between disease explanatory variables (i.e., pseudothecia count and functional foliage mass) and the response variables of interest (i.e., BAI, foliage mass, total NSC, starch, sucrose, glucose and fructose, NSC/BAI) in the trunk, twigs and foliage were made using simple linear regression for all 15 trees, unless otherwise noted. Assumptions of simple linear regression were checked by plotting residuals of the response variable versus fitted values to assess the equality of variance and were also plotted within a normal probability plot to assess normality. Paired t-tests were performed to test for the differences in foliage mass, total NSC, starch, sucrose, and glucose and fructose from June to September for all 15 trees. Assumptions of the paired t-tests were checked by evaluating histograms of the distribution of the sample response variables.

Results

Disease severity assessment

The mean 1-year-old foliage dry mass per growth increment ranged from 0.05 to 0.66 g, and the pseudothecia count ranged from 5 to 36% stomata occluded in the 15 trees studied (Table 1). The maximum observed stomatal occlusion was comparable to the estimated threshold at which needle abscission would likely occur (Manter et al. 2000; Table 1). The mean mass of 1-year-old foliage (averaged across the three sampling dates) was ~0.26 g, whereas the mean growing season mass of 3-year-old foliage was only ~0.04 g (Figure 1). The mean difference in foliage mass between the two age classes was ~0.23 g (95% CI [0.16, 0.30]). There was no relationship between the functional foliage mass and the mean foliage
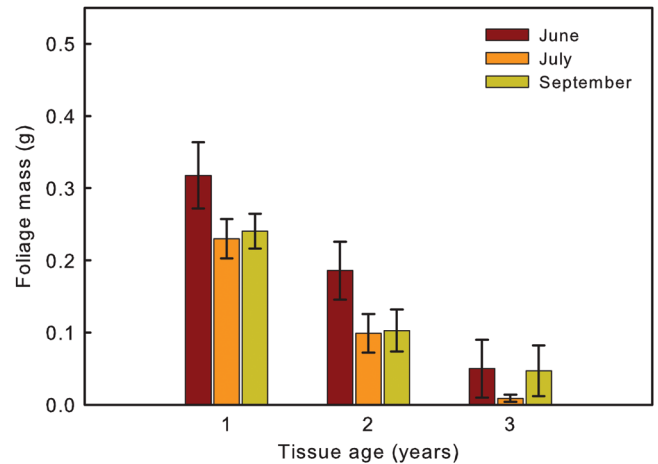

Figure 1. Mean foliage mass per branch growth increment for each sampled foliage tissue age at each sampling date (bars represent one standard error).

mass per increment of new foliage (2012 cohort) at the end of the 2012 growing season (September sampling).

The 15 trees are ordered in Table 1 from the lowest to the highest BAI. There was a significant linear relationship between the pseudothecia count and both mean BAI ($R^2 = 0.58$, $P = 0.0009$) and mean 1-year-old foliage mass ($R^2 = 0.40$, $P = 0.01$), such that a 1% increase in pseudothecia abundance of 1-year-old foliage reflected a 0.58-cm^2 decrease in mean BAI (95% CI [0.29, 0.87]) and a 0.012-g decrease in mean foliage mass per growth increment (95% CI [0.003, 0.02]). There was a very strong relationship between the functional foliage mass and the mean BAI ($R^2 = 0.63$, $P = 0.0004$, Figure 2a). The rest of the analyses described below use functional foliage mass as a measure of disease severity for each tree.

NSC and growth analyses

There was a significant linear relationship between the functional foliage mass and the mean growing season (i.e., averaged over the three sampling dates) trunk NSC content ($R^2 = 0.35$, $P = 0.02$), and without the outlier (Tree 6), the relationship was substantially stronger ($R^2 = 0.54$, $P = 0.003$, Figure 2b). The trend in trunk NSC with functional foliage mass was similar for June and July, though the relationship was not significant in September (data not shown). Only two trees had average sapwood widths slightly less than the trunk sampling depth (i.e., 2 cm). We compared the trunk NSC concentrations of these two trees, as well as between other trees that had similar sapwood widths, and found no relationship between sapwood width and trunk NSC concentration (data not shown). The relationship between the functional foliage mass and the relative mean trunk NSC/BAI was also significant ($R^2 = 0.48$, $P = 0.004$, Figure 2c), indicating that with increasing disease symptom severity and therefore constraints on carbon supply,
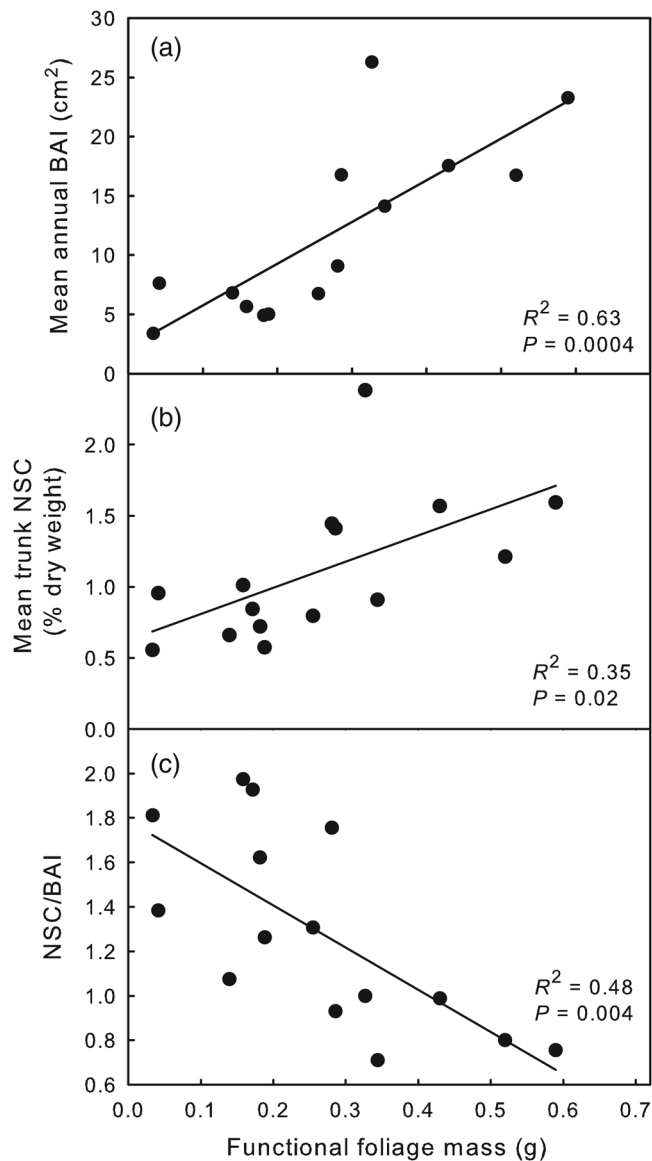
Figure 2. Mean annual 2000–2011 BAI (a), mean trunk NSC content (b) and relative mean trunk NSC/BAI (c) versus functional foliage mass. Each point represents one tree. Simple linear regression analyses are shown. Relative NSC/BAI was calculated by taking the ratio of NSC to BAI after normalizing both measures with respect to their maximum values.

the relative reduction in basal area growth was greater than that of trunk NSC storage. The trend in relative mean trunk NSC/BAI with functional foliage mass was similar for each sampling date (data not shown).

The relationship between NSC concentration and tissue age for twigs and foliage did not differ with disease severity on any of the sampling dates (for data, see Tables S1, S2 and S3 available as Supplementary Data at *Tree Physiology* Online). For this reason, and because the functional foliage mass was only evaluated for prior year foliage, the following analyses with foliage and twigs only include 1-year-old tissues. The mean concentration of NSC was approximately four to five times higher in the crown (twigs and foliage) than in the trunk across trees (Table 2, Figure 3). There were no statistically significant relationships between the functional foliage mass and the mean growing season NSC content of twigs ($R^2 = 0.11$, $P = 0.23$) or foliage ($R^2 = 0.001$, $P = 0.91$, Figure 4a and b) or any individual sampling date (data not shown). However, there were strong positive relationships between the functional foliage mass and the ratio of mean trunk NSC to both twig NSC ($R^2 = 0.33$, $P = 0.02$) and foliage NSC ($R^2 = 0.32$, $P = 0.03$) (Figure 4c and d). These relationships were considerably stronger without the outlying Tree 6 ($R^2 \geq 0.48$, $P \leq 0.006$). The trend in trunk NSC to twig and foliage NSC, respectively, with functional foliage mass was similar for each sampling date (data not shown).

Starch was the largest component of NSC in the trunk, followed by glucose and fructose, and then sucrose (Table 2, Figure 3). In June, there was a significant linear relationship between functional foliage mass and trunk NSC content ($R^2 = 0.38$, $P = 0.02$), as well as with trunk starch content ($R^2 = 0.44$, $P = 0.007$). There were significant positive linear relationships between functional foliage mass and trunk sucrose content near the middle and end of the growing season in July ($R^2 = 0.47$, $P = 0.004$) and September ($R^2 = 0.38$, $P = 0.01$). There was no relationship between functional foliage mass and the mean growing season trunk glucose and fructose content ($R^2 = 0.04$, $P = 0.44$). In contrast to the patterns of NSC constituents observed in trunks, there did not appear to

Table 2. Mean concentrations (% dry weight) of total NSC, starch, sucrose, and glucose and fructose (Gluc/Fruc) of the trunk, and current-year twigs and foliage of the sampled trees from each sampling date.

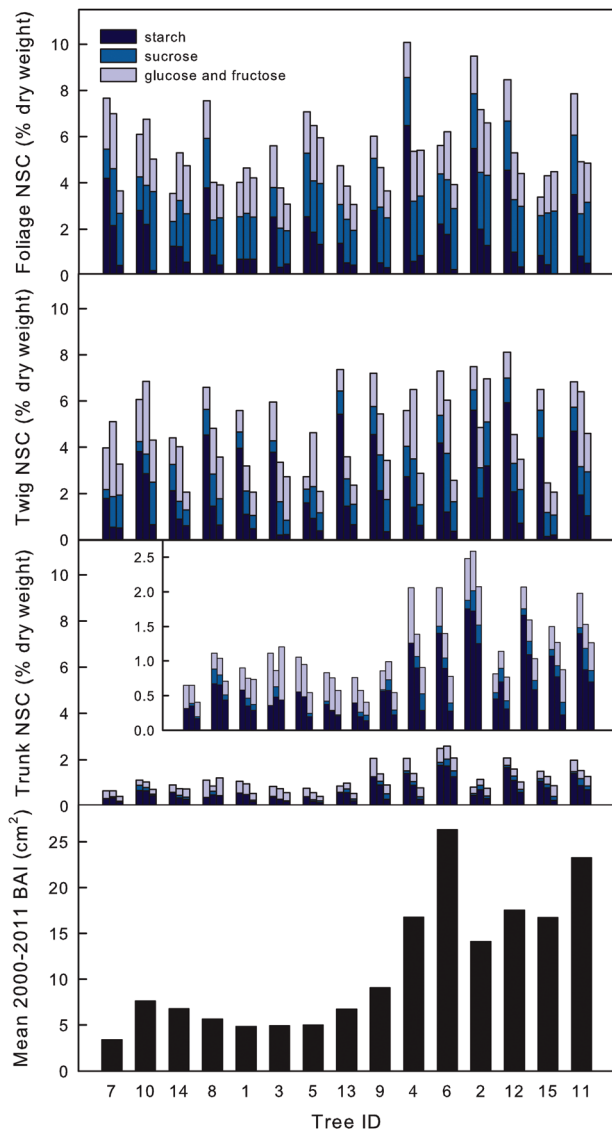|	Trunk				Twigs				Foliage			
	NSC (%)	Starch (%)	Sucrose (%)	Gluc/ Fruc (%)	NSC (%)	Starch (%)	Sucrose (%)	Gluc/ Fruc (%)	NSC (%)	Starch (%)	Sucrose (%)	Gluc/ Fruc (%)
June	1.34 (0.16)	0.85 (0.13)	0.06 (0.02)	0.44 (0.05)	6.11 (0.38)	3.94 (0.35)	0.91 (0.08)	1.26 (0.11)	6.48 (0.53)	3.01 (0.43)	1.92 (0.13)	1.56 (0.10)
July	1.17 (0.13)	0.69 (0.10)	0.14 (0.02)	0.33 (0.03)	4.79 (0.34)	1.34 (0.19)	1.35 (0.12)	2.09 (0.21)	5.32 (0.30)	1.14 (0.17)	2.11 (0.09)	2.07 (0.11)
September	0.85 (0.11)	0.38 (0.07)	0.11 (0.02)	0.36 (0.04)	3.22 (0.34)	0.71 (0.19)	1.17 (0.12)	1.34 (0.11)	4.45 (0.26)	0.55 (0.10)	2.37 (0.14)	1.54 (0.11)

Parentheses contain one standard error from the mean.

Figure 3. Mean foliage, twig and trunk NSC concentrations for current-year foliage and twigs, and trunk tissue, for each sampling date (ordered June, July and September for each tree), and mean 2000–2011 BAI for each sampled tree. Concentrations of NSC components (starch, sucrose, and glucose and fructose) are shown for each bar (see color legend). Trees are ordered by increasing the functional foliage mass. Inset in the trunk NSC panel enlarges the view of trunk NSC values.

be any relationship between NSC constituents in twigs and foliage and disease symptom severity at each sampling date (Table 2, Figure 3). There were no significant relationships between functional foliage mass and mean growing season starch content of twigs ($R^2 = 0.18$, $P = 0.12$) or foliage ($R^2 = 0.007$, $P = 0.77$), or between functional foliage mass and the mean growing season glucose and fructose content of twigs ($R^2 = 0.06$, $P = 0.38$) or foliage ($R^2 = 0.03$, $P = 0.54$). There were marginally significant relationships between the functional foliage mass and the mean growing season sucrose content of twigs ($R^2 = 0.23$, $P = 0.07$) and foliage ($R^2 = 0.20$, $P = 0.09$).

Non-structural carbohydrates fluctuated seasonally in all tissue types (Table 2, Figure 3). Seasonal fluctuation was not related to functional foliage mass, such that there were no statistically significant relationships between functional foliage mass and the percent decrease in mean NSC from June to September in the trunk ($R^2 = 0.03$, $P = 0.57$), twigs ($R^2 = 0.05$, $P = 0.40$) or foliage ($R^2 = 0.001$, $P = 0.92$). From June to September, mean trunk NSC content decreased by 0.49% dry weight (95% CI [0.26, 0.71]), starch content decreased by 0.47% dry weight (95% CI [0.26, 0.68]) and sucrose content increased by 0.05% dry weight (95% CI [0.00, 0.10]). There was no statistically significant change in mean trunk glucose and fructose over the growing season. Mean twig NSC content decreased by 2.89% dry weight from June to September (95% CI [2.05, 3.72]) and starch content decreased by 3.23% dry weight (95% CI [2.54, 3.93]). There was no statistically significant change in mean twig sucrose or glucose and fructose content over the growing season. Mean foliage NSC content decreased by 2.03% dry weight from June to September (95% CI [1.03, 3.03]), foliage starch content decreased by 2.46% dry weight (95% CI [1.58, 3.33]) and foliage sucrose content increased by 0.46% dry weight (95% CI [0.13, 0.79]). There was no statistically significant change in mean foliage glucose and fructose over the growing season.

Discussion

The high degree of disease severity at the site was evident in the reduction in mean growing season foliage mass by ~86% from the 1-year-old cohort to the 3-year-old cohort, when compared with healthier trees in the region, which have been reported to show a reduction in foliage mass of ~25% over the same cohort ages (Weiskittel et al. 2006). In addition, there was no evidence that trees with higher disease symptom severity produced more or less needle mass on individual shoot increments during the 2012 growing season. The relationships between disease severity (previous year functional foliage mass) and Douglas-fir growth and carbohydrate reserves observed in this study indicate that whereas the reduction in carbon assimilation caused by Swiss needle cast results in reduced annual growth and NSC in the trunk, there was no apparent relationship between disease symptom severity and NSC concentrations in twigs and foliage or new foliage growth. Retaining NSC in the crown appeared to have a greater priority than exporting the photosynthate for diameter growth in the trunk (Figures 2 and 4). Thus, the disease appeared to force trees to sacrifice stem growth and stem NSC storage to maintain crown growth under conditions of rapidly abscising foliage. Finally, we also found that carbohydrate reserves were depleted throughout the growing season to different extents in each tissue type (Table 2, Figure 3).
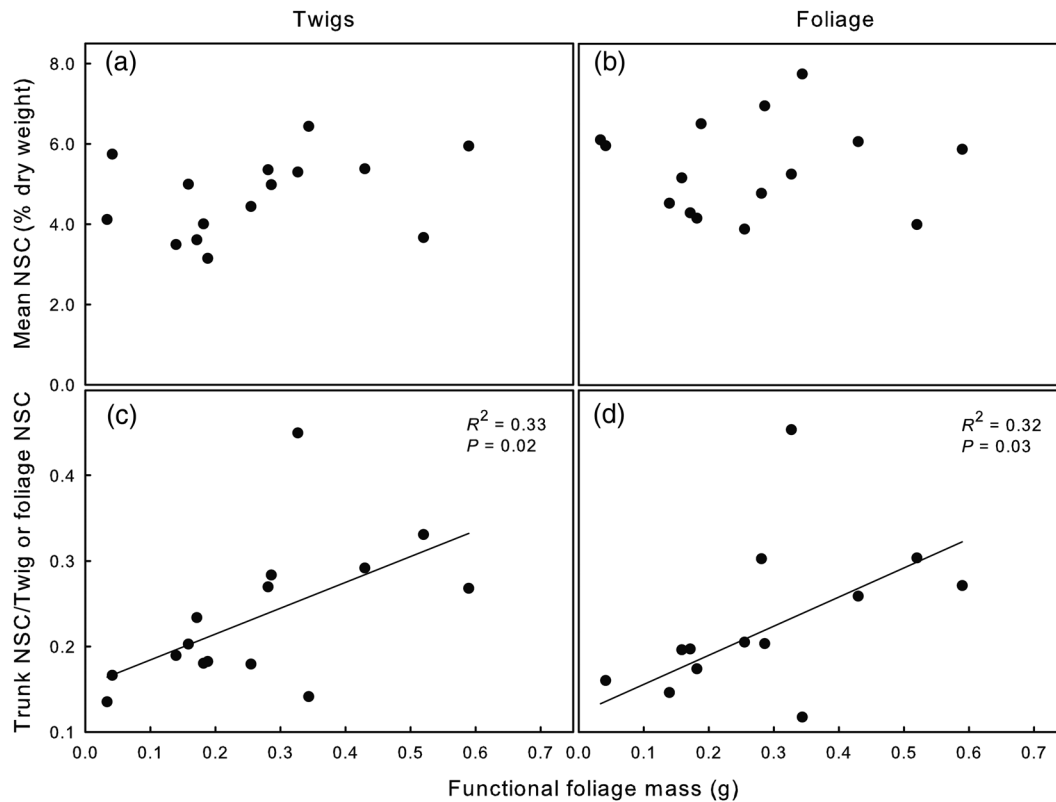
Figure 4. Mean twig (a) and foliage (b) NSC content and mean trunk NSC/twig NSC (c) and trunk NSC/foliage NSC (d) versus functional foliage mass. Each point represents one tree. Simple linear regression analyses are shown in (c) and (d).

The basis for seasonal trends in NSC of conifers is fairly well studied and understood. Starch concentrations are relatively high before bud break and are drawn upon over the growing season to aid in flushing and axial growth, while free sugar concentrations (i.e., sucrose, glucose and fructose) progressively increase and peak in autumn (Chung and Barnes 1980, Hansen and Beck 1990, 1994, Fischer and Höll 1991, Kibe and Masuzawa 1992, Oleksyn et al. 2000, Schaberg et al. 2000, Hoch et al. 2003, Bansal and Germino 2009). Starch is the main carbohydrate storage compound for conifers and is clearly a dynamic pool that accumulates over the winter and ensures that the tree will have adequate carbon reserves for producing a new cohort of needles during the following growing season (Webb 1981). Our results suggested that trunk radial growth was strongly dependent on current-year photosynthate rather than stored NSC. The total amount of NSC stored in the outer 2 cm of sapwood at the beginning of the growing season in June was estimated to be sufficient to supply only ~7% of the carbon required to generate a mean annual radial growth increment at the height where NSC samples were collected (data not shown). Interestingly, this percentage was nearly constant across the range of disease symptom severity in the 15 trees studied.

Although trunk and crown tissues in this study both exhibited the seasonal trend described above, they each behaved differently in response to limited carbon availability under relatively low water stress. There was a clear decrease in trunk NSC with increasing disease symptom severity, primarily due to a decrease in starch, which represented ~45–63% of total NSC over the growing season. In addition, there was a strong positive relationship between functional foliage mass and trunk sucrose at the end of the growing season. Thus, it appears that trunk storage (in the form of starch) was sacrificed with decreasing carbon availability, resulting in lower NSC values in more diseased trees. The higher trunk sucrose concentrations in healthier trees at the end of the growing season could be a consequence of healthier trees having more starch to mobilize at the end of the growing season for stem maintenance. The relative reduction in annual radial growth was greater than that of NSC concentrations with increasing disease-induced reduction in carbon assimilation, implying a priority of storage over growth in the trunk. Considering the lack of a relationship between disease symptom severity and trunk NSC in September, it is possible that all trees maintained a minimum threshold of NSC in the trunk, and trees with higher disease symptom severity reached the threshold earlier in the season due to the reduction in carbon assimilation associated with disease. The decrease in total NSC over the growing season was primarily attributable to the depletion of starch, which made up ~64 and 46% of total NSC in June for twigs and foliage,

respectively, and only ~22 and 12% of total NSC by September for twigs and foliage, respectively. In contrast to the trunk, there was no relationship between NSC and disease severity for twigs and foliage.

Taken together, there seemed to be a greater priority to retain NSC in the crown over exporting NSC to supply diameter growth in the trunk. Figure 4c and d demonstrates how the ratios of trunk NSC to twig and foliage NSC were lower for trees with higher disease symptom severity. Severely diseased trees appeared to be locked into a cycle of maintaining similar concentrations of NSC in twigs and foliage to those of less diseased trees to sustain the construction of new photosynthetic tissue and supporting branches that partially compensate for early needle abscission at the apparent expense of greater stem growth and increased mechanical support in the trunk. This could potentially explain why trees with Swiss needle cast can survive for decades under a chronic reduction in carbon supply. Thus, our results suggest that carbon storage is either partly active and/or a portion of carbohydrate reserves are sequestered, which has implications for our understanding of the role of NSC as a passively accumulated or actively managed pool. Non-structural carbohydrate concentration in a particular tissue does not necessarily indicate that a tree is healthy or has abundant access to carbon, or even that growth is not suffering. Furthermore, we have observed how various regions of a tree behave differently in response to a reduction in carbon supply.

An increase in the priority of carbon storage over other processes like growth during periods of limited carbon supply could have implications for other processes with a possible lower priority than storage. In the case of reduced carbon assimilation caused by Swiss needle cast, previous studies have provided evidence for changes in tree defense and interaction with biotic mortality agents, root NSC storage and mycorrhizal relationships. Kelsey and Manter (2004) found that trees with moderate-to-severe Swiss needle cast had a lower wound-induced oleoresin flow, as well as less ethanol and monoterpene production, compared with healthy Douglas-fir. The authors ascribed these results to reduced availability of photosynthate since carbohydrates are an important building block of defense compounds. Furthermore, the significant level of carbohydrates used by roots for growth, maintenance and mycorrhizal relationships, accounting for ~73% of net primary productivity in coniferous temperate forests (Grier et al. 1981, Fogel and Hunt 1983), should also be affected by reduced carbon supply. There is evidence that a reduction in carbon supply from the crown (e.g., from defoliation) can lead to depleted NSC reserves in roots of conifers (Webb and Karchesy 1977, Oleksyn et al. 2000). Although we currently do not know the effects of Swiss needle cast on Douglas-fir root carbohydrate reserves, our results implying that stem NSC levels are sacrificed to

maintain high concentrations in the crown suggest that roots may also have reduced NSC. Lower root NSC concentrations could have significant implications for root growth, which could restrict soil exploration and the ability to obtain soil water and nutrients. Reduced NSC export might also limit root exudation, which plays a vital role in the cycling of soil organic matter (Millard et al. 2007) and attracting and establishing symbiotic relationships with mycorrhizae (e.g., Graham 1982), which have been shown to receive ~30% of total assimilate from the host tree (reviewed by Soderström 2002). Luoma and Eberhart (2006) observed that Douglas-fir stands with Swiss needle cast have a lower density and diversity of ectomycorrhizal fungi than typically found in healthy stands, suggesting that the reduction in photosynthate associated with the disease could be affecting these below-ground relationships.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

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Conflict of interest

None declared.

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