

Height-related growth declines in ponderosa pine are not due to carbon limitation

ANNA SALA¹ & GÜNTER HOCH²

¹Division of Biological Sciences, The University of Montana, Missoula, MT 59812, USA and ²Institute of Botany, University of Basel, Schönbeinstrasse 6, CH-4056 Basel, Switzerland

ABSTRACT

Decreased gas exchange as trees grow tall has been proposed to explain age-related growth declines in trees. We examined changes of mobile carbon stores (starch, sugars and lipids) with tree height in ponderosa pine (*Pinus ponderosa*) at two sites differing in water availability, and tested the following hypotheses: (1) carbon supply does not become increasingly limited as trees grow tall; rather, the concentration of mobile carbon compounds increases with tree height reflecting greater reductions of carbon sink activities relative to carbon assimilation; and (2) increases of stored mobile carbon compounds with tree height are greater in drier sites. Height-related growth reductions were associated with significant increases of non-structural carbohydrates (NSC) and lipid concentrations in all tissues in the upper canopy and of NSC in the bole. Lipid concentrations in the bole decreased with tree height, but such decrease is not necessarily inconsistent with non-limiting carbon supply in tall trees. Furthermore, we found stronger increases of mobile carbon stores with tree height at the dry site relative to the moist site. Our results provide first direct evidence that carbon supply does not limit growth in tall trees and that decreases of water availability might negatively impact growth processes more than net-photosynthesis.

Key-words: *Pinus ponderosa*; carbon source–sink relations; height-related growth; hydraulic limitation hypothesis; lipids; mobile carbon reserves; non-structural carbohydrate.

INTRODUCTION

Trees are the tallest living organisms on earth with heights exceeding 120 m. The selective pressures driving increases in tree height (e.g. Falster and Westoby 2003) and the challenges of growing tall and against gravity have received much attention in the literature (e.g. Friend 1993; Ryan & Yoder 1997; Mencuccini 2003; Koch *et al.* 2004; Ryan, Phillips & Bond 2006; Niklas 2007). In particular, constraints on water transport as trees grow tall have been the focus of intense recent research (reviewed by Ryan *et al.* 2006; see also Woodruff *et al.* 2007). Such emphasis followed the initial suggestion by Friend (1993) and Yoder *et al.* (1994)

Correspondence: A. Sala. Fax: +1 406 243 6009; e-mail: sala@mso.umt.edu

that as trees grow tall, leaf-level gas exchange becomes increasingly limited due to the higher resistance of the longer hydraulic pathway which, along with the independent negative effects of gravity (e.g. Koch *et al.* 2004; Ishii *et al.* 2008), impose greater xylem tensions and subsequent risk of xylem cavitation. Research was subsequently propelled by the hydraulic limitation hypothesis (HLH) proposed by Ryan & Yoder (1997) stating that tall trees close stomata to a greater extent than short trees, thereby preventing excessive xylem tensions but also reducing their photosynthetic and, presumably, growth potential. As initially stated, the HLH was proposed to explain well-documented age-related decreases in productivity in individual trees and stands after canopy closure (Ryan, Binkley & Fownes 1997). It was advanced as an alternative to the historical view that age-related growth declines were the result of an imbalance between photosynthetic and non-photosynthetic tissues (Kira & Shidei 1967), a view challenged by empirical and modeling results (see Ryan *et al.* 2004). The appeal of the HLH further increased as other alternative hypothesis to explain age- and size- related growth declines (including nutrient limitations and genetic constraints) failed to fully explain this phenomenon (Ryan *et al.* 1997, 2004; but see Martínez-Vilalta, Vanderklein & Mencuccini 2007). Based on the intense body of research that followed, Ryan *et al.* (2006) concluded that in spite of structural, anatomical and physiological mechanisms to offset path length effects and to cope with the negative effects of gravity, hydraulic constraints on gas exchange are very common in tall trees. However, they found no evidence supporting that reductions in carbon assimilation in tall trees are responsible for growth reductions, leaving unresolved the mechanisms responsible for the age-related decline in tree and forest productivity. Although much progress has been made on understanding hydraulic constraints as trees grow tall and the trade-offs associated with subsequent compensating mechanisms (Magnani, Mencuccini & Grace 2000; Sala 2006), the lack of support for the HLH, as initially stated, led to revise the critical assumption that growth is limited by carbon availability (Ryan *et al.* 2006). Indeed, a body of recent research indicates that reduced turgor during cell expansion at the top of tall trees is responsible, at least in part, for reduced cell extension and growth (Bond *et al.* 2007; Greenwood *et al.* 2008; Ishii *et al.* 2008; Meinzer, Bond & Karanian 2008). If so, growth in tall trees may not be limited by

carbon availability but rather by factors that limit cell growth and carbon demand (Bond *et al.* 2007; Greenwood *et al.* 2008).

Consistent with the recent recognition that growth in tall trees may not be limited by carbon availability, a body of parallel but independent research provided evidence that mature trees store large amounts of non-structural mobile carbon compounds (i.e. starch, low molecular sugars, lipids), which are never depleted throughout the season (Hoch, Richter & Körner 2003). Because the concentrations of mobile carbon compounds in plant tissues are indicators of a plant's relative carbon supply (Chapin, Schulze & Mooney 1990), these and other findings led to the hypothesis that tree growth is not carbon limited in natural mature stands at the current atmospheric CO₂ concentrations (Körner 2003, 2006; Millard, Sommerkorn & Grelet 2007). Comparative investigations of mobile carbon reserves have been used to assess the carbon source–sink balance of trees under limited growth. For instance, studies on natural (climatic) alpine tree lines generally show increased concentration of mobile carbon compounds as trees approach their upper distribution limit (e.g. Hoch & Körner 2003; Shi, Körner & Hoch 2008). This pattern has been interpreted to signal a stronger negative effect of cold temperatures on meristematic (growth) activities than on photosynthetic carbon assimilation, leading to an oversupply of carbon at the cold-temperature limit of tree distribution (sink-limitation hypothesis of tree line formation, Körner 1998). Here we propose that, analog to the situation at tree line, the mechanism(s) limiting tree height growth also modify the carbon source–sink balance, thereby altering the supply status of mobile carbon compounds as trees increase in height. According to the original HLH, if carbon acquisition limits growth as trees approach their maximum height, the concentration of mobile carbon compounds should decrease with increasing tree height. On the other hand, if the cessation of tree height growth is directly mediated by turgor or other factors independent of carbon acquisition, the concentration of mobile carbon stores may remain constant or even increase, depending on the relative magnitude of changes in whole tree carbon assimilation versus changes in growth.

Here, we measured growth rates and concentrations of stored mobile carbon compounds in leaves, branches and boles of ponderosa pine (*Pinus ponderosa* Dougl. ex. Laws.) trees of different heights at two sites with slightly different water availability in western Montana, USA. Based on the lack of evidence for the HLH as initially stated, the recent recognition that factors other than carbon availability may limit growth in tall trees, and on the fact that mature trees have large stored mobile carbon pools (see above), we addressed the following hypotheses. Firstly, carbon supply by photosynthesis does not become increasingly limited as trees grow tall. Rather, we predict that the concentration of mobile carbon compounds will increase with tree height reflecting proportionally greater reductions of water-limited cell expansion and growth (C-sink) relative to carbon assimilation (C-source) as trees grow tall. Secondly,

the increase of the concentration of stored mobile carbon compounds with tree height is greater in moderately drier sites relative to moister sites. This is because the negative effects of reduced water availability on cell expansion and growth are expected to be more pronounced at drier sites. Ponderosa pine is relevant for this study because it is particularly vulnerable to water stress-induced cavitation (Piñol & Sala 2000), and decreased gas exchange with increasing tree height is very common (Yoder *et al.* 1994; Hubbard, Bond & Ryan 1999; Ryan *et al.* 2000; Sala 2006). We discuss the physiological and ecological implications of our results and suggest additional research directions.

METHODS

Study area

The study area was located on a private ranch near Missoula, Montana (46.6°N and 113.2°W; 1180 m elevation) on the Blackfoot River drainage. Mean annual air temperature is 7 °C, with mean January and July temperatures of –8.0 and 16.5 °C, respectively. Mean total annual precipitation is 455 mm. The ranch is mostly on rolling hills and dominated by relatively open ponderosa pine forests on the driest sites to mixed forests with Douglas-fir (*Pseudotsuga menziesii* Mirb.) and western larch (*Larix occidentalis* Nutt.) on moister sites. The ranch is on a conservation easement and is subjected to sustainable management with relatively open stands. Ephemeral watercourses on depressions at the lower portion of the ranch provide a mixture of moister sites on depressions near the watercourses and slightly drier sites on the hills and slopes.

After an initial reconnaissance in August 2007, we selected two adjacent sites for sampling. One site was located on flat terrain on a gentle plateau, with an open dry ponderosa pine forest with isolated trees of different heights, and an understory composed of mostly grasses (*Festuca idahoensis* Elmer) and forbs covering only a fraction of the otherwise rocky soil. An adjacent site was located on a depression intersected by an ephemeral watercourse. The stand was also relatively open with significant presence of Douglas-fir in the overstory and an understory mostly dominated by shrubs [*Arctostaphylos uva-ursi* (L.) Spreng. and *Symphoricarpos albus* (L.) S.F. Blake] which covered most of the soil surface. These differences in understory composition are used as indicators of water availability (Pfister *et al.* 1977). We refer to these two sites as 'dry' and 'moist', respectively.

Field sampling and initial sample processing

At each site, we selected 20 trees with five individuals within one of four approximate height classes [A = c. 6 m; B = c. 12 m; C = c. 25 m and D > 30 m (tallest trees, up to c. 40 m); Table 1]. Trees were selected carefully to avoid shading from adjacent trees. For each sampled tree, we measured tree height with a clinometer, diameter at breast height (1.4 m), bark thickness and global positioning system

Site	Height class	Height (m)	Age (years)	$\delta^{13}C$ (‰)	N ($\mu\text{g mg}^{-1}$)
Dry	A	6.3 (0.22)	18.6 (0.45)	-26.4 (0.54)	13.9 (1.31)
	B	12.4 (0.53)	26.2 (0.89)	-26.6 (0.26)	13.1 (0.52)
	C	26.1 (0.83)	78.8 (3.21)	-26.1 (0.30)	13.4 (0.52)
	D	34.5 (2.12)	102.8 (3.4)	-26.0 (0.35)	14.8 (1.63)
	Average	19.8 (2.66) ^a	56.6 (8.38) ^a	-26.3 (0.14) ^a	13.7 (0.44) ^a
Moist	A	5.6 (0.56)	18.2 (1.43)	-27.4 (0.58)	14.7 (1.92)
	B	13.1 (1.43)	28.2 (1.82)	-27.0 (0.04)	11.6 (0.99)
	C	24.2 (1.62)	69.6 (4.35)	-26.7 (0.46)	12.7 (0.72)
	D	37.7 (1.16)	123.6 (20.83)	-25.8 (1.06)	11.7 (1.12)
	Average	20.2 (2.89) ^a	59.9 (10.76) ^a	-26.9 (0.25) ^b	12.7 (0.61) ^a

In parenthesis are standard deviations. Different letters indicate statistically significant site differences. There was an overall significant height effect for $\delta^{13}C$ ($F_{\text{Height}} = 11.9$; $P < 0.01$) but not for N. $n = 5$ for height and age and 3 for leaf traits.

coordinates. Using a hand increment corer (5 mm diameter, Suunto, Finland), one bole core to the pith of each tree was taken at breast height in classes C and D, and slightly lower (c. 1 m above ground) in classes A and B. Cores were wrapped in plastic straws and stored in a cooler over ice until sample processing in the laboratory on the same day. Sun-exposed terminal branches were sampled from the upper third of the canopy of three randomly chosen trees per size class and site. Except for the shortest trees (up to 7 m), which we could reach with a pole pruner, sampling took place with a rifle supported on a tripod. Because correct aiming (particularly to tall branches) was very time consuming and shooting was somewhat risky at the peak of the severe fire season, we restricted the sampling to three randomly chosen trees per size class and site. Branches were stored in plastic bags in coolers over ice for transport until processing in the laboratory on the same day.

Initial sample processing in the laboratory took place immediately after returning from the field (within a maximum of 8 h after sampling). The length of the core (from phloem to pith), the sapwood (visually determined) and that of the 10 most recent tree rings, as well as the number of tree rings in the sapwood and heartwood, were recorded. These measurements were used to calculate age at coring height, total sapwood area and sapwood area increment in the last 10 years. A rough estimate of total aboveground wood volume increment (including all branches) over the last 10 years was calculated by multiplying the corresponding sapwood area by height, assuming a near cylindrical shape for the combined bole and branch xylem. Growth efficiency was calculated as either sapwood area increment or sapwood volume increment in the last 10 years divided by total sapwood area. The sapwood portion comprising the 10 outermost tree rings from the bole core was separated for subsequent chemical analyses.

From each sampled branch, the following two tissues were sampled: one-year-old needles (produced in 2006) and branch wood of the last two years with bark and phloem removed with a sharp blade. Immediately after taking measurements and tissue separation, all samples for chemical analyses (i.e. one-year-old needles, branch wood and the youngest 10-year stem core section) were microwaved for

90 s to stop all enzymatic activity, oven dried for 72 h at 75 °C, and ground to fine powder.

Chemical analyses

Non-structural carbohydrates (NSC) were analysed in needles, branch wood and bole sapwood as described in detail in Hoch, Popp & Körner (2002). About 10 mg of plant powder was extracted with 2 mL distilled water at 100 °C for 30 min. An aliquot of the extract was used for the determination of low molecular carbohydrates after enzymatic conversion of fructose and sucrose to glucose. The concentration of free glucose was determined photometrically after enzymatic conversion of glucose to gluconat-6-phosphate on a 96-well multiplate reader. Following the degradation of starch to glucose by a crude fungal amylase ('Clarase') at 40 °C overnight, NSC was determined as total glucose as described above. The concentration of starch was calculated as NSC minus the free low molecular carbohydrates. All NSC values are given as percent dry matter.

Lipids (acylglycerols) were determined in branch wood and bole sapwood as described in detail in Hoch *et al.* (2002). After saponification of lipids by extraction of about 12 mg wood powder in 1 mL aqueous NaOH for 30 min., the amount of liberated glycerol was determined after enzymatic conversion of glycerol to glycerol-3-phosphate in a 96-well microplate reader. Results are given as μmol glyceride-glycerol (i.e. glyceride-derived glycerol) per gram dry mass.

Carbon isotope ratio and nitrogen analyses in one-year-old needles were performed with a continuous flow mass spectrometer. The ^{13}C signature of carbon is expressed in the δ notation, where $\delta^{13}C$ (‰) = $(R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$, and R sample is the $^{13}C/^{12}C$ ratio of the sample and R_{standard} is the $^{13}C/^{12}C$ ratio of the international Vienna Pee Dee Belemnite carbon standard. Nitrogen concentrations are given as % dry matter.

Statistical analyses

The effects of site and height on growth and tissue chemistry parameters were tested with an analysis of covariance

Table 1. Average height, age at coring depth (see text), and carbon isotope ratios ($\delta^{13}C$) and nitrogen content of mature one-year-old needles from terminal fully exposed branches in the four height classes sampled per site

Table 2. Parameters *a* and *b*, *r*² and slope *P*-values for the regression equations (*y* = *a* + *b**x*) between response variables (leaf carbon isotope ratios, NSC in leaves and branch and bole sapwood and lipids in branch and bole sapwood) and tree height (*x*) in the dry and moist sites

Variable	Tissue	Site	<i>a</i>	<i>b</i>	<i>r</i> ²	<i>P</i>
$\delta^{13}C$	Needles	D	-26.6	0.021	0.14	n.s.
		W	-27.7	0.048	0.57	0.007
NSC	Needles	D	4.77	0.046	0.38	0.014
		W	5.21	0.036	0.16	n.s.
	Branch sapwood	D	1.72	0.114	0.57	0.002
		W	1.73	0.091	0.45	0.01
Bole sapwood	D	1.11	0.023	0.37	0.003	
	W	1.14	0.012	0.10	n.s.	
Lipids	Branch sapwood	D	13.72	0.845	0.50	0.004
		W	16.51	0.184	0.01	n.s.
	Bole sapwood	D	27.74	-0.212	0.24	0.016
		W	21.91	-0.186	0.38	0.002

Quadratic functions did not significantly improve the fit. n.s., not significant; NSC, non-structural carbohydrates.

(PROC GLM; Statistical Analysis Software, version 8.2; SAS, North Carolina), with height class as covariate. Average height per class (Table 1) was statistically undistinguishable between the two sites, and overall average values per class were used (values for classes A through D were 5.9, 12.8, 25.1 and 36.1 m, respectively). We used average height per class rather than individual tree heights to be consistent with our sampling design with discrete height classes. However, the same results were obtained when individual tree heights were used as a covariate (not shown) instead. When height effect was significant, we determined the best fit for each variable as a function of height in each of the two sites. All data were checked to conform with the assumptions of analysis of variance. Results were considered statistically significant at $\alpha < 0.05$.

RESULTS

$\delta^{13}C$ of needles was slightly but significantly higher (less negative) in the dry site relative to the moist site (Table 1; $F_{site} = 4.78$; $p_{site} = 0.04$). $\delta^{13}C$ significantly increased (less negative values) with tree height ($F_{Height} = 11.3$; $P < 0.01$), although analyses by site revealed a significant linear increase only at the moist site ($r^2 = 0.57$; $F = 11.9$; $P = 0.007$; Table 2). Leaf nitrogen content did not vary between the moist and the dry site, or with tree height (Table 1).

Site effects were not significant for any of the growth variables (basal area and volume increment and basal area and volume growth efficiency, $p_{site} > 0.05$ for all variables). In contrast, height was significant for all variables with the exception of volume growth efficiency (Table 3; Fig. 1). Basal area increment over the last 10 years was lowest in the shorter trees at both sites, increased in intermediate trees and either remained constant (moist site) or slightly decreased (dry site) in the tallest height class (Table 3;

Table 3. ANOVA for the effects of tree height and site (dry versus moist) on growth variables

	d.f.	<i>F</i>	<i>P</i>
BAI			
Height	1	9.45	0.004
Site	1	2.2	0.149
Height × site	1	3.6	0.067
VI			
Height	1	63.9	<0.001
Site	1	1.7	0.199
Height × site	1	4.9	0.003
GE_{BA}			
Height	1	319.7	<0.001
Site	1	0.0	0.939
Height × site	1	0.4	0.518
GE_{VI}			
Height	1	0.1	0.752
Site	1	0.2	0.692
Height × site	1	1.0	0.321

BAI, basal area increment; VI, volume increment; GE_{BA} basal area growth efficiency; GE_{VI}, volume growth efficiency; ANOVA, analysis of variance.

Fig. 1a). Volume increment increased from shorter trees to up to 25-m trees at both sites and either remained constant in the tallest class (dry site) or continued to increase (moist site) (significant interaction effect, Table 3; Fig. 1b). As tree height increased, growth efficiency on a basal area basis

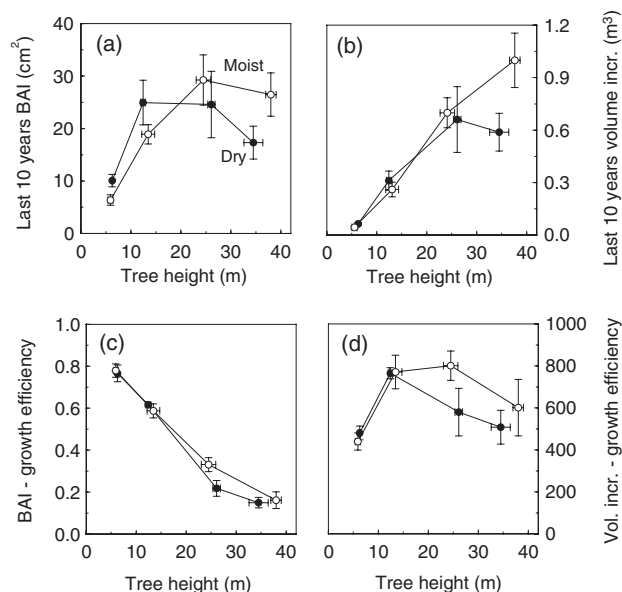


Figure 1. Growth characteristics of ponderosa pine of different height classes (see Table 1) at the dry and moist sites. (a) Last 10 years stem basal area increment (BAI); (b) aboveground wood volume increment of the last 10 years; (c) growth efficiency on a sapwood area basis; (d) growth efficiency on a volume basis. *n* = 5, error bars are standard errors. See Table 3 for analysis of variance results.

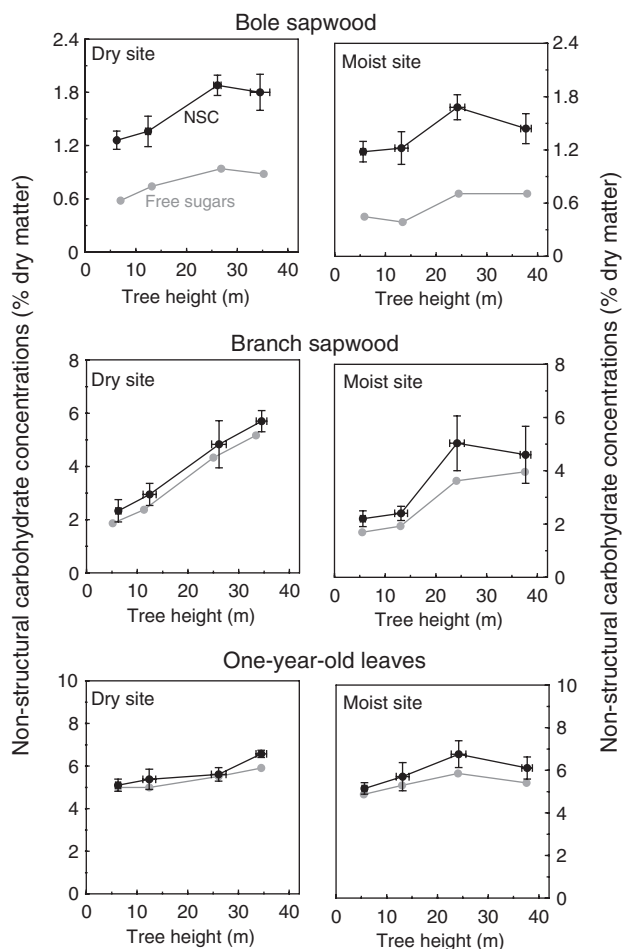


Figure 2. Non-structural carbohydrates (NSC, i.e. sum of free sugars and starch) and free sugar concentrations (gray symbols) in bole sapwood (youngest 10 tree rings), branch sapwood and one-year-old needles of ponderosa pines in different height classes (see Table 1) at the dry and moist site. For clarity, standard errors are given for NSC only. $n = 5$ for stem sapwood, $n = 3$ for branch wood and leaves. See Table 4 for analysis of variance results.

decreased drastically from c. 0.8 in the smallest trees to less than 0.2 in the tallest height class (Fig. 1c; Table 3). The height effect on growth efficiency on a volume basis was not significant (Table 3; Fig. 1d), although there was a significant quadratic correlation at the moist site (results not shown).

Remarkably, NSC in leaves and branches were mostly in the form of low-molecular-weight sugars rather than starch (Fig. 2). In the bole in contrast, starch comprised 50% or more of the total NSC. Overall, NSC in leaves, branches and bole varied as a function of tree height, with values increasing linearly with height (Fig. 2, Tables 2 & 4). Although site and the interaction height \times site were not statistically significant for NSC, analysis of height responses by site showed a significant increase with height for all variables at the dry site, while only branch NSC increased significantly at the moist site (Table 2). These results suggest that increases in NSC with tree height were more apparent at

the dry site compared with the moist site. Overall, changes of low-molecular-weight sugar concentrations with height mirrored those for NSC (results not shown). The sugar : starch ratio in branch sapwood was significantly higher at the dry relative to the moist site (6.7 ± 0.94 SE and 4.3 ± 0.57 SE, respectively; $F = 7.4$; $P = 0.002$). A similar but not statistically significant trend was observed in the bole sapwood (1.4 ± 0.26 SE and 0.8 ± 0.17 SE, respectively; $F = 2.8$; $P = 0.1$).

In contrast to the consistent trend of increasing NSC with tree height in all tissues, height-related patterns of concentrations of glyceride-glycerols (corresponding to the sum of mono-, di- and triacylglycerols, i.e. lipids) differed between bole and branch sapwood (Table 4; Fig. 3). Glyceride-glycerol concentrations in the bole sapwood decreased with tree height (Tables 2 & 4, Fig. 3), with the lowest value in the tallest height class, particularly at the moist site. In contrast, glyceride-glycerol concentrations increased with tree height in the branch sapwood (Table 4), with significant increases at the dry site but not at the moist site (Table 2; Fig. 3). Across all height classes, concentrations of glyceride-glycerols in bole sapwood were higher at the dry site compared with the moist site (Table 4). Although the interaction term height \times site was not significant in either case, analyses of responses by site showed a significant linear increase of glyceride-glycerol concentrations in branches with tree height at the dry site but not at the moist site (Table 2).

DISCUSSION

The significant increase of NSC and lipid concentrations with tree height in all tissues in the upper canopy support our initial hypothesis that carbon supply by photosynthesis does not become increasingly limited as trees grow tall.

Table 4. ANOVA for the effects of tree height and site (dry versus moist) on NSC and lipid concentrations in different tissues

	d.f.	NSC		Lipids	
		F	P	F	P
Bole sapwood					
Height	1	11.5	0.002	5.5	0.025
Site	1	0.0	0.906	13.5	<0.001
Height \times site	1	0.7	0.411	0	0.999
Branch sapwood					
Height	1	27.9	<0.001	14.0	0.001
Site	1	0.0	0.975	0.0	0.853
Height \times site	1	0.3	0.595	3.8	0.064
Needles					
Height	1	9.1	0.007	n.d.	
Site	1	0.5	0.498	n.d.	
Height \times site	1	0.1	0.763	n.d.	

Lipids were measured in woody organs only.

n.d., not determined; NSC, non-structural carbohydrates; ANOVA, analysis of variance.

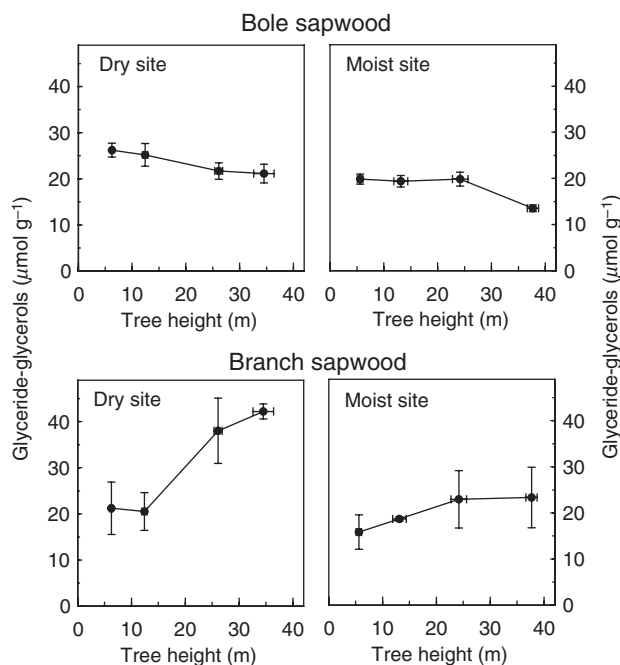


Figure 3. Concentrations of glyceride-glycerols in stem sapwood (youngest 10 tree rings) and branch sapwood of ponderosa pines in different height classes (see Table 1) at the dry and moist site. Error bars are standard errors. $n = 5$ for stem sapwood, $n = 3$ for branch wood. See Table 4 for analysis of variance results.

In contrast to our prediction, however, concentration of lipids in the bole sapwood decreased with tree height. However, we argue that this decline is not necessarily inconsistent with non-limiting carbon supply by photosynthesis in tall trees (see below). Despite small differences in water availability between the dry and moist sites and similar changes of mobile carbon concentration with tree height at the two sites, analysis of height effects by site indicated stronger increases of mobile carbon stores at the dry site relative to the moist site. These results, and the fact that the concentration of lipids in the bole (a longer term carbon storage form) was greater at the dry site relative to the moist site, provide support for our second hypothesis that increases in mobile carbon concentration with tree height are greater at the dry site.

After intense independent research on hydraulic limitations of tree growth on the one hand (reviewed by Ryan *et al.* 2006) and on accumulation of mobile carbon compounds in mature trees on the other (Hoch *et al.* 2003; Körner 2003, 2006; Millard *et al.* 2007), the two research lines have met and the hypothesis that height- and age-related growth declines may not result from carbon limitation on growth has been put forward (Ryan *et al.* 2006; Bond *et al.* 2007; Greenwood *et al.* 2008). Our results directly corroborate this hypothesis. In particular, the strong height-related increase of NSC and lipids in the sapwood of terminal branches (i.e. adjacent to leaves where carbon acquisition by photosynthesis takes place) clearly

suggests that carbon supply by photosynthesis is not limiting growth in tall trees. Therefore, height- and age-related growth declines in ponderosa pine are not due to hydraulic constraints on carbon acquisition. On the contrary, as we hypothesized, our results suggest greater surplus of mobile carbon in the upper canopy of tall trees relative to short trees. Consistent with this, we also found an increase in NSC (with relatively similar proportions of starch and sugars) in the bole sapwood. The remarkably low starch concentrations in leaves and branches may reflect the late sampling date within the season. Starch concentrations are known to be highest immediately before budbreak, decrease throughout the season and reach lowest values during winter (Hoch *et al.* 2003). A significant drought combined with record high temperatures during the summer of 2007 in western Montana may have additionally induced a conversion of starch to low-molecular-weight sugars for osmotic maintenance (Merchant *et al.* 2006).

While the increase of lipid concentration in branch sapwood with tree height is also consistent with our first hypothesis that taller trees are not carbon limited, the decrease of lipid concentration in the bole is not. However, we analysed glyceride-glycerols which include mono- and diacylglycerols (which are not carbon reserves) and triacylglycerols (neutral lipids or reserves). In mature *Pinus sylvestris*, acylglycerols are almost exclusively triglycerols (Fischer & Höll 1992). Unfortunately, we do not know if this is the case for ponderosa pine. The significantly higher lipid concentrations in branch and bole sapwood at the dry site compared with the moist site, however, suggest that, as in *P. sylvestris*, a significant fraction of the acylglycerols are neutral (i.e. reserves; see below). It is also not known whether the fraction of structural (mono- and diacylglycerols) relative to reserve lipids (triacylglycerols) changes with tree age. If such fraction decreases with tree age, it could account for the overall decrease with tree height that we observed without necessarily involving a decrease of storage lipids. Furthermore, we only analysed the outer portion of the sapwood where NSC concentrations are known to be higher (Hoch *et al.* 2003). In *P. sylvestris*, however, the concentration of triacylglycerols has been found to increase slightly from the outermost sapwood toward inner sapwood, and decrease again in the transition zone between the sapwood and heartwood where they are hydrolysed for subsequent fatty acid storage in the heartwood (Saranpää & Nyberg 1987). If this is true for ponderosa pine, lipid storage in the inner sapwood as trees grow tall could have a dilution effect (greater sapwood volume in larger trees) and could explain the decrease of lipid concentration in the outer bole sapwood that we report. Contrary to our results, Saranpää & Piispanen (1994) found similar storage lipid concentrations in young and older trees. Although additional research is clearly needed to understand these patterns, the strong increase of NSC with tree height in all tissues and of lipid concentrations in branches (particularly at the dry site) clearly shows that carbon supply by photosynthesis does not become increasingly limited as trees grow tall.

Although the difference in soil water supply was presumably relatively small between the two sampled sites, we were nevertheless able to detect significant site differences consistent with different water availability. First, the carbon isotope ratio in one-year-old needles was slightly but significantly lower (more negative) in the moist site relative to the dry site. $\delta^{13}\text{C}$ ratios are an integrated measure of the ratio between internal CO_2 concentration in the leaf mesophyll relative to that in the air, which depends on photosynthetic capacity and stomatal conductance (Ehleringer & Osmond 1989). Similar nitrogen concentration at the two sites suggests that maximum photosynthetic capacity was not different (the two factors are usually tightly correlated; Reich, Walters & Ellsworth 1997) and that the slightly higher $\delta^{13}\text{C}$ ratio at the dry site was due to lower time-integrated stomatal conductance. In addition, the dominance of shrubs in understory of the moist site, as opposed to grasses at the dry site, is indicative of more productive habitats with higher water availability (Pfister *et al.* 1977). While overall, growth variables did not differ much between the sites, the tendency for stronger declines of relative growth indicators (growth efficiency) in the tallest tree class in the dry site also suggests lower water availability at this site. Consistent with these results, higher lipid concentrations in bole sapwood of trees at the dry site (particularly in tall trees) suggests greater carbon surplus relative to the moist site. Such surplus at the dry site could be due to either higher overall carbon acquisition or lower carbon demand for growth, defense and reproduction, or a combination. It is unlikely that tree-level carbon acquisition was higher at the dry site. Firstly, nitrogen content of upper canopy one-year-old needles was similar at the two sites. Secondly, the ratio of total leaf area to cross-section of sapwood area in ponderosa pine is known to decrease, not increase, with reduced moisture availability (Callaway, DeLucia & Schlesinger 1994). Consistent with our second hypothesis, the overall higher surplus of mobile carbon at the dry site resulted in stronger increases of NSC concentrations in all tissues and of lipid concentration in the sapwood of upper branches at this site where the height effect was significant in all cases.

If growth in tall trees is not limited by carbon supply by photosynthesis, what explains the well-documented height- and age-related declines of growth in trees and stands (Ryan *et al.* 1997)? Most likely, gravity and path length constraints on water transport in tall trees limit water availability and turgor in the upper canopy (Koch *et al.* 2004; Woodruff, Bond & Meinzer 2004; Ishii *et al.* 2008; Meinzer *et al.* 2008), which has been linked to reduced growth and sink strength to a greater extent than in short trees (Bond *et al.* 2007; Greenwood *et al.* 2008). If so, it remains to be tested whether sink strength in open canopies (not limited by light) increases from the top to the bottom of the canopy, concurrent with diminishing hydraulic constraints on water transport and on reduced effects of gravity. It also needs to be determined whether turgor-limited growth in the upper canopy as trees grow tall translate to decreases in overall tree growth and productivity.

While studies on changes in sink strength with tree height may provide very useful insights, additional factors may need to be considered to explain height-related growth declines. Barnard & Ryan (2003) proposed a very interesting hypothesis that could link hydraulic limitations to growth reductions: lower water potential in taller trees constrains translocation of assimilates. The appeal of this hypothesis is that it highlights for the first time a major potential constraint in tall plants that, remarkably, continues to be overlooked: phloem long distance transport. Very little is known about phloem transport in wild trees (particularly conifers). However, photoassimilates need to be transported throughout the entire length of the tree via the phloem, a process dependent on water and, according to the widely accepted Münch model (Münch 1930), driven by hydrostatic pressure gradients from sources (higher pressure) to sinks (lower pressure). Remarkably, very little thought has been given on whether phloem transport could be involved in height-related growth declines. For instance, higher xylem tensions in the upper xylem and apoplast of tall trees could limit water movement from xylem to phloem (lower phloem pressure at source points), which could impose a bottleneck for photoassimilate distribution throughout the tree.

Another important open question is: why do trees apparently produce and accumulate carbon in excess of what is needed for growth, defense and reproduction? The acquisition of carbon is extremely expensive: not only does it involve an unavoidable water loss and risk of desiccation, but the enzymes, pigments and other metabolites involved in photosynthesis require large amounts of nitrogen (and to a lesser degree phosphorous), which are commonly limiting in nature. Why then do trees continue to assimilate carbon when sink strength (i.e. demand) decreases either due to low temperature, reduced water availability or other growth-limiting factors? Without a doubt, carbon excess may provide a number of benefits, including production of root exudates (Millard *et al.* 2007) or the capacity to buffer episodic major stresses such as herbivore consumption. However, complete depletion of carbon pools in perennial plants, particularly trees, is rare (Chapin *et al.* 1990). For instance, several successive complete defoliations in *Pinus cembra* were insufficient to completely deplete stored carbon pools (Li, Hoch & Körner 2002). Therefore, trees appear to assimilate carbon even in excess of these demands. Given the resource demands involved in carbon acquisition, the maintenance of such expensive process regardless of carbon demand for growth, reproduction and defense suggests either constrains on accessibility to stored carbon and/or alternative long-term benefits. The maintenance of large nitrogen labile pools as Rubisco combined by the production of root exudates for microbial-mediated nutrient cycling (Millard *et al.* 2007) may be some of these benefits. However, whether such benefits are the actual drivers or simply by-products of other major physiological, ecological and evolutionary forces remains unexplored. Clearly, many questions remain unanswered, thus offering a rich avenue for future research.

ACKNOWLEDGMENTS

This research was supported by a grant from the Diettert Preece fund from the University of Montana to A.S. Travel support to G.H. was provided by a Visiting Scholar grant from The University of Montana to A.S. We are very thankful to Kelly Hopping for the NSC analyses. Element and isotope analyses were performed by David Harris at the Stable Isotope Laboratory, University of California Davis, USA. Additional support for chemical analyses was provided by Institute of Botany of the University of Basel. We are very thankful to Carl Fiedler and Hank Goetz for their help with site selection, to Bill Potter for allowing us access to his property, to Frank Mauss for the climate data and to Carl Fiedler because without him, we would still be shooting branches without success.

REFERENCES

- Barnard H.R. & Ryan M.G. (2003) A test of the hydraulic limitation hypothesis in fast-growing *Eucalyptus saligna*. *Plant, Cell & Environment* **26**, 1235–1245.
- Bond B.J., Czarnomski N.M., Cooper C., Day M.E. & Greenwood M.S. (2007) Developmental decline in height growth in Douglas-fir. *Tree Physiology* **27**, 441–453.
- Callaway R.M., DeLucia E.H. & Schlesinger W.H. (1994) Biomass allocation of montane and desert ponderosa pine – an analog for response to climate-change. *Ecology* **75**, 1474–1481.
- Chapin F.S., Schulze E.D. & Mooney H.A. (1990) The ecology and economics of storage in plants. *Annual Review of Ecology and Systematics* **21**, 423–447.
- Ehleringer J.H. & Osmond C.B. (1989) Stable isotopes. In *Plant Physiological Ecology. Field Methods and Instrumentation* (eds R.W. Pearcy, J. Ehleringer, H.A. Mooney & P.W. Rundel) pp. 281–300. Chapman & Hall, London, UK.
- Falster D.S. & Westoby M. (2003) Plant height and evolutionary games. *Trends in Ecology and Evolution* **18**, 337–343.
- Fischer C. & Höll W. (1992) Food reserves of Scots pine (*Pinus sylvestris* L.) II. Seasonal changes and radial distribution of carbohydrate and fat reserves in pine wood. *Trees – Structure and Function* **6**, 147–155.
- Friend A.D. (1993) The prediction and physiological significance of tree height. In *Vegetation Dynamics and Global Change* (eds A.M. Solomon & H.H. Shugart) pp. 101–115. Chapman & Hall, New York, USA.
- Greenwood M.S., Ward M.H., Day M.E., Adams S.L. & Bond B.J. (2008) Age-related trends in red spruce foliar plasticity in relation to declining productivity. *Tree Physiology* **28**, 225–232.
- Hoch G. & Körner C. (2003) The carbon charging of pines at the climatic treeline: a global comparison. *Oecologia* **135**, 10–21.
- Hoch G., Popp M. & Körner C. (2002) Altitudinal increase of mobile carbon pools in *Pinus cembra* suggests sink limitation of growth at the Swiss treeline. *Oikos* **98**, 361–374.
- Hoch G., Richter A. & Körner C. (2003) Non-structural carbon compounds in temperate forest trees. *Plant, Cell & Environment* **26**, 1067–1081.
- Hubbard R.M., Bond B.J. & Ryan M.G. (1999) Evidence that hydraulic conductance limits photosynthesis in old *Pinus ponderosa* trees. *Tree Physiology* **19**, 165–172.
- Ishii H.T., Jennings J.M., Sillett S.C. & Koch G.W. (2008) Hydrostatic constraints on morphological exploitation of light in tall *Sequoia sempervirens* trees. *Oecologia* **156**, 751–763.
- Kira T. & Shidei T. (1967) Primary production and turnover of organic matter in different forest ecosystems of the western Pacific. *Japanese Journal of Ecology* **13**, 70–83.
- Koch G.W., Sillett S.C., Jennings G.M. & Davis S.D. (2004) The limits of tree height. *Nature* **428**, 851–854.
- Körner C. (1998) A re-assessment of high elevation treeline positions and their explanation. *Oecologia* **115**, 445–459.
- Körner C. (2003) Carbon limitation in trees. *Journal of Ecology* **91**, 4–17.
- Körner C. (2006) Plant CO₂ responses: an issue of definition, time and resource supply. *New Phytologist* **172**, 393–411.
- Li M.H., Hoch G. & Körner C. (2002) Source/sink removal affects mobile carbohydrates in *Pinus cembra* at the Swiss treeline. *Trees – Structure and Function* **16**, 331–337.
- Magnani F., Mencuccini M. & Grace J. (2000) Age-related decline in stand productivity: the role of structural acclimation under hydraulic constraints. *Plant, Cell & Environment* **23**, 251–263.
- Martínez-Vilalta J., Vanderklein D. & Mencuccini M. (2007) Tree height and age-related decline in growth in Scots pine (*Pinus sylvestris* L.). *Oecologia* **150**, 529–544.
- Meinzer F.C., Bond B.J. & Karanian J.A. (2008) Biophysical constraints on leaf expansion in a tall conifer. *Tree Physiology* **28**, 197–206.
- Mencuccini M. (2003) The ecological significance of long-distance water transport: short-term regulation, long-term acclimation and the hydraulic costs of stature across plant life forms. *Plant, Cell & Environment* **26**, 163–182.
- Merchant A., Tausz M., Arndt S. & Adams M. (2006) Cyclitols and carbohydrates in leaves and roots of 13 *Eucalyptus* species suggest contrasting physiological responses to water deficit. *Plant, Cell & Environment* **29**, 2017–2029.
- Millard P., Sommerkorn M. & Grelet G.A. (2007) Environmental change and carbon limitation in trees: a biochemical, ecophysiological and ecosystem appraisal. *New Phytologist* **175**, 11–28.
- Münch E. (1930) *Die Stoffbewegung in der Pflanze*. Gustav Fischer, Jena, Germany.
- Niklas K.J. (2007) Maximum plant height and the biophysical factors that limit it. *Tree Physiology* **27**, 433–440.
- Pfister R.D., Kovalchik B.L., Arno S.F. & Presby R.C. (1977) Forest habitat types of Montana. USDA Forest Service, Gen Tech Rep INT-34, Ogden.
- Piñol J. & Sala A. (2000) Ecological implications of xylem embolism of several Pinnaceae of NW United States. *Functional Ecology* **14**, 538–545.
- Reich P.B., Walters M.B. & Ellsworth D.S. (1997) From tropics to tundra: global convergence in plant functioning. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 13730–13734.
- Ryan M.G. & Yoder B.J. (1997) Hydraulic limits to tree height and growth. *Bioscience* **47**, 235–242.
- Ryan M.G., Binkley D. & Fownes J.H. (1997) Age-related decline in forest productivity: pattern and process. *Advances in Ecological Research* **27**, 213–262.
- Ryan M.G., Bond B.J., Law B.E., Hubbard R.M., Woodruff D., Cienciala E. & Kucera J. (2000) Transpiration and whole-tree conductance in ponderosa pine trees of different heights. *Oecologia* **124**, 553–560.
- Ryan M.G., Binkley D., Fownes J.H., Giardina C.P. & Senock R.S. (2004) An experimental test of the causes of forest growth decline with stand age. *Ecological Monographs* **74**, 393–414.
- Ryan M.G., Phillips N. & Bond B.J. (2006) The hydraulic limitation hypothesis revisited. *Plant, Cell & Environment* **29**, 367–381.
- Sala A. (2006) Hydraulic compensation in Northern Rocky Mountain conifers: does successional position and life history matter? *Oecologia* **149**, 1–11.

- Saranpää P. & Nyberg H. (1987) Lipids and sterols of *Pinus sylvestris* L. sapwood and heartwood. *Trees – Structure and Function* **1**, 82–87.
- Saranpää P. & Piispanen R. (1994) Variation in the amount of triacylglycerols and steryl esters in the outer sapwood of *Pinus sylvestris* L. *Trees – Structure and Function* **8**, 228–231.
- Shi P.L., Körner C. & Hoch G. (2008) A test of the growth-limitation theory for alpine treeline formation in evergreen and deciduous taxa of the Eastern Himalayas. *Functional Ecology* **22**, 213–220.
- Woodruff D.R., Bond B.J. & Meinzer F.C. (2004) Does turgor limit growth in tall trees? *Plant, Cell & Environment* **27**, 229–236.
- Woodruff D.R., McCulloh K.A., Warren J.M., Meinzer F.C. & Lachenbruch B. (2007) Impacts of tree height on leaf hydraulic architecture and stomatal control in Douglas-fir. *Plant, Cell & Environment* **30**, 559–569.
- Yoder B.J., Ryan M.G., Waring R.H., Schoettle A.W. & Kaufmann M.R. (1994) Evidence of reduced photosynthetic rates in old trees. *Forest Science* **40**, 513–527.

Received 10 July 2008; received in revised form 30 September 2008; accepted for publication 1 October 2008