

Limitations to photosynthetic carbon gain in timberline *Abies lasiocarpa* seedlings during prolonged drought

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Abstract: Photosynthesis, water status, and associated physiological parameters were measured in chronically drought-stressed seedlings (5 years of below-average precipitation, 107 cm net deficit) of *Abies lasiocarpa* (Hook.) Nutt. above (treeline ecotone site, TS) and below (forest site, FS) a Rocky Mountain timberline. In contrast to normal seasonal patterns reported for timberline conifer trees, xylem water potentials were exceptionally low in early summer and remained low for the rest of the summer. Although photosynthesis was not significantly different between the two sites, early season photosynthesis was greater than late-season photosynthesis, especially at FS. Mean daily values of leaf conductance to water vapor (g_{wv}) and transpiration (E) were also low at the beginning of summer (g_{wv} from 0.01 mol·m⁻²·s⁻¹ to 0.13 mol·m⁻²·s⁻¹ and E from 0.4 μmol·m⁻²·s⁻¹ to 2.9 μmol·m⁻²·s⁻¹) and continued to decrease through summer (an approximate 10-fold decrease in g_{wv} and a 2-fold to 3-fold decrease in E), which resulted in increasing water-use efficiency as summer progressed. Although the slope of instantaneous photosynthesis–intercellular CO₂ concentration curves was reduced (lower carboxylation efficiency) from July to September, the relative stomatal limitation to carbon gain was less than 50% over the entire measurement period. Mean daily intercellular CO₂ concentrations decreased from near ambient levels (approximately 350–360 ppm) to 290 ppm over the course of summer. Overall, nonstomatal limitations appeared to have the largest impact on photosynthetic carbon gain, although seasonal decreases in leaf conductance and a corresponding depletion of intercellular CO₂ indicated that there were also significant stomatal limitations to carbon gain that resulted in a continued regulation of greater water use efficiency.

Résumé : La photosynthèse, le statut hydrique et des paramètres physiologiques associés ont été mesurés sur des semis d'*Abies lasiocarpa* (Hook.) Nutt. stressés par une sécheresse chronique (cinq ans de précipitation sous la moyenne et déficit net de 107 cm) au-delà (station à la limite des arbres) et en deçà (station forestière) de la limite des arbres dans les montagnes Rocheuses. Contrairement aux normales saisonnières disponibles pour les conifères de la limite des arbres, le potentiel hydrique du xylème était exceptionnellement bas au début de l'été et est resté bas pendant le reste de l'été. Bien que la différence de photosynthèse entre les deux stations n'ait pas été significative, elle était plus élevée au début qu'à la fin de l'été et ce, surtout dans le cas de la station forestière. De faibles valeurs journalières moyennes de conductance foliaire de vapeur d'eau et de transpiration ont aussi été mesurées au début de l'été (0,01 mol·m⁻²·s⁻¹ à 0,13 mol·m⁻²·s⁻¹ pour la conductance et 0,4 μmol·m⁻²·s⁻¹ à 2,9 μmol·m⁻²·s⁻¹ pour la transpiration) et ont continué à diminuer au cours de l'été (environ 10 fois moins pour la conductance et 2 à 3 fois moins pour la transpiration), ce qui s'est traduit par une augmentation de l'efficacité d'utilisation de l'eau avec la progression de l'été. Quoiqu'une diminution de la pente de la courbe des valeurs instantanées de la photosynthèse et la concentration de CO₂ intercellulaire ait été observée de juillet à septembre (diminution de l'efficacité de la carboxylation), la limitation relative des stomates au gain en carbone a été inférieure à 50 % pendant l'ensemble de la période de prise de données. Les valeurs journalières moyennes de concentration de CO₂ intercellulaire ont diminué à partir d'une valeur proche du niveau ambiant (entre 350 et 360 ppm) à une valeur de 290 ppm au fil de l'été. De façon générale, les limitations non stomatiques semblent produire le plus grand impact sur le gain photosynthétique en carbone quoique la diminution saisonnière de conductance foliaire et la diminution correspondante de CO₂ intercellulaire indiquent qu'il y a aussi eu des limitations stomatiques significatives au gain en carbone qui ont provoqué la régulation continue d'une plus grande efficacité d'utilisation de l'eau.

[Traduit par la Rédaction]

Introduction

For over a century, the upper altitudinal limit for forest and tree growth has been of interest to scientists (see Smith

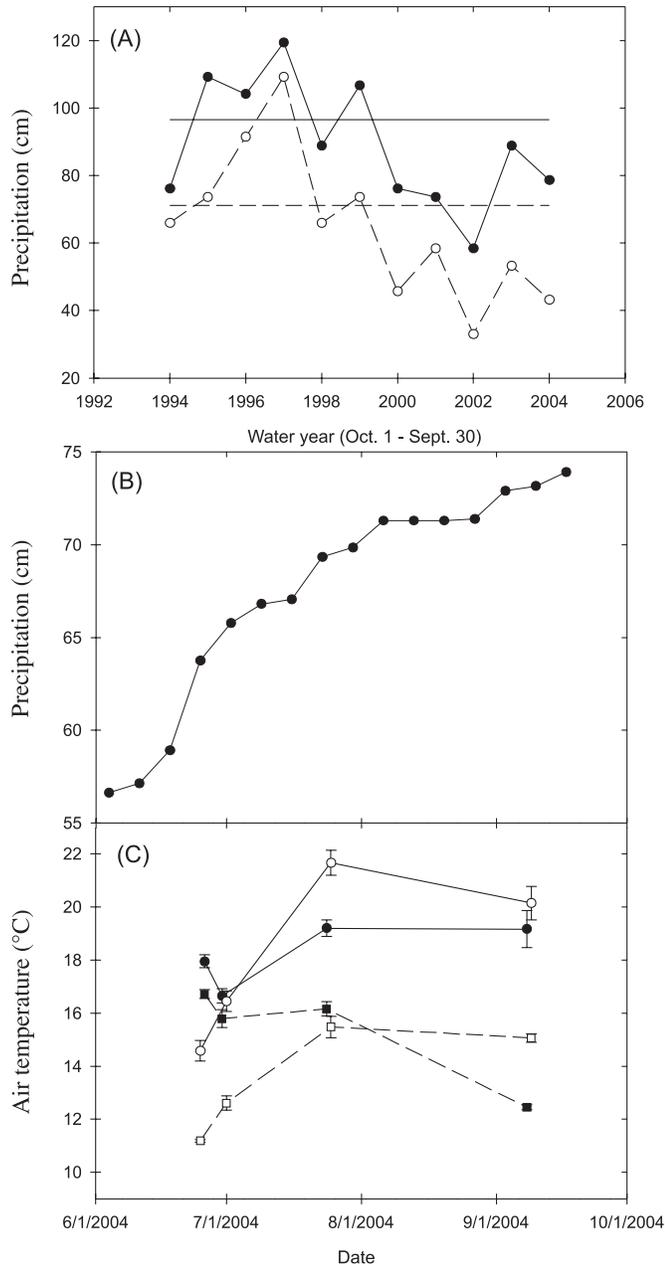
et al. 2003 for review). However, most research concerning the occurrence and stability of this limit has not addressed specific mechanisms but, instead, has focused on temperature regime correlations (Daubenmire 1954; Tranquillini 1979; Grace 1989). For example, although treeline migration to a higher altitude is dependent on new seedling establishment, little work has addressed the mechanisms of seedling establishment in treeline ecotones (Smith et al. 2003). To our knowledge, only a few studies exist that have focused on the specific mechanisms of establishment in very young (current-year) seedlings (Daly and Shankman 1985; Cui and

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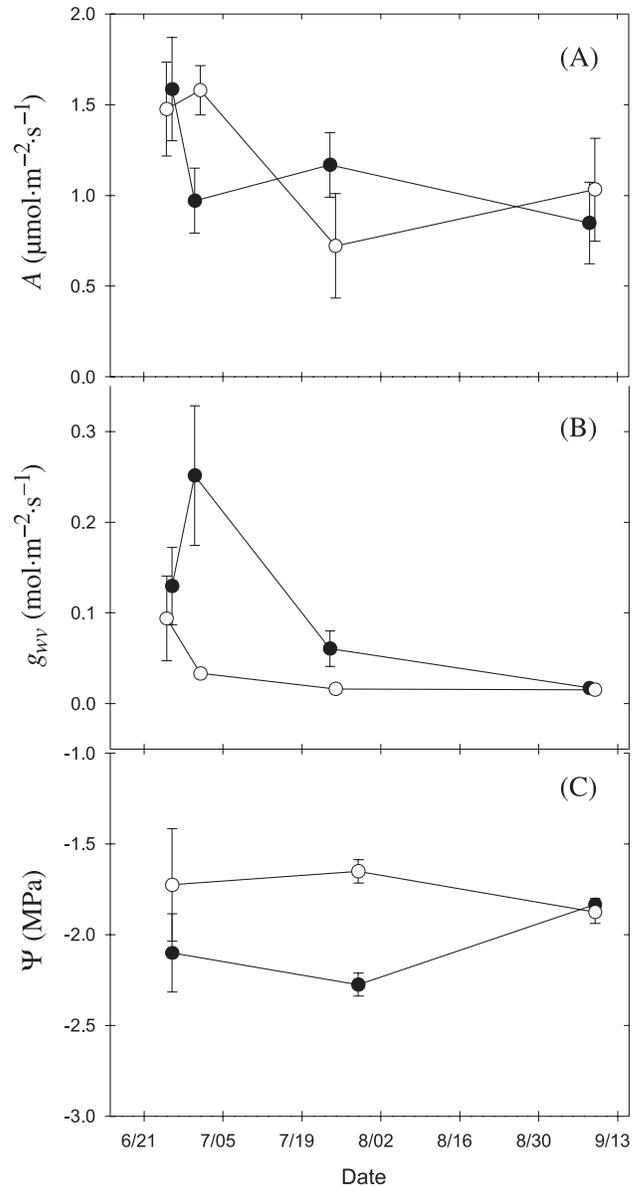
Fig. 1. (A) Annual snowfall (○) and total annual precipitation (●) from 1987 to 2004 (beginning 1 October, ending 30 September) at the National Atmospheric Deposition Program WY00 site. Horizontal solid and broken lines show the mean annual total precipitation and snowfall from 1971 to 2001, respectively. (B) Total accumulated precipitation for the 2004 growth season. (C) Mean daily air temperature and mean air temperature for the 0800 measurement intervals at the forest site (FS: ●, mean daily; ■, mean 0800 measurement) and treeline ecotone site (TS: ○, mean daily; □, mean 0800 measurement).



Smith 1991; Germino et al. 2002) or the ecophysiology of young (less than 5 years old) seedlings at the treeline (Johnson et al. 2004).

An awareness of the mechanisms that govern carbon gain, growth, and survival in young seedlings of the treeline ecotone may be critical for understanding treeline stability

Fig. 2. (A) Mean daily photosynthesis (A), (B) conductance to water vapor (g_{wv}), and (C) xylem water potential (ψ) in seedlings of *Abies lasiocarpa* at the forest site (FS, ●) and treeline ecotone site (TS, ○) during summer 2004. Error bars are SEs. For each date, $n = 20-30$, $20-30$ and $4-6$ for photosynthesis, conductance to water vapor, and xylem water potential, respectively.



(Smith et al. 2003). Abiotic factors that could have strong impacts on seedling carbon gain include water, sunlight (either too much or too little), and temperature (either too high or too low). Each of these abiotic factors could be a limitation to carbon gain at high altitude but, in combination, could be especially stressful. For example, the combination of high sunlight and low temperature resulted in reduced carbon gain and survival in young seedlings of *Picea engelmannii* Parry ex Engelm. (Germino and Smith 2000). Young seedlings of both *Abies lasiocarpa* (Hook.) Nutt. and *P. engelmannii* had reduced carbon gain because of structural shading by tree islands (too little sunlight), low nighttime temperatures, and water stress (Johnson et al. 2004). Also,

Table 1. Light-saturated A ($\text{PAR} > 500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) with corresponding g_{wv} and PAR values for the forest (FS) and treeline ecotone (TS) sites in 2004.

Site	Date	n	A ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	g_{wv} ($\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	PAR ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)
FS	26 June	5	2.15 (0.45)	0.16 (0.18)	1292 (9)
TS	25 June	4	2.96 (0.63)	0.05 (0.07)	1406 (13)
FS	24 July	12	1.44 (0.23)	0.08 (0.07)	877 (7)
TS	25 July	11	1.44 (0.28)	0.02 (0.03)	1634 (6)
FS	8 Sept.	7	1.81 (0.20)	0.03 (0.03)	886 (7)
TS	9 Sept.	7	1.55 (0.23)	0.02 (0.03)	1166 (7)

Note: Values are means with SEs given in parentheses.

desiccation increases significantly with altitude and may result in reductions in carbon gain due to stomatal closure (Smith and Johnson 2006).

Limitations to carbon gain are typically categorized as one of two types: those associated with the stomatal aperture and those associated with the interior portion of the leaf, from the intercellular air spaces to the enzymes responsible for carbon fixation or the photochemistry of the light reactions ("nonstomatal limitations"). Reductions in carbon gain during drought are typically due to stomatal closure and low values of intercellular CO_2 (i.e., diffusion limitations, e.g., Sharkey and Seemann 1989; Lal et al. 1996; Flexas et al. 2004; Grassi and Magnani 2005), although there are also reports of increasing nonstomatal limitations to carbon gain with declining water status, especially under more severe drought (e.g., Kubiske and Abrams 1992, 1993; Bota et al. 2004).

The purpose of the current study was to monitor photosynthetic carbon gain and water status in young seedling populations, both above and below treeline, under chronic drought stress and evaluate quantitatively the relative stomatal and nonstomatal limitations to photosynthesis. It was hypothesized that water conservation and (or) water-use efficiency (WUE) would take priority over carbon gain under these long-term drought conditions (i.e., stomatal closure). Furthermore, nonstomatal limitations to photosynthesis (as interpreted from net photosynthesis and leaf conductance values, as well as instantaneous photosynthesis–intercellular CO_2 concentration ($A-C_i$) curves) would act to further reduce carbon gain, although partial stomatal closure would generate continued carbon gain at greater WUE, in response to such prolonged water stress (e.g., Bota et al. 2004).

Materials and methods

Photosynthetic carbon gain and water status of young seedlings of *A. lasiocarpa* were monitored throughout the summer of 2004, which was also the last year in a consecutive 5 year period of below-average precipitation (data from National Atmospheric Deposition Program, WY00 site; Fig. 1) with a net 5 year deficit of 107 cm of total precipitation. Two study sites, one in a spruce–fir forest and one near the centre of the alpine treeline ecotone (alpine meadow interspersed with tree islands) were located in the Medicine Bow Mountains of southeastern Wyoming. Gas-exchange parameters were both calculated (conductance to water vapor (g_{wv}) and C_i) and experimentally manipulated (ambient CO_2 concentration) to determine the degree of stomatal versus nonstomatal effects on photosynthesis.

Study sites

Tree stature and distribution changes with altitude across the forest–alpine ecotone of the south-central Rocky Mountains, USA. Subalpine forest (at the lowest altitudes) is replaced by ribbon forest, flagged tree islands, large krummholz mats with flagged trees on their downwind sides, and finally by smaller and smaller krummholz mats at the high-altitude limit of the ecotone (Daubenmire 1954; Wardle 1968; Smith et al. 2003). *Picea engelmannii* and *A. lasiocarpa* are the two codominant tree species of the ecotone and are often associated with *Pinus flexilis* James, shrubs (*Salix* spp. and *Ribes coloradense* Coville), and many herbaceous species such as *Erythronium grandiflorum* Pursh and *Caltha leptosepala* DC. (Billings 1969; Peet 1988).

Two 20 m \times 20 m study sites, one in a mixed *A. lasiocarpa* / *P. engelmannii* subalpine forest (FS, 2965 m elevation) and the other in an alpine meadow located near the center of the upper treeline ecotone (TS, 3198 m elevation), were selected for comparison in the treeline ecotone of the Snowy Range of the Medicine Bow Mountains in southern Wyoming, USA (41°20'N, 06°13'W). Although the study only focused on one research site at two different altitudes, these research sites were chosen as representative of the overall forest–alpine ecotone in this region. Seedlings at TS were closely associated with tree islands, whereas seedlings at FS were distributed uniformly throughout the understory (see Johnson et al. 2004 for detailed site descriptions). Soils across the treeline ecotone of the Snowy Range are classified as inceptisols–cryochrepts (Knight 1994; Rahman et al. 1996).

Photosynthetic gas exchange and water status

Photosynthetic carbon gain, along with associated parameters: g_{wv} , transpiration (E), and C_i , was monitored in young seedlings (3 cm to 10 cm in height, approximately 2 years to 6 years old) throughout the summer using gas-exchange measurements at both sites on four dates throughout summer (FS: 26 June, 30 June, 24 July, and 8 September; TS: 25 June, 1 July, 25 July, and 9 September). Dates of measurement were carefully evaluated based on differences in weather conditions. All days of paired sampling dates had very similar weather conditions based on the degree of cloudiness and diurnal air temperature regimes except for 30 June at FS (intermittent clouds) and 1 July at TS (clear). These measurements were initiated five times during the day at approximately 0800, 1000, 1300, 1500 and 1700 (solar time, List 1971). Twelve seedlings at each site (for a total of 24 seedlings) were marked for gas exchange, and each

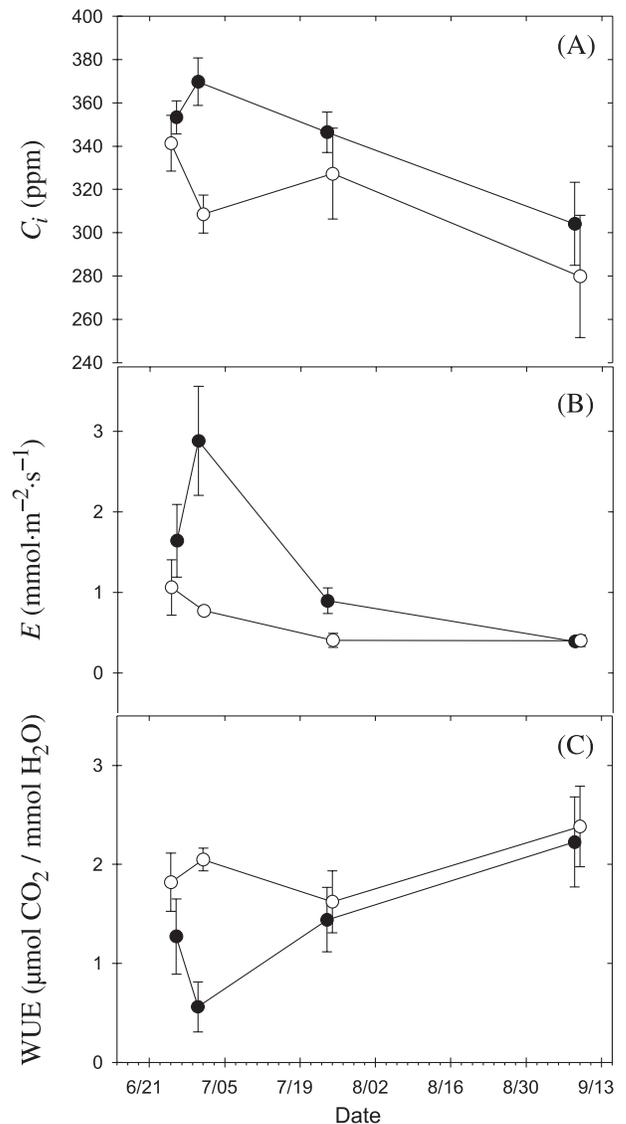
sampling consisted of measurements on four to six individuals randomly selected (among marked seedlings) at each site.

Values for *A* were calculated by averaging all photosynthesis measurements during a day. Photosynthesis was measured using a LICOR-6400 model portable photosynthesis system (LICOR, Lincoln, Nebraska), and seedlings were illuminated with a blue-red LED light source (6400-02B LED; LICOR, Lincoln, Nebraska). For each measurement, the light intensity inside the chamber was matched to the natural sunlight intensity outside the chamber, which was highly variable due to sun angle, structural shading, and cloud effects (see Johnson et al. 2004 for detailed descriptions of light regimes at these sites). Natural orientation of individual seedlings was maintained during all measurements and photosynthetic CO₂ flux densities were computed on a total leaf area basis (Smith et al. 1991). To obtain mean needle area, the length and diameter of 25 random needles for each species were measured and used to compute leaf area. The number of needles per unit of stem length, along with computed needle areas were then used to estimate total needle area inside the cuvette during each photosynthesis measurement. Photosynthesis expressed on a projected or silhouette leaf area basis was approximately three to four times greater than when expressed on a total area basis, as previously reported in Smith et al. (1991). The difference in atmospheric pressure (and CO₂ partial pressure) between FS and TS was minimal (mean atmospheric pressure at FS = 71.7 kPa, at TS = 69.7 kPa, a difference of 1.5%). Also, the difference in pressure between the two sites was taken into account when calculating diffusion coefficients and thus, conductance values. Light-saturated photosynthesis values corresponded to photosynthetically active radiation (PAR) values of greater than 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (the approximate light saturation point for these seedlings; Johnson et al. 2004).

Experimental manipulation of CO₂ concentrations inside the leaf chamber was performed to analyze the instantaneous responses of *A* to *C_i*. Seedlings were placed in the leaf chamber and exposed to 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR. Chamber CO₂ concentrations were varied in the following order: 400 ppm, 500 ppm, 600 ppm, 400 ppm, 400 ppm, 200 ppm, 100 ppm, and 50 ppm; seedlings were allowed to equilibrate for 60 s before each measurement (i.e., change in concentration). Measurements were performed on 28 July and 10 September at the forest site and 29 July and 11 September at the ecotone site. Measurements were made during both morning (from 0800 to 1100) and afternoon time intervals (from 1400 to 1700). Differences in the initial slope of the response of *A* to *C_i* were used as an indicator of changes in nonstomatal limitations to *A* (primarily carboxylation efficiency; Farquhar and Sharkey 1982; Sharkey 1985). The relative stomatal limitation to photosynthesis (*I_g*) was calculated according to Jones (1985) using the equation $I_g = (C_a - C_i)/C_a \times 100$, where *C_a* is the ambient CO₂ concentration.

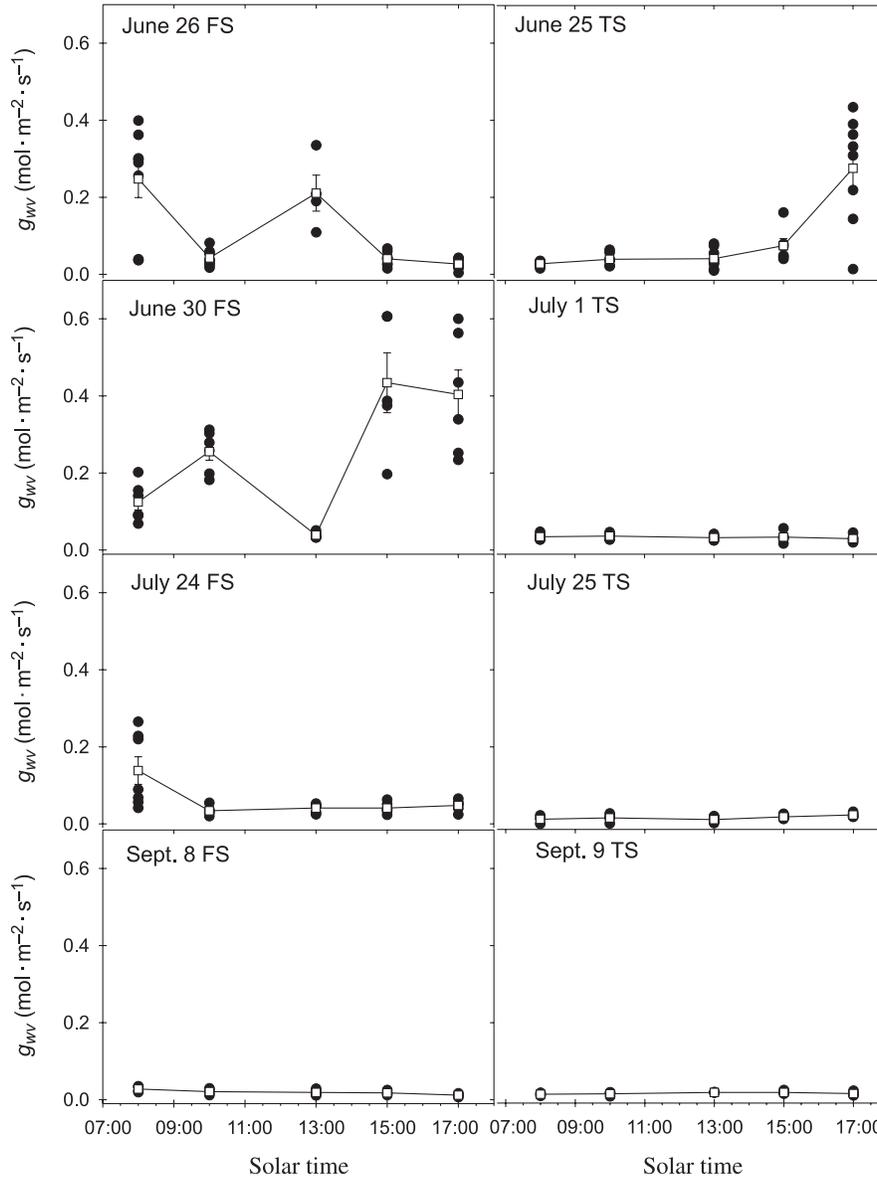
Seedling water status (xylem water potentials, ψ) was measured at intervals throughout the season at both sites. Measurements were performed at five to six week intervals (26 June, 29 July, and 9 September) between 1400 and 1500 solar time during each day. Predawn measurements of seedling water potential were also performed on seedlings on

Fig. 3. (A) Mean daily intercellular CO₂ concentration (*C_i*), (B) transpiration (*E*), and (C) water-use efficiency (WUE) in seedlings of *Abies lasiocarpa* at the forest site (FS, ●) and treeline ecotone site (TS, ○) during summer 2004. Error bars are SEs (*n* = 20–30 for each date).



25 June and 9 September but were not significantly different from afternoon measurements because of large variation in measured predawn values (possibly a product of differences in spatial association of seedlings with larger plants and, thus, differences in exposure to cold night skies and freezing temperatures in June and September). Seedlings marked for gas-exchange measurements were not used for water potential measurements. Instead, nearby (within 5 m of marked seedlings) seedlings were sampled for water potential measurements. Stems were cut with a razor blade and the cut portion was immediately covered with petroleum jelly. Samples were then placed into plastic freezer bags and inside an ice cooler until measurements were completed, approximately 1 h later. Each sample was freshly cut prior to measurement. Measurements of ψ were also taken in the field, within minutes of excision, and compared with shoots trans-

Fig. 4. Daily patterns of conductance to water vapor (g_{wv}) in seedlings of *Abies lasiocarpa* at the forest site (FS, left panels) and treeline ecotone site (TS, right panels). Solid circles and open squares represent individual measurements and hourly means, respectively. Error bars are SEs ($n = 4-6$ for each time point).



ferred to the laboratory. No significant changes in water status occurred because of transfer and storage time during the period of measurement. Xylem water potentials were measured with a Scholander-type pressure bomb (model 1000, PMS Instrument Company, Corvallis Oregon).

Sampling and statistics

Statistical analyses were employed using JMP statistical analysis software (SAS Institute Inc., Cary, North Carolina) and figures were created with Sigmaplot (SPSS Science, Chicago, Illinois). Photosynthesis and water potential ($n = 6$ per measurement interval) were averaged according to time of measurement. Linear least squares regression analysis and standard errors were calculated to test for statistical differences between physiological parameters of seedlings at TS and FS (Students t test; Zar 1999). Best-fit regression

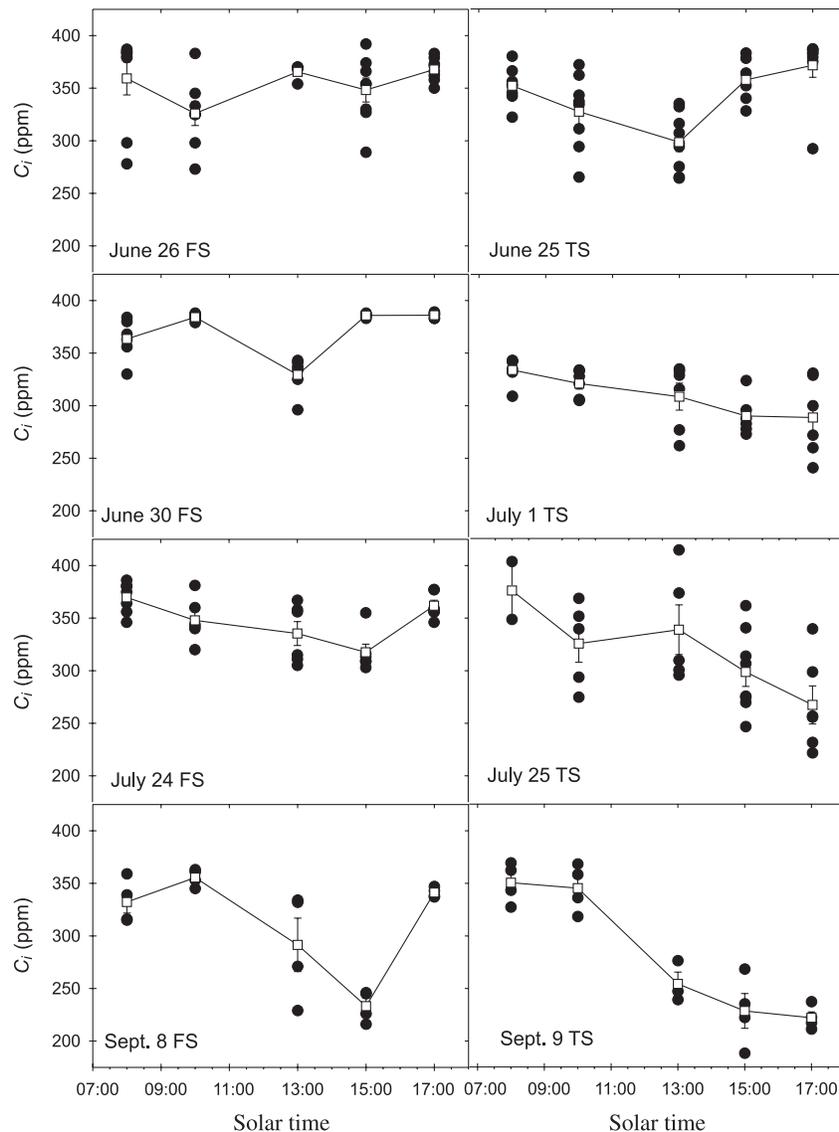
curves and repeated-measures ANOVA were used to determine the relationship between A and C_i (i.e., differences in carboxylation efficiency).

Results

Seasonal trends in photosynthetic gas exchange and water potential

Cumulative precipitation increased seasonally (approximately 17 cm total accumulated precipitation over the measurement period), but there was no measurable precipitation for a 3 week period from approximately 3 August to 25 August (Fig. 1B). Both morning and daily mean air temperatures were lower early in the summer and greater late in the season at TS compared with FS (Fig. 1C). Vapor pressure deficits were similar between sites and were between

Fig. 5. Daily patterns of intercellular CO₂ concentration (C_i) in seedlings of *Abies lasiocarpa* at the forest site (FS, left panels) and treeline ecotone site (TS, right panels). Solid circles and open squares represent individual measurements and hourly means, respectively. Error bars are SEs ($n = 4-6$ for each time point).



0.8 kPa and 1.2 kPa in the morning and 1.9 kPa and 2.2 kPa in the late afternoon.

The A values were similar at FS and TS, with only the 30 June (FS) and 1 July (TS) measurements being different (Fig. 2A; $P < 0.001$). However, 30 June was an overcast day, and the maximum PAR value for the entire day was $209 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (mean PAR = $114 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Overall, A was highest early in the season (25 June–1 July) and then declined and leveled off. The A value for 26 June was significantly greater than 8 September A at FS ($P = 0.03$), although there was no significant difference between 25 June A and 9 September A at TS. Light-saturated A was not statistically different between FS and TS for any of the measurement date pairs (i.e., 26 June at FS vs. 25 June at TS; Table 1).

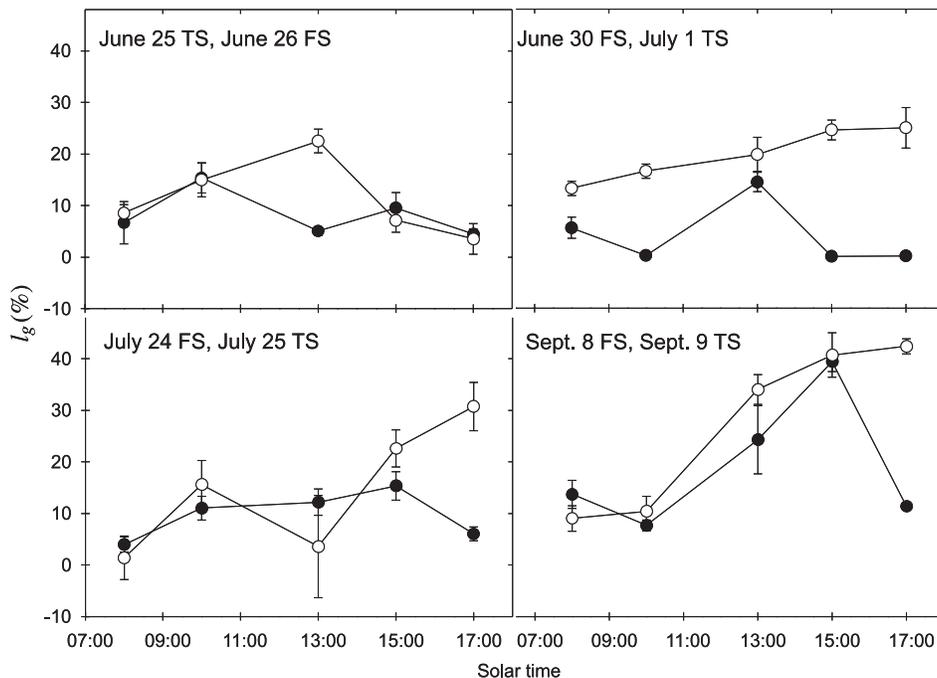
Mean daily g_{wv} was higher for FS seedlings than TS seedlings on 30 June (compared with 1 July at TS; $P = 0.01$; Fig. 2B). There were no significant differences in g_{wv} be-

tween sites for any of the other measurement intervals, as well as no significant difference between mean annual g_{wv} between sites. With the exception of the 30 June FS measurement, mean daily g_{wv} was highest early in the season (25 June at FS; 26 June at TS) and declined steadily over the course of the season. Early season values were significantly greater than late-season values at FS but not at TS (26 June vs. 8 September at FS, $P < 0.01$; 25 June vs. 9 September at TS, $P = 0.06$).

Mean seedling water potentials (Fig. 2C) remained nearly constant (a range of -1.7 to -2.2 MPa) over the summer. There were no significant differences between sites early (26 June) and late (9 September), but water potentials were less negative in TS than FS seedlings on 29 July ($P < 0.001$).

Overall, C_i decreased seasonally at both sites (Fig. 3A). There were no significant differences between sites except on 30 June at FS and 1 July at TS when C_i was much

Fig. 6. The relative stomatal limitation to photosynthesis in seedlings of *Abies lasiocarpa* at the forest site (FS, ●) and treeline ecotone site (TS, ○) for the summer of 2004. Error bars are SEs ($n = 4-6$ for each time point).



greater in FS seedlings ($P < 0.001$). The C_i values were significantly greater early in the summer (25 June at TS; 26 June at FS) than late in the summer (8 September at FS; 9 September at TS) at both FS and TS ($P = 0.03$ and 0.04 , respectively). Early season values ranged from 370 ppm to 310 ppm, whereas late season values ranged from 280 ppm to 310 ppm.

The seasonal pattern in seedling transpiration (Fig. 3B) was very similar to the pattern in g_{wv} . The value of E was significantly greater at FS than TS on 30 June at FS and 1 July at TS ($P = 0.01$), but there were no significant differences on any other date. Overall, mean E decreased over the summer during 2004 (25 June at TS and 26 June at FS were greater than 8 September at FS and 9 September at TS; $P = 0.01$ and 0.04 , respectively). Excluding 30 June (FS), seasonal patterns in E were similar between sites. Values were greatest early in the season ($1.1-1.6 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), then decreased to $0.5-1.0 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in late July and finally decreased to $0.3 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in September.

Although early and late season WUEs were not significantly different at either FS or TS ($P = 0.07$, 0.14 , respectively), WUE appeared to increase slightly over the summer at both sites. WUE was significantly lower at FS on 30 June than at TS on 1 July ($P < 0.001$), but no significant differences were detected on any other date.

Daily patterns in g_{wv} and C_i

Early in the summer (26 June and 30 June), mean g_{wv} reached values of greater than $0.4 \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at the FS (Fig. 4). However, at TS, large values of g_{wv} were restricted to late afternoon on 25 June only. Midseason (24 July at FS; 25 July at TS) values were much lower at both sites, with a maximum hourly mean g_{wv} of $0.14 \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $0.02 \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at FS and TS, respectively (significantly

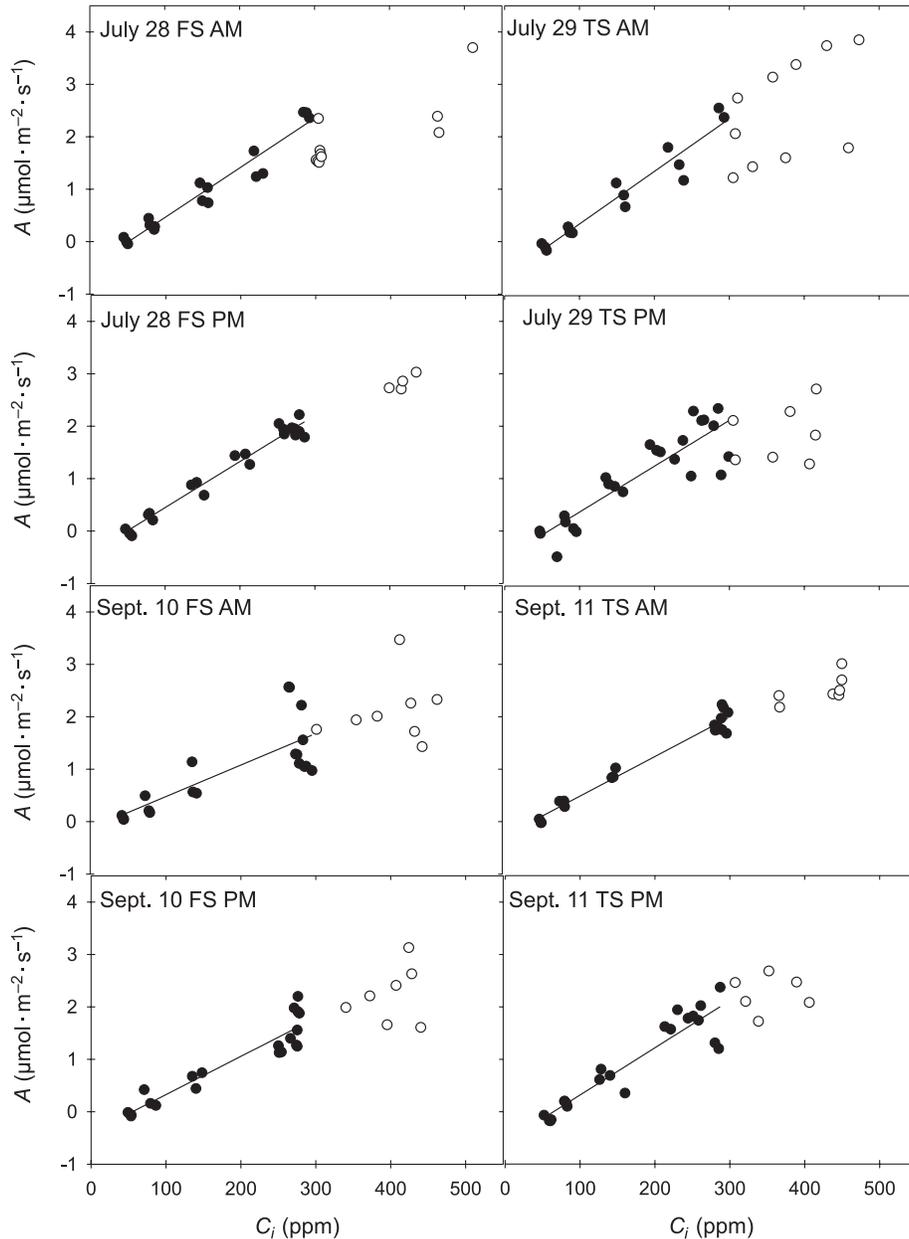
different; $P = 0.007$). At the end of the season, g_{wv} was near zero at both FS and TS.

Overall, there was a large amount of variation in hourly C_i measurements, especially early in the season (Fig. 5). However, mean hourly C_i remained greater than 300 ppm throughout the day on 26 June, 30 June, and 24 July at the FS. Only on 8 September did C_i fall below 300 ppm at the FS, which occurred at 1300 and 1500. C_i remained high at TS on 25 June throughout the day but decreased slightly to approximately 300 ppm at 1300. On 1 July, 25 July, and 9 September, C_i at TS decreased from early morning to late afternoon, with the greatest decreases occurring on 9 September at 1700 (C_i approximately 220 ppm).

Relative stomatal limitation and relationships among A , C_i , and g_{wv}

The calculated stomatal limitation to photosynthesis remained below 50% over the entire season (i.e., nonstomatal limitations dominant; Fig. 6). However, afternoon l_g values were near 40% at the end of the measurement period. Stomatal limitations to photosynthesis were frequently higher at TS than at FS. For example, l_g was significantly greater at TS than FS at 1300 on 25 June (versus 26 June at FS, $p < 0.001$) and at 1700 on both 25 July and 9 September (vs. 24 July and 8 September at FS, respectively, $p < 0.001$ for both comparisons). Instantaneous $A-C_i$ curves remained linear across a range of C_i values from approximately 40 ppm to 300 ppm (Fig. 7) for both morning and afternoon measurements at FS and TS. The linear portion of these curves was analyzed for differences in slope. No significant differences in slope were detected in any of these curves except for pooled July versus pooled September measurements (slope = 0.0090 for July and 0.0073 for September, $p = 0.002$).

Fig. 7. Morning (0800–1000) and afternoon (1400–1600) instantaneous $A-C_i$ curves for seedlings of *Abies lasiocarpa* at the forest site (FS, left panels) and treeline ecotone site (TS, right panels). Solid circles and open circles represent C_i values below and above 300 ppm, respectively. Best-fit regression lines were plotted through the linear portion of the data (below 300 ppm) ($n = 27-35$ for each panel).



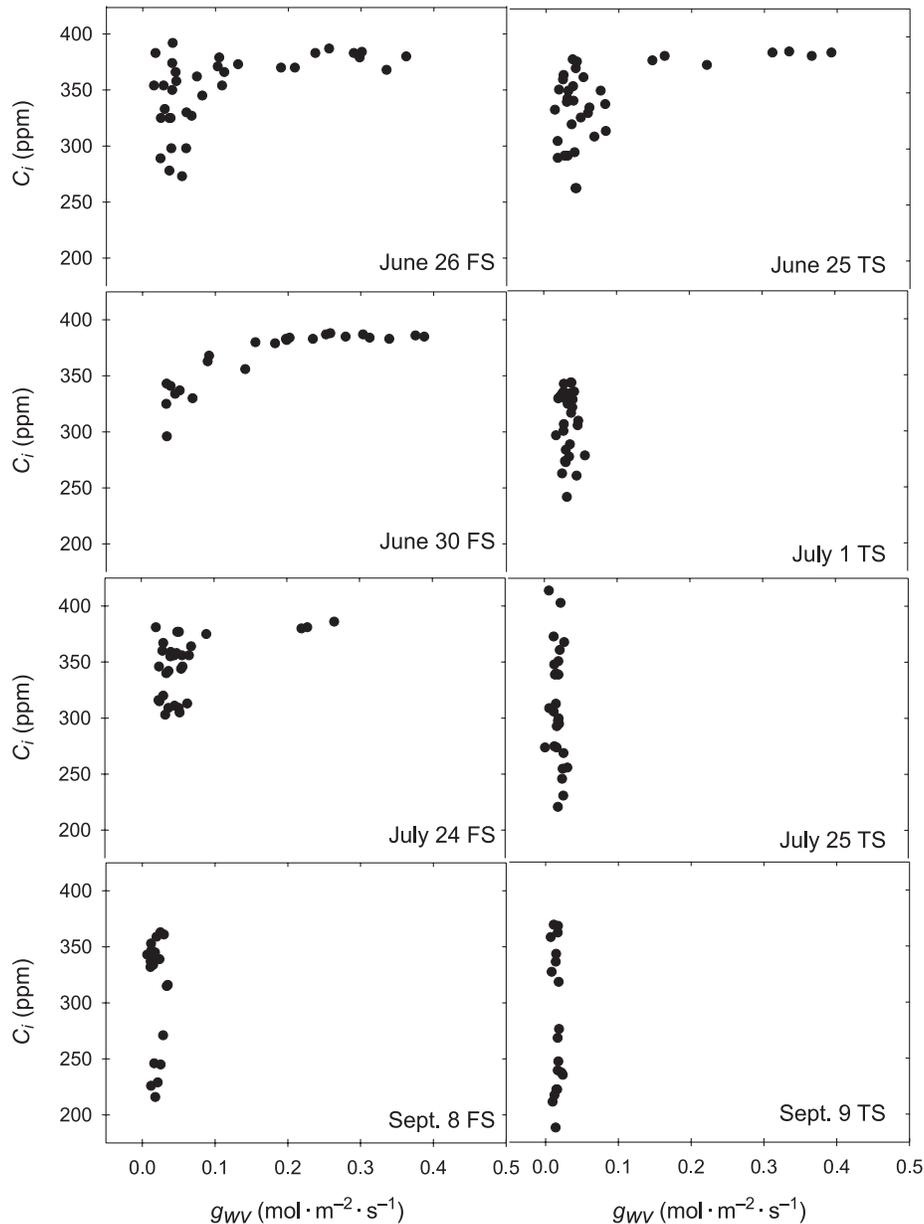
Patterns of C_i versus g_{wv} varied over the course of the summer (Fig. 8). The range of values for g_{wv} was highest early in the summer (from approximately $0 \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ to $0.4 \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; seen also in Fig. 4), whereas the spread in C_i values was greater at the end of summer (from approximately 190 ppm to 370 ppm). Also, values of g_{wv} had a much wider range at FS (especially 30 June and 24 July), whereas the distribution of C_i values was much wider at TS.

Discussion

Drought stress has been shown to decrease photosynthesis in numerous plant species from a variety of habitats (see

Lambers et al. 1998) due to both stomatal and nonstomatal limitations (e.g., Brodribb 1996). Under current scenarios of global change, both increases and decreases in annual precipitation over the next century have been predicted, depending on the model used (Smith et al. 2005). The model developed by the Australian Bureau of Meteorology Research Center predicted a 50 to 100 mm decrease in precipitation in southwestern Wyoming, while the University of Illinois Urbana-Champaign's model predicted a 50–200 mm increase in annual precipitation for the same region. Thus, an understanding of the impact of drought stress on these conifer seedlings at high altitude could provide a particularly sensitive indicator of the health and vigor of the subalpine

Fig. 8. Intercellular CO₂ concentration (C_i) versus conductance to water vapor (g_{wv}) for seedlings of *Abies lasiocarpa* at the forest site (FS, left panels) and treeline ecotone site (TS, right panels) ($n = 20\text{--}30$ for each panel).



forest. Young seedlings with shallow root systems would be particularly vulnerable to future changes in precipitation regimes (Germino et al. 2002).

In the current study, seedling photosynthesis under extreme drought (5 year net deficit of 107 cm; see Fig. 1) decreased throughout the summer of 2004 even with a higher than average rainfall in both 2003 and 2004 (approximately 10 cm greater than average for both years, see Fig. 1). Photosynthesis was not significantly different between the forest and treeline ecotone sites, and this is in contrast to an earlier study (measurements taken in 2002 in the same area as the current study; Johnson et al. 2004) where photosynthesis in young (3–5 years old) *A. lasiocarpa* and *P. engelmannii* seedlings was greater in the subalpine forest than in the higher-elevation treeline ecotone. However, seasonal A_{\max} , June and July xylem water potentials, and total annual

precipitation were much lower in the earlier study ($0.5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, -3.0 to -2.0 MPa, and 58.4 cm, respectively) than measured here. There may be strong physiological effects on seedlings that are already drought stressed because of the frequent frost nights and desiccating effects of high altitude (Smith and Johnson 2006). Although this was not observed in the current study, more extreme drought conditions may make these effects on gas-exchange physiology more apparent (possibly a reason for the differences in photosynthesis between treeline ecotone and forest sites in 2002). Also, Cui and Smith (1991) reported values of A similar to those measured in the current study (A_{\max} approximately $1.5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in young *A. lasiocarpa* seedlings during another drought period (1986–1990; see Fig. 1). Also, during a nondrought year (1995), *A. lasiocarpa* seedlings at alpine treeline had some-

what higher photosynthesis values to seedlings in the current study (A_{\max} approximately $2.0 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; Germino and Smith 1999).

Rapid soil drying following spring snowmelt (typical of the south-central Rocky Mountains) may have large impacts on total seasonal photosynthesis and carbon gain in high-elevation species (see Smith and Knapp 1990 for review). Through the dry summer of 2004, water status of *A. lasiocarpa* seedlings remained low and nearly constant. Although there was little change in seedling water potentials during the summer of 2004, leaf conductance to water vapor decreased at a relatively constant rate as summer progressed. Stomatal closure can act to regulate plant water potentials (Kozlowski and Pallardy 1997; Lambers et al. 1998), maintaining a higher plant water status on a seasonal, and even daily, basis. In a previous study on *A. lasiocarpa* from the Wasatch Mountains of Utah, plants began to lose hydraulic conductivity (via xylem embolism) at water potentials below approximately -2.0 MPa and had lost nearly half of their xylem hydraulic conductivity at -3.0 MPa (Sperry and Sullivan 1992). In the current study, partial stomatal closure may have prevented loss of hydraulic conductivity by maintaining water potentials greater (less negative) than -2.5 MPa. Also, photosynthesis was negligible in seedlings at the same sites when xylem water potentials fell below approximately -2.7 MPa (Johnson et al. 2004). In a similar manner, stomatal control appeared here to lower transpirational water loss in response to declines in carbon gain, generating improved WUE and maintenance of water status. The constancy of water status through stomatal regulation, instead of more rapid photosynthesis followed by complete cessation, might also insure a greater integrated uptake of photosynthetic carbon for the summer growth period.

Reductions in conductance of nonstomatal components in drought-stressed plants can also impact plant carbon gain. Brodrigg (1996) found that initial reductions in stomatal conductance were followed by concomitant increases in nonstomatal limitations for several greenhouse-grown conifer species subjected to experimental drought stress. In another set of greenhouse experiments, Teskey et al. (1986) found that the major limitations to carbon gain in seedlings of *Pinus taeda* L. subjected to water deficits were nonstomatal. However, Bota et al. (2004) found that the primary limitations to photosynthesis in five species (*Rhamnus* (2), *Vitis*, *Phaeolus*, and *Nicotiana*) subjected to drought (also under controlled conditions, i.e., growth chamber) were primarily stomatal and that nonstomatal limitations were not significant until drought stress became severe (when A and g_{wv} are less than 20% of predrought values). Similarly, Grassi and Magnani (2005) observed stomatal limitations as dominant in limiting photosynthesis under drought conditions in *Quercus robur* L. and *Fraxinus oxyphylla* Bieb. In the current study, nonstomatal components were the greatest limitations to photosynthesis and a specific nonstomatal component, carboxylation efficiency, declined from July to September (as inferred from the initial slopes of $A-C_i$ curves). Thus, nonstomatal effects appeared to have the greatest impact on carbon gain compared to stomatal limitations.

Net photosynthesis continued even after g_{wv} had fallen to

near zero late in the season, when the lowest C_i values were also observed. However, C_i also continued to decrease after stomata had essentially closed (g_{wv} approximately $0.01 \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Previous studies have also shown continued photosynthesis following partial stomatal closure, along with a corresponding decrease in C_i (e.g., Kitao et al. 2003). Coordination between stomatal and nonstomatal components (i.e., reductions in stomatal conductance with concomitant increases in nonstomatal limitations to A) should result in a horizontal line in a plot of C_i versus g_{wv} (see Fig. 8). Stomatal and nonstomatal coordination was apparent in early summer but began to disassociate later in summer when g_{wv} became almost constant; however, the variation in C_i was similar to early summer. Thus, there was less change over the summer in the nonstomatal demand component compared to the CO_2 supply component, g_{wv} .

A recent hypothesis concerning the occurrence and maintenance of timberline altitude argues that trees at high altitudes are not limited by photosynthetic carbon gain but are more restricted in their ability to process acquired carbon because of cold temperatures (i.e., respiration limitations, Körner 1998). However, even though stomata were nearly closed all day late in the season (when temperatures were coldest), C_i was between 350 ppm and 400 ppm early in the morning and then decreased over the course of the day. At such low CO_2 uptake rates, it is possible that CO_2 from respiration the previous night could contribute to the higher C_i measured the following morning, although some CO_2 could also diffuse across the nearly-closed stomata.

Seasonal field measurements of gas exchange parameters in young (<5 years) conifer seedlings are rare, especially in high-altitude environments. Because treeline maintenance and upward migration may be strongly dependent on seedling establishment trends (Smith et al. 2003) and because treeline migration is believed to be an early indicator of global change (Little et al. 1994; Rochefort et al. 1994), the physiological responses of treeline seedlings to environmental change is fundamental to any comprehensive understanding of treeline migration, including global change effects. Changes in precipitation are likely under current scenarios of climate change. Thus, an understanding of the responses of young tree seedlings to drought stress will provide a more mechanistic, predictive model of treeline migration under climate change, as well as a potentially early indication of subalpine forest impacts. Future studies should focus on much larger study areas (e.g., similar sites in different mountain ranges) and should employ experimental manipulations of seedling water availability to provide a greater understanding of the impact of drought on seedling carbon gain and subsequent survival at high altitudes, as well as future stability of timberlines.

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References

- Billings, W.D. 1969. Vegetational pattern near alpine timberline as affected by fire-snowdrift interactions. *Vegetatio*, **22**: 192–207.
- Bota, J., Medrano, H., and Flexas, J. 2004. Is photosynthesis limited by decreased Rubisco activity and RuBP content under progressive water stress? *New Phytol.* **162**: 671–681. doi:10.1111/j.1469-8137.2004.01056.x.
- Brodribb, T. 1996. Dynamics of changing intercellular CO₂ concentration (c_i) during drought and determination of minimum functional c_i. *Plant Physiol.* **111**: 179–185. PMID:12226283.
- Cui, M., and Smith, W.K. 1991. Photosynthesis, water relations and mortality in *Abies lasiocarpa* seedlings during natural establishment. *Tree Physiol.* **8**: 37–46. PMID:14972895.
- Daly, C., and Shankman, D. 1985. Seedling establishment by conifers above tree limit on Niwot Ridge, Front Range Colorado, U.S.A. *Arct. Antarct. Alp. Res.* **17**: 389–400.
- Daubenmire, R. 1954. Alpine timberlines in the Americas and their interpretation. *Butler Univ. Bot. Stud.* **2**: 119–136.
- Farquhar, G.D., and Sharkey, T.D. 1982. Stomatal conductance and photosynthesis. *Annu. Rev. Plant Physiol.* **33**: 317–345. doi:10.1146/annurev.pp.33.060182.001533.
- Flexas, J., Bota, J., Loreto, F., Cornic, G., and Sharkey, T.D. 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C₃ plants. *Plant Biol.* **6**: 269–279. PMID:15143435.
- Germino, M.J., and Smith, W.K. 1999. Sky exposure, crown architecture, and low-temperature photoinhibition in conifer seedlings at alpine treeline. *Plant Cell Environ.* **22**: 407–415. doi:10.1046/j.1365-3040.1999.00426.x.
- Germino, M.J., and Smith, W.K. 2000. Differences in microsite, plant form, and low-temperature photoinhibition in alpine plants. *Arct. Alp. Res.* **32**: 388–396.
- Germino, M.J., Smith, W.K., and Resor, A.C. 2002. Conifer seedling distribution and survival in an alpine treeline ecotone. *Plant Ecol.* **162**: 157–168. doi:10.1023/A:1020385320738.
- Grace, J. 1989. Tree lines. *Philos. Trans. R. Soc. London B Biol. Sci.* **324**: 233–245.
- Grassi, G., and Magnani, F. 2005. Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. *Plant Cell Environ.* **28**: 834–849. doi:10.1111/j.1365-3040.2005.01333.x.
- Johnson, D.M., Germino, M.J., and Smith, W.K. 2004. Abiotic factors limiting photosynthesis in *Abies lasiocarpa* and *Picea engelmannii* above and below alpine timberline. *Tree Physiol.* **24**: 377–386. PMID:14757577.
- Jones, H.G. 1985. Partitioning stomatal and non-stomatal limitations to photosynthesis. *Plant Cell Environ.* **8**: 95–104. doi:10.1111/j.1365-3040.1985.tb01227.x.
- Kitao, M., Lei, T.T., Koike, T., Tobita, H., and Maruyama, Y. 2003. Higher electron transport rate observed at low intercellular CO₂ concentration in long-term drought-acclimated leaves of Japanese mountain birch (*Betula ermannii*). *Physiol. Plant.* **118**: 406–413. doi:10.1034/j.1399-3054.2003.00120.x.
- Knight, D.H. 1994. Mountains and plains: the ecology of Wyoming landscapes. Yale University Press, New Haven, Conn.
- Körner, C. 1998. A re-assessment of high elevation treeline positions and their explanation. *Oecologia*, **115**: 445–459.
- Kozlowski, T.T., and Pallardy, S.G. 1997. *Physiology of woody plants*. Academic Press, San Diego, Calif.
- Kubiske, M.E., and Abrams, M.D. 1992. Photosynthesis, water relations and leaf morphology of xeric versus mesic *Quercus rubra* ecotypes in central Pennsylvania in relation to moisture stress. *Can. J. For. Res.* **22**: 1402–1407.
- Kubiske, M.E., and Abrams, M.D. 1993. Stomatal and nonstomatal limitations of photosynthesis in 19 temperate tree species on contrasting sites during wet and dry years. *Plant Cell Environ.* **16**: 1123–1129.
- Lal, A., Ku, M.S.B., and Edwards, G.E. 1996. Analysis of inhibition of photosynthesis due to water stress in the C₃ species *Hordeum vulgare* and *Vicia faba*: electron transport, CO₂ fixation and carboxylation capacity. *Photosynth. Res.* **49**: 57–69. doi:10.1007/BF00029428.
- Lambers, H., Chapin, F.S., and Pons, T.L. 1998. *Plant physiological ecology*. Springer, New York.
- List, R.J. 1971. *Smithsonian meteorological tables*. Smithsonian Institution Press, Washington, D.C.
- Little, R.L., Peterson, D.L., and Conquest, L.L. 1994. Regeneration of subalpine fir (*Abies lasiocarpa*) following fire: effects of climate and other factors. *Can. J. For. Res.* **24**: 934–944.
- Peet, R.K. 1988. Forests of the Rocky Mountains. In *North American terrestrial vegetation*. Edited by M.G. Barbour and W.D. Billings. Cambridge University Press, Cambridge, UK. pp. 63–102.
- Rahman, S., Munn, L.C., Zhang, R., and Vance, G.F. 1996. Rocky mountain forest soils: evaluating spatial variability using conventional statistics and geostatistics. *Can. J. Soil Sci.* **76**: 501–507.
- Rocheffort, R.M., Little, R.L., Woodward, A., and Peterson, D.L. 1994. Changes in subalpine tree distribution in western North America: effects of climate and other environmental factors. *Holocene*, **4**: 89–100.
- Sharkey, T.D. 1985. Photosynthesis of intact leaves of C₃ plants: physics, physiology and rate limitations. *Bot. Rev.* **51**: 53–105.
- Sharkey, T.D., and Seemann, J.R. 1989. Mild water stress effects on carbon-reduction-cycle intermediates, ribulose biphosphate carboxylase activity, and spatial homogeneity of photosynthesis in intact leaves. *Plant Physiol.* **89**: 1060–1065. PMID:16666664.
- Smith, S.J., Thomson, A.M., Rosenberg, N.J., Izaurrealde, R.C., Brown, R.A., and Wigley, T.M.L. 2005. Climate change impacts for the conterminous USA: an integrated assessment. Part 1. Scenarios and context. *Clim. Change*, **69**: 7–25. doi:10.1007/s10584-005-3614-7.
- Smith, W.K., and Johnson, D.M. 2007. Biophysical effects of altitude on gas exchange. In *Biophysical plant ecology: perspectives and trends*. Brown Publishing. In press.
- Smith, W.K., and Knapp, A.K. 1990. Ecophysiology of high elevation forests. In *Plant biology of the basin and range*. Edited by C.B. Osmond, L.F. Pitelka, and G.M. Hidy. Springer-Verlag, Berlin. pp. 87–142.
- Smith, W.K., Schoettle, A.W., and Cui, M. 1991. Importance of leaf area measurement to the interpretation of gas exchange parameters of complex shoots (e.g., conifers). *Tree Physiol.* **8**: 121–127. PMID:14972884.
- Smith, W.K., Germino, M.J., Hancock, T.E., and Johnson, D.M. 2003. Another perspective for interpreting altitudinal limits of alpine timberlines. *Tree Physiol.* **23**: 1101–1112. PMID:14522716.
- Sperry, J.S., and Sullivan, E.M. 1992. Xylem embolism in response to freeze-thaw cycles and water stress in ring-porous, diffuse porous, and conifer species. *Plant Physiol.* **100**: 605–613. PMID:16653035.
- Teskey, R.O., Fites, J.A., Samuelson, L.J., and Bongarten, B.C. 1986. Stomatal and nonstomatal limitations to net photosynthesis in *Pinus taeda* L. under different environmental conditions. *Tree Physiol.* **2**: 131–142. PMID:14975848.
- Tranquillini, W. 1979. *Physiological ecology of the alpine timberline: tree existence at high altitudes with special reference to the European Alps*. Springer-Verlag, New York.

Wardle, P. 1968. Engelmann spruce (*Picea engelmannii* Engel.) at its upper limits on the Front Range, Colorado. *Ecology*, **49**: 483–495. doi:10.2307/1934115.

Zar, J.H. 1999. *Biostatistical analysis*. Prentice-Hall, Englewood Cliffs, N.J.