

## Abiotic factors limiting photosynthesis in *Abies lasiocarpa* and *Picea engelmannii* seedlings below and above the alpine timberline

DANIEL M. JOHNSON,<sup>1</sup> MATTHEW J. GERMINO<sup>2</sup> AND WILLIAM K. SMITH<sup>1,3</sup>

<sup>1</sup> Department of Biology, Wake Forest University, Winston-Salem, NC 27109-7325, USA

<sup>2</sup> Department of Biology, Idaho State University, Pocatello, ID 83209-8007, USA

<sup>3</sup> Corresponding author (smithwk@wfu.edu)

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**Summary** Most research on the occurrence and stability of alpine timberlines has focused on correlations between adult tree growth and mean temperatures rather than on specific mechanisms. Timberline migration to higher altitude is dependent on new seedling establishment in the tree-line ecotone; however, reductions in photosynthetic carbon gain in establishing seedlings have previously been interpreted solely in terms of decreased seedling survival. Our objective was to evaluate the impact of abiotic factors (temperature, light and water) on photosynthetic carbon gain in young seedlings of the two dominant conifer tree species occurring naturally above (tree-line ecotone site, TS) and below (forest site, FS) a Rocky Mountain timberline in southeastern Wyoming, USA. Coincidentally, measurements were made during an unusually dry summer.

Mean daily photosynthesis in seedlings of both *Abies lasiocarpa* (Hook.) Nutt. (subalpine fir) and *Picea engelmannii* Parry ex Engelm. (Engelmann spruce) was less at TS than at FS (19 and 29%, respectively). Minimum nighttime temperatures below 2 °C were more frequent at TS than at FS and were associated with reduced maximum photosynthesis the following day. Low midday water potentials were associated with a reduction in carbon gain at both sites early in the season, prior to snowmelt, as well as late in the season when soils began to dry. However, the lower photosynthetic rates at TS than at FS appeared to be unrelated to seedling water status because seedlings at both sites had similar xylem pressure potentials. Solar irradiance was highly variable at both sites as a result of uneven shading by neighboring trees, although this variation was substantially reduced on cloudy days (44% of all days observed). Compared with sunny days, cloudy days resulted in greater integrated daily carbon gain at both sites (41% increase at TS and 69% increase at FS), based on a simulated photosynthesis model. Photosynthetic responses to temperature, sunlight and water suggest that variable solar irradiance and nighttime temperatures were major abiotic factors limiting photosynthetic carbon acquisition in these young seedlings, especially for seedlings growing in the tree-line ecotone.

**Keywords:** carbon gain, cloud effects, Engelmann spruce, microclimate, subalpine fir, tree line, water.

### Introduction

The upper elevational limit for forest and tree growth has interested scientists for over a century (see review by Körner 1999). Although timberline migration to a higher altitude is dependent on new seedling establishment (Smith et al. 2003), the specific mechanisms of new seedling establishment at the tree line have rarely been studied (Daly and Shankman 1985, Cui and Smith 1991, Germino et al. 2003). The majority of research concerning the occurrence and stability of alpine timberlines has focused on correlations between adult tree growth and mean temperatures (Daubenmire 1954, Tranquillini 1979, Hasler 1982, Grace 1989, Rada et al. 1996). Based on analyses of the ecological factors associated with maintenance of tree line stability of older saplings and mature trees, it has been concluded that photosynthetic carbon gain may not be limiting at the tree line, but that trees are restricted in their ability to process acquired carbon (i.e., growth limitation hypothesis) (Körner 1998, Hoch et al. 2002, Hoch and Körner 2003).

We compared photosynthetic carbon gain between seedlings of two codominant tree species establishing naturally at sites just below and just above an existing timberline, i.e., within the subalpine forest understory and the adjacent tree-line ecotone. We evaluated differences in abiotic factors (temperature, light and water) that might be responsible for measured reductions in carbon gain. In addition, we quantified the effects of shading caused by microsite and cloudy skies to calculate maximum potential photosynthesis at each site under cloudy and clear conditions with and without structural shading from neighboring vegetation. Coincidentally, measurements were made during an unusually dry summer (2002) as a result of low winter snow accumulation and warm spring temperatures that caused an early spring runoff. For the first time in 16 years, no current-year seedlings were found for either of the dominant conifer tree species.

### Materials and methods

As elevation increases from the subalpine timberline across the forest–tundra ecotone to the upper tree line, ribbon forest is first replaced by flagged tree islands, then by large krummholz

mats with flagged trees on their downwind sides and, finally, by progressively smaller krummholz mats that are separated by tens of meters at the high-elevation limit of this tree-line ecotone (Daubenmire 1954, Wardle 1968, Smith et al. 2003). *Picea engelmannii* Parry ex Engelm. and *Abies lasiocarpa* (Hook.) Nutt. are the codominant conifer species that form the timberline and tree-line boundaries of the south-central Rocky Mountains, USA. Other associated species include *Pinus flexilis* James, shrubs (*Salix* spp. and *Ribes coloradense* Cov.), and many herbaceous species such as *Caltha leptosepala* DC., *Helianthella quinquenervis* Hook. A. Gray and *Erythronium grandiflorum* Pursh (Billings 1969, Peet 1988).

We monitored young seedlings of *P. engelmannii* and *A. lasiocarpa* throughout the summer of 2002.

#### Study sites

Two study sites (about 20 × 20 m) were selected for comparison in the tree-line ecotone of the Snowy Range of the Medicine Bow Mountains in southern Wyoming, USA (41°20' N, 106°13' W). One site was located 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 tree-line ecotone (TS, 3198 m elevation) composed of a mosaic of flagged tree and krummholz islands. Seedlings were dispersed uniformly throughout the subalpine forest, whereas seedlings in the tree-line ecotone were associated spatially with tree islands (Germino et al. 2003). Chosen sites were representative of the subalpine forest and tree-line ecotone communities of the Medicine Bow Mountains. Both sites were located on east-southeast (about 106° for FS and about 114° for TS) slopes (slope less than 10° for both sites).

#### Physiological measurements

Photosynthetic carbon gain in young seedlings (3–20 cm in height, mean height = 9.1 cm, SD = 5.5 cm) was monitored throughout the summer by measuring gas exchange at both sites about every 3 weeks (June 8, June 22 and 23, July 12 and 13, July 31 and August 2, August 17 and 19, September 20 and 21). Measurements were made five times during the day at about 0900, 1100, 1400, 1600 and 1800 h solar time (List 1971). Twelve seedlings of each species at each site (a total of 48 seedlings) were marked for gas exchange. At each sampling, six individuals of each species at each site were randomly selected from among the marked seedlings.

Mean daily photosynthetic rate ( $A$ ) was calculated by averaging all photosynthetic measurements for each species and time period during a day. Integrated daily photosynthesis ( $A_{\text{int}}$ ) was computed by averaging values of photosynthesis for each time period (900, 1100, 1400, 1600 and 1800 h), multiplying by the amount of time in each interval (in seconds), and summing for the entire day. Photosynthesis was measured with an LI-6200 portable photosynthesis system (Li-Cor, Lincoln, NE). Natural orientation of individual seedlings was maintained during all measurements, and photosynthetic CO<sub>2</sub> flux densities were computed on a total leaf area basis (Smith et al. 1991). To obtain mean needle area, the mean product of length

and diameter of 25 randomly chosen needles was calculated for each species. The number of needles per unit of stem length, along with computed needle areas was 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 photosynthesis expressed on a total leaf area basis for both species, as previously reported by Smith et al. (1991).

To determine seedling water status, xylem water potential ( $\Psi$ ) was measured at intervals throughout the season at both sites with a Scholander-type pressure chamber (Model 1000, PMS Instrument, Corvallis, OR). Measurements were performed at 3-week intervals between 1400 and 1500 h solar time each day. 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 put in plastic freezer bags and placed in an ice cooler until measurements were completed, about 1 h later. Measurements of  $\Psi$  were also taken in the field, within minutes of excision, and compared with shoots transferred to the laboratory. No significant changes in water status occurred as a result of transfer and storage during the period of measurement.

#### PAR, air temperature and precipitation

Photosynthetically active radiation (PAR) was measured at each site in areas with high seedling densities and where gas exchange measurements were conducted. We recorded PAR every 5 min for 33 days (July 14–August 15) with Li-Cor LI-190 PAR sensors connected to voltage amplifiers (Model UTA, EME Systems, Berkeley, CA) and HOBO data loggers (H8 4-channel logger, Onset Computer, Bourne, MA). Additional measurements of PAR were taken with a hand-held LI-190 PAR sensor during photosynthetic measurements. Hand-held PAR measurements were also used to compare within-site values of PAR at specific seedling locations with PAR values recorded by data loggers at a central location at each site. We also measured PAR in an open area, above tree line, with no obscuring structure. The nomenclature for PAR regimes (sunflecks, sunpatches and sunlight gaps) follows Smith et al. (1989).

Air temperatures at seedling height were measured with three fine-wire (0.02 mm diameter) copper-constantan thermocouples (Omega Engineering, Stamford, CT) shielded from direct solar radiation and placed about 1 cm above the ground at central locations within both sites. Thermocouples were compared (comparisons among three thermocouples and with air temperature measurements taken with a Li-Cor LI-6200 during the photosynthetic measurements) for accuracy and monitored with Campbell Scientific (Logan, UT) 21X data loggers that recorded data every 5 min for a total of 63 days (June 26–July 1, July 5–July 24, August 2–8, August 15–25, August 31–September 21).

Weekly precipitation data were obtained from the National Atmospheric Deposition Program WY00 site (41°23' N, 106°16' W, 3284 m elevation), located less than 1 km from the study sites. Rainfall was measured daily with an Altermeter Belfort rain gauge and summed weekly.

#### Effects of PAR on seasonal photosynthesis

Based on simultaneous photosynthesis and PAR measurements ( $A$  versus PAR) throughout individual days, potential maximum photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) for seedlings was computed and used to predict the potential maximum amount of carbon a seedling could assimilate during a given day (PMC;  $\text{mol m}^{-2} \text{day}^{-1}$ ). Best-fit equations of the association between photosynthesis and PAR (data used were from all sample dates except August 6) were separated into three time intervals (0700 to 1355, 1400 to 1555, and 1600 to 2100 h) for each site (see Table 1). Data for both *A. lasiocarpa* and *P. engelmannii* seedlings were merged into one data set because of close similarities in each species' photosynthetic light response over the given time intervals. Three time intervals were used for each site to account for different abiotic stresses at different times of the day (e.g., potential afternoon depressions in  $A$  as a result of decreases in water potential). This set of six equations (three time intervals  $\times$  two sites) was used to predict PMC for days with clear skies versus days with naturally cloudy conditions. Also, the best-fit equation for 1600 to 2100 h from FS was used to predict PMC under clear and cloudy skies in open microsites with full sky exposure (OS) because this equation predicts reductions in  $A$  at high PAR values (typical for these two species; see Figure 7).

Characteristic PAR values for each site were determined from mean values of measurements taken every 5 min at both sites during typical clear and cloudy (daily maximum PAR < 65% of seasonal maximum PAR) days. Clear days analyzed were July 29, July 30, August 9, August 10 and August 11. Overcast days were July 20, July 25, August 3, August 5 and August 6. Representative days were chosen based on the similarity of their diurnal PAR values to those of the diurnal means (see Figure 6). Values of PAR for these representative days (August 9 was the representative clear day and July 25 was the representative cloudy day) were (1) integrated from 0700 to

2100 h to determine the representative amount of solar radiation received during a typical clear or cloudy day, and (2) entered into the model equations (Table 1) to predict PMC under clear skies, as well as under naturally overcast conditions for seedlings at both sites. The effects of shading caused by microsite (e.g., shading by neighboring vegetation, rocks and downed trees) on seedling sunlight regime and photosynthetic carbon gain were removed by using diurnal PAR values from an open area with no surrounding vegetation and full-sky exposure. The cloudy day analyzed for OS was August 5 and the clear day was July 15. Because of equipment malfunctions at the OS, the same days could not be used at FS, TS and OS. Ratios of PMC to daily integrated PAR were calculated to estimate photosynthetic limitations at each site based on the different sunlight regimes (clear, cloudy, microsite shading).

#### Sampling and statistics

Statistical analyses were performed using JMP Statistical Analysis Software (SAS, Cary, NC). Individual seedling measurements of photosynthesis and water potential ( $n = 6$  for each species at each measurement interval) were averaged for each species according to time of measurement. Linear least squares regression analysis and standard errors were calculated to test for statistical inference (Zar 1999). Best-fit regression curves were used to estimate photosynthetic light response curves, seasonal photosynthetic carbon gain, and seasonal changes in water potential at each site.

## Results

#### Daily and seasonal photosynthesis

Mean daily photosynthesis ( $A$ ) and integrated daily photosynthetic carbon gain ( $A_{\text{int}}$ ) were less at TS than at FS in both *A. lasiocarpa* and *P. engelmannii* (19 and 29% less for  $A$  and 54 and 41% less for  $A_{\text{int}}$ , respectively, Figure 1). Overall trends seemed to follow similar patterns at both sites, with the lowest  $A$  and  $A_{\text{int}}$  values early (June 8) and late (August 17) in the growing season, and maximum  $A$  and  $A_{\text{int}}$  values in midsummer. Seedlings at both sites showed increased photosynthetic rates between August 17 and September 20, following 2 weeks of intermittent rain beginning on September 1 (2.6 cm rainfall; see Figure 3a).

In both species, morning  $A$  was higher at FS than at TS on July 12 and 13 and August 17 and 19. Afternoon values were higher at TS than at FS July 12 and 13, but on August 17 and 19, afternoon  $A$  was similar at both sites (Figure 2). In contrast, late-season (September 20 and 21) carbon gain increased during the day, followed by a decline in the evening (with the exception of TS spruce seedlings, which did not show a decline in  $A$  at 1800 h).

#### Seasonal water status

Seasonally, midday xylem water potential ( $\Psi$ ) increased from June 8 to July 12 (Figures 3b and 3c), presumably because of early snowmelt. Beginning about July 12,  $\Psi$  became increasingly more negative until August 17 (26 and 22% reduction for

Table 1. Light response equations for determining potential photosynthesis at the tree-line ecotone site (TS) and the forest site (FS) with corresponding  $n$  and  $r^2$  values.

Site	Time interval (h)	Best-fit equation	$n$	$r^2$
TS	0700–1355	$0.3012\ln x - 1.0471$	67	0.649
	1400–1555	$0.003x$	29	0.221
	6000–2100	$0.0000007x^2 + 0.0014x + 0.2063$	48	0.291
FS	0700–1355	$0.3606\ln x - 1.3348$	70	0.694
	1400–1555	$0.0000008x^2 + 0.002x - 0.12$	26	0.738
	6000–2100	$0.2802\ln x - 1.0557$	67	0.645

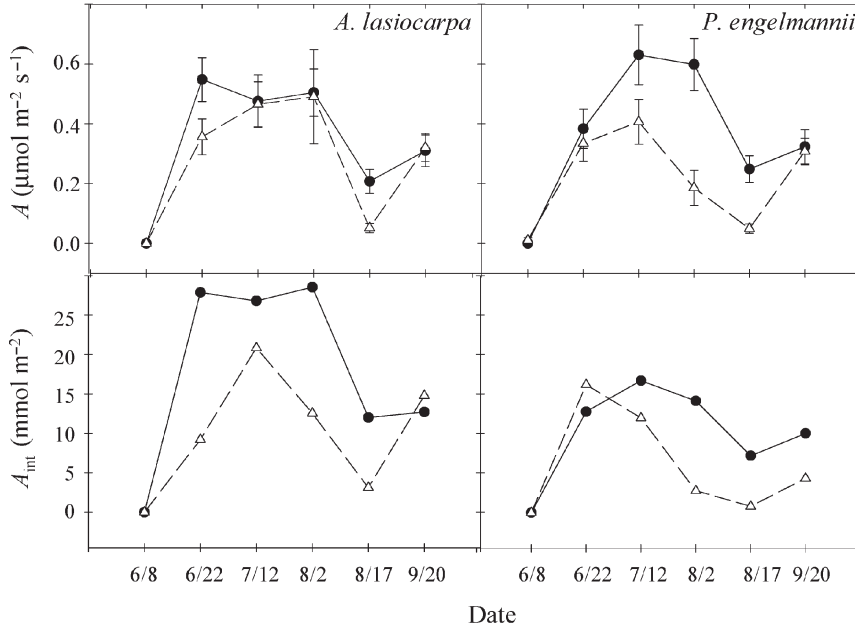


Figure 1. Mean daily photosynthetic rate ( $A$ ) and daily integrated photosynthetic carbon gain ( $A_{int}$ ) of conifer seedlings at the forest (●) and tree-line ecotone (△) sites. Vertical bars are standard errors.

subalpine fir and Engelmann spruce, respectively, at FS, and 15 and 16% reduction for subalpine fir and Engelmann spruce, respectively, at TS), then increased between August 17 and September 20 (27 and 32% increase for subalpine fir and

Engelmann spruce, respectively, at FS, and 14 and 11% increase for subalpine fir and Engelmann spruce, respectively, at TS), possibly in response to 2 weeks of intermittent rain totaling 2.6 cm (Figure 3a). Variations in mean seasonal  $A$  and

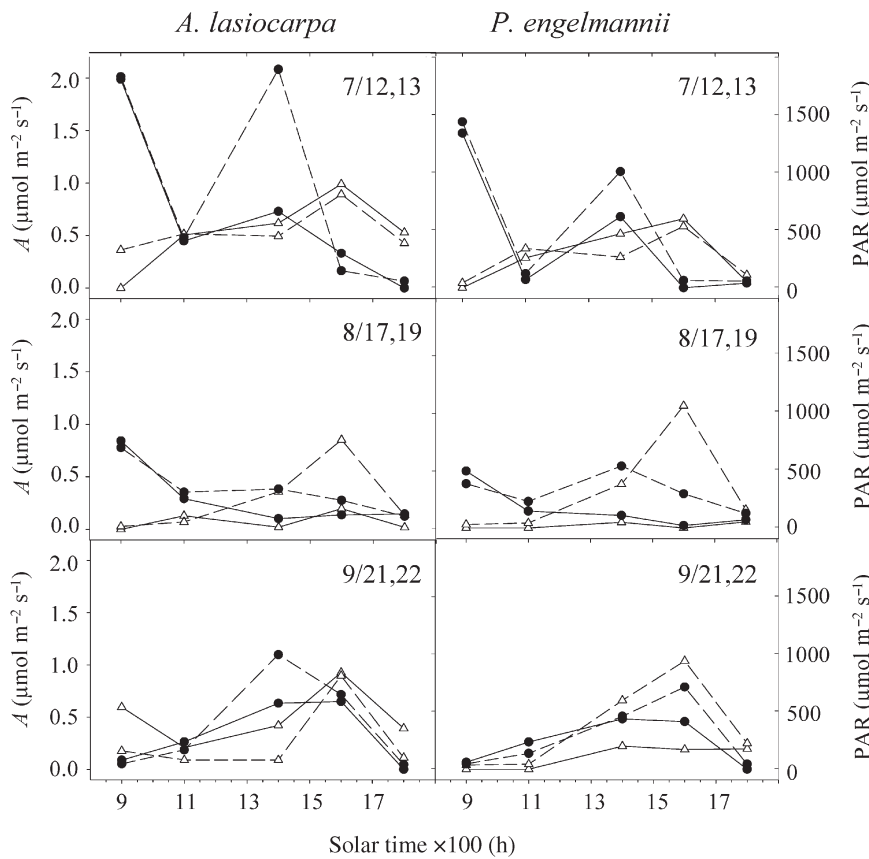


Figure 2. Representative mean daily photosynthetic rates ( $A$ , solid lines) of seedlings and photosynthetically active radiation (PAR) (dashed lines) at the forest (●) and tree-line ecotone (△) sites.

$A_{\text{int}}$  paralleled changes in  $\Psi$ ; high  $A$  and  $A_{\text{int}}$  values were associated with high  $\Psi$ , and low  $A$  and  $A_{\text{int}}$  values were associated with low  $\Psi$ . Mean photosynthesis was negligible at  $\Psi < -2.5$  MPa, which corresponded to measurements on June 8 (Figure 4).

#### Nighttime temperature and maximum photosynthesis

Maximum  $A$  values were less when nighttime temperatures fell below 2 °C (Figure 5) although maximum  $A$  was still positive ( $> 1.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) down to -1 °C (the lowest temperature recorded). The frequency of low ( $< 2$  °C) nighttime minimum temperatures was 44% higher at TS than at FS, and this difference was especially pronounced in July (0 nights at

FS versus 7 nights at TS; Table 2). Mean minimum nighttime temperatures were 2.5 and 3.2 °C at TS and FS, respectively ( $n = 62$ ,  $P = 0.07$ ).

#### Effect of PAR on photosynthesis

Seedlings at the FS and TS sites experienced reduced PAR at certain times of the day compared with full sunlight at an open site with no neighboring adult trees. Seedlings experienced only patchy sunlight on clear days, but within these patches,

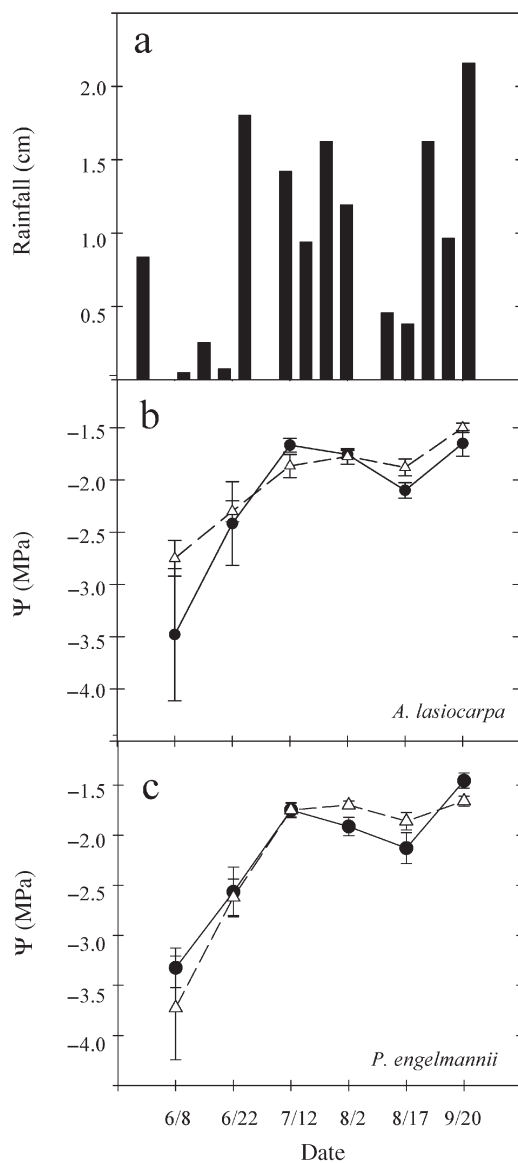


Figure 3. (a) Weekly rainfall for Snowy Range Mountains (WY00 site, National Atmospheric Deposition Program). Mean xylem water potentials ( $\Psi$ ) in (b) *A. lasiocarpa* and (c) *P. engelmannii* at the forest site (●) and tree-line ecotone (△) sites. Vertical bars are standard errors.

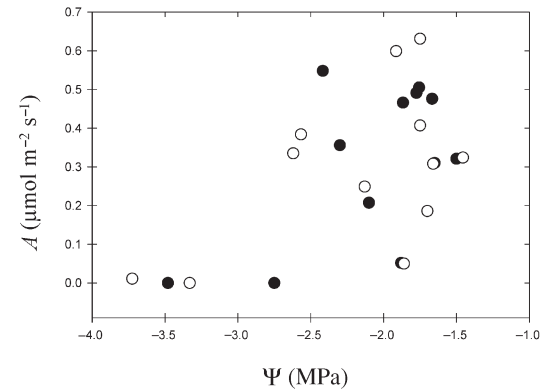


Figure 4. Mean daily photosynthetic rate ( $A$ ) and xylem water potential ( $\Psi$ ) in *A. lasiocarpa* (●) and *P. engelmannii* (○) measured on 12 days over a 3-month period during summer 2002.

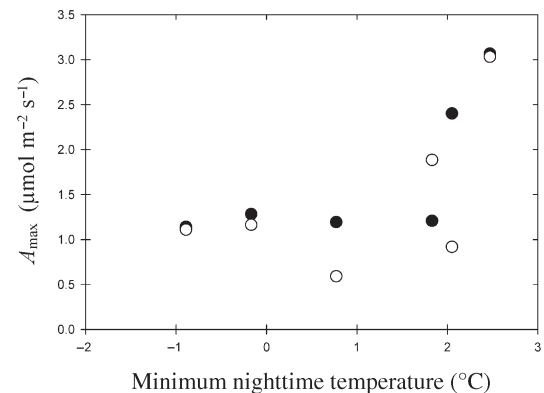


Figure 5. Minimum nighttime air temperature (about 1 cm above ground) and maximum daily photosynthetic rate ( $A_{\text{max}}$ ) for the following day in *A. lasiocarpa* (●) and *P. engelmannii* (○).

Table 2. Proportion of days measured with minimum nighttime air temperatures (1 cm above ground) below 2 °C at the tree-line ecotone site (TS) and the forest site (FS). Numbers in parentheses are percentages.

Site	July	August	September
TS	7/20 (35)	7/19 (37)	12/19 (63)
FS	0/23 (0)	7/19 (37)	11/20 (55)



PAR approached  $1700 \mu\text{mol m}^{-2} \text{s}^{-1}$ . On clear days, PAR in full shade was typically less than  $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ . In contrast, PAR was less variable on overcast days than on clear days, ranging from 55 to  $1341 \mu\text{mol m}^{-2} \text{s}^{-1}$  between 0900 and 2000 h (Figure 6).

At FS, seedlings experienced PAR ranging from  $> 50 \mu\text{mol m}^{-2} \text{s}^{-1}$  to near  $900 \mu\text{mol m}^{-2} \text{s}^{-1}$  in sunflecks on clear mornings (0700 to 1200 h); however, maximum irradiance and its variability were less on cloudy mornings. Early afternoon (1200 to 1600 h) sunlight on clear days at FS was low (mean PAR =  $61 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), with the exception of a few sunflecks (maximum PAR =  $606 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), whereas mean PAR =  $163 \mu\text{mol m}^{-2} \text{s}^{-1}$  on cloudy days. Clear afternoons at FS were characterized by a brief period of intense sunlight (around 1800 to 1900 h, mean PAR =  $612 \mu\text{mol m}^{-2} \text{s}^{-1}$  and maximum PAR =  $1359 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) that was reduced on overcast afternoons (mean PAR =  $81 \mu\text{mol m}^{-2} \text{s}^{-1}$ , maximum PAR =  $159 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Evenings (1700 to 2100 h) at FS had consistently low PAR on clear days (mean PAR  $< 50 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), whereas mean PAR =  $86 \mu\text{mol m}^{-2} \text{s}^{-1}$  on cloudy evenings.

The light environment at TS was less variable than at FS during most of the day. Values of PAR were low on clear days from 0700 to 1700 h (mean PAR =  $58 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) with only a few sunflecks (maximum PAR =  $484 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), which were not observed on cloudy days. A period of relatively high PAR (mean PAR =  $722 \mu\text{mol m}^{-2} \text{s}^{-1}$ , maximum PAR =  $1641 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) from 1700 to 1900 h was measured at TS on clear days, whereas on cloudy days, this pattern was reduced in both irradiance and duration (longest, most intense period was for about 20 min with PAR between 190 and  $1341 \mu\text{mol m}^{-2} \text{s}^{-1}$ ).

Seedlings had reduced photosynthetic rates at full sunlight ( $> 1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), especially in the afternoon when xylem water potentials become more negative (Figure 7). Most seedlings exposed to PAR equal to or below  $50 \mu\text{mol m}^{-2} \text{s}^{-1}$  had

photosynthetic rates at or near zero, whereas more than 50% of seedlings exposed to PAR between 50 and  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$  had detectable photosynthetic rates (Table 3).

Measured integrated sunlight was 63% greater on clear days than on cloudy days at TS (Table 4), whereas FS had only slightly higher integrated PAR values on cloudy days than on clear days (about 2% more on cloudy days). Modeled PMC was higher for cloudy days than for clear days at both sites (41 and 69% increases at TS and FS, respectively). Increases in the ratio of PMC to integrated PAR on overcast days compared with clear days were 64% for TS and 113% for FS. Values of PMC at OS on clear and cloudy days were considerably higher than the corresponding values at TS and FS (303 and 405% higher for clear days at TS and FS, respectively, and 83 and 91% higher for cloudy days for TS and FS, respectively); however, ratios of PMC to integrated PAR at OS were lower than those at TS or FS, with the exception of FS on clear days.

## Discussion

Previous studies of mature trees have reported decreases in photosynthetic carbon gain with increasing elevation (Cavieres et al. 2000, Richardson and Berlyn 2002). In contrast, increases in carbon storage (starch and simple sugars) in older trees have been found with increasing elevation, and have been interpreted as demonstrating limitations to carbon processing, not carbon gain (Hoch et al. 2002, Hoch and Körner 2003). However, an alternative possibility is that increases in stored carbon represent an adaptation for survival in a dynamic, high-stress environment by serving as a reserve for respiratory-driven processes to ensure survival in particularly harsh years (Chapin et al. 1990). Furthermore, it seems unlikely that carbon gain and processing functions could diverge evolutionarily, because they are tightly coupled to one another through feed-forward and feedback interactions (Paul and Peliny 2003, Smith et al. 2003).

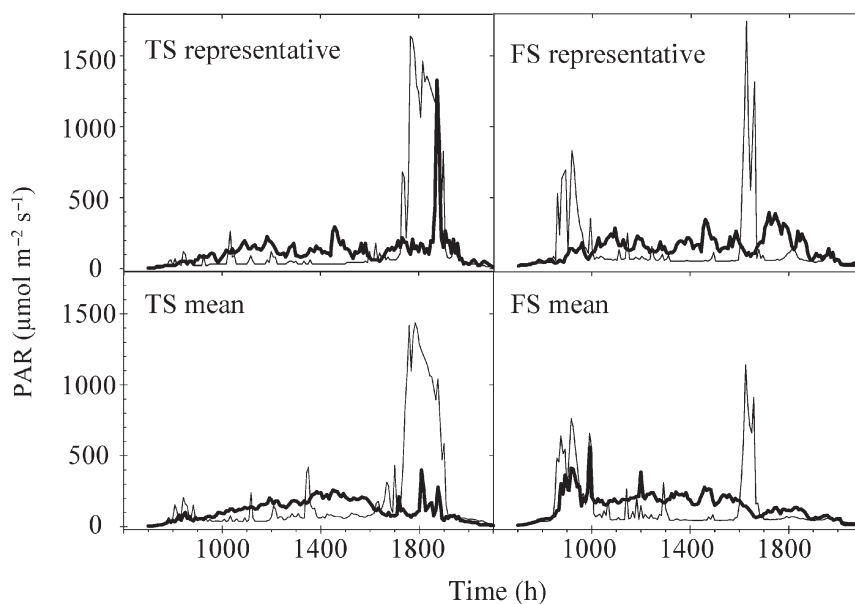


Figure 6. Comparison of mean and representative photosynthetically active radiation (PAR) values for the tree-line ecotone site (TS) and the forest site (FS) on cloudy and sunny days. Thin lines represent sunny days and heavy lines represent cloudy days.

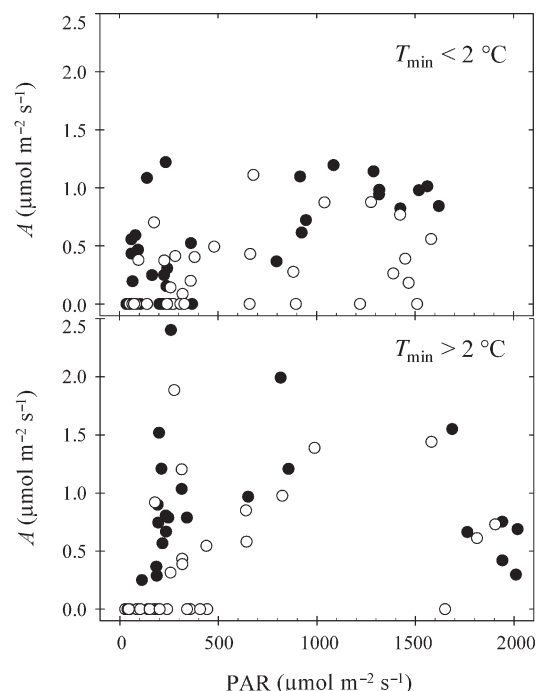


Figure 7. Values of photosynthetically active radiation (PAR) and corresponding photosynthetic rates ( $A$ ) (1300–1700 h) in *A. lasiocarpa* (●) and *P. engelmannii* (○) seedlings in days following nights with minimum temperatures ( $T_{\min}$ ) greater than 2 °C and less than 2 °C. Data from days following nights greater than 2 °C were from July 12 and August 2, and days following nights less than 2 °C were from July 13 and September 20.

Initially, the upward migration of an alpine timberline must begin with seed dispersal, seed germination, and successful seedling establishment into the tree-line ecotone, away from the forest edge (Smith et al. 2003). This new seedling establishment may also be necessary for generating greater ecological facilitation, which leads to even greater seedling establishment and, ultimately, the upward movement of a timberline (and tree line). In addition, a variety of factors limiting seed production, dispersal and germination could inhibit new seedling establishment. However, because small wind-borne seeds such as those of *A. lasiocarpa* and *P. engelmannii* can be dis-

Table 3. Comparison of seedling photosynthetic rates ( $A$ ) at different photosynthetically active radiation (PAR) values. Data from the tree-line ecotone site (TS) and the forest site (FS) were combined.

PAR ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	No. of values > 0	Total no. of values	Percent non-zero (%)	Mean $A$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )
≤ 50	13	81	16	0.026
51–200	59	103	57	0.308
201–500	46	50	92	0.700
501–1000	32	33	97	0.981
1001–1500	26	26	100	1.018
> 1500	25	25	100	0.932

Table 4. Integrated photosynthetically active radiation (PAR) values, seedling potential maximum photosynthesis (PMC) and PMC to integrated PAR ratios (PMC/IP) for representative cloudy and sunny days at the tree-line ecotone site (TS), the forest site (FS) and a site with no structural shading (OS).

Site	Sky condition	Integrated daily PAR ( $\text{mol m}^{-2} \text{day}^{-1}$ )	PMC ( $\text{mmol m}^{-2} \text{day}^{-1}$ )	PMC/IP
TS	Clear	9.09	10.57	0.0012
	Cloudy	5.55	14.89	0.0027
FS	Clear	6.41	8.44	0.0013
	Cloudy	6.54	14.25	0.0022
OS	Clear	57.01	42.64	0.0007
	Cloudy	21.81	22.89	0.0010

persed over large distances, seed source and dispersal may not be a strong limitation to seedling establishment (Arno and Hammerly 1984). In contrast, conifer seed germination and seedling survival appear to be strongly dependent on specific light, water and temperature requirements that are met only within certain microsites (Germino et al. 2003). Several studies reported a high percentage of both newly emerged and older conifer seedlings on fallen logs and moss layers where water retention is prolonged (Knapp and Smith 1982, Duchesneau and Morin 1999).

Seedling mortality is also an important factor in new seedling establishment in the forest understory (Cui and Smith 1991), as well as at tree line (Germino and Smith 2003). Mechanistically, abiotic factors that reduce photosynthetic carbon gain in seedlings are also associated with increased seedling mortality, decreased root growth, and low mycorrhizal infection (Cui and Smith 1991, Miller et al. 1998). These factors include low minimum nighttime temperatures, seasonal soil drying, and suboptimal (both too low and too high) sunlight regimes (Smith 1985, Smith and Knapp 1990, Cui and Smith 1991). For example, seedlings of *P. engelmannii* have higher mortality rates at sites with high sky exposure (cold nighttime skies and high sunlight the following day), a condition that results in low-temperature-induced photoinhibition of photosynthesis (Germino and Smith 2000). Facilitation by surrounding vegetation is associated with increased survival, rather than with less survival as a result of apparent competition for soil water (Germino et al. 2003)

#### Air temperature effects on photosynthesis

Decreases in photosynthesis in high-elevation species can occur in response to the relatively high frequency of low nighttime temperatures throughout summer (e.g., Germino and Smith 1999, Cavieres et al. 2000, Awada et al. 2003). Increases in sky exposure contribute to both lower nighttime temperatures and greater sunlight exposure, as well as low-temperature induced photoinhibition of photosynthesis in *A. lasiocarpa* and *P. engelmannii* (Germino and Smith 2000). Seedlings in microsites with high sky exposure experience in-

creased long-wave thermal radiation losses because of cold nighttime skies, resulting in low minimum leaf temperatures followed by high incident radiation during early morning, a time when water status and photosynthesis are highest for the day. Increases in sky exposure also reduce soil temperatures, which negatively affect photosynthesis in these conifer species (Knapp and Smith 1982). However, soil temperatures tend to remain closer to air temperature than leaf temperature during the day (Germino et al. 2003).

The combination of low leaf temperatures and high solar radiation may result in low-temperature-induced photoinhibition of photosynthetic carbon gain at a time when water limitation is at a minimum for the day (e.g., Öquist et al. 1987). With increasing distance from the forest edge into the tree-line ecotone, sky exposure increases because of the occurrence of fewer and smaller trees (Germino et al. 2003). Cold nighttime temperatures close to the ground surface are a function of altitude (i.e., adiabatic lapse rate) and colder skies (thinner atmosphere), as well as cold air settling as a result of both macro- and micro-topographic effects. In our study, nighttime air temperatures below 2 °C resulted in decreases in maximum  $A$  the following day, and were substantially more frequent during summer at TS than at FS (26 versus 18 nights, respectively).

#### Water effects on photosynthesis

Soil drying in summer is typical of the south-central Rocky Mountains and may have large impacts on seasonal photosynthesis and carbon gain in high-elevation species (see review by Smith and Knapp 1990). In our study, the unusually low winter snowpack (about 40% of the average winter snowpack, USDA SNOTEL program, WY00 site) and several dry years beforehand led to extremely low  $\Psi$  early in summer, followed by a slight recovery by the end of June (Figure 3). These unusually low  $\Psi$  values were characteristic of the entire summer and probably dominated photosynthetic limitations. However, photosynthesis still appeared to be inhibited early in the growing season by low soil temperatures, possibly the result of cold snowmelt water saturating the surface soil layers.

Low water status has been associated with reduced photosynthesis and increased mortality in first-year conifer seedlings of the subalpine forest understory (Cui and Smith 1991). Potentially, insufficient carbon gain can result in reduced root growth, seedling desiccation, reductions in photosynthetic carbon gain and, eventually, increased seedling mortality. Newly germinated seedlings in microsites with good moisture retention, for example fallen logs and mosses, may not need to allocate as much carbon to root growth (Knapp and Smith 1982, Bongarten and Teskey 1987).

The water status of the *A. lasiocarpa* and *P. engelmannii* seedlings was similar at the TS and FS sites, indicating that soil water limitation was not a discriminating stress factor for the two sites during the summer of measurement. When  $\Psi$  values decreased below about -2.5 MPa (June 8 measurements), photosynthesis was negligible at both sites, and decreases in  $\Psi$  corresponded to early- and late-season declines in photosynthesis. It is difficult to estimate the impact that the previous dry

winter had on seasonal photosynthesis of the measured seedlings, but we note that our values of  $\Psi$  and mean  $A$  were substantially lower than those reported in earlier studies (e.g., Cui and Smith 1991).

#### Sunlight effects on photosynthesis

The nature of the daytime sunlight regime is important for maximizing daily carbon gain and is normally tightly correlated to the amount of sky exposure. However, the negative effects of nighttime sky exposure (minimum nighttime temperature) and the potentially positive and negative effects of daytime sky exposure (incident sunlight) are difficult to separate. Nevertheless, sky exposure has been shown to be important for seasonal carbon gain and survival in subalpine fir and Engelmann spruce seedlings at the tree line (e.g., Germino et al. 2003). Similarly, Leakey et al. (2003) found that growth of dipterocarp seedlings was four times greater for seedlings growing under daytime sky exposure conditions characterized by longer periods of direct radiation compared with shorter periods of direct radiation. Higher densities of *Eucalyptus pauciflora* Sieber ex A. Spreng. seedlings were found in areas that minimized both nighttime sky exposure and long periods of intense sunlight rather than either stress alone (Ball et al. 1991). Cui and Smith (1991) found that both too little as well as too much sunlight limited photosynthetic carbon gain and survival in newly emerging conifer seedlings in the forest understory.

Most seedlings in the tree-line ecotone are associated with large tree islands, which generate a highly variable sunlight environment (cf. Hättenschwiler and Smith 1999). In addition, cloud cover plays a large role in determining the amount of incident sunlight in numerous habitats (Roderick et al. 2001, Gu et al. 2002) and is likely important in the south central Rockies where more than 40% of summer afternoons are cloudy or overcast (Young and Smith 1983). Cloud cover increases the amount of diffuse light, generating a more optimal sunlight regime for photosynthesis in understory subalpine species of this area (Young and Smith 1983).

Seedlings at TS received most of their sunlight in the late afternoon (Figure 6), when water potentials were lowest, whereas FS seedlings received most of their sunlight in the morning, when water potentials were less negative. These differences, combined with the higher frequency of freezing nights at TS, may explain why seasonal  $A$  was higher in seedlings at FS than at TS, even though the TS seedlings received more sunlight on clear days (Table 4).

Potential maximum carbon gain was predicted to be greater on cloudy days than on clear days in both FS and TS seedlings as a result of reduced variation and increased penetration of solar radiation. In addition, PMC in the hypothetical site with no surrounding vegetation (OS) was predicted to be higher on clear days than on cloudy days, although the model did not account for the potential photoinhibitory effects of prolonged exposures to high solar irradiances. The ratio of PMC to integrated PAR was substantially greater at both TS and FS on cloudy days compared with clear days, indicating that seedling



photosynthesis may be more efficient under cloudy conditions. Cloud cover may also benefit seedling carbon gain at both TS and FS by reducing leaf temperatures and thus evaporative demand. High evaporative demand is a potentially important characteristic of high altitudes because the lower ambient pressure and more rapid mass diffusion lead to greater transpirational water loss for the same degree of stomatal opening than at low altitudes (e.g., Gu et al. 2002, Smith et al. 2003). Nighttime cloud cover could also provide substantially warmer nighttime skies, warmer minimum leaf temperatures, and a reduced impact of low-temperature-induced inhibition of photosynthesis during the following morning hours (Jordan and Smith 1994, Germino and Smith 1999). Several climate models (e.g., Vuille et al. 2003) have predicted that changes in cloud patterns could impact tree line stability. Increases in seedling carbon gain during cloud cover could result in increased seedling establishment into the tree-line ecotone with increases in cloud frequency, and upward advancement of the current tree line.

### Conclusions

Photosynthetic carbon gain in young seedlings of *A. lasiocarpa* and *P. engelmannii* was reduced in the tree-line ecotone compared with the adjacent forest understory, and appeared to be associated primarily with differences in sunlight and temperature regimes. Reduced water status during this unusually dry year appeared to inhibit carbon gain similarly at both sites early in the season before snowmelt and late in the season when soils began to dry. In contrast, minimum nighttime temperatures below 2 °C were more frequent at the higher-elevation, more sky-exposed site and were correlated with lower maximum photosynthesis the following day. Sunlight regimes were highly variable at both sites as a result of the occurrence of seedlings in the forest understory and beneath tree islands; however, this variability was reduced during cloudy conditions, a characteristic of the afternoons. Cloudy days resulted in greater carbon gain based on a photosynthesis model that predicted potential carbon gain for different microsites and corresponding sunlight regimes. In both the subalpine forest understory and the tree-line ecotone, the abiotic factors resulting in reductions in carbon gain (even during a particularly dry year) appeared to be low nighttime temperatures plus sunlight regimes that were either excessive or inadequate, especially in the tree-line ecotone.

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