

Refugial forests of the southern Appalachians: photosynthesis and survival in current-year *Abies fraseri* seedlings

DANIEL M. JOHNSON¹ and WILLIAM K. SMITH^{1,2}

¹ Department of Biology, Wake Forest University, P.O. Box 7325, Reynolda Station, Winston-Salem, NC 27109-7325, USA

² Corresponding author (smithwk@wfu.edu)

Received December 8, 2004; accepted April 2, 2005; published online August 16, 2005

Summary Fraser fir (*Abies fraseri* (Pursh) Poiret) is an endemic, high-elevation conifer confined to six relict mountain-top communities in the southern Appalachian Mountains, USA. High adult mortality has occurred over the past 50 years, possibly the result of an introduced insect (*Adelges piceae* Ratzeburg), air pollution, or both. Knowledge of the mechanisms of and limitations to seedling establishment may allow reestablishment and perpetuation of this unique community type, notwithstanding global climate change. We monitored seedling emergence and mortality in relation to photosynthetic performance and water relations in microsites differing in canopy openness (sunlight exposure) over the summer of 2004. Abundance of cotyledonous seedlings in early summer was 2.3 times greater (849 versus 366 seedlings m⁻²) in microsites with lower sky exposure (greater canopy closure) than in microsites with greater sky exposure (greater canopy openness). In contrast, late-season abundance and survival were greater in areas beneath more open canopies than in areas beneath less open canopies (3.3 times and 11.7 times greater, respectively). However, newly emerged seedling survival in a completely open site (no overhead canopy) was zero, despite an initial density of 124 seedlings m⁻². Seedling water status was similar in open- and closed-canopy sites (−0.52 and −0.74 MPa, respectively). Photosynthetic carbon gain was higher in newly emerged seedlings at open canopy than at closed canopy sites, especially during early morning. Based on photosynthetic light response curves and measured sunlight regimes, seedlings in open canopy sites were estimated to assimilate 3.3–4.5 times more carbon than seedlings at closed sites. Reductions in carbon gain of closed-site seedlings, primarily a result of limited sunlight, corresponded to substantial increases in seedling mortality (98 versus 79% in open canopy sites). Thus, sunlight exposure, which reflects overstory canopy structure, appears to be an important factor influencing newly emerged seedling survival and distribution.

Keywords: altitude, carbon gain, cotyledon, endemic species, microclimate, refugial populations, stand reestablishment, sunlight regime.

Introduction

Fraser fir (*Abies fraseri* (Pursh) Poiret) is endemic to the southern Appalachian Mountains, USA, where it is restricted to six island populations in North Carolina, southern Virginia and eastern Tennessee (Ramseur 1960). It is the dominant tree species at elevations above about 1850 m and is interspersed with red spruce between 1650 and 1850 m (Whittaker 1956). Over the last several decades, increased adult mortality has been reported (see Eagar 1984 for review) and has often been attributed to the introduced balsam woolly adelgid (*Adelges piceae* Ratzeburg). However, other factors may have contributed to the decline of Fraser fir, including drought (Bruck and Robarge 1988), ice storms (Bruck and Robarge 1988, Nicholas and Zedaker 1989) and deposition of atmospheric pollutants (Hain and Arthur 1985, McLaughlin et al. 1990, 1991, Mohnen 1992). Past records indicate that adult mortality due, for example, to windthrow may have been high before infestation with the adelgid (Crandall 1958).

The decline of Fraser fir over the past 50 years makes understanding limitations to stand regeneration an important goal of conservation research. Successful seedling establishment and subsequent growth under newly formed canopy gaps may be critical for stand reestablishment, as well as for preventing encroachment of shrub species that could out-compete understory fir seedlings, especially *Rubus* (White et al. 1985, Pauley and Clebsch 1990). However, previous studies on Fraser fir seedlings have focused only on abundance and have not examined the factors influencing seedling establishment (Nicholas et al. 1992, Smith and Nicholas 2000).

The purpose of our study was to evaluate newly emerged seedlings, including their abundance, survival and photosynthetic physiology at understory microsites differing in exposure to incident sunlight. Experiments were designed to compare the survival of newly emerged seedlings at natural microsites subject to different solar irradiances. Daily photosynthesis and water relations of seedlings were compared among the sites, and correlated with survival rates to evaluate the potential interactions between sunlight exposure, desiccation and survival. Water status of newly emerged seedlings of tree-line conifers in the Rocky Mountains, USA, has been strongly implicated as a factor influencing seedling mortality

(e.g., Cui and Smith 1991, Germino et al. 2002). We hypothesized that emergent seedling survival is also related to solar irradiance through an effect on photosynthetic carbon gain; too much, as well as too little sunlight, limit photosynthesis, and hence, survival.

Methods and materials

Study sites

Study sites were located in a spruce–fir (*Picea rubens* Sarg. and *Abies fraseri*) mixed forest near Roan High Knob (highest peak in Roan Mountain complex) in Pisgah National Forest, NC, USA (36°5' N, 82°8' W, 1900 m a.s.l.). Roan Mountain is a mosaic of grass and heath balds, northern hardwood forests and spruce–fir forests (Mark 1958), with spruce–fir forests typically found at the highest elevations. This area was monitored about monthly over the summers (May–August) of 2002 and 2003 for the presence of newly emerged seedlings. In 2002, only 31 newly emerged seedlings were found in an area of about 25 m², and no newly emerged seedlings were found in the summer of 2003.

Thirteen, 1-m² permanent quadrats were established in areas differing in canopy openness (Table 1, Figure 1). Four quadrats were established beneath each of three canopy types that differed in sky exposure: open canopy (OC), intermediate canopy (IC) and closed canopy (CC). Plots were distributed across an area of about 1000 m² and no more than two plots of the same type were located within any 25 m² area (e.g., not all OC plots were in the same canopy gap). After the beginning of the study (June 16, 2004), one quadrat was established at an entirely open site (OS, no overstory canopy). The degree of canopy openness for each canopy plot was quantified based on hemispherical photographs of the canopy taken at seedling heights and the software Gap Light Analyzer (Version 2.0, Simon Fraser University, Burnaby, BC, Canada and Institute of Ecosystem Studies, Millbrook, NY). The survival of current-year seedlings was monitored in each plot throughout the summer of 2004. Seedlings were initially counted on May 21 (except at the OS site, where the initial count was made on June 16) and recounted every 1–2 weeks.

Table 1. Study site type, number of quadrats in each site type (*n*), mean % sky exposure and clear-day sunlight. Mean % sky exposure was determined from hemispherical photographs. Photosynthetic photon flux (PPF) was measured with PPF sensors every 5 min and summed to generate cumulative values. Abbreviations: OC = open canopy; IC = intermediate canopy; and CC = closed canopy sites.

| Site type | <i>n</i> | Mean % sky exposure (± SE) | Cumulative diurnal PPF (mol m ⁻² day ⁻¹) |
|-----------|----------|----------------------------|---|
| OC | 4 | 22.6 (1.4) | 1.29 |
| IC | 4 | 19.7 (0.7) | 0.81 |
| CC | 4 | 14.5 (0.6) | 0.30 |

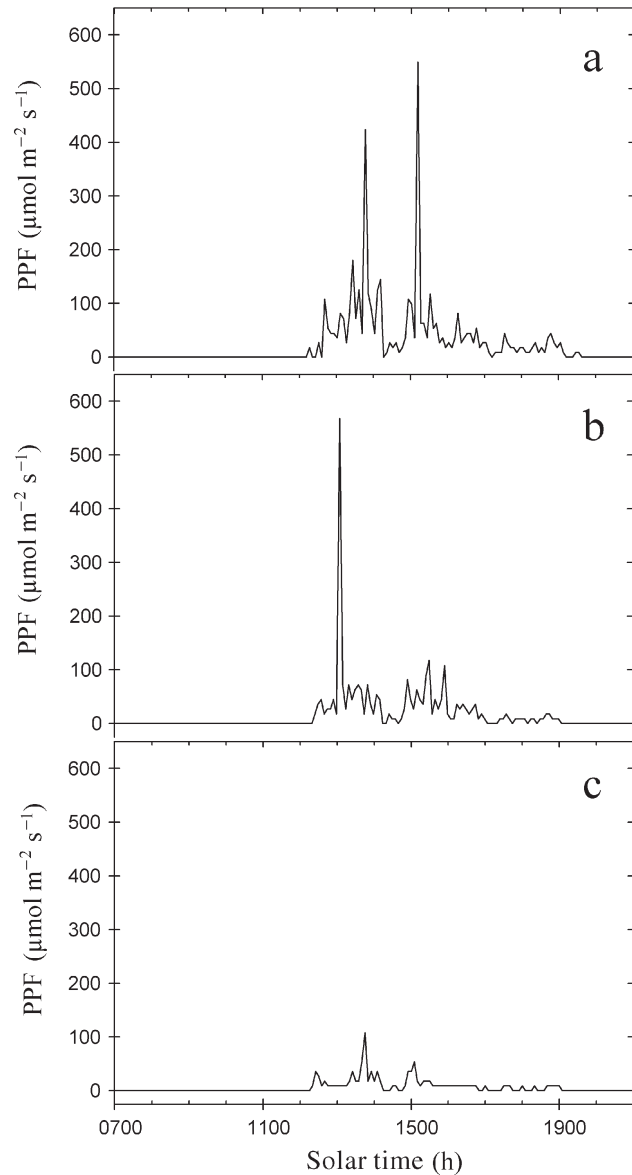


Figure 1. Representative clear-day (August 27, 2004) sunlight regime at the open canopy (a), intermediate canopy (b) and closed canopy (c) sites. Abbreviation: PPF = photosynthetic photon flux.

Measurement of PPF

At each canopy site, photosynthetic photon flux (PPF) was measured every 5 min for 8 days (August 27 to September 3) with LI-190 PAR sensors (Li-Cor, Lincoln, NE) connected to voltage amplifiers (Model UTA, EME Systems, Berkeley, CA) and HOBO data loggers (H8 4-channel logger, Onset Computer Corp., Bourne, MA). Total diurnal PPF for each plot type was calculated from PPF for a representative clear day (August 27) (Table 1, Figure 1). Additional measurements of PPF were made with a handheld LI-190 PPF sensor during photosynthetic measurements. Photosynthetic photon flux measurements made with the hand-held sensor at specific seedling locations were used in conjunction with data-logged PPF measurements made at a central location within each site.

Physiological measurements and predicted daily carbon gain

To determine photosynthetic carbon gain in current-year seedlings (4–5 cotyledons and no primary needles), gas exchange was measured throughout the summer with a Li-Cor LI-6400 model portable photosynthesis system (Li-Cor, Lincoln, NE). Selected seedlings were measured at sites directly adjacent to the quadrats used for the mortality studies, and included the OC and CC sites. Measurements were taken twice each day at about 0800 and 1400 h (Solar Time; List 1971) on days considered representative of the early, middle and late portions of the peak summer growth period (June 16, July 14, August 19). All photosynthetic measurements were made on detached seedlings. Before beginning these measurements, stomatal conductance was monitored in a separate group of detached seedlings, which confirmed that within the 2–3-min time span required for each measurement, there was no change in the stomatal conductance. Natural orientation of individual seedlings was maintained during all measurements by inserting the hypocotyls into holes in a holder which was placed, with the seedlings, inside the chamber. Air temperature and relative humidity inside the chamber were maintained within 5% of ambient values. Seedlings were illuminated with a blue/red LED light source (Li-Cor Model 6400-02B LED). When seedlings were placed in the leaf chamber, PPF was initially 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (a value chosen as being intermediate relative to the daily sunlight regimes experienced by these seedlings; Figure 1). Photosynthetic photon flux in the chamber was then increased (in series: 200, 400, 800, 1000, 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) or decreased (in series: 200, 100, 50, 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$) to construct light response curves for net photosynthesis. Each set of seedlings was used for 4–5 consecutive measurements at different PPF values. Best-fit equations from the photosynthetic light response curves (Table 2) were used to predict seedling carbon gain at each site (see below). Maximum photosynthetic flux (A_{max}) and maximum stomatal conductance to water vapor (g_{max}) were calculated by averaging the five highest values from each set of measurements.

Net photosynthetic rates were computed on a total leaf area basis (Smith et al. 1991). Leaf area inside the sample cuvette was estimated from the number of cotyledons multiplied by the area per cotyledon, cotyledon area being estimated from a regression between length and area determined by the glass bead technique (Hadley and Smith 1987).

The xylem pressure potential of seedlings located adjacent to those used for the gas exchange measurements was measured with a Scholander-type pressure chamber (Model 1000, PMS Instrument Company, Corvallis, OR) between 1400 and 1500 h solar time on the same days that the photosynthetic measurements were made.

An estimate of daily carbon fixation was computed from a model based on the photosynthetic light response curve and light regime (Johnson et al. 2004). Integrated PPF between 0700 and 2100 h on a clear day (August 27) was entered into the model equations (Table 2) to compute the maximum potential carbon gain under clear skies at each site.

Sampling and statistics

Statistical analyses were performed with JMP Statistical Analysis Software (SAS, Cary, NC). Seedling measurements of photosynthesis were averaged by site, PPF and time of measurement. Seedling water potentials were averaged for the OC and CC sites according to date. Least Squares Regression analysis and standard errors were calculated to test for statistical significance among the quantitative comparisons of the OC site versus the CC site (Zar 1999). Statistical differences between measurements at the three canopy sites (OC, IC and CC) were evaluated by the Tukey-Kramer multiple-comparison method. Best-fit regression curves (curvilinear) were used to generate equations of the photosynthetic light response curves at all sites.

Table 2. Light response equations used to predict net photosynthetic carbon gain in seedlings. All equations are of the form $y = a(1 - e^{-bx})$, where y is photosynthesis (A), x is photosynthetic photon flux (PPF) and a and b are constants giving the best fit. Abbreviations: LCP = photosynthetic light compensation point ($\mu\text{mol m}^{-2} \text{s}^{-1}$); OC = open canopy site; and CC = closed canopy site.

| Month | Site | Time | a | b | n | r^2 | LCP |
|--------|------|------|------|-------|-----|-------|------|
| June | OC | 0800 | 2.65 | 0.010 | 15 | 0.95 | 11.3 |
| | | 1500 | 2.27 | 0.013 | 22 | 0.85 | 6.0 |
| | CC | 0800 | 1.93 | 0.019 | 16 | 0.92 | 9.2 |
| | | 1500 | 2.23 | 0.011 | 22 | 0.93 | 8.0 |
| July | OC | 0800 | 2.72 | 0.008 | 31 | 0.83 | 0.0 |
| | | 1500 | 2.17 | 0.016 | 29 | 0.87 | 6.6 |
| | CC | 0800 | 1.02 | 0.022 | 23 | 0.69 | 44.5 |
| | | 1500 | 2.30 | 0.008 | 30 | 0.90 | 9.4 |
| August | OC | 0800 | 1.69 | 0.010 | 24 | 0.74 | 29.6 |
| | | 1500 | 3.13 | 0.007 | 24 | 0.91 | -6.3 |
| | CC | 0800 | 1.34 | 0.019 | 24 | 0.70 | 28.4 |
| | | 1500 | 2.87 | 0.006 | 29 | 0.90 | -2.9 |

Results

Seedling abundance and survival

Moribund seedlings first showed wilting and then blackening of the cotyledons. At first, only a single cotyledon displayed these changes, but others followed until all cotyledons were dark and wilted, at which point the seedling was considered dead.

New seedlings continued to appear from May 21 until

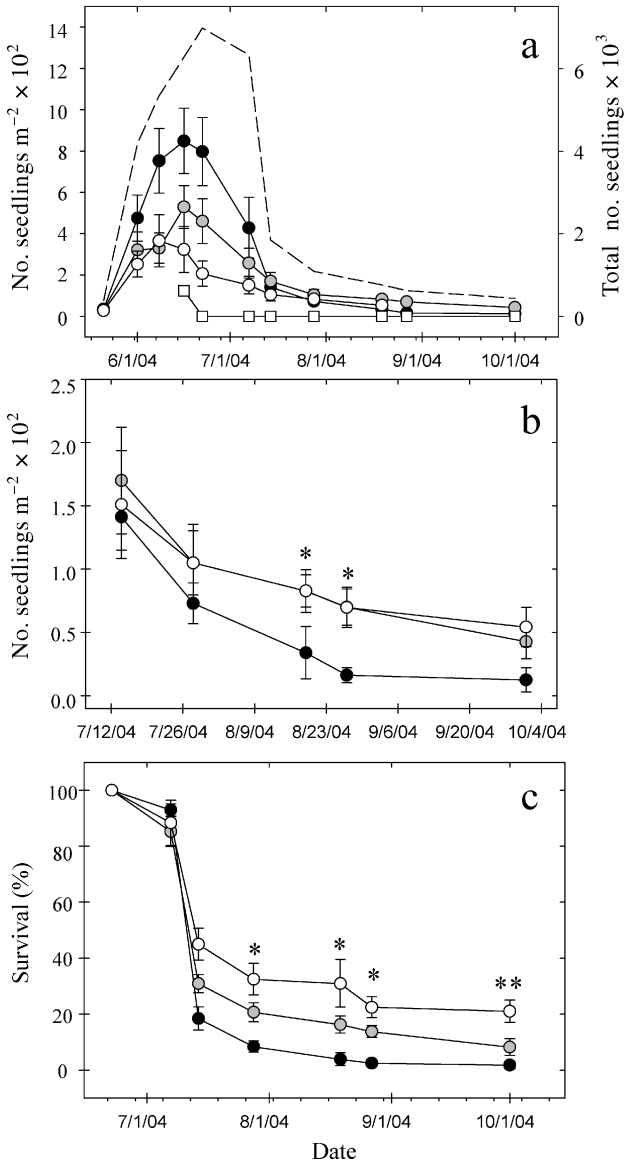


Figure 2. Newly emerged seedling abundance (a, b) and survival (c) in *Abies fraseri*. Symbols: ○ = open canopy site; ● = intermediate canopy site; ■ = closed canopy site; and □ = the most exposed (no canopy) site. In (a), the dashed line represents the total number of seedlings in the study. Statistical significance: * = significant differences ($P < 0.05$, Tukey-Kramer LSD) between open canopy versus closed canopy sites; and ** = significant differences between open canopy versus closed canopy sites and open canopy versus intermediate canopy sites. Vertical bars are standard errors.

June 16 (Figure 2a) and were most abundant at the CC site on June 16, followed by the IC, OC and OS sites. After June 16, seedling abundance began to decline in all plots, decreasing from 124 to 0 m⁻² at the OS site by June 22. Seedling abundance was nearly equal in the other canopy plots by July 14, and continued to decline throughout the summer. Mean seedling abundances on October 1 (Figure 2b) were significantly lower at the CC site than at the OC site (12.5 versus 54.3, $P = 0.031$) when analyzed by t tests, but not when using the Tukey-Kramer method for comparing multiple means. There were no significant differences in abundance on October 1, 2004, between the OC and IC sites or the CC and IC sites using either t tests or the Tukey-Kramer method.

Seedling survival, expressed as a percentage of the maximum number of seedlings in the plot, declined slightly from June 22 to July 7 and then declined rapidly from July 7 to July 14 in all canopy plots (Figure 2c), representing a 50, 64 and 84% reduction in seedling number at the OC, IC and CC sites, respectively. Survival continued to decrease to the end of the study (October 1), although not as steeply as between July 7 and July 14. On October 1, OC seedling survival was significantly different ($P < 0.05$) from that of either IC or CC seedlings, but IC seedling survival did not differ significantly from CC seedling survival. In the canopy plots, higher seedling survival at the end of the study was correlated with greater canopy openness (slope = 0.019% for canopy openness/% survival, $r^2 = 0.56$; Figure 3).

Seedling water status and photosynthesis

Xylem pressure potential remained high (about -0.2 to -0.4 MPa) from June 1 to June 16 (Figure 4) and then declined to about -0.6 MPa in OC seedlings and -0.7 MPa in CC seedlings on July 14. Although seedlings at the OC site had less negative water potentials on August 19, differences between OC and CC seedlings were not statistically significant at any measurement date (smallest P value August 19, $P = 0.079$).

Photosynthetic rates saturated at about 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$

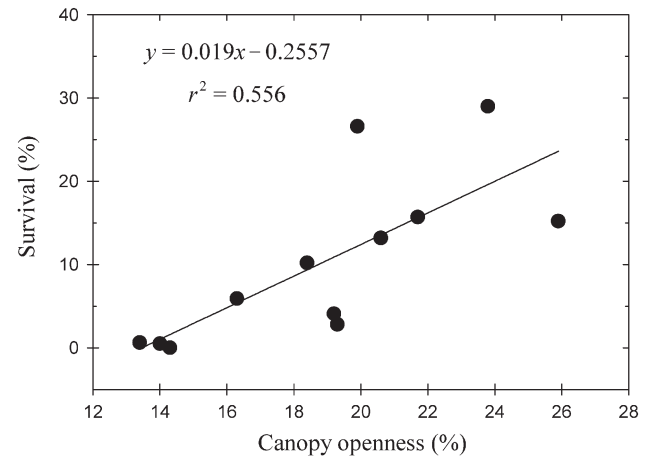


Figure 3. Relationship between percent canopy openness and newly emerged seedling survival in *Abies fraseri* (canopy plots only) at the end of the study period (October 1, 2004).

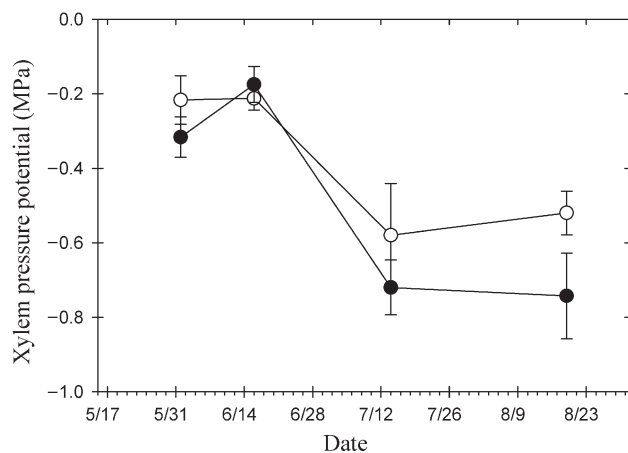


Figure 4. Mean xylem pressure potentials in *Abies fraseri* during the afternoon (1400–1500 h). Symbols: ○ = open canopy site; and ● = closed canopy site. Vertical bars are standard errors.

PPF (Figure 5). Overall, OC seedlings had higher photosynthetic rates than CC seedlings (Figures 5 and 6), especially in the morning. Morning values of saturated photosynthetic

rates were between 1.5 and $2.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ in CC seedlings compared with 2.0 to $3.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ in OC seedlings. Afternoon photosynthetic rates saturated at 2.5 – $3.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ in both CC and OC seedlings. Maximum photosynthetic capacity varied over the course of the season with site type and measurement interval. Morning A_{max} was greater in OC seedlings than in CC seedlings on all measurement dates, whereas afternoon A_{max} was only slightly greater in OC seedlings than in CC seedlings in June and August, and was similar in July (Figure 6). Morning g_{max} was higher in OC seedlings than in CC seedlings on July 14 and August 19, but higher in CC seedlings than in OC seedlings on June 16. Afternoon g_{max} was higher in OC seedlings than in CC seedlings on June 16 and July 14, but higher in CC seedlings on August 19. In OC seedlings, g_{max} increased from June to July and then decreased from July to August in both the morning and afternoon, whereas g_{max} in CC seedlings decreased in the morning and increased in the afternoon throughout the season.

Sunlight regime and simulated carbon gain

Cumulative diurnal PPF was about four times higher at the OC site than at the CC site, corresponding to an 8.1% difference in

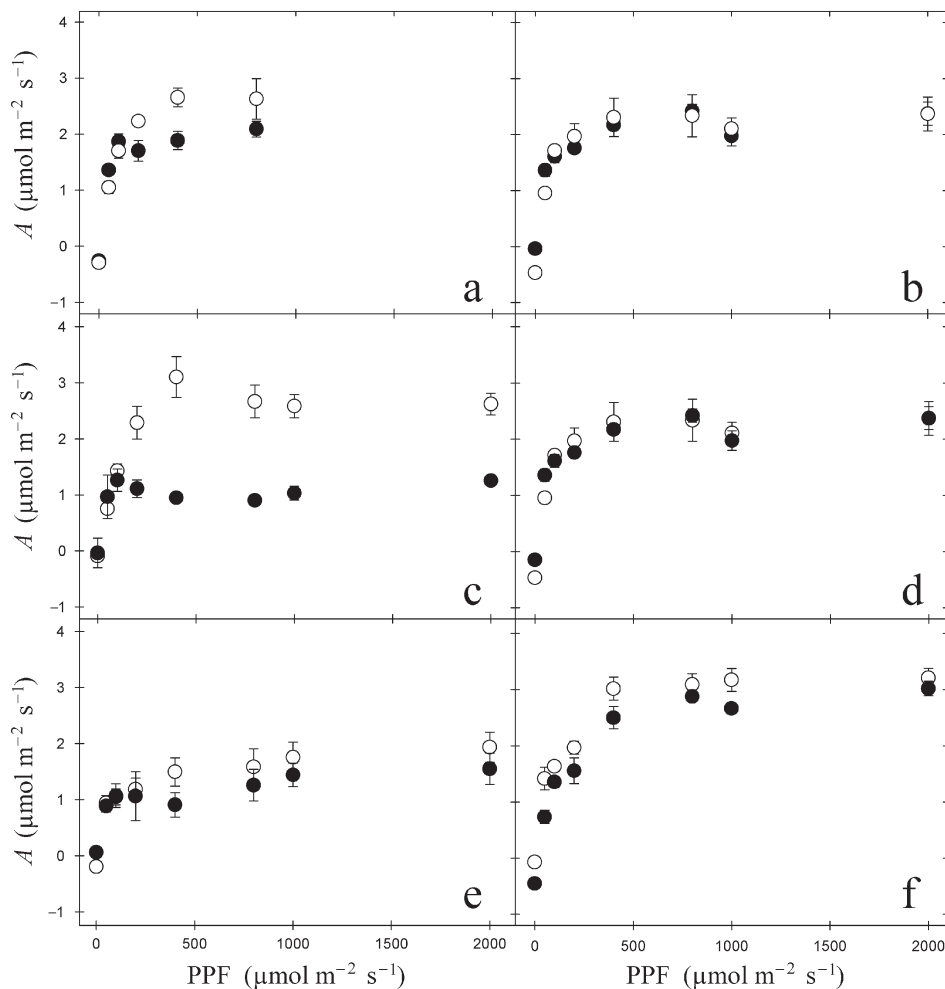


Figure 5. Photosynthetic light response curves (instantaneous) of *Abies fraseri* measured during the morning (0800–0900 h; a, c, e) and afternoon (1400–1500 h; b, d, f) at the open canopy (○) and closed canopy (●) sites. Measurements were made on June 16 (a, b), July 14 (c, d) and August 19 (e, f). Abbreviations: A = photosynthetic rate; and PPF = photosynthetic photon flux.

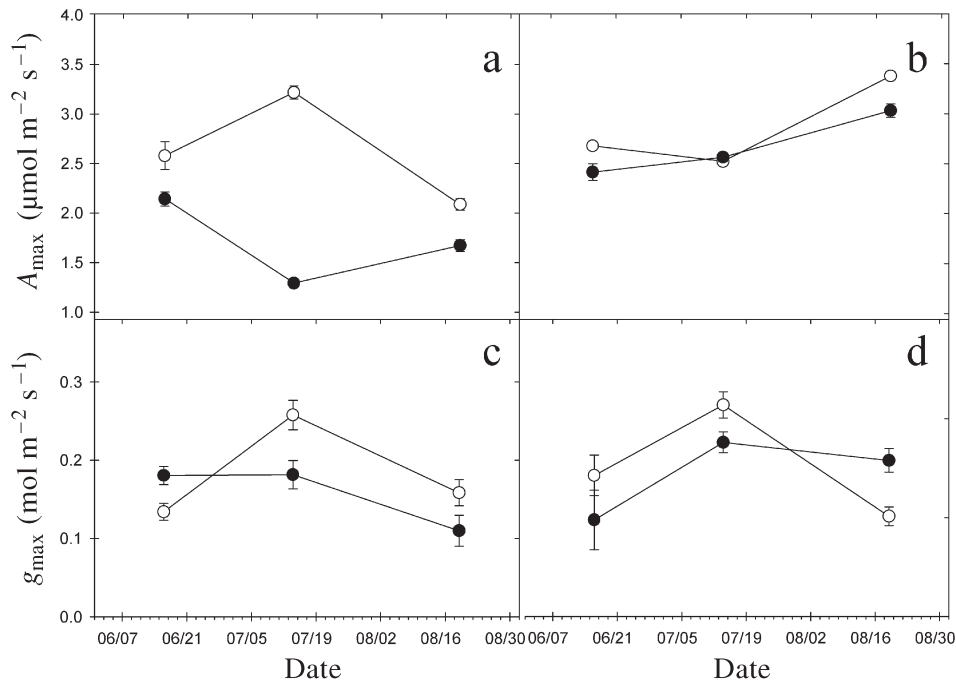


Figure 6. Mean maximum photosynthesis (A_{\max}) (a, b) and stomatal conductance (g_{\max}) (c, d) during the morning (0800–0900 h; a, c) and afternoon (1400–1500 h; b, d). Symbols: ○ = open canopy site; and ● = closed canopy site.

sky exposure between the two plots (Table 1). All detectable sunlight came between 1100 and 2000 h. Irradiance at the OC site peaked at 424 and 549 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in the early and mid-afternoon, respectively, well above the irradiance necessary for saturating photosynthesis (400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF; cf., Figures 1 and 5), whereas irradiance peaked above 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ only once at the IC site and not at all at the CC site.

Based on measured sunlight regimes and photosynthetic light response curves, computed photosynthetic carbon gain by OC seedlings was about 3.3–4.5 times greater than in CC seedlings (22.4 versus 6.7 $\text{mmol m}^{-2} \text{day}^{-1}$ in June, 18.5 versus 5.0 $\text{mmol m}^{-2} \text{day}^{-1}$ in August) and 1.4–2.0 times greater than in IC seedlings (depending on the set of equations used for IC seedlings) (Table 3). Estimated daily photosynthesis decreased each month throughout the summer at all sites.

Discussion

Fraser fir appears to be a relict species that most likely diverged from balsam fir (*Abies balsamea* (L.) Mill.) 7000–8000 years ago during a relatively prolonged warming period

(Myers and Bormann 1963, Jacobs et al. 1984, Clark et al. 2000). During the last glacial period, the genus *Abies* was found throughout North Carolina and Tennessee and extended as far south and east as Alabama and Mississippi (Delcourt and Delcourt 1984). During warmer periods, *Abies* inhabited the cooler, higher elevations of the southern Appalachian Mountains, where it now inhabits only the highest mountaintops (elevations > 1750 m). Ecotypic differentiation appears to have occurred among the six isolated populations known to occur in the southern Appalachians, with documented differences in both needle morphology (Jett et al. 1993) and molecular markers (Ross 1998). It is possible that, with climate change (e.g., warming), this unique community type, which includes numerous endemic species, will be further threatened and could ultimately disappear. Currently, there are large areas of older, standing snags of Fraser fir and red spruce in the southern Appalachians, commonly referred to as the “spruce–fir dieback.” Continued reestablishment of these refugial populations and associated communities is dependent on successful seedling establishment (White et al. 1985, Pauley and Clebsch 1990).

Abundance and survival

Previous studies of abundance of Fraser fir seedlings and saplings showed that the number of new Fraser fir seedlings increased with elevation, from 1500 to 1900 m (Nicholas et al. 1992), and more seedlings (0–10 years old) occurred in areas with lower mortality in the overstory trees (i.e., greater canopy closure) (Smith and Nicholas 2000). In contrast, Duchesneau and Morin (1999) found higher survival of *A. balsamea* in plots with more open canopies compared with closed-canopy plots.

We found that newly emerged seedling abundance was

Table 3. Predicted daily net photosynthetic carbon gain ($\text{mmol m}^{-2} \text{day}^{-1}$) for seedlings at closed canopy (CC), intermediate canopy (IC) and open canopy (OC) sites, based on equations generated from light response curves at either OC or CC (see Table 2 for equation parameters).

| Site | June | July | August |
|-------------------|------|------|--------|
| CC | 6.70 | 5.01 | 4.97 |
| IC (CC equations) | 13.3 | 10.9 | 10.6 |
| IC (OC equations) | 14.7 | 15.7 | 12.4 |
| OC | 22.4 | 22.3 | 18.5 |

greater at the end of the study season at the OC site than at the CC site, although the initial number of newly emerged seedlings during early summer was substantially greater at the CC site than at the OC site (June 16). Thus, newly emerged seedling mortality was much higher at the CC site, which had the lowest PPF. Although this apparent uncoupling between germination and survival seems non-adaptive, the greater abundance of newly emerged seedlings at the CC site may reflect a greater seed bank as a result of a greater abundance of living adult trees in the overhead canopy.

Photosynthesis and water relations

Previous work has shown that, under moderate irradiances (about $500 \mu\text{mol m}^{-2} \text{s}^{-1}$), seedlings previously under low light for extended periods had lower photosynthetic rates than seedlings previously in higher light environments (e.g., Chazdon and Pearcy 1986). Within a species, higher photosynthetic rates in high or moderate light have been documented for sun leaves compared with shade leaves (see Smith et al. 1997 for review). It has also been shown that either too much or too little light reduces photosynthetic carbon gain in seedlings of *Abies lasiocarpa* (Hook.) Nutt. and *Picea engelmannii* Parry ex Engelm. (Johnson et al. 2004) and *Pinus cembra* L. (Tranquillini 1979) and can increase mortality in *A. lasiocarpa*, *P. engelmannii* and *Pinus contorta* Dougl. ex. Loud. (Cui 1990, Cui et al. 1991).

Photosynthesis in cotyledonous seedlings of *A. fraseri* was higher at the OC site than at the CC site at similar PPFs, especially in the morning. During afternoon measurement periods, photosynthesis in CC seedlings was almost as high as in OC seedlings, suggesting an increase in photosynthetic capacity in CC seedlings over the course of the day. Water potentials were higher in OC seedlings than in CC seedlings at the end of the study, perhaps indicating greater rooting depth or increased mycorrhizal infection, either of which could be attributable to greater carbon gain (Nardini et al. 2000, Hasselquist et al. 2005).

Sky exposure

The diurnal sunlight regime experienced by a seedling is closely associated with sky exposure and is important for carbon gain (e.g., Arntz et al. 1998), but high sky exposure can have negative consequences also (Figure 7; Jordan and Smith 1995). Facilitation, through amelioration of seedling microsites, has been shown to be important for survival of congeneric seedlings (*A. lasiocarpa* and *P. engelmannii*) in subalpine regions. Germino et al. (2002) found that microsite facilitation by surrounding vegetation (i.e., reduced sky exposure), which reduced low-temperature photoinhibition, was critical for survival in *A. lasiocarpa* and *P. engelmannii* seedlings at the tree line in the Rocky Mountains. Likewise, newly emerged seedlings of *Eucalyptus pauciflora* Seiber ex A. Spreng. were found in the shade of mature trees that minimized both nighttime sky exposure and the duration of direct sun exposure, thus reducing the risk of low-temperature photoinhibition (Ball et al. 1991; see also Figure 7).

Canopy gaps may facilitate the survival of newly emerged

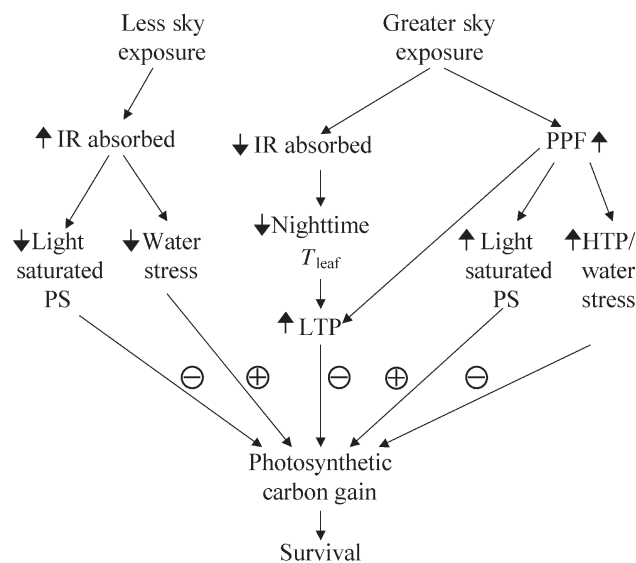


Figure 7. Schematic representation of proposed interactions between sky exposure and seedling photosynthetic carbon gain. Abbreviations: LTP = low-temperature photoinhibition; HTP = high-temperature photoinhibition; IR = longwave infrared radiation from the upper hemisphere; PS = photosynthesis; PPF = photosynthetic photon flux; and T_{leaf} = leaf temperature. The plus and minus signs indicate positive and negative impacts, respectively, on photosynthetic carbon gain and seedling survival.

A. fraseri seedlings that may eventually grow to fill these gaps. However, gaps that are too large might limit seedling establishment because of excessive sky exposure. Newly emerged seedling survival in areas with no overhead vegetation (e.g., grass bald habitats; OS site) was even lower than at the CC site (124 seedlings m^{-2} were observed at the OS site on June 16, but were all dead by June 22). Similarly, Lee et al. (2004) observed 100% mortality in newly emerged seedlings of *Pinus densiflora* Siebold & Zucc. in open areas, but observed surviving seedlings beneath overstory vegetation. Zhu et al. (2003) observed greater survival of newly emerged seedlings of *Pinus thunbergii* Parl. under canopy gaps compared with completely closed canopies.

Seedlings at the OC site were estimated to fix between 3.3 and 4.5 times more carbon than seedlings at the CC site. Predicted clear-day carbon gain in congeneric seedlings (*A. lasiocarpa* and *P. engelmannii*) of the Rocky Mountains (USA) was also higher in areas with greater cumulative diurnal sunlight (Johnson et al. 2004). Cloudy-day carbon gain may be even higher than clear-day carbon gain in newly emerged *A. fraseri* seedlings, especially seedlings in microsites with overhead vegetation. Penetration of relatively high diffuse irradiance in the understory increases in the presence of clouds, often too close to the photosynthetic saturation value (Young and Smith 1983, Roderick et al. 2001, Gu et al. 2002). Predicted carbon gain for seedlings of *A. lasiocarpa* and *P. engelmannii* was greater on cloudy days than on clear days because of increased penetration of diffuse light into the forest understory (Johnson et al. 2004). Diffuse light measured under direct cloud in the

understory at Roan Mountain was more than $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ for long intervals (greater than 10 min, authors' unpublished data), which is well above the light compensation points estimated in our study. Thus, because *A. fraseri*–*Picea rubens* forests experience some degree of direct cloud immersion on more than 65% of all days (68 and 76% of all days at Whitetop, VA, and Mt. Mitchell, NC, respectively; Mohnen 1992), diffuse light from clouds likely impacts carbon gain in *A. fraseri* forests.

Acknowledgments

This work was supported by a National Science Foundation grant to W.K.S. (Ecology and Evolutionary Physiology). The authors thank Michael Sprague and Spencer Bissett for assistance with field data collection.

References

- Ackerly, D.D. and R.K. Monson. 2003. Waking the sleeping giant: the evolutionary foundations of plant function. *Int. J. Plant Sci.* 164: Suppl. S1–S6.
- Arntz, A.M., E.H. DeLucia and N. Jordan. 1998. Contribution of photosynthetic rate to growth and fitness in *Amaranthus hybridus*. *Oecologia* 117:323–330.
- Ball, M.C., V.S. Hodges and G.P. Laughlin. 1991. Cold-induced photoinhibition limits regeneration of snow gum at treeline. *Funct. Ecol.* 5:663–668.
- Bruck, R.I. and W.P. Robarge. 1988. Change in forest structure in the boreal montane ecosystem of Mount Mitchell, North Carolina. *Eur. J. For. Pathol.* 18:357–366.
- Chazdon, R.L. and R.W. Pearcy. 1986. Photosynthetic responses to light variation in rainforest species. I. Induction under constant and fluctuating light conditions. *Oecologia* 69:517–523.
- Clark, C.M., T.R. Wentworth and D.M. O'Malley. 2000. Genetic discontinuity revealed by chloroplast microsatellites in eastern North American *Abies* (Pinaceae). *Am. J. Bot.* 87:774–782.
- Crandall, D.L. 1958. Ground vegetation patterns of the spruce–fir area of the Great Smoky Mountains National Park. *Ecol. Monogr.* 28: 337–360.
- Cui, M. 1990. The ecophysiology of seedling establishment in subalpine conifers of the central Rocky Mountains, USA. Ph.D. Diss., Univ. Wyoming, Laramie, WY, 149 p.
- Cui, M. and W.K. Smith. 1991. Photosynthesis, water relations and mortality in *Abies lasiocarpa* seedlings during natural establishment. *Tree Physiol.* 8:37–46.
- Delcourt, H.R. and P.A. Delcourt. 1984. Late-quaternary history of the spruce–fir ecosystem in the southern Appalachian Mountain region. *In* The Southern Appalachian Spruce–Fir Ecosystem: Its Biology and Threats. Ed. P.S. White. National Park Service, Southeast Region, Gatlinburg, TN, pp 22–35.
- Duchesneau, R. and H. Morin. 1999. Early seedling demography in balsam fir seedling banks. *Can. J. For. Res.* 29:1502–1509.
- Eagar, C. 1984. Review of the biology and ecology of the balsam wooly aphid in the southern Appalachian spruce–fir forests. *In* The Southern Appalachian Spruce–Fir Ecosystem: Its Biology and Threats. Ed. P.S. White. National Park Service, Southeast Region, Gatlinburg, TN, pp 36–50.
- Germino, M.J. and W.K. Smith. 1999. Sky exposure, crown architecture, and low-temperature photoinhibition in conifer seedlings at alpine treeline. *Plant Cell Environ.* 22:407–415.
- Germino, M.J., W.K. Smith and A.C. Resor. 2002. Conifer seedling distribution and survival in an alpine treeline ecotone. *Plant Ecol.* 162:157–168.
- Gu, L.H., D. Baldocchi, S.B. Verma, T.A. Black, T. Vesala, E.M. Falge and P.R. Dowty. 2002. Advantages of diffuse radiation for terrestrial ecosystem productivity. *J. Geophys. Res.* 107: Article No. 4050.
- Hadley, J.L. and W.K. Smith. 1987. Influence of krummholz mat microclimate on needle physiology and survival. *Oecologia* 73: 82–90.
- Hain, F.P. and F.H. Arthur. 1985. The role of atmospheric deposition in the latitudinal variation of Fraser fir mortality caused by the balsam wooly adelgid, *Adelges piceae*: a hypothesis. *J. Appl. Entomol.* 99:145–152.
- Hasselquist, N., M.J. Germino, T. McGonigle and W.K. Smith. 2005. Variability of *Cenococcum* colonisation and its ecophysiological significance for young conifers at alpine treeline. *New Phytol.* 165:867–873.
- Jacobs, B.F., C.R. Werth and S.I. Guttman. 1984. Genetic relationships in *Abies* (fir) of eastern United States: an electrophoretic study. *Can. J. Bot.* 62:609–616.
- Jett, J.B., S.E. McKeand, Y. Liu and W.T. Huxter. 1993. Seed source variation in height and crown traits of Fraser fir Christmas trees. *South. J. Appl. For.* 17:5–9.
- Johnson, D.M., M.J. Germino and W.K. Smith. 2004. Abiotic factors limiting photosynthesis in *Abies lasiocarpa* and *Picea engelmannii* seedlings above and below the alpine timberline. *Tree Physiol.* 24:377–386.
- Jordan, D.N. and W.K. Smith. 1995. Radiation frost susceptibility and the association between sky exposure and leaf size. *Oecologia* 103:43–48.
- Lee, C.S., J.H. Kim, H. Yi and Y.H. You. 2004. Seedling establishment and regeneration of Korean red pine (*Pinus densiflora*) forests in relation to soil moisture. *For. Ecol. Manage.* 199:423–432.
- List, R.J. 1971. Smithsonian meteorological tables. Smithsonian Institution Press, Washington, DC, 527 p.
- Mark, A.F. 1958. The ecology of the southern Appalachian grass balds. *Ecol. Monogr.* 28:293–336.
- McLaughlin, S.B., C.P. Anderson, N.T. Edwards, W.K. Roy and P.A. Layton. 1990. Seasonal patterns of photosynthesis and respiration of red spruce saplings from two elevations in declining Southern Appalachian stands. *Can. J. For. Res.* 20:485–495.
- McLaughlin, S.B., C.P. Anderson, P.J. Hanson, M.G. Tjoelker and W.K. Roy. 1991. Increased dark respiration and calcium deficiency of red spruce in relation to acidic deposition at high-elevation Southern Appalachian Mountain sites. *Can. J. For. Res.* 21: 1234–1244.
- Mohnen, V.A. 1992. Atmospheric deposition and pollutant exposure of eastern U.S. forests. *In* Ecology and Decline of Red Spruce in the Eastern United States. Eds. C. Eager and M.B. Adams. Springer-Verlag, New York, pp 64–124.
- Myers, O. and F.H. Bormann. 1963. Phenotypic variation in *Abies balsamea* in response to altitudinal and geographic gradients. *Ecology* 44:429–436.
- Nardini, A., S. Selleo, M.T. Tyree and M. Vertovec. 2000. Influence of the ectomycorrhizas formed by *Tuber melanosporum* Vitt. on the hydraulic conductance and water relations of *Quercus ilex* L. seedlings. *Ann. For. Sci.* 57:305–312.
- Nicholas, N.S. and S.M. Zedaker. 1989. Ice damage in spruce–fir forests of the Black Mountains, North Carolina. *Can. J. For. Res.* 19: 1487–1491.

- Nicholas, N.S., S.M. Zedaker and C. Eagar. 1992. A comparison of overstory community structure in three southern Appalachian spruce–fir forests. *Bull. Torrey Bot. Club* 119:316–332.
- Pauley, E.F. and E.E.C. Clebsch. 1990. Patterns of *Abies fraseri* regeneration in a Great Smoky Mountains spruce–fir forest. *Bull. Torrey Bot. Club* 117:375–381.
- Ramseur, G.R. 1960. The vascular flora of high mountain communities of the Southern Appalachians. *J. Elisha Mitchell Sci. Soc.* 76: 82–112.
- Roderick, M.L., G.D. Farquhar, S.L. Berry and I.R. Noble. 2001. On the direct effect of clouds and atmospheric particles on the productivity and structure of vegetation. *Oecologia* 129:21–30.
- Ross, R.K. 1988. Patterns of allelic variation in natural populations of *Abies fraseri* (Pursh.) Poiret. Ph.D. Diss., North Carolina State Univ., Raleigh, NC, 102 p.
- Smith, G.F. and N.S. Nicholas. 2000. Size- and age-class distributions of Fraser fir following balsam woolly adelgid infestation. *Can. J. For. Res.* 30:948–957.
- Smith, W.K., A.W. Schoettle and M. Cui. 1991. Importance of leaf area measurement to the interpretation of gas exchange parameters of complex shoots (e.g., conifers). *Tree Physiol.* 8:121–127.
- Smith, W.K., T.C. Vogelmann, E.H. DeLucia, D.T. Bell and K.A. Shepherd. 1997. Leaf form and photosynthesis: do leaf structure and orientation interact to regulate internal light and carbon dioxide? *BioScience* 47:785–793.
- 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, 137 p.
- White, P.S., M.D. Mackenzie and R.T. Busing. 1985. Natural disturbance and gap phase dynamics in southern Appalachian spruce–fir forests. *Can. J. For. Res.* 15:233–240.
- Whittaker, R.H. 1956. Vegetation of the Great Smoky Mountains. *Ecol. Monogr.* 26:1–80.
- Young, D.R. and W.K. Smith. 1983. Effect of cloudcover on photosynthesis and transpiration in the subalpine understory species *Arnica latifolia*. *Ecology* 64:681–687.
- Zar, J.H. 1999. Biostatistical analysis. Prentice-Hall, Upper Saddle River, NJ, 663 p.
- Zhu, J.J., T. Matsuzaki, F.Q. Lee and Y. Gonda. 2003. Effect of gap size created by thinning on seedling emergency, survival and establishment in a coastal pine forest. *For. Ecol. Manage.* 182:339–354.

