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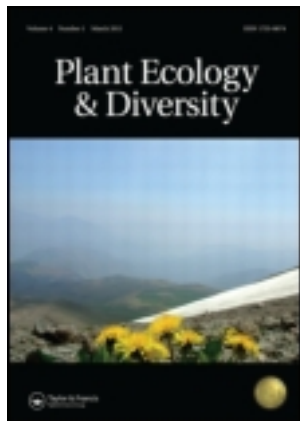
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Conifer seedling recruitment across a gradient from forest to alpine tundra: effects of species, provenance, and site

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Background: Seedling germination and survival is a critical control on forest ecosystem boundaries, such as at the alpine–treeline ecotone. In addition, while it is known that species respond individually to the same suite of environmental drivers, the potential additional effect of local adaptation on seedling success has not been evaluated.

Aims: To determine whether local adaptation may influence the position and movement of forest ecosystem boundaries, we quantified conifer seedling recruitment in common gardens across a subalpine forest to alpine tundra gradient at Niwot Ridge, Colorado, USA.

Methods: We studied *Pinus flexilis* and *Picea engelmannii* grown from seed collected locally at High (3400 m a.s.l.) and Low (3060 m a.s.l.) elevations. We monitored emergence and survival of seeds sown directly into plots and survival of seedlings germinated indoors and transplanted after snowmelt.

Results: Emergence and survival through the first growing season was greater for *P. flexilis* than *P. engelmannii* and for Low compared with High provenances. Yet survival through the second growing season was similar for both species and provenances. Seedling emergence and survival tended to be greatest in the subalpine forest and lowest in the alpine tundra. Survival was greater for transplants than for field-germinated seedlings.

Conclusions: These results suggest that survival through the first few weeks is critical to the establishment of natural germinants. In addition, even small distances between seed sources can have a significant effect on early demographic performance – a factor that has rarely been considered in previous studies of tree recruitment and species range shifts.

Keywords: common garden; climate gradient; local adaptation; *Picea engelmannii*; *Pinus flexilis*; seedling emergence; seedling recruitment; species range boundaries; subalpine forest; treeline

Introduction

Tree establishment depends on both genetic and environmental factors that act on seed availability, germination, the growth of seedlings and saplings, and survival. Understanding the relative importance of these factors to seedling recruitment is critical to understanding the spatial responses of forests, and individual tree species, to landscape-scale disturbances such as climate change, fire and disease. Following the last glacial maximum, tree species migrated into available habitat over hundreds or thousands of years. However, anthropogenic warming will be many times faster (IPCC 2007), and successful responses of subalpine forest species to this rate of climate change may require rapid genetic change resulting from natural selection, and changes in gene expression enabled by phenotypic plasticity (Davis and Shaw 2001; Jump et al. 2008).

Paleoecological studies have established that tree species ranges responded individually to Quaternary climate change (e.g. Prentice et al. 1991; Webb and Bartlein 1992). Thus, the potential for treeline conifer range shifts under future warming is expected to vary by species (Brubaker 1986; Danby and Hik 2007; Schrag et al. 2008). In addition, a rich legacy of provenance tests in common garden experiments has shown that conifer species possess high genetic diversity and site-specific adaptation (e.g. Turesson 1922; Jenson 1993; Rehfeldt et al. 1999; Vitasse et al. 2009; and see Mitton 1995 and Morgenstern 1996 for reviews), with elevation changes of as little as 200 m leading to adaptive differentiation in mountainous terrain (Rehfeldt 1989, 1993). Modelling efforts based on provenance test data across species' ranges have indicated that rapid anthropogenic climate change would likely render populations maladapted in many areas (St Clair and Howe 2007; O'Neill et al. 2008).

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With increasing elevation, tree seedling recruitment becomes progressively more limited by environmental variables such as growing degree days, the availability of suitable microsites, and temperature minima (Cuevas 2000; see Holtmeier 2003 for a review; Smith et al. 2003; Maher and Germino 2006; van Mantgem et al. 2006; Koshkina et al. 2008; Munier et al. 2010). At mid and high latitudes, seedlings at and above treeline are exposed to stressful alpine conditions that include extended snow cover, short growing season and low temperatures (Koerner 2003; Wieser and Tausz 2007). Radiative heat loss at night, followed by high solar irradiance has also been shown to induce physiological stress (Germino and Smith 1999; 2000; Johnson et al. 2004). Thus, although tree seedlings are occasionally observed above treeline, they rarely reach the reproductive stage (Daly and Shankman 1985; Cuevas 2000; Cierjacks et al. 2007).

Tree establishment patterns are commonly derived from a combination of tree-ring and weather station data spanning several decades. However, the main bottlenecks to recruitment occur in the very early life stages, from seed production to seedling survival in the first few years (Gashwile 1971; Christy and Mack 1984; Harcombe 1987; van Mantgem et al. 2006), which are not observable in surveys of older trees. In Tierra del Fuego, Cuevas (2000) found that the fraction of *Nothofagus pumilio* seedlings surviving through one full year dropped from 18% at 450 m above sea level (a.s.l.) to 1% at treeline (690 m a.s.l.). In Rocky Mountain treelines, decades of observations have shown that seedling mortality during the first season is commonly > 90%, but drops steeply over the next two seasons to near zero for 3-year-old seedlings (Alexander 1984; Cui and Smith 1991; Germino et al. 2002; Maher and Germino 2006). While local adaptation of seeds could affect early demographic performance, such population-level effects have not been evaluated in any of these studies.

The objectives of this study were to evaluate seedling success of two co-occurring subalpine tree species within and above their local elevation range, and to test for the influence of local adaptation on early demographic bottlenecks to establishment. We used common gardens in sites that differ in duration of snow cover, sky exposure and elevation to examine seedling emergence and survival within and above the species' current elevation ranges. We compared limber pine and Engelmann spruce (*Pinus flexilis* James and *Picea engelmannii* Parry ex Engelm.) grown from seed collected locally at high and low elevations. We also compared the fate of two different kinds of seedlings: those that emerged from seed sown directly into the research plots the preceding autumn (Germinants) and those that emerged from seed sown indoors and transplanted into the plots as 1–3 week-old seedlings following snowmelt (Transplants). By eliminating limitations imposed by germination and initial survivorship, the use of Transplants provided insight into the importance of these early developmental stages on establishment and also allowed us to evaluate longer-term survivorship.

We predicted that while germination and survival would decline with increasing elevation, low-elevation populations would fare relatively better in the subalpine forest, while high-elevation populations would fare relatively better at treeline and in the alpine tundra, reflecting the effects of local adaptation.

Methods

Species, sites and experimental plot design

In the Colorado Rocky Mountains, USA, the Engelmann spruce–subalpine fir (*Abies lasiocarpa* Nutt.) forest type dominates much of the subalpine forest between 3000 m and 3500 m, depending on aspect and latitude, with limber pine as a minor component on more xeric slopes and ridges. Limber pine is a light- and drought-adapted pioneer species, while Engelmann spruce is shade tolerant and drought sensitive, and known to have vulnerabilities to the frost and bright light that prevail in exposed sites (Ronco 1970a,b; Rebertus et al. 1991; Bansal et al. 2010). At the University of Colorado Mountain Research Station in the Front Range of the Colorado Rocky Mountains, we established three research sites, Forest, Treeline and Alpine, along an elevation gradient (Figure 1).

The Forest site (40° 2' 12" N, 105° 32' 55" W; 3060 m a.s.l.) is dominated by mature Lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) and Engelmann spruce, with limber pine and subalpine fir also present. The forest was logged 100–110 years ago and has since regenerated naturally. Research plots were established within canopy gaps up to 15 m in diameter. The site has a gentle (< 5%) slope with a south-east aspect. The soils, mapped as Leighcan family, developed on igneous and metamorphic residuum or till, and are classified as loamy-skeletal, mixed, superactive Typic Dystrocryepts (Soil-Survey-Staff 2010). The thin soils are covered in a duff layer that averages 2 cm, but can be up to 15 cm in depth. There are occasional shrubs (mainly *Juniperus communis* and *Vaccinium caespitosum*) and minimal herb cover. This site is located within 400 m of the Niwot Ridge AmeriFlux site (40° 1' 58" N; 105° 32' 47" W; 3050 m a.s.l.), where average annual precipitation and temperature are 800 mm and 1.5 °C, respectively (Monson et al. 2002; Table 1, Table 2).

The Treeline site (40° 3' 5" N, 105° 34' 50" W; 3430 m a.s.l.) is at the cool (upper) edge of the local subalpine species range. It is an open meadow dominated by grasses, forbs and sedges, with 8% bare ground and 6% gravels and cobbles. It is surrounded by krummholz mats and flag trees of subalpine fir, Engelmann spruce and a single limber pine. The site has a ca. 13% slope and a south–south-west aspect. The soils, mapped as Leighcan-Moran family, are developed on glaciofluvial deposits or residuum derived from igneous and metamorphic rock, and are classified as lithic Cryorthents. This site receives and traps snow from upwind, and therefore has the highest snowpack (as high as 5 m in some years), latest snowmelt and the shortest growing season of all three sites. At the beginning of this

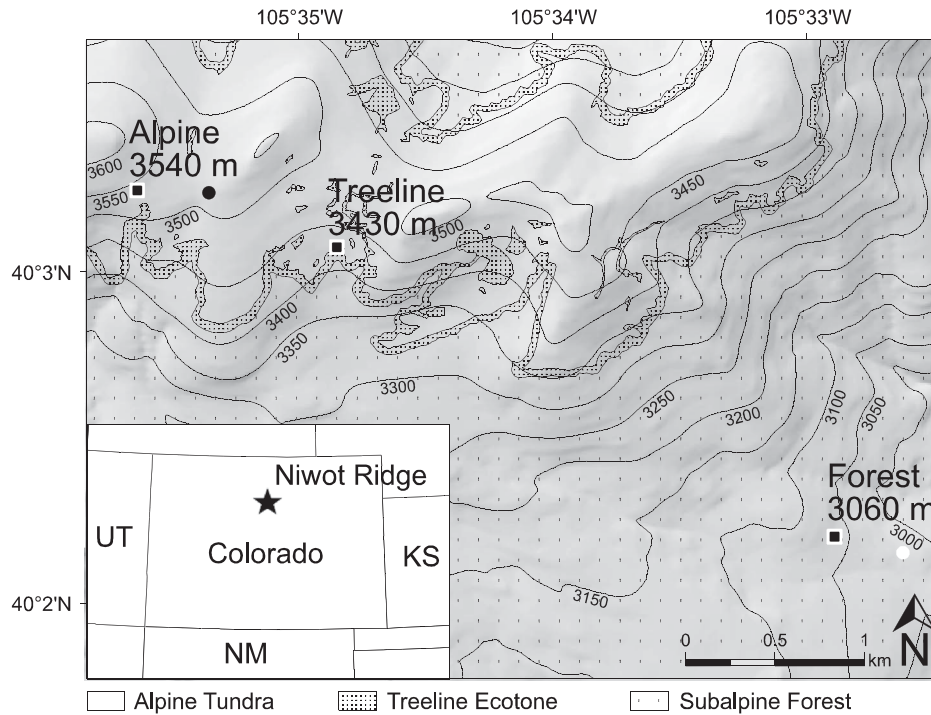


Figure 1. Locations of the three study sites at the Mountain Research Station, Colorado, USA. Niwot Ridge Saddle LTER site is shown as a black circle and the AmeriFlux site is shown as a white circle.

Table 1. Climate data averaged over June–September 2009 and 2010 from weather stations located near the Forest (AmeriFlux) and Alpine (Saddle) study sites. The weather stations are < 500 m away, and within 30 m elevation of these two study sites at Niwot Ridge, Colorado, USA.

Year	Forest (Niwot Ridge AmeriFlux data)		Alpine (Niwot Ridge LTER Saddle data)	
	2009	2010	2009	2010
Precipitation (mm)	185	212		
Air temperature (°C)	9.2 (3.6)	10.9 (3.0)	7.9 (3.8)	9.7 (2.9)
Soil temperature (°C)	7.2 (2.4)	7.7 (2.6)	6.7 (2.7)	7.7 (2.6)
Soil volumetric water content (VWC; m ³ m ⁻³)	0.102 (0.065)	0.121 (0.083)	0.144 (0.090)	0.135 (0.068)
Minimum daily average VWC over the season (m ³ m ⁻³)	0.048	0.045	0.051	0.053
Daytime vapour pressure deficit (kPa)	0.54 (0.34)	0.67 (0.35)	0.50 (0.27)	0.62 (0.29)
Daytime solar radiation (W m ⁻²)	391 (132)	427 (136)	579 (168)	612 (171)

Notes: Precipitation is cumulative for June–September. Mean daily air temperature and vapour pressure deficit are at a height of 2 m in the Forest site and 1.5 m at the Alpine site. Mean daily soil temperature and soil volumetric water content (VWC) are for 5 cm depth. Mean daytime solar radiation is for incoming shortwave radiation at 25.5 m in the Forest site and 300–1100 μm at 1.5 m in the Alpine site. Values in parentheses are standard deviations of daily means.

Table 2. Climate data averaged over June–September 2010, measured at weather stations in the Forest, Treeline, and Alpine study sites at Niwot Ridge, Colorado, USA.

	Forest	Treeline	Alpine
Air temperature (°C)	12.0 (3.0)	9.7 (3.0)	9.1 (3.0)
Soil temperature (°C)	11.0 (2.3)	10.8 (4.1)	10.4 (4.2)
Soil volumetric water content (m ³ m ⁻³)	0.168 (0.068)	0.213 (0.099)	0.221 (0.092)
Minimum daily average VWC over the season (m ³ m ⁻³)	0.062	0.058	0.089
Daytime vapour pressure deficit (kPa)	0.80 (0.38)	0.65 (0.31)	0.60 (0.28)
Daytime solar radiation (W m ⁻²)	na	459 (128)	579 (158)

Notes: Mean daily air temperature and vapour pressure deficit are at a height of 2 m. Mean daily soil temperature and soil volumetric water content (VWC) are for 5–10 cm depth and were averaged across several sensors. Mean daytime solar radiation is for 400–700 μm at 2 m. Values in parentheses are standard deviations of daily means.

study we lacked meteorological data for this site. However, according to data collected in summer 2010, except for the delayed snowmelt, growing season abiotic conditions tend to be intermediate between the Forest and the Alpine (Table 2).

The Alpine site (40° 3' 16" N, 105° 35' 37" W; 3540 m a.s.l.) is located on Niwot Ridge above the local subalpine tree species' current range, and is dominated by forbs, grasses and sedges, with 12% bare ground and 12% gravel or cobbles. The site has a ca. 15% slope and a south-south-east aspect. The soils, developed from the same parent material as the Treeline site, are mapped as Moran family and classified as lithic Cryorthents. Wind and topography interact to produce spatially heterogeneous snow accumulation and melt dates. The organic matter content of the Alpine and Treeline soils is similar, and is about three times greater than Forest soils (unpublished data). The Alpine site is located within 300 m of the LTER Saddle meteorological station (40° 03' 17" N; 105° 35' 21" W; 3528 m a.s.l.), where average annual precipitation and temperature are 930 mm and -2.2 °C, respectively.

At each site, we established up to 20 experimental plots, each 2 m × 2 m. Each plot was divided into four permanently marked 1 m² quadrants – one for each of four local seed provenances: high-elevation limber pine (Pine High), low-elevation limber pine (Pine Low), high-elevation Engelmann spruce (Spruce High) and low-elevation Engelmann spruce (Spruce Low).

Seed collection and sowing

In autumn 2008, we collected seeds from low and high-elevation populations for both limber pine and Engelmann spruce. Pine Low seed was harvested from trees within 400 m of the Forest site, while Spruce Low seed was harvested from squirrel caches within 150 m of the Forest site. Pine High seed was collected between 3370 and 3450 m elevation from krummholz and flag trees on two ridges located 500 m and 900 m east of the Treeline site, while Spruce High seed was collected between 3370 and 3400 m from flag trees located within 400 m south-east to 280 m south of the Treeline site. Cones were dried indoors in well-ventilated areas at low to moderate temperatures (< 28 °C for Pine and < 45 °C for Spruce) until it was possible to extract the seed. Spruce seed were de-winged at the USFS pollen laboratory in Coeur d'Alene, Idaho.

Five seeds were sown into 70 10 cm × 10 cm cells per quadrant (350 seeds per quadrant). Due to a very poor seed crop and limited time between seed maturation and snowfall, we have an uneven design. We did not have adequate seed to directly sow Pine Low in the autumn. Pine High seed was sown into 20 plots at the Forest and Treeline sites but only into 10 plots at the Alpine site. Spruce High and Low seed was sown into 13 plots at the Forest site, four plots at the Treeline site and 10 plots at the Alpine site. After sowing, hardware cloth enclosures were placed over

each quadrant to protect the seeds from predation by birds or small mammals.

Transplants

Seeds for transplants were stored at -20 °C and stratified at 4 °C for 45 days (Pine) and 30 days (Spruce). Following stratification seeds were sown into 2.5 cm diameter Ray Leach 'Cone-tainer' cells lined with coffee filter paper and filled with a mixture of sand and autoclaved potting soil to match the organic matter content of the native soil at each of the three sites. Seeds were processed in three waves in anticipation of different snowmelt dates at each site. The first wave was sown in a greenhouse at the University of Colorado, Boulder (1655 m a.s.l.) and transferred on 26 May to the Marr lab at the Mountain Research Station (2895 m a.s.l.), where the second and third waves were sown. Cone-tainers were watered by hand daily. As seedlings germinated, they were moved outdoors to acclimate to ambient temperature and solar radiation. At least two days prior to transplanting into the Treeline and Alpine sites, these seedlings were carried up to 3500 m a.s.l. and shielded from the sun with window screen netting. At the time of transplanting, seedlings were generally 1–2 weeks old (3 weeks old at most). To transplant, we used a 2.5 cm diameter soil corer to make 10 cm-deep holes, and placed seedling plugs from the Cone-tainers into the holes. Each seedling received 20 ml of water directly following transplanting and twice a week for two more weeks. Transplants of all four seed types were placed into up to 24 pre-designated cells in each quad. Due to insufficient or delayed indoor germination, not all plots at each site received transplants (details below). With the exception of two plots per site, the hardware cloth enclosures used to protect seeds sown in autumn were kept in place to prevent seedling predation.

Surveys

Seedling presence/absence. Following snowmelt, we conducted seedling surveys approximately weekly until seedlings stopped emerging. The last 2009 surveys were conducted at the end of the first growing season on 9 September, 11 September and 28 September 2009, for the Alpine, Treeline and Forest sites, respectively. The first 2010 surveys were conducted following snowmelt at each site and the last 2010 surveys were conducted on 30 Sep and 1 Oct. At each survey, we recorded the number of new or dead seedlings in each 10 cm × 10 cm cell. These data were used to approximate the number and timing of seedling germination and deaths.

Microsite. To determine whether seedlings preferentially germinated or died in certain micro-environments, we categorised the 1 cm radius around each seedling as dominated by bare soil, duff, plant or wood fragment. In addition we categorised the dominant substrate of each 10 cm × 10 cm cell as bare soil, litter/duff or plant.

Data analysis

The period between snowmelt and seedling emergence (germination time), emergence rate as a fraction of seeds sown, and survival of Germinants and Transplants (to the last 2009 survey, first 2010 survey and last 2010 survey) were computed for each seed source within each plot and analysed in factorial ANOVAs, with site, species and provenance as explanatory factors and plot as a random effect. ANOVAs were followed by the Tukey HSD test in cases where differences were significant. Exlosures had significant effects on emergence rates at all sites and on the fraction of transplants that survived to the last survey at the Forest site, so plots lacking exlosures were excluded in these cases. Pine Low seeds were not outplanted (due to insufficient seeds). Therefore, for emergence and Germinant survival, we evaluated the species (Pine compared with Spruce, high elevation only) and provenance (Spruce from high compared with low elevation) hypotheses in two separate ANOVAs. We used the Bonferroni correction to protect against potential false positives with multiple comparisons by conservatively assuming full overlap (rather than 50% overlap) in these pairs of datasets. For Germinant survival, plots where germination for Pine High, Spruce High, or Spruce Low was not observed were excluded, which reduced the sample size (n) to 1 for the Alpine site, which was therefore excluded from the ANOVA (Table 6). Spruce High seedlings were excluded from the Transplant survival analysis due to very low greenhouse germination rates. For Transplant survival we therefore evaluated species (Pine Low vs. Spruce Low) and provenance (Pine Low vs. Pine High) hypotheses in two separate ANOVAs and adjusted for multiple comparisons using the Bonferroni correction, as above.

Table 3. Summary of the datasets available for ANOVA testing by species, provenance, and site for the Forest (F), Treeline (T), and Alpine (A) study sites at Niwot Ridge, Colorado, USA, for each of the following response variables: Lag between snowmelt and emergence (Germ. time); Fraction of sown seeds observed to emerge (Emerg. rate); Germinant survival (to last 2009, first 2010, and last 2010 surveys); and Transplant survival (to last 2009, first 2010, and last 2010 surveys).

Response variable	Pine High	Pine Low	Spruce High	Spruce Low
Germ. time (days)	F, T, A		F, T, A	F, T, A
Emerg. rate (%)	F, T, A		F, T, A	F, T, A
Germinant survival	F, T		F, T	F, T
Transplant survival	F, T, A	F, T, A		F, T, A

Table 3 summarises the information that was available for statistical testing using ANOVAs. Tables 4–6 list uncorrected P values, highlighting those statistically significant at $P < 0.05$ after the Bonferroni correction. The microsite effect was assessed with the Chi-Square test and these data were not averaged by plot. We calculated all statistics using JMP 8.0 (SAS 2008).

Results

Overall patterns of germinant emergence and mortality

The timing of emergence differed strongly by site and seed type (Figure 2): in the Forest, seedling abundance for all species and provenances peaked 6 weeks after snowmelt, around 9 July 2009. Subsequently, Spruce emergence ceased abruptly while Pine emergence continued slowly. In the Alpine, Pine and Spruce emergence proceeded at very low rates throughout the entire season. At Treeline, Pine emergence proceeded at a high rate

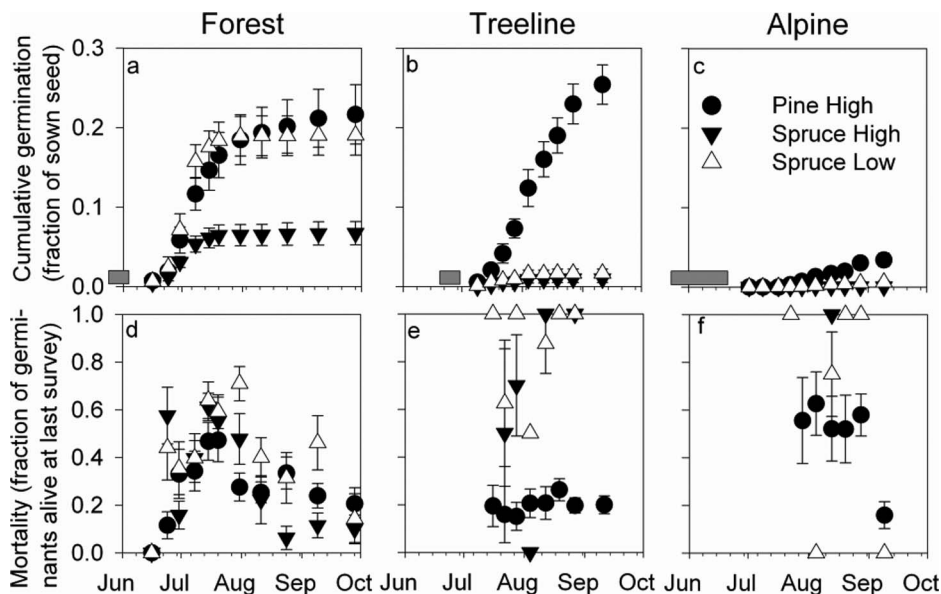


Figure 2. Cumulative emergence (panels a, b, and c) and weekly mortality (panels d, e, and f) by survey date for each study site. Cumulative emergence is calculated as fraction of sown seed. Weekly mortality is calculated as fraction of Germinants alive at the previous survey (panels d, e, and f). Plot mean \pm s.e. $n = 13$ for Forest, $n = 4$ for Treeline, and $n = 8$ for Alpine. Grey boxes indicate the period of snowmelt at each site.

throughout the survey period, while Spruce emergence was almost as low as in the Alpine.

Rates and timing of mortality also differed strongly by site and seed type. In the Forest, weekly Germinant mortality (fraction of seedlings alive in the previous survey) peaked early in the season then declined for all seed types – most significantly for Spruce High (Figure 2). At the Treeline and Alpine sites, most Spruce Germinants died within a week, but Pine mortality hovered around 20% (per week) at Treeline and around 50% in the Alpine.

Emergence

Species. Germination time was longer for Pine than for Spruce (Figure 3, Table 4, $P < 0.01$ for species). Cumulative seedling emergence also was much greater for Pine than for Spruce (Figure 2, Table 4, $P < 0.001$ for species).

Provenance. Germination time was indistinguishable for the two Spruce provenances. Cumulative emergence rates were much greater for Spruce Low than for Spruce High at the Forest site (19% vs. 6%), but the Low provenance advantage was very small at the Treeline site because emergence rates were so low (1.7% for Low vs. 1.0% for High) (Table 4, $P < 0.01$ for site \times provenance).

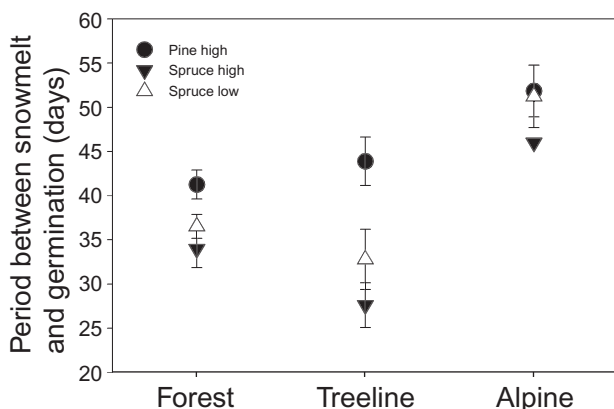


Figure 3. Germination time (the period between snowmelt and emergence) for three seed types at each study site. Plot mean \pm s.e.; $n = 13$ for Forest, $n = 4$ for Treeline, and $n = 8$ for Alpine.

Site and Site \times Species. In 2009, the snowmelt period was 21 May–3 June at the Forest site, 20 May–20 June at the Alpine site and 19 June–29 June at the Treeline site (Figure 2, grey bars). Germination time was 33–42 days following snowmelt in the Forest, 28–44 days following snowmelt at Treeline and 46–52 days following snowmelt in the Alpine (Figure 3, Table 4, $P < 0.1$ for site). Germination times for Pine increased with elevation, but germination times for Spruce were shortest at the Treeline site.

Emergence rates for Pine increased slightly from the Forest to the Treeline and were depressed at the Alpine site. Emergence rates for Spruce decreased sharply from the Forest to both the Treeline ($< 2\%$) and the Alpine ($< 1\%$) sites (Table 4, $P < 0.05$ for Site \times Species). Of over 3000 Spruce High seeds sown in the Alpine site, only one was observed to emerge.

Microsite. For Pine High in the Forest site, emergence was highest in cells dominated by bare soil, intermediate in cells dominated by duff and lowest in cells dominated by plants ($\chi^2 = 17.5$, $P = 0.0002$). In the Treeline and Alpine sites emergence rate was not significantly affected by microsite characteristics.

Survival – first growing season

Germinant survival (as fraction of total emergents) to the last 2009 survey (i.e. end of first growing season) was greatest for Pine High, less for Spruce Low and least for Spruce High (Figure 4(a)–(c), Table 5). With the exception of Spruce Low at the Alpine site, with a $17 \pm 9\%$ survival rate (plot mean \pm s.e.), fewer than 1% of the Spruce seedlings of either provenance survived to the last 2009 survey (Table 5, $P < 0.01$ for species).

Survival of Pine High Germinants to the last 2009 survey was enhanced where the seedlings were within 1 cm of a plant, especially in the Treeline ($\chi^2 = 8.1$, $P = 0.045$) and the Alpine ($\chi^2 = 4.0$, $P = 0.045$) sites. In the Forest site, survival of Pine High Germinants to the last 2009 survey was highest in plant-dominated cells ($\chi^2 = 12.6$, $P = 0.002$). Survival of Spruce was zero or near zero, regardless of the microenvironment.

Table 4. Emergence of limber pine and Engelmann spruce seedlings from local High- and/or Low-elevation seed sources in Forest, Treeline, and Alpine study sites at Niwot Ridge, Colorado, USA. Factorial ANOVA results for analyses of the effect of site, species, provenance, site \times species, and site \times provenance on the lag between snowmelt and emergence (Germ. time) and the fraction of sown seeds observed to emerge (Emerg. rate).

	Pine High vs. Spruce High						Spruce High vs. Spruce Low					
	Site		Species		Site \times Sp.		Site		Prov.		Site \times Prov.	
	F	P	F	P	F	P	F	P	F	P	F	P
Germ. time (days)	3.6	0.041	13.7	0.002	1.3	0.30	8.7	0.002	1.3	0.27	0.2	0.80
Emerg. rate (%)	14.7	<0.0001	31.3	<0.0001	5.0	<i>0.017</i>	29.4	<0.0001	9.3	<i>0.006</i>	9.4	0.001

Notes: The effect of species was only analysed for the High provenances and the effect of provenance was only conducted for Spruce. $n = 13$, $n = 4$, and $n = 8$ for the Forest, Treeline, and Alpine sites, respectively. The raw P values are shown. In addition, **Boldface** indicates significance at $P < 0.01$ and *italics* at $P < 0.05$ after a Bonferroni correction of multiple comparisons ($P \times 2$). Seeds were sown 13 Oct–22 Nov 2008. In 2009/2010 snowmelt began on 21 May/15 May, 19 June/30 May, and 21 May/10 May, and the last surveys were conducted on 28 Sep/1 Oct, 11 Sep/30 Sep and 9 Sep/30 Sep at the Forest, Treeline, and Alpine sites, respectively.

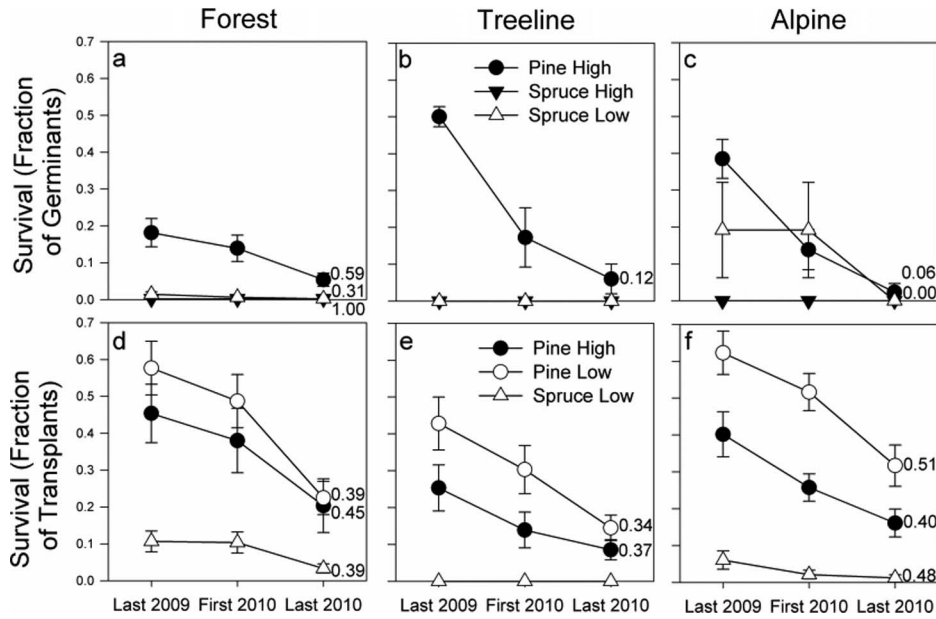


Figure 4. Fractional seedling survival to last 2009 survey, first 2010 survey, and last 2010 survey by site, species, and provenance. Top panels (a, b, c): Germinant survival as a fraction of all seedlings observed to emerge from outplanted seed. Bottom panels (d, e, f): Transplant survival as a fraction of seedlings transplanted into the field. Plot means and s.e. Germinant $n = 13$ for Forest, $n = 4$ for Treeline, and $n = 6$ for Alpine. Transplant $n = 8$ for Forest, $n = 6$ for Treeline, and $n = 6$ for Alpine. The numbers beside the symbols for the last 2010 survey indicate survival to the last 2010 survey as a fraction of seedlings alive at the last 2009 survey.

Table 5. Survival of limber pine and Engelmann spruce seedling that emerged in 2009 from local High- and/or Low-elevation seed sources in Forest, Treeline, and Alpine study sites at Niwot Ridge, Colorado, USA. Factorial ANOVA results for analyses of the effect of site (Forest and Treeline only), species, provenance, site \times species, and site \times provenance on the fraction of Germinants that survived to the last 2009 survey, the first 2010 survey, and the last 2010 survey.

	Pine High vs. Spruce High						Spruce High vs. Spruce Low					
	Site		Species		Site \times Sp.		Site		Prov.		Site \times Prov.	
	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>
Last 2009 survey	13.8	0.0009	68.5	<0.0001	14.2	0.0008	1.2	0.3	0.6	0.4	0.6	0.4
First 2010 survey	0.4	0.5	14.2	0.0008	0.5	0.5	1.0	0.3	0.2	0.7	0.2	0.7
Last 2010 survey	0.2	0.6	8.1	<i>0.008</i>	0.3	0.6	0.6	0.5	0.0	1.0	0.0	1.0

Notes: The effect of species was only analysed for the High provenances and the effect of provenance was only conducted for Spruce. The Alpine site was excluded from the analysis because only one plot had survivors of all three seed types. $n = 13$ and $n = 4$ for the Forest and Treeline sites, respectively. The raw *P* values are shown. In addition, **Boldface** indicates significance at $P < 0.01$ and *italics* at $P < 0.05$ after a Bonferroni correction of multiple comparisons ($P \times 2$). Seeds were sown 13 Oct–22 Nov 2008. In 2009/2010 snowmelt began on 21 May/15 May, 19 June/30 May, and 21 May/10 May, and the last surveys were conducted on 28 Sep/1 Oct, 11 Sep/30 Sep and 9 Sep/30 Sep at the Forest, Treeline, and Alpine sites, respectively.

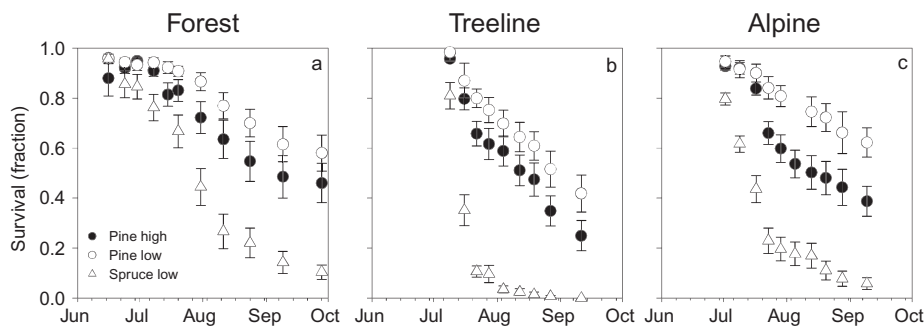


Figure 5. Transplant survival by survey date for each of the study sites. Plot mean \pm s.e.; $n = 14$ for Forest, $n = 12$ for Treeline, and $n = 12$ for Alpine.

Table 6. Survival of limber pine and Engelmann spruce seedlings from local High- and/or Low-elevation seed sources germinated indoors and transplanted after snowmelt into the Forest, Treeline, and Alpine study sites at Niwot Ridge, Colorado, USA. Factorial ANOVA results for analyses of the effect of site, species, provenance, site \times species, and site \times provenance on the fraction of Transplants that survived to the last 2009 survey, the first 2010 survey, and the last 2010 survey.

	Pine Low vs. Spruce Low						Pine High vs. Pine Low					
	Site		Species		Site \times Sp.		Site		Prov.		Site \times Prov.	
	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>
Last 2009 survey	3.4	0.047	53.1	< 0.0001	0.6	0.6	4.0	0.027	4.0	0.053	0.4	0.7
First 2010 survey	5.9	0.006	50.5	< 0.0001	3.0	0.061	6.9	0.031	4.8	0.036	1.4	0.3
Last 2010 survey	6.0	0.006	35.0	< 0.0001	5.0	0.012	3.9	0.031	1.0	0.3	2.3	0.12

Notes: The effect of species was only analysed for the Low provenances and the effect of provenance was only conducted for Pine. $n = 8$, $n = 6$, and $n = 6$ for the Forest, Treeline, and Alpine sites, respectively. The raw *P* values are shown. In addition, **Boldface** indicates significance at $P < 0.01$ and *italics* at $P < 0.05$ after a Bonferroni correction of multiple comparisons ($P \times 2$). Seedlings were transplanted 31 May–11 June, 6–16 July, and 22 June–1 July in the Forest, Treeline, and Alpine sites, respectively. In 2009/2010 snowmelt began on 21 May/15 May, 19 June/30 May, and 21 May/10 May, and the last surveys were conducted on 28 Sep/1 Oct, 11 Sep/30 Sep and 9 Sep/30 Sep at the Forest, Treeline, and Alpine sites, respectively.

The 2009 survivorship curves for the three Transplant types (Pine High, Pine Low and Spruce Low) were broadly similar across sites (Figure 5). Mortality was greater for Pine High than for Pine Low, and declines were steeper for Spruce than for Pine, especially at the Treeline site. Transplant survival to the last 2009 survey was greatest for Pine Low and least for Spruce Low (Figure 4(d)–(f), Table 6, $P < 0.01$ for species). By site, survival was greatest in Alpine, less in Forest and least in Treeline, where Spruce suffered total mortality (Table 6, $P < 0.1$ for site).

Survival – second growing season

Although Pine Germinants at the Treeline site had the highest survival through the first growing season, 50% (Table 5, $P < 0.01$ for species and for site), they also had the highest over-winter mortality (of all species and site combinations), such that survival to the first 2010 survey only differed by species, not by site (Table 5, $P < 0.01$ for species, non-significant for site). By the last 2010 survey (end of the second growing season), fewer than 10% of the original Pine Germinants remained at the Treeline and Forest sites and even fewer remained at the Alpine site (Table 5, $P < 0.05$ for species, non-significant for site).

For Transplants, enhanced survival of Pine over Spruce and of Pine Low over Pine High persisted to the first 2010 survey. But by the last 2010 survey, Pine Low survival dropped to more closely match that of Pine High, and the provenance effect vanished (Figure 4(d)–(f), Table 6). Survival was greatest overall for Pine Low transplants at the Alpine site, above the current species' elevational range limit (Table 6, $P < 0.1$ for site \times provenance).

Discussion

There are at least three bottlenecks to seedling establishment: seed availability, fraction of seeds that germinate and fraction of germinants that survive. We eliminated limitations due to seed availability by planting seeds and excluding seed predators and herbivores, and

focused on the latter stages of establishment. In separate trials, we guarded against lack of germination and survival through the first few weeks by transplanting ca. 2-week-old seedlings. We expected germination and survival to decrease across the elevation gradient, particularly for seeds from low-elevation seed sources. We observed these expected patterns for Spruce Germinants but not for Pine Transplants.

Species differences

In the Colorado Rockies, post-fire forest succession typically starts with colonisation by limber pine, which is very drought tolerant (Tomback and Linhart 1990). The establishment of Engelmann spruce and the associated subalpine fir require the amelioration of light and wind conditions, with the delay between pine and fir establishment being as long as 100 years (Rebertus et al. 1991). Consistent with a successional relationship in which pine facilitates establishment of other species, in a previous study at treeline in the Rocky Mountains of Wyoming, we found that in the initial years following germination survival was greater for Whitebark pine (*Pinus albicaulis* Engelm.) than for Engelmann spruce or subalpine fir (Maher and Germino 2006). Rebertus et al. (1991) found that once overtopped by spruce and fir, recruitment of the shade-intolerant limber pine effectively halts – older pine stands are only found in xeric sites.

A similar successional dynamic occurs between *Pinus sylvestris* and *Picea abies* at high altitudes in the Swedish Scandes. Following stand-destroying fires towards the end of the Little Ice Age in the eighteenth century, *Betula pubescens* formed dense subalpine stands. As a typical pioneer species, pine was unable to re-establish under the canopy, and became relegated to rocky outcrops at lower elevations. In contrast, spruce did eventually re-invade and establish, forming the current spruce–birch subalpine forest association (Kullman 1996). More recently, following exceptionally warm summers since 1997, *P. sylvestris* saplings are spreading up to 500–700 m above the current treeline (Kullman 2007).

Our findings confirm contrasting environmental requirements for the two species we studied (see, for example, Alexander and Shepperd 1990; Steele 1990; Rebertus et al. 1991). While Engelmann spruce is known to recruit in the forest understory, our Forest plots cover areas that are in canopy gaps, and these may have had fewer favourable microsites for spruce recruitment. As Rebertus et al. (1991) concluded based on adult tree ages, we found that Engelmann spruce seedlings could not recruit into exposed sites, but that limber pine seedlings could – in fact the Pine Transplants performed better in the exposed high-elevation sites than they did in the Forest. The different response of the two species to the same environmental cues showed that their environmental requirements differed for all early stages of seedling establishment, in ways that may influence their geographic range or population response to disturbance or change in climate.

In recent decades, the upper elevation limit of limber pine has expanded both upslope above bristlecone pine (*Pinus longaeva* D.K. Bailey) and downslope along cold-air drainages, in response to warmer summer nights in the White Mountains of California, USA (Millar et al. 2007). An important question for future research is how information on differences in seedling establishment such as those reported here can help predict response of species ranges to warming.

Provenance differences

Evidence for local adaptation was partially supported by the fact that, from the Forest to the Treeline, Spruce emergence rates fell over ten-fold for the low-elevation seed source (from 19% to < 2%) but only six-fold for the high-elevation seed source (from 6% to 1%).

Work by others indicating that seed quality tends to decrease towards the species upper limit (e.g. Oleksyn et al. 1998; Cuevas 2000; Johnstone et al. 2009) was supported by results for the first growing (2009) season, with the low- largely outperforming the high-elevation seed sources. X-ray analyses of the cleaned Spruce seed indicated similar viability rates (85% for low vs. 90% for high elevation seed sources). Yet in the field as well as in growth chamber trials (data not shown), emergence rates were 2–5 times larger for the low- compared with the high-elevation seed source across all sites. In addition, Reinhardt et al. (2011) found that during the first growing season, specific leaf area, shoot:root ratios, photosynthesis and conductance were significantly greater ($P \leq 0.08$) for the Low compared with the High Pine Transplants.

For both species, average seed mass was 10% greater for the high- compared with the low-elevation seed sources, which does not support the possibility of a low-elevation maternal advantage. It is possible, however, that low-elevation environments allow trees to better provision their seeds with nitrogen or more mobile forms of carbohydrates, which could explain higher success of first-year low-elevation Spruce Germinants and Pine Transplants at all sites.

However, over the second (2010) growing season, and in spite of more severe temperature and radiation conditions (Table 1), the advantage of the low- over the high-elevation provenances tended to diminish. For example, first-year Pine Transplant survival was greater for Low compared with High, but second year survival of Pine Low and Pine High was indistinguishable at both Forest and Treeline sites (Figure 4(d)–(e)). These results suggest that while low-elevation populations may be selected for faster growth and initial survival, high-elevation populations may be selected to endure harsher conditions and may, in addition, have higher tolerance to a wider range of stressors. Compared with Engelmann spruce, subalpine fir has lower seedling emergence as a fraction of seed sown (Germino et al. 2002) and weaker physiological tolerance (Germino and Smith 1999, 2000). Yet similar survival of the two species has been observed in naturally occurring germinants at the treeline (Germino et al. 2002). Considered together, these findings suggested that germination of subalpine fir seeds is especially sensitive to temperature and moisture conditions, such that a microsite that is favourable for germination is also more likely to meet the niche requirements of the resulting seedling. A similar effect may be occurring with respect to high vs. low-elevation provenances in this experiment. For example, although <1% of the emerged high-elevation seedlings survived their first year, all of those seedlings survived at least one more year, even in the warmest, driest site (Figure 4(a)). In contrast, fewer than one-third of the first-year low-elevation Spruce Germinant survivors lasted another full year. Thus, in spite of the apparent potential for substantial pollen and seed dispersal over the short distances involved, provenance-level genotypic differences – that are more typically reported as differences in seedling and sapling morphology (e.g. Rehfeldt 1989, 1993) – may also be instrumental in seedling survival and, hence, establishment.

Site differences

The Forest is the warmest and driest of the three experimental sites, with the longest growing season. Seedling emergence was higher there than at any other site. In addition, although the mortality rate for Spruce was >99%, most likely due to drought and/or heat girdling (Alexander and Noble 1971; Alexander and Shepperd 1990), the relatively shaded Forest was the only site where Spruce Germinants survived in substantial numbers. In addition, this site had the highest overall second-year Germinant survival, primarily due to low over-winter mortality.

At the Treeline site, Spruce emergence was extremely low, perhaps because the snow depth was too great (Hattenschwiler and Smith 1999), and survival to the end of 2009 was nil. We surmise that this is the worst site for frost-induced photoinhibition, to which Spruce is especially vulnerable (Germino and Smith 1999; Johnson et al. 2004). While the Treeline and Alpine sites are equally exposed to the sky, the Treeline site is protected from the wind by krummholz tree forms. This limits convective

heat transfer from relatively warm wind to the land surface, making it effectively colder for seedlings experiencing radiative heat loss during clear nights. In addition, Moyes et al. (2012) found that at critically low soil moisture (volumetric water content $< 0.08 \text{ m}^3 \text{ m}^{-3}$), soil surface temperatures were higher at the Treeline than at the Alpine site, pointing to desiccation as another probable mechanism for high Spruce mortality. Conversely, Reinhardt et al. (2011) found that Pine Transplants had higher mid-season stem water potential here than at the Alpine site. Accordingly, we found that the establishment of the relatively deep-rooted Pine species was limited not by survival through the first season but by over-winter survival (Figure 4(b)), possibly a result of root infection by pathogenic fungi (Holm 1994; Pluess et al. 2005), although no observations of fungal infection were made.

While the Alpine site had by far the lowest Germinant emergence, it had moderate first-year survival, and the highest overall second-year survival of both Pine and Spruce Transplants. This suggests that, at least once seedlings surpass a minimum size threshold, the growing environment is less severe than that of the Treeline site. In spite of lower snowpack and earlier snowmelt, the 2010 soil sensor data indicated that soils at the Alpine site did not dry down as much as those at Treeline (Table 2, minimum daily soil water content). This reduced moisture stress may provide seedlings the opportunity to produce protective tissue to survive over-winter wind and frost damage. Indeed, in 2009, Reinhardt et al. (2011) found that the ratio of photosynthesis to dark respiration was higher in the Alpine than the other two sites, and higher for Pine Low than for Pine High. At Alpine, greater survival of Pine Low over Pine High persisted into the second year (Figure 4(f)). These results suggest the potential for synergy – as opposed to a trade-off – between initial seedling growth and subsequent survival.

Therefore, in the absence of increased summer precipitation, expected warmer summer temperatures would make the Forest site warmer, drier, and thus less hospitable, particularly for Spruce. In contrast, warming may ameliorate cold conditions at the Alpine and especially Treeline sites. As was observed by Daly and Shankman (1985) in the vicinity of the Alpine site, our study demonstrates that establishment in the alpine is possible even under present conditions. We hypothesise that establishment in the alpine will be further facilitated if global warming leads to earlier snowmelt and a longer growing season.

Transplants vs. Germinants

While many conifer seedling studies rely on transplants, and they can be useful for investigating some questions, our results show that they ought not to be used to study seedling establishment because they do not necessarily behave like seedlings that germinate in situ. In this study, survival was higher for Transplants than for Germinants and site had less of an effect on Transplants than on Germinants. In fact, the Alpine site had the highest Transplant survival.

These differences highlight the fact that demographic sorting across species, provenances and sites occurred soon after germination – at a stage that was skipped by the < 3 -week-old Transplants, which were also assisted by watering for the first 2 weeks in the field. Our results reinforce the idea that carbon gain and growth in the first few weeks are particularly strong bottlenecks to establishment in the alpine (Bansal and Germino 2008; Reinhardt et al. 2011).

Interannual variability

Weather patterns affect all stages of reproduction and establishment from seed production and quality to seedling emergence and survival. Seed maturation is a function of thermal accumulation during the summer (Fedorkov 2001), and Kullman (1996, 2007) observed that seed viability in both *Pinus sylvestris* and *Picea abies* is strongly positively correlated to summer temperature, which helps to explain recent advances of pine above treeline in the Swedish Scandes. Seed production of both Engelmann spruce and limber pine can be highly variable from year to year (Alexander and Shepperd 1990; Steele 1990). Furthermore, seedling establishment is critically dependent on daily patterns of soil moisture and temperature minima and maxima (Moyes et al. 2012). Thus, interannual climate variability will critically modulate the effect of global warming on seedling establishment within and above the current range of the subalpine forest. It follows that our results are to some degree sensitive to normal interannual weather and climate variability. For example, the unusually mild summer of 2009 (see Table 1) may have facilitated establishment in the Alpine, while harsher temperatures and solar radiation, over the course of the second growing season, may have promoted the higher-than-expected mortality experienced by second-year seedlings. Multiple years of observations with varied combinations of seed lot (year of production), winter snow and growing season weather are thus required to more definitively assess the relative importance of species, provenance, site and micro-environment on patterns of seedling establishment.

Implications

The spatial responses of forests to landscape-scale disturbances such as climate change, fire and disease depend upon recruitment abilities of individual tree species and provenances across a mosaic of habitats. Direct observation of the controls underlying seedling emergence and initial establishment are therefore critical to accurately modelling present tree species range limits, as well as potential range shifts.

We have found that, while seed provenance leads to differences in seedlings' capability to grow and survive across a range of environments, these differences do not always reflect local adaptation. For example, in spite of low phenotypic plasticity (Schoettle and Rochelle 2000), limber pine seeds can germinate and seedlings survive across a 500 m elevational difference from the low end to above the current species range (3060–3540 m), and low-elevation Pine seed sources in particular can thrive in the alpine, well

above treeline. Also, in both the Forest and the Treeline, we observed that the Low provenances generally grew and survived better in their first year, but the High provenances survived better in their second year. This suggests that genetic differences between the two provenances (such as selection for growth vs. survival) may, in the long run, balance each other out.

The tendencies we observed in the relative performance of Germinants vs. Transplants and Pine vs. Spruce point to size and/or rooting depth as a potential underlying control on seedling survival through stressful events such as over winter and during periods of drought. In addition, morphological and physiological observations by Reinhardt et al. (2011) in the High vs. Low elevation Pine provenances suggest that the Low provenances are selected not only for growth but also for first-year survival. Thus, whereas it is commonly understood that a tradeoff exists between growth and stress survival (e.g. for Low vs. High provenances), our findings, combined with those of Reinhardt et al. (2011), suggest that absent critical limitations to growth in the initial life stages, there may be a positive relationship between growth and survival through ensuing stressful events.

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Bettina Weibel led the microsite characterisation effort as part of her graduate research at the University of Zurich, Switzerland.

Lara M. Kueppers, lead investigator of the Alpine Treeline Warming Experiment, uses field experiments, observations, and regional climate and vegetation models to study ecological consequences of and feedbacks to climate change.

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