

## Comparative Seedling Ecology of Eight North American Spruce (*Picea*) Species in Relation to their Geographic Ranges

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• **Background and Aims** Allowing for dispersal limitation, a species' geographic distribution should reflect its environmental requirements. Comparisons among closely related species should reveal adaptive differentiation in species characteristics that are consistent with their differences in geographic distribution. This expectation was tested by comparing characteristics of seedlings of spruce species in relation to environmental factors representative of their current natural ranges.

• **Methods** Seedlings were grown from a total of 34 populations representing eight North American spruce (*Picea*) species in a controlled environment chamber for 140 d. Traits related to the potential of seedling establishment, including tolerance to stress events (high temperature, desiccation) were evaluated. Correlations were sought between these characteristics and modal values of latitude, aridity and continentality in the geographic range of each species.

• **Key Results** Many seedling traits changed significantly in response to stress events, but only the response of chlorophyll concentration differed significantly among species. Components of seedling growth were good correlates of species distribution. Seedling relative growth rate (RGR) and specific leaf area (SLA) were positively correlated with latitude, and leaf weight ratio (LWR) negatively correlated with aridity. Seed mass was negatively correlated with latitude.

• **Conclusions** Relationships found between seedling traits and geographical variation in environmental conditions suggest that factors such as temperature regime, water availability and perhaps litter depth affect species range in North American spruces. Seedling characteristics appear to be elements in a reasonably distinct environmental niche for each spruce species at the continental scale.

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**Key words:** Relative growth rate, specific leaf area, leaf weight ratio, seed mass, seedling, stress response, distribution, *Picea*.

### INTRODUCTION

Why the natural ranges of plants differ and what has led each species to its current distribution pattern have always been focal questions in biogeography (Cox and Moore, 1993; Brown and Lomolino, 1998). There is also an important contemporary concern in these questions because of the need to predict the future distribution of species and vegetation under global climate change (Huntley, 1991; Thompson, 1993). There has long been a broad consensus that two conditions must be met if a species is to occur in a locality: (1) propagules of the species must reach the locality; and (2) the species must be able to establish and persist in the local environment. The paleoecological record of the ranges of trees in eastern North America during the Holocene offers ample illustration of how contemporary distribution is contingent on both dispersal and ecological adaptation (Jackson and Overpeck, 2000; Jackson *et al.*, 2000). Mechanisms that may underlie distributional patterns have been sought in correlations between environmental factors and species ranges (Iverson *et al.*, 1999; Shafer *et al.*, 2001), and plant ecophysiological responses to environmental factors have been incorporated into models that predict geographical vegetation patterns (Woodward, 1987; Prentice *et al.*, 1992). These lines of inquiry all build

on and support the supposition that, making allowance for dispersal limitation, a species geographic distribution reflects its environmental requirements.

This premise can be further tested through a related line of inquiry that is less well explored: the evolutionary and ecological diversification of closely related species. Rather than investigate species with broadly different evolutionary histories and disparate adaptations as components of a vegetation type, it is useful to compare the basis for the geographic distribution of species within a single evolutionary lineage. Species within a lineage share the same basic form and function, varying quantitatively rather than qualitatively one from another. Assuming good dispersal over time, a correspondence between the values of traits known to affect species distribution and measures of the environment characterizing contemporary ranges is expected. This line of inquiry offers two advantages: (1) it allows a more refined evaluation of the possible mechanistic basis in species adaptations that underlie patterns of geographic distribution; (2) it may reveal the redundancy of species at the ecosystem level, a topic of concern among conservationists (Tilman, 1999; Schwartz *et al.*, 2000). If closely related species have distinct patterns of variation in traits that affect their distribution and abundance, and these patterns of trait variation map onto geographic scale climatic patterns, then it can be inferred that species have evolved distinct adaptations (niches) and may not be substitutable one for another in terms of ecosystem function under global change.

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This comparative approach can be taken to examine a number of factors at the seedling stage that may determine the species-specific distribution patterns of eight of the ten species of spruce (*Picea*) occurring in North America. These represent almost one-quarter of the extant spruces (Schmidt-Vogt, 1977). The current distribution patterns of North American spruce (Burns and Honkala, 1990; Weng and Jackson, 2000) are largely the result of post-glacial range expansion; the species that now dominate the northern part of the continent were restricted to south of the ice margin at the last glacial maximum (Graumlich and Brubaker, 1995; Jackson *et al.*, 2000). Assuming all the North American spruces have had ample opportunity for range expansion during the Holocene, it is expected that these species occur in the regions where their ecological characteristics are well-suited to environmental conditions. More specifically, it should be possible to observe quantitative differences among the North American spruces in traits that affect seedling establishment and survival, and these differences should be explicable in relation to geographic differences in environmental factors important at the seedling stage. In this study, two inter-related sets of traits are emphasized: (1) seed size as an index of maternal investment potentially important in early seedling establishment; (2) the components of seedling growth as a measure of early performance. We compare seedlings of eight North American spruce species grown under both favourable and stressful conditions.

Seed size influences early seedling growth and establishment (Wang *et al.*, 1994, 1995), but somewhat surprisingly, seedling relative growth rate (RGR) actually decreases with seed mass (see references in Swanborough and Westoby, 1996; Westoby *et al.*, 2002). Relative growth rate is an important measure of plant performance that integrates critical aspects of seedling form and function (Hunt, 1990; Hunt and Cornelissen, 1997). The RGR is a function of net assimilation rate (NAR) and the leaf area ratio (LAR), which in turn is a function of specific leaf area (SLA) and leaf weight ratio (LWR). Since species that have inherently low RGR tend to occur in adverse habitats (Lambers and Poorter, 1992), low RGR has often been considered to be an adaptation to unfavourable conditions (Grime and Hunt, 1975; Chapin, 1988). However, the suggested ecological advantages of such conservative growth are questionable (Lambers and Dijkstra, 1987; Poorter, 1989; Lambers and Poorter, 1992). An alternative explanation is that components linked with RGR, rather than RGR itself, have been the target of selection (Lambers and Dijkstra, 1987; Poorter, 1989; Poorter and Remkes, 1990; Lambers and Poorter, 1992; Saverimuttu and Westoby, 1996). If this is the case, and local environments favour species that have appropriate SLA or LWR values, then the geographic ranges of species should be correlated with interspecific variation in these components of seedling growth.

Any correlations between these seedling characteristics (growth-related traits, seed mass) and environmental factors may be disrupted or even overridden by species responses to stress events (Guttschik and BassiriRad, 2003). Distributional limits may be set not by responses to modal environmental regimes but instead by tolerance

of occasional extreme events. High temperatures and reduced water availability in particular can stress seedlings. High temperature adversely affects plant growth, and often limits the distribution of coniferous species (Woodward, 1995). Desiccation is one of the greatest causes of seedling mortality in spruce, restricting seedling growth in both natural and planted stands (Grossnickle, 2000). If responses to abruptly increased temperature and drought differ among species, these differences may influence species distributions. In this paper, therefore, seedling characteristics (growth, seedling architecture, foliar chlorophyll, chlorophyll *alb* ratio, photosynthetic capacity, seed mass) of North American spruce species grown with and without stress are compared. The relationships are considered between these species-specific traits and three geographic factors (latitude, climatic dryness and Continentality Index) that broadly characterize the environmental regime typical of each North American spruce species. Answers to the following questions are sought: What seedling characteristics correlate with environmental attributes of the geographic ranges of these North American spruce species? Do stress events alter seedling characteristics differently from species to species? To what degree do seedling attributes suggest niche differentiation within the lineage that might influence the contemporary biogeography of spruce species in North America?

## MATERIALS AND METHODS

### *Seed sources and stratification*

Seeds of eight spruce species, represented by a total of 34 populations (three to six per species) were mostly obtained from seed companies. *Picea chihuahuana* seeds from three populations and one population of *P. engelmannii* was a gift from David R. Johnson (USDA Forest Service, Placerville, CA, USA). Seeds were stored in a dark, dry room at 8 °C until the experiment. Seeds were stratified at 4 °C, under moist conditions for 2 weeks before planting. Unfortunately, no seeds of *P. martinezii* or *P. mexicana* were available. These two species survive as relict populations in the mountains of eastern Mexico, sometimes in high elevation conifer stands but also in stands at mid-elevation that include hardwood trees (Ledig *et al.*, 2000a, b, 2002). The only representative of the interesting relicts at the southern extent of spruce in North America included in the present study is *P. chihuahuana*, which occurs in the mountains of western Mexico (Gordon, 1968; Taylor *et al.*, 1994; Ledig *et al.*, 1997, 2000b).

### *Growing conditions*

Peat, topsoil and sand (1 : 2 : 2, v/v/v) were mixed in a soil mixer (H.C. Davis, Bonner Spring, KS, USA) with some water until the entire batch of soil was uniformly moistened. A single batch of 250 L of soil was used to fill 30 nursery containers (IPL 15-320; IPL, St-Damien, Quebec, Canada), each container having 15 cells for a total of 450 seedlings. The volume of each cell was 320 cm<sup>3</sup>. Cells were randomly assigned to ten replicates for 34 populations of the eight

species. Among the 450 cells, 90 were randomly assigned to *P. abies*, which was used as a space filler and check on the spatial consistency of conditions in the growth chamber.

Deciding the growing conditions under which these spruce species from widely different regions should be compared posed a difficulty. Only a single growth regime could be simulated, but growing any species in an environment very different from its natural habitat had to be avoided. An intermediate temperature regime was therefore chosen, which did not differ more than 4 °C from July temperatures typical within any species' range (see 'Geographical data' section). The phytotron chamber (PGW36-M10, Conviron, Winnipeg, Manitoba, Canada) was set at an 18/14 °C (day/night) temperature regime for 25 d, until the seeds had germinated, and then changed to 16/14 °C (day/night). Throughout the experiment, the chamber was set at 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  irradiance with a 16 h photoperiod and 80 % relative humidity level. The phytotron chamber set at this growth regime is referred to as the 'control chamber' to distinguish it from another phytotron chamber (stress chamber) used for the episodic stress treatment described below. Seedlings were grown for 140 d and then harvested. Seedlings were watered every day with a mist sprayer, and the entire container was soaked once a week in deionized water; fertilizer was applied at the same time by adding 20–20–20 N : P : K fertilizer (Plant Products Co. Ltd, Brampton, Ontario, Canada) in the soaking water at a concentration of 0.5 g L<sup>-1</sup>. It is believed that this growth regime allows a meaningful, though not comprehensive, comparison of seedling traits potentially relevant to species distribution.

Drought-temperature stress treatment was imposed 100 d after sowing, and repeated twice more, 112 and 115 d after sowing, respectively. Half of the containers, which had been randomly assigned beforehand to the stress treatment, were moved from the 'control chamber' and placed in an identical chamber. There water was withheld, relative humidity level was reduced to 50 %, and temperature was set at 25/22 °C (day/night) then 30/25 °C for the first event, and 27/22 °C for the second and third events. The stress treatment was stopped when about 10 % of seedlings started to wilt; this took 72 h for the first event, 50 h for the second and 31 h for the third. The stressed seedlings were immediately watered at the end of the stress treatment, and the 'stress chamber' was gradually cooled down to 16 °C in 9–16 h. The containers were then moved back to the 'control chamber'. A few seedlings did not recover from wilting after the stress treatments, and about 5 % of the stressed seedlings eventually died. Both control and stressed seedlings were harvested 18 d after the third stress event.

#### Measurements

The mean seed weight of each population was measured by averaging the weight of ten randomly selected seeds. The photosynthetic capacity of seedlings was assessed just before harvest using the protocols of Wang *et al.* (1995). The branch number and each branch length were measured 115 d after sowing and again just before the harvest. After harvest, projected needle area and needle length were

measured on ten needles from middle positions of each seedling by capturing images with a Panasonic CCTV Camera WV-CD20 (Matsushita Communication Industrial Co., Ltd, Japan) and scanning them with Microsoft Photo Editor 3.0. Digitized image area and maximum length for each needle were then calculated with the UTHSCSA Image Tool for Windows, Version 1.28. Roots from each seedling were carefully washed and maximum root length measured. Roots and shoots were then dried at 70 °C for at least 3 d, and the dry mass of root, needle and stem measured (to the nearest 0.01 mg).

#### Geographical data

Maps showing the habitat ranges of North American spruce species were obtained from Burns and Honkala (1990), and from Gordon (1968) for *P. chihuahuana*. The modal latitude of the range of each species was estimated by sampling from every degree of latitude and longitude, and averaging the latitude of the points within the habitat range. July mean temperature, July precipitation and annual temperature range values were obtained from comparable maps in the Climatic Atlas of North and Central America (Steinhauser, 1979) and the modal value of each variable for each species was obtained in the same manner as for latitude. Potential evapotranspiration (PE) was calculated by the equation from Pereira and de Camargo (1989). A Dryness Index was calculated by subtracting July precipitation from PE. A Continentality Index was calculated using the equation proposed by Conrad (1946), which gauges the contrast in winter and summer temperature adjusted for latitude. Weng and Jackson (2000) provide informative, up to date range maps for all the North American spruces, including the Mexican relict species; a report by Thompson *et al.* (1999), which unfortunately only became available after the design of our experiment, gives more complete characterizations of the climatic envelopes for the North American spruces.

#### Data analysis

The RGR was calculated by setting the emergence day as growing day zero, and seed weight as the initial biomass. Therefore, RGR in this paper is:  $(\log M_f - \log M_s)/t$ , where  $M_f$  is final biomass,  $M_s$  is seed mass, and  $t$  is growing time in days, and thus represents a mean value throughout the entire first year growth period, rather than the maximum RGR that is often calculated using the first 3 weeks of growth (Hunt and Cornelissen, 1997). This first year mean RGR allows stronger comparisons of seedling function among species that differ in seed mass, and hence in possible maternal influences. In this growth analysis, NAR cannot be calculated because the initial leaf area is zero. Hunt (1990) provides background on these growth parameters and their biological significance. A principal component analysis (SAS system for Windows, version 6.12, PROC PRINCOMP) was conducted to create an index of form based on branch number, total length, mean length, maximum length, and minimum length of two measurements. The first PC axis represented 47 % of the variation in these

TABLE 1. Mean, standard deviation and sample size for each trait for control and stressed seedlings pooled across all species (listed traits are the ones that had a significant treatment effect)

Trait	Control		Stressed		Effect of stress (%)	P
	Mean	n	Mean	n		
Traits decreased by stress treatment						
Total dry weight (g)	2.01 ± 0.85	162	1.63 ± 0.65	157	-16.1	<0.0001
Stem dry weight (g)	0.381 ± 0.17	162	0.292 ± 0.13	157	-23.5	<0.0001
Needle dry weight (g)	1.12 ± 0.52	163	0.911 ± 0.40	157	-18.8	<0.0001
Root dry weight (g) <sup>c</sup>	0.496 ± 0.25	164	0.426 ± 0.20	157	-14.1	0.0057
Height (cm)	11.3 ± 3.7	165	10.1 ± 3.0	157	-10.8	0.0006
1 needle area (mm) <sup>b</sup>	20.0 ± 9.0	163	18.2 ± 7.9	155	-9.3	0.0002
First root length (cm) <sup>a</sup>	18.3 ± 5.8	164	16.9 ± 5.0	157	-7.6	0.0007
1 needle length (cm) <sup>a</sup>	1.93 ± 0.55	163	1.82 ± 0.50	155	-6.1	0.0002
Branchiness	0.454 ± 2.4	109	-0.544 ± 2.1	91	-	0.0018
Stem/needle ratio	0.356 ± 0.11	162	0.337 ± 0.10	157	-5.4	0.0286
RGR (mg/mg/week)	0.362 ± 0.057	162	0.351 ± 0.052	156	-3.0	0.0009
Traits increased by stress treatment						
Leaf chlorophyll content (mol g <sup>-1</sup> ) <sup>a</sup>	953 ± 330	154	1070 ± 350	154	11.9	<0.0001
Chl <i>a/b</i> ratio	3.15 ± 0.65	156	3.32 ± 0.60	154	5.6	0.0004
A <sub>mass</sub> (μmol g <sup>-1</sup> s <sup>-1</sup> )	0.054 ± 0.011	154	0.056 ± 0.012	147	4.1	0.0203

The effect of stress is the percentage decrease or increase in stressed seedlings relative to control; P is the probability of a significant difference between control and stressed samples, tested by nested ANOVA.

Symbols a, b, and c indicate transformations for ANOVA (tabulated means are not transformed): a = logarithmic transformation, b = reciprocal transformation, c = square root transformation. A<sub>mass</sub> = net photosynthesis per unit mass.

traits, with branch number and total branch length contributing the most (eigenvectors 0.40, 0.43, respectively). This first PC axis was used as a branchiness index, larger values indicating longer and more branches.

The probability of significant differences in trait values among species was tested by ANOVA (SAS system, PROC GLM). Population was considered as a random effect, and nested within species; therefore, population effect was used as an error term to test the species effect. Mean values for each trait were calculated by averaging the population replicates for each species. Regressions (SAS system, PROC REG) were weighted by the number of populations. Correlation matrices were generated (SAS system, PROC CORR) with species mean values.

## RESULTS AND DISCUSSION

### *Effects of stress on seedlings and spruce distribution*

Although the stress regime had substantial effects on the spruce seedlings (Table 1), the species did not differ significantly in their stress responses. The only exception is needle chlorophyll concentration, which had a marginally significant species × stress interaction effect ( $P = 0.043$ ). In fact, all species had increased chlorophyll concentration under stress but differed significantly in the magnitude of the response; *P. breweriana* showed the highest increase (42.7%) and *P. engelmannii* the lowest increase (2.6%). The differences in magnitude of this stress response among species did not correlate with the modal latitude, dryness or continentality characteristic of the species range (data not shown). Except for this response in chlorophyll concentration, the high temperature and drought stress regime

affected all the North American spruce species more or less equally. Although more prolonged or more severe stress events may play a role in determining distribution, this sort of short-term stress on seedlings cannot be regarded as part of the explanation for the present biogeography of North American spruces.

### *Relationship between geographical factors and growth analysis components*

Seedling growth components, on the other hand, show important correlations with species distribution. There was a positive correlation between latitude and RGR, latitude and SLA; there was a negative correlation between dryness and LWR (Table 2 and Fig. 1). Might any of these correlations reflect effects on seedling establishment and survival that underlie the continental distributions of the North American spruces?

The higher RGR of northern species could confer an advantage on seedlings in a relatively shorter growing season. After snow melts in late spring, newly germinated seedlings must grow rapidly and develop needles fully before an early autumn frost (Wang and Lechowicz, 1998; Grossnickle, 2000). Seedling survival will be higher if the seedling can grow over a longer period and also develop well-cutinized needles as protection against winter desiccation (Wilson *et al.*, 1987; Grossnickle, 2000). However, this adaptive rationale cannot explain the relatively lower RGR in southern species. All things being equal, faster-growing species should survive better than slower-growing species in the south as well. The ecological significance and selection pressures underlying this latitudinal trend in RGR may lie in the components of RGR, rather than in RGR itself.

TABLE 2. Correlation matrix among growth analysis components and geographical factors. The upper half of the matrix contains Pearson correlation coefficients and P-values, and lower half the Spearman rank correlations

	Latitude	Dryness	K*	RGR	LAR	SLA	LWR
Latitude		0.5669	-0.0074	<b>0.8243</b>	0.6051	<b>0.8551</b>	-0.0426
		0.1428	0.9862	<b>0.0118</b>	0.1120	<b>0.0068</b>	0.9202
Dryness	0.0714		-0.1387	0.1906	0.1031	0.4528	-0.5750
	0.8665		0.7433	0.6511	0.8080	0.2599	0.1359
K	0.2619	-0.3333		0.1902	0.1007	-0.1696	0.2815
	0.5309	0.4198		0.6520	0.8124	0.6881	0.4995
RGR	<b>0.8333</b>	-0.2619	0.3333		<b>0.8210</b>	<b>0.8145</b>	0.2648
	<b>0.0102</b>	0.5309	0.4198		<b>0.0125</b>	<b>0.0138</b>	0.5262
LAR	0.5238	-0.4524	0.3571	<b>0.8333</b>		0.6771	0.6733
	0.1827	0.2604	0.3851	<b>0.0102</b>		0.0651	0.0672
SLA	<b>0.8333</b>	0.2857	-0.0238	<b>0.7619</b>	0.4524		-0.0853
	<b>0.0102</b>	0.4927	0.9554	<b>0.0280</b>	0.2604		0.8409
LWR	0.1667	- <b>0.8571</b>	0.4524	0.3333	0.5476	-0.2381	
	0.6932	<b>0.0065</b>	0.2604	0.4198	0.1600	0.5702	

Bold numbers indicate significant correlation.

\* Continentality Index.

Specific leaf area, which strongly correlates with RGR (Table 2), also had a positive relationship with latitude (Fig. 1B). The higher SLA in northern species may help maintain denser foliage structure and protect seedlings from low temperatures. Reich and Walters (1992) consider SLA an index of leaf construction cost per area, where higher SLA indicates a less costly leaf. Northern spruce species can then potentially produce needles in greater numbers, since their needles are less costly and smaller in terms of biomass than those of southern species (Fig. 2A and B). More needles and more branches in northern species (Fig. 2C) will create denser foliage structure compared with southern species with long but sparse needles and fewer branches. Wind velocity at the needle surface will be more reduced if needle density along branches is greater, and this will increase foliage temperature. Vowinckel *et al.* (1975) reported needle surface vs. air temperature differences of up to 8 °C under natural conditions in *P. mariana* when there is little air disturbance, whereas only 2–4 °C differences under conditions of constant wind speed of 4 m s<sup>-1</sup>. Hadley and Smith (1987) observed an extreme value of 23.7 °C above air temperature in sunlit needles of krummholz *P. engelmannii*; the measurement was made 10 cm below the canopy surface, where very low wind velocities (<4 cm s<sup>-1</sup>) occurred. Keeping this sort of warm microenvironment within foliage is important in the north, both during the growing season, when the air temperature is often below the optimum for photosynthesis, and during winter to protect needles from frost injury. In southern regions, on the other hand, since the temperature is high already, species should perform better if they have less dense foliage structure and let wind cool down needle temperature. Together with needle size and branchiness, SLA contributes to determining foliage density. These needle morphology and shoot structure variations are likely to be key traits under selection along the latitudinal gradient. This trend may hold in mature trees as well. Having shorter

needle length in species with more northern distributions is also observed in spruce trees growing in natural forests (Fig. 3).

Unlike RGR and SLA, LWR was correlated best with mid-summer dryness within the species range (Table 2 and Fig. 1C). LWR did not correlate with RGR (Table 2), but is still an important factor contributing to RGR variation, since LAR (the product of SLA and LWR) correlates better with RGR than SLA alone (Table 2). The lower LWR observed in species from summer-dry regions seems reasonable from an ecological perspective. LWR is the proportion of needle in the total biomass, and lower LWR indicates that seedlings allocate less of their resources to needles. Since seedlings lose water by transpiration mainly from needles, seedlings can conserve water by having a lower proportion of needles. Investing more in stem and root biomass, and extending deeper roots also characterized species from drier regions (Fig. 4A–C). While needles transpire and lose water, stems can store water and conduct water upward from roots (Kozłowski and Pallardy, 1997). Trees with extensive, much-branched root systems are known to survive droughts much better than those with shallow or sparsely branched root systems (Kozłowski and Pallardy, 1997). In dry condition, allocating more to stem and root to postpone desiccation may be a priority. In more mesic environments, investing large amounts of resources to stem and root is not necessary; the species allocating more to needles, which leads to more carbon gain, should be favoured.

#### The effect of seed mass

Seed mass might influence these results through an effect on growth components, or it might have adaptive significance in its own right (Westoby *et al.*, 2002). Seed mass did correlate with latitude (Fig. 5A), as did growth components. Seed size has been reported to have negative correlations with RGR and SLA (Swanborough and Westoby, 1996),

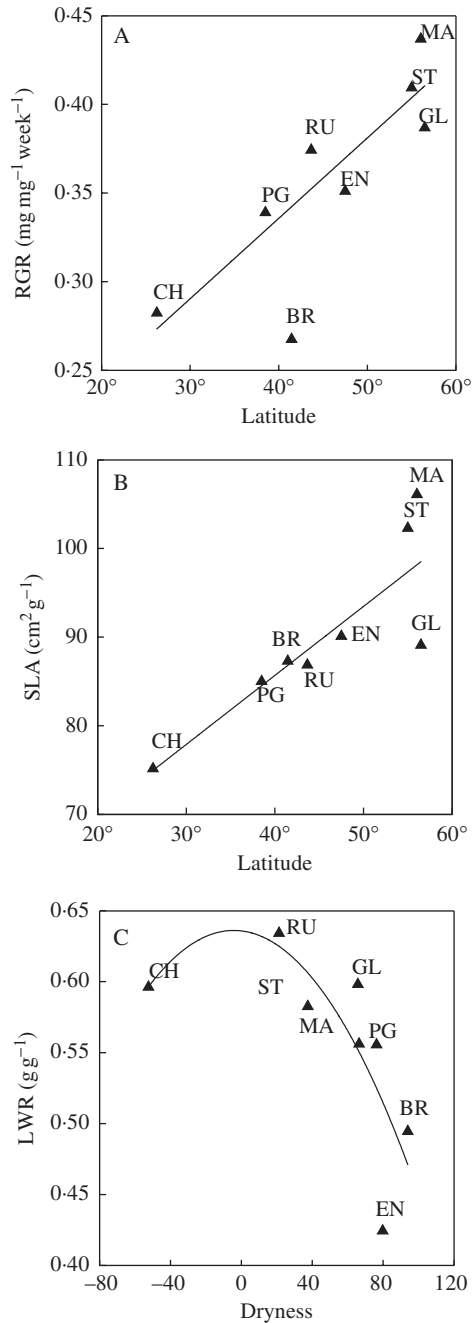


FIG. 1. Relationships between geographic factors of species' natural ranges and mean growth components. For geographic factors, see 'Geographical data' section. Alphabetical symbols represent the following spruce species: BR, *P. breweriana*; CH, *P. chihuahuana*; EN, *P. engelmannii*; GL, *P. glauca*; MA, *P. mariana*; PG, *P. pungens*; RU, *P. rubens*; ST, *P. sitchensis*. The same symbols are used in all other figures. (A) Relative growth rate: fitted regression is  $y = 0.1540 + 4.493 \times 10^{-3}x$ ,  $r^2 = 0.705$ ,  $P = 0.009$ . (B) Specific leaf area:  $y = 54.54 + 0.7785x$ ,  $r^2 = 0.600$ ,  $P = 0.010$ . (C) Leaf weight ratio:  $y = 0.6331 - 1.352 \times 10^{-5}x - 1.402 \times 10^{-5}x^2$ ,  $r^2 = 0.804$ ,  $P = 0.038$ .

which is also the case in this study (Fig. 5C and D), but the reason smaller seeds have higher RGR or SLA is as yet unknown (Westoby *et al.*, 1996). Grotkopp *et al.* (2002) compared 29 pine (*Pinus*) species and found invasive

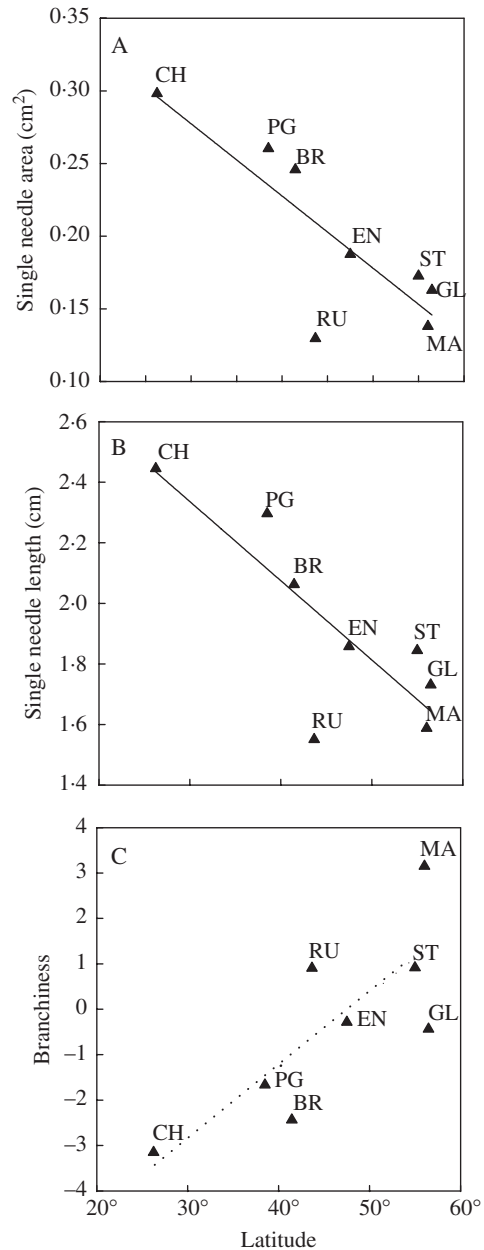


FIG. 2. Relationships between modal latitude of a species natural range and seedling traits. Solid regression line indicates significant correlation ( $P < 0.05$ ). (A) Average needle area per single needle:  $y = 0.4264 - 4.970 \times 10^{-3}x$ ,  $r^2 = 0.702$ ,  $P = 0.009$ . (B) Average needle length:  $y = 3.121 - 2.616 \times 10^{-2}x$ ,  $r^2 = 0.676$ ,  $P = 0.012$ . (C) Branchiness: branchiness is the first principal component of branch traits (branch number, total branch length, mean length, maximum length and minimum length). Larger values indicate longer and more branches. *P. chihuahuana* (CH) did not make any branches. See Materials and Methods for details.  $y = -7.759 + 0.1618x$ ,  $r^2 = 0.452$ ,  $P = 0.098$ .

species to have higher RGR, SLA and smaller seeds. In addition to the apparent advantage of higher RGR (through higher SLA) held by successful invaders, they pointed out that the smaller seeds can be dispersed for longer distances, which also facilitates the spread of invasive species. It is interesting that the spruce species that are more widespread are found at higher latitudes. This may indicate that the

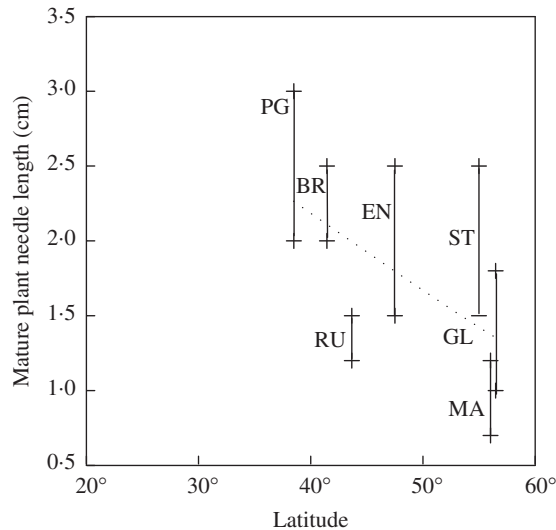


FIG. 3. Relationships between modal latitude of a species natural range and range of needle length on mature trees; data from Vidakovi (1991). The bar in each plot indicates the maximum and minimum needle length.  $y = 4.151 - 4.90 \times 10^{-2}x$ ,  $r^2 = 0.278$ ,  $P = 0.053$ .

species that spread north during the post-glacial period had more invasive characteristics compared with those that remained at lower latitudes, thus creating the latitudinal gradient in seed mass observed today. Alternatively, a correlation between seed mass and latitude might also have arisen in adaptive response to latitudinal gradients in environmental factors.

One working hypothesis is that the decrease in seed mass at higher latitudes may be an adaptation to latitudinal trends in litter depth. Species with larger seeds have a longer hypocotyl (Fig. 5B). The hypocotyl elongates as the seedling germinates, and the elongation ceases when the seed coat is shed to let the needles unfold. Since the amount of litter increases in lower latitudes (Lonsdale, 1988), pushing through deeper litter at lower latitudes may require the maternal reserves invested in larger seeds; smaller seeds at higher latitudes need not penetrate as deep a litter layer and can deploy needles sooner. It is generally the case that tree species in the colonization stage are affected by litter depth and that the influence of litter depth on establishment decreases at higher latitudes (Xiong and Nilsson, 1999). Validation of this interpretation would require data on latitudinal trends in litter depth under spruce stands.

#### Summary and implications for the distributional response to climate change

It is clear that there are relationships of growth components, seed mass and other seedling traits with some geographical factors in the environment (Fig. 6) that suggest a role for seedling ecology in the biogeography of spruce species in North America. Temperature regimes and water availability, both of which are used in predicting vegetation types, appear to be associated with species range. On the other hand, the Continentality Index did not show any particular relationship with growth analysis

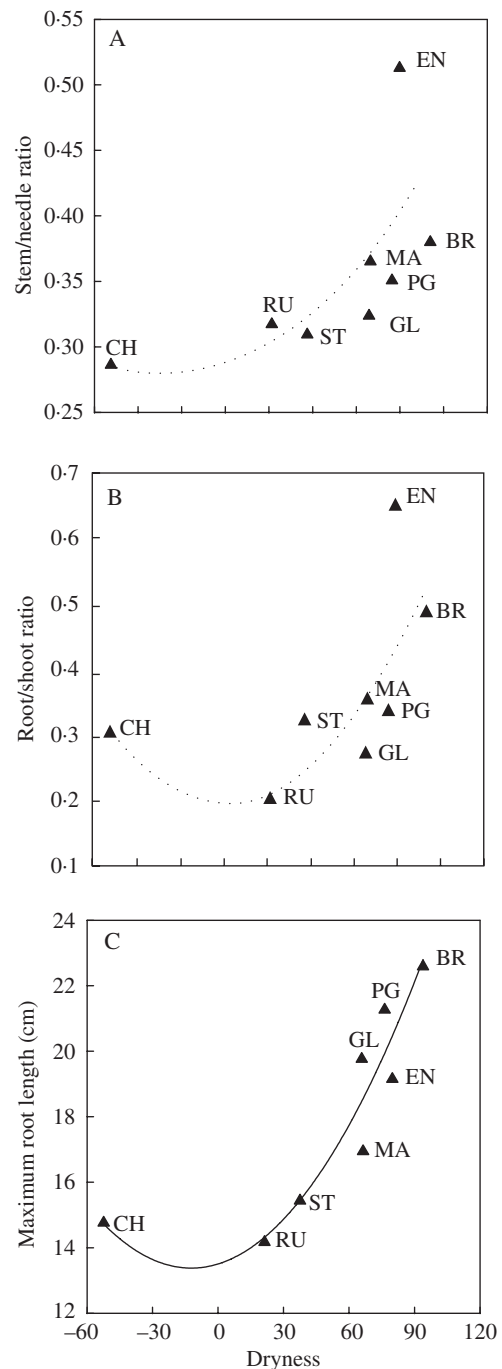


FIG. 4. Relationships between July Dryness Index and seedling traits. Solid regression line indicates significant correlation ( $P < 0.05$ ). (A) Stem : needle ratio:  $y = 0.2867 - 5.050 \times 10^{-4}x - 9.969 \times 10^{-6}x^2$ ,  $r^2 = 0.415$ ,  $P = 0.262$ . (B) Root : shoot ratio:  $y = 0.1961 - 1.180 \times 10^{-4}x - 3.903 \times 10^{-5}x^2$ ,  $r^2 = 0.491$ ,  $P = 0.185$ . (C) First root length (the longest root of a seedling):  $y = 13.50 + 2.049 \times 10^{-2}x + 8.350 \times 10^{-4}x^2$ ,  $r^2 = 0.857$ ,  $P = 0.008$ .

components (Table 2) or with other seedling traits (data not shown). Temperature and moisture regimes appear to be more important than seasonal extremes of climate in terms of the prediction of species range in the North American spruces.

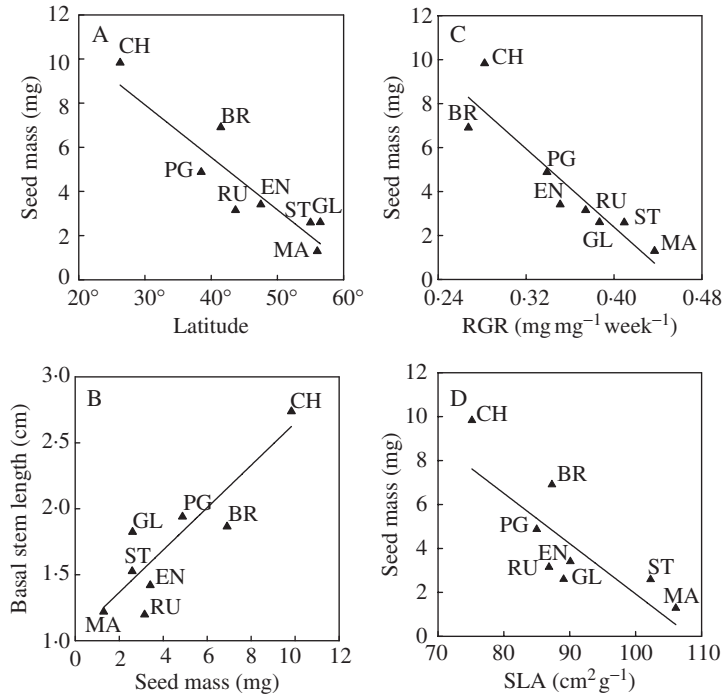


FIG. 5. Relationship between seed weight of spruce species and (A) latitude ( $y = 15.05 - 0.2377x$ ,  $r^2 = 0.838$ ,  $P = 0.001$ ); (B) basal stem length ( $y = 1.028 + 0.1602x$ ,  $r^2 = 0.795$ ,  $P = 0.003$ ); (C) RGR ( $y = 0.4387 - 1.893 \times 10^{-2}x$ ,  $r^2 = 0.843$ ,  $P = 0.001$ ); (D) SLA ( $y = 102.4 - 2.936x$ ,  $r^2 = 0.671$ ,  $P = 0.013$ ).

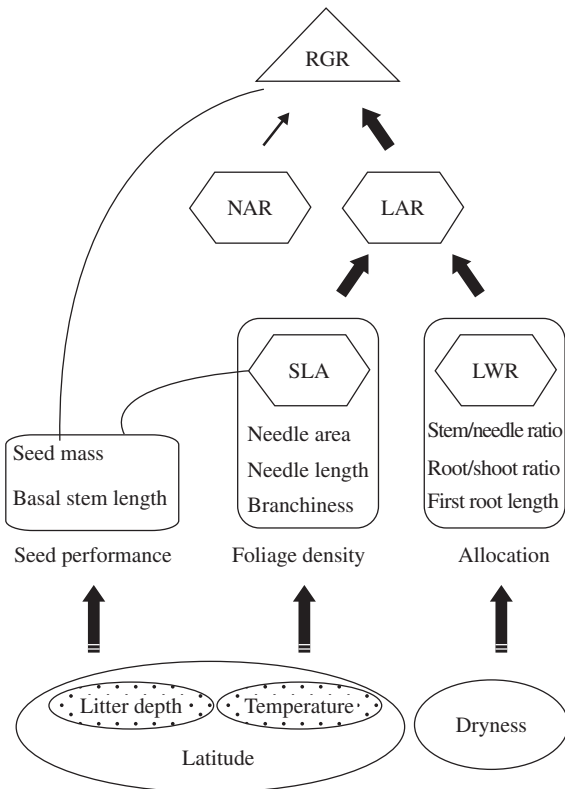


FIG. 6. An outline of the postulated relationships among growth components and environmental factors associated with the North American ranges of spruce species. Readers unfamiliar with the biological interpretation of growth analysis are referred to Hunt (1990).

Under global warming, a simple northward shift along present latitudinal gradients in temperature and moisture regimes cannot be expected to influence spruce distribution. Temperature regimes will not be constant across regions, seasons or day/night cycles. Temperature increases will be greatest in northern latitudes during winter (Katterberg *et al.*, 1996), and increases in night-time minimum temperatures will exceed increases in daytime maxima (Karl *et al.*, 1993). The temperature regime and growing period of northern regions in the future therefore will not simply be equivalent to conditions in southern regions today. Furthermore, precipitation regimes will shift, creating drier and also wetter areas but not in any simple latitudinal pattern (Katterberg *et al.*, 1996). Some spruces may be unable to accommodate this complex shift in environment and be at risk of extinction. *Picea critchfieldii*, which was a dominant spruce in south-eastern North America during the last glacial maximum, became extinct for unknown reasons about 9000 years ago (Jackson and Weng, 1999). It is possible that some of the extant North American spruces could suffer the same fate under global change. The southernmost spruces in North America, *P. chihuahuana*, *P. mexicana* and *P. martinezii*, survive only as isolated populations at mid- to high-elevation sites in the Sierra Madre mountains of Mexico; these relict species may be especially vulnerable to extinction (Ledig *et al.*, 1997, 2000a, b, 2002).

Considering the results of this study, the difference in seedling survivorship under stressful conditions associated with global change may depend less on adaptation to stress *per se* than on the intrinsic characteristics of each species that have arisen in the evolution of the spruce lineage.

These seedling characteristics correspond broadly to the geographical variation in environment across the present ranges of the different North American spruces, suggesting that, in spruce, genostasis rather than rapid adaptive evolution prevails during periods of climatic change (Bradshaw and McNeilly, 1991; Wang *et al.*, 1994). The potential future distribution of spruce species in North America then will be adjusted by migration and shifts in range provided that the rate of climate change or loss of habitat do not preclude establishment of a new range in equilibrium with the future climate. In other words, the extant spruce species in North America each has a fairly distinct environmental niche and, given sufficient time and opportunity, the biogeography of spruce in North America will continue to reflect interspecific differences that developed in the deeper evolutionary history of the genus *Picea*.

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