

Morphometric, allometric, and developmentally adaptive traits in red spruce and black spruce.

I. Species and seed-source variation

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Abstract: The study objective was to compare intraspecific seed source and interspecific variation of red spruce (*Picea rubens* Sarg.) and black spruce (*Picea mariana* (Mill.) BSP) in a number of morphometric, allometric, and adaptive traits. Analyses of variance for cotyledon number, root dry weight, shoot to root ratio, and seedling water balance revealed significant species effects. Germination time, total height, diameter, needle and stem wood dry weight, and survival had significant species effects and species \times region interactions. Potential inbreeding depression effects were reflected in a positive relationship between height growth and percent germination and a negative relationship between height growth and germination time; these effects may have partly contributed to the species \times region interactions. On average, 66% of the height growth difference between the species may be attributable to earlier germination and the other 34% to faster growth. Covariate allometric analysis showed that black spruce had a 39% higher shoot to root ratio than red spruce. Red spruce allocated 25% more dry weight (per unit needle weight) towards roots than did black spruce. When the resource sinks (stem wood and roots) are summed, black spruce is 8.6% more efficient at converting resources into sink biomass than is red spruce.

Résumé : Les auteurs ont comparé la diversité intraspécifique de sources de graines d'épinette rouge (*Picea rubens* Sarg.) et d'épinette noire (*Picea mariana* (Mill.) BSP) et leur diversité interspécifique pour un certain nombre de caractères liés à la morphométrie, l'allométrie et l'adaptation. Les analyses de variance ont permis de mettre en évidence des effets significatifs reliés à l'espèce pour le nombre de cotylédons, le poids sec des racines, le coefficient système foliacé/système racinaire et le bilan hydrique des semis. Des effets significatifs reliés à l'espèce et aux interactions espèces \times régions ont été notés pour le délai de germination, la hauteur totale, le diamètre, le poids sec des aiguilles et celui du bois de la tige ainsi que la survie. Une relation positive entre la croissance en hauteur et le pourcentage de germination et une relation négative entre la croissance en hauteur et le délai de germination sont le reflet des effets potentiels d'une dépression de consanguinité qui pourrait avoir contribué en partie aux interactions espèces \times régions. En moyenne, 66 % de la différence de croissance en hauteur entre les espèces est attribuable à une germination hâtive et 34 % à une croissance plus rapide. L'analyse de covariance allométrique a permis de mettre en évidence un coefficient système foliacé/système racinaire 39 % plus élevé chez l'épinette noire que chez l'épinette rouge. L'épinette rouge alloue 25 % plus de poids sec (par unité de poids d'aiguilles) aux racines que l'épinette noire. Lorsque les puits de ressources (tige ligneuse et racines) sont confondus, l'épinette noire est 8,6 % plus efficace pour convertir les ressources en puits de biomasse que l'épinette rouge.

[Traduit par la Rédaction]

Introduction

Red spruce (*Picea rubens* Sarg.) is a valuable timber species and an important component of the late-successional forests of eastern Canada. However, red spruce has declined substantially over most of its geographic range. This decline has been associated with past forestry practices, such as

selective removal and clear-felling, particularly during the 1800s and 1900s (Korstian 1937; Gordon 1996), atmospheric pollution (Johnson and Siccama 1983), climate change (McLaughlin et al. 1987; Hamburg and Cogbill 1988), and hybridization with black spruce (*Picea mariana* (Mill.) BSP), which is sympatric with red spruce across its Canadian range (Morgenstern and Farrar 1964; Manley 1972; Gordon 1976). The geographical range of red spruce extends, primarily as isolated montane remnants, along the Appalachian Mountains from North Carolina and Tennessee to Prince Edward Island in the north (Blum 1990) and from the Atlantic coast of Nova Scotia across the Maritimes into the Laurentian Mountains of Quebec. The westernmost extension of red spruce occurs as disjunct, fragmented remnant stands associated with the Algonquin Highlands of southern Ontario (Mosseler et al. 2000). Current site occupancy of red spruce has been estimated at between one tenth (Korstian 1937) and one fifth (Gordon 1996) of its former extent in terms of population sizes, numbers, and geographical distri-

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bution. The area of continuous distribution and the center of the range are now limited to southern portions of New Brunswick, Nova Scotia, and the northern New England States (McLaughlin et al. 1987). Red spruce has declined to a point where it is becoming increasingly uncommon across large portions of its former range.

Black spruce, also a valuable pulp and paper species, is one of two North American transcontinental spruces. It grows on organic soils, and productive stands are found on a variety of upland and peatland soil types (Viereck and Johnston 1990). Black spruce is often a postfire pioneer species and forest stands are generally even aged. It is often difficult to clearly distinguish between red and black spruce, particularly in areas where natural hybridization occurs. Adequate benchmark information on morphometric, allometric growth, and developmental traits is generally lacking for the species, compounding identification and management problems for these otherwise ecologically distinct species (Manley 1972; Beylor 1999). Measures of seedling trait variation and growth performance, along with their allometric relationships, can serve as indicators of important ecophysiological adaptations and life history traits (Grossnickle and Major 1994; McConnaughay and Coleman 1999). To understand red spruce and its associated genetic complexity, a better account of the genetic variation of the species across various seed sources and comparisons with black spruce would be useful.

The objective of this study was to examine and compare intraspecific seed source and interspecific variation of red and black spruce in a number of morphometric, allometric, and adaptive traits. Our hypothesis is that, as distinct species, red and black spruces possess morphometric, allometric, and developmentally distinct traits that reflect adaptations to their different ecological niches. Species \times region interaction, particularly for the small, isolated populations, may be of special interest. We examined and compared a number of morphometric, allometric, and developmentally adaptive traits including cotyledon numbers, height and height growth rates, needle, stem wood, and root dry weights, allometric growth relationships, and percent germination and germination times in natural populations of red and black spruce from three different regions (Ontario, New Brunswick, and Nova Scotia) and from two locations in each region. Moreover, potential effects of inbreeding depression were determined by testing height growth in relation to percent germination and germination time. Results are discussed with respect to the ecological role of both species. A companion paper (Major et al. 2003a) presents the results of our study of morphometric adaptive traits of interspecific and intraspecific controlled hybrid crosses of red spruce, black spruce, and hybrid spruce.

Material and methods

Species, regions, locations, and populations examined

The genetic and hierarchical organization of the experiment includes two species (red and black spruce), three regions (Canadian provinces of Nova Scotia (NS), New Brunswick (NB), and Ontario (ON)), and two locations within each region. Thus, each location includes one selected population of red spruce and a nearby black spruce population.

The red spruce populations were used in previous assessments of population genetics, reproductive traits, and mating system (Mosseler et al. 2000; Rajora et al. 2000). Seed source is a general inclusive term that may refer to region, location, or population of seed origin.

Red spruce cones were collected from 15–20 dominant and codominant trees from a number of specific populations in the northern part of its range in NS, NB, and ON during September 1996 (Mosseler et al. 2000). The sampled populations were all located within a similar range of latitude and elevation (Table 1). Populations in the ON region consisted of small, remnant stands or patches, often with fewer than 40–50 widely spaced trees per stand within upland hardwood forest mixtures. The NB populations generally consisted of medium-sized stands of up to several hundred mature trees. The NS red spruce populations consisted of several thousand individuals in relatively undisturbed, old-growth forest stands dominated by red spruce of all ages. These populations were selected for specific attributes such as their old-growth characteristics or because they represent the most northern location (e.g., Blythe Township in ON).

Black spruce seeds were obtained from previous bulked seedlots collected from dominant and codominant trees and stored at the Canadian Forest Service's National Tree Seed Centre. The geographically closest black spruce populations were selected for detailed examination and comparison with the red spruce populations. Seeds were extracted and stored according to International Seed Testing Association (1996) protocols. Thus, in our present study, one red spruce and one black spruce population were used from each of two locations from the three regions, ON, NB, and NS, for a total of 12 seedlots (Table 1). No seed stratification treatments are required for these species (Edwards 1987; International Seed Testing Association 1996).

Greenhouse conditions

Seeds were sown in hydrated 36-mm JiffyTM peat pellets (Jiffy Products Ltd., N.B.) (90 mL full volume) at the beginning of June 1998. Each population was sown in three replicate trays containing 84 Jiffy peat pots per replicate and located randomly in a greenhouse compartment at the Canadian Forest Service - Atlantic Forestry Centre Greenhouse in Fredericton, N.B. (45°52'N, 66°31'W). Seedlings were watered and fertilized twice weekly from June 11 to 29 with 11:41:8 N-P-K plus micronutrients at 50 ppm N (Plant Products Co. Ltd., Brampton, Ont.), from July 1 to September 7 with 20:8:20 at 100 ppm N two or three times weekly, and from September 15 to October 28 with 8:20:30 at 35 ppm N twice weekly. Temperature and humidity were set to 26°C and 70% relative humidity from June 1 to 29, 25/18°C and 60/50% relative humidity (day/night) from July 1 to September 30, and 10/5°C to November 30. Natural light was supplemented by sodium vapor lamps with an 8-h blackout period starting from September 8. Bud initiation began September 18.

Morphometric parameters

Cotyledon emergence (germination) was assessed every 2–3 days for 32 days after sowing. Cotyledon emergence was recorded when the seed coat was discarded and cotyledons were fully exposed. The parameter germination time is

Table 1. Geographic coordinates and elevations for red and black spruce populations.

| Seed source | Location designation | Coordinates | Elevation (m) |
|----------------------|----------------------|------------------|---------------|
| Ontario | | | |
| Black spruce | | | |
| North Bay | ON-1 | 46°19'N, 79°27'W | 201 |
| Alice | ON-2 | 45°45'N, 77°17'W | 182 |
| Red spruce | | | |
| Blythe Township | BT-1 | 46°32'N, 79°32'W | 380 |
| Centennial Ridge | CR-2 | 45°34'N, 78°25'W | 510 |
| New Brunswick | | | |
| Black spruce | | | |
| Trouser Lake | NB-1 | 47°00'N, 66°58'W | 394 |
| Crown Point Brook | NB-2 | 46°48'N, 66°25'W | 30 |
| Red spruce | | | |
| Blowdown Brook | BB-1 | 46°41'N, 67°31'W | 380 |
| Quiddy River | QR-2 | 46°31'N, 65°12'W | 100 |
| Nova Scotia | | | |
| Black spruce | | | |
| Goshen | NS-1 | 45°11'N, 62°58'W | 152 |
| Cross Lake | NS-2 | 45°31'N, 62°27'W | 61 |
| Red spruce | | | |
| Rossignol Lake | RL-1 | 45°08'N, 65°14'W | 100 |
| Abraham Lake | AL-2 | 45°10'N, 62°38'W | 185 |

the number of days to reach or exceed 90% of final germination (day 32). From each population, a total of 24 randomly selected seedlings were tagged and then measured periodically for height growth. Seedling height was measured every 2–3 weeks beginning on day 43 and ending on day 190 from sowing. Percent survival was measured as the percentage of seeds sown that resulted in live seedlings on day 190.

On day 66 (harvest 1), 20 seedlings from each seedlot were randomly selected and measured for: shoot height, root collar diameter, hypocotyl height, epicotyl height, cotyledon number, and cotyledon surface area. Cotyledon surface area was determined using the WinSeedle™ program (Regent Inc., Quebec, Que.) and a Hewlett Packard 6100C scanner. On days 113 and 191 (harvests 2 and 3), 20 seedlings from each seedlot were randomly selected and measured for shoot height, root collar diameter, needle, stem wood, and root dry weights, and shoot and root surface area. Dry weights were measured after oven drying the seedling components at 65°C for 48 h. Surface areas were determined using a LI-3100 area meter (LI-COR Inc., Lincoln, Nebr.). From the morphological data, the following parameters were calculated: shoot to root ratio (dry weight) and seedling water balance index (= total shoot surface area/root collar diameter × root surface area), similar to Grossnickle and Major (1994).

Experimental design and statistical and allometric analyses

The study was established as a completely randomized experimental design with trays of populations randomized across greenhouse benches. Sampling, for morphometric assessments, was random across all trays. Species, region, and location were all considered fixed effects. ANOVA was used to test the effects of species, region, species × region interaction, locations nested within region, and species × location

(within region) interaction using the residual error for all tests. Tukey's post hoc test ($p = 0.05$) was used to determine the statistical significance of differences between specific regions.

Because trait relationships can change due to differences in size, ANCOVAs were also used to examine one seedling morphometric trait in relation to another trait (allometry) (Ledig et al. 1970; Johnsen and Bongarten 1991). The method used was analogous to the ANCOVA of female effects presented by Major and Johnsen (1996) using the model

$$Y_{ij} = B_0 + B_{0i} + B_1X_{ij} + B_{1i}X_{ij} + e_{ij}$$

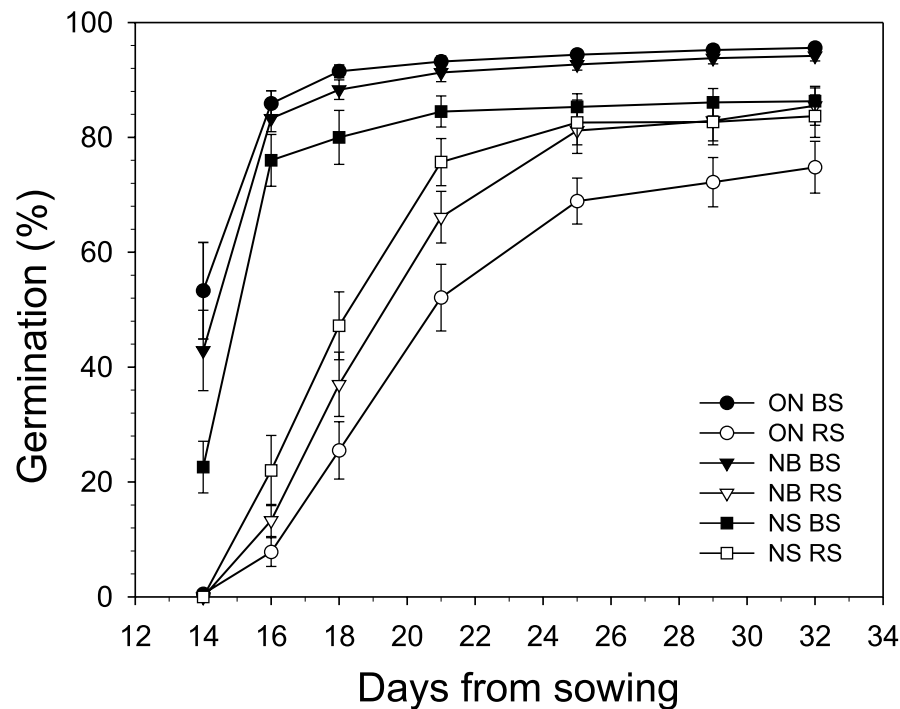
where Y_{ij} is the seedling trait of the j th tree of the i th species, B_0 and B_1 are average regression coefficients, B_{0i} and B_{1i} are the species-specific coefficients, X_{ij} is the independent variable (seedling trait), and e_{ij} is the error term. In these analyses, three sources of variation were studied: (i) seedling trait (covariate), (ii) species, and (iii) species × covariate. Significant species effects indicate differences in species means (e.g., differences in B_{0i} coefficients, if B_{1i} coefficients are similar) and significant species × covariate effects indicate differences in the slopes (B_{1i} coefficients) between the species. To determine correlations between mean height and percent germination, data from intraspecific black and red spruce families from a separate hybrid spruce experiment (Major et al. 2003a) were included to increase the number of seedlots from six to 10 for each species. Spline curves were fitted to the mean absolute growth rates and cotyledon frequency data.

Results

Germination

By day 14, none of the red spruce showed any germina-

Fig. 1. Germination (mean \pm SE) by days from sowing for black spruce (BS) and red spruce (RS) populations from Ontario (ON), New Brunswick (NB), and Nova Scotia (NS).



tion; however, black spruce germination ranged from 22 to 54% (Fig. 1). By day 16, there were significant species and species \times region interaction effects on germination (not shown). For the remainder of the assessment, red spruce germination was greatest in NS and lowest in ON (i.e., NS > NB > ON). This order was reversed, however, for black spruce (ON > NB > NS). Statistically, the results were the same up to and including the last date of assessment, where species effects remained significant, region effect was not significant, and species \times region interaction was significant (Fig. 1). Also, location nested within region was often significant across measurement dates. Most often, species \times location was not significant and, if it was significant, it was due to changes in magnitude rather than rank.

Germination time and survival

Average germination time was 16.9 and 23.7 days for black and red spruce, respectively (Table 2). ANOVA of germination time data showed significant differences due to species, region, and species \times region interaction (Table 2). Black and red spruce mean survival percentages were 94.5 and 86.8%, respectively (Table 2). Regional differences were not significant, but there was a significant species \times region interaction. The ON region had a significantly different percent survival for black and red spruce (98.2 and 80.3%, respectively) ($p = 0.002$). The NB region was also different: 96.4 and 91.8% survival ($p = 0.053$) for black and red spruce, respectively. However, for the NS region, the survival of black spruce was similar to that of red spruce at 88.0% ($p = 0.576$).

Covariate analysis of mean seedling height, using mean percent germination as a covariate and testing for species effect, had a significant species \times percent germination interac-

tion ($p = 0.028$) but no consistent species effect ($p = 0.100$, $r = 0.903$) (Fig. 2A). Covariate analysis of seedling height, using germination time as a covariate, had no significant germination time \times species interaction ($p = 0.733$) and species effect had a low degree of significance ($p = 0.080$) (Fig. 2B). The germination time effect was significant ($p < 0.001$, $r = 0.902$).

Harvest 1 (age 66 days)

Hypocotyl height was 2.5 mm greater for red spruce than it was for black spruce ($p < 0.001$) (Table 2). A significant region effect was found in which the mean hypocotyl height of the NS region was significantly greater than that of the ON region, but neither the NS nor the ON region was significantly different from the NB region for this trait (i.e., the NB region ranked intermediate). In contrast, mean epicotyl height was 34.5 and 23.9 mm for black and red spruce, respectively. Total height was significantly greater for black spruce (4.92 cm) than for red spruce (4.12 cm) ($p < 0.001$) (not shown). Seedling height for the NB region was significantly higher than that of the NS region, but neither was significantly different from that of the ON region. There were no significant species \times region interactions for the above traits. For epicotyl height, there was a significant species \times location effect, which was due to changes in magnitude rather than rank.

Cotyledon number was significantly different ($p < 0.001$) between black and red spruce, with an average of 4.5 and 5.9 cotyledons per seedling, respectively (Table 2). There was no significant region effect or species \times region interaction for this trait. Of the traits examined in the first harvest, the ANOVA for cotyledon number resulted in the second highest coefficient of determination ($r^2 = 0.512$). Area per

cotyledon was 4.5 and 5.8 mm² for black and red spruce, respectively ($p < 0.001$), with the NS and NB regions having greater values than the ON region (not shown). Total cotyledon area had the highest coefficient of determination ($r^2 = 0.678$) and had significant differences due to species and region effects but no species \times region interaction (Table 2). The cotyledon area for the NB region was significantly greater than those of the ON and NS regions ($p = 0.050$). The frequency distributions of cotyledon numbers from the ON region had strong peaks at 4 and 6 cotyledons, with some overlap between species (Fig. 3A). The NB region had broader peaks centered at 4.5 and 6 cotyledons per seedling for black and red spruce, respectively (Fig. 3B). The NS region had the narrowest peak centered at 4 and 6 cotyledons for black and red spruce, respectively (Fig. 3C).

Height growth

The average 43-day height was 2.46 and 2.27 cm for ON black and red spruce, respectively, resulting in a modest (8.0%) difference between the species (Fig. 4A). Between days 43 and 66, black spruce more than doubled its growth rate to 1.33 mm/day (Fig. 5A), whereas red spruce growth rate increased by approximately 50% to 0.77 mm/day, resulting in a 37% height difference between the two species on day 66 (5.52 versus 4.03 cm) (Fig. 4A). The growth rates continued to increase until maxima were reached between days 88 and 113, where mean growth rates were 3.04 and 2.08 mm/day for black and red spruce, respectively. Nonetheless, the height differences between the species remained fairly constant (between 30 and 40%) and statistically significant ($p < 0.001$) during this time. After approximately day 130, declines in growth rate were similar for both species.

A similar height growth pattern was observed for black and red spruce from NB (Fig. 4B). However, there were no significant species differences at day 43 and the red spruce growth rate was closer to that of black spruce during the first third of the season (Fig. 5B).

The NS region had no significant differences in height growth between the two species at any age, except on day 43, when red spruce height was slightly higher (7.1%) than that of black spruce (2.55 versus 2.38) (Fig. 4C). The growth rate curve for black spruce was narrower and had a higher, more defined peak than that of red spruce (Fig. 5C). This pattern was consistent across regions, but with varying intensities. For black spruce, the peak growth rate was similar across regions, except that the growth rate for NS black spruce was slightly lower. For red spruce, the peak growth rates ranked from the highest to lowest as follows: NS > NB > ON.

Harvests 2 and 3 (age 113 and 191 days)

Because the ranking results of the second harvest were similar to those of the third harvest, the second harvest data are not reported. Morphometric measures from harvest 3 had no significant location effect or species \times location interaction (Table 3). Harvest 3 total heights were 20.6 and 17.1 cm for black and red spruce, respectively ($p < 0.001$), a 20% difference. There was no significant region effect but a significant species \times region interaction. For the NB and ON regions, heights were significantly different between the species ($p < 0.001$), whereas for the NS region, heights of red and

Table 2. Species by region (Ontario, New Brunswick, and Nova Scotia) means and overall species means and standard errors for seed morphometric traits and results from ANOVA with sources of variation including species, region, species \times region, location(region), and species \times location(region).

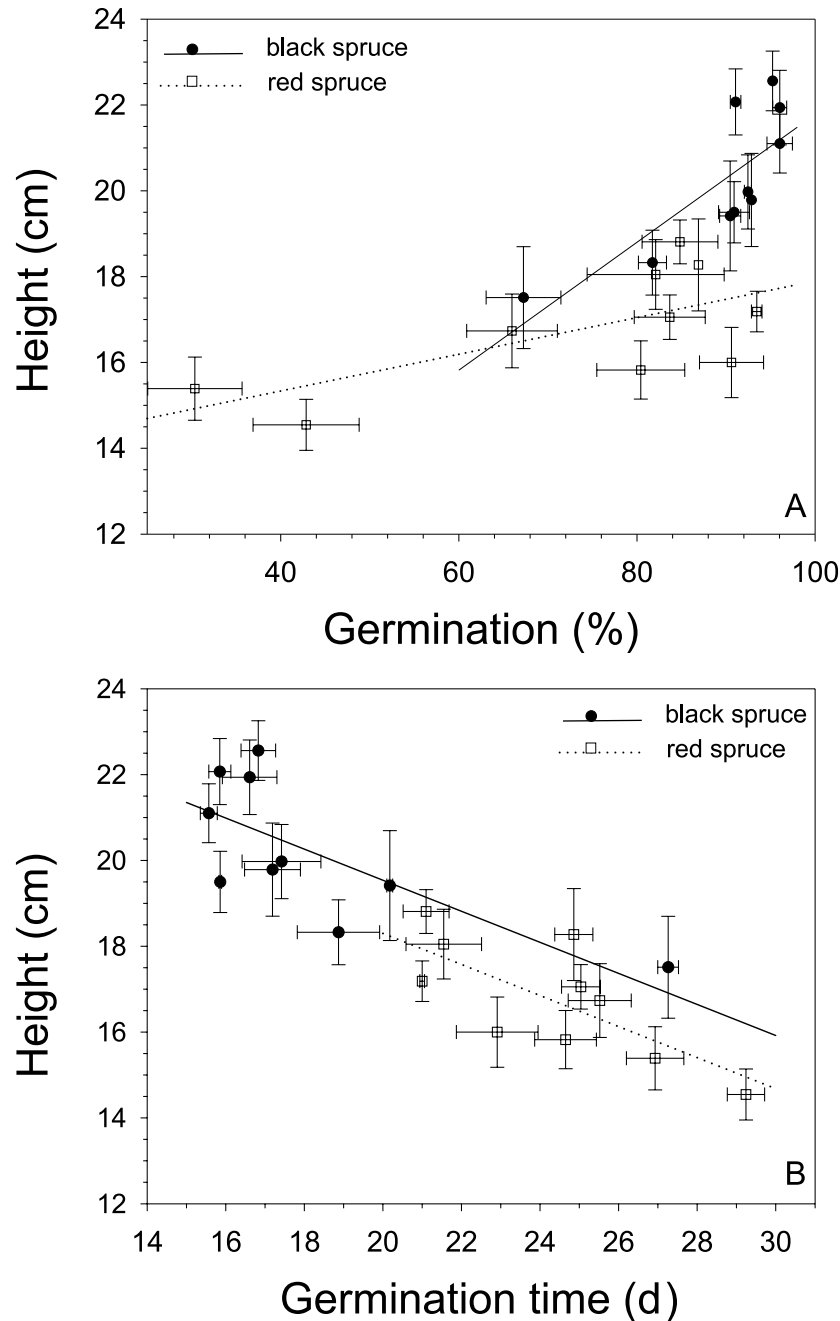
| Trait | Species | Ontario | New Brunswick | Nova Scotia | Overall | n | S | R | S \times R | L(R) | S \times L(R) | r^2 |
|---|---------|------------------|------------------|------------------|------------------|-----|--------|--------|--------------|--------|-----------------|-------|
| | | | | | | | | | | | | |
| Germination time (days) ^a | BS | 16.2 \pm 0.4 | 17.0 \pm 0.6 | 17.4 \pm 0.8 | 16.9 \pm 0.4 | 36 | <0.001 | 0.050 | <0.001 | 0.111 | 0.185 | 0.906 |
| | RS | 25.3 \pm 0.4 | 23.8 \pm 0.7 | 21.3 \pm 0.5 | 23.7 \pm 0.5 | 36 | 0.002 | 0.120 | 0.010 | 0.050 | 0.137 | 0.603 |
| Survival (%) ^a | BS | 98.2 \pm 0.5 | 96.4 \pm 0.7 | 88.9 \pm 2.5 | 94.5 \pm 1.3 | 36 | 0.002 | 0.120 | 0.010 | 0.050 | 0.137 | 0.603 |
| | RS | 80.3 \pm 4.6 | 91.8 \pm 2.3 | 88.6 \pm 4.0 | 86.8 \pm 2.3 | 36 | 0.002 | 0.120 | 0.010 | 0.050 | 0.137 | 0.603 |
| Harvest 1 ^b | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| Hypocotyl height (mm) | BS | 14.3 \pm 0.4 | 14.9 \pm 0.4 | 15.6 \pm 0.4 | 14.9 \pm 0.2 | 240 | <0.001 | 0.012 | 0.732 | 0.486 | 0.061 | 0.224 |
| | RS | 16.5 \pm 0.6 | 17.8 \pm 0.5 | 18.0 \pm 0.4 | 17.4 \pm 0.3 | 240 | <0.001 | 0.012 | 0.732 | 0.486 | 0.061 | 0.224 |
| Epicotyl height (mm) | BS | 35.5 \pm 1.6 | 37.5 \pm 1.3 | 30.5 \pm 1.2 | 34.5 \pm 0.8 | 240 | <0.001 | <0.001 | 0.818 | <0.001 | <0.001 | 0.426 |
| | RS | 23.6 \pm 1.1 | 25.9 \pm 1.5 | 22.1 \pm 2.0 | 23.9 \pm 0.9 | 240 | <0.001 | <0.001 | 0.818 | <0.001 | <0.001 | 0.426 |
| Cotyledon number | BS | 4.50 \pm 0.12 | 4.68 \pm 0.13 | 4.22 \pm 0.10 | 4.47 \pm 0.07 | 240 | <0.001 | 0.140 | 0.243 | 0.056 | 0.558 | 0.512 |
| | RS | 5.93 \pm 0.16 | 5.98 \pm 0.11 | 5.91 \pm 0.10 | 5.94 \pm 0.08 | 240 | <0.001 | 0.140 | 0.243 | 0.056 | 0.558 | 0.512 |
| Total cotyledon area (mm ²) | BS | 19.06 \pm 0.83 | 22.14 \pm 0.83 | 19.17 \pm 0.55 | 20.12 \pm 0.45 | 240 | <0.001 | <0.001 | 0.115 | 0.126 | <0.001 | 0.678 |
| | RS | 30.90 \pm 1.11 | 37.39 \pm 0.92 | 34.51 \pm 1.43 | 34.25 \pm 0.70 | 240 | <0.001 | <0.001 | 0.115 | 0.126 | <0.001 | 0.678 |

Note: BS, black spruce; RS, red spruce; n, total sample number; S, species; R, region; L, location; r^2 , coefficient of determination; p values <0.05 are in bold type.

^adf: S = 1, R = 2, S \times R = 2, L(R) = 3, S \times L(R) = 3, error = 24.

^bdf: S = 1, R = 2, S \times R = 2, L(R) = 3, S \times L(R) = 3, error = 228.

Fig. 2. (A) Relationship between seedling height and percent germination; equation from ANCOVA: black spruce, $y = 6.877 + 0.149x$; red spruce, $y = 13.632 + 0.043x$; $r = 0.903$. (B) Relationship between seedling height and germination time; equation from ANCOVA: black spruce, $y = 26.78 - 0.36x$; red spruce, $y = 25.54 - 0.36x$; $r = 0.902$.

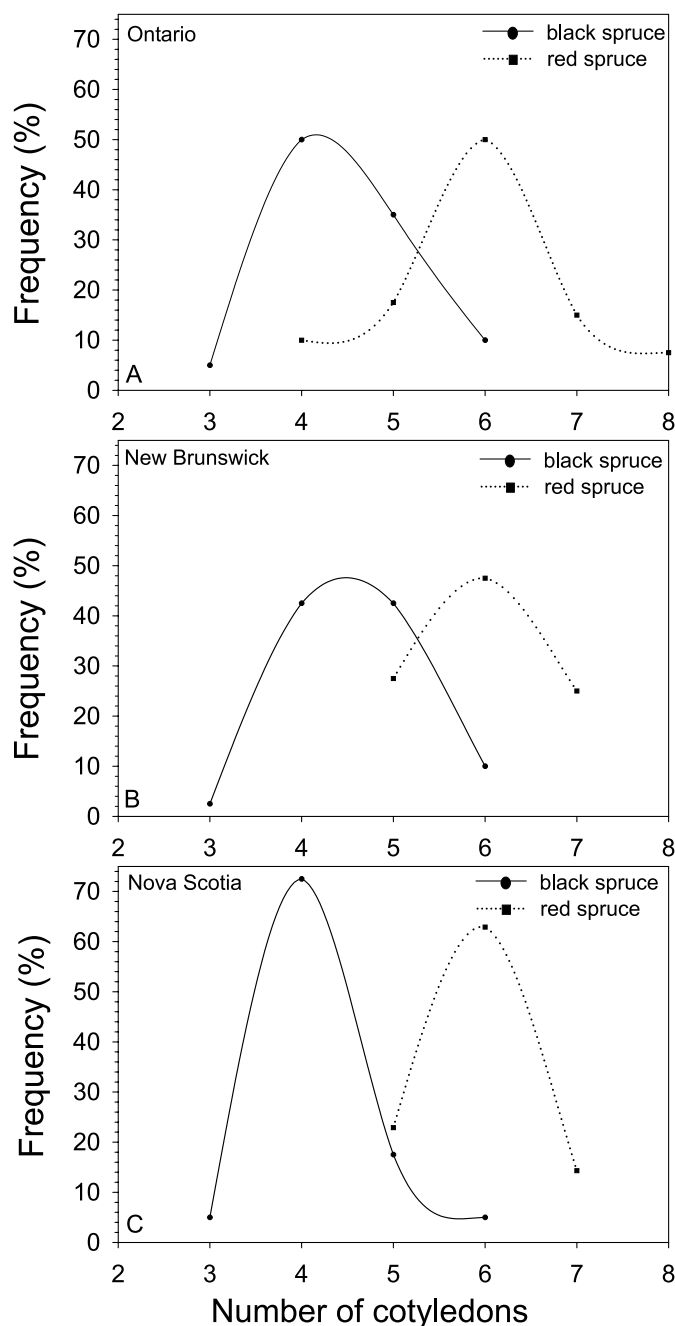


black spruce were similar. Statistically, results were similar for diameter, needle dry weight, and stem wood dry weight (Table 3). Root dry weight and the ratio of shoot to root by dry weight had no significant species \times region interaction but maintained consistent species differences across all regions. Covariate analysis for shoot dry weight, using root dry weight as a covariate and then testing for species differences, had no significant root dry weight \times species interaction ($p = 0.451$). Further analysis showed a significant species effect ($p < 0.001$) and root dry weight effect ($p < 0.001$), with

black spruce having an average 0.230 g (39%) higher shoot weight per unit root weight than red spruce ($r^2 = 0.829$) (Fig. 6).

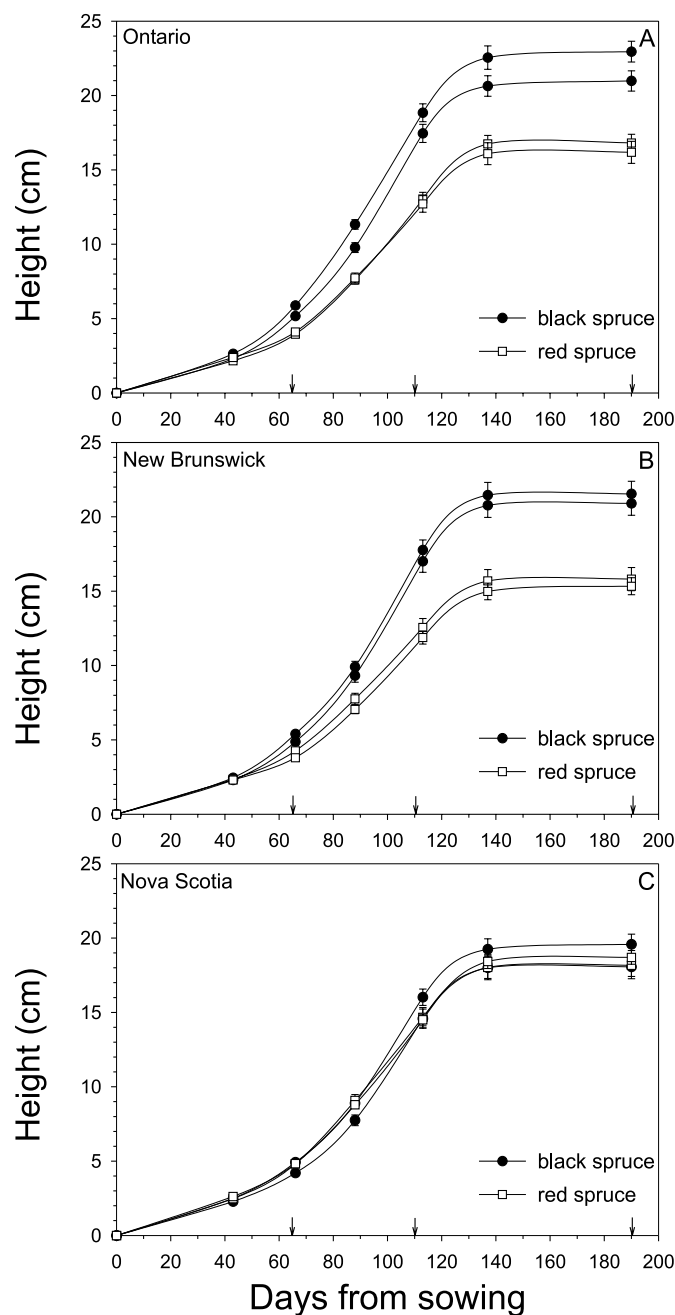
Covariate analysis of stem dry weight, using needle dry weight as a covariate and testing for species effect, had no significant species \times needle dry weight interaction ($p = 0.678$). Further analysis showed a consistent significant species ($p < 0.001$) and needle dry weight effect ($p < 0.001$), with black spruce having 45.2 mg (20%) more stem dry weight per unit needle dry weight than red spruce ($r^2 =$

Fig. 3. Frequency distribution of cotyledon numbers for black and red spruce populations from (A) Ontario, (B) New Brunswick, and (C) Nova Scotia.



0.907) (Fig. 7A). Covariate analysis of root dry weight, using needle dry weight as a covariate and testing for species effect, had no significant species \times needle dry weight interaction ($p = 0.466$). Further analysis had a consistent significant species ($p < 0.001$) and needle dry weight effect ($p < 0.001$), with red spruce having 18.2 mg (25%) more root weight per unit needle weight than black spruce ($r^2 = 0.791$) (Fig. 7B). Covariate analysis of stem wood plus root dry weight, using needle dry weight as a covariate and testing for species effect, had no significant species \times needle dry

Fig. 4. Height (mean \pm SE) by days from sowing for black and red spruce populations from (A) Ontario, (B) New Brunswick, and (C) Nova Scotia. Arrows on the x axis indicate the three dates for sample harvest.

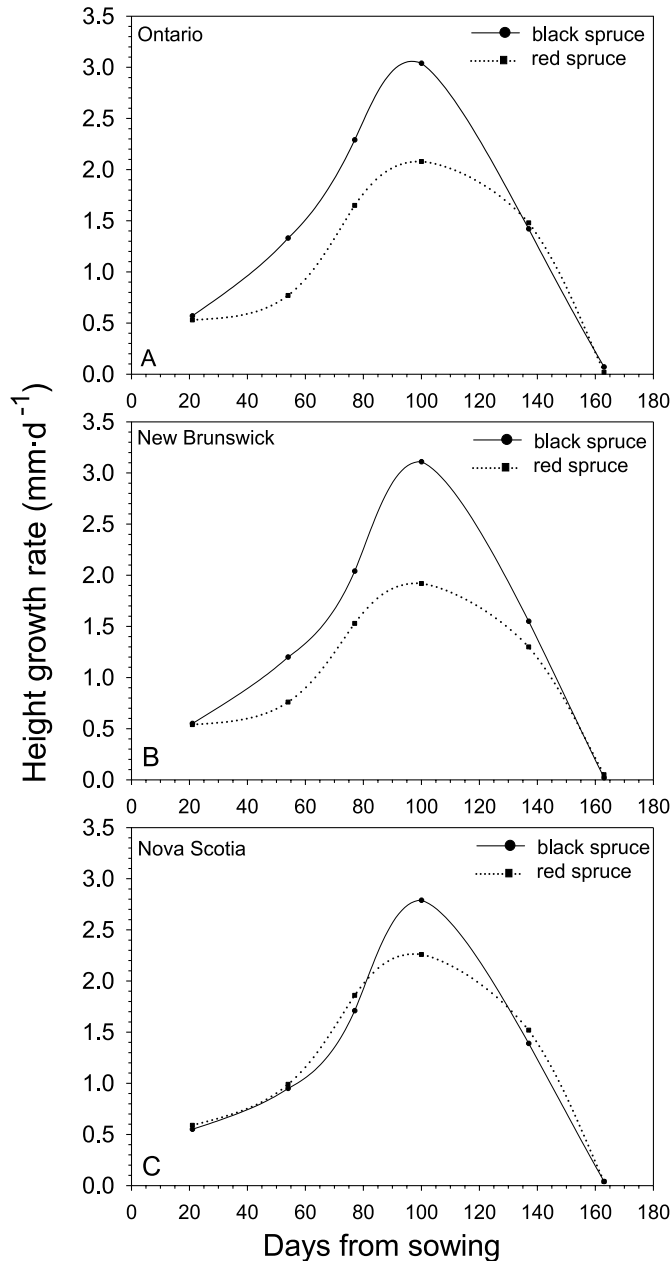


weight interaction ($p = 0.949$). Further analysis had a consistent significant species ($p = 0.001$) and needle dry weight effect ($p < 0.001$), with black spruce having 27.0 mg (8.6%) more root plus stem wood dry weight per unit needle weight than red spruce ($r^2 = 0.927$) (Fig. 7C).

Discussion

These results support the general observation that higher juvenile growth rates are associated with early successional

Fig. 5. Absolute height growth rate for black and red spruce populations from (A) Ontario, (B) New Brunswick, and (C) Nova Scotia.



species (Bazzaz 1979). Black spruce had higher growth rate than red spruce (Fig. 5). Rapid early growth would facilitate a quick escape from the strong interspecific competition normally associated with newly disturbed sites. The largest growth rate differential between the species occurred between approximately day 40 and day 66, after which the percent height difference between the species remained at approximately 30–40% (Fig. 5). Although the heights were similar at the first harvest (day 66), black spruce had proportionally more epicotyl height than red spruce and perhaps more needles available for photosynthesis. Black spruce has a narrower peak growth period, reflecting the pattern of growth required in an environment dominated by a short growing season and characterized by a moist spring followed by a

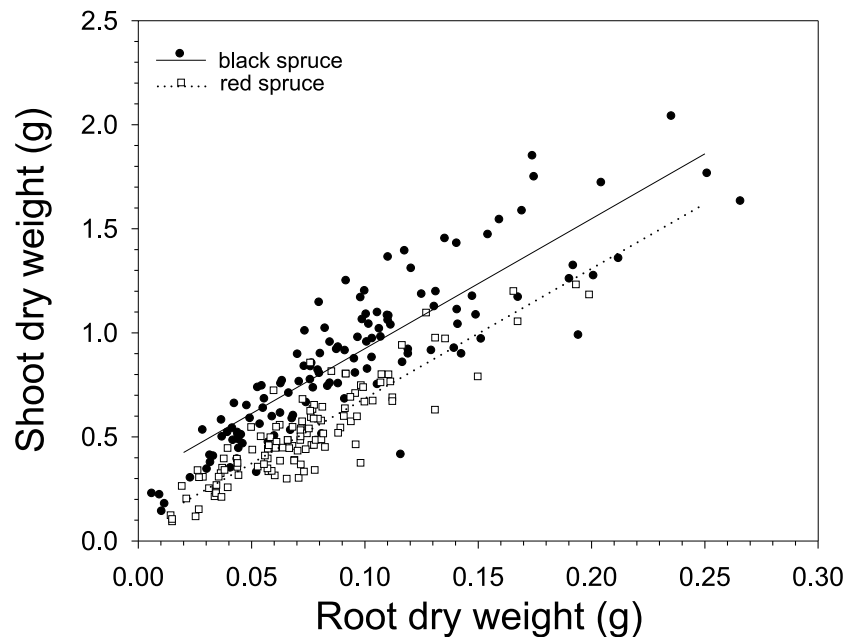
Table 3. Species by region (Ontario, New Brunswick, and Nova Scotia) means and overall species means and standard errors for seedling morphometric traits at 191 days after sowing (harvest 3) and results from ANOVA with sources of variation including species, region, species \times region, location(region), and species \times location(region).

| Trait | Species | Ontario | New Brunswick | Nova Scotia | Overall | S | R | S \times R | L(R) | S \times L(R) | r^2 |
|---|---------|------------------|------------------|------------------|------------------|--------|-------|--------------|-------|-----------------|-------|
| Total height (cm) | BS | 21.81 \pm 0.50 | 21.00 \pm 0.63 | 18.91 \pm 0.52 | 20.59 \pm 0.33 | <0.001 | 0.185 | <0.001 | 0.434 | 0.206 | 0.310 |
| | RS | 16.90 \pm 0.50 | 15.91 \pm 0.52 | 18.43 \pm 0.48 | 17.08 \pm 0.30 | | | | | | |
| Diameter (mm) | BS | 2.14 \pm 0.07 | 2.11 \pm 0.07 | 1.94 \pm 0.06 | 2.06 \pm 0.04 | <0.001 | 0.699 | 0.004 | 0.777 | 0.647 | 0.246 |
| | RS | 1.67 \pm 0.05 | 1.61 \pm 0.06 | 1.79 \pm 0.05 | 1.69 \pm 0.03 | | | | | | |
| Needle dry weight (mg) | BS | 597.7 \pm 41.9 | 561.8 \pm 47.6 | 469.5 \pm 34.2 | 547.0 \pm 24.7 | <0.001 | 0.124 | 0.019 | 0.494 | 0.170 | 0.291 |
| | RS | 351.2 \pm 22.1 | 284.7 \pm 20.3 | 373.1 \pm 27.7 | 333.0 \pm 13.6 | | | | | | |
| Stem wood dry weight (mg) | BS | 368.5 \pm 24.4 | 329.2 \pm 26.1 | 292.4 \pm 25.1 | 332.3 \pm 14.8 | <0.001 | 0.294 | 0.016 | 0.057 | 0.374 | 0.385 |
| | RS | 163.2 \pm 11.7 | 157.8 \pm 12.6 | 194.3 \pm 16.8 | 169.7 \pm 7.8 | | | | | | |
| Root dry weight (mg) | BS | 98.9 \pm 8.4 | 100.0 \pm 9.2 | 83.9 \pm 6.6 | 94.3 \pm 4.7 | <0.001 | 0.655 | 0.246 | 0.394 | 0.871 | 0.086 |
| | RS | 72.1 \pm 4.8 | 70.3 \pm 5.6 | 75.7 \pm 5.9 | 80.7 \pm 6.8 | | | | | | |
| Shoot to root ratio (dry weight) | BS | 10.86 \pm 0.53 | 10.48 \pm 0.86 | 10.16 \pm 0.36 | 10.51 \pm 0.35 | <0.001 | 0.323 | 0.083 | 0.619 | 0.056 | 0.268 |
| | RS | 7.35 \pm 0.29 | 6.56 \pm 0.21 | 7.78 \pm 0.37 | 7.23 \pm 0.18 | | | | | | |
| Seedling water balance index ^a | BS | 4.93 \pm 0.68 | 4.46 \pm 0.36 | 5.25 \pm 0.43 | 4.88 \pm 0.29 | 0.001 | 0.275 | 0.798 | 0.871 | 0.167 | 0.081 |
| | RS | 3.62 \pm 0.18 | 3.66 \pm 0.25 | 4.13 \pm 0.23 | 3.80 \pm 0.13 | | | | | | |

Note: df: S = 1, R = 2, S \times R = 2, L(R) = 3, S \times L(R) = 3, error = 228; total sample number = 240. BS, black spruce; RS, red spruce; S, species; R, region; L, location; r^2 , coefficient of determination; p values <0.05 are in bold type.

^aSeedling water balance index = shoot area/(diameter \times root area).

Fig. 6. Relationship between shoot and root dry weight from harvest 3; equation from ANCOVA: black spruce, $y = 0.30 + 6.25x$; red spruce, $y = 0.06 + 6.25x$; $r^2 = 0.829$.



dry summer. Red spruce has a flatter growth profile, which is expected of a species adapted to a milder, more extended growing season in which moisture is less limiting throughout the season. Red spruce is believed to be adapted to, and to have originated from, a rather narrow coastal strip along the Atlantic Ocean. This area served as red spruce's glacial refugium. Its movement into ON and Quebec was probably a relatively recent occurrence, dating back no more than 2000 years (Morgenstern and Farrar 1964).

The cotyledon number distribution patterns revealed the following about the three regions. In NS, the black and red spruce distributions are very distinct, which may indicate strong ecological and reproductive barriers. The ON region had a moderately higher degree of species overlap than did the NS region for cotyledon number. This is a noteworthy finding considering red spruce in ON is found in only a few isolated populations. Both black and red spruce populations from NB have flatter distributions for cotyledon number than do those from the other two regions, and the NB region has the highest degree of species overlap. This is consistent with the hypothesized levels of higher natural interspecific hybridization in the eastern lowlands of NB (Manley 1972). Seed weight data strongly suggest that the females were true to species type (not shown) but some of the offspring may be the result of interspecific hybridization. Cotyledon numbers also suggest that most of the introgression is found in the black spruce as opposed to the red spruce populations.

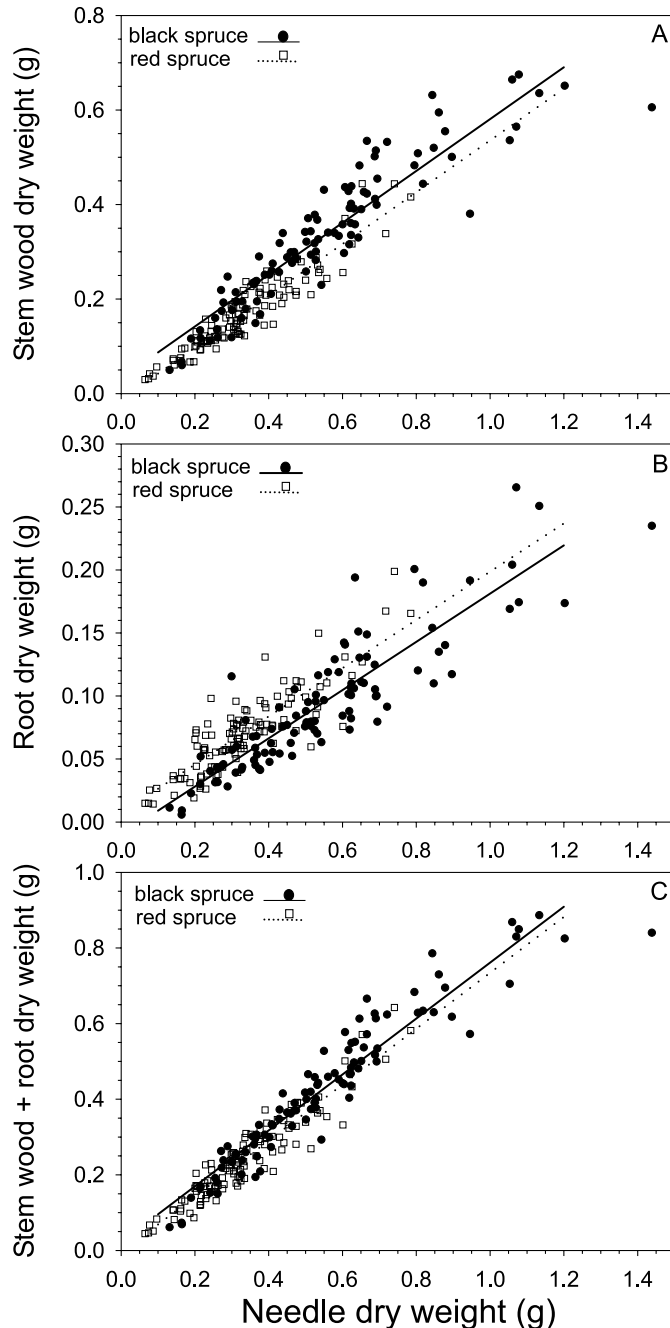
Our study clearly demonstrates that red and black spruce can be differentiated by their germination patterns (Fig. 1). The same germination patterns were seen with a set of controlled crossed black \times black spruce and red \times red spruce originating from NS (Major unpublished) and NB (Major et al. 2003a). The early emergence of black spruce cotyledons and the rapid early growth of its seedlings are consistent with its ecological role as an early successional species in a highly competitive growth environment (Cater and Chapin

2000). The slower emergence of red spruce may result from the greater number and size of the cotyledons. The large cotyledon area associated with red spruce may be an adaptation to competition or survival under an established forest canopy in more shaded, late-successional forests where it may provide increased photosynthetic surface under low-light environments.

Earlier germination has been correlated with greater growth for black spruce but not for jack pine (*Pinus banksiana* Lamb.) (Wang and Lechowicz 1998). The same correlation exists for both black and red spruce, with 3.6 mm more height growth per day of earlier germination. The fact that black spruce germinated 6.6 days earlier than red spruce can partially explain the species' height differences. However, given the same date of germination, black spruce was 12.4 mm taller than red spruce. Thus, 66% of the growth difference in first-year growth was attributable to earlier germination, with the other 34% attributable to higher overall shoot growth. However, it should be noted that germination had a consistent and significant species \times region interaction for each date (Fig. 1). Interestingly, germination time was highly correlated with percent germination for both species ($r = 0.911$ and 0.816 for black and red spruce, respectively). Inbreeding depression may be a common cause for this correlation.

Inbreeding depression was not directly measured in this experiment and is not the only cause of reduced germination. Nevertheless, it may partly explain the slower growth and slower emergence time of red spruce compared with black spruce for the ON and NB regions. Increased inbreeding in small populations can depress traits such as net photosynthesis and growth in jack pine (Blake and Yeatman 1989), survival and growth in black spruce (Johnsen et al. 1999), and fecundity in red spruce (Mosseler et al. 2000). The ON populations of red spruce are small and isolated and appear to have increased rates of inbreeding based on a combination

Fig. 7. (A) Relationship between stem wood and needle dry weight from harvest 3; equation from ANCOVA: black spruce, $y = 0.0324 + 0.5483x$; red spruce, $y = -0.0128 + 0.5483x$; $r^2 = 0.907$. (B) Relationship between root and needle dry weight; equation from ANCOVA: black spruce, $y = -0.0101 + 0.1913x$; red spruce, $y = 0.0073 + 0.1913x$; $r^2 = 0.791$. (C) Relationship between stem wood plus root and needle dry weight; equation from ANCOVA: black spruce, $y = 0.0215 + 0.7396x$; red spruce, $y = -0.0055 + 0.7396x$; $r^2 = 0.927$.



of seed traits and allozyme data (Mosseler et al. 2000; Rajora et al. 2000). Black spruce populations remain robust in ON and NB but are found in more isolated pockets in NS and are known to be susceptible to the effects of inbreeding de-

pression (Morgenstern 1972). Compared with red spruce, it appears that black spruce does not tolerate inbreeding effects as well. Red spruce displayed a 4.3-mm decrease in height with every 10% decrease in germination, whereas black spruce had three times the reduction, or a 14.9 mm decrease in height, for every 10% decrease in germination. Higher tolerance for inbreeding in red spruce may be related to low inherent genetic diversity and relatively high inbreeding rates in this species (DeHayes and Hawley 1992; Rajora et al. 2000).

Allometric analysis demonstrated that black spruce had a significantly higher (39%) shoot to root ratio than red spruce and that black spruce produced and (or) allocated 20% more dry weight (per unit needle weight) to the stem than did red spruce. This contrasts with the results of Beck (1985) who compared early growth between red and black spruce from New England and found that, although red spruce had the lightest root system, it had a greater shoot to root ratio. In our study, further allometric analysis demonstrated a 25% increase in allocation to root growth for red spruce over black spruce. When the sinks are summed together, black spruce was 8.6% more efficient in converting resources into biomass compared with red spruce. The greater efficiency of black spruce may be due to higher net photosynthesis per unit needle dry weight. However, increased net photosynthesis was not observed in mature trees (Johnsen et al. 1998) but was observed in 1-year-old seedlings based on measurements of chlorophyll fluorescence (Major et al. 2003b).

The higher allocation of biomass to roots in red spruce than in black spruce was not expected. It was originally hypothesized that black spruce would have higher allocation to roots to withstand drier above- and belowground early successional environments. This result may need to be viewed with respect to the life history of each species; red spruce lives to a greater age and size than black spruce. As trees age and increase in size, it has been found that growth is reduced due to increasing hydraulic resistance (Yoder et al. 1994; Ryan and Yoder 1997; Ryan et al. 1997). Although we only measured allocation in 1-year-old seedlings, there was a significant correlation of 1-year-old seedling height with 22-year-old height in mature trees of black and red spruce intra- and interspecific hybrids grown on two sites ($r = 0.918$ and $r = 0.968$) (Major et al. 2003a). We hypothesize that this allocation pattern may also persist and that greater root allocation in red spruce acts to buffer the hydraulic stresses found in large trees in late-successional forests. Obviously, this hypothesis is speculative and we plan to quantify shoot to root allocation in adult stands using ground-penetrating radar to assess root biomass (Butnor et al. 2001, 2003).

Although similar to shoot to root partitioning, the seedling water balance index provides a better representation of the water supply and demand tension because it incorporates the absorbing surface area, the conduit size (diameter), and the transpiring area. Variation in water balance can affect productivity and survival (Grossnickle 2000). It appears that red and black spruce have adapted to different ecophysiological norms. For instance, black spruce has a higher seedling water balance index than red spruce, which may reflect natural selection pressures on aboveground growth in highly competitive and variable early successional environments (Bazazz

1979; Cater and Chapin 2000). In contrast, the typical red spruce environment in the protected understory and high humidity of the Maritimes or in the cloud-capped upper slopes of the Appalachians (Johnson and Siccama 1983) represents a milder, more stable growing environment. The higher seedling water balance and the constant exposure to drier atmospheric conditions experienced in typical black spruce environments suggest that drought tolerance traits would be more strongly selected for in black spruce than in red spruce to balance this strategy. This hypothesis will be explored in future studies.

There were a number of species-specific traits, such as cotyledon number, shoot to root ratio, root dry weight, and seedling water balance. Other traits, such as germination time, height, diameter, needle dry weight, and stem wood dry weight, also had significant species effects that interacted with region. These were due to magnitude changes or possible inbreeding effects. Compared with red spruce, black spruce possessed morphometric, allometric, and developmentally distinct adaptive traits that were consistent with early successional demands. Greater allocation to roots may be a key feature of red spruce's competitive advantage. Traits such as those described here are relatively easily measured and are scientifically meaningful with respect to ecological adaptations in trees (Ledig et al. 1970; Bazzaz 1979). It is important to understand the adaptive biology of forest tree species, particularly in late-successional species such as red spruce, to develop management prescriptions for optimizing natural regeneration and stand productivity and maintaining population viability.

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