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THE GENECOLOGY OF SEEDLING ROOT GROWTH, STOMATAL FREQUENCY AND SEED MASS IN *Pinus banksiana* Lamb.

by

© ROBERT A. WRIGHT

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN

PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF FOREST SCIENCE

EDMONTON, ALBERTA

SPRING, 1994

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled The Genecology of Seedling Root Growth, Stomatal Frequency and Seed Mass in *Pinus banksiana* Lamb. submitted by Robert A. Wright in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY

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ABSTRACT

Seedling root growth and stomate numbers of populations on a 1000 km north-south transect through the boreal forest were compared in common garden and reciprocal transplant experiments. Northern seedlings possessed higher root/shoot biomass ratios, lower shoot biomass and fewer stomata per mg leaf tissue than southern seedlings. Significant garden and block-within-garden effects were present, indicating two spatial scales of environmental variance in root growth traits. Survivorship in the first growing season followed a power function model and a negative exponential model in the second year. Most first year mortality was due to herbivory, while drought stress caused more deaths in the second year. Lower stomatal frequencies and greater investment in the root compartment may give northern populations of jack pine a greater ability to survive the increasing drought stress expected with climate change.

In the second experiment, seeds from adjacent wet- and dry-site jack pine stands were planted in a common growth chamber and seedlings were grown with and without drought. Seedlings originating from the wet site had significantly more tertiary roots than those originating from the dry-site. Drought inhibited secondary and tertiary but not primary root growth. Seed mass was significantly correlated with seedling size.

The pattern of seed mass variation in jack pine was also examined. Seed mass was 45% greater in the south than in the north. Seed mass was negatively correlated (R=0.91) with latitude. Dry-environment seed mass exceeded wet-environment seed mass in both northern and southern populations.

Narrow-sense heritability of seed mass was higher in the southern population (0.75 vs.0.34). Dry-environment genotypes in the south showed variation in reaction norm to a 4-year increase in April-June precipitation.

Seedling survivorship was positively correlated (R=0.98) with seed mass. Survivorship among seedlings was best described by a power function. Seed weight among dry-environment trees in the southern population was positively correlated (R=0.94) with April-June precipitation.

The heritability of seed mass and the positive correlation between seed mass and survivorship suggested an opportunity for the natural selection of seed mass.

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CHAPTER I

INTRODUCTION TO THE THESIS

Introduction to the Thesis

Jack pine (*Pinus banksiana* Lamb.) is the dominant tree species over extensive portions of the western Canadian boreal forest. It is found primarily in sandy, rapidly-drained ecosystems although it does occur in mesic ecosystems with well- to imperfectly-drained, medium-textured soils. Occasionally it is found as a minor component of black spruce (*Picea mariana*) forests on peaty poorly-drained soils in treed fen or bog ecosystems. Its modal occurrence is within ecosystems that are prone to develop low soil water potential during periods of low or no rainfall.

This propensity to occur in drought-prone ecosystems, plus a curious ability to persist in low numbers in edaphically wet ecosystems, prompted my research into the ecology and genecology of seedling drought resistance in jack pine. I had become intrigued with the possibility that jack pine had developed both ecotypic and clinal forms of genetic differentiation for drought resistance. My fascination with this possibility arose from an odd juxtaposition of personal observations on the distribution of jack pine in northern Saskatchewan and my Masters research on seedling root growth of pioneer shrubs on volcanic cinder in Hawaii. Several other factors contributed to my decision to probe the genecology of seedling drought resistance in jack pine. One was a growing interest in the interface between the ecology, genetics and evolution, in effect the evolutionary ecology, of plants. The second was the apprehension that the genecology of root growth in seedling jack pine had received scant attention in the forestry and ecological literature. The third motivational factor was a curiosity about the interaction between the genetic structuring of important species within the boreal forest and the various scenarios presented for the possibility of rapid anthropogenic climate change. Finally, I felt, from my work in Hawaii, that root growth parameters presented a family of potentially evolutionarily labile traits exposed to a high probability of strong selection for drought resistance during the seedling life stage.

I decided to attack the problem at two spatial scales:

1) At the scale of ecotypic differentiation between populations of jack pine occupying sandy, well-drained uplands and peaty, poorly-drained lowlands immediately adjacent to one another.

2) At the geographically broad scale of clinal variation from the southern edge of the species range in western Canada, at the ecotone between the boreal forest and grassland biomes, to the northern edge of the species range 1000 km away near the ecotone between the boreal forest and the subarctic woodland east of Yellowknife, Northwest Territories.

To accomplish this goal I collected seed from stands in adjacent wet and dry environments near Prince Albert, Saskatchewan and from eight populations located along a 1000 km south-north transect through the boreal forest from Prince Albert, Saskatchewan to Yellowknife, Northwest

Territories. Pairs of populations were sampled from each of four regions along the transect (south, south-central, north-central, and north regions) to obtain an estimate of the between population variance within regions.

Common garden experiments were conducted under controlled conditions in the greenhouse and University of Alberta Phytotron and under field conditions. A reciprocal transplant experiment involving two gardens was also undertaken. Root growth parameters, such as root length and numbers and root/shoot biomass ratio, were examined along with the variance of stomatal frequency among populations. The rationale for choosing these traits was based on their likelihood of exposure to strong selection during dry weather because of their direct effects on the water budget and survival of the seedling.

I will not be engaging in a review of the literature in this introduction to the thesis because it would simply constitute a needless recapitulation of the reviews contained in the three papers which make up the body of the work. Instead, I offer the following short, explanatory guide to the papers themselves.

Chapter II is a paper on the evidence for clinal variation in root growth and stomatal traits among populations of jack pine scattered along a 1000 kmnorth-south transect throught the boreal forest of western Canada. Chapter III is a paper dealing with the question of ecotypic differentiation for root growth traits between adjacent wet- and dry-environment stands of jack pine. The fourth chapter of the thesis is a paper that interweaves the spatial pattern and heritability of seed mass in a treatment of the ecology and evolution of seed mass in jack pine. The thesis closes with a short synthesis chapter.

Chapter II will be submitted to the Canadian Journal of Forest Science. Chapter III was published in Forest Science 38:777-785, in 1992. Chapter IV will be submitted to Ecological Applications.

CHAPTER II

CLINAL VARIATION IN SEEDLING GROWTH AND STOMATAL CHARACTERISTICS AMONG POPULATIONS OF JACK PINE

Introduction

Jack pine (*Pinus banksiana* Lamb.) often grows on sands in the boreal forest (*e.g.* dune fields, eskers, sandy plains, Rowe 1972) where low soil water potential during seedling establishment may favour the evolution of enhanced drought-avoidance capability. Plants often experience heavy mortality in the seedling life stage (Oppenheimer 1960, p. 114, Hett and Loucks 1976), and drought-induced mortality in jack pine is known to be highest in young seedlings (Shirley 1934). Because of heavy mortality, and the attendant possibility of high selection intensity, the seedling is the life stage in which natural selection ought to be strongest for traits that enhance drought avoidance.

Although the correlation between seedling root growth and survival has not been investigated in jack pine, there is evidence that drought avoidance in this species occurs by root extension (*e.g.* McClain 1973, Thomas and Wein 1985). We use drought avoidance here, in the sense described by Levitt (1972, p. 353), to mean traits that lead to the avoidance of water deficits within the living tissue of the plant. In loblolly pine (*Pinus taeda* L.) the survival of outplanted seedlings has been correlated with root length, root number and root/shoot biomass ratio (Larsen *et al.* 1986), and drought-resistant provenances of loblolly pine have deeper, more heavily branched rooting patterns (van Buijtenen et al. 1976). Drought resistance implies an ability to resist the deleterious effects of drought via a strategy of avoidance, tolerance or escape (Levitt 1972). Such differences in seedling root length and root/shoot biomass ratio are known to be heritable in some conifers (see Khalil 1985). We have shown (Wright et al. 1992) that differentiation in root growth traits occurs between stands of jack pine on adjacent wet and dry sites. The pattern of seedling root growth may also vary between populations in the same geographic region (e.g. root horizontality in larch-*Larix laricina* (Du Roi) K. Koch, Rehfeldt 1970). Common garden experiments by Yeatman (1966) have provided some evidence of a decrease in above-ground size of germinants with increasing latitude of origin.

The rate at which plants lose water during photosysnthesis directly influences their capacity to avoid drought. The frequency (i.e. # stomata/unit mass oven-dry leaf tissue) and the aperature of stomata are likely to affect conductance and the rate of water loss via transpiration. Conductance is known to be correlated with stomatal frequency in some species (e.g. Nerkar et al. 1981). An inverse relationship between the frequency and size of stomata (Jones 1987) may negate the effect of increased stomatal frequency on the rate of transpiration. Stomatal frequency is known to decrease with increasing latitude in *Pinus strobus* (Mergen 1963). Measurements on loblolly pine from a common garden experiment showed that 2-year old trees from a dry-climate provenance had fewer stomata/mm^2 than 2-year old trees from a provenance of more mesic macro-climatic conditions (Knauf and Bilan 1974). Stomatal frequency and size are also often ighly heritable (e.g. Liang et al. 1975). Based on these findings, the frequency and size of stomata are worth considering in a study of the geographical pattern and intensity of differentiation for drought-avoidance traits among disjunct populations of jack pine.

The objective of our work was to investigate the strength and pattern of population differentiation for seedling root system architecture, biomass allocation, and stomatal characteristics among eight provenances of jack pine sampled from along a 1000 km north-south transect through the boreal forest of western Canada. These traits were chosen because of their importance as indicators of comparative seedling drought-avoidance capability. We believe that spatial structuring of the gene pool for traits that enhance drought avoidance could play an important role in directing the demographic response of jack pine to the drier, warmer conditions anticipated in the wake of anthropogenic climate change.

Population Selection and Sampling

Seed was collected from four broad regions (Figure II-1) along a 1000 km north-south transect through the boreal forest of western Canada. Two populations were sampled from each region (south region - near Prince Albert, Saskatchewan - 53°15 N, 106°3 W and near Bruderheim, Alberta - 53°52,N, 112°56,W; south-central region - Fort Hills, Alberta - 57°22 N, 111°33 W and near Fort McKay, Alberta - 57°20 N, 111°39,W; north-central region - near Peace Point, Alberta - 59°8 N, 112°16 W and Sandy Lake, Northwest Territories - 60°32 N, 114°34,W; north region - near Mosquito Creek, Northwest Territories - 62°30 N, 116°28,W and near Reid Lake, Northwest Territories - 62°30 N, 113°35 W). Seed was collected from 40 maternal families in each population. Collections were restricted to stands of jack pine growing on sandy, well-drained sites to avoid confounding patterns of differentiation between adjacent soil moisture types (see Chapter III)with the broader geographical patterns of differentiation. The term population indicates a stand of trees found in the same physical locale, in the case of this study an area of <1 km^2, in all cases.

Experimental Design and Analysis

Common Garden Greenhouse Experiment

A randomized complete block design was used for this experiment. The blocks were designed to isolate unwanted variation between the east and west sides of the glasshouse bench. Four regions, two populations per region, forty families per population and one seed per family appeared in each block. Twenty families (and therefore twenty seeds) per population were randomly assigned to the drought treatment and 20 families to the well-watered treatment within each block. The watering treatment was included to test for differential population response to water stress. The ANOVA model for the experiment was:

$$Y_{ijklm} = \mu + B_i + R_j + B\ R_{ij} + P_{k(j)} + BP_{ik(j)} + T_l + BT_{il} + RT_{jl} + PT_{k(j)l} +$$
$$BRT_{ijl} + BPT_{ik(j)l} + S_{m(ijkl)},$$

with $i=2$, $j=4$, $k=2$, $l=2$, $m=20$, and total d.f.$=2(4)(2)(1)(20)-1=639$, where μ = overall experimental mean, B = block (fixed), R = region (fixed), P = population (random), T = watering treatment (fixed), S = individual seedling effect (residual error). Because the estimate of heritabilities or genetic correlations was not the objective of this experiment, the maternal family factor was not treated explicitly in the model.

Each seed was randomly assigned to and planted in a 30 cm deep plastic pot, of 5cm diameter, filled with medium-textured, washed commercial sand. The watering schedule for the two treatments was based on knowledge of the desorption curve and drying properties of the potting medium acquired in a separate experiment (Chapter III). On planting day, the well-watered plants were given 60 ml (wetting front penetration to 15 cm depth after 48 hours) and the drought treatment plants 50 ml (wetting front penetration to 9 cm after 48 hours) of a nutrient mix developed for the growth of pine seedlings in a greenhouse environment. The well-watered plants were watered to original wet weight every week (based on the mean water loss from a subsample of pots in each block). The drought treatment plants were watered with 20 ml of nutrient solution at the first sign of wilting, as indicated by laxness of the foliage and wrinkling of the needle surface (generally every 10-14 days). Daylengths was maintained at 18 hours using supplementary lighting with mercury and sodium vapor high intensity discharge lamps. Day-night temperatures were 25 and 15°C.

Seedlings were harvested 70 days after emergence. We measured, to the nearest mm, primary, secondary and tertiary root lengths as well as the numbers of secondary and tertiary roots (>1mm in length) and oven-dry (70°C) root and shoot biomass to the nearest 0.1 mg.

Seedling growth is correlated with seed mass in jack pine (see Chapter III and Radsliff 1981), and heavier seeds are known to produce heavier seedlings with longer roots in other pine species (e.g. *Pinus brutia*, Isik 1986). For this reason we employed seed mass as a covariate (after the manner of Kolb and Steiner 1989) to avoid confounding population differences in seedling growth rate with seedling size differences due to variations in maternal investment in the seed or population-level genetic differences in seed mass. In the analyses of covariance (ANCOVA), the term $\beta\ (x_{ijk} - \bar{x})$ was added to the model to adjust for covariance between seed mass and seedling growth (β = linear regression coefficient of growth parameter y on seed mass (x); $\bar{x}$ = overall mean seed mass). Covariance analysis was used only if the slopes of the seedling size vs. seed mass regression lines for the populations were homogeneous (i.e. no population X seed mass interaction, see Freund *et al.* 1986, p. 154). The data were also examined using multivariate analysis of covariance, with seed mass employed as the covariate (Freund et al. 1986, p. 170). In all of our analyses, observed departures from homoscedasticity and normality did not jeopardize the power of the F-test according to the criteria of Scheffé (1959) and Wilcox (1987).

Reciprocal Transplant Experiment

This experiment consisted of a common garden field experiment replicated in the four regions described under Population Selection and Sampling. The four sites selected for gardens were: 1) south region - Bruderheim, 2) south-central region - Fort Hills, 3) north-central region - Sandy Lake, 4) north region - Reid Lake. The Bruderheim common garden consisted of four blocks, each of which contained four sub-blocks. Approximately 20 seeds from each of the regions (approximately 10 from each population in each region) were planted within each sub-block. The other three common gardens consisted of four blocks each but with only two sub-blocks per block. These sub-blocks were of the same design as those described for the Bruderheim garden.

The ANOVA model for the experiment was:

$$Y_{ijklm} = \mu + G_i + B_{j(i)} + S_{k(ij)} + R_l + GR_{il} + BR_{jl} + SR_{kl} + P_{m(l)} + GP_{im} + ,$$

$$BP_{jm} + SP_{km} + T_{n(ijklm)}, \text{ with } i=4,\ j=4,\ k=2\&4,\ l=4,\ m=2.$$

where μ = overall experimental mean, G = garden (fixed), B = block (random), S = sub-block (random), R = region (fixed), P = population (random), T = individual seedling effect (residual error). Total degrees of freedom at the start of the experiment were (1(4)(4)(4)(2)(10)) + (2(4)(2)(4)(2)(10)) = 2560-1 = 2559. The first member of the left side of the degrees-of-freedom equation represents the garden at Bruderheim with 4 sub-blocks per block, and the second member on the left side represents the gardens at Reid Lake and Sandy Lake with 2 sub-blocks per block. It proved impossible to harvest the garden at the Fort Hills, and that garden does not appear in the analysis. Mortality caused a great diminution in the degrees of freedom in the other gardens by the end of the experiment.

The gardens were planted in June, 1989. All sites chosen were in areas of medium-textured sands (aeolian deposits at Bruderheim and Fort Hills, glacio-fluvial deposits at Sandy Lake and Reid Lake). In each garden two of the blocks were located in open, unshaded spots and two were located in relatively shady micro-environments protected from direct sunlight for most of the day. This was done to provide a range of safe sites for germination that would ensure the emergence of at least some of the seedlings under a variety of summer weather conditions. (e.g. hot and dry to wet and cool conditions). The blocks were located at least 200 m apart to minimize the risk of catastrophic loss from windthrow of trees or disturbance by animals. The LFH layer was removed from the soil of each 1 m^2 sub-block, all roots and debris were removed from the top 30 cm of mineral soil, the surface was raked and levelled and the seeds were planted at a depth of 1 cm in a 10X10 cm square pattern with the aid of a planting frame. The identity of the seed at each planting point was recorded and the selection of seeds for planting was done at random from a mix of 80 seeds (10 from each population, therefore 20 per region) for each sub-block. The sub-blocks were separated from each other by 0.5 meters of undisturbed soil. All sub-blocks received a 2 cm watering just prior to planting to promote imbibition and to re-consolidate the disturbed soil surface. The anchor points for the frame were marked with stakes to allow relocation and identification of the seedlings for survivorship monitoring and harvest.

The seedlings of the southern garden emerged in the growing season of 1989. Two hot, dry weeks in June and July, 1989, prevented seedling emergence in the northern (Reid Lake), north-central (Sandy Lake), and south-central (Fort Hills) gardens. The seeds in those gardens overwintered and the seedlings emerged during the 1990 growing season. Absolute seedling sizes in the Reid Lake and Sandy Lake gardens could be compared to each other but not to the Bruderheim garden. An early snowfall prevented harvest of the Fort Hills garden; this effectively rendered the Bruderheim site a common garden experiment and left the Sandy Lake and Reid Lake gardens as the reciprocal transplant experiment.

The gardens were harvested in late September (Sandy Lake and Reid Lake) and mid-October (Bruderheim) of 1990. The seedlings were carefully excavated, using squirt bottles and water, to prevent breakage or loss of roots, then labeled and stored in plastic bags for subsequent measurement in the laboratory.

The frequency of stomata (i.e. # stomata/mg oven-dry leaf tissue) was also measured on a sample of six seedlings from each population from the Bruderheim garden. The estimation of stomatal frequency was accomplished by counting all the stomata on a 1 cm long section from the middle of each of three needles per seedling. The 1 cm long leaf sections were then oven dried at 70°C, weighed, and stomatal frequency was expressed as stomata/mg of oven-dried leaf tissue.The ANOVA model for the analysis of stomatal frequency was:

$$Y_{ijkl} = \mu + R_i + P_{j(i)} + S_{k(ij)} + L_{l(ijk)}, \text{ with } i=4,\ j=2,\ k=3,\ l=4,$$

where R = region, P = population within region, S = seedling within population, and L = needle within seedling. Region was the only fixed factor.

The length of the stoma was also measured on another sample of seedlings from the Bruderheim Garden, but only for one population per region. The populations were: Bruderheim from the south region, Fort Hills from the south-central region, Peace Point from the north-central region, and Mosquito Creek from the north region. Stoma length was measured on four seedlings per population, three needles per seedling, and six stoma per needle (with the exception of

Bruderheim, for which only three seedlings were sampled). The ANOVA model for the analysis of stoma length was:

$$Y_{ijkl} = \mu + P_i + S_{j(i)} + L_{k(ij)} + T_{l(ijk)}, \text{ with } i=4,\ j=4,\ k=3,\ l=6,$$

where P = population, S = seedling within population, L = needle within seedling, and T = stoma within needle. Population was the only fixed factor.

Results

Common Garden Greenhouse Experiment

Univariate analyses of covariance of root/shoot biomass ratios (Table II-1) showed that jack pine seedlings of north region populations allocated a greater proportion of total biomass to the root compartment that did jack pine originating from more southerly populations. Note that seed mass was employed as a covariate in order to focus on seedling size differences independent of the influence of seed mass. The south region showed the lowest mean root/shoot ratio although the latitudinal trend was complicated by the similarity in biomass allocation between the south-central and north-central regions. A clear-cut latitudinal trend in absolute root mass was not evident. The north and south-central populations possessed significantly heavier root systems than seedlings from north-central populations and although north region root biomass was numerically greater than south region root biomass, the difference was not statistically significant. The dichotomy between north and south was clearer for shoot biomass with the two southern regions having significantly heavier shoot systems than the seedling samples from the two northern regions.

A MANCOVA (multivariate analysis of covariance, with seed mass as the covariate) incorporating shoot and root biomass and root/shoot biomass ratio demonstrated significant regional, treatment and population-within-region effects in the model (Table II-2). All regions differed significantly in regard to the multivariate complex of biomass allocation and magnitude (Table II-3). The pre-planned, orthogonal tests among regions indicated that the most distinct differences occured between the northern region and all the others. Differentiation between the two central regions and between the south and central regions was relatively weak.

Univariate covariance analyses of root architecture (primary, secondary, and tertiary root lengths and number of secondary and tertiary roots) showed no evidence of significant differences among regions for these morphological facets of root growth (results not shown). A MANCOVA of root architecture indicated some differentiation among regions for these aspects of root form (F=1.79, numerator df=15, denominator df=798.2, Prob>F=0.032 (based on Wilk's Lambda)). Only the pre-planned orthogonal comparison between the south and north-central regions proved to be significant for the constellation of root architecture variables. The MANCOVA provided no evidence of a significant variance component among populations within regions.

We used canonical discriminant analysis (Luginbuhl et al. 1987, p.173-188) to summarize and explore the between-region variation in root architecture and biomass partitioning. A two-tiered approach was employed to enable an examination of the discriminatory power of biomass and architecture variables alone and then in combination.

Biomass variables were effective in discriminating among regions (R^2 values in Table II-4). From an examination of the raw canonical coefficients it was clear that root/shoot biomass ratio, and to a lesser extent shoot biomass, played dominant roles in the correlation between region and the first canonical variable (Table II-4). Differences in the absolute magnitude of root biomass played a very minor role in discriminating among seedlings of the four regions. The first canonical variable yielded most of the discrimination among regions. The discriminatory power of the complex of architectural variables was also statistically significant but much weaker than that of the biomass variables (R^2 values in Table II-4). Secondary and primary root length made the largest contribution of any of the root architecture variables to the discriminatory power of the first and second canonical variables. Canonical variable 2 was largely a function of within-region differences among seedlings in root biomass and primary and secondary root length.

A discriminant analysis employing both biomass and root architecture variables showed that the simultaneous consideration of both types of variables produced slightly better discrimination among regions (R^2 values in Table II-4). Root/shoot biomass ratio and shoot biomass were the dominant variables underlying the discriminatory power of the first canonical variable in the combined analysis. These two variables played a pre-eminent role in discriminating between seedlings of northern and southern provenances (Figure II-2 and Table II-5). Shoot mass contributed a negative coefficient to the composition of the first canonical variable. Seedlings from the southern populations had significantly greater mean shoot biomass than seedlings originating from northern gene pools and this was reflected in the largely negative scores for southern seedlings on CAN1 (Figure II-2). Northern seedlings possessed higher mean root/shoot biomass ratios than seedlings from southern populations and, as root/shoot ratio contributed a positive coefficient to the construction of CAN1, northern seedlings generally showed positive scores on the first canonical variable.

The discriminatory power of the first canonical variable, for this data set, rests upon the polarity in shoot biomass and root/shoot biomass ratio between the northern and southern populations in the boreal forest. Because of the similarity in root/shoot biomass ratio and shoot biomass for both central regions, the first canonical variable was ineffective in distinguishing between seedlings of north-central and south-central populations (Figure II-3 and Table II-5). The squared Mahalanobis distance between regions (Table II-5) was positively correlated with the latitudinal separation between regions ($R = 0.937$, R being significantly greater than 0 at Prob <

0.01). Regional means on the canonical variables showed the same rank order for all three discriminant analyses, north and south at the extremes, with north-central adjacent to north and south-central adjacent to south.

Drought-treatment seedlings had significantly shorter and fewer roots, and lighter root and shoot systems, than well-watered seedlings (Table II-6). Biomass ratios remained unaltered by drought, highlighting the stability of the ratio between investment in the root and shoot even among seedlings that experienced drought. No significant interaction occurred between region or population and drought treatment for any of the variables.

Reciprocal Transplant and Common Garden Field Experiments

Reciprocal Transplant Experiment

High seedling mortality in the Sandy Lake and Reid Lake gardens necessitated the amalgamation of blocks for data analysis. In both gardens the two blocks located in open, sunny micro-environments were combined and the two most heavily-shaded blocks were combined. Consideration was given to irradience levels in the amalgamation of blocks, not to establish another fixed factor in the model, but rather to maximize the similarity of the amalgamated blocks. Block remained a random factor in the model and differences between blocks were of interest only for the information they carried concerning the spatial scale of interactions between regional gene pools and their growing environments.

ANOVA of root/shoot biomass ratio (Table II-7) of jack pine seedlings grown under field conditions showed that southern populations had less invested in the root compartment than north and south-central region populations. The north-central mean biomass ratio was very similar to the north and south-central regional means and close to being significantly different from the south region mean (Prob > F less than 0.10). On this basis, it appears that the three northern regions did not differ in the allocation of biomass to the root and shoot compartments. This regional pattern was similar to that found in the greenhouse common garden experiment (Table II-1). While the regional pattern was similar, the actual root/shoot biomass ratios were very different between the field- and greenhouse-grown seedlings. In the field, the root compartment was the dominant sink for biomass, whereas in the greenhouse the shoot received the largest proportion of photosynthate investment (Table II-1). The field seedlings were much larger overall but were also about 40 days older, at harvest, than the greenhouse seedlings.

The larger size of the shoot in the south region seedlings grown in the field was consonant with the results of the biomass ratio analysis (Table II-7) and parallelled the behavior of shoot biomass in the greenhouse experiment (Table II-1). There were no significant differences in root biomass among the regions. Again, this result was consistent with the results from the greenhouse experiment in which root biomass differences among regions showed no definite north-south trend. Total root length was significantly higher in the south region than in the north-central region, and there appeared to be a latitudinal trend with greater total root length in the two southern regions. Primary root lengths were essentially the same across regions; the difference in total root length was accounted for by the greater mean length of secondary roots in the south (153 mm) and south-central (131mm) regions compared to the north (114mm) and north-central (118mm) regions. There were no significant interactions between region and garden or block for any of the variables considered in the univariate analyses.

Garden was a significant factor in the analysis of shoot biomass, total root length, and total number of roots, while block was a significant factor in the analysis of root mass, shoot mass, and root/shoot biomass ratio. Environmental variation between gardens apparently affected somewhat different aspects of seedling morphology than did environmental variation between blocks, within gardens. Note that seedlings grown in the more southern garden (Sandy Lake) were larger, on average, than seedlings grown in the more northern garden (Reid Lake)(Table II-8). Root/shoot biomass ratio did not vary between gardens and this demonstrated again the stability of root/shoot ratio across environments.

Seed mass was not used as a covariate in the analysis of the reciprocal transplant experiment because time constraints allowed the measurement of original seed mass only for seedlings planted in one sub-block of each block. Sample analyses of covariance with the seedlings, for which original seed mass was known, did show that seed mass was not a significant covariate under field conditions. The regional pattern of biomass allocation to the root and shoot was similar for the greenhouse and field experiments despite the absence of seed mass as a covariate in the analysis of the reciprocal transplant experiment.

A MANOVA (multivariate analysis of variance) incorporating shoot and root biomass, root/shoot biomass ratio, total root length , and total number of roots as dependent variables demonstrated significant garden, block, and regional effects (Table II-9) in the reciprocal transplant experiment. As with the univariate analyses, there were no significant interactions between region and garden or block. The north and south region and the north-central and south regions showed significant differences in the multivariate complex of biomass allocation and root architecture parameters (Table II-10). This regional pattern was consonant with the results of the tests among regions in the greenhouse experiment.

We undertook a canonical discriminant analysis to determine the utility of biomass and architecture variables for distinguishing among the four regional gene pools in the reciprocal transplant experiment. Consideration of root/shoot biomass ratio, shoot and root biomass, total root length and total number of roots produced only a weak (R^2 = 0.10) correlation between the first canonical variable and region. Examination of the raw canonical coefficients showed that the first canonical variable was strongly influenced by shoot biomass but only weakly affected by the other variables in the analysis. Examination of the squared Mahalanobis distances (Table II-11) showed that the regions were less distinct under the field conditions of the reciprocal transplant experiment than under the controlled conditions of the greenhouse experiment (see Table II-5). Nevertheless, the regions were distinguishable on the basis of the genetic information carried by the biomass and root architecture variables examined in both the greenhouse and field experiments.

Common Garden Field Experiment

Univariate analyses of variance and multivariate analyses of variance showed no significant differences among regions for root or shoot biomass or root/shoot biomass ratio. There were no significant interactions between region and block. There was a significant variance component for block in the analysis of root biomass, indicating that micro-environment did have an effect on the absolute size of the root compartment.

We also examined the variance in stomatal characters among regions according to the protocol and statistical model discussed previously under Experimental Design and Analysis. ANOVA showed significant variation among regions for stomatal frequency (Table II-12). The magnitude of variance at the population level was substantial but only significant at $\alpha=0.076$. The probable cause of the variance among populations was the large difference between one of the southern populations (Bruderheim, $\bar{x} = 1587$ stomata/mg) and the extreme northern population (Reid Lake, $\bar{x} = 732$ stomata/mg). The variance among seedlings, within population, was highly significant and demonstrates the existence of a large variance in stomatal frequency among different genotypes within populations.

We delved further into the nature of the regional variation in stomatal frequency with a multiple-stage test of differences among regional means (Tukey's Studentized Range or HSD Test, Luginbuhl and Schlotzhauer 1987, page 599). Figure II-4 shows that the north and south regions were significantly different but that all other pairs of means were statistically homogeneous. Provenances at the northern and southern edges of the range showed differentiation for stomatal frequency - low in the north, high in the south. The two populations of the south-central region had virtually identical mean values for stomatal frequency (989 vs. 986 stomata/mg). Those populations were separated by a straight-line distance of only 15 km.

The macro-climates under which these populations exist in nature differ in several respects (Table II-13). Reid Lake jack pine are exposed to the lowest mean annual total precipitation and the lowest mean growing season (May-Sept.) precipitation of any of the populations. The Bruderheim population receives the second highest level of precipitation. The Bruderheim parent population exists in the warmest environment and Reid Lake jack pine occupy the coolest climate.

We also examined the effect of overall leaf size on stomatal frequency in order to ascertain whether or not variation in stomatal frequency might have simply been a function of variation in whole leaf mass. Analysis of covariance, with total leaf dry mass as the covariate, showed that stomatal frequency was not correlated with the mass of the whole leaf. This finding eliminated the possibility that regional differences in stomatal frequency were merely a function of differences in leaf size.

To investigate further the notion that differences in stomatal frequency may be ecologically and evolutionarily consequential, we tested the hypothesis that stomatal frequency was negatively correlated with stoma length. This question of correlation is relevent because if changes in the frequency of stomata are counterbalanced by inversely proportional changes in the mean aperature of stomata (and the mean perimeter and cross-sectional area), then the net change in conductance and drought resistance may be zero. Measurements of stoma length were taken on seedlings originating from one population per region. ANOVA of these data showed no differences in stoma length among regions (Table II-14). Stoma size remained constant regardless of the latitude of the seed source from which the seedlings were derived. Stoma size did vary among seedlings, within region, and among individual leaves taken from the same seedling. The predominance of the among-seedling variance component may be considered as preliminary evidence that much of the genetic variation in stoma size in jack pine manifests at the spatial scale of tree to adjacent tree, within populations.

The survivorship of cohorts of seedlings from the individual populations and regions was monitored over the course of the experiment at the Bruderheim garden. Regional survivorship curves are shown in Figure II-5. Two models were tested for their goodness-of-fit to the survivorship data; a negative exponential model (Deevey type II curve) and a power function model (Deevey type III curve) (Deevey 1947, Silvertown 1982, Ch. 3). If survivorship is Deevey type II then the proportion of the population dying per unit time remains constant. If survivorship is Deevey type III then a decreasing proportion of the population dies, per unit time, as the cohort ages.

We used linear regression analysis to examine the relationship between log survivorship and time (to linearize a negative exponential curve) and log survivorship and log time (to linearize a power curve). Because the log-log regressions had higher R^2 values for each of the regional survivorship curves, we concluded that the survivorship of all regional cohorts was better described by a power function. Fewer and fewer plants died per unit time as the cohorts aged. However, the mathematical description of the form of the survivorship curves, with a single function, did not adequately characterize the temporal trend in mortality. Survivorship in the first growing season, up to October 19, 1989 (day 142), was well described by the power function but the first winter and the second growing season were characterized by a constant proportional mortality, a pattern of survivorship suggestive of a negative exponential model. These quantitative descriptions of the survivorship curves are tentative because of the minimal number of data points used in the mathematical examination of curve shape.

The power function regression lines of the regional cohorts were compared using the methods of Weisberg (1985, p. 180-84). The south-central cohort had significantly higher survivorship (or significantly lower mortality) than the north region cohort. All of the other between-region comparisons indicated homogeneity of regression lines and survivorship. We also grouped the south with the south-central cohort and the north with the north-central cohort and compared the survivorship curves of these composite northern and southern regions. The synthetic regions had homogeneous survivorship curves at $\alpha=0.05$ but the southern region did show significantly higher survivorship at the less stringent $\alpha=0.10$.

Mortality, monitored by cause, was split into three categories (Table II-15). If seedlings were severely wilted or shrivelled, the cause of death was recorded as drought. If seedlings had been browsed, or the stem cut, the cause of death was recorded as herbivory. Some seedlings were killed through burial by rain-spattered soil particles. With one exception, every cohort showed a very even split between herbivory and drought as the cause o[redacted] death. The exception was the north-central cohort which suffered substantially greater mortality due to herbivory. From these data it was clear that differences in the cause of mortality could not be invoked to explain the significant difference in overall mortality between the north and south-central regional cohorts at Bruderheim.

Figure II-6 shows the temporal trend in sources of mortality over two seasons at the Bruderheim garden. Herbivory was the dominant source of post-emergence mortality during the first growing season and drought was the major cause of death in the second growing season.

Discussion

Our results provided evidence of genetic differentiation between northern and southern populations of jack pine. The constancy of the relative order among regions, across both field and controlled-environment experiments, was particularly clear from an examination of the squared Mahalanobis distances among regions in both types of experiments. This multivariate approach to summarizing genetic differentiation along a cline was first employed by Squillace (1966) in his work with slash pine (*Pinus elliottii*). The correlation between squared Mahalanobis distances and latitude indicated that the magnitude of geographical separation was an indicator of the extent of genetic differentiation between populations.

Northern populations invested comparatively more in root biomass than did southern populations. The general lack of significance of individual root system morphological variables was consistent with the prime difference between the northern and southern regions being the larger shoot systems among provenances from the south. Greater root/shoot ratios were apparent in the north with (in the greenhouse experiment) or without (in the reciprocal transplant field experiment) the use of seed mass as a covariate, demonstrating that underlying population genetic differences were not obscured by seed mass effects in the field experiment.

The stability of root/shoot biomass ratios under drought stress in the greenhouse experiment showed that jack pine seedlings did not respond to drought with greater investment in the root compartment. Rather, jack pine depends on a strategy of avoiding desiccation by investing in root growth prior to the onset of drought. The lack of difference in mean biomass ratio between the two reciprocal transplant gardens exemplifies the stability of biomass allocation even between widely separated and environmentally heterogenous field locations.

The lack of interaction between region or population and watering treatment indicated that the comparative drought resistence of regional gene pools may be constant under both well-watered and drought conditions. A consequence of this lack of genotype X environment interaction is that populations currently possessing a greater development of characteristics that enhance drought resistence are likely to maintain their rank order for these traits, compared to other populations, in the face of a trend to greater aridity in the boreal forest.

The importance of field experiments as checks on the reliability of results from greenhouse experiments was highlighted by the differences between field and greenhouse root/shoot biomass ratios. We expect that field-grown seedlings probably possessed a closer correspondence to the plant material actually exposed to natural selection across the boreal forest. Work on the effects of photon flux density (PFD) on growth have shown that PFD can have significant effects on the root/shoot biomass ratio of young birch (*Betula* spp.) seedlings (Wayne and Bazzaz 1993). Differences in irradience regimes between our field and greenhouse experiments may have led to the observed differences in biomass allocation. Variation in the species of mychorrizal fungi, and their degree of colonization of the root systems, between greenhouse and field environments may also have influenced the comparative root/shoot biomass ratios in the two experimental environments.

Evidence was presented that northern populations had fewer stomata per unit leaf mass than southern populations. This latitudinal trend in jack pine was similar to that found for number of stomata/mm of leaf in *Pinus strobus* (Mergen 1963). We also demonstrated that stoma size was not correlated with stomatal frequency so that any physiological effects of increased frequencies would probably not be vitiated by counter-balancing effects from decreases in mean stomal aperture. The frequency of stomata was of interest because of the role of stomata in defining the upper bounds of conductance for both CO_2 and H_2O. These conductances set the upper limits for rates of photosynthesis and water loss. The north region populations had remarkably low stomatal frequency plus relatively high biomass investment in the root compartment. Both features should contribute to enhanced seedling drought resistance among jack pine in the far north. The high stomatal frequency and relatively low investment in root biomass in south region populations should confer relatively less resistance to drought.

What can we infer about the evolution of drought resistance traits in the north and south regions of the boreal forest? We believe our results indicate that one or more features of the far northern environment have selected for suites of traits that confer enhanced drought resistance ability. It may be that the greater aridity of the north has been enough, by itself, to drive the evolutionary trajectory in the direction observed. However, it is important to recall that Wright (1978) has shown that populations with continuous distributions will undergo genetic differentiation purely as a result of isolation by distance (i.e. without the action of natural selection) if gene dispersal is sufficiently restricted.

Climate change has profound evolutionary implications for boreal forest species that are genetically structured for traits contributing to drought resistance. If winters become milder, with less snowfall and spring runoff, then establishing populations will be under increased selection for high drought resistance. An increase in mean growing season temperatures may also increase selection intensities for traits that promote greater efficiency in the use or conservation of water. A rapid increase in the aridity of the boreal forest climate might contribute to the extirpation of local populations of jack pine. Based on our data, jack pine populations in the north appear to be better equipped than southern populations to withstand additional drought stress during early establishment and growth.

Solomon (1992) has argued that rapid climate change should favour trees that, like jack pine, reproduce at an early age and are shade intolerant; trees that are able to reproduce and grow in the open, savanna-like vegetation that may develop as the boreal forest is transformed by climate change. It has also been suggested that climate change may cause an increase in fire frequency in the boreal forest (e.g. Wein 1990). While most discussion of the ecological effects of climate change centres around the response of whole species or biomes, genecologists may note that the intraspecific effects of rapid environmental change have as many interesting ramifications for populations and genetic structuring as for species and merit the same consideration as species-level questions.

The lack of regional gene pool-by-environment interaction in the reciprocal transplant experiment, at both the garden and the block within garden levels, is evidence that the comparative growth of regional populations is probably constant across sites at several spatial scales in the boreal forest. This is a fundamentally important point for understanding the evolutionary consequences of genetic structuring within the gene pool of jack pine. Strong interaction between region or population and garden or block could lead to a unique ordering of the comparative growth (and potential survival) of the regional cohorts depending on the characteristics of the micro- (i.e. block) and/or macro- (i.e. garden) environment (including climate). The constancy of the growth of cohorts, relative to one another, simplifies our expectations of the evolutionary pathway during environmental change to one which is congruent with the stable ordering of the comparative growth of cohorts seen in the experiments.

The far north has a more arid climate than the far south in the present-day boreal forest of western Canada (Environment Canada 1981).

If we take our cue from the existence of current clinal differences in drought resistance traits, then precipitation, rather than temperature or degree growing days, would seem to have been the selective factor in evolution. The climate of western Canada appears to have been more arid than at present for significant periods during the last 10,000 years (see Zoltai and Vitt 1990 or MacDonald 1987). The amelioration of the climate in the middle to late Holocene produced the current climatic gradient of increasing dryness from south to north in the boreal forest. This gradient was favorable to the development of, and the current maintenance of, accentuated seedling drought resistance characteristics in the north.

Our data provide some evidence that cohorts of seedlings from the home garden region or the adjacent regions experienced lower mortality than cohorts from regions geographically remote from the reciprocal transplant garden. We interpret this as evidence of adaptation to site. This result corroborates the findings of Jeffers and Jensen (1980), for 20-year-old cohorts, that seed sources from near the garden site have the highest rate of survival in reciprocal transplant experiments with jack pine.

The conformation of survivorship in the first season to a power function model was consistent with the hypothesis that density-dependent factors dominated mortality in the first summer of growth. The preponderance of herbivore-caused deaths in the first growing season also supported this hypothesis. When the cohorts of seedlings first emerged, their densities were high and herbivores probably required little effort to consistently find the small trees. As the density of pine seedlings fell, herbivores would have required more time to locate seedlings and the proportion of seedlings dying per unit time would have decreased, as predicted by a power function model. Survivorship in the second growing season was more accurately described by a negative exponential model. Drought stress surpassed herbivory as the major cause of death in the second season. Drought stress is largely a density-independent source of mortality and we would expect a constant proportional loss per unit time as death tends to occur as a random event when mortality is independent of seedling density. We suggest that survivorship in young jack pine may not be adequately explained by a single model of mortality but might be more accurately characterized by a more complex mathematical description with greater fidelity to the unfolding of actual biological events. The temporal shift in the principal source of mortality found in our study was opposite to that found by Waters et al. (1991) for Douglas-fir. Weather effects dominated first-year mortality, and herbivory and disease dominated mortality in the second year for Douglas-fir. This contrast in results emphasizes the need to bear in mind that the origin of mortality is likely to vary depending on species and environment.

The delay of seedling emergence by two high temperature-low precipitation events, of 4 to 5 days duration each, at the Reid Lake and Sandy Lake gardens illustrates one of this species' responses to drought. The delay to the following growing season occurred despite the overall above-average precipitation for the growing season of 1989. It appears that short-duration weather events may be instrumental in controlling important demographic events, like emergence, in jack pine. These short-lived climatic events may play an important role in determining the demographic response of plant species to anticipated climate change in the boreal forest.

Tables

TABLE II-1. Differences among regional cohorts according to univariate covariance analyses of the greenhouse common garden experiment (covariate=seed mass). Regional means with one or more following letters in common were not significantly different (Prob>F=0.05). All comparisons were pre-planned and orthogonal. Total number of seedlings used in analysis = 317.

		Regional Least Square Means	
Variable	Region[1]	Linear Scale[2]	Log 10 Scale ± SE
Root Biomass (mg)	N	13.1 a	1.117 ± 0.028
	NC	10.6 b	1.026 ± 0.023
	SC	12.8 a	1.106 ± 0.024
	S	11.4 ab	1.059 ± 0.026
Shoot biomass (mg)	N	28.4 a	1.453 ± 0.020
	NC	28.7 a	1.458 ± 0.017
	SC	32.4 b	1.510 ± 0.018
	S	33.2 b	1.521 ± 0.019
Root/Shoot Biomass Ratio	N	0.47 ± 0.014 a	-
	NC	0.39 ± 0.012 bc	
	SC	0.41 ± 0.012 b	
	S	0.36 ± 0.013 c	

[1] N = North (62.5°N), NC = North-central (59.8°N), SC = South-central (57.4°N), S = South (54.0°N).

[2] Analyses for root and shoot biomass performed on $\log_{10}$ transformed values, back-transformed means presented for ease of interpretation. Analysis of biomass ratio performed on untransformed values.

Table II-2. Results of multivariate covariance analysis (covariate=seed mass) from the common garden greenhouse experiment. Dependent variables were root biomass, shoot biomass, and root/shoot biomass ratio. The F, DF, and Prob>F shown are for Wilk's Lambda, but the other common eigenvalue tests in MANCOVA were in agreement where Wilk's Lambda showed a significant factor. All three-way interactions were non-significant. Total number of seedlings used in analysis = 317.

Factor	Numerator DF	Denominator DF	F	Prob > F
BLOCK	3	290	2.6	0.05
REGION	9	706	5.2	0.0001
BLK X REG	9	706	1.0	0.46
TREATMENT	3	290	18.0	0.0001
BLK X TRT	3	290	0.8	0.49
REG X TRT	9	706	0.9	0.49
POP (REGION)	12	768	2.3	0.0065
BLK X POP	12	768	1.2	0.27
TRT X POP	12	768	0.9	0.53

Table II-3. Pre-planned, orthogonal tests between regions for the significance of differences in the multivariate complex of biomass variables from the common garden greenhouse experiment. DF for all contrasts were numerator = 3 and denominator = 290.

Regional Contrast	F	Prob > F
North vs. South	13.0	0.0001
North vs. South-central	5.1	0.002
North vs. North-central	8.0	0.0001
North-central vs. South-central	2.6	0.05
South vs. North-central	2.7	0.05
South vs. South-central	3.5	0.02

Table II-4. Raw canonical coefficents contributing to the canonical variables CAN1 and CAN2 from the canonical discriminant analysis of biomass variables, root architecture variables, and all the biomass and root architecture variables together, from the common garden greenhouse experiment. Correlations between canonical variables and region were significant at Prob >F = 0.0006 or less for CAN1 and 0.036 or less for CAN2. The correlation between CAN3 and region was not significant (Likelihood Ratio Test, Luginbuhl et al. 1987, p. 183). Variables within variable complexes are arrayed in descending order of the absolute value of their contribution to the canonical variable.

Variable Complex	Individual Variables	Raw Canonical Coefficient 1 (R^2 with Region)	Raw Canonical Coefficient 2 (R^2 with Region)
BIOMASS VARIABLES	Rt/St Biomass Ratio[1]	-6.76	13.24
	Shoot Biomass	3.50	8.58
	Root Biomass	0.83	-4.97
		(R^2=0.22)	(R^2=0.036)
ROOT ARCHITECTURE VARIABLES	Secondary Root Length	3.57	-1.22
	Primary Root Length	2.19	-2.42
	# of Tertiary Roots	-0.36	0.18
	# of Secondary Roots	-0.26	0.55
	Tertiary Root Length	-0.17	1.37
		(R^2=0.069)	(R^2=0.047)
COMBINED BIOMASS & ROOT ARCHITECTURE VARIABLES	Shoot Biomass	-6.67	-1.00
	Rt/St Biomass Ratio	4.70	-1.88
	Root Biomass	0.25	4.17
	Secondary Root Length	-0.76	-3.01
	# of Tertiary Roots	0.37	0.23
	Primary Root Length	0.35	-3.86
	Tertiary Root Length	0.12	1.19
	# of Secondary Roots	0.10	0.58
		(R^2=0.26)	(R^2=0.062)

[1] Root/shoot biomass ratio.

Table II-5. Squared Mahalanobis distances between regions in the greenhouse common garden experiment. Distances were derived from a canonical discriminant analysis based on a multivariate complex of both biomass and root architecture variables.

From Region	Squared Mahalanobis Distance to Region			
	North	North-central	South-central	South
North	0	0.783	1.24	2.87
North-central	–	0	0.334	1.21
South-central	–	–	0	0.955
South	–	–	–	0

Table II-6. Extent of the inhibition of root growth by exposure to drought in the common garden greenhouse experiment. Sample sizes were n = 112 for the drought treatment and n = 207 for the well-watered treatment.

Variable	Watering Treatment (mean ± SE)		Prob > F
	Drought	Well-watered	
Primary Root Length (mm)	52 ± 2.4	110 ± 3.7	0.0001
Secondary Root Length (mm)	29 ± 2.6	47 ± 3.1	0.0001
Tertiary Root Length (mm)	2.3 ± 0.6	5.4 ± 0.9	0.003
Number of Secondary Roots	13 ± 0.8	28 ± 1.4	0.0001
Number of Tertiary Roots	4.6 ± 0.83	11.7 ± 1.16	0.0001
Root Biomass (mg)	12 ± 0.6	15 ± 0.5	0.0001
Shoot Biomass (mg)	30 ± 1.4	39 ± 1.2	0.0001
Root/Shoot Biomass Ratio	0.41 ± 0.01	0.39 ± 0.008	0.17

TABLE II-7. Differences among regional populations according to univariate analyses of variance of reciprocal transplant data from the Reid Lake and Sandy Lake gardens. Regional means with one or more letters in common were not significantly different (Prob>F=0.05). All comparisons were pre-planned and orthogonal. Mean root/shoot biomass ratio was calculated from untransformed biomass values and cannot be derived using mean values for shoot and root biomass given in this table. The total number of seedlings used in the analysis was 231.

		Regional Means	
Variable	Region	Linear Scale[2]	Log 10 Scale ± SE
Root Biomass (mg)	N	88.5 a	1.947 ± 0.043
	NC	95.7 a	1.981 ± 0.029
	SC	104.5 a	2.019 ± 0.025
	S	104.7 a	2.020 ± 0.026
Shoot Biomass (mg)	N	70.2 a	1.846 ± 0.026
	NC	79.6 a	1.900 ± 0.017
	SC	84.6 ab	1.927 ± 0.019
	S	96.1 b	1.983 ± 0.017
Root/Shoot Biomass Ratio	N	1.33 ± 0.0756 a	–
	NC	1.32 ± 0.0705 ab	–
	SC	1.33 ± 0.0676 a	–
	S	1.16 ± 0.0482 b	–
Total Root Length (mm)	N	228 ± 14.4 ac	–
	NC	229 ± 10.2 ab	–
	SC	245 ± 11.8 abc	–
	S	265 ± 13.5 c	–

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[1] N = North (62.5°N), NC = North-central (59.8°N), SC = South-central (57.4°N), S = South (54.0°N).

[2] Analyses for shoot biomass performed on $\log_{10}$ transformed values, back-transformed means presented for ease of interpretation. Analysis of biomass ratio and total root length performed on untransformed values.

TABLE II-8. Differences in mean growth parameters between the Sandy Lake and Reid Lake reciprocal transplant gardens according to univariate analyses of variance. Garden means followed by different letters were significantly different (Prob>F=0.05). Mean root/shoot biomass ratio was calculated from untransformed biomass values and cannot be derived using mean values for shoot and root biomass given in this table.

		Garden Means		
Variable	Garden (n)	Linear Scale[2]	Transformed Scale ± SE	
Root Biomass (mg)	Sandy Lake (76)	130.3 a	2.115 ± 0.023	($\log_{10}$)
	Reid Lake (163)	87.3 a	1.941 ± 0.016	
Shoot Biomass (mg)	Sandy Lake (76)	101.9 a	2.008 ± 0.017	($\log_{10}$)
	Reid Lake (163)	76.4 b	1.883 ± 0.011	
Root/Shoot Biomass Ratio	Sandy Lake (76)	1.38 ± 0.0595 a	–	
	Reid Lake (163)	1.23 ± 0.0388 a	–	
Total Root Length (mm)	Sandy Lake (95)	286 ± 12.4 a	–	
	Reid Lake (171)	220 ± 6.2 b	–	
Total Number of Roots	Sandy Lake (94)	128 ± 6.8 a	10.8 ± 0.34	(Sq.Root)
	Reid Lake (171)	88 ± 3.1 b	9.1 ± 0.18	

[1] Analyses for shoot and root biomass performed on $\log_{10}$ transformed values, analysis for total number of roots on square root transformed values. Back-transformed means presented for ease of interpretation. Analysis of biomass ratio and total root length performed on untransformed values.

Table II-9. Results of multivariate analyses of variance, employing root biomass, shoot biomass, root/shoot biomass ratio, total root length, and total number of roots (i.e. sum of all secondary and tertiary roots + 1) as the dependent variables, for the reciprocal transplant data from the Reid Lake and Sandy Lake gardens. The F, DF, and Prob>F shown are for Wilk's Lambda, but the other common eigenvalue tests in MANCOVA were in agreement where Wilk's Lambda showed a significant factor. The sparseness of the data set did not allow the inclusion of interactions between garden or block and population within region. The number of seedlings used in the analysis was 231.

Factor	Numerator DF	Denominator DF	F	Prob > F
GARDEN	5	210	5.7	0.0001
BLOCK (GARDEN)	5	210	5.6	0.0001
REGION	15	580	2.1	0.0076
GARDEN X REGION	15	580	0.94	0.519
BLOCK X REGION	15	580	0.87	0.595
POPULATION (REGION)	20	697	0.68	0.850

Table II-10. Pre-planned, orthogonal tests between regions for the significance of differences in the multivariate complex of biomass and root architecture variables for the reciprocal transplant data from the Reid Lake and Sandy Lake gardens. DF for all contrasts were numerator = 5 and denominator = 210. The total number of seedlings used in the analysis was 231.

Regional Contrast	F	Prob > F
North vs. South	3.6	0.0035
North vs. South-central	1.1	0.382
North vs. North-central	1.8	0.111
North-central vs. South-central	0.8	0.522
South vs. North-central	3.5	0.0046
South vs. South-central	1.9	0.102

Table II-11. Squared Mahalanobis distances between regions in the reciprocal transplant experiment. Distances were derived from a canonical discriminant analysis based on a multivariate complex of both biomass and root architecture variables.

From Region	Squared Mahalanobis Distance to Region			
	North	North-central	South-central	South
North	0	0.305	0.268	0.836
North-central	_	0	0.133	0.589
South-central	_	_	0	0.300
South	_	_	_	0

Table II-12. ANOVA of stomatal frequency across the boreal forest with region as the fixed geographical factor in the model. Seedlings were grown in the Bruderheim common garden field experiment.

Source	DF	SS	MS	F	Prob >F
Region	3	2471108	823703	3.13	0.045
Population (region)	4	2563092	640773	2.43	0.076
Seedling (population)	23	6054041	263219	6.30	0.0001
Leaf (seedling) (residual error)	62	2588887	41756	_	_

Table II-13. Climatic normals (1951-1980) for the eight populations sampled along a north-south transect through the boreal forest. Source, Environment Canada (1981).

Population	Daily Mean Temp. (°C)	Extreme Max. Temp (°C)	Mean Total Ppt. (mm)	Mean GS[1] Ppt. (mm)	G.D.[2] Days over 5°C
Reid Lake & Mosquito Creek	-5.4	32.2	267	142	1027
Sandy Lake	-3.6	35.6	340	175	1050
Carlson Road	-3.3	35.0	349	203	1131
Bitumont Tower	-0.2	36.1	472	311	1290
Fort McKay	-0.2	36.1	472	311	1290
Prince Albert	0.1	37.8	398	265	1412
Bruderheim	2.3	35.6	423	296	1470

1 GS = growing season.

2 G.D. = Growing Degree

Table II-14. ANOVA of regional differences in stoma length along a north-south transect through the boreal forest. One population was sampled per region. Seedlings were grown in the Bruderheim common garden field experiment.

Source	DF	SS	MS	F	Prob >F
Region	3	0.000150	0.000050	0.02	0.997
Seedling (region)	11	0.031006	0.002819	4.41	0.0012
Leaf (seedling)	24	0.015339	0.000639	1.71	0.0236
Stoma (leaf) (residual error)	228	0.085020	0.000373	-	-

Table II-15. Sources of seedling mortality, by region, at the Bruderheim Garden from May 30, 1989 to October 18, 1990.

Regional Cohort	Source of Mortality		
	Drought[1]	Herbivory	Rain-spatter
South	30	31	3
South-central	28	28	0
Noth-central	28	47	3
North	24	25	0

[1] May include some pathogen-induced mortality.

Figures

FIG. II-1. Location of the sampled regions (circled numbers) and populations within regions (2 letter abbreviations). Regions were, 1=north, 2=north-central, 3=south-central, 4=south. Populations were, RL=Reid Lake, MC=Mosquito Creek, SL=Sandy Lake, PP=Peace Point, FM=Fort McKay, FH=Fort Hills, BR=Bruderheim, PA=Prince Albert.

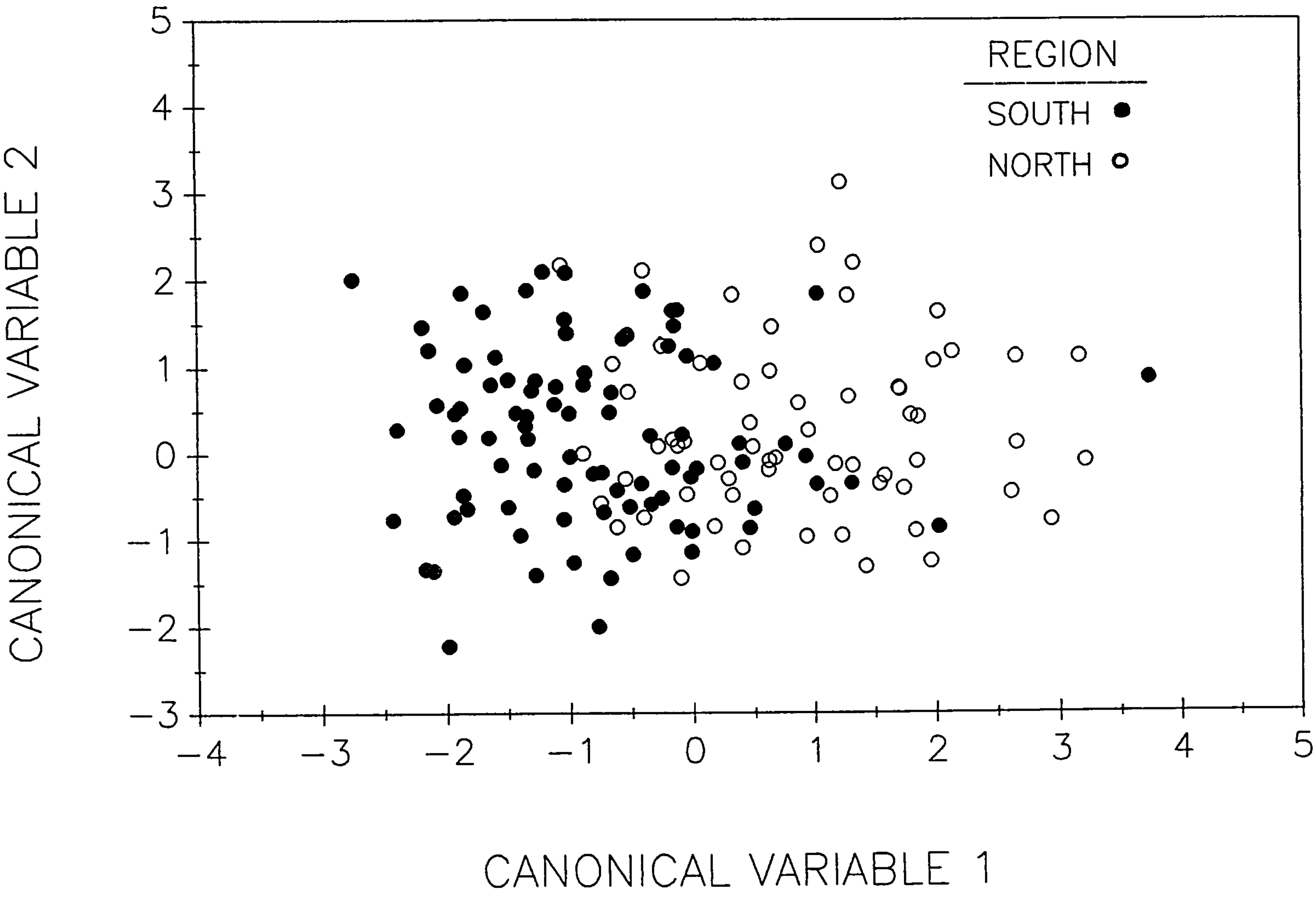

FIG. II-2. Seedlings of the south and north region cohorts from the common garden greenhouse experiment plotted on canonical variables 1 and 2 from the canonical discriminant analysis. Shoot biomass contributed the dominant negative coefficient and root/shoot biomass ratio the dominant positive coefficient to the construction of canonical variable 1.

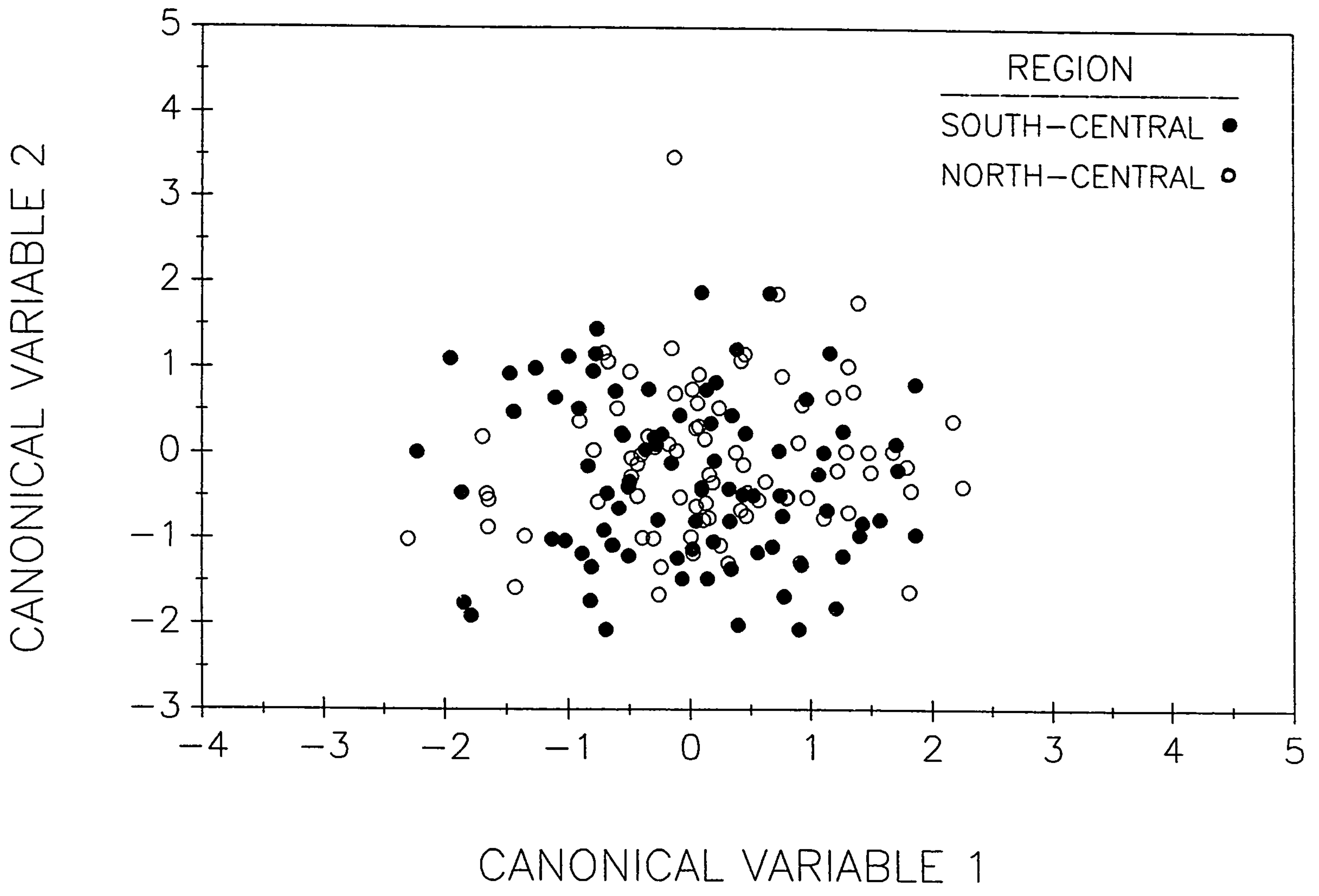

FIG. II-3. Seedlings of the south-central and north-central region cohorts from the common garden greenhouse experiment plotted on canonical variables 1 and 2 from the canonical discriminant analysis. Shoot biomass contributed the dominant negative coefficient and root/shoot biomass ratio the dominant positive coefficient to the construction of canonical variable 1.

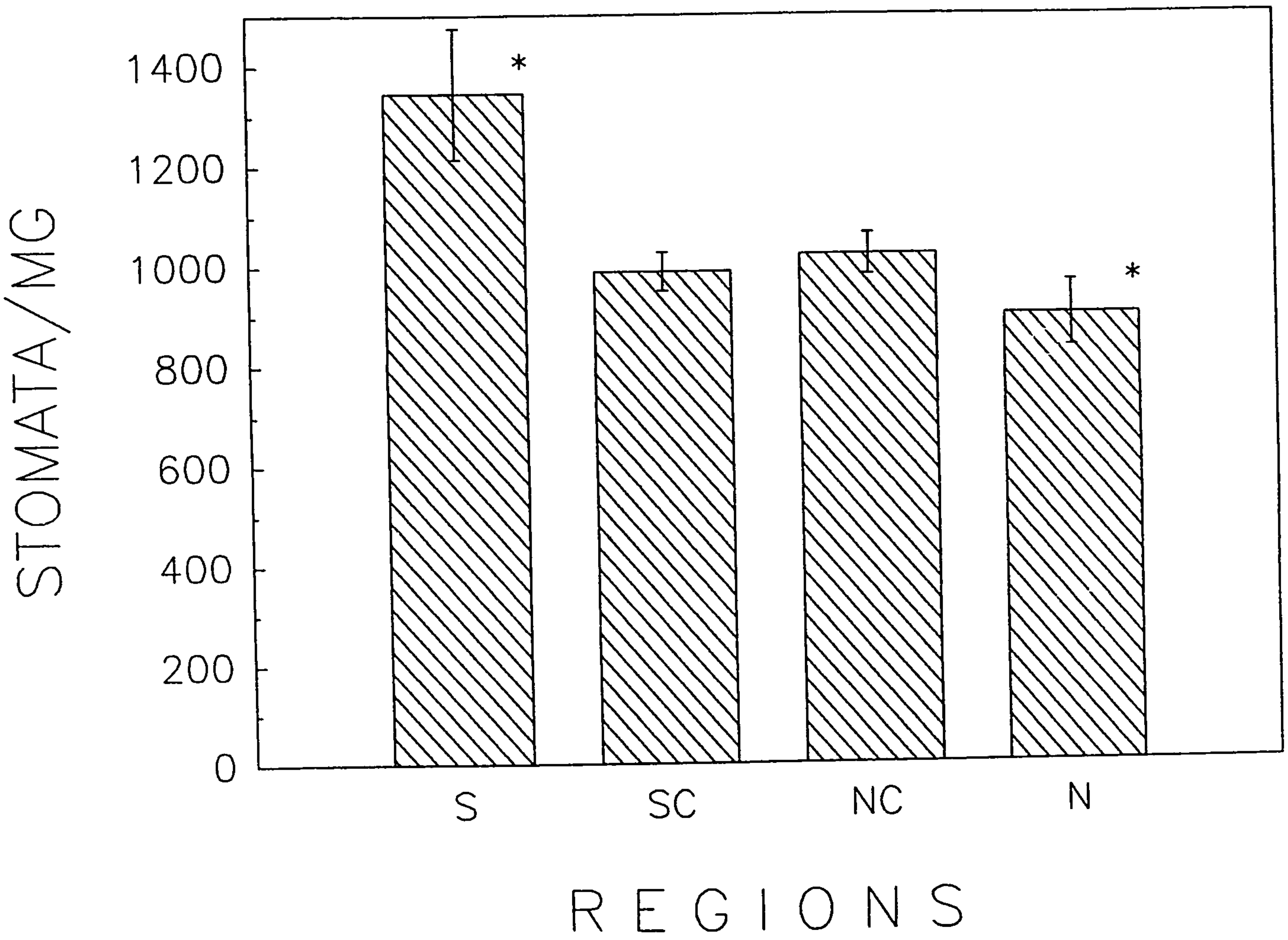

FIG. II-4. Comparative mean stomatal frequency (stomata/mg oven dry leaf tissue) of the four regional cohorts from the Bruderheim common garden field experiment. Error bars are ± 1SE. The south and north regional cohorts (asterisk) were significantly different (Prob>F<.05).

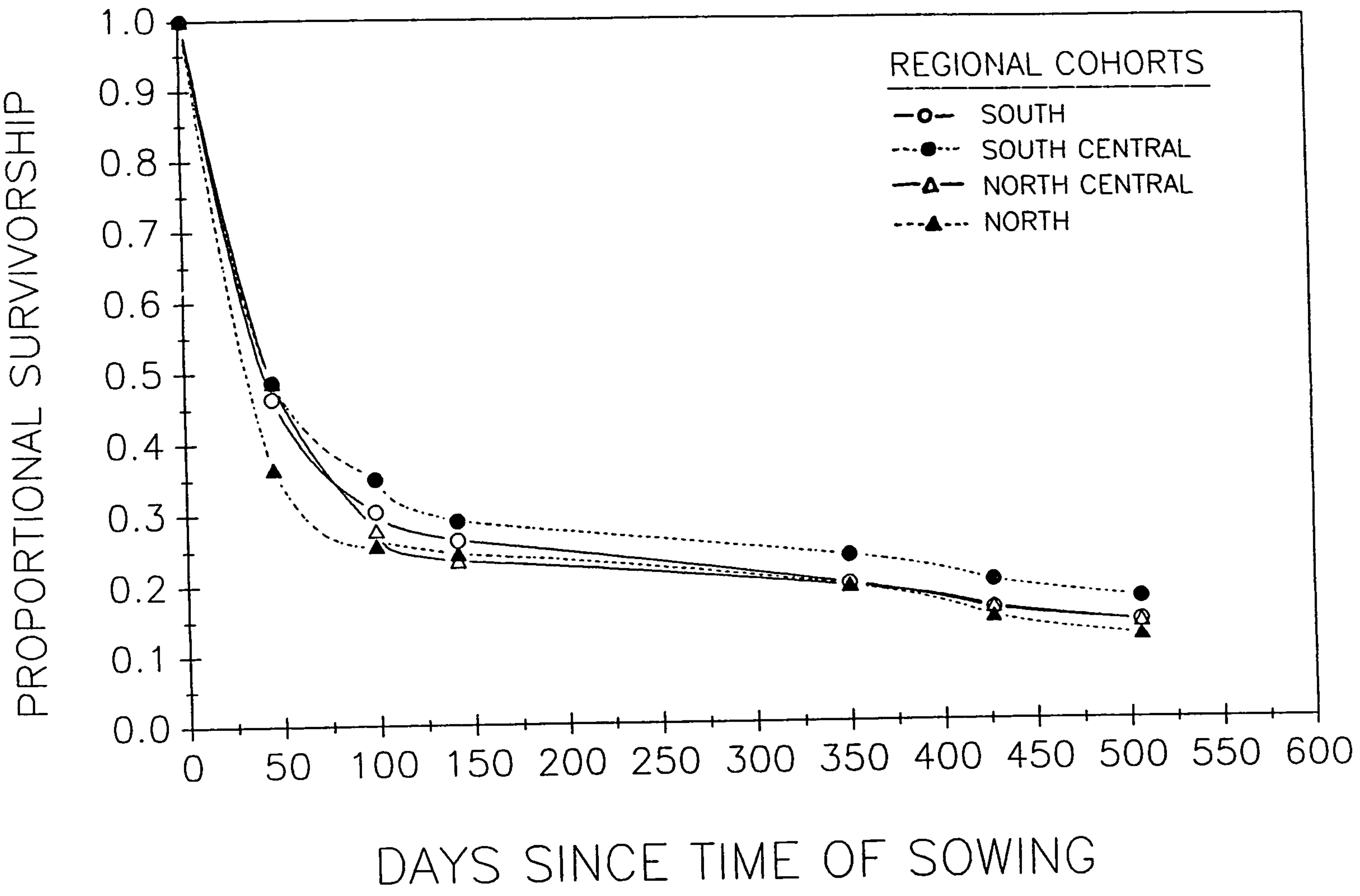

FIG. II-5. Survivorship curves for the regional cohorts from the Bruderheim common garden experiment, May 30, 1989 to October 18, 1990. Population sizes on May 30, 1989 were n=323 (north), 320 (north-central), 320 (south-central), 319 (south).

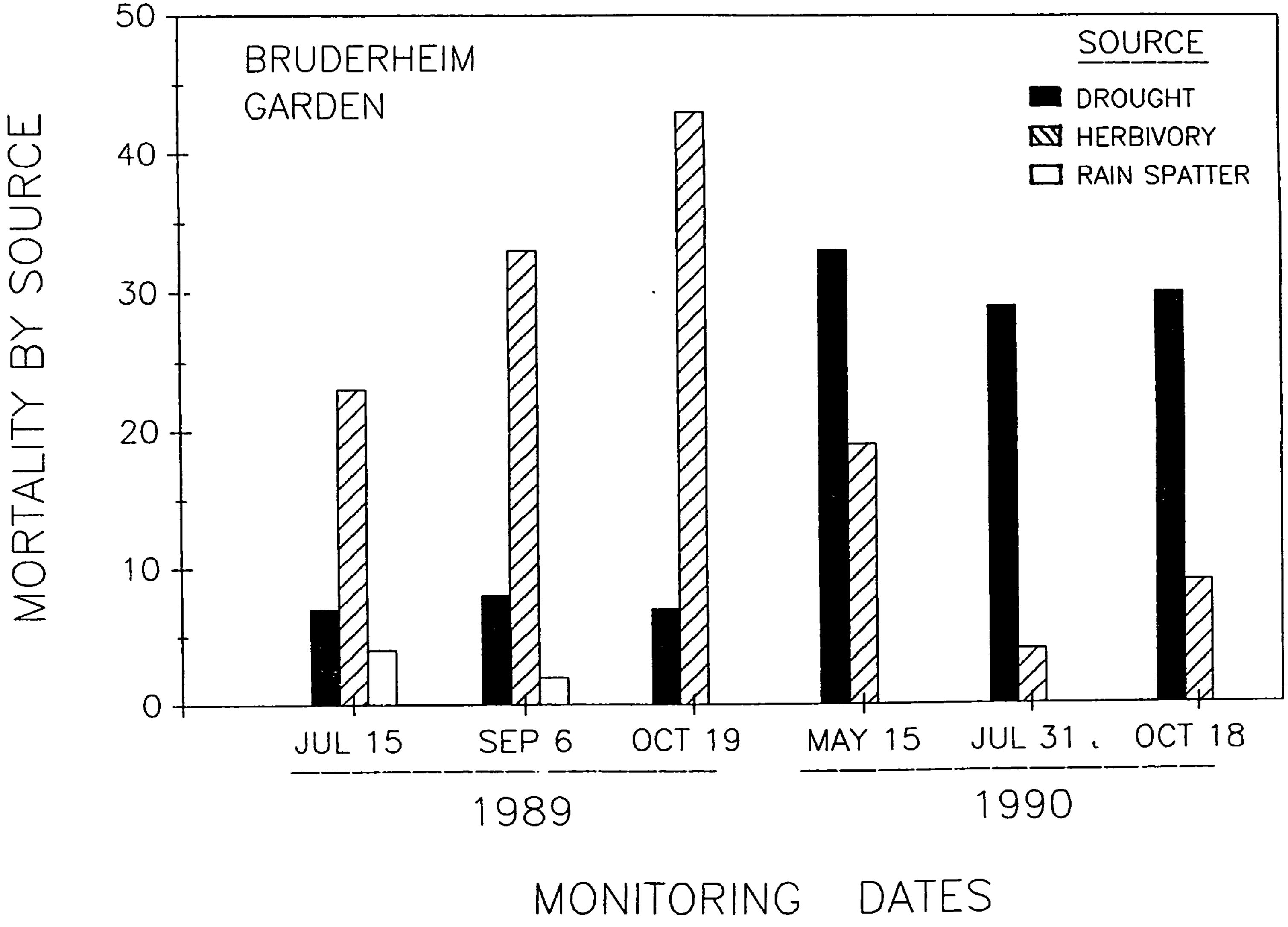

FIG. II-6. Sources of mortality in the Bruderheim common garden experiment.

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CHAPTER III

POPULATION DIFFERENTIATION IN SEEDLING RO[illegible] BETWEEN ADJACENT STANDS OF JACK PIN[illegible]

Introduction

Jack pine often grows on sands in the boreal forest, *e.g.* dune fields, eskers, sandy plains (Rowe 1972) where low soil water potential during seedling establishment may favour the evolution of drought-avoidance traits. In this paper we test the hypothesis that seedlings of dry-site mother trees produce larger root systems than offspring of adjacent wet-site trees.

Although the correlation between seedling root growth and survival has not been investigated in jack pine there is evidence (*e.g.* McClain 1973, Thomas and Wein 1985) that drought avoidance (*sensu* Levitt 1972, p. 353) in this species occurs by root extension. In loblolly pine (*Pinus taeda* L.) the survival of outplanted seedlings has been correlated with root length, root number and root/shoot biomass ratio (Larsen *et al.* 1986) and drought-resistant provenances of loblolly pine have deeper, more heavily branched rooting patterns (van Buijtenen *et al.* 1976). Such differences in seedling root length and root/shoot biomass ratio are known to be heritable in some conifers (see Khalil 1985). Habeck (1958) has shown that upland (dry-site) provenances of white cedar (*Thuja occidentalis* L.) produce much longer roots than lowland (wet-site) populations. The pattern of seedling root growth is also known to vary between populations in the same geographic region (*e.g.* root horizontality in larch (*Larix laricina* (Du Roi) K. Koch), Rehfeldt 1970).

From a tree-improvement perspective, Fayle (1978) has contended that emphasizing differences in the specifics of rooting intensity, as opposed to simply measuring root biomass, should prove more useful in efforts to genetically manipulate root development. Rehfeldt (1984) has also argued that our knowledge of the spatial pattern of habitat-related micro-evolution can contribute to improving the definition of seed zones and breeding units. The study of spatial variation in seedling drought resistance could also enlarge our understanding of how natural and silvicultural regeneration of jack pine might be affected under the new environmental conditions expected to arise (*e.g.* Kellogg and Zhao 1988) from anthropogenic climate change.

Population Selection and Experimental Design

We located jack pine growing in adjacent wet and dry environments at the southern edge of the species' range near Prince Albert, Saskatchewan, Canada. This most drought-prone (see Harrington and Flannigan 1987) part of the western Canadian range was chosen to maximize potential differences between wet and dry soil moisture environments. The dry-site soil was stabilized dune sand. The wet-site soil was sand, overlain by 5-15 cm of peat. Representative mid-growing season water contents (% oven dry, 105°C) of dry (d)- and wet (w)-site soils were: 5-6% (d) and 50-115% (w) between 0-5 cm ; 2-5% (d) and 95-220% (w) at the 5-10 cm depth. Cones were collected from the middle and upper parts of the crowns of ten trees at each site (≥ 30 m between sampled trees). The wet- and dry-site trees were separated by 50-250 m.

The 1986 seed crop (the most recent seed year at the time of collection) was used in the experiment. We randomly selected twenty-five seeds from each of the ten trees per site and then bulked the seed by site (bulked seed was used because our interest lay in population-level differentiation and not in the estimation of genetic parameters). Seeds selected at random from the bulked samples were individually weighed then sown in washed commercial sand in 30 cm lengths of 5 cm diameter PVC pipe. Day length in the controlled environment room was 17 hours (sodium and mercury vapour lamps, PPFD ≃ 320 μmol $m^{-2}s^{-1}$). Pots were randomly re-positioned twice weekly. Day and night temperatures were 25 and 15°C.

The seedlings were randomly assigned to either a well-watered treatment or a drought treatment (included to test for differential population response to water stress). The watering schedule for the two treatments was derived from preliminary pressure plate and desorption curve analysis. The well-watered plants were watered with 100 ml and the drought treatment plants with 50 ml on planting day. The well-watered plants were watered to original wet weight the second day after emergence and thereafter every four days until harvest on day 14 or 35. The drought treatment plants harvested on day 14 were watered to original weight on days 2 and 6. The drought treatment plants harvested on day 35 were watered to original weight on days 2, 6, and 14. Drought treatment plants experienced soil water potentials as low as -1.5 MPa while well-watered seedlings experienced soil water potentials as low as -0.2 MPa.

Seedlings were harvested at 14 and 35 days, post-emergence, to enable a comparison of the populations at two ages in the early establishment phase of the life cycle. Plants often experience heavy mortality in the seedling life stage (see Oppenheimer 1960, p. 114, Hett and Loucks 1976) and drought-induced mortality in jack pine is known to be highest in young seedlings (e.g. Shirley 1934). Because of heavy mortality, and the attendant possibility of high selection intensity, the seedling is the life stage in which natural selection ought to be strongest for root traits that enhance drought avoidance. Accordingly, we measured several root growth variables as indicators of comparative drought-avoidance capability that may have undergone disruptive selection in adjacent wet and dry environments. The variables measured were primary, secondary and tertiary root lengths (to the nearest mm, by ruler), the numbers of secondary and tertiary roots (>1mm in length) and oven-dry (70°C) root and shoot biomass. The ANOVA model for each harvest was:

$$Y_{ijk} = \mu + P_i + D_j + PD_{ij} + S_{k(ij)} \left\{ \begin{array}{l} i=1,2 \\ j=1,2 \\ k=1,2,\ldots,11\text{-}33 \end{array} \right. ,$$

where μ = overall experimental mean, P = population (fixed), D = watering treatment (fixed), S = individual seedling effect (residual error). Variation in (k) was due to mortality from water stress in the drought treatment.

Seedling growth is correlated with seed weight in jack pine (Yeatman 1966, Radsliff 1981) and heavier seeds are known to produce heavier seedlings with longer roots in *Pinus brutia* (Isik 1986). For this reason we employed seed weight as a covariate (after the manner of Kolb and Steiner 1989) to avoid confounding population differences in seedling growth rate with seedling size differences due to variations in maternal investment in the seed. In the analyses of covariance (ANCOVA), the term $\beta\ (X_{ijk} - \bar{X})$ was added to the model to adjust for covariance between seed weight and seedling growth (β = linear regression coefficient of Y on seed weight (X); $\bar{X}$ = overall mean seed weight). Covariance analysis was used only if the slopes of the seedling size vs. seed weight regression lines for the populations were homogeneous (*i.e.* no population X seed weight interaction, see Freund *et al.* 1986, p. 154).

We recognize that in using this procedure we are assuming either that seed weight and seedling traits are genetically independent or that seed weight is strongly influenced by environment. We have no information on the strength of genetic correlation between seed weight and seedling growth but we did find that seed weight in the dry site was significantly correlated (R=0.94, Prob>|R| <0.06) with April to June precipitation in the year of seed maturation. A similar, although statistically not significant, trend was observed in the wet-site population (Wright 1992, unpublished manuscript). Although the assumption that seed weight and seedling growth are genetically uncorrelated may be invalid, ignoring seed weight effects carries with it an equally problematic assumption - that seed weight and seedling growth are genetically correlated or that seed weight effects are absent. Our strategy will be to present the results of univariate analyses that first omit and then include seed weight as a covariate. This enables the reader to interpret the data and results under either assumption. We emphasize that the extraction of seed weight effects from post-emergence seedling growth is especially critical if, as is entirely possible, the magnitude of seed weight differences is largely controlled by environment rather than genetics.

We also tested for population differences in the parameters of the root size vs. whole plant size regression lines using the methods of Weisberg (1985, p.179-185). In this case we tested a model in which the populations were assumed to have the same intercepts and slopes (the null model) against an alternative model in which different intercepts and slopes were assumed. If there was a significant increase in residual sums of squares from using the null model, compared to the alternative, then we concluded that the populations were best described by separate regressions and thus differed in their relative investment in the root system. In all of our analyses, observed departures from homoscedasticity and normality did not jeopardize the power of the F-test according to the criteria of Scheffe (1959) and Wilcox (1987).

Results

Drought profoundly inhibited secondary and tertiary root growth (Table III-1). Note that some of the initial inhibition had disappeared by day 35. Significant correlations between primary length and secondary length (r=0.48, Prob>|r|=0.002 (day 14); r=0.44, Prob>|r|=0.004 (day 35)) in the drought treatment, but not the well-watered treatment, suggests that evasion of surface drought, via primary root exploration of deeper, wetter sand layers, was responsible for enhanced secondary root growth in larger 14-day-old seedlings and in the deeper-rooted 35-day-old seedlings. There was no evidence that exposure to water stress stimulated root growth in any way.

Seed weight was correlated with several root and shoot variables(Table III-2). Dry-site mean seed weight (4.2 mg) was also greater (ANOVA, Prob>F= 0.0003) than wet-site mean seed weight (3.9 mg). Accordingly, we employed seed weight as a covariate in multivariate and univariate analyses to control for its influence on the comparative growth of the two populations of seedlings. Because growth was strongly inhibited by drought (Table III-1) the populations were also compared using only the well-watered treatment data in some of our analyses. Correlation analysis, by treatment, showed that about one-half of all possible correlations between root growth variables and seed weight were significant in the well-watered treatment but only one-fifth were significant in the drought treatment (Prob>|r|≤0.05, not tabulated). Water stress apparently overwhelmed the maternal effect and masked its effect on seedling performance.

For the 35-day-old seedlings, multivariate analysis of covariance (MANCOVA, Freund *et al.* 1986, p. 170), employing seed weight as the covariate, indicated significant population and treatment effects but only a weak population by treatment interaction (Table III-3). MANCOVA of the 14-day-old material showed a significant treatment effect but little evidence for a population effect and no indication of a population by treatment interaction. Subsequent univariate analysis of variance (ANOVA) revealed significant differences in secondary root length and shoot biomass between the populations at day 14 but these differences disappeared when the effect of seed weight was controlled in analysis of covariance (ANCOVA) (Table III-4). However, the mean number of tertiary roots per secondary root (TSratio-a measure of the intensity of tertiary rooting) at day 35 remained significantly higher in the wet-site population even after control of the seed weight effect. Consideration of the seed weight effect also revealed a significantly higher number of tertiary roots in the lighter-seeded wet-site population (Table III-4). There was some indication that dry-site seedlings possessed greater numbers of secondary roots by day 35 but the evidence was weak (Prob>F=0.06). The populations did not differ in primary root length or root:shoot biomass ratio. No significant population X treatment interaction was found in any of the univariate analyses.

We caution the reader that our use of covariance analysis must carry with it the assumption that either seed weight and subsequent seedling relative growth rates are genetically uncorrelated or that variation in seed weight is largely a function of environment. Those readers unwilling to make either assumption should place more emphasis on the results of the univariate analyses that do not adjust for the effects of seed weight on seedling performance (column ANV, Table III-4).

The preceding analyses were designed to test the question of whether or not dry- and wet-site seedlings differed in the absolute size of their root systems. A related and equally important question was whether or not the populations differed in the size of their root systems relative to whole plant size. This problem was addressed by testing for population differences in the parameters of the root size vs. whole plant biomass regression lines (Weisberg 1985, p.179-185). We used secondary root length and root biomass (day 14 harvest) and number of secondary and tertiary roots and tsratio (day 35 harvest) to compare the relative size of the dry- and wet-site root systems.

The comparison of regression lines showed that tsratio (mean number of tertiary roots per secondary root) in the day 35 harvest was the only variable for which the population regression lines (*i.e.* tsratio vs. total seedling biomass) were significantly different (Prob>F=0.005). The slope for the dry-site population was not significantly different from zero (*i.e.* the mean number of tertiary roots per secondary root did not change significantly with seedling size, up to age 35 days; r^2=0.04, Prob>F=0.19). The wet-site regression (r^2=0.25, Prob>F=0.002) had a positive slope indicating that as wet-site seedlings grew there was a significant increase in the mean number of tertiaries per secondary root.

Discussion

The results of multivariate and univariate analyses both indicated that differentiation in root growth has occured between the two populations. The univariate analyses demonstrated that the higher number of tertiary roots and the higher ratio of tertiary to secondary roots in the wet-site population were the only variables that were, by themselves, responsible for a significant difference between the populations. Root size vs. whole seedling size regressions provided evidence that the number of tertiary roots per secondary root increased with seedling size in the wet-site but not the dry-site population. 14-day-old seedlings do not have tertiary roots and this may explain why differentiation between the populations was only very weakly expressed in 14-day-old seedlings but clearly evident in the 35-day-old material. The higher number of tertiary roots in the wet-site population and the absence of any real differences in other root variables speaks against our hypothesis that natural selection by drought has fostered the evolution of larger seedling root systems in the dry-site population. From an adaptationist perspective, the greater intensity of tertiary rooting in the wet-site population could conceivably enhance nutrient or oxygen uptake in the spring when the soil is likely to be water-logged and anoxic in the wetter micro-environment. In this regard, the number of roots may be more critical than root length because most water, oxygen and nutrient uptake probably occurs near the unsuberized root tips.

The observed lack of genetic differentiation between the dry- and wet-site populations for most root growth variables may be due to several factors. The differential between environments in selection coefficients for root growth traits may be too small to overcome the homogenizing influence of gene flow between sites. Another possibility is that drought may be such a strong stress that dry-site seedlings depend on escape rather than tolerance or avoidance (*sensu* Levitt 1972) to survive dry periods. Escape could be in time (*i.e.* only seedlings established in drought-free periods survive the first growing season) or space (*i.e.* only seedlings in shaded or moist micro-environments survive drought periods) or both.

Differences in the vigor of the wet- and dry-site pollen pools may also be inhibiting genetic divergence. Recent work in wild radish (*Raphanus raphanistrum* L.) has shown that pollen from plants of low-nutrient environments fertilizes fewer ovules than pollen from high-nutrient environment plants when the two pollen pools are in competition (Young and Stanton 1990). A similar phenomenon in jack pine could mitigate against genetic differentiation between wet- and dry-site populations as wetland and dryland sites typically show differences in soil nutrient levels. Differentiation would be inhibited by enhanced gene flow as pollen from the richer environment would be favored in the fertilization of trees in the more nutrient-poor environment, relative to poor-site pollen.

Forest fires may also have played an evolutionary role in preventing differentiation. If the wet area burned less frequently than the dry, then wet-site jack pine might eventually disappear from the site because they are shorter-lived than the dominant black spruce. The next fire to burn both wet and dry environments would release seed from the dry-site trees (jack pine have serotinous cones). With only upland jack pine seed available for colonization of the wet site, the micro-evolutionary clock would be set back to zero.

Baker (1972) and Salisbury (1974) both proposed that evolution in dry environments has favored heavier seed weights and their attendant larger root systems. Our results confirmed this pattern. We emphasize that the advantages to root growth of large-seededness were most strongly expressed when water stress was weak or absent. If seed size plays a role in determining relative drought resistance it apparently will do so by influencing seedling root size prior to the onset of water stress. Consequently, the maternal effect would be operative in the sense of enabling drought avoidance (by root extension prior to stress) as opposed to drought tolerance (by continued growth during stress).

Tables

Table III-1. Effect of watering treatment on root and shoot size. Prob>F refers to H_0: $\text{mean}_{\text{Drought}} = \text{mean}_{\text{Well-watered}}$. See Experimental Design for description of ANOVA model.

		Day-14 Harvest			Day-35 Harvest		
Variable	Treatment	Mean (SE)	n	Pr>F	Mean (SE)	n	Pr>F
Primary Root Length (mm)	Drought	40 (1.9)	41	0.21	107 (2.5)	42	0.16
	Well-w[a]	37 (0.8)	63		111 (2.1)	60	
Secondary Root Length (mm)	Drought	1 (0.4)	41	0.0001	69 (3.5)	40	0.0001
	Well-w	9 (0.8)	63		166 (7.7)	57	
Number of Secondary Roots	Drought	0.5 (0.19)	41	0.0001	31 (1.7)	40	0.21
	Well-w	3.3 (0.26)	63		33 (1.0)	57	
Tertiary Root Length (mm)	Drought	-[b]			0.1 (0.04)	40	0.0001
	Well-w	-			5 (1.0)	57	
Number of Tertiary Roots	Drought	-			6 (1.3)	40	0.0001
	Well-w	-			40 (2.7)	57	
TSratio[c]	Drought	-			0.17 (0.04)	40	0.0001
	Well-w	-			1.25 (0.01)	57	
Root Biomass (mg)	Drought	1.4 (0.09)	32	0.0001	7.2 (0.28)	35	0.02
	Well-w	2.0 (0.07)	44		8.4 (0.30)	54	
Shoot Biomass (mg)	Drought	3.8 (0.18)	32	0.002	10.8 (0.45)	34	0.12
	Well-w	4.9 (0.27)	44		11.8 (0.47)	48	
Root/Shoot Biomass Ratio	Drought	0.38 (0.015)	32	0.03	0.71 (0.03)	31	0.76
	Well-w	0.45 (0.025)	44		0.71 (0.02)	46	

[a] Well-watered treatment.

[b] No tertiary roots at 14 days.

[c] Number of tertiary roots/number of secondary roots.

Table III-2. Partial correlation coefficients between seedling size variables and seed weight, corrected for the fixed effects in the model, at ages 14- and 35-days.r = partial correlation coefficient.Prob>|r|= prob>|r| under H_0:rho=0. D.F.=71.

	Day 14 Harvest		Day 35 Harvest	
Variable	r	(Pr>|r|)	r	(Pr>|r|)
Secondary Root Length	-0.08	(0.50)	0.58	(0.0001)
Number of Secondary Roots	0.25	(0.03)	0.02	(0.86)
Tertiary Root Length	-[a]		0.33	(0.005)
Number of Tertiary Roots	-		0.46	(0.0001)
TSratio[b]	-		0.54	(0.0001)
Root Biomass	0.37	(0.001)	0.24	(0.04)
Shoot Biomass	0.24	(0.04)	0.50	(0.0001)
Root/Shoot Biomass Ratio	0.17	(0.32)	-0.38	(0.0009)

[a] No tertiary roots on 14-day-old seedlings.

[b] Number of tertiary roots/number of secondary roots.

Table III-3. Multivariate analyses of covariance (MANCOVA) for seedling root and shoot size variables. D.F. for 14-day-old seedlings = 7 (numerator), 65 (denominator); for 35-day-old seedlings D.F. = 10 (numerator), 62 (denominator). Covariate was seed weight.

Age at Harvest	Model[a] Effect	F	Prob>F[b]
14 Days	Pop	1.9	0.087
	Trt	26.2	0.0001
	Pop X Trt	0.5	0.81
	Seedwt	2.7	0.018
35 Days	Pop	2.7	0.008
	Trt	13.7	0.0001
	Pop X Trt	2.0	0.044
	Seedwt	7.3	0.0001

[a] Dependent variables in the model were: 1) 14 days - length of primary root, depth of primary, length and number of secondaries, root and shoot biomass and root:shoot biomass ratio, 2) 35 days - same as 14 days with addition of length and number of tertiaries and ratio of number tertiaries: number of secondaries.

[b] Wilk's lambda, Pillai's trace, the Hotelling-Lawley trace and Roy's greatest root all had the same F, degrees of freedom and Prob>F in this model.

Table III-4. Significance of differences in root and shoot size between dry- and wet-site populations under univariate analysis of variance (ANV, Prob>F) and analysis of covariance (COV, Prob>T, comparing least squares means). Covariate = seed weight. Statistical models are described under Experimental Design.

			Day 14			Day 35		
Variable	Trt[a]	Pop	Mean (SE)	ANV	COV	Mean (SE)	ANV	COV
Secondary Root Length (mm)	W	Dry	10.2 (0.20)	0.04	0.14	124 (9.9)	0.78	0.74
		Wet	7.4 (0.95)			128 (9.9)		
Number of Secondary Roots	W	Dry	3 (0.3)	0.53	0.88	35 (1.4)	0.06	CNS[b]
		Wet	2 (0.3)			31 (1.5)		
Number of Tertiary Roots	B	Dry	-[c]	-	-	22 (3.0)	0.13	0.03
		Wet	-			30 (3.8)		
TSratio[d]	B	Dry	-[c]	-	-	0.6 (0.08)	0.02	0.002
		Wet	-			1.0 (0.14)		
TSratio	W	Dry	-[c]	-	-	1.0 (0.09)	0.01	0.0001
		Wet	-			1.5 (0.16)		
Root Biomass (mg)	B	Dry	1.9 (0.10)	0.08	0.52	7.8 (0.26)	0.60	-[e]
		Wet	1.7 (0.10)			8.1 (0.36)		
Shoot Biomass (mg)	B	Dry	5.0 (0.31)	0.05	0.23	11.6 (0.42)	0.49	0.87
		Wet	4.1 (0.21)			11.2 (0.54)		

[a] Treatment, W=well-watered only; B=both well-watered and drought.

[b] Covariate not significant.

[c] No tertiary roots on 14-day-old seedlings.

[d] Number of tertiary roots/number of secondary roots.

[e] Slopes of root biomass vs seedwt regressions were heterogeneous for dry- and wet-site populations.

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CHAPTER IV

THE ECOLOGY AND EVOLUTION OF SEED MASS IN JACK PINE *(PINUS BANKSIANA)*

Introduction

The basic theme of our paper is that genetic variation in seed mass is evolutionarily and ecologically significant because of its influence on seedling survivorship and the heritability of seed mass. The early growth of jack pine seedlings is positively correlated with the mass of the seeds from which they arise (Wright *et al.* 1992, Yeatman 1966, Radsliff 1981). Seed mass is also known to influence germination rate and early growth in loblolly pine (Dunlap and Barnett 1983) as well as early growth in *Pinus brutia* (Isik 1986). Any early differences in seedling size resulting from inequalities in starting capital (i.e. seed reserves) are likely to be accentuated over time by logarithmic growth (see Hunt 1982) unless counteracted by other genetic or environmental effects. Thus, seedlings originating from heavier seeds may have a natural selective advantage in environments where larger size has the potential to enhance survival (e.g. droughty sites).

Within the genus *Pinus*, a correlation between maternal environment and seed mass has been reported in at least one species (*Pinus brutia*, Sefik 1965). Genetic differentiation between wet- and dry-site populations, for traits other than seed mass, is known from other conifers (e.g. micro-geographic differentiation in allozymes in *Picea engelmannii*, Mitton *et al.* 1989). Differences in seed mass have also been found among populations of Douglas-fir of different slope aspects (e.g. north vs. south) in west-central Oregon (Campbell 1979).

The analysis in Chapter III showed that rooting performance in jack pine (i.e. the length, number and biomass of roots) is positively correlated with seed mass. The extent of correlation between the size of the seedling root system and survival is unknown for jack pine but the species does appear to avoid drought by root extension (McClain 1973, Thomas and Wein 1985). In loblolly pine the survival of outplanted seedlings has been positively correlated with root length, root number and root/shoot biomass ratio (Larsen *et al.* 1986). Drought-resistant provenances of loblolly pine are also known to have deeper, more heavily branched rooting patterns (van Buijtenen *et al.* 1976). It seems plausible that jack pine populations with consistently larger seeds could well exhibit superior root growth and greater drought-resistance compared to lighter-seeded populations.

Although the interaction of genotype with environment has received much attention from quantitative geneticists (e.g. Namkoong 1979, Falconer 1981, p. 123, Skrøppa 1984) a broader interest in the problem has been re-kindled by zoologists working on genotype "reaction norms" (e.g. Scharloo 1989). In this context, norm is used in the sense of pattern or type, not in the sense of normalacy or usualness. The advantage of examining how individual genotypes react to environmental variation lies in the opportunity to illuminate the potential comparative evolutionary versatility of populations. For example, the level of diversity of reaction norms within a population could be an indicator of its range of possible responses to climate change.

Analyses of the pattern, extent, and heritability of genetic variation in seed mass (and any correlated variation in seedling growth) are evolutionarily relevent for several reasons. Genetic variation in seedling root growth and drought resistance may produce differential regeneration within or among families and populations facing the possibility of permanent changes in the soil moisture regime produced by anthropogenic climate change (see Kellogg and Zhoa 1988). Spatial heterogeneity in family or population genetic composition and fitness has profound implications for survival and evolution in a rapidly changing environment (e.g. Ayala 1965a & b and see Crow 1986, Ch. 7, especially pages 194-200). From an applied forestry perspective, Rehfeldt (1984) has argued that patterns of habitat-related micro-evolution should be considered in defining seed zones and breeding units. It has also been shown that conclusions arising from tree improvement experiments on early selection may be altered by adjusting for seed mass effects (Radsliff 1981).

Our work had three objectives, 1) to investigate the magnitude, pattern and cause of seed mass variation between jack pine of adjacent wet and dry environments and among populations of jack pine located along a 1000 km north-south transect through the boreal forest of western Canada, 2) to explore the relationship between seed mass and seedling survival in jack pine and 3) to examine the effects on seed mass of the climatic conditions in the year of seed maturation.

Population Selection and Sampling

We located jack pine populations growing in adjacent wet and dry environments near Prince Albert, Saskatchewan (53°15' N, 106°3 W) and at Sandy Lake, Northwest Territories (60°32 N, 114°34 W), Canada (see Figure II-1). The Prince Albert population was found at the southern edge of the species' range in western Canada while the Sandy Lake trees were found approximately 800 km to the northwest on the northern boundary of Wood Buffalo National Park. Climatic data indicate that Prince Albert is much droughtier (i.e. higher probability of extended dry spells) than Sandy Lake (Harrington and Flannigan 1987). The dry-environment trees of both populations were growing on very well-drained, vegetated sand dunes. The wet-environment trees near Prince Albert were growing on 10 cm of poorly-drained peat underlain by wet sand. The Sandy Lake wet-environment trees were found growing on deep peat underlain by permafrost in a palsa field (i.e. a degrading peat plateau). Cones were collected from various positions within the crowns of nine trees (i.e. families) in each environment for the southern population and from five families in each environment for the northern population (at least 30 m between sampled families). All of the seeds collected from one tree were therefore from the same maternal family and open-pollinated.

For the examination of the spatial pattern of seed mass variation along a 1000 km north-south transect through the boreal forest of western Canada eight populations (see Figure II-1) were sampled (near Prince Albert, Saskatchewan- 53°15' N, 106°3' W; near Bruderheim, Alberta- 53°52' N, 112°56' W; Fort Hills, Alberta- 57°22' N, 111°33' W;

near Fort McKay, Alberta- 57°20′ N, 111°39′ W; near Peace Point, Alberta- 59°8′ N, 112°16′ W; Sandy Lake, Northwest Territories- 60°32′ N, 114°34′ W; near Mosquito Creek, Northwest Territories- 62°30′ N, 116°28′ W; near Reid Lake, Northwest Territories- 62°30′ N, 113°35′ W). These populations were all growing in upland areas on aeolian or glacial-fluvial sands of medium texture. For the test for variation in seed mass across the boreal forest, seeds from 40 maternal families from each population were pooled and between 200 and 1800 seeds were sampled from each of the eight population pools. All seed samples were cleaned with a moving air column seed cleaner to remove unfilled seed.

Experimental Design and Analyses

Components of Seed and Cone Mass Variation

For our analysis of seed mass variation between wet and dry environments, we obtained the mass of seeds from three cones per year for 4 to 6 seed years for each tree. The age of the cone and its seeds was determined from position on the branch. Jack pine generally form a single pair of cones per branch per year. The youngest cones are closest to the branch tip. The serotinous cones remain closed and attached to the branch for many years. The number of seeds weighed per cone ranged from 4 up to the arbitrarily chosen maximum of 10. The full ANOVA model was:

$$Y_{ijk} = \mu + E_i + Y_j + EY_{ij} + F_{k(i)} + YF_{jk(i)} + C_{l[jk(i)]} + S_{m(ijkl)} ,$$

with $i=1,2;\ j=1,\ldots,4,5 \text{ or } 6;\ k=1,\ldots,5 \text{ or } 9;\ l=1,2,3;\ m=4,\ldots,10$,

where μ was the overall experimental mean, E_i was environment (fixed, wet or dry), Y_j was year (fixed), $F_{k(i)}$ was family within environment (random), $C_{l[jk(i)]}$ was cone within the year by family interaction (random), and $S_{m(ijkl)}$ was the residual error (individual seed) effect. We also undertook an analysis of cone mass using a similar model (three cones per year per tree) in which C was the residual error effect.

Reaction Norms

To study the comparative diversity of genotype reaction norms between populations we adopted an approach described by Falconer (1981) of the analysis of environmental sensitivity. To test for differences in reaction norms we undertook a regression analysis of genotype mean seed mass for specific years on the environmental value for the years. Environmental value of each of the yearly climates was calculated as the mean mass of all seeds from all genotypes from a specific soil moisture environment for that year.

Heritability of Seed Mass

Seed mass can be considered a progeny trait amenable to quantitative genetic analysis, because the embryo (i.e. the progeny) makes up a portion (10%, Kremer and Larson 1983) of the whole seed. Most workers (e.g. St. Clair and Adams 1991) have chosen to view seed mass as a maternal trait but in so doing have overlooked the influence of the diploid genotype of the embryo on seed mass. In order to discern the effects of the embryonic genotype on megagametophyte mass, and to isolate the genetic variance in embryo mass, we chose an alternative strategy and controlled for environmental variation in the non-embryonic tissues. This was accomplished by including the soil moisture environment, year and cone factors, and the year x family and the environment x year interactions in our statistical model. For the analysis of heritabilities (described below) we operated under the assumption that the residual variance in seed mass among maternal families, after statistically controlling for the environmental effects described above, was attributable to variation in either the embryo mass or the effect of the embryonic genotype on megagametophyte development.

Because non-genetic environmental variances appear in the denominator of the equation for narrow-sense heritability ($\underline{h}^2$), $\underline{h}^2$ is at least partially a function of the environmental context in which the development of the experimental subjects occurs (Narrow-sense heritability is calculated as the ratio between additive genetic variance and phenotypic variance (Falconer 1981). Additive genetic variance is the sort of genetic variation that can be passed from parents to their offspring). It is therefore often fruitful to examine the heritability of a trait in different environmental contexts to obtain a deeper understanding of the variation in this genetic parameter which helps to contour the evolutionary landscape through which a population might travel over time. To achieve this end we estimated $\underline{h}^2$ at three different spatio-temporal scales. The analyses were based on the assumption that the seeds from one maternal family were half-sibs (i.e. the seeds from a particular tree have the same mother and are assumed to have different fathers). The three demographic levels at which heritability was estimated (see Becker 1968 for the derivation of the equations) were:

1) The whole population level (i.e. all trees and years at either Prince Albert or Sandy Lake): $\underline{h}^2_i = \dfrac{2.8\,\sigma^2_f}{\sigma^2_f + \sigma^2_{yxf} + \sigma^2_c + \sigma^2_s}$

2) The whole environment within population level (i.e. all trees and years within an environment within a population): $\underline{h}^2_i = \dfrac{2.8\,\sigma^2_f}{\sigma^2_f + \sigma^2_{yxf} + \sigma^2_c + \sigma^2_s}$

3) The year within environment level (i.e. all seeds from a particular year, within an environment, within a population):

$$\underline{h}^2_i = \frac{2.8\,\sigma^2_f}{\sigma^2_f + \sigma^2_c + \sigma^2_s}$$

where σ^2_f is the among-tree (i.e. family) variance, σ^2_{fxy} is the family by year interaction variance, σ^2_c is the among-cone within year by family variance and σ^2_s is the among-seed within cone (error) variance. In equation 1 the fixed factors were 3 - region, environment and year. In equation 2 there were only 2 fixed factors - environment and year. Because the fixed main effects do not appear in these equations (only random sources of variance were included), equations 1 and 2 are the same except for the number of families included in the analysis.

In the abscence of inbreeding, σ^2_f, in the numerator, is multiplied by four in the calculation of individual tree heritabilities (Falconer 1981). Natural selfing among jack pine (Cheliak *et al.* 1985) may also produce a certain proportion of full sibs among open-pollinated progeny. This bias in the estimation of $\underline{h}^2$ can be compensated for by decreasing the constant by which σ^2_f is multiplied in the numerator. With family sizes of 80-120, a selfing rate of 0.12 (from Cheliak *et al.* 1985) and assuming approximately 20 pollen parents per family the appropriate constant is ≈ 2.8 (F. Yeh 1991, unpublished tables). Values of $\underline{h}^2$ range between 0 and 1.

Seed Mass and Seedling Survivorship

To test for a relationship between seed mass and seedling survivorship we undertook to monitor the survivorship of seedlings originating from seeds of known mass. Seeds from the eight populations listed in the description of the geographical transect experiment were planted in 3 common gardens located in naturally occurring jack pine forests on stabilized sand deposits near Bruderheim, AB, Sandy Lake, NWT and Reid Lake, NWT (latitude and longitude as listed above). Each garden consisted of 4 blocks of ≈ 10 seeds of known mass per population per block. The blocks were separated by at least 50 m to minimize the probability of all the seeds or seedlings being destroyed by chance events (e.g. grazing, tree fall or unfavorable micro-environmental conditions). The seedbed was prepared by removing the surface duff, excavating the soil to 20 cm depth, removing the existing root networks, replacing the mineral soil (but not the surface duff layer) and carefully levelling the surface. Seeds were planted at a depth of 1.5 cm in a 10 X 10 cm square grid pattern and then watered once. The survivorship of 8 seed mass classes (≤2.6, 2.61-2.9, 2.91-3.2, 3.21-3.5, 3.51-3.8, 3.81-4.1, 4.11-4.4, ≥4.41 mg) was monitored from May 1989 to October 1990 although a drought at the Sandy Lake and Reid Lake gardens delayed emergence until the spring of 1990 and restricted the monitoring of survival, in those gardens, to 1990.

Our analysis of the relationship between seed mass and seedling survival consisted of:

1) a regression of seedling survivorship on the seed mass classes described above.

2) a comparison of the survivorship curves of subpopulations of seedlings derived from light (i.e. the four lightest seed mass classes combined) and heavy seeds (i.e. the four heaviest seed mass classes combined).

Effect of Climatic Variation on Seed Mass

For our regression analysis of the correspondence between seed mass and climatic variation we obtained precipitation, temperature and heating degree days from meteorological stations 25 km to the east of Prince Albert and 35 km to the north of Sandy Lake. The year was divided into three ecologically significant divisions (October-March, April-June, July-September). The rationale for these divisions is that the mass of seeds produced in any one year could be affected by:

1) the quantity of water available from the melting of the snow pack accumulated over the previous winter (hence the October-March period).

2) the precipitation falling in the period of greatest seed growth, the early growing season (hence the April-June period).

3) the relative droughtiness during final maturation of the seeds in the summer and fall (hence the July-September period).

We tested for a seed mass-climate relationship with a regression analysis of seed mass on climatic variables for each year X environment combination within each population. For all of the analyses discussed in the following results, observed departures from homoscedasticity and normality did not jeopardize the power of the F-tests (criteria of Scheffé (1959) and Wilcox (1987)).

Results

Components of Seed and Cone Mass Variation

Our analysis revealed both spatial and temporal patterns of variation in seed mass. The mean dry-environment seed mass was significantly heavier than the wet-environment mean at both Prince Albert (4.4 vs. 4.0 mg) and Sandy Lake (3.7 vs. 3.2 mg) (Table IV-1 and Figure IV-1). At Prince Albert the significant year-to-year variation across (see Table IV-1) and within environments (Table IV-2) was mainly due to a 32% decline in seed mass in the dry environment between 1984 and 1987 (Figure IV-1). This temporal trend was more weakly expressed, but still significant (Table IV-2), in the wet environment. Year-to-year variation in the Sandy Lake population was not statistically significant (Tables IV-1 and IV-3, and Figure IV-1). A significant environment X year interaction (Table IV-1) in the southern population reflects the diminishing difference between dry- and wet-environment means between 1984 and 1986 and then the reversal in rank order in 1987 (Figure IV-1). No significant E X Y interaction was evident at Sandy Lake.

Partitioning of variance among the random factors (family, cone and individual seed) showed significant among-family and among-cone components in both populations at the whole-population level (Table IV-1). Additive genetic variance in seed mass (Family in Table IV-1) at Prince Albert was more than double that found at Sandy Lake (both absolutely and proportionately). At the environment within population level (Tables IV-2 and IV-3) both the family and cone variance components were significant at Prince Albert. The among-family variance component was not significant at Sandy Lake at the environment level in the model. The total within-environment additive genetic variance at Prince Albert (Family in Table IV-2) was greater in the wet environment than in the dry., both absolutely and as a proportion of total variance. Change in the ranking of years among families and in the ranking of families among years (Figures IV-2 and IV-3) produced a significant year by family interaction in both populations (Table IV-1). Analysis at the whole-environment level showed that this interaction took place principally among the dry-environment trees (Tables IV-2 and IV-3). We interpret this effect as a genotype by environment interaction.

At Sandy Lake the mother trees showed a great diversity of reaction to the environment presented in any one year. For example, family #2 (dry environment) produced its heaviest mean seed mass in 1984 but that year saw only light to medium mean seed mass in the other trees of that environment (Figure IV-2). The same pattern of heterogeneity in family response to climate held for the 1985 seed crop. Family #1 produced its heaviest mean seed mass in that year but, in contrast, 1985 was the year of the lightest mean seed mass in trees # 3 and 4. Close scrutiny of Figure IV-2 shows that no year elicited a uniform response in seed mass from all the Sandy Lake trees. We interpreted this as evidence that a strong interaction had occurred between maternal genotype and climate in the dry-environment portion of the Sandy Lake population.

The dominant pattern of temporal variation in seed mass at Prince Albert was noticeably different than at Sandy Lake. Two thirds of the dry-environment families had their heaviest mean seed mass in 1984 and their lightest in 1987 (compare families, within a specific year, in Figure IV-3). In other words the ranking of years, by mean seed mass, was quite consistent among families, especially among dry-environment families. The families at Prince Albert were more homogeneous than the Sandy Lake families in their response to the environment presented by any particular year.

Mean seed mass ($\bar{x}$ ± SE) at Prince Albert (4.15 mg ± 0.023, n = 1274) was significantly greater (t-test, Prob > t <0.001) than mean seed mass at Sandy Lake (3.44 ± 0.017, n = 1297). Analysis of a separate sampling of seed mass from eight populations along a 1000 km north-south transect through the boreal forest showed that this observation conformed to the significant geographical trend of decreasing jack pine seed mass to the north (Figure IV-4). Latitude explained about 82% of the variation in seed mass among populations.

The large cone variance component highlighted the considerable influence of the cone micro-environment, within the crown, on the mature mass of seeds produced. This fact, plus the significant correlation of cone and seed mass (Sandy Lake, R=0.23, n=1297, Prob >|R| <0.0001; Prince Albert, R=0.52, n=1274, Prob >|R| <0.0001) prompted us to examine the pattern of spatio-temporal variation in cone mass. Mean cone mass ($\bar{x}$ ± SE) at Prince Albert (7.5 g ± 0.19, n=139) was significantly heavier (Prob > F = 0.038) than at Sandy Lake (7.0 g ± 0.23, n=134). This result was consonant with the geographic pattern in mean seed mass. The environments did not differ in mean cone mass at either Prince Albert or Sandy Lake (Table IV-4). Cone mass varied significantly among years in the southern population but not in the northern population. Again, this pattern was concordant with the result for seed mass. A significant environment X year interaction was evident at Prince Albert but absent at Sandy Lake, parallelling the result from the analysis of seed mass.

Partitioning of variance in cone mass among the random factors showed significant variance among family and cones in both populations (see variance estimate and confidence limits in Table IV-4). The among-family component was particularly dominant at Sandy Lake where approximately three quarters of the total variance was attributable to among-family variance (Table IV-4). The significant year X family component in both populations indicated significant variance in family cone mass among years.

Reaction Norms

In the seed mass analysis the existence of a significant year X family interaction in the dry environments was our motivation for comparing the genotype (i.e. maternal family) reaction norms of the populations at Prince Albert and Sandy Lake. The reader should be cognizant that this analysis requires the assumption that differences in family response across years are products of the maternal genotype rather than the maternal microenvironment (i.e. variation in soil, slope aspect, insolation, etc.). Figure IV-5 shows the regressions of genotype mean seed mass against annual environmental means for Prince Albert (recall that the environmental mean for a particular year was calculated as the mean seed mass of all the genotypes for that year).

Three general patterns of reaction and sensitivity to yearly climate change were found among the genotypes (i.e. families). Genotype 3 (R^2=0.81, Prob>F=0.10), genotype 5 (R^2=0.67, Prob>F=0.18) and genotype 6 (R^2=0.96, Prob>F=0.02) had regression coefficients around 1, indicating a constant response to environmental change; in other words, the ratio of genotype mean to environmental mean remained constant no matter what the value of the environment. These genotypes showed stability in the face of environmental cahnge. With regression coefficients greater than 1, genotype 2 (R^2=0.95, Prob>F=0.03), genotype 8 (R^2=0.88, Prob>F=0.06) and genotype 9 (R^2=0.99, Prob>F=0.004) showed a positive reaction to the value of the environment (i.e. their mean values, relative to the environmental mean, increased as the value of the environment increased). Genotype 1 (R^2=0.80, Prob>F=0.10), genotype 4 (R^2=0.81, Prob>F=0.10) and genotype 7 (R^2=0.86, Prob>F=0.07) had regressions with slopes less than 1. This group reacted negatively to environmental change (i.e. as environmental mean values increased, the ratio between the individual genotype mean and the environmental mean decreased).

By way of a numerical example, the mean seed mass of genotype 1 was 126% of the environmental value in 1987 but only 107% of the environmental mean seed mass in 1984. Contrast this with genotype 2 whose mean was 110% of the environmental value in 1987 but 122% in 1984. These genotypes reacted in completely opposite fashions to the same change in climate. The foregoing reaction norms can be contrasted with that for genotype 5 (99% in 1987 and 100% of environmental mean in 1984) with its constant proportional tracking of fluctuations in annual climate.

The same analysis of reaction norms among the five dry-environment trees at Sandy Lake showed that only one tree had a significant regression of genotype on environment mean seed mass. This is reflective of the fact that there was no significant year effect among dry-environment trees at Sandy Lake.

Heritability of Seed Mass

The significant among-tree variance component suggested that differences in seed mass between mothers may have had a genetic component. Accordingly, we calculated the narrow-sense heritability ($\underline{h}^2$) of seed mass at the whole-population and environment within population level and at the year within environment level (Table IV-5). Our objective in examining heritabilities was to test for spatial and temporal patterns in heritabilities that might affect the efficiency of natural selection and the rapidity of evolution in seed mass at a variety of spatial and temporal scales across the landscape (see Experimental Design and Analyses, above).

The broadest spatio-temporal scale for which heritability was estimated was the sample of all families, across environments and years, within each population. At that scale, seed mass was most highly heritable at Prince Albert (Table IV-5). The dominant factors in this between-population difference were the proportionately larger year X family variance component (appearing in the denominator of $\underline{h}^2$) and proportionately smaller among-family component (in the numerator) at Sandy Lake (see Table IV-1, % of random factor variance).

At the environment within population scale seed mass was clearly most heritable among the wet-environment trees at both Prince Albert and Sandy Lake (Table IV-5). The proportion of random factor variance ascribable to among-family variance was much higher in the wet environment (% variance for family, Tables IV-2 and IV-3). In the dry environment, much more of the environmental variance was concentrated at the among-cone level (i.e. increased sensitivity to micro-environmental variation along branches) and at the year by family interaction level. Both of these variance components appear in the denominator of the heritability ratio and along with the lower proportion of additive genetic variance (i.e. among family variance) account for the lower heritability of seed mass in the dry environment.

At Prince Albert there was also extensive year-to-year variation (within environment) in the estimates of seed mass heritability (Table IV-5). At this environmental scale the only discernable, consistent relationship between heritability and environment was the negative correlation between heritability in the dry environment at Prince Albert and early growing season precipitation (i.e. April-June) in the year in which the seeds matured (R = -0.93, Prob> R =0.066). The standard error of $\underline{h}^2$, expressed as a percentage of $\underline{h}^2$, was positively correlated with early growing season precipitation (R=0.93, Prob> R =0.066). As the amount of precipitation decreased, heritability increased and its standard error (%) decreased. This result is somewhat counter-intuitive as environmental stress seems to have accentuated the expression of genetic potential and decreased the uncertainty surrounding the estimate of $\underline{h}^2$.

Seed Mass and Seedling Survivorship

The probability of survival during germination, emergence and early post-emergent growth was positively correlated with seed mass in Garden 1 (Bruderheim, AB) (Figure IV-6). The influence of seed mass on early survival was most evident in the fall of the first year of growth (Figure IV-6b,c). Variation in seed mass explained 84% of the variation in survivorship up to October of the first year (1989). The relationship between seed mass and survival at Garden 1 had weakened by the following spring (Figure IV-6d). By October of 1990 (Figure IV-6f) there was no evidence that seedlings from heavier seeds had a greater chance of survival than seedlings derived from lighter seeds.

To test for the correlation of seed mass with survival in a larger database we combined the survivorship data from Garden 3 and 4 with that from Garden 1. A positive relationship between seed mass and survival in the first year of life was clearly evident in the enlarged sample (Figure IV-7a,b). Because it was only possible to monitor Gardens 3 and 4 for one year we were not able to combine the second year pattern from Garden 1 with data from the other gardens.

In order to separate the effects of seed mass on pre- and post-emergent survival we also calculated survivorship from the date by which all emergence was completed in Garden 1 (September 1989). These results showed that post-emergent survival in the fall of the first year was also clearly related to seed mass (Figure IV-8a,b) and that this relationship had weakened and disappeared by the spring of the second year of growth (Figure IV-8c).

The more frequent monitoring and longer time span of the experiment at Garden 1 produced data that were amenable to survivorship curve analysis. The four heaviest seed mass classes were grouped into one heavy seed class and the four lightest classes were grouped into a single light class. The survivorship of seedlings derived from these two classes is shown in Figure IV-9. The separation between the curves raised the question of whether or not the mortality rate of the seedlings derived from heavy seeds was significantly lower than that for seedlings arising from lighter seeds.

Survivorship curves for plants are often Deevey (1947) type II (negative exponential) or Deevey type III (power) curves (for examples see Silvertown 1982, Ch. 3). The equation of a negative exponential curve is $y_i = y_0 e^{-bx}$ where y_i is the number of individuals in any age class x, y_0 is the initial size of the population, b is the slope of the curve (the mortality rate) and e is the base of the natural logarithms. A semilogarithmic plot of this function yields a straight line with slope b, implying that the proportion of the population dying per unit time remains constant. The equation for a power curve is $y_i = y_0 x^{-b}$ (definitions of parameters as in the negative exponential). Because this curve is linear on a log-log plot, any specific proportional (i.e. percentage) increase in time is accompanied by a constant and proportional (percentage) mortality. A consequence of the constant ratio between proportional mortality and proportional time is that the proportion of mortality per unit arithmetic time (e.g. per year) decreases in a curvilinear fashion over time. A decreasing proportion of the population dies, per unit time, as the cohort ages.

We undertook two regression analyses, one of log % survivorship on time (the appropriate strategy for linearizing a negative exponential curve), the other of log % survivorship on log time (the appropriate strategy for linearizing a power curve). Because the log-log regressions (Figure IV-9b) had much higher R^2 values for both subpopulations of seedlings we concluded that survivorship in both groups of seedlings was best described by a power function. Fewer and fewer plants died per unit time as both the heavy-seeded and light-seeded cohorts aged. This is the same pattern of mortality found among young balsam fir (*Abies balsamea*) and eastern hemlock (*Tsuga canadensis*) by Hett and Loucks (1976).

We compared the regression lines and coefficients shown in Figure IV-9b using the methods of Weisberg (1985, p. 180-84) for the whole-line comparison and those of Sokal and Rohlf (1981, p. 505) for the comparison of regression coefficients. Both tests indicated that the lines were significantly different (Prob>F <0.001). This is strong evidence that we were dealing with statistically distinct populations and, most importantly, that the mortality rate was lower for seedlings derived from heavy seeds than for seedlings derived from light seeds.

Effect of Climatic Variation on Seed Mass

The difference between dry- and wet-environment seed mass and the significance of year-to-year variation in the dry environment at Prince Albert suggested that precipitation or air temperature might have been influencing seed mass by differentially affecting soil moisture and conditions for growth in the two environments. We examined the correspondence between temperature, precipitation and heating degree days (Anonymous 1983-1987) and seed mass, for three periods - October to March, April to June, July to September - as described under Experimental Design.

The correlation analyses indicated that only early season precipitation (April to June) was related to the time trend in seed mass at Prince Albert. The regression of seed mass on early growing season precipitation was significant in the dry environment at Prince Albert (Figure IV-10A). A similar trend in wet environment seed mass was apparent in Figure IV-10B but was not statistically significant.

There was no evidence of a relationship between seed mass and early growing season precipitation at Sandy Lake. This analysis indicates that a drought of several years duration is required to affect seed mass. Representative mid-growing season (1988) water contents (% oven dry, 105°C) of dry (d)- and wet (w)-environment soils at Prince Albert were: 5-6% (d) and 50-115% (w) between 0-5 cm; 2-5% (d) and 95-220% (w) at the 5-10 cm depth. Higher soil moisture levels in the wet site apparently buffered the trees against the effects of low rainfall in the drought years of 1986 and 1987.

Discussion

Natural Selection for Seed Mass

We have established that dry-environment jack pine had heavier seeds and that seedlings originating from heavier seeds were more likely to survive the first growing season. On this basis, it seems probable that increased seed mass would facilitate post-fire or post-harvest establishment of jack pine seedlings. That trees growing in the wet and dry environments produced seeds apparently pre-adapted to the cultural conditions that the seedlings would experience after germination may have been fortuitous but we suspect something more. Within the context of our assumptions about the statistical control of maternal influences on seed mass, we have presented evidence that seed mass was highly heritable. The heritability of seed mass sets the stage for the play of disruptive selection between jack pine populations in adjacent, but hydrologically dissimilar, environments.

The analysis in Chapter III showed that larger seeds produced seedlings with larger root systems. In the current paper we have demonstrated that seedling survivorship over the first growing season, under dry-environment conditions, was positively correlated with original seed mass. These factors should lend a selective advantage to trees producing heavier seeds, and larger seedlings, in edaphically dry environments. This superior survivorship may result from the ability of larger seedlings to avoid water stress by producing larger root systems that occupy correspondingly larger volumes of soil, thereby accessing greater quantities of water. Partial leaf loss to herbivores is also likely to be less disastrous to larger seedlings. Burial by rain-splash deposited soil may also be less frequent among larger seedlings. The demographic consequences of the positive correlation between seed mass and seedling survivorship, in the first growing season, will remain in effect throughout the life cycle of the cohort. A related question was why the seed mass effect on survivorship was only present during the first growing season.

The answer to this question seems to depend, at least partially, on the quality of the first winter and the second spring of life. Winter and early spring mortality may be due to factors such as excavation and breakage of roots by soil frost heaving, dehydration during warm-weather excursions when the soil is still frozen, and herbivory. If these causes of mortality are uninfluenced by seedling size or negatively correlated with seedling size then the initially higher survivorship of seedlings derived from larger seeds may dissipate over winter and during the second growing season. Our data did show higher mortality of seedlings derived from large seeds over the first winter and the second growing season. That trend is reminiscent of density-dependent mortality and it is possible that the more common seedling classes derived from heavier seeds had a greater chance of falling prey to herbivores or disease in the second growing season simply because they were more common and more visible than smaller seedlings. This is consonant with the evidence on mortality in outplanted Douglas-fir seedlings (Waters *et al.* 1991) that weather-related effects dominate mortality in the first year of growth but are on a par with disease and predation in the second and third years.

The Evolutionary Autecology of Seed Mass

Baker (1972) and Salisbury (1974) both proposed that evolution in dry environments has favored heavier seed mass and its attendant larger root systems. Our data followed the expected pattern with heavier seed mass in the dry environments. We have argued that there are good reasons to conclude that seed mass differences between the jack pine of dry and wet environments could be due, in part, to genetic differentiation between the subpopulations. At the same time, our results provide ample evidence of a strong environmental component to the determination of seed mass. For example, in the southern population dry-environment trees were quite sensitive to trends in precipitation. This sensitivity is of particular ecological interest because it may well foreshadow some of the possible ecosystem-level effects of climate change in the boreal forest. Consider the following scenario.

Reduced precipitation leads to lower seed mass among the climatically-sensitive jack pine of dry environments. Lower seed mass accentuates the difficulty of re-establishment under increasingly xeric conditions. The coupling of several such stresses (e.g. seed mass decreases, increased drought stress, elevated fire frequency) causes widespread extirpation of the species in the dry, sandy environments it currently dominates. In contrast, individuals inhabiting hydrological refugia (e.g. jack pine on wetter soils) are buffered against the effects of adverse climate change. Climatic shifts are extreme and the populations of jack pine in these currently atypical sites may come to constitute the bulk of the surviving species' gene pool.

The decrease in seed mass as one goes north in western Canada corroborates the pattern found by Yeatman in his 1966 study of the geographic pattern of seed mass variation in jack pine. How might the decrease in seed size with increasing north latitude be accounted for? One intriguing hypothesis is that light-seeded pine may have been favoured over their heavy-seeded cohorts in the occupation of the post-glacial landscape due to the potentially greater mobility of their lighter offspring. The difference in mean seed mass between northern (Reid Lake) and southern (Bruderheim) jack pine was greater than 40%. If these differences were accentuated by geographical differences in projected surface area of the seed and wing then significant variation in vagility between northern and southern jack pine would be possible (see discussion in Augspurger and Franson 1987). It may be that the heavier-seeded biotypes in the western Canadian population have simply not had sufficient time to migrate into the northern part of the range thus producing a depletion of biotypes at the northern edge of the range. A test for differences in vagility of seeds from northern (light) and southern (heavy) populations would require dispersal experiments and calculations of seed variables, such as wing-loading (force of gravity/projected surface area), for each population.

The fact that additive genetic variance in the Prince Albert population was well over double that found in the Sandy Lake population (both absolutely and proportionately) is congruent with the argument that the gene pool of jack pine may have become impoverished during the migration into northern Canada. The attenuation of variance from the wet to the dry environment at Prince Albert supports the notion that dry environments might prove to have greater evolutionary inertia than wet environments during periods of rapid climate change. Populations in hydrologically anomalous wet ecosystems may be poised to contribute disproportionately to the gene pools of boreal forest species in the aftermath of climate change.

Given the plausibility of ongoing climate change, it would seem prudent to increase the effort to determine the levels of population genetic structuring for climatically-sensitive traits in widespread or keystone species. We need to pay special attention to the genetics of those populations now occupying hydrologically atypical habitats because they have the potential to dominate their species' gene pools under conditions of rapid climate change. It is conceivable that major climatic shifts may instigate large shifts in the composition of gene pools by destroying populations of typical habitats and selecting for genetically distinct populations of atypical environments. Profound climate-mediated changes in the ecological and evolutionary landscape may also allow species to overcome genostasis (Bradshaw 1984) and display significant changes in ecological amplitude. It is important to bear in mind that the increase in atmospheric CO_2 concentration driving the greenhouse effect and climate change may complicate the issue by promoting the production of heavier seeds in some species (e.g. *Abutilon theophrastii,* Garbutt and Bazzaz 1984). If the climate of the western boreal forest becomes drier and the survival advantages accruing to seedlings arising from heavier seeds disappears, then the demographic advantage of upland jack pine would gradually be erased. Ironically, a progressively drier macro-climate might also present concurrent barriers to regeneration for the lighter-seeded seed bank held in cones on jack pine of wet environments.

A high level of heterogeneity in reaction and sensitivity to annual climatic fluctuation was apparent among the dry-site jack pine at Prince Albert. If we consider that seed mass does influence seedling fitness for survival, then the relative magnitudes of the genotypic mean seed mass are evolutionarily consequential. Our data showed that some genotypes produced relatively heavy seeds under dry conditions but relatively light seeds under wet conditions. This may be the most adaptive response in the face of a permanent drying trend in the climate. On the other hand, the production of relatively low-mass seed under dry conditions and high-mass seed under wet conditions may be maladaptive. What is of particular evolutionary interest is that the Prince Albert population contained the full gamut of response to climatic variation while Sandy Lake was rather depauperate in its diversity of genotypic responses. This hints that southern populations may have a greater breadth of genetic response to draw on in coping with climate change. The occurence of greater breadth of response in the south is concordant with the biogeographic principle that the diversity of biotypes is greatest near the centre of a species' range.

Two possible causes of loss of genetic variance in reaction norm during the migration of jack pine into the post-glacial landscapes of northern Canada are genetic drift caused by chance sampling effects and strong selection. We must emphasize that the year by family interaction accounted for a greater proportion of variance at Sandy Lake compared to Prince Albert. This indicates that while the breadth of response to climate change may be expected to be greater at Prince Albert, the potential for heterogeneity of response to climate change among families may be greater at Sandy Lake. Whether sheer breadth of response or great heterogeneity of response will be more important for the survival and evolution of species during climate change is a worthwhile question awaiting the attention of plant demographers and evolutionary biologists. Our results are preliminary but we believe they highlight some of the features of the ecosystem that need to be closely examined in attempts to predict the ecological and evolutionary effects of climate change.

The large and significant proportion of variance attributable t cone in both populations suggested that the position of a maturing seed within the tree crown strongly influences final mass. The quantity of photosynthate available for cone and seed development is likely to be a function of the photosynthetic photon flux density reaching the photosynthetic tissues of the developing cone and adjacent leaves. This PPFD will be a function of the compass direction faced by the cone's branch, the height of the cone in the canopy and the density of the surrounding stand of trees.

Our analysis showing that dry-environment seed mass was more sensitive to early growing season precipitation supports the hypothesis that seed mass variation was partially controlled by climate. The concordance between the year of lowest seed mass in the dry environment and the 1985 peak of a jack pine budworm outbreak in the Prince Albert area (Volney 1988) also points to herbivory as a possible influence on seed mass. It cannot be assumed that low or medium levels of herbivory will necessarily lead to [illegible] seed mass because very high levels of herbivory are required to decrease seed mass in some coniferous species (e.g. *Abies alba*, Schroeder 1989).

The lower heritability of seed mass at Sandy Lake was a product of a much larger interaction of maternal genotype with annual climatic environment (see family x year variance, Table 1). Other things being equal, selection for seed size would likely be less efficient at Sandy Lake because of that population's lower heritability for seed mass and its greater heterogeneity in genotypic reaction to annual climatic variation. Higher heritability of seed mass in the southern population could prove to be a definate evolutionary asset to that population if it were exposed to fast-paced climate change.

The possible causes of $\underline{h}^2$ estimates greater than unity are worth speculating upon. Recall that we have argued that our experimental design and statistical model have adequately controlled for environmental variance. If this was in fact not the case, the estimates of among-family variance may have been upwardly biased by the inclusion of unspecified environmental variance (e.g. variation in soils among families) or maternal bias. An unspecifed maternally-induced upward bias in the estimate of additive genetic variance could account for the heritabilities in excess of unity reported for years within site X environment.

Heritability was higher in the wet than in the dry environment at Prince Albert. More favourable growing conditions likely facilitated a clearer expression of genetic potential in the wet-environment population which could provide a greater opportunity for the play of natural selection. Ironically, the intensity of natural selection may be higher in the dry environment but, because of the lower heritability of seed mass, selection is likely to be less efficient. Silvertown (1989) has pointed out that one could conceivably interpret this sort of pattern of results as evidence that seed mass is a phenotypically plastic trait. The evolutionary emphasis would then shift from the concept of differentiation between populations to one of phenotypic plasticity across populations. Both evolutionary pathways (differentiation vs. plasticity) could be successful routes for crossing the adaptive landscape through which a species must pass over time and we ought to bear in mind that differentiation and plasticity are not necessarily mutually exclusive but may be complementary responses to different sets of evolutionary circumstances. In any case, it is important to remember that the comparison of heritabilities between disjunct populations should be approached with some caution because of the likelihood of changes in genetic variances and covariances over time (Mitchell-Olds 1986) and between environments.

Conclusions

The evidence we have presented lends support to the hypothesis that disruptive selection may have caused genetic differentiation in seed mass between wet and dry environments. The patterning of seed mass variation across edaphic environments was consistent at the south and north ends of the species' range in western Canada. Seed mass clearly affected fitness for survival and was a heritable trait. In contrast to Silvertown's (1989) general conclusions we believe our results show a pattern of, and impetus for, differentiation for seed mass among naturally occuring populations. The differences in the magnitude and heritability of seed mass at micro- and macro-spatial scales highlights the meshing of ecological and evolutionary time that must be integral to the process of evolution. As the pace of evolution approaches the time scale of ecological events, as we might expect during periods of rapid climate change, the spatial patterning within gene pools will play an increasingly crucial role in defining the consequent genetic structure of species.

We also consider it important to comment on Silvertown's notion that between-environment variation in seed mass is likely to be the product of developmental constraints. In our data, the presence of significant differences in seed mass between wet and dry environments, despite the abscence of significant variation in cone mass between the environments, indicates that seed mass differences between environments are not the product of developmental constraints imposed by cone size.

A significant conclusion arising from our work is that seed mass variations in time and space may bias work on intra-specific population differentiation and efforts at tree improvement. If early growth and survivorship are positively genetically correlated with seed mass, then the accuracy of early selection (natural or artificial) will depend upon the mix of years and environments-of-origin in the seed rain or collection. The problem of bias due to between-year variation could be avoided by bulking equal numbers of seed from a series of years or by restricting seed collection to one seed year. To avoid bias due to spatial variation, equal numbers of seed from defined environments should be bulked or collection should be restricted to one habitat type. If the spatio-temporal pattern of collection cannot be controlled then use of seed mass as a covariate is essential for statistical control of non-random seed mass variations and their effects on subsequent seedling growth.

With regard to estimating genetic parameters, using multi-year seed from some mothers may lower their contribution to the family level variance, while employing single-year seed from other mothers may inflate their contribution to the estimate of family variance (if year X family variance is not included as an environmental factor in the model). Bias of the estimate of V_{fam} away from the parametric value has serious implications for the estimate of heritability ($\underline{h}^2$). The strong relationship between seed mass and early growing season precipitation underscores the necessity of either using seed of the same year when the family level variance is to be estimated or of including a variance component for temporal variation in the analytical model. Ecologists and silviculturalists should be cognizant of the risk of genetic impoverishment (i.e. exclusion, from experiments, of families with particularly heavy or light seeds) that could arise from any procedure that grades seeds by mass (see Hellum 1976).

Tables

Table IV-1. Variance of seed mass at Prince Albert and Sandy Lake. Variance of random factors was estimated by Satterthwaite (1946) approximation. Total variance for Prince Albert was 0.492, for Sandy Lake, 0.349. Environment and year were fixed factors.

Pop	Source	DF	SS	F[a]	Prob>F	Variance Estimate (lcl,ucl)[b]	% of RF Variance[c]
Prince Albert	Environment	1	76.27	4.25	0.056	–	–
	Year	3	316.16	36.24	<0.001	–	–
	E X Y	3	116.36	13.35	<0.001	–	–
	Family(F)	16	283.63	6.13	<0.001	0.137 (0.08, 0.33)	28
	Y X F(E)	48	135.60	1.66	0.012	0.042 (0.02, 0.14)	9
	Cone(EYF)	144	240.78	11.75	<0.001	0.171 (0.14, 0.21)	35
	Seed(EYFC)	1736	247.07	–	–	0.142 (0.13, 0.15)	28
Sandy Lake	Environment	1	68.52	7.23	0.028	–	–
	Year	5	19.08	1.07	0.399	–	–
	E X Y	4	12.02	0.83	0.517	–	–
	Family(E)	8	73.56	2.57	0.029	0.043 (0.02, 0.40)	12
	Y X F(E)	31	106.53	2.51	<0.001	0.080 (0.04, 0.20)	23
	Cone(EYF)	88	116.51	13.72	<0.001	0.130 (0.14, 0.21)	37
	Seed(EYFC)	1189	114.73	–	–	0.096 (0.09, 0.10)	28

[a] Satterthwaite F ratio.

[b] Lower and upper confidence limits for variance estimates; $\alpha = 0.10$.

[c] Random factor variance (i.e. excluding fixed-factor variation).

Table IV-2. Variance of seed mass in dry and wet environments at Prince Albert. Variance of random factors was estimated by Satterthwaite (1946) approximation. Total variance in the dry environment was 0.568, in the wet environment, 0.419. Year was a fixed factor.

Pop - Envir	Source	DF	SS	F[a]	Prob>F	Variance Estimate (lcl,ucl)[b]	% of RF Variance[c]
Prince Albert	Year	3	450.87	35.06	<0.001	–	–
Dry	Family	9	141.44	3.65	0.005	0.112 (0.05, 0.53)	20
	Y X F	24[d]	100.44	1.78	0.032	0.067 (0.03, 0.36)	12
	Cone(YF)	74	172.19	15.16	<0.001	0.236 (0.18, 0.33)	41
	Seed(YFC)	913	140.10	–	–	0.153 (0.14, 0.17)	17
Prince Albert	Year	3	48.57	10.51	<0.001	-	-
Wet	Family	8	161.80	13.25	<0.001	0.176 (0.09, 0.58)	42
	Y X F	24	35.79	1.52	0.090	0.019 (0.01, 0.24)	4
	Cone(YF)	72	69.42	7.45	<0.001	0.095 (0.07, 0.13)	23
	Seed(YFC)	850	109.98	-	-	0.129 (0.12, 0.14)	31

[a] Satterthwaite F ratio.

[b] Lower and upper confidence limits for variance estimates; α = 0.10.

[c] Random factor variance (i.e. excluding fixed-factor variation).

[d] Only 1987 cones available for tree 6, thus D.F. for YXT = (4-1)(9-1) = 24.

Table IV-3. Variance of seed mass in dry and wet environments at Sandy Lake. Variance of random factors was estimated by Satterthwaite (1946) approximation. Total variance in the dry environment was 0.377, in the wet environment, 0.306. Year was a fixed factor.

Pop - Envir	Source	DF	SS	F^a	Prob>F	Variance Estimate (lcl,ucl)[b]	% of RF Variance[c]
Sandy Lake Dry	Year	5	12.16	0.53	0.750	–	–
	Family	4	33.13	1.80	0.170	0.024 (0.01, 39.3)	6
	Y X F	20	87.80	2.97	<0.001	0.115 (0.06, 0.32)	31
	Cone(YF)	54	76.42	15.14	<0.001	0.145 (0.11, 0.21)	38
	Seed(YFC)	695	64.97	–	–	0.093 (0.09, 0.10)	25
Sandy Lake Wet	Year	4	18.94	2.73	0.090	-	-
	Family	4	40.44	5.90	0.010	0.079 (0.03, 0.79)	26
	Y X F	11	18.73	1.41	0.210	0.019 (0.00, 17.3)	6
	Cone(YF)	34	40.09	11.71	<0.001	0.107 (0.07, 0.18)	35
	Seed(YFC)	494	49.76	-	-	0.101 (0.09, 0.11)	33

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[a] Satterthwaite F ratio.

[b] Lower and upper confidence limits for variance estimates; $\alpha = 0.10$.

[c] Random factor variance (i.e. excluding fixed-factor variation).

Table IV-4. ANOVA of cone mass over space and time. For random factors, variance components were estimated by Satterthwaite (1946) approximation. Total variance for Prince Albert was 3.35, for Sandy Lake, 7.68. Environment and year were fixed factors.

Pop	Source	DF	SS	F[a]	Prob>F	Variance Estimate (lb, ub)[b]	% of RF Variance[c]
Prince Albert	Environment	1	5.90	0.43	0.520	_	_
	Year	3	217.91	29.42	<0.001	_	_
	E X Y	3	46.56	6.29	0.001	_	_
	Family(E)	16	217.56	5.51	<0.001	1.411 (0.78, 3.46)	42
	Y X F(E)	48	118.04	1.76	0.015	0.541 (0.27, 1.68)	16
	Cone(EYF)	70	97.69	_	_	1.396 (1.08, 1.89)	42
Sandy Lake	Environment	1	107.74	1.40	0.271	_	_
	Year	5	7.85	0.56	0.728	_	_
	E X Y	4	9.37	0.84	0.512	_	_
	Family(E)	8	619.21	27.81	<0.001	5.583 (2.82, 17.23)	73
	Y X F(E)	30	82.05	1.59	0.051	0.375 (0.15, 2.42)	5
	Cone(EYF)	86	148.17	_	_	1.723 (1.36, 2.26)	22

[a] Satterthwaite F ratio.

[b] Lower and upper confidence limits for variance estimates, $\alpha = 0.10$.

[c] Random factor variance (i.e. excluding fixed factor variation).

Table IV-5. Narrow-sense heritabilities (h^2) at the population, environment within population, and year within environment levels. SE = standard error of h^2 according to the methods of Becker (1968, p. 10 & 11). SE% is the SE expressed as a percentage of h^2. * indicates that h^2 values were calculated based on an among-family variance component with a Prob>F greater than 0.05.

HERITABILITY - $\underline{h}^2$ ± SE (SE%)

PRINCE ALBERT		SANDY LAKE	
0.78 ± 0.198 (25%)		0.34 ± 0.148 (43%)	
DRY ENVIRONMENT	WET ENVIRONMENT	DRY ENVIRONMENT	WET ENVIRONMENT
0.55 ± 0.218 (40%)	1.18 ± 0.347 (30%)	0.18 ± 0.1[illegible] * (73%)	0.72 ± 0.391 * (54%)
1984	1984	1984	1984
0.55 ± 0.258 (47%)	1.67 ± 0.351 (21%)	0.96 ± 0.490 (51%)	NA
1985	1985	1985	1985
0.71 ± 0.297 (42%)	0.86 ± 0.327 (38%)	1.79 ± 0.479 (27%)	NA
1986	1986	1986	1986
0.98 ± 0.348 (36%)	1.27 ± 0.372 (29%)	0.45 ± 0.321 * (72%)	0.33 ± 0.314 * (96%)
1987	1987	1987	1987
1.41 ± 0.346 (25%)	1.27 ± 0.368 (29%)	1.92 ± 0.441 (23%)	2.05 ± 0.492 (22%)
1988	1988	1988	1988
NA	NA	0.34 ± 0.271 * (80%)	0.84 ± 0.499 (47%)
1989	1989	1989	1989
NA	NA	1.30 ± 0.522 (40%)	0.54 ± 0.355 (66%)

Figures

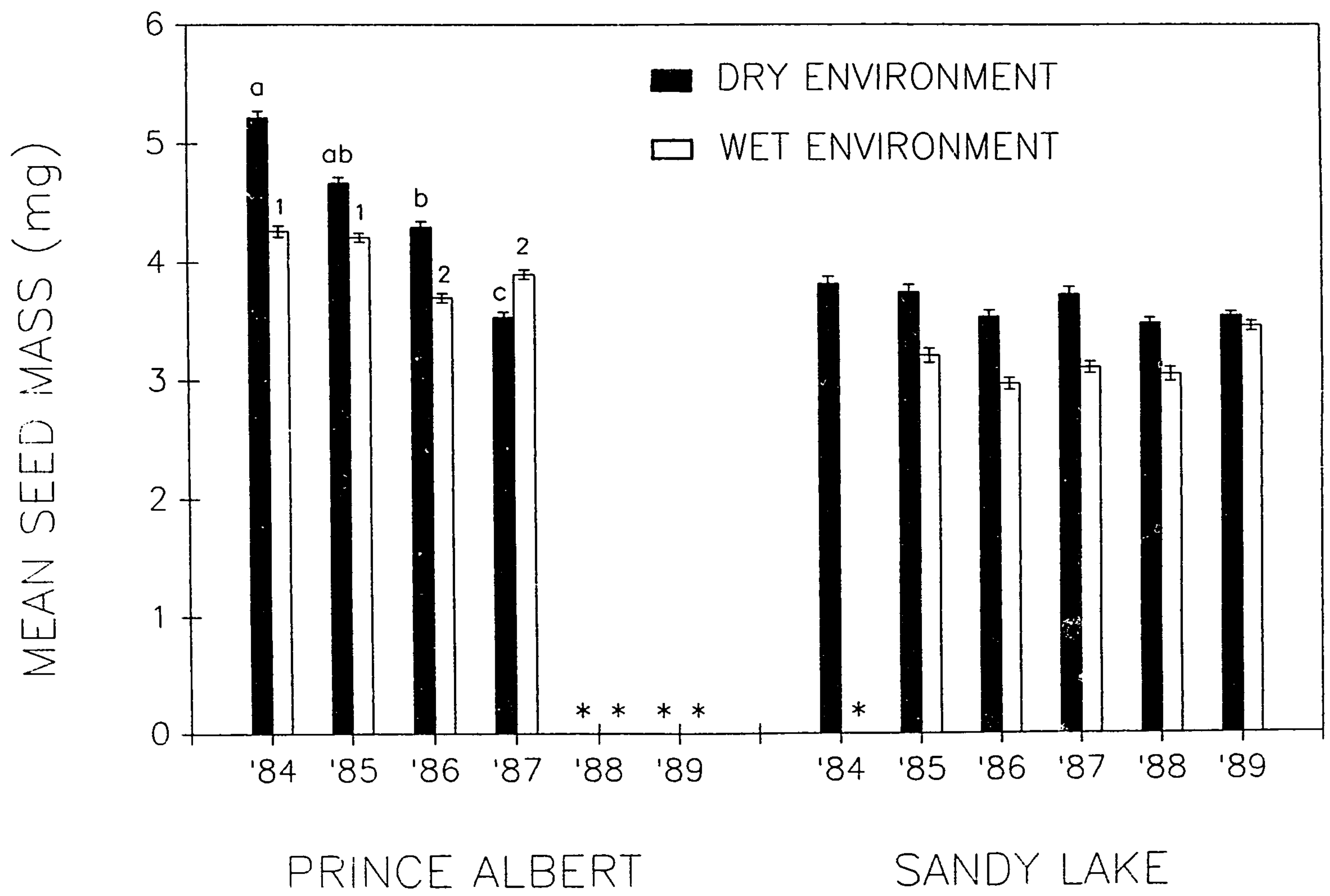

FIG. IV-1. Comparison of seed mass in the southern (Prince Albert) and northern (Sandy Lake) populations, between soil moisture environments in each population and among years within each soil moisture environment. For Prince Albert, years in the dry environment with different letters, and years in the wet environment with different numbers, are significantly different (Tukey-Kramer procedure for pairwise comparison of means, $\alpha=0.01$). The * indicates that no seed collections were available for the year indicated. Error bars are ± 1 SE.

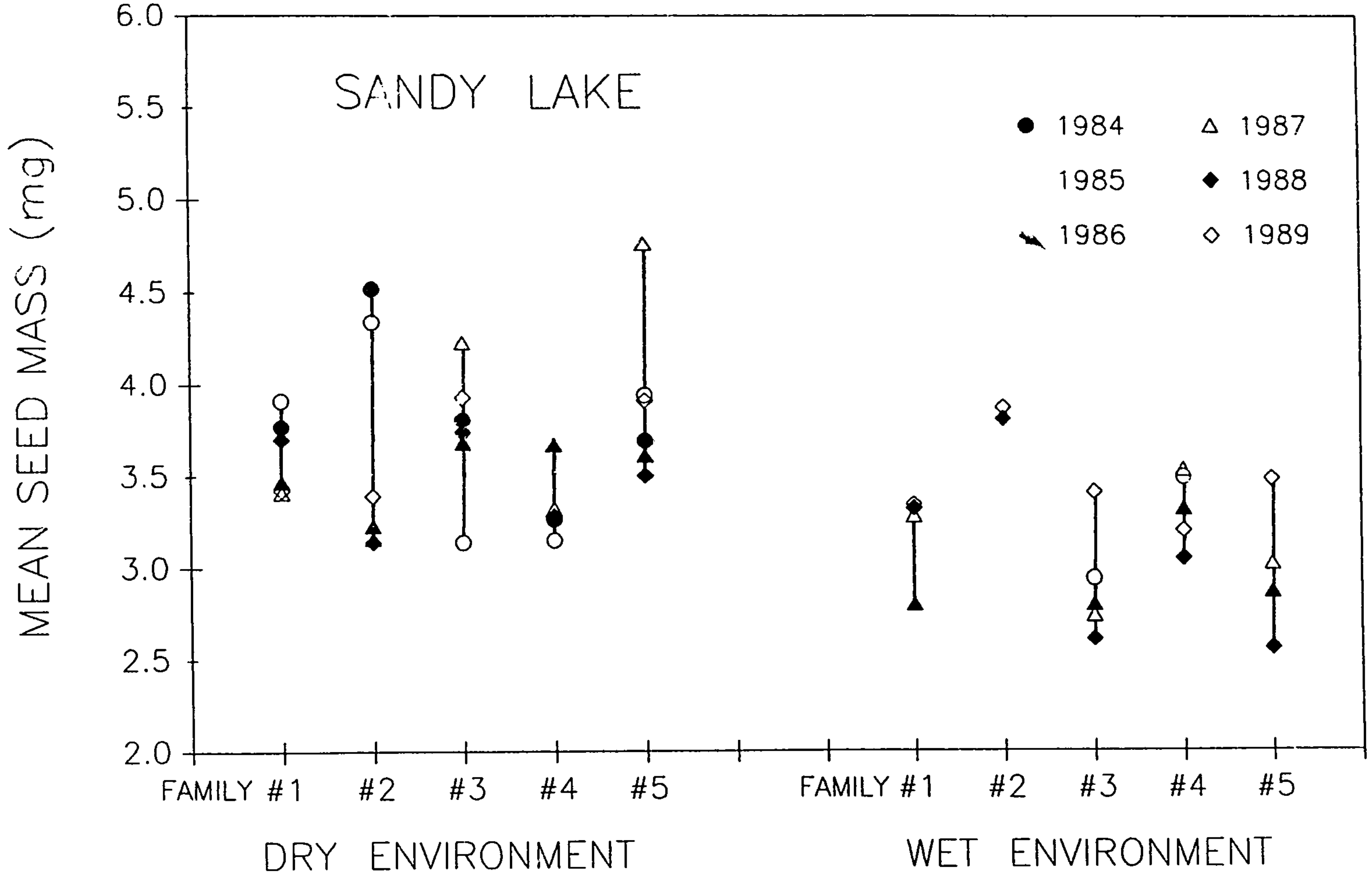

FIG. IV-2. Variation in mean seed mass within maternal families, among years, in both soil moisture environments in the northern population (Sandy Lake).

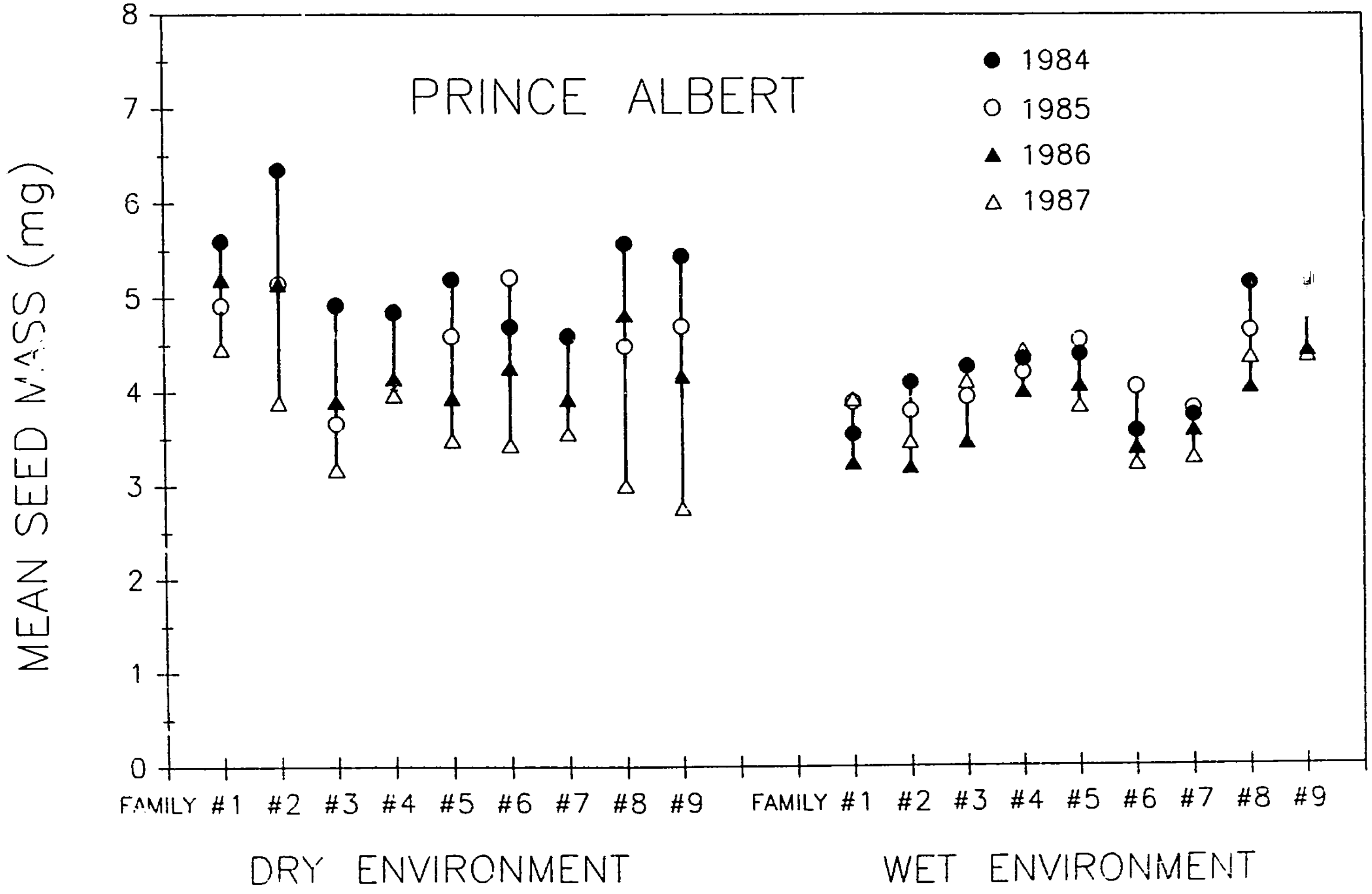

FIG. IV-3. Variation in mean seed mass within maternal families, among years, in both soil moisture environments in the southern population (Prince Albert).

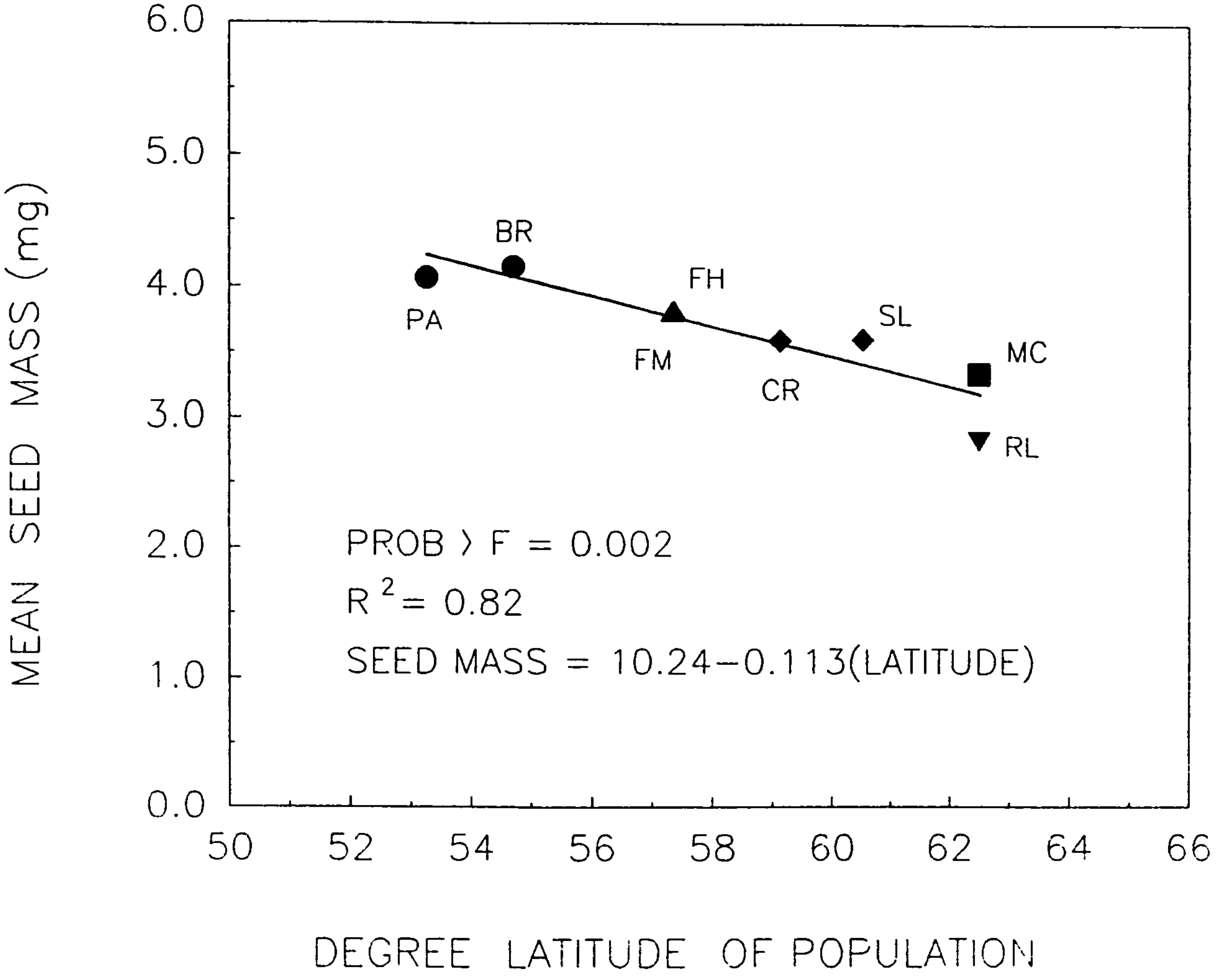

FIG. IV-4. Decrease in seed mass with increasing northern latitude. PA=Prince Albert, BR=Bruderheim, FH=Fort Hills, FM=Fort McKay, CR=Carlson Road, near Peace Point, SL=Sandy Lake, MC=Mosquito Creek, RL=Reid Lake (see Population Selection and Sampling for exact latitudes and longitudes). Population means having different symbols are significantly different by the Tukey-Kramer procedure for pairwise comparison of means, $\alpha=0.05$.

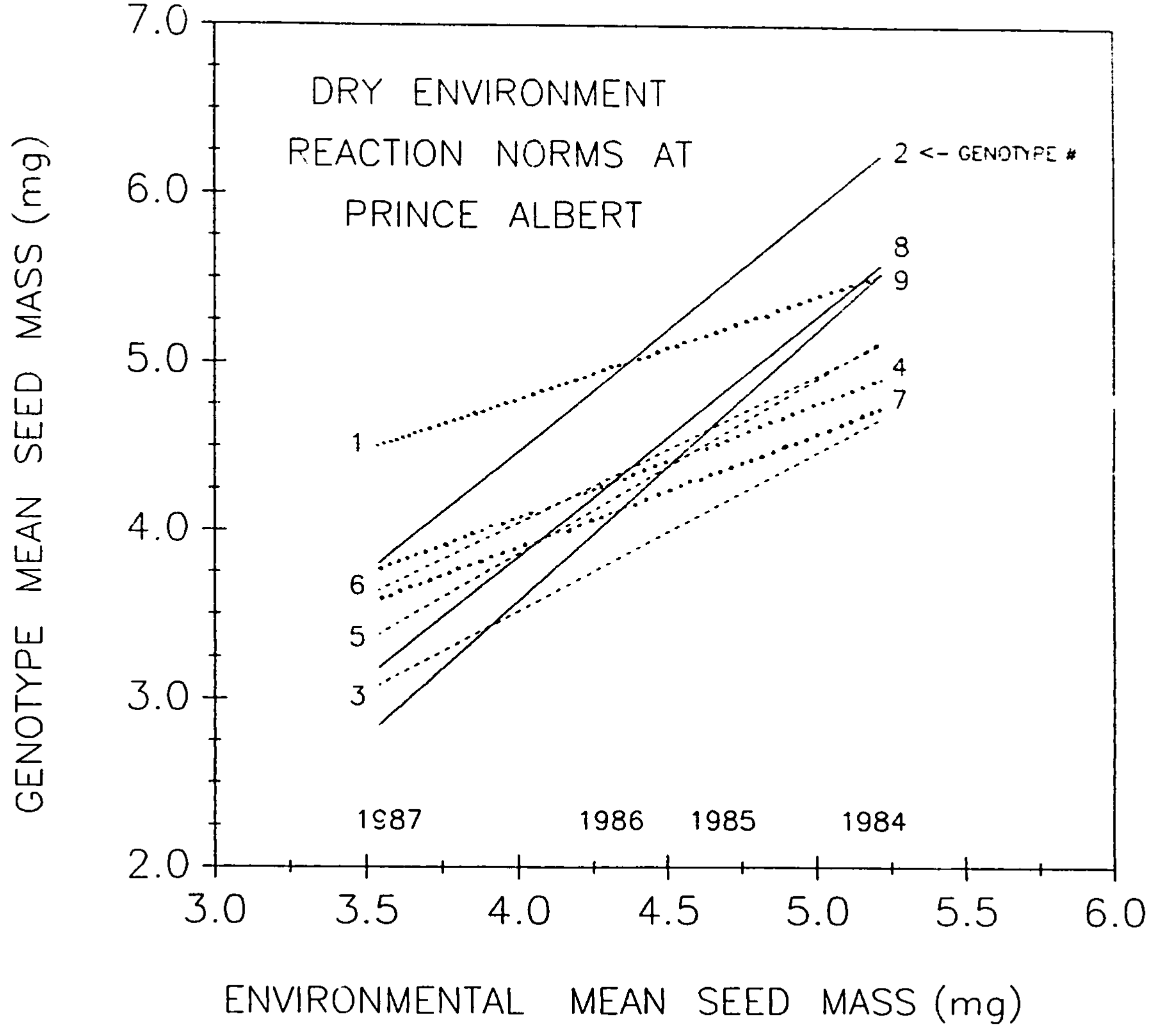

FIG. IV-5. Variation of genotype means relative to environmental mean seed mass (i.e. the mean of all genotypes for the year in question). The dates are placed directly above the environmental mean for the year. See Figure 10A for the April-June precipitation in each year.

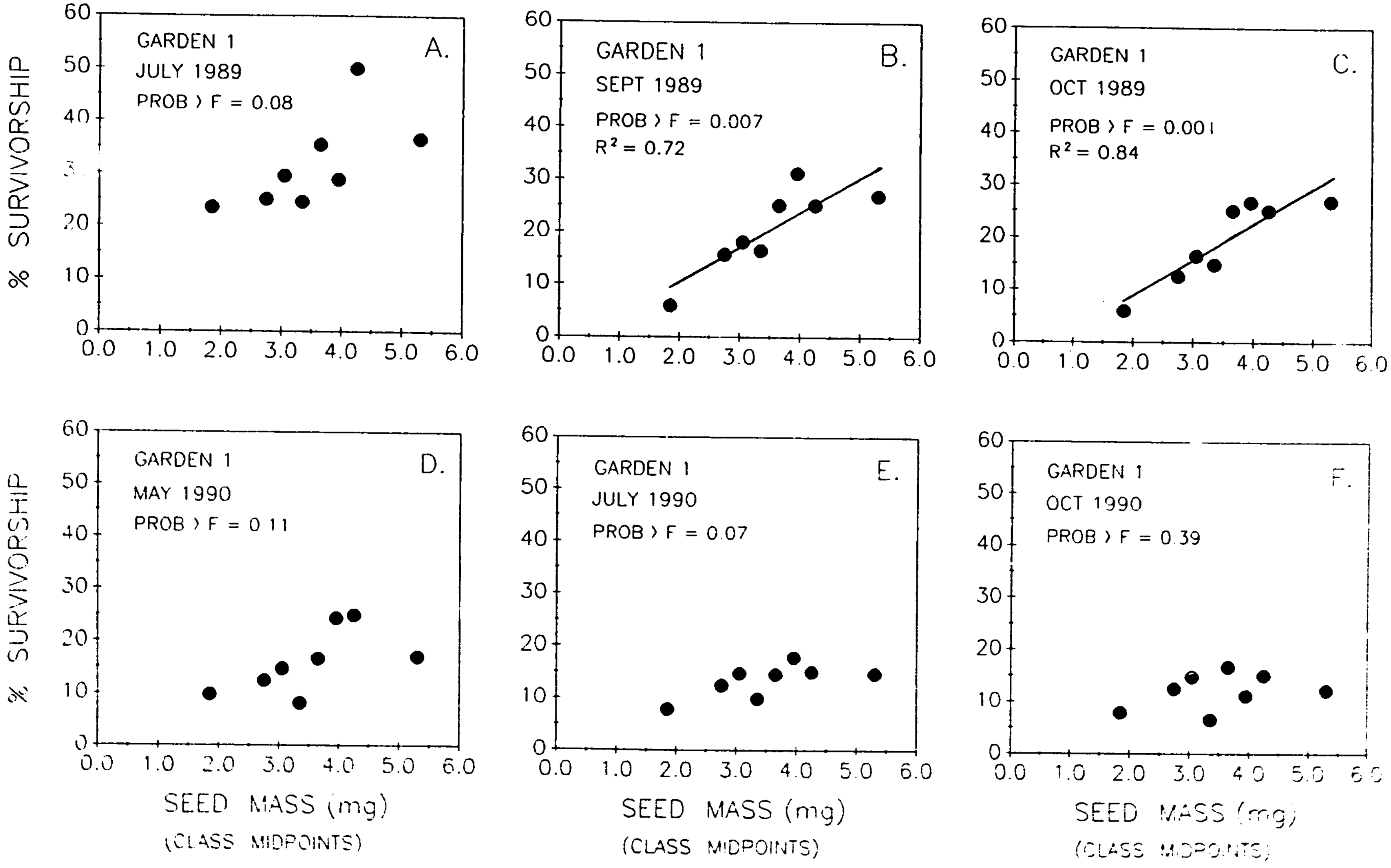

FIG. IV-6. Temporal trend in the relationship between seedling survivorship and seed mass in the experimental garden at Bruderheim, Alberta. n_0 = 359.

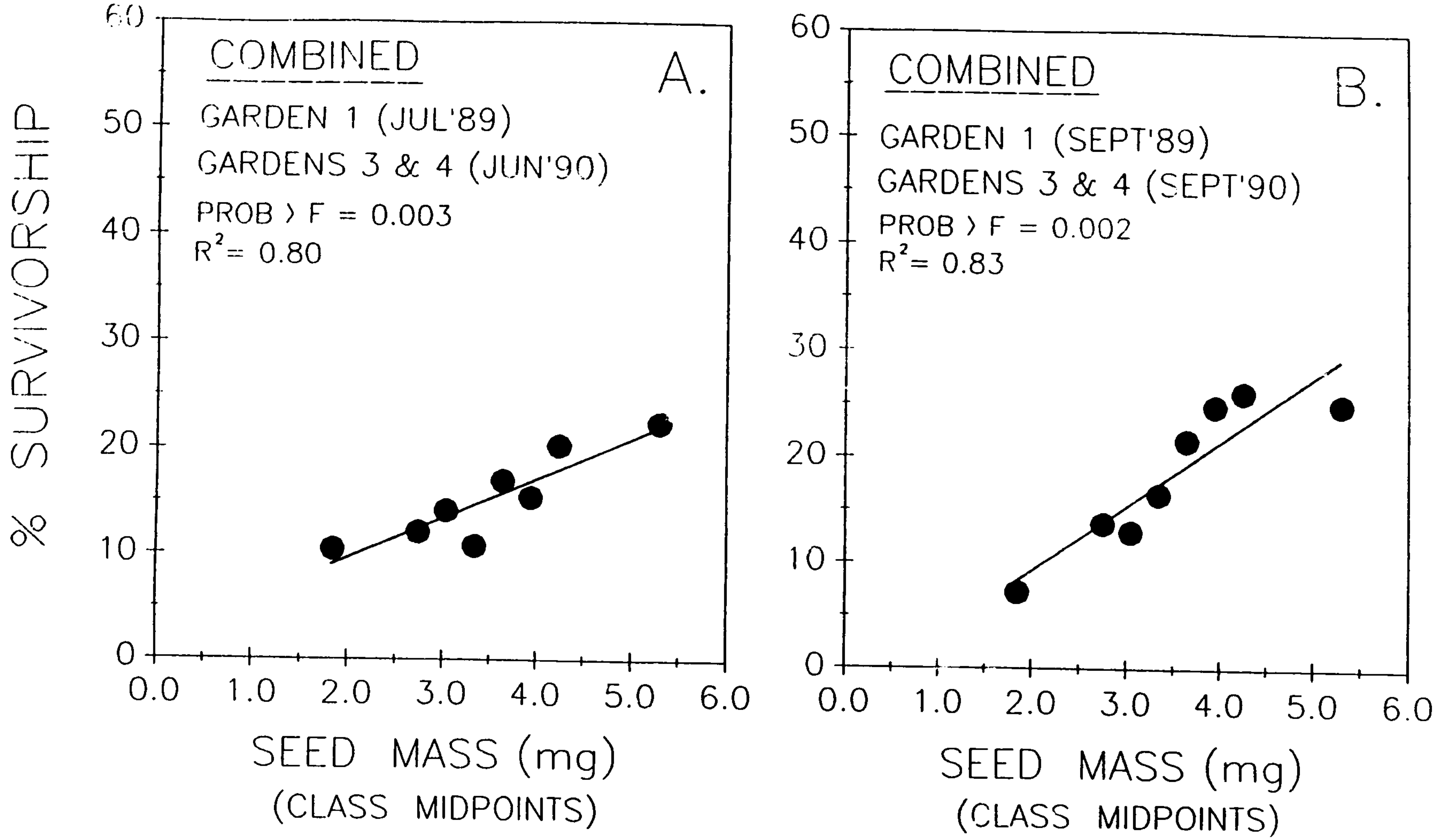

FIG. IV-7. Relationship between seedling survivorship and seed mass for the combined sample from the experimental gardens at Bruderheim (Garden 1), Sandy Lake (Garden 3) and Reid Lake (Garden 4). n_0 = 1078.

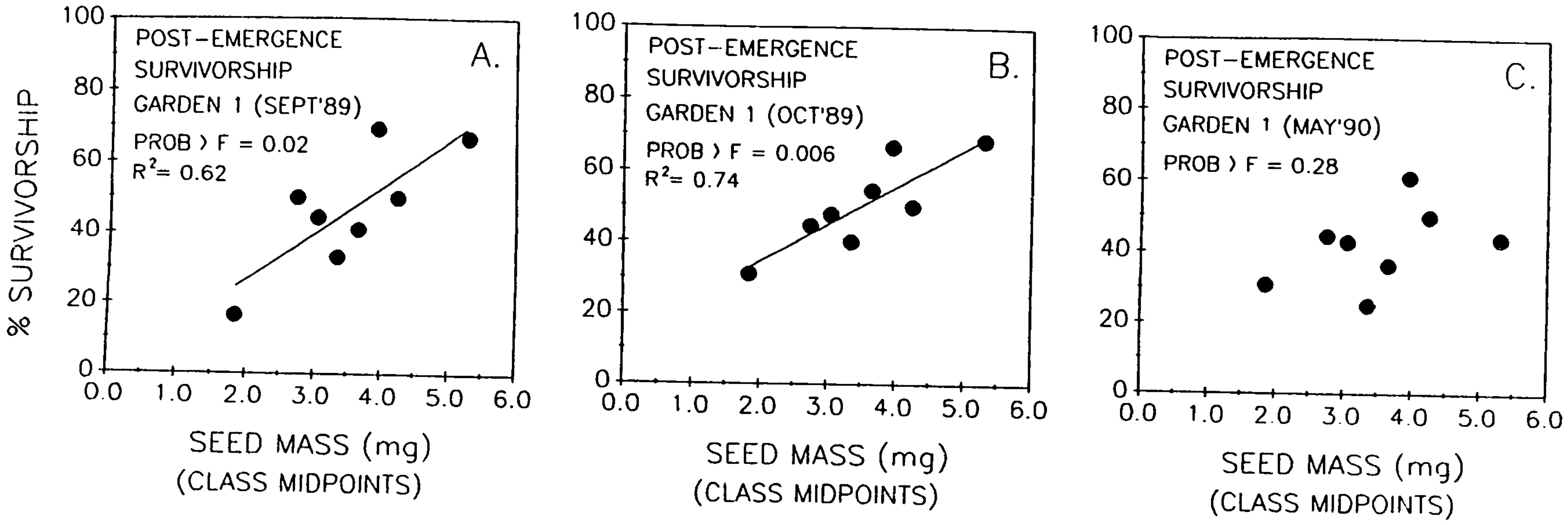

FIG. IV-8. Temporal trend in the relationship between post-emergence seedling survivorship and seed mass in the experimental garden at Bruderheim, Alberta. n_0 (Sept'89) = 108; n_0 (Oct'89) = 129.

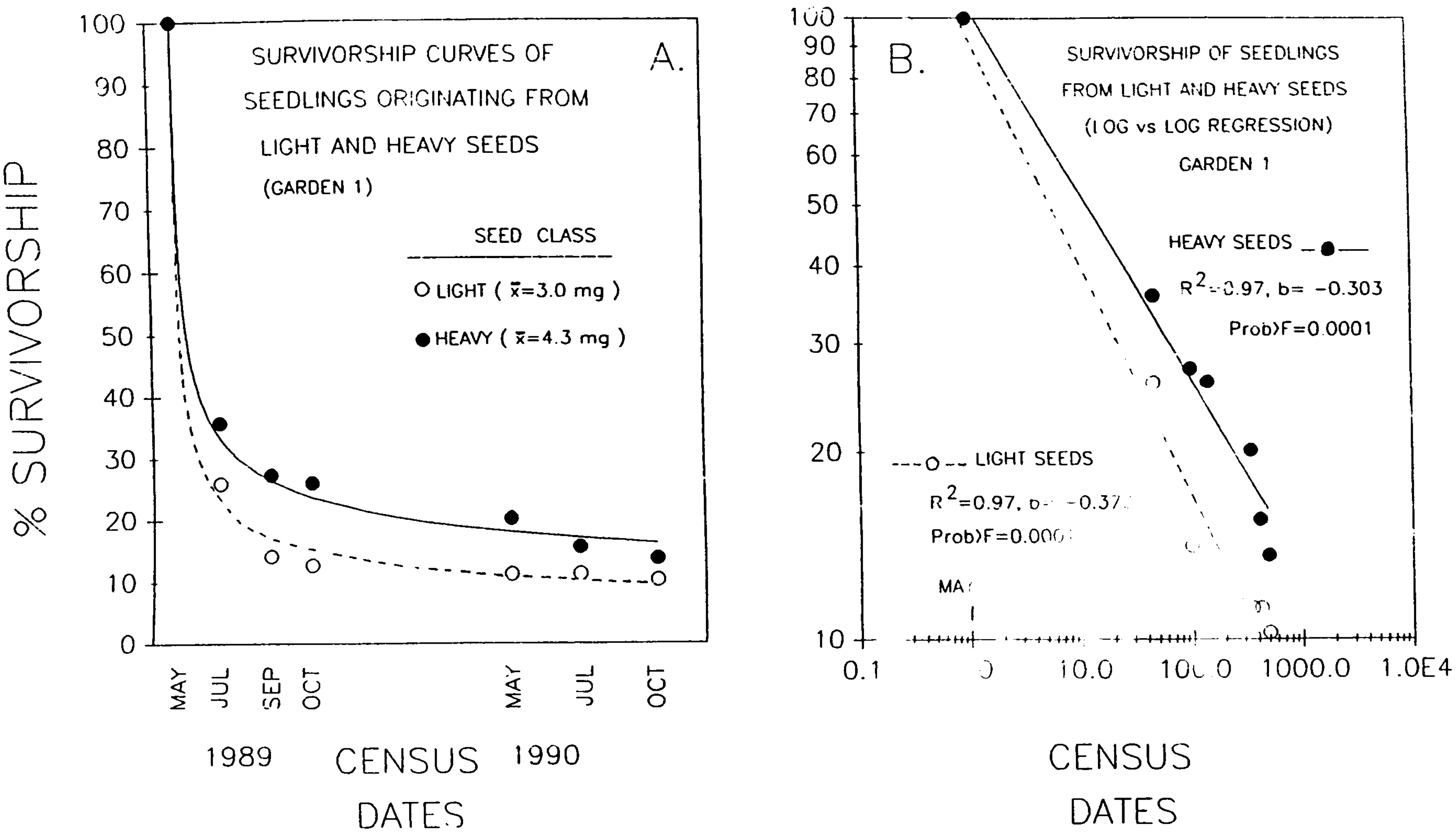

FIG. IV-9. Survivorship curves of seedlings derived from heavy and light seeds, plotted on linear (A) and log-log axes (B).

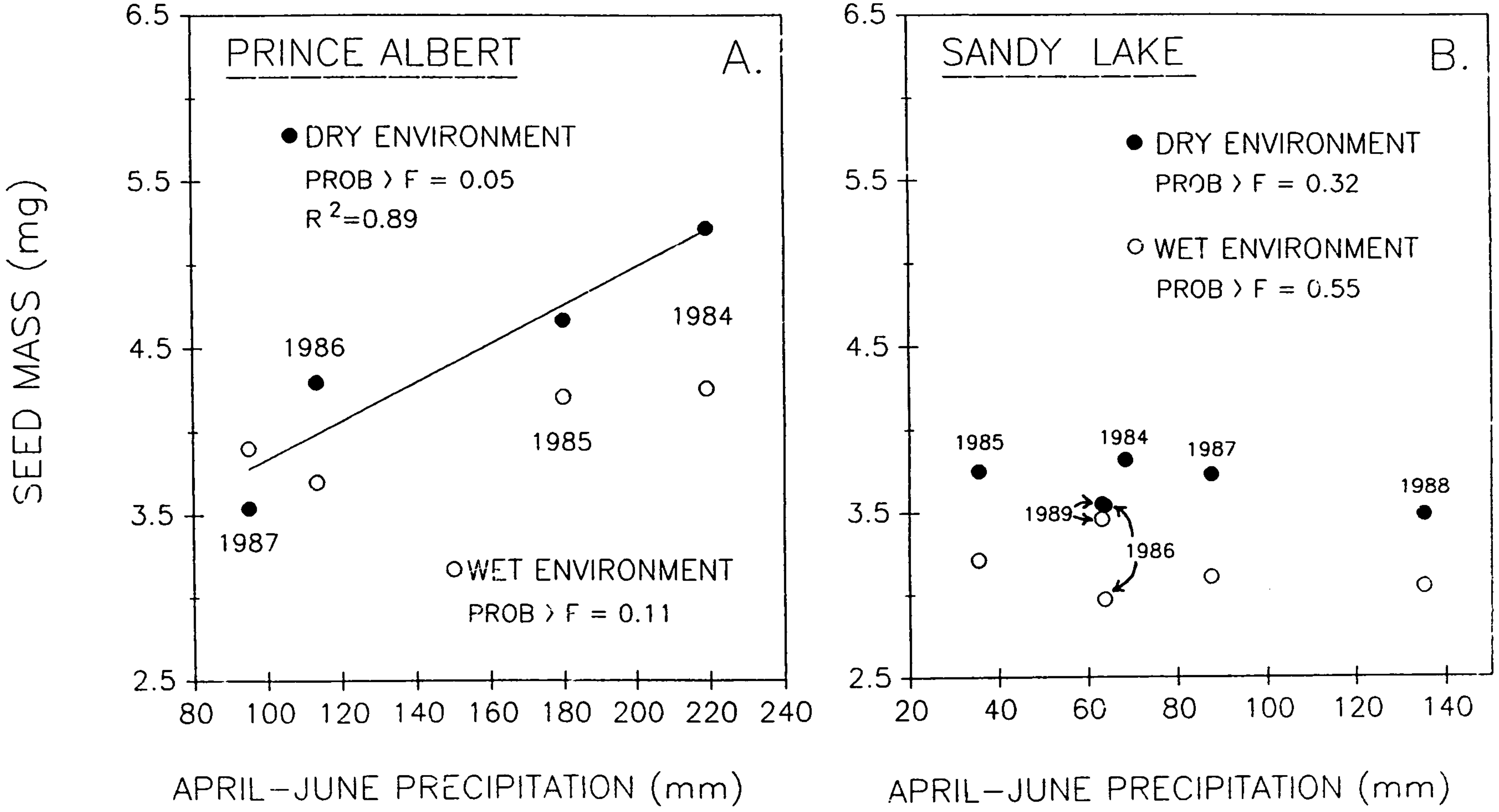

FIG. IV-10. The extent of correlation of seed mass with April-June Precipitation in the southern population at Prince Albert (A) and the northern population at Sandy Lake (B).

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CHAPTER V

SYNTHESIS

Seedlings derived from northern populations invested proportionately more in root biomass than seedling progeny of southern regions. A larger root system contributes to drought avoidance by providing more water-absorptive root surface per unit leaf area. The larger root surface supplying each unit of the photosysnthetic apparatus increases the likelihood that the root system will be able to meet the water needs of the aboveground portion of the plant and prevent the development of tissue water deficits. This characteristic, by itself, has the potential to confer enhanced drought-avoidance abilities on north-region seedlings, compared to southern seedlings. The incorporation of root architectural variables into the comparison of regional populations of jack pine strengthened the conclusion that populations display a significant clinal increase in root growth characteristics that enhance drought resistance from south to north across the boreal forest. Similarly, the lower stomatal frequency of northern populations reinforced the picture of north-region jack pine as that fraction of the species with the greatest overall adaptation to the resistance of drought in the western Canadian boreal forest.

The ecological and evolutionary significance of potential changes in mean temperature and precipitation can be addressed by observing the existing relationship between patterns of population differentiation and climatic gradients. Our analysis has shown that northern populations are better equipped to contend with the increase in the frequency or the intensity of drought that might result from anthropogenic climate change. This pattern of genetic differentiation for drought resistance traits across the boreal forest appears to be correlated with the increase in annual and growing season precipitation from north to south in western Canada. On this basis, our data support the conclusion that the dominant factor, in the development of the cline in seedling drought resistance characters across the boreal forest, has been increasing aridity to the north. If a trend to greater aridity develops in western Canada the gene pool of northern populations may well increase in importance within the species as a whole.

Genetic variance is the raw material for evolution and a widespread species with high spatial variance in genetic composition is better positioned to survive periods of rapid change in the nature and intensity of natural selection. The traits we have measured parallel several dimensions of the regeneration niche of jack pine. The heterogeneity of population means for those traits across the boreal forest, at two very different spatial scales, is one indicator that jack pine possesses the genetic versatility needed to cope with a rapid climate-mediated shift in the regeneration niche. Times of rapid change in the direction or intensity of natural selection may present opportunities for species to overcome genostasis and make the shift to new adaptive optima that appear in the shifting evolutionary landscape.

The existence of genetic variation for seedling morphological traits suggests that tree improvement for drought resistance may be possible in jack pine. The strength and spatial distribution of genetic differentiation indicate that populations of jack pine in the boreal forest possess the variation necessary to make a meaningful contribution to tree improvement efforts. Seedling drought resistance traits could be combined in a selection index that incorporates traditional commercial factors such as adult form and growth rate. The advantage of including seedling and even adult drought resistance traits in tree improvement programs stems from the risk-reduction value they bring to plantation forestry in times of extreme environmental uncertainty. Plantations established with stock selected to perform optimally in the present environment, with no consideration of the probability of a rapid drying and warming trend in the boreal forest, are exposed to a potentially unacceptably high risk of plantation failure. Predictions of future climates should not be allowed to drive tree improvement programs but the knowledge we possess about the spatial genetic structuring in species gene pools should be brought to bear in a fashion that increases the likelihood of success in the field of tree improvement and plantation forestry.

The positive correlation of seed mass with seedling survivorship presents another important dimension of the ecology and evolution of seedling drought resistance traits in jack pine. The subtleties of seed mass variation should receive further attention from foresters and evolutionary biologists because of the pervasive influence of seed mass on early growth in trees and because of the intriguing questions that remain unanswered concerning spatial variation in seed mass and its relationship to the demography and evolution of important tree species in the boreal forest.