ENVIRONMENTAL CONTROL OF SPRUCE SEEDLING GROWTH AND SHOOT DEVELOPMENT

By

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ABSTRACT

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The photoperiodic protraction of indeterminate (free) growth is important in commercial seedling production. Free growth varies among species and with age. In spruce, the mature growth pattern is determinate. Only young seedlings free grow. The objective of this study was to improve the understanding of seedling development during this growth-pattern change.

Free growth dynamics of spruce seedlings were followed for 24 weeks. Relative biomass growth rates were constant, suggesting exponential growth. Height growth was also studied. Relative height growth rates declined rapidly.

Apical meristem development was also followed. Measurements were made with a digitizer and a volumetric computer program. Meristem volumes increased up to 10 weeks; cytohistological zone proportions continued to change. The pith-rib meristem relative volume increased at the expense of the others zones.

Shortened growth cycles (SGC) were used to determine the effects of cyclic growth on free growth potential. All species could free grow, even after four cycles. SGC seedlings were smaller than normally cycled seedlings. The buds were also smaller than normal. SGCs seemed to impede development.

John William Heckman, Jr.

Seedling age and size effects on free growth were tested. Blue spruce seedlings of three sizes, grown by "accelerated growth" (AG) and "conventional nursery" (NUR), methods were compared under long days (LD) and short days (SD). Within each size class, AG seedlings were less developed than NUR seedlings.

Photoperiod had no effect on flushing. NUR seedlings grew faster and more than AGs, during the flush, but had shorter internodes.

NUR seedlings free grew less than AGs, all of which free grew under LD. Some NUR seedlings did not free grow. Reduced growth potential, perhaps due to root disturbance and low light levels, was more important than seedling size.

Normal bud development occurred under SD. By the last sample, SD meristems had no cytohistological zonation and cell walls appeared shrunken. SD meristem cells had smaller vacuoles and more starch grains than LD cells.

Free growing meristems had distinct zonation and were like active meristems previously described in spruce.

Seedlings not free growing under LD had meristems like those normally seen during late bud scale initiation. Prolonged bud scale formation seems to occur in seedlings not free growing under LD.

TABLE OF CONTENTS

CHAPTER Page

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The growth of trees in containers has been studied at least since the investigations of Van Helmont, in 1648 (in Kimball, 1969). Since that time, growing trees in containers has progressed from a botanical curiosity to a major source of seedlings for a variety of silvicultural and horticultural endeavors. It is estimated that over one half billion containerized forest tree seedlings are produced annually, in the northern hemisphere (Hulton, 1974; Reese and Scarratt, 1982).

One of the major discoveries facilitating containerized seedling production was that, in the early part of this century, of photoperiodism in forest tree seedlings (see Wareing, 1956 for early review). By combining the optimum climate of a properly regulated greenhouse, a controlled growing medium, and an altered photoperiod, years can be stripped off the the normal nursery period for many tree species (e.g. Hanover et al., 1976; Tinus and McDonald, 1979).

In order to prudently alter the photoperiodic regimes, it is essential to understand the response of the developing seedling to differing photoperiods.

Spruce (Picea sp.) is a genus of Northern Hemisphere forest trees generally found in the cooler climatic regions. Several species are commercially important. As a mature tree, spruce exhibits a determinate form of shoot growth.

All of the needles and their subtending internodes for a given year are preformed during the preceding summer. They lie dormant, as preformed shoots in the vegetative buds, through the winter.

The spruce embryo has no preformed leaves, except the cotyledons. The preformed shoot thus consists only of a primordial apical meristem and the hypocotyl. Therefore, the spruce seedling must change from a completely indeterminate form of growth to the cyclic determinate form mentioned above. This appears to occur within the first few years for spruce grown under natural conditions (Pollard and Logan, 1976; Young and Hanover,1976). It is during this early development, especially in the first year, that the greatest advantages of containerized spruce seedling production are realized.

Blue spruce (Picea pungens Engelm.), Norway spruce (P. abies (L.) Karst.), and white spruce (P. glauca (Moench.) Voss) are species of commercial importance, in northern temperate zones. Greenhouse production of seedlings of these species, using containers and artificially extended photoperiods, is also increasing.

The long day (or short night) photoperiodic induction and control of indeterminate or "free" growth in spruce germinant seedlings is well documented and seems to hold for all species (e.g. Hanover et al., 1976). Less is known about their growth and developmental dynamics. As more and greater investments are made in containerized seedling production and

energy costs increase, a knowledge of how seedlings grow, under various photoperiodic regimes, will have increasing economic importance. The role of photoperiod in the whole of seedling development, in addition to the induction of free growth, is being increasingly realized as a critical factor in the production of seedlings that continue to perform well, after they leave the greenhouse (Glerum, 1982).

In contrast to germinant seedlings, and regardless of the photoperiod, older spruce seedlings do not free grow. For example, blue spruce seedlings three years old, or older, will not grow continuously under extended photoperiods while younger seedlings will (Young and Hanover, 1976). This change in shoot growth habit is important from standpoint of nursery management and plantation establishment. For example, understanding shoot growth phenology is important in controlling seedling frost hardiness which dramatically affects subsequent field survival and performance (Glerum, 1982).

As Romberger (1963) pointed out, although meristems are small, the whole course of a tree's development depends on the actions of these growth centers. In addition to being the ultimate source of the entire shoot, above the cotyledons, the shoot apical meristem is instrumental in the control of the growth and development of the subtending shoot (Kramer and Kozlowski, 1979). Thus, the shoot apical meristem is a potentially productive first place to look for ontogenetic and environmentally induced developmental changes.

The overall development of the spruce shoot and its responses to some environmental stimuli have been studied before (e.g. Dormling et al., 1968; Pollard and Logan, 1977; Young and Hanover, 1976,1977a,1978). There appear to be no investigations of the initial developmental anatomy of the apical meristem in spruce, except the works of Burley (1966a,b), Gregory and Romberger (1972a,b), Cannell (1978a). None of these of these investigations involved comparison controlled environmental effects.

This investigation attempted to quantify some of the environmentally affected developmental changes in the early ontogeny of spruce seedlings that might be related to photoperiodically induced free growth response. Since the apical meristem plays a major part in the free growth phenomenon, the relationship between its developmental anatomy and the rest of seedling growth and development was a major focus of this study.

Because size is one characteristic of a spruce seedling that changes greatly during the period of photoperiodic free growth responsiveness, seedling size and growth rate dynamics were investigated along with concomitant changes in shoot apical meristem anatomy. This investigation was conducted under uniform environmental conditions, with continuous irradiation, in order to limit environmental effects on these dynamics.

Older nursery-grown seedlings, that will no longer free grow in response to long photoperiods, have also experienced

sequential physiological cycles. A series of shortened growth cycles was created by controlling shoot activity with altered photoperiods and artificial chilling-regimes. This was used in an attempt to determine if the accumulation of growth cycles was a discrete factor in the loss of the free growth response.

Lastly, the intermediate photoperiodic response of two-year-old blue spruce seedlings was investigated. The importance of seedling size, morphology and apical anatomy on free growth were compared with in two-year-old nursery-grown and one-year-old accelerated-growth seedlings.

By developing a satisfactory model of the growth and development of the spruce seedling, during the ontogenetic period from germination to the loss free growth response, better allocation of nursery resources should be possible. In addition, such a model would provide a strong framework for subsequent investigations into the underlying physiological mechanisms of environmentally controlled seedling growth and development.

CHAPTER 1

THE GROWTH DYNAMICS OF SPRUCE SEEDLINGS IN A UNIFORM ENVIRONMENT

ABSTRACT

The growth dynamics of three species of spruce and an interspecific hybrid between two of them were studied for 24 weeks under conditions promoting indeterminate growth. Interspecific differences were seen in seedling emergence and in cumulative biomass at each measurement time. There were, however, no significant interspecific differences in mean relative biomass growth rates during this period. Root to shoot biomass distribution also differed among species but there was no net change over time.

Seedling height growth was also studied. Height growth differences existed among species at all measurement times, and increased over time. Some of these differences were attributable to differences in internode length. Seedling mean relative height-growth rates, declined rapidly but asymptotically over time. Interspecific differences in relative height growth rate declined with age. Thus, height growth appeared to approach a constant rate under the environmental conditions used.

INTRODUCTION

Dormancy delay by the photoperiodic protraction of indeterminate growth in spruce (*Picea sp.* A. Dietr.) germinant seedlings is well established. This phenomenon, termed "free growth" (Jablanski, 1971), exists for seedlings of many conifers; it is seen in young seedlings of all tested spruce species (*e.g.* Downs, 1962; Dormling *et al.*, 1968; Hanover and Reicosky, 1972; Pollard and Logan, 1977). Many investigations have been made to quantify the environmental and biological factors involved in the free growth of spruce. In addition to photoperiodic control, light quality and intensity (Young and Hanover, 1977a; Arnott, 1979), nutrients and water (Young and Hanover, 1978), geographic seed source (Vaartaja, 1959; Dormling, 1973; Cannell and Willett, 1976; Pollard and Logan, 1974a; Pollard and Ying, 1979) and seedling age (Neinstaedt, 1966; Jablanski, 1971; Young and Hanover, 1976; Powell, 1982) all affect this response. Less is known about the dynamics of free growth in spruce seedlings.

Indeterminate shoot growth in conifer seedlings occurs in two general forms. In the case of some pines, such as lodgepole pine (*Pinus contorta* Dougl.), Scots pine (*P. sylvestris* L.), and longleaf pine (*P. palustris* Mill.), indeterminate growth occurs in a saltatory manner, often with a resting bud formed between successive flushes (Downs and Borthwick, 1956; Lanner, 1976; Wheeler, 1979). In some pines

the photoperiodic free growth response appears to be more limited. With white pine, for example, Fowler (1961) found that even under 24 hr photoperiods most greenhouse grown seedlings set bud after 5 to 9 months of culture. He also reported that the heights were no more than 4 cm taller than the natural photoperiod controls, all of which set bud within two months.

Spruce germinant seedling shoot-growth, under optimum environmental conditions, is continuous. Foliar primordia, formed at the apical meristem, develop directly into needles and internode elongation is continuous (Gregory and Romberger, 1972a,b, 1977; Jablanski, 1971; Powell, 1982). Typically, the initial growth rate increases (i.e. the plastochron duration decreases) during the first weeks of free growth in germinant seedlings (Gregory and Romberger, 1972a,b; Cannell, 1978a).

Spruce seedling biomass growth, under long days, has also been studied for some species. Jarvis and Jarvis (1964) reported relative growth rates for Norway spruce (Picea abies (L.) Karst.) grown under long days (18 hr). Grime and Hunt (1975) calculated similar rates for Norway and Sitka spruce (P. sitchensis (Bong.) Carr) seedlings grown under the same photoperiod.

Similar types of growth analyses have been used to model dry weight accumulation and photosynthate partitioning in spruce and other species of tree seedlings under nursery conditions (van den Driessche, 1968; Ledig, 1974; Cannell and

Willett, 1976). The use of growth analysis techniques has been suggested as an approach to early genetic selection (see for example Ledig, 1976). In another sense, growth analysis is also suggested for monitoring greenhouse seedling production (Tinus and McDonald, 1979).

The quantitative aspects of seedling height growth, on the other hand, have received much less attention. Height growth rate also appears to increase, under optimal conditions for free growth, early in the seedling development. A plot of height against age is initially curvalinear. Generally, however, within a few weeks of growth under long photoperiods, height growth appears nearly linear (e.g. Dormling et al., 1968; Heide, 1974a; Young and Hanover, 1977a; Logan, 1977; Cannell and Cahalan, 1979).

The objective of this investigation was to study the growth of spruce germinant seedlings under optimal conditions for indeterminate growth to better understand their growth dynamics. Information of this kind should be useful to people involved in planning and managing seedling production operations and to others interested in seedling developmental physiology.

MATERIALS AND METHODS

Seed source and cultural system

Three spruce species and one interspecific hybrid spruce, hereafter considered as an individual species for convenience, were used in this experiment. Blue spruce seed

was collected from a single, non-isolated tree on the campus of Michigan State University. White spruce came from border row trees in a white spruce racial test and the Norway spruce from a individual tree source, both at Kellogg Experimental Forest, near Augusta Michigan. The hybrid spruce seed was from an experimental cross between the single blue spruce mentioned above (male) and one of the border row trees in the white spruce test (female) (described by Hanover and Wilkinson, 1969). All seed was cleaned then stored at about 4°C prior to use.

Seeds were sown in milk cases of 5 x 5 and 10 x 10 x 27 cm, polyethylene-coated, paper plant-bands, filled with a 1:1:1 sphagnum peat : perlite : vermiculite mixture. The bands were initially fertilized with a soluble fertilizer (Peter's) (19:18:17 (NPK)), at 1.2 g / case, and Peter's trace mineral solution (STEM), at 0.6 g / case, then watered to field capacity. Each case also received an equal amount of additional fertilization at two week intervals throughout the experiment. Water was applied twice daily during germination and thence as needed to maintain uniform soil moisture.

The cases were arranged on a three-tiered growth-frame in a 20-25°C room. Irradiation was provided by T96 high-output cool-white, two-bulb fluorescent fixtures. Three fixtures were used on each level of the frame. The photosynthetic photon flux density (PPFD), in $\mu M\ m^{-2}\ sec^{-1}$, in the 400 to 700 nm spectrum was measured for each band at

the soil level. Three blocks, each with 72 seedlings of each species, were arranged based on PPFD gradients. The PPFD ranged from 48 to 250 $\mu M\ m^{-2}\ sec^{-1}$.

Growth measurement

Four weeks after sowing, and at 20 weekly intervals thereafter, the height of each remaining seedling shoot, from the cotyledons to the tip of the distal most developing needle, was recorded. For height growth analysis, block means were used. These were calculated from seedlings not sampled during the experiment and thus represent repeated measurements of the same seedlings. In addition, each week, 12 seedlings, one of each species from each block, from the smaller bands were randomly selected for morphological and anatomical analysis in a concomitant experiment. From these seedlings the number of main stem needles and primordia (plastochron index) was recorded. Seedlings were then oven dried at 60°C and shoot and root dry weights were recorded for biomass analysis.

Analytical methods

Species cumulative biomass growth and height growth were evaluated weekly by analysis of variance of block means for each species. Overall growth dynamics were analyzed by least squares regression techniques, using several classes of models. The data were first logarithmically transformed to reduce the heterogeneity of variance, associated with the large differences in weight, encountered over the course of development.

Species weekly mean relative growth rate ($\overline{RGR}$) was calculated using weekly mean biomass, by the following standard equation:

$$\overline{RGR} = \frac{\text{Ln } W_2 - \text{Ln } W_1}{t_2 - t_1}$$

Where Ln W_1 is the natural logarithm of a species mean weekly-sample biomass at the initial measurement time and Ln W_2 is that of the next period. The values t_1 and t_2 are the weeks over which the rate is being averaged.

By counting main stem needles and needle primordia of the sampled seedlings, plastochron index (PI) was calculated. Plastochron index models, based on seedling height and species, were then developed by linear regression. Further analyses were preformed using calculated plastochron indices.

RESULTS

Initial observations

Seedling emergence was first observed ten days after sowing. Blue and Norway spruce seeds germinated first, followed by white and lastly by the hybrid seeds. Most bands, of all species, had one or more germinant seedlings by the third week; bands were thinned to one seedling by the fourth week. In general the blue and Norway spruce germinant seedlings were larger than those of the hybrid and white spruce. All seedlings grew indeterminately for the whole

experiment; no terminal and few lateral buds were seen on any of the seedlings.

Biomass growth

Seedling biomass for all species increased slowly at first then rapidly later in the experiment (Figure 1.1A). Cumulative biomass differences, especially in the first 10 weeks of growth, are more clearly seen when expressed logarithmically with respect to time (Figure 1.2B).

Sampled Norway spruce seedlings were significantly heavier than blue spruce seedlings. Blue and Norway spruce both produced more biomass than either white or hybrid spruce. There was no significant overall difference between white and hybrid spruce biomass (Table 1.1).

Table 1.1. Selected morphological characteristics of blue, hybrid, white, and Norway spruce germinant seedlings after 24 weeks of growth under 24 hr photoperiod.

Characteristic	Blue	Hybrid	White	Norway
Shoot Height (cm) (final sample)	20.1a[1/]	9.6b	11.8b	20.8a
Biomass (g) (final sample)	4.75a	1.40b	2.40b	4.38a
Biomass (g) (average)	1.3a	0.55b	.70b	1.82a
Root:Shoot Ratio (average)	.25a	.18b	.20b	.29a

1/ Means, within a characteristic, followed by the same letter are not significantly different ($p < .05$ Tukey's HSD). Means listed as average are pooled over 24 weeks.

In addition to differences in cumulative biomass, there were interspecific differences in root to shoot dry weight

Figure 1.1 Cumulative biomass growth of blue, hybrid, white and Norway spruce seedlings grown for 24 weeks under 24 hr photoperiod.

A) Weekly mean oven-dry biomass values.

B) Weekly mean natural logarithm of seedling oven-dry biomass values.

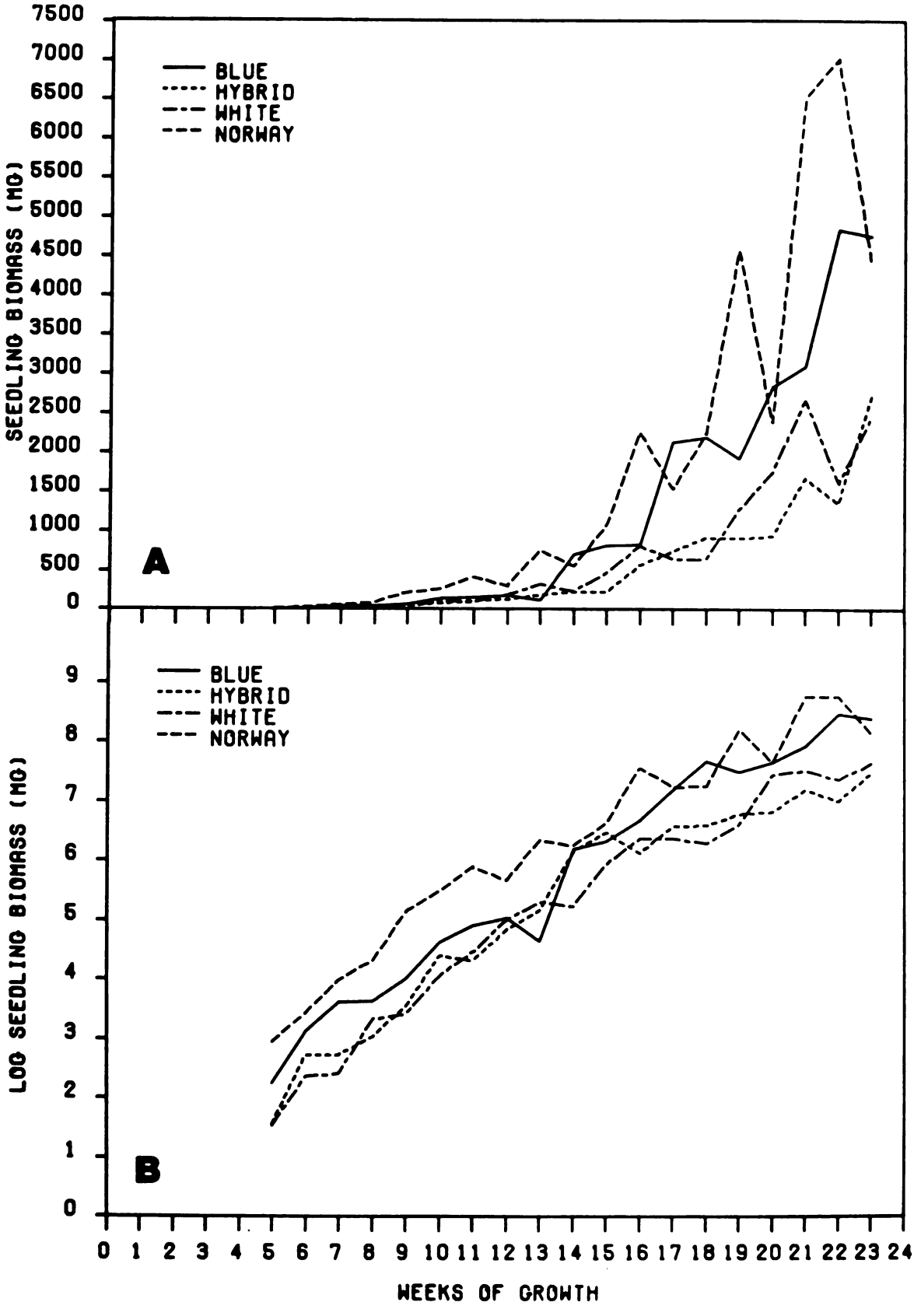
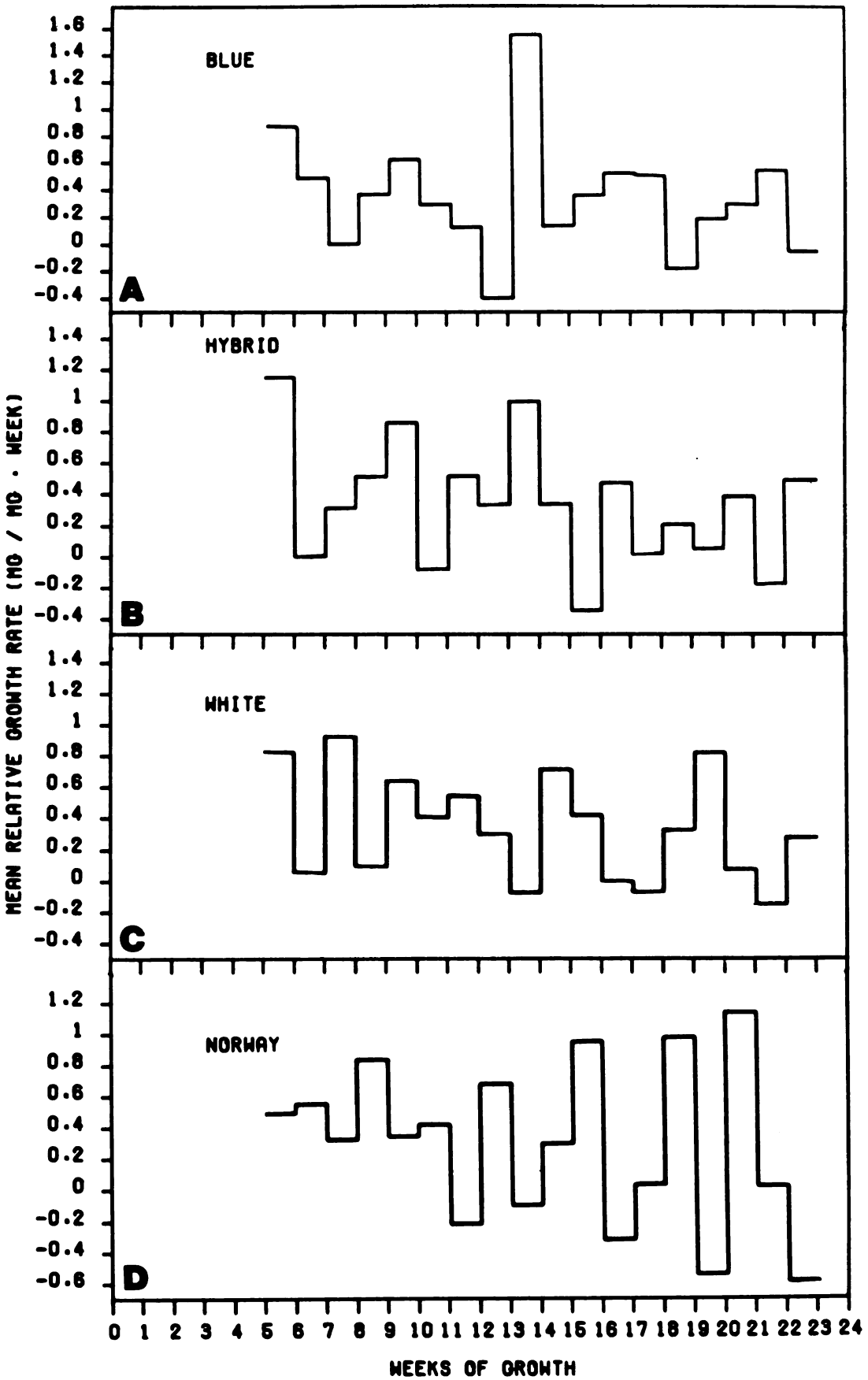

BLUE
HYBRID
WHITE
NORWAY
SEEDLING BIOMASS (MG)
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
A
BLUE
HYBRID
WHITE
NORWAY
LOG SEEDLING BIOMASS (MG)
9
8
7
6
5
4
3
2
1
0
B
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
WEEKS OF GROWTH

Figure 1.2 Weekly mean relative growth rates ($\overline{RGR}$) for spruce seedlings grown for 24 weeks under 24 hr photoperiod. A) blue spruce, B) hybrid spruce, C) white spruce, and D) Norway spruce.


BLUE
A
HYBRID
B
WHITE
C
NORWAY
D
MEAN RELATIVE GROWTH RATE (MG / MG · WEEK)
WEEKS OF GROWTH
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

distribution (Table 1.1). Norway spruce root to shoot ratios were not significantly higher than blue spruce. Both blue and Norway spruce had higher root to shoot ratios than white and hybrid spruce (Table 1.1). Hybrid and white spruce were not significantly different. Root to shoot ratios for each species remained relatively stable over the course of the experiment showing no significant upward or downward trends.

The various growth models and their respective tests of goodness of fit are presented in Table 1.2. From the test criteria in Table 1.2, it can be seen that little additional goodness of fit is afforded by polynomial models higher than the first order. This is especially true for the differences in fit between the second and third order models. That there is a slight improvement in fit with the second order equation is expected given the apparent slight curve in the original data (Figure 1.1B). There is no evidence of a third inflection in the data, and hence little real improvement in overall fit with the third order equation.

Seedling weekly mean relative growth rate ($\overline{RGR}$), calculated by using the weekly sample means (for biomass), is illustrated for each species in Figure 1.2. When calculated on a weekly basis these $\overline{RGR}$ values are the slopes of the line segments connecting the data points presented in Figure 1.1B.

The regression coefficients of the first order regression equations used to fit the biomass data (Table 1.2), by virtue of the logarithmic transformation used, also represent the average relative growth rate over the course of

Table 1.2. Regression models tested for comparing seedling biomass growth for blue, hybrid, white, and Norway spruce, under 24 hr photoperiod, for 24 weeks.

Species	n	Regression model[1/]	RMSE[2/]	D[3/]	R^2
Blue spruce	57	$LnY = 1.0845 + .3363A$	.662	.969	.884
		$LnY = 0.3400 + .4619A - .0045A^2$	.651	.970	.885
		$LnY = 1.4765 + .1569A + .0195A^2 - .0006A^3$	.647	.970	.885
Hybrid spruce	55	$LnY = 0.8149 + .3112A$	.648	.969	.868
		$LnY = -0.7779 + .5825A - .0099A^2$	.595	.974	.887
		$LnY = -1.3494 + .7372A - .0220A^2 + .0003A^3$	.594	.974	.885
White spruce	56	$LnY = 0.6942 + .3237A$	.793	.957	.831
		$LnY = -0.9732 + .6053A - .0101A^2$	.745	.962	.848
		$LnY = -1.3069 + .6951A - .0171A^2 + .0002A^3$	.745	.962	.845
Norway spruce	55	$LnY = 2.1721 + .2955A$	.732	.962	.819
		$LnY = 0.5078 + .5720A - .0098A^2$	.687	.967	.838
		$LnY = 0.1869 + .6564A - .0164A^2 + .0002A^3$	.686	.967	.835

1/ Where: Y = Total seedling biomass.
A = Seedling age in weeks.
n = Number of seedlings measured.
2/ RMSE Root mean square error (Willmott, 1982).
3/ D Index of agreement (Willmott, 1982).

the experiment. When the values for each species were compared to that of a pooled regression (Variation among regressions / Average within regressions) by an F test (Sokal and Rohlf, 1981) no significant difference was found. This implies that the relative growth rates for the four species tested, averaged over the growth period of the experiment, were the same.

As mentioned above, the second order regressions (Table 1.2) describe most of the curved nature of the logarithmically transformed data. The slopes of their first derivatives, therefore, describe the overall rate of change of relative growth rate. In all cases these values are negative and very slight. The least squares fit of these lines through the $\overline{RGR}$ data, however, never yielded a significant regression. This was likely due to the high variability in weekly $\overline{RGR}$ values.

Shoot height growth

Blue and Norway spruce main-stem height was significantly greater than hybrid and white spruce at each measurement time. In addition, these differences increased throughout the experiment (Figure 1.3A). There was no significant difference between blue spruce and Norway spruce cumulative height at any time during the experiment. Differences in height growth between white and the hybrid spruce, although increasing throughout the experiment, were never significant. The mean final cumulative heights are presented in Table 1.1.

Figure 1.3 Cumulative height growth of blue, hybrid, white and Norway spruce seedlings grown for 24 weeks under 24 hr photoperiod.

A) Weekly means of seedling heights.

B) Natural logarithms of weekly means of seedling heights.

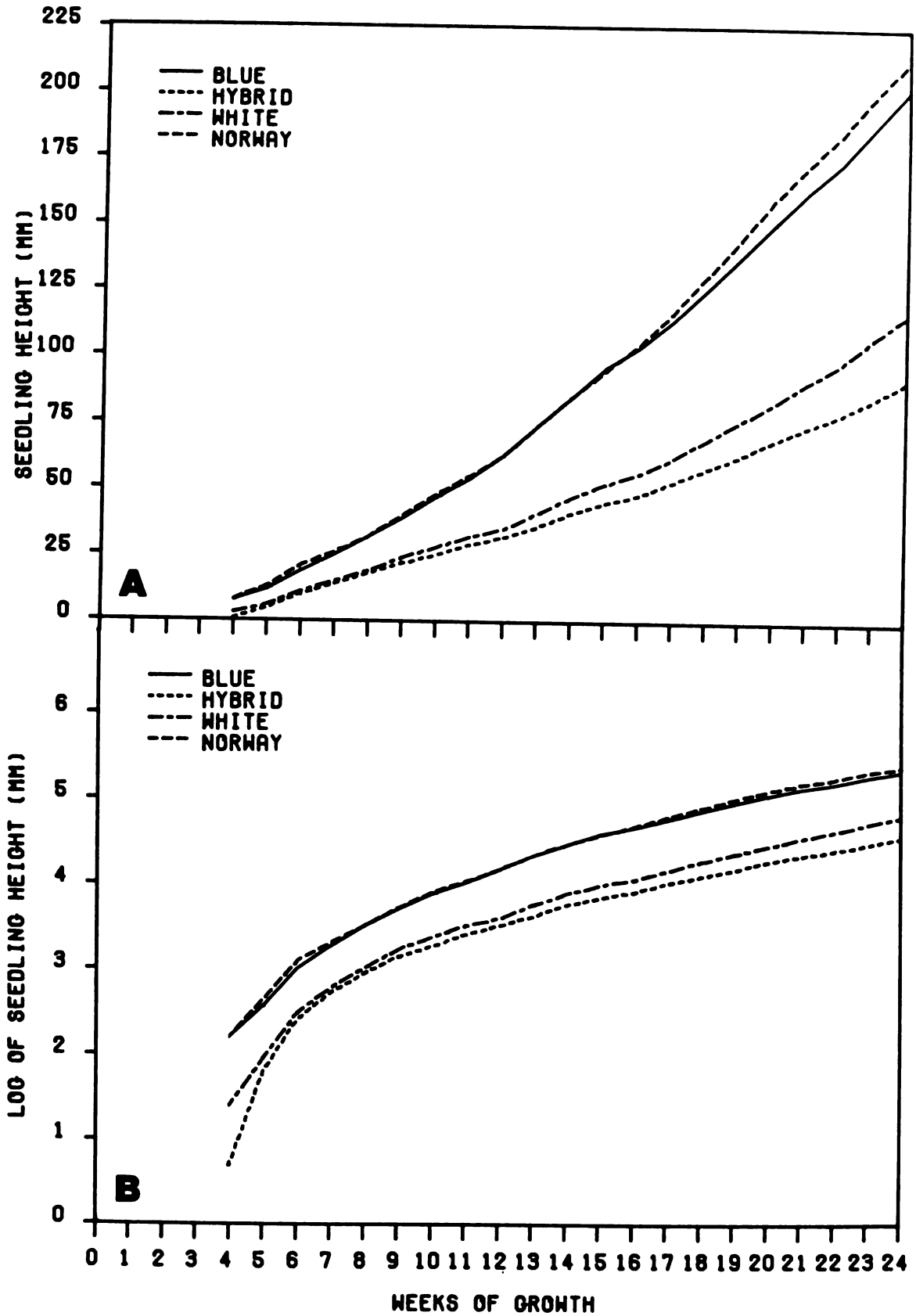

BLUE
HYBRID
WHITE
NORWAY
SEEDLING HEIGHT (MM)
225
200
175
150
125
100
75
50
25
0
A
BLUE
HYBRID
WHITE
NORWAY
LOG OF SEEDLING HEIGHT (MM)
6
5
4
3
2
1
0
B
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
WEEKS OF GROWTH

Table 1.3. Regression models tested for comparing seedling height growth for blue, hybrid, white, and Norway spruce, under 24 hr photoperiod, for 24 weeks.

Species	n	Regression model[1/]	RMSE[2/]	D[3/]	R^2
Blue spruce	63	$LnY = 2.2555 + .1400A$	.261	.977	.912
		$LnY = 1.2113 + .3235A - .0066A^2$	.150	.993	.970
		$LnY = 0.4556 + .5400A - .0240A^2 - .0004A^3$	.132	.994	.977
Hybrid spruce	63	$LnY = 1.5484 + .1386$	.379	.951	.827
		$LnY = 0.1161 + .3903A - .0090A^2$	.240	.982	.930
		$LnY = -1.7621 + .9284A - .0523A^2 + .0010A^3$	.162	.992	.967
White spruce	63	$LnY = 1.6964 + .1399A$	.290	.972	.893
		$LnY = 0.5685 + .3381A - .0071A^2$	.176	.990	.960
		$LnY = -0.7915 + .7278A - .0384A^2 + .0007A^3$	.120	.996	.981
Norway spruce	63	$LnY = 2.2882 + .1402A$	.248	.979	.920
		$LnY = 1.3582 + .3036A - .0058A^2$	.158	.992	.967
		$LnY = 0.6904 + .4949A - .0212A^2 + .0004A^3$	.145	.993	.972

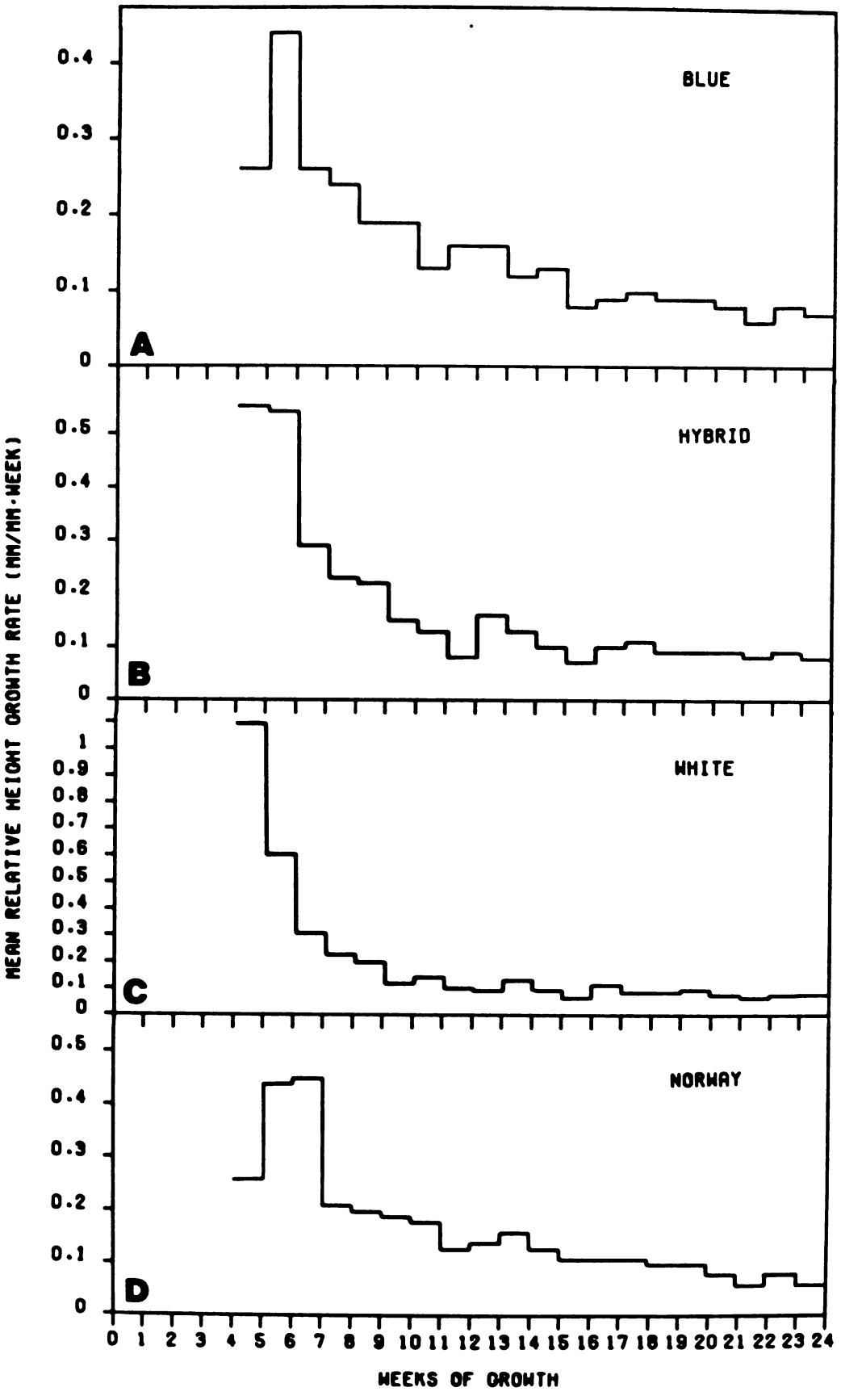
1/ Where: Y = Seedling main-stem height above cotyledons.
A = Seedling age in weeks.
n = Number of seedling height mean values used in the regression.
2/ RMSE Root mean square error (Willmott, 1982).
3/ D Index of agreement (Willmott, 1982).

As with the biomass data, a series of least squares polynomial regressions was developed to compare the height growth dynamics of the seedlings (Table 1.3). The original data was, again, log-transformed to homogenize sample variance among weeks. The result of these transformations is seen in Figure 1.3B. In contrast to the biomass data, these height growth data represent the block means of repeated measurements of the same plants and the sampling error was, therefore, greatly reduced.

The goodness of fit of regression for height increased considerably with the order of the fitted polynomial. There was a large and consistent decrease in the RMSE, which describes overall regression bias, with increasing polynomial order. This reduction in bias was greatest for hybrid and white spruces and was attributable to the greater change in curvature of their log-transformed data over time (Figure 1.3B). The improvement in fit afforded by the third order equation was largely due to removal of the continuing downward trend due to the (negative) second order regression coefficient, at later measurement times.

The seedling weekly mean relative height-growth rate ($\overline{\mathbf{RHR}}$) was calculated using weekly mean heights, illustrated in Figure 1.3A, by the same formula used to describe $\overline{\mathbf{RGR}}$, substituting the natural logarithm of mean heights for those of biomass. These values are plotted in Figure 1.4. and represent the weekly-interval slopes of the log-transformed height data presented in Figure 1.3B.

Figure 1.4 Weekly mean relative height growth rates ($\overline{RHR}$) for spruce seedlings grown for 24 weeks under 24 hr photoperiod. A) blue spruce, B) hybrid spruce, C) white spruce, and D) Norway spruce.


BLUE
A
HYBRID
B
WHITE
C
NORWAY
D
MEAN RELATIVE HEIGHT GROWTH RATE (MM/MM·WEEK)
WEEKS OF GROWTH
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

The slopes of the first derivatives of the second order polynomials show an experimentwise decline in $\overline{RHR}$. This decline is significant over the course of the experiment. This decline appears to be more asymptotic than linear, as implied by the better fit of the first derivative of the third order polynomial, which is a parabolic function.

Linear regression models of needle number (PI) per unit height for each species were calculated (Table 1.4). PIs were derived for the unsampled seedlings, for each height measurement, using these models. Using these, interspecific growth rate differences appear smaller (Figure 1.5A). Since these models are linear, the PI growth dynamics (Figure 1.5B) follow the same form as height (Figure 1.3B). These results indicate interspecific differences in internode elongation existed over the course of the experiment.

Table 1.4. Regression equations predicting plastochron index from height for each species.

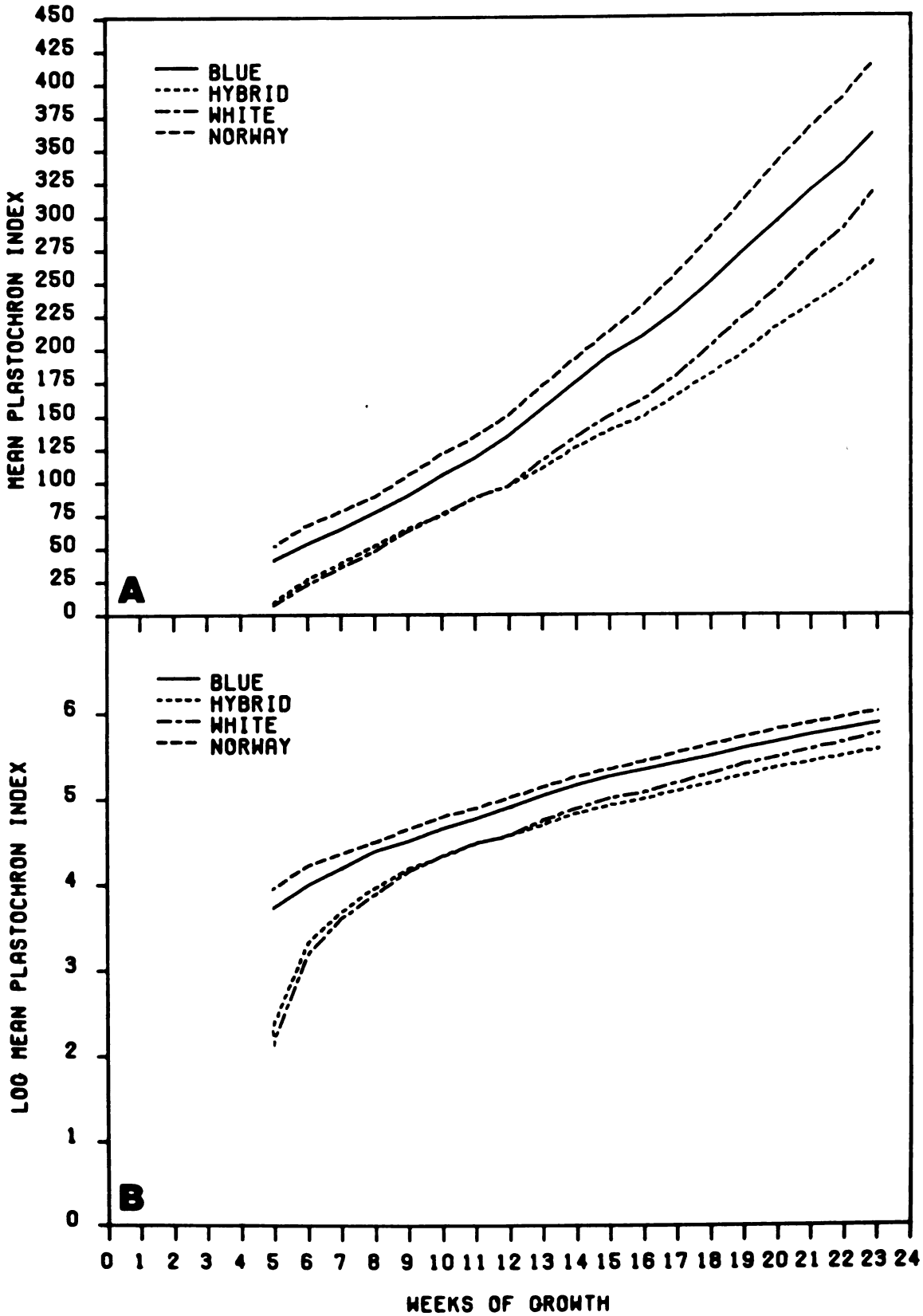
Species	n	Regression model[1/]	(r^2)
Blue spruce	46	Y = 18.58 + 1.84H	.919
Hybrid spruce	51	Y = -5.64 + 3.22H	.918
White spruce	51	Y = -10.57 + 3.08H	.905
Norway spruce	46	Y = 26.61 + 1.96H	.921

1/ Where: Y = Calculated plastochron index.
H = Seedling height in mm above cotyledons.
n = Number of seedlings measured.

Figure 1.5 Seedling plastochron index (PI) for blue, hybrid, white, and Norway spruce seedlings grown for 24 weeks under 24 hr photoperiod.

A) Mean weekly calculated (PI).

B) The natural logarithm of weekly mean calculated plastochron index (PI).


MEAN PLASTOCHRON INDEX
450
425
400
375
350
325
300
275
250
225
200
175
150
125
100
75
50
25
0
BLUE
HYBRID
WHITE
NORWAY
A
LOG MEAN PLASTOCHRON INDEX
6
5
4
3
2
1
0
BLUE
HYBRID
WHITE
NORWAY
B
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
WEEKS OF GROWTH

DISCUSSION

The biomass and height data presented here, indicate that two different sets of dynamics are involved in their respective growth, over the same period of time.

For biomass, over the course of the experiment, the $\overline{RGR}$ was essentially constant. This indicates an exponential rate of biomass growth: $W = W_o \cdot e^{rt}$

Where: W = weight of the plant at time t.

W_o= initial weight of the plant.

t = the time of measurement.

r = is the intrinsic growth rate.

These types of equations imply that the growth rate of the organism increases in direct proportion with the mass. Thus, even small initial differences in mass have the same proportions at any stage. They tend, therefore, to provide a reasonable fit of the data only for small highly meristematic organisms. Since, however, no growth rate asymptote could be inferred from the data (Figure 1.2), use of biologically more "realistic", sigmoid functions, such as the Richards function (Causton and Venus, 1981), or splined polynomial regressions (Hunt, 1982) was precluded.

The $\overline{RGR}$ values obtained in this study (Table 1.1) averaged .32 $week^{-1}$. This value is between those obtained by Grime and Hunt (1975) for Sitka spruce (.22 $week^{-1}$) and Norway spruce (.33 $week^{-1}$) germinant seedlings, for their initial five weeks of growth. Jarvis and Jarvis (1964)

reported different $\overline{RGR}$ for germinant seedlings and 2-year-old seedlings of Norway spruce. For the germinant seedlings, the $\overline{RGR}$ for the first 35 days after the cotyledon stage was .45 $week^{-1}$ in nursery grown seedlings, the rate was .03 $week^{-1}$ during the period of shoot elongation. The initial dry weights of their two-year-old seedlings were less than those of 17th weekly sample weight of Norway spruce seedlings studied here, indicating that past growth history may have a major effect on $\overline{RGR}$. Thus, the exponential growth phenomenon appears to be restricted to the continuous, early growth phase in spruce.

Assuming that the $\overline{RGR}$ was not different among species, and an apparent exponential growth over the course of the experiment, the most likely causes of the great differences in biomass are seed weight and germination time.

Seed weights for blue and Norway spruce are generally higher than those of white and the hybrid. In spruce, seed size and embryo size are positively related (Burley, 1965). If an exponential growth rate is observed, these values become W_o in the equation above and, by definition, the $\overline{RGR}$ is the same and constant, the biomass differences at any time are directly proportional to the seed weight differences. Long lasting seed weight effects are a fairly common observance (Sweet and Wareing, 1968; Evans, 1972; Hanover and Reicosky, 1972).

Differences in germination rapidity also have a similar effect in the case of true exponential growth. By starting

their growth earlier, faster germinating seeds have a higher weight at whatever common time is chosen as t_o.

In contrast to the biomass growth, seedling height did not follow an exponential pattern. From the second measurement on, seedling $\overline{RHR}$ declined. The asymptotic nature of the curve suggests that seedling height growth rate increases but, at a decreasing rate until some constant rate is achieved. A similar relationship between height growth and biomass growth was reported in Scots pine (Pinus sylvestris L.) grown from seed under 18 hr photoperiods (Oden and Dunberg, 1984).

Main stem height is a function of the number and elongation of stem units (Doak, 1935), which are composed of needles and their corresponding internodes. Gregory and Romberger (1972a, 1972b) have shown that, for Norway spruce, the rate of needle formation and internode development rise towards a maximum during the first 160 days. They also note that internode development, at least in terms of vascular differentiation, seems to occur at a relatively constant chronometric rate (Gregory and Romberger, 1977).

Under the cultural conditions used here, the average internode length for the hybrid and white spruce was significantly less than that of the blue and Norway seedlings. Other than the obvious possibility of genetic differences in this trait, one possibility is that the internal water potential varied among species, due to differing responses to the environmental conditions used.

Water potential deficits of less than 20%, induced by root pruning, have been associated with a 39% reduction in shoot elongation in 6-year-old white spruce (Marquard, 1983).

If the interspecific root to shoot ratio differences seen in this study (Table 1.1) gave rise to a similar water stress effect, this might explain the shorter internodes. The shoot height differences seen may be largely due to interspecific differences in root growth potential in the environment used rather than inherent differences in internode growth potential. Within a species, differences in spruce shoot growth, in a common environment, seem more strongly related to stem unit number than internode elongation (Cannell, et al, 1976; Pollard and Logan, 1979).

In conclusion, under optimum conditions, spruce seedlings of at least the three species and hybrid tested undergo an essentially exponential growth in biomass for at least 6 months. Although there seems to be no difference among the species in $\overline{RGR}$, there seems to be considerable interspecific plasticity, in seedling shoot morphology, and root to shoot biomass allocation. Height growth follows a different pattern with its increase tending towards a constant rate. This may be related to development of a constant rate of needle initiation and a finite potential for internode expansion.

CHAPTER 2

THE DEVELOPMENT OF THE SPRUCE SHOOT APICAL MERISTEM IN A UNIFORM ENVIRONMENT

ABSTRACT

The development of the shoot apical meristem of blue, white x blue hybrid, white, and Norway spruce was studied. Seedlings were grown, in a controlled temperature room, for 24 weeks under a 24 hr photoperiod. Qualitative and quantitative measurements were made from median longitudinal sections by light microscopy. Quantitative measurements were made using a electronic digitizer and a volumetric computer program.

The ontogenetic progression in apical development was similar for all species. Embryo apical meristems had fewer but larger cells that also differed qualitatively from those of the growing seedlings.

In general, Norway spruce had the largest apices. There were no overall size differences among those of the other types. Apical meristems of all species increased in overall volume with age, apparently asymptotically. There were no significant species x age interactions. The relative volumes of the apical meristems in the apical initial-central mother cell zone, pith-rib meristem and peripheral zones changed during development. The relative

volume of the pith-rib meristem increased at the expense of those of the central mother cell and peripheral zones. Imbalances in the cytohistological zones of the meristem might lead to growth restrictions.

INTRODUCTION

Blue spruce (Picea pungens Engelm.), Norway spruce (P. abies(L.) Karst.), and white spruce (P. glauca (Moench.) Voss) are spruce species that are of considerable commercial importance in the northern temperate zone. Seedlings of these species are often grown in greenhouses, using containers, frequently with artificially extended photoperiods. Use of these systems allows spruce germinant seedlings to be be grown to field planting size with a substantial savings of time over conventional nursery methods (e.g. Hanover et al., 1976; Tinus and McDonald, 1979). To use these systems to their greatest advantage, it is necessary to understand how seedlings develop in controlled environments and to what degree different species differ in their responses.

The photoperiodic protraction of indeterminate growth in spruce germinant seedlings is well documented (e.g. Downs, 1962; Dormling et al., 1968; Hanover and Reicosky, 1972). Less is known about the alterations in growth and development induced by these treatments. One of the most obvious alterations occurring under greenhouse culture is that of the ontogeny and phenolgy of the shoot apex. In nursery culture there are seasonal constraints on the development of the shoot apex. In order for the seedling to overwinter, shoot growth must stop and a bud must form as part of the overwintering process (Glerum, 1976,1982). The protracted indeterminate growth of spruce germinant

seedlings raised under long photoperiods allows more time for uninterrupted shoot formation and apical meristem development than either conventional nursery practices or natural field conditions.

While the complete yearly phenology of mature spruce shoot apices, for some species, has been well described (Fraser, 1966; Owens and Molder, 1976; Owens et al., 1977; Pillia and Chacko, 1978; Thompsett, 1978; Harrison and Owens, 1983), the initial development of germinant seedling shoot apices, under natural conditions, has received less attention (Burley, 1966a). Much of the investigative work in this area has concerned bud development subsequent to seedling growth acceleration (Pollard and Logan, 1974b; Pollard, 1974a; Pollard and Logan, 1977; Young and Hanover, 1977a; Cannell and Cahalan, 1979).

In spruce, there are fewer studies of the ontogenetic development of the apical meristem itself (Gregory and Romberger, 1972a,b,1977; Cannell, 1978a). The apical meristem is traditionally defined as the undifferentiated tissue distal to the most recently formed foliar primordium.

In their studies, the preceding authors developed mathematical models for apical meristem development and plastochron duration. Gregory and Romberger (1972a) also indicated that there was an ontogenetic progression in the development of the cytohistological zonation typical of conifers (sensu Foster, 1938).

There have been many more investigations of germinant

seedling apical meristem development, especially with respect to cytohistological zonation, in the pines (Pinus sp.) (Fosket and Miksche, 1966; Riding, 1972; Riding and Gifford, 1973; Mia and Durzan, 1974; Cecich, 1977; Kremer, 1984). In the pines, quantitative changes in zonation are seen as the seedling ages (Kremer, 1984) and in mature trees, during their annual growth cycle (Hanawa, 1966; Owestón, 1969). In mature pines there also appear to be differences within the crown of the tree in the size and internal structure of the dormant apical meristems (Tepper, 1963).

As spruce seedlings develop under the lengthened photoperiods used in most greenhouse accelerated-growth seedling production systems, they undergo size rlated changes in growth rate and apical activity (Chapter 1). This experiment investigated the quantitative changes in apical meristem size and histological development of three species and one interspecific hybrid of spruce to test for relationships between apical meristem development and seedling shoot development.

MATERIALS AND METHODS

Seed source and cultural system

Blue, white, white x blue hybrid, and Norway spruce seedlings were used in this experiment. The sources were the same as those described in Chapter 1. As before, the hybrid will hereafter be considered as a fourth species, for

convenience.

Seeds were sown in plastic cases of 5 x 5 x 27 cm polyethylene coated paper plant bands, filled with a 1:1:1 sphagnum peat: perlite: vermiculite mixture. These were distributed randomly within the blocks of a larger growth study (Chapter 1). Nutrient application and maintenance were as described in Chapter 1.

Gross morphology

At weekly intervals, starting 5 weeks after sowing, 12 seedlings (one of each species in each block) were randomly selected for morphological and anatomical analysis. After excision of the apical region for anatomical analysis, the root systems were gently washed free of potting medium and cut from the shoot. Roots and shoots were both oven dried at 60^{o}C then weighed to the nearest mg , for total biomass and root to shoot ratio determination (Chapter 1). Main stem needle and primordia number (Plastochron Index (PI)) were also recorded.

Microtechnique

The shoot apical meristem and upper 3 to 5 mm of stem of each of the seedlings measured above were excised and immersed in FAA (formalin:acetic acid:alcohol:water), to kill and fix the tissues. This was done under partial vacuum to remove trapped air. They were then dehydrated in a water:ethanol:t-butanol series (Johanson, 1940) followed by infiltration with Paraplast embedding medium. Serial longitudinal sections were made at 10 μm and those passing

through the apical meristem were collected and mounted on glass slides. The dewaxed slides were progressively stained with hemalum (Sass, 1958) and regressively with safranin (Johanson, 1940). In addition to the sampled seedlings, three embryos of each of the species used were also excised from dry seeds seeds and prepared as above.

Median longitudinal sections were identified for measurement. Photomicrographs were taken with a Leitz Ortholux microscope using a 10X plano objective and a 10X wide field occular with a Wilde 35mm camera system. Constant magnification enlargements, at 330 X, were made from these negatives for digital analysis. Selected material was also photographed with the same microscope system usng a 4 x 5 inch plate camera. In all cases, calibration photomicrographs were made using a micrometer slide.

Volumetric analysis

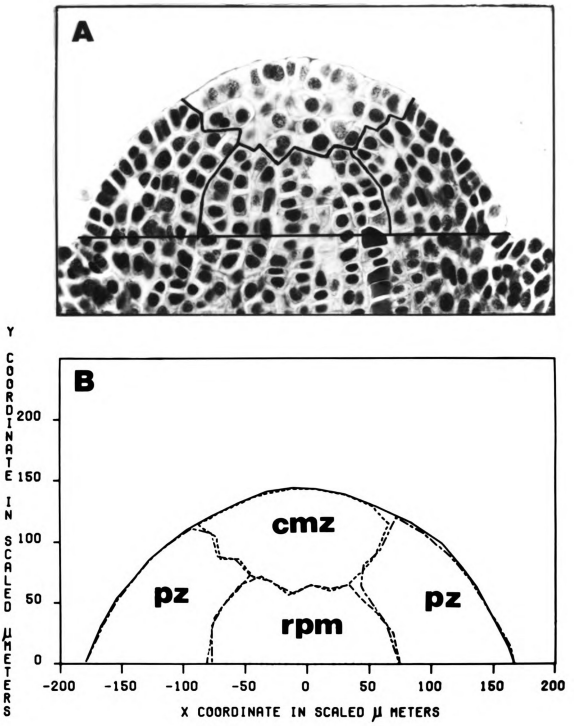
Digitizing of the apical meristem photographs was done with a GTCO micro-datizer digitizer coupled to a CDC Cyber 750 computer, by "tracing" the outlines of the whole meristem and pictured cytohystological zones with the digitizing cursor. This initial process yielded two dimensional coordinate data with a Y value recorded for each 10 scaled micrometers (3.3 mm) in the X direction (Figure 2.1).

Total meristem volume and cytohistological zone volumes were calculated from these data with a FORTRAN program, on the same computer.

Figure 2.1 Median longitudinal section through the shoot apical meristem of a representative 16-week-old Norway spruce seedling.

A) Photomicrograph (330X). The meristem base, as determined by the level of the last formed primordium, and the cytohistological zones have been highlighted to show their boundaries.

B) A digital rendition of the same meristem generated by plotting the raw data from tracing the highlighted areas in (A). The digitizer was set to record the ordinate "Y" value on every change of 10 scaled µm in the abscissa "X" direction during tracing. The procedure was repeated for the boundary of each zone, cmz (central mother cell zone), rpm (pith-rib meristem), and the pz (peripheral zone).


A
B
cmz
pz
pz
rpm
Y COORDINATE IN SCALED μ METERS
200
150
100
50
0
-200
-150
-100
-50
0
50
100
150
200
X COORDINATE IN SCALED μ METERS

Volumes of the whole meristem and those of the individual cytohystological zones, were calculated by summing mean conic frustra calculated from the digitized data. In the case of the total meristem volume, for example, the program calculates the volumes of two series of frustra.

The first series progresses acropetally, starting on the left side (negative most X value) at the level of the last formed needle primordium and ending with a broad cone at the top of the meristem. The second is a basipetal series starting at the top center of the meristem and progressing downward to the same base line. The incremental volumes of each series are halved and summed to accommodate asymmetrical apices and variation in Y axis positioning. The algorithm uses the following equation to calculate incremental half frustra:

$$V = \sum_{i=Xmin}^{Xmax} 1/2 \left(1/3 \pi h \left(R_1^2 + R_2^2 + R_1 \times R_2\right)\right.$$

Where:

Xmin = The X coordinate of the left most point of intersection of the curve of the apical meristem surface and the base line described above.

Xmax = The X coordinate of the right most point of intersection.

h = The height of the frustrum.

R_1 = The radius of the bottom of the frustrum (The first value is thus Xmin).

R_2 = The radius of the top of the frustrum (The first value is thus Xmin + 10).

The volumes of the cytohistological zones were calculated by decomposition into sums of similar series. The apical initials and central mother cell zones were considered as one zone (central mother cell zone), based on their position and morphological similarity (Owens et al., 1977; Mauseth and Niklas, 1979). Measurements of mean basal radius and apical height were also recorded.

RESULTS

Initial observations

The apical meristems of the dry embryos were strikingly different from those of the first sampling of germinant seedlings.

The cells of all embryo apices were larger (based on cross sectioned area) than those of the first sampled germinant seedlings. The cytoplasm was filled with numerous and relatively large (1 to 6 μm) weakly staining bodies (Figure 2.2A), which did not stain darkly with iodine, as would starch. The nuclei, while staining deeply with safranin, were smaller and had a distorted appearance. The cell walls also appeared wrinkled and stained more densely

Figure 2.2 Median longitudinal sections through the apical meristems a of a spruce embryo and 5-week-old seedling.

A) Representative spruce embryo (blue spruce). Note large, irregular cells with densely staining cellwalls, frequently distorted nuclei, and largeweakly staining cytoplasmic bodies (seearrow). Bar = 50 µm.

B) Representative 5-week-old seedling (hybrid spruce). Note smaller cell size, more regular nuclei, loss of cytoplasmic bodies, mitotic activity (seearrow) and smoother, lighter staining cell walls. Bar = 50 µm.

A
B

than those after germination.

There were few qualitative differences observed among the cells of the embryo meristems. The cells in upper center of the meristem lacked the vacuolization seen in the apical initials and central mother cells of mature apices. Those cells along the flanks of the meristem were slightly smaller and less elongate than those along the vertical axis. The alignment of the cell walls was, in general, suggestive of pattern of zonation typically seen in apical meristems of actively growing conifers.

Growth and development

By the first sample, 5 weeks after sowing, meristems of all species were composed of more, but smaller, mitotically active cells. There were pronounced files of pith-rib meristem cells and in some cases, a distinguishable central mother cell zone, with lighter staining nuclei and a vacuolated appearance was evident. The large, weakly staining bodies seen in the embryo meristems were completely absent (Figure 2.2B). The nuclei and cell walls, as well, were more normal in appearance.

Cytohistological zonation became increasingly distinct for all species through the first 8 to 12 weeks after which there appeared to be little change (Figures 2.3-2.6).

There was one significant interspecific difference in total apical meristem volume. Norway spruce apical meristems were significantly larger then the other species, when averaged over the course of the experiment ($p<.05$).

Figure 2.3 Median longitudinal sections through the shoot apical meristems of blue spruce seedlings at different times during their development: A) From an embryo, excised from a seed. B) 5 weeks after sowing. C) 6 weeks after sowing. D) 8 weeks after sowing. E) 12 weeks after sowing. F) 16 weeks after sowing. G) 20 weeks after sowing. H) 24 weeks after sowing. Bar length = 100 µm. Sections were cut at 10 µm.

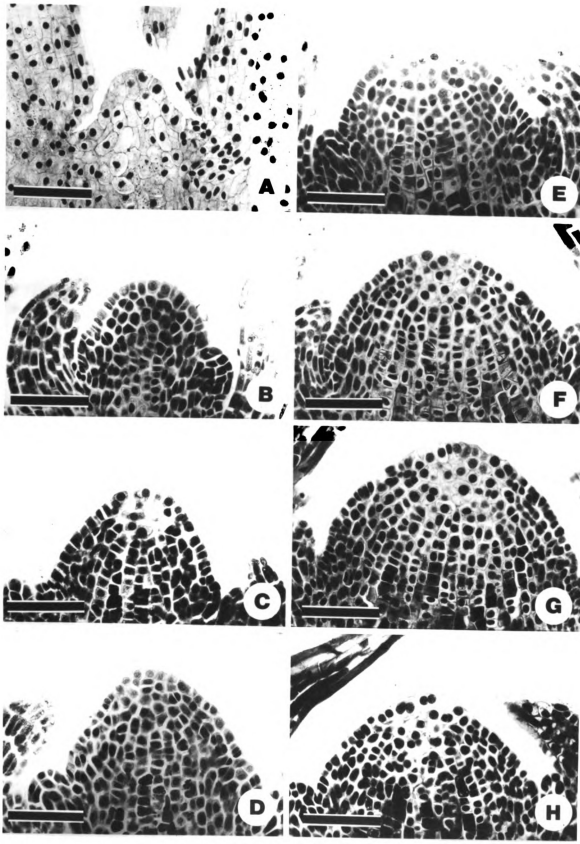

A
E
B
F
C
G
D
H

Figure 2.4 Median longitudinal sections through the shoot apical meristems of hybrid spruce seedlings at different times during their development: A) From an embryo, excised from a seed. B) 5 weeks after sowing. C) 6 weeks after sowing. D) 8 weeks after sowing. E) 12 weeks after sowing. F) 16 weeks after sowing. G) 20 weeks after sowing. H) 24 weeks after sowing. Bar length = 100 µm. Sections were cut at 10 µm.

A
E
B
F
C
G
D
H

Figure 2.5 Median longitudinal sections through the shoot apical meristems of white spruce seedlings at different times during their development: A) From an embryo, excised from a seed. B) 5 weeks after sowing. C) 6 weeks after sowing. D) 8 weeks after sowing. E) 12 weeks after sowing. F) 16 weeks after sowing. G) 20 weeks after sowing. H) 24 weeks after sowing. Bar length = 100 µm. Sections were cut at 10 µm.

A
E
B
F
C
G
D
H

Figure 2.6 Median longitudinal sections through the shoot apical meristems of Norway spruce seedlings at different times during their development: A) From an embryo, excised from a seed. B) 5 weeks after sowing. C) 6 weeks after sowing. D) 8 weeks after sowing. E) 12 weeks after sowing. F) 16 weeks after sowing. G) 20 weeks after sowing. H) 24 weeks after sowing. Bar length = 100 µm. Sections were cut at 10 µm.

A
E
B
F
C
G
D
H

There was no detectable overall difference among the blue, white, or hybrid apical meristem volume. In addition, there was no significant age x species interaction, indicating that the course of apical development was similar for all the species under the environmental conditions used. The volumetric data was, therefore, pooled over species for further analysis.

Apical meristem volume increased most, early in the experiment. With the exception of the mean value for week 21, there were no significant differences in volume after week 10. Total meristem growth, thus, occurred in an apparently asymptotic manner (Figure 2.7A). Meristem volume was more highly correlated with seedling plastochron index (PI) than chronological age (Table 2.1). This is also supported by the more stable maximum volume, usually 5 to 6 million μm^3, shown in Figure 2.7B. The overall shape of the apical meristem also changed over time, increasing more radially than in height through the first 10 weeks or PI 200 (Figure 2.7C-D). The meristem thus became broader during early development (Figures 2.3-2.6) and radius was a better linear indicator of apical development (Table 2.1) than height.

Within the meristems themselves, the proportion of tissue in each cytohistological zone changed continuously. The biggest change was seen between the peripheral zone and the pith-rib meristem relative volumes (Figure 2.8A-D). The relative volume of the pith-rib meristem increased and that

Figure 2.7 Spruce seedling shoot apical meristem volume, radius and height development under 24hr photoperiod.

A) Total apical meristem volume averaged over blue, hybrid, white, and Norway spruce species for the first 24 weeks.

B) Total apical meristem volume averaged over blue, hybrid, white, and Norway spruce species at intervals of 50 plastochrons.

C) Apical meristem basal radius and height averaged over blue, hybrid, white, and Norway spruce species for the first 24 weeks.

D) Apical meristem basal radius and height averaged over blue, hybrid, white, and Norway spruce species at intervals of 50 plastochrons.

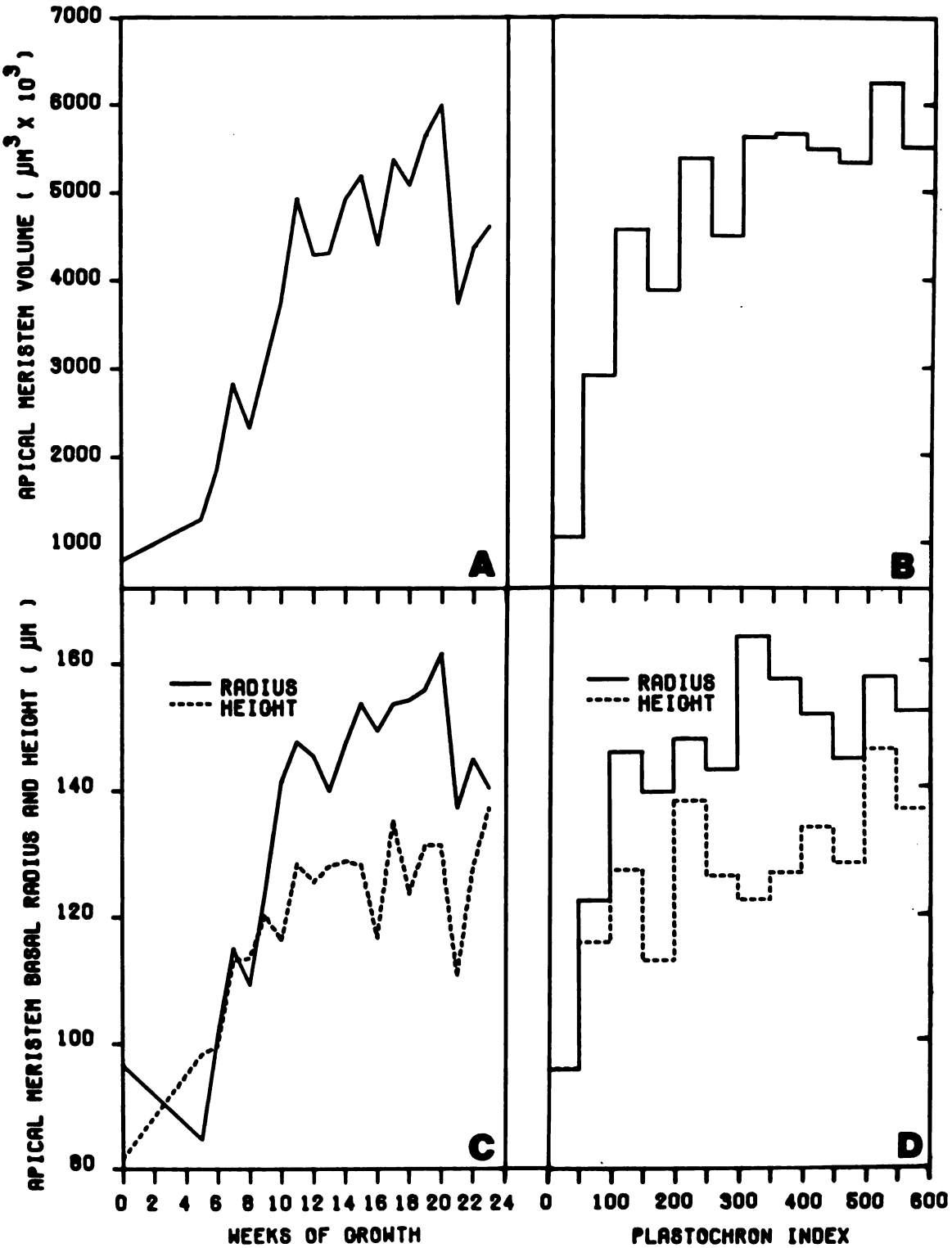
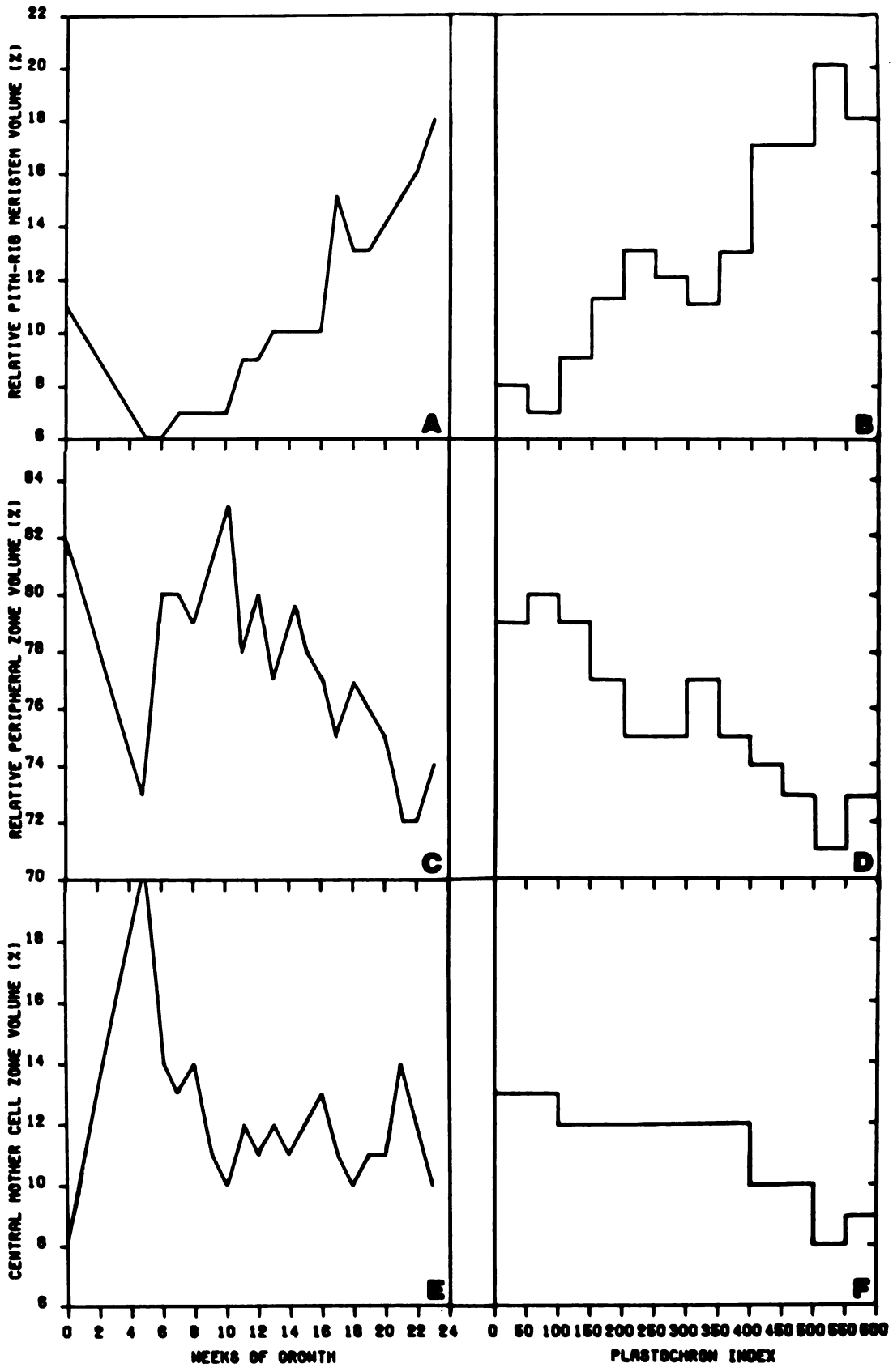

APICAL MERISTEM VOLUME (μM³ X 10³)
7000
6000
5000
4000
3000
2000
1000
A
B
APICAL MERISTEM BASAL RADIUS AND HEIGHT (μM)
160
140
120
100
80
RADIUS
HEIGHT
C
D
0 2 4 6 8 10 12 14 16 18 20 22 24
WEEKS OF GROWTH
0 100 200 300 400 500 600
PLASTOCHRON INDEX

Figure 2.8 Relative volumes of cytohistological zones within the developing shoot apical meristems of blue, hybrid, white and Norway spruce grown for 24 weeks under 24 hr photoperiod.

A and B) Relative rib-pith meristem volume related to age and PI respectively.

C and D) Relative peripheral zone volume related to age and PI respectively.

E and F) Relative central mother cell zone volume related to age and PI respectively.


RELATIVE PITH-RIB MERISTEM VOLUME (%)
RELATIVE PERIPHERAL ZONE VOLUME (%)
CENTRAL MOTHER CELL ZONE VOLUME (%)
A
B
C
D
E
F
WEEKS OF GROWTH
PLASTOCHRON INDEX

of the peripheral zone decreased significantly.

Table 2.1 Spearman rank correlations between shoot apical meristem developmental characteristics and selected growth indices for pooled spruce apical meristem data.

Meristem Characteristic	PI	Age	Meristem Volume	Growth Rate 1/
Total Volume	.62 ***	.54 ***	- - -	.53 ***
PRM / Total 2/	.61 ***	.64 ***	.48 ***	.47 ***
PZ / Total 3/	-.46 ***	-.45 ***	-.16 ns.	-.42 ***
CMZ / Total 4/	-.09 ns.	-.11 ns.	-.32 ***	-.01 ns.
Basal Radius	.58 ***	.50 ***	.94 ***	.50 ***
Height	.43 ***	.38 ***	.80 ***	.39 ***

*** - significant at the P = .001 level; ns. not significant ($P \geq .05$)
1/ Previous week's mean height growth rate.
2/ Relative pith-rib meristem volume.
3/ Relative peripheral zone volume.
4/ Relative central mother cell zone volume.

There was no significant decrease in the central mother cell zone relative volume, over the course of the experiment. At higher PI however, there may be a loss, as indicated in Figure 2.8F.

Based on the volumetric approach used here, apical meristem development is more strongly related to PI than chronological age, under the continuous growth conditions used. In addition, while correlations between the volumetric attributes measured here and height growth rate (in the week prior to harvest) are similar to those for PI and age, they are weaker. When the relative cytohistological zone volumes are compared with the total

apical meristem volume, a reversal of the significances in relative volume loss between the central mother cell zone and the peripheral zone is seen. In other words, central mother cell zone relative volume loss is more strongly associated with increasing total meristem volume than it is to time or PI (Table 2.1).

DISCUSSION

The changes seen between the dry embryo apices and those of the first sample (at 5 weeks) are similar to those previously reported during germination in Norway spruce and other conifers (Tepper, 1964; Foskett and Miksche, 1966; Gregory and Romberger, 1972a; Riding, 1972).

The large, weakly staining bodies prevalent in the embryonic apices showed no birefringence under polarized light and did not stain positively for starch, with iodine. Although they did not stain very darkly with hemalum (hematoxylin) they otherwise fitted the description of the inclusions seen before in spruce embryos. These appear to be protein-like in nature; probably storage proteins (Gregory and Romberger, 1972a). Similar types of bodies were reported in the embryos of Monterey pine (Pinus radiata Laws.) by Riding and Gifford (1973) and in ponderosa pine (P. ponderosa) by Tepper (1964). Mia and Durzan (1972) investigated the ultrastructural changes in germinating jack pine (P.banksiana) embryos and noted that there was a progressive loss of protein-like bodies in the meristematic

cells during germination. These bodies are 50% digested within 48 hr of imbibition (Cecich and Horner, 1977).

The condition of the embryonic nuclei, suggestive of an inactive state, appears to have been similar to that seen by Riding and Gifford (1973) in Pinus radiata. The distortion seen in their study and this one were less apparent in several other studies on germinating conifers (e.g. Gregory and Romberger, 1972a; Mia and Durzan, 1974). This may have been due to the different fixatives used or seed storage conditions. The FAA used here and in Riding and Gifford's study may have caused less swelling during fixation due to the ethanol content. Some swelling, as indicted by elongation of the excised embryos, was present even with the fixative used here. In contrast, the cells of growing meristems show some shrinkage due to FAA fixation (e.g. Figure 2.2B). Since all meristems were treated alike, however, these differences should not affect comparative differences (Gregory and Romberger, 1972a).

The great reduction in cell size seen by the first sample time appears to be largely due to the exhaustion of the protein bodies and the resumption of mitotic activity associated with needle initiation.

In general, the first few days of post imbibition development seem to be more involved with "reactivation" of the shoot meristem rather than active primordia initiation.

The occurrence of an apparently asymptotic form of growth of the apical meristem has been seen previously in

spruce. In Gregory and Romberger's study (1972a), the basal radius of the meristem increased, but at a slowing rate, for 168 days under continuous growth conditions. Cannell (1978a) reported similar growth dynamics in the apical meristems of Sitka spruce (Picea sitchensis) grown under long days. The results presented here are in closer agreement with those of Gregory and Romberger. Their data indicate that a relatively stable basal diameter of about 300 μm is reached within 100 days. While Cannell (1978a) reported somewhat larger sizes for Sitka spruce, he suggests that they may have been due to higher light intensities. As seen in Table 2.1, basal radius appears to be the best linear estimator of apical volume.

In their work with Monterey pine Riding and Gifford (1973) reported that there was no observed difference in apical meristem histochemistry from 84 days until the end of their experiment at 12 months, using greenhouse culture. Previously, Riding (1972) reported that cytohistological zonation as established by 60 days after sowing remained the same for at least the next 18 months. Total volume, after an initial decline during the first 10 days increased during the rest of the experiment. Kremer (1984) found, however, using measuring techniques similar to those employed in this study, that the volume of the apical meristems of jack pine (Pinus banksiana) increased up to about 60 days after sowing then decreased in volume for the remaining 28 days. He mentions that this was perhaps due to a growth chamber

misfunction. In his experiment, the reduction in total meristem volume was associated with an increase in plastochron duration (lower stem growth rate).

In the first 24 weeks of growth, spruce seedlings, grown under optimal conditions, appear to initiate needles in an asymptotically increasing manner (Chapter 1). This implies a corresponding asymptotic decrease in plastochron duration occurs (Gregory and Romberger, 1972a,b, Cannell, 1978a; Cannell and Cahalan, 1979). Shoot growth, based on the formation and development of stem units at the apical meristem, is thus controlled partly by the size of the meristem.

The formation of a primordium at the meristem results in the loss of tissue from the peripheral zone which must be replaced, by mitotic activity, in order to perpetuate the meristem. Thus, the meristem limited rate of shoot growth is also determined at least in part by the tissue volume partitioned into the primordia (Romberger and Gregory, 1977; Cannell, 1978a), the peripheral zone volume and peripheral zone cell cycle time.

An interesting developmental trend that has not been addressed in previous studies of spruce germinant seedlings, is that of the continuing change in apical meristem zonal distribution seen in this study. These were similar to those seen in jack pine (Kremer, 1984). In that study, apical meristem volumes were calculated using parabolic periods of revolution, an alternative approach to the "disk

method" used here. Kremer found that the peripheral zone relative volume decreased and the pith-rib meristems relative volume increased for the first 68 days.

While PI was the growth index most closely associated apical meristem volume (Cannell, 1978a; Gregory and Romberger, 1972a), its relationship with the cytohistological zone trends seen in this study is less clear. Changes in the central mother cell zone, seem more strongly related to overall meristem volume than PI although the trends are the same. To a lesser extent, rib-pith meristem relative volume may be more strongly related to age.

The relative volumes of the cytohistological zones in the shoot apical meristem may be involved in hormonal output from the shoot apical meristem (Mauseth, 1979). This may also affect the determination of primordium fate and other aspects of the seedlings physiology. In addition, if the rate of peripheral zone loss were to continue according to the trend reported here, it might present a physical limit to primordium initiation and thus to continued free growth.

CHAPTER 3

THE EFFECT OF SHORTENED GROWTH CYCLE NUMBER ON SPRUCE SEEDLING APICAL DEVELOPMENT AND FREE GROWTH POTENTIAL

ABSTRACT

Seedlings of blue, white x blue, white and Norway spruce were grown in a shortened growth-cycle (SGC) regime which exposed them to five, consecutive, artificially shortened, cycles of shoot growth and bud formation. Seedlings of all species were able to resume free growth in a propitious environment, even after four such cycles.

Seedling development under SGCs was much reduced compared to normal nursery-grown seedlings or seedlings grown under accelerated conditions for less than one year. The average seedling height at the end of five SGC was less than that of an average production-run two-year-old (2+0) seedling.

The main stem buds, sampled at the end of each growth cycle, were smaller than normal seedling buds. The stage of phenological development at the end of each cycle seemed to decline with cycle number. While meristem volume increased, there were progressively fewer needle primordia in the buds. This may indicate that as seedlings get bigger more time is needed for complete bud formation.

INTRODUCTION

Mature spruce (Picea sp. (A.) Dietr.) trees undergo an annual growth cycle in which the annual shoot growth results from the elongation and expansion of preformed stem units existing in the overwintering bud. A stem unit consists, collectively, of an internode, a node, and the nodal appendages (needles or other primordia) at its distal extremity (Doak, 1935). This determinate growth pattern is common to all species of spruce investigated thus far (Fraser, 1962, 1966; Owens and Molder, 1976; Owens et al., 1977; Harrison and Owens, 1983; Bongarten, 1978; Pillia and Chacko, 1978).

In germinant or young seedlings, this growth cycle is modified to include the formation of additional stem units which develop directly into needles and internodes, at the end of the period of preformed shoot elongation. This occurrence has been termed "free growth" (Jablanski, 1971). Since the spruce embryo, within the seed, has no preformed shoot or needle primordia, it is also the sole pattern of shoot growth in the germinant seedling.

Free growth in young spruce seedlings is maintained by long photoperiods. The response follows the action spectrum of a phytochrome mediated response (Young and Hanover, 1977a). Free growth, in spruce, can be curtailed by a number of stressful environmental conditions, even under long photoperiods (e.g. Dormling et al., 1968; Robak and

Magnesen, 1970; Malcolm and Pymer, 1975; Young and Hanover, 1978). It is induced, however, only when a critical night length, which may vary with seedling size is broken by light with a red (≤ 600-680 nm) spectral component (e.g. Downs and Borthwick, 1956; Downs, 1962; Pollard, 1973; Pollard et al., 1975; Young and Hanover, 1977a).

In nursery grown seedlings of white spruce (Picea glauca (Moench.) Voss.) free growth often occurs in the second year. It often accounts for most of the years shoot growth (Jablanski, 1971; Pollard and Logan, 1976; Powell, 1982). By four years, howver, white spruce shoot growth appears to be determinate (Neinstaedt, 1966). In black spruce (Picea mariana (Mill.) B.S.P.), Pollard and Logan (1974) also found evidence of free growth occurring in seedlings up to four years old. For blue spruce (Picea pungens Engelm.) seedlings, free growth is reported in seedlings up to three years old (Young and Hanover, 1976).

Developmental changes take place, as well as an increase in size, as spruce seedlings age. During the first three years in blue and Engelmann spruce (Picea engelmannii Parry), for example, there are qualitative and quantitative differences in needle structure (Heckman et al., 1982). In blue spruce, Young and Hanover (1977b) found that the number of preformed needle primordia in overwintering buds increased in the first few years of growth. The average size of the apical meristem also appears to increase in the first years of development (Heckman, unpublished data).

Older seedlings, that have been grown outdoors, differ from germinant seedlings in non-morphological ways as well. They have been exposed to a series of physiological fluctuations associated with their annual growth cycles. These cycles involve a series of endogenous and environmentally triggered developmental events which enable seedlings to survive over winter. There is considerable latitude and variability in the time required for many of these events, allowing their experimental modification.

The rapidity of bud-set in spruce germinant seedlings can be varied by light intensity and quality, photoperiod, nutrients, and temperature. In Norway spruce, 8 hr photoperiods will precipitate bud formation in as few as 6 days, with faster responses seen under warmer temperatures (Dormling et al., 1968). Under 12 hour days and warm temperatures, buds were visible within 5 weeks in blue spruce and low intensity light-breaks did not prevent dormancy (Young and Hanover, 1977a). In Sitka spruce (Picea sitchensis (Bong.) Carr) bud scales were visible after 14 days under 10 hr photoperiods. Primordia formed at the apex were destined to become scales by the second day (Cannell and Cahalan, 1979). In white spruce, as well, bud initiation was evident in about 14 days (Pollard, 1974a).

Bud maturation can also be controlled by environmental alteration. Temperature affected the rate of bud growth in Sitka spruce (Malcolm and Pymer, 1975). Light intensity, temperature, soil moisture, and nutrients all affected the

rate and extent of bud development in white spruce (Pollard and Logan, 1977). Bud maturation in white spruce, under 8 hr photoperiod, warm temperatures and good fertilization, was complete in 6 to 12 weeks (Pollard, 1974a). This process appears to take about 4 weeks for some provenances of Norway spruce (Dormling et al., 1968).

The chilling period, required after bud-set, to permit reflushing, is also variable. The time to budbreak of spruce seedlings when returned to propitious conditions declines exponentially with increasing chilling time (Sorensen, 1983). Two-year-old and five-year-old white spruce seedlings, for example, required only eight weeks of chilling to allow subsequent normal bud break under conditions conducive to bud flushing (Neinstaedt, 1966). Eight weeks of chilling was also found to be sufficient time for one-year-old seedlings of several other spruce species to break bud normally (Neinstaedt, 1967).

The time and rate of bud-flushing are also environmentally variable. In mature white spruce, for example, Owens et al. (1977) found that temperature sums were better indices of budburst phenology than was date. Indeed, many species of woody plants may be "forced" to break bud after their dormancy breaking chilling requirement has been received, by placing them in a warm environment (Kramer and Kozlowski, 1979).

Thus, the actual time biologically required for the annual cycle of growth appears to be less than one year.

This principal has been pursued in attempting to shortened the time to flowering in Norway spruce (Picea abies (L.) Karst.) (Dormling et al., 1968), by subjecting germinant seedlings to a series of 3 to 4 contracted cycles per year.

Since the propensity for free growth is a juvenile characteristic in spruce, a similar series of growth cycles might be useful in separating the chronological factors from cyclic developmental changes occurring as spruce seedlings loose the ability for free growth.

I report here the cumulative effect of shortened growth-cycles (SGC) on the propensity for seedlings of blue, white, white X blue (hybrid), and Norway spruce to undergo free growth. The effects of these shortened cycles on shoot apical development were also addressed.

MATERIALS AND METHODS

Blue, white x blue hybrid, white and Norway spruce seedlings, from seed sources described elsewhere (see Chapter 1), were compared using five artificially shortened growth cycles. Seeds were sown in 5 x 5 x 27 cm, polyethylene-coated, paper plant-bands, held in cases of 36. These were filled with a perlite : vermiculite : sphagnum peat (1:1:1 by volume) mixture. Peter's soluble fertilizer (20:19:18 NPK) was applied at the rate of 1.2 grams/case, twice during each growth cycle. A Peter's STEM minerals solution was also at 0.6 grams/case after the second cycle. Water, insecticide, and fungicide, applications were

uniformly made to all seedlings, as needed, during the experiment. All seeds were germinated for two weeks, under a 24 hr photoperiod, prior to the beginning of the first growth cycle.

Up to five, sequential, shortened growth-cycles (SGC) were applied to seedlings of all species. Growth cycles consisted of two weeks of growth under continuous fluorescent irradiation at approximately 100 $\mu Mm^{-2}sec^{-1}$ PPFD (400-700 nm) at 20 to 22°C followed by 6 weeks of 8 hr photoperiods at the same light level then chilling for 6 weeks at 4° C ($\pm 1^{o}$) under 8 hr photoperiods at approximately 30 $\mu Mm^{-2}sec^{-1}$ PPFD. The total cycle, thus, took 14 weeks to complete.

At the end of the chilling period of each of the five cycles, all SGC seedlings were placed under 24 hr photoperiod in the growth room to induce flushing. At the end of two weeks, three cases were removed from the controlled growth room and placed in a greenhouse under a photoperiod extended to 24 hrs by fluorescent lamps. Those remaining in the growth room were returned to 8 hr photoperiods. These conditions served to test for the inducibility of free growth.

Prior to bud burst, in each cycle, all remaining seedlings were measured from the level of their cotyledons to the distal end of the longest needle at the shoot tip. In addition, 3 to 4 seedlings of each species were removed from the cases scheduled for free growth testing. The

terminal shoot bud of each was excised and fixed in FAA, under vacuum, for microscopic examination.

For a partial comparison of the apical development of the SGC material to natural development, considering blue spruce as representative, dormant terminal buds of one-year-old (1+0), two-year-old (2+0) and three-year-old (2+1) commercial nursery-grown blue spruce and upper-crown primary-branch terminal buds from 3 mature blue spruce tree were also prepared as described above.

The terminal buds were processed for light microscopy, photography and digital analysis by the same techniques used in Chapter 2.

Height growth and apical meristem volume differences among SGC material were evaluated by analyses of variance.

To test for the presence of free growth, the within species pooled heights of the cyclically grown material were compared with those of the seedlings placed under 24 hr photoperiods, at the beginning of the next cycle.

RESULTS

Shoot growth phenology

At the beginning of the experiment, during the initial germination period, blue and Norway spruce seeds germinated first. White and the hybrid germinated later. Most seedlings had initiated and developed needles above their cotyledons prior to budset.

Most seedlings, of all species, resumed free growth

even after five SGC (Figure 3.1). Although the amount of additional shoot growth in the first 8 weeks after transfer to the 24 hr photoperiod decreased after the last cycle the seedlings were still significantly taller under long days. Most seedlings did not set a terminal bud under long days in the cycle following their transfer, but continued to grow (Figure 3.2). In addition, many of the seedlings forming buds subsequently broke bud and resumed free growth.

Under long days, interspecific differences existing at the beginning of the free growth testing, after each cycle, diminished over time. At the beginning of the second cycle, for example, blue and Norway spruce seedlings were taller than either hybrid or white spruce ($p=.05$) within the same free growth test lot. By the end of the experiment, after 56 weeks under 24 hr photoperiod, there were no interspecific height differences among those seedlings.

Under short days there was a progressive increase in height with cycle number for all species (Figure 3.1). The overall incremental shoot growth was greatest for the second and third cycles and declined for the fourth and fifth cycles (Figure 3.2). Under the shortened growth cycles, blue and Norway spruce were significantly ($p=.01$) taller than white and hybrid spruce at the end of each cycle. The relative magnitude of these differences decreased with cycle number.

For SGC seedlings, remaining under the 8 hr photoperiod of the shortened cycles, bud-scales were visible within two

Figure 3.1 Mean heights of seedlings of blue, hybrid, white and Norway spruce at the end of 1 to 5 shortened growth cycles under short days (SD) and their response to long days (LD):

A) 1 cycle all SD.

B) 2 cycles SD and LD response after 1 SD cycle.

C) 3 cycles SD, LD response after 2 SD cycles and continued LD response to 1 SD cycle.

D) 4 cycles SD, LD response after 3 SD cycles and continued responses of previous treatments.

E) 5 cycles SD, LD response after 4 SD cycles, and continued LD responses of previous treatments.

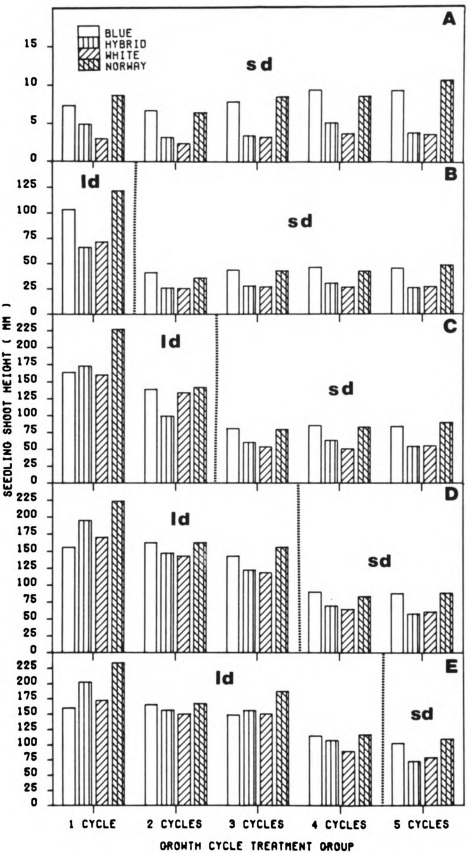

BLUE
HYBRID
WHITE
NORWAY
A
B
C
D
E
sd
ld
SEEDLING SHOOT HEIGHT (MM)
0
5
10
15
25
50
75
100
125
150
175
200
225
1 CYCLE
2 CYCLES
3 CYCLES
4 CYCLES
5 CYCLES
GROWTH CYCLE TREATMENT GROUP

Figure 3.2 Average height growth of spruce seedlings under 24 hr photoperiod after 1 to 4 shortened growth cycles.

SEEDLING SHOOT HEIGHT (MM)
225
200
175
150
125
100
75
50
25
0
CYCLE 1
CYCLE 2
CYCLE 3
CYCLE 4
CYCLE 5
ONE SD CYCLE
TWO SD CYCLES
THREE SD CYCLES
FOUR SD CYCLES
FIVE SD CYCLES

weeks. Buds were seen on all SGC seedlings by the fourth week of the cycle. Thus, all seedlings had terminal buds formed, prior to the start of the chilling period of the cycles, and height growth had ceased.

Apical development

No significant interspecific differences in the total apical meristem volume were observed among SGC seedlings. There were, however differences in total apical meristem volume among the first four sequential cycles ($p=.05$). The buds from the fifth cycle were accidentally dried and were thus useless for apical meristem volumetric measurement. There was an increase in total meristem volume with cycle number (Figure 3.3, Table 3.1). No significant differences were seen, either among cycles or species, in the relative volumes or the cytohistological zones. Cytohistological zonation was indistinct within the dormant meristems.

The apical meristems of SGC seedlings, even after four cycles, were significantly smaller than those of the two-year-old and older nursery grown and mature blue spruce apical meristems (Table 3.1). The SGC meristems were not significantly larger than the one-year-old nursery grown seedling apical meristems even after four growth cycles (c.f. Figures 3.4 and 3.5).

Figure 3.3 Representative median longitudinal sections through the terminal shoot bud of blue spruce seedlings after 1 to 4 shortened growth cycles. A) one cycle, B) two cycles, C) three cycles, and D) four cycles. Bar = 300 µm. Thickness= 10 µm.

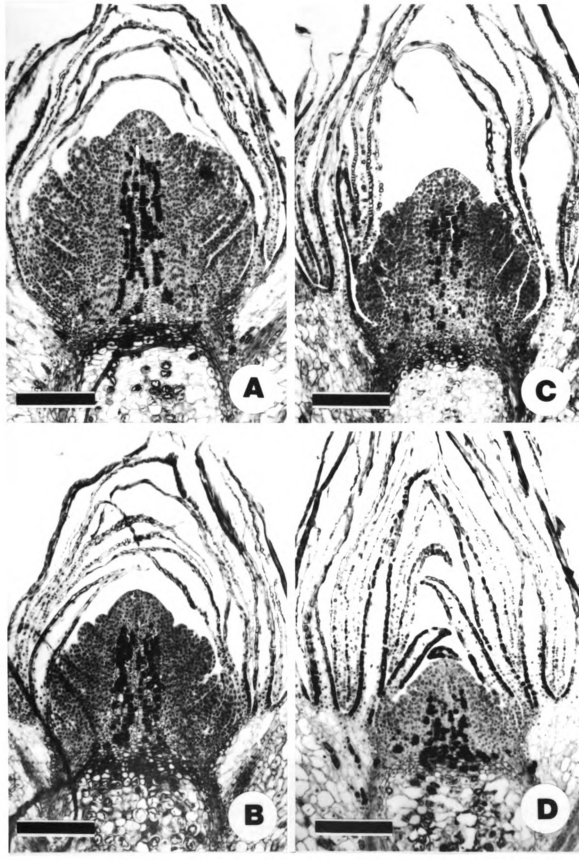

A
C
B
D

Figure 3.4 Representative median longitudinal sections through buds of spruce seedlings after four shortened growth cycles. A) Blue spruce, B) Hybrid spruce, C) White spruce and D) Norway spruce. Bar = 300 μm. Thickness = 10 μm.

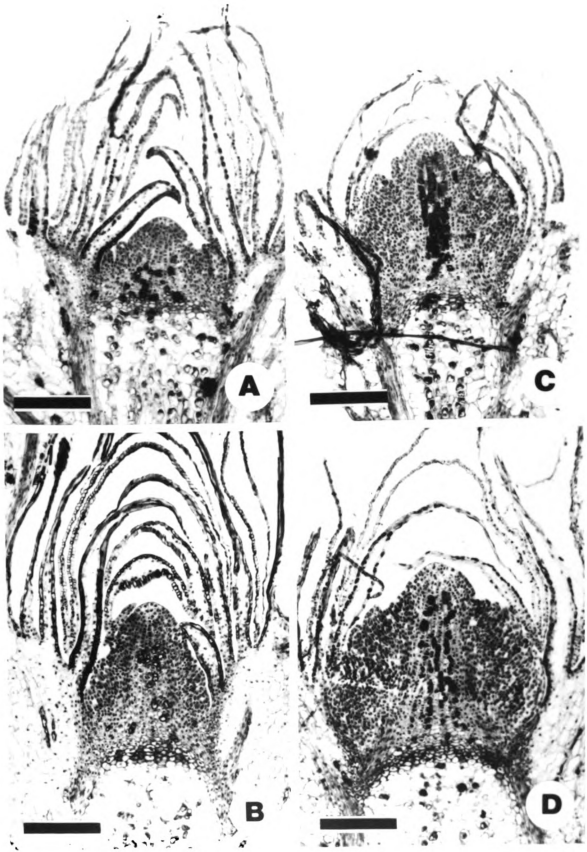

A
C
B
D

Figure 3.5 Representative median longitudinal sections through naturally-cycled, dormant blue spruce buds. A) 1+0 nursery grown. B) 2+0 nursery grown. C) 2+1 transplant. D) Mature tree (upper crown). Bar = 500 µm. Thickness = 10 µm.

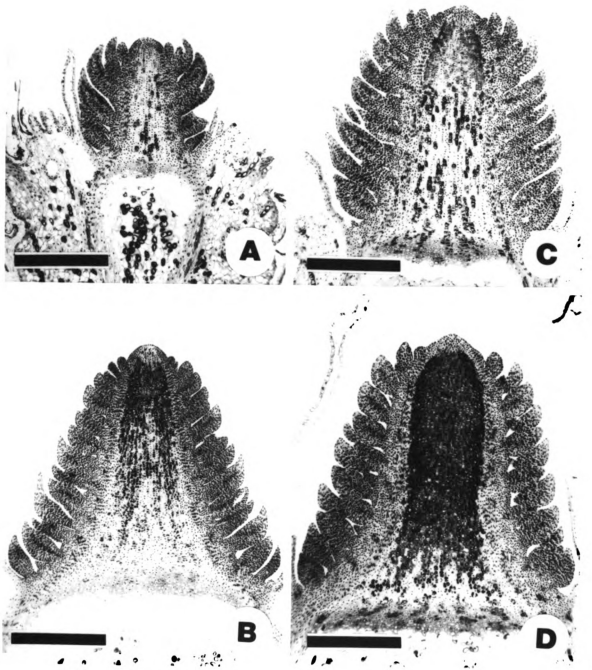

A
C
B
D

Table 3.1 Apical meristem volumes and needle primordia seen in spruce buds developed under SGC and in nursery-grown and mature buds.

All Species Pooled (shortened cycles)			Blue Spruce (nursery grown)		
Cycle Number	Meristem[1] volume	Needles[2] in bud	Seedling age	Meristem volume	Needles in bud
1	1148a[3]	13.2ab	1+0	2658a	14.7
2	2258ab	15.5b	2+0	6019b	24.9
3	1574ab	12.6a	2+1	4140	26.0
4	2597b	9.0a	50+	8745	28.0
5	----	8.4a	---	----	----

1/ Meristem volumes expressed in $\mu m^3 x 10^6$.
2/ Needles seen in median longitudinal section.
3/ Means within a column followed by the same letter are not significantly different at the (p =.05) level, Duncan's multiple range test.

With the exception of the first cycle, the number of needle primordia within the buds, seen in cross section, declined with successive cycle number (Table 3.1, Figure 3.3). In buds from all cycles, Norway spruce had significantly more needles than any of the other species. There were no other interspecific differences. All species responded similarly to the sequential cycles. The number of needle primordia within the buds of the SGC blue spruce was never significantly more than that seen in one-year-old, blue spruce, nursery grown seedlings (Table 3.1, Figure 3.3). In addition, the number of needle primordia in two-

year-old and older nursery grown blue spruce was greater than that of the one-year-old seedlings ($p<.01$) or any SGC seedling.

DISCUSSION

The average shoot growth under the long-day greenhouse conditions (free growth test) appeared to be asymptotic in nature (Figure 3.2). Under optimal conditions, spruce shoot height-growth often increases in a nearly linear manner (cf. Dormling et al., 1968; Young and Hanover, 1977a; Cannell and Cahalan, 1979; Chapter 1). The decline in growth rate seen in the free-growth test material here (Figure 3.2) may have been induced by the high temperatures and/or water stress encountered in the green houses during the summer. Temperatures in mid and late summer were often in excess of 30°C. These have generally been found to be too high for optimal spruce growth (e.g. Young and Hanover, 1978). This combined, with a higher light intensity, made maintenance of uniform water status difficult.

Based on the shortened growth cycle system used in this study, the developmental factors which control the free growth propensity in spruce seedlings do not seem to be bound to the number of growth cycles, per se. There were, however, a number of quantitative differences between SGC seedlings and those cyclically grown under natural

conditions, which need to be considered when interpreting the SGC effects.

First, although the seedlings in this experiment were subjected to up to 5 phenologically complete growth cycles, the mean height of the seedlings averaged only 88 mm at the end of the fifth cycle (SD). This is considerably smaller than the height of an average 5-year-old nursery-grown spruce seedling of any of the species used. For blue spruce, even the tallest of the fifth cycle seedlings (132 mm) was only about average for two-year-old (2+0) commercial nursery stock (cf. Chapter 4).

In most past studies of free growth inducibility in various ages of spruce, seedling morphological or developmental characteristics, other than age, have not been reported (Nienstaedt, 1966; Young and Hanover, 1976; 1977b). It was illustrated by Pollard (1974b) that seedlings, in a free growing state, became increasingly sensitive to a dormancy inducing stimuli (an intervening two weeks of 8 hr photoperiods) as they aged. However, the relationship between height and dormancy (no resumption of shoot growth) appeared to be stronger (r^2=.90) than that of age and dormancy (r^2=.72). The results reported here indicate that the consideration of seedling height, in age-class seedling development tests, may warrant further attention.

A second major difference seen between the development of the SGC seedlings and those of nursery-grown and mature blue spruce, is that of terminal bud development. In

naturally grown spruce buds, seedlings or trees older than one year generally have far more preformed needles in the buds (Table 3.1, Figure 3.5) than do one-year-old seedlings (Young and Hanover, 1977b; also cf. Burley, 1966a and Owens and Molder, 1976).

With the SGC seedlings, as indicated in Table 3.1 and Figures 3.3-3.5, there was an overall trend among all species towards fewer needle primordia with each successive cycle. This suggests that with progressive cycles, either more time was necessary for bud scale formation prior to needle primordia initiation or the needles were initiated at a lower rate. From the number of scales seen in longtudinal sections, it appeared that more primordia developed as bud scales in early part of later cycles (cf. Figures 3.3A and 3.3D), supporting the former of these two possibilities.

The dormant apical meristems in the SGC material were generally smaller than that of typical one-year-old nursery-grown blue spruce (Table 3.1). They were also smaller than those reported in one-year-old nursery-grown Sitka spruce (Burley, 1966a) or four-year-old dormant white spruce (Cecich and Miksche, 1970). They never attained the size of any of the older naturally grown blue spruce meristems. The SGC meristems were also smaller than meristems from six-month-old spruce, from the same seedlots, growing under free growth conditions (Gregory and Romberger, 1972a; Cannell and Cahalan, 1979; Chapter 1).

It is important to note, with regard to the last observation, above, that there are fluctuations in meristem size during the natural growth cycle. In the annual cycles of the spruce species that have been observed, the apical meristem reaches its maximum size between the end of bud scale initiation and late needle-primordia initiation. This is followed by a decline until late autumn, when the meristem is at its smallest size until the first bud scales are initiated prior to budbreak the next year (Cecich and Miksche, 1970; Owens and Molder, 1976; Owens et al., 1977; Pillia and Chacko, 1978; Thompsett, 1978). A similar increase and decrease in meristem size was seen, during photoperiodically induced budset, in Sitka spruce germinant seedlings (Cannell and Cahalan, 1979). Thus, it is important to consider the phenological stage of the material observed, when making quantitative comparisons of apical meristem volume.

In this experiment, in light of the decreasing number of needle primordia seen within the buds, the size increase of the apical meristem seen may or may not reflect the actual ontogenetic developmental progression. If primordia formation were interrupted by the beginning of the chilling treatment used in this experiment, it might have left the apical meristem larger than if needle initiation had proceeded to completion.

Finally, the bud burst phenology of the SGC seedlings also differed from that seen in spruce seedlings having the

equivalent number of natural cycles. Through out the experiment, budburst had occurred on all seedlings prior to the return to 8 hr days in each cycle.

In one-year-old (1+0) through seven-year-old (2+2+3), completely chilled, white spruce seedlings brought under 18 hr photoperiods, budburst ranged from 26 to 39 days (Nienstaedt, 1966). Young and Hanover (1977b) found a similar age-related increase in time to budbreak in blue spruce seedlings.

However, when seedlings of white, blue, and Norway spruce were grown under long photoperiods (20 hr) for 4 months, allowed to set bud for 2 months under 13 hr photoperiod then chilled for 6 weeks under 13 hr photoperiods the buds broke 14, 16, and 25 days later, respectively, when the seedlings were returned to favorable growth conditions (Nienstaedt, 1967). Similar results were seen with blue spruce when the initial growth time was varied from 2 to 6 months (Young and Hanover, 1977).

The results of this study are in general agreement with these latter observations, indicating that perhaps the artificial shortening of the bud development period effects the depth of the subsequent dormancy as well as shoot growth potential.

In conclusion, while spruce seedlings can be "forced" through almost 4 artificially shortened growth cycles in the time filled by one natural cycle, there are quantitative differences in the phenological changes usually associated

with these cycles. Growth-cycles accumulated, *sensu stricto*, do not appear to be developmental characteristics intrinsically involved with loss of free growth in spruce. Since size alone does not appear to be a limiting aspect (Dormling *et al.*, 1968; Chapter 1), one possibility is that the developmental stage past which free growth will not occur in spruce involves some interaction of age and size.

CHAPTER 4

EFFECTS OF SEEDLING SIZE, AGE AND CULTURAL METHOD ON SUBSEQUENT PHOTOPERIODICALLY-MEDIATED GROWTH IN BLUE SPRUCE SEEDLINGS

ABSTRACT

Blue spruce seedlings of three size classes, and two cultural methods, "accelerated growth" (AG) and "conventional nursery" (NUR), were growth-tested under 24 hr long day (LD) and 12 hr short day (SD) photoperiods for 16 weeks, in a temperature controlled room.

AG seedlings initially had lower overall weight, lower plastochron index, smaller stem caliper, and fewer preformed needles in their buds than did NUR seedlings, within size classes. AG seedlings broke bud sooner than the NUR seedlings, perhaps due to their age or the storage conditions.

Seedling growth phenology was evaluated in two phases, flush growth and subsequent growth. Photoperiod and seedling initial height had no significant effect on the time to bud-break or shoot flush rate. NUR seedlings grew faster during the flush phase and had greater total flush height. The internode elongation was less than that of the AG seedlings, however. After the flush phase AG seedlings of all sizes grew faster than NUR seedlings, under LDs. The smaller two size classes of NUR seedlings grew faster than

the largest, which showed no significant post flush height growth.

Fewer free growth needles were formed on NUR than AG seedlings, all of which free grew under LDs. Some NUR seedlings did not resume free growth. Lack of free growth, as determined by needle counts, did not appear to be related to initial height. It appeared that a lower growth potential, largely due to root disturbance and low photosynthetic photon density, more strongly affected free growth induction than did initial seedling size or age.

INTRODUCTION

Along with increased size, aging spruce seedlings show changes in shoot development and phenology. Under nursery conditions, spruce germinant seedlings grow indeterminately in their first year until environmental stimuli, largely photoperiod and temperature, induce the formation of an over-wintering bud (e.g. Burley, 1966b; Arnott, 1974; Powell, 1982). In following years, spruce seedling shoot growth changes from this completely indeterminate pattern of growth ("free growth" (Jablanski, 1971)), to a completely determinate type of shoot growth (Jablanski, 1971; Pollard and Logan, 1976; Young and Hanover, 1976; Powell, 1982). In this mature growth pattern, all of a years needles and their internodes exist, in a "telescoped" manner, within the over-wintering bud formed in the previous year (Fraser, 1966; Owens and Molder, 1976; Owens et al., 1977; Bongarten, 1978; Pillia and Chacko, 1978; Harrison and Owens, 1983).

This change of growth pattern, in the species in which it has been observed, is usually completed within two to five years (Nienstaedt, 1966; Jablanski, 1971; Young and Hanover, 1976; Powell, 1982). In blue spruce (Picea pungens Engelm.), for example, seedlings in the nursery appear to have a completely determinate growth pattern by their third growing season. This seems to be the case for most spruce species, although some sources of black spruce (Picea mariana (Mill.) B.S.P.) may retain the free growth

characteristic for up to 12 years (Pollard and Logan, 1976).

In addition to the loss of free growth under natural conditions, there seems to be a concomitant change in their response to extended photoperiods. For example, germinant seedlings and one-year-old blue spruce seedlings resume free growth when placed under 24 hr photoperiods. Three-year-old and older seedlings set bud at the end of shoot elongation (Young and Hanover, 1976). A similar lack of free growth response was reported in five-year-old white spruce and thirty-year-old grafts (Neinstaedt, 1966).

Under extended photoperiods, two-year-old, nursery-grown blue spruce seedlings are intermediate in their shoot growth response. They exhibit three patterns of shoot growth. After flushing and elongation of the preformed shoot, these seedlings either continue needle formation and stem elongation (free growth), set bud then flush and resume free growth, or set bud and remain dormant (Young and Hanover, 1976). Similar shoot growth patterns were seen in white spruce (Watt and McGregor, 1963; Nienstaedt, 1966) and Norway spruce (Farrar, 1961) of a similar age. These responses imply that such seedlings are in a transitional stage in the development of their photoperiodic response.

In order to determine which, if any, of the observable developmental changes seen in early seedling ontogeny are involved in the changes in photoperiodic response, it is necessary to separate and test them. Accumulation of growth cycles, alone, does not seem to be the limiting factor

(Chapter 3). Size, alone, also does not seem to limit free growth, since accelerated seedlings can be grown to larger sizes than typical non-responsive nursery seedling (e.g. Dormling et al., 1968; Hanover and Reicosky, 1972; Heide, 1974a; Chapter 1).

Since two-year-old nursery-grown seedlings have shown an intermediate response, they are an appropriate population to analyze for developmental attributes associated with free growth propensity. In addition, since one-year-old, similar-sized, accelerated-growth seedlings are available, some insight can be gained into interactions of shoot size and age by comparing them to similar sized nursery-grown seedlings.

This study was designed to test the relationships between size and age on photoperiodically-induced shoot free growth and seedling development in blue spruce.

MATERIALS AND METHODS

Blue spruce seedlings, probably from southern Colorado seed sources, were obtained from a commercial nursery in lower Michigan. Two-year-old (2+0) conventional nursery stock (NUR), fall-lifted and stored bare-rooted, was used along with one-year-old, accelerated-growth, container-grown plug seedlings (AG). The seedlings had been in protected cold-storage, since lifting in the fall. All seedlings were sorted into three size classes, 75-150 mm, 150-225 mm, and

225-300 mm, based on height from the cotyledons to the tip of the terminal bud. Individual seedling heights, to the nearest 5 mm, were also recorded.

The seedlings were received on March 31 and stored at about 4°C until April 4 when all seedlings were transplanted into 10 x 10 x 27 cm, polyethylene-coated paper plant-bands filled with a peat:perlite:vermiculite (1:1:1 by volume) mixture. Each band then received an equal amount of fertilizer (Peter's 19:18:17 (NPK) and STEM trace minerals) solution, and the whole experiment was watered to drainage. Additional fertilizer and water were periodically applied during the experiment, uniformly and simultaneously to all seedlings, as needed.

Two photoperiods, short day (SD) 12 hr and long day (LD) 24 hr were used in this experiment. Irradiation was provided by 96 inch VHO fluorescent lights. Two lamp fixtures (with two tubes each) were used in the (SD) photoperiod while a single two-tube fixture was used in the (LD) photoperiod treatments. The photosynthetic photon flux density (PPFD, 400-700 nm), measured at the soil surface in the center of each band, averaged 75 $\mu M\ m^{-2}sec^{-1}$ in the SD treatments and 35 $\mu M\ m^{-2}sec^{-1}{}_m$the LD treatments. Overall, there was no significant difference in total daily quantum fluxes between the two photoperiodic treatments.

The experiment was grown indoors, in a controlled temperature room. The temperature was maintained between 24 and 26°C and did not vary significantly between the two

photoperiods. The treatment combinations, (i.e. photoperiod, size class, and cultural method (age)) were arranged factorially in a split-plot randomized complete block design. Photoperiod formed the main plots. Cultural method, and size class formed the sub-plots. There were 3 main-plot replications with 6 sub-plot treatment combinations. Six seedlings of each treatment combination were randomly arranged within each main plot replication, for a total of 216 seedlings.

Shoot growth phenology was recorded weekly, for 16 weeks. After budburst (when needles were first evident between the expanding bud scales), the shoot height from the bud base to the distal tip of the vertical most needle (flush height) was recorded for each seedling each week. Height growth rate differences among the treatments during the two different growth phases, flushing and subsequent shoot growth, were evaluated by comparisons linear regression slopes (Sokol and Rohlf, 1981).

In order to assess gross morphology and development, samples were taken at five times during the experiment. The timing of the samples corresponded with observable changes in the shoot-growth phenology. Samples were taken: (1) of dormant stock, as received; (2) during budburst, at two weeks after planting; (3) at the end of preformed shoot elongation, four weeks later; (4) six weeks after that, during needle primordia initiation under short days; (5) and a last time, one month later, at the end of the experiment.

At each sampling time one seedling, of each of the 12 treatment combinations, was randomly selected from those remaining in each of the three main-plot replications. Subtending primordia were first counted, then the apical meristem and the upper few mm of the shoot were excised for microscopy, in a concomitant experiment. The rest of the main stem needles and needle primordia were then counted, diameter at cotyledon level recorded, and the shoots and roots separated. The seedlings were then oven-dried at 70°C and weighed.

In order to test for total free-growth, needles were counted on the new formed shoots of the unsampled seedlings at the end of the experiment. These data were pooled with the data from the last sample, which was taken on the last day of the experiment. Free growth was determined as increases over means of SD shoot needle number in the LD seedlings of each corresponding sub-plot treatment combination.

RESULTS

Initial observations

Within each size class, there was no initial difference in shoot height between the NUR and the AG seedling types. NUR seedlings did, however, have more mainstem needles per mm (i.e. higher plastochron index (PI)), greater stem diameter, and higher total dry weight than did the AG seedlings (Table 4.1). There were no significant initial

differences in root to shoot ratio.

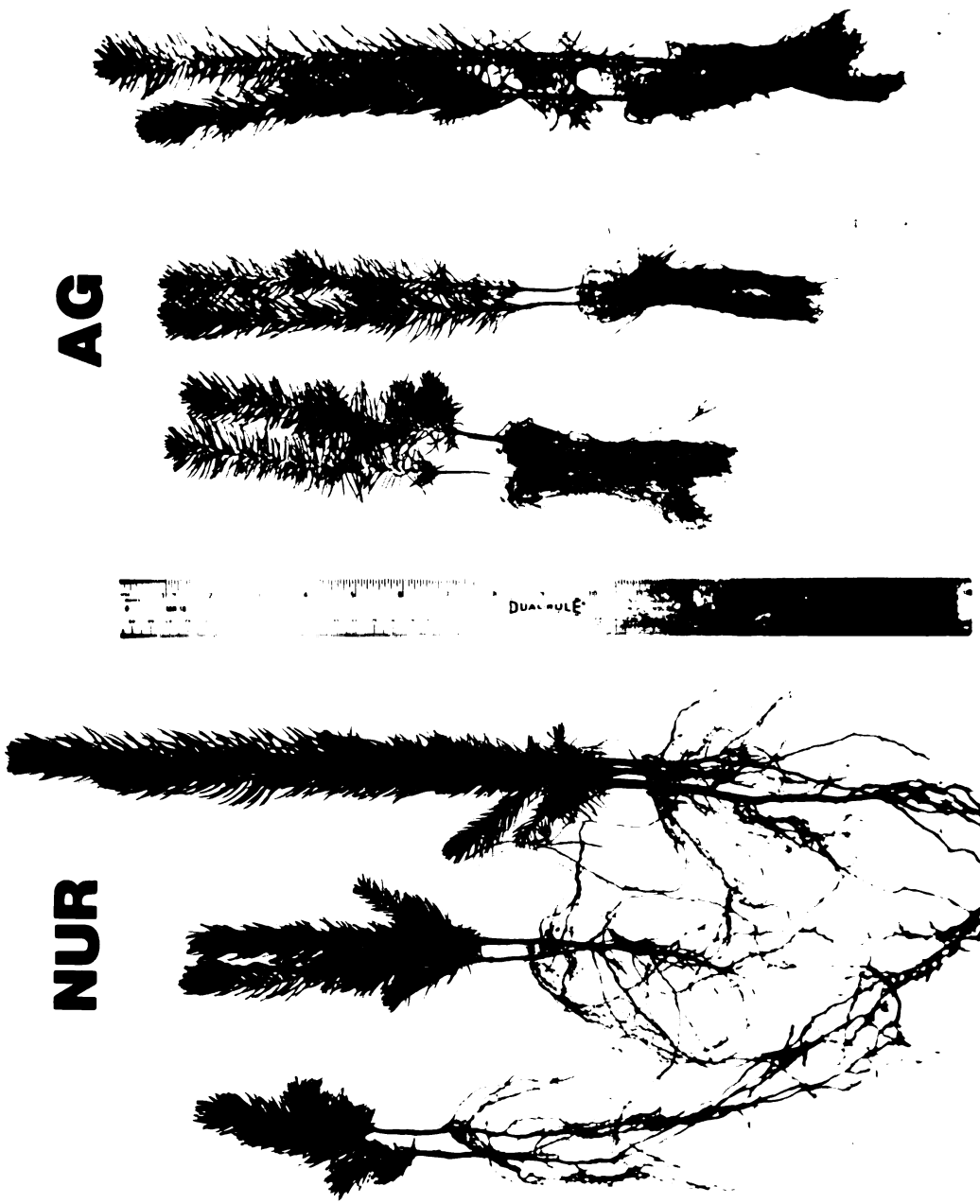
In addition to these quantitative differences, the shoots of the AG seedlings were morphologically different from the NUR seedlings, in ways not quantified. In general, the NUR seedlings had more lateral branches. These appeared to result mainly from the extension of lateral buds formed during the first year. The main stem buds of the NUR seedlings were larger, externally, than the AG buds. The AG needles were not as stiff as the NUR needles and were more normal to the stem axis (Figure 4.1).

Table 4.1 Selected initial characteristics of commercial blue spruce nursery stock grown under conventional methods (NUR) for two years (2+0) and under accelerated growth conditions (AG) for one year.

Type	Size Class (mm)	Mean Height	Plastochron Index	Dry Weight	Root/Shoot Ratio	Stem Diameter
AG	75-150	153a[1/]	275a	0.73a[2/]	.64a	1.9a[3/]
	150-225	195b	302a	1.00a	.37a	2.6a
	225-300	270c	370b	1.12a	.45a	2.4a
NUR	75-150	139a	257a	0.83a	.36a	2.5a
	150-225	200b	379b	2.10b	.62a	4.3b
	225-300	260c	488c	3.49c	.65a	4.8b

1/ Means within a column, followed by the same letter, are not significantly different ($p \leq .05$) Duncan's Multiple Range Test.
2/ Oven dry weights in grams.
3/ Stem diameters, at the cotyledon level, in mm.

Figure 4.1 Representative blue spruce seedlings of the three size classes and two nursery culture methods, tested for subsequent free growth potential.


AG
NUR

The form of the NUR root system also differed from AG seedlings. The NUR root systems had fewer and coarser roots. They also had a more pronounced tap root than AG seedlings. The lateral roots of the AG seedlings were reflexed downward, about 90°, where they had encountered the wall of the container. On some AG seedlings there were indications of spiral roots forming. Also, the prominent tap root seen in the NUR seedlings was missing (Figure 4.1).

Flushing and height growth

There was no significant difference between the LD and SD treatments in the time to budburst, averaged over all seedling types. There was a difference, however, between the cultural methods. On the average, the AG seedlings broke bud and began shoot elongation within 1.5 weeks. The average for the NUR seedlings was 2.4 weeks. Within these two groups there were no differences in budburst date among size classes, nor were there any significant size by cultural-type interactions.

Shoot elongation proceeded rapidly for all types of seedlings, under both photoperiods (Figure 4.2). There was no significant difference in the average height-growth rate, during the first 6 weeks, between the LD and the SD treatments among size classes. There were, overall, significant height-growth rate differences between the NUR and the AG seedlings during this phase ($p=.001$). NUR seedlings average height-growth rate was 42% greater. Preformed shoot growth, under short days, was complete by

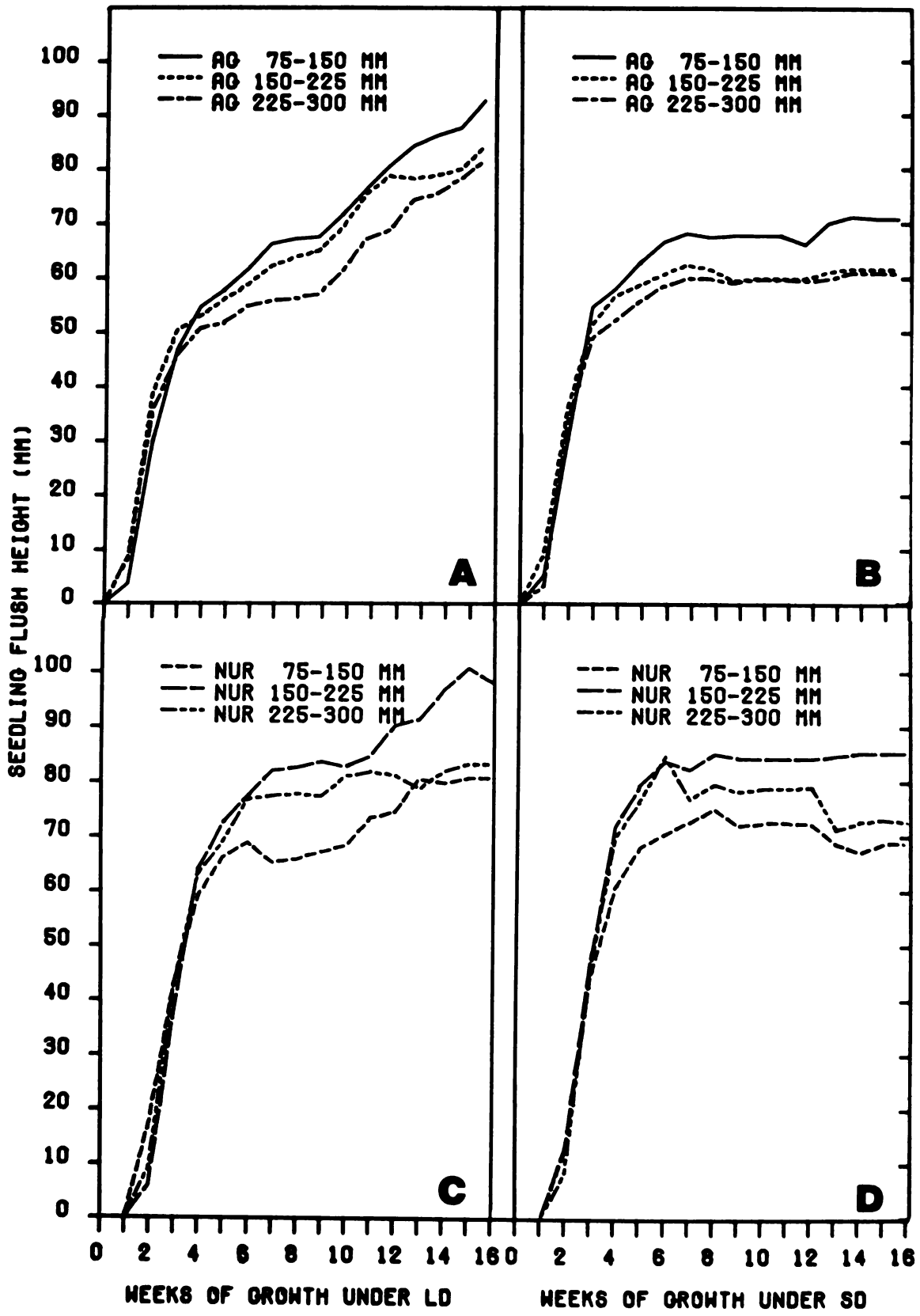
Figure 4.2 New flush heights for AG and NUR seedlings grown for 16 weeks under two photoperiods.

A) Cumulative flush height curves for three size classes of AG blue spruce seedlings grown under 24 hr photoperiods.

B) Cumulative flush heights curves for three size classes of AG blue spruce seedlings grown under 12 hr photoperiods.

C) Cumulative flush height curves for three size classes of NUR blue spruce seedlings grown under 24 hr photoperiods.

D) Cumulative flush height curves for three size classes of NUR blue spruce seedlings grown under 12 hr photoperiods.


AG 75-150 MM
AG 150-225 MM
AG 225-300 MM
NUR 75-150 MM
NUR 150-225 MM
NUR 225-300 MM
A
B
C
D
SEEDLING FLUSH HEIGHT (MM)
WEEKS OF GROWTH UNDER LD
WEEKS OF GROWTH UNDER SD

the sixth week of the experiment. By this time, the average NUR seedling flush height was 28% greater than that of the AG seedlings (Figure 4.2).

Post flushing growth

Under SD, all seedlings had set dormant buds by the end of shoot elongation. There was no overall change in shoot height during the last 10 weeks of the experiment for any of the NUR or AG SD seedlings (Figure 4.2B,D).

There was significant shoot height-growth, over the last 10 weeks of the experiment, for both AG and NUR seedlings, under LD. AG seedlings grew 75% faster than the NUR seedlings, averaged over size classes, during this period. Under LD all AG seedlings grew at the same rate. There were significant differences among size classes within the LD NUR seedlings. While the smaller two size classes, 75-150 and 150-225 mm, did not differ, their average growth rate was 67% greater than the 225-300 mm size class (Figure 4.2A,C).

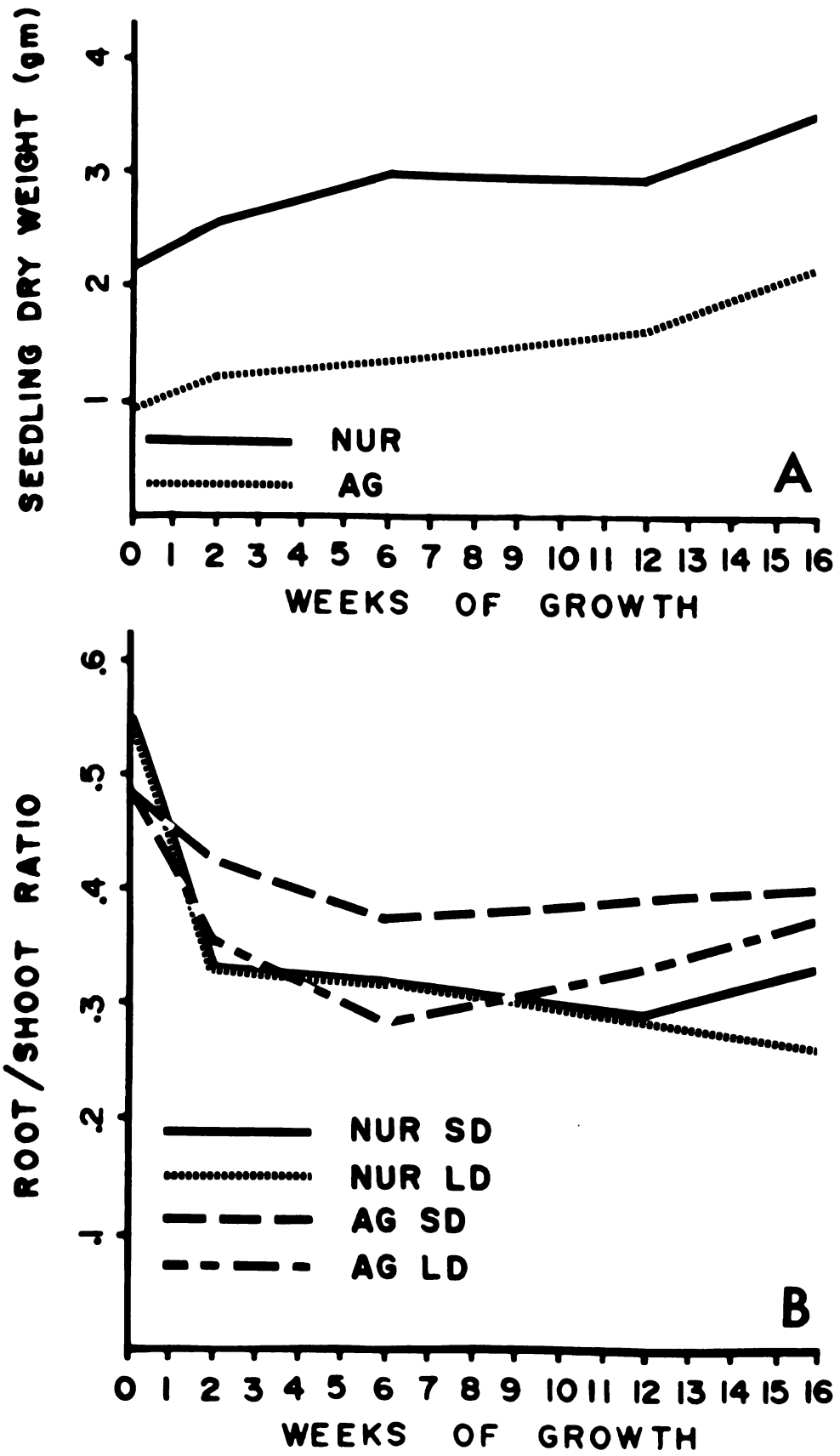
Total seedling dry weight also increased throughout the experiment for both NUR and AG seedlings. There was no overall photoperiodic effect on total weight increase (Figure 4.3A).

In contrast to total dry weight, there were significant differences in the root to shoot ratio between the photoperiods. LD seedlings had significantly lower root to shoot ratios than SD seedlings (.32 vs. .36, $p=.05$). Overall, the AG seedlings had higher root to shoot ratios

Figure 4.3 Biomass accumulation and distribution in NUR and AG blue spruce seedlings grown for 16 weeks under 12 hr and 24 hr photoperiods.

A) Total seedling dry weight for AG and NUR seedlings averaged over size class and photoperiod.

B) Root to shoot ratios of AG and NUR seedlings averaged over size class for both 12 hr and 24 hr photoperiods.


SEEDLING DRY WEIGHT (gm)
1
2
3
4
NUR
AG
A
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
WEEKS OF GROWTH
ROOT/SHOOT RATIO
.1
.2
.3
.4
.5
.6
NUR SD
NUR LD
AG SD
AG LD
B
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
WEEKS OF GROWTH

than the NUR seedlings. There were also differences in root to shoot ratio among the sample times. Root to shoot ratio was highest among the seedlings prior to their potting at the beginning of the experiment. It declined overall until the end of shoot elongation then began to increase under SD but not under LD (Figure 4.3B).

Final observations

After 16 weeks of growth under LD, all size classes of AG seedlings had significantly more needles than did their SD counterparts. On the average, 50% of the AG LD flush needles were neoformed or "free growth". There were also significant differences in the final flush-height, between photoperiods, in the AG seedlings. LD flush height averaged 30% greater than SD flush height.

Under LD there was less, but still significant, free growth in the NUR seedlings, as well. NUR seedling shoots, on the average, had produced only 22% of their needles by free growth by the end of the experiment. The NUR free growth was highest on the 225-300 mm seedlings on which 32% of the flush needles were formed by free growth. Although the means of all the LD NUR treatments had more stem units than the corresponding SD treatments, this was the only individual treatment combination which showed significant free growth. In contrast to the AG seedlings, there was no significant flush height difference between LD and SD for the last sampled NUR seedlings, due to the extremely short internodes of the free growth stem units.

Table 4.2 Selected characteristics of 2-year-old blue spruce nursery stock (NUR) and one-year-old, accelerated-growth (AG) stock after flushing and growth for 16 weeks under 12 hr (SD) and 24 hr (LD) photoperiods.

Type	Size Class (mm)	Flush Height[1]	Plastochron Index[2]	Dry Weight	Root/Shoot Ratio	Stem Diameter
			LD			
AG	75-150	93 *[3]	225 *	1.35 a[4,5]	.41 cd	2.9 a[6]
	150-225	86 *	210 *	2.00 abc	.34 abc	2.8 a
	225-300	82 *	193 *	2.79 abc	.32 abc	3.0 ab
			SD			
AG	75-150	73 *	113 *	1.53 ab	.44 d	2.8 a
	150-225	63 *	106 *	1.71 ab	.41 cd	2.8 a
	225-300	61 *	97 *	3.04 bc	.30 ab	3.7 bc
			LD			
NUR	75-150	81 ns.	257 ns.	1.67 ab	.27 ab	2.7 a
	150-225	99 *	284 *	3.61 cd	.25 a	3.8 bc
	225-300	84 ns.	271 ns.	4.64 def	.25 a	4.0 c
			SD			
NUR	75-150	69 ns.	220 ns.	1.57 ab	.36 bcd	3.0 ab
	150-225	86 *	190 *	4.14 cde	.33 abc	4.1 c
	225-300	79 ns.	211 ns.	5.67 f	.31 abc	4.4 c

1/ Height of the new formed terminal shoot in mm.
2/ Number of needles and needle primordia on the new shoot.
3/ Means followed by *, within a type and size class, are significantly different (p=.05, LSD) between photoperiods.
4/ Means within a column, followed by a common letter, are not significantly different ($p \leq .05$) Duncan's Multiple Range Test.
5/ Oven dry weights in grams.
6/ Stem diameters, at the cotyledon level, in mm.

By the last sample, the average dry weight was 82% greater than the initial value. The AG seedlings increased dry weight by 2.18 times while the NUR seedlings increase was only 69% of the initial value. There was no significant photoperiodic effect on final dry weight (Table 4.2).

The root to shoot ratio was greater, in the SD seedlings than in the LD seedlings at the last sample time. There were still significant differences among the size classes. Overall, the tallest seedlings had the lowest root to shoot ratios.

The stem diameter had also increased significantly during the experiment. While the NUR seedlings had the greatest stem diameters at all sample times, diameter growth was only significant among the AG seedlings. Photoperiod had no effect on stem diameter growth (Table 4.2).

DISCUSSION

The AG seedlings used in this investigation initially differed quantitatively and qualitatively from the similar sized NUR seedlings used. Therefore, age was not the only difference between cultural treatments among size classes. From the responses observed, it seems likely that some of these differences had a strong effect on the free growth propensity of these seedlings.

The earlier budburst in the AG seedlings was most likely due to the more advanced phenology of their buds, as they were received. This was investigated in a concomitant

experiment (Chapter 5). Differences in the bud phenology may be due to the winter storage environment. Photoperiod and temperature affect bud phenology, in cold stored conifer seedlings (Arronson, 1975; Lavender, 1980), and they were not known. Since the NUR seedlings were stored bare rooted and the AG seedlings had a "plug" of moist growing medium surrounding their roots, the storage environment may have affected the two classes differently.

That the average height-growth rate during the flush phase was greater for the NUR seedlings than the AG seedlings and was not significantly different between the LD and SD treatments is not surprising. Temperature, rather than photoperiod, is generally regarded as the most important environmental stimulus of budburst, of a wide variety of woody plants (Kramer and Kozlowski, 1979). This effect of temperature may be cumulative, as seems to be the case in mature white spruce (Owens et al., 1977). Since the NUR seedlings had more preformed needles in their buds, they would be expected to have a greater flush growth rate. If there was an equal rate of internode extension, flush growth rate should be directly proportional to the number of preformed stem units within the bud.

The internode extension was not, however, uniform. While the NUR buds had nearly twice as many needle primordia as those of the AG seedlings, the average height-growth rate for the NUR seedlings was only 72% greater than the AG seedlings. In established spruce trees, the number of

preformed primordia accounts for up to 92% of the variation in shoot growth rate, within a given site (Cannell et al., 1976).

The loss of internode elongation "vigor" in the NUR seedlings may have resulted from a number of external factors rather than any endogenous change in shoot growth-rate potential. The reduction in the following year's growth, after transplanting, in bare-rooted nursery stock (planting check, (Sutton and Tinus, 1983)) is commonly observed in spruce (Burdett et al., 1984). Burdett and others (1984) also found that plug seedlings of Engelmann and white spruce, similar in size to those used in this investigation, suffered less from transplanting than bare root nursery stock.

The combined effects of root damage, during lifting and storage, may have lowered the potential stem growth rate by lowering the seedling water potential during the shoot extension phase. In field planted ponderosa and lodgepole pine (Pinus ponderosa Laws. and P. contorta Doug.), transplanting effects can lower the water potential for at least three years after planting (Baldwin and Barney, 1976). Root pruning lowers spring water potential and shoot elongation, in older blue spruce, in the field (Marquard, 1983).

Photoperiod had no apparent effect on internode or needle elongation, during the flushing phase. This implies that the the meristematic tissues involved in the final

development of these organs, i.e. within the preformed stem internodes and needle primordia, may not be governed by the photoperiod. If they are, it is not as distinct as the photoperiodic control of organ differentiation and development, at the shoot apical meristem, under free growth conditions (cf. Cannell, 1978b).

Throughout the experiment, a 12 hr photoperiod was assumed to induce no free growth. Since no significant increase in the number of needles on the developing shoot was observed at any sample time under SD, the number of needles on the short day shoot was considered to be the same as that in the bud (Pollard and Logan, 1977). In most studies involving spruce germinant seedlings, shoot growth stopped under 12 hr photoperiods well before seedlings were as old or big as any used in this investigation (e.g. Heide, 1974a; Pollard et al., 1975; Young and Hanover, 1976).

Shoot growth continued or resumed under LD, after shoot elongation, in all seedling types. However, the rate of shoot growth was much lower than that observed previously in germinant and young seedlings of blue spruce, grown under similar conditions (e.g. Young and Hanover, 1976, 1977b, 1978; Chapter 1). Here, as with the flush growth phase, the internode distance was less in the NUR seedlings. In the AG LD material in this study, the average height growth rate after the elongation of the preformed shoot was 2.9 mm/week while for the NUR LD seedlings it was 1.7 mm/week.

The reduction in root to shoot ratio during the early

part of the experiment may have arisen from at least two processes. The loss may have been due to the translocation of storage materials, from the roots, to the shoot during budburst and flushing (Kramer and Kozlowski, 1979). A second possibility is that the relative weight loss was caused by the preferential allocation of new photosynthate to the shoot (Loach and Little, 1973). Since there was some dry weight increase of the roots by last sample, in most treatment combinations, both may have occurred but at different rates during the experiment.

The higher root to shoot ratios seen under SD, overall, probably arose in a similar manner. Shoot growth stopped at the end of shoot elongation in these seedlings and relatively more photosynthate could then have been available for translocation to the roots.

The principle reasons for the low growth rates seen in this experiment appear to be transplanting shock and low PPFD. The former reason is substantiated, but not proved, by both the lowered flush vigor and lower weight increase in the NUR seedlings, which had received far more mechanical disruption of their roots, in their previous culture, than the plug AG seedlings.

That PPFD was also limiting is suggested by the comparison of the average shoot height-growth rates and the PPFDs of the germinant seedlings grown for the experiment in Chapter 1 with those of the AG LD seedlings here (which had received relatively little root disturbance). Both the

average height-growth rate and PPFD were about three times greater for seedlings in that experiment than those seen in this investigation. The average light intensity used under LD in this investigation was less than 10% of the photosynthetic saturation value for blue spruce seedlings, which is less than that needed for optimum growth (Tinus and McDonald, 1979).

In conclusion, seedling heights within the range of 75 to 300 mm seem to have no effect on the ability of spruce seedlings to free grow, in the first two years. In older seedlings, the size and age may, or may not, have independent or collective effects on the ability to free grow. In order to objectively evaluate this phenomenon, in light of the long term effects of root disturbance on seedling shoot growth and physiology seen here and elsewhere (Baldwin and Barney, 1976; Burdett et al, 1984), further comparisons should be made on undisturbed material grown either entirely in pots or directly in the ground.

CHAPTER 5

THE EFFECTS OF PHOTOPERIOD ON SHOOT APICAL DEVELOPMENT, ANATOMY, AND ULTRASTRUCTURE IN BLUE SPRUCE SEEDLINGS.

ABSTRACT

The shoot apical development of three size classes of blue spruce (Picea pungens Engelm.) seedlings, grown under long and short days was studied. The seedlings had previously been grown for either two years (2+0) under conventional nursery practices (NUR) or less than one year under accelerated-growth greenhouse culture (AG). All seedlings grown under the short day (SD) treatment set buds at the end of shoot elongation. Under long days (LD), the response was less clear. Most seedlings of all types resumed "free growth" under LD treatment. Some nursery grown seedlings set buds which did not break during the course of the experiment.

Normal bud development followed bud set in all cases under short day treatments. After 16, and in many cases by 12 weeks, the apical meristem of the SD treatment seedlings lost a well defined cytohistological zonation and cell walls appeared shrunken. In addition, the cells of of all zones of these meristems had smaller vacuoles and more starch grains. These changes were associated with the development of a normal preformed shoot within the resting bud.

Under long days, those seedlings which resumed free growth had apical architecture similar to those previously observed in spruce germinant seedlings, grown under long days, that had fully developed apical meristems. Zonation was well developed and primordia formed from the peripheral zone tissue developed directly into needles. In contrast to developing germinant seedling meristems, and perhaps due to low photosynthetic photon flux density levels, total meristem volume decreased over time.

Those seedlings setting bud and not resuming free growth under long days had large conical meristems similar to those seen in naturally grown material late in the bud scale formation period. Prolonged bud scale formation seems to occur under LD in seedlings not exhibiting free growth. This suggests a change, rather than a loss, of photoperiodic response as in older spruce seedlings.

INTRODUCTION

The annual cycle of shoot growth and bud development in mature spruce (Picea sp. (A.) Dietr.) trees, growing under natural conditions, has been well described for a number of species (Fraser, 1962, 1966; Owens and Molder, 1976; Owens, et al., 1977; Pillia and Chacko, 1978; Thompsett, 1978; Harrison and Owens, 1983). In germinant seedling and young seedlings, grown under natural conditions, the determinate growth pattern seen in mature trees is modified by the inclusion of photoperiodically induced, indeterminate growth (Jablanski, 1971; Powell, 1982). While few anatomical studies of bud development and phenology have been made on young spruce seedlings (Burley, 1966b; Gregory and Romberger, 1972; Cannell, 1978a), considerable work has been done on the environmental control of bud development in germinant and older seedlings (e.g. Fraser, 1966; Dormling, et al., 1968; Pollard, 1973; Heide, 1974a; Pollard and Logan, 1974b,1977; Young and Hanover, 1977b; Cannell and Cahalan, 1979; Pollard and Logan, 1979). No previous investigations have compared controlled-environment effects on spruce apical meristem anatomy and ultrastructure.

Spruce seedlings loose the propensity for indeterminate or "free" growth as they age. This loss includes both the free growth response under natural photoperiods and the response to artificially extended photoperiods. In blue

spruce, for example, germinant and one-year-old seedlings grow indeterminately under 24 hr photoperiods. Two year old seedlings appear to be at a transitional stage, some resuming free growth and others not (Young and Hanover, 1976).

While three-year-old and older blue spruce will not grow indeterminately, spruce that has passed through more than three artificially shortened growth cycles will (Chapter 3). The free growth phenomenon, therefore, does not seem to be linked, specifically, to the cyclic changes in meristematic activity and bud development seen during the first three years.

Since blue spruce seedlings can be grown, under optimum conditions, larger than most two-year-old seedlings (e.g. Hanover and Reicosky, 1972; Chapter 1), shoot size per se does not appear to be a limiting attribute either.

Blue spruce seedlings grown under accelerated-growth (AG) conditions, using extended photoperiods, for several months then allowed to form buds prior to chilling all resumed free growth when returned to warm temperatures under long days. Two-year-old (2+0), conventional nursery-grown seedlings (NUR), of the same shoot height classes, were intermediate in their response. Some NUR seedlings appeared to resume free-growth. Other seedlings formed resting buds and never resumed shoot growth. No clear relationship was found between the reinitiation of free growth and any single, measured characteristic of the NUR seedlings,

although root damage in nursery handling was suggested (Chapter 4).

The objectives of this experiment were to determine if there were photoperiodically induced differences in bud development and anatomy between similar sized AG and NUR blue spruce seedlings. Also, it was hoped to gain further understanding into the role of the photoperiod in the development of dormant buds in a uniform environment. In addition, an attempt was made to determine if there were developmental, anatomical, or ultrastructural differences in the apical meristems, associated with the presence or absence of free growth, under 24 hr photoperiod.

MATERIALS AND METHODS

The apical regions of blue spruce seedlings, of the age, size and cultural treatments sampled for the investigations of Chapter 4 were used in this experiment. Two photoperiods were used, long days (LD), 24 hr of continuous irradiation and short days (SD) of 12 hr of irradiation at twice the daily quantum flux as the LD treatments (see Chapter 4).

Five samples with three replications from each of the treatment combinations were taken throughout the experiment. These sample times, corresponding with gross phenological changes in the seedlings, were: (1) dormant buds as the seedlings were received; (2) during budburst two weeks after

planting; (3) at the end of preformed-shoot elongation; (4) at the end of bud-scale initiation (as seen under short day treatments); and (5) late needle initiation (also short days), after 16 weeks of growth.

At each sample time, the main-stem apical meristem and any preformed, unexpanded primordia associated with it were excised and plunged immediately in to ice-cold, PIPES buffered (Piperazine-N-N'-bis (2-ethane sulfonic acid)), glutaraldehyde/paraformaldehyde fixing solution (Cecich, 1977). When the apical meristem and preformed shoot were covered by bud scales, most of these were removed first. The tissue was then exhausted under vacuum and allowed to fix overnight under refrigeration (4°C). After five 10 to 15-min washes in plain PIPES buffer, the material was post fixed with osmium tetroxide as described by Cecich (1977).

The material was then dehydrated in an acetone:water series with 25% concentration steps, changed hourly. After two additional changes of 100% acetone, the last one overnight, the apices were infiltrated, with agitation, with Spurr's medium-hard resin (Spurr, 1969). A similarly graded acetone:resin series with 24 hr between steps was used. The specimens were finally given three changes of 100% resin. Following the last resin change, the specimens were cast in blocks and the resin polymerized for about 72 hr at 60°C.

For light microscopy, the apices were sectioned, longitudinally, up to the median plane at 2 μm thickness on a Porter-Blum ultramicrotome using a glass knife. By

monitoring sectioning progress every 5 to 10 sections the, median plane could be detected to within one cell thickness of dead center.

The sections were mounted on glass slides dried, then stained with methylene blue-azure A with or without basic fuschin counterstain (Berlyn and Miksche, 1976). After drying, cover slips were affixed with Permount. The same photographic and digitizing techniques used in Chapter 2 were then used in quantifying apical meristem development. In addition to the volumetric data, relative zonation was scored on a scale of 0 to 3, based on increasing clarity of cytohistological zonation.

Selected apices were also sampled for transmission electron microscopy (TEM). For TEM pale gold (90 nm) and thinner sections were made with a diamond knife. These sections were mounted on copper mesh grids and stained with uranyl acetate (Stempak and Ward, 1964) and lead citrate (Reynolds, 1963). The lead procedure was modified by the addition of barium chloride, to precipitate carbonate, to the washing solutions.

These grids were then viewed in a Philips TM 300 or a TM 201 transmission electron microscope operating at 80 or 60 kV respectively. Micrographs of selected areas at various magnifications were made on Kodak 4489 electron imaging film.

At both the initial and final samples, apical regions from representative seedlings were also viewed with a

scanning electron microscope (SEM). These samples were mounted on aluminum stubs with graphite cement and viewed fresh, in an ISI Super III scanning electron microscope, operating at 15 kV.

RESULTS

Initial observations

Buds of the NUR seedlings at the initial sample had more than twice the performed preformed needle primordia in their buds than the AG seedlings (Table 5.1, Figure 5.1). There were no significant differences among size classes within the two nursery culture techniques. The NUR buds were also larger in external appearance.

Table 5.1 Selected initial characteristics of the terminal buds of commercial blue spruce nursery stock grown under conventional methods (NUR) for two years (2+0) and under containerized, accelerated-growth conditions (AG) for one year.

Cultural Method	Preformed[1/] Needles	Meristem[2/] Volume	Height (μm)	Diameter (μm)
AG	102[3/]	2891	103	276
NUR	203	6780	136	350

1/ Needles elongated under SD treatments.
2/ In $\mu m^3 \times 10^3$, calculated from median longitudinal sections.
3/ All means, between cultural methods, were significantly different ($p \leq .05$).

Clear phenological differences were also apparent between the AG and the NUR seedlings at the initial sample.

The AG seedling buds had progressed further towards budbreak than those of the NUR seedlings (Figure 5.1). The start of vascular differentiation in the proximal cortex of the preformed shoot had progressed acropetally to nearly the top of the expanding preformed shoot. Needles of the AG buds were further developed, completely over-arching the apical meristem (Figure 5.1A). Most of the buds of the NUR seedlings, while not as far advanced as those of the AG seedlings had started to elongate (Figure 5.1B,E,F). Both needle elongation and vascular development had started in these as well.

There were also anatomical differences in the apical meristems of the seedlings of the two cultural methods, at the initial sample. The apical meristems of the AG seedlings were smaller, overall, than those of the NUR seedlings. This difference was due to both increased height and diameter of the NUR seedlings (Table 5.1).

The meristems of the less advanced buds were anatomically different from those in buds further advanced towards bud break. These differences were observable at light-microscopy resolution and at the TEM level.

The most obvious difference between the less and further advanced apical meristems was the shrunken appearance of the cells within the less advanced meristems. This shrinkage, visible at the light microscopy level, did not involve the separation of the plasmalemma from the cell wall (Figure 5.2). As seen in Figure 5.2A, the shrinkage

Figure 5.1 Preformed shoots from buds of AG and NUR blue spruce seedlings.

A) Scanning electron micrographs of the preformed shoot in an AG seedling terminal bud. Bar = 1 mm.

B) Median longitudinal sections through the preformed shoot in an AG bud. Embedded in epoxy and sectioned at 2 µm. Bar = 500 µm.

C) Median longitudinal section through the shoot apical meristem in the bud of an AG seedling. Bar = 100 µm.

D) Scanning electron micrographs of the preformed shoot in a NUR seedling terminal bud. Bar = 1 mm.

E) Median longitudinal sections through the preformed shoot in a NUR bud. Embedded in epoxy and sectioned at 2 µm. Bar = 500 µm.

F) Median longitudinal section through the shoot apical meristem in the bud of a NUR seedling. Bar = 100 µm.

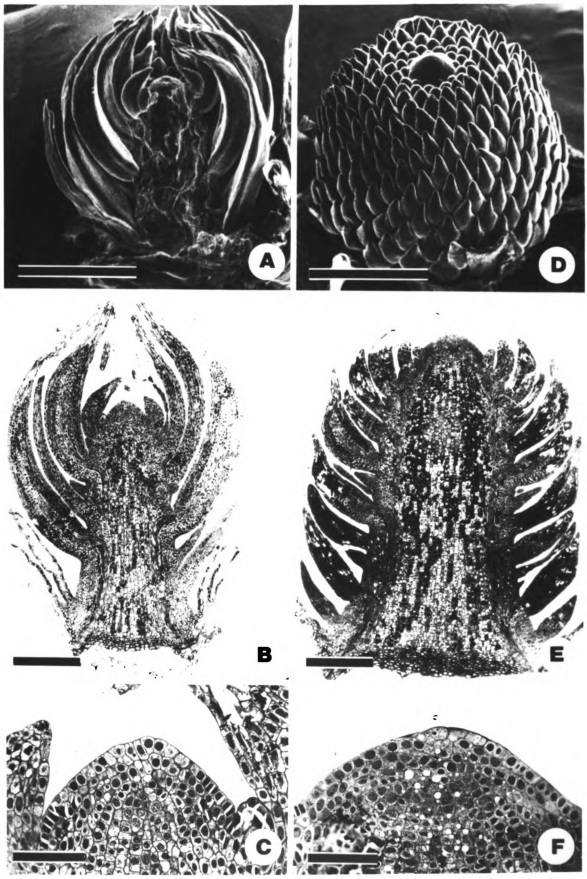

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Figure 5.2 Light and transmission electron micrographs of the shoot apical meristem of a dormant blue spruce seedling, prior to the start of elongation.

A) Light micrograph of a median longitudinal section of the apical meristem. Note low contrast of the nuclei and shrunken appearance of cells. Bar = 75 µm. This condition existed through out the preformed shoot, note last formed needle primordium (np).

B) Higher magnification light micrograph of the central mother-cell zone area of the same apical meristem. Note small vacuoles and high concentration of refractile starch grains. Bar = 15 µm.

C) Transmission electron micrograph of an apical-initial central mother-cell zone cell. Note convoluted cell walls, heterochromatin (hc) in the nucleus and starch free plastids (p). Bar = 2 µm.

D and E) Central mother-cell zone cells showing contorted cell walls, numerous starch filled plastids and osmiophillic (lipid) bodies. The vacuoles (v) present in the these cells often had membranous inclusions or infoldings. Bar = 2 µm.

F) Plastids and inclusions in the central mother-cells. The plastids had large starch grains (s) and little lammelar development. Lipid bodies (lb) were common. They were not seen to be associated with endoplasmic reticulum cisternae but but were observed in association with some vacuoles. The dense cytoplasm in these cells appeared to be due to large numbers of ribosomes (r). Bar = 500 nm.

np
A
B
p
hc
C
D
v
E
s
lb
F

was not restricted to any area of the meristem. The degree of shrinkage appears uniform throughout the meristem and subtending needle primordia, but did not appear to extend into the sub-crown cortex or pith.

In all meristems, the central mother-cell zone appeared to have more vacuoles then the surrounding tissues. The vacuoles were smaller in the less advanced meristems than those observed in typical active meristems. In addition, the membranes of many of the vacuoles appeared to have lipid inclusions or associations and infolded membranes, conditions not seen in the vacuoles of active meristems (5.2D,E).

The cells of the less advanced meristems, especially those of and near the central mother-cell zone, were richer in two types of "storage" compounds. The plastids of these cells had more and larger starch grains (Figure 5.2). In addition, they contained more osmiophillic (presumptively lipid) bodies. These bodies ranged in size up to about 1 um in cross section (Figure 5.2F). They did not appear to be membrane bound nor to have any association with endoplasmic reticula. At the light-microscopy level, these bodies appeared to be non-birefringent and non-refractile. Starch grains were non-birefringent but refractile, depending on the plane of focus (Figure 5.1A,B). These small starch grains also stained pink with basic fuschin as did the large grains seen later in the cells of the developing pith of the SD buds. Both types of bodies were nearly absent in the

further developed meristems (cf. Figure 5.1C,F and Figure 5.2A,B).

Lastly, the nuclei of the central mother-cell zone of the less advanced meristems were more heterochromatic than the more advanced ones. The lower contrast between the nuclei and the surrounding cytoplasm in the less advanced meristems, seen at both the light microscopy and TEM levels appeared to be due mainly to a higher concentration of ribosomes in the cytoplasm of these cells (Figure 5.2f).

Bud break and shoot elongation

Bud-break was significantly earlier in the AG seedlings. The AG buds broke, on the average, in 1.5 weeks while the NUR seedlings took 2.4 weeks. There were no significant differences among size classes within cultural treatments. In addition, there appeared to be no photoperiodic influence on time to bud break (Chapter 4).

The volume of the apical meristems, averaged over all seedling sizes and nursery treatments, declined throughout the experiment. There were no overall differences in meristem volume among the different seedling size classes within the nursery culture techniques nor were there any significant differences between the NUR and AG meristems averaged over the course of the whole experiment. There were, however, three significant interactions.

The meristems of the NUR seedlings, averaged overall sample times did not differ significantly between the LD and SD treatments while the AG seedlings did. The average SD,

NUR meristem volume was 4.67 x 10^6 μm^3 while under LD it was 4.33 x 10^6 μm^3. For the AG seedlings the corresponding means were 5.00 x 10^6 and 2.812 x 10^6 μm^3, for the SD and LD treatments respectively.

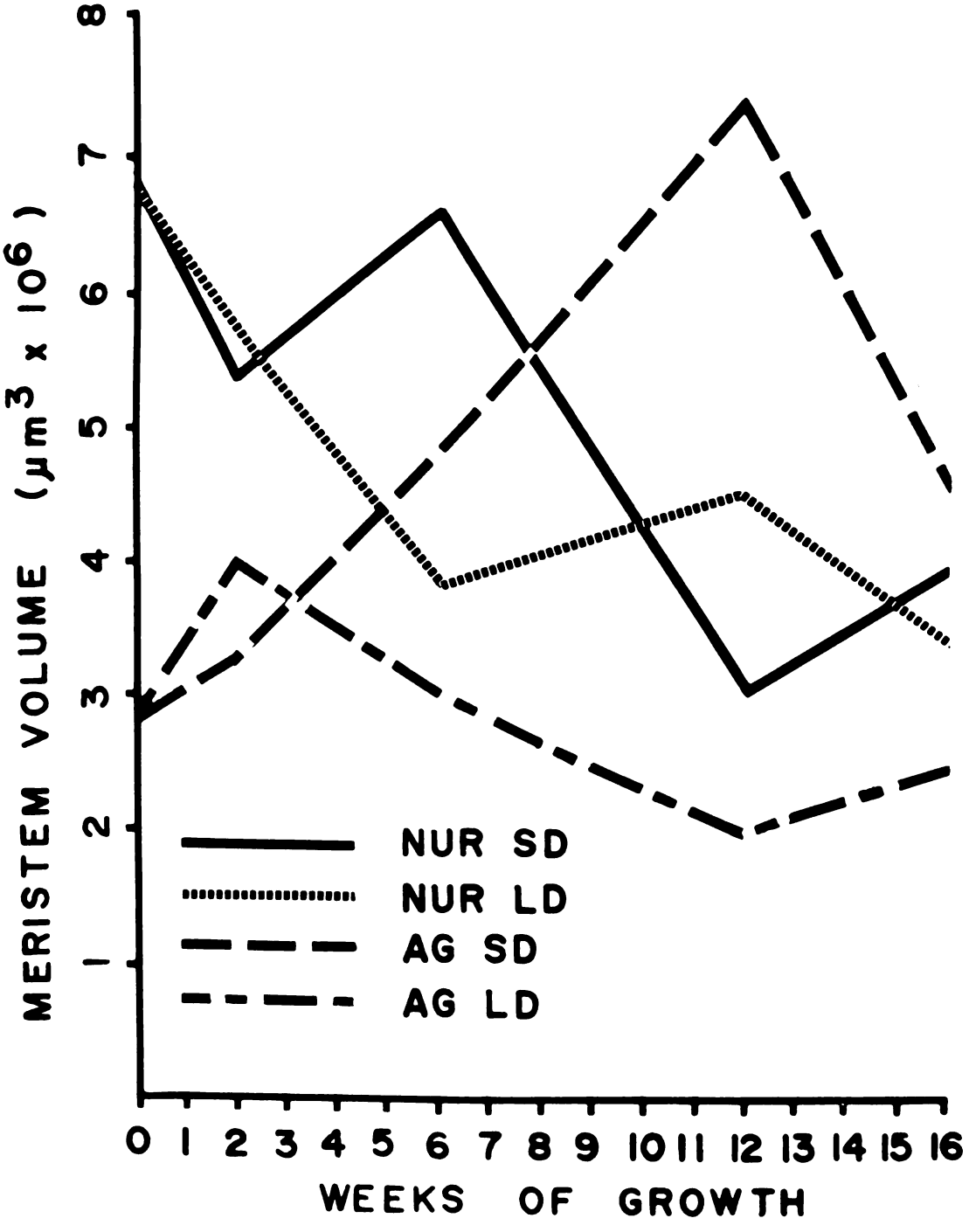
The second differential response was that of the two photoperiod averages at the four sample times. Under LD, there was an overall decline in meristem volume while under SD there was an overall increase followed by a decline.

The greatest differential response was that between the two nursery treatments and photoperiods at different sample times (Figure 5.3). Its significance results from the later and greater increase in meristem volume seen in the AG seedlings under SD.

There were significant differences in the distinctness of the cytohistological zonation between the two photoperiods. The meristems of all types had more distinct zonation under LD. The distinctness of the zonation declined over the course of the experiment after peaking at the second sample time.

In addition to the quantitative changes in apical meristem volume and zonation, there were changes in the types of derivatives of the meristems. These differed with photoperiod and sample time and are related to the quantitative changes described above. At the first sample, the last formed primordia of all types of seedlings appeared to be needle primordia (Figure 3.1C,E). By the second sample, during bud burst, most of the meristems appeared to

Figure 5.3 Average total apical meristem volumes for AG and NUR blue spruce seedlings, pooled over heights, grown under LD and SD photoperiods for 16 weeks.


MERISTEM VOLUME ($\mu m^3 \times 10^6$)
1
2
3
4
5
6
7
8
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
WEEKS OF GROWTH
NUR SD
NUR LD
AG SD
AG LD

have primordia differentiating into bud scales on their flanks. This was true under both LD and SD treatments and for all sizes of NUR and AG seedlings. At the end of shoot elongation under SD, photoperiodic differences first became apparent.

Under long days, apical activity followed two different routes. After differentiating several bud scale primordia, many meristems returned to the production of needle primordia which subsequently developed directly into needles. This was visible by the third sample time. Some meristems, all of the NUR nursery treatments, differentiated only bud scales.

Final observations

By the end of the experiment, after 16 weeks of growth under the two photoperiodic treatments, major differences were evident among the apical meristems and developing primordia of the 12 treatment combinations. All seedlings grown under the SD treatment had well developed buds. Under LD, two types of apical development were observed. Seedlings under long days were either free growing and had not recently formed bud scales (FGLD), or had formed visible buds and were not free growing (NGLD)

The SD buds of all size classes of NUR and AG seedlings had developed morphologically complete performed shoots (Figures 5.4 and 5.5). In all cases, the needle primordia were less developed and shorter than those in the buds at the beginning of the experiment. No vascular development

Figure 5.4 Apical regions of AG blue spruce seedlings after flushing and growth under 12 hr and 24 hr photoperiods.

A) Scanning electron micrograph of the apical region of an AG seedling grown under LD. Bar = 500 µm.

B) Median longitudinal section through the apical region of an AG seedling grown under LD. Bar = 500 µm.

C) Median longitudinal section through the shoot apical meristem of an AG seedling grown under LD. Note the large vacuoles and large lightly staining nuclei in the central mother-cell. Bar = 100 µm.

D) Scanning electron micrographs of the apical region of an AG seedling grown under SD. All but two bud scales have been removed. Bar = 500 µm.

E) Median longitudinal section through the apical region of an AG seedling grown under SD. Note crown formation at base of preformed shoot. Bar = 500 µm.

F) Median longitudinal section through the shoot apical meristem of an AG seedling grown under SD. Note the less distinct zonation, shrunken cells, smaller vacuoles and abundant dense inclusions. Bar = 100 µm.

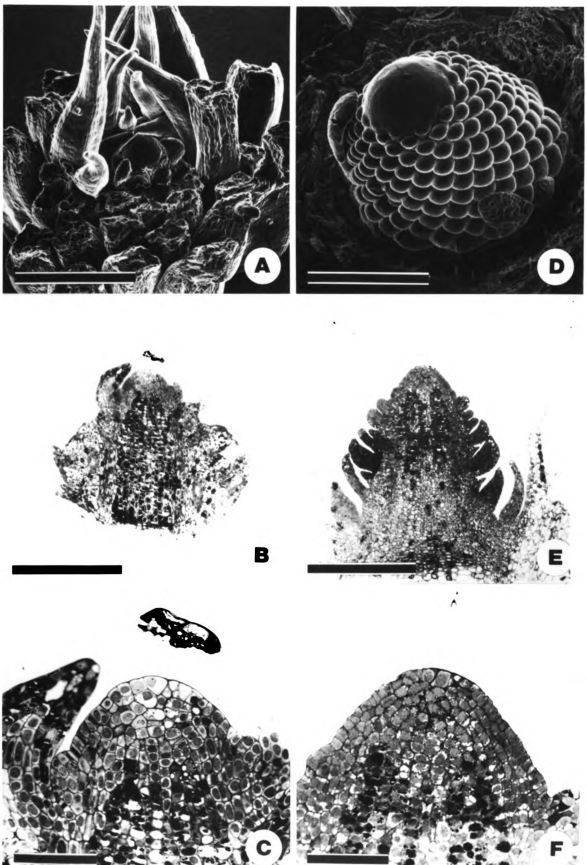

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Figure 5.5 Apical regions of NUR blue spruce seedlings after flushing and growth under 12 hr and 24 hr photoperiods.

A) Scanning electron micrograph of the apical region of an NUR seedling grown under LD. Bar = 500 µm.

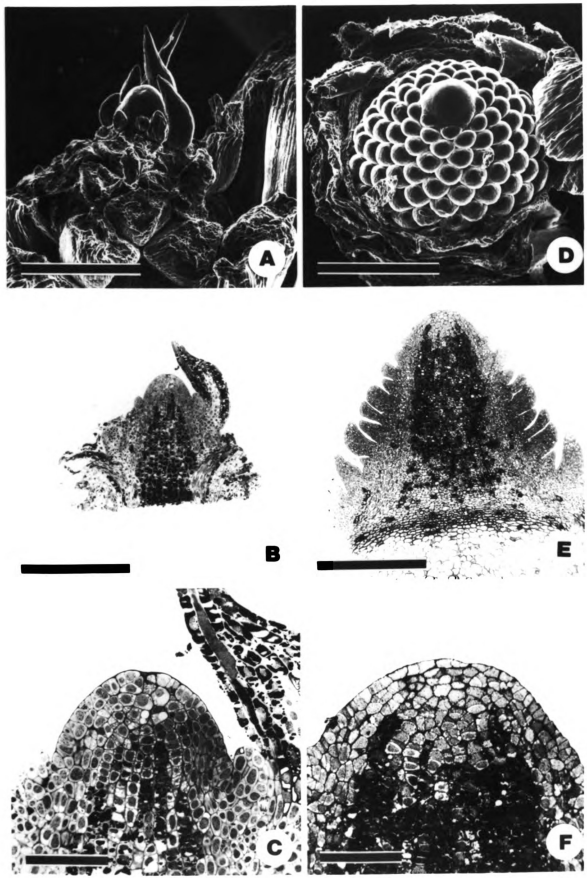
B) Median longitudinal section through the apical region of an NUR seedling grown under LD. Bar = 500 µm.

C) Median longitudinal section through the shoot apical meristem of an NUR seedling grown under LD. Note the large vacuoles and large lightly staining nuclei in the central mother-cell. Bar = 100 µm.

D) Scanning electron micrographs of the apical region of an NUR seedling grown under SD. All but two bud scales have been removed. Bar = 500 µm.

E) Median longitudinal section through the apical region of an NUR seedling grown under SD. Note crown formation at base of preformed shoot. Bar = 500 µm.

F) Median longitudinal section through the shoot apical meristem of an NUR seedling grown under SD. Note the less distinct zonation, shrunken cells, smaller vacuoles and abundant dense inclusions. Bar = 100 µm.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was evident in the cortical regions of the preformed shoots. Although counts of total needle primordia were not made, the buds of the AG seedlings appeared to have more primordia than those of the NUR seedlings (cf. Figures 5.4D,E and 5.5D,E). In median longitudinal section, the buds of the AG seedlings had 36% more needle primordia than the NUR bud preparations.

At the base of all SD buds, just proximal to the bud scale receptacle, a well developed crown region had formed (Figures 5.4E and 5.5E). The pith cells immediately proximal to the crowns were large and lightly staining but had not broken down. Thus, no cavity had formed.

The cytohistological zonation in the SD apical meristems was less distinct than that of the FGLD meristems. At the light microscopy level, the nuclei stained much less distinctly and the cytoplasm much more darkly. These factors, combined with the fewer and smaller vacuoles reduced the overall within and between cell contrast, compared to the FGLD meristems (Figure 5.4C, 5.5C). In the central mother-cell zone and upper pith-rib meristem areas, concentrations of starch grains were seen. Vacuoles in the apical initials and central mother-cell zone were small and less numerous then in the FGLD meristems. The cell walls of the SD meristems appeared wrinkled.

At the TEM level of resolution, further differences were apparent. The walls of the cells bounding the SD meristems were wrinkled, but the sunken appearance, with

the walls protruding only at the junction with adjoining cell walls (cf. Figure 5.2C and Figure 5.6A), was not generally apparent. This type of cell wall distortion was seen throughout the SD meristems, and their subtending primordial shoots (Figure 5.4F and Figure 5.5F).

In the central mother-cell zone of the SD meristems, the vacuoles were small, often with infolded membranes. The plastids had starch grains, but the grains did not appear to be quite as big as those seen in the less advanced initial samples (cf. Figures 5.2D and 5.6D). Lipid bodies, also most obvious in the central mother-cell zone, were more abundant in the SD meristems. They also appeared to be smaller than those seen in the less advance meristems of the initial sample. The nuclei of the central mother-cell zone were more heterochromatic than those seen under the FGLD seedlings. They appeared, however, to lack the large, contiguous aggregations of heterochromatin seen in the less advanced initial material. Heterochromatic nuclei of similar description were seen in the pith-rib meristem and the peripheral zone (Figure 5.6E). Again, as with the initial samples, the darker staining of the cytoplasm was largely due to a high density of ribosomes.

Under LD, two types of apical development and activity were seen by the end of the experiment. The apical meristems of most LD seedlings appeared to be free growing (FGLD) (Figure 5.4A, 5.5A). Some meristems, all from the NUR seedlings, had formed only bud scales under LD and were not

free growing (NGLD), even after 16 weeks (Figure 5.7A,B).

The FGLD seedling meristems differed qualitatively and quantitatively from the SD. The average meristematic volumes were significantly smaller (Figure 5.3). The FGLD meristems also had more clearly defined cytohistological zonation than did the SD meristems. Meristematic cell turgor also appeared to be greater in FGLD seedlings, as the cell walls of these apical meristems were less wrinkled than those of the SD meristems (Figures 5.4, 5.5, 5.7). This was especially evident along the free margin of these meristems (Figure 5.7 A,D,E).

The cytohistological zonation, at the light microscopy level, in the FGLD meristems was largely the result of the differential distribution of vacuoles, differences in nuclear size and staining, the contrast between the nuclei and the cytoplasm, and the alignment of the cell walls. These observations were also related to ultrastructural characteristics. In general, the differences in cell contents among cytohistological zones were greater within the meristems of FGLD seedlings than within the SD seedlings (cf. Figures 5.6, 5.7).

The FGLD central mother-cell zone cells were lightly staining. On an ultrastructural level this appeared to be due to more and larger vacuoles, large euchromatic nuclei and a lower density of ribosomes in the cytoplasm (Figure 5.7). The vacuoles in the central mother cell zones of free-growing meristems often comprised the major part of the

Figure 5.6 Transmission electron micrographs of cells of the shoot apical meristem of a representative AG seedling grown under SD.

A) Apical initial cells showing contorted cell walls (w) and some shrinkage along the upper meristem boundary (mb). Bar = 1 µm.

B) Central mother-cell zone. Note contorted cell walls and starch grains forming in plastids. Bar = 1 µm.

C) Plastid (p) in central mother-cell zone. Starch grain is relatively small and has a distinct lammelar structure surrounding it. The plastids generally appeared less electron lucent than mitochondria (m) which were about the same size. Bar= 500 nm.

D) Central mother cell zone showing plastids with larger starch grains (s) and numerous lipid bodies. The nuclei of these cells had some heterochromatin (hc) evident. Bar = 2 µm.

E) Peripheral zone cells. These cells had large numbers of ribosomes in their cytoplasm yielding the dark image. Cell walls were perforated by numerous plasmodesmata (p), as were those of the other zones. Cell walls were also contorted in this zone. Bar = 5 µm.

F) Rib-pith zone cells. Some cells of this region had large tannin filled vacuoles (tv). The plastids of the rib-pith meristem also had large starch grains in many of them. The nuclei in this zone were highly heterochromatic. Bar = 1 µm.

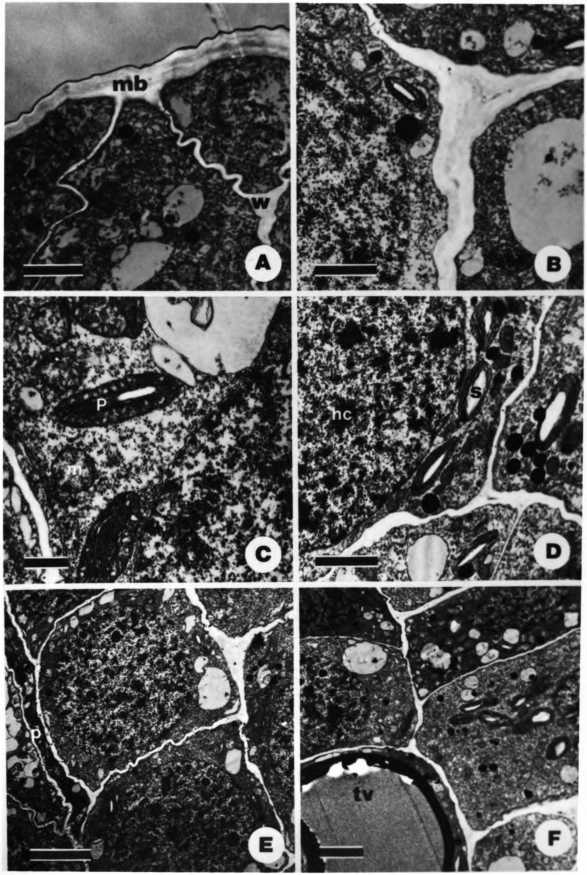
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Figure 5.7 Transmission electron micrographs of cells of the shoot apical meristem of a representative NUR seedling that showed free growth under LD.

A) Apical initial cells showing turgid cell walls and large vacuoles (v) without membranous inclusions. Bar = 3 μm.

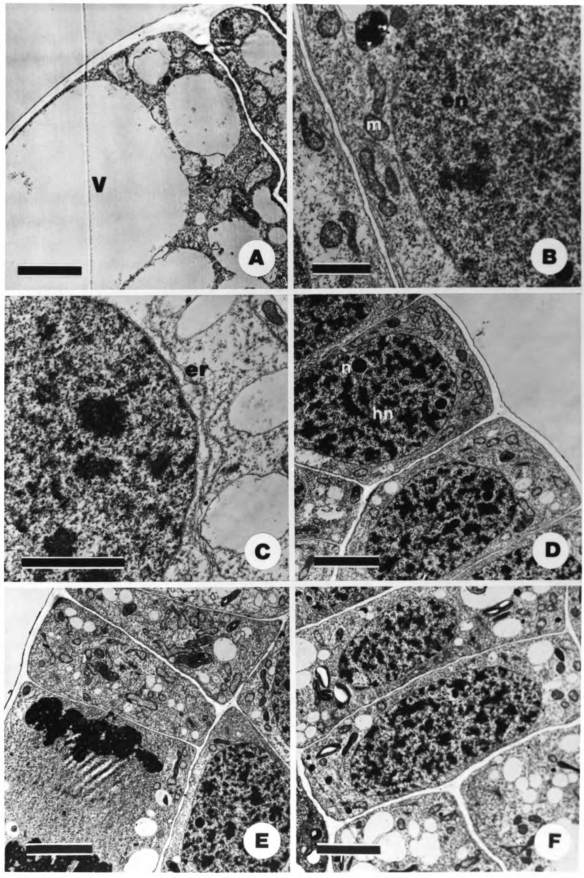
B) Central mother-cell zone. Note contortion free cell walls and large euchromatic nucleus (en) Bar = 3 μm.

C) Central mother-cell zone cell. Some heterochromatin was evident in some central mother-cell zone cells. The cytoplasm is less densely staining apparently from a lower concentration of ribosomes. Endoplasmic reticula (er) was more easily seen in these cells. While most of the central mother-cell zone cells had large nuclei, some had a number of smaller separate vacuoles. Bar= 2 μm.

D) Peripheral zone cells showing plastids without starch grains. The vacuoles in the peripheral zone cells were smaller than those in other regions. turgid cell walls were apparent throughout this region. The nuclei of these cells were highly heterochromatic (hn) with prominent nucleoli (n). Bar = 4 μm.

E) Peripheral zone cells showing telophase mitotic figure. The cytoplasm of the peripheral zone appeared to be richer in ribosomes than that of the central mother-cell zone. Bar = 4 μm.

F) Rib-pith meristem cells. The plastids of the rib-pith meristem had large starch grains in many of them. The nuclei in this zone were highly heterochromatic. Most of the cells had numerous small vacuoles. Bar = 4 μm.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cell. There were other ultrastructural differences seen in the apical-initial central mother-cell zone as well. Fewer plastids were seen in the free growing meristems and most of those seen were devoid of starch and had no organized lamellar structure. The osmiophillic (lipid) bodies of the type seen in the dormant and SD apical meristems were small and scarce in the FGLD meristems.

While the nuclei in the central mother-cell zone were large and euchromatic, those in the peripheral zone and rib-pith meristem were highly heterochromatic. These nuclei appeared to be smaller and more elongate or flattened as well. The vacuoles in the pith-rib meristem and the peripheral zone cells were fewer and smaller than those of the central mother-cell zone. There were fewer starch grains seen in the plastids of the peripheral zone cells than in the pith-rib meristem cells (Figure 5.7D,F). Both the pith-rib meristem and the peripheral zone cells had denser cytoplasm than the central mother-cell zone cells, this appeared to be due to a higher ribosome density.

A few sampled seedlings, all from NUR treatments, had set bud and had not reflushed under LD (NGLD). While the buds of these seedlings had the same external appearance as the SD buds, no preformed shoot was found within. Some contained a few partly elongated needles and some contained only bud-scales. In all cases, the last formed primordia appeared to be bud-scales (Figure 5.8). The meristems in these buds were larger than either those of the SD

Figure 5.8 Light and transmission electron micrographs of the shoot apical region of a NUR seedling not resuming free growth under LD photoperiod.

A) Light micrograph of a median longitudinal section of the apical meristem and last formed bud scale primordia (bs). Note lack of vascular differentiation in these primordia. There are relatively few tannin filled cells in the pith region . Bar = 250 µm.

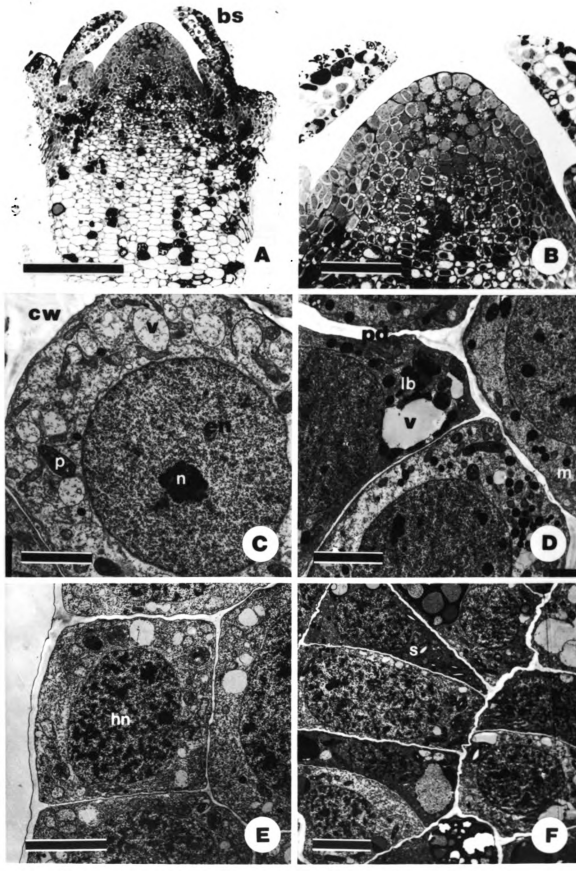
B) Higher magnification light micrograph of same apical meristem. Note small vacuoles and high concentration of refractile bodies. Bar = 80 µm.

C) Transmission electron micrograph of an apical-initial central mother-cell zone cell. Note small vacuoles (v), euchromatic nucleus (hn) with prominent nucleolus (n) and starch free plastids (p) The light area (cw) in the upper left corner is a thick region of the cell wall. Bar = 5 µm.

D) Central mother-cell zone cells showing slightly contorted cell walls, numerous plastids devoid of starch grains and numerous osmiophillic (lipid) bodies (lb). The vacuoles (v) present in these cells were small and sometimes had inclusions. Plasmodesmata (pd) were evident in the thinner areas of the cell walls. Mitochondria (m) were also abundant in these cells. Bar = 5 µm.

E) Peripheral zone cells. These cells had heterochromatic nuclei (hn), small vacuoles, and no starch in the plastids. Bar = 5 µm.

F) Rib-pith meristem cells. Cell wall distortion was most evident in the rib-pith meristem. Plastids in this region also had fairly large starch grains (s). Bar = 5 µm.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treatments or the FGLD seedlings (cf. Figures 5.5 and 5.7), at the end of the experiment. They appeared to be quite similar to those SD meristems sampled during bud-scale initiation.

The zonation of NGLD meristems was not as distinct as that of the FGLD meristems. Their fine structure was roughly intermediate between the FGLD meristems and those of the SD seedlings. The cells of the central mother-cell zone, while having large euchromatic nuclei, had smaller vacuoles than those of the FGLD seedlings. The plastids had few starch grains, but there was an abundance of lipid bodies. These were sometimes aggregated on the margins of vacuoles (Figure 5.8). Nuclei of the cells of the peripheral zone and pith-rib meristem were heterochromatic as with the other treatments. The cell turgor seemed to be intermediate as well. The lower turgor was more evident in the pith-rib meristem cells and the peripheral zone cells, where some cell wall wrinkling was seen (Figure 5.8E,F).

DISCUSSION

Initial observations

The time to bud-break in this study was considerably shorter than that reported previously for spruce seedlings, whose chilling requirement was fulfilled, placed in conducive environments (e.g. Nienstaedt, 1966; Young and Hanover, 1977b). The anatomical differences between the last sample (Dormant, unchilled) and initial sample which

had been chilled in storage indicate that the first sample buds were far from dormant.

In mature Engelmann spruce, for example, mitotic activity in the apical meristem and elongation of the preformed shoot precedes visible bud break by more than a month (Harrison and Owens, 1983). This is also the case in Sitka and white spruce and many other determinate-growth northern conifers (Owens and Molder, 1973a,b, 1976; Owens, et al., 1977; Owens, 1984). Mitotic activity is, perhaps, the best currently applicable indicator of growth resumption in tree meristems (Cecich, 1980).

Physiological changes probably precede observable mitotic activity in most conifer species. In the case of the cells in the pith of the preformed shoot of Scots pine (Pinus sylvestris L.) buds, for example, there are changes in the carbohydrate content or composition detectable about seven days prior to resumption of mitotic activity (Hejnowicz, 1979). Starch accumulation and/or redistribution is also seen throughout seedlings of Scots pine prior to bud-break (Ericsson, 1984). Histochemical analyses, such as those performed by Vanden Born (1963) and Fosket and Miksche (1966) also indicate that considerable physiological changes occur without mitotic activity in apical meristems.

Although the seedlings in this study, as received, all had completely closed buds, considerable post dormancy activity had been underway within them. This advanced

development, in conjunction with the warmer than natural temperatures in the growth room, probably precipitated the rapid bud break.

While the more advanced development of the of the AG seedlings might have resulted from several interacting cultural or storage (Arronsson, 1975; Lavender, 1980) conditions, it may have resulted partly from their age. One-year-old nursery grown spruce seedlings undergo more post differentiation needle development in the fall than do older seedlings (Burley, 1966a,b; Chapter 3.). They also tend to break bud sooner under conducive conditions (Nienstaedt, 1966; Young and Hanover, 1977b).

The higher number of starch filled plastids in the apparently less advanced meristems seems unusual. In Scots pine buds, starch usually decreases in the fall, is lowest in the winter, then is detectable again in the spring (Hejnowicz, 1979). A similar trend is seen in the needles (Martin and Oquist, 1979). I have noticed a similar trend during the annual cycle in mature blue spruce, growing in lower Michigan (Heckman, unpublished data). Small starch grains were abundant in the central mother cell zone and developing pith in the elongating shoots prior to bud break. By mid November, very few were present. The latter observations are consistent with the theory developed early in this century by Lidforss (1896, 1907) and briefly discussed by Steponkus (1984) that starch is hydrolized during dormancy induction to provide sugars which have a

cryoprotectant role. The former observation, that of this investigation, may have resulted from the disruption of the natural bud phenology, especially with respect to the temperature regime, caused by nursery storage.

In the initial sample, the less advanced meristems also appeared to be less hydrated than those more advanced towards bud-break. Evidence of this is given by the wrinkled appearance of the cell walls, denser cytoplasm, and small vacuoles (Figure 5.2). This may have been a residual characteristic of the hardened, post-dormant state. Lower intracellular cellular water content is a commonly observed attribute of chilling-resistant dormant plant material (Weiser, 1970; Levitt, 1980). Since the dehydrated appearance was not seen in all initially sampled meristems, and all meristems at each sample time were processed identically, it does not appear to be a preparation artifact. Chemically-caused dehydration damage in plant microtechnique is usually characterized by the separation of the plasma membrane from the cell wall and this was not seen. Further, the ultrastructural differences between the advanced and less advanced buds, associated a wide range of biochemical and physiological processes would be hard to induce quickly and simultaneously as artifacts.

Cecich, (1977, Figures 1 and 2) shows the shoot apical meristem of Pinus banksiana seedlings prepared for light microscopy by CRAF fixation and paraffin techniques and prepared for electron microscopy essentially as described

above. In the TEM preparation, the cells have a similar appearance, where as the paraffin preparation shows no indication of wrinkled cell walls. This effect of paraffin technique may explain why a shrunken appearance in dormant spruce meristem cells has not been reported previously, in spite of many excellent phenological studies (e.g. Owens and Molder, 1976; Owens, et al., 1977; Harrison and Owens, 1983).

The heterochromatic content of the nuclei and nuclear size also varied with the apparent phenological status of the initially sampled meristems. Heterochromatin has been reported to be relatively inactive in RNA synthesis (Berlowitz, 1965). This is consistent with the dormant condition. The heterochromatin seen in the nuclei in the dormant meristems may be constituative, however, and perhaps due to smaller nuclear volumes seen in these meristems (Owens and Molder, 1973; Riding and Gifford, 1973; Cecich, 1977).

Bud burst and shoot elongation

The phenology of the buds grown under SD was similar to that seen in spruce, and many other conifers with similar shoot phenology, in the natural environment.

In the natural growth cycle of these trees, bud-scales are formed during budburst and shoot elongation in mature spruce, (Owens and Molder, 1976; Owens,et al., 1977; Harrison and Owens, 1983), Douglasfir (Pseudotsuga menzeseii) (Sterling, 1946; Owens and Molder, 1973a),

western hemlock (Tsuga heterophylla (Raf.) Sarg.) (Owens and Molder, 1973b), and the true firs (Abies sp.) (Parke, 1959; Owens and Singh, 1982; Owens, 1984). Throughout the ranges of these species, this occurs under the longest photoperiods of the year.

In the SD material in this experiment, grown under 12 hr photoperiod, most seedlings had visible bud scales by the second sample and all did by the third, taken at the end of shoot elongation. These were also the sample times during the experiment when the shoot apical meristem had reached or was approaching its maximum volume under SD (Figure 5.4). Thus, bud initiation was not altered by the short photoperiods.

The pattern of apical development under LD treatment was completely different. It also differed from that reported in germinant seedlings grown under LD (Gregory and Romberger, 1972a; Cannell, 1978; Chapter 2). In these studies, and other studies of the initial development of the conifer meristem (e.g. Tepper, 1964; Fosket and Miksche, 1966; Riding, 1972), the meristem gets bigger over time. In this study there was an overall decline. This may be attributed to both endogenous and environmental factors.

The most probable environmental factor was the low PPFD. Under the LD treatment, the mean PPFD at the soil level was 35 $\mu Mm^{-2}sec^{-1}$ or about 225 foot-candles. This is less than 10% of the saturation photon-flux-density for Engelmann spruce seedlings (Ronco, 1970; Tinus and McDonald,

1979). Blue spruce seedlings are reported to have similar or even higher saturation point (Tinus, 1979). While the PPFD was greater at mid-canopy of the seedlings, it was never greater than 100 μMm^{-2} sec^{-1}. This corresponds to considerably less total energy input (photon flux) than previously used for such experiments, even considering the 24 hr photoperiod.

Shoot elongation in conifers is a major sink for the stored and new photosynthesis of the previous years shoot (Dickmann and Kozlowski, 1970; Loach and Little, 1973; Little, 1974; Chapter 4). The expansion of free growing stem units also represents a likely metabolic sink. Under the low light intensities used here these demands may not have been met. If the carbohydrate requirement for free grown stem unit elongation and that needed for meristem "reinvestment" sensu Gregory and Romberger (1972a) were greater than that formed de nova, the development of either or both might suffer. Needle differentiation without tissue replacement might reduce the size of the undifferentiated meristem in a manner analogous to that seen during late needle formation in mature spruce trees and seedlings (e.g. Owens, 1976; Owens et al., 1977; Cannell and Cahalan, 1979; Harrison and Owens, 1983).

Since there is less post initiation development in primordia formed under SD and almost no stem elongation (i.e. within the forming bud), one would expect a lower photosynthate demand. The lower than natural total quantum

flux might then have less of an effect the development of these buds.

Final observations

By the end of shoot elongation, in mature spruce trees, the apical meristem is covered by a protective bud. At this time in the annual cycle, needle primordia are initiated but do not elongate. This development of needle primordia continues until late autumn, a period of up to 4 months, after which the apical meristem is at its dormant size, mitotic activity ceases, and it shows the least zonation (Owens and Molder, 1973a, 1976; Owens, et al., 1977; Harrison and Owens, 1983). In germinant seedlings of white spruce, grown under AG conditions for 3 months, Pollard (1974a) found that most provenances had completed needle primordia initiation by 12 weeks when the seedlings were placed under short days with optimum temperatures and nutrients. In that study, Pollard also showed that most of the needle primordia formation occurred in the first six weeks of bud development.

In this experiment, buds formed under SD treatments had begun to form scales by the sixth week, allowing somewhat less than 10 weeks for needle primordia initiation prior to the end of the experiment. The preformed shoots within the buds had formed well developed crowns by the last sample. In the determinate conifers that have this structure (see Lewis and Dowding, 1924; Venn, 1965), this occurs during late needle initiation (Parke,1959; Owens and Molder, 1973b,

1976; Owens, et.al., 1977; Pillia and Chacko, 1978; Harrison and Owens, 1983). This implies that the buds in this study had nearly all their preformed needle primordia differentiated. The differences in needle primordia number between the NUR and AG buds were probably due to the transplanting effects (Chapter 4).

The apical meristems of the SD buds at the end of the experiment were similar to that of the less advanced buds at the beginning. The qualitative differences between the SD and FGLD seedling meristems were similar to those between the meristems of the more and less advanced buds at the initial sample. Apparently photoperiod can alter the carbohydrate, lipid and water status of the meristem itself in addition to (or conjunction with) controlling the fate and development of the primordia initiated. These characteristics, combined with the mentioned changes in the cytoplasmic density and nuclear staining seem to be responsible for the loss of distinct cytohistological zonation reported in dormant conifer meristems (Owens and Molder, 1973a; Cecich, 1977).

The shrinkage of the vacuoles and wrinkled appearance of the cell walls seem to indicate that the meristem is at a lower state of hydration. The chemical binding or export of free water is often associated with the development of frost hardiness (Weiser, 1970; Levitt, 1980). It was not determined if these apparent changes of hydration were present throughout the seedling or just within the bud,

although cells of the bud-receptacle region (when not trimmed away) did not appear similarly shrunken. If the latter were true, it would further support the idea of the crown structure as a physiological barrier (Lewis and Dowding, 1924; Chalupa and Durzan, 1973; Jansson et al., 1983). If the former were true, a developmental photoperiodic effect on either root uptake and transport or a similar hardening mechanism through out the plant would be implied.

The higher number of starch filled plastids in the cells of the SD meristems is incongruous with that seen in the apical meristems and preformed shoots of mature blue spruce in the late fall. As mentioned with respect to the initial sample, starch grains are more prevalent in the spring and disappear in the fall. This is thought to be related to the increase of free sugars (by hydrolysis) associated with cryoprotection (Weiser 1970; Arronsson et al., 1976; Levitt, 1980).

In tobacco (Nicotiana tabacum) leaves. Far red light lowers the size and number of starch grains seen and increases the amount of soluble sugar. Red light has the opposite effect, implying a phytochrome mediated response (Kasperbauer and Hamilton, 1984).

In the material in this investigation starch appeared to accumulate under SD conditions. Since phytochrome reverts to its red absorptive form (i.e. a similar effect to the far-red treatment described above) in the dark (Song,

1984), the SD response seen in this investigation suggests that the seasonal hydrolysis of starch to sugars in spruce might be triggered by temperature. This may in turn be a factor in the interdependence of photoperiod and temperature on the development of spruce seedling winter-hardiness (Arronsson, 1975; Christersson, 1978; D'Aoust and Cameron, 1982).

In the lower elevation Himalayas, where the climate is seasonal but mild, Pillia and Chacko (1978) found that starch increased in October and decreased in January and February in Indian spruce (Picea smithiana (Wall.) Bioss.) buds. Other than this aspect, the rest of the bud phenology they described was similar to those reported for North American spruce species (loc. cit.). Indian spruce also appears to be less cold hardy than blue spruce (Rehder, 1940), further suggesting a relationship between the starch -sugar balance and the development of cold hardiness.

At the end of the experiment, all LD AG meristems were free growing (FGLD). These meristems as described above were smaller than those of the initial sample. This was also the case in most of the FGLD NUR meristems. It would appear that for free growth to have continued in these seedlings, either the rate of primordia formation would have needed to decrease or the volume of undifferentiated tissue in the meristems would have needed to increase.

The NGLD meristems (Figure 5.8) were anatomically similar to the SD meristems, sampled during bud-scale

initiation. By the end of the experiment, however, the buds were similar in external appearance to those formed under short days. Removal of the bud scales revealed no preformed shoot formation. Either the meristem had stopped differentiating primordia after the normal number of bud scales had been formed and they had continued to grow or the primordia differentiated remained bud scales.

Although (unfortunately) no counts were made of the individual bud scales of either LD or SD buds, it appeared as though the latter was the case. The bud cavities were filled with scales.

If the continued production of bud scales in the NGLD seedlings did occur and it was not an effect of transplant shock, it may represent the mature response of the spruce shoot apical meristem to photoperiod. Under natural conditions, mature spruce apical meristems produce bud scales during the days with the longest photoperiod (loc. cit.). Since under short days, the first neoformed primordia always differentiated into bud scales, the mature photoperiodic response may involve the inhibition of needle primordia initiation, rather than the induction of bud scales.

In conclusion, shoot height, with in the range of the material used in this investigation, has no main effect on apical meristem phenology. Shoot apical meristem phenology appears to change as the seedlings age, although transplant shock may have affected this both quantitatively and

qualitatively. Rather than the loss of photoperiodic response, as seedlings mature, there appears to be a change in the photoperiodic effect on organogenesis at the apical meristem. Under short photoperiods, preformed shoot development appears to proceed to completion even in the absence of limiting temperatures but some histological changes, associated with chilling hardiness, do not seem to occur, under warm growth-room conditions.

CONCLUSIONS AND RECOMMENDATIONS

The results of this study indicate that spruce germinant seedlings, of a diverse genetic origin, appear to grow at nearly the same relative growth rate under propitious conditions. The large interspecific differences in dry weight appear to be due to initial seed size and germination vigor even after 6 months of culture.

For at least the first 6 months, the growth of a relatively unrestricted spruce seedling is nearly exponential, its biomass growth-rate, at any time, being proportionate to its weight. That this rate cannot continue is also obvious. Its reduction is foreshadowed by the apparently asymptotic increase in height growth rate. In order for growth to proceed at an exponential rate, either all of the plant's mass must increase at the same unit rate i.e. remain totally meristematic, or the unit rates of the meristematic areas must continue to increase. While neither of these possibilities makes biological sense, the observed rates do present some interesting implications for both containerized seedling production and an understanding of spruce seedling development.

From the standpoint of greenhouse culture, the concept of even an initial exponential-growth rate, has some strong implications. The "lag" phase commonly observed during early development is apparently due to the low $\overline{RGR}$ of about

extremely important at this point to avoid any circumstances that even slightly reduce vigor, as the reduction might be proportionally expressed throughout the rotation period.

The results also imply that regardless of the species grown, efforts to secure the fastest germinating, highest weight seed should be made, for the fastest absolute growth.

From the standpoint of further research, the most immediately promising areas would seem to lay at either end to the sample time reported here. Little information is available on the initial dynamics of germination in spruce, or what affects its initial growth rate. The very early growth-rate dynamics are often different than the average rates over periods such as this investigation (Hunt, 1982). Such information would perhaps be useful to those involved with small propagule sizes in vitro.

At the other extreme, the existence of a nearly exponential growth rate after six months of growth, in light of the stabilizing height growth-rate and changes in tissue distribution in the apical meristem, also warrants further investigation. While the seedlings in this experiment never completely filled their containers with roots, the effects of root and shoot environment on long term experiments requires careful consideration in experimental design. A root medium that could be replaced periodically or continuously, such as a hydroponic system, might be advantageous in this respect, as would higher light intensities.

The shoot growth rate, on the other hand, approaches a nearly constant value, after about six months of accelerated growth. Plastochron index and height were very closely related in the time span of this experiment indicating a relatively uniform internode elongation, within each species. Since differences in height-growth rate between species, in this study, appeared to be more related to internode elongation than needle number, plastochron duration in spruce may be nearly the same under accelerated growth conditions. Growth analyses techniques of this type may prove useful in developing greenhouse or nursery crop monitering programs, in addition to basic information on how seedlings grow.

Past work in spruce has indicated that the rate of needle initiation is strongly tied to the volume (or basal area) of the apical meristem in Norway spruce (Gregory and Romberger, 1972a,b) and Sitka spruce (Cannell, 1978a) in these studies, the apical volume increased asymptotically over time. The anatomical results here indicate that a similar response is seen in other spruces. Later work by Gregory and Romberger (1977) and showed that vascular differentiation, as indicated by protoxylem initiation, occurred with chronometric uniformity rather than morphometric. This implies a possibly different "control system" for internode elongation than primordia initiation at the apex. The results from Chapter 4, that photoperiod did not affect internode elongation during flushing, in the

blue spruce seedlings tested, lends further credence to this hypothesis.

It is interesting to note that the general effect of shortened growth cycles appeared to be retard seedling development rather than accelerate it. After more time under shortened growth cycles than it takes for two growth seasons of nursery culture, the apical development was no greater than a one-year-old seedling. The results of the flowering studies using similar shortened growth cycles by Dormling et al. (1968) have not, to my knowledge, been reported. From the standpoint of accelerating maturity in spruce, it would appear that systems allowing the most uninterrupted growth, hold more promise (e.g. Young and Hanover, 1976).

Even in very short growth cycles, progressively more time is seems to be needed for bud formation. This may also be the case for free grown seedlings of progressively increasing size. In white spruce Pollard (1974b) found that taller seedlings within an age class had more bud needles after 10 weeks of short days, but that the relative advantage declined with age. If the older, taller seedlings took longer to complete bud set, they may have had less time left for needle initiation. Thus, the greenhouse practitioner may need to consider the coordination of three stem growth components, initial free growth height, the time to complete bud scale formation, and the time to initiate needle primordia.

Within 75 to 300 mm tall two-year-old (2+0) blue spruce, seedling height does not appear to limit free growth. Seedlings in the middle of this range were the only ones to show, quantitatively, free growth. However, the overall mean was significantly greater than the non-inductive control as well. Since all of the AG seedlings in this height range free grew under long days, height, alone, within this range, does not seem to affect free growth.

Comparison of the shoot growth development in the nursery-grown seedlings to that of the AG seedlings illustrated some equivocality in the use of transplants in such studies. While the shoots of the NUR seedlings were larger in most measured characteristics, and had more shoot growth potential their out-growth performance was relatively less than the AG "plugs" when grown indoors under either continuous or short day photoperiods. Ideally, this aspect of the change in photoperiod response should be clarified by using various aged seedlings, grown from seed in large pots.

That growing transplants in the test pot for the year prior to the experiment (e.g. Nienstaedt,1966) may not be sufficient to remove transplanting effects is suggested by the field work of Burdett and others (1984). In this experiment, transplanted (2+1) seedlings appeared to develop fewer primordia than AG seedlings under short day.

Anatomically, there were many differences between the apical regions of seedlings grown under long and short days. The short day response was the most varied less with the

size or age of the seedling than the long day treatments.

Under short days, bud development followed the same general phenology as seen in mature spruce. Use of TEM preparation techniques yielded considerable new information at light and TEM levels. While these results should be replicated with material grown under more "normal" lighting, it appears that photoperiod, either directly or indirectly, alters the water and carbohydrate status of the bud as it forms.

Starch accumulation occurred in the dormant SD buds. This is opposite of what is usually seen in northern temperate zone conifers under natural conditions. In light of the implications in seedling hardiness and growth potential chemical analyses should be undertaken along with further anatomical observations.

Under long days, the apical meristem took on two different types of morphology related to the presence or absence of free growth. While the anatomy of the free growing meristems was qualitatively similar to the paraffin image, the TEM preparation technique, in spruce, suggests that the light staining seen in the CMZ of active meristems is not a lipid extraction artifact (cf. Cecich, 1980).

Those seedlings not free growing under long days appeared to be in a state of prolonged bud set. There may be a change in photoperiodic response as the seedling ages, rather than a loss. Long days in mature spruce may inhibit needle primordia initiation.

BIBLIOGRAPHY

BIBLIOGRAPHY

Arnott, J. T. 1979. Effect of light intensity during extended photoperiods on the growth of amabilis fir, mountain hemlock, and white and Engelmann spruce. Can. J. For. Res. 9: 82-89.

Arronsson, A. 1975. Influence of photo- and thermoperiod on the initial stages of frost hardiness and dehardening of phytotron-grown seedlings of Scots pine (Pinus sylvestris L.) and Norway spruce (Picea pungens (L.) Karst.). Stud. For. Suec. 128: 1-20.

Baldwin, V. C. and C. W. Barney. 1976. Leaf water potential in planted ponderosa and lodgepole pines. Forest Sci. 22: 344-350.

Berlowitz, L. 1965. Correlation of genetic activity, heterochromatin, and RNA metabolism. Proc. Nat. Acad. Sci. 53: 68-73.

Berlyn, G. P. and J. P. Miksche. 1976. Botanical Microtechnique and Cytochemistry. Iowa State University Press, Ames, Iowa. 326pp.

Bongarten, B. C. 1978. Genetic and environmental variation in shoot growth and other traits of blue spruce. Ph. D. Dissertation. Michigan State University. 104 p.

Burdett, A. N., Herring, L. T., and C. F. Thompson. 1984. Early growth of planted spruce. Can. J.For. Res. 14: 644-651.

Burley, J. 1965. Variation in seed characteristics of Sitka spruce. Ad. Front. Plant Sci. 10:11-24.

Burley, J. 1966a. Genetic variation in seedling development of Sitka spruce (Picea sitchensis (Bong.) Carr.). Forestry. 39(1): 68-94.

Burley, J. 1966b. Provanance variation in growth of seedling apices of Sitka spruce. Forest Sci. 12(2): 170-175.

Cannell, M. G. R. 1978a. Analysis of the shoot apical growth of Picea stichensis seedlings. Ann. Bot. 42: 1291-1303.

Cannell, M. G. R. 1978b. Components of conifer shoot growth. Proc. 5th. Nor. Amer. For. Biol. Wrkshp. C. A. Hollis and A. E. Squillace. eds. Pp. 313-318.

Cannell, M. G. R., S. Thompson, and R. Lines. 1976. An analysis of inherent differences in shoot growth within some north temperate zone conifers. Pp. 174-205. In Cannell, M. G. R. and F. T. Last (ed.) Tree physiology and yield improvement. Academic Press. London, New York, San Francisco.

Cannell, M. G. R. and S. C. Willett. 1976. Shoot growth phenology and matter distribution and root:shoot ratios of provenances of Populus trichocarpa, Picea sitchensis, and Pinus contorta growing in Scotland. Silvae Genetica 25(2): 49-59.

Cannell, M. G. R. and C. M. Cahalan. 1979. Shoot apical meristems of Picea sitchensis accelerate following budset. Ann. Bot. 49: 209-214.

Causton, D. R. and J. C. Venus. 1981. The Biometry of Plant Growth. Edward Arnold Ltd. London. 307 p.

Cecich, R. A. 1977. An electron microscopic evaluation of cytohistological zonation in the shoot apical meristem of Pinus banskiana. Amer. J. Bot. 64: 1263-1271.

Cecich, R. A. 1980. The apical meristem. In Little, C. H. A. (ed.) The Control of Shoot Growth in Trees. IUFRO Joint Wrkshp. Fredericton, New Brunswick.

Cecich, R. A. and J. P. Micksche. 1970. The resopnse of white spruce (Picea glauca (Moench.) Voss.) shoot apices to exposures of chronic gamma radiation. Rad. Biol. 10: 457-467.

Cecich, R. A. and H. T. Horner. 1977. An ultrastructural and microspectrophotometric study of the shoot apex during the initiation of the first leaf in germinating Pinus banksiana. Amer. J. Bot. 64(2) 297-222.

Chalupa, V. and D. J. Durzan. 1973. Growth and development of resting buds of conifers in vitro. Can. J. For. Res. 3:196-208.

Christersson, L. 1978. The influence of photoperiod and temperature on the developmenet of frost hardiness in seedlings of Pinus sylvestris and Picea abies. Physiol. Plant. 44: 288-294.

D'Aoust, A. L. and S. I. Cameron. 1982. The effect of dormancy induction, low temperatures and moisture stress on cold hardiness of containerized black spruce seedlings. Proc. Can. Containerized Tree Symp. Glerum, C. and C. A. Plexman (eds.) p 153-161.

Doak, C. C. 1935. Evolution of foliar types, dwarf shoots, and cone scales of Pinus. Ill. Bio. Mono. 127-230.

Dickmann, D. I. and T. T. Kozlowski. 1970. Mobilization and incorporation of photoassimilated ^{14}C by growing vegetative and reproductive tissues of adult Pinus resinosa Ait. trees. Plant Physiol. 45: 284-288.

Dormling, I. 1973. Photoperiodic control of growth and growth cessation in Norway spruce seedlings. Proc. IUFRO symposium on dormancy in trees. Kornik, Sept. 5-9, 1973.

Dormling, I., A. Gustafsson, and D. von Wettstein. 1968. The experimental control of the life cycle in Picea abies (L.) Karst. I. Some basic experiments on the vegetative cycle. Silvae Genetica 17: 44-64.

Downs, R. J. 1962. Photocontrol of growth and dormancy in woody plants. Pp. 133-148 In Kozlowski, T. T. (ed.) Tree growth. Ronald Press. NY.

Downs, R. J. and H. J. Borthwick. 1956. Effects of photoperiod on growth of trees. Bot. Gaz. 117: 310-326.

Driessche van den, R. 1968. Growth analysis of four nursery-grown conifer species. Can. J. Bot. 46: 1389-1395.

Ericsson, A. 1984. Effects of low temperature and light treatment, following winter cold storage, on starch accumulation in Scots pine seedlings. Can J. For. Res. 14: 114-118.

Evans, G. C. 1972. The quantitative Analysis of Plant Growth. The University of California Press. Berkley and Los Angeles. 734 p.

Farrar, J. L. 1961. Induced variation in the pattern of shoot extension in five seed sources of Picea abies. Proc. 8th. Nteast. For. Tree. Imp. Conf. pp 14-20.

Fosket, D. E. and J. P. Miksche. 1966. A histochemical study of the seedling shoot apical meristem of Pinus lambertiana. Amer. J. Bot. 53(7): 694-702.

Foster, A. S. 1938. Structure and growth of the shoot apex in Ginko biloba. Bull. Tor. Club. 63: 531-556.

Fowler, D. P. 1961. The effect of photoperiod on white pine seedling growth. For. Chron. 37: 133-143.

Fraser, D. A. 1962. Apical and radial growth of white spruce (Picea glauca (Moench) Voss.) at Chalk River, Ontario, Canada. Can. J. Bot. 40: 659-668.

Fraser, D. A. 1966. Vegetative and reproductive growth of blask spruce (Picea mariana(Mill.) BSP.) at Chalk River, Ontario, Canada. Can. J. Bot. 44: 567-580.

Glerum, C. 1976. Frost hardiness in forest trees. In Cannell, M. G. R. and F. T. Last. (ed.) Tree Physiology and Yield Improvement. Academic Press London, New York.

Glerum, C. 1982. Frost hardiness and dormancy in conifers. Proc. Neastern. Area Nurserm. Conf. Halifax, Nova Scota. p 37-46.

Gregory, R. A. and J. A. Romberger. 1972a. The shoot apical ontogeny of the Picea abies seedling. I. anatomy, apical dome diameter and plaastochron duration. Amer. J. Bot. 59(6): 587-597.

Gregory, R. A. and J. A. Romberger. 1972b. The shoot apical ontogeny of the Picea abies seedling. II. growth rates. Amer. J. Bot. 59(6): 598-606.

Gregory, R. A. and J. A. Romberger. 1977. The shoot apical ontogeny of the Picea abies seedling. IV. Protoxylem initiation and the age of the internodes. Amer. J. Bot. 64(6): 631-634.

Grime, J. P. and R. Hunt. 1975. Relative growth-rate: Its range and adaptive significance in a local flora. J. Ecol. 63: 393-422.

Hanawa, J. 1966. Growth and development in the shoot apex of Pinus densiflora. I. growth periodicity of the terminal vegetative shoot apex. Bot. Mag. Tokyo. 79: 736-746.

Hanover, J. W. and R. C. Wilkinson. 1969. A new hybrid between blue spruce and white spruce. Can. J. Bot. 47: 1693-1700.

Hanover, J. W. and D. A. Reicosky. 1972. Accelerated growth for early testing of spruce seedlings. Forest Sci. 18(1): 92-94.

Hanover, J. W., Young, E., Lemmien, W. A., and M. Van Slooten. 1976. Accelerated-Optimal-Growth: A new concept in tree production. Michigan State University Ag. Exp. Sta. Res. Rep. 317. 16 p.

Harrison, D. L. S. and J. N. Owens. 1983. Bud development in Picea engelmannii. I. Vegetative bud development, differentiation, and the early development of reproductive buds. Can. J. Bot. 61: 2291-2301.

Heckman, J. W., Epstein, E. R. and J. W. Hanover. 1982. Developmental anatomy of nursery and greenhouse grown blue and Engelmann spruce needles. Proc. 7th North Amer. For. Biol. Wrkshp. (Abstract) p 432

Heide, O. M. 1974a. Growth and dormancy in Norway spruce ecotypes (Picea abies) I. Interaction of photoperiod and temperature. Physiol. Plant. 30: 1-12.

Heide, O. M. 1974b. Growth and dormancy in Norway spruce ecotypes (Picea abies) II. After effects of photoperiod and temperature on growth and developmetn in subsequent years. Physiol. Plant. 31: 131-139.

Hejnowicz, A. 1979. Tannin vacuoles and starch in the development of Scots pine (Pinus sylvestris) vegtetative buds. Acc. Soc. Bot. Pol. 48(2) 193-213.

Hulton, H. 1974. Containerization in Scandinavia. Proc. N. Amer. Cont. Forest Tree Seedl. Symp. p 20-28.

Hunt,R. 1982. Plant growth curves: the functional approach to plant growth analysis. 248 p. Edward Arnold (Publishers) Unlimited, London.

Jablansky, A. 1971. Changes due to age in apical development in spruce and fir. Can. For. Serv., Bi-mo. Res. Notes. 27(2): 10.

Janson, E., Jensen, P. and C. H. Bornman. 1983. Effect of the crown on uptake and transport in embryonic shoots of Picea abies. Physiol. Plant. 59: 521-527.

Jarvis, P. G. and M. S. Jarvis. 1964. Growth rates of woody plants. Physiol. Plant. 17: 654-666.

Johansen, D. A. 1940. Plant Microtechnique. McGraw-Hill, N.Y.

Kasperbauer, M. J. and J. L. Hamilton. 1984. Chloroplast structure and starch grain accumulation in leaves that received different red and far-red levels during devvelopment. Plant Physiol. 74: 967-970.

Kimball, J. W. 1969. Biology 2nd. ed. Addison-Wesley, San Francisco.

Kramer, P. J. and T. T. Kozlowski. 1979. Physiology of Woody Plants. Academic Press, N.Y. 811p.

Kremer, A. 1984. Distribution of relative growth rates and variation of cytohistological zonation in apical meristems of seedlings of two contrasting open pollenated jack pine (Pinus banksiana) families. Can. J. For. Res. 14: 297-310.

Lanner, R. M. 1976. Patterns of shoot development in pines. In Cannell, M. G. R. and F. T. Last. (ed.) Tree Physiology and Yield Improvement. Academic Press London, New York, San Francisco.

Lavender, D. P. 1980. Effects of the environment upon the shoot growth of woody plants. In Little, C. H. A. (ed.) Control of Shoot Growth of Trees Proc. IUFRO Joint Wrkshp. Fredericton N.B.

Ledig, F. T. 1974. Concepts of growth analysis. In Reid, C. C. P. and G. H. Fechnner (eds.). Proc. 3rd. North Amer. For. Biol. Wrkshp. p. 166-182.

Ledig, F. T. 1976. Physiological genetics, photosynthesis, and growth models. In Cannell, M. G. R. and F. T. Last. (ed.) Tree Physiology and Yield Improvement. Academic Press London, New York, San Francisco.

Levitt, T. 1980. Response of Plants to Environmental Stresses. Avademic Press. N.Y.

Lewis, F. J. and E. S. Dowding. 1924. The anatomy of the buds of coniferae. Ann. Bot. 38: 217-228.

Lidforss, B. 1896. Zur physiologie und biologie dur wintergrunen flora.In Steponkus, P. L. 1984. Role of the plasma membrane in freezing injury and cold acclimation. Ann. Rev. Plant Physiol. 35: 543-584.

Lidforss, B. 1907. Die wintergrunen flora.In Steponkus, P. L. 1984. Role of the plasma membrane in freezing injury and cold acclimation. Ann. Rev. Plant Physiol. 35: 543-584.

Little, C. H. A. 1974. Relationship between the starch level at budbreak and current shoot growth in Abies balsamea L. Caan. J. For. Res. 4: 268-273.

Loach, K. and C. H. A. Little. 1973. Production, storage, and use of photosynthate during shoot elongationin balsam fir (Abies balsamea). Can. J. Bot. 51: 1161-1168.

Logan, K. T. 1977. Photoperiodic induction of free growth in juvinile white spruce and black spruce. Can. For. Ser. Bi-mon. Res. Notes. 33: 29-30.

Malcolm, D. C. and C. F. Pymer. 1975. The influence of temperature on the cessation of height growth of Sitka spruce (Picea sitchensis (Bong.) Carr.) provenances. Silvae Genetica. 24: 5-6.

Martin, J. B. and G. Oquist. 1979. Seasonal and experimentally induced changes in the ultra structure of chloroplasts of Pinus sylvestris. Physiol. Plant. 46: 42-49.

Marquard, R. D. 1983. Floral enhancement of the Picea genus through hormonal and cultural treatments. Ph. D. Dissertation. Michigan State University. 121 p.

Mauseth, J. D. 1979. Cytokinin-elicited formation of the pith-rib meristem and other effects of growth regulators on morphogenesis of Echinocereus (Cactaceae) seedling shoot apical meristem. Amer. J. Bot. 66: 446-451.

Mauseth, J. D. and K. J. Niklas. 1979. Constancy of relative volumes in shoot apical meristems in cactaceae: implications concerning meristem size, shape, and metabolism. Amer. J. Bot. 68(8): 933-939.

Mia, A. J. and D. J. Durzan. 1974. Cytochemical and subcellular organization of the shoot apicalmeristem of dry and germinating jcak pine embryos. Can. J. For. Res. 4: 39-54.

Nienstaedt, H. 1966. Dormancy and dormancy release in white spruce. For. Sci. 12(3): 374-384.

Nienstaedt, H. 1967. Chilling requirements for seven Picea species. Silvae Genetica. 16: 65-68.

Oden, P-C. and A. Dunberg. 1984. Abscisic acid in shoots and roots of Scots Pine (Pinus sylvestris) seedlings grown under long day and short day environments. Planta. 161: 148-155.

Owens, J. N. 1984. Bud development in grand fir (Abies grandis). Can. J. For. Res. 14: 575-588.

Owens, J. N. and M. Molder. 1973a. A study of DNA and mitotic activity in the vegetative apex of Douglas-fir during the annual cycle of growth. Can. J. Bot 51: 1395-1409.

Owens, J. N. and M. Molder. 1973b. Bud development in western hemlock. I. Annual growth cycle of vegetative buds. Can. J. Bot 51: 2223-2231.
vegetative buds and shoot elongation as they relate to date and temperature sums. Can. J. Bot. 55: 2728-22453.

Owens, J. N. and M. Molder. 1976. Bud development in Sitka spruce. I. Annual growth cycle of vegetative buds and shoots. Can. J. Bot. 54: 313-325.

Owens, J. N., Molder, M. and H. Langer. 1977. Bud development in Picea glauca. I. Annual growth cycle of vegetative buds and shoot elongation as they relate to date and temperature sums. Can. J. Bot. 55: 2728-2245.

Owens, J. N. and H. Singh. 1982. Vegatative bud development and the time and method of cone intiation in subalpine fir (Abies lasiocarpa). Can. J.Bot. 60: 2249-2262.

Oweston, P. 1961. The shoot apex in eastern white pine: its structure, seasonal development and variation within the crown. Can. J. Bot. 47: 1181-1188.

Parke, R. V. 1959. Growth periodicity and the shoot tip of Abies concolor. Amer. J. Bot. 46: 110-118.

Pillia, S. K. and B. Chacko. 1978. Growth periodicity and structure of the shoot apex of Picea smithiana (Wall.) Boiss.): An anatomical and histochemical study. Flora, Bd. 167. S. 528-543.

Pollard, D. F. W. 1973a. Provanance variation in phenology of needle initiation in white spruce. Can. J. For. Res. 3: 589-593.

Pollard, D. F. W. 1974a. Bud morphogenesis of white spruce in a uniform environment. Can. J. Bot. 52: 1569-1571.

Pollard, D. F. W. 1974b. Effect of age of white spruce seedlings on dormancy inducing treatment. Can. For. Ser. Bi-mon. Res. Notes. 29: 27.

Pollard, D. F. W. and K. T. Logan. 1974a. The role of free growth in the differentiation of provenances of black spruce (Picea mariana (Mill.) B.S.P.). Can. J. For. Res. 4: 308-311.

Pollard, D. F. W. and K. T. Logan. 1974b. Seedling size and age as factors of morphogenesis in white spruce (Picea glauca (Moench.) Voss.) Buds. Can. J. For. Res. 4: 97-100.

Pollard, D. F. W., Teich, A. H., and K. T. Logan. 1975. Seedling shoot and bud development in Provanances of Sitka spruce (Picea stichensis (Bong.) Carr). Can. J. For. Res. 5: 18-25.

Pollard, D. F. W. and K. T. Logan. 1976. Inherent variation in "free" growth in relation to numbers of needles produced by provanances of Picea mariana. In Cannell, M. G. R. and F. T. Last. (ed.) Tree Physiology and Yield Improvement. Academic Press London, New York, San Francisco.

Pollard, D. F. W. and K. T. Logan. 1977. The effects of light intensity, photoperiod, soil moisture potential and temperature on bud morphogenesis in Picea species. Can. J. For. Res. 7: 415-421.

Pollard, D. F. W. and K. T. Logan. 1979. The response of bud morphogenesis in black spruce and white spruce provenances to environmental variables. Can. J. For. Res. 9: 211-217.

Pollard, D. F. W. and C. C. Ying. 1979. Variation in response to declining photoperiod among families and stands of white spruce in southeastern Ontario. Can. J. For. Res. 9: 443-448.

Powell, G. R. 1982. A comparison of early shoot development of seedlings of some trees commonly raised in the northeast of North America. pp. 1-24. Proc. Nteast. Area. Nur. Conf. Halifax, Nova Scotia. July 25-29, 1982.

Reese, K. H. and J. B. Scarratt. 1982. Canadian containerized tree seedling symposium. COJFRC Symp. Proc. o-p-10. 460 p.

Rehder, A. 1940. Manual of cultivated trees and shrubs. McMillin, Co. N.Y.

Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17: 208-212.

Riding, R. T. 1978. Within-crown variation in dormant long-shoot apicies of mature trees of Pinus resinosa. Can. J. Bot. 56: 141-147.

Riding, R. T. 1972. Early ontogeny of seedlings of Pinus radiata Can. J. Bot. 50: 2381-2387.

Riding, R. T. and E. M. Gifford, Jr. 1973. Histochemical changes occuring at the seedling shoot apex of Pinus radiata. Can. J. Bot. 51: 501-512.

Robak, H. and S. Magnesen. 1970. Contribution to the knowledge of the ecology of growth termination in spruce seedlings of Norwegian and Central-European provenances. Silvae. Genetica. 19: 188-190

Romberger, J. A. 1963. Meristems, growth and development in woody plants. U.S.D.A./F.S. Tech. Bull. 1293. 214 p.

Ronco, F. 1970. Influence of high light intensity on survival of planted Engelmann spruce. For. Sci. 16: 331-339.

Sass, J. E. 1958. Botanical Microtechnique, 3rd. Iowa State College Press, Ames.

Sokal, R. R. and F. J. Rohlf. 1981. Biometry, 2nd ed. Freeman. San Francisco. 859 pp.

Song, P-S. 1984. Phytochrome. In Advanced Plant Physiology. Wilkins, M. B. (ed.) Pittman Publishing Limited. London.

Sorenson, F. C. 1983. Relationship between logarithms of chilling period and germination or budflush rates is linear for many tree species. For. Sci. 29(2): 237-240.

Spurr, A. R. 1969. A low-viscoscity epoxy resin embedding medium for electron microscopy. J. Ultrastruc. Res. 26: 31-43.

Stempack, J. C. and R. T. Ward. 1964. An improved staining method for electron microscopy. J. Cell Biol. 22: 697-701.

Steponkus, P. L. 1984. Role of the plasma membrane in freezing injury and cold acclimation. Ann. Rev. Plant. Physiol. 35: 543-584.

Sterling, C. 1946. Organization of the shoot of Pseudotusga taxifolia (Lamb.)Britt. I. Structure of the shoot apex. Amer. J. Bot. 33: 742-750.

Sutton, R. F. and R. W. Tinus. 1983. Root and root system terminology. For. Sci. Monogr. No. 24. (Supplement to For. Sci., Vol. 24(4).

Sweet, G. B. and P. F. Wareing. 1968. A comparison of the rates of growtrh and photosynthesis in first-year seedlings of four provenances of Pinus contorta Dougl. Ann. Bot. 32: 735-751.

Tepper, H. B. 1963. Dimensional and Zonational variation in dormant shoot apices of Pinus ponderosa. Amer. J. Bot. 50(6): 589-596.

Tepper, H. B. 1964. Ontogeny of the shoot apex of seedlings of Pinus ponderosa. Amer. J. Bot. 51(8); 859-865.

Thompsett, P. B. 1978. Studies of the growth and flowering in Picea sitchensis (Bong.) Carr.) 2. Initiation and development of male, female, and vegetative buds. Ann. Bot. 42: 889-900.

Tinus, R. W. and S. E. McDonald. 1979. How to grow tree seedlings in containers in greenhouses. U.S.D.A./F. S. Gen. Tech. Rep. RM-60.

Vaartaja, O. 1959. Evidence of photoperiodic ecotypes in trees. Ecolog. Monogr. 29(2): 91-111.

Vanden Born, W. H., 1963. Histochemical studies of enzyme distribution in shoot tips of white spruce (Picea glauca) buds. Can. J. Bot. 41: 1509-1527.

Watt, R. F. and W. H. D. McGregor. 1963. Growth of four northern conifers under long and natural photoperiods in Florida and Wisconson. For. Sci. 9(1): 115-128.

Wareing, P. F. 1956. Photoperiodism in woody plants. Ann. Rev. Plant Physiol. 6: 191-214.

Weiser, C. J. 1970. Cold resistance and injury in woody plants. Science. 169: 1269-1278.

Willmott, C. J. 1982. Some comments on the evaluationof model performance. Bull. Amer. Meterol. Soc. 63(11): 1304-1313.

Wheeler, N. 1979. Effect of continuous photoperiod on growth and development of lodgepole pine seedlings and grafts. Can. J. For. Res. 9: 276-283.

Young, E. and J. W.Hanover. 1976. Accelerating maturity in Picea seedlings. Acta. Hort. 56: 105-114.

Young, E. and J. W. Hanover. 1977a. Effects of quality, intensity, and duration of light breaks during a long night on dormancy in blue spruce (Picea pungens Engelm.). Plant Physiol. 60: 271-273.

Young, E. and J. W. Hanover. 1977b. Development of the shoot apex of blue spruce (Picea pungens). Can. J. For. Res. 7: 614-620.

Young, E. and J. W. Hanover. 1978. Effects of temperature, nutrient, and moisture stresses on dormancy of blue spruce seedlings under continuous light. Forest Sci. 24(4): 458-467.