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Ecological Genetics of Pinus contorta from the Rocky Mountains (USA): a Synthesis

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Abstract

Studies of population differentiation in *Pinus contorta*, which were conducted in common gardens, are summarized according to the growth, freezing tolerance, and the periodicity of shoot elongation of seedlings from 173 Rocky Mountain (USA) populations. Regression models accounted for 45 to 77% of the variance among populations, coordinated the results of previous studies and described clinal patterns of variation that generally reflect elevational and geographic gradients in the frost-free period.

Key words: Population differentiation, genecology, adaptive variation.

Zusammenfassung

Studien über Differenzierungen bei *Pinus contorta*-Populationen, die in öffentlichen Parks durchgeführt wurden, werden zusammenfassend dargestellt. Es wurde das Wachstum, die Frosttoleranz und die Periodizität der Sproßstreckung an Sämlingen von 173 Rocky Mountain (USA) Populationen untersucht. Regressionsmodelle erklärten 45 bis 77% der Variation zwischen Populationen, koordinierten die Resultate vorausgegangener Studien und beschrieben klinale Variationsmuster, die allgemein geographische und Höhengradienten der frostfreien Periode reflektierten.

Introduction

Population differentiation in *Pinus contorta* var. *latifolia* from the Rocky Mountains (USA) occurs along clines that parallel gradients in the frost-free period (Rehfeldt, 1983; 1985a, b, c; 1987). As a result, clines are elevationally steep and geographically gentle. The clines also involve a coadaptive complex of traits, many of which are components of an annual sequence of developmental events that begins with shoot elongation in the spring and culminates with cold acclimation in the fall. Adaptation to spatially heterogeneous environments thus reflects a balance between selection for a suite of traits that provide a high growth potential in mild environments and selection for a suite that provide high tolerance to early autumn frosts in severe environments.

Both the development and perpetuation of the clines are strongly influenced by the ecology and demography of the species. In the Rockies, *P. contorta* is a seral species that occupies almost any forest habitat (Pfister and Daubenmire, 1973). The species is distributed across more than 2,000 m of elevation and thus occupies extremely heterogeneous environments that differ, for example, by 100 frost-free days (*Fig. 1*). Even-aged natural populations commonly develop under repetitive cycles (Lotan *et al.*, 1984) that include: (1) wildfire, (2) profuse reproduction of as many as a half million seedlings/ha from either serotious or open cones, (3) intense natural thinning to less than 1,000 trees/ha, (4) epidemics of the mountain pine beetle, which supplement competitive mortality to provide the fuel for (5) wildfire. As a result, conditions are provided that are ideally

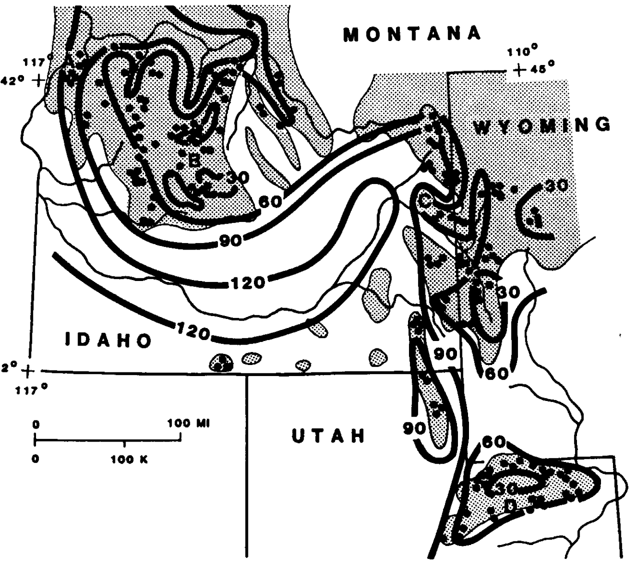

Figure 1. — Region of study showing the distribution (shading) of *P. contorta* (from Little, 1971), location of populations (dots), and average frost-free period (from U. S. Department of Commerce, 1968). Localities A to D position the elevational clines of *Figure 2*.

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suited to the development of pronounced clines in adaptive traits: intense juvenile selection, exposure to environmental gradients that vary continuously with elevation and geography, and establishment of populations on the sites on which their ancestors grew.

This note synthesizes common garden studies of 173 seedling populations (*Fig. 1*) representing the geographic and ecologic distribution of the species in three geographic areas: central Idaho (Rehfeldt, 1985a), eastern Idaho and western Wyoming (Rehfeldt, 1985b), and Utah (rehfeldt, 1985c). Results of all studies are combined to describe adaptive variation for the entire region.

Procedures

For each of the three geographic regions, seedling populations had been compared in separate tests of (1) growth and development of 3-year-old trees in field environments at both 900 and 1,500 m elevation on the Priest River Experimental Forest in northern Idaho, (2) periodicity of shoot elongation of 2-year-old trees in a greenhouse at Moscow, Idaho, and (3) freezing tolerance conducted in early autumn at the Moscow laboratory. Study 1, Utah, tested seedlings representing 45 localities; study 2, central Idaho, tested 89 populations; and study 3, eastern Idaho, tested 60 populations. Studies 1 and 2 had two populations in common; studies 2 and 3 had four in common; and studies 1 and 3 had eight in common. Of the total populations tested, 173 were from the region of study (*Fig. 1*).

The original studies detected differences among populations for numerous intercorrelated variables, five of which are used here to describe adaptive variation across the entire region: 3-year height and leaf length in the field, the rate of elongation and time of shoot growth cessation in the greenhouse, and freezing injury in the laboratory. In all tests, interactions of genotype and environment for these five variables were due to scale effects.

The three studies, however, not only involved a different set and number of populations but also were completed in different years. Consequently, transformation and scaling were necessary to obtain a data set within which the performance of a population tested in one study was directly comparable with the performance of populations tested in other studies.

Table 1. — Derivation of scaling factors (SF), the difference in mean performance of populations common between studies.

	Variable				
	Cessation of elongation	Rate of elongation	Freezing injury	Height	Leaf length
	- - - - - - - - - Standard	deviates	- - - - - - - - -		
Scaling Factor 1[a]					
Study 3	0.05	-0.15	0.06	-0.40	-0.06
Study 1	0.08	0.31	-0.06	0.43	-0.49
SF1	-0.03	-0.46	0.12	-0.83	0.43
Scaling Factor 2[b]					
Study 3	-0.32	-0.21	0.09	-0.97	-0.26
Study 2	-0.18	-0.07	-0.05	-0.18	0.11
SF2	-0.14	-0.14	0.14	-0.79	-0.37

[a] Based on 8 populations represented in studies 1 and 3.

[b] Based on 4 populations represented in studies 2 and 3.

Table 2. — Scaled values for two populations that were common to studies 1 and 2 and thereby could be scaled to study 3 by means of different scaling factors (SF). The difference in scaled values relative to the standard error of the mean ($s_{\bar{x}}$) reflects scaling effectiveness.

	Variable				
	Cessation of elongation	Rate of elongation	Freezing injury	Height	Leaf length
	- - - - - - - - - Standard	deviates	- - - - - - - - -		
Mean in study 1 + SF1	-0.20	-0.23	0.07	-1.03	-0.19
Mean in study 2 + SF2	-0.36	-0.12	0.31	-0.78	-0.10
$s_{\bar{x}}$	0.18	0.25	0.19	0.24	0.31

In order to eliminate the effect of different testing environments data wihin each sudy were transformed to standard normal deviates:

$$Z_{ijk} = (X_{ijk} - \bar{x}_j)/\sigma_j$$

where Z_{ijk} is a standard deviate for seedling k from population i tested in study j; X, $\bar{x}$ and σ are an original observation, a mean, and a standard deviation.

Next, population means ($\bar{Z}_{ij}$) were scaled to those from study 3 according to the differential performance of populations common to the various studies. Thus, a mean standard deviate ($\bar{Z}_i^{\prime}$) was defined such that:

Table 3. — Independent variables screened by stepwise regression and coefficients for those variables represented in the best-fitting models. EL = elevation, LT = latitude, LN = longitude, NW = northwest departure, SW = souhwest departure, EI =eastern Idaho, CI = central Idaho.

Independent variable	Cessation of elongation	Rate of elongation	Freezing injury	Height	Leaf length
Intercept	-398.4541	-271.1376	1.1780	2628.6660	2.2255
EL	-0.0013	-0.0011	-0.0009	-0.0021	-0.0014
$(EL)^2$					
LT		13.0908			
LT in EI		2.1648			-1.1321
LT in CI	1.5763			12.9027	
LN	7.2170			-46.2287	
LN in EI					
LN in CI				-1.3679	
NW	-0.0852			0.1439	0.0243
NW in EI	0.1142		0.1085		0.1981
NW in CI		-0.1093		0.3507	-0.0402
SW			-0.0062		-0.0339
SW in EI		0.0649			
SW in CI		0.1136		0.1989	0.0638
$(LT)^2$		-0.1564			
$(LT \text{ in } EI)^2$	-0.0030		-1.3126	0.0023	0.0216
$(LT \text{ in } CI)^2$	-0.0375	-0.0482		-0.2068	
$(LN)^2$	-0.0320			0.2031	
$(LN \text{ in } EI)^2$			0.0040		
$(LN \text{ in } CI)^2$					
$(NW)^2$	0.0004		0.0001	-0.0007	
$(NW \text{ in } EI)^2$	-0.0006	-0.0001	-0.0007	-0.0004	-0.0010
$(NW \text{ in } CI)^2$		0.0003		0.0007	0.0004
$(SW)^2$				-0.0011	
$(SW \text{ in } EI)^2$		-0.0006	0.0011		
$(SW \text{ in } CI)^2$	0.0011		0.0001		

(1) if j = 3 (eastern Idaho), then $\bar{Z}_i^{\prime} = \bar{Z}_{i3}$

(2) if j = 2 (central Idaho), then $\bar{Z}_i^{\prime} = \bar{Z}_{i2} + SF2$

(3) if j = 1 (Utah), then $\bar{Z}_i^{\prime} = \bar{Z}_{i1} + SF1$

where the scaling factor, SF2, is the average difference between the test 2 and the test 3 performance of four populations common to both tests (*Table 1*); SF1 is the difference between the test 1 and test 3 performance of eight populations common to both tests.

Because studies 1 and 2 were scaled to study 3 independently of each other, the performance of the two populations tested in both studies 1 and 2 can be used to judge the effectiveness of scaling. In *Table 2*, the mean performance of these two populations tested in study 1 and scaled by SF1 is compared with their performance in sudy 2 and scaled by SF2. For all but freezing injury, the difference between alternative scalings is either less than or essentially equal to the standard error of the mean ($s_{\bar{x}}$). This suggests that bias introduced by scaling lies well within the tolerances associated with sampling and experimental errors.

Scaled values of the five variables were then used as dependent variables in multiple regression analyses, the objectives of which were to describe elevational and geographic patterns of genetic variation rather than to test causal effects of individual environmental factors. Consequently, stepwise regression models for maximizing R^2 (SAS, 1982) were used to screen 26 independent variables describing the location of the seed source: elevation, latitude, longitude, northwest departure, southwest departure and their squares (*Table 3*). Northwest and southwest departures were obtained by rotating the grid of latitude and longitude by 45°. To accommodate the possibility of contrasting regional patterns, independent variables were nested within the three geographic regions. The three disjunct populations from south central Idaho (*Fig. 1*) were not assigned to a geographic region. Elevation was considered without geographic interaction because preliminary analyses showed that only for the rate of elongation did the slope of the regressions vary significantly between geographic regions (*Fig. 2*).

The stepwise regression was of the general form:

$$Y_i = \beta_0 + \beta_1 E_i + \beta_2 E_i^2 + \sum_{j,k} \gamma_{jk} X_{ijk} + \sum_{j,k} \delta_{jk} X^2_{ijk}$$

where Y_i is the performance of population i; E_i is the elevation of population i; X_{ijk} is geographic variable k for

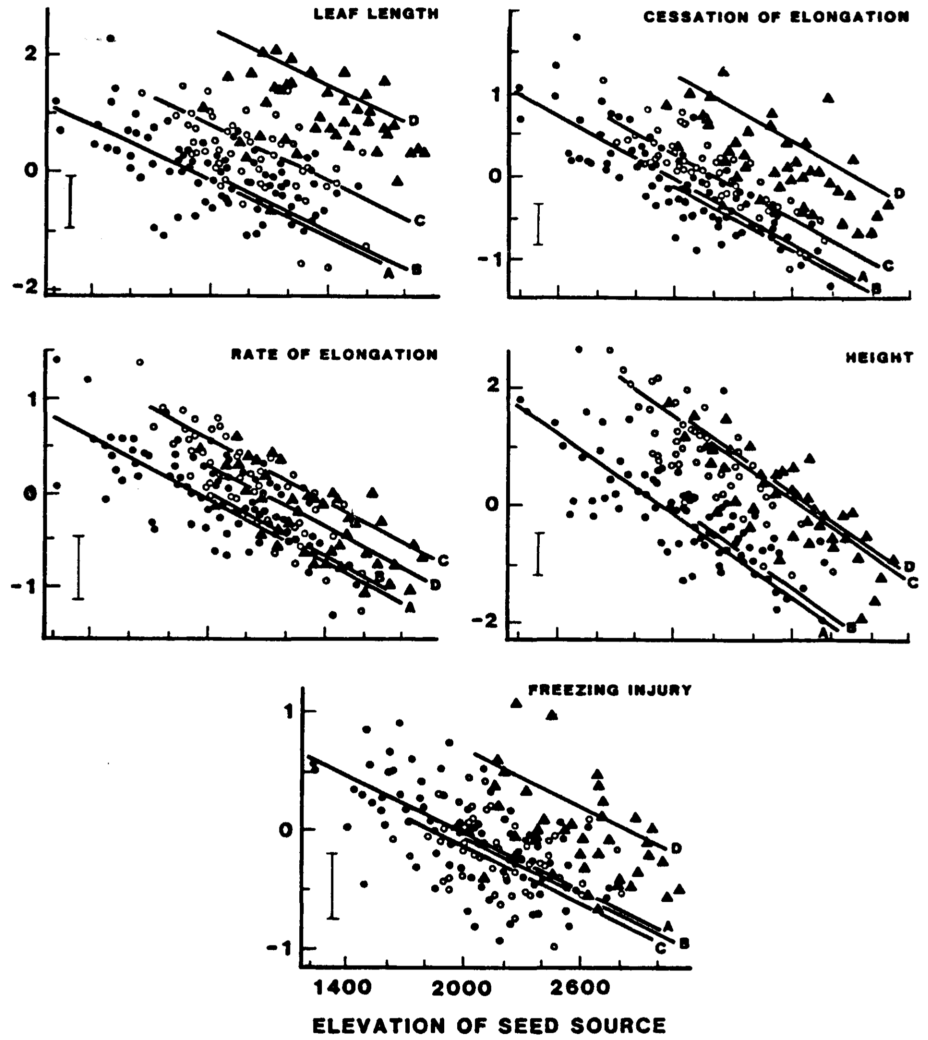

Figure 2. — Mean performance of populations plotted by elevation of the seed source. Brackets quantify *lsd* (0.05); ● = central Idaho, ○ = eastern Idaho and adjacent Wyoming, ▲ = Utah. Localities A to D are keyed to *Figure 1*. A, C and D represent populations of the highest growth potential for each region; B is a population of low growth potential (see *Fig. 3*).

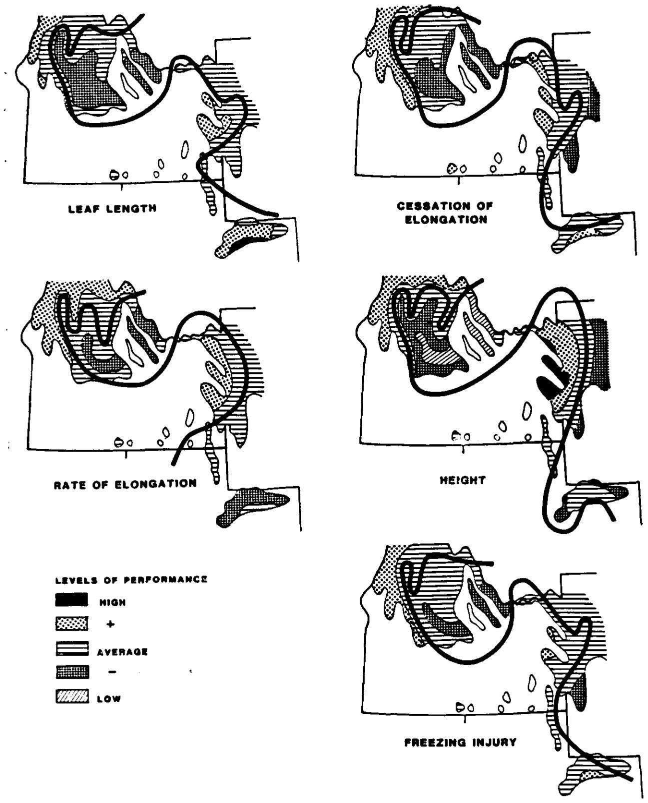

Figure 3. — Geographic patterns of variation predicted for populations located at base elevations, the lowest elevations supporting *P. contorta*. Intervals between bands of equal performance (shadings) are scaled to a value of *lsd* (0.05) and are plotted with reference to the mean of all populations (heavy, unbroken isopleth) from which contouring was begun.

population i in geographic region j; and β_0, β_1, β_2, γ_{ik}, and δ_{jk} are regression coefficients, $j = 1 \ldots 4$, $k = 1 \ldots 4$. The best stepwise model was defined as that which was statistically, significant, produced the lowest residual mean square, and exhibited no relationship between residuals and independent variables (Draper and Smith, 1981).

Results and Discussion

The best fitting regression models accounted for 69, 66, 43, 77, and 58% of the variance between populations for cessation of shoot growth, rate of shoot elongation, freezing injury, 3-year height, and leaf length, respectively. However, these models used 9 to 16 independent variables to describe genetic variation (*Table 3*) and are, therefore, subject to overfitting, the fitting of independent variables to individual populations rather than to the group as a whole (Draper and Smith, 1982). For this reason, differentiation along both the elevational (*Fig. 2*) and geographic (*Fig. 3*) clines is evaluated relative to the value of the least significant difference (Steel and Torrie, 1960) at the 0.05 level of probability — *lsd* (0.05). Values of *lsd*, calculated from analyses of variance, are quantified in *Figure 2* and represent the intervals between isopleths in *Figure 3*. Thus, populations separated by a distance equaling the geographic interval between isopleths (*Fig. 3*) or by an elevational interval that subtends a mean difference greater than *lsd* (*Fig. 2*), are expected to differ with a probability of about 0.95.

Regression models described elevational clines (*Fig. 2*) that are typical for montane populations of *P. contorta*. Populations from low elevations have a high growth potential that is expressed in mild environments by long leaves, a tall stature, a rapid rate of shoot elongation and a late cessation of elongation; but in severe environments, these same populations are susceptible to injury from early fall frosts. By contrast, populations from high elevations have a low growth potential and high cold hardiness. Elevational clines are so steep that populations separated by as little as 300 m of elevation within the same geographic region are expected to differ genetically (95% level). Because the frost-free period declines by about 80 days across an elevational interval of 1,000 m, (Baker, 1944) detectable genetic differentiation is associated with an environmental difference of about 24 frost-free days.

Mean values predicted for base elevations (*Fig. 3*), the lowest elevation at which the species occurs in individual drainages, follows a pattern that unmistakably parallels that of the frost-free season (*Fig. 1*). In fact, mean values for all populations (heavy lines of *Fig. 3*) tend to trace the path of the 60-day isopleth. These geographic clines further illustrate that populations from the mildest environments are of highest growth potential but are least tolerant of early fall frosts.

Because a given frost-free period is associated with a different elevation in separated localities, the geographic and elevational clines are interdependent. In fact, much of the geographic variation in *Figure 3* actually reflects the elevational cline. The pronounced genetic differences (*Fig. 3*) that occur between base populations at localities A and B (*Fig. 1*) actually are due to the elevational cline (*Fig. 2*). Indeed, a single, long elevational cline of 2,000 m in central Idaho encompasses all of the genetic variation within the entire region (*Fig. 2*) for all traits except leaf length.

Interdependence of the two clines also means that similar genotypes recur at different elevations in separated localities. On the average, a given level of performance in populations from central Idaho will recur in populations from eastern Idaho at an elevation that is 500 m higher and recur in populations from Utah at an elevation approximately 1,000 m higher (*Fig. 2*). Thus, for a constant elevation, the amount of geographic variation in all variables except leaf length is equivalent to that which occurs within 1,000 m of elevation at a single locality (*Fig. 2*). The long leaves of populations from Utah are remarkably inconsistent with general patterns for other variables.

Microevolution in *P. contorta* has produced populations that are physiologically specialized for particular segments of the environmental gradient. Genetic variation is closely associated with the frost-free period. Clines tend to be steep elevationally but relatively gentle geographically. The clines, however, are interdependent; similar genotypes recur at different elevations in separated localities. The existence of numerous populations each of which is specialized thus accounts for the large geographic and broad ecologic distribution of the species (Rehfeldt, 1987). Perpetuation of the specialized populations occurs readily in response to cycles of even-aged reproduction that are established on sites that supported their progenitors. Nevertheless, genetic variability within populations is pronounced (Rehfeldt, 1985d; Fries and Lindgren, 1986), a probable response to either or both long distance pollen migration and temporal variation in selection pressures.

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Promotion of Flowering in Black Spruce using Gibberellins

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Abstract

Enhanced flowering of 20 year old black spruce (*Picea mariana* MILL. B. S. P.) of seedling origin was observed after spraying with a gibberellin A4/7 (GA) mixture at concentrations of 200 and 800 mg/l. Addition of Naphthaleneacetic acid (NAA) and GA_9 to the mixture had little effect. A slight reduction in seed quality or quantity per cone was more than compensated for by the increased number of cones per tree.

Key words: Black spruce, Gibberellin, A4/7, A_9, NAA, seed yield.

Zusammenfassung

Bei 20 Jahre alten *Picea mariana* MILL. (B. S. P.)-Sämlingen, wurde nach dem Besprühen mit einer Gibberellin-Säuremischung GA 4/7 in Konzentrationen von 200 bis 800 mg/l ein verstärktes Blühen beobachtet. Die Zugabe von Naphtyl-Essigsäure (NAA) und GA_9 zu der Mischung hatte nur einen geringen Effekt. Eine leichte Abnahme der Samenqualität oder -quantität pro Zapfen wurde durch die erhöhte Zapfenanzahl je Baum mehr als kompensiert.

Introduction

Genetically improved seed from seed orchards of black spruce is in great demand for reforestation purposes but despite high levels of investment; flowering levels are low or variable in quantity. Failure of some parent clones to flower also reduces genetic diversity of the seed and erratic flowering causes delays and interruptions in research and reforestation programs. If early and consistent flowering could be assured then the efficiency of seed production programs would be increased.

DUNBERG (1974) found that clones of Norway spruce *Picea abies* (L.) KARST. that flowered had higher endogenous levels of GA - like substances in meristematic tissue at the time of flower bud initiation/differentiation than clones producing few or no flowers. In many *Pinaceae* species, including the spruces, enhanced flowering has resulted after treatment with gibberellins primarily mixtures of GA_4 and GA_7 (PHARIS and ROSS 1986a, 1986b; PHARIS *et al.*, 1986).

Attempts at promotion of flowering in the *Pinaceae* have centered on the commercially valuable conifers particularly Douglas-fir *Pseudotsuga menziesii* (MIRB.) FRANCO, Scots pine (*P. sylvestris* L.) Norway spruce and the southeastern hard pine group (PHARIS and ROSS, 1984). Studies have also been conducted on boreal species, jack pine (*P. banksiana* LAMB.) (CECICH 1981, 1983) and white spruce *P. glauca* (MOENCH) VOSS (MARQUARD and HANOVER, 1984a, 1984b, 1985; HARE, 1984; CECICH, 1985). The other major boreal conifer, black spruce has received little attention. Since it is the major reforestation species in Newfoundland and tree improvement programs concentrate on this species (HALL, 1981), a study on the promotion of flowering was initiated in black spruce in 1982. The objective was to determine if flowering in black spruce could be enhanced by the foliar application of GA4/7, GA_9 and the auxin, (NAA).

The most frequently used GA in Pinaceae flowering studies has been the GA4/7 mixture, applied as a foliar spray or injected into the pith or cambium (PHARIS and ROSS, 1986b). The effects of GA4/7 have often been enhanced by its combination with GA_9 and/or NAA (DUNBERG, 1980; PHARIS and ROSS, 1986a). The concentration of growth hormone can also affect its efficacy. A direct relationship between concentration and flowering level was reported in jack pine (CECICH, 1983) white spruce (MARQUARD and HANOVER, 1984a, 1984b), and Sitka spruce (*P. sitchensis* (BONG.) CARR.) PHILIPSON 1985, Douglas-fir (ROSS, 1983) and western hemlock (*Tsuga heterophylla* (RAF.) SARG.) ROSS *et al.* 1981.

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