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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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The time of application is also important and is usually related to the stage of vegetative shoot development in the year of initiation/differentation of the flower bud. It has been suggested that the GA's be applied on the early developing shoots in Norway spruce (Luukkanen, 1980); early to late in shoot development in loblolly pine (*Pinus taeda* L.) (Schmidtling, 1983) and two weeks before cessation of shoot growth in white spruce (Marquard and Hanover, 1984b). The expression of sex of flower buds can also be influenced by the timing of the application of the GA4/7; application in May-June enhanced male flowering while application in July-August enhanced female flowering (Chalupka, 1984; Marquard and Hanover, 1984b).

Methods

The study trees were part of a natural black spruce stand which originated after wildfire in 1961. The selected trees had not previously produced female flowers but may have produced male flowers. The trees averaged 3m in height.

The following hormonal treatments and concentrations were tested:

1. Control - 60% ethanol
2. GA4/7 @ 200 mg/l
3. GA4/7 @ 800 mg/l
4. GA4/7 @ 200 mg/l + GA_9 @ 10 mg/l
5. GA4/7 @ 200 mg/l + NAA @ 100 mg/l

The growth regulators were dissolved in absolute ethanol and stock solutions were made up in 60% ethanol. Then 1 ml/l of the surfactant "Aromox C12W"[1]) (0.1% active ingredient) was added to the ethanolic solution. The substances were applied as a foliar spray, with each tree receiving about 400 ml of solution.

The treatments were applied during two stages of shoot development; when elongation about half complete (7 July) and 95% complete (4 August). Each of the five treatments was applied to four randomly selected trees on each date for a total of 40 trees. The statistical design was a randomized complete block (Steel and Torrie, 1960).

Height of the sample trees was measured before and after treatment. A direct relationship between height and flowering has been reported in black spruce (Simpson and Powell, 1981). Numbers of male and female flowers were counted on each tree the next year (June 1983). Cones were collected in October 1983 to assess effects of treatments on seed quantity and quality.

[1]) Aromox C12W supplied courtesy of Armak Chemicals Limited, 100 University Avenue, Toronto, Ontario, Canada.

Results and Discussion

The GA4/7 treated trees produced many more flowers than the controls (*Table 1*). More flowers were produced from the July treatments than the August ones and more males were produced than females. The difference between treatment dates were statistically significant but differences among treatments were not. This seemed to be caused by the large tree-to-tree variation in response levels; a feature also reported in Norway spruce (Dunberg, 1980). The major result — that of promotion of flowering after treatment with GA4/7 — has also been reported to occur in 18 other conifers (Pharis and Ross, 1986a, 1986b).

The later spraying (August) did not promote female flowering which indicates that the optimal time for promotion of female flowers is earlier than for males. Similar, if unusual, results have been reported in white spruce (Marquard and Hanover, 1984b).

Comparing the various treatments, female flowering was promoted only by GA4/7 and there was no response attributable to concentrations. Philipson (1985) also found no dosage-related response in Sitka spruce, but dose-related flowering responses have been reported by Tompsett *et al.* (1980). The reported synergistic effects of GA_9 and/or NAA (Dunberg, 1980; Pharis and Ross, 1984) did not occur in black spruce, however the GA_9 level used was very low and this may be the reason no response was observed. Male flowering was increased by GA4/7 at the higher concentration and when NAA was used. It is possible that the GA4/7 triggers female flowering at a low concentration and male flowering at a higher concentration. A similar situation has been reported to occur in Douglas-fir (Pharis *et al.*, 1980). If this differential response can be repeated then it could be exploited in a breeding program. Use of the auxin, NAA, together with GA4/7 in this study promoted male but not female flowering. It has been reported to stimulate female and/or male flowering in loblolly pine (Hare, 1984). Douglas-fir (Ross, 1976; Ross *et al.*, 1980; Pharis *et al.*, 1980) and Scots Pine (Luukkanen, 1980). It inhibits flowering in slash pine *P. elliottii* Engelm.), loblolly pine and longleaf pine (*P. palustris* Mill.) (Hare, 1984). In Japanese larch NAA used with GA4/7 was found to inhibit both male and female flowering (Hall, 1977). Results with NAA for black spruce thus most clearly resemble the effects in Douglas fir; enhancement of male flowering (Pharis *et al.*, 1980). It is possible that the dose of NAA applied to black spruce was supraoptimal for female flowering thus inhibiting female flowering.

Continued increased flowering in the years following application of GA4/7 has been reported to occur in Japanese

Table 1. — **Number of flowers (mean and range) on black spruce one year following treatment.**

Growth substance	Concentration mg/l	Number of Flowers: Male, July, Mean	Range	Male, August, Mean	Range	Female, July, Mean	Range	Female, August, Mean	Range
Control	-	1.8	0-7	1.8	0-7	1.0	0-4	7.8	0-31
GA4/7	200	7.2	1-16	2.2	0-9	27.8	0-67	0	-
GA4/7	800	118.2	0-261	0	-	28.2	0-79	0.2	0-1
GA4/7 GA_9	200 10	22.5	3-74	2.8	0-7	2.0	0-4	0	-
GA4/7 NAA	200 100	43.8	2-88	47.2	0-155	8.0	0-20	2.5	0-10

and European larch (BONNET-MASIMBERT, 1982) and Douglas-fir (PHARIS *et al.*, 1980). In this study nearly all trees — treated and control — flowered in the second year (*Table 2*). The difference between times of application was significant in the first year after treatment but not in the second year. When comparing control and treated trees it appears that there may even have been some inhibition of flowering in the second year on treated trees.

The increase in male flowering in the second year after treatment could be attributed, in part, to the gradual increase in the male flowering zone as new shoots are formed and as females are initiated on new shoots which have not been treated. If growth hormones act in a manner to divert nutrients from vegetative to reproductive buds as has been reported (ROSS, 1979) then in the year after treatment the tree might be over-compensating for this temporary diversion and act to reverse the process. This might also explain the greater flowering of controls in the second year. The effects could act differently for male and female and for early and late applications. An alternative (and simpler) explanation is that in year one the environmental conditions were more conducive to flower initiation than in the subsequent two years.

Height measurements of the sample trees showed there to be no effects of treatment on leader growth in the same or subsequent year. Inhibition of terminal shoots has been reported to occur in jack pine (CECICH, 1983) and stimulation of vegetative growth has been shown to occur in loblolly pine (HARE, 1979) and Douglas fir (WEBBER *et al.*, 1985). There was no correlation between tree height and number of flowers per tree, a further indication that the increased flowering could not be attributed to tree height. This contrasts with the results reported by SIMPSON and POWELL (1981) who showed significant correlations between tree height and levels of flowering in young black spruce.

Both seed quantity and quality were reduced in the year after treatment on treated trees (*Table 3*). Yields on trees treated with GA4/7 at 800 mg/l and GA4/7 + NAA were particularly low. (The data are based on few trees and tree to tree variation was large). No deterioration of seed quality after treatment was reported for western hemlock (ROSS *et al.*, 1981), Douglas-fir (ROSS *et al.*, 1980) Sitka spruce (TOMPSETT *et al.*, 1980) or *whithe spruce* (MARQUARD and HANOVER, 1984b). However, PURITCH *et al.* (1979) reported a reduction in filled seed per cone in Douglas-fir after treatment with GA4/7. Overall seed yield was increased because of the large numbers of GA4/7-induced cones. Thus the reduction of about 20% in filled seed per cone (*Table 3*) was more than compensated by the 28-fold increase in cones produced (*Table 1*). The data on seed yield are insufficient to determine if the reductions are a result of treatment or merely the heavy cone crop. It is a subject which should be investigated, however, because of the indication that gibberellin-induced cones apparently produce a lower percentage of viable seeds than "natural" cones.

Table 2. — Neumber of flowers tree for each treatment one, two, three years after treatment.

Growth substance	Conc. mg/l	Years after treatment	Number of Flowers Per Tree: Male	Female
Control	-	1	1.8	4.4
		2	81.8	45.2
		3	-*	2.4
GA4/7	200	1	4.7	13.9
		2	29.5	38.4
		3	-	2.4
GA4/7	800	1	59.1	13.9
		2	98.8	25.1
		3	-	4.8
GA4/7	200	1	12.6	1.0
GA_9	10	2	55.6	19.8
		3	-	.6
GA4/7	200	1	45.5	5.2
NAA	100	2	77.8	7.0
		3	-	4.6

*) Numbers of male flowers were not determined in year three.

Table 3. — Seed yield (quantity and quality) in treated and untreated trees.

Treatment: Hormone	Concentration	No. of seeds per cone: Total	Filled	Viable	Average cone wt. (g)	wt. 1000 seeds (g)
Control		37.1*	30.3	29.6	1.96	1.27
GA4/7	200 mg/l	46.5	24.5	23.6	1.36	.99
GA4/7	800 mg/l	38.8	24.3	17.2	2.60	1.09
GA4/7 GA_9	200 mg/l 10 mg/l	-**	-	-	-	-
GA4/7 NAA	200 mg/l 100 mg/l	28.2	21.8	16.8	1.77	1.12

*) Data for controls, one year after treatment is from adjacent natural trees.
**) No data available.

Conclusions

(i) Both male and female flowering was promoted in terms of the number of flowers per tree and the proportion of trees producing flowers.

(ii) Application of hormone in early July produced significantly more flowers than did application in early August. Use of GA4/7 + NAA promoted flowering when sprayed in August.

(iii) Response was greater for male flowering than for female flowering.

(iv) Female flowering was promoted equally at 200 and 800 mg/l of GA4/7 but male flowering was significantly promoted only at 800 mg/l or when GA_9 or NAA was combined with 200 mg/l of GA4/7.

(v) There is an indication that the treatments had a positive effect beyond the first year for female flowering, but not for male.

(vi) The treatments did not affect terminal shoot growth either in the year of application or he subsequent year. Flowering levels were not related to tree height before or after treatment.

(vii) Seed quality per cone was reduced in the year following treatment but the increased number of cones induced by the GA4/7 treatment more than compensated for the reduced quality of seed.

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Changes in the Genetic Control of Growth of Radiata pine to 16 Years and Efficiencies of early Selection

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Summary

Additive genetic and phenotypic variances and covariances were estimated for height and basal area at about 2½, 4½, 6½, 10½ and 16 years after planting in an open-pollinated progeny trial of *Pinus radiata* in South Australia. At about 11 years the trial was thinned to 45% original stocking. Heritability and additive variance of annual height increments were high between 2½ and 6½ years after planting but declined sharply from 6½ years (following stand closure) to thinning. Heritability and additive variance of basal area increments increased steadily with time, particularly following thinning at 11 years. The phenotypic variance of growth increments increased with time despite the fact the actual rate of height growth diminished.

Indirect selection of individual trees on either height increments to 6½ years (when trees were 10 m tall) or a restricted index combining height and basal area increments to 6½ years may be expected to produce over 60% more gain per year in volume at 16 years, compared with later direct selection on volume 16 itself. Indirect selection on traits measured at 10½ years produced less gain per year because of the extended generation interval. For early traits having high heritabilities indirect individual selection proved more efficient than indirect family selection.

Key words: Heritability, juvenile-mature correlations, early selection, indices.

Zusammenfassung

Anhand einer Prüfung frei abgeblühter Nachkommenschaften von *Pinus radiata* in Südaustralien wurden additiv genetische und phänotypische Varianzen und Kovarianzen für Höhe und Grundfläche des Stammfußes etwa 2½, 4½, 6½, 10½ und 16 Jahre nach dem Auspflanzen geschätzt. Im Alter von etwa 11 Jahren wurde der Versuch bis auf 45% seiner Ursprungsdichte durchforstet. Die Heritabilität und die additive Varianz des jährlichen Höhenzuwachses waren in der Zeit zwischen 2½ und 6½ Jahren nach dem Auspflanzen hoch, nahmen aber nach 6½ Jahren (nach Bestandesschluß) bis zur Durchforstung schnell ab. Die Heritabilität und die additive Varianz der Zuwächse im Bereich der Grundfläche des Stammfußes stieg mit der Zeit beständig an, besonders nach der Durchforstung im Alter von 11 Jahren. Die phänotypische Varianz der Höhenzuwächse erhöhte sich mit der Zeit, trotz der Tatsache, daß sich die aktuelle Höhenzuwachsrate verringerte.

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