EFFECTS OF AGE OF ORTET, TIME OF CUTTING COLLECTION AND GENOTYPE ON ROOTING BLACK SPRUCE CUTTINGS[1]

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Abstract.--Results of three stem cutting propagation experiments indicate that the rooting response in black spruce (Picea mariana (Mill.) B.S.P.) is genetically variable at the individual tree level and the response declines significantly between ortet ages 3 and 7 years. Time-of-cutting collection also effects the rooting response, but to a lesser degree.

Additional keywords: Vegetative propagation, maturation, genetic variation.

The potential contributions of clonal propagation to forest tree improvement and deployment have long been recognized (Grace 1939, Deuber and Farrar 1940, Larson 1955, Burdon and Shelbourne 1974, Kleinschmit 1974, Libby 1974). Advantages of clonal versus seed propagation include; increased genetic gain through clonal testing, selection and utilization, and decreased or controlled within variety genetic variance. In addition to increased improvement and deployment possibilities, clonal propagation should also enhance basic research in quantitative genetics, physiology, pathology, entomology and biometry by providing genetically homogeneous plant material.

This paper reports results of three recently completed black spruce rooting experiments. These experiments are part of an ongoing research effort aimed at more clearly defining problems associated with clonal propagation systems and developing baseline data for materials being used in in vitro propagation studies and/or clonal tests. Specific objectives of these three experiments were as follows: (1) determine the effects of age-of-ortet and date-of-cutting collection on rooting and number of roots per rooted cutting; (2) investigate the family and tree within family variability of rooting and number and length of roots per rooted cutting; and (3) determine the effects of clone and an IBA quick-dip treatment on rooting percent of mature (age 12 years), field tested and selected (height growth) genotypes.

Past work with black spruce has indicated a significant effect of ortet age on stem cutting rooting, with seedlings rooting and older trees failing to root. Phillion (1983), Girouard (1970) and Rauter (1971) reported 97, 66 and 44 percent rooting for ortets of ages 23 weeks, 4 and 5 years, respectively. Many other ortet-related factors, including genotype and collection time have also been shown to effect rooting (Girouard 1974, Rauter 1979). Rauter (1971) found variation in rooting percent among and within natural black spruce populations of Canadian origin, and recommended collecting hardwood cuttings just prior to bud break (Rauter 1977).

MATERIALS AND METHODS

Experiment I

Cuttings were collected from trees of four ages-- 3, 7, 11 and 15 years from seed-- on three dates in late winter of 1986-- February 14, March 21 and April 18. The cuttings were

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taken from the lowermost branches and consisted of the recent year's (1985) terminal growth. Six cuttings were collected from each of eight trees per age-date treatment combination, excluding the age 3 treatments. Fewer cuttings per tree and more trees were sampled in the age 3 treatment combinations. In the age 7, 11 and 15 treatments, the same eight trees were sampled on each collection date, while different trees were sampled on each date in the age 3 treatments.

All sampled trees were located within six miles of Grand Rapids, Minnesota. Trees in the age 11 and 15 treatments were growing in two University of Minnesota genetic test plantings. Trees in the age 3 and 7 treatments were growing in the Blandin Company nursery and a commercial planting, respectively. All sampled trees were selected at random.

The fresh cuttings were transported on ice to St. Paul, Minnesota, where they were stored at 4°C. First, second and third collection date cuttings were prepared and stuck on February 18, March 24 and April 21, 1986, respectively. The preparation consisted of randomly selecting 32 of the 48 collected cuttings per treatment combination, making a fresh cut at the basal end and reducing cutting length to a maximum of 12 cm. In all cases, only the basal portion of each shoot was utilized as the prepared cutting (Runquist and Stefansson 1973).

The prepared cuttings were stuck 3 to 5 cm deep in a 2:1 by volume washed sand and perlite rooting medium. The medium, 10 cm deep over a 5 cm deep gravel (6 to 19 mm) base, was located on a greenhouse bench in a polyethylene covered, intermittent mist chamber (day, 15 s / 2 h; night, 15 s at I am). The rooting medium and gravel base were separated with a layer of synthetic filter fabric, which allowed water to drain into the gravel.

To reduce daytime light intensity and temperature, a 51 percent shade cloth was draped over the chamber roof and upper-half of the side walls. Temperature of the rooting medium remained fairly constant, 20°C over the course of the experiment, while air temperature varied from 22° to 30°C. Banrot fungicide (3.3g/Gal) and Diazinon insecticide (9.2g/Gal) were applied to the rooting medium, following each date of sticking and on June 30 and July 29.

A randomized complete block experimental design was utilized, with 4 replications and 12 treatment combinations (4 ages x 3 dates). Each experimental unit (plot) consisted of 8 non-contiguously arranged cuttings. Number of roots per cutting was recorded, 18 weeks after sticking. Binomial rooting data (1 =rooted, 0=unrooted) and number of roots per rooted cutting, transformed to the square-root scale, were analyzed with the following fixed effect model (Steel and Torrie 1980):

$$Y_{ijkl} = \mu + r_i + a_j + d_k + ad_{jk} + \epsilon_{ijkl}, \qquad (1)$$

where μ = overall mean, r_i = effect of replication i, a_j = effect of age j, d_k = effect of date k, ad_{jk} = effect of age j x date k interaction and ϵ_{ijkl} = random error.

Experiment 2

Twenty wind-pollinated black spruce seedlots were randomly selected from the remnant seed of a 1974 Minnesota statewide single tree seed collection (Hyun 1981, Nelson 1988). The seed were stratified for 16 hours, then sown in Promix BX filled Ray Leach pine cells on December 20, 1985. Ten seeds were sown per cell and twenty cells per wind-pollinated family. Germination was generally good but variable. Seedlings were thinned and transplanted, where possible, to one vigorous seedling per cell on January 15 and 16, 1986.

The seedlings were grown in a greenhouse under extended photoperiods and weekly fertilizer applications. On June 1 (23 weeks), eight trees from five families were selected for use as stock plants in a separate rooting experiment (not published). The remaining seedlings were maintained in pine cells, without fertilization; until August 15, when twice-weekly fertilizer applications were begun.

On August 30 (32 weeks), six cuttings were taken from each of three seedlings in 20 families and immediately stuck in 15 x 30 cm flats containing 2:1 by volume washed sand and perlite. The flats were placed on coarse gravel, in an intermittent mist chamber as described in Experiment 1. The cuttings were stuck in a randomized complete block experimental design, with three replications, 20 families as treatments and six cutting-row plots. Pairs of cuttings by tree-in-family were randomized within plots. The photoperiod during the rooting phase was extended to 24 h with incandescent lamps (low intensity).

Number of roots and lengths of the longest and shortest roots were recorded, 11 weeks after sticking. Total root length was estimated for each rooted cutting by multiplying the number of roots by the midpoint of the longest and shortest roots. For cuttings with only one root, the length of that root was used as total root length. Binomial rooting data (1=rooted, 0=unrooted), and number of roots and total root length per rooted cutting, transformed to square-root scales, were analyzed with the following random effect model:

$$Y_{ijkl} = \mu + r_i + f_j + rf_{ij} + t(f)_{jk} + rt(f)_{jik} + \epsilon_{ijkl}, \qquad (2)$$

where μ = overall mean, r_i = effect of replication i, f_j = effect of family j, rf_{ij} = effect of replicate i x family j interaction, $t(f)_{jk}$ = effect of tree k in family j, $rt(f)_{jik}$ = effect of replicate i x tree k in family j interaction and ϵ_{ijkl} = random error.

To examine the relationships between rooting and number and length of roots, simple correlation coefficients of family and clone means were calculated. Relationships between rooting and early field height and flowering were investigated with family mean correlation estimates between each of the rooting variables and tree height and a flowering index at age 12 (Nelson and Mohn 1988). Height and flowering data from a field test at Cloquet, Minnesota were utilized for these correlation analyses.

Experiment 3

In late April 1987, winter-dormant cuttings were collected from the lower branches of 16, twelve-year-old black spruce trees. The trees had been selected for age 10 total tree height, with a combined family plus individual tree index (Nelson 1988), and were growing in any one of four wind-pollinated family tests. All selected trees were unrelated and each had flowered (female strobili) at least once during the previous three years. The cuttings were transported, stored and prepared as described in Experiment 1.

Immediately prior to sticking, 24 cuttings from each clone were quick-dipped in a 75 percent ethanol solution with 5000 ppm IBA (indole-3-butyric acid) and 24 cuttings in 75 percent ethanol only. The cuttings were stuck on April 28, 1987, in a randomized complete block design with four replications, 16 clones and two IBA levels as factorially arranged treatments and six-cutting row plots. Flats and media were the same as described in Experiment 2 and intermittent mist, photoperiod and pesticide regimes were the same as described in Experiment 1.

Numbers of rooted cuttings per plot were recorded at 12 and 16 weeks from sticking. Rooted cuttings were saved and potted for further observation and use as stock plants for additional rooting work. Proportion of rooted cuttings per plot at 16 weeks, transformed to the arcsine square-root scale, was subjected to analysis of variance with the following fixed effect model:

$$Y_{ijkl} = \mu + r_i + c_j + h_k + ch_{jk} + \epsilon_{ijk}, \qquad (3)$$

where μ = overall mean, r_i = effect of replication i, c_j = effect of clone j, h_k = effect of hormone dip k, ch_{jk} = effect of clone j x hormone dip k interaction and ϵ_{ijk} = random error.

RESULTS

Experiment I

Overall, 24.5 percent of the cuttings had rooted at 18 weeks. Table I lists the mean (across replications) percent rooted for each treatment combination, marginal means for each level of age-of-ortet and date-of-cutting collection, and multiple comparisons (LSD, Steel and Torrie 1980) within and across levels of age and date. Analyses of variance and F-test results for rooting (binomial) and number of roots per rooted cutting are presented in Table 2.

Table 1. Treatment combination and main effect means of percent rooting in Experiment 1.

Age-of-Ortet	Date-of-Collection February	March	April	Over Dates
3	59.4a	56.3a	90.6a	68.8a
7	21.9b	9.4b	15.6b	15.6b
11	3.1c	3.1b	12.5b	6.3b
15	0.0c	6.3b	15.6b	7.3b
Over Ages	21.1	18.8	33.6	

Note: age-of-ortet means connected by lines and date-of-collection means followed by the same letter, respectively, are not significantly different (p=.05)

Table 2. Analyses of variance and F-test results for rooting and transformed number of roots per rooted cutting in Experiment 1.

Source	df	MS	F	p
		Rooting		
Rep	3			
Age	3	9.86	76.4	≤.0001
Date	2	.82	7.3	≤.001
Age x Date	6	.25	2.3	≤.05
Error	369	.11		
		Number		
Rep	3			
Age	3	3.18	12.4	≤.0001
Date	2	.25	1.0	>.05
Age x Date	5	.32	1.2	>.05
Error	80	.26		

Across and within all dates of cutting collection, a statistically significant decline in rootability was detected between ages 3 and 7. After age 7, rooting appeared to decline gradually although not significantly, except within the February collection date (Table 1). Across ages of ortets, a significant increase in rootability was detected between the two early dates of collection

and the latest date, April. This trend was only significant within age 3, although for practical applications it appears equally important for trees 11-years-old and older.

Number of roots per rooted cutting averaged 4.38 over all treatment combinations (data not shown). The effect of age-of-ortet was significant (Table 2) with cuttings from age 3 trees producing significantly more roots than cuttings from the older trees (5.3 per cutting versus 2.1). Date-of-cutting collection was not a significant factor in number of roots initiated per rooted cutting (Table 2).

Experiment 2

To maximize the family variation in the binomial rooting data, cuttings were evaluated relatively early (11 weeks). Previous experiments with black spruce seedling cuttings had indicated that 50 percent rooting occurs between nine and 11 weeks from sticking. Analyses of variance and F-tests for rooting (binomial data) and number of roots and total root length per rooted cutting are presented in Table 3. Interaction effects involving replications (model 2) were not significant in any analysis, so they were pooled into one experimental error term for the analysis of each dependent variable.

Table 3. Analysis of variance and F-test results for rooting and transformed number of roots and transformed total root length per rooted cutting in Experiment 2.

Source	df	MS	F	p
		Rooting		
Rep	2			
Family	19	.388	1.34	>.05
Tree(Family)	40	.289	1.43	≤.05
Error	298	.202		
		Number		
Rep	2			
Family	19	.151	1.37	>.05
Tree(Family)	39	.100	0.93	>.05
Error	167	.110		
		Length		
Rep	2			
Family	19	13.69	1.22	>.05
Tree(Family)	39	11.68	1.03	>.05
Error	167	11.20		

Through 11 weeks, 63 percent of the cuttings had rooted. Ranges in family and clone means for each of the rooting characters are listed in Table 4. Differences among trees within families were significant in rooting, but not for number of roots or total root length per rooted cutting (Table 3). Family differences were not significant ($p<.05$) for any character.

Family and clone mean correlation estimates between rooting characters are listed in Table 5. All correlations were positive and significant (Table 5), except the family mean correlation between number of roots and total root length per rooted cutting. Apparently, genotypes that root well also initiate more roots per rooted cutting, but the relationship between total root length and number of roots is less clear.

Table 4. Range in family and clone means of percent rooted and number and total length of roots per rooted cutting in Experiment 2.

	Rooted (%)	Number of roots	Length of roots (cm)
Family	39 - 89	1.3 - 2.6	2.3 - 8.6
Clone	0 - 100	1.0 - 3.7	0.2 - 11.2

Table 5. Family (above diagonal) and clone (below) mean correlation coefficient estimates between percent rooted and transformed number and total length of roots per rooted cutting in Experiment 2.

	Percent rooted	Number of roots	Length of roots
Percent rooted	---	.54 *	.46 *
Number of roots	.37 *	---	.30
Length of roots	.39 *	.38 *	---

* significant at $p \leq .05$

Family mean correlation estimates between the rooting characters and age 12 total tree height and age 10 through 12 female flowering were near zero or negative and not significant at p=.05 (data not shown). For each rooting character the correlation with flowering was more negative than with height, suggesting that selection for height may not have a negative impact on rooting potential.

Experiment 3

Through 16 weeks, 13.3 percent of the cuttings had rooted. Clone means (over replications and IBA levels) ranged from 0 to 37.5 percent. The analysis of variance and F-tests for proportion rooted per plot (transformed) is given in Table 6. The clonal effect on proportion rooted was significant, but the effects of IBA and clone x IBA interaction were not.

Table 6. Analysis of variance and F-test results for transformed proportion rooted per plot in Experiment 3.

Source	df	MS	F	p
Rep	3			
Clone	15	.314	6.04	$\leq .0001$
IBA	1	.009	.17	>.05
Clone x IBA	15	.040	.77	>.05
Error	93	.052		

Multiple comparison tests (LSD) revealed that the 16 sampled clones could be grouped into two statistically distinct classes (Table 7). The higher rooting class consisted of five clones and averaged 30.8 percent rooted, while the lower class contained the remaining 11 clones and averaged 5.3 percent rooted. In addition to the clone rooting means, Table 7 includes female strobili incidence data for the clones (ortets) in the previous three years. Both rooting groups

contain lesser flowering ortets, suggesting that rooting and flowering are not mutually exclusive events.

Table 7. Percent rooted and previous three year female strobili incidence data for each clone (ortet) in Experiment 3.

Clone	Rooted (%)		Age[a] 10	11	12
265	37.50	[b]	0[c]	1	1
276	33.33		0	1	0
278	29.17		1	1	1
281	27.08		1	0	1
335	27.08		1	1	1
313	12.50		1	1	1
283	10.42		1	1	1
245	8.33		0	0	1
224	6.25		1	-[d]	-
126	6.25		1	-	-
174	4.17		1	1	1
254	4.17		1	-	-
50	2.08		1	0	0
223	2.08		0	1	1
69	2.08		1	-	-
252	0.00		1	1	1

[a] years from seed
[b] means connected by lines are not significantly different (p=.05)
[c] 0 = no female strobili, 1 = one or more female strobili
[d] data were not available

IBA-treated cuttings averaged 12.5 percent rooted versus 14.1 percent for non-treated cuttings. The cuttings were also evaluated at 12 weeks and at that time more IBA-treated cuttings had rooted, but the difference was not significant (data not shown). This observation supports the hypothesis that exogenous auxin may only act to enhance the rate of root initiation and not the probability (Struve 1982, Hess 1961).

DISCUSSION AND CONCLUSIONS

The rooting of seedling materials of black spruce is apparently routine and can be very useful in producing genetically homogeneous research materials or increasing the number of plants in a proven cross (Burdon and Shelbourne 1974, Armson et al. 1980, Phillion 1983). Our studies confirm that ortet age is limiting the rooting of black spruce at a practical rate (>50 percent). Under the conditions of Experiment 1, the rooting response declined significantly between ortet ages 3 and 7 years, which is consistent with results of Phillion (1983), Girouard (1970) and Rauter (1971).

Understanding the "juvenile-to-mature" transition in rootability is critical to preserving and recovering juvenility and the eventual utilization of mature, tested genotypes in clonal forestry

systems. Both the trends associated with time-of-cutting collection and the apparent genetic variability in rooting may have implications in the development of improved propagation systems and dealing with recalcitrant materials.

In the black spruce population we examined, genetic variation in rootability was substantial and appears to be of greater significance between trees within families than between families. Individual tree selection (clonal) may well be effective for improving the rooting response and the lack of significant correlations between rooting and height growth are encouraging from the point of view of developing black spruce for silviculture systems based on clonal forestry.

The success of rooting several field tested and selected clones at greater than 25 percent may also have implications for the use of rooted cuttings instead of grafts for establishing clonal seed orchards. Werner (1977) investigated this possibility with Norway spruce (Picea abies) and found no difference between cuttings and grafts in time-to-flower and flower production. Growth of the rooted cuttings in Experiment 3 appears normal under greenhouse conditions, suggesting that the use of relatively young octets (age 12 years) may have reduced the loss of vigor that is frequently associated with asexually propagated materials from older trees.

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