MASS PRODUCTION ALTERNATIVES FOR FAST-GROWING SPRUCE HYBRIDS

Hans Nienstaedt[1]

ABSTRACT.--A reçiptrocal crossing program of *Picea glauca* and *P. omorika*, and *P. mariana* and *P. omorika* with adequate intraspecific control crosses was carried out. Yields of full seed/cone ranged from 0 to 2.6 for the *P. glauca-P. omorika* combination. These low seed sets and a 9-day difference in female receptivity rule out producing this hybrid via seed. The full seed/cone yields of the *P. mariana-P. omorika* combinations averaged approximately half the yield of the intraspecific crosses. However, receptivity of the two species is well synchronized and in spite of reduced yields, mass production via seed is a practical option. A seed orchard design is outlined and a 4-acre grafted orchard may produce at least 75,000 plantable seedlings on 6- to 8-year-old grafts. Results of a rooting trial of selected hybrid seedlings are described. A practical mass production program for the *P. glauca-P. omorika* combination based on rooted cuttings is described. The program would be based on juvenile selection among 4-year-old seedlings, cloning, and the simultaneous establishment of clonal trials and hedges. The hedges would be a source of cuttings for future mass production--a hedge 400 feet long by 5 feet high would produce a minimum of 20,000 cuttings annually.

In 1959 a single cross between native *Picea mariana* and *P. omorika* was produced at Rhinelander, Wisconsin. Seed set was high and height growth of the hybrid was superior to the native species.

A *P. omorika* x *P. glauca* cross was first produced in 1964. In the greenhouse, mean early height growth of the hybrid exceeded the growth of *P. glauca* by 45 percent, and in 1975 in a field test exceeded the open-pollinated progeny of the *P. glauca* by 14 percent. The 1964 control cross *P. omorika* x *P. omorika* produced 9.8 full seeds per cone; in comparison the interspecific cross yielded 7.4

[1]Chief Plant Geneticist, USDA, Forest Service, Institute of Forest Genetics, Box 898, Rhinelander, Wisconsin 54501.

seeds/cone. Both values are low, because up to 60 to 80 filled seeds per cone have resulted from other P. omorika x P. omorika crosses. Comparison among the 1964 crosses, however, suggested that the two species were cross compatible and that P. omorika x P. glauca crosses generally would result in high seed yields.

In 1971 both crosses were repeated on a larger scale with adequate controls to verify the superior growth of the hybrids and to determine the potential for mass production by seed. This trial clearly showed that P. omorika x P. mariana can be readily produced by seed on a large scale but that other means of reproduction must be found for the P. omorika x P. glauca hybrid. In 1975 a study was begun to test the possibility of vegetatively propagating P. omorika x P. glauca by means of cuttings.

It is the purpose of this paper to compare the sexual mass production with the vegetative mass propagation of these two promising spruce hybrids. The emphasis will be on propagation potential; performance of the hybrids will not be discussed.

SEXUAL MASS PRODUCTION OF HYBRIDS

Methods

Three individuals of each of P. glauca, P. omorika, and P. mariana were selected for vigor and the combinations P. glauca-P. omorika and P. mariana-P. omorika were tested. Intraspecific crosses were used as controls.

The selected P. glauca parents have been included in many tests to date and their progenies have consistently been top performers. The 1971 pollinations were made on 1959 grafts growing near Lake Tomahawk, Wisconsin.

P. mariana and P. omorika selections were made in a 1936 planting of spruce species growing on an upland site in Forest County, Wisconsin. Vigor of the trees was the only selection criterion.

The crossing scheme, including reciprocals, should have yielded the following number of crosses:

Combination	No. of crosses
glauca - omorika	18
mariana - omorika	18
glauca - intraspecific	6
mariana - intraspecific	6
omorika - intraspecific	6
	54

Due to lack of female conelets, particularly on *P. omorika*, only 44 of the crosses were attempted and of those 42 actually produced seed. In addition the three *P. omorika* trees were used as male parents in six crosses with two additional *P. omorika* genotypes and the three *P. glauca* genotypes were crossed with a random mixture of *P. glauca* pollen.

For *P. glauca* and *P. mariana*, fresh 1971 pollen collections extracted and cleaned at the laboratory were used. Pollen collected in 1970 and stored under various conditions were used for *P. omorika* crosses on *P. glauca* and on the two *P. omorika* genotypes. However, because no meaningful storage effects could be identified on either inter- or intraspecific crosses, the seed yield data were combined. A mixture of pollen from the various storage conditions was used to pollinate the three *P. omorika* and the three *P. mariana* trees in the Forest County, Wisconsin, test plantation.

Bagging of *P. glauca* was on May 10, 1971, and the later-developing species were bagged when the female strobili could be recognized. The following schedule shows the time of pollinations, debagging, and cone harvest:

♀ Species	Pollination	Debagging	Cone Harvest
		1971 Date	
P. glauca	5/21	6/4 - 6/8	9/1
P. omorika	5/30	6/11	9/21
P. mariana	5/30	6/11	9/9 - 9/21

For each pollination bag harvested, cones were counted and each individual cone extracted by hand to assure that *all* seeds were extracted. After dewinging and cleaning, the total sample was X-rayed and the number of full seeds counted on the X-radiographs. Last, the total number of seeds was sampled from eight bags on each of the three *P. glauca* and two to three bags in the case of *P. mariana* and *P. omorika*. From these counts the average total number of seed and full seed per cone were determined.

Unpollinated control bags were placed on all trees and only one bag on *P. omorika* yielded seed.

Results and Discussion

Seed yields in intraspecific crosses

The three *P. glauca* crosses (two diallels and one with mixed pollen) averaged 34, 43, and 47 full seeds per cone (for 1885, 1887, and 1889, respectively). These values compare favorably with previously published values for the same clones (Nienstaedt and Jeffers 1970, King *et al*. 1970). For individual crosses, yield of full seed ranged from 22 to as high as 73 (table 1). The latter value is higher than any previously published (Nienstaedt 1972). The results of the individual crosses suggest that clone 1889 is more productive than either 1885 or 1887; this substantiates previously published results (Nienstaedt and Jeffers 1970, King *et al*. 1970).

In the three P. mariana trees the two diallel crosses per tree averaged 27, 38, and 14 full seeds per cone for trees 5343, 5344, and 5340 respectively, and ranged from 11 to 38 full seeds per cone for individual crosses (table 1). These results are good because P. mariana cones usually average 43 full seeds per cone.

Table 1.--Yield of full seed from diallel crosses of three selected trees

Picea glauca

Female parents	Male parents: 1885	1887	1889	Random mix
1885	0 2/	32/127	37/83	23/88
1887	22/29 1/	0	54/54	39/18
1889	58/38	42/79	0	73/37

Picea mariana

Female parents	Male parents: 5343	5344	5340
5343	0	27/78	27/90
5344	38/75	0	38/99
5340	17/36	11/36	0

Picea omorika

Female parents	Male parents: 5342	5347	5341
5342	0	46/39	74/22
5347	0	0	0
5341	68/4	78/2	0

1/ 22 = No. of full seed per cone.
29 = *No. of cones harvested.*

2/ 0 = Cross not performed.

The sample of 20 cones from the three P. omorika trees averaged a total of 87 seed per cone. The diallel crosses were only completed on 2 of the 3 trees and on the average yielded 56 and 71 full seed per cone on trees 5342 and 5341, respectively. The overall average was 57 full seed per cone. For individual crosses, yields ranged from 46 to 78 full seed per cone (table 1). This also shows that pollen quality and timing of pollination was good.

Seed yields in interspecific crosses

Contrary to expectation, the crosses between P. glauca and P. omorika yielded little or no seed regardless of the direction of the cross (tables 2 and 3).

Table 2.--Yield of full seed from diallel interspecific spruce crosses

Picea glauca and Picea omorika

Female parents: of Picea glauca	Male parents Picea omorika 5342	5347	5341	Female parents: of Picea omorika	Male parents Picea glauca 1885	1887	1889
1885	0.06[1] / 839	0.04 / 615	0.11 / 666	5342	0.68 / 57	2.61 / 31	0.43 / 46
1887	.14 / 503	.01 / 272	.15 / 154	5347	.00 / 7	0[2]	0
1889	.04 / 479	.04 / 292	.05 / 756	5341	1.67 / 3	2.33 / 3	.00 / 1

Picea mariana and Picea omorika

Female parents: of Picea mariana	Male parents Picea omorika 5342	5347	5341	Female parents of Picea omorika	Male parents Picea mariana 5343	5344	5340
5343	11 / 99	13 / 70	22 / 140	5342	49 / 13	44 / 49	74 / 4
5344	14 / 42	18 / 70	21 / 72	5347	0	0	0
5340	5 / 44	5 / 53	7 / 26	5341	0	0	0

1/ .11 = No. of full seed per cone.
666 = *No. of cones harvested.*

2/ 0 = Cross not performed.

Table 3.--Average yields of full seed/cone from diallel intra- and interspecific spruce crosses

Cross combination	Cones	Full seed	Ave. full seed/cone	Ave. seed/cone[1/]	Full Seed
	- - - - - -	- - - -	-Number-	- - - - - - -	Percent
P.omorika x P.omorika	256	7,695	30.1	86.67	35
P.omorika x P.glauca	66	3,090	46.8	90.55[3/]	52
P.mariana x P.mariana	414	12,130	29.3	43.03[2/]	68
P.mariana x P.omorika	612	8,907	14.6	43.03[2/]	34
P.omorika x P.glauca	148	152	1.0	86.67	1
P.glauca x P.glauca	553	21,564	39.0	71.33	55
P.glauca x P.omorika	4,576	312	.07	71.33	.1

[1/] Unless footnoted, the derivation of these values is explained in the text.

[2/] Based on counts of all bags with P. mariana x P. mariana crosses -- a total of 414 cones.

[3/] Based on counts of 11 cones from tree 5342 -- the only tree in the cross.

Crosses between P. mariana and P. omorika, on the other hand, were successful. The average yield of full seed per cone when P. mariana was the female parent was 14.6 seed, about half the amount of seed obtained in the intraspecific P. mariana crosses (table 3). Mean full seed per cone yields of individual mother trees were 16, 18, and 5 seeds per cone for trees 5343, 5344, and 5340 as compared to the 27, 38, and 14 full seeds obtained from the intraspecific crosses. Individual crosses yielded from 5 to 22 full seeds per cone (table 2). Lowest yields were on tree 5340, but the cones on this tree contain only 19 seeds per cone as compared to 43 for 5343 and 53 for 5344. Nevertheless, 5340 appears to be the least cross compatible of the three trees--full seed yield was 27 percent of total seed yield as compared to 37 and 34 percent for the other trees. Unfortunately, it was only possible to perform the P. mariana crosses on one of the P. omorika trees--5342. This tree is characterized by a total yield of 91 seeds per cone and yielded an average of 56 full seeds per cone in intraspecific crosses--62 percent of total seed set. The seed yield for P. mariana crosses was almost as good (tables 2 and 3) averaged 47 seeds per cone (52 percent of total seed set).

Discussion

The above yields of seed clearly show that sexual mass production of hybrids between P. glauca and P. omorika is impractical. The 9-day difference in the phenology of the two species would necessitate an artificial pollination technique. But even if a practical mass pollination technique could be developed, the low seed yield would present an insurmountable obstacle.

It is, on the other hand, feasible to sexually mass produce hybrids between P. omorika and P. mariana. Phenologically, the species are well synchronized and even though the seed sets are reduced, they are still substantial.

The three crosses on the P. omorika parent (5342) suggest that it would be best to use P. omorika as the female. Other evidence supports this conclusion. In 1960 seed was collected from nine P. omorika trees growing near Eagle River, Wisconsin. Plots of P. mariana are located close by in the same test.[2/] The progenies were planted in 1965 near Lake Tomahawk. In 1975, 129 of the original 180 trees had survived and of the survivors, 86 were identified as hybrids between P. omorika and P. mariana. Allowing for differential survival, we estimate that between 50 and 60 percent of the original trees were of hybrid origin. Hybrids were found in every progeny and must have represented from about 30 to as much as 90 percent of the trees in individual families. Thus, P. omorika 5342 isn't an isolated case of high cross compatibility between the two species. All the nine trees in the progeny test must have been phenologically well synchronized with the adjacent P. mariana and generally are cross compatible.

VEGETATIVE MASS PRODUCTION OF HYBRIDS

Vegetative propagation has been suggested as an alternative to sexual propagation of cross incompatible species combinations (Roulund 1971)and several schemes have been suggested for mass propagation of juvenile selections of various Picea species (Rauter 1974, Kleinschmit 1974, Kleinschmit et al. 1973, and Roulund 1976).

King et al. (1965) have shown that juvenile selections in 2-2 P. glauca retained superiority 7 years after field planting; more recent measurements at 22 years have shown that the selected seedlings are still 30 percent taller than the control trees.

In 1974 a study of selected juvenile P. glauca mass propagation by cuttings was begun and in 1975 it was extended to include the hybrid progenies described in the previous section.

2/ The P. omorika and P. mariana parents of the crosses were from the same tests.

Source of Material

The seed was cleaned by blowing and for some samples by submerging in 95 percent ethanol to remove empty seed. Seeds were sown in perlite in glass-covered stainless steel trays on Jan. 29 and 30, 1973. The trays were kept in the greenhouse at 20-25°C and received 18-20 hours of light each day. The major period of transplanting was February to March, but lower than expected germination and unexplained seedling mortality necessitated resowing and replacement of some progenies in early May. Seedlings were transplanted into Ontario 5/8 inch tubes in a 1:1:1 sand-soil-peat mixture. In August the seedlings were transplanted to the nursery. Shade was provided initially.

The tallest 104 seedlings were selected during the summer of 1976. They represented all inter- as well as intraspecific combinations except *P. omorika* x *P. omorika*. In addition, six *P. glauca* and seven *P. mariana* seedlings of average heights were selected as controls. The mean height superiority of the selected seedlings is expressed by the following values:

Parent combination	Mean ht. superiority over mean ht. of control
	percent
P. glauca x *P. glauca*	103
P. glauca x *P. omorika*	78
P. mariana x *P. mariana*	39
P. mariana x *P. omorika*	52

Cuttings were taken from the plants with pruning shears. They were 7-1/2-to 9-cm-long first and second order branch terminals. The top whorl was too coarse to be used. Cuttings were immediately put in plastic bags on ice in a cooler and within 24 hours were placed in the rooting bench.

Rooting environment

Rooting was on an open greenhouse bench provided with an automatic mist system, bottom heat, and an incandescent floodlight system to extend the photoperiod.

Air-temperature was set at 21°C (70°F) but substantially exceeded this on sunny days.

Media temperature was set at 26 to 29°C (79 to 84°F) but reached 32° (90°F) on sunny days, which damaged the cuttings.

Mist was applied for 15 seconds every 15 minutes during the day and 15 seconds every 30 minutes during the night.

The aim was to keep the cuttings constantly wet without excessive run-off but on sunny days the tops of the cuttings were dry on part of the bench. Also, because the water was hard, it covered cuttings with salts.

Media was gravel - peat - medium grade perlite, 1:1:1. Gravel was obtained by sifting pit run sand through 1/8-inch hardware cloth. pH was 4.0 initially and gradually increased to 6.0, a more favorable level.

Light regime was 19 hours photoperiod.

Fertilizer was applied weekly after rooting by drenching with Peter's Fertilizer (20-20-20) at a 1:128 ratio.

Cooling-off period consisted of reducing mist to 15 seconds every 30 minutes during the day and 15 seconds every 45 minutes during the night.

Newly transplanted cuttings were also kept under mist for a short period of time.

Experimental design

Containers were "Fives" rootrainers by Spencer-LeMaire[3/]. One book of five cavities constituted a plot. Plots were arranged in five randomized complete blocks on a greenhouse bench.

Rooting schedule

September 27 and 28, 1976	Collection of cuttings
September 28 and 29, 1976	Sticking of cuttings
December 6, 1976	Cooling off period begun
December 23, 1976	Fertilizer treatment begun
January 7 to 26, 1977	Root evaluation and transplanting to 4 inch clay pots.

Results and Discussion

Rooting of clones of intraspecific crosses and control seedlings

In total, we rooted 16 clones of *P. glauca* (10 from selected seedlings and 6 from controls) and 29 clones of *P. mariana* (22 selected for vigor and 7 controls).

3/ Mention of trade names does not constitute endorsement of the products by the USDA Forest Service.

The mean rooting percent for selected P. glauca and P. mariana clones was close to 50 percent. P. glauca controls did not root as well (42 percent) as the P. mariana controls (63 percent) (table 4).

Table 4.--Cuttings rooted from selected Picea glauca and P. mariana clones
(In percent)

Picea glauca

Female parent	Male parent 1885	Male parent 1887	Male parent 1889
1885		40.0 / 3[1]	48.0 / 1
1887			54.0 / 2
1889	54.0 / 4		

All 10 clones $\bar{x}$ = 49 percent
Control
4885[2] 42.0 / 6

Picea mariana

Female parent	Male parent 5340	Male parent 5343	Male parent 5344
5340			58.7 / 3
5343	48.0 / 4		
5344	49.0 / 8		

All 15 clones $\bar{x}$ = 51 percent
Select
7169[3] 49.1 / 7

Control
7169[2] 62.9 / 7

1/ *Number of clones included in the mean values.*

2/ Open pollinated unselected control clones.

3/ Open pollinated seedlings selected for vigor and cloned.

Variation among individual clones was large and statistically significant. P. glauca rooting percentages ranged from 20 to 88 for selected clones and from 8 to 76 for control clones. Comparable values for P. mariana were 16 to 80 and 44 to 76 percent for selected and control clones, respectively. Rooting equaled or exceeded 60 percent in 30 percent of the selected P. glauca and 36 percent of the selected P. mariana clones.

Rooting of clones of interspecific crosses

Mean rooting of four P. glauca x P. omorika and five clones of reciptrocal crosses was 67 and 56 percent, respectively. For individual clones in the two groups, rooting percentages range from 36 to 92 and from 36 to 68 percent, respectively (table 5). The data suggest that the hybrids are better rooters than *P. glauca* crosses and controls, but the differences are not statistically significant. The number of roots per cutting showed a similar and significant trend.

Table 5.--Cuttings rooted from selected clones of hybrids of *Picea omorika*, *P. glauca*, and *P. mariana*

(In percent)

P. glauca and *P. omorika*

Female parent of *Picea glauca*	Male parent of *Picea omorika*			Female parent of *Picea omorika*	Male parent of *Picea glauca*		
	5342	5347	5341		1885	1887	1889
1885				5342		52/2	
1887		92/1 1/	70/2	5347			
1889			36/1	5341		59/3	
All 4 clones $\bar{x}$ = 67				All 5 clones $\bar{x}$ = 56			

P. mariana and *P. omorika*

Female parent of *Picea mariana*	Male parent of *Picea omorika*			Female parent of *Picea omorika*	Male parent of *Picea mariana*		
	5342	5347	5341		5343	5344	5340
5343	62/5 1/	76/1	74/2	5342	65/3	55/11	65/28
5344	48/2	47/3	48/6	5347			
5340	50/2			5341			
All 21 clones $\bar{x}$ = 55				All 42 clones $\bar{x}$ = 63			

1/ *Number of clones included in the mean values.*

Overall, the rooting of the clones of the P. mariana and P. omorika combinations were comparable. Forty-two P. omorika x P. mariana clones had a mean rooting value of 63 percent and 21 clones of the reciprocal combination had a mean rooting value of 55 percent. The ranges in rooting percentages within these two groups were large; from 36 to 96 percent for the crosses in which P. omorika was the female parent and from 12 to 84 percent when the mother was P. mariana. The data in table 5 suggest that the ability to root is under strong genetic control; however, although the differences in rooting percent of individual clones were highly significant, the analysis of variance could not demonstrate significant parent tree effects. The number of roots per cutting was also determined and for this characteristic parent tree as well as clonal effects were significant.

Discussion

Because the environmental control was inadequate, rooting was less than anticipated for juvenile material. Two blocks on one end of the greenhouse bench had particularly poor rooting success. Therefore, the rooting in the three other blocks may be a better indication of the rooting potential of the clones. As an example, for all 5 blocks the rooting in 52 (47 percent) of the clones equaled or exceeded 60 percent. In the 3 better blocks, 82 (75 percent) of the clones attained this level of rooting. It is likely that even better rooting can be achieved if optimum conditions could be attained for an entire test (Kleinschmit 1974).

Mass production is only practical if large numbers of propagules can be produced for an extended period of time. But time connotes aging and rooting ability declines as stands mature. Hedging may be one answer to the problem (Libby 1974).

CONCLUSION

P. omorika x P. mariana seed production will require grafted seed orchards. Two orchard designs are possible:

(1) A simple two clone design -- in such an orchard, seed would be harvested on both the P. omorika and the P. mariana clone and, except for a small portion of selfed seed, would all be expected to be of hybrid origin.

(2) Orchards of one P. omorika clone and several P. mariana clones--hybrid seed would be harvested only on P. omorika. Whether or not the seed on P. mariana would be harvested would depend on the intraspecific general combining ability of the P. mariana clones. Good combiners could yield improved P. mariana seed. A small admixture of hybrid seed could probably be expected, but should not deter from the quality of the material.

The performance of the progenies involved in the studies I have discussed indicate the desirability of control-pollinated progeny testing before or concurrent with seed orchard establishment. Only three parents of either species were tested, but heights of progenies clearly showed that one _P. mariana_ was a superior general combiner. This consideration may favor the orchard with many _P. mariana_ clones. Inferior clones could be rogued on the basis of progeny test results.

Early productivity of seed orchards can perhaps be roughly estimated. _P. omorika_ is not a prolific cone producer, but it might produce 10 cones per ramet at 6- to 8-year from grafting. With this assumption, a 4-acre orchard with 1,250 grafts (10 x 12 foot spacing)--250 _P. omorika_ and 1,000 _P. mariana_ (20 clones of 50 ramets each)--would produce 117,000 full seed (46.8/cone, table 3) which may yield approximately 75,000 plantable seedlings.

P. glauca x _P. omorika_ or reciprocal hybrids would have to be vegetatively mass produced. A practical program may be as follows:

(1) Use 20 to 30 select _P. glauca_ and 5 to 10 select _P. omorika_ to produce 150 to 200 crosses between the two species. The majority of crosses should be on _P. glauca_ and each cross should involve a minimum of 500 conelets. A program of this magnitude would probably produce from 3,000 to 6,000 hybrid seed.

(2) Fall sow in greenhouse and spring plant in the nursery at 18 x 18 inch spacing. If enough seedlings are available, an adequate statistical design should be used.

(3) At the end of the fourth season in the nursery, select the best 6 seedlings in the best 20 families.

These 120 seedlings will form the basis for the following clonal testing and mass propagation scheme:

First year. In the fall, stick 40 cuttings per clone. Discard all clones with less than 25 rooted cuttings.

Second year. In the fall, plant

(1) short-term clonal nursery test using single tree plots with 15 replications.

(2) at least 10 ramets for each clone selected on the basis of rooting, in double-row hedges with 1 foot between plants and 1 foot between rows (hedges would be 8 feet from center to center).

Third year. In the fall, stick 75 cuttings per clone--3 from each ramet in hedge as well as test.

The program in subsequent years may either seek the earliest possible production of stock for field planting or may aim at rapid build-up of the hedges. The clones will be evaluated at 5 years after the establishment of the test (7 years from the beginning of the program).

During the same period, it should be possible to establish 400 to 500 feet of double row hedges of each clone. If five cuttings could be produced per square foot of hedge, a 400-foot hedge 5 feet high would produce 20,000 cuttings, which would be 12,000 to 16,000 plants for field planting annually.

The productivity of the total program would, or course, depend on the final number of clones included in the hedge collection. If 30 clones of the original 120 were retained, we could expect from 360,000 to 480,000 plants from approximately 2-1/4 acres of hedges.

The program as outlined will be time-consuming and probably involve a minimum of selections. It could readily be increased without increase in the rooting facility if new clones were added each year by winter propagation. Profitability will depend on the gains in growth and frost resistance.

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