

### Zusammenfassung

Bei *Pseudolarix amabilis* (NELS.) REHD. wurde die Entwicklung der weiblichen und männlichen Blütenanlagen bis zum Zeitpunkt der Blüte untersucht. Im Raum Connecticut waren sowohl die weiblichen als auch die männlichen Blütenanlagen bereits im September des der Blüte vorausgehenden Jahres zu erkennen. Die Meiose fand dann etwa Mitte April statt. Der Pollen wurde etwa in der zweiten Maihälfte entlassen. Die weiblichen Blütenanlagen zeigten bis zum November nur eine geringe Entwicklung und blieben danach bis zum April inaktiv. Sie waren dann wenige Wochen später voll entwickelt.

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## Interspecific Hybridization in Pines with the special Reference to *Pinus rigida* X *taeda*<sup>1)</sup>

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### Introduction

In order to improve pitch pine (*Pinus rigida* MILL.) which has long been extensively used for reforestation in Korea by combining the cold hardiness of pitch pine and the rapid growth rate and better timber quality of loblolly pine (*P. taeda* L.), hybridization program has been carried out using pitch pine as seed parent and loblolly pine as pollen parent for nearly twenty years since 1954.

F<sub>1</sub>-hybrid seeds were mass produced by large scale controlled pollination, test plantations of pitch-loblolly hybrid pine were then laid out with pitch pine check, and from these test plantations the F<sub>2</sub> wind pollinated seeds were produced in a commercial scale. And through controlled pollinations, F<sub>2</sub>-hybrid seeds and *P. rigida* X F<sub>2</sub> back cross hybrid seeds were also produced.

The growth performances of these hybrids have been examined at different geographic localities. From the results obtained so far, the strategy for increasing the superiority of X *Pinus rigida taeda* hybrid in the advanced generations and the means of mass production of hybrid seeds were discussed.

### Selection of The Parent

As it has been clearly demonstrated that the hybrid performance of X *Pinus rigida taeda* differ distinctly due to the seed source of the parent, it is essential to select the best seed source of the parent in the pitch-loblolly hybridization program. On an average, the pollen parent of the best seed source (New Jersey) gave 24.4 percent more growth than the pollen parent of the poorest seed source (Florida) in the height growth of the F<sub>2</sub>-hybrid mainly due to the better cold hardiness of the New Jersey source (Fig. 1).

And, as it is also proven that the combining ability of parental species differ due to the individuals of the parent even within the same seed source, it is also needed to select the best parent individuals giving the highest com-

binning ability within the selected seed source at the outset of utilizing desirable F<sub>2</sub>-hybrid.

In the mass production program of pitch-loblolly hybrid pine, young commercial pitch pine plantations, 8 to 10 years in age, were used as maternal stands flowered when small

### 5 years after planting

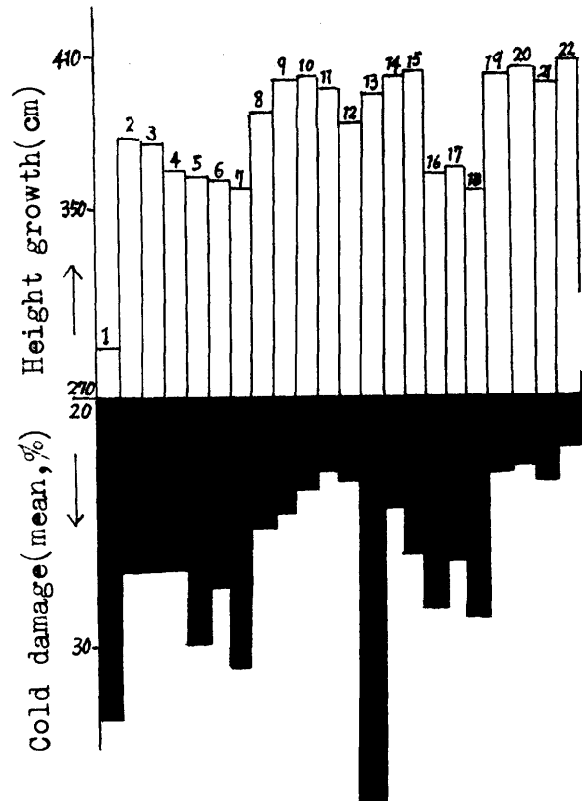


Fig. 1. — Histogram showing height growth and cold damage of X *Pinus rigida taeda* hybrid of different pollen sources in the field at five years after planting. 1-3: Fla., 4-7: Tex., 8-9: Miss., 10-15: N.C., 16-18: Arkansas, 19: Va., 20-22: N.J.

<sup>1)</sup> A dedication article to the anniversary issue of *Silvae Genetica* in honour of professor LANGNER.

Table 1. — Percentage of fertile seed yield.

Crossing	Period	Number of pollen source used	Number of conelet pollinated	Mean percentage of conelet drop (%)	Mean percentage of fertile seed yield (%)
<i>P. rigida</i> × <i>rigida</i>	1968—1970	22	1,175	60.52	81.94
× <i>P. rigida.taeda</i> -F <sub>1</sub>	1954—1970	86	55,450	74.12	27.83
× <i>P. rigida.taeda</i> -F <sub>2</sub>	1961—1970	14	5,929	54.56	50.03
× <i>P. rigida.taeda</i> -F <sub>1</sub> × wind	1962—1966	27	—	—	92.41
F <sub>1</sub> × <i>P. rigida</i>	1959—1970	6	2,075	62.80	45.32

and so could be bagged and pollinated from ground or short ladders. 20,000 to 30,000 pollination bags were mounted on these maternal stands every spring for controlled pollination. And, due to the variation among the individuals of those plantations, the hybrid progenies produced were of considerable variation.

For the production of hybrid seed of uniformity, clone gardens in which clones of selected individuals of selected seed sources of parent species are planted in separate by clones are to be established to facilitate the controlled pollination between the selected parental combinations.

#### Seed Setting and Performances of Hybrids

Since 1954 numerous control pollinations of *Pinus rigida* × *taeda* have been conducted every year for experimental purposes using *Pinus rigida* plantations, 8 to 10 years in age, as maternal stands and pollinating it with the pollen of *Pinus taeda* introduced from various seed sources of *Pinus taeda* in U.S.A. and from these pollinations various amount of *P. rigida* × *taeda*-F<sub>1</sub> hybrid seeds were produced.

Along with those pollinations, *Pinus rigida* × *taeda* F<sub>2</sub> crossings and × *Pinus rigida.taeda* F<sub>1</sub> × *P. rigida* back crossing were also conducted using × *Pinus rigida.taeda*-F<sub>1</sub> plantations established in various localities of the country as maternal stand and pollinating these with the pollens of × *Pinus rigida.taeda*-F<sub>1</sub> produced by *Pinus taeda* of different seed sources for the F<sub>2</sub> crossings and with the pollen of *Pinus rigida* for the back crossings.

With those crossings, conelet drop and seed yield were observed. And, at the same time, the seed yield from × *P. rigida.taeda*-F<sub>1</sub> × wind was also observed for comparison.

And, as seen in the following table, the fertile seed yield was highest in the F<sub>1</sub> × wind as well as *P. rigida* × *rigida*, and the F<sub>2</sub> gave much higher fertile seed yield than the F<sub>1</sub> did.

The test plantations of × *Pinus rigida.taeda*-F<sub>1</sub> which were laid out in different geographic areas of south Korea with the check of *Pinus rigida* started coning from the age of around six. In those test plantations, × *Pinus rigida.taeda*-F<sub>1</sub> and *Pinus rigida* were planted in each four to nine row plot with alternate replicates.

It was presumed therefore, that the seed produced from the F<sub>1</sub> plots of those test plantations could be the F<sub>2</sub>-seed mixed with the F<sub>1</sub> × *P. rigida* back cross seed in some extent. And, as it was found that the progenies of the F<sub>1</sub> wind pollinated seed (F<sub>1</sub> × wind) reveal promising growth performance in their early age, the F<sub>1</sub> × wind seeds are now being produced in a commercial scale.

The growth performances of those hybrids in the field are differ according to the climatic condition of the area.

In the test plantation of Kwangjoo area located in the southern latitude where the winter is mild enough causing no cold damage to the all hybrid types tested, both of the F<sub>1</sub> and the F<sub>2</sub> are distinct in growth superiority and no

actual difference in the mean growth rate between the F<sub>1</sub> and F<sub>2</sub> was recognizable (Table 2). And, the fact that the coefficient of variation of the stem volume was not much larger in the F<sub>2</sub> than in the F<sub>1</sub> seemed to indicate that the F<sub>2</sub> is not inferior at all to the F<sub>1</sub> for the practical use in the southern area, i.e. south of the latitude of 35° 00' N.

And, in around the middle latitude of south Korea where the winter is not as mild as Kwangjoo area too, both of the F<sub>1</sub> and the F<sub>2</sub> outgrow *P. rigida* by far. But the F<sub>2</sub> was suffered by cold damage a little (Table 3) and was apparently inferior to the F<sub>1</sub> in the stem volume growth. And, it seemed that the unfavorable winter cold had depressed the growth of the segregated components of the F<sub>2</sub> which are close to the type of *P. taeda*, the pollen parent. However, the fact that in both of DBH and hight growth the F<sub>2</sub> is outgrown by the F<sub>1</sub> only by a little and the coefficient of variation of the stem volume is not much larger in the F<sub>2</sub> than in the F<sub>1</sub> seemed to indicate that the F<sub>2</sub> is utilizable as much as the F<sub>1</sub> in the area of around the middle latitude of 35° 50' N.

And, even in the plantation of Yongin (Table 4) located in the northern latitude of south Korea, the inferiority of the F<sub>2</sub> in growth is not so distinct and the coefficient of variation of the stem volume is not particularly larger in the

Table 2. — Growth performance at the Kwangjoo test plantation (11 year old) (Latitude: 35° 09' N)

Species	Stem Volume (cm <sup>3</sup> )			Cold damage
	Mean	ratio	C.V.	
<i>P. rigida</i>	12.498	100	0.34	0
× <i>P. rigida.taeda</i> -F <sub>1</sub>	37.087	297	0.43	0
× <i>P. rigida.taeda</i> -F <sub>2</sub>	37.082	297	0.52	0
F <sub>1</sub> × wind	40.632	325	0.45	0

The difference between the species is significant at 1% level.

Table 3. — Growth performance at the Wanjoo test plantation (10 year old) (Latitude: 35° 49' N)

Species	Volume (cm <sup>3</sup> )			Cold damage
	Mean	ratio	C.V.	
<i>P. rigida</i>	6,837	100	0.74	0
× <i>P. rigida.taeda</i> -F <sub>1</sub>	29,672	434	0.51	0
× <i>P. rigida.taeda</i> -F <sub>2</sub>	27,180	398	0.63	3.5
F <sub>1</sub> × wind	18,344	268	0.50	0

The difference between the species is significant at 1% level.

Table 4. — Growth performance at the Yongin test plantation (11 year old) (Latitude: 37° 15' N)

Species	Volume (cm <sup>3</sup> )			Cold damage
	Mean	ratio	C.V.	
<i>P. rigida</i>	17,138	100	0.72	0
× <i>P. rigida.taeda</i> -F <sub>1</sub>	36,973	216	0.61	8.6
× <i>P. rigida.taeda</i> -F <sub>2</sub>	34,766	203	0.67	12.0
F <sub>1</sub> × wind	22,602	132	0.59	10.0

The differences between the species are significant at 1% level

F<sub>2</sub> than in the F<sub>1</sub>, thus indicating that in the low land around the northern latitude of south Korea, too, the utilization of the F<sub>2</sub> for the F<sub>1</sub> is quite feasible.

From above facts, it was concluded that in the pine hybridization program aiming at the combining of desirable traits of different species based on the high combining ability between species, F<sub>2</sub> can be utilized favorably in the area where the environment is favorable.

### Mass Production of Hybrid Seed

#### 1). Through controlled pollinations

Controlled pollinations in the interspecies crossings generally yield less seed per cone than wind pollination due to genetic incompatibility between parents, unadequate pollen handling, failure of pollination in the most receptive stages and over heating female flowers by pollination bag.

In the interspecies crossings, interspecies crossability is the major factor affecting the seed yield.

Genetic incompatibility can be overcome by using pollens mixed with mentor (compatible) pollen, the killed pollen by gamma-irradiation (STETTLER 1968) or more successfully by using pollens mixed with recognition pollen, the killed pollen by freezing and thawing (KNOX, WILLING and PRYOR 1972).

In mass controlled pollination of *Pinus rigida* × *taeda*, synthetic sausage casings, 20 cm × 50 cm in size, were used as pollination bag and pollination was effected by using hypodermic syringe which is fitted with rubber bulb and pollen agitator as recommended by Duffield.

Two to three pollinations were needed for each bag to complete pollination as the female flowers do not all reach the receptive stage simultaneously. On an average, 0.3cc (0.1 gram) of pollen were used for each pollination bag to complete the pollination and sixty seven mandays were needed to pollinate 10,000 pollination bag.

The pollen amount was not important factor affecting hybrid seed yield showing the fact that from 0.3cc to 1.0cc per bag gave no significant difference in seed yield provided the viability of pollen was not too low. Thirteen sound seeds were obtained per cone on an average.

The cost for the hybrid seeds by the controlled pollination was low enough and the mass production of hybrid seed through large scale controlled pollination was economically feasible in the 1950s, however the increased labour cost in recent years made this unfeasible.

#### 2). Through open pollination

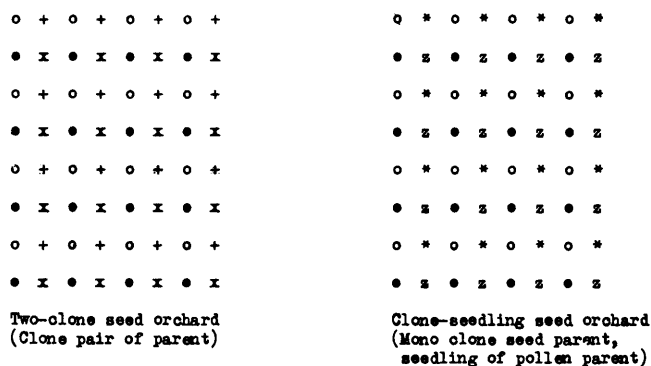
##### a. F<sub>1</sub>-hybrid seed orchard

Hybrid seed production through open pollination in a F<sub>1</sub>-hybrid seed orchard in which both parent species interplanted is most desirable. However, seven to ten days discrepancy in flowering between *Pinus rigida* and *P. taeda* has unabled this scheme. And, in order to overcome this barrier, early flowering pitch pine and late flowering loblolly pine were selected to lay out several biclonal (two-clone) seed orchards with the clone-pairs of parents which are synchronous in flowering and to use the bulked seeds from the biclonal seed orchards for planting (Fig. 2).

Interplanting of early flowering mono clone of pitch pine with the seedlings of selected loblolly pine forms an alternate way of producing hybrid seeds through open pollination. In the later case, seeds can only be collected from the seed parent of mono clone.

##### b. From the F<sub>1</sub>-test plantations

F<sub>1</sub> wind pollinated seed wich is to be the F<sub>2</sub>-seed mixed



key : o Seed parent clone to be removed at thinning  
 ● Seed parent clone to be preserved  
 x Pollen parent clone to be removed at thinning  
 + Pollen parent clone to be preserved  
 z Pollen parent seedling to be removed at thinning  
 \* Pollen parent seedling to be preserved  
 (Planted at 4m x 4m)

Fig. 2. — Hybrid Seed Orchard.

with the F<sub>1</sub> × *P. rigida* back cross seed has been producing in a commercial scale from the F<sub>1</sub>-test plantations laid out with pitch pine check, and the F<sub>2</sub> seeds are being produced through open pollination from those F<sub>1</sub>-test plantations from which pitch pine check were removed.

Thus, the F<sub>1</sub>-test plantations are playing roles as one type of hybrid seed orchard.

### Increasing the Superiority of the Hybrid in the Advanced Generation

As the level of the superiority of a hybrid for trait combination is depended upon the combining ability between parents as the case of *Pinus rigida* × *taeda*, a modification of the reciprocal recurrent selection suggested by Comstock et al is recommendable in developing advanced generation of breeding population for increasing the superiority of the hybrid.

Under this modification, two groups of tree selected among two original populations of parental species based on the superiority of the trait to be improved form the two foundation sources, A (seed parent species) and B (pollen parent species). Selected trees from source A are outcrossed to randomly chosen several individuals of source B as tester. Similarly selected trees from source B are outcrossed to randomly chosen several individuals of source A as tester through controlled pollination in a prepared clone bank in which clones of selected trees from both parental source populations are planted altogether.

When the test cross evaluation data become available, a group of tree of source A which reveals superior test cross performance is intermated to produce source A<sub>1</sub> (seed parent species). Similarly, a group of tree of source B which reveals superior test cross performance is intermated to produce source B<sub>1</sub> (pollen parent species).

A<sub>1</sub> and B<sub>1</sub> then serve as the two parental source populations for new cycle of selection of hybrid parent.

And, it is no doubt that the A<sub>1</sub> × B<sub>1</sub> should produce hybrid of increased superiority due to the increased species

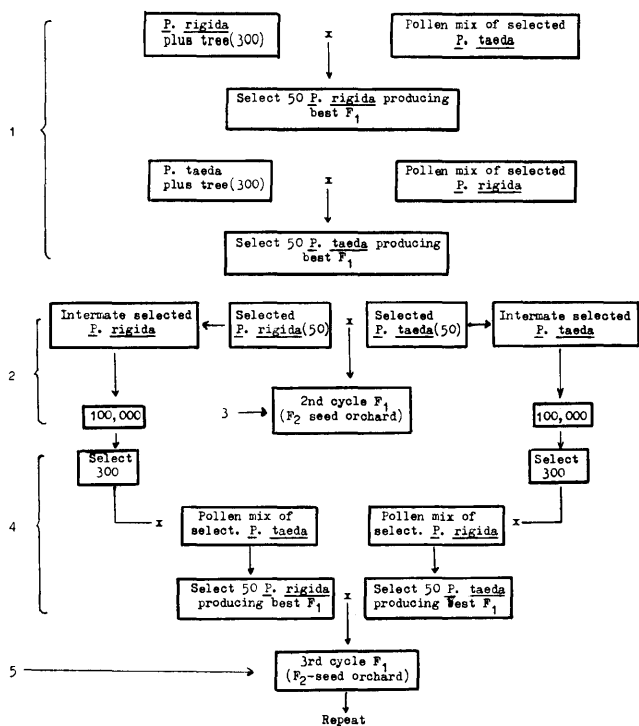


Fig. 3. — The Scheme of the improvement of interspecies hybrid through reciprocal recurrent selection.

combining ability between  $A_1$  and  $B_1$ . Therefore,  $(A_1 \times B_1)$ - $F_1$  hybrid progenies shall be produced by controlled pollination and then  $F_2$  shall be mass produced by open pollination of the  $F_1$  for practical use (Fig. 3).

Thus, (1) reciprocal recurrent selection for the parental species — (2) production of  $F_1$  through controlled pollination between the selected breeding populations of the parents — (3) mass production of  $F_2$  seeds through open pollination between the  $F_1$  progenies for commercial use would be the future strategy for the improvement of  $\times$  *Pinus rigitaeda* hybrid in the advanced generation.

#### Summary

Based on the 20 years experiences on  $\times$  *Pinus rigida*. *taeda* hybridizing programme, following facts were concluded.

1. The hybrid performance differs significantly due to the seed source of the parent and to the individual of the parent within the same seed source.

2. As the superiority of the  $F_1$  was not reduced at all in the  $F_2$  and the  $F_2$  gives much higher fertile seed yield than  $F_1$ , the utilization of  $F_2$  seeds for practical use is recommendable for the low land region of south Korea.

The  $F_1$  wind pollinated progeny from the  $F_1$ -plantation with pitch pine as check is rather cold hardier than the straight  $F_1$  progeny in the northern region of south Korea.

3. The feasibility of mass production of hybrid seed through controlled pollination depends upon the wage rate and the crossability between the parents.

4. For mass production of hybrid seed through open pollination, two-clone seed orchards which are constituted of clone pairs of parent which are synchronous in flowering, or interplanting seed parent of mono clone with the seedlings of selected pollen parent are recommended.

5. For increasing the superiority of the hybrid and its mass production in the advanced generation, developing breeding population of the parents through reciprocal recurrent selection followed by production of  $F_1$ -hybrid progenies by controlled pollination between selected parents and leading it to a  $F_2$ -seed orchard for mass production of  $F_2$  seed for practical use is recommended.

Key words:  $\times$  *Pinus rigitaeda*, Hybrid seed orchard,  $F_1 \times$  wind seed, Reciprocal recurrent selection.

#### Zusammenfassung

In Kreuzungsprogrammen mit *Pinus rigida*  $\times$  *Pinus taeda* und umgekehrt, während der letzten 20 Jahre, konnten folgende Erfahrungen gemacht werden: 1. Die Wachstumsüberlegenheit der  $F_1$  verringert sich bei der  $F_2$  nur wenig. Die  $F_2$  hatte höhere Saatguternten mit mehr keimfähigen Samen als die  $F_1$ . Für eine Massenproduktion von Saatgut in Samenplantagen werden Klonpaarungen mit synchroner Blütezeit empfohlen.

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