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Interspecific Hybridization in Pines in the Subsection *Sylvestres* LOUD

By

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Summary : This paper is a progress report on an interspecific hybrid[3] study within *Pinus* subsection *Sylvestres* LOUD. from 1971 to 1983. Sound seeds from 28 crosses were obtained, and those from 26 of the crosses produced some plantable seedlings. It was concluded that there are greater possibilities to make crossings between Asiatic species than in other combinations except in few cases, but all crosses can produce some seeds. At five years of age, the hybrid vigor in height growth was observed in all four combinations which were made with a *P. thunbergii* PARL. female parent. The best one was a *P. thunbergii* × *P. massoniana* hybrid. The experiment was also successful in creating new varieties with resistance to the pine-wood nematode, *Bursaphelenchus xylophylus* (STEINER and BUCHRER) NICKLE. Through resistance and preference tests made by artificial inoculations with nematodes and feedings by the vector insect, *Monochamus alternatus* HOPE, respectively, four hybrids of all cross combinations were recognized as being considerably resistant to the nematodes, but significantly there was no difference in the preference of the vector among the hybrids. From these results, the future role of interspecific hybridization is discussed, and it is suggested that hybridization can play an important role together with other breeding methods in pine tree improvement.

1. Introduction

This species hybridization work in the *Pinus* subsection *Sylvestres* LOUD. began in 1971 at the Kanto Forest Tree Breeding Institute in Ibaraki Prefecture, Japan. This subsection, which includes 19 species[2], is fairly-well distributed naturally around the world in northern latitudes. Many hybridizations have been attempted at several research institutes, especially in the United States[3][7][17][25].

In Japan, except in Hokkaido, there are two important pine species, *P. densiflora* SIEB. and ZUCC. and *P. thunbergii* PARL. These species cover mainly montane and costal areas as secondary forests. It is seems that genetic influences have pointed towards their extinction and their loss of genetic variation by imperceptible degrees compared with continental species for the following two reasons : ① their natural populations have been influenced by urban development and other human activities, as well as sever insect damages, since the dawn of history, and ② these two endemic species have had no chance to produce any introgressive hybridizations

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(3) The terms "hybrid" and "interspecific hybrid" have been used in this paper. However, Dr. W. B. CRITCHFIELD, USA, has suggested that we use the term "putative interspecific hybrid", pointing out that in the work at Placerville, California, at least 20 percent of the offspring obtained from interspecific crosses turn out to be of the maternal species and not hybrids. While we attempted to establish hybrid identity by means of non-pollination and morphological tests of the crossed offspring, we were not able to obtained full verification in this regard. Hereinafter, our use of the term "hybrid" includs some putative hybrids.

with other crossable pine species because they have been isolated from other *Sylvestres* species of the Eurasian continent. Although we cannot say so definitely, this general tendency seems logical. Based on this viewpoint, we consider it necessary to make breeding plans, throughout the field of interspecific cross-breeding, to produce new breeding materials for future programs. Therefore, this work was done to enrich the genetic variation of the domestic pines by means of tnterspecific crosses between these domestic pines acting as the females and the other species of *Sylvestres*.

The cross combinations were limited to this subsection's species in this experiment because it has been reported by some of the literatures on pine crossability that considerably greater success is obtained with crosses within the same subsection than between species of different subsections (Wright[24][26]).

With the assessment of each hybrid, we should attach importance to the characteristics of resistance to various types of damage, as well as to hybrid growth. Recently, much importance has been attached to the problem of Japanese pine species being severly attacked by the pine-wood nematode, *Bursaphelencus xylophilus* (Steiner and Buhrer) Nickle. Therefore, resistance tests dealing with this nematode are necessary.

2. **Materials**

The female parents used in these crossing experiments were planted as ramets of selected plus-tree clones of *P. densiflora* and *P. thunbergii* at the Kanto Forest Tree Breeding Institute, in central Honshu, Japan. These 15-year-old parent trees were maintained as low-crown types, 3 to 5 m high, by trimming and topping. The various pollens were obtained from foreign countries, especially from many of the pollen lots growing at the Eddy Arboretum, Institute of Forest Genetics, Pacific Southwest Forest and Range Experiement Station, US Department of Agriculture, in California. In addition, pollens from *P. nigra* Arnold and its varieties were obtained from Station D'amelioration Des Arbres Forestiers, Nancy, France, and *P. mugo* Turra and *P. heldreichii* Christ pollen was obtained from the Instituto Nazionale Per Piante Da Legno Giacomo Piccarole, Trino, Italy. Of the several Asiatic species, we collected ourselves *P. luchuensis* Mayr, *P. massoniana* Lamp., and *P. tabulaeformis* Carr. from domestic arboreta. The initial plan was to use two domestic species and 17 foreign species of *Sylvestres* to produce 34 hybrids. Actually we obtained pollen lots of 13 of the 17 foreign species, missing on *P. tropicalis* Moreleu, *P. hwangshanensis* Hsia, *P. insularis* Endl., and *P. merkusii* Jungh. and De Vriese. That of *P. heldrichii* was lost in a refrigerator accident at our lavoratory. Eventually, 28 combination crosses were made during the three years from 1972. Other combinations have not been achieved to date.

3. **Methods and Procedures**

Foreign pollen lots were obtained from the USA in 1972 and from Italy and France in 1972 and 1973. Domestic pollen was collected from nearly-ripe catkins in the spring of each year. All pollen lots were kept in cotton-stoppered vials at a relative humidity of 25% or less and at temperatures of 3° to 4°C until pollination time. The pollinations were made in 1972, 1973, and 1974 as shown in Table 1. Each year, branches bearing female strobili were bagged after emasculation and before flower-bud expansion, that is, *P. thunbergii* in the middle of April and *P. densiflora* in early May. As a general rule, the pollen was applied twice at an interval of three to four days. The pollination bags were removed when flower development

Table 1. Development of hybridization and establishment of test plantations

1972	1973	1974	1975	1976	1977	1978	1979
1st pollination,	Seed collection, 2nd pollination,	Seed storege, Seed collection, 3rd pollination,	Seed storage, Seed storage, Seed collection,	Sowing Sowing Sowing	Trans-planting or potting	Outplanting in Ibaraki and Shizuoka Prefectures	Outplanting in Chiba Prefecture

Table 2. Summary of pollination years

	Male parent	Female parent	
		Pinus densiflora	*Pinus thunbergii*
1	*Pinus resinosa*	1974	1974
2	*P. tropicalis*	Not yet	Not yet
3	*P. nigra*	1974	1974
4	*P. heldreichii*	Not yet	Not yet
5	*P. mugo*	1973, 1974	1973, 1974
6	*P. pinaster*	1974	1972, 1973, 1974
7	*P. halepensis*	1974	1974
8	*P. brutia*	1972, 1974	1973, 1974
9	*P. sylvestris* L.	1972, 1974	1973, 1974
10	*P. densiflora*	1972, 1973, 1974	1972, 1973, 1974
11	*P. thunbergii*	1972, 1974	1972, 1973, 1974
12	*P. massoniana*	1972, 1973, 1974	1972, 1973, 1974
13	*P. taiwanensis* HAYATA	1974	1974
14	*P. luchuensis*	1973, 1974	1973
15	*P. hwangshanensis*	Not yet	Not yet
16	*P. tablulaeformis*	1972, 1973, 1974	1972, 1973, 1974
17	*P. yunnanensis*	1972, 1974	1972, 1973, 1974
18	*P. insularis*	Not yet	Not yet
19	*P. merkusii*	Not yet	Not yet

reached the stage where scales had grown together and bracts no longer were visible. In the next fall of each pollination year, cones were collected, and the seeds were extracted at air temperatures. Seeds were cleaned with empty and full seeds separated by surface color, that is, pale brown seeds were deemed empty and dark ones were deemed sound. The sound seeds were stored dry in a refrigerator by sowing year.

In April 1976, all seed lots were sown directly into two replicated nursery seedbeds at a density of 1000 or less seeds per square meter. The germination percentage was estimated two months after sowing. Prior to transplanting, needle color was investigated in the fall and reported by the authors[20][21]. All 1-0 seedligs were transplanted into unreplicated transplant beds in the spring of 1977. At the same time, some were potted in a greenhouse for convenient transport to distant plantations. Permanent hybrid test plantation were established in 1978 and 1979 on the Pacific Coast side of central Honshu as shown in Figure 1. They are described as follows :

Fig. 1. Location of field test plantations.
No. 1 Kanto Forest Tree breeding Institute, Ibaraki Prefecture ; No. 2 Hamamatsu, Shizuoka Prefecture ; No. 3 Chiba, Chiba Prefecture.

No. 1. Kanto Forest Tree Breeding Institute, Kasahara-cho, Mito-shi, Ibaraki Prefecture.
Established in 1978; area 0.84 ha; altitute 30 m; annual precipitation 1312 mm; annual mean temperature 14.0°C.

No. 2. Hamamatsu Forest District Office, Shinozukayama National Forest, Shizuoka Prefecture.
Established in 1978; area 1.72 ha; altitute 80 m; annual precipitation 2132 mm; annual mean temperature about 14.0°C.

No. 3. Chiba Forest District Office, Kotagaya National Forest, Chiba Prefecture.
Established in 1979; area 1.03 ha; altitute 110～210 m; annual precipitation 2032 mm; annual mean temperature about 13～14°C.

The experimental layout was of incomplete randomized blocks with unequalsized plots, and 1–1 stock was planted at a 1.8 m×1.8 m spacing. It was possible to reach valid conclusions regarding the significance of differences of characteristics because most cross-combinations were planted in three or four replicated plots. After planting, the survival rates and the tree heights were estimated each fall from 1978 to 1983.

The resistance study dealing with the pine-wood nematode was completed in July, 1978. Hybrid families whose 1–1 transplants were produced in sufficient numbers, were selected and artificially inoculated with cultured nematodes in the greenhouse. *P. rigida-taeda* recognized as a hybrid higher-resistant to the nematode[16] was used as a check. At the same time, the 1–1 transplants of hybrids and their parental wind-pollinated offsprings were planted in an insectarium constructed of metal screening. Japanese pine sawyers (*Monochamus alternatus* Hope), the nematode vector, were placed in the insectarium in May. Two months later, the survival rate of the inoculation test and the number of bitten traces per plant were recorded. Hybrid resistance to the nematodes and preference of the vectors for the hybrids were compared in the fall. We published progress reports of both tests[10)20)].

4. Results

The procedure described in Tables 1 and 2 produced hybrid seeds from 28 combinations (30, when including three varieties of *P. nigra*). Table 3 describes data for the seeds of each hybrid used to establish a crossability index for each hybrid. Plantable hybrid seedlings were produced by 26 of the 28 crosses. Exceptions were *P. densiflora*×*P. pinaster* Ait. and *P. halepensis* Mill. The offsprings from these crosses have been growing in each locality as shown in Figure 2. Survival rates and height-growth indices at five years of age are summarized for all three experimental plantations as shown in Table 4. The results of the inoculation and feeding tests are shown in Figure 3.

1) Crossability patterns

A perusal of field records on controlled pollinations and cone collections indicates that there is little or no difference of cone set among pollen parents of all species, but there is a great difference in the number of sound seeds per cone, that is, in spite of intraspecific crosses of *P. densiflora* and *P. thunbergii* producing 10.4 and 10.3 as mean values, respectively, all interspecific crosses produced a fewer number, not more than 2.7 seeds with some producing less than 0.1. However, it is noted that there were no cases of all seeds being empty within any of the 28 interspecific crosses. These may be broken down as follows :

a. Intraspecific crosses of the two domestic pines produced more than 10 sound seeds per cone.

b. Interspecific crosses, between *P. densiflora* and *P. nigra*, *P. mugo*, *P. thunbergii*, *P. massoniana*, *P. luchuensis*, and *P. tabulaeformis*; and between *P. thunbergii* and *P. nigra*,

Table 3. Data for crossability computation and their indices

	Pollen parent species	①Sound seeds per cone (number)		②Sound seeds obtained (number)		③Germination capacity in seed bed (%)		④Crossability** index	
		P. densiflora	*P. thunbergii*	*P. densiflora*	*P. thunbergii*	*P. densiflora*	*P. thunbergii*	*P. densiflora*	*P. thunbergii*
1	*P. resinosa*	0.1	0.1*	7	4	66.7	100.0	0.7	0.3
3	*P. nigra*	1.6	0.8	1,980	653	72.2	77.2	13.4	6.8
5	*P. mugo*	2.0	0.1	689	171	74.5	94.0	16.5	1.0
6	*P. pinaster*	0.1*	0.1*	14	63	0.0	81.8	0.0	0.6
7	*P. halepensis*	0.1*	0.3	2	7	0.0	88.0	0.0	2.6
8	*P. brutia*	0.2	0.1*	127	12	84.3	93.3	1.9	0.4
9	*P. sylvestris*	0.1	0.1*	28	16	85.5	83.5	1.1	0.3
10	*P. densiflora*	10.4	1.4	32,465	505	87.2	77.8	100.0	12.0
11	*P. thunbergii*	1.1	10.3	249	31,144	89.5	84.8	10.9	100.0
12	*P. massoniana*	0.8	1.0	349	603	82.3	84.2	6.9	10.0
13	*P. taiwanensis*	0.1*	0.1*	40	28	82.0	97.0	0.5	0.5
14	*P. luchuensis*	2.7	2.3	29	442	71.0	79.8	20.9	20.6
16	*P. tabulaeformis*	2.3	0.8	2,308	820	88.2	80.4	22.4	7.3
17	*P. yunnanensis*	0.1*	1.7	34	1,588	73.7	79.6	0.8	15.7

* Fewer than 0.1

** Mean values from $I = [(Sh \times Gh)/(Sf \times Gf)] \times 100$.

P. densiflora, *P. massoniana*, *P. luchuensis*, *P. tabulaeformis*, and *P. yunnanensis* FRANCH. produced 1 to 3 sound seeds per cone.

c. Fewer than 0.2 sound seeds per cone were produced by the other combinations, and nine of them produced less than one per 100 cones.

The total number of sound seeds from all the interspecific crossings ranged from 2 to 2308. Germination percentages were above 70% in all combinations except *P. densiflora*×*P. pinaster* and *P. halepensis*. There were no significant differences among them.

To express an indicator (*I*) of crossability, we used the following calculation for each interspecific combination of each pollinaton year :

$$I = [(Sh \times Gh)/(Sf \times Gf)] \times 100$$

where : Sh = Number of sound seeds per cone of hybrid.

Gh = Germination rate of the above seeds.

Sf = Number of sound seeds per cone of the intraspecific cross of the female species in the same pollination year.

Gf = Germination rate of the above seeds.

The result of the calculation is denoted in Table 3 as the mean value of all results. There was a great difference among combinations of species. All interspecific crosses have indices of less than 23, corresponding to their female parents' intraspecific indices of 100. Among interspecific crosses, crossability indices of *P. densiflora*×*P. nigra*, *P. mugo*, *P. thunbergii*, *P. massoniana*, *P. luchuensis*, and *P. tabulaeformis*; and *P. thunbergii*×*P. densiflora*, *P. massoniana*, *P. luchuensis*, and *P. yunnanensis* were high at more than 10.

2) Growth performances in field-test plantations

The second object of this study was to investigate the pattrns of growth in the field. The

Table 4. Summary of growth performance in field-test plantations

Pollen parent species		Seedlings obtained for out-planting		Survival rates after out-planting		Height growth index at 5 years	
		P. densiflora	*P. thunbergii*	*P. densiflora*	*P. thunbergii*	*P. densiflora*	*P. thunbergii*
		Number	Number	%	%		
1	*P. resinosa*	7	4	100	100	74*	93*
3	*P. nigra*	1,164	390	96	83	75	85
5	*P. mugo*	530	126	98	96	91	83
6	*P. pinaster*	0	30	—	42	—	83
7	*P. halepensis*	0	4	—	60	—	77*
8	*P. brutia*	85	6	84	100	96	102*
9	*P. sylvestris*	14	1	100	100	90*	101*
10	*P. densiflora*	776	376	99	100	100	116
11	*P. thunbergii*	174	709	98	99	99	100
12	*P. massoniana*	260	441	98	96	91	118
13	*P. taiwanensis*	26	2	100	90	88*	99*
14	*P. luchuensis*	25	312	100	85	94*	114
16	*P. tablaeformis*	1,683	354	98	89	88	104
17	*P. yunnanensis*	12	780	100	79	82*	91

* Value excluded from consideration because of lack of enough samples.

** Indices were calculated by following formula ;

Index = mean value of (height of hybrid/height of intra-cross female species of each plantation) × 100 mean survival rates of the hybrids in the three plantations showed no significant differences except for the two hybrids *P. thunbergii*×*P. pinaster* and *P. mugo*. At present we do not find any of unsound hybrids among those planted because mortalities at five years of age are less than 20% just as in Japanese domestic pine plantations. Height-growth curves of the three test plantations during the 5-year period are shown in Figure 2. The figure confirms that many of the hybrids with a *P. thunbergii* female parent are taller than those with a *P. densiflora* female parent with but few exceptions. There are highly significant differences at the 5% level between female parents in many cases, especially in crosses with male parents of *P. densiflora*, *P. massoniana*, and *P. tabulaeformis*. However, in other cases it was disruption occurred from differences of age, male parent, and plantation environment.

On the basis of the above growth performances, a standard index was established for evaluation of all hybrid growth by the following formula :

$$\text{Index} = \frac{\text{Mean height-growth of each hybrid}}{\text{Mean height-growth of intraspecific offspring of the female parent}} \times 100 .$$

The index was calculated for each test plantation hybrid, and a mean value for each hybrid was derived from those of all plantations. The indices at five years of age are shown in Table 4. The results confirm that there are some vigorous hybrids in the case of crosses with

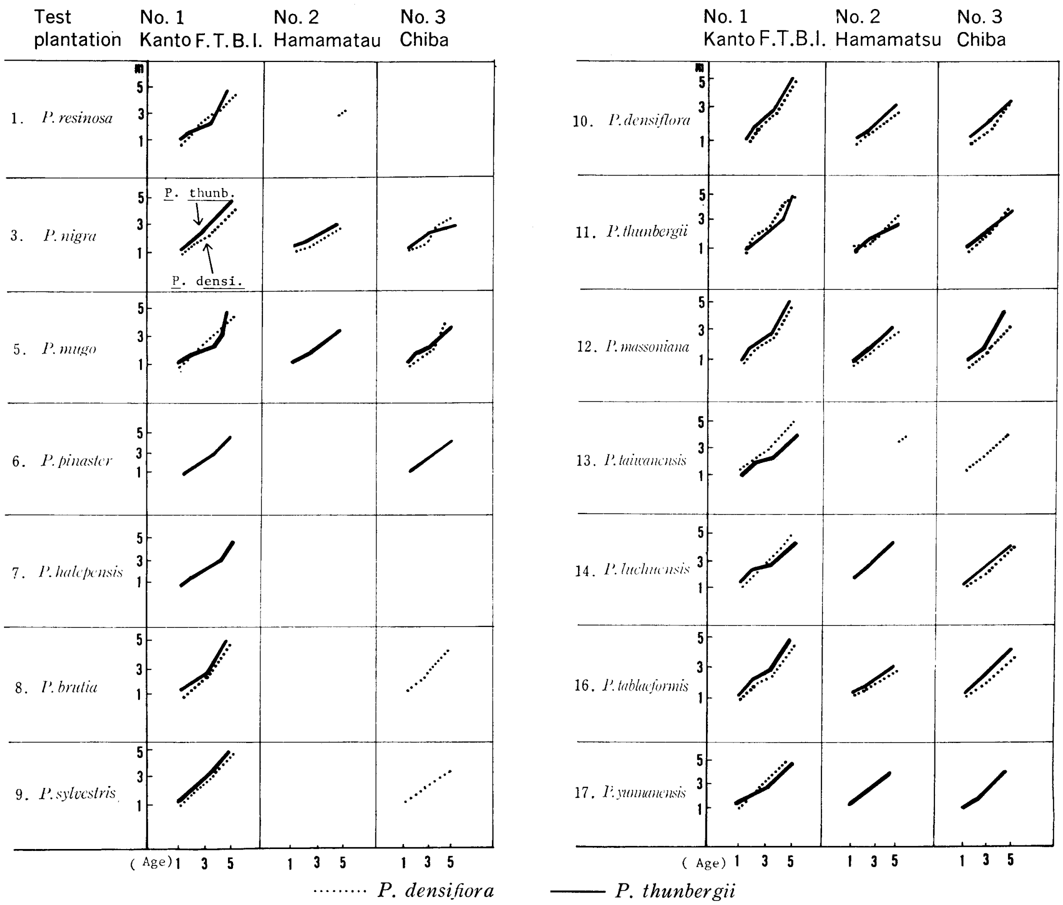

Fig. 2. Height growth performances of each plantation

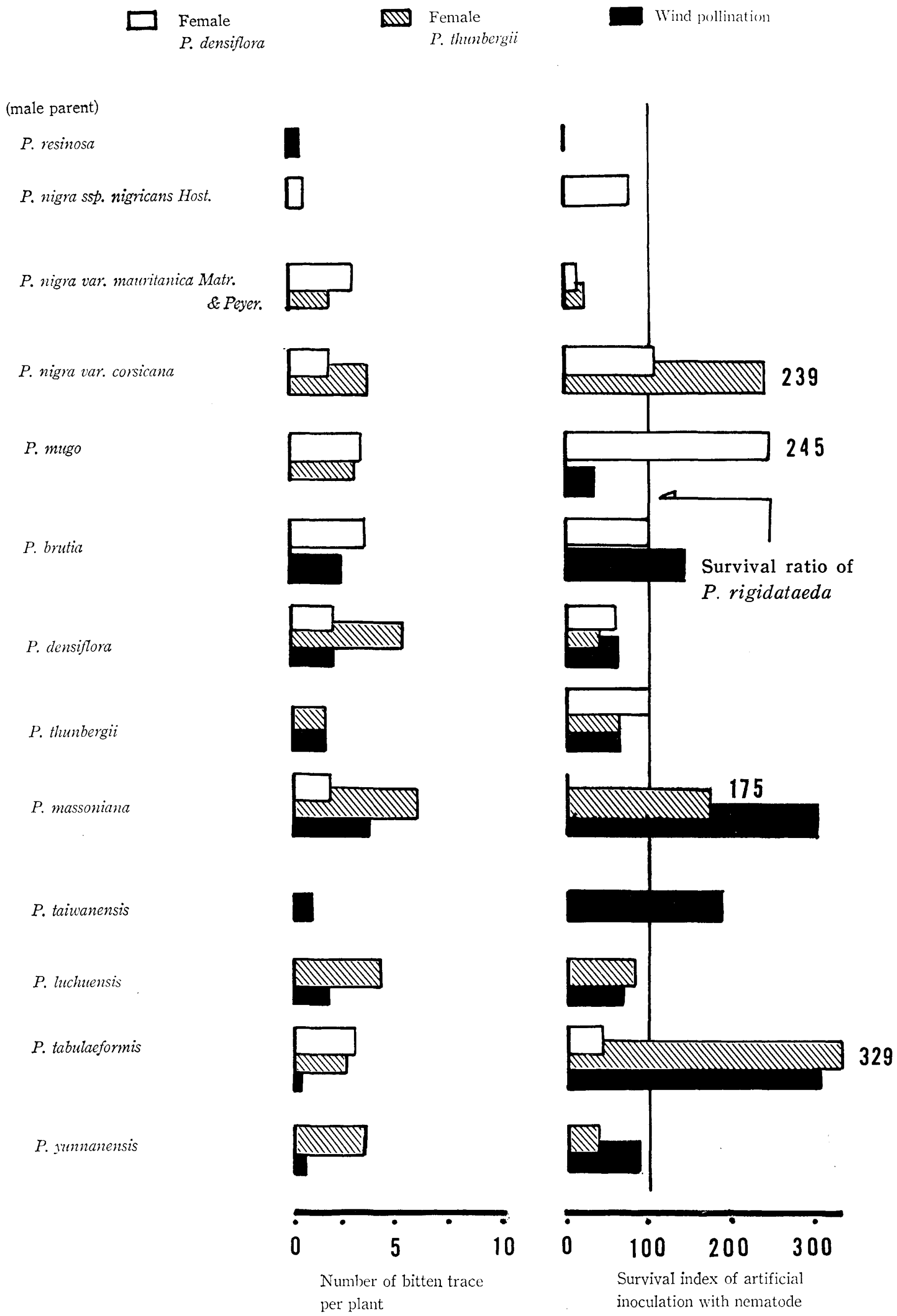

Fig. 3. Differences of feeding by insects and of resistance to nematodes

a *P. thunbergii* female, but there are none that are vigorous in the case of crosses with a *P. densiflora* female. There is a significant separation between the two groups, above 100 and below 100, respectively. The good growth group exceeding index 100 includes four interspecific hybrids, namely, *P. thunbergii*×*P. densiflora*, *P. luchuensis* and *P. tabulaeformis* with *P. thunbergii*×*P. massoniana* the best. The distinctive statistical differences are evident in only one case, that between the *P. thunbergii* intraspecific (100) and the *P. thunbergii*×*P. massoniana* cross (118) within the good-growth group. It is clear that all good-growth hybrids (indices more than 100) are derived from parents of Asiatic species which are distributed in Japan and China.

3) Resistance against disease and insect attack

Concerning resistance to pine-wood nematodes, results of both experiments are presented in Figure 3. The survival rates estimated by means of inoculation tests were significantly different among cross combinations. The rates of the following four interspecific hybrids showed extremely-high survival rates, and there are significant differences at the 5% level between each hybrid and the check, *P. rigida-taeda.*

P. densiflora×*P. mugo*
P. thunbergii×*P. nigra* var. *corsicana* Loud.
P. thunbergii×*P. massoniana*
P. thunbergii×*P. tabulaeformis*

These rates were more than 1 to 3 times that of the check hybrid. However, the insect feeding preference test indicated that there was no significant difference in the number of bitten traces per plant among all the hybrids tested and between the wind-pollinated or intraspecific offsprings of female parents and their hybrids.

The results were not indicative of the degree of resistance of the hybrids, but we assumed that the resistance of hybrid offsprings of *Sylvestres* subsection are very different. On the other hand, we repeated the same inoculation tests for several exotic species and their hybrids, cooperating with many collaborators, and reached a conclusion that *P. massoniana* and *P. thunbergii*×*P. massoniana* have strong resistance to the pine-wood nematode[9)10)16)20)].

Regarding diseases, needle cast, *Lophodermum pinastri* (Schrad.) Chev. was found in the No. 1 test plantation and may has been an influence on the growth of *P. thunbergii* and its crossing with *P. massoniana* and *P. yunnanensis*; it was not the cause of mortality.

5. Discussion and Conclusions

The 90-odd species of *Pinus* in the world are grouped into three subgenera, five sections, and 15 subsections by Critchfield and Little[2)]. *Sylvestres* is one subsection of 15 and includes 19 species of two-needled pines. It is classified in the subgenus *Pinus* and in the section *Pinus.* The species belonging to *Sylvestres* are distributing widely in Eurasia and North America and include ten Asiatic, six European, one Eurasian, and two American species as denoted in Figure 4 from Little and Critchfield[13)]. Wright[24)~26)] reported that a large percentage of the crosses between species in the same subsection have succeeded, whereas almost all crosses between species from different subsection have failed. Our results confirm the former because there were no cases where cross-combination seeds were all sterile. Perhaps some of these seed lots included a few none-hybrid seeds as stated in footnote 3). However, the crossability patterns within the subsection show great differences among male parents and between female parents with indices ranging from less than 1 to 22.4. In Table 3 and Figure 4 the indices are

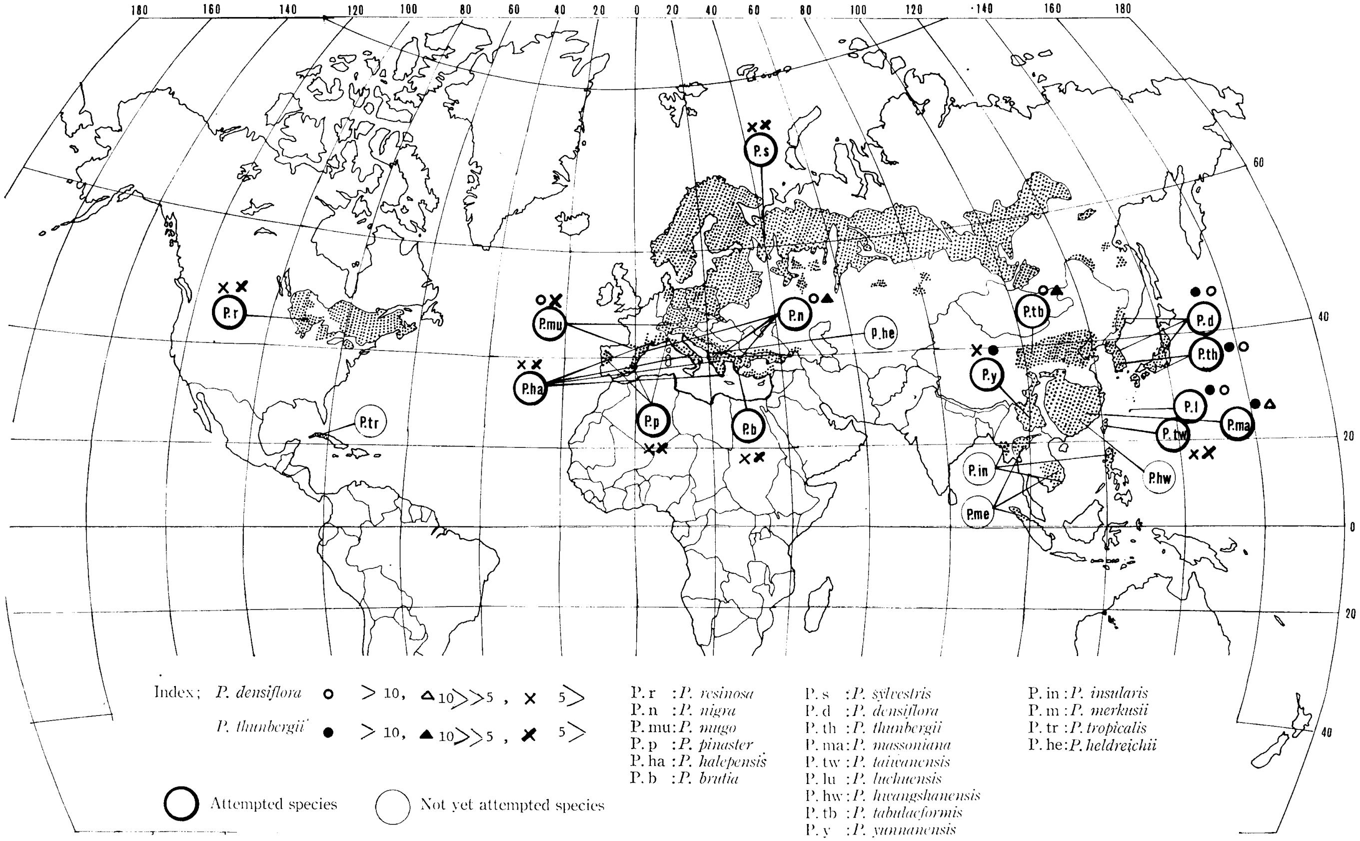

Fig. 4. The distribution of *Sylvestres* subsection and the crossability of its species (Distribution map from Little and Critchfield[13])

ranked according to the crossabilities of all hybrids. In the case of Japanese domestic species as the female, a cross with a species distributed relatively nearby has a larger index than that of a cross with a species having more distant distribution. Crosses of Japanese species with seven Asiatic species have indices larger than 5 except in three cases, *P. densiflora*×*P. taiwanensis* and *P. yunnanensis*, and *P. thunbergii*×*P. taiwanensis*, but in cases of crosses with other more distant species, only three of 14 cross-combinations are such larger indices. However this tendency does not always occur, for example, the indices of *P. densiflora*×*P. nigra* and *P. mugo* are larger than 10. According to our results, that empirically all crosses produced some sound seeds, it appears that crosses within *Sylvestres* have considerable high-crossabilities. Mirov[14], who discussed the role of genetic barriers and incompatibility, said that some pine isolated for a long time have not had any differences in their genetic mechanism that would have made them incompatible to their relatives which were distant both in space and in time. Following his argument, it can be assumed that isolated distributions of *Sylvestres* species could has resulted in the weaker development of genetic barriers and in higher crossability. The hypothesis coincides with the above mentioned two crosses. With regard to reciprocal crossings, the crossability of *P. densiflora*×*P. thunbergii* is larger than that of its reciprocal cross. This might be related to the difference of flowering season of the two species, that of *P. densiflora* being considerably later than that of *P. thunbergii*.

In general, hybrid vigor or heterosis[5)18] has been proven in many tree species at a young age. In most cases, it seems that the data have not been sufficient regarding their dominance and which were evaluated at too young an age. It is difficult to judge the effect of hybrid vigor on forest tree species. Even if their juvenile growth was vigorous, it does not always follow that this characteristic links with the yield volume at the final cutting-age. In our results, the hybrid vigor of height growth was recognized in four hybrids limited to crosses with a *P. thunbergii* female parent. Especially, *P. thunbergii*×*P. massoniana* which was best, exceeded that of three other vigorous hybrids, and there was a significant difference at the 1% level in the height growth between the hybrid and its parents' offspring under these experiemental conditions at five years of age. At present, it is too early to discuss their adaptability of hybrid vigor because they are too young.

Regarding the resistance to pine-wood nematodes examined in the 1-1 transplant stage, five hybrids were recognized as highly resistant varieties, but the characteristic does not always associated with height growth. The *P. thunbergii*×*P. massoniana* hybrid (so-called "Wakamatsu") may be a suitable combination because it was observed to have two valuable traits, that is, rapid growth in the juvenile stage and resistance to wood nematodes.

Toda[23] suggested that interspecific hybrids have no ecological niche in the ecosystem and that the new varieties would not be allowed to exist there for a long time. Duffield and Snyder[6] stated that adaptability to many ecological settings and pest-resistance are of considerable importance in hybridization. Brown[1] also concluded from many previous reports on this subject that hybridization is not so useful a tool in forest-tree improvement. When the niche which a species has made naturally for itself is eliminated by some biotic factor, it should be replaced by other silvicultural developments that may be produced by breeding countermeasures. For this purpose, our efforts will be payed for by the production of any fundamental materials for resistance breeding, such as pest and disease resistance or cold hardiness, and especially so if a characteristic indispensable to forestry, such as pine-wood nematode resistance, does not exist within a population of the planting species.

Interspecific hybridization would become a useful tool for combining specific attributes[1)7)8)]. Of course, it is a very rare case in past experiments where hybrid seedlings themselves become actual planting stock, but it is possible for them to be the foundation for future breeding. Therefore, we support the suggestion that hybridization has an important place, together with other methods of tree improvement, in coping with two problems, adaptability and pest resistance (DUFFIELD and SNYDER[6)]).

In general, hybridization does create new gene combinations within either parent species. These available genes can be captured by each through interspecific hybridization. In this case, it is better if the characteristics can be controlled by a few dominant major genes. Moreover, it is hoped that the objective chracteristics aimed at by cross breeding can be achieved with short-term tests at a younger age. On this points, the *P. thunbergii*×*P. massoniana* hybrid meets such conditions; the resistance to pine-wood nematodes can be controlled by major-genic inheritance[10)] and the inoculation test is achieved easily in the 1-1 seedling stage[16)]. The hybrid is the most interesting one from our experiments from the viewpoint of practical use because it has three valuable traits, good viability of seeds, high resistance to pine-wood nematodes, and fast growth in the juvenile stage.

The fact that resistance to pine-wood nematodes is controlled by a single, dominant gene was suggested by one of our previous reports[10)]. Of course, there also are several deficiencies such as yellowish-colored needles, wide crowns, forked stems, and other inferior traits, but what we need at present are the nematode resistance and good juvenile-growth. In Japan, the hybrid seeds resulting from artificially controlled pollination are at present providing one of the countermeasures for pine-forest damage, and the resulting seedlings will be used as planting stock in regions where pine trees are damaged severely by the pine-wood nematode.

Hance, there is a difficult problem in that such controlled pollination may be very expensive in producing hybrid seeds. In the future, until more effective methods of propagation, such as mass pollination or mass propagation, that is, tissue culture or micro-propagation[11)12)], can be developed, such hybridization can play an important role for pine-tree improvement.

Supplemental Note

The identifies of many of our hybrids were verified several times, but the identifies of some of them have not been verified completely to date. Dr. W. B. CRITCHFIELD suggested that for some our interspecific combinations we should provide good evidence of their identity before we should be convinced that we succeeded in obtaining hybrid offspring from the crosses, namely for

P. densiflora×*P. resinosa* AIT.,
P. densiflora×*P. brutia* TEN.,
P. thunbergii×*P. resinosa*,
P. thunbergii×*P. pinaster*,
P. thunbergii×*P. halepensis*, and
P. thunbergii×*P. brutia*,

In our non-pollination tests, we obtained a few seeds in two of nine cases of non-pollinations, but only a very few germinable seeds were obtained in only one case with *P. densiflora*[19)]. Therefore, we assumed that contaminations with the pollens of female parent species were rare in our experiments. On the other hand, our morphological investigations on needle and winter-bud colors and forms in the 1-1 transplant stage convinced us that seven of our all combinations were true hybrids[21][22]. However, we agree with Dr. CRITCHFIELD's skepticism, and believe that in the future it will pay us to probe more deeply into hybrid identification by other methods for example, isoenzyme and monoterpene analyses.

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Literature Cited

1) BROWN, A. G. : The role of the hybrid in forest tree breeding. Document, IUFRO Genetics-SABRAO Joint Symposia, Tokyo. C-1(1), (1792)

2) CRITCHFIELD, W. B., and E. L. LITTLE, JR. : Geographic Distribution of the pines of the world US Dept Agri Misc Pub 991, 97 pp., (1966)

3) CRITCHFIELD, W. B. : Crossability and relationships of the closed-cone pines. Silvae Genet **16** : 89～97, (1966)

4) CRITCHFIELD, W. B. : Impact of the pleistocene on the genetics structure of the North American conifers. *In* Proc 8th No Am For Bio Workshop, (R. M. LANNER ed) Utah State Univ. 196 pp., (1984)

5) DOBZHANSKY, T. : Nature and origin of heterosis. *In* Heterosis (J. W. GOWEN ed) 218～223. Hafner, New York, (1964)

6) DUFFIELD, J. W. and E. B. SNYDER : Benefits from hybridizing American forest tree species. J For **56**(11) : 805～815, (1958)

7) FOWLER, D. P. and C. W. YEATMAN : Symposium on interspecific and interprovenance hybridization in forest tree. Frederiction, N. B. Proc 14th Meet Can Tree Improv Assoc Part 2. 142 pp., (1973)

8) FOWLER, D. P. : Population improvement and hybridization. Unasylva **30**(119～120) : 21～26, (1978)

9) FURUKOSHI, T. and M. SASAKI : On the studies of hybridization among the species belong to *Sylvestres* subsect.—A capacity for hybridization. Trans 90th Annual Meet Jap For Soc. (1979)**

10) FURUKOSHI, T. and M. SASAKI : Hybridization among pine species belong to subsect. Sylvestres and their resistance to wood nematode including future cross-breeding strategy. Kanto Forest Tree Breed Inst Annu Rep. (16) : 195～225, (1984)*

11) KLEINSCHMIT, J. A. : A program for large-scale cutting propagation of Norway spruce. N Z J Forest Sci **4** : 359～366, (1974)

12) LIBBY, W. J. : Clonal option. Norsk Inst For Skogforskning, Norway. 32 pp., (1983)

13) LITTLE, E. L. and W. B. CRITCHFIELD : Subdivisions of the Genus *Pinus* (Pines). US Dept Agri Misc Pub. 1144, 51 pp., (1969)

14) MIROV, N. T. : The genus *Pinus*. Ronald Press, New York, 602 pp., (1967)

15) Nakai, I. and others : Studies on the cross-breeding of the pines (1). Interspecific pollination in *Pinus thunbergii*, and the cross-ability between *Pinus thunbergii* and the other several pines. Bull Kyoto Univ. Forests (39) : 125～143, (1967)*

16) Ohoba, K. and others. : Difference of survival ratios among pine half-sib families inoculated with pine-wood nematodes. Trans 30th Kyushu For Assoc 67～68, (1977)**

17) Righter, F. I., and J. W. Duffield : Interspecific hybrids in pines, a summary of interspecific crossings in the genus *Pinus* made at the Institute of Forest Genetics. J. Heredity **62**(2) : 75～80, (1951)

17) Righter, F. I. : Evidence of hybrid vigor in forest trees. Tree growth (T. T. Kozlowski, ed) Ronald Press, New York. 442 pp., (1962)

19) Sasaki, M. and T. Furukoshi : Interspecific hybridization among species of sub-section *Sylvestres* (1)—Crossability of each combination and their seed weight—. Kanto Forest Tree Breed Inst Annu Rep. (11) : 107～133, (1974)*

20) Sasaki, M. and T. Furukoshi : The initial performances of interspecific hybrids,—Cone setting, seed quantity, seedling growth and resistance for timber-nematodes—. Trans 87th Annu Meet Jap for Soc 183～184, (1976)**

21) Sasaki, K. and T. Furukoshi : On the study of the hybridization among the species belong to *Sylvestres* subsect. (II).—Phenotypic characteristics of hybrid seedlings at 1-0 stage. Trans 90th Annu Meet Jap For Soc 233～234, (1979)**

22) Sasaki, M. and T. Furukoshi : On the studies of hybridization among the species belong to *Sylvestres* subsect. (III).—Phenotypic characters of hybrid seedlings 1-1 stage—Trans 90th Annu Meet Jap For Soc 235～236, (1979)**

23) Toda, R. : Forest genetics up-to-date. Norin Syuppan Co., Ltd. Tokyo, 230 pp., (1979)*

24) Wright, J. W. : Species crossability in spruce in relation to distribution and taxonomy. Forest Sci **1** : 319～349, (1955)

25) Wright, J. W. and W. J. Gabriel : Species hybridization in the hard pines, series *Sylvestres*. Silvae Genet. **7**(4) : 109～115, (1958)

26) Wright, J. W. : Genetics of tree improvement. FAO, Rome, 399 pp., (1962)

* Japanese with English summary
** Only in Japanese

Sylvestres 亜節内の種間交雑に関する研究

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摘　要

本報告は，マツの *Sylvestres* 亜節内でアカマツとクロマツを母親として他の17種を組合せた種間交雑に関する研究のうち1971年から1983年までの研究成果をまめたものである。この研究では34組合せのうち28の組合せを実行し，これらすべてから健全種子を得ることに成功した。そのうち26の組合せからは山出し苗を得た。この結果から，交雑稔性はアジア産の種同士を組合せた場合には，その他の地方のものとの組合せよりも高い稔性が得られるという傾向を認めた。しかし，全く健全種子の得られない組合せはなかった。本州中部の太平洋岸で茨城，千葉，静岡の各県に1カ所ずつこれらの家系の試植地をつくり，生存率と樹高生長を測定した。その結果，生在率は全供試家系とも80%以上であり，アカマツ及びクロマツと比べても大差はなかったが，樹高生長には著しい差があった。この3試験地を通して，クロマツを母親にした4つの交雑家系の中にクロマツより生長の優れた雑種があったが，アカマツを母親として用いた交雑種にはアカマツより優れたものはなかった。この中で最も生長の旺盛なのはクロマツ×馬尾松（和華松と命名）であった。一方，1-1苗の段階でマツノザイセンチュウ抵抗性を人工接種により検定した結果は，4つの組合せで著しい抵抗性を示す雑種が見出された。また網室を用いてマツノマダラカミキリの食害状況を比較したところ，家系間には大差がなかった。これらの成果をもとに，林木の雑種強勢とマツ類の育種に対する種間交雑の役割について論議し，種間交雑は他の育種法と併用して特殊形質を導入するための手段として有効であることをのべた。

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