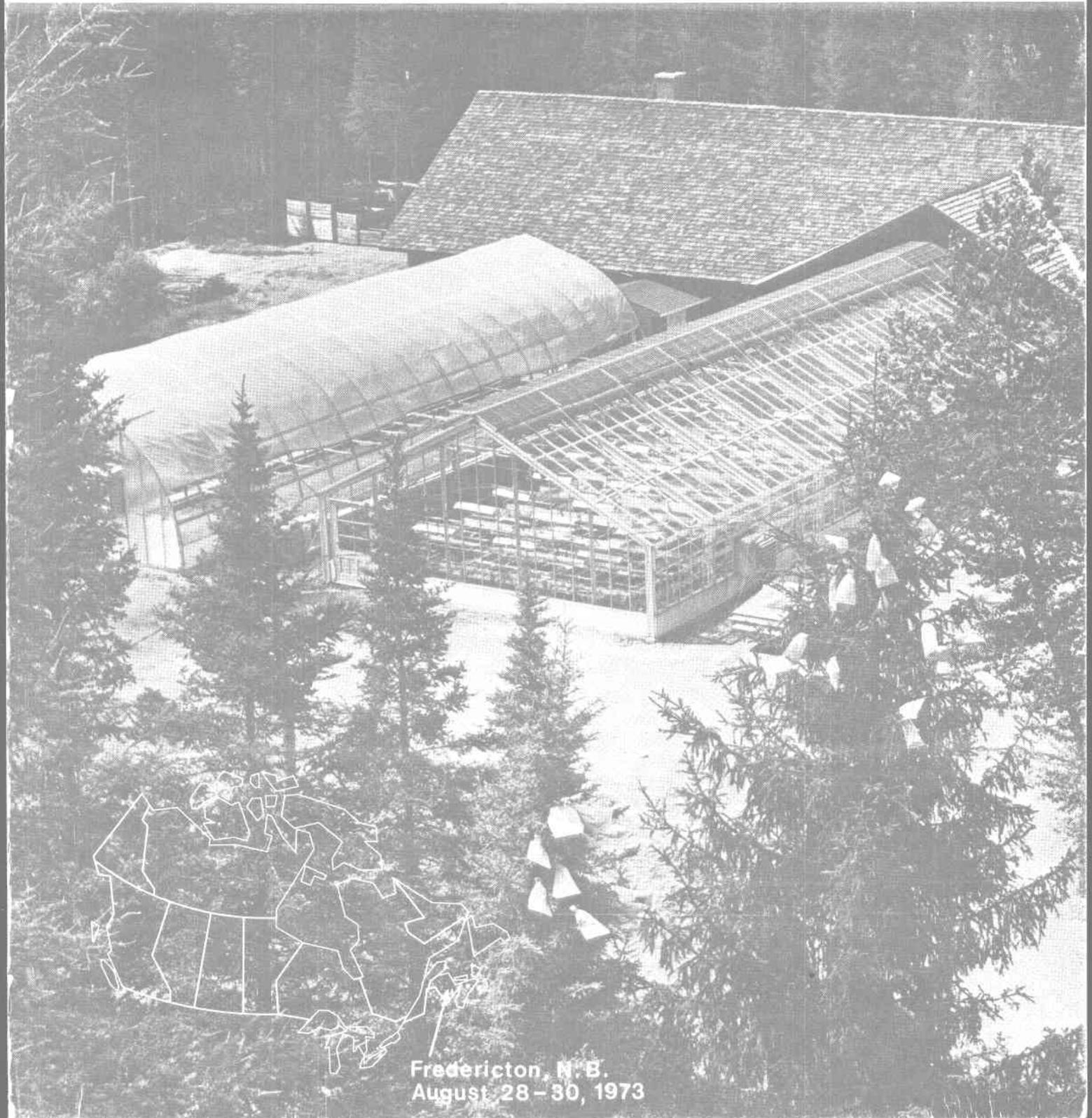


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Proceedings of the fourteenth  
meeting of the Canadian tree  
improvement association:  
Part 2

Comptes rendus de la  
quatorzième conférence de  
l'Association canadienne pour  
l'amélioration des arbres:  
Partie 2



Fredericton, N. B.  
August 28 - 30, 1973

PROCEEDINGS OF THE FOURTEENTH MEETING OF  
THE CANADIAN TREE IMPROVEMENT ASSOCIATION

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The Fifteenth Meeting of the Association will be held at the  
Petawawa Forest Experiment Station, Chalk River, Ont., in August 1975.  
Canadian and foreign visitors will be welcome. Detailed information will  
be distributed early in 1975 to all members and to others upon request.

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TO: Mr. K. Illingworth, Executive Secretary, Canadian Tree Improvement  
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PROCEEDINGS OF THE FOURTEENTH MEETING OF  
THE CANADIAN TREE IMPROVEMENT ASSOCIATION\*

PART 2

Symposium on  
Interspecific and Interprovenance Hybridization  
in Forest Trees

Fredericton, New Brunswick, August 28-30, 1973

Editors: D.P. Fowler and C.W. Yeatman

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\*Formerly the Committee on Forest Tree Breeding in Canada.

- Part 1 Minutes and Members' Reports was distributed to Association members and is available to others on request.
- Part 2 Symposium, Interspecific and Interprovenance Hybridization of Forest Trees, received worldwide distribution to persons and organizations actively engaged or interested in forest genetics and tree improvement.
- 1<sup>re</sup> partie Procès-verbaux et Rapports de membres distribués aux membres de l'association. Distribution générale sur demande.
- 2<sup>e</sup> partie Colloque sur l'hybridation interspécifique et interprovenances des arbres forestiers. Distribution à l'échelle mondiale aux personnes et organisations activement engagées ou intéressées dans la génétique forestière et l'amélioration des arbres.

Produced by the Canadian Forestry Service  
Department of the Environment for the  
Canadian Tree Improvement Association  
Ottawa, 1975

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Cover photograph: Research greenhouses and spruce pollination  
at the Acadia Forest Experiment Station, Canadian Forestry  
Service, Fredericton, New Brunswick

## INTRODUCTION

The participants in the Symposium on Interspecific and Interprovenance Hybridization in Forest Trees covered a range of topics; they said much about the promise, or lack of it, in interspecific and intraspecific hybridization; they talked about incompatibility barriers and the problems they create. But, no new workable schemes were presented to capitalize on specific combining ability on either the inter- or intraspecies level. Until that problem is solved, research on hybrid performance will remain more academic than practical.

What methods are now available in forestry to produce high-yielding progeny of specific crosses? Schemes in use include: 1) the tedious and expensive pollination of individual strobili or inflorescences by hand; 2) bicultural seed orchards composed of pairs of self-incompatible clones; 3) the use of the  $F_2$  produced in orchards of hybrid individuals. The control of flowering is another possibility and, although it is promising, technology is not sufficiently advanced to permit application. What is desired is a chemical agent to influence the sex ratio or, in the ultimate, a chemical emasculant so that all seed produced on treated clones will be the result of cross-pollination. Similar results might be achieved by physically locating seed orchards in climatic zones where pollen production of one of the parents is suppressed.

The most exciting possibility lies in vegetative propagation. Ernst Schreiner has for decades advocated vegetative propagation as a method of fixing and utilizing hybrid vigor. However, techniques now being developed by cell physiologists go beyond what even Ernst Schreiner prophesied. The techniques of the cell biologists not only offer the possibility of vegetative multiplication but could enable forest tree breeders to overcome incompatibility problems such as those mentioned in this symposium by Critchfield and by Kriebel. These techniques are tissue culture, cell culture, isolation of protoplasts and, finally, parasexual hybridization - a bypassing of sex that will overcome several of the barriers that prevent the production and utilization of hybrids.

In parasexual hybridization, undifferentiated cells of two species may be joined to produce an almost unlimited number of replicas of a specific hybrid plant. Only in the last few years has it been possible to isolate plant cells without cell walls, naked protoplasts. Eventually, such research culminated in the fusing of protoplasts from different species, the production of hybrids without crossing. Peter S. Carlson has already produced a tobacco hybrid by asexual means. The hybrid is similar in all respects to hybrids produced by sexual crosses between members of the same species. Other hybrids will soon be forthcoming, but extensive research will be required before the techniques can be applied to forest trees.

Because of the potential of parasexual hybridization, readers of this symposium on hybridization can be expected to react with a great deal more enthusiasm than was possible only a few years ago. Great changes are

on the horizon. Some of the participants were justifiably influenced by the present impracticality of hybrid production, and their assessment of cost-benefit ratios indicated that hybrids had little value. Others, more optimistic, stressed only the promise of hybrids, could they but be produced in quantity. Those interested in tree improvement are advised to evaluate the arguments carefully; to be skeptical but to retain an open mind. Future developments in what to forestry are still exotic fields may change our present evaluation of hybrids in a revolutionary way.

F. Thomas Ledig, Chairman  
Symposium on Interspecific and  
Interprovenance Hybridization in  
Forest Trees

# RECENT RESULTS OF INTERPROVENANCE CROSSES IN SWEDEN AND THE IMPLICATIONS OF BREEDING

Bo Nilsson

*Royal College of Forestry  
Stockholm, Sweden*

In an earlier paper (Nilsson and Anderson 1969) the peculiarities of the migration history of Norway spruce and Scots pine into Scandinavia are emphasized. This migration history is the basis of the hypothesis of genetic differences between populations of the two species that could result in heterosis when individuals from different populations are crossed.

There are two different research approaches to find the best combinations for practical use. One is to use pollen mixes from different provenances on mother trees from different populations. Another is to make individual crosses between trees from different populations. The first method is based on the assumption that the interracial hybrid variances are greater than the individual ones. The advantage of the second method is better information on individual tree variation that can be used as a basis for selection. The number of individual combinations needed in the second approach can be reduced by using a partial diallel crossing system.

This method was used in my research in provenance hybridization between 1954 and 1970. In the first three crossing experiments in spruce (1954, 1964 and 1967) a few Swedish mother trees were used to test many different foreign father trees; in the last experiment (1970) a partial diallel system was used in accordance with Hinkelmann and Stern (1960). In pine, a partial diallel crossing system (Andersson unpublished) was followed in 1966. The origins of the pollens used during the 16-year period, which include second generation material from southern Sweden, are presented in Fig. 1.

## NORWAY SPRUCE HYBRIDS

### *1954 Crosses*

Six female testers of central Swedish origin were crossed, the pollen used being collected from second-generation central European stands growing in southern Sweden (Nilsson 1958). Four field experimental areas and four pilot areas were established; the latter were studied until they were 8 years of age. The growth data of Swedish, foreign and hybrid combinations at this age, supplemented with data from international provenance experiments between latitudes 50 to 64°N, are summarized in Fig. 2. At age 8 years the hybrids are most promising for locations between latitudes 59 and 62°N, viz the region of origin of the northern parents. Studies in the four field experiments were continued longer. The last measurements in one of these experiments in central Sweden were made in 1971. The relative growth during the years

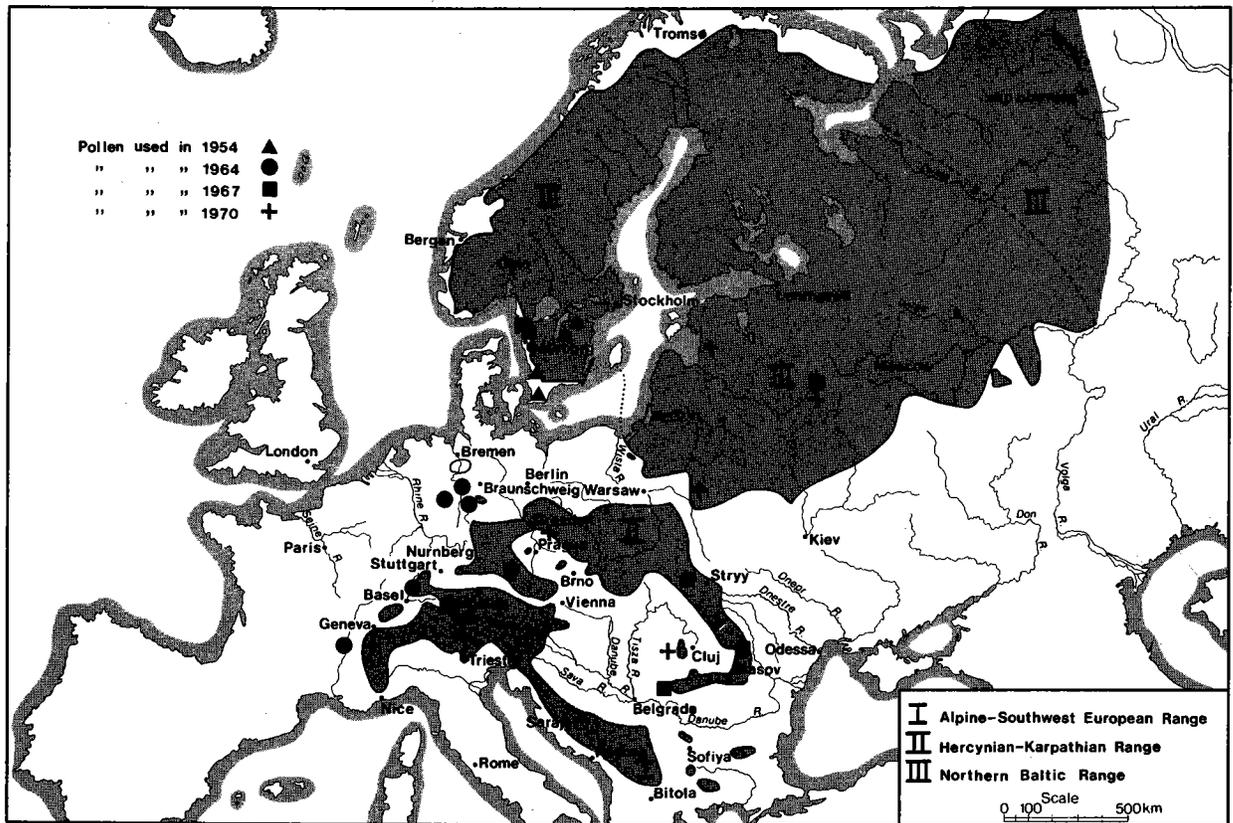


Figure 1. Range of Norway spruce in Europe (from Rubner 1960).

1956-71 for Swedish, foreign and hybrid combinations is presented in Fig. 3. The superiority of the hybrids over the northern provenances continues to be evident. The continued decline of the central European provenances is also of interest.

The decline of the central European provenances was thought to be due to a lack of hardiness. To test this hypothesis, the growth change between 1957 and 1971 in individual progenies in a 6 x 6 diallel system (Fig. 4) was studied. As can be seen from the figure, the foreign provenances (central European) were inferior as well as the hybrids with foreign male WG 45. The latter were the best in 1957 but the poorest in 1971, and both these hybrids and the foreign provenances were damaged by frost in 1956-57. The same hybrids also had relatively low dry matter content in their needles (Fig. 5) during the autumn and lower survival (Fig. 6). On the basis of the most recent data (Fig. 4), hybrids with the Swedish female M 19 performed best and also scored well in respect to branch length and stem straightness (Fig. 7).

The data from the field experiments, replicated in two main and two validation field sites, are of special interest. To date only one of the main experiments and the two validation experiments have been measured. This main experiment is located in central Sweden at 59°N. The validation experiments are at 61°N and 56°N. The 1971 growth data (Blomquist 1973, personal

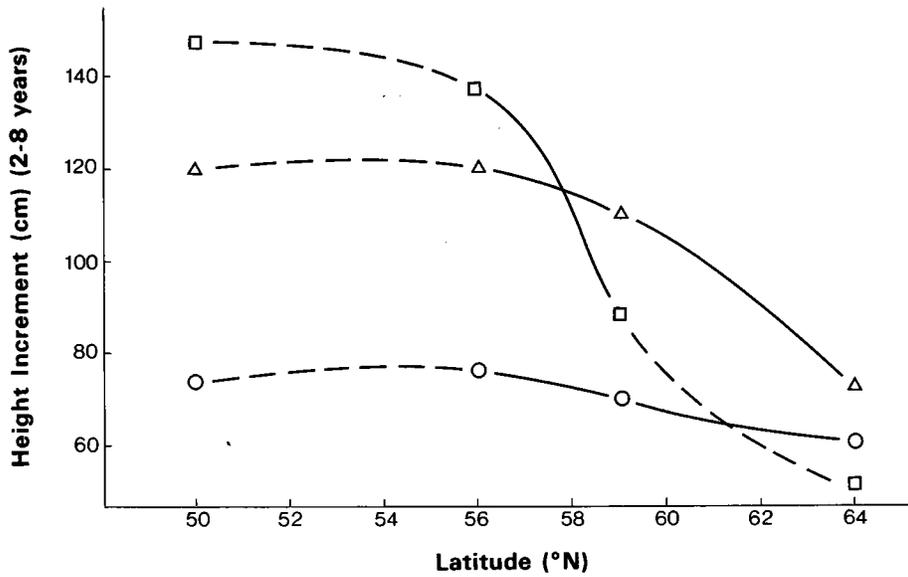


Figure 2. Interaction between latitude and height growth for Swedish (o), central European (□) and hybrid combinations (Δ) in Norway spruce pilot experiments at age 8.

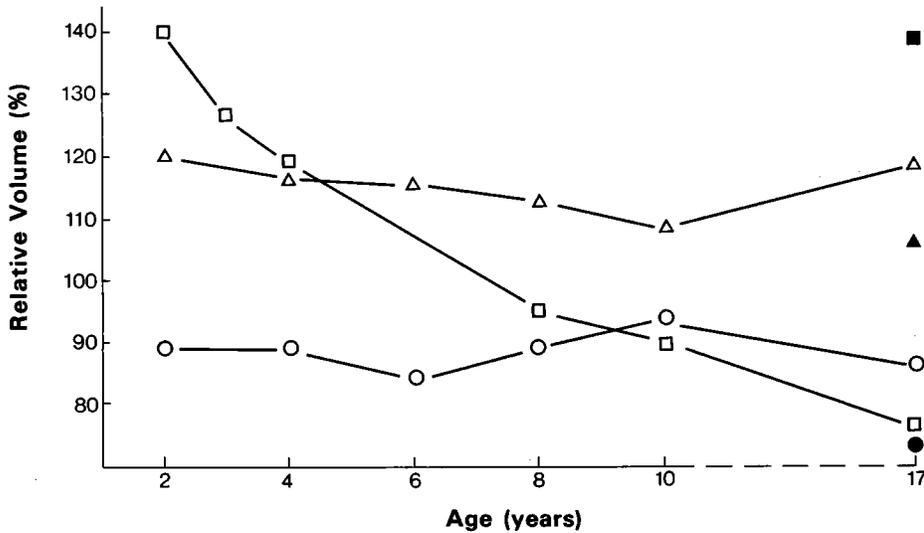


Figure 3. Interaction between age - height growth (age 2-10) and volume growth (age 17) for Norway spruce combinations at latitude 59°N. Filled symbols = latitude 56°N. Legend cf. Figure 2.

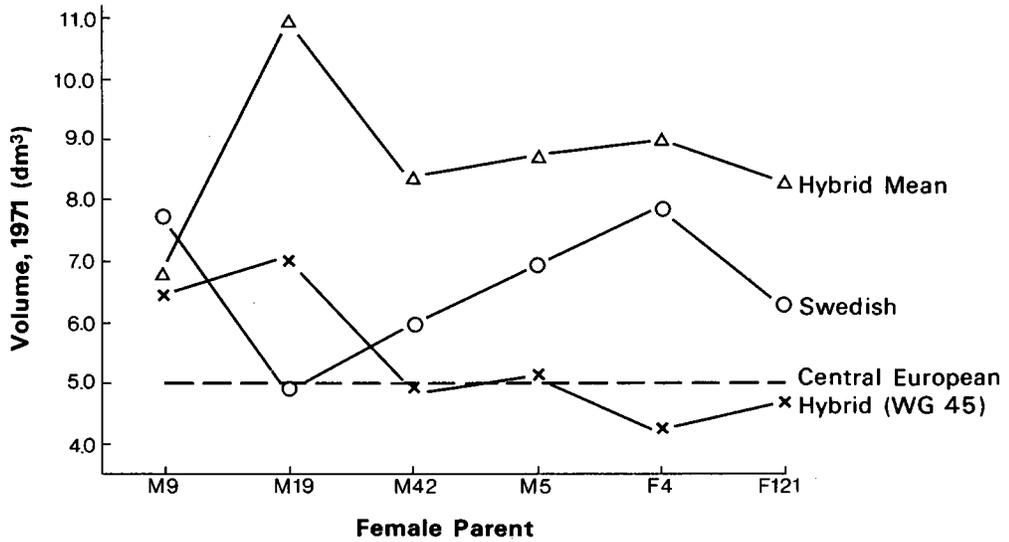
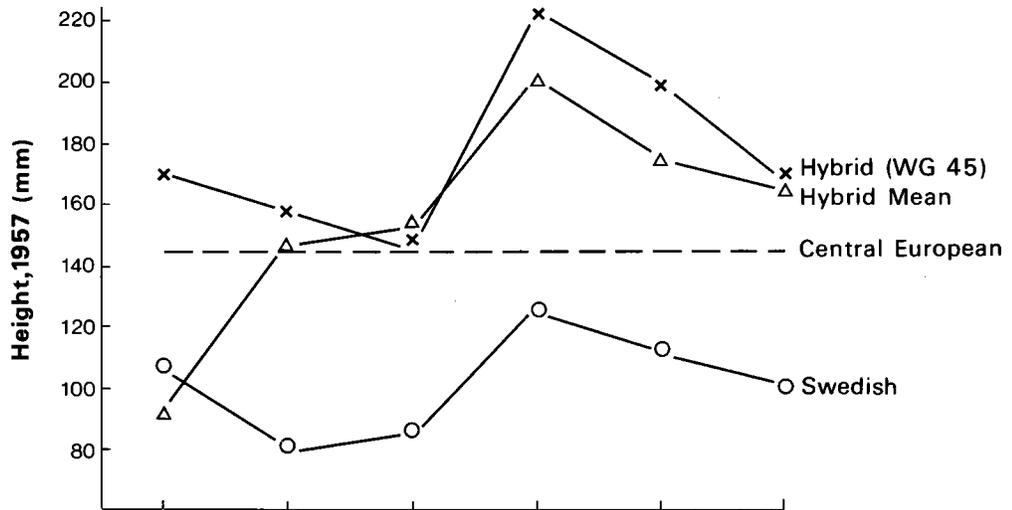


Figure 4. Height growth 1957 (age 3) and volume growth 1971 (age 17) for different Norway spruce combinations with six common female testers.

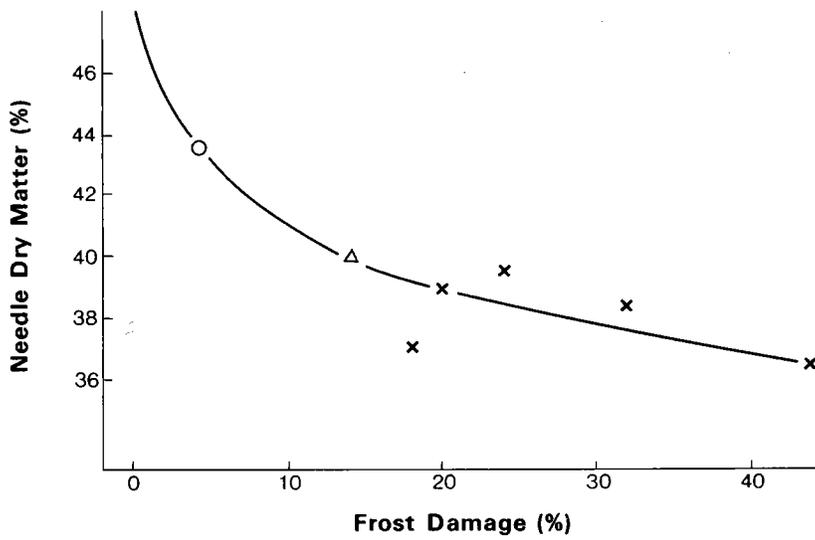
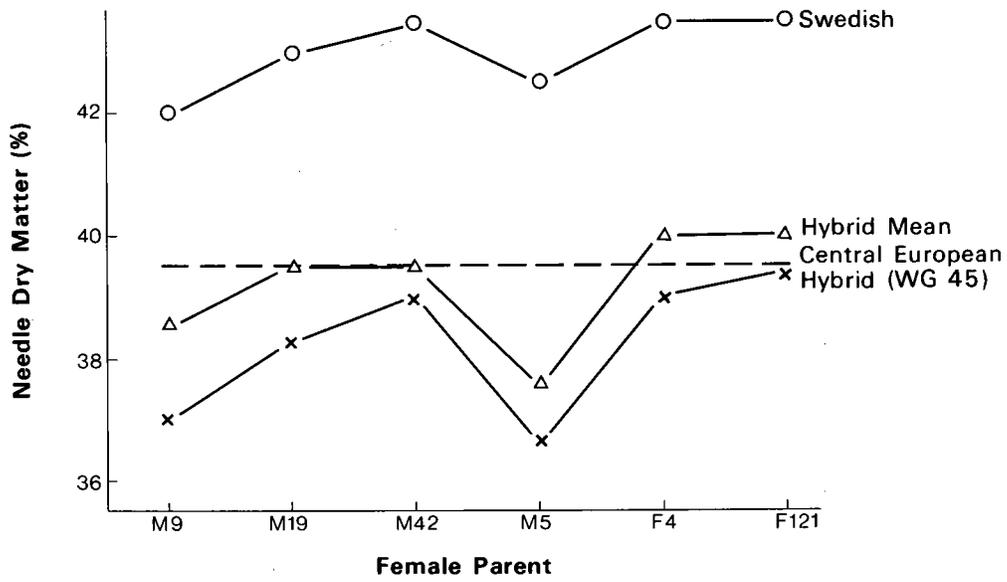


Figure 5. Relative dry matter content and frost damage 1957 for different Norway spruce combinations. Legend, cf. upper section.

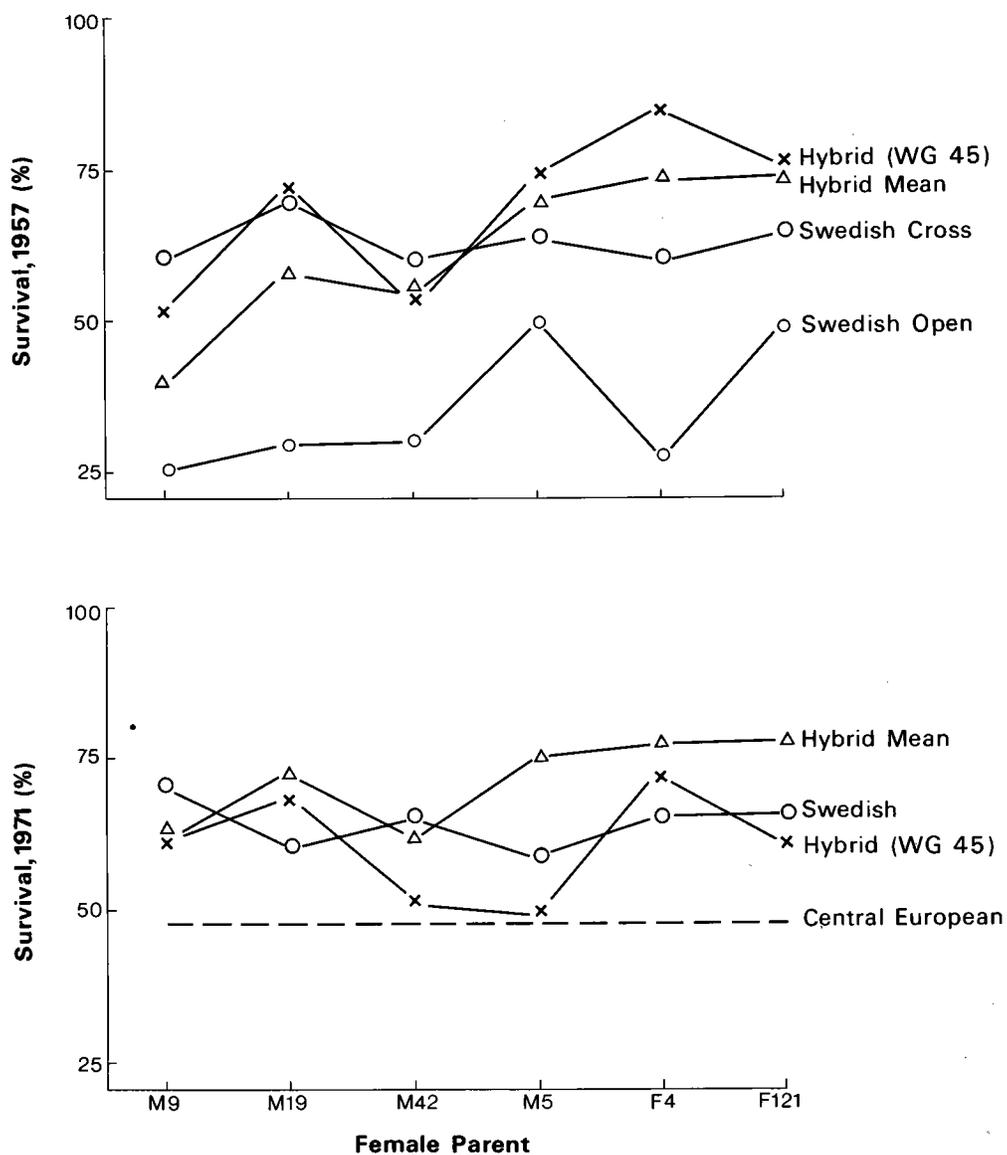


Figure 6. Survival in the nursery 1957 (age 3) and in the field 1971 (age 17) for different Norway spruce combinations.

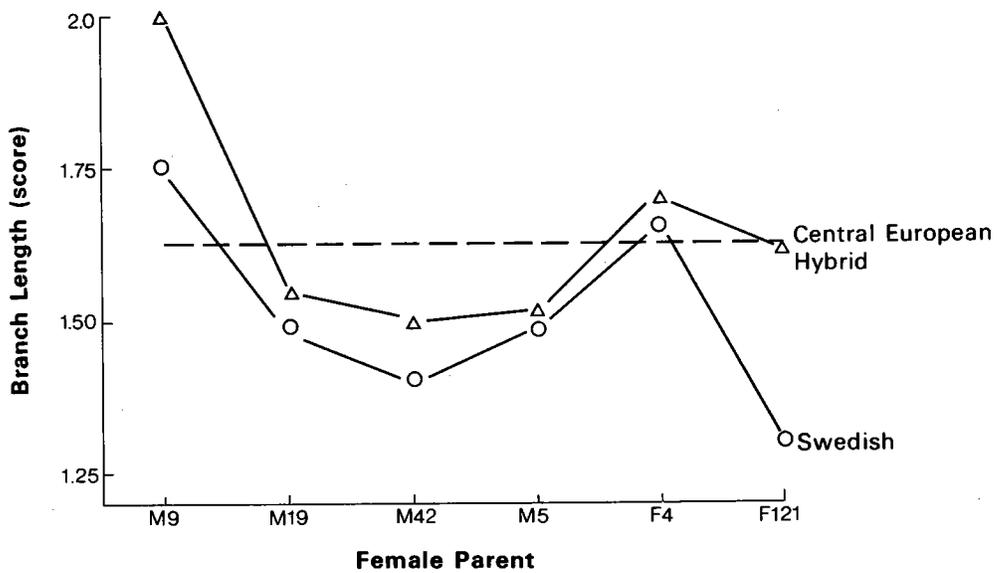
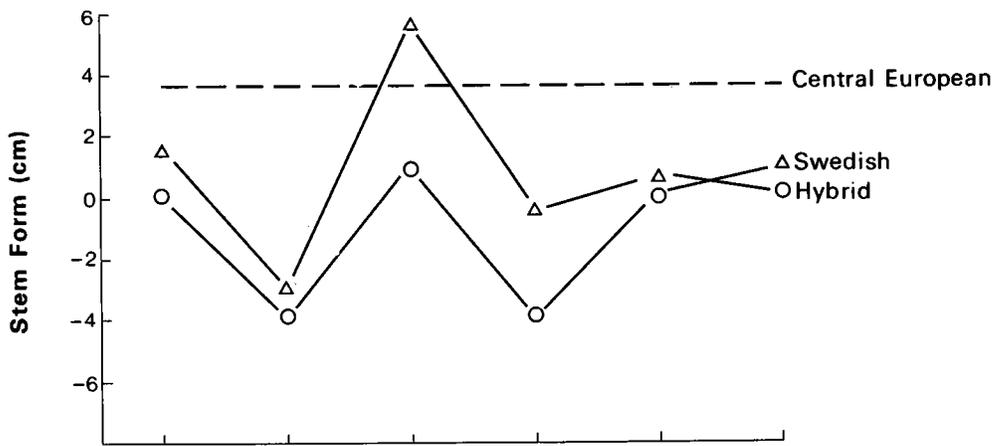


Figure 7. Trunk crookedness (scores 1 - 3) and branch length in relation to the function branch length on tree height for different Norway spruce combinations at age 17.

communication) from the northern validation site (61°N) correspond well with the data from the main experiment. The rank correlation coefficient between the different combinations is 0.86.

The ranks of the combinations in 1971 (age 17) in the main site in central Sweden and in the second validation site, situated in southern Sweden (56°N), are, however, quite the reverse (Werner 1972, personal communication). At the latter site the growth of the hybrids is intermediate and the picture looks quite similar to that at age 8 (Fig. 2). This result supports the hypotheses that the best area for these hybrids is central Sweden and that the reason for the growth superiority of the hybrids in this area is their superior hardiness in relation to the southern parental combinations.

#### *1964 Crosses*

Seven female testers from central Sweden and pollen collected from grafted individuals selected in central European stands were used in 1964. Growth data were collected after the first summer in the nursery (1965) and in the field (1969). There were some similarities in the reactions of this material compared with the material of 1954. After the first summer in the nursery the foreign provenances outgrew the local ones with the hybrids intermediate. After 3 years in the field the advantage of the foreign provenances disappeared as it did with some of the hybrids. This change could be related to a greater frost sensitivity of the declining combinations, e.g., the hybrids of the female S3364 in the following table:

COMBINATION	ACCUMULATED GROWTH %		FROST DAMAGE
	1965	1969	SCORE, 0 - 1 1965-1969
Central European	160	100	0.45
Hybrids (S3364)	130	90	0.40
Hybrid mean	110	100	0.25
Central Sweden	100	100	0.25

Further data will be recorded in the autumn of 1973 to study the development of these changes. In this connection it should be emphasized that in a given year bud development in the spring, budsetting, lignification and prolepsis are often affected by random climatic influences that are not correlated with frost damage over a longer period.

#### *1970 Crosses*

Nine grafted female testers selected from southern, central and northern Sweden were combined in a partial diallel cross design with pollen from eastern Europe collected from individuals in Danish provenance experiments (Wellendorf, personal communication). Growth data recorded from the 1-year-old seedlings, not yet exposed to frost, showed the common picture: the foreign provenances outgrow the hybrids, which outgrow the local

provenances. A new finding from this experiment was that progenies from tester trees selected farther north (in order to give the central Sweden test area a more intermediate position between the parent provenances used) were not superior at this early stage (Fig. 8). Perhaps this will change in the future owing to the better hardiness of the northern parent.

### Conclusions of the Spruce Hybridization Work

Young spruce hybrids between Swedish and central-east European provenances do not show any general heterosis (defined as outgrowing both parent provenances); they outgrow the Swedish parent population but are inferior to the southern sources. In time the hybrids can take advantage of the adaptation of the northern parent to the climate of central Sweden, where they were planted - e.g., by reason of frost hardiness - and outgrow both parental provenances. Among the hybrids excellent combinations can be selected and used for practical application. Other combinations will not perform so well.

The best hybrid combinations could be selected after they have been adequately tested under different climatic conditions. It may be possible to predict adaptability to an area of application by recording early growth performance and variables associated with frost hardiness for several years.

The success of hybridization is not only a question of luxuriance of growth: a very important aspect is to combine the vigor of the foreign parent with the hardiness and climatic adaptability of the local one.

### SCOTS PINE HYBRIDS

Since the last report on Scots pine racial hybrids in northern Europe given at a meeting of the working group on quantitative genetics in Raleigh, N.C. (Nilsson and Andersson 1969), many new crossing experiments have been made (Andersson, personal communication). The progenies are still too young to provide a basis for any general conclusions, and collected data have not yet been analyzed in detail. However, some cases of heterosis have been observed in Scots pine (as well as in Norway spruce). Some information of Scots pine inter- and intraprovenance hybrid variances is available from 2-year-old material published by Nilsson (1970). Crosses between and within different Swedish provenances show strong additive effects and intermediate hybrid performance (Fig. 9).

Several cases of superiority of spontaneous provenance hybrids and artificial intraprovenance crosses over open-pollinated progenies in the natural stands have been recorded. As an example, the following populations from open and artificial pollination on the same mother trees (Nilsson 1967) at 8 years of age (Samuelsson 1973) can be mentioned:

#### RELATIVE VOLUME %

1. Open pollinations in the natural stands..... 100

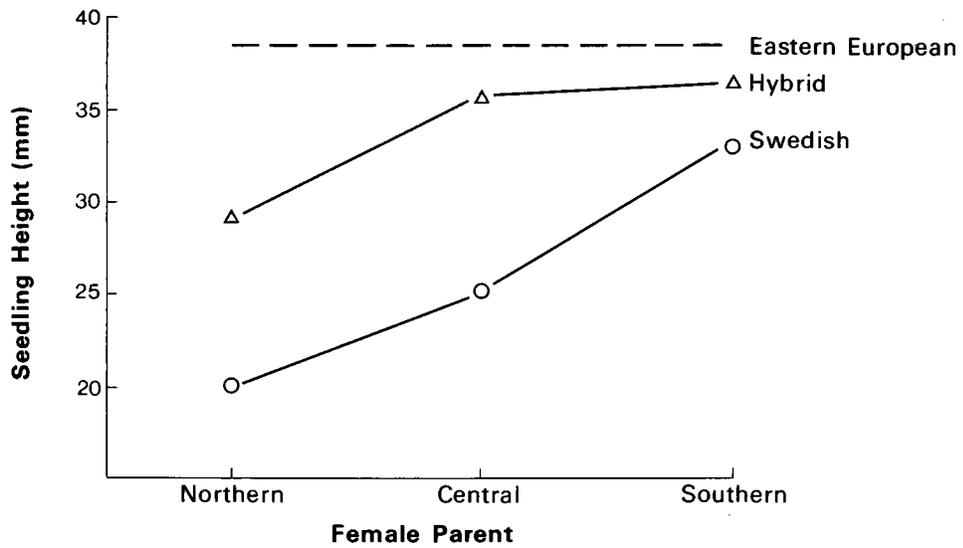


Figure 8. Northern, central and southern Sweden Norway spruce testers in Swedish and eastern European combinations.

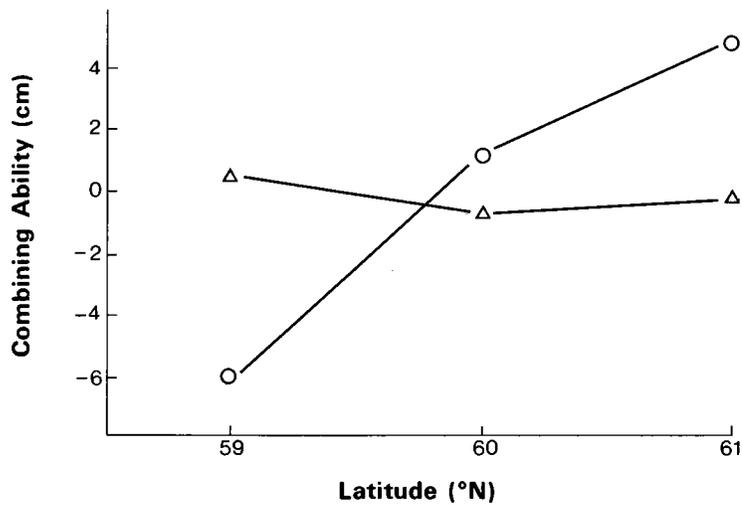


Figure 9. Height growth general combining abilities of 2-year-old Scots pine hybrids ( $\Delta$ ) in relation to intra-provenance crosses (o) in three different latitudes.

2. Open pollinations in young seed orchards in non-native regions and with sparse male flowers (spontaneous interprovenance hybrids)..... 119 - 168
3. Intraprovenance controlled pollinations between selected trees..... 121 - 174

The results look very similar to those obtained in the spruce hybrid experiments of 1954 presented, e.g., in Fig. 6 (cf. also Nilsson 1964 and 1967). Open-pollinated progenies are inferior to both Swedish and hybrid combinations in the same female tree. The inferiority of the open-pollinated progenies is probably the result of in-breeding depression by combinations between close relatives. It has been shown (Park 1972) by isozyme studies that the neighbors of a tree in natural stands are probably relatives. Close neighbors also contribute the major part of the pollination of a tree in natural stands of spruce and pine (Langner 1952-53, Fendrik 1965).

The absence of general hybrid superiority in interprovenance combinations of Scots pine, in contrast to Norway spruce, may have different reasons. First, pine is more resistant to frost damage than spruce; second, the two species have quite different histories of migration into Scandinavia (Nilsson and Andersson 1969); third, plant material of spruce artificially moved somewhat north outgrows the local provenance while the contrary is the case for pine (Bergman 1965, Steffansson and Sinko 1967); and, fourth, interprovenance crosses in Norway spruce show more specific combining effects than Scots pine (Nilsson 1967 and 1970, Nilsson and Andersson 1969).

#### IMPLICATION FOR BREEDING

In Norway spruce the specific combining effects of interprovenance crosses would be best utilized by using two-clone seed orchards or artificial crosses and propagation of the best hybrid individuals by vegetative techniques, e.g., cuttings.

In Scots pine recurrent selections of intra- or close interprovenance combinations by multiclonal regional seed orchards appear to be most useful. In Norway spruce the rapid growth of central or east European parents could be combined with the local adaptability - e.g., frost resistance - of the native parent. In Scots pine outstanding qualities of selected individuals of the local or a moderately northern provenance could be used.

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## DISCUSSION

- Ledig: What is the "snow mold" you mentioned in your paper (as delivered)? Does this attack only the basal portion of the stem or is the attack more general than that?
- Nilsson: It is a fungus that will attack the plants under the deep snow in northern Sweden. In southern Sweden, south of latitude 60°, it is not very important.
- Morgenstern: In your test design, did you have an opportunity to investigate the epistatic effect?
- Nilsson: No, we did not.
- Lester: When you refer to "frost provenances" in Norway spruce, are you referring to late spring frost or winter damage?
- Nilsson: I think it is both. I have found very severe autumn frost damage on the central European provenances; but in our provenance studies of central European and east European provenances, it is very often that the central European provenances will also be sensitive to spring frost damage. The east European provenances begin to develop later than the Swedish ones, so they would not be subject to spring frost.
- Ledig: Why does spruce behave differently from pine? Why do you get such a response from intraprovenance hybridization with spruce - that is, high specific combining ability - while with pine you get very little specific combining ability? Do you have any ideas or speculation about this?
- Nilsson: I think the reason for this is still unknown. It could be the frost. Pine is not sensitive to frost on the shoots. They are sensitive, perhaps, to mechanical damage by snow and ice.
- Kriebel: I noticed that on one of your charts, the advantage over open-pollinated spruce was about the same for interracial hybrids and intraprovenance crosses. If this is generally true, would there be any advantage of one over the other?
- Nilsson: The local crosses are equal in survival but inferior to the hybrids in growth.

## INTERPROVENANCE CROSSES OF LOBLOLLY PINE

R.A. Woessner

*Texas Forest Service, College Station, Texas*

Economically important differences have been found among geographic provenances of all the southern pines that have been adequately studied (Evans 1971, Wells and Wakeley 1966, Burns 1968). Thus, hybridization between provenances could conceivably produce beneficial results in all species. A survey of the current status of interprovenance hybridization in the southern pines reveals that this technique is currently being used only with loblolly pine (*Pinus taeda* L.). Loblolly is a logical choice because of its racial diversity and because it is the most widely planted of the southern pines.

### HABITAT

Loblolly has the widest geographic spread of the southern pines. Its range stretches some 800 air miles in the Coastal Plain and Piedmont from Delaware and central Maryland south to central Florida and then some 1,200 miles west to eastern Texas. The climate is humid with long, hot summers and mild winters. Average annual rainfall varies from 30 to 65 inches a year; it is least in Maryland and Delaware and at the western end of the range in east Texas. The frost-free period varies from 6 to 10 months. July temperatures average more than 75°F and frequently exceed 100°; January temperatures average 36°F to 63°F and occasionally go down to -10°F in the northern and western part of the range. Loblolly grows from near sea level to elevations up to and over 2,000 feet (Fowells, 1965).

### RACIAL VARIATION

The 15-year results (Wells 1969) from the 18 plantations of the loblolly pine phase of the Southwide Pine Seed Source Study reveal well-developed patterns of variation in survival, in rust infection by the southern fusiform rust (*Cronartium fusiforme*) and in height growth. On an average for all plantings, trees from two sample areas west of the Mississippi River had a 10% higher survival rate than trees from the other sources. Rust infection was light in trees from Maryland, southeastern Louisiana, and sources west of the Mississippi River. Height growth in the majority of the plantings indicated that trees grown from seed collected in areas with high summer rainfall and mild winters were tallest, except in the two coldest planting locations, Maryland and Tennessee. In these plantings, trees from areas with low summer rainfall and cold winters performed best.

Along the southeastern coast in North and South Carolina, indications are that Coastal Plain seed should be planted in the Coastal Plain and Piedmont seed in the Piedmont.

Loblolly varies geographically. Unfortunately for the tree breeder, there is little or no correlation among the three important traits; that is, trees with the genetic potential for high survival, fast growth, and rust resistance do not occur in the same area.

#### INTERPROVENANCE CROSSING OF LOBLOLLY

Wright (1964) suggested that crossing of different races within a tree species could lead to hybrid vigor in the  $F_1$  hybrids as does crossing between inbred lines of maize. Also, desirable parental traits peculiar to the individual parents could be expected to be combined in the  $F_1$  hybrid. Likewise, new genetic combinations useful in broadening the genetic base would be created in subsequent generations when the  $F_1$  hybrids crossed among themselves.

An interprovenance crossing program was initiated in 1968 by some members of the North Carolina State Tree Improvement Program (Woessner 1972a). Seed orchard selections indigenous to three different areas of the loblolly range - the Coastal Plain, the Piedmont, and west of the Mississippi River (Fig. 1) - were crossed according to mating design II of Comstock and

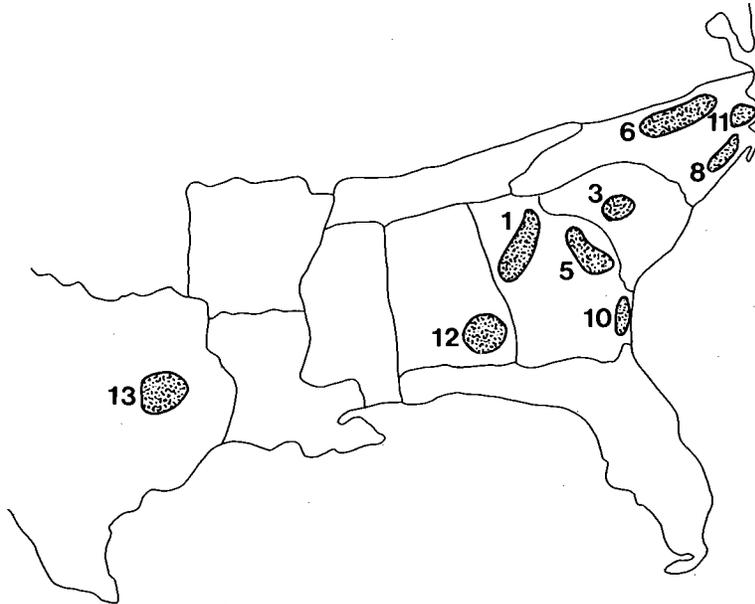


Figure 1. Southern states showing the origin of the trees used as female and male parents. The shading delineates the areas within which the ortets were selected.

Robinson (1948). Four female parents of Piedmont origin were crossed with 5 males from the Piedmont, 10 males from the Coastal Plain, and 4 males of Western origin. Four female parents of Coastal Plain origin were crossed with 6 males from the Piedmont, and 4 males of Western origin. An examination of Fig. 1 indicates that a large part of the loblolly range was sampled.

One-year height results indicated that Coastal parents produced the tallest crosses, followed by Piedmont, and then by Western with the shortest (Table 1). The better performance of Coastal sources has been frequently reported elsewhere (Wells and Wakeley 1966, Kraus 1967, Lantz and Hofmann 1969).

TABLE 1. ACTUAL 1-YEAR HEIGHT AND THE HEIGHT DEVIATION OF THE INTERPROVENANCE CROSSES AS A PERCENT OF THE MIDPARENT VALUE

Cross	Actual height (cm)	Height as a percentage deviation from the midparent
Soil A <sup>a</sup>		
Piedmont females		
X Piedmont males	35.9	4.6
X Coastal males	36.2	1.7
X Western males	35.3	-3.8
X Piedmont females	36.4	4.3
Coastal females		
X Piedmont males	37.5	1.3
X Western males	36.5	-4.5
X Coastal females	39.4	3.1
Soil B <sup>b</sup>		
Piedmont females		
X Western males	18.6	4.3
Coastal females		
X Western males	19.8	.3

<sup>a</sup>Soil A was fertile and well watered.

<sup>b</sup>Soil B was of low fertility and watered infrequently.

The study was designed so that the interprovenance crosses could be evaluated as a deviation from the average of the wind-pollinated progeny of the two parental trees. The heterosis deviation was presented as a percentage of the average of the wind-pollinated progeny of the two parental trees. The 1-year height results examined in this fashion indicated there was little positive heterosis above that obtained from within-provenance crosses when the materials were grown under near ideal growing conditions

(Table 1). However, under poorer growing conditions, the interprovenance crosses with the Western source changed from a negative to a positive heterosis.

The height results for the near ideal growing conditions are generally in agreement with the results from interpopulation crossing of maize (Moll et al. 1965). Maize heterosis increased with increased divergence within a restricted range of divergence, but extremely divergent crosses resulted in a decrease in heterosis. The poor performance of the interprovenance crosses could thus be interpreted as being due to extremely divergent crosses.

Cress (1965) also supplies a possible explanation for either a negative heterosis or a small heterotic response when the measurement is from midparent values. His results indicate that with more than two alleles per locus, negative contributions to heterosis are to be expected at certain loci and that the net effect may be a hybrid genotypic value equal to or below that of the midparent. This can occur even when the alleles in pairs have partially dominant, completely dominant, or over-dominant effects. When all loci in a genotype are considered, the positive contributions to heterosis will generally be larger in magnitude than the negative.

The height results for the poor growing conditions are also in agreement with maize results. The interprovenance crosses with the Western parents change from a negative heterosis under good growing conditions to a positive heterosis under the poor growing conditions. Thus, the more severe the environmental conditions, the better the performance of the interprovenance cross when judged in relation to the midparent value. Similar results were found in maize by McWilliam and Griffing (1965). They found additional heterosis in hybrids when the plants were grown at temperatures above their growth optimum. Crosses between races of mimulus were found by Hiesey (1965) to be suited to environments unsuitable for either parent. The indications here are that some interprovenance loblolly crosses might be suitable for sites not suited to either parent.

Although little heterosis was observed, there is good evidence that desirable parental characteristics have been combined in the hybrids. Examination of the seasonal distribution of growth (Table 2) of Coastal x Coastal, Piedmont x Piedmont, and Piedmont x Coastal crosses from the same latitude indicated that the early season growth of the Coastal source was combined in the  $F_1$  hybrid with the later sustained growth of the Piedmont source (Woessner 1972a). Similarly, Piedmont North Carolina x Western crosses grew more than Coastal North Carolina x Western crosses late in the growing season (Woessner 1972b) in a good soil, but less than Coastal North Carolina x Western crosses in a poor soil. In both these cases, the interprovenance crosses parallel the performance of the intra-provenance wind-pollinated families.

It is in order to speculate on the genetic situation that underlies the performance of the loblolly interprovenance crosses. For heterosis

to occur, there must be some degree of directional dominance and also genetic diversity between the parents for the alleles controlling the characteristic of interest (Falconer 1960). Since these crosses show little heterosis, perhaps one or both of these features are completely lacking in these F<sub>1</sub> interprovenance crosses. Evidence from selfing six of the eight female parents used in this study (Table 3) indicates there is some degree of directional dominance. The Piedmont females averaged an 8.4% inbreeding depression for 1-year height while the Coastal females averaged a 12.7% depression.

Divergence of gene frequency would be expected to occur in isolated populations undergoing selection under different environmental conditions or as the result of gene exchange with related species. The populations sampled in this study were certainly growing under different environmental conditions and the seed-source-study results definitely indicate racial differentiation in growth potential. Genetic differences could also arise from introgression between loblolly and shortleaf pine (Zobel 1953, Mergen et al. 1965).

The information available at present would cause one to conclude that the heterosis observed can be accounted for on the basis of an additive model with partial to complete dominant types of gene action at some loci. A reasonable explanation for the negative heterosis is found in the work of Cress (1965). Alleles as members of a multiple allelic series can show a

TABLE 2. COMPARISON OF PERIODIC GROWTH RATES OF INTRA- AND INTERPROVENANCE CROSSES FROM NORTH CAROLINA

Cross	Height increments (cms)				Total height
	0-7 wk	7-15	15-27	27-45 wk	
Piedmont x Piedmont	12.5	10.0	7.4	6.5	36.4
Piedmont x Coastal	12.2	10.1	8.1	6.7	37.1
Coastal x Coastal	14.1	12.0	7.4	5.9	39.4

TABLE 3. PERCENTAGE INBREEDING DEPRESSION FOR HEIGHT FOR SIX OF THE EIGHT SEED PARENTS

Clone	Piedmont clones	Clone	Coastal plain clones
6-7	15.1	8-33	24.1
6-9	+1.5	8-68	6.2
6-20	11.6	8-76	7.7
mean	8.4	mean	12.7

negative heterosis even when the alleles in pairs have partially dominant, completely dominant, or overdominant effects. The generally better or poorer growth of the trees from certain areas and the different growth patterns may be explained as resulting from favorable genes fixed or at high frequency in one area and completely lacking or at low frequency in another area.

#### SUMMARY

Assessment of juvenile height of the loblolly interprovenance crosses did not indicate that any heterotic response of the type found for yields in varietal crosses of crop plants (15 to 25% over the midparent value) is manifested in height growth response of  $F_1$  hybrids. Additive rather than dominant types of gene action provide an explanation for the performance of these interprovenance crosses. There was evidence that desirable parental characteristics peculiar to individual provenances were combined in the hybrid in an additive fashion.

A crossing program employing a design similar to that used in this study has recently been initiated by Wells (1973, personal correspondence) to take advantage of this possibility. Parental provenances were deliberately chosen for their outstanding characteristics. Livingston Parish Louisiana Coastal Plain loblolly (fast growth, rust resistant) will be crossed with Coastal Carolina (fast growth, frost hardiness) and Marion County Florida loblolly (fast growth, rust resistant). It is hoped that a fast-growing, rust-resistant, frost-hardy hybrid will result.

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## DISCUSSION

Zsuffa: As I see from the tables, the differences you work with are relatively small, and I didn't think you said anything about the significance of the differences and of the possible effect of seed size on early growth of seedlings.

Woessner: I think in Figures 3 and 4 (delivered text) you can see where the actual tests of significance were made. As far as seed size is concerned, I think that if you looked at individual crosses, combinations like family A x family B, you would find some sizeable effect. However, the seed-weight average of the Coastal parents and the seed-weight average of the female Piedmont parents were essentially the same. So, in this case, you are comparing different males on the same female. I don't think, unless you look at individual full-sib families, that seed size is important for the comparisons being made. It is unfortunate that these are only 1-year results, but that is all we have. Some 15 plantations were established throughout the South and we will analyze them at an older age.

Morgenstern: I note with interest your interpretation in which I was waiting for the word "epistasis" because it seems to have some bearing on the problem.

Woessner: No, I don't think any design used in forestry is really sensitive enough to detect epistasis. In the design we used there would be no way to actually determine an epistatic effect. Those of you who were in Raleigh heard Dr. Stuber give a presentation on breaking down components of variance in wide crosses. What it amounts to is the theory that we are presently using in quantitative genetics was developed for within-provenance materials that are at a Hardy-Weinberg equilibrium situation. This theory is not directly applicable to the between-provenance crossing situations.

Fraser: Do you have any data on the root production for these trees such as the root form in relation to soil moisture?

Woessner: No, I am sorry I did not have anything on roots, but past research has shown that material from Texas has the ability to put down a somewhat larger root on the average over a given period of time under field conditions.

Cech: I am wondering if the western male (Texas) material might be from the "lost pines" region?

Woessner: Well actually, it was from Grimes County, which is on the western edge of the main range, which would not be in the

"lost pines" region. These materials from Grimes County were growing on quite deep sand. We would expect that there was some drought hardiness.

Cech: Were these selected trees?

Woessner: Yes, everything used in this study was material that had been phenotypically selected. It was being used in the seed orchards.

Schmitt: In order to properly evaluate the hybrids then you probably have to sample in the midpoint of range, and I am wondering how you define your population. For example, you have a north, south, Coastal, and Piedmont. You lead us to think you need more information from the midpoint of the range.

Woessner: Well, I think I might have misled you. I was trying to make a broad generalization to other species. What I was trying to say was that if a species had a definite north-south growth variability pattern and if material from the north was crossed with material from the south and this hybrid acted at various locations, I don't think this would prove anything unless open-pollinated material or control-pollinated material from along this transect was included for comparison.

Schmitt: If you had sampled material from the northern end of the range and the southern end of the range, would you feel satisfied that you have adequately checked what is happening in the center part of the range?

Woessner: Yes, if it is a clinal-type variation pattern. In the case of loblolly, I think we have adequately sampled the loblolly range south of Maryland and can now see what we will get with loblolly if you are talking of heterosis per se.

## EARLY RESULTS OF PROVENANCE HYBRIDIZATION IN BLACK SPRUCE

E.K. Morgenstern

*Canadian Forestry Service  
Petawawa Forest Experiment Station  
Chalk River, Ontario*

Provenance hybridization is an interesting research problem but has so far found little application in practical breeding. Provenances have been crossed to study the genetic control of crown and stem form (Dengler 1939, Scamoni 1955), to achieve heterosis (Johnsson 1956), or to combine several desirable qualities and greater tolerance to stress conditions (Woessner 1972). It appears that in some species and in particular habitats favorable results have been obtained in juvenile populations.

However, early results are not reliable indicators of performance over a complete rotation period. Furthermore, the designs of experiments were often insufficient to interpret all the theoretical aspects of results. The genetic theory commonly applied to provenance crosses usually dealt with additive and dominant genes only, neglecting the role of epistatic interactions between different gene loci (Falconer 1960). These interactions become increasingly important in wide crosses, and have led to a loss in fitness even if a gain was obtained in other characters, such as growth rate in plants. Populations in different parts of a species' range differ not merely in gene frequencies but in the makeup of whole gene complexes whereby epistasis plays an important role. The coadaptation or integration of such complexes in native populations is an essential phenomenon assuring their survival. Falconer (1960, p. 262) writes: "If two populations adapted to different ways of life are crossed, the cross-bred individuals will be adapted to neither, and consequently, will be less fit than either of the parent populations." Experiments designed to investigate the role of all genes (including epistatic interactions) are highly complex, and require the study of F<sub>2</sub> populations and backcrosses; to interpret them genetically is difficult, because the standard assumptions cannot be made. For example, it is unlikely that a linkage equilibrium exists in the F<sub>1</sub> generation (Stern 1964, Stuber 1970).

The experiments reported here were initiated to test the hypothesis that the fast growth of southern and the hardiness of northern provenances of black spruce could be combined. The goal was thus practical and the aim was not to achieve results that would lend themselves to detailed genetic explanation. The provenance hybrids and control lots were tested in two environments, and results to age 5 are given in this paper.

## MATERIALS AND METHODS

In May 1966 M.J. Holst of Petawawa Forest Experiment Station crossed individual mother trees in 10 black spruce provenances with a pollen mixture from other provenances; all the male and female parents were situated in plantations at the Station. Nine of the provenances were fertilized with Petawawa pollen from two stands with 5 to 10 trees each; the tenth provenance received pollen from Little Pic River, Ontario, as shown in Table 1. The female flowers were isolated with double Kraft paper bags. There was good seed set and most crosses yielded 10 to 20 full seeds per cone.

The hybrid seed was sown in February 1968 in the greenhouse together with seed from the sources used as control (Table 1), which included the seed from several trees in the general area of the original stands that produced the female parents. All seedlings were transplanted to individual clay pots and arranged in plots of 10 to 20 trees in two randomized blocks, with the half-sib families from the same provenance and control lots grouped together in a compact family-block design (Snyder 1966). A 20-hour photoperiod was applied until 2 months before outplanting. Survival and development were satisfactory, and total height measurements were made in December 1968 (age 10 months) and June 1969 (16 months).

In July 1969 a short-term plantation test was established with this material in the nursery at 3 x 3 foot spacing, with five-tree plots and six randomized blocks, again with families and controls from the same area grouped together. The sandy soil of the test site was fertile, but the local climate maintained the snow cover for a long period in the spring and exposed the trees to winter drought. This was rated in the spring seasons of 1970 (age 2 years) and 1972 (4 years) in five classes:

CLASS	DESCRIPTION
1	<u>no damage</u>
2	<u>light damage</u> - a few needles desiccated along upper whorls, all buds healthy
3	<u>moderate damage</u> - many needles desiccated and some buds
4	<u>severe damage</u> - most needles in exposed part of the tree desiccated as well as some buds
5	<u>very severe damage</u> - all needles and buds in upper portion killed and leader dry

A height measurement in the fall of 1972 (age 5 years) was the last assessment.

TABLE 1. LIST OF CROSSES MADE AND CONTROL SEEDLOTS USED FOR COMPARISON

NO.	Female parents		CROSS Origin of		Male parents		CONTROL Seedlot origin	
	Lat. °N	Long. °W	Lat. °N	Long. °W	Lat. °N	Long. °W	Lat. °N	Long. °W
1	Dog River, Ont. 49.4	89.9	Chalk River, Ont. 46.0	77.5	Chalk River, Ont. 46.0	77.5	Dog River, Ont. 49.4	89.9
2	Thunder Bay, Ont. 48.3	89.3	Chalk River, Ont. 46.0	77.5	Chalk River, Ont. 46.0	77.5	Thunder Bay, Ont. 48.3	89.3
3	Kapuskasung, Ont. 49.4	82.4	Chalk River, Ont. 46.0	77.5	Chalk River, Ont. 46.0	77.5	Kapuskasung, Ont. 49.4	82.4
4	Chalk River, Ont. 46.0	77.5	Chalk River, Ont. 46.0	77.5	Chalk River, Ont. 46.0	77.5	Chalk River, Ont. 46.0	77.5
5	Rimouski, Qué. 48.0	68.0	Chalk River, Ont. 46.0	77.5	Chalk River, Ont. 46.0	77.5	Matapedia, Qué. 48.0	67.0
6	St. Zenon, Qué. 46.5	73.7	Chalk River, Ont. 46.0	77.5	Chalk River, Ont. 46.0	77.5	St. Zenon, Qué. 46.5	73.7
7	St. Faustin, Qué. 46.1	74.5	Chalk River, Ont. 46.0	77.5	Chalk River, Ont. 46.0	77.5	St. Zenon, Qué. 46.5	73.7
8	Acadia FES, N.B. 46.0	66.4	Little Pic River, Ont. 49.0	86.0	Little Pic River, Ont. 49.0	86.0	Kedgwick River, N.B. 47.7	67.4
9	Mt. Pearl, Nfld. 47.5	52.8	Chalk River, Ont. 46.0	77.5	Chalk River, Ont. 46.0	77.5	Sandy Brook, Nfld. 48.7	56.1
10	Avalon Peninsula (?), Nfld. <sup>1</sup> 48.0	53.0	Chalk River, Ont. 46.0	77.5	Chalk River, Ont. 46.0	77.5	Sandy Brook, Nfld. 48.7	56.1

<sup>1</sup>Origin on the Avalon Peninsula is likely but could not be confirmed.

The objectives of the analysis were:

1. to survey the variation found in the material as well as the correlations among the five measured characters and latitude and longitude;
2. to compare hybrid performance with the performance of local controls;
3. to test the possibility of heterosis (hybrid vigor) by comparing the hybrids with values intermediate between local controls representing female and male parents (these intermediate values are not true midparent values because the parents and local controls were not identical);
4. to see how the variation in the hybrids is distributed among levels of sampling, i.e., provenances and progenies in provenances.

For the analyses of variance, sources of variation and degrees of freedom are shown in Table 2. There were 10 provenance means each for the hybrids, controls and intermediate values, and 52 individual hybrid progeny means. The first analysis in Table 2 is that of a completely randomized experiment based upon the provenance means where the difference between the hybrids and the controls and hybrids and intermediates is tested for significance. The second analysis examines the distribution of variation among the hybrid progenies, employing a hierarchal subdivision of all progenies into provenances and progenies within provenances.

## RESULTS

The correlation analysis (Table 3) indicated a relation between growth of the hybrid progenies and latitude of origin of the female parents significant at the 1% level. Winter drying was negatively associated with longitude; i.e., the descendants of eastern mothers in milder climates suffered more than their western counterparts, as one would expect (5% level).

In general, the hybrids exceeded the control populations and calculated intermediate values in growth but were inferior or just equal to them in the ability to withstand winter drying.

A detailed examination of the results shows that the hybrids exceeded the controls at every age in height, as indicated by the analysis of variance based upon provenance means (Table 4), the difference being significant at the 1% level. The hybrids were usually less resistant to winter drying, but these differences were significant (5% level) only at 2 years of age, i.e. after the first winter of exposure to outdoor conditions. Fig. 1 gives two separate regressions for the growth of hybrids and controls.

When the hybrids and calculated intermediate values are compared (Table 5), the results are very similar: again the hybrids are significantly different in height at all ages but for winter drying only at age 2.

TABLE 2. ANALYSES OF VARIANCE. THE CHARACTER USED AS EXAMPLE IS "TOTAL HEIGHT, AGE 5 YEARS"

Source of variation	Degrees of freedom	Derivation	Example
<u>1. Analysis of hybrids, controls, and intermediate values based upon provenance means</u>			
Between groups		$g - 1$	$2 - 1 = 1$
Within groups		$g(a - 1)$	$2(10 - 1) = 18$
Total		$ga - 1$	$20 - 1 = 19$
<u>2. Analysis of hybrids, based upon the 52 individual progenies, with hierarchal subdivision of the progeny</u>			
Replications		$r - 1$	$6 - 1 = 5$
Progenies		$p - 1$	$52 - 1 = 51$
Provenances of female parents		$a - 1$	$10 - 1 = 9$
Progenies in provenances		$p - a$	$52 - 10 = 42$
Error (Prog. x Reprs.)		$(r - 1)(p - 1)$	$(6 - 1)(52 - 1) = 255$
Total		$rp - 1$	$311$

TABLE 3. CORRELATION COEFFICIENTS CALCULATED FROM THE 52 HYBRID PROGENIES (50 D.F.)

Character	$x_1$	$x_2$	$x_3$	$x_4$	$x_5$	$x_6$	$x_7$
$x_1$ Height, age 10 months	1.00	+0.62**	+0.06	+0.24	+0.19	+0.41**	+0.01
$x_2$ Height, age 16 months		1.00	+0.42**	+0.05	+0.42**	+0.49**	+0.13
$x_3$ Winter drying, age 2 years			1.00	-0.05	+0.13	+0.16	-0.29*
$x_4$ Winter drying, age 4 years				1.00	-0.53**	-0.22	-0.30*
$x_5$ Height, age 5 years					1.00	+0.49**	+0.48**
$x_6$ Latitude, female parent						1.00	+0.40**
$x_7$ Longitude, female parent							1.00

Significance levels: \*, 5%; \*\*, 1%.

TABLE 4. COMPARISON OF HYBRIDS WITH LOCAL CONTROLS BASED UPON THE 10 PROVENANCE MEANS IN EACH GROUP (THE UNDERLINED % INDICATES SUPERIORITY). SIGNIFICANCE LEVELS AS SHOWN IN TABLE 3

Character	$\frac{\text{Hybrid total}}{\text{Control total}} \times 100 = \%$ superiority or inferiority	F-value
Height, 10 months	$\frac{182.9}{123.6} \times 100 = \underline{148}$	16.59**
Height, 16 months	$\frac{467.8}{402.2} \times 100 = \underline{116}$	29.23**
Winter drying, 2 years	$\frac{24.0}{20.6} \times 100 = 116$	8.28*
Winter drying, 4 years	$\frac{29.9}{29.8} \times 100 = 100$	0.20
Height, 5 years	$\frac{961.1}{832.1} \times 100 = \underline{115}$	20.15**

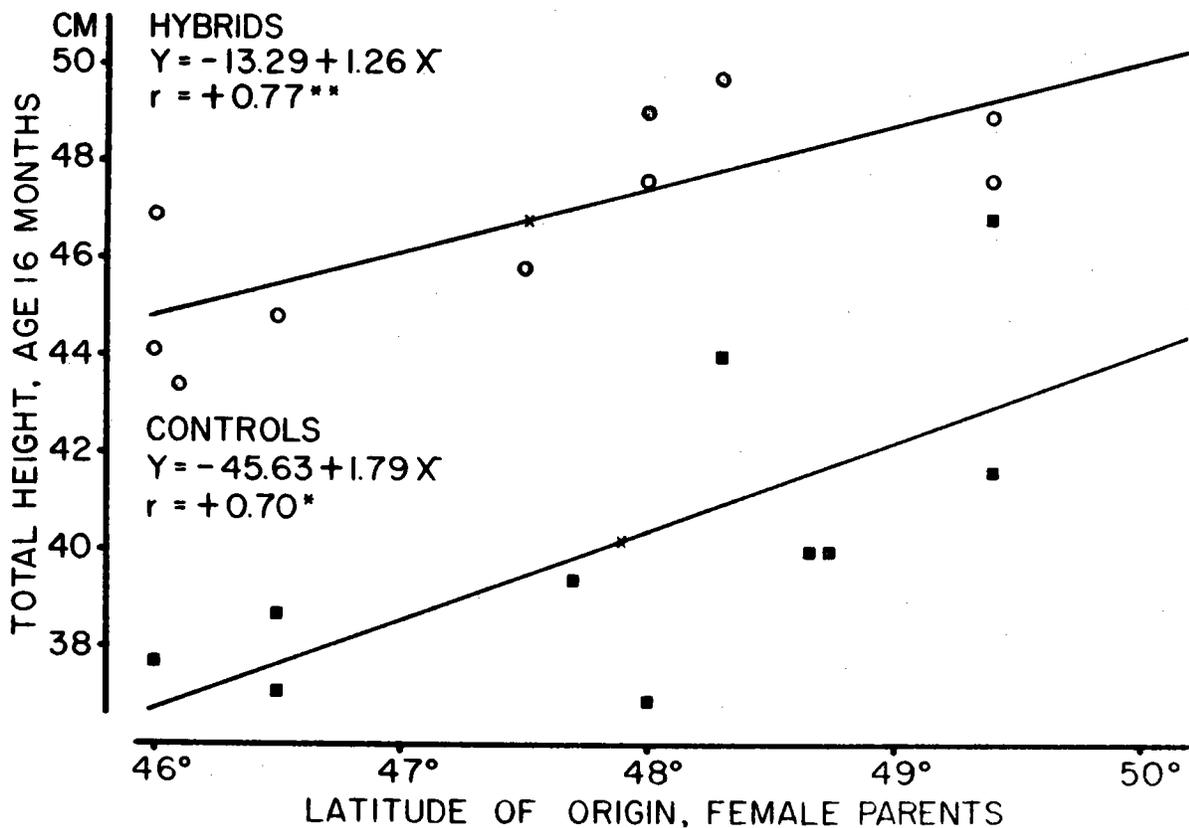


Figure 1. Regressions of total height on latitude of origin of the female parents for the hybrids and local control populations at 16 months of age.

TABLE 5. COMPARISON OF HYBRIDS WITH INTERMEDIATE VALUES BASED UPON THE PROVENANCE MEANS. SIGNIFICANCE LEVELS AS IN TABLE 3

Character	$\frac{\text{Hybrid total}}{\text{Control total}} \times 100 = \% \text{ superiority or inferiority}$	F-value
Height, 10 months	$\frac{182.9}{107.7} \times 100 = \underline{170}$	54.58**
Height, 16 months	$\frac{467.8}{392.5} \times 100 = \underline{119}$	71.77**
Winter drying, 2 years	$\frac{24.0}{21.4} \times 100 = 112$	6.80*
Winter drying, 4 years	$\frac{29.9}{28.6} \times 100 = 104$	3.22
Height, 5 years	$\frac{961.1}{892.9} \times 100 = \underline{108}$	10.24**

Comparisons between local controls, hybrids and the calculated intermediate values at the same time are facilitated by Fig. 2. At 16 months of age the hybrids often exceeded the calculated intermediate values in height, but at age 5 this is evident mainly in the northern Ontario and two of the Quebec provenances. No. 4 in this figure is the local Chalk River material. The hybrids in this provenance are better only at 16 months. In comparison with the other provenances, this one is average, being exceeded mainly by the ones from northern Ontario.

The final analysis based upon the 52 hybrid progenies (Table 6) revealed differences among progenies for all five characters that were significant at least at the 5% level. Much of the variation was contributed by provenances, but variation among progenies in provenances was significant as well, except for winter drying at age 4.

#### DISCUSSION

In general, the analyses have shown that provenance hybrids grow rapidly but that their superiority diminishes with age, and that they are often more strongly subject to winter desiccation than the controls. There is one exception: at age 5 years, the tallest hybrid provenance, with female parents from Dog River, Ontario, was also least susceptible to desiccation at age 4 (Fig. 2); thus the two desirable qualities, growth and hardiness, have been combined. The significant variation at the level of progenies in a provenance would offer an opportunity to select the best progeny within that provenance. It is interesting to note that Dog River is the most

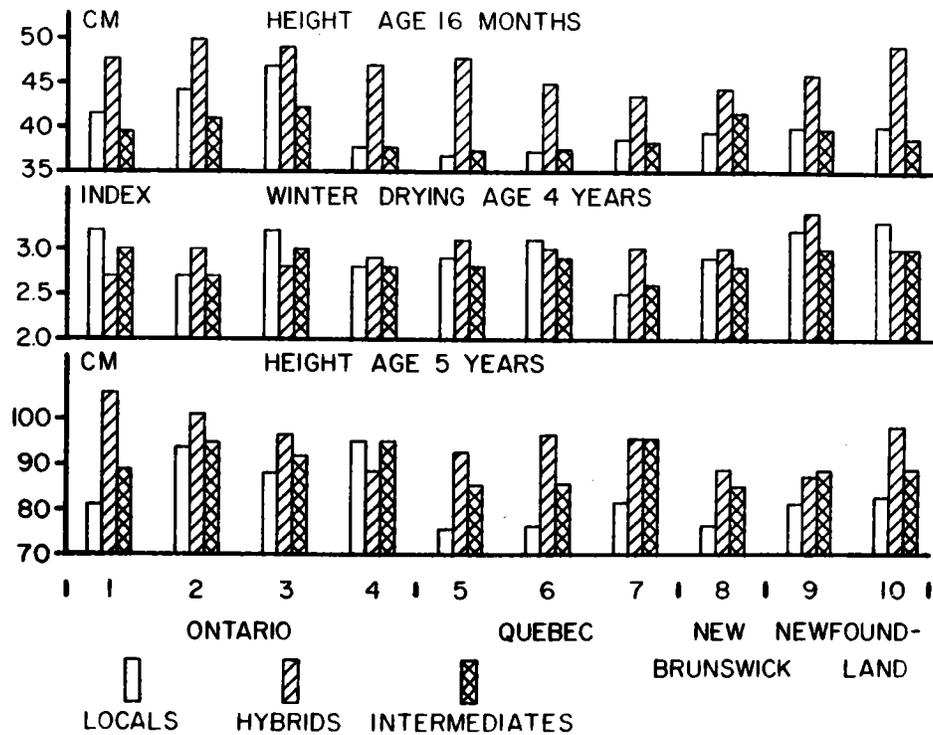


Figure 2. Comparisons of the local control, hybrids and calculated intermediate values for three characters.

northwesterly parent provenance and has the most pronounced continental and coldest climate (Chapman and Brown 1966).

Heterosis, or hybrid vigor, is a phenomenon observed when inbred lines are crossed (Falconer 1960). Woessner (1972) applied the term to material exceeding the midparent value. In this study the calculated intermediate values (which are probably of similar magnitude as true midparent values) were in fact exceeded by the hybrids and on this basis heterosis would seem to exist.

However, one complication that arises here is the difficulty of comparing open-pollinated controls with control-pollinated hybrid material. Work in Sweden, where many provenance hybrids have been made, indicates that such comparisons may be biased in favor of the hybrids because of a lower seed quality in the open-pollinated controls, as well as a low level of inbreeding in natural stands (Nilsson and Andersson 1970). The reduction of inbreeding produces more vigorous progeny in seed orchards than in natural stands; therefore gains in growth may be obtained to supplement the benefits of plus-tree selection. The gain achieved by provenance hybridization would have to be substantial to make it attractive as an additional breeding method. The observed 15% increase in growth could have resulted from a higher seed quality in the hybrids (young parent trees growing at a suitable spacing on

TABLE 6. ANALYSIS OF VARIANCE OF 52 HYBRID PROGENIES. SIGNIFICANCE LEVELS AS IN TABLE 3

SOURCE OF VARIATION	MEAN SQUARES				
	Height, 10 months	Height, 16 months	Winter drying, 2 years	Winter drying, 4 years	Height, 5 years
Replications	121.00	2.68	6.88	0.49	3007
Progenies	24.61**	28.09**	0.92**	0.62*	319**
Provenances of female parents	72.72**	59.65*	2.60**	0.88	941**
Progenies in provenances	14.30*	21.33**	0.56**	0.57	186**
Error (Prog. x Reps.)	7.39	11.78	0.28	0.41	30

an upland site) and a little inbreeding in the controls. The results of this study, therefore, do not conclusively demonstrate heterosis for growth in black spruce.

At the same time, it is clear that the material must be studied for a longer period and in additional environments. To meet these requirements, field experiments were established at Petawawa in 1971 and by Université Laval and Ministère des Terres et Forêts in Quebec in 1972. If the hybrids show promise in a greater number of environments, perhaps a whole new series of crosses based on more detailed experimental designs should be made.

#### ACKNOWLEDGMENT

I wish to thank M.J. Holst who initiated the project and the greenhouse experiment reported here.

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# SELFING AND PROVENANCE HYBRIDIZATION IN RED PINE

M.J. Holst and D.P. Fowler

*Canadian Forestry Service, Petawawa Forest Experiment Station  
Chalk River, Ontario, and Maritimes Forest Research Centre,  
Fredericton, New Brunswick*

The present paper reports on the results of field testing of red pine (*Pinus resinosa* Ait.) selfings, provenance hybrids and their controls. This material was tested earlier in a short-time (two-month) greenhouse test by Fowler (1965). His main conclusions at that time were that red pine, both as individual trees and as a species, was homozygous for most alleles, self-fertile, self-compatible, and that seedlings resulting from self-pollination exhibit little or no inbreeding depression. The interprovenance hybrids exhibited no heterosis at that time. Our purpose is therefore to investigate whether there is any inbreeding depression due to selfing or any hybrid vigor due to provenance hybridization in red pine when it is grown under normal field conditions.

## MATERIALS AND METHODS

In 1961, controlled pollinations were made on different provenances in a U.S. Forest Service trial at Kane, Pa., and in two natural stands (Watabeag Lake and Lake Abitibi) in northern Ontario. Fig. 1 shows the locations where the pollinations were made and the origins of the provenances pollinated at Kane. The pollinations at Watabeag Lake were made by C.W. Yeatman of the Petawawa Forest Experiment Station, while the pollinations at Kane and Lake Abitibi were made by D.P. Fowler, who was then employed by the Ontario Department of Lands and Forests. For a detailed description of this work the reader is referred to Fowler (1965).

In all, 45 trees representing 16 seed sources in the Lake States, one source in Massachusetts and the two northern Ontario sources were used as female parents for controlled pollinations. The following pollinations were made on each tree: a) self-pollination (self), b) intraprovenance pollination (local) and c) interprovenance pollination (Maine). The intraprovenance pollinations were made with a pollen mix from 10 unrelated trees of any particular provenance. The interprovenance pollinations were made with a pollen mix of 20 trees from a Maine provenance. In addition five trees at Lake Abitibi and eight trees at Watabeag Lake were used as female parents in crosses with pollen from Maine, Minnesota, New York, Pennsylvania, Wisconsin and central Ontario. The seeds obtained from the controlled pollinations were used to establish two experiments.

*Experiment 1* compared the selfings, the intraprovenance hybrids and the interprovenance hybrids in which the Maine provenance was the male

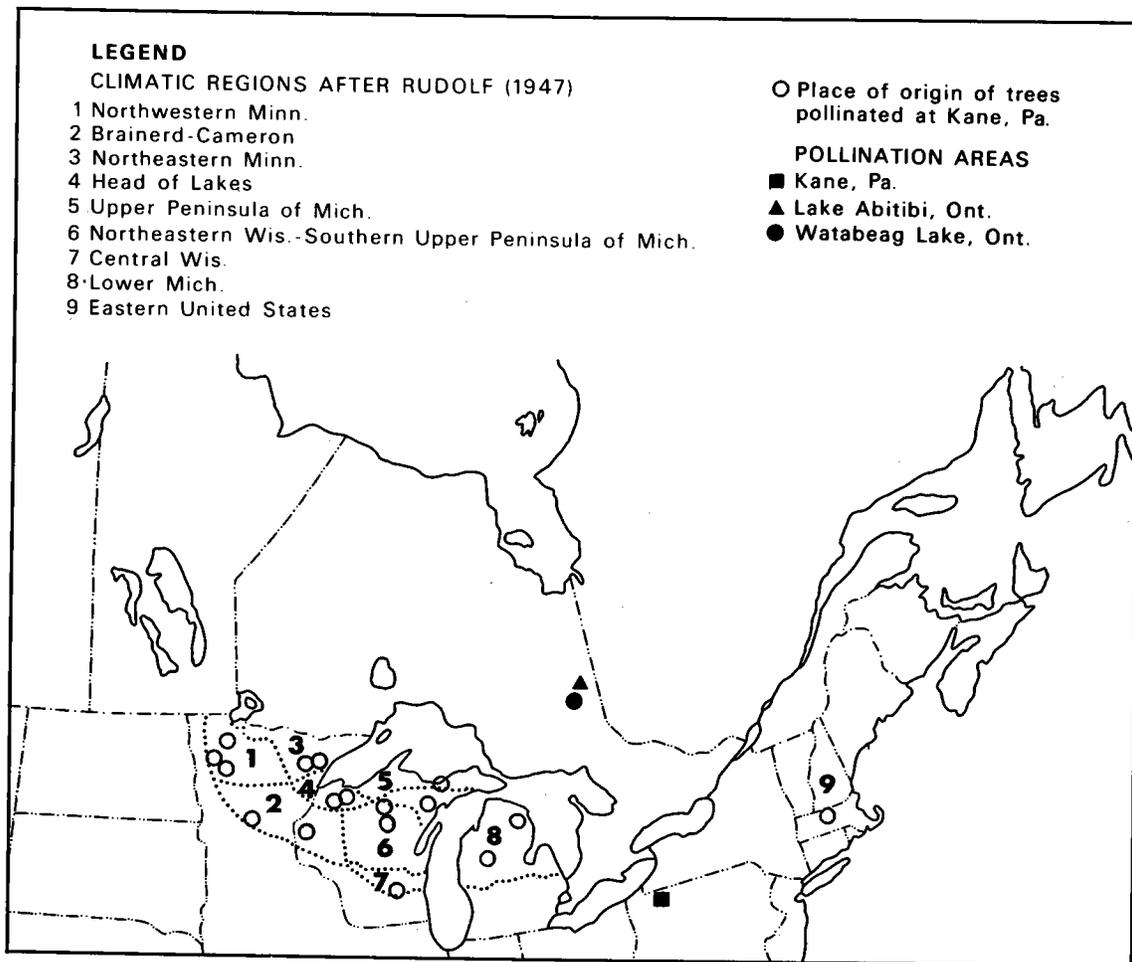


Figure 1

parent. This experiment was carried through the greenhouse and nursery phases at Maple, Ontario, and was field-planted by M.J. Holst at Petawawa in the spring of 1966. Four-year-old seedlings totalling 2,155 (755 selfs, 702 intraprovenance and 698 interprovenance) were planted in a completely randomized, single-tree plot design. In the fall of 1970, when the plants had completed their ninth growing season, total height was measured for all surviving seedlings.

*Experiment 2: Part 1.* The seedlings were raised through the greenhouse and nursery phases at Maple, Ont., and transplanted to the Acadia Forest Experiment Station nursery in 1966. In the spring of 1969, 227 six-year-old hybrids and controls were field-planted at Acadia in a completely randomized single-tree plot design. Ten-year height was measured during the summer of 1973 (height to the 1972 internode).

*Part 2.* Seed for the experiment was sown in the spring of 1963 in the Petawawa nursery. The 411 seedlings were field-planted as 2-2 stock in the spring of 1967 at Petawawa in a completely randomized single-tree plot

design within a checkerboard arrangement of controls. Positions 1 and 3 of the checkerboard were planted with seedlings from a Petawawa general collection while positions 2 and 4 received seedlings from single-tree progenies from Watabeag Lake and Lake Abitibi respectively. The seedlings were measured in the fall of 1970 after completion of the eighth growing season from seed.

## RESULTS AND DISCUSSION

*Experiment 1.* Table 1 shows the numbers of surviving plants and their heights at age nine years. Survival is 88, 89 and 91% for selfs, intraprovenance (x local) and interprovenance (x Maine) respectively. The data comparing selfing and intraprovenance crossing are summarized in Table 2. The mean height for all selfed materials is 157 cm and for intraprovenances crosses 171 cm. The mean reduction in height (inbreeding depression) due to selfing is 14 cm or 8 percent. A positive correlation ( $r = .72$ ,  $n = 15$ ,  $P < .05$ ) exists between the height of selfs and the height of intraprovenance seedlings. Those provenances that produce the large seedlings from intraprovenance pollinations also produce the largest selfed seedlings.

It is possible to examine the within-population relationship between selfing and intraprovenance crossing of three provenances. There is a positive (not significant) correlation for Watabeag Lake ( $r = .76$ ), Lake Abitibi ( $r = .03$ ) and Kilbourne, Wis. ( $r = .72$ ).

In Table 3 the data comparing the intraprovenance and interprovenance hybrids are presented. The mean heights for the intraprovenance (171 cm) and interprovenance (171 cm) hybrids are similar. There is, however, a significant negative correlation in respect to height ( $r = -.78$ ,  $n = 14$ ,  $P < .01$ ). The nine slow-growing provenances (with heights less than 175 cm) increased in height growth by an average of 4.3% when crossed with Maine, while the five fast-growing provenances (with heights over 175 cm) decreased in height growth by an average of 7.6%. The difference of 11.9% between the two groups is highly significant ( $t = 7.28$ ,  $P < .01$ ). These results can perhaps be explained if we consider Maine poorly adapted in the continental climate of the test site at Petawawa. The other provenance from the Atlantic Seaboard (Winchendon, Mass.) had very poor growth at Petawawa (160 cm). At Ely, Minn. it had the poorest height growth of all the provenances tested (Nienstaedt 1964) and at Kane, Pa. it ranked 12th of the 50 provenances tested (Hough 1967). This suggests that provenances from an Atlantic climate show poor adaptation in continental climates. Unfortunately, seedlings from the Maine provenance were not included in the test, and thus it was not possible to make direct comparisons between the growth of the interprovenance hybrids and both parent provenances. The Maine provenance is, however, represented in the provenance trial at Kane, Pa., where it ranked 40 of 50 tested at 25 years of age (Hough 1967). Thus in the continental climate at Petawawa, Maine may have poorer growth potential than Winchendon, Mass.

TABLE 1. NINE-YEAR HEIGHT OF SELFINGS (X SELF), INTRAPROVENANCE (X LOCAL) AND INTERPROVENANCE (X MAINE) PLANTED AT THE PETAWAWA FOREST EXPERIMENT STATION

Provenance	Clima- tic region (Rudolf 1947)	Number and height of plants					
		x Self		x Local		x Maine	
		Number	Ht cm	Number	Ht cm	Number	Ht cm
Watabeag Lake, Ont.		12	156	8	169	23	162
" " "		25	164				
" " "		15	119	21	138	23	160
" " "		14	137	28	168	24	172
" " "		18	143	26	149	15	142
Mean (4 lots)			139		156		159
Lake Abitibi, Ont.		25	158	17	148	25	171
" "		25	162	27	164	25	168
" "		25	139	28	157	28	164
" "		24	145	24	142	26	156
Mean (4 lots)			151		153		165
Itasca State Park, Minn.	1	19	146				
Chippewa Nat. For., Minn.	1			5	158		
Bagley, Minn.	1	12	149	27	177	23	163
Onamia, Minn.	2	24	154	10	174	14	179
Turtle Lake, Wis.	2	26	166	25	179	10	190
Turtle Lake, Wis.	2			22	166	26	180
Aurora, Minn.	3			1	206		
" "	3			3	157		
" "	3	25	167	20	191	17	175
Virginia, Wis.	3	18	148	13	167	2	140
Cedar, Wis.	4	20	170				
" "	4			8	153		
" "	4	29	164	21	180	24	162
Iron River, Wis.	4	22	167			1	168
Munising, Mich.	5	8	159				
" "	5	24	141	22	162	24	162
" "	5	16	161	25	161	16	172
" "	5	3	119				
Mean (2 lots)			151		162		167
Trout Lake, Wis.	6	11	183				
" "	6	23	170	18	172	26	174
" "	6	9	137	18	180	27	164
Mean (2 lots)			154		176		169
Irma, Wis.	6	22	158			21	174
Rapid River, Mich.	6	9	162	20	168	12	179
" " "	6			8	168	18	192

TABLE 1. NINE-YEAR HEIGHT OF SELFINGS (X SELF), INTRAPROVENANCE (X LOCAL) AND INTERPROVENANCE (X MAINE) PLANTED AT THE PETAWAWA FOREST EXPERIMENT STATION (Cont'd.)

Provenance	Clima- tic region (Rudolf 1947)	Number and height of plants					
		x Self		x Local		x Maine	
		Number	Ht cm	Number	Ht cm	Number	Ht cm
Kilbourn, Wis.	7	23	148	17	167	21	174
" "	7	3	113	21	166		
" "	7	25	162	24	183	13	171
" "	7	24	158	18	167	26	184
Mean (3 lots)			156		172		176
Cadillac, Mich.	8	24	171	9	165	26	168
Curran, Mich.	8	27	184	25	191	28	175
Winchendon, Mass.	9	24	152	22	161	27	175
" "	9	7	135	29	158	22	164
" "	9	1	178	15	172		
" "	9					25	161
Mean (2 lots)			144		160		170
Total number of living plants		661		625		638	
Total number of plants planted		755		702		698	
Percent survival		88		89		91	

TABLE 2. COMPARISON OF SELFINGS (X SELF) AND WITHIN-STAND CROSSES (X LOCAL) WITH DATA TAKEN FROM TABLE 1

Provenance	Number of crosses	Mean height of all plants		Inbreeding depression, percent
		x Self cm	x Local cm	
Watabeag Lake, Ont.	4	139	156	11
Lake Abitibi, Ont.	4	151	153	1
Bagley, Minn.	1	149	177	16
Onamia, Minn.	1	154	174	11
Turtle Lake, Wis.	1	166	179	7
Aurora, Minn.	1	167	191	13
Virginia, Wis.	1	148	167	11
Cedar, Wis.	1	164	180	9
Munising, Mich.	2	151	162	7
Trout Lake, Wis.	2	154	176	12
Rapid River, Mich.	1	162	168	4
Kilbourn, Wis.*	3	156	172	9
Cadillac, Mich.	1	171	165**	-4
Curran, Mich.	1	184	191	4
Winchendon, Mass.***	2	144	160	10
Mean		157	171	8

\*Excluded tree no. 10 because only three plants in selfing.

\*\*Only nine plants.

\*\*\*Excluded tree no. 3 because only one plant in selfing.

If genetic variation in the red pine system were completely additive one would expect the heights of the interprovenance hybrids to be intermediate between those of the two parent provenances. It would then be possible to estimate the height of the Maine provenance from the heights of the intraprovenance and interprovenance hybrids. Estimates of the height of the Maine provenance range from 144 cm for Cedar, Wis. (which had the biggest reduction in growth) to 204 cm for Rapid River, Wis. (which had the highest increase in growth) compared with an estimate of 170 cm when all provenances are used. A test for normality of these estimates indicates a normal distribution and thus supports the proposition of a predominantly additive system.

Although these experiments were designed primarily to examine the effects of inbreeding in red pine and not as provenance studies, it is interesting to note the considerable variation in height growth between provenances. Table 4 is a comparison of provenance heights at Petawawa, Kane, Pa. (Hough 1967), Cass Lake, Minn. (Buckman and Buchman 1962), and Ely, Minn. (Nienstaedt 1964).

The slow growth of the two northern Ontario provenances (155 cm) likely results from adaptation to the short growing season and severe climate

TABLE 3. COMPARISON OF WITHIN-STAND CROSSES (X LOCAL) AND BETWEEN-STAND CROSSES (X MAINE) WITH DATA TAKEN FROM TABLE 1

Provenance	Number of crosses	Mean height of all plants		Relative to x Local, percent
		x Local cm	x Maine cm	
Watabeag Lake, Ont.	4	156	159	102
Lake Abitibi, Ont.	4	153	165	108
Bagley, Minn.	1	177	163	92
Onamia, Minn.	1	174	179	103
Turtle Lake, Wis.	2	173	185	107
Aurora, Minn.	1	191	175	92
Virginia, Wis.	1	167	(140)*	(84)
Cedar, Wis.	1	180	162	90
Munising, Mich.	2	162	167	103
Trout Lake, Wis.	2	176	169	96
Rapid River, Mich.	2	168	186	111
Kilbourn, Wis.	3	172	176	102
Cadillac, Mich.	1	165	168	102
Curran, Mich.	1	191	175	92
Winchendon, Mass.	2	160	170	106
Mean (excluding Virginia, Wis.)		171	171	100

\*Only two plants in x Maine.

of northern Ontario. The slow growth of the Winchendon, Mass. provenance (160 cm) may result from poor adaptation of an Atlantic provenance to continental conditions.

Growth of provenances from the Lake States area reveals a rather complex situation (Table 4), in which the random occurrence of fast- and slow-growing provenances is evident. The data from this experiment indicate that provenances from the Upper Peninsula of Michigan and from the extreme western range of red pine in the Lake States are relatively slow growing at Petawawa. Provenances from Lower Michigan, eastern Minnesota and mid-Wisconsin are faster-growing. This is not supported by the results of the trials at Kane, Pa., while the height growth at Cass Lake and Ely, Minn. shows better correspondence with the Petawawa results.

*Experiment 2.* The results of this experiment are presented in Tables 5 and 6. Survival was 94 and 95% at Petawawa and Acadia respectively and was not related to parentage.

The interprovenance hybrids from Watabeag Lake were 6% taller than the open-pollinated controls at Petawawa and 2% taller than the controls at Acadia. The Lake Abitibi provenance hybrids were 6% taller than the

TABLE 4. HEIGHTS OF PROVENANCES (X LOCAL) GROWN AT PETAWAWA FOREST EXPERIMENT STATION, COMPARED WITH HEIGHTS OF PROVENANCES GROWN AT KANE, PA., CASS LAKE, MINN., AND ELY, MINN.

Collection number (Hough 1952)	Seed zone (Rudolf 1957)	Provenance	Petawawa F.E.S., Ont., 9-year height x Local, cm	Kane, Pa., 25-year height, feet	Cass Lake, Minn., 27-year height, feet	Ely, Minn., Mean annual height increment, feet
<u>Northern Ontario</u>						
-	-	Watabeag Lake, Ont.	156	-	-	-
-	-	Lake Abitibi, Ont.	153	-	-	-
		Mean	155			
<u>Atlantic Seaboard</u>						
123	-	Winchendon, Mass.	160	40.10	-	.88
293	-	Maine	-	39.46	-	-
		Mean		39.76		
<u>Upper Peninsula Michigan</u>						
54	4E	Rapid River, Mich.	168	41.65	-	1.32
246	4D	Munising, Mich.	161	39.76	-	-
		Mean	165	40.71		
<u>Extreme Western Range in Lake States</u>						
181	4B(3A)	Bagley, Minn.	177	38.95	39.7	1.04
74	3C	Onamia, Minn.	174	37.98	-	1.23
159	2C	Turtle Lake, Wis.	173	40.12	-	1.27
		Mean	175	39.02		1.18
<u>Eastern Minnesota and Mid-Wisconsin</u>						
36	4B	Aurora, Minn.	191	41.17	41.1	-
165	3D	Cedar, Wis.	180	39.25	38.3	1.26
10	4C	Trout Lake, Wis.	176	39.91	39.9	-
60	2D(1E)	Kilbourn, Wis.	172	41.27	37.1	1.24
38	4B	Virginia, Wis.	167	39.60	39.5	1.26
		Mean	177	40.24	39.1	1.25

TABLE 4. HEIGHTS OF PROVENANCES (X LOCAL) GROWN AT PETAWAWA FOREST EXPERIMENT STATION, COMPARED WITH HEIGHTS OF PROVENANCES GROWN AT KANE, PA., CASS LAKE, MINN., AND ELY, MINN. (Cont'd.)

Collection number (Hough 1952)	Seed zone (Rudolf 1957)	Provenance	Petawawa F.E.S., Ont., 9-year height x Local, cm	Kane, Pa., 25-year height, feet	Cass Lake, Minn., 27-year height, feet	Ely, Minn., Mean annual height increment, feet
<u>Lower Michigan</u>						
213	3E	Curran, Mich.	191	42.96	-	1.25
210	3E	Cadillac, Mich.	165	42.28	-	-
		Mean	178	42.62		

TABLE 5. INTERPROVENANCE HYBRIDS MADE AT WATABEAG LAKE, ONT., SHOWING 8-YEAR HEIGHT AT PETAWAWA FOREST EXPERIMENT STATION AND 10-YEAR HEIGHT AT ACADIA FOREST EXPERIMENT STATION

Parent		Petawawa F.E.S. Number of plants			Acadia F.E.S. Number of plants		
Female	Male	Planted	Living	Mean ht cm	Planted	Living	Mean ht cm
ST 1350	Pa.	20	20	95	9	9	122
ST 1350	Open	6	5	103	5	4	121
ST 1351	Minn.	-	-	-	13	13	126
ST 1351	Open	15	15	98	10	10	128
ST 1352	Minn.	41	40	86	8	7	152
ST 1352	Open	15	12	79	4	3	122
ST 1353	Wis.	60	58	90	11	10	120
ST 1353	Open	15	15	82	12	11	108
ST 1354	Maine	30	27	85	5	5	140
ST 1354	Open	15	13	80	3	3	126
ST 1355	Pa.	20	20	95	6	6	108
ST 1355	Open	4	3	103	8	7	137
ST 1356	Maine	30	30	89	15	14	128
ST 1356	Open	15	14	89	16	15	131
ST 1357	New York	30	30	98	6	6	138
ST 1357	Open	15	14	88	11	10	126
Weighted mean							
	hybrids			91*			128
	controls			86			125
	hybrid/controls (percent)			106			102

\*Excluding ST 1351.

open-pollinated controls at Petawawa, but 1% shorter than the controls at Acadia. These differences in height were not significant at the 5% level. The correlation between the height of the open-pollinated controls and the hybrids from the same mother trees was low and nonsignificant for Watabeag Lake and Lake Abitibi materials at both Petawawa and Acadia.

#### CONCLUSIONS

The conclusion drawn by Fowler (1965) that red pine shows little or no inbreeding depression after selfing must be modified. At age 9 years from seed the red pines in this experiment exhibited a significant, 8 percent, depression in growth. This inbreeding effect is still remarkably small, considering that selfing in red pine resulted in normal seed set, germination and survival.

TABLE 6. INTERPROVENANCE HYBRIDS MADE AT LAKE ABITIBI, ONT., SHOWING 8-YEAR HEIGHT AT PETAWAWA FOREST EXPERIMENT STATION AND 10-YEAR HEIGHT AT ACADIA FOREST EXPERIMENT STATION

Parent		Petawawa F.E.S.			Acadia F.E.S.		
		Number of plants		Mean	Number of plants		Mean
Fe- male	Male	Planted	Living	ht cm	Planted	Living	ht cm
40	Pa.	-	-	-	8	8	115
40	Open	8	8	105	8	8	109
42	Maine	12	11	79	15	15	111
42	Local	4	4	104	-	-	-
42	Open	8	8	93	7	6	129
43	Maine	15	15	82	-	-	-
44	Trout Lake, Wis.	-	-	-	6	5	116
44	Open	20	18	102	11	11	111
45	Petawawa F.E.S., Ont.	20	19	106	9	8	121
45	Open	20	20	87	6	6	115
46	Maine	3	3	95	-	-	-
46	Open	20	20	92	-	-	-
47	New York	30	30	96	9	9	114
47	Open	20	19	91	6	6	122
Weighted mean							
hybrids				96*			115
controls				90*			116
hybrids/controls (percent)				106			99

\*Excluding trees 40, 43 and 44.

In general, the growth rate of interprovenance hybrids of red pine is intermediate between the growth rates of the parent provenances. This experiment provided no evidence of heterosis. On the contrary the significant positive correlation between the height of selfings and intraprovenance hybrids and the significant negative correlation between the height of intra- and interprovenance hybrids support the supposition that the system governing the growth of red pine is primarily additive. Interprovenance hybridization does not appear to be a very promising method of increasing the growth of red pine.

Although this study was not designed as a provenance trial, it is evident that relatively small but significant differences in growth exist between provenances. The two fastest-growing provenances at Petawawa were from Aurora, Minn., and Curran, Mich., and were 10% taller than average at 9 years of age.

The ability to establish new stands after fire from pioneer single trees or a few scattered trees seems to be a characteristic of red pine.

Such a reduction in the local breeding population results in increased inbreeding, reduces the frequency of deleterious recessive genes and favors the ability to self-pollinate. Thus red pine has good set of sound seed after self-pollination, and the resultant selfings are healthy with only a moderate reduction in growth. In a species that is highly homozygous a high degree of uniformity is expected. This is true of red pine for most of the traits studied.

Red pine shows broad regional adaptation and broad regional differences as well as random occurrence of traits. This model explains the random occurrence of fast- and slow-growing provenances within seed zones in the Lake States (Rudolf 1957). Provenance hybridization is not a promising method of increasing growth and yield, but further testing of provenances is. Preferably these should be carefully executed stand tests based on single-tree progeny tests.

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## DISCUSSION

- Gabriel: I would like to ask both Morgenstern and Fowler if they recall what criteria they used in making the parental selection within provenances and for selecting the seed trees they used for their controls.
- Morgenstern: The basic criterion in black spruce was the production of female flowers. There was no random selection.
- Gabriel: Yet you were studying height-growth difference?
- Morgenstern: No, we did not select the parents from the point of view of looking for good features. We were simply looking for the parents that produced female flowers and male flowers.
- Fowler: Our work with red pine material was basically the same. The criterion for selection at Kane, Pa., was that the selected trees have enough strobili to work with. Most of the trees that met this requirement were broad-crowned "border" trees.
- Khalil: What do the figures in your table represent and what are the "classes"?
- Morgenstern: It was the proportion of the tree that was subjected to winter drying. I have a more detailed description. It ran something like this: Class 1, no damage; Class 2, light damage; some of the needles and buds were killed in the upper portion of the tree that was exposed to drying winds; and this went all the way up to Class 5 where all the upper needles and buds were killed.
- Zsuffa: It seems to me normal to expect such large differences in growth and other characteristics depending on the test location in the species with such a wide range as black spruce. I would think that if such interprovenance crosses were made, they should be made for certain locations.
- Morgenstern: The conditions under which the black spruce were grown were somewhat unusual and perhaps not typical of Petawawa. The greenhouse experiment was conducted under a 20-hour photoperiod; so this environment in a way simulated a northern environment, although perhaps the temperatures were too high. The local climate in the nursery seems to simulate a northern environment as well. On the whole, I certainly have to agree that we need to make hybrids for specific environments and that this is the only chance of utilizing provenance hybridization as one of the breeding methods.
- Valentine: These comparisons in most cases have been based upon the means of hybrid progeny in relation to those of control groups. I

was wondering how the amount of variation in the hybrids compares with that of the controls, and how this would affect the selection in the second generation in which one might expect to find trees with superior growth. Under some conditions hybridization doesn't look promising initially, but a second or later generation might prove worthwhile.

Morgenstern: I think they might be promising, but one has to weigh the alternatives.

Valentine: Was the variance considerably larger in the hybrids?

Morgenstern: No, it wasn't. Most of the variation was contributed by the provenance component, but the variance component of progenies in provenances was still large. So there is a possibility for selection; and if one thinks that this was promising, one has to weigh the degree of effort. At the moment our effort here is limited. If we had additional funds and personnel, we could intensify the effort; but I would certainly emphasize that now we are not prepared to do so.

Valentine: One reason why I asked this question is based on personal observations of *Populus* hybrids at Harvard Forest, both interspecific and interprovenance hybrids. In most cases one wouldn't think this method worthwhile, but one or two trees from one of the interspecific crosses are so obviously much better than their full siblings or unrelated hybrids that it seems worthwhile to select the individual progeny rather than to select on the basis of a parent contribution to a progeny group.

Ledig: I want to ask a question of Don Fowler and Mark Holst that is similar to your question. Was the variance in the interprovenance hybrids greater than the variance within provenances? In other words, was interprovenance hybridization useful in increasing the amount of variance in red pine; that is, individual tree variance?

Fowler: There appears to be a slight increase in variance in the interprovenance hybrids. This results, at least in part, from a small increase in the numbers of "abnormal" (usually stunted) seedlings. It may be possible to create populations that have a greater potential for selection, but the possibilities are still rather limited.

Critchfield: I would like to ask any of the speakers if they encountered any cases of crossing barriers between provenances with species that have been discussed.

Morgenstern: There did not seem to be any crossing barrier in black spruce; in fact, the seed set was quite high - 10 to 20 or 30 filled

seed, per cone, and that was the average we got for black spruce under a normal situation.

Woessner: With loblolly, I would say there was none. The wide-cross material was screened in the greenhouse and actually there were no more mutants than in the open-pollinated plots. There was no indication at all that we had any trouble making the crosses themselves.

Nilsson: I would say that the variance in the provenance crosses was very small as compared with the local crosses in which you also have small variation; but if you have open-pollination, there was a very big variation in the nursery and this is probably inbreeding effects.

Yeatman: There is no evidence of crossing barriers in jack pine. In the early years of predominantly female flowering the seed per cone from all combinations was quite high for provenances growing at Petawawa. But if I can comment briefly on the point concerning variation, I do have tabular values for coefficient of variation on tree heights for one of the experiments. They have not been analyzed, but it is clear that the better provenances, including the local Petawawa source, have low values for coefficient of variation. Higher values are associated with the slower-growing provenances from high latitudes and the Maritimes. There is a negative correlation between coefficient of variation and growth at Petawawa. Among the provenance hybrids the variation is no greater and no less than in the provenance controls. Some of the hybrids have generally low values in the order of 10 to 12%; others have relatively high values, around 20%. So there doesn't appear to be any great difference in the variability within provenance hybrids as compared with the controls.

Klein: This is a speculative question. I have always thought that hybrids might have the greatest value for producing double hybrids or crosses with hybrids because you will release the variability that comes together in the first hybridization, and I wondered if any of the speakers have contemplated making crosses using the hybrid trees in the experiment. You get, I think, a lot of variation among the gametes from any parent because of the recombination.

Fowler: We have been involved with some work with *Larix* at the species level. One of our most promising crosses is (*Larix laricina* x *L. leptolepis*) x *L. decidua*. The variance of the hybrid material is large (probably resulting from recombination in the female parent). We appear to get a normal increase in variance plus up to 25% abnormal (often dwarf seedlings). Only if the "dwarfs" are removed from the hybrid and control population do the hybrids appear heterotic. The populations

do provide good possibilities for individual tree selection, especially if they can be propagated vegetatively.

Woessner:

I just have a general comment. What we are seeing in the F-1 might not be what we are going to see in the F-2 because you have to realize that all genes that are fixed in each population are not giving you any variance in that population; and when you cross them to make the F-1, you are not getting any variance. When you cross these F-1's among themselves, you might really get an explosion, and release a tremendous amount of variability. I think I would say not to give up yet on interprovenance hybrids.

Teich:

I think one of the previous points has not really been emphasized. When you perform a hybrid cross, you may on the average get only a small amount of heterosis, say 5 or 6 to 10%; but if you looked at the individual hybrids within some provenance crosses, you might find some with much more. This is essentially what happens with hybrid corn. When hybrid vigor was first examined in corn, the average of these hybrids was only about 15%. But some individual crosses had tremendous amounts of hybrid vigor. It would be interesting to see in the hybrid data of forest trees how the individual hybrids within the provenance cross compared with the average of the parents; and it may be that certain crosses have remarkable heterosis and in some cases this could be perpetuated on a production basis by vegetative propagation.

Morgenstern:

I must say I am very skeptical about provenance hybridization. Falconer in his book points out that one makes the assumption that heterosis is the result of dominance variance and that one eliminates inbreeding when crossing populations. Therefore, the focus of our attention is dominance, and epistasis is neglected. He points out that even though we may get an improvement in characters like growth, plants are complex organisms and they are really not dependent only upon growth. There are many other factors that we must watch. Things like survival, hardiness, resistance to insect pests, and this is, I think, the big problem that we haven't touched. We also have no experiments that make a complete interpretation possible. We don't know what effect epistasis has, and epistasis will no doubt have a very large effect if we consider organisms like forest trees that have to cope with a large number of environmental situations even at the same site. This is quite a different situation than in corn, where we have an annual crop that goes through an almost predictable type of environment in one year. So I think we should be very skeptical until we have a much better tool to work with on the complete genetics of the species.

Lester:

I will talk a little more about some of these points this afternoon, but let me add to Chris Morgenstern's comment to

emphasize that much of what we have heard this morning has dealt with growth as one variable. If we look at the results of hybridization for a variety of traits, we will find that intermediacy is something that may be extremely useful to us; and I point to the Korean experiments with *Pinus rigida* x *taeda*. Hybridization produces progeny which are intermediate; yet the improvement of form as compared with *P. rigida* and improvement of hardiness as compared with *P. taeda* represent significant genetic gains.

Woessner: We do have some data as far as comparing the percentage superiority within source crosses is concerned. This is on the basis of individual full-sibs and the largest superiority with the Piedmont females was 16%; in other words, an individual full-sib within provenance cross exceeded the average of the wind-pollinated parents by 16%. Looking at the between-provenance crosses, the largest was 15%. So with the Piedmont females, you really haven't gained anything. I would say the same situation occurred in the coastal females since the full-sib within provenance crosses had the largest positive deviation.

Fraser: I would like to direct this question to Kris. Perhaps the other speakers would add their comments about the evaluation of the criteria we are using. The term growth, for the most part, is applied to apical growth, but there are perhaps also differences in distribution of photosynthates. Have you evaluated your material in terms of total productivity?

Morgenstern: We have not done so.

Yeatman: One of the real values of the early provenance hybrids in jack pine will be to provide information on the effects of natural hybridization following transfer of seed or plants over a latitudinal or climatic difference. This will be of direct use to the operational foresters either in permitting them flexibility in seed movement or as a warning that certain movements should be avoided to prevent the generation of less productive hybrids within indigenous populations.

Nienstaedt: I wonder if any of the speakers have considered or have been able to consider in their studies the genotype-environment interaction, and to compare the responses of the hybrid in different environments?

Morgenstern: I have not considered genotype-environment interaction since I was dealing with only one environment at a time. I certainly agree that if we made a very systematic study, this is what we would have to do.

Ledig: Ron, in your short-term experiment in which you used three or four artificial environments, you did get a genotype-environment interaction, correct?

- Woessner: Yes, if you recall Fig. 4 (oral presentation), you can see evidence of the genotype-environmental interaction because of the switching that takes place.
- Fowler: Probably one of the greatest benefits from that loblolly material would be just the utilization of rust resistance and incorporation of that into the better-adapted population.
- Woessner: Right, and I also think that, for those individuals who would like to plant loblolly in possibly the top of zone 6 and into Tennessee, by crossing material from the southern part of the range with material from the northern part they could generate useful crosses similar to those described in spruce. Here, you are using a hybrid outside that area in which you would naturally grow the parents.
- Yeatman: The hybrid environment interactions in the jack pine material were quite extreme and very obvious. Any hybrid with a southern parent will be subject to cold damage when planted in the north. It seems to be a fairly additive situation rather than heterotic, at least in the F-1 generation. What will happen in the F-2 I don't know. If I can just make a brief comment on the Korean situation, which will be discussed this afternoon. The *Pinus rigida* x *taeda* hybrids are hardy and grow well in central south Korea, but they are of no value in the north, where it is too cold, nor in the south, where *P. taeda* is preferred. This is a situation where it is a hybrid of exotic species which fits the intermediate environment. A very different idea from the testing of provenance hybrids within the area of natural distribution of the species concerned.
- Lester: But again it is a multivariate situation. We may accept some compromise in growth to offset gains in other traits through hybridization. You have to look at more than one variable.

## INTERSPECIFIC HYBRIDIZATION IN FOREST TREES: POTENTIAL NOT REALIZED

D.M. Schmitt

*U.S. Forest Service  
Northeastern Forest Experiment Station  
Upper Darby, Pennsylvania*

I am aware that scientists are supposed to be objective. Indeed, objectivity is admirable in any discussion, but more often honored in the breach than in the observance. In any case my administrative status should relieve me, in part, of responsibility for strict objectivity. I call this to your attention because my title asserts a conclusion; and, of course, it is simply a personal one.

It has been said that if one uses material from a single source, it is plagiarism; but if one uses two or more, it is research. Since I hope to develop a contrapuntal theme of interspecific hybridization research and application, it will be necessary to review briefly interspecific hybridization research; so at least the charge of plagiarism can be avoided.

Many, including some plant geneticists, think that genetics research began with Father Mendel. In fact, leaving aside the Assyrians and the Egyptians and their date palms, experimental genetics became conceptually possible in the late 17th century when Camerarius proposed an essentially correct theory of sexuality in plants. But it was left to Linnaeus to attempt plant hybridization experimentally. His attempts were partially successful. Two of his successful species crosses produced completely sterile hybrids - and thus began a fund of information on interspecific hybridization.

The immediate purpose of Linnaeus' investigations, however, was to demonstrate sexuality in plants, a concept that nevertheless was not fully accepted until the late 18th century. But his observations and those of his students on hybrid performance, including failure of attempted crosses, partial to complete sterility of hybrid progeny, and poor hybrid vigor when the plants were subjected to competition, served a secondary purpose: to support the unique species creation theory.

Thus, as an outgrowth of the effort to relate experimentally sound data to an erroneous concept of the origin of species, a curious, almost modern, approach to plant taxonomy developed. On finding that two species crossed readily, producing viable progeny, pre-Mendelian botanists were prone to question the specific status of one or both parents. Not to do so would admit that crossability and hybrid fertility contributed to a speciation process. Since the conventional wisdom of the time postulated a single act of creation, crossability information was constructed into a crude but recognizable paradigm of a modern biospecies concept.

Conventional wisdom is remarkably persuasive, yet requires continuous reinforcement. Consequently investigators between 1700 and 1900, when Mendel was rediscovered, developed a substantial literature on plant hybridization, including interspecific hybridization. Focke provided a bibliographic treatment, including Mendel's papers, in 1890.<sup>1</sup> Without in any way disparaging in the many contributions of this period, I will mention only Kolreuter, because he spent a lifetime in the field and had summarized by 1949 most of the empirical observations in interspecific hybridization, which many of us, I included, have published again; and Thomas Andrew Knight because he very nearly anticipated Mendel's discoveries.

Kolreuter's observations on species crossing may be grossly summarized as follows: the difficulty of producing hybrids crudely approximates the taxonomic affinities of the parental species; i.e., the more closely related they are, the more likely a successful cross; hybrid characters are generally intermediate between the same characters in the parents; interspecific hybrids are frequently, though not always, sterile; fertile hybrids produce less seed than the parent species; hybrids are often considerably more vigorous than the parent species in some traits; intercrossing fertile hybrids produces extremely variable progeny.

Knight's contributions to plant hybridization deserve mention because they illustrate how zeal to apply research in the short run can have the effect of foreclosing long-term possibilities. His plant hybridization work began about 1820. Like Mendel, he recognized the value of peas as an experimental organism; like Mendel, but unlike any of his predecessors, he was able to produce and handle sizeable populations; like Mendel, he noted individual plant characters; like Mendel, he intercrossed hybrid progeny and observed recombination and segregation in the second generation. Unlike Mendel, however, he did not attempt to explain his observations; rather he recognized a splendid opportunity to make selections from the large array of recombinations at his disposal. And this is what he did, extending the principles to other species of agricultural or horticultural interest in England.

The foregoing information on pre-Mendelian plant hybridization was culled from Roberts' (1929) fascinating book on the subject. The commentaries are my own, but Roberts does state the pre-Mendelian research on plant hybridization was devoted largely to taxonomy, incompatibility, and pollination mechanisms, and hardly at all, except the contributions of Knight and the Vilmorin family, to breeding possibilities and their development.

#### INTERSPECIFIC HYBRIDIZATION RESULTS IN FOREST TREES

I will refrain from attempting to draw a parallel between past research interest in interspecific hybridization and that which has prevailed since 1910, except to suggest that such a parallel is at least possible. But

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<sup>1</sup>Cited by Roberts (1929).

Shull's and Jones' contributions to hybrid corn firmly fixed hybridization and heterosis in the plant breeder's lexicon.

Early North American forest tree breeders, with few exceptions, knew how they would produce improved trees - by interspecific hybridization. In retrospect it seems they had little understanding of heterosis or the operant conditions leading to it. Since the mechanism of heterosis is still unclear, they can hardly be faulted for not understanding it. However, the operant conditions inducing heterosis were sufficiently well known by 1912 that East and Hayes (1912) were able to propose the first of several theories concerning the genetic basis of heterotic behavior.

In addition to faith that interspecific hybridization would result in heterosis, the early tree breeders maintained what appears now to be a rather simple definition of heterosis, viz hybrid superiority in vigor relative to both parents. Consequently, the early tree hybridization experiments consisted of a more or less indiscriminate crossing of commercial tree species. Moreover, these efforts as well as most present ones were cursed with the same problem that afflicted pre-Mendelian hybridizers - difficulty in producing hybrid populations large enough to evaluate as populations rather than as individuals. The published results of these early efforts (e.g., Bey and Lorenz 1970, Minckler 1952, Schmitt 1968, Derr 1966, Little and Somes 1951, Johnson 1955, Shearer 1966, Stark 1964, Wright 1959, Zobel et al. 1956, Rehfeldt and Lotan 1970) tended to dispel early hopes of utilizing heterosis in tree-breeding. There were and are some striking exceptions to the monotonous tenor of these reports, notably the case of Austrian x Japanese red pine hybrids in Michigan (Wright et al. 1969) and that of poplar hybrids (Schreiner 1952). Strictly speaking, the latter are not truly the products of heterosis, but rather of selection in hybrid populations.

Must we then abandon heterosis in tree-breeding? I do not think we can answer this question now. But we cannot afford 30 to 40 more years of survey research in species hybridization, in the hope of hitting some jackpots.

Prudence suggests that tree hybridizers take advantage of (1) the fairly substantial and excellent body of crossability and incompatibility information that exists now - thanks to the same 30 to 40 years of hybrid research - and (2) the admittedly theoretical, but nevertheless useful, constructs of the mechanism of heterosis contributed by crop, but not forest, geneticists.

Quantitative geneticists, for example, define a heterotic response as a deviation of the mean population hybrid from the midparent value. Forest-tree hybridizers, by searching only for a positive deviation from both parents, have consistently overlooked one-third and possibly two-thirds of potential heterotic responses, thereby significantly diminishing their opportunities to understand heterosis and to exploit that knowledge.

Cress (1966) utilized a crop breeder's definition to develop a model for evaluating heterotic response based on parental genetic diversity (differences in the frequencies of alleles in the two parental populations and their

genotypic values). Whether it is a reasonable model, I cannot say; but if forest-tree breeders intend to utilize heterosis, they must develop guides for achieving it, techniques for recognizing the full range of heterotic response, and breeding programs that incorporate heterotic breeding at one or more points in the total program.

Before we leave heterosis, it is worth noting that the one or two well-documented cases in forest trees (Wright et al. 1969, Jeffers 1971) involve samples of parental populations known with a high degree of confidence to be genetically diverse on a population basis. Subsequently the hybrid progenies have been evaluated on sites alien to at least one and usually both parent populations. The contribution of genetic diversity in parent populations (which can crudely be assumed to exist in the absence of any opportunity for gene exchange) to heterosis has been observed often enough to be used as a guide for heterotic breeding: we are less certain concerning the utility of the "hybridized habitat" for this purpose.

Anderson's (1949) monograph on introgressive hybridization introduced the hybridized habitat concept to explain more or less persistent populations of natural hybrids. Subsequently the concept has been employed, though often less convincingly, for the same purpose. But forest geneticists have not yet advanced a theory to explain gene-environment interactions. In short, empirical results can be and are explained in terms of the hybridized habitat when this condition can reasonably be presumed to exist; but the theory in its present form cannot predict what will constitute a hybrid habitat in advance of the projected cross. Fortunately some of the recent provenance research (e.g., Fryer and Ledig 1972, Roche 1969) is designed to provide a much better understanding of gene-environment interaction.

If efforts to achieve useful heterosis in hybrids of forest trees were not particularly successful, they also were not without value. It has been amply demonstrated that desirable characteristics of one parent species, lacking in the other, can be transmitted to the hybrid progeny. Disease resistance (Derr 1966, Jewell and Mallett 1964, Dinus 1972) and insect resistance (Libby 1958) can, at least in some cases, be readily transmitted to hybrid progeny. Adaptability of a desirable species can also be improved by hybridization with a hardy but less desirable species (Hyun 1969). Tree breeders have also observed that the effects of selection in populations prior to hybridization are reflected in the progeny (Nilsson and Andersson 1970, Schmitt 1968, Righter and Duffield 1951). By themselves these observations may seem trivial to a modern geneticist, but they are potentially important for a breeder. Interspecific hybridization is an obvious mechanism for transferring desirable genes into a native population that lacks them. And parental selection is a useful hedge against introducing undesirable genes into the same population.

#### PROBLEMS IN THE APPLICATION OF INTERSPECIFIC HYBRIDIZATION IN TREE-BREEDING

On balance, then, our thumbnail sketch of interspecific hybridization in forest trees has revealed some disappointments as well as useful research

accomplishments; yet very few forest tree-breeding programs utilize interspecific hybridization. At first, of course, there was a well-justified concern that the hybrids would prove to be sterile. By 1958, however, Stebbins could generalize that in woody plants, hybrid inviability and sterility were poorly developed. And by the 1960's it was apparent that many interspecific hybrids of forest trees were quite fruitful.

But if hybrid sterility in its most aggravated form is not a problem, a related problem appears in the form of reduced seed set in  $F_1$  crosses (Snyder and Squillace 1966, Hanover and Wilkinson 1969). When this problem appeared in a breeding program for pine reproduction weevil resistance, the geneticist resorted to backcrossing to one of the parents with much improved seed yields and little or no loss in insect resistance (Libby 1958). The example illustrates not just a plausible and, in this case, realistic solution to the seed-set problem, but a refusal to balk, like a faulty steeplechase runner, at a hurdle common to all forest tree breeding - the relatively long interval between generations of forest trees.

Though there are several excellent forest tree breeding programs in North America, forest-genetics research has been chiefly directed to what, for lack of a better term, I will call "the now generation". The result has been some good research, but few long-term research programs and consequently a relative absence of long-term breeding plans. Gradually we are finding that the generation interval is not quite as long as we thought it might be, and there are strong indications that, if we choose to do so, we can reduce it substantially through breeding (Stern 1961, Greene 1967).

But if the long generation problem is not as critical as it once seemed, there still remain some formidable practical problems related to the production of sizeable populations of interspecific hybrids. When there are large differences in flowering phenology between parents, one must resort to the use of stored pollen and controlled pollinations. There have been relatively few mass pollination attempts, and the one I am aware of was not very successful (Wakeley et al. 1965). Long-term storage techniques (exceeding 1 year) are available for many coniferous species (Snyder 1961, Callaham and Steinhoff 1966, Fechner and Funsch 1966), but more research on pollen storage of hardwood species is required. Results to date are interesting (Pyanitskii 1947, Ledig et al. 1971) but still fall short of the need for maintaining hardwood pollen viability for 1 or more years.

There is still another potential problem in producing populations of interspecific hybrids. For some species combinations there will be an absolute restriction on the choice of seed or pollen parent. Species crosses may succeed in one direction only. If it is assumed, that reciprocal crosses have no genetic differences, difficulties will occur when the availability of seed parents or pollen dictates the direction of the cross.

#### UTILIZING HYBRIDS

Thus far I have suggested that hybridization, especially interspecific hybridization, could be employed in tree-breeding programs. One

can conceive of three broad possibilities: (1) as a tool in support of breeding in which hybridization is not an important part of the total program; (2) as a means of producing hybrid populations that are intended to be the source of improved material; and (3) as an essential component of a long-range breeding program.

Hybridization as a tool in support of a breeding program would likely be considered when a desirable character is present in one segment of the gene resource available but absent from an otherwise improved population. Disease and insect resistance, adaptability, chemical or cytoplasmic properties, etc., are examples of characters that come to mind in this context; or a particular problem in a breeding program, such as poor seed production, may be solved by hybridization. The pine reproduction weevil case, already cited, melded both these approaches. In peanut-breeding, interspecific hybridization is employed to bypass an incompatibility barrier (Banks 1973). Similar possibilities exist in forest trees. Sonderegger pine, for example, a rather undistinguished hybrid of longleaf and loblolly pine, crosses readily with other southern pines (Schmitt 1968).

More often, however, the hybridization process has been considered as a major component of the breeding program, and the hybrid population is intended to be the source of improved material. With this objective, at least two approaches are possible. In one, hybridization is merely a sexual process employed to assemble an extremely diverse population, which is then subjected to intensive single-tree selection. Selected trees are subsequently cloned to produce populations of improved material. Hybridization presumably produces a wider array of material from which to select than is available in native populations. For species that are easily propagated vegetatively - ease of vegetative propagation is a requirement that is a major restriction - this approach would lend itself well to subsequent line-breeding for type, and breeders with a number of diverse breeding objectives might find it interesting.

A second approach in the use of hybridization for improvement would be a repetition of the original hybridization cycle. Whether this would be efficient or how soon it would become grossly inefficient is not known. We are currently reviewing the possible alternatives for further improvement in the Northeastern Station's hybrid poplar program, which was founded on intensive selection of hybrid poplar populations.

Most forest species, however, are not readily propagated vegetatively. Programs intending to employ hybrids expected, as mentioned, heterotic  $F_1$  hybrids from seed. More conventional approaches are possible. Intensive selection in parent populations before hybridization would probably result in considerable improvement. Alternatively, intensive selection in  $F_1$  populations resulting from nonselected parents could be employed to form a superior foundation population. Clearly both could be combined in a single program. Unfortunately, the research that would tell the breeder what he could expect has never been done. What is worse, in the absence of sizeable populations of advanced generation hybrids, the necessary research will not be possible.

Finally, hybridization may be used as a component in a total breeding program. In this context its primary function would be to restore vigor (take advantage of heterosis) and to provide opportunities for selection in recurrent selection schemes. Some of the possibilities are outlined in Andrus (1963).

#### CONCLUSION

To be candid, it is unlikely that operational solutions for some of the hybridization problems mentioned will appear in the near future. It would be tragic, however, if research organizations were to allow efficiency considerations to deter them from genetics, particularly population genetics, research on hybrid populations. Unfortunately, short-term, approachable research has been the rule, and the rule seems to persist.

The practical result has been that forest-genetics research, with few exceptions (Shelbourne 1969) has not contributed to the formulation of complete breeding programs. And it is in this context that hybridization will make a critical contribution to the genetic improvement of forest trees.

In concluding a 1958 paper on the same subject Duffield and Snyder (1958) said, "Benefits to American forests credited to hybridization are few and not too impressive." Almost 20 years later little can be added to this statement.

Perhaps we are now at the stage where long-range, well-thought-out programs of research on hybrid populations can be developed. I hope so, because the experience of crop breeders suggests that hybridization will be required by forest-tree breeders after several cycles of breeding; more important, fundamental information concerning the nature of genetic variance and its transmission and potentiation by the environment could be employed in the development of efficient breeding programs any time the information became available, and genetic analysis of hybrid populations could expedite in an important way the formulation of such information.

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## INTERSPECIFIC INCOMPATIBILITY AND INVIABILITY PROBLEMS IN FOREST TREES

H.B. Kriebel

*Ohio Agricultural Research and Development Center  
Forestry Department  
Wooster, Ohio*

Interspecific hybridization extends the limits of variability in the gene pool beyond those of any one species. Thus it is particularly valuable in tree-breeding when a trait needs to be augmented or diminished or when it is desirable to combine two or more traits. Moreover, heterosis is fairly common in forest tree hybrids and can have practical value.

However, utilization of genetic divergence often introduces the problem of overcoming hybridity barriers that have developed through the process of evolution. The use of a third species as a bridge may be practical, but it can also extend the time required to develop a new variety or introduce undesirable characteristics that reduce the value of the hybrid.

There are two general types of internal, genetically controlled barriers to hybridity in forest trees. These are incompatibility and inviability.

Incompatibility, in the broad sense but not including vegetative incompatibility, is a genetically controlled failure or partial failure in some process leading to fertilization (Snyder 1972). This definition includes both self- and cross-incompatibility. Both types are gene-controlled, but whereas self-incompatibility involves a reaction following mating of gametes with one or more similar alleles, cross-incompatibility involves the mating of excessively dissimilar genotypes.

Hybrid inviability includes any block occurring after zygote formation up to the time of reproductive maturity. It has been defined as the lowering of somatic vigor due to aberrant developmental processes in hybrids (Rieger et al. 1968). Only embryo inviability will be considered in this paper, covering the period from fertilization to germination.

Both these types of hybridity barriers have been identified in forest trees. The term "crossability" as used in tree-breeding applies to both types and may be defined as the relative success in obtaining viable offspring from cross-pollinations, especially from interspecific ones (Snyder 1972).

The physiological basis of a crossability barrier depends on the stage at which developmental inhibition occurs. Therefore, it is first necessary to establish precisely the point in seed development at which the block occurs after pollination. Subsequent investigation of the biochemistry of the incompatibility or inviability phenomenon may indicate the possibility of overcoming the barrier.

## INCOMPATIBILITY

The first major type of hybridity block, incompatibility, is caused by genetic information carried by the alleles in the cell nucleus. The incompatibility genes are present in both the male and the female tissues. In all incompatibility reactions, the information stored in the male and female cells is the cause of the specificity of the reaction, the induction of the reaction, and the energetic and metabolic background of the reaction. These finally lead to processes that prevent normal fertilization. The intensity of the metabolic pathway leading to the reaction is affected by modifying factors of the environment (Linskens 1965).

We know much more about self-incompatibility than we do about cross-incompatibility. However, some recent studies of incompatibility in angiosperm plants, including trees, show certain similarities in the genetic mechanism of self- and cross-incompatibility. Thus we cannot ignore the information derived from the genetic and physiological study of self-incompatibility in considering problems of interspecific hybridization.

### Sporophytic vs. Gametophytic Incompatibility

Two types of self-incompatibility are recognized in the homomorphic flowering plants. In one type, the incompatibility reaction of the pollen is determined by the genotype of the parent plant; i.e., there is sporophytic determination. Alternatively, it may depend on the genetic constitution of the pollen itself; i.e., it is determined by the gametophyte (Allard 1960).

In systems with sporophytic incompatibility, the specificity of the reaction is imposed on the diploid mother cells, or earlier, and inhibition occurs on the stigma surface. Some pairs of alleles exhibit dominance, others act individually, in both the male and the female organs. In systems with gametophytic incompatibility, the specificity of the reaction is imposed on the haploid pollen grain and inhibition occurs during pollen-tube growth through the style. There is always individual action of alleles in the style (Lewis 1954, 1956, Crowe 1964).

Brewbaker (1957) observed a correlation between pollen cytology and the type of incompatibility in homomorphic flowering plants. The sporophytic type, with inhibition on the stigma surface, occurs primarily in species with trinucleate pollen grains. Gametophytic-type incompatibility, with inhibition occurring during pollen-tube growth in the style, is typical of species with binucleate pollen grains.

### Stigmatic Reactions

Linskens (1965) concluded that the sporophytic type of incompatibility, occurring on the stigmatic surface, follows an immunological reaction. This relates to Sampson's (1962) hypothesis that the incompatibility reactions depend on the combination of complementary sites of pollen and stigma molecules, and that a molecular code exists whereby compatible pollen and stigmas

"recognize" each other. The reaction must take place at the contact surface between stigmatic papillae and the coat of the pollen grain.

Proteins and enzymes are rapidly leached from the pollen grain onto the germinating medium, even before tube growth begins (Mäkinen and Lewis 1962, Stanley and Linskens 1965). These diffusible proteins have been found in a wide range of angiospermous plants and in pines. Initially, they were believed to be localized principally in the intine cell wall of the pollen grain (Knox and Heslop-Harrison 1971, Knox 1971), but more recently, Knox and Heslop-Harrison (in press) have shown that such proteins, derived from the tapetal layer, may be localized in the cavities within the pollen exine.

The study of protein diffusion supports Sampson's conclusion that a recognition phenomenon occurs at the pollen-stigma interface. Knox and Heslop-Harrison (1971) showed that pollen of *Phalaris* released antigens, which spread over the stigmatic surface adjacent to the grains within 5 to 10 minutes of arrival. In this period, pollen tubes penetrated the stigma in compatible matings but failed to do so in incompatible matings.

The same phenomenon was demonstrated in various other genera, including *Populus*. Fluorescent micrographs of stigmas of *Populus deltoides* showed that pollen tubes of *Populus yunnanensis* (a compatible mating) had penetrated the stigma 24 hours after pollination, whereas the tubes of *Populus alba* (an incompatible mating) had not. However, killed pollen of the compatible type, in mixture with pollen of the incompatible type, furnished diffusible recognition substances to the stigma surface, enabling penetration and growth of the incompatible pollen tubes (Knox et al. 1972).

The results extend Stettler's (1968) success with "mentor" pollen in overcoming the incompatibility barrier in poplar crosses and interpret the role of the inviable pollen as the supplier of recognition substances. Destruction of the generative function of the pollen does not affect the wall proteins that are the source of the recognition phenomenon.

Recognition or mentor pollen has been successfully produced from compatible pollen by three methods: (1) freezing to  $-18^{\circ}\text{C}$  and thawing, the process being repeated 6 to 12 times; (2) gamma irradiation at doses up to 100,000 rads; (3) 2-minute exposure to ether or anhydrous methanol (Stettler 1968, Knox et al. 1972).

The technique was also applied successfully to overcome sporophytic self-incompatibility in *Cosmos* and significantly increase seed set from selfing (Knox et al. 1972). This suggests a functional similarity between cross- and self-incompatibility systems affecting pollen germination inhibition.

#### Pollen Tube Inhibition in Conifers

Knox and Heslop-Harrison (1970) also found diffusible extracellular pollen wall proteins in the genus *Pinus* but made no *in vivo* experiments with "killed" pollen. Vidaković is currently investigating pollen-tube inhibition

in the incompatible crosses *Pinus nigra* x *sylvestris* and its reciprocal by use of the mentor or recognition pollen technique at various levels of gamma irradiation.

Some qualitative differences in proteins exist in pine pollens of different species, though electrophoretic and serological studies indicate that the major protein differences are between the two subgenera (Bingham et al. 1964, Hagman 1967a). Differences have also been found between nucellar extracts of incompatible species of pines in the concentrations of various amino acids (McWilliam 1959) and sugars (Chira and Berta 1965), but the biological significance of the differences, if any, has not been determined.

Self-incompatibility, though probably common in angiosperms, is apparently nonexistent in the conifers (Hagman 1967b). In conifers, the reaction to self-pollination, when it occurs, takes place after fertilization, usually at a very early stage of embryo development, resulting in a high proportion of so-called "empty" seed in the cones.

In the pines, interspecific incompatibility has been verified by microtechnique in ovules of at least seven species of hard pines in four of the six subsections of Subgenus *Pinus* (Diploxylon pines). The nine species combinations include two reciprocals and several crosses between subsections. All six subsections are involved as either females or males (McWilliam 1959, Hagman 1964a, Hyun and Lee 1964, Krugman 1970, Vidaković and Jurković-Bevilacqua 1970). In one of these hard pine crosses, *Pinus elliottii* x *nigra*, the pollen germinated, but no penetration of the nucellus was observed. In every other hard-pine cross, pollen-tube failure occurred in the nucellus.

In the soft-pine studies to date, there is no convincing evidence of interspecific incompatibility. I have observed arrested pollen-tube growth in ovules of *P. strobus* crossed with *P. cembra*, *P. koraiensis* and *P. flexilis*. But evaluation of a large array of crossing data showed that the frequency of incomplete pollen-tube penetration of the nucellus was no greater in the species crosses than it was in the intraspecific controls (Kriebel 1972). Likewise, in *Pinus peuce* crosses, a block occurs after fertilization (Hagman and Mikkola 1963) and earlier breakdown does not seem to be attributable to genetic incompatibility.

The positive identification of the inhibition stage during ovule development in pines presents particular problems from the standpoint of field and laboratory technique. Controlled pollination is especially difficult in the soft pines, and collection and histological work must wait until the following year. The timing and frequency of ovule collection are critical, and large numbers of ovules and of serial sections within ovules must be mounted, stained and sequentially examined. Thus a substantial effort is required to verify the specific dependence of a reaction on a particular species combination.

In the spruces, Mikkola's (1969) work indicates that each incompatible species combination has its own mean and frequency distribution with regard to the distance the pollen tubes penetrate the nucellus. The only

statistical tests were of crosses within *Picea abies* and *P. glauca*, and between the incompatible *P. abies* x *glauca* and its compatible but inviable reciprocal. In both cases, significant differences were found in the degree of penetration of the nucellus by the pollen tubes.

In certain species combinations of spruce reported by Mikkola (1969, 1972) and Kossuth and Fechner (1973), proembryos or embryos were observed in some ovules of each cross, whereas pollen-tube failure was seen in others. The extent to which fertilization and embryo development occurred varied with the species cross. Though more close-interval, chronologically sequential comparisons are needed between the hybrids and intraspecific controls, the results seem to indicate that two major types of barrier can exist in the same cross. This apparent redundancy of isolation mechanisms in spruce hybrid crosses contrasts with the single mechanism found in pines, i.e., incompatibility in hard pines and inviability in soft pines.

#### Intra-stylar Inhibition in Hardwoods

In hardwood tree species, arrested pollen-tube development after initial stylar penetration has been reported in several genera. The system was identified in *Betula* by Hagman, whose work is particularly significant because of its biochemical implications. Hagman thought that the application of serological methods to incompatibility in plants by Mäkinen and Lewis (1962) could be extended to forest trees. He therefore investigated antigen-antibody reactions in pollen-tube inhibition in birches. With the aid of fluorescent antibody technique, he was able to localize serologically-active substances in or on the tip of the pollen tube. His results seemed to confirm the idea that some of the substances involved in the incompatibility reaction are situated on the pollen-tube membrane (Hagman 1963, 1964b, 1967a).

Hagman speculated that the incompatibility reaction is dependent on a highly specific system of interaction between the enzymes of the pollen tube and the substrate in which it is growing. Both the formation of the enzyme and the structure of the substrate would allow very specific reaction patterns to be involved. The products of an enzymic reaction can inhibit the reaction by being bound specifically to the active site of the enzyme molecule. Thus he speculated that the progress of an enzymatic reaction associated with the penetration of the pollen tube could be dependent on the genetically controlled stereochemical structure of the enzyme as well as the complementary formation of its substrate.

In *Alnus*, interspecific incompatibility exists and is of the type found in *Betula*. In crosses between *Alnus glutinosa* (L.) and both *Alnus incana* (L.) Moench and *Alnus rubra* Bong., pollen-tube growth is retarded in comparison with intraspecific crosses (Hagman 1970). When *A. glutinosa* is pollinated with *A. incana* pollen, viable seed yield is either nil or nearly so, whereas a small percentage of viable seed can be obtained from the reciprocal cross. The cross *A. glutinosa* x *rubra* is completely sterile, whereas, again, the reciprocal cross is slightly fertile. The incompatibility varies with individual trees and environmental conditions (Ehrenberg et al. 1955, Hagman 1970).

We know little about crossability barriers in other deciduous forest trees. Most families, including the Fagaceae and Aceraceae, have binucleate pollen grains and probably have a gametophytic system of self-incompatibility. Retarded pollen-tube growth was reported in hybrids of *Quercus* by Piatnitsky (1947). The nature of the reactions underlying the incomplete crossability barriers in *Castanea* (Jaynes 1964) has not been investigated.

#### Methods of Overcoming Arrested Pollen-tube Growth

Pollen-tube inhibition due to self-incompatibility can sometimes be overcome by irradiation of the pollen or of the style. Linskens et al. (1960) were able to break down the self-incompatibility reaction of *Petunia* by X-irradiation of the pollen, leading to mutation of the S-allele, or by irradiation of the style immediately prior to selfing. The stylar irradiation was most effective at 2,000 R, nearly half the irradiated flowers yielding seed. Irradiation of the style 24 hours after pollination was ineffective. The stylar effect was regarded as biochemical rather than mutational.

Vidaković and Jurković-Bevilacqua (1970, 1971) used irradiation in attempts to overcome the incompatibility barrier between *Pinus nigra* and *Pinus sylvestris*. By histological analysis of crosses in both directions, they had previously found that pollen-tube growth was arrested in the nucellus early in the second year. Low-level gamma radiation at levels up to 5,000 R was therefore applied to the pollen on the possibility that physiological "stimulation" of tube growth might lead to fertilization. At 5,000 R fewer ovules had degenerated by 20 June of the second year than at 0 R.

Induced mutation in the pollen may be more practical in breaking down self-incompatibility than in overcoming cross-incompatibility, because it would be easier to increase heterozygosity than homozygosity. If, as Linskens et al. (1960) believe, stylar irradiation has a biochemical rather than a genetic effect, it might have more promise than pollen irradiation for interspecific hybridization. Tree breeders would have logistic problems in the application of stylar irradiation, however, except when crossing can be accomplished on grafts or cut branches.

Another method used successfully in some flowering plants to overcome gametophytic self-incompatibility is high-temperature treatment. Before or after selfing, the plants are subjected to a temperature in the 32° to 34°C range for 24 to 48 hours. In clover, the resulting development of viable seed has been shown to be the effect of specific temperature-sensitive genes on the S-alleles (Hecht 1964; Hopper et al. 1967; Townsend 1966, 1970). It is possible that there could be a similar alteration of the allelic interaction that leads to pollen-tube inhibition in interspecific crosses.

#### EMBRYO INVIABILITY

Embryo inviability, the second major cause of hybrid breakdown within the developing seed, may be in either of two systems. One is the inability

of an otherwise viable embryo to develop to maturity in the female gametophyte, presumably due to the absence of a specific growth factor in the surrounding nutritive tissue. The second is a metabolic disruption at some stage of embryo development attributable to the malfunction of one or more allelic pairs in the genome.

If the problem is a communication failure between the embryo and the surrounding ovule tissue, or inability of the embryo to break through the seed coat, excision of the embryo and *in vitro* culture is a feasible method of obtaining hybrids. The technique has been used successfully in tomato-breeding to introduce wilt-resistance genes from the wild tomato into domestic varieties (Smith 1944). An example in forest tree breeding is the successful production of sugar pine hybrids by Stone and Duffield (1950). In this case, the embryos were fully developed when excised and were not actually cultured *in vitro*. The seed coat was removed and embryo and endosperm were cultured together.

The more common inviability problem in forest trees may be that concerned with the pairing of genes and chromosomes. Since major changes in chromosome structure of pines are not considered important in establishing reproductive barriers, except perhaps in subsection *Sylvestres* (Saylor 1969), the problem in pines may be functional at one or more gene loci.

#### Inviability Systems in Pines

Hagman and Mikkola (1963) investigated the total block in crosses of *Pinus peuce* Griseb. with *P. koraiensis* and *P. cembra*. After the time of fertilization in intraspecific crosses, all observed ovules of *P. peuce* x *koraiensis* and most ovules of *P. peuce* x *cembra* contained at least one fertilized archegonium. In both crosses, the maximum developmental stage observed before degeneration was the proembryo, usually at the four-nucleus stage but in a few ovules at the six- or eight-nucleus stage. No cell walls were seen in the proembryos.

Hagman and Mikkola concluded that the hybrid nucleus was able to function as long as no organization of the new zygote was necessary. It seemed that at the first stage of differentiation, when the four nuclei divide and cell wall formation begins at the base of the oocyte, the nuclei were no longer able to communicate with the surrounding protoplasm.

Subsequently, in my own experiments with five-needle pines, I found that fertilization and early embryogenesis also occur in the blocked crosses *P. strobus* x *koraiensis*, *P. strobus* x *cembra* and *P. strobus* x *flexilis*. In fact, a large-scale study of late-stage ovules showed that there was no inhibition at the proembryo stage. Cell walls formed in the proembryo, which subsequently pushed out of the archegonium into a corrosion cavity, exactly as in the intraspecific cross. Abortion occurred after the development of from one to several suspensor cells and up to eight cells in the embryo initials. I also found that corrosion cavities previously believed to have developed without embryos had, in fact, previously contained embryo remnants and were the result of (1) earlier failure to make daily collections at this

late stage and (2) loss of contents during microtechnique (Kriebel 1967, 1968, 1972).

This was the first evidence that embryogenesis in some blocked pine hybrids proceeds to a stage of cell differentiation. It is possible that inviable *P. peuce* hybrids may also be capable of developing an early embryo.

It appears that there is a general pattern of pollen-tube incompatibility in hard-pine hybrids and of embryo inviability in soft-pine hybrids. Arrested pollen-tube growth can be seen in soft pine crosses, but it appears to occur in no greater frequency in interspecific crosses than in intraspecific crosses.

Culture of early-stage pine embryos, either from intraspecific or interspecific crosses, has so far been unsuccessful. Ovule culture is also difficult. I have attempted, with limited success, the culture of entire strobili of *P. strobus* collected as early as late June. The usefulness of the technique for the culture of hybrid cones is still undetermined (Kriebel 1970). Thus we do not know whether normally inviable hybrid pine embryos can mature *in vitro*.

#### The Physiology of Embryo Inviability

My work seems to support the hypothesis of Hagman and Mikkola (1963) that breakdown in soft pine crosses occurs when communication is required between the hybrid nucleus and the cytoplasm of the embryo. But rather than occur at the point of wall formation in the proembryo, the critical point seems to occur later, at the stage when transition is required from one-dimensional growth of the suspensor system to two- to three-dimensional growth, especially that involving cell proliferation in the embryonal apex. This is the point when the archegonial cytoplasm is depleted and ceases to be a growth substrate, the suspensors degenerate, and development comes under the control of the hybrid nuclei of the embryo itself.

In both plants and animals, increased cooperation, as evidenced by a sharp rise in protein concentration, is required when a major phase shift in growth occurs. In plants, this has been noted at the onset of two-dimensional growth in ferns (Hotta and Osawa 1958, Hotta et al. 1959). In animals, a critical stage occurs at the beginning of gastrulation in hybrid sea urchins. At this point, new templates are required for protein synthesis, but transcription of messenger RNA is blocked by allelic imbalance in the hybrid genome (Brachet et al. 1963, Gross and Cousineau 1964, Gross and Moyer 1964).

In white pine hybrids, I have found a deep red safranin staining of aborted suspensor cells in prepared slides, which suggests an accumulation of nucleic acids accompanying a block of protein synthesis. However, Shafer, who is working at Wooster on the biochemistry of embryo inviability, found no accumulation of DNA or RNA in aborted embryos after critical histochemical staining (Shafer 1973).

Shafer is currently working on the hypothesis that the early embryo development of inviable conifer hybrids is directly dependent on "preformed" messenger RNA. If this is so, initial protein synthesis and cell differentiation in the embryo are not dependent on messenger RNA derived from the zygote itself. Instead they utilize the mRNA formed during oögenesis and stored in the egg cytoplasm. At the point of phase shift in embryo development, mRNA for subsequent growth must begin to be transcribed by the hybrid nuclei. It is the inability of these nuclei to function that leads to the death of the embryo. The concept is an extension of the demonstrated role of preformed mRNA coding in sea urchin development (Gross and Moyer 1964) and of evidence that such coding may occur in the egg cells of ferns (Jayasekera and Bell 1971, 1972). Its role in embryogenesis has not been investigated in other plants.

#### Embryo Inviability in Other Trees

In *Picea*, as previously mentioned, embryo inviability has a significant role as a block to hybridity; but we have very little histological information about inviability in deciduous trees. It is interesting to note that Melchior and Seitz (1968) reported that the cross *Populus nigra* x *deltoides* leads to zygote formation and the beginning stages of embryo development before degeneration occurs. This is in sharp contrast to the very early stage barrier due to failure of pollen tubes to penetrate the stigma, found by Knox et al. (1972) in the *P. deltoides* x *alba* cross.

#### Problems in Overcoming Embryo Inviability

It seems at this time that the prospects of overcoming interspecific crossability barriers are better in cases involving incompatibility than when embryo inviability exists. This is because it has been shown to be possible by special techniques to sustain pollen-tube growth and effect fertilization. In the successful experiments, there was no further barrier to embryo development. In crosses involving embryo inviability, however, it seems possible that the block is in the hybrid genome itself. If it is not, or if gene action can be modified, this barrier may also be bypassed.

We have as yet made little use of genetic selection of individual trees for crossability in hybrid combinations. In early crossability experiments, Wright (1953) found that wide variability exists in the hybrid seed yield of individual trees. The most rapid progress in overcoming hybridity barriers will be obtained by combining genetic selection for fertility with some modification of the functional sequence at the predetermined critical point in development.

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## DISCUSSION

Gabriel: Howard, are you calling postzygotic abortion embryo inviability?

Kriebel: Yes, any breakdown after fertilization on is classified as embryo inviability.

Gabriel: Since the embryo hasn't really developed, it would seem to be postzygotic abortion rather than embryo inviability, since the latter term implies that the embryo completed its growth.

Kriebel: We have no evidence in trees of genetically controlled breakdown at the time of fertilization. In conifers, the only evidence we have is of breakdown occurring after zygotic division and some organization at the base of the archegonium. Embryo inviability has also been found in hardwoods. But in only one genus of flowering plants has breakdown been observed at the time of fertilization.

Schmitt: Howard, just as a matter of information, is the stain that you mentioned specific for messenger RNA or just RNA?

Kriebel: It is a general indicator of RNA, not specific for any one type. This staining technique requires a comparison. In one series of slides, you use the combination methyl green and pyronine, and in another series you follow the staining by incubation in ribonuclease. The ribonuclease removes RNA, thus taking away the red or pink color produced by the pyronine reaction, if this color is actually due to the presence of RNA. The methyl green will similarly indicate the localization of DNA if the color is removed after incubation in DNAase.

Schmitt: Are you people or anybody else doing any work with electron microscopy in this connection?

Kriebel: We are not. Knox and his associates are using electron microscopy, but not with pines.

Nienstaedt: The possibility of selecting parents that were in fact compatible could be considered. Is there any reason to think that a parent that is highly self-compatible would also be cross-compatible with another species?

Kriebel: I think there is, but I don't have much to go on. In a few cases I have observed high conelet retention and later high seed yield in individual white pines that are also highly self-compatible. In fact, they were able to retain their cones in the absence of pollination, a phenomenon which occurs in a rather small percentage of *Pinus strobus* trees. I think we should investigate the possibility that there is a correlation between self-fertility and cross-fertility. It may be that

there is a general physiological basis of fertility which applies to both self- and cross-compatibility.

Critchfield: At Placerville, we don't have very much reason to think that a particular tree that is highly crossable with another species would also be highly crossable with a second other species. In other words, a Jeffrey pine that readily crosses with Coulter may or may not cross readily with ponderosa. It is a real difference.

Kriebel: It is quite possible that one cannot generalize, or that hard and soft pines differ. As I say, we have indications to the contrary in *Pinus strobus*, but as yet no firm data.

Kossuth: You said that *Pinus aristata* and *P. balfouriana* were highly compatible in California and suggested that the bristlecone pine that grows in California and that which grows in the Rocky Mountains might be given species status because of some differences suggested by Bailey. Maybe differences between the California and Rocky Mountain bristlecone pine would also show up if intraspecific crosses were made from these two distributions as well as interspecific crosses between Rocky Mountain bristlecone pine and California foxtail pine. Has this been tested?

Critchfield: No, it hasn't, Sue. Our crosses have all been made between the western race, or subspecies, or species, of bristlecone pine and *P. balfouriana*.

Gabriel: In the case of the pines that you were talking about, in which you had incompatibility between the north and south populations, how far has the morphological differentiation within the species gone in these two areas?

Critchfield: It is not very well correlated at all. I don't want to go into that, but it is not. Incidentally, I would not say that we had incompatibility. I am getting very gingerly about using this word since it has been preempted for barriers prior to fertilization through constant use in relation to angiosperms. So we have almost a complete lack of crossability whether it is incompatibility or embryo inviability.

Gabriel: Did you try artificial pollinations between the north and south populations to see if barriers were causing the lack of seed set?

Critchfield: Yes, we tried that; but without any interesting stuff like irradiating it.

Gabriel: Bill, I think the story about the *resinosa* x *nigra* hybrids is worth telling.

work, and I never published it. Still, it was clear, based on probability calculations for numbers of gametes per nucellus compared to filled seed resulting from pollen mixes, that the technique would give very few, if any, *resinosa* hybrids. We grew progenies in the greenhouse for one year. They all looked like either *resinosa* or *nigra*. We transplanted them to the nursery and, in the process of changing environments, things didn't work out too well. Though my results were incomplete, I would say that mentor pollen is not a promising technique for producing numerous hybrids between *P. resinosa* and *P. nigra*.

Critchfield: I wish I had done like you, Don, and never published on it.

Critchfield: The only hybrids that were ever obtained from this combination were in what was observed as a contaminated pollination; that is, we harvested 40 to 50 seeds of this combination and most of them turned out to be *Pinus nigra*; so it was a contaminated cross and the suggestion was that somehow the presence of Austrian pine pollen in the Austrian pine ovules made it possible for the red pine pollen to germinate, grow, fertilize, and produce embryos. But then we tried this later, deliberately contaminating by making pollen mixes of the two species, and we didn't get anything.

Ledig: Bill, what do you think about the *Pinus rigida* x *radiata* cross? Have you seed of Hyun's material from Korea?

Critchfield: Hyun has sent us two or three tiny lots of seed of that doubtful cross, and they have never appeared to anybody who has seen them growing in the nursery to be intermediate between *rigida* and *radiata* in any respect. I think they are hybrids of some other southern pine. Hyun has published quite a bit now on that cross; but honestly, I am completely unconvinced.

Gabriel: He did have some pictures of cones at one time, I thought.

Critchfield: They were unconvincing, too. I think he sent us some cones, which were really unconvincing. I would like to know the flowering time of their putative hybrids because *rigida* and *radiata* have a 4-month difference in pollen shedding time; and if they have a good hybrid there, they should get some interesting phenological data on when it flowers. Hybrids are almost always intermediate in flowering time.

Gabriel: I thought that *resinosa* and *nigra* were impossible to cross. So how did they get their hybrids?

Critchfield: Well, they didn't claim that it was a good hybrid. They just presented these data and they seemed very inconclusive; but I must say that they have done very careful work -- I visited them several years ago, and I was impressed. I also read their reports regularly. I have been impressed by the quality of the work, and it encourages me that they are picking up a few *nigra* x *resinosa* hybrids. It gives me more confidence that ours might be good hybrids. On the other hand, something that gives me less confidence about the identity of our hybrids is some work that Duane Zinkel has been doing at the Forest Products Lab. He has been doing work on resin acids in the leaves. His work is very preliminary, but it certainly doesn't support their *resinosa* parentage.

Lester: Several years ago I did a series of pollination experiments on *P. resinosa* x *nigra* using irradiated pollen and nonirradiated pollen in various combinations. It wasn't particularly careful

## THE ROLE OF INTERSPECIFIC HYBRIDIZATION IN FOREST TREE BREEDING

D.T. Lester

*Forestry Department  
University of Wisconsin  
Madison, Wisconsin*

Recent reviews of interspecific hybrids and their potential in forestry have been published by Wright (1964), van Buijtenen (1970), and Brown (1972). My comments will therefore emphasize genetic considerations, which have been less discussed. The opportunity to consider this topic was particularly welcome, for it coincided with my own summary and future research planning after 5 years of interspecific hybridization in elms (*Ulmus* sp.).

In keeping with the symposium title, my discussion emphasizes interspecific hybridization. I assume that in terms of total genetic differentiation between parents, interspecific hybridization represents combinations of greater diversity than interprovenance hybridization, though we know so little about the genome of any forest tree that this assumption cannot be substantiated at present. Moreover, in certain cases is it not possible that provenance crosses within species represent combinations of greater genetic diversity than crosses among species? Examples of the latter possibility may be subalpine fir (*Abies lasiocarpa* Nutt.) and balsam fir (*A. balsamea* [L.] Mill.) or jack pine (*Pinus banksiana* Lamb.) and lodgepole pine (*P. contorta* Dougl.). One recent discussion of the classification problem proposed that the terms "taxonomic species" and "biological species" usefully illustrate the necessary distinctions: a taxonomic species includes individuals of similar morphology and a biological species is a reproductively isolated system of breeding populations (Grant 1971). Perhaps the unifying concept here is a scale of genetic diversity between parents ranging from the single locus variation by which geneticists define hybridization to the combination of extreme diversity implied in the term "wide crosses".

In many respects interspecific hybridization as a breeding method should stimulate dreams of remarkable achievements among tree breeders. Consider, for example, the range of genetic variation present at the genus level in *Pinus* and *Populus*. Moreover, new techniques for breaking crossing barriers between species promise easier access to a fantastic number of gene combinations (Knox et al., 1972). With such apparent potential for remaking our commercial forest tree species, why is interspecific hybridization so rarely given emphasis in breeding programs? Some notes on genetics will serve to temper general enthusiasm for the method.

Results from interspecific crossing are difficult to predict *a priori*. The knowledge of inheritance patterns and genetic variances gained from intraspecific breeding is often of little value when unusual new gene combinations are made. Moreover, the array of F<sub>1</sub> genotypes available to the

breeder may be restricted by gametic or genotypic selection against certain gene combinations. Reduced seed set in interspecific crosses is certainly common, but I know of no studies in forest trees on whether the actual array of viable genotypes is a random or a biased sample of the potential array. It has been hypothesized that there is general linkage between genes controlling plant morphology and those affecting growth and vigor (Grant 1967). Perhaps closer attention by tree breeders to details of morphological variation in  $F_1$  hybrids would provide a clearer picture of what genetic combinations we are sampling.

The sampling question is also important in relation to  $F_1$  progeny sizes in advanced-generation breeding schemes. The  $F_1$  is traditionally regarded as relatively genetically uniform. While this assumption holds for highly inbred field crops, we generally assume that forest trees are heterozygous, and that traits of interest are polygenically inherited, several loci probably recombining within linkage groups. For forest trees, then, many genetically different gametes can be produced in each parent and a large number of hybrid genotypes is possible. Under these circumstances, adequate sampling of  $F_1$  genotypes would probably require much larger  $F_1$  progenies than are suggested by Wright (1964). Published evidence of wide variation within  $F_1$  interspecific hybrid progenies includes data on *Picea omorika* x *jezoensis* (Langner 1970), *Populus deltoides* x *alba* (Knox et al. 1972), and *Ulmus rubra* x *pumila* (Lester and Smalley 1972). Experiments designed to estimate the components of variance among and within  $F_1$  progenies would be useful in interspecific hybridization programs to allow more efficient choice of number of parents and progeny size required for a breeding program.

## $F_1$ HYBRIDS

Looking first at  $F_1$  hybrids as breeding products for commercial use in forestry, we may say that the number of interspecific combinations regarded as worthy of immediate development is remarkably small. Perhaps tree breeders have placed too much emphasis on single-trait evaluation of hybrids. Hybrid vigor, i.e., progeny vigor exceeding that of both intraspecific progenies, has not been the common result of interspecific hybridization. Although genetic diversity between parents is a prerequisite for hybrid vigor, the converse is not true and *a priori* prediction of heterotic performance remains highly speculative at best. In some cases,  $F_1$  hybrid performance may be worse than intraspecific progeny performance as illustrated by response to *Hypoxyylon pruinatum* (Klotzsch) Cke. in progenies of *Populus tremuloides* x *grandidentata* (Berbee and Rogers 1964).

Where hybrid vigor is the breeding objective, it might be worthwhile to apply cost-benefit analysis for estimation of how much growth increase is needed to offset breeding costs. Cost-benefit analyses of intraspecific breeding programs have generally shown that small to modest growth improvement offsets breeding costs (e.g., Carlisle and Teich 1970). It is probable that modest growth gains from seed orchards for open-pollinated hybrid seeds would similarly offset costs. Perhaps even the establishment of unselected parents

in orchards would be worthwhile. Less certain are the cost-benefit relationships that might be present in more complex breeding programs involving recurrent selection. Although data from which to estimate benefits is lacking for many species and all of the more complex selection schemes, an evaluation of expected costs would point out the approximate magnitude of improvement required.

When more than one trait is considered, the frequent intermediacy of  $F_1$  hybrid progeny appears in a different perspective. Ideally the most favorable traits of each parental species would be dominant in the hybrid; yet even with intermediacy for all traits, trade-offs between traits can result in major improvement. The Korean experience with *Pinus x rigitaeda*, in which intermediacy of growth rate, stem form, and cold resistance represent a major advance in quality of planting stock, is a classical example (Hyun 1972). By contrast, intermediacy of ornamental traits and disease resistance in elm hybrids resulted in progenies of practically no commercial value (Lester and Smalley 1972).

#### ADVANCED-GENERATION CROSSING

When we consider interspecific hybridization beyond the  $F_1$  generation, prospects depend in large part on how we view the architecture of the parents' germ plasm. The classical view, formulated by Mendel, is that genes will recombine and assort independently during meiosis in  $F_1$  hybrids to give a broad array of genotypes in the  $F_2$  generation. Theoretically, production of a large enough  $F_2$  generation would result in the appearance of every possible gene combination, although for highly diverse parents an adequate  $F_2$  population might require more growing space than is available on the Earth. In practice, the classical view of independent assortment is modified by linkage and by gene action. The mechanics of meiosis restrict gene recombination to a maximum of half the amount required for random assortment at each locus. The result is a nonrandom array of genotypes forming what has been described as the recombination spindle in a multidimensional space of potential gene combinations. As an extreme example, assume that two species differ by 100 genes. Then  $F_2$  populations from  $F_1$  interspecific hybrids would potentially contain only three different genotypes if one nonrecombining linkage group was present and  $3^{100}$  genotypes if each differing gene was on a different chromosome. Another way of viewing the mechanical restriction of gene recombination is illustrated by the calculation that with a haploid chromosome number of 12 and an average of two to three chiasmata per chromosome (assumptions quite compatible with available information on conifers), the cohesive tendency of multiple factor linkage with 100 gene differences between species would restrict recombination to less than 1/500,000 the amount of potential free recombination (Anderson 1949).

Pleiotropy, or the influence of one gene on several traits, may also complicate the production of desired trait combinations. The prerequisite studies by which to distinguish linkage and pleiotropy for most genes in forest trees would be difficult if not impossible.

Nonrandom elimination of gametes or genotypes is another restricting factor. Incompatibilities or genetic imbalance involving chromosomes, genes, cytoplasm, plastids, and embryo-endosperm relationships in  $F_1$  hybrids may block access to certain gene combinations. For example, about 50% of the individuals produced from crossing *Populus deltoides* with *P. alba* have been described as dwarf or of reduced vigor (Knox, et al. 1972). Unfortunately useful gene combinations might be present in the dwarfs, but it would be difficult to recognize them.

Sterility in  $F_1$  hybrids, though an obvious obstacle for advanced-generation breeding, may be a useful result in  $F_1$  hybrids are the breeding goal. Fortunately, hybrid sterility is not commonly associated with reduced vigor of vegetative growth. This point could be especially important if genetic contamination of adjacent stands or plantations by interspecific hybrids is a potential problem.

Hybrid breakdown in the  $F_2$  may further restrict access to desired gene combinations. Again hybrid failure can be the result of vegetative weakness or sterility. The appearance of genetic imbalance in advanced generations has been given a variety of genetic interpretations. With these interpretations we can summarize views of germ plasm architecture and outline implications for interspecific hybridization in forest trees. Three levels of organization, genic, chromosomal, and genomic must be considered. Returning first to the principle of independent assortment, we say that  $F_2$  failure may represent disharmonic interaction between genes in new combinations produced during meiosis or between genes and cytoplasm of  $F_1$  hybrids. This interpretation emphasizes genes and may involve specific loci promoting  $F_2$  failure. In other cases of  $F_2$  failure, nonhomologous chromosome pairing is the consequence of species differences in chromosome arrangements, and the chromosome level of organization is emphasized. A third level of interpretation, and one that has received less attention in hybridization studies, is the view that in most species the genome is rather closely integrated by coadaptive gene complexes. Hybrid inviability is then the result of breaking up gene complexes essential to survival.

To illustrate the potential importance of coadaptive gene complexes, assume that two species differ allelically in five unlinked genes that control survival in their respective habitats. If the alleles are coadapted to produce a cohesive gene complex, 97% of the  $F_2$  segregants are expected to be genetically unbalanced and only 3% will have maintained the parental or  $F_1$  allelic combinations necessary for adequate development in a hybrid habitat or either parental habitat (Grant 1971). Linkage and reduced cohesion within the gene complex would reduce  $F_2$  loss, whereas increased numbers of gene differences would increase  $F_2$  loss. Results of interspecific hybridization in a variety of plants have been interpreted as evidence for coadapted gene complexes (Clausen and Hiesey 1960, Nielson and Smith 1968, Grant 1966). The wealth of data on ecotypic variation suggests the possibility that adaptive gene complexes are present in most forest tree species but the  $F_2$  populations necessary for confirmation are rarely available.

The genetic consequences of restrictions on arrays of gene combinations can be summarized in terms of changes in frequency distributions

expected in the F<sub>2</sub> generation. We usually expect a relatively uniform F<sub>1</sub> generation and great diversity in the F<sub>2</sub> and subsequent generations. With independent assortment, recovery of parental types in the F<sub>2</sub> is possible though improbable unless few genes or very large populations are involved. Linkage and pleiotropy decrease kurtosis (peakedness) as does transgressive segregation. Nonrandom elimination of gametes or genotypes has less predictable effects, although nonrandom assortment involving genic, chromosomal, or genomic levels of organization can be expected to increase the frequency of genotypes more closely resembling the parental species. Perhaps an increasing degree of hybrid failure would first result in a platykurtic frequency distribution, then in increasingly bimodal distributions until, in the extreme case, only types could be recovered.

#### BREEDING METHODS

For forest trees, I find few detailed studies from which to judge the potential of various advanced-generation breeding methods. Theoretically we expect great variation in F<sub>2</sub> populations, some loss of hybrid vigor if hybrid vigor was present in the F<sub>1</sub>, and nonrandom genotypic arrays if we have genetic imbalance. The expectations suggest that F<sub>2</sub> populations may not be highly useful for forest production, though nongenetic considerations such as cost of F<sub>1</sub> hybrid seed production might increase the attractiveness of F<sub>2</sub> seed collection from F<sub>1</sub> hybrid plantations. Evidence on actual F<sub>2</sub> performance is rare. For *Pinus x rigitaeda* in Korea, relative performance of parental species, F<sub>1</sub> hybrids, backcrosses, and F<sub>2</sub> populations was closely tied to climate of the planting site. Vigor was maintained in F<sub>2</sub> populations only in southern Korea, where mild weather presumably allowed development of segregants more similar to cold-sensitive *Pinus taeda* (Hyun 1972). Thus, the environment in which hybrids will be used may dictate the crossing strategy.

Where vegetative propagation for forest production is potentially feasible, advanced-generation crossing immediately becomes more attractive. Development and utilization of desirable gene combinations is then limited only by the restrictions on genotypic arrays available to the breeder. Breeding for transgressive segregation may be one possibility in hybrid combinations suitable for vegetative propagation.

Gene transfer by backcrossing is another advanced-generation breeding method of perhaps some utility in forest tree breeding. Greatest efficiency for the method accrues when the trait to be transferred has high heritability. Extensive backcrossing may not be required, as illustrated by the success of first backcrosses of *Pinus x rigitaeda* to *P. rigida* in environments too severe for F<sub>1</sub> and F<sub>2</sub> progenies. By contrast, first backcrosses in *Castanea* sp., in which disease resistance is the trait to be transferred, have not produced commercially useful progenies, although promising individuals were present (Clapper 1952). Here again the usefulness of vegetative propagation as a means of extracting worthwhile gene combinations from intermediate steps in hybridization is apparent.

Perhaps detailed study of introgressive hybridization will aid in showing us something of the potential for backcrossing, although foresters are interested in only a few of the components of fitness. Unfortunately there are few reports in which the direction of gene flow in natural hybrid forms has been studied in detail. In an analysis of *Pinus* at one Louisiana site, Namkoong (1963) concluded that reduced site disturbance (reduced grazing and improved fire control) was reducing the establishment of interspecific hybrids, and that gene flow was occurring from hybrids toward both parental species. Maintenance of an apparently larger array of genotypes from interspecific hybridization has been reported for *Quercus* in central California, though not as a consequence of broad adaptation (Benson et al. 1967). Rather, evolutionary sorting was occurring rapidly on specific slopes, but the range of environments available within relatively short distances apparently provided adequate sites for most hybrid genotypes. For interspecific hybrids of red and black spruce, advanced-generation natural crossing has been reported but possibly with less gene flow to parental species than in the *Quercus* or *Pinus* examples (Manley 1972).

#### QUANTITATIVE GENETICS IN INTERSPECIFIC HYBRIDIZATION

Little effort has been devoted to the theory, estimation, or utility of quantitative genetic parameters in interspecific hybridization. The principal practical question is the determination of total genetic variance and the subsequent partitioning into additive and nonadditive types. I know of no interspecific hybridization studies in which such estimates have been published. For provenance hybrids, dominance variances in corn ranged from 75% to 140% of additive variances (Moll and Robinson 1966). By contrast, provenance crosses of Scandinavian and central European Norway spruce showed specific combining abilities from high for plant weight to zero for bud set (Nilsson and Andersson 1970). Reports of  $F_1$  hybrid performance from provenance and interspecific crosses in forest trees generally indicate intermediacy for most traits. Unfortunately the term intermediacy is not necessarily synonymous with the term midparent. For example, published diagrams of mean values for parental species and  $F_1$  hybrids of *Eucalyptus* (Mergen, et al. 1966), and *Pinus* (Mergen and Furnival 1960) show largely intermediate hybrid performance but many deviations from midparent values for a wide range of characters. Means from such studies cannot be interpreted in terms of genetic variance parameters, and we are thus left without variance estimates.

The utility of estimates of genetic variance relates to choice of breeding methods. When  $F_1$  hybrid production is the breeding goal, estimates of additive variance within each parental population are required for predicting progress by any of the recurrent selection schemes. For such breeding approaches, a large literature is available on several traits of economic interest in many tree species. For selection within the  $F_1$ , additive variance estimates can be derived but may be seriously biased by included dominance variance (Stuber 1970). Estimates of potential selection progress among  $F_1$  interspecific tree hybrids have never been made to my knowledge. Some indication of what tree breeders might expect in terms of additive genetic variances

or realized genetic gains in hybrid programs would be useful as a guide to evaluating alternative breeding approaches.

#### HYBRIDS AND ENVIRONMENT

Having successfully negotiated the potential problems of reproductive biology in interspecific hybridization, the tree breeder may turn to consideration of environments in which the hybrids would be useful. Much of the outlook on this point derives from the idea that hybrids require a hybrid environment. In terms of response to natural selection for traits of fitness, the hybrid environment hypothesis is well substantiated; yet two thoughts occur to me that might modify the utility of hybrids in relation to environments available for planting. First, in many cases where interspecific hybridization is proposed, the predominant purpose for hybridizing may be genetic protection against a single ubiquitously distributed factor. If a pest or climatic problem is severe, perhaps some sacrifice in other traits is justified. Second, there may be situations in which major elements of natural-selection pressure are eliminated by cultural practices so that hybrids can perform well in a new but not necessarily hybrid environment. As an example, hybrids among species of differing ability to tolerate competing vegetation might be highly successful where early vegetational competition is removed. Perhaps some of the larches are examples of this. Similarly, hybrid vigor of *Pinus monticola* x *strobis* reported as definite at Placerville, Calif., and Spokane, Wash., was regarded as uncertain in central Wisconsin and northern Idaho plantings (Bingham et al. 1956). This may reflect the absence of certain climatic limitations in the far western locations. Winter kill of needles in Wisconsin and snow damage in northern Idaho were serious problems for the hybrids. By contrast, where genetic differences between species involve adaptation to major differences in climatic or edaphic factors largely unmodifiable by silvicultural practice, the constraints of hybrid habitat may apply more strongly. Experimental evidence on this point would be useful particularly since we understand so little about which environmental factors are exerting major selection pressures on species and hybrids.

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## APPENDIX - INTERSPECIFIC HYBRIDIZATION AS A BREEDING METHOD IN ELMS

After 15 years of screening worldwide seed collections at the University of Wisconsin, it has been found that the best combination of high resistance to Dutch elm disease (*Ceratocystis ulmi* [Buism.] C. Moreau) and fairly satisfactory ornamental quality is in an F<sub>1</sub> hybrid progeny (*U. pumila* L. x *japonica* [Rehd.] Sarg.) from northern Japan. Whereas identification and transmission of apparent resistance in the native American elm (*U. americana* L.) has been difficult, crosses among the Asian species have shown a consistent relationship in which resistance increases and ornamental value decreases as the proportion of germ plasm from *U. pumila* increases (Table 1). A similar relationship between *U. pumila* and the North American species *U. rubra* Mühl. has been found, although the introduction of genes from *U. rubra* markedly increases the range of variation in susceptibility and ornamental traits such as leaf size, branch diameter, and crown spread.

The present breeding objective is to find a combination of two or three species that will produce at least 90% symptom-free individuals with acceptable ornamental qualities. Results from crossing can be summarized in a triangular graph representing various combinations of the three species (Fig. 1). The shaded area includes species combinations that may yield the desired result. It is in this area that crossing is now concentrated.

Some crosses to achieve a variety of species combinations are illustrated in Table 2. The elm-breeding program in Wisconsin is now in the second and third generations for several combinations. Natural hybrids of flowering age have been used to advance the program quickly.

A time scale for production of disease-resistant, ornamental elms is shown in Table 3. The specific breeding objective is an identification of parents that is usable in a grafted seed orchard for commercial seed production. So far, emphasis has been on average performance of species combinations represented by at least a few different parents. In initial studies, species effects have dominated, although variation among full-sib families within species combinations has been significant in some cases. Crossing in the second and third generations will be done only with highly resistant individuals so that genotypes chosen for commercial seed production will reflect genetic advance from species, family, and individual selection. As the level of commercial acceptance is approached, performance of individual full-sib families will probably become important, but the genetic base for a commercial seed orchard is expected to remain highly heterogeneous for traits other than resistance to Dutch elm disease.

During the period that genotypes suitable for a commercial seed orchard are being identified, outstanding individuals are being tested and released for commercial propagation and distribution. One genotype from the outstanding Asian F<sub>1</sub> hybrid progeny is at present in the process of being officially announced and released. Several other clones are near the completion of testing.

In this example, difficulties in developing resistance in the preferred species (*U. americana*), availability of natural hybrids, and relatively short periods of reproductive immaturity combine to make interspecific hybridization an attractive breeding method.

TABLE 1. SAMPLE SIZE, MEAN RESPONSES TO INOCULATION WITH *CERATOCYSTIS ULMI*, AND MEAN LEAF LENGTH FOR SIX TYPES OF ELM PROGENIES

Species combination <sup>a</sup>	Sample size (no.)		Frequency of symptomless seedlings (%)	Disease intensity (%) <sup>b</sup>	Leaf length (cm)
	Progenies	Individuals			
j x j	1	80	64	14	-
j x pj	3	89	74	6	-
pj x pj	3	142	85	2	4.8
p x pj	8	337	90	6	4.0
p x p	8	350	93	2	3.6
a x wind	4	156	1	48	10.8

<sup>a</sup>j = *Ulmus japonica*, p = *U. pumila*, a = *U. americana*.

<sup>b</sup>Data are percent of crown killed 6 weeks after inoculation in trees with visible crown damage.

TABLE 2. A SAMPLE OF CROSSING SCHEMES TO PRODUCE DIFFERENT PROPORTIONS OF GERM PLASM FROM THREE ELM SPECIES

Generation	Cross	Percent of germ plasm <sup>a</sup>		
		j	p	r
1	p x j, p x r, r x j			
2	pj x r, pr x j, jr x p			
3	(pj x r) x j	62.5	12.5	25
	(pr x j) x j	75	12.5	12.5
	(jr x p) x p	12.5	75	12.5
	(jr x p) x j	62.5	25	12.5

<sup>a</sup>j = *Ulmus japonica*, p = *U. pumila*, r = *U. rubra*.

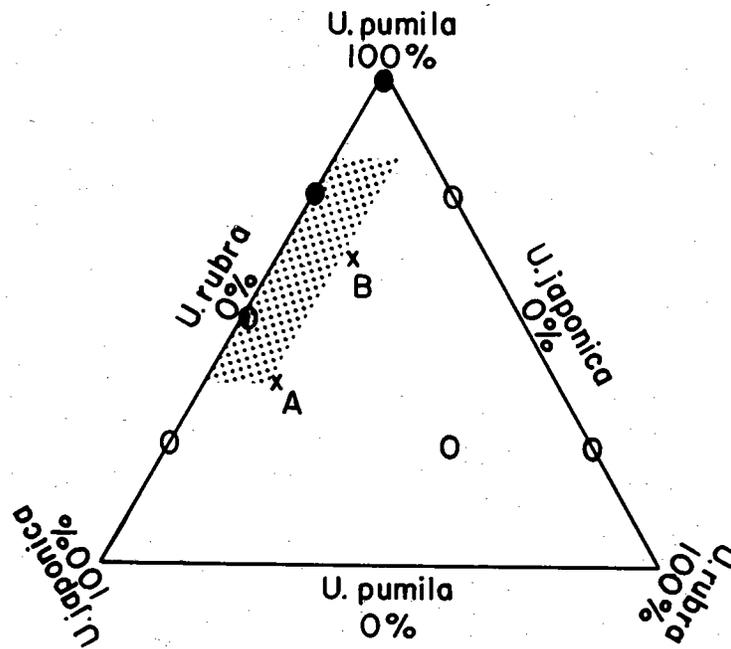


Figure 1. Summary of results from various combinations among three species (0 = symptom frequency in progeny exceeded 10%; 0 = symptom frequency was less than 10%) and area in which future crossing will be concentrated (hatched). Points A and B represent distributions of germ plasm achievable in three generations of crossing.

TABLE 3. TIME SCALE OF GENETIC IMPROVEMENT WITH ADVANCED GENERATION PROGENIES OF INTERSPECIFIC ELM HYBRIDS

Year	Activity	
1	Crossing, seed harvest and sowing	
2	Field transplanting	
3	Inoculation and evaluation	
5	Inoculation and evaluation	
6	Selection	
	<u>Seed propagation</u>	<u>Clonal propagation</u>
7	Advanced-generation crossing or grafted seed orchard establishment	Ramet production
8		Field transplanting
9		Seasonal inoculation and evaluation
10		Commercial release
11		
12	Advanced-generation crossing or commercial seed production	

## INTERSPECIFIC HYBRIDIZATION IN PINUS: A SUMMARY REVIEW

W.B. Critchfield

*U.S. Forest Service,  
Pacific Southwest Forest and Range Experiment Station,  
Berkeley, California*

The pines and other groups of temperate forest trees often fail to conform to widely accepted principles of plant evolution that are based primarily on observations of herbaceous plants. For example, interpreters of plant evolution tend to equate the fertility or sterility of interspecific hybrids with the magnitude of the genetically controlled reproductive barriers limiting the production of hybrids. This tendency has been articulated by Grant (1958, p. 353): "This is assuming that incompatibility barriers and sterility barriers, two distinct phenomena in the physiological sense, tend to run parallel in most groups, an assumption which despite a few exceptions is generally supported by the facts." The common corollary to this assumption is that vigorous, fertile interspecific hybrids reflect the absence of well-developed barriers to hybridization. In *Pinus*, neither Grant's assumption nor its corollary is supported by the facts summarized in this paper.

Because of the economic importance of many pines, species hybridization has been explored more fully in this genus than in most other plant genera. This work has sought to establish the limits of genetic material available for the improvement of a wild species - the starting point of nearly all tree-improvement programs.

The first authentic pine hybrid was produced nearly 60 years ago, and the first continuing program of exploratory hybridization was begun in the late 1920's at the Institute of Forest Genetics (then the Eddy Tree Breeding Station) at Placerville, Calif. The results of the work done at Placerville, at the Northeastern Forest Experiment Station, at Maple, Ont., and elsewhere have been summarized by Wright (1962), Critchfield (1963b, 1966, 1967), and Bingham et al. (1972). The generalizations about pine hybridization advanced in this paper are based on these earlier summaries and on unpublished data of the Institute of Forest Genetics, at Placerville.

About four-fifths of the 95 to 100 species in *Pinus* have been involved in one or more hybridization attempts. Of the more than 4,500 possible combinations of species, an estimated several hundred have been attempted. From these attempts, about 95 successful hybrid combinations - a conservative estimate - have been produced (Table 1).

Pine species have proved to vary widely in their crossing ability. Some pines (e.g. *Pinus pinea*, the Italian stone pine) have not been successfully crossed with any other species, nor are they likely to be

TABLE 1. SUCCESSFUL INTERSPECIFIC HYBRIDIZATIONS WITHIN AND BETWEEN SUBSECTIONS OF *PINUS*.

Subsection(s) <sup>1</sup>	Number of species	Estimated number of hybrid combinations
<i>Cembrae</i> (white pines)	5	1
<i>Cembrae</i> x <i>Strobi</i>		2
<i>Strobi</i> (white pines)	14-15	18
<i>Cembroides</i> (pinyon pines)	8	6
<i>Balfouriana</i> (foxtail pines)	2-3	2
<i>Sylvestres</i> (Eurasian hard pines)	19	19
<i>Australes</i> (southern and Caribbean hard pines)	11	15
<i>Australes</i> x <i>Contortae</i>		2
<i>Contortae</i> (small-cone pines)	4	2
<i>Sabiniana</i> (big-cone pines)	3	2
<i>Sabiniana</i> x <i>Ponderosae</i>		2
<i>Ponderosae</i> (western and Mexican hard pines)	13-15	16
<i>Ponderosae</i> x <i>Oocarpae</i>		2
<i>Oocarpae</i> (closed-cone pines)	7	6

<sup>1</sup>The eight species in the other five subsections have no verified interspecific hybrids.

without a major advance in the manipulation of reproductive processes. In contrast, some of the western and Mexican yellow pines, southern pines, white pines, and Eurasian hard pines can be crossed directly with as many as six other species and linked indirectly to still others.

Although the exploratory hybridization of *Pinus* is incomplete, the available information provides a sufficient basis for several generalizations about the pines:

(1) With very few exceptions, there are no marked barriers to crossing between different races of a species. The Sierra Nevada and coastal races of lodgepole pine (*P. contorta*), for example, are fully crossable, although the morphological differences between them are so great that they are still occasionally regarded as different species.

A minor exception to this generalization is ponderosa pine (*P. ponderosa*). Crosses between its Pacific and Rocky Mountain races produce somewhat reduced yields of sound seed (Krugman 1970), indicating weak barriers to crossing. A more remarkable exception is bishop pine (*P. muricata*) of the California coast. Its northern and southern races are isolated by nearly absolute barriers to crossing (Critchfield 1967), although both can be crossed with a geographically intermediate race.

(2) With a few exceptions, pine species are partly or completely isolated from each other by genetic barriers. The magnitude of these barriers

can be expressed quantitatively as *crossability*: the yield of sound, germinable seed from crosses between two species, expressed as a percentage of the seed yield from within-species crosses.

The crossability of those pine species that have been successfully hybridized ranges from 100% to nearly zero. There appear to be no reproductive barriers between foxtail pine (*P. balfouriana*) and the western race of bristlecone pine (*P. aristata*), which have a crossability of close to 100%. The closed-cone pines *P. attenuata* and *P. radiata* have a crossability of 69 to 85% (Critchfield 1967). Examples of crossabilities approaching zero are the closed-cone pine cross *P. patula* x *P. radiata*, and the big-cone pine cross *P. sabiniana* x *P. coulteri*, each of which has yielded a single hybrid tree (Critchfield 1966, 1967). Most of the crossable combinations of pine species are in the lower part of the 0 to 100% range, with crossabilities of less than 40%.

(3) Crossing is usually impossible among the 15 groups of species (subsections) currently recognized as making up the genus. These groupings, reflecting ideas about relationships among the pines, are subject to continuing reevaluation as new morphological, chemical, cytological, and crossing data are accumulated. Such taxonomic groupings provide a necessary working hypothesis in the exploratory hybridization of a large genus like *Pinus*, with its numerous possible species combinations.

Shaw's (1914) monograph on *Pinus* supplied the working hypothesis for the early investigations of pine hybridization. The results obtained were the basis for Duffield's (1952) reappraisal of the hard pines (subgenus *Pinus*). His new groups, designated by Roman numerals, were more coherent morphologically, chemically, and geographically than the groups they replaced. Duffield's groups, with minor changes, have recently been named and more fully described by Little and Critchfield (1969).

The white pines (subgenus *Strobus*) have not received a recent reappraisal like that of the hard pines. One of the problems in this subgenus is the poorly defined subsection *Cembrae*, which is distinguished chiefly by its "indehiscent" cones. It may eventually be combined with *Strobi*, a group that includes nearly all of the familiar five-needled white pines (*P. strobus*, *P. monticola*, etc.). Two verified hybrids have been produced from *Cembrae* x *Strobi* crosses (Table 1), and other probable *Cembrae* x *Strobi* hybrids have been reported (Bingham et al. 1972). In this instance, the production of hybrids between species in different groups may reflect the unsatisfactory state of white pine taxonomy.

In other instances, hybrids between species in different groups may be true links between otherwise well-defined groups. Two successful *Sabinianae* x *Ponderosae* crosses (Table 1), both involving Jeffrey pine (*P. jeffreyi*), are an example of such a link. Jeffrey pine so closely resembles the more widespread ponderosa pine that field identification is often difficult, and both species are in the same subsection (*Ponderosae*). In its resin chemistry, however, Jeffrey pine is much more like the three big-cone pines in subsection *Sabinianae* (Mirov 1961). And in its crossing

behavior Jeffrey pine links the *Ponderosae* and *Sabinianae* (Critchfield 1966). It has been successfully crossed with several other species in *Ponderosae*, including ponderosa pine. It has also been hybridized with two big-cone pines, *P. coulteri* and *P. torreyana*. The problem posed by Jeffrey pine cannot be resolved taxonomically, and it seems best to consider the *Ponderosae* and *Sabinianae* as groups that have diverged somewhat less in the course of evolution than most of the other groups now classified as subsections.

Subsection *Contortae* is another exception to the general rule that pines can only be hybridized with other members of their groups. This morphologically similar group of small-cone pines includes two northern species, lodgepole and jack pines (*P. contorta*, *P. banksiana*) and two southeastern species, Virginia and sand pines (*P. virginiana*, *P. clausa*). Lodgepole and jack pines hybridize naturally where they overlap in western Canada, and are readily crossable under controlled conditions (crossability about 30%). The two southeastern pines are also highly crossable (Critchfield 1963b). Efforts to cross the northern and southern species have failed to produce any verified hybrids. But sand pine has been successfully crossed with two southern pines in subsection *Australes*: *P. elliottii* (Saylor and Koenig 1967) and *P. taeda* (Critchfield 1963b). The limits to crossing of the pines in *Contortae* have not been fully explored, but this group appears to be a notable exception to the rule that morphology and crossing ability tend to go together in the pines.

(4) The two major groupings in *Pinus*, the white pines and the hard pines (subgenera *Strobus* and *Pinus*), differ greatly in several aspects of species hybridization. The two groups are completely isolated from each other; the magnitude of the reproductive barriers separating them is reflected in high levels of conelet abortion and often in much reduced numbers of hollow seed when white and hard pines are crossed (Krugman 1970).

The most conspicuous difference between the breeding behavior of white and hard pines is related to geography. White pines of the Eastern and Western Hemispheres hybridize readily; half of the 21 species combinations in the *Cembrae/Strobi* (Table 1) are interhemisphere crosses. The most unusual example of this lack of association between crossing ability and geographical separation is sugar pine (*P. lambertiana*), a California montane species. Although it is a fairly typical white pine in its morphology, it has not been successfully crossed with any of the other American white pines. It has, however, been hybridized with two east-Asian white pines, *P. armandii* and *P. koraiensis*, both of them very different in morphology from sugar pine and from each other.

The hard pines, by contrast, exhibit strict geographic limits to crossing. The only successful Eastern x Western Hemisphere cross between hard pines is the difficult and as yet unrepeated *P. nigra* x *P. resinosa* (Critchfield 1963a). Among the North American hard pines, even crosses between species of different geographic regions are uncommon. The northern and southern species of *Contortae*, already discussed, are an example. Geographic limitations to crossing are also illustrated by two rather

similar groups of American hard pines: subsection *Australes*, of the southeastern U.S. and the Caribbean region, and subsection *Ponderosae*, of western and southern North America. Most of these pines were grouped together by Shaw (1914). They were segregated into two groups by Duffield (1951-52), partly because of the failure of all attempts to cross the eastern and western species.

The white and hard pines also differ in the nature of the reproductive failures that occur in species crosses. The genetic barriers between white pines are usually expressed relatively late in the reproductive process, after fertilization has taken place (Kriebel 1972). In crosses between hard pines, however, reproductive failures may occur at almost any stage, from the failure of the pollen to germinate to the development of the embryo (McWilliam 1959, Krugman 1970).

(5) Most interspecific pine hybrids are highly viable and highly fertile. Hybrid inviability is very rare, and the only two reported examples need to be repeated and verified. They are the southern pine crosses *P. taeda* x *P. clausa* and *P. elliottii* x *P. glabra*. These combinations have produced only seedlings (some of them albino) that died soon after germination (Critchfield 1963b). With these possible exceptions, the growth and development of pine hybrids show few major departures from the growth and development of nonhybrids.

The reproductive capacity of pine hybrids is also comparable to that of nonhybrids, although critical comparisons are mostly lacking. Hybrids do have generally higher levels of meiotic irregularities than do nonhybrids (Saylor and Smith 1966). Pollen abortion levels are definitely higher in some hybrid combinations, but most show the same low levels (0 to 5%) as nonhybrids. Many of the interhemispheric white pine hybrids studied by Saylor and Smith had fairly high levels of aborted pollen: 10 to 22%. The largest amounts of pollen abortion, however, have been found in some lodgepole x jack pine hybrids, one of the more crossable combinations of hard pines. Many individuals of one hybrid lineage produced 30 to 40% aborted pollen (Saylor and Smith 1966, and Saylor 1972 [personal communication]). Despite these sometimes high levels of pollen abortion, lodgepole x jack pine hybrids of the F<sub>1</sub> to F<sub>3</sub> generations produce large amounts of sound seed in most combinations.

The failure of meiotic irregularities and pollen abortion to seriously depress the reproductive capacity of pine hybrids may be related to the reproductive system of pines. Although a pine seed usually contains only a single embryo at maturity, the ovule from which it develops can accommodate several pollen grains and ordinarily contains several eggs. With this margin for reproductive failures, it seems probable that only high levels of reproductive disturbance would significantly influence the reproductive capacity of pine hybrids.

In conclusion, *Pinus* does not appear to be very different from many other plant genera in the ability of its species to cross with one another. In most instances crossing is possible only between those species

that resemble each other most closely, although the exceptions have been emphasized in this paper. *Pinus* does differ from many herbaceous plant genera, however, in that most interspecific hybrids are vigorous and fertile. Reproductive sterility is sufficiently common among herbaceous plant hybrids to have been used by Clausen, Keck, and Hiesey (1940) as a major criterion in defining their still widely used biosystematic categories of ecotype, ecospecies, and coenospecies. These categories are not applicable to *Pinus* and similar woody genera, in which hybrid sterility is both uncommon and seemingly unrelated to the magnitude of crossing barriers between species.

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# A SUMMARY REVIEW OF INTERSPECIFIC BREEDING IN THE GENUS POPULUS L.

L. Zsuffa

*Ontario Ministry of Natural Resources,  
Forest Research Branch,  
Maple, Ontario*

The interspecific hybrids of poplars, created by man's action and cultivated by man, are a first among forest trees. According to Muhle Larsen (1960) the history of *P. deltoides* x *nigra* hybrids goes back 300 years, to the time when the first trees of eastern cottonwood were imported into France from southeastern Canada. There they freely interbred with the native black poplar (*P. nigra* L.) and gave rise to hybrid seedlings noted for fast growth, good form and ease of vegetative propagation by cuttings. The description of the earliest cultivar of this hybrid dates back to 1775 (*P. serotina* Hartig). Soon, the descriptions of others followed and the collective name of *P. x canadensis* Moench (1795) was given to *P. deltoides* x *nigra* hybrids. They spread fast through many countries as ornamental trees, trees of row plantations and timber trees. The interest in poplars increased; species and varieties were studied and breeding programs initiated.

## CLASSIFICATION OF POPLARS

The genus *Populus* L. includes approximately 30 species distributed throughout the temperate regions of Eurasia and America. The poplar species are grouped in five sections (Table 1): the economically most important species are in sections *Leuce*, *Tacamahaca* and *Aigeiros*. The wide geographic distribution of some species, the frequent introgressive hybridization, the centuries-old cultivation and the ease of vegetative reproduction of poplars caused much confusion in the nomenclature of poplar species; numerous synonyms exist and many times hybrids and cultivated varieties have been named as species.

## REPRODUCTION

The poplars are normally dioecious; however, abnormal floral organizations, with monoecious trees bearing unisexual or bisexual flowers, occur.

The flower buds are borne in the late summer on short shoots. The flowers are in pendulous catkins, which appear before the leaves. The light seed matures in capsules in 2 to 8 weeks. Each capsule bears numerous seeds surrounded at the base by long silky hairs.

TABLE 1. CLASSIFICATION OF *POPULUS*

Section	Species	Geographic distribution
<i>TURANGA</i> Bge	<i>euphratica</i> Olivier <i>pruinosa</i> Schrenk	W. and C. Asia, N. Africa C. Asia
<i>LEUCE</i> Duby	<i>adenopoda</i> Maxim. <i>alba</i> L. <i>dauriana</i> (Dode) Schneid. <i>grandidentata</i> Michx. <i>sieboldii</i> Miq. <i>tomentosa</i> Carr. <i>tremula</i> L. <i>tremuloides</i> Michx.	China Europe, Asia, N. Africa N.E. Asia N. America Japan, Korea Asia Europe, Asia N. America
<i>LEUCOIDES</i> Spach	<i>ciliata</i> Wall. <i>heterophylla</i> L. <i>lasiocarpa</i> Oliv. <i>wilsonii</i> Schneid.	C. Asia S.E. USA China China
<i>TACAMAHACA</i> Spach	<i>angustifolia</i> James <i>balsamifera</i> L. <i>cathayana</i> Rehd. <i>koreana</i> Rehd. <i>laurifolia</i> Ledeb. <i>maximowiczii</i> Henry <i>simonii</i> Carr. <i>suaveolens</i> Fisch. <i>szechuanica</i> Schneid. <i>trichocarpa</i> Hook. <i>yunnanensis</i> Dode	N. America N. America N.E. Asia Korea Siberia N.E. Asia, Japan Asia Asia China N. America China
<i>AIGEIROS</i> Duby	<i>deltoides</i> Marsh. <i>fremontii</i> S. Wats. <i>nigra</i> L. <i>sargentii</i> Dode <i>wislizenii</i> Sarg.	N. America N. America Europe, Asia, N. Africa N. America N. America

The flowering age is between 5 and 15 years. Environmental factors influence the process of aging, and juvenile periods may recur in cycles (Kopecky 1962a). Grafting on young or mature stock induces juvenility or flowering (Kopecky 1966a).

All poplars, except those of section *Leuce*, sprout vigorously from the stump and root collar. Coppicing has been occasionally reported on young aspen. Reproduction by formation of adventitious shoots on roots (suckers) is another common phenomenon of poplars. However, *Aigeiros* and *Leucoides* poplars do not form suckers as readily as the others.

Stem cuttings from the poplar species which belong to sections *Tacamahaca* and *Aigeiros* root with relative ease; however the rooting is poor in sections *Leuce*, *Leucoides* and *Turanga*.

## TECHNIQUE OF CROSSING

The simplest method of controlled pollination of poplars is on twigs placed in a water culture (Wettstein 1930). Twigs with flower buds of 50 to 100 cm in length are placed in pots with tap water. In the warm greenhouse the twigs with male buds flower in a few days, and the pollen can be collected and the twigs disposed of. However, the twigs bearing female flowers have to stay longer (Fig. 1). Where seed requires more than 3 weeks to ripen, as in the case of *Aigeiros* and *Tacamahaca* poplars, the small amounts of nutrients in the twigs often appear insufficient to produce fully matured seed. As an alternative the technique of bottle-grafting twigs with several female buds is widely used and gives satisfactory results (Muhle Larsen 1960) (Fig. 2).

## CROSSABILITY OF *POPULUS* SPECIES

Extensive work on crossing poplars comprising the *Leuce*, *Tacamahaca* and *Aigeiros* sections has been carried out for many years. However, few data are available on the crossability of poplars belonging to the sections *Turanga* and *Leucoides*.

Crosses between species of the same section are not difficult to obtain (Table 2). The intrasectional crosses are usually very fertile and in many cases result in vigorous hybrids.

Certain species crosses are sometimes more successful than their reciprocals. The *P. deltoides* x *nigra* cross is very fertile. However, Melchior and Seitz (1968), and Steenackers (1970) failed with the reciprocal cross *P. nigra* x *deltoides*.

The intersectional crosses show different degrees of compatibility. The *Aigeiros* x *Tacamahaca* crosses are easy to make. However, the crosses between *Leuce* and *Aigeiros* poplars and *Leuce* and *Tacamahaca* poplars show pronounced incompatibility. These crosses usually result in dead seed or dwarfed seedlings (Johnsson 1956, Kopecky 1962b). Moreover, the offspring usually resembles the female parent (Kopecky 1956). Yet, occasionally, promising single hybrids are reported. A good *P. alba* x *trichocarpa* hybrid has been reported by Wettstein (1933), a *P. alba* x *deltoides* by Sekawin (1963), and a *P. tremula* x *deltoides* by Catalan (1963). Recently, a vigorous *P. alba* x *deltoides* hybrid was also produced by Zsuffa at Maple, Ont. As for the other sections, Richens (1945) recorded a successful *Aigeiros* x *Leucoides* hybrid (*P. nigra* x *lasiocarpa*) and Sekawin (1969) failed to cross *P. euphratica* (section *Turanga*) with *Leuce* and *Tacamahaca* poplars. According to Sekawin (1963) Kasartzev also failed with *Turanga* x *Tacamahaca* and *Turanga* x *Aigeiros* crosses but succeeded in crossing *P. euphratica* with a *P. alba* hybrid.

By using interspecific hybrids instead of pure species as parents, some difficult intersectional crosses have become feasible (Zufa 1968). In

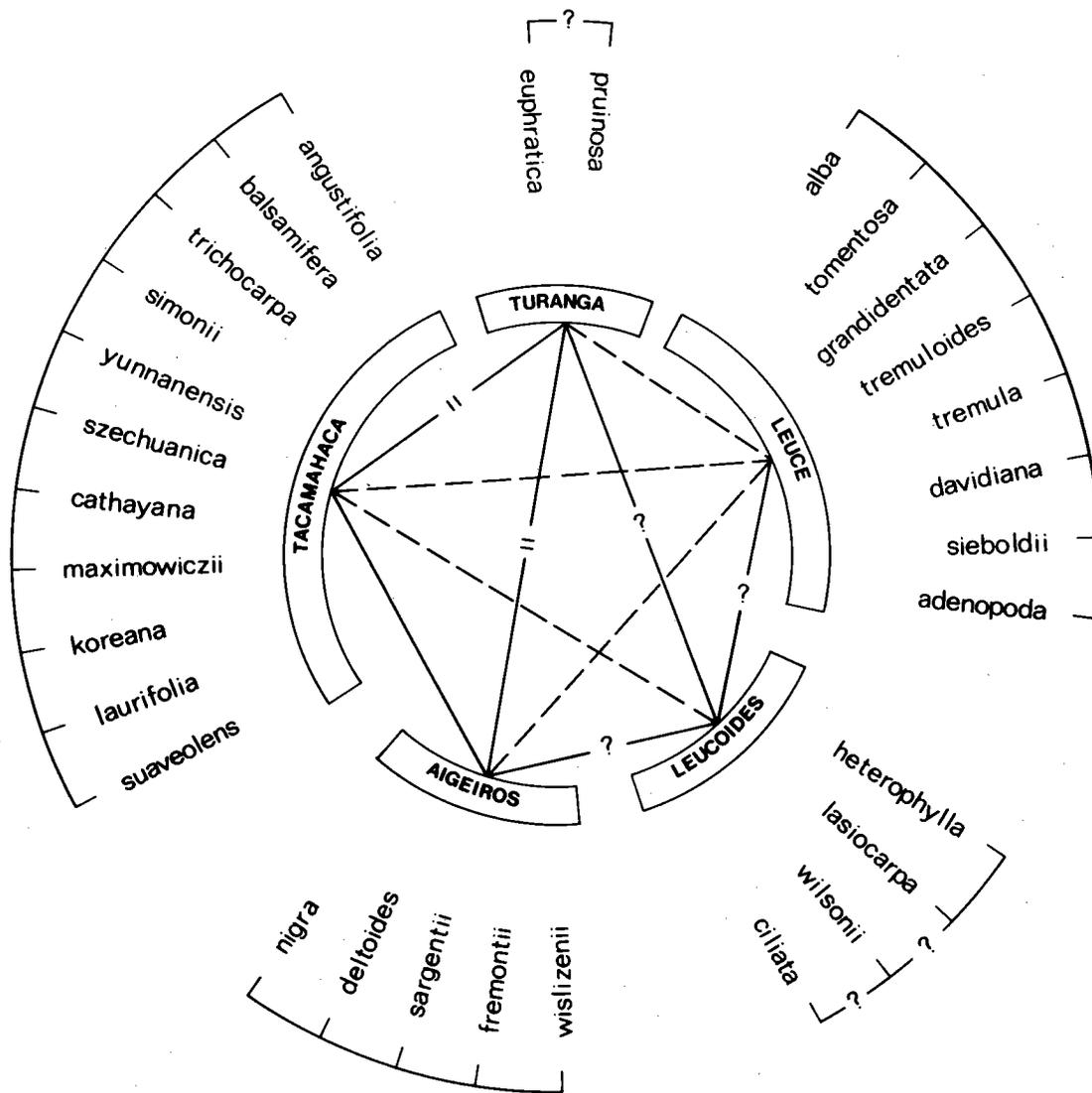


*Figure 1. Twigs of a female P. canescens in water culture. Seed catkins are maturing on the twigs after artificial pollination in the greenhouse.*



*Figure 2. Bottle-grafted twigs of a female P. deltoides. The twigs are bearing seed catkins as the result of artificial pollination with P. nigra.*

TABLE 2  
CROSSABILITY OF POPULUS SPECIES



Fertile Crosses —————

Difficult Crosses - - - - -

Incompatible — || —

No Information — ? —

such crosses the segregated  $F_2$  hybrid generation is not as uniform and dwarfed as the  $F_1$  intersectional offspring was but is heterogeneous with some vigorous seedlings. Heimbürger (1940) reported a successful *Leuce* x *Aigeiros* cross of this type; it involved *P. (alba x grandidentata) x euramericana* cv. *Eugenii*. Similar successful intersectional crosses were realized by Heimbürger in 1967 (*P. canescens* x *Petrowskyana*) and by Zsuffa in 1968 (*P. canescens* x *jackii* and *P. tremula* x *jackii*).

An incompatibility system characterized by inability of the pollen tube to penetrate the stigma has been found in some poplar crosses. The block has been overcome by mixing fresh incompatible pollen with recognition pollen, i.e. killed pollen of the compatible type produced usually by gamma radiation. Using recognition pollen, Stettler (1968), Zufa (1968 and 1971a) and Knox et al. (1972) obtained good results with otherwise difficult crosses of *Leuce* with *Aigeiros* and *Tacamahaca* poplars.

#### THE SIGNIFICANCE OF INTERSPECIFIC BREEDING IN POPLARS

Interspecific breeding, paired with vegetative propagation of the selected ortets, has been a powerful tool in producing poplar strains and clones of vigorous growth, improved disease resistance and better rooting ability of stem cuttings. By interspecific breeding, hybrids can also be produced for different ecological environments and with a wider tolerance for site conditions.

#### Heterosis in Interspecific Poplar Hybrids

Related poplar species, when crossed, have often produced offsprings exhibiting hybrid vigor or "heterosis." The vigor of growth of hybrids depended (i) on the parental species, (ii) on the selection of individual parent trees, and (iii) on the filial generation of hybrids.

(i) The parental species that came from geographically distant areas or from regions otherwise isolated produced heterotic hybrids most frequently. Heterosis also occurred in hybrids of the parental species adapted to different ecological environments. A list of interspecific hybrids expressing heterosis is given in Table 3, on the basis of a review of papers by Wettstein (1938), Heimbürger (1940, 1958 and 1968), Johnsson (1956), Kopecky (1956, 1962b, and 1971) Hyun and Hong (1959), Muhle Larsen (1960), Sekawin (1963), Stecki (1965), Takayama (1968), Zufa (1968), Steenackers (1970), Benson (1972), and Chung and Son (1972).

Many crosses of *Leuce* species evolved in well-separated areas have been noted for vigorous growth. The *P. tremula* x *tremuloides* hybrids drew attention on account of heterosis more than 30 years ago (Wettstein 1938, Johnsson 1956). Hybrid vigor has been also commonly observed with *P. alba* x *grandidentata*, *P. alba* x *dauriana*, *P. alba* x *sieboldii*, *P. alba* x *tremuloides*, *P. grandidentata* x *dauriana*, *P. grandidentata* x *sieboldii*, *P. tremuloides* x *adenopoda* and *P. tremuloides* x *dauriana*. Hybrids of

TABLE 3. INTERSPECIFIC POPLAR HYBRIDS NOTED FOR HYBRID VIGOR (HETEROSIS)

Section	Cross*
LEUCE	<i>tremula</i> x <i>tremuloides</i> <i>tremuloides</i> x <i>adenopoda</i> <i>tremuloides</i> x <i>dauriana</i> <i>sieboldii</i> x <i>grandidentata</i> <i>dauriana</i> x <i>grandidentata</i> <i>alba</i> x <i>grandidentata</i> <i>alba</i> x <i>dauriana</i> <i>alba</i> x <i>sieboldii</i> <i>alba</i> x <i>tremula</i> <i>alba</i> x <i>tremuloides</i>
AIGEIROS	<i>deltoides</i> x <i>nigra</i>
TACAMAHACA	<i>maximowiczii</i> x <i>trichocarpa</i> <i>maximowiczii</i> x <i>laurifolia</i> <i>maximowiczii</i> x <i>simonii</i> <i>simonii</i> x <i>yunnanensis</i>
AIGEIROS X TACAMAHACA	<i>deltoides</i> x <i>balsamifera</i> <i>deltoides</i> x <i>trichocarpa</i> <i>deltoides</i> x <i>yunnanensis</i> <i>deltoides</i> x <i>laurifolia</i> <i>deltoides</i> x <i>maximowiczii</i> <i>nigra</i> x <i>maximowiczii</i> <i>nigra</i> x <i>trichocarpa</i> <i>nigra</i> x <i>koreana</i> <i>nigra</i> x <i>simonii</i>

\*Or reciprocal cross.

these types showed fast growth and high yields in experimental plantations on good poplar sites in southern and central Ontario (Zufa 1971b) (Fig. 3). The yield of hybrids was compared with the yield of native aspen on site class I (Table 4). The average heights and diameters (DBH) of hybrid trees in 7- to 15-year-old plantations were equivalent to those in natural stands at 35 to 50 years of age. The mean annual increments (MAI) in the plantations were between 103 and 292 cubic ft/acre while the MAI of the unmanaged natural stands reached a maximum of 109 cubic ft/acre at 55 years of age.

The *P. deltoides* x *nigra* hybrid of North American and European *Aigeiros* poplars is well known for its heterotic growth. The clonal varieties of this hybrid have spread all over the world and have given rise to a new timber culture (Fig. 4). An international Poplar Commission of FAO of the United Nations was formed after the Second World War to deal with the different problems of breeding, management and utilization of the wood of what was essentially this hybrid, which seemed promising for solving the



*Figure 3. P. alba x grandidentata in plantation. Witchurch, southern Ontario. Age 12 years. Average diameter at breast height 6 inches (15.2 cm), average height 52 feet (16.1 m).*

TABLE 4. COMPARISON BETWEEN THE YIELD OF INTERSPECIFIC HYBRIDS OF LEUCE POPLARS IN PLANTATIONS AND THE YIELD OF NATIVE ASPEN ON SITE CLASS I IN UNMANAGED ASPEN STANDS (ON THE BASIS OF YIELD TABLES BY PLONSKI 1960)

	Plantations of hybrids	Unmanaged aspen stands
Average annual diameter growth (DBH) at 7-15 years of age at 20 years of age	0.50-0.61 in. (1.3-1.5 cm)	0.18 in. (0.5 cm)
Average annual height growth at 7-15 years of age at 20 years of age	3.3-5.7 ft (1-1.7 m)	1.9 ft (0.6 m)
Mean annual increment (MAI) at 7-15 years of age at 20 years of age at 55 years of age (maximum)	103-292 cu ft/acre (7.2-20.5 m <sup>3</sup> /ha)	65 cu ft/acre (4.5 m <sup>3</sup> /ha) 109 cu ft/acre (7.6 m <sup>3</sup> /ha)



*Figure 4. Plantation of P. x euramericana cv. "I-262" in northern Italy. Age 10 years. Average diameter at breast height 15.7 inches (40 cm).*

pronounced shortage of timber in many countries of the world. In 1950 the name *P. x euramericana* (Dode) Guinier, followed by the name of the cultivar, was accepted by the International Poplar Commission for *P. deltoides x nigra* and its advanced crosses and backcrosses.

Many of the *Tacamahaca* hybrids of distant species have been noted for vigorous juvenile growth (Table 3). The vigor in these hybrids seemed to depend to a larger degree on the selection of individual parent trees. Thus, Stout and Schreiner (1933) and Steenackers (1970) reported on the good growth of *P. maximowiczii x trichocarpa* hybrids, while Hyun and Hong (1959) observed poor performance for the same cross.

The fertile intersectional *Aigeiros x Tacamahaca* crosses gave rise to many vigorous hybrids. The *P. deltoides x trichocarpa*, *P. nigra x trichocarpa*, *P. deltoides x maximowiczii*, *P. nigra x maximowiczii* and *P. koreana x nigra* crosses of geographically remote parent species seem to be those on which breeding work is concentrating (Stout and Schreiner 1933; Steenackers 1970; Chung and Son 1972).

Crosses between species with overlapping ranges have given various results. Heterosis has not been observed in *P. grandidentata x tremuloides* hybrids. However, the vigorous growth of *P. x canescens* (Ait.) Sm., a hybrid swarm of *P. alba* and *P. tremula*, and of *P. x jackii* Sarg., a hybrid swarm of *P. deltoides* and *P. balsamifera*, is well known. The vigor of these hybrids is probably due to the adaptation of the parent species to different ecological environments.

(ii) The success of interspecific breeding depends not only on the choice of parent species but also to a great degree on the choice of the individual parent trees. Johnsson (1956) compared a number of *P. tremula x tremuloides* hybrids with the native *P. tremula* and observed that, while the best performing hybrid progeny grew twice as much as the best *P. tremula*, the poorest hybrid progeny was only slightly better. Similar observations were made by Muhle Larsen (1960) and Kopecky (1966b). The latter compared the growth rate of several *P. deltoides x nigra* hybrids with that of the parents. The most vigorous hybrids outgrew the parents by more than 50%, but the least vigorous hybrids were slower than the parents.

The ecological conditions of the area from which the parent trees were chosen, especially the length of the growing season, also influence the growth of the progeny. This is another reason why some hybrids have no superiority over the parents on certain sites but show very vigorous growth under other conditions. Heimburger (1958) obtained variable results by crossing *P. tremuloides* with different European sources of *P. tremula* pollen. On the test site in southern Ontario the pollen from southern latitudes produced the most vigorous hybrids.

(iii) Heterosis occurs especially in the  $F_1$  generation of interspecific hybrids. This first filial generation of related species is usually fairly uniform in its morphological and physiological characters. But the crosses made between interspecific hybrids and the backcrosses of

interspecific hybrids to parental species result in heterogeneous progenies. They segregate and the characteristics of the parental species show in offspring. According to Kopecky (1962b), such progenies are of decreased capacity for growth: in the segregated population only a few individuals reach the vigor of parents. Yet, according to Muhle Larsen (1960), it is possible to operate with advanced crosses of interspecific hybrids and with their backcrosses to parental species. Such crosses may result in progenies in which some desired characteristics, such as disease resistance, form and branching of the trees, or rooting ability, are strengthened. In poplars, it is enough to have only a portion of the progeny with desired characteristics. The selected individuals can be easily propagated vegetatively and developed into monoclonal or multiclinal varieties. Most of the Italian *P. x euramericana* clones widely cultivated in many countries originated from advanced crosses and subsequent vegetative propagation (Piccarolo 1948). Heimbürger (1968) and Zufa (1968 and 1969) reported outstanding individual trees in advanced crosses of *Leuce* poplars, for example in *P. canescens* x (*alba* x *grandidentata*), *P. (alba* x *dauriana*) x (*alba* x *grandidentata*), and *P. canescens* x (*alba* x *dauriana*). This work has now reached the stage where the development of clonal varieties is under way.

#### Disease Resistance in Interspecific Poplar Hybrids

Different poplar species show various degrees of resistance to diseases. According to Steenackers (1972) *P. deltoides* is known to be resistant to diseases caused by *Marssonina* spp., *Melampsora* spp., *Septoria* spp., and *Dothichiza populea*. However, about 90% of the individuals in tested populations of *P. deltoides* are susceptible to bacterial canker (*Aplanobacter populi*). *P. nigra* is generally susceptible to *Melampsora* spp. and to *D. populea*, but it is extremely resistant to bacterial canker. Thus the *P. deltoides* x *nigra* hybrids, if derived from properly selected parent trees, can unite the resistance of both parent species and give rise to clonal varieties of multiple resistance. Another example, showing the potential of interspecific breeding of poplars for inducing disease resistance, is in *Aigeiros* x *Tacamahaca* crosses. The balsam poplars are known to be highly susceptible to *Septoria* canker (*Septoria musiva*), to which *P. deltoides* and *P. nigra* are resistant. The crosses of balsam poplars with either one of these *Aigeiros* species thus offer a possibility for the selection and vegetative propagation of resistant individuals.

Unfortunately, increased susceptibility of hybrids to diseases as compared with parent species may also occur in some cases. Thus susceptibility to *Plagiostoma populi* was registered by Heimbürger (1966). The fungus is causing severe blight and dieback on *P. alba* hybrids, such as *P. alba* x *grandidentata*, *P. alba* x *tremuloides*, *P. alba* x *tremula*. Yet the fungus is rarely found on the parent species of the hybrids observed. It occurs occasionally on the *P. grandidentata* x *tremuloides*, and *P. tremula* x *tremuloides* hybrids. To explain the increased susceptibility of *P. alba* hybrids, Heimbürger (1966) postulated a system of major genes conditioning susceptibility.

## Improving the Rooting Ability of Poplars by Interspecific Breeding

Some poplar species of the sections *Aigeiros* and *Tacamahaca* show very good rooting ability from stem cuttings. However, the rooting ability of *P. deltoides* varies and sometimes, under unfavorable field conditions, large portions of its cuttings perish. *P. nigra* and *P. balsamifera* cuttings root more readily. These species transmit their better rooting ability to hybrids with *P. deltoides*. In fact, cuttings of *P. x euramericana* and *P. x jackii* clones commonly root even under adverse field conditions.

Within the poplars of the *Leuce* section, the European white poplar (*P. alba*) is the only species easy to propagate with cuttings. *P. alba* creates fertile and vigorously growing hybrids with aspen species and at the same time transmits its rooting ability to the hybrids, as reported for example by Heimbürger (1958 and 1968) for crosses of *P. alba* with aspen species, such as *P. grandidentata*, *P. tremuloides*, *P. tremula*, *P. davidiana* and *P. sieboldii*. By backcrossing the hybrids to *P. alba* and by producing multiple hybrids of unrelated parents, such as *P. canescens* x (*alba* x *grandidentata*), *P. (alba x davidiana) x (alba x grandidentata)*, heterogenous progenies with outstanding individuals were produced, in which the rooting ability was strengthened (Heimbürger 1968, Zufa 1968 and 1969).

### BREEDING PROGRAMS

The first program of interspecific poplar breeding aimed at producing hybrid varieties for commercial timber was initiated by the Oxford Paper Company, with Stout and Schreiner, in 1924 (Stout and Schreiner 1933). Soon poplar-breeding started in Austria (Wettstein 1930), the USSR (Albenskiy and Delicina 1934), Canada (Heimbürger 1936) and Italy (Piccarolo 1948). After the Second World War, poplar breeding programs sprang up in many countries. The literature in this field became so abundant that it is impossible to review it here. The interested reader should refer to the publication entitled *Poplars in Timber Production and Land Use* (FAO 1956), the reports of the International Poplar Commission (FAO, Rome, Italy), reports of the IUFRO Working Group on Breeding Poplars (IUFRO, Vienna, Austria), and the reports of the specialized poplar research institutions.

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## DISCUSSION

Fowler: Louis, you mentioned differences in your results with reciprocal crosses. I was wondering how common this is. What sort of explanation do you have for this? I would also like to ask Bill Critchfield what his results are in terms of differences between reciprocal crosses.

Zsuffa: It wasn't I who noticed that. I was citing someone else. However, it has been noticed repeatedly that while *Populus deltoides* female trees can cross very easily with male *P. nigra*, the reciprocal cross is very difficult to make. There is a very interesting paper published by Knox, Willing and Pryor (*Silvae Genetica* 21(3-4):65-69) on this problem. Similar problems were not described in other interspecific poplar crosses.

Fowler: It isn't a common thing?

Zsuffa: No, it is an isolated phenomenon.

Critchfield: We have never looked very critically at that, Don. It is my general impression that there isn't much difference between pine species in reciprocal combinations. I'd say I have only looked at it critically on a couple of trees.

Valentine: We have made a large number of crosses between *tremuloides* and *grandidentata* and backcrosses of natural hybrids and known interspecific hybrids to each of these species. There does appear to be some type of incompatibility between these species and the hybrids, at least in certain cross combinations. Our data, however, have not been summarized; so I cannot say whether a general pattern of incompatibility exists or whether the failures of certain crosses were due to certain individual trees. Results from our crossing program do indicate that there is some difference between these aspen species that interferes with or prevents crossing.

Yeatman: I would like to ask Dan how he is going to persuade research men to undertake long-term research programs, such as with the white pine, in the present research climate, especially if you cannot come up with a pretty strong cost-benefit analysis to persuade management it is going to pay off.

Schmitt: Well, I think Dr. Yeatman realizes that nobody really has an answer to that. Furthermore, it is very obvious to me, and I am sure it has been to everyone here, that the research climate, especially in the past 5 years, has been of the type which has placed a rather high priority on - I call them grave problems but of a short-term nature. Now, in my own organization in the

past 2 or 3 years there has been a realization, thanks to our environmental problems, of the need for data coming from long-term studies. I am not talking particularly about genetics research in this case. When researchers sought the information, they found it wasn't available. They also found that the long-term studies had actually been installed 15-20-25-30 years ago. The investigations were passed on in one form or another, the schedule of measurements was not kept up, and utility of data that could have been made available was lost. Just as sort of a side comment, I know most of the people here are involved in genetics research or its application, but data somehow acquire a unique value of their own. Frequently, they can be used for many, many different purposes. As I said before, our organization is beginning to realize the value of long-term research. Getting back to the immediate subject, I think that everybody has been concerned about formulation of breeding programs on a long-term basis; and as a postscript to the talk, I will say in all fairness, the within-species breeding programs that have been developed since the early 1950's have indicated that there is plenty of natural variation, and we didn't need to go to hybridization. It was probably a pretty valid approach. But nevertheless I think, again personal opinion, that interspecific hybridization - analyzing species crosses on a population basis rather than on an individual basis - this is a point that cropped up two or three times - will be a useful tool in developing breeding programs. It has a possibility of providing information which may not be readily obtained any other way. It is just a gut feeling on my part, and I feel that quantitative geneticists should start looking at the problems that have been pointed out today in the analysis of these populations.

Nienstaedt: In view of Dan's comments, I wonder what the reaction of the group is to Don's suggestion that we need cost-benefit analyses in connection with interspecific hybridization programs. Would anybody care to comment on this?

Schmitt: I can't give you any kind of definitive answer, but I think that in the area of interspecific hybridization, with our present state of knowledge, it would be a mistake to undertake this type of analysis. I think that the cost-benefit analysis has pointed out which segments of the program are more expensive, what kind of a return you can get from the total program. In other words, an analysis devoted to the components of the program can provide important clues to decide critical areas in order to, say, reduce the cost of a certain part of the program. For example, in one of the few analyses I have seen it was indicated that initial selection in the parental population was more expensive than the progeny-testing stage. This is interesting because in the past few years I have been aware of growing concern about the merits of intensive individual tree selection and comparison trees. I suppose it could be

debated for quite a little time depending on which side of the fence you stand on, but certainly the cost-benefit analysis is quite helpful where you have an on-going program. But when you have no program or when you are thinking in terms of value of research which is going to back up a program, I suppose the thing to do would be to run the analysis; and if it didn't come out too good, just quietly put it in your desk.

Lester:

In the context of cost-benefit analysis, I have never sat down to figure out what might be a reasonable estimate of additional costs in handling two or more species for an interspecific hybrid program or costs of relatively sophisticated recurrent selection schemes. I don't think we have any idea whether it would be a factor of 2, 5, or 10. We don't have much biological data to estimate the benefit side of the equation, but if it turns out that costs for a reciprocal recurrent selection program are 10 times the costs for a within-species program, the chances are that benefits are unlikely to be large enough in most cases.

Hunt:

I think we are going to be pushed into a cost-benefit squeeze regardless of whether we do it ourselves or not, and I see it coming about this way. If you want to buy hybrid seed on the market, there are a few places where it is available - where it is authentic and not just a putative hybrid. The one example was Langner selling (*Larix leptolepis*) seed here. His price was 50 marks per kilogram, that is before the devaluation of the dollar. It came out to something like \$8.00 per 50 gram sample, which is a few ounces. Hyun reports he is the only one with seed from the pitch pine x loblolly cross, and the only seed that he will send us is F-2 seed. He reports it has performed as well in Korea as F-1, and although we don't know where he got his material for the first cross, we have been very happy to accept the seed even to the point of sending him whatever he suggests in return. There are states in the East that are just crying for seed because their foresters have talked up the need for the seed and Hyun's material in the U.S. is not even performing as well as Si Little's material grown in New Jersey from selected pitch pine and loblolly, at least in the early years. It would then be fitting if we could mass-produce suitable pitch pine x loblolly hybrids for the eastern part of the U.S. and indeed Si Little is giving thought to this. The cost-benefit ratio then is to consider all the factors and get the cheapest possible improved seed into production. We can measure our improvement by how much of the seed used the nursery is "improved". This is the only way we have of saying that our improvement program is actually bearing fruit; and if you want to do it on the very little seed we are getting from our research, we can't afford to do it. We have to do it on an optimistic view that our orchards are actually going to produce quite a bit of seed and the seed is going to be quite a bit improved or planted on a large scale so that

there is a slight improvement over many acres. Otherwise, we have no justification on which to sell our program.

Fraser:

In the last issue of the *Annual Review of Plant Physiology*, Bob Braver from Duke evaluated the results of over 40 years of research in this field. What he proposed is also worth consideration in the field of genetics. The time has come to evaluate what has already been produced in the literature - the variety, its importance and its application. I don't know of anyone who has managed to get money for such a review. Funds are usually forthcoming to start a new aspect of research, yet it is certainly essential to evaluate what has been done in the past and to establish a good basis for future research. I know a few years ago Abe Teich produced a cost-benefit analysis on certain aspects of tree-breeding, and perhaps we should hear from him.

Teich:

After what Clyde Hunt said I don't really have a great deal to add. We have a great need for improved seed right now, and the type of programs that will produce improved seed rapidly will not necessarily come up with tremendous genetic gain. Rapid means like mass selection and provenance-testing won't produce sophisticated publications and won't gain a person world fame, but will produce some slight improvement very rapidly. Although the benefit is very small, it doesn't take very much benefit to justify these types of programs. If we are going to stay in the business of tree-breeding, we have to show we can produce improved seed fast. There are very few people who are willing to wait 40, 50, or 100 years to get benefit out of tree-breeding. They want to get something right now. I think some simple program that came up with 2 or 3 or 4% increase in production, but in which seed would be available rapidly, is of far more benefit than the sophisticated program that would take 50 or 100 years to produce a 40 or 50% gain. So, while we shouldn't try to negate the long-term programs, it is a question of balance. We need seed right now, and we will need seed in the second and third generation also. It is not a question of saying let's jump with both feet into intraspecific or interspecific hybridization: it is a question of how we would like to allocate our resources. I think the first priority is to find the methods which are going to produce a large volume of seed in the very near future. At the same time, we should also allocate a smaller proportion of our resources to the frontiers of breeding.

Nienstaedt:

I think what we have to do now is to mass-produce the most promising material we have and use that to justify the long-term work. Improved material planted now must sell our long-term work as well as the carefully designed breeding programs of the future. We have to get something out now or we are going to lose the credibility we need. We have been riding

along with the South; we can't continue to do that very much longer. I might mention one approach that we plan to take in this respect right now at Rhinelander. We have produced the hybrid between Serbian and black spruce. It is about 15 years old now from seed and it is still a good 25% superior to the black spruce parent and definitely superior to the Serbian spruce. We are going to attempt some small two-clone orchards for the mass production of the seed. We are also going ahead mass-producing - by control pollination mind you - the best families of white spruce. We probably can produce at a fairly low cost 50,000 to 100,000 sound seed a year of families that outproduce seed production area seed by 25 to 30%. I have to do this sort of thing in order to gain continued support for the work at Rhinelander.

Hunt:

I think, Hans, if we don't do this, we are going to find ourselves superseded by private nurserymen. As spin-off look what has happened to Schreiner's clones. We have people planting them on spoil banks, we have people planting them in cities as shade trees, we have all sorts of people propagating them because it is a spin-off where they haven't had to go back to the breeding room and repeat the cross. In Europe, you have chaps like Kleinschmitt, whose method was to take a bunch of small seedlings at age 3 or 5 and begin vegetatively propagating them and put them on big blocks with 50 or 100 trees per plot. The forstmeister could build a little tower and take his boss out there and show him all these great big blocks and point out that one clonal source was 50 or 100% greater, and if he was really in doubt, he could sacrifice 10 trees and do a fiber analysis and find out indeed that it was actually producing so much more fiber that we couldn't afford not to keep producing this tree. I am not saying that you should give up hybridization, but if your Serbian x black spruce cross is so good, why not grow some from cuttings: We have good response in some of our spruce work from cuttings. I know the nut growers were propagating all sorts of grafted walnuts long before the researchers got to the point of saying it was a superior timber tree. We have all sorts of walnut clones being produced by nurserymen and being sold in the trade much like apples, etc. Somebody is going to get on the bandwagon.

Nienstaedt:

In spruce I think it should be possible for us to produce populations of 12,600 superior seedlings by controlled pollination with very little work. This should be enough for a 10-acre planting - enough to interest a forest manager.

Cook:

I am not a geneticist in the usual sense of the word. Rather, I am a manager, a silviculturist, and in the field of tree improvement, a robber of squirrel hoards. As a long-time nurseryman, I feel that the group is taking too narrow a view of what we are trying to do. The cost of GOOD seed is seldom

consequential. In the nursery business, it is axiomatic that the cost of seed is rarely a significant part of the cost of planting stock. The difference between GOOD seed and POOR seed can be of the magnitude of 12- to 15-fold. If it be POOR seed, the nursery costs go up and the value of the succeeding plantation may be vastly diminished.

To the forest manager (and he is our ultimate consumer), there is NOTHING more expensive than poor seed. Let me cite just one example. In 1950, I planted at Cooxrox Forest Plantation No. 35, alternate rows of Japanese and European larch spaced 10 feet between the rows, 6 feet between plants, on an area I had appraised as Site I+ for larch. The European turned out to be of high Alpine source; it never got started. The Japanese simply grew away from it, ultimately suppressed and killed it. So I end up with a stand of pure Japanese larch spaced 20 feet by 6 feet - and doing okay.

If anyone had set out a pure plantation of that European, he would have been sadly disappointed. I'm sure many did - and were!

Another thing we sometimes fail to appreciate - we have a captive market. Most tree seed is going into state or provincial nurseries; so you have to make your sales pitch to only one or two men. Ten years ago, Eli (the late E.J. Eliason) and I sat down and went over all the seed lots of larch that were growing on state forests. We said to the district foresters, "Collect from these, and from no others". That one act raised the quality of our larch stock very substantially and eliminated all the poor stuff.

I feel very strongly that, if the tree improver can produce really top quality seed, he should not be unduly concerned about this production cost!

I have shown you slides of Larsen's Own, an F-1 hybrid larch. The cost of the seed was high. But look what we got. Let's be of courage!

Plummer:

I don't know if I should be on my feet - I am one of those who is nearly done in this field of tree-breeding. I haven't been very strong in the genetic area, but I don't think you ought to sell yourselves short, because industry should be very interested in what you are doing. May I go back 2 or 3 years. When a large paper company in Maine decided at the top echelon that it needed wood, they had to have it "fast". What did they do? They turned to hybrid poplar. I don't know what has happened to the program, but one spokesman for the company said they would need 150 cords per day in the shortest time possible. I believe they obtained cuttings of hybrid poplar and started

several plantations. I can't tell you anything of the current status as I haven't heard from them this year. I don't know if we have any industrial people here - we have had them at past meetings. (Encouragement by invitation may be helpful if sent to the forestry departments of our wood industries.)

Nienstaedt: From the discussions this afternoon, I think it is the feeling that there is a definite place for interspecific hybridization in tree-breeding programs. Perhaps I can add my own view to that. I think we should look at some of the work done in the past and make sure that we haven't overlooked opportunities that should be followed up. With that, I will thank our speakers for their contributions this afternoon and turn the meeting back to the Chairman.

# PROCEEDINGS, PART 1

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ACTIVE MEMBERS  
1974  
CANADIAN TREE IMPROVEMENT ASSOCIATION

Dr. M.G. Boyer	York University Department of Biology 4700 Keele Street Downsview 463, Ont.
Mr. R. Calvert	Manitoba Mines Resources and Environmental Management Research Branch Winnipeg, Man. R3C 0V8
Dr. A. Carlisle	Canadian Forestry Service Petawawa Forest Experiment Station Chalk River, Ont.
Dr. L.P. Chiasson	St. Francis Xavier University Antigonish, N.S.
Mr. A. Corriveau	Canadian Forest Service Laurentian Forest Research Centre BP 3800, Ste. Foy Quebec 10, Que.
Dr. W.H. Cram	Superintendent P.F.R.A. Tree Nursery Department of Regional Economic Expansion Indian Head, Sask.
Mr. B.W. Dance	Ontario Ministry of Natural Resources Research Branch Southern Research Station Maple, Ont.
Dr. D. D'Aoust	Canadian Forestry Service Laurentian Forest Research Centre BP 3800, Ste. Foy Quebec 10, Que.
Dr. D.J. Durzan	Canadian Forestry Service Forest Ecology Research Institute Montreal Road Ottawa, Ont.

Mr. W.G. Dyer	Ontario Ministry of Natural Resources Forest Management Branch Parliament Buildings Toronto, Ont. M7A 1W7
Mr. K.C. Eng	Ontario Ministry of Natural Resources Forest Management Branch Angus, Ont.
Mr. E.R. Falkenhagen	University of British Columbia Faculty of Forestry Vancouver, B.C.
Dr. J.L. Farrar	University of Toronto Faculty of Forestry Toronto, Ont.
Dr. D.P. Fowler	Canadian Forestry Service Maritimes Forest Research Centre P.O. Box 4000 Fredericton, N.B.
Dr. D.A. Fraser	Sir George Williams University Department of Geography 1435 Drummond Street Montreal 107, Que.
Dr. R. Girouard	Canadian Forestry Service Laurentian Forest Research Centre BP 3800, Ste. Foy Quebec 10, Que.
Dr. A.G. Gordon	Ontario Ministry of Natural Resources Forest Biology Laboratory Box 490 Sault Ste. Marie, Ont.
Dr. J.W. Green	Laurentian University Sudbury, Ont.
Mr. J.C. Heaman	British Columbia Forest Service Research Division Victoria, B.C.
Mr. C. Hewson	British Columbia Forest Service Reforestation Division Red Rock Nursery Prince George, B.C.

Mr. M.J. Holst	Canadian Forestry Service Petawawa Forest Experiment Station Chalk River, Ont.
Dr. M. Hubbes	University of Toronto Faculty of Forestry Toronto 5, Ont.
Mr. K. Illingworth	British Columbia Forest Service Research Division Victoria, B.C.
Mr. I. Karlsson	British Columbia Forest Service Research Division Cowichan Lake Forest Experiment Station Mesachie Lake P.O., B.C.
Dr. M.A.K. Khalil	Canadian Forestry Service Newfoundland Forest Research Centre P.O. Box 6028 St. John's, Nfld.
Mr. G.K. Kiss	British Columbia Forest Service Red Rock Forest Nursery R.R. 5, 15-Mile Road Prince George, B.C.
Dr. J.I. Klein	Canadian Forestry Service Northern Forest Research Centre 5320-122 Street Edmonton, Alta.
Dr. J.L. Ladell	1176 Tecumseh Park Drive Mississauga, Ont.
Mr. M. Lalor	Alberta Department of Lands and Forests Natural Resources Building Edmonton, Alta.
Mr. Yves Lamontagne	Ministère des Terres et Forêts La Pépinière Forestière Berthierville, Qué.
Mr. C.H. Lane	Ontario Ministry of Natural Resources Forest Management Branch Parliament Buildings Toronto, Ont. M7A 1W7

Mr. C. Larsson	Ontario Ministry of Natural Resources Research Branch Maple, Ont.
Mr. C.H. Lindquist	Department of Regional Economic Expansion P.F.R.A. Tree Nursery Indian Head, Sask.
Mr. K.T. Logan	Canadian Forestry Service Petawawa Forest Experiment Station Chalk River, Ont.
Mr. H.G. MacGillivray	Canadian Forestry Service Maritimes Forest Research Centre P.O. Box 4000 Fredericton, N.B.
Mr. J.A. McPherson	Kimberley Clark Canada Limited Longlac, Ont.
Mr. M.D. Meagher	British Columbia Forest Service Reforestation Division Koksilah Nursery 5847 Chesterfield Street Duncan, B.C.
Mr. S. Manley	Canadian Forestry Service Maritimes Forest Research Centre P.O. Box 4000 Fredericton, N.B.
Dr. E.K. Morgenstern	Canadian Forestry Service Petawawa Forest Experiment Station Chalk River, Ont.
Dr. B. Mullick	Canadian Forestry Service Pacific Forest Research Centre 506 West Burnside Road Victoria, B.C.
Dr. L. Parrot	Université Laval Faculté de Foresterie et de Géodésie Québec, Qué.
Dr. R.F. Piesch	Canadian Forestry Service Pacific Forest Research Centre 506 West Burnside Road Victoria, B.C.

Dr. D.F.W. Pollard	Canadian Forestry Service Petawawa Forest Experiment Station Chalk River, Ont.
Dr. G.R. Powell	University of New Brunswick Faculty of Forestry Fredericton, N.B.
Rose Marie Rauter	Ontario Ministry of Natural Resources Research Branch Southern Research Station Maple, Ont.
Mr. J.B. Santon	Canadian Forestry Service Petawawa Forest Experiment Station Chalk River, Ont.
Mr. D.A. Skeates	Ontario Ministry of Natural Resources Research Branch Maple, Ont.
Dr. C.R. Sullivan	Canadian Forestry Service Forest Insect Laboratory P.O. Box 490 Sault Ste. Marie, Ont.
Dr. O. Sziklai	University of British Columbia Faculty of Forestry Vancouver 8, B.C.
Dr. A.H. Teich	Canadian Forestry Service Petawawa Forest Experiment Station Chalk River, Ont.
Dr. G. Vallée	Ministère des Terres et Forêts Service de la Recherche 875 rue St. Amable Québec 4, Qué.
Mr. B.S.P. Wang	Canadian Forestry Service Petawawa Forest Experiment Station Chalk River, Ont.
Dr. C.W. Yeatman	Canadian Forestry Service Petawawa Forest Experiment Station Chalk River, Ont.
Dr. L. Zsuffa	Ontario Ministry of Natural Resources Research Branch Southern Research Station Maple, Ont.