Inter-racial Hybridization in Scotch Pine: Geographic Flowering Patterns and Crossability

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Early results from testing inter-racial matings in Scotch pine *(Pinus sylvestris)* have been reported by Dengler (1939), Wettstein and Daubinet (1941), Scamoni (1950), Ehrenberg (1973), and Nilsson and Anderson (1970). Heterosis is one possible benefit from inter-racial hybridization. Some of Dengler's Scotch pine hybrids were taller at age nine than the offspring of matings within the parent populations. They became intermediate between parent populations by age twenty (Scamoni, 1950), but still surpassed mid-parent values. Other possible benefits would include combining of desirable traits of different populations or creating greater variability within populations for selection purposes. These findings suggest that a closer look at inter-racial hybridization in Scotch pine would be worthwhile.

We set out several years ago, in cooperation with J. W. Wright of Michigan State University, to explore systematically the possibilities of hybridization among range-wide populations of Scotch pine. To do this, geographic variation in the amount and timing of flowering was studied along with seed yields from inter-population matings.

Materials and Methods

Amount and timing of flowering were studied in a Scotch pine provenance plantation established at Michigan State University's Kellogg Experimental Forest in southern Michigan. The 113 provenances planted there in 1961 were a range-wide sample coming from six western, four eastern, and four southern European countries; several widely separated regions in the U.S.S.R.; and from Norway, Sweden, and Finland. For a more detailed description of these provenances see Wright and Bull (1963). The design of the plantation was ten randomized complete blocks, with four-tree plots. Five blocks were mainly east facing; three mainly west facing; and two level. The two level blocks were on Hillsdale sandy loam, while the rest were on Oshtemo loamy sand.

In 1969 and 1970 the following variables were recorded: the number of trees in each plot producing female flowers, the number of trees in each plot producing male flowers, the number of female strobili on each tree, and the average phenological stage of females on each tree. Data were collected soon after flowers of the earliest populations had started to emerge. Phenological stages were: 1, floral buds closed; 2, strobili emerging from bud scales; 3 and 4, receptive period; 5, scales swollen shut.

The numbers of female strobili per four-tree plot were subjected to analysis of variance, fixed effects model with randomized complete blocks. Other flowering variables were dealt with in terms of means and ranges.

Crossability was studied by examining both the number of filled seed per cone and seed weight from intra- and inter-population matings. Polycross matings were made among trees from the same Kellogg Forest plantation from which flowering data were obtained (*Table* 1). These trees were randomly chosen from the populations they represented, except for the restrictions that they produced 40 or more female strobili or at least 10 clusters of male strobili. In 1969, 48 individual trees used as females were mated factorially with eight pollen mixtures, and in 1970 there were 45 females and six mixtures. Nine females were identical and five pollen mixtures were similar in both years. Pollen mixtures were composed of equal volumes of pollen from 6 to 10 trees. All matings were done using the mini-bag technique (Gerhold, 1968).

Table 1. — Number of trees per provenance used in polycross matings at the Kellogg Forest.

Provenance	1969	1970	Identical in both years
Number of Females			
201 Norway	4	0	0
212 France	4	1	0
214 Turkey	2	0	0
221 Turkey	0	1	0
238 France	3	2	0
240 France	4	3	2
316 France	3	4	2
242 Yugoslavia	2	2	1
243 Greece	4	0	0
245 Spain	1	1	0
246 Spain	0	4	0
250 Germany	3	2	1
261 USSR	0	3	0
262 USSR	4	1	1
267 Scotland	0	2	0
268 Scotland	4	4	2
269 England	3	4	1
318 Belgium	3	3	2
541 Sweden	0	2	0
542 Sweden	1	0	0
550 Sweden	0	1	0
556 Italy	3	4	1
557 Italy	0	1	0
Number of Males In Pollen Mixtures			
212 France	7	7	5
213, 220, and 221 Turkey	0	6*	0
243 Greece	3	0	0
250 Germany	9	10	2
261 USSR	0	10*	1
262 USSR	5		
269 England	6	0	0
316 France	5	0	0
318 Belgium	9	9	2
556 Italy	9	9	7

• Provenances from same locality combined.

The number of filled seeds per cone and seed weight were average values calculated for each mating. A two-way analysis of variance, fixed effects model, completely

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randomized design, was computed for these two variables. The data for each year were analyzed using individual females and pollen mixtures as treatments. The data were analyzed a second time after grouping individual females into the provenances they represented. For mother trees and pollens common to both years, a final analysis was done using years as an additional main effect.

Results and Discussion

Provenances, blocks, years and all two-way interactions were found to significantly influence the number of female flowers per four-tree plot. The main effects were greater than all interactions, as indicated by the relative size of the F-ratios *(Table 2)*.

Table 2. — Analysis of variance for number of female flowers per four-tree plot.

Source	D.F.	F-Ratios
Provenances	112	19.0*
Blocks	9	13.6*
Years	1	86.0*
P × B	1008	2.6*
P × Y	112	2.0*
B × Y	9	3.3*
Error	1008	

* Significant at .05 level.

The relatively small provenance by block interaction is consistent with other reports. The pattern among the provenances in amount of flowering was consistent over the four plantations in southern Michigan studied by Wright *et al.* (1966 b). Gerhold (1966) found that flowering in 23 of 34 families studied remained proportional to mean flower production at each locality, but certain families deviated, demonstrating large interactions of families with environments.

Heavier flowering was observed in those blocks having sandy loam soil. Most likely this was due to the larger size of trees on more fertile soil, and helps explain the block effect indicating variation due to site factors at one locality. Variation among localities in the amount of flowering had previously been reported. Wright *et al.* (1966 b), studying four-year-old Scotch pine plantations, found flowering in southern Michigan but none in northern Michigan. There was also variation among sites in southern Michigan. Gerhold (1966) found variation among three planting locations in Pennsylvania for 34 open pollinated families of unknown provenance and one precocious commercial variety of Scotch pine.

Regional patterns of variation in the observed flowering variables paralleled those for other characters reported by Wright (1966 a). However, the flowering of Scottish and some French sources resembled central European sources more than southern European provenances. Provenances of the central European region had the highest percentage of provenances with abundantly flowering trees. Southern Europe and southwest Asia had the next highest, and Scandinavia and Siberia had the lowest. The number of trees per provenance with male flowers and the number with female flowers followed the same pattern *(Table 3)*. Certain provenances from France, Italy, Germany, and Belgium were especially prolific. Others from Finland, Norway, Sweden, Siberia, and Greece had no trees in either year with enough flowering to be used for breeding. Those from Scotland had more trees with female strobili than male strobili, while the reverse was true for some Greek and Turkish provenances. In general, the number of trees with male strobili was similar to the number with female strobili. A breeder could use these regional means to determine in which provenances adequate flowering would be produced on young trees.

Table 3. — Regional means of flowering of Scotch pine provenances[a].

Region	No. trees with		Average Flower Stage	Range in ♀ Flower Stages
	≥40 ♀ Flowers	♂ Flowers		
Scandinavia and Siberia	.9	.8	3.5	2.4—4.2
Central Europe	6.8	6.7	1.9	1.0—3.7
Southern Europe and Southwest Asia	2.2	3.6	2.0	1.2—3.0

[a] Means based on number of trees that met flowering abundance criteria out of 40 planted per provenance.

The low flower production in Scandinavian and Siberian provenances is probably due in part to the smaller sizes of these trees at the ages studied. Effects of size of tree on quantity of flowering have been reported by Gerhold (1966), Sarvas (1962), and Wright *et al.* (1966 b).

The quantity of flowering for these geographic regions follows the same pattern at the Kelogg Forest as it did for a plantation in eastern Nebraska, composed of 36 of the same provenances (Read, 1971). The pattern was also similar to that at a plantation of 70 provenances in Missouri (Polk, 1967) and other plantations in Michigan (Wright *et al.*, 1966 b).

Neither topographic aspect nor soil type was clearly associated with flower phenology. Populations from central Europe and from southern Europe and southwest Asia were similar in their average stage of female strobili development, while those from Scandinavia and Siberia flowered 4 to 10 days earlier, depending on weather conditions *(Table 3)*. This is in agreement with data on many of the same provenances in Missouri (Polk, 1967) and with Marcet (1951). The ranges of female flower stages show that some overlap in the flowering times of these three regions did exist. Certain widely separated provenances almost coincided. For example, 554 Italy, 272 Greece and 541 Sweden had developmental stages of 2.9, 3.0 and 2.8 respectively. Furthermore, provenances near each other may differ considerably in flowering stage. For example, the stages of 273 and 274 Norway were 3.8 and 3.0, 202 and 321 Germany were 1.6 and 3.7, 554 and 557 Italy were 2.9 and 2.0, and 218 and 219 Spain were 2.0 and 1.2 More often flowering times within small regions were similar, however. The breeder can therefore predict in most cases the phenological relationships among provenances based on their region of origin.

In those cases where the flowering times of the desired provenances do not coincide, some change in phenology can be obtained by planting at different locations. In 1966, Scotch pine in Missouri (Polk, 1967) flowered sixteen days earlier than it did in southern Michigan and central Pennsylvania. Late flowering provenances at the early location could be made to coincide with early provenances at a late location. Any phenological barriers to artificial hybridization could easily be overcome in this manner, or by storing pollen.

Weight per seed and number of filled seeds per cone were influenced by pollen parents and also by females for matings made in 1969. Only female effects were significant again in 1970. Provenances were also found to differ in their seed weight. Trees from Spain and Georgia, U.S.S.R., produced seeds weighing 25 to 30 percent more than most others. In 1969 provenances were found to differ in number of filled seeds per cone, but not in 1970. Inter-population matings gave yields comparable to those obtained from intra-population matings.

When the matings common to both years were analyzed, years, females, and the year-by-female interactions were found to influence both weight per seed and number of filled seeds per cone. Pollens also influenced number of filled seeds per cone but not seed weight. The seed weight F-ratio for individual females was much larger than for pollens in all analyses *(Table 4).*

Table 4. — Analysis of variance of seed yields for mother trees and pollen used in both 1969 and 1970.

Source	D.F.	Mean Squares Seed Weight	Mean Squares No. Filled Seeds	F-Ratios Seed Weight	F-Ratios No. Filled Seeds
Years	1	3228	447	43.8*	21.3*
Pollens	4	142	94	1.9	4.5*
Females	8	3773	273	51.2*	13.0*
Y × P	4	137	35	1.9	1.7
Y × F	8	264	119	3.6*	5.7*
P × F	32	44	18	0.6	0.9
Error	32	73	21		

* Significant at .05 level.

The performance of the nine trees used in both years shows an association between the stage of female strobili development at the time of pollination and the number of filled seeds per cone. The overall mean number of filled seeds per cone in 1969 was approximately 50% greater than it was in 1970. However, for five of the trees the 1969 value differed from the 1970 value by a factor of two or three. The smaller value for all but one of these five trees was obtained in the year when some of the female strobili were at a non-optimal stage at the time of pollination. Timing of pollination was therefore important, and likely was partially responsible for differences among females in number of filled seeds per cone.

Dengler (1932) demonstrated with five provenances that quantities of filled seed could be obtained from inter-population matings that were comparable to these from intra-population matings. The present experiment shows the same to be true for 189 inter-population combinations. The failure of any mating to yield a typical quantity of seed could be explained by pollination at a non-optimal time or by squirrel damage, as indicated by inspection of the field records.

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Summary

Geographic flowering patterns in Scotch pine *(Pinus sylvestris* L.) and seed yields from intra- and inter-population matings were studied to determine the feasibility of hybridizing diverse populations. Quantity of flowering (the number of female flowers per tree, the number of trees with female flowers, and the number of trees with male flowers) and phenology of flowering were examined in 113 provenances grown in a plantation in southern Michigan. Data were obtained during the ninth and tenth years after the trees had been planted. Polycross matings among certain of these provenances produced 197 different population combinations.

Provenances and environmental variables were found to influence quantity of flowering, while the interaction of provenances with environment was small. This would indicate that the amount of flowers produced by a provenance, relative to other provenances, would not vary greatly over different sites and years. The provenances were grouped as follows: central Europe; southern Europe and southwest Asia; Scandinavia and Siberia. Provenances from central Europe had the highest percentage of trees with at least 40 female flowers, the minimum number needed for our systematic breeding. Southern Europe and southwest Asia had intermediate abundance of flowering, and Scandinavia and Siberia had the lowest percentage. Pollen production followed the same pattern.

Phenology of flowering was also influenced by provenance and environment, and again the interaction was small. Most provenances from central Europe, southern Europe, and southwest Asia flowered within a few days of each other. Scandinavian and Siberian provenances generally flowered four to ten days earlier depending on the weather conditions in different years. Greek and Turkish provenances also tended to flower earlier.

The number of filled seeds per cone and seed weight were affected by both parents in 1969 but only by females in 1970. Female effects were much larger than male effects in both years. The difference between the years could be explained by deviations from optimal flower stage at pollination. All failures of any matings to yield seed could also be ascribed to flower stage at pollination or squirrel damage. There are techniques for coping with phenological differences. The amount of flowering should continue to increase as trees grow larger. Therefore, we conclude that inter-population hybridization is feasible among most or all Scotch pine populations.

Key words: Pinus sylvestris L., Inter-racial Hybridization, Geographic Flowering, Seed-Yield.

Zusammenfassung

In den Jahren 1969 und 1970 wurden in einem Kiefernprovenienzversuch *(Pinus sylvestris* L.) im Kellogg Experimental Forest im südlichen Michigan der Zeitpunkt und die Quantität des Blühens und der Samenertrag untersucht. Die 113 Provenienzen, die im Jahre 1961 ausgepflanzt worden waren, stammen aus 6 west-, 4 ost- und 4 südeuropäischen Ländern, einige aus weit gestreuten Gebieten in der USSR, sowie aus Norwegen, Schweden und Finnland. Sie wurden bei der Untersuchung in folgende Gruppen zusammengefaßt: Zentraleuropa, Südeuropa, Südwest-Asien, Skandinavien und Sibirien.

Den höchsten Prozentsatz an weiblichen Blüten hatten die Kiefern aus Zentraleuropa, den niedrigsten diejenigen aus Skandinavien und Sibirien, wobei die Pollenproduktion ähnlich verteilt war. Das Erscheinen der Blüten wurde sowohl durch die Provenienz bestimmt als auch durch den Standort. Die Anzahl der vollen Samen pro Zapfen und das Samengewicht wurden im Jahre 1969 durch beide Elternteile, im Jahre 1970 nur durch den weiblichen Elternteil bestimmt, wobei der weibliche Einfluß in beiden Jahren größer war. Alle *Pinus sylvestris*-Populationen ließen sich miteinander kreuzen.

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Ceroplastes rubens Maskell Damage of Pinus caribaea Morelet with Notes on the Scale's Preference of Certain Clones as Host Material (Hemiptera: Coccidae)

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Introduction

The red wax scale, *Ceroplastes rubens* Maskell (Hemiptera: Coccidae), a pest of citrus, *Pinus taeda* L. and *P. radiata* D. Don (Browne, 1968), is now causing damage to certain clones of *Pinus caribaea* Morelet in Papua New Guinea. Damage was first noted when an examination was carried out to determine factors causing various clones to have noticeably sparse and dark foliage. The greatest damage, as a result of scale attack, arises from the aggravation of the host by sooty-molds which live on honey dew secretions produced by the scale (Blumberg, 1935). Tausman and Hart, 1974, reported citrus leaves with sooty-mold fungus had less absorption of light than clean leaves.

First records of *P. caribaea* as a host of *C. rubens* are reported herein. The described investigations were carried out to determine the preference of the scale to different species of *Pinus* and various clones of *P. caribaea*. Of further interest was the extent of insect damage found on *P. caribaea*.

Materials and Methods

The study was carried out in the Bulolo and Watut River Valleys of the Morobe District, Papua New Guinea, from September 1974, to March 1975. Red wax scale damage was first noted in a *P. caribaea* seed orchard located 1.2 km S.E. of Bulolo at 700 m elevation. The seed orchard was established between June 1968, and July 1971, by randomly planting grafted ramets of clones taken from Bowenia, Queensland, Australia. In the orchard, no two ramets of the same clone were planted adjacent to one another.

The sampling of scale populations of trees within the seed orchard was carried out to determine relative attack frequencies on trees of 23 clones of *P. caribaea*. A count of scales was obtained on samples of thirty needles taken from the lower, middle and upper crown of all trees in the orchard.

Due to the uneven number of trees per clone, only 18 of the 23 clones could be analyzed statistically for attack frequencies. All clones with three or more trees were included in the test. Those clones with more than three trees were represented by a randomly selected sample of three trees. The statistical analysis included 57.5 percent of the sampled trees.

Tree heights were monitored in October 1975, and six months later, to determine height increment. Clones were rated according to their height increment for this period, as well as for a yearly period in 1969-70 (Holesgrove and Howcroft 1970).

Examination of stands of many species of *Pinus* was carried out to determine the scale's preference of various species.

Results and Discussion

Samples of attack frequencies on needles (*Table 1*) from different portions of the crown and from different clones revealed that the most heavily attacked portion of the tree was the upper crown (*Fig. 1 and 2*). The mid-crown portion was more heavily infested than the lower portion. The samples also revealed a great difference (0.5 percent probability level) of attack frequencies among clones. When a Student-Newman-Keul's test was performed, the only significant difference (5 percent probabilitylevel) was between clones No. 21, 55 and 65, and the remainder. These three clones exhibited a greater amount of attacks than did the other clones (*Table 2*).

No correlation was found between height of trees and amount of scales on samples within or among clones. This

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