REPORT ON THE CROSS PINUS RESIONSA X P. TROPICALIS[1]

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ABSTRACT.-- Interspecific hybridization was attempted between *Pinus resinosa* and *P. tropicalis*, the only two New World members of the *Pinus* group Sylvestres. Thirteen P. *resinosa* trees at Madison, Wisconsin, and nine trees near Fredericton, New Brunswick, were used as female parents in crosses with P. *tropicalis* pollen. A total of 504 pollinated strobili yielded 115 mature cones, but only 2 putative hybrid seeds contained filled embryos as determined by X-ray photography. The two seeds failed to germinate.

Probably no other commercially important tree species has less intraspecific variability and is more difficult to hybridize with other species than red pine (*Pinus resinosa* Ait.). Fowler and Lester (1970) summarized the small differences among provenances that have been reported for several characteristics of red pine, and they point out that the induction of greater genetic variation through interspecific hybridization has been almost impossible to obtain. Although some genetic gains can probably be made through inter- and intraprovenance selection and breeding, the rate of progress will be much slower than for other species. However, despite past disappointments in interspecific hybridization with red pine, the full range of possible crosses has yet to be adequately tried.

Red pine belongs to the *Pinus* subsection called Sylvestres (Critchfield and Little 1966) or Lariciones (Shaw 1914). All the other members of this taxon are restricted to the Eastern Hemisphere except *Pinus tropicalis* Morelet (tropical pine) which is restricted to two small areas in the Caribbean, the low mountains of western Cuba, and the Isle of Pines (Critchfield and Little 1966).

Much effort has been expended in attempting to cross red pine with other Sylvestres pines (Fowler and Lester 1970). One of the repeated attempts to cross Austrian pine (*Pinus nigra* Arnold) with red pine

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resulted in a few confirmed hybrid seedlings (Critchfield 1963); no success has been achieved with other species. Apparently there have been no previous attempts at crossing red pine with *Pinus tropicalis*.

Saylor's (1964) karyotype study of the Sylvestres group indicates that the chromosomes of *P. resinosa* resemble those of *P. tropicalis* more than they do any other pine. The most striking evidence for this is the similarity in arm ratios for the two smallest chromosomes in each species. This observation, coupled with the lack of other geographically close relatives, suggests the cross with *P. tropicalis* as a logical step in trying to hybridize red pine.

Little information is recorded on the biology of *Pinus tropicalis*, but this species must be adapted to a different set of climatic conditions than is red pine. *P. resinosa* x *P. tropicalis* hybrids, if produced, would probably not be hardy within the natural range of red pine, but might be directly useful in more southerly latitudes. However, the main advantage would be the introduction of new germplasm that could eventually be incorporated into a hardy organism by back crossing to red pine or by further hybridization with temperate-zone pines of the Sylvestres group.

P. tropicalis has long needles (up to two times longer than *P. resinosa*), distinctively large needle resin ducts (Shaw 1914), and a seedling "grass" stage (Mirov 1967). These traits should be helpful in hybrid identification. The presence of a grass stage in this species also leads to speculation on possible past hybridization of *P. tropicalis* with other southern and tropical pines that might make it a particularly rich species in terms of germplasm diversity (Mirov 1967). Aside from the applied tree improvement benefits that the cross *P. resinosa* x *P. tropicalis* might yield, it should provide some insight to the evolution of the Sylvestres subsection.

MATERIAL AND METHODS

Pinus tropicalis pollen was obtained from plantations in South Africa and distributed in the United States and Canada by Dr. P. W. Garrett of the USDA Forest Service.

In May 1972, 13 red pine trees growing at the University of Wisconsin Arboretum at Madison and 9 trees growing near Fredericton, New Brunswick, were used as female parents for crosses with *P. tropicalis* and red pine. An unpollinated control was also included. Cones were harvested in the fall of 1973, the seed extracted, and X-ray photographs taken to determine which seeds were filled and carried a normally developed embryo. Filled seeds were germinated without any pretreatment.

In May 1974, an additional set of five red pine trees at the Holst State Forest in central Iowa were used as female parents. The timing on these pollinations was somewhat delayed, and the peak of strobili receptivity had passed by the time pollen was applied. Subsequent cone development was observed in the fall of 1974 and the summer of 1975. Cones that mature will be harvested in the fall of 1975.

RESULTS

No hybrid seedlings have been produced to date. A reasonably good cone set resulted from the *P. tropicalis* pollinations, although the number of seeds formed per cone was less than for strobili pollinated with red pine pollen (table 1). In Wisconsin a total of 276 seeds was produced from *P. tropicalis* pollinations, but X-ray analysis revealed that only one of these seeds contained a morphologically normal embryo. All other seeds were empty. The one normal "hybrid" seed failed to germinate.

Table 1.--Results of 1972 red pine pollinations in Wisconsin and New Brunswick

Item	Location	Pollen applied	
		P. tropicalis	*P. resinosa*
Strobili pollinated[1] (No.)	Wisconsin	71	12
	New Brunswick	433	117
Mature cones produced (No.)	Wisconsin	37	9
	New Brunswick	78	50
Seeds per cone			
Range	Wisconsin	1-38	34-58
Average	Wisconsin	7.5	39.8
Average	New Brunswick	5.2	21.5
Seeds produced (Total No.)	Wisconsin	276	358
	New Brunswick	409	1,075
Filled seeds[2] (No.)	Wisconsin	1	304
	New Brunswick	1 + 103[3]	954
Germination (%)	Wisconsin	0	70
	New Brunswick	0 + 98[3]	94

[1] Excluding strobili lost to insect damage or breakage.
[2] Determined from X-ray photographs.
[3] Two cones in one pollination bag produced 103 full seeds. These seeds proved to be nonhybrids in subsequent tests (see text).

In New Brunswick, pollinations with *P. tropicalis* yielded 409 seeds, of which 104 were full. However, 103 of these full seeds were obtained from 2 cones in a single pollination bag and their hybrid authenticity was dubious. Those putative hybrid seeds, along with suitable control seeds of red pine and *P. tropicalis*, were germinated and transplanted into a greenhouse test. In August 1974 the surviving putative hybrids were examined and compared to the parent species. They were all nonhybrids and indistinguishable from red pine. It was concluded that they resulted from use of a defective pollination bag or the inclusion of male strobili in the pollination bag. The one supposedly "legitimate" hybrid seed failed to germinate.

The 1974 pollinations performed in Iowa have even less promise of success based on the percent of pollinated strobili that were still maturing as cones in midsummer of 1975. Only 3 of 126 pollinated strobili were continuing to develop, and those were somewhat smaller than the open-pollinated cones on the same trees.

DISCUSSION

The results observed to date are not encouraging. *P. tropicalis* pollen can stimulate conelet and seed coat development in red pine, but it appears that embryogenesis occurs rarely, if at all. Based on a sample of two crossing trials, one in Wisconsin and one in New Brunswick, the viability of the hybrid is also questionable. However, the possibility that red pine can be crossed with *P. tropicalis* should not be abandoned on the basis of this work alone. In these trials only one source of 1-year-old *P. tropicalis* pollen was used and although this pollen showed "normal" germination in distilled water, this is not conclusive evidence that it had retained the ability to function normally *in vivo*.

McWilliam (1959) studied the course of events during the breakdown of seed formation in incompatible pine hybridizations. His work focused on the cross *P. nigra* x P. resinosa. In most cases of incompatibility, he observed some penetration of the nucellus by the foreign pollen tubes. In some cases this pollen tube growth halted within the first few months and rapid breakdown of the megaspore followed. Cone abortion was common. When initial pollen tube growth was vigorous, development of the nucellus and megaspore proceeded normally through the first 12 months. But in all cases studied, normal pollen tube growth did not resume in the second spring leading to deterioration of the gametophyte and empty seeds. The failure to complete fertilization was attributed to an adverse metabolic environment for foreign pollen tube growth.

The results of the *P. tropicalis* pollinations indicated that many pollen grains are functioning during the first season, but nearly all fail to complete development the second spring. It was observed that some female parents yielded a higher percentage of matured cones and seeds per cone. For example, 1 female in the 1972 Wisconsin pollinations provided only 4 of the strobili that were pollinated (out of a total of 71), but all 4 strobili matured and yielded an average of 20+ seeds per cone including the 1 filled seed. Much tree-to-tree variation in seed set was also observed among the New Brunswick trees. Such tree- to-tree differences favor the "metabolic imbalance" hypothesis. It is also possible that failure of the *P. tropicalis* pollen to fertilize red pine results from the inability of the germinated pollen to survive the rigorous climates of Wisconsin and New Brunswick.

The fact that the percent of pollinated strobili to continue development in the second year was much lower in the Iowa study is probably more a result of delayed pollination than of tree-to-tree variation or climatic differences.

At this point in the effort to hybridize red pine with tropical pine, several approaches remain to be explored, including: (1) repeating the cross _P. resinosa_ x _P. tropicalis_ using pollen from South African plantations or preferably from other sources as these become available, and pollinating as many female parents as possible (manipulation of male parentage is not so easy to arrange because all pollen has to be supplied by foreign cooperators); (2) concentrating further effort on those female parents that have previously given good cone and seed set when pollinated with _P. tropicalis_ pollen; (3) taking steps to reduce the harsh, over-winter environment that the _P. tropicalis_ pollen tubes are exposed to (e.g., using grafted red pine females grown in the greenhouse); (4) making the reciprocal cross on either the native _P. tropicalis_ stands or the South African plantations; and (5) using _P. resinosa_ - _P. tropicalis_ pollen mixes to pollinate either _P. tropicalis_ or _P. resinosa_.

ACKNOWLEDGMENTS

The authors extend their thanks to Dr. P. W. Garrett of the USDA Forest Service, Northeastern Forest Experiment Station, for providing the _Pinus tropicalis_ pollen for this study, and to Dr. T. D. Rudolph of the USDA Forest Service, North Central Forest Experiment Station, for X-raying the Wisconsin seed.

LITERATURE CITED

Critchfield, W. B. 1963. The Austrian x red pine hybrid. Silvae Genet. 12:187-192.

Critchfield, W. B., and E. L. Little, Jr. 1966. Geographic distribution of the pines of the world. USDA For. Serv. Misc. Publ. 991, 97 p.

Fowler, D. P., and D. T. Lester. 1970. The genetics of red pine. USDA For. Serv. Res. Pap. WO-8, 13 p. Gov. Print. Off., Wash., D.C.

McWilliam, J. R. 1959. Interspecific incompatibility in _Pinus_. Am. J. Bot. 46:425-433.

Mirov, N: T. 1967. The genus _Pinus_. 602 p. Ronald Press, New York, New York.

Saylor, L. C. 1964. Karyotype analysis of _Pinus_-group Lariciones. Silvae Genet. 13:165-170.

Shaw, G. R. 1914. The genus _Pinus_. 96 p. Riverside Press, Cambridge, England.