the lethal are separated by about 25 crossover units or less (Figure 1). The probability that the single marker gene and 1 of the 10 lethals are in the same half-arm of a chromosome (there are 48 half-arms) is 0.19. If these assumptions are valid, then, among individuals which carry marker genes, about one in five would carry it within approximately 25 crossover units of a lethal gene. An additional one individual in five would carry the lethal and the marker more loosely linked. Thus, one would not have to deal with many recessive marker genes before he could expect some deviant ratios to occur because of this linkage. This, of course, applies only to species with a fairly high incidence of lethals. Where there are few, for example in Pinus resinosa (FOWLER 1965 a), it would be most improbable bhat linkage with a lethal would be the cause of a distorted segregation ratio of a marker.

In some cases, it may be desirable to know the cause of an abnormal ratio and/or genetic basis of a recessive trait which shows up in an unexpected proportion in some selfed progeny. Both embryo abortion and pregermination selection would usually (perhaps always) result in fewer deviant seedlings than expected, whereas in the case of linkage with a lethal either excesses or deficiencies could be expected. Hence, where there is a well-based ratio giving an excess of deviants, linkage should be suspected as the cause. Where excesses of normal seedlings are obtained, the cause may be elucidated by use of the technique of FOWLER (1964), by knowledge of the frequency of embryonic lethals, and in some rare cases by crossing two trees which both carry the same marker but not the same lethal. The last approach. perhaps largely unusable now, may gain utility with time as the inventory of marker-carrying individuals is built up in the more thoroughly studied species.

Summary

In this paper, an attempt has been made to estimate the frequency with which the observed segregation of marker genes may differ from the expected due to linkage with an embryonic lethal. It should be stressed that the estimate is based partially on unverified assumptions. However, the findings serve to point out that investigators working with segregation ratios in selfed progenies of forest trees can expect such linkage from time to time. A frequency of such linkage is estimated for coastal Douglas-fir.

References

BARNER, H., and CHRISTIANSEN, H.: The formation of pollen, the pollination mechanism, and the determination of the most favourable time for controlled pollination in Pseudotsuga *menziesii*. Silvae Genetica 11, 89–102 (1962). — FOWLER, D. P.: Pre-germination selection against a deleterious mutant in red pine. Forest Sci. 10, 335–336 (1964). — FOWLER, D. P.: Effects of inbreeding in red pine, Pinus resinosa, AIT. II. Pollination studies. Silvae Genetica 14, 12—22 (1965 a). — FOWLER, D. P.: Effects of inbreeding in red pine, Pinus resinosa, AIT. IV. Comparison with other Northeastern Pinus species. Silvae Genetica 14, 76–81 (1965 b). — FOWLER, D. P.: Natural self-fertilization in three jack pines and its implications in seed orchard management. Forest Sci. *11*, 55–58 (1965 c). — HAGMAN, M.. Incompatibility in Pinus. Hereditas 52, 245 (Abstract only) (1965). — MERGEN, F., BURLEY, J., and FURNIVAL, G. M.: Embryo and seedling development in Picea glauca (MOENCH) Voss after self-, cross-, and wind-pollination. Silvae Genetica 14, 188–194 (1965). — ORR-EWING. A. L.: A cytological study of the effects of self-pollination on Pseudotsuga menziesii (MIRB.) FRANCO. Silvae Genetica 6, 179–185 (1957). — SARVAS, R.: Investigations on the flowering and seed crop of Pinus *sylvestris*. Comm. Inst. Forest Fenn. 534, 1962. — SNYDER, L. H., and DAVID, P. R.: The principles of heredity. D. C. Heath and Company, Boston, 5th ed., 1957. — SQUILLACE, A. E., and KRAUS, J. F.: The degree of natural selfing in slash pine as estimated from albino frequencies. Silvae Genetica 12, 46–50, (1963).— STURTEVANT, A. H., and BEADLE, G. W.: An introduction to genetics. Dover Publ. Inc., New York, 1962.

The Slash x Sand Pine Hybrid[1])

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Interspecific hybridization has been recognized for several years as a potentially useful technique in forest tree improvement. Consequently, many organizations have incorporated some type of hybridization in their breeding programs, particularly when trying to develop material for use on problem sites. The successful cross of Pinus elliottii ENGELM. var. elliottii (slash pine) and P. clausa (CHAPM.) VASEY (sand pine) made in 1962 by the Union Camp Paper Corporation is the result of such an endeavor. Although difficulties in making the cross undoubtedly will restrict its commercial use, this hybrid is of interest for taxonomic study and for transferring characters between species by backcrossing. Of particular signifioance is the fact that it represents one of the few successful inter-Group crosses in the genus Pinus.

The hard pines of southeastern United States were arranged by SHAW (1914) into two Groups. Pinus elliottii (listed as P. caribaea by SHAW) was placed along with most of the other major pines of the region in the Group Australes, while P. clausa was placed in the *Insignes*. Later DUFFIELD (1952) regrouped the hand pines primarily on the basis of results obtained from hybridization studies. In this revision, all of the southern pines were grouped together except P. *clausa* and P. *virginiana*. They were placed with P. banksiana and P. contorta in a sepanate Group because of their morpholagieal similarity.

Pinus elliottii and P. clausa occur sympatrically over extensive areas in central Florida, but they are easily identified because of striking differences in rnorphological features. For example, slash pine occurs as a medium- to large-sized tree generally of good form with long needles (12—25 cm.) and medium-sized cones (5—15 cm.) that open at maturity (SHAW, 1914; HARLOW and HARRAR, 1941). Sand pine, on the other hand, is commonly found as a small, low spreading tree of poor fonm with small needles (5—9 cm.) and cones (5—8 cm.) that are usually serotinous.

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Both slash and sand pine have been artificially crossed with other species of pine (CRITCHFIELD, 1963). The number of successful crosses is much greater for slash pine, however, than it is for sand pine. *Pinus elliottii* has been crossed with *P. caribaea, P. echinata, P. taeda, P. palustris*, and *P. glabra. Pinus clausa* has only been crossed with *P. virginiana*. Seed has been obtained from a cross between *P. clausa* and *P. taeda* on two different occasions (CRITCHFIELD, 1963; GODDARD *et al.*, 1962), but the seedlings all died soon after germination. Because of these results and the inability to cross *P. virginiana* with either *P. banksiana* or *P. contorta*, CRITCHFIELD (1963) suggested that *P. virginiana* and *P. clausa* may be more closely related to the other southern pines than to the non-southern pines with which they have been grouped. This paper describes the characteristics of five hybrids obtained from the cross of *P. elliottii* × *P. clausa*.

Material and Methods

In 1962, the Woodlands Research Department of Union Camp Paper Corporation and the N. C. State Industry Cooperative Forest Tree Improvement Program initiated a hybridization project. The primary objective of this program was to develop material for use on exceptionally dry and wet problem sites where trees could not be grown commercially. Several species combinations were chosen. Some were known to be producible, and some, like the slash-sand pine combination, were highly speculative. Although sand pine has a relatively restricted range and little is known about its variability, it was selected as a possible source of drought resistance because of its known tolerance to poor site conditions. It is most commonly found on light, sandy, infertile soils derived from deep deposits of marine sand and clay.

Test crosses of *P. elliottii* × *P. clausa* and the reciprocal were made in 1962 and 1963. The slash pine parents for these crosses were grafted trees located in the Union Camp seed orchard at Savannah, Georgia. The ortets of the seed orchard ramets were select trees located in three different counties in southeastern Georgia. The sand pine parents, located in the Ocala National Forest in north-central Florida, had been selected by St. Regis Paper Company for possible use in their tree improvement program.

In 1962, three trees of both slash and sand pine were used in a combination of 15 crosses; pollen was not available from one tree. These same trees were crossed again in 1963 in all 18 possible combinations.

In April 1964 seeds from one of the two successful 1962 crosses were sown in the greenhouse at Raleigh, North Carolina, along with open-pollinated seed from each of the two parental trees. After one month of growth, the seedlings were transferred from the germination bed to clay pots. At the end of the first year, they were placed outside in an open cold frame.

Various measurements were taken at periodic intervals of growth to determine the hybridity of the seedlings. For the needle analysis, only secondary needles of comparable age were used; measurements were taken from five needles per plant.

Oleoresin samples were obtained by collecting the exudate that formed on the branch tips when the buds were removed. The volatile fractions were analyzed with an F and M gas chromatograph equipped with an eight-foot copper column packed with 10 percent b'b'-oxidipropionitrile on acid-washed chromosorb W (70/80 mesh). Samples of 0.2 to 0.8 μl. were injected into a chamber maintained at a temperature of 123° C. The column and detector temperatures were 60° C and 145° C, respectively.

Crossability

Of the 18 different combinations tried, only two were successful in producing sound seed, and both of these had the same female slash pine parent *(Table 1)*. Although the seed set averaged less than one seed per cone, these combinations were successful both times they were tried.

The results of all crosses made in the two seasons were quite poor. Of the 302 slash × sand conelets present when the pollination bags were removed, 67 percent survived the first year, but only 46 percent were actually harvested. Average seed yield was a meager 0.5 sound seeds per cone. Results of the reciprocal crosses were even poorer. Only 26 percent of the conelets present at the time of bag removal remained after one year, and only 6 percent were harvested. No sound seed were recovered. A large number of hollow seeds were obtained from all the cones; in fact, some cones yielded only wings or empty seeds.

Breakdown of cone and seed development apparently occurred at several different stages. This was most evident when the slash × sand pine crosses were compared to the reciprocal crosses. Although there was some difference beween years, a greater percentage of cones was harvested for the slash × sand pine crosses (42—50 percent) than for the reciprocal crosses (5—6 percent). Many of the cones of the reciprocal crosses were lost the first year before fertilization occurred. No sound seeds were obtained from the sand × slash pine crosses. These results indicate that fewer barriers exist to making this hybrid when slash pine is the female parent and that barriers to crossing appear early in cone development when sand pine is the female parent.

Table 1. — Cone and seed yield for controlled crosses.

Parents		Number of live conelets at bag removal		Number of 1-yr. conelets		Number of cones collected		Number of sound seed	
Female	Male	1962	1963	1962	1963	1962	1963	1962	1963
slash-97 ×	sand-2	55	11	20	11	20	7	15	5
slash-97 ×	sand-6	62	31	21	31	21	24	25	10
slash-97 ×	sand-8	—	22	—	22	—	5	—	0
slash-85 ×	sand-2	17	14	4	14	4	4	0	0
slash-85 ×	sand-6	4	13	4	13	4	7	0	0
slash-85 ×	sand-8	—	11	—	11	—	8	—	0
slash-77 ×	sand-2	10	21	9	16	9	3	0	20
slash-77 ×	sand-6	15	16	10	16	10	12	0	0
slash-77 ×	sand-8	—	*)	—		—		—	
	Total	163	139	68	134	68	70	40	35
sand-2 ×	slash-97	5	10	0	7	0	2	0	0
sand-2 ×	slash-85	3	12	1	10	1	1	0	0
sand-2 ×	slash-77	5	12	2	9	2	1	0	0
sand-6 ×	slash-97	7	3	0	0	0	0	0	0
sand-6 ×	slash-85	10	13	1	0	0	0	0	0
sand-6 ×	slash-77	6	8	0	0	0	0	0	0
sand-8 ×	slash-97	5	1	0	0	0	0	0	0
sand-8 ×	slash-85	10	6	0	2	0	0	0	0
sand-8 ×	slash-77	7	1	0	0	0	0	0	0
	Total	58	66	4	28	3	4	0	0

*) Lost indentity.

Characteristics of the Hybrids

Soon after the primary needles emerged, the general appearance of the hybrids was strikingly similar to the sand pine control seedlings. This resemblance, which continued throughout all subsequent stages of development, was manifested primarily in the texture of the needles, branches and stem. These structures were noticeably thicker in the slash pine seedlings than in either the hybrid or sand pine seedlings *(Figure 1).* Because sand pine was the male parent, the initial comparisons indicated that the seedlings obtained from the cross were quite likely *bona fide* hybrids and not contaminants.

In contrast to some other interspecific crosses that were made, hybrids from this cross were generally more uniform in appearance than open-pollinated seedlings of either parent. In six of nine characteristics studied, the hybrids were the least variable; however, their range of variability was greatest for two traits. Slash pine seedlings were the most variable of all three groups.

Seedling Heights and Diameters

Seedling heights were measured after eight, sixteen and twenty-two months of growth. Noticeable changes occurred in the pattern of height growth during the 22-month period. At eight months, slash pine seedlings were significantly taller than either the hybrid or sand pine seedlings *(Table 2).* After 16 months of growth, however, the slash pine seedlings had been passed by both the hybrid and sand pine seedlings, with the latter definitely being the tallest. Differences in height beween the groups of seedlings were no longer statistically significant at 22 months, but definite trends could be detected. The hybrids were the tallest, and the slash pine seedlings were still the shortest. At this time all of the seedlings had terminated growth.

Differences in stem and branch diameters were the most striking of all the characteristics analyzed. Stems of the slash pine seedlings were much thicker than any of the others. The hybrids were truly intermediate, with highly significant differences being found among all three groups. The hybrids were also intermediate for branch diameter, but they were very similar to the sand pine seedlings.

Leaves

Needles of slash and sand pines differ in several characteristics. For example, slash pine has longer and thicker needles than sand pine, and these differences are manifest at an early stage of seedling development. Needles of the hybrids were intermediate between the parental types but closely resembled those of sand pine. Slash pine needles were both significantly longer and thicker than needles of the hybrids and sand pine.

Length of the fascicle sheath was the most useful needle characteristic for distinguishing between the hybrid and parental seedlings. This characteristic and stem diameter were the only two traits for which highly significant differences were found between all three groups. Fascicle

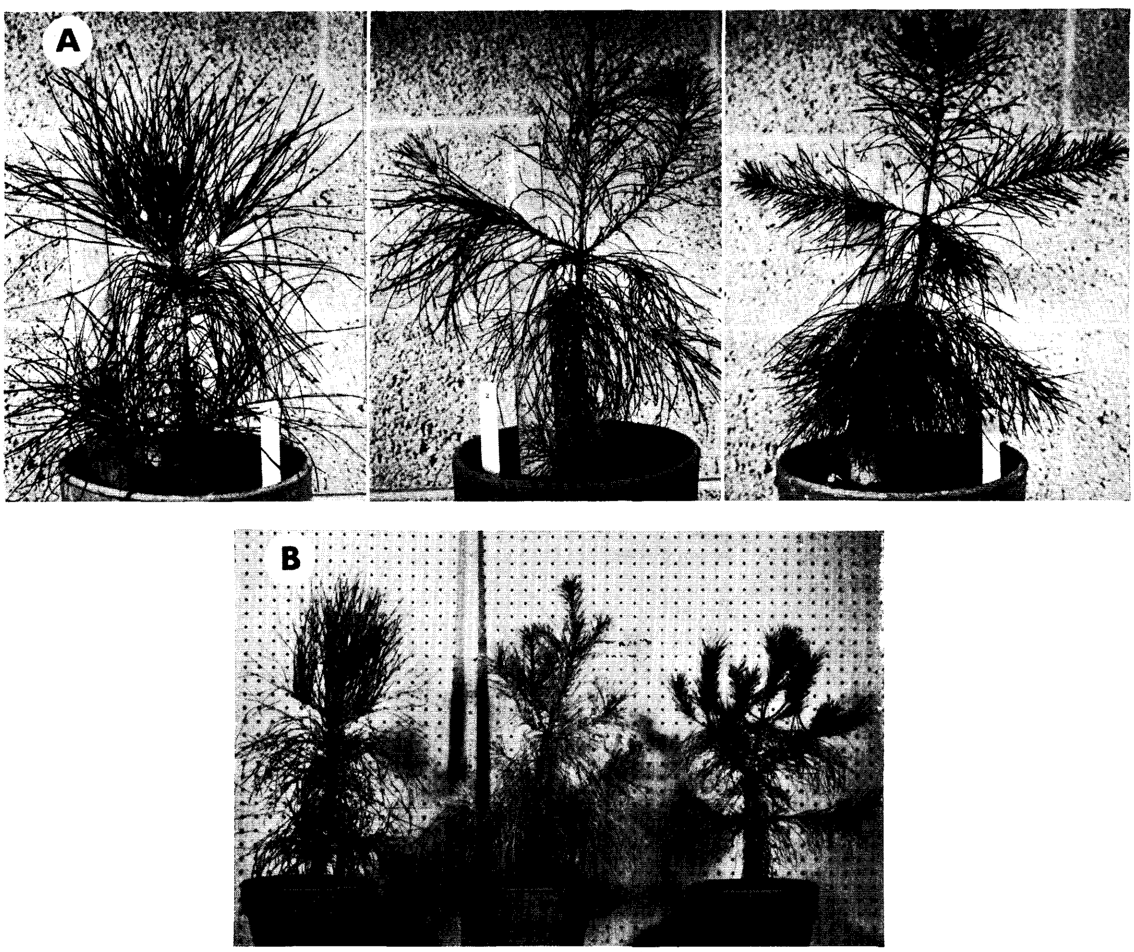

Figure 1. — Slash × sand pine hybrid (middle) and open-pollinated progenies of the slash (left) and sand pine parents. — (A) Sixteen months from germination. — (B) Twenty-two months from germination.

Table 2. — Characteristics of hybrids and open-pollinated offspring of the parents.

Characteristics	Mean Measurements			Mean Differences		
	slash	slash × sand	sand	slash minus sand	slash minus hybrid	sand minus hybrid
	8 Months From Germination					
	(20 trees)	(8 trees)	(20 trees)			
Height (cm.)	23.2	14.3	15.6	7.6**	8.9**	1.3
	16 Months From Germination					
	(4 trees)	(5 trees)	(5 trees)			
Height (cm.)	27.7	30.2	37.7	—10.0*	—2.5	7.5*
Diameter (mm.)	8.3	5.1	4.3	4.0**	3.2**	—0.8**
Diameter largest branch at 1/2 height (mm.)	3.4	2.1	1.8	1.6*	1.3*	—0.3
Needle length (cm.)	16.0	10.5	10.3	5.7**	5.5**	—0.2
Needle thickness (1/1000 in.)	23.8	20.3	18.6	5.2**	3.5**	—1.7*
Fascicle sheath length (mm.)	14.2	9.7	7.1	7.1**	4.5**	—2.6**
Stomatal rows on abaxial surface of needle	11.8	10.5	9.9	1.9**	1.3**	—0.6
Stomata per 1.5 cm. of needle length	50.1	47.3	54.6	—4.5**	2.8*	7.3**
Serrations per 1.5 cm. of needle length	20.1	18.3	22.8	—2.7**	1.8*	4.5**
Cell layers of hypodermis	2	1	1			
Resin canals per needle	3	2&3	2			
	22 Months From Germination					
	(6 trees)	(5 trees)	(6 trees)			
Height (cm.)	46.1	54.6	51.6	—5.5	—8.5	—3.0

* Significant at the 5 percent level
** Significant at the 1 percent level

sheaths of the hybrids were intermediate in length between the longer sheaths of slash pine and the shorter sheaths of sand pine.

The three groups of seedlings differed in the number and location of stomata. The hybrids were intermediate but closely resembled sand pine in number of stomatal rows on the abaxial needle surface. Stomatal rows were most numerous in slash pine, differing significantly from both the hybrid and sand pine values. The pattern of variation changed, however, for numbers of stomata and serrations per unit length of needle. For these characteristics, the hybrids were not intermediate but had the lowest values, while those for sand pine were the highest. These were the only two characteristics for which the hybrid material was the most variable.

Several anatomical features of the needles were studied, but the variation was such that they were only of limited value in establishing hybridity. For nearly every characteristic studied the hybrids strongly resembled the sand pine controls. The most useful character was the number of resin canals. Needles with both two and three canals were found in the hybrids, while slash pine needles had exclusively three canals, and sand pine needles had only two canals *(Figure 2)*. Slight differences were also found in the hypodermis of the needles. In the hybrid and sand pine needles, the hypodermis consisted primarily of one row of thin-walled cells. In contrast, slash pine needles frequently had two layers of cells in the hypodermis, and cell walls of the innermost layer were generally very thick.

Location of the resin canals and presence of Casparian strips in the endodermis were also studied. Distinctive patterns for these features were not found in any group, but certain trends were evident. Resin canals in hybrid and sand pine needles were almost always in a medial position, while those in slash pine needles were mostly internal. Similarly, Casparian strips were present in the endodermis of nearly every hybrid and sand pine needle examined, but they were frequently absent in the slash pine needles *(Figure 2)*.

Oleoresins

Analysis of the composition of the volatile fraction of the cortical oleoresin provided results that were not easily interpreted. For the major components, α- and β-pinene, the range of hybrid values clearly transgressed those of the parents *(Table 3)*. For these components the hybrids were most similar to the slash pine seedlings. A relationship to sand pine is suggested, however, by the presence of myrcene and the absence of β-phellandrene in the hybrids. Metabolic systems can be hypothesized to explain such results, but considerably more information would be required to prove them. At least for this study, the value of these data must remain limited.

Conclusions

All of the slash × sand pine seedlings analyzed were considered to be true hybrids. Although the small sample size requires caution to be used for any conclusions, certain relationships were very apparent. The hybrid seedlings were more uniform in appearance than the open-pollinated seedlings of either parent. The hybrids were intermediate between the parental types in all but two morphological characteristics. There was a strong tendency for the hybrids to resemble the sand pine controls.

Table 3. — Percent composition of volatile fraction of cortical oleoresin from 18-month-old seedlings.

Tree	α-pinene	β-pinene	camphene	myrcene	β-phellandrene
slash					
E—1	54.7	43.8	1.5	0	0
E—4	55.0	42.1	1.0	0	1.9
E—5	51.7	41.8	1.2	0	5.3
E—7	62.3	34.5	1.3	0	1.9
E—12	58.6	39.3	1.2	0	0.9
E—14	52.1	47.9	0	0	0
x̄	55.7	41.6	1.0	0	1.7
sand					
S—3	44.8	55.2	0	0	0
S—5	38.9	59.1	1.0	1.0	0
S—6	33.2	66.3	0.5	0	0
S—10	37.9	60.0	1.4	0.7	0
S—13	41.1	57.8	0.7	0.4	0
S—15	39.5	58.8	1.0	0.7	0
x̄	39.2	59.5	0.8	0.7	0
slash × sand					
H—6	65.3	33.3	1.0	0.4	0
H—7	63.4	35.4	0.8	0.4	0
H—8	62.0	36.9	1.1	0	0
H—10	60.2	38.3	1.3	0.2	0
H—11	63.7	33.4	1.4	1.5	0
x̄	62.9	35.5	1.1	0.5	0

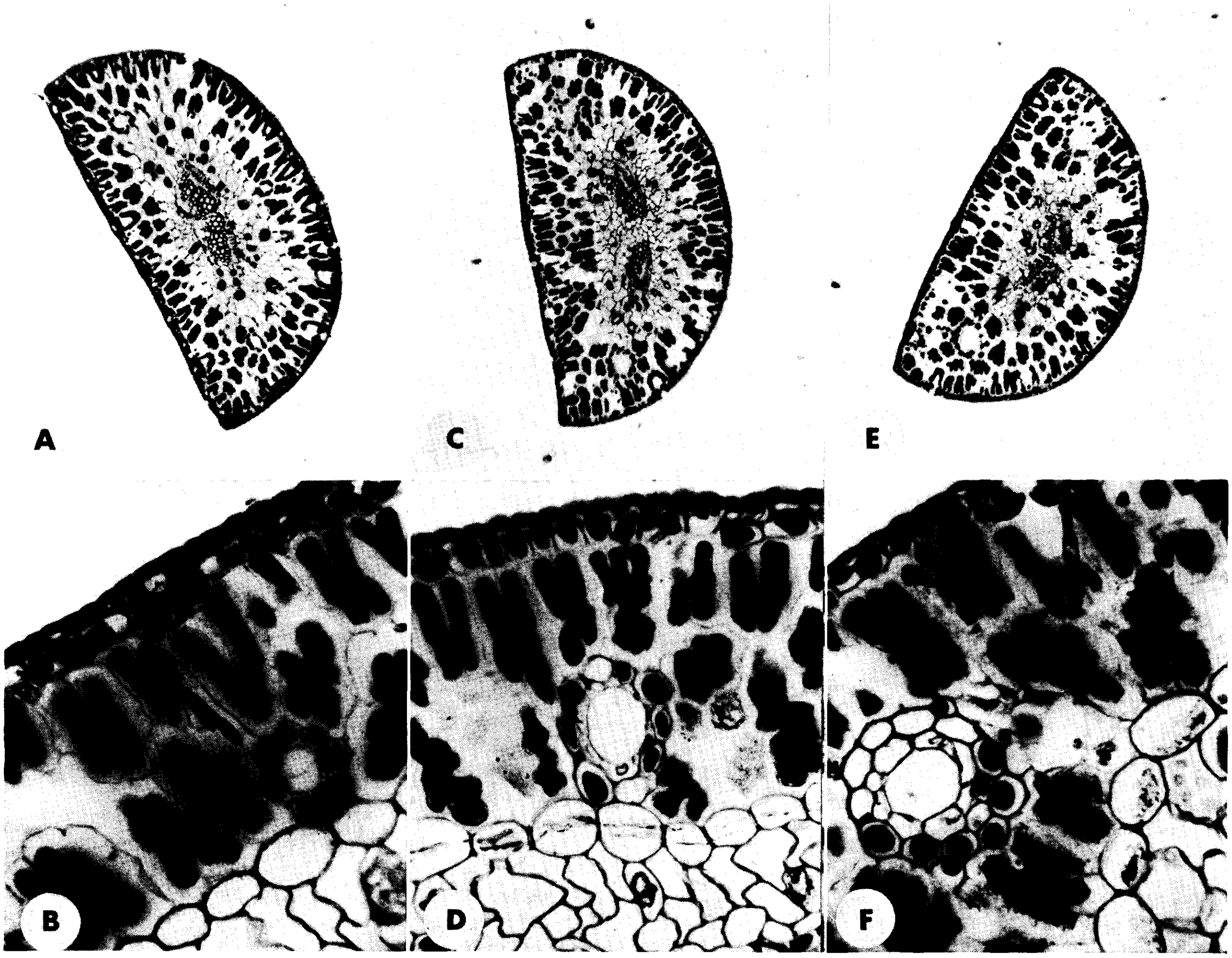

Figure 2. — Needles of 16-month-old seedlings in transection (50× and 470×). — (A—B) Slash pine, (C—D) Slash × sand pine hybrid, (E—F) Sand pine.

According to present classifications, the slash × sand pine combination must be considered an inter-Group cross. As CRITCHFIELD (1963) has pointed out, however, crossability data may prove that sand and virginia pines are more closely related to the southern pines than to jack and lodgepole pines with which they are grouped. Results of the present study provide data to support this possibility.

Summary

Pinus elliottii var. *elliottii* (slash pine) and *P. clausa* (sand pine) were successfully crossed in 1962 and 1963 at the Union Camp Paper Corporation seed orchard in Savannah, Georgia. This cross is one of the few successful inter-Group crosses in the genus *Pinus,* and represents the first well documented hybrid between a species of *Australes* and species from any other Group. Five hybrids were analyzed and compared to open-pollinated seedlings from the parental trees at different times over a 22-month period. The hybrids were intermediate between their parental controls in seven of nine morphological characteristics studied, but they closely resembled the male parent (sand pine), especially during the early stages of development.

Literature Cited

CRITCHFIELD, W. B.: Hybridization of the southern pines in California. Southern Forest Tree Improvement Committee Publication No. 22. Proceedings of a Forest Genetics Workshop, Macon, Georgia, 1963. — DUFFIELD, J. W.: Relationships and species hybridization in the genus *Pinus* Z. Forstgenetik 1: 93—100 (1952). — GODDARD, R. E., PETERS, W. J., and STRICKLAND, R. K.: Cooperative Forest Genetic Research Program, 4th Progress Report. University of Florida, School of Forestry Research Report No. 7, 1962. — HARLOW, W. M., and HARRAR, E. S.: Textbook of Dendrology. McGraw-Hill Book Co. Inc., New York, 1941. — SHAW, G. R.: The genus *Pinus.* Arnold Arboretum Publication No. 5, 1914.