of viable seed among the parents used two years earlier to produce full-sib families by conventional methods of controlled pollination on the trees. Cuttings with unopened male buds were collected from the same three trees, and from one additional tree, on the same date.

All cuttings were treated with 8,000 ppm IBA to stimulate rooting, and placed in rooting benches on 13 January 1965. All other treatments prior to their placement in the rooting bench, and their environment in the rooting bench, were as described in LINHART and LIBBY (1967), with one exception: to preclude possible damage to the strobili, the cuttings were not washed with Clorox. Cuttings with female strobili were put in a bench 15 meters away from the cuttings with male strobili. As male strobili started to shed, the cuttings bearing them were taken out of the benches to prevent the mist-watering system from wetting and washing off the mature pollen. They were kept in jars with the cutting bases in water, about 15 meters away from the female strobili.

Pollinations were made in a headhouse adjoining the greenhouse during the period 20–25 January 1965. Several pollination techniques were used. Some female strobili of each clone (see *Table 1* for exact numbers) were pollinated by rubbing shedding male strobili on them. Other strobili were pollinated with a pollen mixture from all four clones: some were dipped in this pollen; others had pollen brushed between the open scales with a camel's-hair brush; and still others were dipped in a suspension (either water, or a 10% sucrose solution) of the pollen. Following pollination, the cuttings were replaced in the rooting bench. The mist watering equipment was turned off for at least six hours after pollination, to prevent the mist from washing the pollen out of the strobili.

Three types of unpollinated controls were included: (1) female buds were bagged in small sausage casings, to prevent all pollination; (2) others were left unbagged among the cuttings bearing pollinated female strobili; and (3) cuttings with unbagged female strobili were inserted among the cuttings bearing male strobili.

Cuttings bearing forty female strobili were collected on 6 February 1965 from the same three donor trees, after open-pollination in the field had occurred. Ripe cones were collected from the same three trees in October.

Cuttings were checked for rooting at monthly intervals from February to October. Rooted cuttings were potted in soil and removed from the mist to a greenhouse table. Cones were collected from the cuttings when they were judged ripe, i. e., when a cone color change from green to light brown was accompanied by a parting of the cone scales.

The seeds were extracted, counted, and sown in pots containing perlite in January 1966. Seedlings were measured in February and potted in soil. The initial measurements included stem length (hypocotyl plus epicotyl), root length, and cotyledon length. Further measurements of stem length, number of leaves, and number of branches were made six and twelve weeks after potting.

Pots with seedlings from cones which matured in the greenhouse and from cones which matured on the tree should have been randomized relative to their location on the bench. Instead, seedlings from greenhouse seeds were kept together, next to but not interspersed with seedlings from tree seeds. All seedlings were potted in peat pots 10 cm. in diameter, using soil from the same container, and were subsequently watered on the same schedule. Nevertheless, the lack of randomization may have affected the comparisons of post-germination growth.

Results

Some cuttings rooted and at least one cone was brought to maturity in all three clones. However, clone 8 was far superior to the other two in both rooting and cone maturation *(Table 1).*

Rooting of a cutting did not ensure that the strobili on the cutting would mature, nor was rooting a necessary

Table 1. — Rooting of Cuttings and Maturation of ♀ Strobili (by Pollination Technique and Clone)

Pollination Technique	Clone Number											
	8				10				21			
	Cuttings		♀ Strobili		Cuttings		♀ Strobili		Cuttings		♀ Strobili	
	Number Set	Number Rooted	Number of Open Buds	Cones Matured	Number Set	Number Rooted	Number of Open Buds	Cones Matured	Number Set	Number Rooted	Number of Open Buds	Cones Matured
Dry Pollen:												
Single ♂ Strobili	14	8	43	25*)	16	1	68	1*)	8	4	38	0
Pollen Mixture Brushed in	4	3	17	14*)	6	0	22	0	6	1	22	0
♀ Strobili Dipped In Pollen Mixture	—	—	—	—	6	0	22	0	4	0	8	0
Pollen Mixture Suspension:												
In Water	14	7	23	3	8	1	9	0	8	0	19	0
In 10% Sucrose			23	3			9	0			19	0
Controls:												
Bagged	5	2	12	1	4	0	10	0	1	0	1	0
Unbagged Among ♀ Strobili	5	1	10	1	4	0	9	0	1	0	1	0
Unbagged Among ♂ Strobili	4	1	6	1	4	0	6	0	4	2	8	3*)
Totals:	46	22	134	48*)	48	2	155	1*)	32	7	116	3*)

*) Includes cones which matured on unrooted cuttings.

Table 2. — Cone and Seed Data for Mature Cones

Pollination Technique	Clone used as ♀	Number of Cuttings [1]	Number of ♀ Strobili [2]	Number of Mature Cones	Average Cone Length (mm)	Seeds per Cone	Seeds Sown	Number Germinated	Germination Percent
1965 Experiments				*Greenhouse Pollinations: Bench Matured*					
Single ♂ Strobili	10	1*)	4	1	9.0	5	5	0	—
Single ♂ Strobili	8	2*)	10	8	10.9	49	392	0	—
Single ♂ Strobili	8	8	33	17	10.7	43	730	6	0.8%
Pollen Mixture Brushed In	8	1*)	2	1	13.0	60	60	0	—
Pollen Mixture Brushed In	8	3	15	13	11.0	49	639	11	1.7%
Pollen Mixture Suspension	8	2	6	6	11.6	42	255	0	—
				Greenhouse Controls: Bench Matured					
Bagged	8	1	3	1	12.0	78	78	0	—
Unbagged among ♀ Strobili	8	1	2	1	10.0	50	50	0	—
Unbagged among ♂ Strobili	21	1*)	3	3	10.0	33	97	1	1.0%
Unbagged among ♂ Strobili	8	1	3	1	11.0	70	70	0	—
				Tree Pollinated: Bench Matured					
Wind: Collected February	8	1*)	10	8	12.0	57	460	29	6.3%
Wind: Collected February	8	1	8	6	11.2	56	340	1	0.3%
1963 Collections				*Tree Pollinated: Tree Matured*					
Wind: Collected October	8	—	—	10	17.8	81	300	70	23.3%
8 × 12 Controlled Cross	8	—	44	44	18.0	61	300	47	15.7%
8 × 15 Controlled Cross	8	—	47	47	18.2	106	600	197	32.8%

[1]) Number of cuttings which had at least one cone mature.

[2]) Restricted to the ♀ strobili on those cuttings which matured at least one cone.

*) These cuttings had not rooted, but were potted in wet soil and removed from the mist shortly before the cones became ripe. Unstarred numbers in this column refer to cuttings rooted and established in soil prior to cone maturation.

prerequisite to cone development. However, cones matured more frequently on cuttings which rooted than on cuttings which did not root during the period of cone development.

In clone 8, the application of dry pollen to the strobili was associated with maturation of 65% of the strobili thus pollinated (61% of the cuttings rooted), while only 13% of the strobili dipped in water or sucrose suspensions of pollen matured (50% of the cuttings rooted), and 11% of the unpollinated strobili matured (29% of the cuttings rooted). Unfortunately, clone 8 was not used to test dipping the female strobili in abundant pollen, although all 30 strobili thus treated in the other two clones failed to mature (none of the cuttings rooted). Summed over all three clones, 13% (46 of 342) of the pollinated strobili matured. By contrast in 1963 we had pollinated 839 strobili on nine trees, and harvested 768 (91%) from the trees as mature cones.

Table 2 is a summary of all the results pertaining to mature cones and seeds obtained from our pollinations. It was not necessary for cuttings to root to produce cones with viable seed. Cone size and seed yield per cone were comparable between bench-pollinated cones and cones from cuttings collected soon after open-pollination in nature. Cones which completed their development on the tree were larger (*Figure 2*) and produced more seeds per cone than cones which developed in the rooting bench.

The use of dry pollen resulted in six viable seeds when single male strobili were rubbed on the female strobili, and in eleven viable seeds when the pollen mixture was brushed into open female strobili. Pollinations with pollen suspensions in either water or sucrose solution did not yield viable seed. The germination percentages of seeds pollinated in nature but matured on cuttings were similar to those of seeds from the successful bench-pollinations. However, germination percentages of seeds matured on cuttings were an order of magnitude lower than comparable percentages of wind-pollinated and control-pollinated seeds which matured on the tree. (The data on tree-matured seed in *Table 2* are from experiments conducted two years earlier, as the data for the 1965 tree-matured seeds were lost. However, the germination percentage and measurements of the 1965 cones and seeds were of the same order of magnitude as the 1963 data in *Table 2.*)

The cones which matured on cuttings yielded seeds that were smaller than those from tree-matured cones. Shortly after germination, stem, root, and cotyledon length were shorter among seedlings from greenhouse-matured seed than among seedlings from tree-matured seed (*Table 3*).

Table 3. — Average Growth and Development of Seedlings from Cones Matured in the Greenhouse and on the Tree

	Origin of Seedling (♀ tree 8 - all seeds)		
	Greenhouse-Matured Seed		Tree-Matured Seed
	Control Pollinated	Wind Pollinated	Wind Pollinated
Number of Seedlings	15	16	10
Date cuttings with ♀ strobili or cones removed from tree	Jan. 65	Feb. 65	Oct. 65
At Planting			
Stem Length (cm)	1.13	1.10	1.61
Root Length (cm)	1.12	1.13	2.44
Cotyledon Length (cm)	1.16	1.16	1.21
At 6 Weeks			
Growth 0—6 wks (cm)	2.62	2.42	2.61
Total Height (cm)	3.75	3.52	4.22
Number of Leaves	15.8	15.7	14.0
At 12 Weeks			
Growth 6—12 wks (cm)	4.75	3.69	4.19
Total Height (cm)	8.50	7.21	8.41
Number of Leaves	27.0	27.0	29.8
Number of Branches	3.9	2.8	3.5

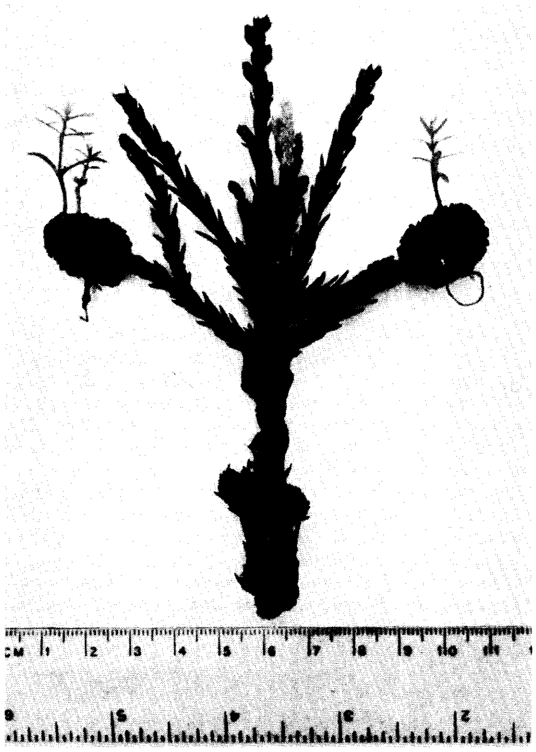

Figure 1. — Seedlings which have germinated in cones matured on an unrooted redwood cutting.

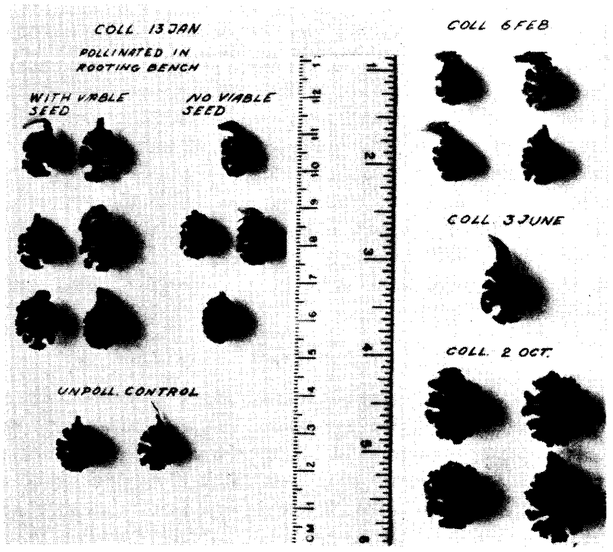

Figure 2. — Relative size of cones from tree 8. Cones on left matured on cuttings detached from the tree prior to opening of the strobilus buds. Cones on upper right were pollinated on the tree, but completed their development on detached cuttings. Cones on lower right matured on the tree.

During the subsequent 12 weeks, growth rate and development were comparable for wind-pollinated seedlings of tree-matured and greenhouse-matured seed, and the total height of the seedlings resulting from greenhouse pollinations had actually surpassed that of the tree-matured seedlings.

Discussion and Conclusions

The rooting of cuttings appears to be an important, although not essential, factor in cone and seed development. A rooted cutting which is potted in soil can supply more nutrients to the cone than an unrooted cutting kept in a state of internal water balance by mist. Furthermore, we observed that cones in late stages of devlopment on unrooted cuttings frequently became molded, damaging or aborting the cones when the cuttings were kept in the rooting bench under intermittent mist. The mist also stimulated the germination of seeds which had matured in cones kept closed by the high moisture *(Figure 1)*. To prevent molding and premature seedling germination, we potted unrooted cuttings in wet soil several weeks before cones opened and obtained viable seed *(Table 2)*.

Contamination by air-borne redwood pollen entering the greenhouse through open windows and vents seems unlikely, since all seeds from unpollinated controls kept among the female strobili failed to germinate. (The maturation of unpollinated redwood strobili is consistent with our observations in 1963. Of 53 strobili bagged and unpollinated on 3 redwood trees, 53 mature cones containing no viable seeds were harvested.) However, if pollen-bearing strobili are allowed in the greenhouse near receptive female strobili, fertilization can occur, as evidenced by the single seedling from the unbagged strobilus of clone 21 *(Table 2)*. To reduce the possiblility of contamination without bagging the female buds, it should be possible to collect cuttings early, force them in the warm greenhouse, and obtain mature pollen and receptive strobili before natural pollen shedding occurs in the wild.

Cones matured in the greenhouse were about two thirds the size of the tree-matured cones *(Figure 2)*, and the seeds were also smaller and fewer per cone. The relative seed size was reflected by the seedling measurements at the time of planting. If seedlings obtained from greenhouse-pollination were permanently dwarfed, the technique would lose much of its value for genetic research. However, in spite of the failure to randomize the seedlings, it is clear that the small size of greenhouse-pollinated seedlings is temporary and can be rapidly overcome by adequate growing conditions after germination *(Table 3, Figure 3)*.

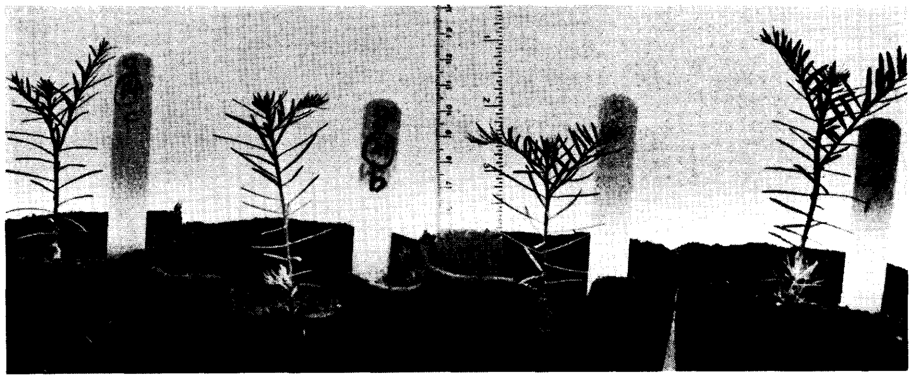

Figure 3. — The two seedlings on the left are of bench-pollinated origin. The two on the right are from wind-pollinated strobili. The seedling on the far right is from tree-matured seed, and the seedling second from the right is from bench-matured seed. Photo taken 15 weeks after planting.

This experiment has established that normal healthy seedlings can be obtained by bench-pollinating redwood strobili on detached cuttings maintained in the rooting bench. The technique is not yet ready for large-scale use, but improvements in methods of pollination and subsequent culturing of the cuttings may make it more efficient, as well as much safer, than controlled crossing accomplished on the tree. Using only the most successful clone and pollination technique as an example, 18 cuttings of clone 8 bearing 64 strobili produced 15 healthy seedlings. This already is an acceptable level of success to produce small families for genetic research.

Summary

Cuttings with unopened female strobili were collected from three Coast redwood trees. The cuttings were kept under intermittent mist in a greenhouse bench. When the female strobili became receptive, they were pollinated by various methods using pollen shed from male strobili kept in the same conditions. Additional cuttings bearing female strobili were collected shortly after pollination occurred in nature. Finally, seeds were extracted from the cones which had remained on the parent trees until mature.

Forty-six of 342 greenhouse-pollinated female strobili developed into cones, 11 of which yielded one or more viable seeds. Seventeen seeds germinated, producing 15 healthy seedlings. These were compared to seedlings obtained from tree-pollinated cones matured in the greenhouse, and from tree-pollinated cones matured on the tree. Seedlings from seeds which matured in the greenhouse were smaller than those from tree-ripened seeds. However, subsequent growth rates of the three types of seedlings were comparable.

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Results of the 25 Years' Provenance Experiment established by using 16 Scotch Pine of European and 1 of Native Provenances in Turkey

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A. Lay-out

1. General

The natural distribution of *Pinus silvestris* in Turkey generally covers the North and North West Anatolia. In this large forest region of Turkey, it occupies the high Southern slopes of the mountains and also reaches the margin of dry inland region. In central Anatolia it occurs in regions up to 2000 meters in eastern Turkey, up to 2600 meters. The southern limit of it reaches to 38° 34' latitude

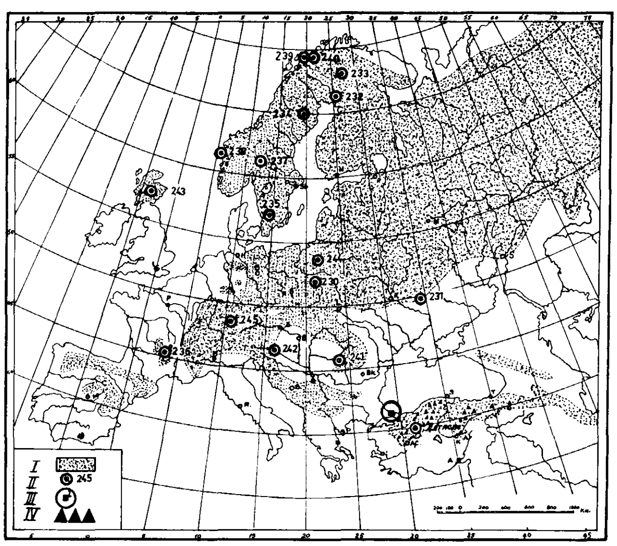

Fig. 1. — The natural distribution of *Pinus silvestris* according to Dengler (I). — Provenances and their origin numbers (II). — Places of experiment plots (III). — Natural distribution areas of *Pinus silvestris* in Turkey according to Bernhard and Kayacik (IV).

Fig. 2. — A dense *Pinus silvestris* stand, composed of phenotypically good quality trees. Kizilcahamam, Altitude 1545 m. (Photo S. Ürgenç).