The direction of changes in habit characters in pine is coincident with the direction of intensification of continental features in the climate of Poland, what may suggest a mutual relationship between these phenomena.

### Summary

The paper contains results of studies on the variation in habit characters in Scots pine (Pinus silvestris L.) on the area of Poland.

For studies were used seed producing stands of pine with the following pattern of taxation characters: tree cover and density within limits of 07–08, site index – most frequently I and II, number of trees per 1 ha – amounting to 150–200 pieces.

Study areas have been established within homogeneous vegetation stands considering their representative appearance for the whole stand. On each area 50 trees were subjected to measurements of characters describing the habit in pine (crown type, length and width of crown, thickness of 1st order branches, angle of branching, range of cracked bark). Arithmetic mean values for individual characters within each area yielded the material for statistical processing, in which the spatial, site, and age variation in study objects were taken into consideration. The existence of correlation between crown type and remaining characters was found. This made possible the identification of two habit types in pine: 1) crown type A, with thin branches, arising at acute angle, with long and narrow crown, with low reaching thick bark. Trees classified to this type are higher and thinner; 2) crown type B, with thicker branches, with crown shorter and broaderwhen compared with the previous one, with higher proportion of branches set at right angle. This type has a higher proportion of trees with panel - like bark. Trees classified to this type are lower and thicker in d.b.h. It was found that within limits of differentiation from Vaccinio-Pinetum to Querco-Carpinetum habit characters failed to reveal any relation with site quality.

Age had an obviously differentiating effect upon habit characters in pine. At the same time, within even-aged groups of stands there was marked a spatial variation which leads to the division of study objects into two main groups: western and eastern. The boundary runs along Vistula River.

In the north-eastern part of Poland, in the region corresponding with the Northern Section distinguished by SZAFER, in stands exceeding 111 years of age the number of trees classified to the type A is contained within limits of 50—88%. On the remaining area, belonging to Baltic Section, the proportion of trees in this type decreases to 21—34%

# **Literature Cited**

Anderson, T. W.: An introduction to multivariate analysis, J. Wiley, New York, 1960. - Emberger, L.: Bulletin du Service de la Carte Phytogeographique avant propos. Serie C, 1956. - FISHER, R. A.: The design of experiments. Oliver and Boyd, Edinburgh 1947. - HOTELLING, H.: The generalization of Students ratio. Am. Math. Stat. 2: 360-378 (1931). - Matusz, S.: Nowa metoda przyrządy do oznaczania pokroju drzew. (Neue Methode und Instrumente zur Bestimmung der Umrißform von Bäumen.) Prace Inst. Bad. Leśnictwa, Warszawa (1963). - NAWROCKI, Z.: Zarys metodyki doświadczeń rolniczych. PWN. Warszawa, 1963. - Steven, H. M., and CARLISLE, A.: The native pine woods of Scotland. Edinburgh, 1959. — Szafer, W. (red.): Szata roślinna Polski. I, II. Warszawa, 1959. — Tyszkiewicz, S.: Wybór sosnowych drzewostanów nasiennych. (Selection of pine seed stands.) Sylwan 7: 73-75 (1960). VANSELOW, K.: Wuchsformen der Kiefer in Deutschland. Allg. Forst- und Jagdzeitung (1933; 1934). - Weber, E.: Grundriß der Biologischen Statistik. G. Fischer. Jena, 1961. - ZAJĄCZKOWSKI, M.: Studia nad sosna zwyczajna w Tatrach i Pieninach. (Studies on the common pine in the Tatra and Pieniny Mountains/Western Carpathians.) Kraków, 1949.

# Control/Pollination Seeds from Cuttings of Coast Redwood')

By W. J. LIBBY, Y. T. KIANG and Y. B. LINHART<sup>2</sup>)

# Introduction

In 1964, we observed the maturation of viable seeds on cuttings detached from Coast redwoods (Sequoia sempervirens D. Don [Endl.]) shortly after pollination. In 1965, further investigation showed that the cuttings could be taken before the male and female strobili opened, and controlled pollinations could be performed successfully in the glasshouse. Seedlings from seeds matured on cuttings were smaller at germination than seedlings from seeds matured on the tree but these differences disappeared after 12 weeks' growth. Success varied with pollination technique, and with the clones used as females. Most of the viable seeds were produced by one pollination technique and were from females of a single clone. Thus, the 1964–66 observations and experiments established that normal healthy seedlings can be obtained from controlled crosses on detached redwood cuttings, but the technique needed further development to be practical (LINHART and LIBBY, 1967).

During 1966—68, we conducted a more extensive series of experiments to investigate the effects of several variables on yield of viable seed: position of the cutting in the crown; storage of both cuttings and pollen; number of female strobili per cutting; levels of contamination with unbagged strobili; and differences between clones.

# **Materials and Methods**

All the cuttings were collected on 23 December 1966 from six planted trees (20 to 30 years old) growing on the Russell Reservation 15 km east of Berkeley. Approximately half were placed in cold storage (4° C) in moist partly-opened polyethylene bags. The rest were placed in rooting benches in the glasshouse, in a perlite rooting medium with bottom heat (22° C), at ambient temperatures from about 7° C to 33° C and averaging about 20° C, with the photoperiod extended to 16 hours by incandescent lights, and intermittent mist irrigation during the day. The occasional male strobilus occurring on a cutting with female strobili was removed, and the cuttings with male and female strobili were placed in separate rooting benches 15 meters apart.

Of the six trees used in 1966—68, clones 8 and 10 had been used as females and clones A, B, 8 and 10 had been used as males in the 1965 experiments (LINHART and LIBBY, 1967).

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Pollen was shed from male strobili on cuttings in the glasshouse during the period 1—12 January, and from the parent trees on the Russell Reservation during the period 4—19 January. Cuttings with opening male strobili were moved into the warm dry headhouse 24 hours prior to pollen collection, to prevent wetting of the pollen and to hasten pollen shedding. It was possible to collect several cc of pollen per cutting by tapping the strobili into a petri dish. Pollen was collected from trees by placing washed twigs with opening strobili in paper bags for one or two days in a warm dry room, shaking the bags, and sifting the pollen. Pollen not used immediately was stored in capped vials in the fruit drawer of a kitchen refrigerator. Pollen collected from tree B early in 1966 was stored for a year in this drawer.

All pollinations were made by brushing dry pollen into the open scales of the female strobili with a small brush. Pollinations were done in the evening, and thus the newlypollinated strobili remained dry overnight until the mist sprays came on the following morning. Most female-bearing cuttings had several strobili. Those cuttings with more than three female strobili had different pollens and sometimes an unpollinated control randomly assigned to groups of two or more strobili on the same cutting.

# Results

Difference between clones. Pollen from all six clones produced viable seeds. However, pollinated female strobili from only four of the six clones produced viable seeds (Table 1).

Pollen and cutting history. We assumed that pollen matured on the tree and used shortly after collection would be most effective. However, such pollen collected from trees B and D produced no viable seeds, and from trees 8 and 10 produced 24 viable seeds, while pollen forced from

Table 1. - Differential Production of Seedlings by Clones Used as Males and Females

23 December Experiment (Fresh Cuttings)												
Clone:	A		В		С		D		8		10	
	\$	ð	9	ð	φ	ð	9	3	9	ð	9	3
Number of cuttings	3		4		8		14		11	_	4	
Number of strobili pollinated <sup>1</sup> )	6	39	15	123	51	25	145	34	62	65	31	24
Number of germinated seeds pe	r											
pollinated strobilus	0	7.5	6.0	2.0	0.8	14.8	8.1	1.7	0.9	5.3	0	1.0
3	Ma	rch 1	Experi	ment	(Store	d Cutt	ings)					
Number of cuttings	3	_	5	_	9		16	_	6	_	3	_
Number of strobili pollinated <sup>1,2</sup> )	5	37	20	53	83	63	202	67	50	34	50	60
Number of germinated seeds per												
pollinated strobilus	0	0	0	0	1.0	0.3	0.4	0	0.2	0.4	0	0.7

 $<sup>^{1}</sup>$ )  $\circlearrowleft$ : Number of female strobili of this clone receiving pollen of various sources.

Table 2. — Pollen History.

	23 December Experiment (Fresh Cuttings)							
		Number of	Mr b am af	Germinated Seeds				
Pollen	Number of Nu Strobili Ha		Number of Seeds Germinated	As <sup>0</sup> / <sub>0</sub> of Harvested Seeds	Per Pollinated Strobilus			
FRESH¹)								
Tree-collected	57	7	24	5%	0.4			
Forced from cuttings STORED	97	42	564	18%	5.8			
1—2 weeks, forced from								
cuttings²)	84	46	783	20%	9.3			
1 year (tree B)	46	15	0	0%	0.0			
UNPOLLINATED CONTROL	, 68	6	0	0%	0.0			
		3 Mar	rch Experiment (Sto	red Cuttings)				
FRESH								
Forced from cuttings STORED	159	23	45	3%	0.3			
6 weeks, tree-collected³) 8—10 weeks, forced from	81	13	84	9%	1.0			
cuttings4)	141	29	32	2%	0.2			
UNPOLLINATED CONTROL	218	47	0	0%	0.0			

<sup>1)</sup> Used within 6 days of extraction.

 <sup>5:</sup> Number of female strobili of various clones receiving pollen from this clone.
2) Including female strobili pollinated by wild-tree pollen which was capable of producing viable seeds (see Table 2).

<sup>&</sup>lt;sup>2</sup>) Pollen stored 3 weeks was used in 3 crosses on 11 strobili, which all failed to mature.

<sup>3)</sup> A bulk collection made from a population shedding pollen on 30 January.

<sup>&#</sup>x27;) Forced pollen from the 23 December experiment.

cuttings and used fresh or within two weeks produced 1347 viable seeds (*Table 2*). No viable seeds were recovered from crosses using the year-old pollen available from tree B.

The cuttings stored in the cold room allowed us to further investigate pollen storage. These cuttings were placed in the rooting bench in early March, under conditions similar to those of the December experiment (ambient air temperature was higher and true day length longer). All three types of pollen used in the March experiment produced viable seeds, with freshly-forced pollen producing seedlings at a frequency similar to stored pollen forced from cuttings two months earlier. A mixed batch of pollen collected from late-shedding trees on 30 January and stored six weeks performed considerably better than the two forced pollens (Table 2).

The number of seedlings produced per strobilus by the best of these storage techniques is far lower than the seedlings per strobilus produced by pollen forced and used without storage of either cuttings or pollen. Some of the poorer performance in the March experiment with stored cuttings is due to increased strobilus abortion. In addition, germination percentages of recovered seeds are lower than those achieved with forced pollen in the December experiment.

Contamination levels with unbagged strobili. The pollination operation is much more efficient if female strobili need not be bagged prior to and following pollination. Contaminating pollen blowing into the glasshouse, or from cuttings in the glasshouse or headhouse, could make such protection necessary. As a test for such contamination, 286 female strobili were left unpollinated. No viable seeds were produced by the 53 unpollinated strobili which matured (Table 2).

Cutting position in the crown. In nature, it has been observed that germination percentages are greater from seed collected high in the crown of redwood trees than from lower in the crown³). Four of our six trees were fertile over a sufficient area of crown to allow us to collect female-bearing cuttings from the upper and middle parts of the crown. One was clone A, which aborted all female

strobili before cones matured. In the remaining three clonal comparisons in the December experiment, germination from upper-crown cuttings was 675 of 4545 seeds (15%) (4.9 viable seeds per pollinated strobilus), while germination from middle-crown cuttings was 696 of 4080 seeds (17%) (5.4 viable seeds per pollinated strobilus). On the basis of this small sample, it appears there is no advantage in requiring cuttings to be from the upper crown.

Number of strobili per cutting. Redwood can produce large numbers of female strobili per branch. In these experiments, the numbers of female strobili per cutting (10 to 20 cm long) ranged from 1 to 53. We wanted to determine whether the number of female strobili per cutting would affect the production of viable seeds (Table 3). The percentage of strobili aborting ranged from 58% to 75%with fresh cuttings, and from 79% to 100% with stored cuttings, and seems uncorrelated with the original number of strobili per cutting. Among fresh cuttings, the number of seeds per harvested cone appears inversely related to the number of strobili per cutting, but the germination percent of harvested seeds was, if anything, higher from cuttings with many female strobili. These opposing effects served to make viable seed per pollinated strobilus relatively unrelated to number of strobili per cutting. Since the effort to collect strobilus-bearing cuttings and space occupied in the rooting bench are important operational considerations in this technique, while the time to pollinate additional strobili on a cutting in hand is negligible, the number of viable seeds per cutting is the most important statistic in Table 3. Thus, it seems useful to take cuttings with large numbers of female strobili, and to pollinate them all.

#### Discussion and Conclusions

The technique of making controlled pollinations on detached cuttings of Coast redwood cannot guarantee success with every cross attempted. As with pollinations done on trees, some individual trees or clones may be unsuitable as females or males for a variety of reasons. In 1965, trying several techniques we produced 15 viable seeds from 444 pollinated strobili, or 0.03 seedlings per pollinated strobilus. In 1967, using the best technique from the 1965 experiment

Table 3. — Effect of Number of Female Strobili Per Cutting.

23 December Experiment (Fresh Cuttings)										
Ctmobili		Number of			Germinated Seeds					
Strobili Per Cutting	Number of Cuttings	Pollinated Strobili	% Cones Harvested	Seeds Per Cone	As % of Harvested Seeds	Per Pollinated Strobilus	Per Cutting³)			
1—3	12	27	41%	97	10%	4.1	9			
4—6	11	55	22%	98	12%	2.6	13			
7—9	6	48	31%	82	11%	2.8	22			
10—12	11	93	35%	83	18%	5.2	44			
13+¹)	4	87	40%	69	21%	5.8	126			
		;	3 March Expe	riment (Stored	Cuttings)					
1—3	9	15	0%	0	0%	0.0	0			
4—6	10	49	12%	73	6%	0.6	3			
79	7	53	19%	70	7%	1.0	7			
1012	4	34	21%	96	7%	1.4	13			
13+2)	12	259	15%	63	1%	0.1	3			

<sup>1)</sup> Range 13-43; Average 28.5.

<sup>3)</sup> Personal communication, S. Krugman, Timber Management Research, Forest Service, USDA, Washington D.C.

<sup>2)</sup> Range 13-53; Average 25.9.

<sup>5)</sup> Uncorrected for the number of unpollinated controls on some of these cuttings, and therefore the figures are conservative for cuttings with greater than three strobili.

(dry pollen brushed into receptive strobili), we produced 1532 viable seeds from 720 pollinated strobili, or 2.1 seedlings per pollinated strobilus. Considering only the fresh cuttings of female-fertile clones and recently-forced pollen, we produced 1347 viable seeds from 181 pollinated strobili, or 7.4 seedlings per pollinated strobilus.

The germination percentages achieved are comparable to those commonly obtained from tree-matured redwood seed (Fowells, 1965). However, few female strobili abort from trees while half or more abort from detached cuttings. Because of this higher abortion rate, the production of viable seed per pollinated strobilus is lower than for controlled pollinations on the tree.

As previously reported, many of the cuttings do not root before ripe cones with viable seeds are harvested. However, sooner or later most do root, and these continue to produce male and female strobili in increasing numbers when planted (Figure 1). We have recovered viable seeds from these plants of cutting origin. Similar initiation of strobili has been reported for first-year rooted cuttings of the related Dawn redwood, Metasequoia glyptostroboides (Longman, 1970).

Thus, the success, convenience, and physical safety of pollination on detached cuttings recommends the technique for the production of redwood families by control pollination. With the ability to root redwood cuttings and continue pollinations on the rooted cuttings at ground level for several years, plus the possibility of forcing advanced-generation offspring into early flowering (Pharis and Morf, 1969), the future of redwood genetics looks much brighter than it did a few years ago.

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#### Summary

The technique of making controlled pollinations on detached cuttings of Coast redwood is now developed to a point where it is practical for tree breeding and genetics work with this species. The best success was obtained by removing cuttings from the trees shortly before opening of the strobili, and continuing their development in the rooting bench immediately following collection. Pollen shed from such cuttings and used shortly after shedding was more satisfactory than fresh pollen collected from trees, or pollen stored a few weeks from either cuttings or trees. The pollinations were best accomplished by brushing dry pollen between the open scales of the female strobili, and efficiency of the operation is increased by using cuttings with many strobili. The cuttings which root continue to produce abundant male and female strobili, and viable seeds, in subsequent years.

#### Literature Cited

Fowells, H. A.: Silvics of forest trees of the United States. Agriculture Handbook No. 271, p. 665 (1965). — Linhart, Y. B. and W. J. Libby: Successful controlled pollination on detached cuttings of Coast redwood. Silvae Genetica 16: 168—172 (1967). — Longman, K. A.: Initiation of flowering on first year cuttings of Metasequoia glyptostroboides. Nature 227: 299—300 (1970). — Pharis, R. P. and W. More: Precocious flowering of coastal and giant redwood with gibberellins A<sub>3</sub>, A<sub>4/7</sub>, and A<sub>13</sub>. Bioscience 19: 719—720 (1969).

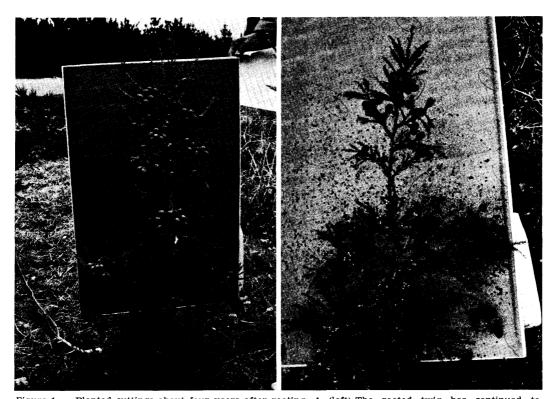


Figure 1. — Planted cuttings about four years after rooting. A. (left) The rooted twig has continued to grow horizontally, with largely bilateral symmetry, and abundant male and female strobili. A radially-symmetrical vertical shoot has initiated near the base of the cutting, and in its second growing season contains mostly female strobili, here shown as nearly-ripened cones. B. (right) No radially-symmetrical vertical shoot has yet initiated from this cutting. Note abundant male strobili near the base and ripe cones further out on the twigs, with freshly-shed seed visible on the board placed under this horizontally-growing plant.