Literature Cited

DAVIS, L. S.: Investments in loblolly pine clonal seed orchards; production costs and economic potential. J. For. 65 (12): 882—887 (1967). — FREEMAN, A. M. III, HAVEMAN, R. H. and KNEESE, A. V.: The economics of environmental policy. John Wiley & Sons, Inc., New York, 184 p. (1973). — HYUN, S. K.: Interspecific hybridization in pines with special reference to *Pinus rigida* × *taeda*. Silvae Genet. 25: 188—191 (1976). — LITTLE, S. and TREW, I. F.: Pitch × loblolly pine hybrids: loblollies for the North? J. For. 77 (11): 709—713 (1979). — MILLS, T. J. and CAIN, D.: Timber yield and financial return performance of the 1974 Forestry Incentives Program. USDA For. Serv. Res. Pap. RM-204, 56 p. (1978). — PHELPS, R. B.: The demand and price situation for forest products 1974—1975. U. S. Dep. Agric. Misc. Publ. 1315, 85 p. (1975). — PORTERFIELD, R. L., ZOBEL, B. J. and LEDIG, F. T.: Evaluating the efficiency of tree improvement programs. Silvae Genet. 24: 33—44 (1975). — POWERS, H. R., Jr., McCLURE, J. P. KNIGHT, H. A. and DUTROW, G. F.: Incidence of financial impact of fusiform rust in the South. J. For. 72 (7): 398—401 (1974). — ROW, C.: Probabilities of financial returns from southern pine timber growing. Ph. D. Diss. Tulane Univ., New Orleans, La., 428 p. (1973). — SHERMAN, L.: Trends in forest product prices. For. Farmer 26 (5): 40—41 (1977). — SMALLEY, G. W. and BAILEY, R. L.: Yield tables and stand structures for loblolly pine plantation in Tennessee, Alabama, and Georgia highlands. USDA For. Serv. Res. Pap. SO-96, 81 p. (1974a). — SMALLEY, G. W. and BAILEY, R. L.: Yield tables and stand structure for shortleaf pine plantations in Tennessee, Alabama, and Georgia highlands. USDA For. Res. Pap. SO-97, 57 p. (1974b).

Study of the pollination pattern in a Scots pine seed Orchard by means of Isozyme Analysis

By H.-H. SHEN[1], D. RUDIN[2] and D. LINDGREN[2]

(Received July / November 1980)

Summary

A genotype analysis of three loci — LAP B, GOT A and B, using some two thousand seeds originating from three ramets of a marker clone and ten grafts from surrounding clones was carried out in a seed orchard close to Umeå. A significant difference with regard to frequency of genotypes from seeds derived from the three ramets was found, and there was variation among sectors at different levels of a crown within a ramet due to differences in the fertilizing pollen cloud. Pollen dispersal seems to be dependent on the coincidence of the time of flowering of male and female trees, the distance from a pollen tree to a mother tree as well as wind direction during flowering. A neighbouring tree with all favourable conditions received 31 per cent of the fertilizing pollen from the marker ramet. The average frequency of self-pollinated seeds from the marker clone was roughly estimated at 6 per cent with the lowest frequency at the top of the crown.

Key words: Pinus sylvestris, isozymes, selfing, genetic marker, clone, fertilization, pollen dispersal

Zusammenfassung

Isozymatische Untersuchungen über die Befruchtungsverhältnisse in einer Kiefernplantage.

An den Samen einer Kiefernplantage in der Nähe von Umeå wurden Isozymanalysen für drei Loci: LAP B, GOT A und GOT B durchgeführt. Drei Pfropflinge eines Markörklons und umgebene Propflinge anderer Klone wurden untersucht.

In verschiedenen Höhenzonen und Richtungssektoren der Krone konnten signifikante Unterschiede in der Frequenz verschiedener Isozymmuster festgestellt werden. Diese Unterschiede sind auf unterschiedliche Zusammensetzung des Pollenangebotes während der Blütenperiode zurückzuführen. Die Pollendispersion des Markörklons wurde näher untersucht: Der Pollenbeitrag ist abhängig vom gleichzeitigen Blühtermin männlicher und weiblicher Blüten, dem Abstand zwischen Vater- und Mutterbaum und der Windrichtung zum Blühtermin.

Unter günstigen Verhältnissen konnten 31% der befruchtungsfähigen Pollen auf eine direkte (unmittelbare) Nachbarschaft des Vaterklones zurückgeführt werden.

Die Rate der Selbstungen wird an Hand des Markörklons auf durchschnittlich 6% geschätzt. Sie ist im obersten Kronenteil am niedrigsten.

Introduction

The establishment of seed orchards based on selected trees has become common all over the world during the last twenty years. In many countries they are now the essential way of supplying genetically improved forest seeds. In order to obtain a greater genetic gain it is important to know whether the original prerequisites for the seed orchards are really fulfilled. Some of the main principles for the establishment and function of seed orchards are the following:

1. Selection of the best phenotypes within a homogeneous climatic region
2. Grafting and random plantation in a seed orchard
3. Localization of the seed-orchards in site with favourable climate and soil which encourage flowering and seed-ripening
4. Isolation against dominating pollen from outside the seed-orchard
5. No clones to dominate the pollen production and fertilization
6. The frequency of selfing to be kept at a minimum level

The three first prerequisites are in many cases fulfilled today. However, until recently it has been very difficult to get hold of good information about the last three.

By analysing differences in empty seed frequencies between selfed, outcrossed and open pollinated seeds from a Scots pine seed orchard, JOHNSSON (1972) found that less than 1 per cent of the viable seeds were due to selfing. Biochemical markers such as isozymes and monoterpenes now make it much easier to study these questions. The perspectives opened up by these methods for research into seed orchards were pointed out by RUDIN and LINDGREN (1977). MÜLLER (1976). GREGORIUS and MÜLLER (1975) discussed methods to calculate the pattern of pollination in seed orchards. MÜLLER-STARCK (1979) found a selfing frequency as high as 12-14% in a 12-15-year-old Scots pine seed orchard containing 36 clones (with 25 ramets from

[1]) Peking College of Forestry, Peking, The People's Republic of China

[2]) Swedish University of Agricultural Sciences, Department of Forest Genetics and Plant Physiology, S-901 83 Umea, Sweden

each clone) in Western Germany. RUDIN and LINDGREN (1977) found that one marker clone each in two separate Scots pine seed orchards in Sweden did not dominate more than expected. The frequencies of selfing in these two seed orchards, comprising 21-25 clones, were estimated at 2-5 per cent. Comparing isozyme genotypes at two levels in the crowns of ramets in a Scots pine seed orchard comprising 25 clones HADDERS (1971), RUDIN and LINDGREN (1977) found indications of a higher outcrossing frequency at the top level. These studies were based on empty seed frequencies and isozyme gene markers respectively.

The purpose of this study was to obtain information about the net effect of pollen dispersal to different sectors of the crown of ramets and between ramets with special interest focused on the selfing frequency in a Scots pine seed orchard. For this purpose the seeds originating

Table 1. — Genotype frequencies of embryos from seeds originating from different sectors of crowns of three ramets of AC 1065 and from pollen fertilizing the corresponding female flower. The numbers analysed and proportion within each sector are indicated. The genotypes of AC 1065 is LAP-B_2B_2 GOT-A_1A_2, B_2B_2.

Ramets		Frequencies of genotypes from embryos												Total	Frequencies of genotypes from pollen						
	LAP-	B_2B_2	B_2B_2	B_2B_2	B_2B_2	B_2B_2	B_1B_2	B_1B_2	B_1B_2	B_1B_2	B_2B_3	B_2B_3	B_2B_3	analyses	B_2	B_2	B_1	B_1	B_3	B_3	B_2**
	GOT-	A_2A_2	A_2A_2	A_1A_2	A_1A_2	A_1A_1	A_1A_2	A_2A_2	A_1A_2	A_2A_2	A_2A_2	A_2A_2	A_1A_2	number	A_2	A_2	A_2	A_2	A_2	A_2	A_1
	Sector	B_2B_2	B_2B_3	B_2B_2	B_2B_3	B_2B_2	B_2B_2	B_2B_2	B_2B_3	B_2B_3	B_2B_2	B_2B_3	B_2B_2	of	B_2	B_3	B_2	B_3	B_2	B_3	B_2
AC 1065 A	N_T	13	9	3	11		4	1	2					43	16	20	5	2			
		.30	.21	.07	.26		.09	.02	.05						.37	.47	.12	.05			
	E_T	8	7	7	10			1	1	1				35	15	17	1	2			
		.23	.20	.20	.28			.03	.03	.03					.43	.43	.03	.06			
	E_M	13	9	4	8	1	1	2		2				40	17	17	3	2			1
		.32	.22	.10	.20	.03	.03	.05		.05					.43	.43	.07	.05			.02
	E_B	4	1		2									7	4	3					
		.57	.14		.29										.57	.43					
	S_T	14	14	5	11	1				1	1			47	18	25		1	1		2
		.30	.30	.11	.23	.02				.02	.02				.38	.53		.02	.02		.04
	S_M	8	10	10	13	3		1		1				46	17	23	1	1			4
		.17	.22	.22	.28	.07		.02		.02					.37	.50	.02	.02			.09
	S_B	5	11	9	7	1								33	12	18					3
		.15	.33	.27	.21	.03									.36	.55					.09
	W_T	7	5	6	8	1					1	1		29	13	13			1	1	1
		.24	.17	.21	.20	.03					.03	.03			.45	.45			.03	.03	.03
	W_M	2	7	8	4					1				22	10	11		1			
		.09	.32	.36	.18					.05					.45	.50		.05			
	W_B	6	5	4	3									18	10	8					
		.33	.28	.22	.17										.56	.44					
	Sum	80	78	56	77	7	5	5	3	6	2	1		320	132	155	10	9	2	1	11
		.250	.244	.115	.241	.022	.016	.016	.009	.018	.006	.003			.412	.484	.031	.028	.006	.003	.034
AC 1065 B	N_T	8	4	8	8								2	30	16	12			2		
		.27	.14	.27	.27								.06		.53	.40			.07		
	N_M	11	4	15	2									32	25	6					1
		.34	.13	.47	.06										.78	.19					.03
	E_T	9	10	7	4	1				1				32	16	14		1			1
		.28	.31	.32	.13	.03				.03					.50	.44		.03			.03
	E_M	8	9	6	3	3						2		31	14	12				2	3
		.26	.29	.17	.10	.10						.06			.45	.39				.06	.10
	S_T	5	11	12	5									33	17	16					
		.15	.33	.37	.15										.52	.48					
	S_M	11	9	5	3	2								30	16	12					2
		.36	.30	.17	.10	.07									.53	.40					.07
	S_B	11	1	14	1	1			1				1	30	25	2		1	1		1
		.37	.33	.47	.03	.03			.03				.03		.83	.07		.03	.03		.03

from three ramets of one marker clone and ten grafts from other clones in Östteg Scots pine seed orchard were used.

Materials and Methods

Cones were collected in the autumn 1979 in the seed orchard at Östteg, which is located close to Umeå, Sweden, at latitude 63°40', longitude 20°15' and altitude 10 m. The seed orchard was established during the years 1956-1964. The present investigation was concerned with only the older parts. It may be noted that the study was carried out at a time when there was an unusually bad seed crop. There was a total of 51 clones and the original spacing was 5 m. At present the distance between the grafts is irregular because of thinning and damage caused by voles. There are around 150 trees/ha. The grafts are 6 to 7 m high. The seed orchard is still fairly open and there are

Ramets		Frequencies of genotypes from embryos													Frequencies of genotypes from pollen						
	LAP-	B_2B_2	B_2B_2	B_2B_2	B_2B_2	B_2B_2	B_1B_2	B_1B_2	B_1B_2	B_1B_2	B_2B_3	B_2E_3	B_2B_3		B_2	B_2	B_1	B_1	B_3	B_3	B_2**
	GOT-	A_2A_2	A_2A_2	A_1A_2	A_1A_2	A_1A_1	A_1A_2	A_2A_2	A_1A_2	A_2A_2	A_2A_2	A_2A_2	A_1A_2		A_2	A_2	A_2	A_2	A_2	A_2	A_1
	Sector	B_2B_2	B_2B_3	B_2B_2	B_2B_3	B_2B_2	B_2B_2	B_2B_2	B_2B_3	B_2B_3	B_2B_2	B_2B_3	B_2B_2		B_2	B_3	B_2	B_3	B_2	B_3	B_2
AC 1065 B	W_T	9	7	8	6	1								31	17	13					1
		.29	.32	.26	.19	.03									.55	.42					.03
	W_M	5	9	13	4					1				32	18	13		1			
		.16	.28	.40	.13					.03					.56	.41		.03			
	W_B	7	4	14	3	1						1		30	21	7				1	1
		.33	.43	.47	.10	.03						.03			.70	.23				.03	.03
	Sum	84	68	102	39	9			1	2		3	3	311	185	197		3	3	3	10
		.27	.219	.328	.125	.029			.003	.006		.010	.010		.595	.344		.010	.010	.010	.032
AC 1065 C	N_T	3	4	2	2									11	5	6					
		.27	.36	.18	.18										.45	.55					
	E_T	7	3	12	8		1							31	19	11	1				
		.32	.10	.39	.36		.03								.61	.36	.03				
	S_T	9	5	7	6	1								28	16	11					1
		.32	.18	.25	.21	.04									.57	.39					.04
	W_T	10	9	12	5									36	22	14					
		.38	.35	.33	.14										.61	.39					
	Sum	29	21	33	21	1	1							106*	62	42	1				1
		.274	.148	.311	.198	.009	.009								.585	.396	.009				.009

Abb N_T = North top, E_T = East top, N_M = North middle, E_M = East middle, S_T = South top, E_B = East bottom, S_M = South middle, W_T = West top, S_B = South bottom, W_M = West middle, W_B = West bottom

* After completing this study another 102 embryos together with corresponding mactogametophyte from AC 1065 were analysed for another purpose. From these embryos four carrying the LAP B_1 allele, two carrying LAP B_3 and one selfed were found. These data are included in calculations of selfing frequencies.

** This column is derived from the GOT A_1A_1 and some of the GOT A_1A_2 genotypes with an A_2 allele from the mother.

Table 2. — Contribution of the pollen from the marker ramet AC 1065 C to the pollination of neighbouring grafts and relevant data.

Clone		AC 1026	AC 1043	AC 3006	AC 1038	AC 2011	AC 1015	AC 4210	Z 3001	AC 1014	AC 1060	Sum
Facing side	*	9/ 51	6/ 62	2/ 51	2/ 53	1/ 57	1/ 53	1/ 50	0/ 50	0/ 52	0/ 50	22/551
	%	17.7	9.8	3.9	3.8	1.8	1.9	2.0	0	0	0	4.1
Reverse side	*	7/ 51	1/ 25	2/ 53	2/ 52	1/ 51	0/ 51	0/ 51	1/ 52	0/ 50	0/ 51	14/501
	%	13.7	4.0	3.8	3.8	2.0	0	0	1.9	0	0	2.9
Total	%	15.7	8.0	3.9	3.8	1.9	1.0	1.0	1.0	0	0	3.4
Contribution		31.4	16.0	7.8	7.6	3.8	2.0	2.0	2.0	0	0	6.8
Flowering time		early	early	medium	medium	medium	early	late	medium	early	late	
Distance to marker tree m.		5	19	10	12	10	19	8	7	12	22	

* = Ratio of the number of seeds with the marker allele to the total number of investigated seeds.

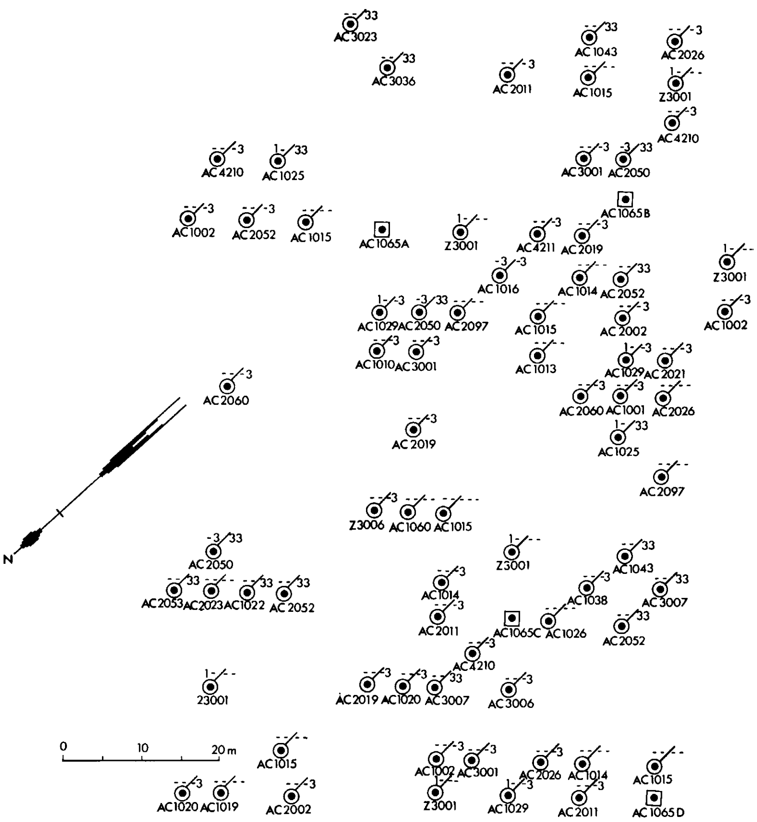

Figure 1. — The general view of the older parts ot the Östteg seed orchard and the isozyme type of some ramets planted in block I. This block is situated in the SW corner of the older part of the seed orchard. The arabic numerals in front of the slanted lines denote the alleles of LAP B locus, and after the lines — those of the GOT B locus. The bar indicates allele 2 in both loci.

considerable open areas within it. The old part of the seed orchard is divided into seven blocks. The seeds were derived from block 1. The distribution of some ramets in this block is shown in *Figure 1*.

To facilitate the choice of a suitable marker clone, a survey of LAP (leucine aminopeptidases) and GOT (glutamate oxalacetate transaminases) zymograms of all 51 clones was carried out by analysing 7 endosperms of the seeds from every clone. This means that the probability that one of the two alleles in a heterozygote is not detected is $1/2^6 = 0.016$. By this means the AC 1065 clone was found to be suitable because it has a GOT A_1 band which is unique for this seed orchard.

The LAP-B and GOT-B genotype of some ramets in block I are shown in *Figure 1*.

The AC 1065 clone has a somewhat smaller diameter than average. It is of average height and pollen production does not seem to be below average. It has red male flowers. There were four ramets of the clone in block I.

AC 1065 A has no close competing neighbours and has at least 10 m of space in all directions.

AC 1065 B seems to be a little suppressed by its closest neighbours. It is a somewhat below average pollen producer.

AC 1065 C is growing at a distance of 5 m from its closest neighbour AC 1026. The crown is thin but tall.

AC 1065 D is located at the north-western boundary of the seed orchard and looks rather weak. It was not included in the study.

The cones were collected from 12 sectors of a crown separately. Four directions and three levels were used for this purpose. There were only a few or even no cones to be collected from some of the sectors. In order to study the contribution of pollen from the AC 1065 C marker ramet to neighbouring grafts, cones were collected from two sectors, one facing the marker tree and the other facing away from it. Seeds from the marker ramets were extracted from some cones separately, from others only from sectors separately. Where available at least 30 seeds from every sector were analysed.

For the separation of the LAP and GOT isozyme, embryos and endosperms were homogenized separately and starch gel electrophoresis in a modified discontinuous buffer system was performed (Ashton and Braden 1961). Patterns of inheritance were checked by Rudin, D. (1975, 1976) and 1:1 segregation of alleles to endosperms were checked in this study.

The LAP-A locus contains the alleles A_1 and A_2, the LAP-B locus the alleles B_1, B_2 and B_3, the GOT-A locus, the alleles A_1 and A_2 and, as we have discovered the GOT-B locus the alleles B_{18}, B_2, B_{22} and B_3. It was rather difficult to distinguish between the band positions for B_{18}, B_2 and B_{22} because they were very close to one another. In this situation, rather than running the risk of getting unreliable results and even though we thereby lose some information, the B_{18}, B_2 and B_{22} bands were pooled into one group called B_2.

The data concerning the time of flowering were provided by the Northern District Research Institute for Tree Improvement at Sävar, Sweden. In their study the development of male and female strobili was divided into four phases including the pollen shedding and receptive phases. 5—6 ramets for every clone in the seed orchard were checked for strobili development at intervals of 4 days during the time of flowering.

The meterological material was provided by the Swedish Weather Forecasting Agency. The measurements were carried out at a station at Umeå Airport 500 m from the seed orchard.

Results and Discussion

1. Genotype frequencies of embryos from different sectors of the crowns

The distribution of genotypes from seeds derived from different sectors of the crowns of three ramets of AC 1065 are presented in *Table 1* and *Figure 2*.

The data show that the genotype frequencies of embryos vary greatly among these ramets. The difference in the genotype frequencies is highly significant. The chi square value of differences among the ramets was calculated to be 28.91 (8 df = 21.96 $p < 0.995$). The eight classes with low frequencies were pooled in this analysis.

The fluctuation of the frequencies in some sectors within one ramet, particularly for rare genotypes, seems to be fairly great, although it was not statistically significant.

It is interesting to note that many cones, particularly those which contain a large number of seeds (17—19 seeds), cover 6-7 types.

2. Segregation of GOT A_1 and $-A_2$ Alleles in the Macrogametophytes of the marker clone

There are several factors which cause the diversity of genotypes. One of them is segregation of the mother tree. In our experiment the marker clone AC 1065 was heterozygous in the GOT-A locus (A_1, A_2). According to the analysis of a total of 841 seeds, 399 seeds belong to A_1 and 422 to A_2. The chi square test of 1:1 segregation gave a value of 2.10^{ns}. This indicates that there seems not to be any lethal factors linked to these alleles. However, in some cases the ratios of A_1 and A_2 for different samples derived from single cones or sectors of crowns are insignificantly deviating from 1:1 segregation.

The macrogametophytes (endosperms) from a total of 79 cones were analysed separately. There were two cases in which the ratio of GOT $A_1:A_2$ was 0 to 7, and in one case it was 1 to 9. Within one sector even a ratio of 13 to 27 was found.

In some cases the uneven segregation to macrogametophytes in a single cone or even in a sector caused a diversity of the frequencies. For example, in sectors S_t and S_m of AC 1065 A, the frequencies of the genotypes LAP- B_2B_2, GOT- A_2A_2, B_2B_2 and LAP- B_2B_2, GOT-A_1A_2, B_2B_2 are somewhat different, but if all the corresponding data are added together no significant differences are found (see *Tab. 1*). To sum up, no statistically significant deviations from the expected 1:1 Mendelian segregation could be established.

3. Selfing in the marker clone

When analysing embryos and macrogametophytes from the same seeds the genotype of the fertilizing pollen can be determined. As there is only one clone carrying the GOT A_1 allele and as this allele is generally rare outside the seed orchard, it may be concluded that almost all cases, when you are picking cones on the marker clone where the father is GOT A_1 this must be due to selfing. As AC 1065 is heterozygous GOT A_1/A_2, 50 per cent of the selfings will pass undetected. Thus, to get the frequency of selfing, the frequency of embryos containing the allele GOT A_1 from the father has to be doubled.

The frequency of GOT A_1 in different sectors and in different ramets is summarized in Fig. 3. When analysing the data it ought to be kept in mind that pollination from other ramets of the same clone may have been a contributory factor. There were four AC 1065 ramets in block 1.

As AC 1065 is a normal pollen producer, a contribution of around 6 per cent to the general pollen cloud seems to be possible. This is in agreement with our observations (6.8 %) mentioned in *Table 2*.

There is a significant difference between overall averages of levels ($\chi^2 = 7.58$, 2 df, $0.99 > p > 0.975$). The lowest values were found at the top of the trees. This is in agreement with other investigations (cf RUDIN and LINDGREN, and others). It is also logical, because there are few male flowers at the top of the tree and since the wind can easily blow

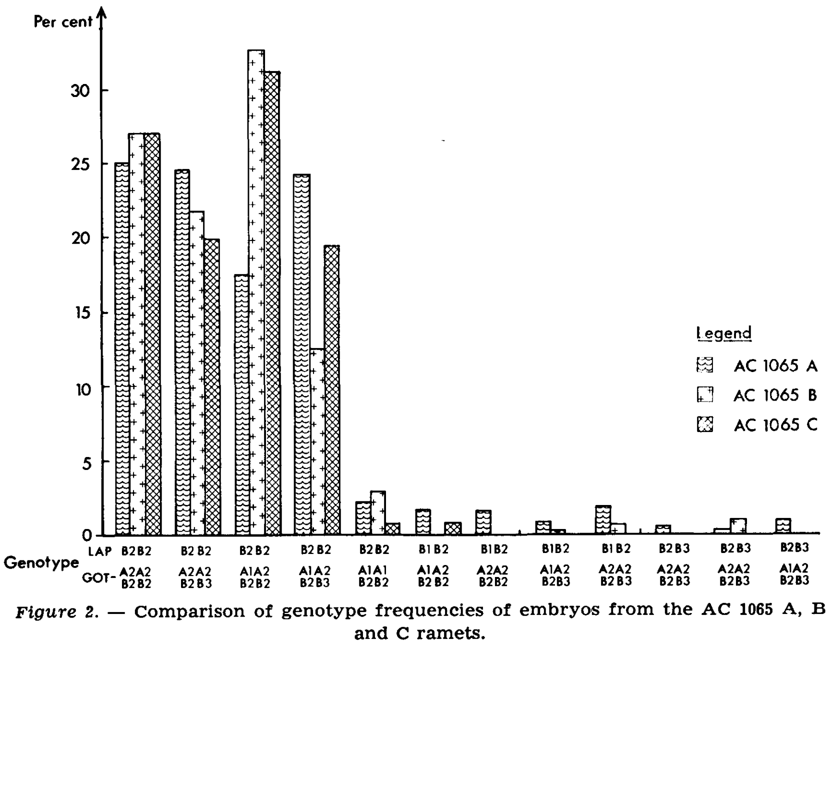

Figure 2. — Comparison of genotype frequencies of embryos from the AC 1065 A, B and C ramets.

away selfing pollen there. There seems to be more selfing in the middle than at the bottom of the grafts which is somewhat surprising. The reason for this might be:

- statistical fluctuations
- in AC 1065 there are as many male flowers in the middle of the graft as at the bottom, thus there is no reason to expect a higher degree of selfing at the bottom
- the distance between the cones and the trunk of the tree may be larger at the bottom than in the middle. Thus, the lowest cones may be situated more periferally
- during the time of pollen dispersal upward air streams may occur in a seed orchard on a sunny day

There seem to be striking differences in spontaneous selfing frequencies, depending on direction, in individual ramets. In the AC 1065 A ramet most incidences of selfing took place in the southerly direction and in the B ramet to the South and the East (see *Fig. 3)*.

When fertilizing pollen from all three ramets was pooled, no significant difference was noted between the four directions but a good indication on differences. The high frequencies of GOT-A_1 are found to the direction S-E, which is no clear cut correlation with the prevailing winds during the whole average flowering period. The marker clone, however, is early flowering and during this period the impact from northern winds is more heavy than later which coincides with results.

Furthermore, the frequencies of self-pollinated seed (fertilized by GOT A_1 pollen) derived separately from a total of 75 cones, which were chosen at random, from all sectors were investigated. There were no selfed seeds in 61 of 75 cones, One selfed seed was found in each of 9 cones containing 2 to 15 seeds. Only in one cone, which contained 19 seeds were two selfed seeds detected.

The bionomial probability of getting 2 or more selfed seeds in a cone containing 19 seeds is 7.6 per cent when the average percentage of embryos carrying the GOT A_1 allele from pollen is 2.4 per cent. Thus, there does not seem to be any tendency for the selfed seed to "cluster" in certain

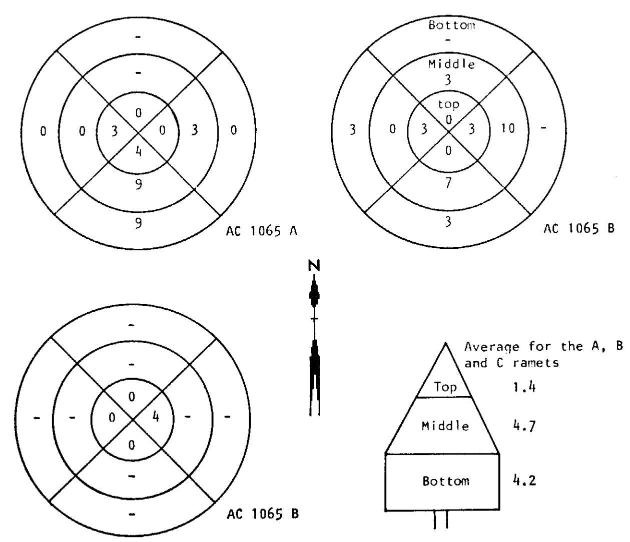

Figure 3. — Percentual distribution of GOT A_1 in different sectors of three ramets of AC 1065. The selfing frequency is obtained by doubling the indicated values (see text). For instance, selfing frequencies on average for the top, middle and bottom of three ramets are 2.9, 9.4 and 8.5 per cent respectively.

cones. This might be explained by the fact that all embryos of a cone are not receptive at the same time.

4. The outcrossing of the marker clone

In *Figure 4* the pattern of LAP B and GOT B in the different sectors is represented. When percentages were cal-

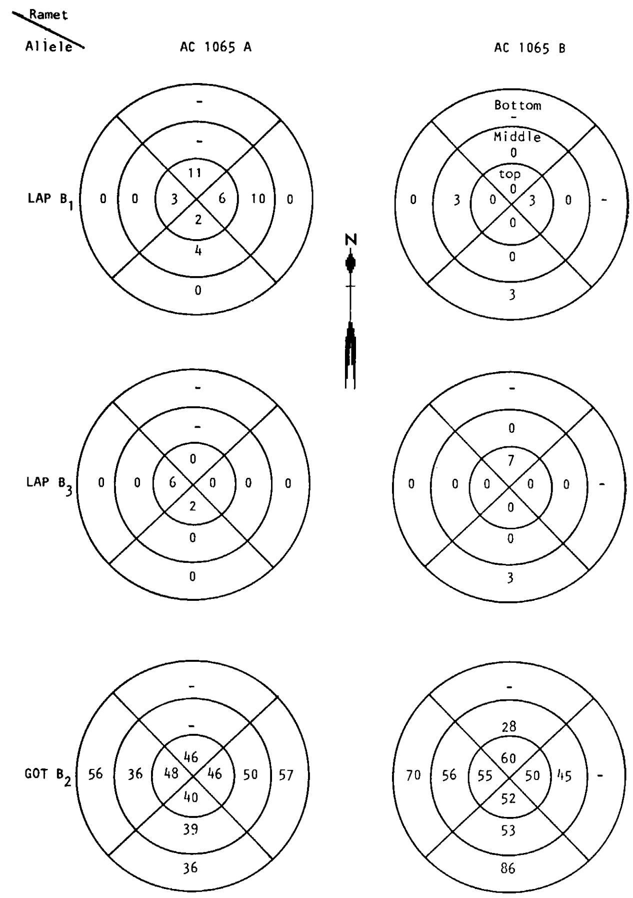

Figure 4. — Percentual distribution of three alleles in different sectors.

culated the selfing pollen carrying the GOT A_1 allele was excluded, while selfing pollen carrying GOT A_2 remained. As AC 1065 C is represented only by the top level and rather few seeds, it is not included in *Figure 4*.

If all fertilizing pollen in the different sectors is pooled, there are significant differences among the tree ramets for both LAP B (on the 5 % level) and for GOT B (on the 0.001% level).

No overall differences between levels or directions could be verified. However, there seems to be a clearly recognizable pattern within ramets *(Fig. 4)*. This is logical because each ramet has different neighbours.

In AC 1065 A there is a pattern of high frequencies of LAP B_1 at the top towards the North-East and LAP B_3 in the South-West part of the top. The high incidence of LAP B_1 in ramet A is logical as two of its three closest neighbours carry this allele (cf. *Fig. 1)*. But this raises two questions, namely, why is the incidence high at the top and low in the western sectors. The pollen may have originated from the sixth closest neighbours, of which AC 1025 plays an important role. There are also two LAP B_3 sources among the 7 closest neighbours.

For AC 1065 B the pattern of fertilization is rather irregular. AC 1065 C has five seeds carrying LAP B_1 and two carrying LAP B_3 alleles out of a total of 208 seeds.

As was the case with self-pollination the data is not enough to show any precise tendency for pollen dispersal during the flowering time. In general, the LAP B_1 and LAP B_3 alleles detected in the analysed seeds seem to be fewer than would be expected.

5. *Influence of pollen from the marker ramets on the pollination of neighbouring grafts*

In order to study the dispersal of the pollen from the marker ramets and its influence on the neighbouring grafts, AC 1065 C was chosen. Ten grafts surrounding this ramet at different distances and in various directions were selected (see *Fig. 5*). The data concerning frequencies

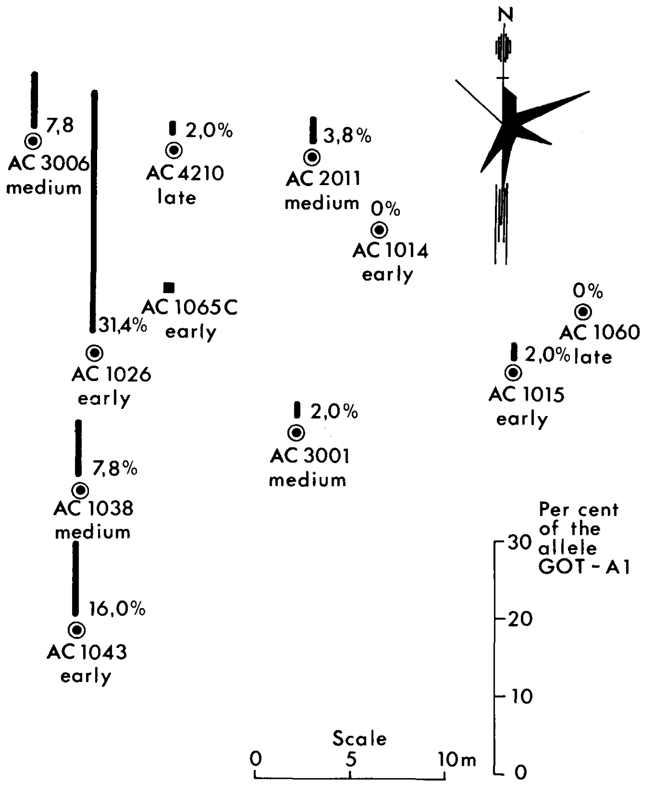

Figure 5. — Observed contribution of the marker GOT A_1 from AC 1065 C in the open pollinated seed from neighbouring grafts. The relative time of flowering is also indicated. On the North arrow, a diagram of a combined expression of temperature and directions from which the wind blows is shown (see text).

of GOT A_1 in neighbouring grafts, time of flowering, distance from the marker ramet and wind direction are summarized in *Table 2*.

The results show that, according to the frequencies of GOT A_1, the neighbouring grafts can be roughly divided into three groups *i.e.* (1) Ac 1026, 31.4% (2) AC 1023, AC 3006, AC 1036, 16-7.6%; (3) AC 2011, AC 4210, AC 1015, AC 1040, AC 1060, Z 3001, 3.8-0%.

The results also indicate that in most cases the percentage of seeds with the marker allele derived from the side facing the marker tree is higher than from the corresponding opposite side. The mean occurrence of the former for ten grafts is 1.43 times the latter, although the difference is not significant.

The pattern of pollination within a seed orchard depends mainly on the coincidence the abundance, the female and male flowering, temperature and moisture in the air, together with the wind directions and velocities.

With respect to the time of flowering, the pollen shedding of all clones in the seed orchard was fairly short, lasting only about one week. The evident demarcation line for the time of pollen shedding among clones was not found because of the rather long interval between observations. But the receptive phase of female stroboli was longer and continued for about two weeks. The discrepancy in receptive time between early and late flowering clones was somewhat more than a week. Thus, the grafts of different clones can easily be divided into three groups, *i.e.* early, medium and late. The time of pollen shedding for AC 1065 coincided with the receptiveness of the early and medium groups.

The time and output of pollen also depends strongly on the temperature during the pollen shedding period. In order to explain the pattern of dispersal of pollen from the marker clone the pollen dispersal diagram on the arrow found in *Figure 5* was constructed. It is based on data concerning directions, velocity and temperature. Registrations were made four times per day at 01, 07, 13 and 19 hours. From these data the daily mean temperature and the wind directions and velocities at 13 and 19 hours were chosen because these data seem to have the most pronounced impact on pollen dispersal. The diagram in *Figure 5* was built up by means of a summation of wind velocity and corresponding temperature from the 1 st to 15th of June 1978. The maximum temperature measured during this period was 22.9° C. Therefore, no correction for overoptimal temperature for pollen production is necessary. From the diagram in *Figure 5* there seems to be a correspondence between a "line" of trees with a high capture of marker pollen and directions of the prevailing winds.

It is reasonable that AC 1026 should have the highest ratio because the pollen impact from AC 1065 C on this ramet is heavy. The grafts of the second group coincide with the flowering time of AC 1065 too. They are located to the west of the marker ramet at distances of 10—19 m. All grafts of the third group are located up-wind (prevailing winds) from the marker ramet at distances of 7—22 m.

The discrepancy in the time excluded the possibility of pollination of AC 4210 from AC 1065 C, even though conditions were otherwise favourable. On the other hand, conditions were favourable for AC 1014 as regards flowering time and distance, but not as regards location, in relation to prevailing wind direction, and therefore the ratio became lower than expected.

It is questionable whether the neighbouring grafts were pollinated only by AC 1065 C or by any other ramet of this clone. In general it is true that every graft in a seed orchard is under the influence of many ramets of the same clone. However, in this case the importance of the other ramets may be considered to be limited due to their distance , and to the fact that the wind was blowing so that AC 1065 could hardly spread pollen from other sources than AC 1065 D which is a small ramet with a limited pollen production. There are the impact of other ramets of AC 1065 to the observed results shown in *Figure 5* is negligible.

General Discussion

There are two questions which need to be discussed on the basis of the above mentioned results. One concerns the diversity of genotypes of seeds originating from different ramets and from various sectors of a crown within a ramet of the same clone. The other is the question of net effect of pollen dispersal in the seed orchard.

First, our investigation indicates that the seeds originating from different ramets and from various sectors of a crown within a ramet of the same clone show a different genotype composition. Differences between ramets are significant but for sectors the level of significance is not always reached.

It might be of interest to point out that in our investigation of seed weight variation (unpublished), using the same material as in this study, the average weights of seeds derived from AC 1065 A and B differ significantly from one another. The former is 6.67 ± 0.56 mg, (mean ± standard deviation) while the latter is 5.18 ± 0.64 mg. The seeds from AC 1065 C have an intermediate value, 6.15 ± 0.58 mg. The average weight of seeds derived from different sectors also shows significant differences in some cases.

The results indicate that the fertilizing pollen varies between different sectors, and selfing seems to be more common at low levels. Therefore sampling of open pollinated seeds for progeny testing does not seem to be satisfactory at the level of "breast height" and from only one sector of the crown. Open pollinated seeds for progeny testing purposes should be collected from several ramets, several sides of the ramet and preferably from the top of the tree. In practice it is common to leave cones at the top of the trees if there are a sufficient number of cones in the more accessible lower part of the crown. From a genetical point of view this may mean a loss of wood production capacity, as the degree of selfing is lowest at the top of the tree.

The second question to discuss is the net effect of pollen dispersal from and to one marker clone. As pointed out in the section 5 in this paper if a mother tree is located up-wind from the pollen-tree, even at a distance of 10—15 m, the contribution of the pollen tree was limited (see AC 2011, AC 1014 in *Fig. 5)*. The following observations illustrate this question. There are grafts carrying the LAP B_1 allele which surround AC 1065 A. These are AC 1025 on the east side and AC 1029 on the west side. The distances from these grafts to AC 1065 A are 18 and 13 m, respectively. The times of flowering coincide with one another. The frequencies of LAP B_1 in seeds derived from the northern, eastern and southern sectors were higher than from the western sector. They are 6.3, 9.8, 2.4 and 1.5 per cent, respectively.

The fact that the frequencies of LAP B_1 for seeds from the North, East and South are higher than from the West is probably connected with the prevailing wind direction during the time of flowering.

The frequencies of LAP B_1 in the AC 1065 A ramet are higher than in the other two ramets. They are 6.1 and 2.5 times those of the B and C ramets, respectively. The reason for this may be the fact that LAP B_1 sources situated upwind from AC 1065 B are not as close as they are to AC 1065 A. For instance, the distance from Z 3001 carrying LAP B_1 on the North-East side is 33 m. Since Z 3001 grows only 10 m to the East. The frequency of LAP B_1 in AC 1065 C is somewhat lower than expected.

Furthermore, three ramets of AC 2050 and AC 1016 carrying the LAP B_3 allele are to be found to the East and South East of AC 1065 at distances of 40, 46 and 49 m. This means that AC 1065 C is located downwind of these grafts. In addition, the flowering time for these clones is rather early, and thus coincides with the receptive phase of AC 1065. Only about 0.5 percent of embryos with LAP B_3 allele corresponding to one per cent of the pollination was found. However, it was previously noted that the frequencies of LAP B_3 in the AC 1065 A and B ramets are also lower than might be expected from the clonal composition of the seed orchard. It can be stated that pollen sources situated at a distance of 40 m or more may play a role in pollination. If half of the pollen originated from greater distances than 40 m, it does not seem probable that 2 out of 51 clones would make such a small contribution as 1 per cent of the pollinations, as was found in this case. Therefore it seems that most of the pollen comes from neighbours at a short distance. JONSSON *et al* (1976) found that the flowering intensity is the main factor which influence the genotype distribution in a seed orchard. Of course the true situation and natural mechanism cannot be completely elucidated with only one marker clone available therefore we are working hard to find more marker clones in this seed orchard.

Authors who have discussed pollen dispersal including selfing frequencies in seed orchards are e.g. HADDERS (1971), KOSKI (1975) and MÜLLER-STARK (1978). KOSKI states that the pollen dispersal and selfing frequencies within a seed orchard are dependent on such factors as the total and clonal pollen production within and outside a seed orchard and the coincidence in time of male and female flowering within and outside a seed orchard. He states that above 7 m of height and a breast-height diameter of 16 cm you will have a sufficient amount of pollen to get a good pollination of pollen coming from the orchard itself.

In the studied seed orchard the diameter mentioned has been mostly reached but the average height is not yet 7 meters. The seed orchard, however, is well isolated with at least 2 km to the nearest Scots pine stand. Therefore the impact of any invading rare alleles might be expected to be small.

The intensity of male flowering was analysed by HADDERS (1971) in relation to empty seed frequencies. No correlation was found in the top of the ramets but at the lower parts it was obviously positively correlated. This indicates that you might find a certain amount of selfing in the lower parts of the male flowering clones. The same tendency was found in the same material by RUDIN and LINDGREN (1977) by means of isozyme gene markers. These results are in accordance with the results of the present study.

The frequency of viable inbred seeds from seed orchards, however, varies very much between different authors from less than one per cent (JOHNSSON 1972) to 12—14% (MÜLLER-STARCK 1978). As was mentioned above our mean value was estimated at 6% which is intermediate to above mentioned figures.

The reasons for these discrepancies between different investigations are probably connected to such factors as the design of the seed orchard,the number of clones and lethal factors in actual clones, the level of cone-collection, the shape of the crowns of the ramets, the intensity and coincidence of male and female flowering and the turbulence within the seed orchard. That a co-operation of all these varying factors will result in different levels of selfing frequencies is obvious.

Concluding remarks

1) The gene frequencies of seed originating from different ramets of the same clone differ. Considerable variations within different sectors of a crown can also be detected in some cases. It seems to be difficult to expect random mating in the seed orchard. The diversity in the gene frequencies is not much due to the segregation of the mother trees but to the various composition of neighbours.

2) The dispersal of pollen is mainly depending on the coincidence of the time of flowering, the distance between parent trees and the wind direction during pollen shedding. The neighbour to the marker ramet, with all mentioned conditions favourable, received 31.4 per cent of fertilizing pollen to that ramet. It seems, as if in the prevailing wind-direction trees within 10 m contributed considerably to the fertilizations, trees at a distance of 10—20 m to a large extent, but less than trees within 10 m. Trees at a distance of more than 40 m and up-wind only make a small contribution.

3) The frequency of self-pollinated seeds originating from one clone in the Scots pine seed orchard was roughly estimated at 6 per cent with the lowest frequency in the top of crown. This points on the risks of mainly picking cones in the lower parts of the crowns both on practical and scientifical purposes.

Acknowledgements

We wish to express our thanks to Miss G. Lindkvist and M.. L. G. Lejdebro for their assistance and friendly cooperation during the work in the laboratory, to Miss H. Risberg for kindly typing the manuscript and to M. A. Dunberg for valuable discussions. For the correction of our English we are indepted to Miss K. Ryde and Mr. N. Shrimpton. We are also grateful to the Northern District of the Institute for Forest Improvement and the Swedish Weather Forecasting Agency for supplying us with relevant data.

References

Ashton, G. C. and Braden, A. W. H.: Serum β-globlin polymorphism in mice. Austr. J. Biol. Sci. 14: 248—253 (1961). — Dixon, W. J. and Massey, F. J.: Introduction to statistical analysis (1969). — Gregorius, H. R. and Müller, G.: Genetic structure in finite, open-pollinated plant populations. Theor. and Appl. genetics 46, 295—305 (1975). — Hadders, G.: Pollineringssituationen i tallplantager. (The situation of pollination in seed orchards of Scots pine (*Pinus sylvestris* L.). With English summary). — Fören Skogsträdsförädl. Inst. Skogsförbättr Arsbok 1971, 111—139 (1971). — Johnsson, H.: Självpollineringsfrekvensen i en tallfröplantage (The frequency of self-fertilization in a pine seed orchard). (Prelim Rep in mineo) (1972). — Jonsson, A., Ekberg, I. and Eriksson, G.: Flowering in seed orchard of *Pinus sylvestris* L. Studia Forestalia Suecica No. 135. 38 p. (1976). — Koski, V.: Natural pollination in seed orchards with special reference to pines p 83—91 in Seed orchards ed R. Faulkner. Forestry Commission Bulletin 54 (1975). — Müller, G.: A simple method of estimating rates of self-fertilization by analysing isozyme in tree seeds. Silv Gen 25, 15—17 (1976). — Müller-Starck, G.: Estimates of self- and cross-fertilization in a Scots Pine seed orchard. Proceedings of the Conference on Biochemical Genetics of Forest Trees, Umea, Sweden 1978: 170—179 (1979). — Rudin, D.: Inheritance of glutamate-oxalate-transaminases from needles and endosperms of *Pinus sylvestris*. Hereditas 80: 296—300 (1975). — Rudin, D.: Leucine-amino-peptidases from needles and macrogametophytes of *Pinus sylvestris* L. Hereditas 85, 219—226 (1977). — Rudin, and Lindgren: Isozyme studies in seed orchards. Studia Forestalia Suecica No 139, 23 p. (1977).

Inbreeding depression in selfs of redwood

By W. J. Libby, B. G. McCutchan and C. I. Millar

Departments of Genetics, and of Forestry & Resource Management,
University of California, Berkeley, Calif. 94720, USA.

(Received August /November 1980)

Summary

Given the polyploid chromosome constitution of *Sequoia sempervirens*, there was reason to question whether it would exhibit inbreeding depression. Preliminary results from studies of self and related outcross families are reported as a guide to the selection of trees for redwood seed-orchards and breeding-orchards. The data indicate that, compared to outcrosses, selfing produced no additional cone abortion, no consistent effect on number of seeds per cone, and variable effects on germination. Consequently, the relative proportions of inbred and outcrossed offspring produced are normally maintained.

Under good nursery conditions, survival of selfs and outcrosses was both high and similar. Under stress nursery conditions, survival was lower for both, but selfs had a much lower survival rate than outcrosses. The selfs were consistently 65—80% the height of the outcrosses in the nursery, and also after one year in the field. Then inbreeding depression appears to become much more severe. After fourteen years in the field, in the single family available, selfs averaged only 42% the height and 29% the diameter of related outcrosses. It thus appears prudent to restrict inbreeding in redwood seed-orchards.

Key words: Cone abortion, Diameter, Germination, Height, Nursery practice, Polyploidy, Seed-orchards, *Sequoia sempervirens*, Stress, Survival.

Résumé

Etant donné la constitution chromosomique polyploïde de *Séquoia sempervirens* on pouvait se demander si l'autofécondation chez cette espèce s'accompagne de perte de vigueur. Les résultats préliminaires des études de descendances dérivant d'autofécondations ou de sujets apparentés, sont rapportés pour fournier un guide pour le choix des arbres à placer en vergers à graines ou parcelles d'amélioration génétique. Les faits montrent qu'en comparaison à des croisements non apparentés, l'autofécondation ne provoque pas d'excès d'avortement de cônes et n'a pas d'effet significatif sur le nombre de graines par cônes. Les effets sur la germination sont variables. Il résulte de ceci que les proportions relatives entre descendants consanguins et non apparentés on pu être normalement maintenues.