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A CLONE BANK OF DOUGLAS FIR

by

A.L.Orr-Ewing

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ABSTRACT

In 1956 a program was initiated by the B.C. Forest Service with the objective of selecting Douglas-fir for grafting in seed orchards. A clone bank was also established at Lake Cowichan for both conserving the germplasm of selected trees and for crossing. This paper describes the development of the clone bank with particular reference to those clones grafted between 1958 and 1963 and which have already been used in crosses. In general, the production of female and male strobili has been extremely variable both between clones and between the ramets of individual clones. There is a definite tendency, however, for higher production of male rather than female strobili. Delayed graft failure from the overgrowth of scion to rootstock is already causing considerable damage in the clone bank and, at present, only temporary measures have been developed for alleviating it. Crosses were made on the more productive clones in 1966 and 1968. Frost and high winds were the main factors responsible for the variable loss of strobili during the pollination period. The number of seeds per cone varied from 36.6 to 72.1 in the 16 clones used in 1968.

A study in which both clonal and half-sib progenies from 12 trees were grown side by side in four replicated blocks showed that at 10 years from establishment, the latter were producing more female strobili. Survival was also much higher, mortality among the clones being largely due to overgrowth. Further losses are anticipated. The results confirm the opinion that the establishment of clonal seed orchards is not to be recommended at this time. It is further suggested that those already established should be converted to seedling seed orchards where survival will be higher and seed production should be at least as good and probably better.

INTRODUCTION

In 1956 a program was initiated by the B.C. Forest Service with the objective of selecting Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, for grafting in seed orchards. In addition scions from the selected plus trees were grafted in 1958 in order to establish a clone* bank at Lake Cowichan in southern Vancouver Island. The objectives were to both conserve the germplasm** of the selected trees and for crossing the clones once enough female and male strobili*** were being produced. Early and abundant production of strobili together with high survival of the clones themselves are necessary conditions for a seed orchard and breeding program. The results from the clone bank at Cowichan Lake, however, have already shown that such conditions are by no means as easily fulfilled as was at first anticipated. The information has been mainly obtained from those clones grafted between 1958 and 1963 and, in particular, with those used in the 1966 and 1968 crossing program.

* Clone. A group of plants derived from a single individual (ortet) by asexual reproduction (Snyder, 1959).

** Germplasm. The material that is the physical basis for inheritance (Snyder, 1959).

*** Strobilus. The male or female inflorescence (cone) of a conifer (pl strobili), (Wright, 1962).

THE CLONE BANK

1. Strobili Production

It was clearly desirable that a crossing program should be initiated in the clone bank at the earliest opportunity so attempts were made to induce the production of strobili. In April 1965 and again in 1967 ammonium nitrate was applied at the rate of 60, 120 or 180 grammes per ramet* on the 74 clones grafted between 1958 and 1963. The strength of fertiliser used depended on the size and vigour of the ramet. These treatments were only partially successful, in 1966, for example, 12 of the 74 clones produced sufficient female strobili for pollinations to be made and then only when ramets from other clone banks were added. Two clones only at Lake Cowichan produced more than 100 female strobili, the number considered necessary if two or more crosses were to be made.

The results were more satisfactory in 1968 as 15 of the clones had more than 100 female strobili. In both 1966 and 1968, however, the production of male strobili was much the heavier. This is of interest as both Arnborg et al (1957) and Fielding (1964) working with Scots and Monterey pine respectively found that more female than male strobili were being produced at eight and ten years. The scarcity of female strobili is certainly the more serious with Douglas-fir as sufficient pollen can still be collected from the selected trees themselves, the majority of which are not yet logged. On the other hand, controlled pollinations on these trees would be extremely difficult and quite impractical.

Production by ramets of the 23 clones used for the 1966 and 1968 crosses is shown in Table 1. In 1968, the female strobili were all counted in the spring whilst the production of male strobili was assessed as either scattered when no pollen collection was warranted or as adequate when the quantity was sufficient. Details of the ages and elevations of the ortets** themselves are also shown, all were located on Vancouver Island with the exception of No. 53 which is on the lower mainland. The clones have all been ranked by year of grafting.

The figures in the table on the production of strobili raise several points. Firstly, there is no question that at Lake Cowichan the clones of some ortets are much more productive than others. This, moreover, appears to be unrelated to either the age and elevation of the ortet or to the number of years since grafting. Ortet numbers 35, 98, 102 and 160, for example, were all under 63 years of age, their elevations ranged from 550 to 3,300 feet and the clones were only grafted in 1962.

* Ramet. An individual member of a clone (Snyder, 1959).

** Ortet. The original ancestor of a vegetatively propagated clone (Wright, 1962).

The respective ramets were producing both female and male strobili in 1966, just four years after grafting and by 1968 all of them were productive. In other clones such as No. 25 however, only four of the 21 ramets were producing female strobili.

Secondly, there is much variation in production between the ramets of a single clone, the 29 ramets of No. 28 for example were extremely variable and 11 of them have yet to produce a female strobilus. There is little doubt that a great deal more research is needed on the use of fertilisers for inducing production of strobili. At the present time, applications of ammonium nitrate have been singularly ineffective on these unproductive ramets. The complete lack of information as to how soon a clone will become productive in a particular clone bank and whether all or just a few of the ramets can be used for subsequent crossing emphasises the necessity of establishing at least 15 ramets per clone. Careful records on production by ramets must also be kept. Nilsson et al (1967) have pointed out that wide spacing is extremely important for optimum production in a clone bank. Some thinning, therefore, is inevitable once the ramets develop and it is vital to know which are the more productive otherwise they may be unwittingly removed.

TABLE 1

STROBILI PRODUCTION OF THE TWENTY THREE CLONES 1968

Ortet No.	Age in Yrs.*	Elev: in ft.	Years of Grafting	No. of Ramets	No. of Ramets by Female Strobili Classes				No. of Ramets By Male Strobili Classes		
					0	1-50	51-100	101+	0	Scattered	Adequate
33	104	1,900	1958, 60	4	2	1		1		3	1
28	45	2,250	1958,60,61	29	11	16	1	1	7	8	14
37	50	1,900	1959,60,61	13	8	5			5	6	2
25	58	2,300	1959,60,61	21	17	4			1	15	5
36	46	1,600	1959,60,63	23	19	4			13	8	2
47	75	2,000	1960,61	18	13	5			11	5	2
55	130	900	1960,61	12	7	5			6	4	2
60	61	100	1961	12	7	4		1	10		2
62	56	200	1961	18	11	6		1	4	7	7
79	147	800	1961	6	2	2	2		1	1	4
53	75	1,200	1961,63	4	3			1	3		1
69	140	1,200	1961,63	8	7	1			1	5	2
35	62	2,800	1962	6		5	1				6
86	64	2,850	1962	5	1	4					5
87	64	2,850	1962	4	1	3			1		3
93	84	1,850	1962	8	5	2		1	4		4
98	58	550	1962	3		1	2				3
102	56	700	1962	4		2		2		2	2
105	61	700	1962	2		1		1	1	1	
107	62	700	1962	3		1		2		1	2
109	64	700	1962	1		1					1
151	83	1,300	1962	3		2	1		1	2	
160	60	3,300	1962	6		3	3			1	5

* Age when first propagated.

2. Survival

The 23 clones used for the 1966 and 1968 crosses had all been grafted by the methods outlined by Orr-Ewing et al (1959). The origin of the rootstock was known but the genetic constitution of course would vary widely. Survival of these clones since planting is shown in Table 2. There have been heavy losses in some clones such as Nos. 53, 87, 93, and 109. This has been largely caused by incompatibility between rootstock and scion. Graft failure from incompatibility in the Douglas-fir was first reported by Duffield et al (1964). They recognised two main types, graftability or failure to make an initial union and delayed graft failure where the scion grew more rapidly than the rootstock. This latter type, characterised by the typical overgrowth of scion to rootstock, is already widespread in the clone bank and was briefly reported by Heaman (1959). At present, the only method used for controlling incipient overgrowth is the cutting technique developed by Karlsson (1966). Unfortunately, this is a temporary measure only and repeated cutting has been necessary on some clones. As can be seen from Table 2, already 188 of the 213 ramets involved have had to be cut at least once for overgrowth.

There is no question that this is a serious problem and will occur irrespective of grafting technique. It may take from 10 to 12 years to develop and an apparently healthy ramet can die in a matter of months. The ramets which have been cut, moreover, are often weak at the union between rootstock and scion and unless secured to stakes are readily broken by high wind and snow. There are numerous examples where cutting has been carried out too late and as a result the strobili produced are greatly reduced in size. Such ramets are never used in crosses. The specific cause of overgrowth in the Douglas-fir is little understood at this time but it is of interest that there are already examples in the clone bank of what might be termed "undergrowth" where the rootstock develops more rapidly than the scion. This condition has not resulted in any losses in the clone bank and would appear to be the normal development from grafting. Unfortunately, the incidence of overgrowth is much higher at the present time. The development of "undergrowth" has been well illustrated by Dimpflmeir (1954) in his photographs of 55 year-old interspecific grafts in Pine.

TABLE 2

SURVIVAL AND INCIDENCE OF OVERGROWTH IN THE TWENTY THREE CLONES 1968

Clone	No. of Ramets Planted	No. Alive in 1968	No. Cut by 1968
33	8	4	4
28	31	29	25
37	20	13	13
25	25	21	21
36	31	23	23
47	19	18	13
55	13	12	12
60	13	12	11
62	20	18	15
79	7	6	5
53	15	4	3
69	11	8	8
35	8	6	5
86	7	5	3
87	8	4	3
93	10	8	4
98	7	3	3
102	6	4	4
105	6	2	2
107	6	3	3
109	6	1	1
151	5	3	2
160	6	6	5

3. Cone and Seed Yields

The results to-date of the 1968 pollinations on 16 clones have been summarised in Table 3, the clones being ranked by the number of seeds per cone. Several points need emphasis. Firstly, the difficulty of pollinating sufficient strobili for any one cross to ensure that enough seed is produced is quite evident. The number of strobili which are bagged, unfortunately, do not all develop into cones. In certain clones such as Nos. 53, 87 and 93 the losses have been high. Such losses are mainly caused by low temperatures and high winds. In 1968, the strobili were first bagged (enclosed with pollination bags) from March 22nd. on as there is always considerable variability in development between different clones. The pollinations were started on April 16th. and completed by the 25th. During this period, the minimum temperatures registered at the weather station which is within a mile of the clone bank dropped to 29° and 32° F. on the 29th. and 30th. of March. In April, temperatures dropped to 31°, 27°, 31°, 31°, 32°, 31° F. on the 11th., 12th., 16th., 17th., 19th., 21st., and 22nd., considerable damage being caused by the low of 17°F. on the 11th. The strobili were either emerging or fully emerged from the bud scales at this time and were particularly susceptible. Losses from frost can be expected to vary from year to year but from past experience (Orr-Ewing, 1966) they can occur as late as May when the strobili have already developed into immature cones.

High winds can also cause considerable damage during the crucial pollination period. The clone bank is exposed on the north and west where it is bounded by Lake Cowichan and winds sweeping down from the northwest have even delayed bagging on occasions. The pollination bags themselves are very exposed and their violent motion in high winds can either result in the strobili being crushed against the sides or the branchlet itself may be broken off. In 1966 and in 1968, every bag was either secured to the ramet or to posts by lengths of string. This entails considerable extra work especially when many hundreds of bags are involved but it has certainly helped in reducing losses. The bags are removed once the scales on the developing cones have sealed and are replaced with much lighter ones made of dacron for protection against insects and squirrels. In 1968, the replacement of bags began on May 6th. and was completed on the 15th. Considerable maintenance is still required up to the time that the cones are collected as they become so heavy that even the ramet itself may have to be supported. It is evident from Table 3, however, that the major loss of strobili occurs in March and April. From past experience with crossing on both young trees and clones there is no question that the former are easier to work with as the branches need much less support.

Secondly, although the figures are only based on a single year, there was a surprisingly wide range of from 36.6 to 72.1 seeds per cone in the 16 clones as indicated in Table 3. No comparisons with the 1966 yields of seed from the five clones also used in 1968 have been made as ramets from other clone banks were used to increase the number of strobili. This introduces another factor particularly when there had been severe frost damage. It is interesting, however, to compare the 1966 yields of the ortet with those of the clone even though they are in different years. The footnote in Table 3 shows the number of seeds per cone from four of the ortets whose clones were used in the 1968 crossing program. It would be expected that the yields from protected cones in a clone bank should be higher than those of the ortet and this was the case with clones 35, 102 and particularly 160. It is somewhat surprising, however, to find that the yield from ortet No. 28 at an elevation of 2,250 feet is still higher than that of the clone at Cowichan.

TABLE 3

CONE AND SEED YIELDS 1968

Clone	No. of Ramets Used	No. of Strobili Bagged	No. of Strobili Pollinated	No. of Conelets At Bag Changing	No. of Cones Collected	No. of Seeds Per Cone
87	3	75	54	34	34	72.1
86	4	63	62	62	59	71.9
60	5	111	90	90	89	61.8
105	2	129	121	121	121	61.2
35	5	80	74	68	63	59.9 *
151	3	103	99	82	73	59.5
102	3	180	145	145	143	53.7 *
53	1	113	97	72	70	51.9
79	4	107	84	67	65	50.6
98	4	132	123	123	121	49.3
47	5	70	65	58	58	49.0
160	4	125	124	124	124	45.8 *
62	5	201	201	147	141	43.7
28	17	319	265	247	247	41.7 *
93	3	109	75	58	58	40.9
33	2	169	147	137	128	36.6

* In 1966, the number of seeds per cone from ortets 28, 35, 102 and 160 were 54.4, 52.2, 33.4 and 17.9 respectively.

STROBILI PRODUCTION ON CLONES AND PROGENIES

There have been numerous arguments among tree breeders regarding the respective advantages of clonal and seedling seed orchards. Two factors have to be considered with the Douglas-fir, production and survival. In regards to the former, Zobel et al (1964) have stated that the grafts of most species flower at an early age and that horticulturists cite the rule that a graft will flower in half the time required for a seedling. There have, however, been few opportunities to test this hypothesis with different species. Fortunately however such an opportunity was presented in an older study at Lake Cowichan. In brief, scions were grafted from 12 trees in 1956 and the ramets were planted in four replicated blocks in 1957. Cones from these trees were collected in 1954, the seed was sown the following year and in 1958 the half-sib* seedlings were established in the same four replicated blocks. Seedlings and ramets with a common ancestor, were thereby planted side by side in each block (Orr-Ewing, 1967).

No detailed checks were made before 1968 but more cones were observed on the half-sib progenies in both 1965 and 1966. In May, 1968, the number of female strobili were counted on the half-sib and clonal progenies whilst the production of male strobili was assessed using the same two classes described previously. Both survival and strobili production of the respective progenies are shown in Table 4. As regards the former, it is obvious that there has been far more mortality in the clonal than in the half-sib progenies. This has been largely caused by overgrowth and is still continuing. The comparisons of strobili production between the half-sib and clonal progenies certainly do not indicate that the latter are the more productive. On the otherhand, the results show that at 11 years from establishment the half-sib progenies are producing as large and in some cases larger numbers of female strobili than those of the clones planted one year earlier. There was again a tendency for the clones to produce more male than female strobili.

It was interesting to find that although two of the clones, namely Nos. 20 and 22, had produced no female strobili at all, the ortets which were growing less than 5 miles away had many hundreds of strobili. This certainly demonstrates the unreliability of cone production being used as a criterion in the selection of trees for clonal seed orchards.

* Half-sib. Seedlings with a known female parent. Male parent unknown.

TABLE 4

SURVIVAL AND STROBILI PRODUCTION ON THE HALF-SIB AND CLONAL PROGENIES 1968

Parent Tree	Origin of Progenies	No. Planted	No. Alive	No. of Half-Sibs and Ramets by Female Strobili Classes					No. of Half-Sibs and Ramets by Male Strobili Classes		
				0	1-50	51-150	151-250	251+	0	Scattered	Adequate
13	Half-sib	48	45	4	4	7	19	11	5	17	23
	Ramets	27	3		3						3
14	Half-sib	48	46	9	11	10	12	4	22	15	9
	Ramets	27	9	1	2	2	2	2	2		7
15	Half-sib	48	47	6	13	8	11	9	13	18	16
	Ramets	25	9		5	1	1	2	1	3	5
16	Half-sib	48	44	22	12	3	6	1	27	10	7
	Ramet	25	6	1	2	3				1	5
17	Half-sib	48	46	8	13	10	7	8	19	13	14
	Ramets	28	7	3	3	1			2	1	4
18	Half-sib	48	44	15	13	10	5	1	20	14	10
	Ramets	26	9	5	1		1	2	4	2	3
19	Half-sib	48	43	17	9	9	5	3	21	11	11
	Ramets	23	12	10	2				9	2	1
20	Half-sib	48	47	20	14	6	4	3	30	8	9
	Ramets	26	3	3					3		
21	Half-sib	48	47	12	15	12	6	2	22	15	10
	Ramets	26	4	2	1	1			2	1	1
22	Half-sib	17	16	7	3	4	1	1	6	4	6
	Ramets	27	14	14					8	4	2
23	Half-sib	48	42	13	12	9	3	5	16	21	5
	Ramets	21	3	1	2				1	2	
24	Half-sib	48	48	22	14	6	5	1	32	11	5
	Ramets	25	11	4	1	1	3	2	1	1	9

DISCUSSION

Although it has not been the main purpose of this paper to discuss the relative merits of clonal and seedling seed orchards for Douglas-fir, certain conclusions must be made. Duffield (1962) has previously pointed out that in the haste to establish seed orchards it has been virtually impossible to evaluate the periodicity of cone production of the selected plus trees. There is further the problem in determining how the seed and pollen production of a mature tree may be transformed when scions from it are grafted onto young rootstock. Two examples of this latter point have been given in the previous section. It is, therefore, inevitable that some unproductive trees have already been widely grafted in coastal seed orchards in B.C. By 1968, for example, 72 of the 109 ramets from five clones grafted between 1958 and 1961 at Lake Cowichan and known to be established in several seed orchards have yet to produce a single female strobilus.

There is little consolation either to be gained in the hopes that heavier production will follow once the clones are older. In 1968, for example, 55 of the 70 ramets used in the pollinations had already been cut at least once to alleviate incipient overgrowth. It is by no means improbable that some clones will die out completely before any pollinations are possible. It can only be concluded that when the problem of overgrowth is added to that of irregularity in early production the establishment of clonal seed orchards can hardly be recommended at this time. A method for detecting incompatibility in two year old grafts has indeed been discovered (Copes, 1967). These early tests however, require additional work time and more ortets for replacement if some of the original selections for the seed orchard prove incompatible. The decision has, therefore, to be made as to whether it might not be best to establish seedling seed orchards which have the advantages of high survival, strobili production at 10 years and much easier maintenance. Increased research in strobili production may indeed reduce the period from establishment to production. As regards clonal seed orchards which have already been established, it would certainly appear advisable that they be gradually replaced by seedling orchards.

REFERENCES

Arnborg, T. and G. Hadders, 1957. Studies of some forestry qualities in clones of Pinus silvestris. Medd. 87, Sällskapet praktisk skogsförädling. Uppsala, 125-157.

Copes, D.L. 1967. A simple method for detecting incompatibility in 2-year-old grafts of Douglas-fir. Res. Note, PNW-70; 1-8.

Dimpflmeier, R. 1954. Pfropfungen an Waldbäumen durch Professor Dr. Heinrich Mayr vor 55 bis 66 Jahren. Z. Forstgenet 3; 122-125.

Duffield, J.W., 1962. Forest tree improvement: old techniques and the new science of genetics. H.R. MacMillan Lectureship, Univ. British Columbia, 1-16.

Duffield, J.W. and J.G. Wheat, 1964. Graft failures in Douglas-fir. Jour. For. 62; 185-186.

Fielding, J.M., 1964. Notes on a Monterey pine seed orchard on Tallaganda State Forest in New South Wales, Austr. For. 28; 203-206.

Heaman, J.C., 1965. Differential growth of scion and rootstock in Douglas-fir. For. Res. Rev. B.C. For. Serv., 41-42.

Karlsson, I., 1966. A possible remedy for overgrowth in Douglas-fir. For. Res. Rev., B.C. For. Serv. 80.

Nilsson, B. and S. Wiman , 1967. Om Kottsättning påympar och ursprungstrad av gran. Fören. Skogsträdföradl. Årsbok, 59-68.

Orr-Ewing, A.L. and D.C. Prideaux, 1959. Grafting methods for the Douglas-fir. For. Chron. 35; 192-202.

Orr-Ewing, A.L., 1966. Frost damage to immature Douglas-fir cones. For. Res. Rev., B.C. For. Serv. 77-80.

Orr-Ewing, A.L., 1967. A progeny test of Douglas-fir to demonstrate the importance of selection in forest practice. Res. Note 43, B.C. For. Serv. 1-23.

Snyder, E.B., 1959. Glossary for forest tree improvement workers. South For. Expl. Stat. 22 p.

Wright, J.W., 1962. Genetics of forest tree improvement. Rome. F.A.O. For. and For. Prod. Stud: 16 399 p.

Zobel, B. and R.L. McElwee, 1964. Seed orchards for the production of genetically improved seed. Silv. Genet., 13; 4-11.