Flowering and Seed Production in Grafted Clones of Scots Pine

By IAN R. BROWN

Department of Forestry, University of Aberdeen

Introduction

The progress of a tree improvement programme depends upon a plentiful supply of viable seed from controlled crosses and wind pollinations. In Britain low yields of viable seed from controlled crosses on Scots pine grafts have been causing concern because the low yields slow down the establishment of progeny trials from test crosses and increase the cost of the work. The investigations discussed in this are the first stages of a study designed to measure the variation in seed and cone yield of Scots pine grafts, to determine the factors affecting this yield and, if possible, to suggest methods of raising the levels of cone and seed production. Methods of increasing the production of male and female primordia are not included in the study.

The final cone and seed yields from controlled crosses may be influenced by breakdown of any one of the processes of pollination, pollen grain germination, pollen tube growth, fertilization and embryo development. A further loss of seed is occasioned by premature abscission of the cones from the tree.

Losses at Pollination:

Seed yield is reduced if insufficient pollen is applied to the female strobilus. Unpollinated ovules degenerate during the first growing season and only the wings continue to develop (MCWILLIAN, 1959; SARVAS, 1962). In addition, it has been demonstrated (SARVAS, 1962) that in the wind-pollination of Scots pine there is a negative correlation between the abscission of conelets[1]) in the first growing season and the density of the pollen cloud. It appears that a threshhold value is reached at about twenty per cent conelet loss, when further increase in the density of the pollen cloud has no effect. It may be that beyond this threshhold value for pollen density, factors such as lack of synchronous development of male and female strobili results in inadequate pollination of a certain proportion of ovules, but it is also possible that factors unconnected with pollen supply are operating to bring about the abscission of conelets.

For maximum yield of seed the female strobili must be pollinated when fully receptive to pollen, that is, when the axis of the strobilus is fully elongated and the ovuliferous scales are separated from each other. This stage was defined by EHRENBERG and SIMAK (1957) who also demonstrated that reduced seed yield and increased cone loss were associated with the pollination of immature female strobili.

Female strobili are frequently damaged by handling when being isolated or pollinated, or by abrasion against the sides of isolation bags during windy periods. It has been observed in Britain that such damage, especially at an early stage in conelet development, is associated with increased cone loss.

Losses related to Pollen viability:

The pollen chamber of the ovule of Scots pine may contain up to five or more pollen grains depending upon the density of the pollen cloud and the size of the pollen chamber. When pollen viability is low seed production will be critically affected when the number of pollen grains per pollen chamber is small or the number of archegonia per ovule is low. It is normal practice in Britain to use one-year-old pollen dried after collection and stored at a low temperature (WORSELY, 1959). It is therefore important to know the effect of storage on pollen. Unfortunately, none of the methods of testing pollen in vitro indicate precisely its fertilising ability. Germination counts from in vitro tests are not related to seed yields (EHRENBERG, 1960; STANLEY, 1962; LIVINGSTON and CHING, 1967). Chemical tests of respiratory activity (OWEZARZAK, 1952; COOK and STANLEY, 1966) do not appear to be more reliable than germination tests in predicting seed yields.

Losses at Fertilisation *and* embryo developrnent:

Empty seed and seed of low viability are examples of the reduction in seed potential caused by breakdown in embryogeny. This aspect of seed yield has been extensively studied and efforts have been made to quantify and trace the origin of these developmental failures. SIMAK and GUSTAFSSON (1953a and b) developed an X-ray technique for the non-destructive radiographic examination of the embryo and endosperm. These authors and others (MULLER-OLSEN and SIMAK 1954) defined five embryo and two endosperm classes. Class 0 seed had no endosperm or embryo; Class I had endosperm but no embryo; Class II had endosperm and one or more small embryos no larger than one half the length of the embryo cavity; Class III seed had endosperm and one embryo, one-half to one-third the length of the cavity and Class IV seeds were fully formed. The two endosperm classes were A- where the endosperm fills the seed cavity and absorbs X-ray wells and B — where the endosperm is incompletely developed, shrunken and deformed.

DOGRA (1967) has shown by a microscopic study of Scots pine seeds that the seed classes defined above are not related to any particular stage in embryo breakdown. The embryological situation in any one class of seed is, in fact, very difficult to ascertain from rediographs. In general, DOGRA found that Class 0 seeds do contain endosperm remains and 10 to 50 per cent contain remains of embryos. The stage of endosperm development, however, in somc seeds lacking embryos indicated that they did have embryos at first and that degeneration occurred at the pro-embryo stage. DOGRA also found that seed classes 0 and I result from degeneration of endosperrn and/or embryo from pre-pollination to the early stages of late embryogeny. Class II seeds are the consequences of inhibition of development during late embryogeny and the stage of organ differen-

[1]) Strobilus and cone are synonomous terms but in this paper the word "strobilus" will be used to refer to the young fleshy condition before and just after pollination. The word "conelet" will be used to describe the stages following pollination to the start of the second growing season, when the ovuliferous scales become woody and "cone" will be used to describe the stage of growth from the beginning of the second growing season until the time of harvest.

tiation in the embryo. Breakdown at later stages gives rise to Class III seed.

Such developmental failures may be due either to environmental or genetic influences. It is well-known that the proportion of Class IV seed decreases with increasing altitude and latitude of the seed sources (Buszewicz *et al.*, 1955; Muller-Olsen and Simak, 1954; Ehrenberg *et al.*, 1955; Simak and Gustafsson, 1954; Andersson, 1965). In material collected in Southern Finland in 1956 severe damage to the female gametophyte was reported by Sarvas (1962) who thought that the most probable cause was severe frost at the end of May. With respect to genetic factors an important potential cause of developmental breakdown is the occurrence of lethal and semi-lethal genes. Self-pollination increases genetic homozygosity and increases the chance of obtaining homozygous combinations of recessive deleterious genes which affected the developing embryo. Selfing of Scots pine increases the proportion of seed of Class 0—III. (Ehrenberg and Simak, 1957; Plym Forshell, 1953; Ehrenberg *et al.*, 1955).

The number of empty seed per cone is influenced in yet another way. As pointed out by Sarvas (1962) Scots pine ovules may contain one, two or three archegonia, all capable of receiving a pollen tube, when as a result of a plentiful supply of pollen multiple pollination occurs archegonial polyembryony results. The number of arborted embryos resulting from the union of lethal genes will be smaller than the number of empty seed in this case since the chances are that if one embryo fails, there will be another in the same ovule which can develop. Thus, the more pollen reaching the ovule the smaller the empty seed percentage. Given a constant embryo mortality of 30 per cent, the empty seed percentage where ovules have only one archegonium would be 30%; where two arechegonia per ovule occur the empty seed percentage would be only 9 per cent.

Losses due to Cone Abscission:

It has already been noted that premature cone drop increases as the pollen supply is reduced. Cone loss varies greatly between clones even when one pollen source and one isolation procedure have been used. Forestry Commission records show that between pollination and cone collection up to 95 per cent of cones may be lost by some clones. Loss of cones following the 1965 pollinations in the Scots pine tree bank at Newton averaged 35 per cent.

Cone abscission occurs at almost any time from pollination onwards although there appears to be peak periods of drop. Katsuta and Satoo (1964) showed for *Pinus thunbergii* that cone drop in the first season of development occurs after pollination in May and June. In the second season cone drop occurs from May to October. The authors state that second season cone drop appeared to have no connection with the degree of fertilisation and embryo development. Lester (1967) suggested that the existing crop of second year cones has a great short term demand for nutrients which affects the development of first year cones and also the initiation of flower primordia.

Seed yields in Scots pine:

Few data are available concerning seed yield from controlled pollination in Scots pine although data for open-pollination are available. Approximate seed yield figures for Southern Sweden (Johnsson *et al.*, 1953) show that total number of seed per cone averages 15 to 20 with a range of 4 to 32. The number of full seed per cone averages 15 with a range of 2 to 30. Unpublished Forestry Commission data show that similar figures are obtained in Britain for both wind and controlled pollinations with wind pollinations yielding marginally higher total numbers of seed and full seed per cone.

Objectives

The immediate objectives of the experiments discussed below were varied. The first aim was to assess the efficiency of controlled pollination in delivering pollen to the ovules of the strobilus. Secondly, experiments were devised to determine the effects of variations in pollen quality and quantity on seed yields. The effect on seed yield of enclosing female strobili to prevent contamination by foreign pollen, was also studied.

Finally, information on cone loss was gathered from these experiments to identify the factors affecting premature cone abscission and to study the relationship between premature cone loss and the final yield of seed from ripe cones.

General Material and Methods

The experimental work in these investigations was carried out in the Scots pine tree bank at the Forestry Commission Research Division nursery at Newton, near Elgin, Morayshire during the years 1965 to 1967. The tree bank was established in 1957 and contains grafted material from putative plus trees selected from all parts of Britain. Each ortet was originally represented by five ramets planted in line at 5 feet spacing. The lines of grafts are spaced 20 feet apart.

Before the ovuliferous scales of the strobili became visible a celluloid tube 5.5 cm in length and 1.5 cm in diameter was slipped over the tip of the current year's shoot and plugged at both ends with polystyrene sponge. The top plug was easily removable for pollination with a pollen gun. When the strobili were judged secure from contamination by foreign pollen the isolation tubes were removed. The pollen applied was a mixture from 10 parent trees and had been cold stored at —5° C since the previous spring.

Cones were collected in mid-December of the following year and their lengths and diameters measured before drying for seed extraction. The seeds were extracted cone by cone and the normal sized seeds separated into full and empty on the basis of their colour — the full seed being very much darker in colour than the empty seed. This method was checked by pentane flotation (McLemore 1965) and by X-ray radiography, revealing an error of less than 1 per cent in separating empty seed (Class O) from full seed (Classes I—IV).

In the results the seed yields are given as "total seed yield per cone" which includes both full and empty seed and also as "full seed per cone". Since unpollinated ovules do not develop into full-sized seed the "total seed yield per cone" is a measure of pollination success while the number of "full seed per cone", if the amount and quality of pollen applied is constant, gives some measure of the success of fertilisation and subsequent development of the pollinated ovules.

Experimental Methods and Results

Distribution of pollen in the ovules:

It is difficult to assess the efficiency of controlled pollination methods from the subsequent yields of seed and from the cones which develop to maturity because pollen viability is variable and not all ovules which are pollinated develop into seeds. It was decided, therefore, to obtain a direct estimation of the efficiency of controlled pollination methods by counting pollen grains in the pollen chambers of ovules following the application of pollen by hand.

To increase the visibility of the pollen on the nucellus of ovules the grains were stained with methylene blue and re-dried over Calcium chloride before being applied to strobili isolated by celluloid tubes in the normal way. After two weeks, when the micropyle had closed, the conelets were collected for examination. One spiral of scales was selected on each cone, both ovules on each scale on the spiral were dissected and the pollen grains counted. In Scots pine the degree of development of both ovule and scale varies along the length of the cone and it was important to exclude from the counts those ovules which did not contain pollen because of their lack of development. It is easy to reject ovules on the small basal scales of the cones because of their small flattened appearance but as one proceeds further up the cone it becomes more difficult to grade the ovules on their macroscopic appearance alone. Arbitrarily, then, all scales on which both ovules lacked pollen were rejected. When a pollen grain was found in one or both of the ovules the pollen count was started. At the distal end of the cone the transition to undeveloped ovules was more abrupt and such ovules were easier to identify and exclude from the count.

In this experiment two clones, numbers 46 and 900, were used and seventy cones and one thousand ovules were sampled. *Table 1* shown shows the observed distribution of pollen grains in ovules for both the clones, and *Fig. 1* is a combined frequency distribution of pollen grains in ovules in the two clones. It can be seen that the largest class in both clones is that where no pollen grains were found in the ovules. This occurred despite the fact that during dissections of the ovules it was observed that the scales and central axis of the strobili were liberally dusted with pollen.

Effects of pollen viability on seed yield:

Using the data given in *Table 1* it is possible to predict the effects on "total seed yield per cone" of applying pollen of varying viability. The curve relating seed yield to pollen viability is given in *Fig. 2*. It has been assumed that viable and non-viable pollen grains are equally likely to reach the pollen chamber of the ovule. The average potential yield of seed from a cone has been taken as 50. This

Table 1. — Frequency distribution of pollen grains on the pollen chamber of ovules.

No. of grains per ovule	Ovules with pollen grains: Clone 900 No.	Clone 900 %	Clone 46 No.	Clone 46 %	Total %
0	154	23.7	67	21.2	22.9
1	57	8.8	49	15.5	11.0
2	119	18.3	90	28.5	21.7
3	112	17.3	55	17.4	17.3
4	74	11.4	29	9.2	10.7
5	80	12.3	20	6.3	10.4
6	44	6.8	5	1.6	5.1
7	8	1.2	1	0.3	0.9
8	1	0.1	—	—	0.1
Total	649		316	100.00	100.00

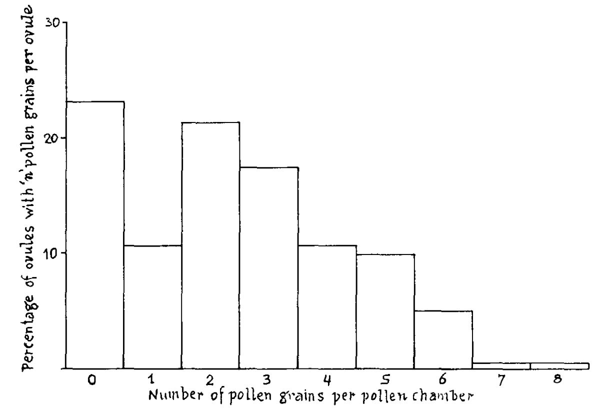

Fig. 1. — Frequency distribution of pollen grains in ovules of clones 46 and 900.

figure was obtained from the results of dissections of first year conelets from three clones where the average number of apparently fully developed ovules was estimated by inspection.

The number of apparently fully developed ovules ranged from 18 to 76 and the standard error of the mean was 1.82. These dissections were done towards the end of the first growing season when the tissues of ovules which had not originally been fully developed were showing clear signs of degeneration. However, any variation in this assumed maximum seed potential does not alter the shape of the response curve depicted in *Fig. 2* which shows that pollen

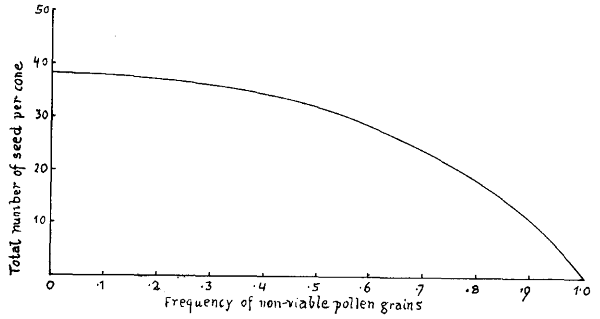

Fig. 2. — Seed yield (full and empty) response to increasing frequency of non-viable pollen grains.

viability has small influence on seed yield until viability is reduced to 50 per cent or less. It can also be seen that with completely viable pollen the average yield of seed per cone would be 38.

Comparison of seed yield using fresh and stored pollen:

Published data comparing the seed yields using freshly collected and stored pollen are rather sparse. A small test of fresh and one-year-old pollen from the same sources was carried out by the Forestry Commission Genetics Section and it can be seen from the results in *Table 2* that in clones 296 and 363 fresh pollen produced a greater "total seed yield per cone", a higher yield of "full seed per cone" and less cone drop. In clone 210 stored pollen produced a slightly higher "total seed yield per cone". A polymix containing different pollen sources was used for clones 210 and the seeds were extracted by hand rather than by the bulk extraction method used for other clones. The differences in "total seed yield per cone" between fresh and stored pollen in clones 296 and 363 may be due

Table 2. — Seed and cone yields from crosses using fresh and one-year-old stored pollen from the same source

Clone No.	Pollen	No. of cones	Mean Total No. Seed per cone	Mean No. full seed per cone	Full seed per cone (%)	Cone loss (%)
296	stored	214	16.0	7.0	43.8	67.3
	fresh	216	26.5	16.5	62.2	35.2
363	stored	207	2.1	0.8	38.1	71.0
	fresh	208	6.8	3.6	52.9	33.6
210	stored	33	19.9	3.4	17.0	32.7
	fresh	24	16.2	6.4	37.0	45.5

to the fact that empty seed are very much harder to extract than full seed and that stored pollen did in fact produce more empty seed per cone than the extraction process revealed. The decreased percentage of full seed resulting from application of stored pollen suggests that storage at 7 per cent moisture content and —5° C affects the ability of the pollen to achieve fertilisations leading to normal embryo development.

Effects of concentration of pollen on seed yield:

If the density of the pollen cloud is reduced, the seed yield in comparison to that from a standard pollen application should indicate whether or not an excess of pollen is normally applied. The effect of pollen concentration on cone retention can also be investigated.

Two methods of reducing the concentration of pollen were considered, both involving diluting the applied pollen with an inert carrier. Dead pollen as a diluent seems an obvious choice but it is possible that dead pollen contains a diffusible active substance which could affect cone retention. It was decided, therefore, to use talc as the carrier since it is likely to be physiologically inert. It was hoped that even if the talc was not taken into the pollen camber it would adhere to the micropylar arms, blocking sites for pollen grains and reducing the number of grains taken into the pollen chamber by the pollen drop mechanism. (For a description of the operation of this mechanism see McWilliam, 1958).

Pollen was applied in mixture with talc at four concentrations 100, 75, 50 and 10 per cent to the strobili of three clones. Each pollen/talc mix was applied to 50 strobili per clone.

The results in *Table 3* show that in all three clones there is a tendency for "total seed yield per cone" to fall when the concentration of the pollen/talc mixture is reduced to 10 per cent pollen. There are rather wide differences in the variances of the treatment means which present a more detailed analysis of total seed yield figures. A X^2 test on the raw data of treatment differences in the frequencies of full and empty seed is significant at the 5 per cent level in clone 50 only. Similar analysis for clones 741 and 50 show that differences between treatments in cone drop are significant. The regression coefficient of the trend in increasing cone loss with decreasing pollen concentration is significant at the 5 per cent level in 741 and 50.

Table 3. — Seed and cone yields following application of pollen/Talc mixtures of varying proportions.

Treatment	No. strobili isolated	No. cones harvested	Mean Total No. Seed per cone	Mean No. full seed per cone	Full seed per cone (%)	Cone Loss (%)
Clone 741						
T_1	56	31	27.2 ± 1.9	8.2	30.2	44.6
T_2	45	13	22.1 ± 2.5	6.0	27.2	71.1
T_3	55	8	25.2 ± 1.3	5.5	21.7	85.5
T_4	40	7	17.1 ± 4.3	4.7	27.5	82.5
					$X^2 = 6.0$	$X^2 = 26.35$**
Clone 50						
T_1	59	32	25.8 ± 1.3	10.4	40.3	45.8
T_2	54	21	19.0 ± 2.0	9.0	47.5	61.1
T_3	52	27	27.5 ± 1.4	11.9	42.7	48.1
T_4	39	6	13.7 ± 2.5	7.5	54.9	48.6
					$X^2 = 10.75$*	$X^2 = 17.07$**
Clone 985						
T_1	39	29	26.6 ± 1.8	15.7	59.1	25.6
T_2	68	52	28.7 ± 1.3	15.6	54.5	23.5
T_3	40	33	35.3 ± 2.6	20.6	58.2	17.5
T_4	39	30	17.5 ± 1.1	9.5	54.0	23.1
					$X^2 = 7.07$	$X^2 = 0.85$

X^2 for 3 degrees of fredom = 7.81 for all three clones
T_1 — 100% Pollen No Talc
T_2 — 75% Pollen 25% Talc
T_3 — 50% Pollen 50% Talc
T_4 — 10% Pollen 90% Talc

Table 4. — Seed and cone yields from wind and controlled pollinations.

No. of cones extracted	Mean Total No. of seed per cone	Mean No. of full seed per cone	Full seed per cone (%)	Cone loss (%)	Mean No. of full seed per pollination
Clone 970					
Control 71	23.6	11.1	47.0	60.9	4.3
Wind 23	29.9	14.2	47.4	83.3	2.6
Clone 221					
Control 25	22.1	4.8	21.9	46.9	2.6
Wind 22	30.5	16.2	53.1	72.2	4.6
Clone 900					
Control 87	8.1	2.3	29.0	22.5	1.8
Wind 59	9.2	2.8	30.7	57.1	1.2

Yield of seed from controlled and wind pollination:

Experience has shown that wind pollination results in cones with a greater number of full seed than does controlled pollination. This was confirmed on three clones when a comparison between wind and controlled pollination was made. The results given in *Table 4* show that both the "total seed yield per cone" and the number of "full seed per cone" are higher after wind pollination. Cone loss, however, is much greater after wind pollination than after controlled pollination. The reasons for this difference in cone drop will be discussed in a later section.

Effect of pollen pretreatment on seed yield:

Stored pollen is dried to a moisture content of 7 per cent and is applied to the strobili at or near this level. In an attempt to test the effect of rehumidification of pollen before use, on seed yield, stored pollen was exposed to the air for two periods of four and six days raising the moisture contents respectively from 7 per cent to 10 and 11 per cent. The results are given in *Table 5*. Total seed yield appears to be unaffected but in two clones a X^2 test on the raw data showed that there are significant differences between treatments in the proportions of full and empty seed. There are, however, no consistent trends in the responses. Only clone 46 shows significant differences between treatments in cone drop.

Effect of length of isolation period on seed yield:

Strobili were pollinated under four isolation regimes to test the effect on seed yield and cone loss. Celluloid tubes were used to isolate the strobili before they became receptive and were removed immediately on pollination and on one, two and four days after pollination. Normally, isolation tubes may be left on the shoots for a week but it was not possible to continue the experiment for this length of time.

The results in *Table 6* show that there are no differences in "total seed yield per cone" but in three clones a X^2 test on the raw data showed that there are significant differences between treatments in yield of "full seed per cone". There is, in fact, a trend of decreasing yield of full seed with increase in length of isolation period. In all four clones the regression coefficient of this trend was found

Table 5. — Seed and cone yields following application of pollen exposed to the air for varying times.

Treatment	No. strobili isolated	No. Cones harvested	Mean total No. Seed per Cone	Mean No. full Seed per Cone	Full Seed per Cone (%)	Cone Loss (%)
Clone 982						
T_1	61	25	18.9 ± 1.7	4.1	21.5	59.0
T_2	68	26	24.6 ± 1.9	7.8	31.8	61.8
T_3	75	33	23.2 ± 1.9	7.6	32.6	56.0
					$X^2 = 20.3^{**}$	$X^2 = 0.48$
Clone 743						
T_1	68	56	15.1 ± 1.0	5.5	36.3	17.6
T_2	66	52	14.2 ± 1.0	5.8	40.8	21.2
T_3	70	58	12.4 ± 0.9	4.7	37.9	17.2
					$X^2 = 3.52$	$X^2 = 0.43$
Clone 46						
T_1	76	49	33.3 ± 1.3	16.9	50.8	35.5
T_2	74	58	34.6 ± 1.3	16.0	46.4	21.6
T_3	67	40	30.7 ± 1.5	14.3	46.4	40.3
					$X^2 = 8.44^{*}$	$X^2 = 6.18^{*}$

X^2 for 2 degrees of freedom is 5.66 for all three clones.

	Moisture content per cent
T_1 — 0 days exposure and air	7.0
T_2 — 4 days exposure and air	10.0
T_3 — 6 days exposure and air	11.0

Table 6. — Seed and cone yields following different periods of isolation.

Treatment	No. strobili pollinated	No. Cones harvested	Mean total No. Seed per Cone	Mean No. full Seed per Cone	Full Seed per Cone (%)	Cone Loss (%)
Clone 959						
T_1	52	37	33.4 ± 1.3	18.3	54.8	28.8
T_2	56	34	37.5 ± 1.1	19.0	50.8	39.3
T_3	50	37	35.0 ± 1.0	18.1	51.7	26.0
T_4	56	31	36.2 ± 1.0	18.1	49.8	44.6
					$X^2 = 6.75$	$X^2 = 4.08$
Clone 105						
T_1	60	49	31.2 ± 1.7	8.1	26.0	18.3
T_2	56	42	38.1 ± 1.3	8.1	21.2	25.0
T_3	35	26	30.9 ± 2.1	6.1	19.6	25.7
T_4	37	29	30.7 ± 1.6	6.0	19.3	21.7
					$X^2 = 16.97^{**}$	$X^2 = 1.04$
Clone 44						
T_1	41	26	31.7 ± 1.6	19.5	61.5	36.6
T_2	55	36	35.2 ± 1.1	19.7	56.1	34.6
T_3	47	29	36.6 ± 1.1	19.4	52.9	36.3
T_4	43	19	27.0 ± 1.9	13.8	50.9	55.8
					$X^2 = 19.46^{**}$	$X^2 = 5.29$
Clone 22						
T_1	49	40	17.5 ± 0.7	8.1	46.3	18.4
T_2	47	40	15.9 ± 0.9	6.5	40.7	14.9
T_3	30	27	17.2 ± 1.0	7.8	45.2	10.0
T_4	49	45	13.6 ± 1.6	5.2	38.6	8.2
					$X^2 = 10.41^{*}$	$X^2 = 2.92$

X^2 for 3 degrees of freedom = 7.81 for all three clones
T_1 — Celluloid isolation tube removed on day of pollination
T_2 — Celluloid isolation tube removed 1 day after pollination
T_3 — Celluoid isolation tube removed 2 days after pollination
T_4 — Celluloid isolation tube removed 4 days after pollination

to be significant at the 50 per cent level. The length of the isolation period has no significant effect on cone loss.

Effect of the isolation method on seed yield:

In 1965 the Forestry Commission made extensive use of two isolation methods. The first employed the colluloid tube already described and the second a 45 cm long 9 cm broad tube manufactured from non-woven "Terylene" fabric and fitted with a poly-vinyl-chloride window 15 cm by 1.5 cm. The "Terylene" tube covers the current year's shoot and is sealed at the base by a plug of cotton wool wrapped around the top of the previous year's growth.

In the experiment the strobili on each clone were isolated by both methods and the same pollen mixture used throughout. When the seeds were harvested only full seeds were counted and the results in *Table 7* show that the differences in full seed per cone between treatments were small. There was a larger difference in cone drop between treatments and the yield of full seed per strobilus pollinated is therefore higher for celluloid tubes than for terylene tubes.

Observations on cone abscission:

It has already been noted that cone drop tends to increase with decreasing pollen supply *(Table 3)* and is greater after wind pollination than controlled pollination *(Table 4)*. In the latter case it appears that pollen supply is the important factor affecting cone drop since if strobili are not isolated but simply pollinated when receptive the resulting cone loss is less than with wind pollination. *Table 8* shows the results of one such comparison. It can be seen that only in clones 47 and 86 did loss of conelets following controlled pollination exceed that following open pollination. Also in this study cone drop was measured relative to position of cones in the crown and as can be seen from *Table 9* the loss of cones on the north side of the crown is twice that on the south side. This difference holds for both wind and controlled crosses. There is also some indication, (see *Table 2*), that use of fresh pollen rather than stored pollen decreases cone loss.

Cone loss does not appear to be confined to any particular stage of cone development as can be seen in *Fig. 3*

Table 7. — Seed and cone yields resulting from isolations using terylene tubes and celluloid tubes.

No. of flowers isolated	Type of isolation	No. cones harvested	Mean No. of full seeds per cone	Cone loss (%)	Mean No. of full seed per strobilus pollinated
3651	terylene tube	2189	10.7	40.0	6.4
2879	celluloid tube	1976	11.0	31.4	7.5

Table 8. — Cone loss in the first sixteen months following wind and control pollination.

Clone No.	No. of strobili pollinated		No. of conelets after one year		Loss of conelets	
	Conrol	Wind	Control	Wind	Control (%)	Wind (%)
6	36	41	30	31	16.7	24.4
12	23	50	19	29	17.4	42.0
32	55	54	45	43	18.2	20.4
40	45	50	31	30	31.1	40.0
47	27	52	17	36	37.0	30.1
86	78	68	65	60	16.7	11.8
118	35	46	29	28	17.1	39.1
182	24	52	19	17	20.8	60.8
201	28	56	14	22	50.0	60.7
229	24	54	14	15	41.7	72.2
263	23	53	14	19	39.1	44.4
264	45	54	39	31	13.3	42.6
281	49	48	35	33	28.6	31.2
Total	492	678	371	394	24.6	41.9

where the course of cone loss is shown for clones 985, 741 and 50 used in the pollen concentration experiment. In addition *Table 10* shown loss of cones from three clones concentration experiment. In addition *Table 10* shown loss of cones from three clones where both wind pollinations and controlled pollinations were studied. In most of the clones examined a large proportion of the drop occurs immediately after pollination. Conelets continue to fall during the summer and following winter but it is not possible to say when the abscission layers of such conelets were formed. This is because when counts were made during this period conelets became detached from the shoots when handled and such conelets were invariably found to be desiccated and in an advanced state of deterioration. It is obvious then that a long period of time may elapse between formation of the abscission layer and loss of the cone from the tree. Observations made, in 1967 only, have shown that once the cones recommenced growth in the spring of their second year there was little further loss until the winter months preceding harvest in December when in some clones there was a fairly high loss.

Table 9. — Effect of aspect on cone loss sixteen months after pollination.

Pollination	Aspect of strobili in crown North (% cone loss)	South (% cone loss)	X^2 Significance
Controlled	34.55	19.42	1%
Wind	55.88	28.86	1%

Due to the deterioration of the abscissed conelets it was not possible to ascertain the numbers of pollinated and non-pollinated ovules they contained although this is a simple matter in healthy cones. It was found, however, that "total seed yield per cone" of the crop remaining at harvest time was correlated with the degree of cone drop. The data were obtained from the ten clones used in the experiment on pollen concentration, pollen exposure and isolation time where the same pollen source was used for all pollinations. With the exception of clones 22 and 743 high cone drop was associated with low "total seed yield per cone" in the final cone crop and as cone drop decreased there was a corresponding increase in seed yield per cone. In the case of "full seed per cone" there was a similar relationship for all clones except 22, 743 and 105. In an attempt to account for the differences between the two groups of clones cone volume was taken into account. It became evident that where numbers of "full seed per cone" were concerned the data fell into two populations giving two regression lines *(Table 11)* in which cone loss increased as the ratio of volume of cone to number of full seed in the mature cones increased. This relationship is significant at the 1 per cent level in the first population and significant at the 10 per cent level in the second population comprising clones, 22, 105 and 743. When, in the expression for x, "total seed yield per cone" is used in the denominator a significant regression is also found in population 1 but in the second population no significant relationship is found.

Table 10. — Cone loss following wind and control pollination.

Clone No.	Pollination	Loss of cones 1 month after pollination (%)	1 year after pollination (%)	at time of harvest (%)
970	Control	48.3	60.4	60.9
	Wind	73.2	81.9	83.3
221	Control	13.6	29.7	46.9
	Wind	22.2	45.4	72.2
970	Control	8.2	9.8	22.5
	Wind	27.8	34.1	57.1

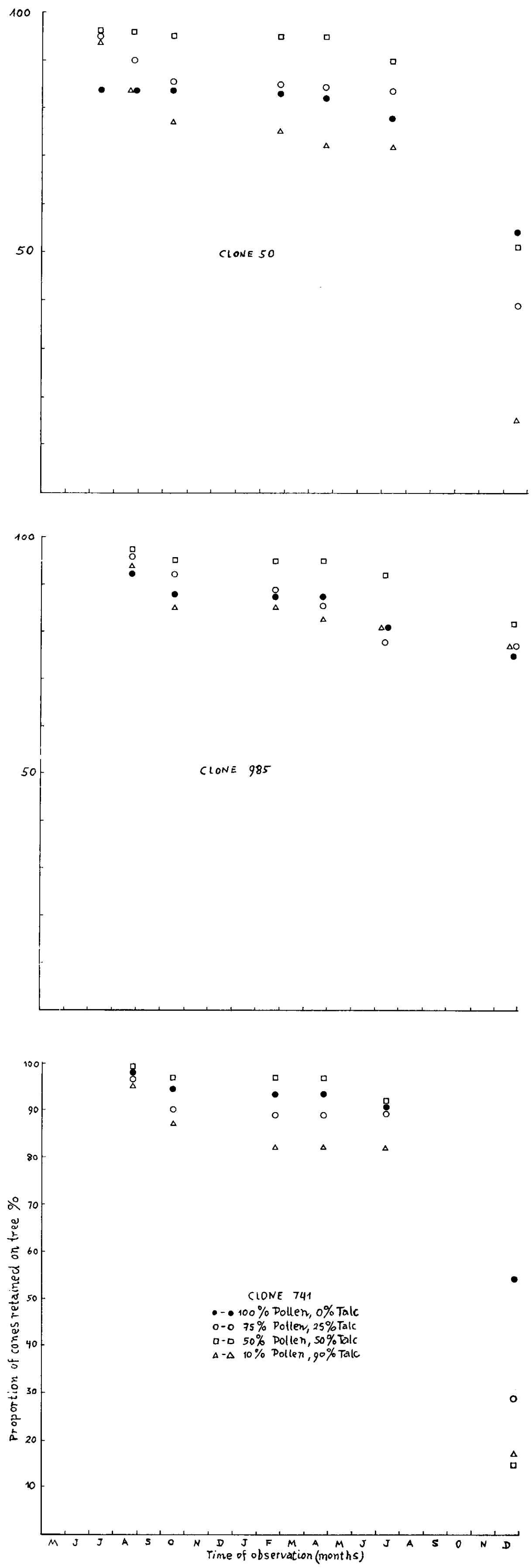

Fig. 3. — Loss of cones in three clones following application of four concentrations of a pollen/talc mixture.

Seed yield:

An examination of the data in terms of cone loss and cone size gives an opportunity of assessing the importance of these factors in relation to yield of seed per cone. The two populations mentioned in the previous section were separately subjected to a multiple regression analysis with "total seed yield per cone" and percentage of "full seed per cone" as the dependent variables and total cone loss, percentage of cone loss occurring in the first year and cone volume as the independent variables. The correlation coefficient of "total seed yield per cone" on per cent "full seed per cone" is 0.464. The results of this analysis are given in *Table 12*. Clone 105 is difficult to classify into either the first or second population since it has attributes belonging to both populations. It was included in the second population, however, because in terms of cone loss and percentage "full seed per cone" it was similar to clones 22 and 743.

In the case of the first population the full regression accounts for 49.8 per cent of the variation in "total seed yield per cone" and 44.7 per cent of the variation in per cent "full seed per cone". But when only the term for total cone drop is included there is, in both cases, little reduction in the amount of variation accounted for. Percentage "full seed per cone" in the second population, however, is related to other factors, cone volume being the most important and accounting for half the variation.

Discussion

The yield of seed following controlled pollination:

The distribution of marked pollen grains in ovules of the strobili of two clones of Scots pine showed that, following controlled pollination,about twenty per cent of ovules remain unpollinated. The occurrence of empty ovules after controlled pollination may be due, firstly, to the use of a pollen gun which drives the pollen in one direction and, secondly, to lack of circulation of pollen inside the isolation tube. Concerning the second point, heavy condensation can often be seen inside the isolation tube and this may dampen the pollen and hinder its movement.

A third possible reason for the occurrence of empty ovules after controlled pollination is incorrect timing of the application of pollen and two sets of observations point to the importance of this aspect of pollination technique. Assuming fully viable pollen, the observations on pollen distribution and numbers of ovules per cone indicate that the expected average total number of seed per cone in Scots pine will be about thirty eight. The results presented in *Tables 3—6* show that even when pollen stored for one year was used, only three clones (22, 743 and 900) gave "total seed yields per cone" of less than twenty. If is further assumed that all full-sized seeds, whether full or empty, result from pollinated ovules, then the total number of seeds in a cone provides some measure of the effectiveness of the method used to apply pollen. The same pollen mixture was applied by the same pollination method to all the thirteen clones included in *Tables 3—6* and the differences in "total seed yield per cone" within clones were small while the differences between clones were larger. If the supply of pollen to the strobili is assumed to have been constant the most likely cause of the differences between clones in "total yield of seed per cone" is that in some clones the most receptive period of strobilus development

Table 11. — Cone loss regression.

y = Cone loss %	*Cone volume* x_1 = No. of full seed per Cone	*Cone volume* x_2 = Total Seed per Cone
Regression	Correlation coefficient	Level of significance of regression
Population 1 7 clones	26 observations	
$y = 17.14 + 11.44\,x_1$	0.7897	1%
$y = -6.91 + 53.33\,x_2$	0.7213	1%
Population 2 3 clones	11 observations	
$y = 9.51 + 2.7\,x_1$	0.5167	10%

is difficult to recognise and the applications of pollen were mistimed.

There is a fourth possible reason for the occurrence of empty ovules after controlled pollination, namely, that the environment of the isolation tube may inhibit the pollen-drop mechanism. In later experiments done in the Scots pine tree bank at Newton nursery, small thermistor thermometer probes were inserted into the colluloid isolation tubes. The probes revealed that air temperatures inside the tubes could be two or three degrees Centigrade higher than outside air temperatures during the day and two or three degrees lower at night. A direct check of impairment of the pollen drop mechanism could be made by comparing, on a population of strobili given the same pollination treatment, the distribution of pollen on the micropyle or arms of one sample with the distribution of pollen in the pollen chambers of a subsequent sample, but this comparison has not yet been made.

A fifth possible reason for the occurrence of empty ovules after controlled or open pollination lies in clonal variation in flower structure and development. Only two clones were used in the observations on the distribution of marked pollen grains so nothing can be said about the possible effects of clonal differences in flower structure on the distribution of pollen. This point is, however, worthy of further study.

Seed yield and embryogeny:

Another cause of reduction in the yield of seed by Scots pine clones is failure in embryo development and consequent formation of empty seed. In the clones used in these studies the percentage of "full seed per cone" ranged from twenty to sixty per cent. The experiment on the duration of isolation of strobili from foreign pollen contamination shows that the proportion of full seed per cone is influenced by the environment of the isolation tubes. With increasing length of isolation the proportion of "full seed per cone" was reduced. The treatment could have affected the pollen and/or the ovule because damage to both pollen and ovule will cause abnormal embryo development. In another experiment fresh pollen and pollen stored for one year but from the same source was tested on three clones and the use of fresh pollen resulted in proportionately more full seed than the stored pollen. In this instance the function of the male gamete appears to have been affected by the storage conditions.

Table 12. — Multiple regression analysis of factors affecting seed yield.

y_1 = Total No. of seed per cone; y_2 = Percentage full seed per cone	x_1 = Total cone drop; x_2 = First year drop as percentage of total drop; x_3 = Cone volume	
Significance level of partial regression coefficients	Regression	Coefficient of determination
Population 1 7 clones, 26 observations		
10%	$y_1 = 28.53 - 0.23x_1 - 96.01x_2 + 0.39x_3$**	49.79%
	$y_1 = 27.89 - 0.22x_1 + 0.39x_3$	49.67
5%	$y_1 = 38.28 - 0.23x_1$**	43.14
1%	No change	
	$y_2 = 6.48 - 0.41x_1 - 20.71x_2 - 1.57x_3$**	44.73
10%	$y_2 = 63.90 - 0.41x_1$	44.72
1%	No change	
Population 2 3 clones, 11 observations		
10%	$y_2 = 64.58 - 0.51x_1$* $- 0.12x_2 - 0.64x_3$	71.05
	$y_2 = 63.97 - 1.12x_3$	52.6
5%	No change	

** Regression significant at 1% level
* Regression significant at 5% level

Differences in the proportion of "full seed per cone" between clones treated with the same pollen are large in comparison to the differences within clones. The clonal differences may be due to one or several unfavourable environmental factors but are also likely to have a genetic component.

The yield of "full seed per cone" is correlated with the "total yield of seed per cone". This correlation can be explained as follows: — As the amount of pollen applied to a strobilus increases it can be expected that the number of ovules pollinated and the numbers of pollen chambers filled to capacity will also increase. There is then a corresponding rise in the possibility that all archegonia in the ovule are fertilised and that one of the resulting embryos will dominate and a viable seed be produced. Thus, with increasing amounts of pollen applied or increasing efficiency of pollen application there will be an improvement in both "seed yield per cone" and the proportion of "full seed per cone".

Premature drop of conelets and cones:

The third major loss of seed in Scots pine is caused by conelet and cone drop. Large clonal differences were observed and drop following controlled pollination ranged from twenty to seventy per cent of those strobili pollinated.

It was found that dilution of pollen with talc decreased the number of mature cones harvested whereas the use of fresh rather than stored pollen increase the number of strobili developing to maturity[2]). In ten clones high conelet and cone loss was associated with low seed yields in relation to the size of the cones in the final crop.

The retention of cones to maturity appears to some extent to be independent of the formation of embryos. Many experiments in hybridisation in *Pinus* have shown that in interspecific crosses, whether or not an embryo is ultimately formed, strobili are retained to maturity although frequently losses are greater than those found in intraspecific crosses. In a current series of tests at Newton nursery it has been found that, sixteen months after pollination of Scots pine strobili with *Pinus contorta* pollen conelet loss is only ten per cent greater than in crosses where Scots pine pollen was applied.

At Newton nursery wind pollination is followed by relatively high loss of cones before maturity and a high yield of seed per cone in the final harvested crop. It may be that there is an abnormal distribution of pollen due to persisting prevailing winds, resulting in good pollination of strobili on the windward side of the tree crown while strobili on the lee side receive little pollen and subsequently absciss. Alternatively, the period over which individual strobili become receptive may be such that many strobili are receptive either before or after the main period of pollen dispersal. If it is assumed that retention of developing cones depends on the total amount of pollen reaching the strobilus without regard to whether it is on the scales, the central axis or in the micropyle a third possible reason for the observed effects of wind pollination appears. It is almost certain that there is a greater mass of pollen in a strobilus after controlled pollination than after wind pollination and thus a greater quantity of some hypothetical abscission inhibitor could diffuse into the conelet tissue. When stored pollen is used in controlled pollinations fewer of the pollen grains reaching the nucellus are germinable (when compared with fresh wind-borne pollen) and this results in fewer full seed per cone. That dead non-germinable pollen may be biologically active was shown by McWilliam (1959) who pollinated strobili of Austrian pine with heat-killed pollen which delayed ovule degeneration by about one month. Callaham (1967) showed that the dilution of viable pollen with heat-killed pollen did not influence cone set in interspecific pine crosses but dilutions containing ten or twenty per cent of viable pollen produced fewer full seeds per cone.

In the present study, dilution of pollen with talc to fifty and twentyfive per cent appeared to have little effect on the "total yield of seed per cone" but cone loss increased markedly in two clones following the application of diluted pollen. Thus, while a half and half mixture of pollen and talc provides as many effective pollen grains per pollen chamber as does undiluted pollen, the mass of pollen reaching the strobilus is reduced by half. It is possible that this reduction in the total mass of pollen is associated with a corresponding reduction in the amount of the hypothetical cone abscission inhibitor and this is the cause of the greater loss of cones.

On the assumption that pollen produces some active substance which prevents cone abscission the use of a number of plant growth substances to retard cone abscission has been tested by Hagman and Mikola, 1963 and McWilliam 1959. However, they reported little success in their attempts to reduce cone drop.

Finally, there is the problem of cone drop during the second season of development. Shedding of cones after they reach full size and before they are harvested in December of the second year may represent a high proportion of the total cone loss in some clones. We will collect and examine such cones to determine the cause of drop. It is possible that a minimum number of active embryos or endosperms are required to prevent premature fall of cones just prior to harvesting but it is already obvious that very wide clonal differences exist.

During the period from pollination to September of the second year cone drop is correlated with aspect. Cone drop on the north side of the crowns of Scots pine grafts at Newton nursery was almost double the drop on the south side. This applies, not only to wind-pollinated cones where the effect could be confounded with direction of pollen supply, but also to un-isolated control-pollinated cones where there is less reason to suspect that variation in pollen supply could affect the results. It will not be known until the cones are harvested whether there are low seed yields corresponding to the high cone drop on the north side of the crowns.

Thus it is obvious that a number of factors influence the retention of developing cones and in this study variations in pollen supply, cone size, and aspect of the strobilus in the crown have been found to be associated with variations in drop of developing cones. The precise role and interaction of these factors must be determined before variation in cone drop between clones can be explained and increase achieved in the proportion of pollinated strobili surviving to maturity.

Yield of seed per cone:

When the relationships between seed yield and total conelet and cone drop, conelet drop during the first growing season, and cone volume was studied, two sub-popula-

[2]) Other tests carried out by the Forestry Commission (R. Faulkner, personal communication) confirmed the effect of fresh pollen on the majority of cones tested.

tions appeared among the clones examined (see *Table 11*). In the first sub-population of clones approximately forty-four per cent of the variation in both "total number of seed per cone" and the per cent "full seed per cone" was associated with cone drop during the period from pollination to maturation. In the second, smaller, sub-population of clones, fifty per cent of the variation in the per cent "full seed per cone" was associated with cone volume.

Because cone drop and cone volume are characteristics of individual clones there may not be a causal relationship between all the clones. The success of these "polycross" and cone drop has been shown to increase when the supply of pollen to the strobili is reduced. Also cone drop is increased by the application of stored rather than fresh pollen. Thus, the quality and quantity of the pollen applied would be expected to be directly related to the final yield of seed. Just how closely conelet and cone drop are related to pollination conditions is another matter because conelet and cone drop varied from clone to clone when the pollination treatments were apparently identical. Moreover, within clones, conelet and cone drop are influenced by environmental factors such as the aspect of the strobili on the crown of the grafts. Further experiments are planned to define more precisely the relationship between the quantity and quality of the applied pollen and the final yield of full seed. The modifying effect of cone drop and clonal variation on this relationship will also be investigated.

Conclusions

The method commonly used to improve Scots pine by the application of genetics and breeding is to select parent trees, produce clones from them and put the clones in a seed orchard laid out to improve the possibility of crosses between all the clones. The success of these "polycross" seed orchards is largely dependent on an equal contribution of pollen and seed from each clone. Thus, knowledge of the variation between clones in the process of pollination, fertilisation and embryo development is essential. This knowledge will also be of value when attempting to exploit specific combining ability by crossing certain clones.

Although, in Britain, work on the variation in the seed and cones of Scots pine clones is at an early stage some general findings are emerging which point the way to improving seed yields.

During dissection of conelets to measure the distribution of marked pollen in ovules it was observed that even in conelets where there was a relatively high number of ovules without pollen there were masses of pollen on the central axis of the conelet and on the cone scales. From these observations and the results of pollen dilution experiments it seems unlikely that increasing the rate of pollen application will have an appreciable effect on the yield of full seeds from controlled crosses. It appears that what is required is a more effective delivery of pollen at the time of maximum receptivity of the strobili. McWilliam (1959) reported that, in Austrian pine, non-pollinated strobili remained receptive for longer than did pollinated strobili. It commonly happens that strobili are pollinated before they become fully receptive and are revisited and pollinated again. It may be that premature pollination results in maturation of the strobilus without further extension of the central axis and containing a low number of pollinated ovules. If this were the case one application of pollen ensuring maximum coverage of the strobili at their most receptive stage should give the best results. Both the total number of seed per cone and the proportion of full seed per cone would increase.

Fresh pollen produces a greater proportion of full seed per cone than does pollen stored for one year. The fresh pollen also reduces premature conelet and cone drop. In a large crossing programme use of fresh pollen may not be practicable and there is, therefore, need for improved pollen storage techniques.

Other workers have shown that the environmental conditions under which the mother trees are grown may influence the proportion of full seed produced. In the study reported in this paper it was seen that the duration of the period of isolation of the strobili by the isolation tubes also affected the proportion of full seed produced. It may prove of benefit to reduce the duration of isolation or improve the materials and methods used to isolate the strobili. A point to be cleared up is that after an adequate coverage of the strobilus by the applied pollen the chances of contamination by foreign pollen may be very much reduced so that the isolation tubes could be removed immediately after pollination without prejudicing the results of subsequent progeny trials.

At Newton, conelet and cone drop was found to be correlated with the aspect of the strobili on the crowns of the grafted trees. It is possible that low temperatures or exposure to wind affects conelet and cone retention. In the tree bank at Newton the crowns of many clones are noticeably more sparsely provided with foliage on the north side of the crown than on the south side and provision of shelter on this exposed site could help to increase cone retention.

The findings of this preliminary study are being followed up on a larger sample of forty clones. One of the first objects is to confirm that the Scots pine clones are a mixed population with respect to the correlation between conelet and cone drop and the yield of full seed per cone. The variation within and between the clones is being measured and the consistency of the yield of full seed from year to year is also being studied. Some clones which have been replicated in seed orchards throughout Britain have been pollinated with the same pollen mixture to allow measurement of environmental effects on seed development. In addition, each of the forty clones has been selfed in an effort to determine the relative differences in the load of deleterious genes carried by each clone.

In consistently low yielding clones studies will be made on such characters as strobilus development and ovule maturity at time of pollination, the length of the receptive period and size of the pollen chamber.

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Summary

The efficiency of methods of controlled pollination was assessed by observing the distribution of marked pollen grains in the pollen chambers of ovules.

About twenty per cent of ovules remained unpollinated and the expected total yield of full and empty seed per cone is around thirty-eight. This figure is seldom reached and some clones produce less than twenty full and empty seed per cone. It is suggested that one important reason for low total seed yields after controlled pollination is mistiming of the pollen application. Dilution of the amount of pollen applied by adding talc had little effect on the yield of full seed until the proportion of pollen fell to ten per cent. Conelet and cone loss significantly increased as the proportion of pollen was reduced.

The percentage of full seed per cone varied between clones from twenty to sixty. The use of fresh pollen rather than pollen stored for one year increased the proportion of full seed per cone. Lengthening the period of isolation of stro-

bili with celluloid tubes increased the proportion of empty seed in the final crop.

Premature abscission of conelets and cones caused serious loss of seed. A large proportion of conelets were shed in the weeks following pollination with losses continuing at a low rate until just before harvest in December of the following year when the rate of cone loss often increased. Between seventeen and eighty-five per cent of strobili pollinated abscissed before reaching maturity. Following wind pollination there was usually a greater loss of conelets and cones than after controlled pollination using stored pollen. After both wind and controlled pollination the rate trees was twice that on the south side.

A high rate of cone and conelet loss was associated with a high ratio of cone volume to number of seed per cone in the final crop. In this respect the clones studied appeared to form two sub-populations. In one, containing the longest number of clones, approximately forty-four per cent of the variation in total seed per cone and per cent full seed per cone was associated with cone loss. In contrast, in the other population where there was a smaller increase in cone and conelet loss as the ratio of cone volume to number of seed per cone increased, fifty per cent of the variation in "percent full seed per cone" was associated with cone volume.

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Growth and Nutrient Uptake of Aspen Hybrids Using Sand Culture Techniques

By Dean W. Einspahr[1])

Introduction

Projected forest industry raw material requirements and predicted forest land use trends have created considerable interest in intensive forestry practices. These projections emphasize the need for the production of species that will do well on low-quality sites and respond to improvements in soil fertility and soil moisture conditions. The study described is part of a larger comprehensive study under way aimed at evaluating the nutrient requirements of several types of aspen hybrids. The basic approach involves the use of sand culture techniques to compare the growth and nutrient requirements of aspen hybrids with comparable seedlings of the "parent species".

Recent tree nutrition studies, primarily with cottonwood (*P. deltoides*) and hybrids in the black poplar group (section *Aigeiros*) emphasizes that large growth increases result when optimum nutrient levels are maintained. Little data are available on the nutrient requirements of aspen and aspen hybrids. Nutrient deficiency symptoms described by Hacskaylo and Vimmerstedt (1967) have demonstrated the essential nature of a number of nutrient elements to the growth of cottonwood. Bonner and Broadfoot (1967) found cottonwood seedlings grew best when nutrient solutions contained 100 ppm N, 75 ppm P, and 100 ppm K. Similar optimum levels of N and K and a lower level of P (18—25 ppm) were reported by Phares (1966) for cottonwood grown using sand culture techniques under greenhouse conditions. Curlin (1967), in a study on 22 cottonwood clones, reported large increases in growth as a result of nitrogen fertilization and a strong "clone × fertilizer" interaction.

Satoo (1960) in N, K, and P sand culture studies with *P. davidiana* demonstrated the absence of nitrogen had the most serious influence on growth. Also of interest is the work of Shumakov (1963) who pointed out that aspen assimilates three to four times as much N, five times as much Ca, and four times as much P as pine. Cultivated poplars are reported to enrich the soil with humus and assimilated bases, such as Ca and Mg, but deplete the soil of N. Other Russian work by Slukhai (1962) with poplar

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