Seed development after self-pollination and cross-pollination of Scots pine, Pinus sylvestris L.

Fröutveckling efter självpollinering och korspollinering av tall, Pinus sylvestris L.

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Abstract

ODC 181.521/522: 174.7 Pinus sylvestris

The development of pollen tubes and ovules and the formation of embryos and endosperm after different types of pollination: self-pollination, cross-pollination and wind-pollination were studied in ten clones of Pinus sylvestris.

Pollination and fertilization took place in all types of pollination. At a certain stage of development, however, embryos in some seeds began to degenerate. This degeneration was followed by a breaking down of the endosperm and resulted in empty seeds. The percentage of degenerated seeds was highest after self-pollination, considerably lower after wind-pollination and smallest after cross-pollination. The results indicate that inviable zygotes, homozygous for lethal or sub-lethal genes, are formed after self-pollination and explain the high percentage of empty seeds usually found after this type of pollination. Wind-pollination may to some extent involve self-pollination.

No clone was found to be quite self-sterile, but the differences in self-fertility between the clones were rather great.

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Contents

1 Introduction

The purpose of the investigation was to study seed development in the Scots pine, *Pinus sylvestris,* for different types of pollination: self-pollination, cross-pollination and wind-pollination.

Seed setting after different types of pollination has been studied in a number of contexts. Embryologic development in particular has been studied in species of *Pinus, Picea* and *Pseudotsuga* and others (Hagman & Mikkola 1963; Kriebel 1967; Krugman 1970; McWilliam 1959; McWilliam & Mergen 1958; Mergen *et al* 1965; Mikkola 1969; Orr-Ewing 1957 a; Sarvas 1962).

If pollination was satisfactory, a low yield, or no yield whatsoever, of full seed may be caused either by failure to fertilize or by the zygote breaking down at a relatively early stage. Here the first case is described by the term *incompatibility* and the second case by the term *sterility* (cf. Bingham & Squillace 1955; Hagman 1967; Krugman 1970 and others). Sterility may be absolute when no embryo reaches full development, or partial when embryos are developed in some seeds. The term *inviability* is sometimes used in the same sense as sterility. In his analyses of seed setting Sarvas (1962) prefers to calculate *the embryo mortality.*

Incompatibility has not been found in connection with self-pollination of gymnosperms. The pollen tubes grow down and reach the egg cell, fertilization takes place and the zygote continues its development.

The incompatibility in angiosperms, on the other hand, is an effective system of preventing undesirable inbreeding of plants which are capable of cross-fertilization (Bateman 1952). Incompatibility in gymnosperms occurs mainly after crosses between species. Sterility between species has also often been found, i.e. fertilization takes place, but the embryo dies at the 4—8-

nucleate stage (Hagman 1967; Hagman & Mikkola 1963; Klaehn & Wheeler 1961; Kriebel 1967, 1970; Li 1964; McWilliam 1959; Mikkola 1969; Ueda *et al* 1961; Vidakovic 1963). It was found that in relation to the large number of different combinations of species tested, only a small number produced full seed. In 1959, Schütt published a detailed survey of the combinations carried out up to that date, and Kriebel (1970) has reported on more recent experiments with crosses between species. Occasional individuals may, however, produce hybrid seed after a combination which was considered to be incompatible (Mikkola 1969).

The seed yield after a more or less extreme inbreeding of conifers is normally considerably lower than after hybridisation. In the opinion of most scientists, this is the result of a homozygotisation of recessive lethal genes. The embryos are unable to develop and die at a fairly early stage ("sterility", "inviability", "embryo mortality" Hagman 1967; King *et al* 1970; Koski 1971; Krugman 1970; Mergen *et al* 1965; Orr-Ewing 1957 a; Sarvas 1962). Diekert (1964) considered the possibility of an "intolerance system" ("Unverträglichkeitsystem"), similar to the incompatibility systems with multiple sterility genes, but effective only after fertilization.

Gymnosperms are in other words more or less self-sterile. There are, however, exceptions to this. Fowler (1962, 1964, 1965 a, b), amongst others, has found that *Pinus resinosa* is a species which is almost free from the defects of inbreeding. In his view this is explained by the fact that the present stock of *Pinus resinosa* is derived from a limited number of trees which have survived after forest fires or drastic felling. Inbreeding of the trees has made successive

generations more and more homozygotic and uniform. Recessive homozygotes having been unable to survive, lethal genes have gradually been weeded out.

Seemingly contradictory information is given by Johnson (1945), who found that seed setting was considerably lower after self-pollination than after cross-pollination and wind-pollination of *Pinus resinosa*. However, these results referred to only one tree. Langner (1957, 1959) showed in experiments with *Picea omorika* that this species is highly self-fertile; the progeny after self-pollination did, however, contain a comparatively high percentage of chlorophyll mutants.

For this work, cones after self-pollination were examined at different stages of development in order to determine more closely the stage at which breaking down of the embryo occurs. As a comparison, an examination was made of the seed development in cones after wind-pollination and after controlled crosses. Further, controls were set up—in other words, development was studied after isolation without pollination, after pollination with interspecific pollen and with genetically dead pollen, and after injection of the female flowers (female inflorescences[1]) with growth hormone.

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[1] Instead of the term "female inflorescence" (strobilus), the term "female flower" will be used for the remainder of this report.

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2 Material and methods

Grafts from 10 clones in an orchard at Tjuttorp, in the province of Östergötland, were selected as trees for the experiment. The clones were free flowering and represented mother trees from different localities, as follows:

	Locality	Latitude	Longitude	Altitude above sea level m
Orchard	Tjuttorp	58°48′	15°50′	55
Mother tree:				
E 3003	Mottorp	58°47′	15°52′	55
E 3004	Brevik	58°46′	16°18′	85
H 1007				
H 1008				
H 1010	N:a Kvill	57°45′	15°33′	170
H 1011				
S 3098	Vägsjöfors	60°22′	12°63′	170
W 1015	Dalfors	61°16′	15°28′	250
W 3123	Östanvik	61°11′	15°21′	270
W 3124	Östanvik	61°	15°13′	250

Longitude calculated East from Greenwich.

Crosses were carried out during 1964—1966 (Table 1). Within each clone the number of isolations was spread over five graft trees. The flowers were isolated by means of butyrat tubes with foam plastic stoppers.

The crosses carried out in 1964 included all ten clones crossed with six father clones, besides wind-pollination and controls. Studies made on this material covered the development stages during the first and the beginning of the second vegetation period, i.e. the growth of male and female gametophytes, fertilization and zygote formation. From the crosses carried out in 1965 and 1966 sample cones were collected at certain set times during July—August 1966 and 1967, and above all, a study was made of the development of embryos and endosperms during the latter half of the second vegetation period. The combinations compared were self-pollination, cross-pollination with a common pollen, wind-pollination and controls.

Isolation without pollination—"open control"—meant that the stopper of the isolation tube was removed for a few seconds at the time when the flowers were considered to be receptive. *Pinus banksiana,* which belongs to a different group of the sub-genus *Diploxylon* from *Pinus sylvestris* and does not hybridize with this species, was chosen as the interspecific pollen. In 1964 genetically dead pollen was produced by heating, but for the 1965 controls 10-year-old pollen was used. Germination tests *in vitro* showed that the pollen was unable to germinate, 0.01% indole-acetic acid was used as a hormone solution. The open control was intended to determine the reliability of the method of isolation and pollination which had been used. The purpose of the other

Table 1. Crosses carried out during 1964—1966.

♀ \ ♂	E 3003	E 3004	H 1007	H 1008	H 1010	H 1011	S 3098	W 1015	W 3123	W 3124	wind-pollination	open control	dead pollen	hormone	P. banksiana
E 3003	1964 1965 1966	1964 1966	1964	1964	1964	1964	1965				1964 1965	1964 1965	1964 1965	1964 1965	1965
E 3004	1964 1966	1964 1965 1966	1964	1964	1964	1964	1965				1964 1965	1964 1965	1964 1965	1964 1965	1965
H 1007	1964	1964 1966	1964 1965 1966	1964	1964	1964	1965				1964 1965	1964 1965	1964 1965	1964 1965	1965
H 1008	1964	1964	1964	1964 1965 1966	1964	1964	1965				1964 1965	1964 1965	1964 1965	1964 1965	1965
H 1010	1964	1964 1966	1964	1964	1964 1965 1966	1964	1965				1964 1965	1964 1965	1964 1965	1964 1965	1965
H 1011	1964	1964 1966	1964	1964	1964	1964 1965 1966	1965				1964 1965	1964 1965	1964 1965	1964 1965	1965
S 3098	1964	1964 1966	1964	1964	1964	1964	1965 1966	1965			1964 1965	1964 1965	1964 1965	1964 1965	1965
W 1015	1964	1964 1966	1964	1964	1964	1964	1965	1965 1966			1964 1965	1964 1965	1964 1965	1964 1965	1965
W 3123	1964	1964 1966	1964	1964	1964	1964	1965		1965 1966		1964 1965	1964 1965	1964 1965	1964 1965	1965
W 3124	1964	1964 1966	1964	1964	1964	1964	1965			1966	1964 1965	1964 1965	1964 1965	1964 1965	1965

three control series was to try to stimulate cone and seed formation without normal fertilization.

During the sampling one cone per combination of crosses examined, usually from one grafted tree per clone, was collected and fixed. The material was then bedded in paraffin, sectioned and dyed, usually with safranin and fast green.

3 Development during the first vegetation period

The samples taken during the first vegetation period (crosses carried out in 1964) showed a certain variation in the development stages of male and female gametophytes. There was, however, no lasting difference in rate or mode of development either between types of pollination (self-pollination, cross-pollination and wind-pollination) or between clones. Development followed a normal course in all the combinations. The tissues of the control cones collected after open control, pollination with dead pollen and spray application of hormone solution were however, in the process of decomposition and degeneration.

4 Development during the second vegetation period

4.1 Crosses in 1964

H 1010 was selected as mother clone for the analysis of seed development during the early part of the second vegetation period. Within the clone, a comparison was made between self-pollination, crosses with trees from the same population (H 1008), crosses with trees of a different provenance (E 3004), and wind-pollination. In all these combinations, second year growth started at the end of May. Frequent samples taken during June showed variations in the rate of development between seeds from within one cone, between different cones on the same graft and between cones from different grafts. The variations were, however, quite random; the summing up of the observations up to the formation of the zygote showed no differences between combinations. Seed after both self-pollination and other crosses developed entirely in accordance with what is considered normal.

4.2 Crosses in 1965

A report of the material collected during the summer of 1966 is given in Table 2. The sampling times were chosen with a view to charting the course of development of the seeds during the latter part of the second vegetation period.

4.2.1 4th July 1966

During the observations made on 4th July, polyembryony (Figure 1: 1—2) was found in all combinations. Polyembryony arises either through the pollination of several archegonia (polyzygotic polyembryony), or through the cleavage polyembryony which occurs in pine. In this several independent embryos are formed from one zygote. At this stage the suspensors were still visible and the embryos small. Starch had been laid down in the female gametophyte tissue which from this stage on is called endosperm. Primary embryo cavities had formed where the reserve food material of the archegonium had been used up by the growing pro-embryo, or where the unfertilized archegonium had degenerated. Secondary embryo cavities had gradually formed as the embryos penetrated into the endosperm.

The stages of development of seeds in different clones and pollination types are shown in Figure 2.[2] There were also occasional seeds at the early zygote stage or even at pre-fertilization stages.

The material as a whole showed no difference in seed development between one type of pollination and another. The frequency of class Ib seed was slightly lower after self-pollination than after cross-pollination and wind-pollination, but no definite difference can be demonstrated statistically.

Similarly, no difference could be found in the rate of seed development between cross-pollination and wind-pollination within the separate clones. The clones did however differ as regards the relation between these pollination types on the one hand and self-pollination on the other. Seed development had progressed further after self-pollination

[2] In Figures 2—12 the stages of seed development have been divided into uniform classes. Figure 1 gives an explanation of the different classes, together with references to the corresponding photos. The number of seeds per cone both within and between clones varied considerably. In spite of this, and in spite of the occasional lack of information regarding combinations, the average for each type of pollination has been calculated and compared on each occasion when sampling took place.

Table 2. Crosses in 1965. Samplings during the summer 1966.

Combination	4th July	20th July	7th August	Combination	4th July	20th July	7th August
E 3003 × E 3003	1	1	1	H 1011 × H 1011	1	1	1
× S 3098	1	1	1	× S 3098	1	1	1
wind pollination	1	1	1	wind pollination	1	1	1
hormone		1		× P. banksiana	(1)	1	1
× P. banksiana	(1)		(1)	S 3098 × S 3098	1	1	1
E 3004 × E 3004	1	1	1	× W 1015		1	1
× S 3098	1	1	1	wind pollination	1		1
wind pollination	1	1	1	× P. banksiana	(1)	1	(1)
hormone		1		W 1015 × W 1015	1	1	1
× P. banksiana	(1)	1	(1)	× S 3098	1	1	1
H 1007 × H 1007	1		1	wind pollination		1	
× S 3098	1	1	1	× P. banksiana	(1)	(1)	(1)
wind pollination	1	1	1	W 3123 × W 3123	1	1	1
dead pollen		(1)		× S 3098	(1)	1	1
hormone		1		wind pollination	1		1
× P. banksiana	(1)	1	1	× P. banksiana		1	1
H 1008 × H 1008	1		1	W 3124 × S 3098	1	1	1
× S 3098	1	1	1	wind pollination	1		1
wind pollination	1	1	1	× P. banksiana		1	(1)
× P. banksiana		(1)					
H 1010 × H 1010	1	1					
× S 3098	1	1	1				
wind pollination	1	1	1				
dead pollen		(1)					
× P. banksiana	1	1	(1)				

(1) = cone without seeds.

Figure 1. Stages of seed development during the second vegetation period.

Photo No   Seed class

I. *Polyembryonic seeds*
1   a. Early zygote stages
2   b. Small embryos, $< \frac{1}{2}$ embryo cavity
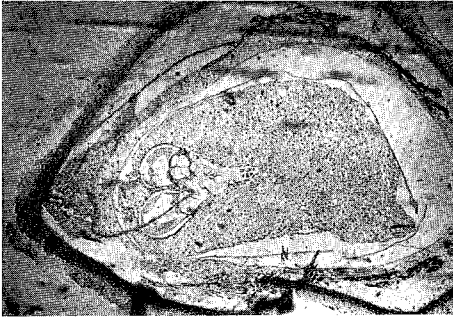
II. *Well developed seeds*
3   a. Large, dominant embryo, not totally differentiated
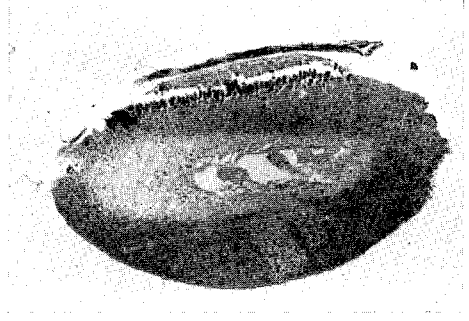4   b. One, well differentiated, embryo

III. *Degenerating seeds*
5   a. Degenerating embryos, normal endosperm
6   b. Degenerating embryos, degenerating endosperm
7   c. No visible embryos, degenerating endosperm

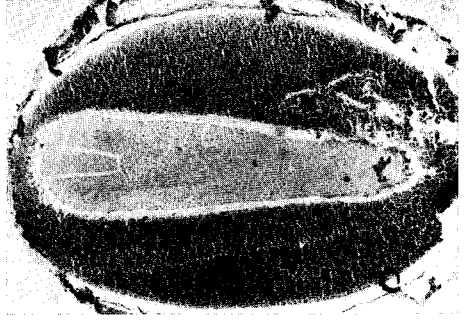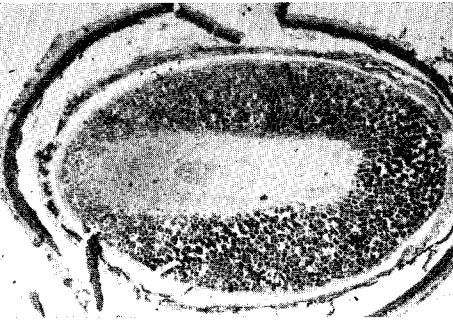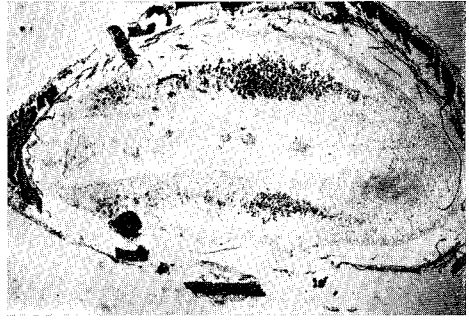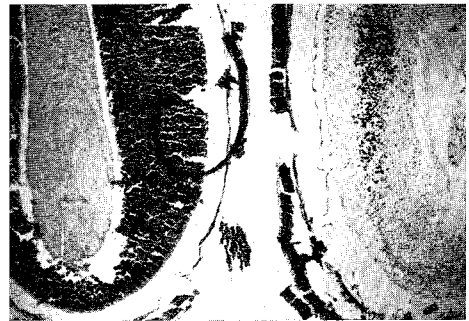8   Well developed seed (IIb) and degenerating seed (IIIb) in August 1967.
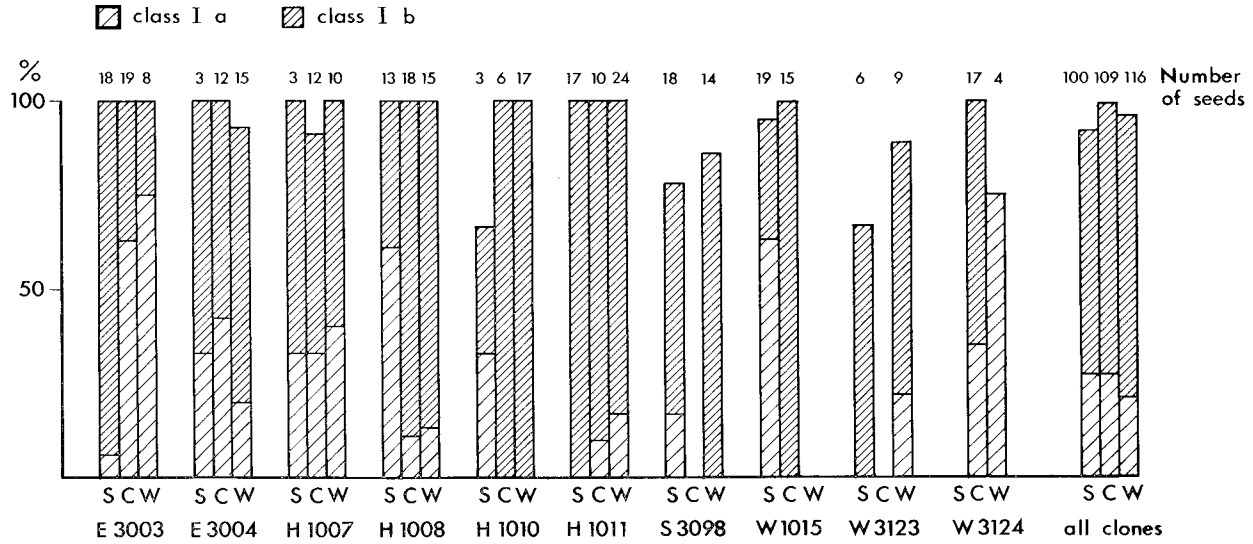
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Figure 2. Crosses in 1965. Seeds in different stages of development (seed classes) on *4th July 1966*.

than after cross-pollination and wind-pollination within clone E 3003, whilst the reverse was the case in, for example, clones H 1008, H 1010 and W 1015.

4.2.2 *20th July 1966*

Seeds from cones collected on 20th July 1966 still showed polyembryony, usually with one or two dominant embryos. Many embryos had started to differentiate into apical meristem and cotyledons. At this time the endosperm was rich in starch. It is however obvious that the development of some seeds did not follow this pattern. The embryos in these seeds had ceased to develop and had started to decompose. The degeneration was followed by the breaking down of the endosperm. The fact that some seeds had deteriorated embryos but a fairly normal endosperm implies that the degeneration of the embryos is the primary occurrence. In seeds where the degeneration of the tissues had gone so far that the embryo structure could no longer be distinguished, the cells in the endosperm were vacuolized and contained hardly any protoplasm (Figure 1: 7).

The stages of seed development for different pollination types and clones are shown in Figure 3.

On this occasion too, as in the case of the cones collected on 4th July, the number of seeds per cone varied considerably.

As regards the frequency of seeds at different stages of development, obvious differences between types of pollination were found. In the case of *self-pollination* a comparatively low percentage of seeds—16 %—was found in the most advanced embryo and endosperm stages (class II). The corresponding value for *cross-pollination* was 83 %, and for *wind-pollination* 75 %. Degenerating seeds (class III) accounted for a good 40 % after self-pollination but less than 10 % after cross-pollination and wind-pollination. The frequency of seeds in which the embryos were still very small (class Ib) gives some idea of the rate of development of the seeds. After self-pollination approx. 40 % were at these stages, after wind-

pollination approx. 20 %, and after cross-pollination, where the development appeared to have been most rapid, the figure was approx. 10 %.

After self-pollination the percentage of well developed seeds did not reach the values for the two other types of pollination in any clone. Clones E 3003, H 1010 and W 3123 had 20—30 % seeds with large dominant embryos, whilst in the remaining clones the percentage varied between 4 % and 16 %. The frequency of degenerating seeds (class III) was highest in clones H 1010 and H 1011 (50 % and 76 % respectively). This can be explained by the fact that these two clones, which at the time the sample was taken had the lowest percentage of undeveloped seeds after self-pollination, were on an average at the most advanced stage of development. As has been previously pointed out, seed development after cross-pollination was fairly rapid. Clone H 1008, which had 81 % undeveloped seeds (class I), was however a striking exception to this. After wind-pollination, too, this clone had a comparatively high percentage of seeds at an early stage.

4.2.3 *7th August 1966*

On 7th August, when the last samples of the summer of 1966 were taken, seed development had progressed sufficiently far for most seeds either to contain one, well differentiated, embryo, or to show obvious signs of degeneration (Figure 4, Figure 1: 8). Seeds from cones after *cross-pollination* either were well developed and had differentiated embryos (class IIb) or were completely degenerated (class IIIc). The same applied to seeds after *wind-pollination*, with the exception of a few degenerating seeds in which the embryos had not yet completely decomposed (class IIIb). Seeds resulting from *self-pollination* showed almost all stages of development (see Figure 5).

It was even more obvious now than previously that the development of the seeds after self-pollination differed from that after cross-pollination and wind-pollination. It
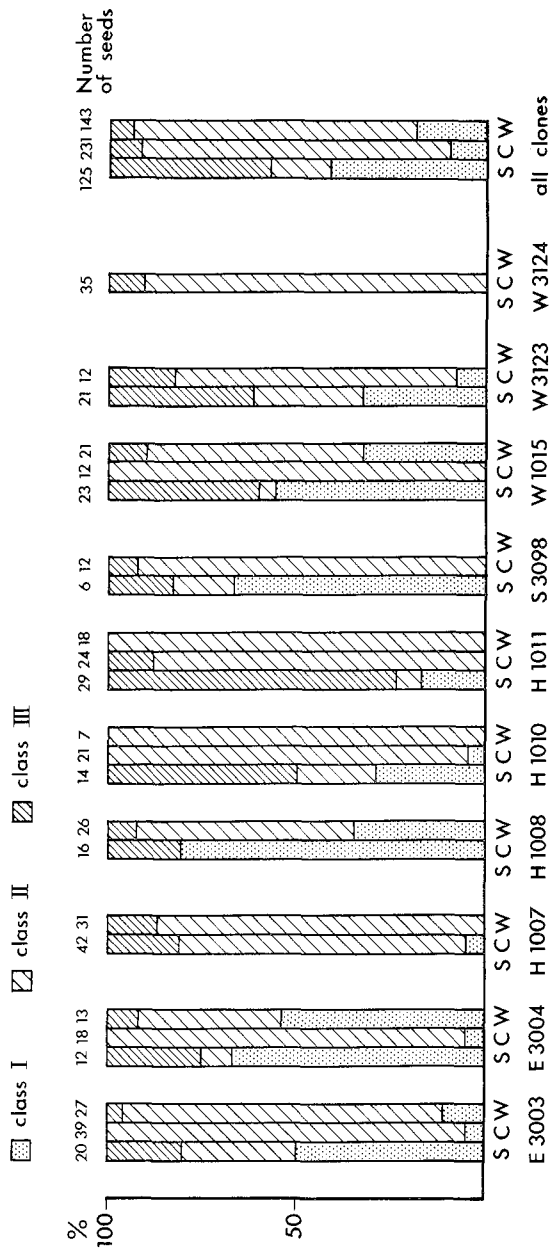
Figure 3. Crosses in 1965. Seeds in different stages of development (seed classes) on *20th July 1966.*
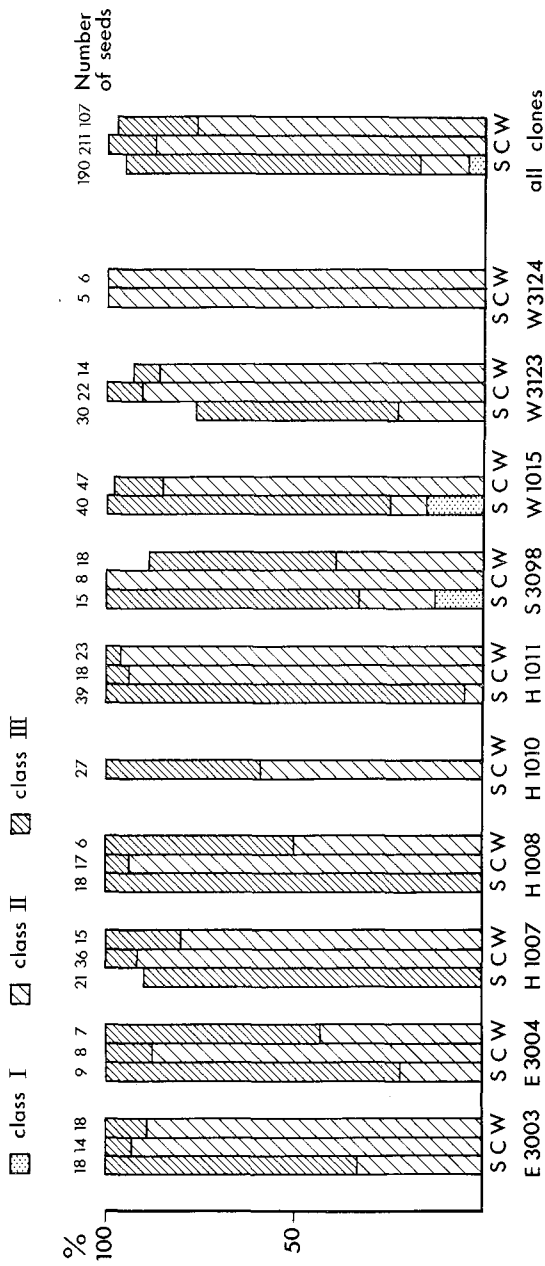
Figure 4. Crosses in 1965. Seeds in different stages of development (seed classes) on *7th August 1966.*
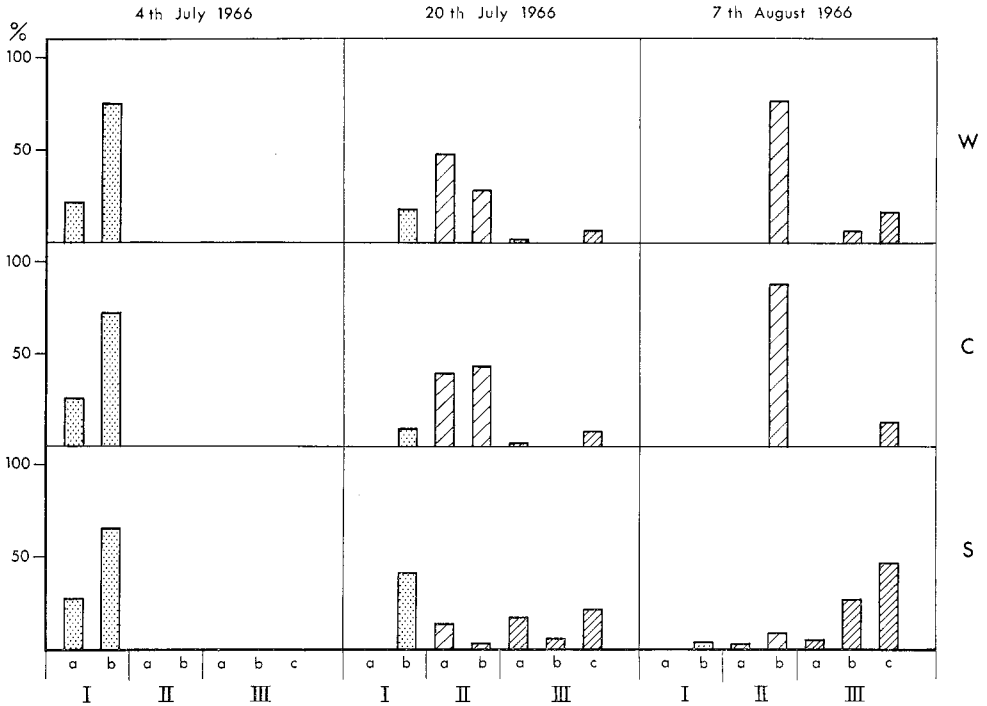
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Figure 5. Crosses in 1965. Seeds in different stages of development (seed classes) at samplings during the summer 1966. Averages of all clones.

was also possible to trace a tendency towards inferior seed quality after wind-pollination as against cross-pollination.

The percentage of well developed seeds (class II) within each type of pollination did not differ to any great extent from the results of the previous sampling (20th July, Figure 3), but within the class there had been a shift towards obviously differentiated embryos (class IIb). The frequency of seeds with embryos and endosperms in the process of breaking down was however considerably greater at this later date, after self-pollination in particular. It is probable that most of the seeds which had been classified as undeveloped on 20th July, had stopped growing and later degenerated.

A *comparison between the individual clones* showed certain differences in the seed development within the different pollination types. In clone E 3003 self-pollina-

tion resulted in more than 30 % well developed seeds, while these were completely lacking in, for example, clones H 1007 and H 1008. In these all the seeds had degenerated (class III). In nine of the clones examined the frequency of seeds with well developed embryos and endosperms (class II) after cross-pollination was between 85 % and 100 %. After wind-pollination, on the other hand, the quality of the seed varied greatly from one clone to another. At this time half or more of the seeds from clones E 3004, H 1008 and S 3098 had degenerated (class III), whilst the other clones had a degeneration frequency of between 4 % and 20 %. Occasional seeds with abnormal cells occurred in all three types of pollination. One or two cell formations of this kind had been recorded during earlier samplings, and the frequency was particularly high after hybridisation with *Pinus banksiana*.

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This year the average total number of seeds per cone[a] appeared to be the same after self-pollination as after cross-pollination. The average after wind-pollination was lower. This could indicate a more complete pollination of the isolated flowers as compared to those where wind-pollination was allowed (compare however with the result of the following year). In all types there was a very large variation in the number of seeds within and between clones and between samplings.

Figure 5 gives a summary of the results from the three samplings undertaken during the summer 1966.

4.3 Crosses in 1966

Seed development during the second vegetation period was again studied during the summer of 1967. From all 10 clones, cones after self-pollination and after crossing with clone E 3004 were collected on 17th, 24th, and 31st July and on 14th August. To enable a comparison between self-pollination and cross-pollination to be made in clone E 3004 as well, this clone had been crossed with E 3003.

4.3.1 *17th July 1967*

Seed development, which is to a large degree dependent on the weather throughout the year, was slower this year than during the previous one. Seeds collected on July 17th (Figure 6) showed polyembryony and were still at stages of development which had been passed at the same time the year before. Occasional seeds showed signs of degeneration. As was the case on July 4th 1966 (page 11) it was difficult to determine whether there were any definite differences between pollination types and clones.

4.3.2 *24th July 1967*

On July 24th (Figure 7) polyembryony was still general, but it was observed that in most seeds one embryo had developed further than the others. Occasional dominant embryos had differentiated into rudiments of apical meristem and cotyledons.

The difference between the two types of pollination was more marked now than a week earlier. For the whole material the frequency of undeveloped seeds (class I) was approximately the same for both pollination types, but whilst an average of only 9 % of the seeds had well-developed embryos and endosperms after *self-pollination*, the corresponding figure for *cross-pollination* was 46 %. The difference is also illustrated by the proportion of degenerating seeds (class III), which was 48 % after self-pollination, as compared to 15 % for the crossing with E 3004. The *variation between clones* was also considerable. After self-pollination, E 3003 and S 3098 had a fairly high frequency of well-developed seeds, whilst for example H 1008 had 67 % degenerating seeds and the remainder undeveloped. A high proportion of undeveloped seeds was found in clone W 3123. After cross-pollination the seed quality in clone H 1010 was markedly poorer than in the other clones. In this clone 61 % of the seeds were declining and the remainder developed late. In clones H 1008 and W 1015 development was fairly slow with many seeds in early stages.

4.3.3 *31st July 1967*

Figure 8 shows the position one week later, on 31st July. After *self-pollination* the frequency of degenerating seeds had increased from 48 % to 73 %, whilst on the other hand after *cross-pollination* there was an increase in the frequency of well-developed seeds. In the latter type of pollination 82 % of the seeds now had large dominant embryos, most of them differentiated. Judging by the results from the previous year it could be assumed that a large proportion of the seeds which were still at the polyembryonic stages would not develop further.

[a] The number of seeds shown in the bar charts signifies the number of classifiable seeds, which in some cases differs from the total number of seeds per cone due to accidents during the preparation of specimens.
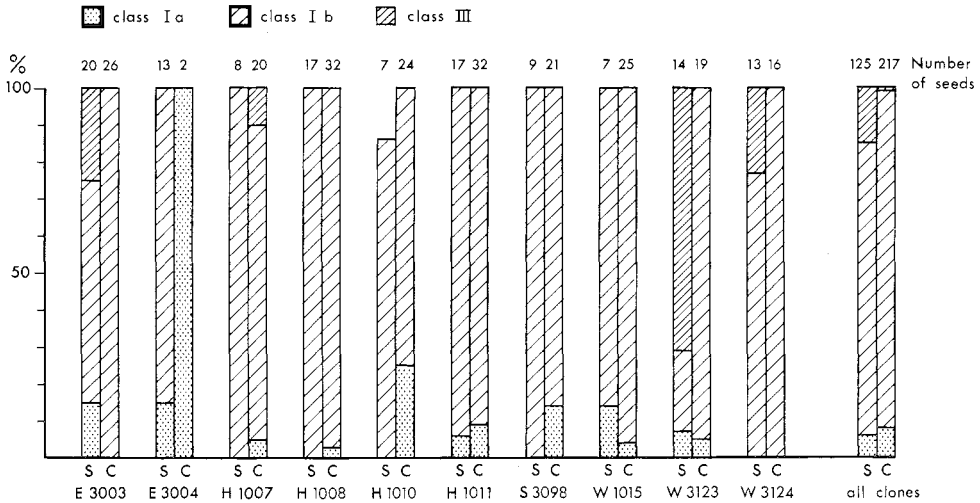
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Figure 6. Crosses in 1966. Seeds in different stages of development (seed classes) on *17th July 1967*.
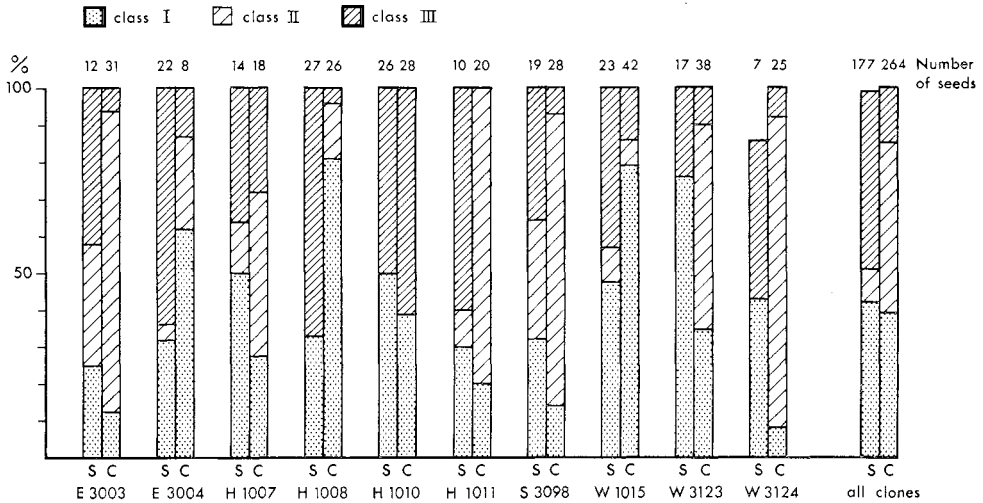
Figure 7. Crosses in 1966. Seeds in different stages of development (seed classes) on *24th July 1967*.

The *difference between the clones* as regards the frequency of seeds at different stages was obvious, especially after self-pollination. In clone E 3003 almost half of the seeds had dominant embryos. As at earlier samplings, the frequency of well developed seeds in clone S 3098, was comparatively high (42 %) and in clones H 1010 and W 3123 it was 23 %. Other clones were characterised by a very high percentage of degenerating seeds. The quality of the seed in clone W 1015 was exceptionally poor, with only undeveloped or degenerating seeds. After cross-pollination, between 80 % and 100 % of seeds with well developed embryos were usually obtained, most of them differentiated into rudiments of cotyledons and apical meristems (class IIb). The
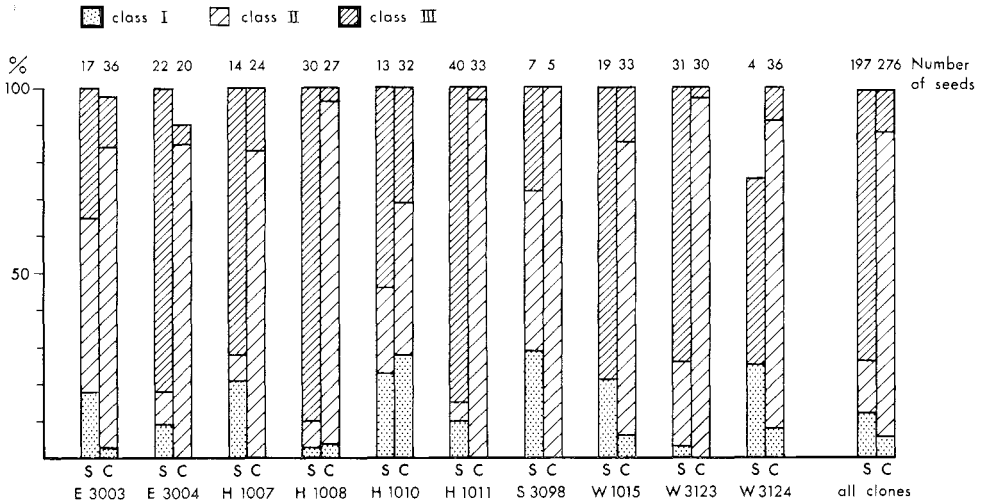
Figure 8. Crosses in 1966. Seeds in different stages of development (seed classes) on *31st July 1967*.
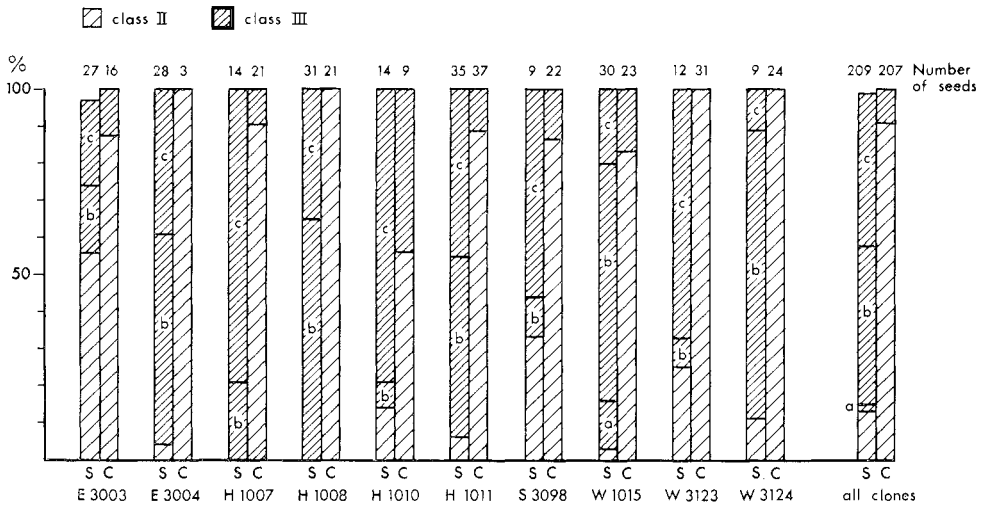
Figure 9. Crosses in 1966. Seeds in different stages of development (seed classes) on *14th August 1967*.

exception was clone H 1010 in which only 41 % of the seeds had dominant, but as yet undifferentiated embryos (class IIa).

4.3.4 *14th August 1967*

The appearance of the seeds on 14th August (Figure 9) indicated that the morphological development was almost complete. This was particularly obvious after cross-pollination where the seeds contained either one well differentiated embryo (class IIb) or the remains of degenerated tissue (class IIIc). The slower development of seeds after self-pollination was apparent from the fact that in more than half of the degenerating seeds (class III) the embryo and endosperm tissue had not yet completely decomposed. A com-

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Table 3. Crosses in 1965. Analysis of variance of the total number of seeds per cone. Self-pollination and cross-pollination.

Source of variation	D.f.	Mean Square	F
Pollination types	1	720	9.2*
Clones	8	864	11.1***
Error	180	78	

Table 4. Crosses in 1966. Analysis of variance of the total number of seeds per cone. Self-pollination and cross-pollination.

Source of variation	D.f.	Mean Square	F
Pollination types	1	6 024	95.6***
Clones	9	1 455	23.1***
Error	752	63	

parison with the results from 31st July was interpreted to mean that what were then undeveloped seeds (class I) after cross-pollination had to a great extent developed normally, whilst the seeds after self-pollination had degenerated.

The results of a *comparison between the clones* at the time of this last sampling corresponded well with the results obtained earlier in the summer. After self-pollination the highest incidence of well developed seeds with differentiated embryos—clearly above average—was found in clones E 3003, S 3098 and W 3123. Five of the clones had a very low percentage of seeds in the optimum seed development class. Clones W 3124 and H 1010 occupied an intermediate position. As had previously been the case, after cross-pollination this latter clone, through its low percentage of seeds with well developed embryos, showed a marked difference from the other clones, which had 80 % or more of full seeds.

As in the previous year, the total number of seeds per cone varied within a clone as well as from one clone to another. In the latter year, however, it was found that the

number of seeds was almost invariably lower after self-pollination than after cross-pollination. The exception was clone E 3004, where the reverse was the case. An analysis of variance by clone and pollination types of the number of seeds per cone was therefore carried out. The values for samples obtained during the summer of 1966 were analysed at the same time. To ensure more accurate conclusions, the results from the collection of mature cones during the autumns of 1966 and 1967 were also included. Both years showed significant differences between clones. The difference between pollination types was marginally significant in 1966 but quite definite in 1967 (Tables 3 and 4).

A summary of the test sampling results during the summer of 1967 is given in Figure 10.

4.4 Controls for the 1965 crosses

The same series of controls was carried out for the 1965 crosses as for 1964. Further, the 10 mother clones were crossed with pollen from *Pinus banksiana*. Except where inter-species crosses had taken place, only very few cones remained at the start of the second vegetation period (Table 5), and this number was further reduced during the course of the summer.

After *open control* two cones from clone H 1007 and one cone from clone S 3098 were collected in the autumn of 1966. It appeared that the latter contained full seeds. One of the cones from H 1007 contained no seeds, the other contained one empty seed.

After pollination with *dead pollen*, two cones were collected on 20th July 1966, one cone from clone H 1007 and one from clone H 1010. Neither cone contained a seed. A cone from H 1007, collected in the autumn of the same year, contained an empty seed. The remaining cones showed no further development.

Of the cones remaining after *hormone spray-application*, which were collected on 20th July, one each from clones E 3003 and E 3004 contained full seeds. In the cone

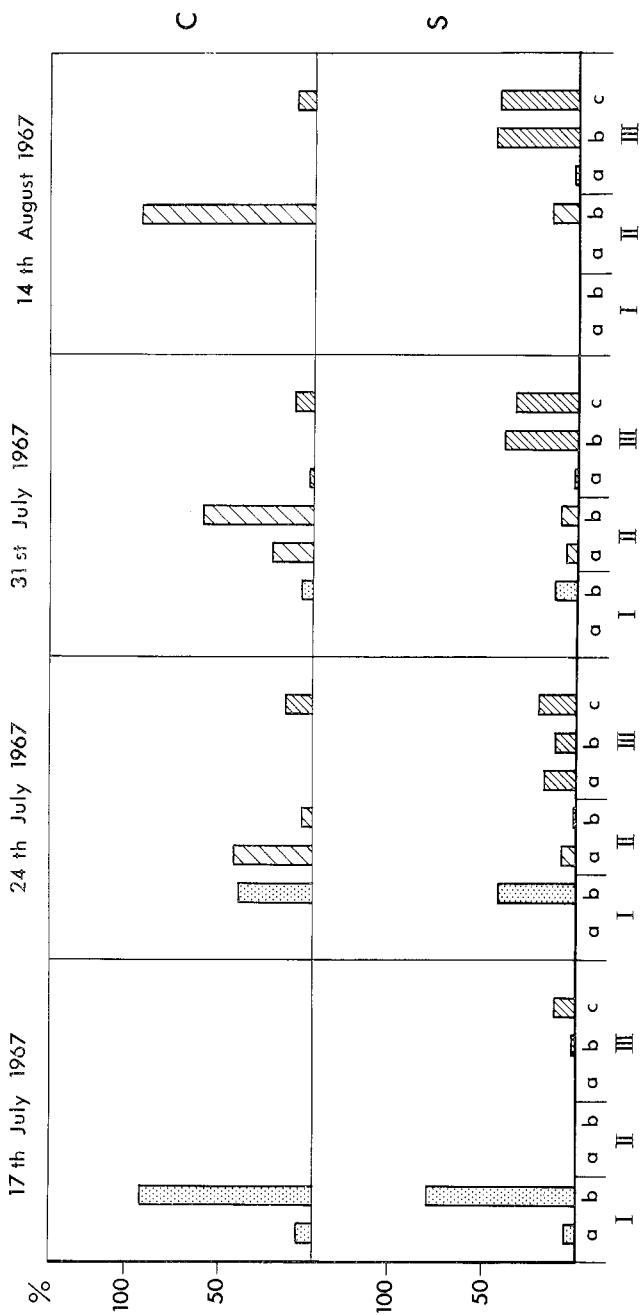Figure 10. Crosses in 1966. Seeds in different stages of development (seed classes) at samplings during the summer 1967. Averages of all clones.

Table 5. Crosses in 1965. Controls. Number (%) of remaining cones in May 1966.

Motherclone	Open control	Dead pollen	Hormone	×P. banksiana
E 3003			1(4)	7(15)
E 3004			1(5)	39(88)
H 1007	4(20)	3(15)	1(6)	36(92)
H 1008				3(7)
H 1010		2(8)	3(12)	36(78)
H 1011				30(77)
S 3098	1(4)	1(4)		26(55)
W 1015				17(41)
W 3123				21(46)
W 3124				25(61)

from clone H 1007 an empty seed was found. The graft in clone H 1010 in which the three control cones had been recorded in May 1966 was dead at the July sampling, and the cones were dry.

During the summer a total of 25 cones from the cross with *Pinus banksiana* were collected. 14 of these proved to lack seeds (Table 2), whilst most of the other cones contained a small number of seeds. Approximately half of the seeds from all clones were small and undeveloped. In the seeds of normal size which had formed, the female gametophyte had begun to emerge. Pollen grains were apparent in the pollen chamber, but they had not swelled or developed pollen tubes. Thus no fertilization had taken place, nor had any embryos developed. A cone which was collected on 4th July contained two seeds with apparently normal endosperms. In other seeds, collected later in the summer, the endosperm tissue had thinned out and was in the process of decomposing.

From Tables 2 and 5 it can be seen that the clones reacted differently to pollination with *Pinus banksiana*. Clones with few remaining cones at the start of the second vegetation period, e.g. E 3003 and H 1008, lacked seeds when samples were taken later in the summer. It appeared that if a larger number of cones remained, more cones also contained seeds.

The result from the collection of mature cones in the autumn showed that the total percentage of cones without seeds, in all clones, had gone down from 56 % to 40 %. This indicates that the cones which had dropped during the vegetation period were those in which no seeds had formed.

5 Discussion

In the material examined pollination and fertilization took place within all pollination types and in all clones. The course of the resulting seed development was "normal" during the early embryonic stages but the rate of development varied to some extent. Successive observations showed the development to be similar up to some time in July during the second vegetation period. After this time a degeneration of the embryos of some seeds occurred, followed by the breaking down of the endosperm. From observations made during August it was apparent that the degeneration continued until the seeds were empty.

5.1 Differences between types of pollination

The degeneration of embryo and endosperm occurs in all types of pollination. The types differ mainly in the varying frequency of degenerating seeds. This frequency is greatest after self-pollination. It is probable that sterility as defined on page 5 depends in this case on the homozygotisation of sublethal genes which leads to the further development of the embryos being arrested. There is a physiological interaction between embryo and endosperm, since the existence of an embryo appears to be the pre-requisite for the endosperm to stay alive. A higher frequency of seeds with decomposing tissues was found after wind-pollination than after cross-pollination. This difference in the amount of degeneration is explained by the fact that wind-pollination, whether it concerns a single tree or a clone in a seed orchard, may involve some degree of self-pollination. This question is frequently discussed in connection with the assessment of orchard seed, and many efforts have been made to estimate the risks of self-pollination in this and other types of

plantations and stands. As an example it may be mentioned that Sarvas (1962) estimates that approximately 26 % of the pollen which surrounds a tree in a normal stand of Scots pine (*Pinus sylvestris*) consists of the tree's own pollen. Only 7 % of full seeds after wind-pollination are however the result of self-pollination. Franklin (1969) considers the main effect of spontaneous self-pollination in seed orchards to be a reduced seed yield. His investigation of *Pinus taeda* showed that the proportion of seed plants resulting from self-pollination in comparison with plants after cross-pollination is so small as to be negligible. In his experiments with *Pinus resinosa,* Fowler (1965 c) estimated that less than 10 % self-pollination occurs in an orchard where trees are closely spaced; in a small isolated stand the corresponding figure is approximately 20 %. However, Fowler stresses most strongly of all the differences in self-pollination frequency between different parts of the head of a tree. This frequency may be twice as high in the lower part of the head as in the upper part. In an extensive examination of grafts in an orchard, Hadders (1971) found that 35 % of the seeds from cones on the lower branches, where there are plenty of male flowers, were empty, whilst only 15 % of the seeds from cones on higher branches were empty. Bearing in mind that the cones after wind-pollination which are included in the present investigation were normally gathered from the centre and lower parts of the head, self-pollination could have had some influence on the seed yield.

In a natural population where adjacent trees are often related, homozygotisation of sub-lethal factors may be the cause of empty seeds occurring. In the case of artificial pollination between assumedly un-

related individuals, this no doubt occurs less often. The explanation in this case may be that after certain combinations, zygotes are formed which react "negatively" with the endosperm, with the consequence that seed development is checked. Empty seeds can develop after cross-pollination even if no fertilization takes place. The reason is that it is possible for non-viable pollen to stimulate the integument tissue to develop a seed coat. Observations of the seed development at different times showed very few seeds from cross-pollination in stages of early degeneration. It is difficult to determine whether the female gametophyte in the nearly empty seeds had decomposed for lack of fertilization or whether the early stages had already passed when the samples were taken. A fourth possible reason for the occurrence of empty seeds could be a purely physiological one: the position of the seed in the cone may be such that it does not obtain sufficient nourishment, and hence normal development is checked. The points outlined above are relevant also to explain part of the frequency of empty seeds after self-pollination and wind-pollination.

In a number of investigations it has been found that the total number of seeds per cone is specific to each mother tree, irrespective of the combination of crosses. However, the result of the analyses of variance made for this particular investigation covering the total number of seeds per cone after self-pollination and cross-pollination showed that there are in fact differences between pollination types. One of the factors influencing the number of seeds in cones after wind-pollination is pollen density (Sarvas 1962), and this category of seeds was therefore not included in the comparison. In most clones the number of seeds was lower after self-pollination, but of the ten clones which were examined in 1966 and 1967, four contained more seeds after cross-pollination one year and after self-pollination the next. Fowler (1965 b) found in *Pinus banksiana* that two out of five trees examined had more seeds per cone after self-pollination than after cross-polli-

nation. He was, however, of the opinion that as a rule there is no difference in the total number of seeds per cone between these two types of pollination. Brown (1970), who examined 25 clones of *Pinus sylvestris* and Franklin (1969) both found a lower number of seeds in cones after self-pollination as compared with cross-pollination. In his investigation of *Pinus taeda,* Franklin came to the same conclusion as McWilliam (1959), namely that the explanation may be metaxenia, i.e. the pollen and the protruding pollen tube influence the development of the female tissues in the cone. There is no reason why as a general rule the number of seeds per cone should vary between different combinations. The variation in the receptivity of the ovules at the time of pollination naturally influences seed development. This may be one of the reasons why the same combination of crosses produces different results in different years. One pollination type produces the highest number of seeds one year, the other the next. An example of this is the results from artificial crosses carried out on one and the same tree for five years (Plym Forshell, unpublished). One year both combinations had the same total number of seeds. For two years the largest average number of seeds was found in cones after cross-pollination and for two years in cones after self-pollination.

5.2 Differences between clones

The ten clones included in the experiment showed consistent differences year after year. The chances of a definite evaluation of characteristics, where the differences between clones—if they exist at all—are fairly slight, are diminished by the necessity to limit the material to one cone per sampling and per combination.

As has been pointed out earlier, the total number of seeds per cone is specific to each mother tree and clone. As could be expected, an analysis of variance of the number of seeds showed a significant difference between clones.

The degree of self-fertility varied between

clones (Figures 11 and 12). Clone E 3003 was fairly self-fertile, whilst clones H 1007 and H 1008 approached total self-sterility. In no clone did the frequency of well developed seeds after self-pollination reach the same level as after cross-pollination and wind-pollination. A parallel variation in the self-fertility of single trees was found in an earlier investigation of *Pinus sylvestris* (Plym Forshell 1953) and in, amongst others, *Pinus monticola, Pinus glauca* and *Pseudotsuga menziesii* (Bingham & Squillace 1955; Mergen *et al* 1965; Orr-Ewing 1957 a). According to King *et al* (1970) the degree of self-fertility is correlated to the number of recessive lethal genes in the mother tree. In a tree, embryo mortality as a result of homozygotisation of these genes is constant after self-pollination (Sarvas 1962). The observed difference from year to year in the percentage of empty seeds within clones would then be explained by the fact that a varying number of non-viable embryos could be housed inside each seed. Hence embryo mortality would be a better measure of self-sterility than the number of empty seeds.

As regards the frequency of well developed seeds, the differences between clones were less after cross-pollination than after either wind- or self-pollination. Clone H 1010, however, differed from the others in the fairly high percentage of degenerating seeds in August, a difference which was not found in cones collected in the autumn. The larger variation after wind-pollination is explained partly by the fact that some self-pollination is most probably included, and partly by the effects of chance, in other words dependent on pollen density etc. Dieckert (1962, 1964, *Picea* and *Larix*), King *et al* (1970, *Picea*) and Sarvas (1962, *Pinus*) found a similarity in the yield of full seed after self- and cross-pollination of single trees or clones. High self-fertility corresponded to high fertility after cross-pollination. This means that also after cross-pollination the seed yield would be proportional to the number of recessive lethal genes. Possibly because of the relatively small material examined, it has not been possible to establish this connection between self-pollination and cross-pollination within the limits of this investigation. On the other hand, the averages of the frequency of well developed seeds on four sampling occasions (Figure 12) showed a certain connection between cross-pollination and wind-pollination (r = 0.7). In the above-mentioned unpublished investigation of the results from earlier artificial crossings with *Pinus sylvestris,* it has also been possible to trace a parallel trend in seed yield between cross-pollination and wind-pollination. The results after self-pollination of the corresponding mother trees do not appear to be correlated with the results after other types of pollination.

5.3 Controls

In the pine the seed coat is already fully developed before fertilization. Even if fertilization does not take place, pollination alone may result in an empty seed. Out of 750 control-isolated flowers, only three developed into cones with full seeds. The reliability of the isolation method cannot be judged only from this result, as cones containing an empty seed too indicate that one or two pollen grains got into the isolation tube and initiated the development of a seed coat. Pollen grains were also observed in some pollen chambers during the first vegetation period. Unlike, for example, the *Picea* family (Klaehn & Wheeler 1961; Mergen *et al* 1965; Mikkola 1969), species of *Pinus* do not even develop seed coats without prior pollination (Buchholtz & Stiemert 1945; Hagman 1967; Hagman & Mikkola 1963; McWilliam 1959; Sarvas 1962). One of the reasons for this difference is connected with the fact that *Picea* species complete their seed development in one summer whilst the *Pinus* species generally require two vegetation seasons for their seeds to develop fully. Where this is not the case, it is possible that empty seeds develop from non-pollinated ovules in individual examples of *Pinus halepensis* and *Pinus cembra* (Sarvas 1962).
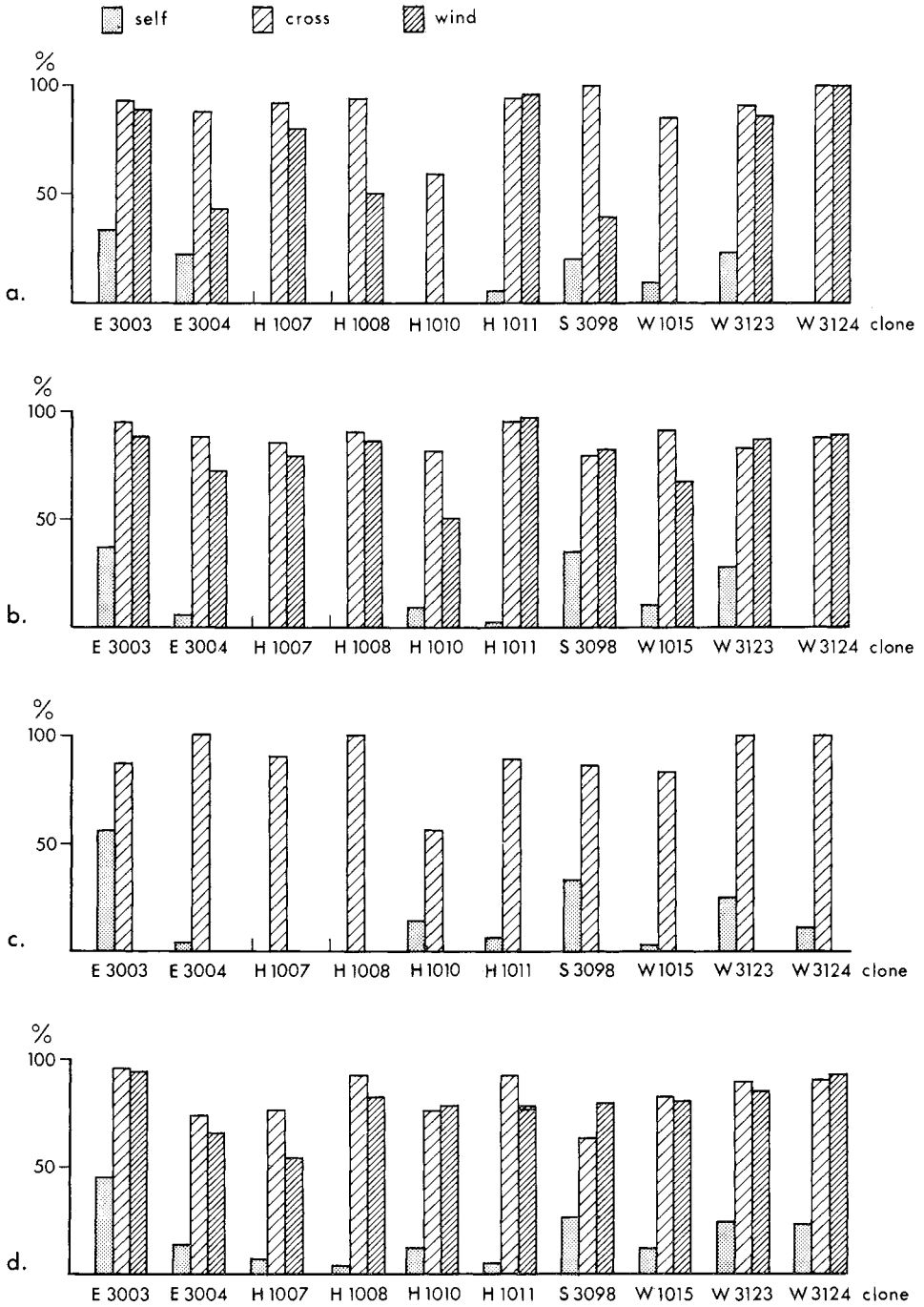
Spray application of hormone solution

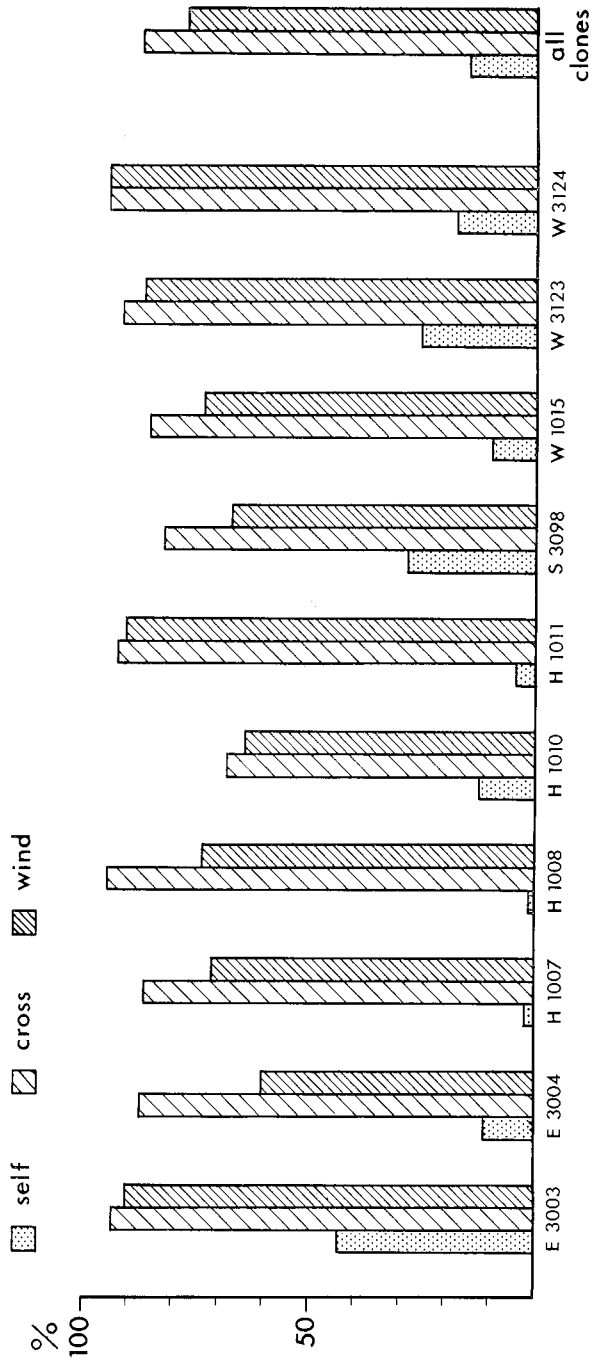Figure 11. Frequency of well developed seeds. a.: August 1966; b.: autumn 1966; c.: August 1967; d.: autumn 1967.

Figure 12. Averages of the frequency of well developed seeds. Samplings from August and autumn 1966 and 1967 respectively.

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and dead pollen did not stimulate the ovules to develop either seeds or cones. McWilliam (1959) did not succeed in initiating cone development in *Pinus nigra* either by spray application of auxins or of ether or alcohol extract of pollen, or by means of pollination with dead pollen. In an examination of *Pinus peuce,* Hagman and Mikkola (1963) found that treatment of the female flowers with gibberellic acid or indole-3-acetic acid started off the development of the ovules, but that degeneration followed fairly rapidly, resulting in cone loss. Spray applications of hormone and dead pollen were judged to be ineffective, as cones without full seeds had developed in two clones only, H 1007 and H 1010. Further, as clone H 1007 had developed cones without seeds after open control as well, these cones are considered rather to be signs of parthenocarpy. Compare Allen (1942, *Pseudotsuga*), Langner (1959, *Picea omorika*), Orr-Ewing (1954, 1957 b, *Pseudotsuga*), Plym Forshell (1953, *Pinus sylvestris*).

Amongst the controls the cross with *Pinus banksiana* is a special case on its own. It is apparent that its pollen is able to initiate both cone and seed development in *Pinus sylvestris* in spite of the fact that no pollen tubes appear. Without fertilization and embryo formation the female gametophyte declines, and the result is an empty seed. Using the terminology of this article, this "control cross" is rather an example of incompatibility between species. These are so closely related that pollen grains from *Pinus banksiana* are able physiologically to influence the development of the other species (cf. Krugman 1970). This means that a mechanical stimulus alone, for example by dead pollen, is not sufficient. Obvious differences have been found between clones, both as regards the frequency of developed cones and the proportion of cones with seeds. In this control series too, clone H 1007 differed by its high percentage of cones remaining at the beginning of the second vegetation period. As was pointed out on page 24 the cones which dropped during the second vegetation period usually lacked seeds, which agrees well with Brown's (1970) observations. He connects the loss of cones with the absence of embryo in the seeds of the fallen cones.

6 Summary

The purpose of the investigation was to study seed development in the Scots pine, *Pinus sylvestris,* for different types of pollination—self-pollination, cross-pollination and wind-pollination.

The development of seed was also observed in different types of controls:
1. open control
2. pollination with old, genetically dead, pollen
3. pollination with a pollen from a different species (*Pinus banksiana*), and
4. spray application of growth hormone (0.01 % indole-3-acetic acid).

Grafts of ten clones in a pine orchard were selected as trees for the experiment, and artificial crosses were carried out during 1964—1966.

The course of development was found to be similar for seeds of all three pollination types during the first and the beginning of the second vegetation period. Nor was it possible at this stage to find any difference between clones. In the investigation maximum attention was therefore concentrated on seed development during the latter half of the second vegetation period.

From the stage of polyembryony which was reached in all the combinations, development continued mainly along two lines. One line led to well developed, full seeds due to the fact that a single embryo had grown at the expense of others whilst starch was being stored in the endosperm. In the second type of seed all the embryos degenerated, and this process was followed by the breaking down of the endosperm tissue. The degeneration continued until only thin remains of the original contents of the seed were visible; the seed could in other words be classified as empty.

6.1 Differences between types of pollination

Repeated observations of seed development during July and August showed increasingly obvious differences between the types of pollination (Figures 5 and 10). *Cross-pollination* led mainly to well-developed seeds with viable embryos. Seeds after *self-pollination,* on the other hand, were characterised by the gradual decomposition of embryos and endosperms. The degeneration of embryos after self-pollination is most certainly caused by a homozygotisation of lethal factors. The physiological interaction with the endosperm is disturbed, whereupon this too disintegrates.

There was a slightly higher frequency of seeds with decomposing tissues after *wind-pollination* than after cross-pollination. Since wind-pollination to a certain extent may mean self-pollination, homozygotic sub-lethal genes are also in this case a probable cause of empty seeds.

6.2 Differences between clones

The greatest variation between clones as regards the frequency of well-developed seeds occurred after self-pollination (Figures 11 and 12). Three clones (E 3003, S 3098, and W 3123) were fairly self-fertile, whilst clones H 1007, H 1008 and H 1011 were almost completely self-sterile. The differences between clones were smallest after cross-pollination.

The assumed element of self-pollination most probably explains to some extent the variation between clones in the percentage of well-developed seeds after wind-pollination.

The self-sterility is correlated to the number of recessive lethal genes in the mother tree. The connection between seed yield after self-pollination and cross-pollination,

established in other investigations, was not confirmed, possibly due to the limited material. On the other hand there appeared to be a slight correlation between the percentage of well-developed seeds after cross-pollination and after wind-pollination.

This investigation showed the total number of seeds per cone to be specific to each mother clone. One year, however, the variation within the clones was large enough to make it possible to differentiate also between types of pollination.

6.3 Controls

Samples taken during the first vegetation period from control combinations 1, 2 and 4 (crosses of 1964, open control, pollination with dead pollen and spray application of hormone), showed that the gametophyte tissues were in the process of decomposing.

At the beginning of the second vegetation period (crosses carried out in 1965), there were only one or two cones left from the control series, with the exception of the crosses with *Pinus banksiana*. The results of cone collection during the summer showed that application of hormone solution and dead pollen were incapable of initiating the formation of cones and seeds, but they gave rise to the assumption that, of the ten clones, H 1007 had the capacity for parthenocarpy. Pollination with *Pinus banksiana* resulted in cones both with and without seeds. Fertilization never occurred; all the seeds were empty. This "control cross" could be taken as an example of incompatibility between species. Obvious differences between clones were found both as regards the frequency of developed cones and the proportion of cones with seeds.

Acknowledgements

Sammanfattning

Syftet med undersökningen var att studera fröutvecklingen hos tall, *Pinus sylvestris,* vid olika pollineringstyper: självpollinering, korspollinering och fri avblomning.

Fröutvecklingen observerades även efter olika typer av kontroller:

1. öppen kontroll
2. pollinering med gammalt, genetiskt dött, pollen
3. pollinering med artfrämmande pollen (*Pinus banksiana*) samt
4. besprutning med tillväxthormon (0.01 % indolylättiksyra).

Som försöksträd utvaldes ympar av 10 kloner i en tallplantage där artificiella korsningar utfördes under åren 1964—1966.

Utvecklingsförloppet befanns vara likartat för frön efter alla tre pollineringstyperna under första och början av andra vegetationsperioden. Ej heller mellan kloner kunde någon skillnad konstateras under detta skede. Största intresset ägnades därför i denna undersökning fröutvecklingen under senare delen av andra vegetationsperioden.

Från det stadium av polyembryoni som uppnåddes hos alla kombinationer fortsatte utvecklingen efter i huvudsak två linjer. Den ena linjen ledde fram till välutvecklade matade frön genom att endast ett embryo tillväxte på de andras bekostnad alltmedan i endospermet upplagrades stärkelse. I den andra typen av frön degenererade alla embryoner och denna process följdes av en nedbrytning av endospermvävnaden. Degenerationen fortskred tills endast tunna rester av det ursprungliga fröinnehållet var synliga; med andra ord fröet kunde klassificeras som tomt.

Skillnad mellan pollineringstyper

Upprepade observationer av fröutvecklingen under juli och augusti visade allt tydligare skillnader mellan pollineringstyper (figurerna 5 och 10). *Korspollinering* ledde mestadels till välutvecklade frön med livskraftiga embryoner. Hos frön efter *självpollinering* var däremot den successiva nedbrytningen av embryoner och endosperm karakteristisk. Degenerationen av embryoner efter självpollinering beror säkerligen på en homozygotisering av letalfaktorer. Det fysiologiska samspelet med endospermet rubbas varefter även detta bryts ner.

Frön med vävnader i upplösning uppträdde i något högre frekvens efter *fri avblomning* än efter korspollinering. Eftersom fri avblomning i viss utsträckning kan innebära självpollinering är homozygota subletalgener även här en trolig förklaring till uppkomsten av tomma frön.

Skillnad mellan kloner

Variationen mellan kloner i frekvensen välutvecklade frön var störst efter självpollinering (figurerna 11 och 12). Tre kloner (E 3003, S 3098 och W 3123) var relativt självfertila medan klonerna H 1007, H 1008 och H 1011 var nästan självsterila. Klonskillnaderna var minst efter korspollinering. Det förmodade inslaget av självpollinering kan sannolikt förklara en del av variationen mellan kloner i procent välutvecklade frön efter fri avblomning.

Självsteriliteten är korrelerad med antal recessiva letalgener hos moderträdet. Det samband mellan fröutbytet efter självpollinering och korspollinering som fastställts i andra undersökningar kunde, kanske på grund av materialets begränsning, ej konstateras. Däremot syntes en svag korrelation mellan procent välutvecklade frön efter korspollinering och fri avblomning föreligga.

Totalantalet frön per kotte visade sig i

denna undersökning vara specifikt för varje moderklon. Ett år var variationen inom klonerna dock så stor att skillnader även mellan pollineringstyper kunde påvisas.

Kontroller

Provtagningar under första vegetationsperioden av kontrollkombinationerna 1, 2 och 4 (1964 års korsningar, öppen kontroll, pollinering med dött pollen och besprutning med hormon) visade att gametofytvävnaderna var på väg att upplösas.

Vid början av andra vegetationsperioden (1965 års korsningar) fanns med undantag för korsningen med *Pinus banksiana* endast enstaka kottar från kontrollserierna kvar. Resultaten av kottinsamlingen under sommaren visade att besprutning med hormonlösning och dött pollen ej kunde initiera någon kott- och fröbildning men gav anledning förmoda att av de 10 klonerna H 1007 har förmåga till partenokarpi. Pollineringen med *Pinus banksiana* resulterade i kottar både med och utan frön. Någon befruktning kom aldrig till stånd, fröna var alla tomma. Denna "kontrollkorsning" är snarast ett exempel på inkompatibilitet mellan arter. Tydliga klonskillnader iakttogs både beträffande frekvensen utbildade kottar och andelen kottar med frön.

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