Observations on Cross-, Self-, and Interspecific Pollinations in Pinus peuce Griseb.

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(Received for publication April 8, 1963)

Introduction

The increasing field of forest tree breeding has during the last thirty years paid more and more attention to the production of fast growing hybrids of different forest tree species. Although this work has already met with much success, there is still a great demand for the knowledge of the breeding mechanisms of forest trees and the nature of the barriers that might occur, for the successful application of the in- and outcrossing methods of plant breeding, which have been used for years in the breeding of agricultural and horticultural crops.

The general course of fertilization and the development of the embryo after free pollination in Pines has been fairly thoroughly studied by many authors (FERGUSON, 1904; HÅKANSSON, 1956; MCWILLIAM and MERGEN, 1958; SARVAS, 1962).

Experimental studies after controlled pollinations have been made by DENGLER (1932, 1942), BUCHHOLZ (1944), PLYM FORSHELL (1953), WRIGHT (1953, 1959), EHRENBERG et al. (1955), BINGHAM and SQUILLACE (1955), SQUILLACE (1957), MCWILLIAM (1959, 1960), BARNES et al. (1962).

This study is an attempt to follow the pollen germination, fertilization, and first development of the embryo after 4 different kinds of controlled pollination in the Macedonian pine (Pinus peuce GRISEB.). Observations were also made on unpollinated controls. Our work was done at the Forest Tree Breeding Station, Maisala, of the Forest Research Institute of Finland. This study is a part of a research program on the genetics of the flowering of forest trees: the problem of fertilization.

Material and Methods

The mother trees (No E 1940, E 2061 and E 2062) used in this study are growing in the Ruotsinkylä experimental area close to the forest tree breeding station. They belong to plantations established in 1931 with 7 year old plants. The origin of the seed was the Rino Planino mountain region of Bulgaria.

For cross pollinations, pollen of tree No E 1940 was used. In the interspecific crosses we used as father trees one Pinus cembra L. (No E 1938) growing at the tree breeding station and one Pinus koraiensis SIEB. & ZUCC. (No E 1675). The latter is growing at the Solböle experimental area from seed originating from Hozan, Korea. The origin of the P. cembra is unknown.

A total of 464 strobili were isolated on 8—9 June, 1961, when the flower buds were still completely covered by the bud scales. The isolation was made with double pergamyn-paper bags. The pollination was carried out from the 13th to the 15th of June. As controls 208 isolated strobili were left unpollinated.

The microscopical studies were began at the beginning of the second growing season in spring, 1962. A total of 431 ovules were fixed in NAVASHIN, embedded in paraffin, and cut. Usually the sections were 12—20 micr. in thickness. The preparates were stained in crystal violet and in some cases FEULGEN was also used. In addition, 168 ovules were studied under a preparative microscope.

Results

Cross- *and* Self-Pollination

There was no difference in the germination of the pollen in the cross- and self-pollinated ovules. In samples fixed on June 20th, 1962, the pollen tube had grown ⅓ to ½ of the distance through the nucellus (Fig. 1). The pollen tubes were at this time enlarged at the apex and showed an abundance of starch grains. The generative cell and the stalk-cell had wandered into the pollen tubes. In most of the pollen tubes the generative cell was already above the stalk-cell. In some preparations, they were in the act of passage.

The fertilization and the first development of the embryo were also very similar in cross- and self-pollinated ovules (Fig. 2). In most of the ovules 1 to 3 archegonia were fertilized; the modal number was two. In most of the ovules at least one archegonium remained unfertilized. In some of the self-pollinated, and also in some of the

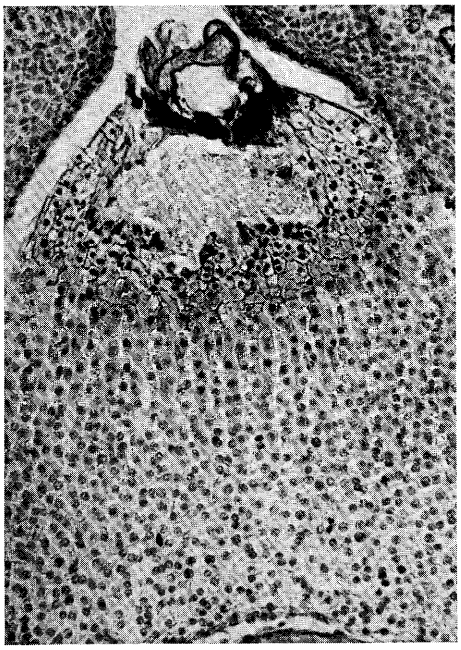

***Fig.* 1. — Nucellus and pollen chamber of a cross-pollinated ovule at the first half of the second growth season, June 20. 1962. The apex of the pollen tube is considerably enlarged and contains starch grains. Magnification: × c. 90.**

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*) The research reported here was supported by Grant No FG-Fi-113-60 from the U.S. Ministry of Agriculture.

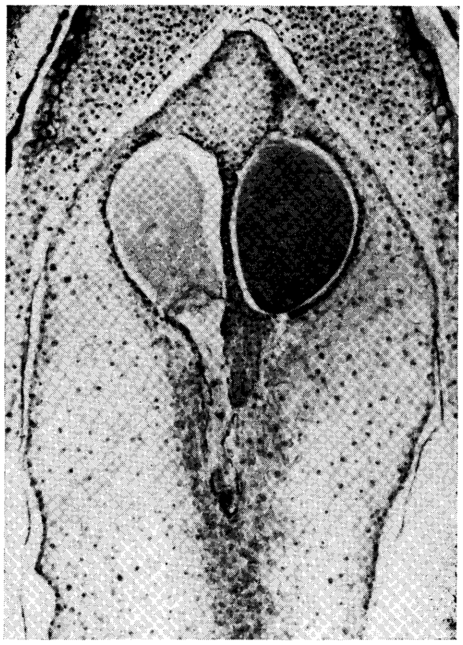

Fig. 2. — In a self-pollinated ovule the suspensor cells of the embryo have grown longer and the embryo is growing into the developing embryo cavity. The archegonium to the right is unfertilized. Its plasma looks more dense, since the cavities containing protein grains are still in evidence. The egg nucleus of this archegonium is not more clearly detectable. July 19. 1962. Magnification: × c. 41.

cross-pollinated ovules all of the archegonia remained unfertilized. In these ovules the pollen tubes had not grown with sufficient rapidity through the nucellus.

In those archegonia in which no fertilization had taken place one or several depressions were formed in the egg-nucleus *(Fig.3)*. At a later stage of development the outlines of the nucleus are growing vague. At the time when in the normal ovules the embryo starts growing into the corrosion cavity of the prothallium the egg-nuclei of the unfertilized archegonia already have totally disappeared into the plasma.

When the pro-embryo is at the four-cell stage the proteid vacuoles disappear almost completely from the plasma of the archegonium (Ferguson, 1904, p. 125). In unfertilized archegonia they are still present at the moment when it is no longer possible to recognize the egg-nucleus *(Fig. 2, 3 and 5)*. According to this, it is possible, from observing the plasma, to distinguish fertilized archegonia which have developed to the four-cell stage or further, from ones which have not.

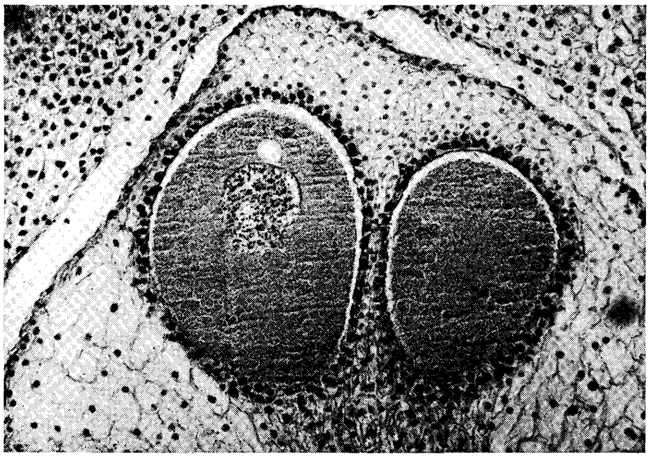

Fig. 3. — Two unfertilized archegonia on July 19. 1962. In the archegonium to the left is an egg nucleus in which there already is a small impression in the lower right part. Above the egg nucleus is a pollination vacuole. The plasma of the ovules contains protein cavities. Magnification: × c. 71.

Interspecific Pollinations

A. Pinus peuce × *P. cembra*

The growth of the pollen tube was, at the beginning, quite normal. On the 20th June 1962 the pollen tubes had grown nearly half of the way through the nucellus. In a small number of the ovules, however, all the archegonia remained unfertilized, because the growth of the pollen tube had slowed down during the later stage, or perhaps stopped *(Fig. 4)*.

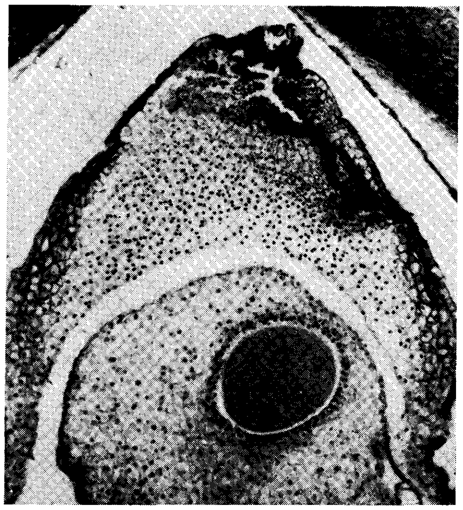

Fig. 4. — In the archegonium the egg nucleus is not anymore visible. The pollen tube of *P. cembra* has not grown completely through the nucellus. July 19. 1962. Magnification: × c. 38.

In most of the ovules at least one archegonium was fertilized in the normal way. The development of the pro-embryo was likewise normal at first. However, no ovule was found in which the embryo had grown out from the archegonium into the embryo cavity. In all observed ovules it was destroyed before that stage. The embryo cavity was

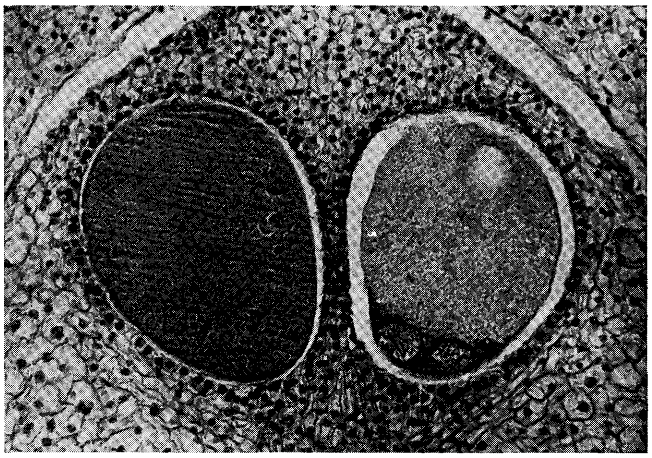

Fig. 5. — In the archegonium to the right the four nuclei of the hybrid embryo (father: *P. cembra*) are at the bottom of the oösphere. Two of them are seen in the picture. The plasma around the nuclei is dense, as is usual in this stage. The other archegonia are unfertilized. Its egg nucleus has already disappeared into the plasma. July 17. 1962. Magnification: × c. 71.

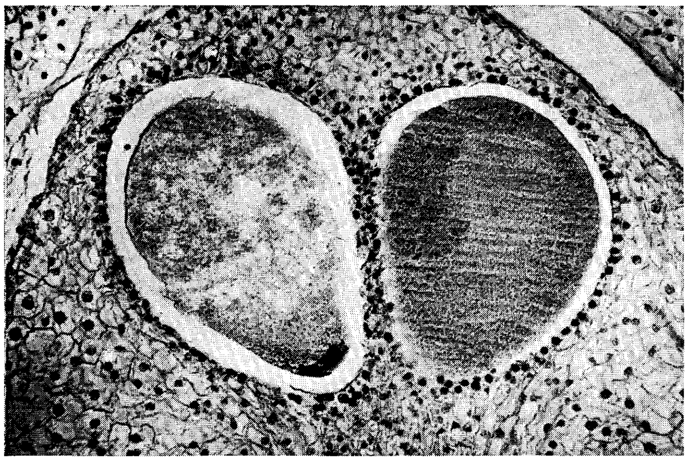

Fig. 6. — Two archegonia from an ovule pollinated with *P. cembra.* In the left one the fertilization has, judget from the plasma, taken place and the embryo has developed at least to the four nucleus-stage. No nuclei are, however, visible in the archegonium. At the bottom of the archegonium there is a mass of very dense plasma, which is perhaps the rest of the degenerated embryo. The other archegonium is unfertilized. July 19. 1962. Magnification: × c. 75.

formed but remained empty. The development of the endosperm was also still normal on the 30th of July. By the 7th of August it had, however, stopped its development. At this time the endosperm did not continue to fill out the cavity inside the seed coat. In the development of the seed coat there were no differences compared with the seed after cross- and self-pollination.

On the 19th of July there were, in most of the fertilized archegonia studied, four nuclei which had already wandered down to the apex of the egg cell *(Fig. 5).* In two archegonia there were eight nuclei, but no signs of the formation of cell walls were to be seen. In one archegonium, which, judged from the number of "protein vacuoles", was a fertilized one, no nuclei could be observed *(Fig. 6).* At the bottom of the archegonium, however, there was a very dense quantity of plasma. This undetermined mass perhaps was the rest of a pro-embryo which succumbed at the four- or eight-nucleus stage.

In those archegonia which had remained unfertilized, no egg-nucleus could more be seen, although in the fertilized archegonia of the same ovule the embryos were only in the four-nucleus stage.

In a normal cross-pollination, the embryo usually has already started its growth into the embryo cavity at the time when in the unfertilized archegonia of that same ovule the egg-cell nuclei can no longer be seen. Thus it is obvious that the development had slackened if not in most cases stopped at the four-nucleus stage.

B. Pinus peuce × P. koraiensis

The pollen tube of *P. koraiensis* had grown very vigorously through the nucellus. In all ovules studied at a later stage, there was at least one fertilized archegonium. In most cases three archegonia were fertilized, and in some cases four.

The fertilization took place as in the intraspecific cross *(Fig. 7).* Also the pro-embryo developed in the normal way at least to the four-nucleus stage *(Fig. 8).* In one of the archegonia studied there were, however, six nuclei instead of four. They were of the same size as the nuclei of the four-nucleus stage, when those move to the bottom of the archegonium.

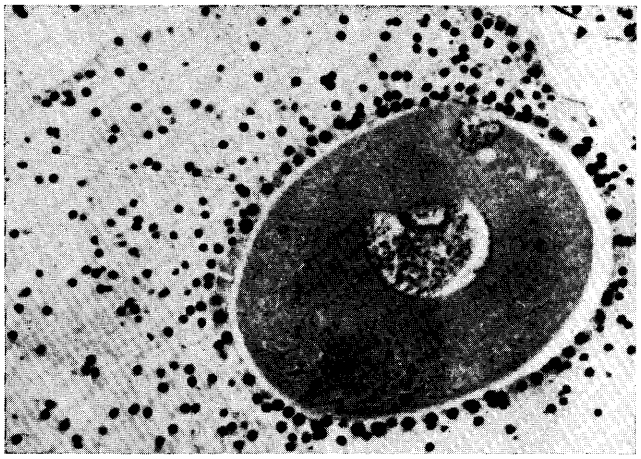

Fig. 7. — The sperm nucleus of *P. koraiensis* in contact with the egg nucleus. In the upper part of the archegonium the other sperm nucleus is seen. July 11. 1962. Magnification: × c. 105.

There is no certain observation available concerning the development of the pro-embryo beyond the four-nucleus stage, because the series of fixations were incomplete due to the unexpectedly great differences in the developmental stages of the cones. In any case, in none of the ovules studied at the later stage of development was there any sign of an embryo which had grown out of the archegonium. All of the embryos were destroyed at an earlier stage.

The embryo cavity was formed and the endosperm developed in a normal way, just as in the cross *P. peuce × P. cembra (Fig. 9).*

Unpollinated Controls

At the beginning of the second growth season, the cones were counted on the 5th of June, 1962. All the 83 unpollinated cones of tree No E 2061 had dropped. The development of the control cones in the tree No E 2062 (total 23) had also stopped. On one twig all eight cones were still left, but they were dry, and were attached to the shoots merely due to the pitch which had developed. In tree No E 1940 there were 12 cones remaining from the 18 cones which were unpollinated. In this tree the control cones were left on only five isolated twigs. On every twig there was at least one cone left. These cones were not dried, and their

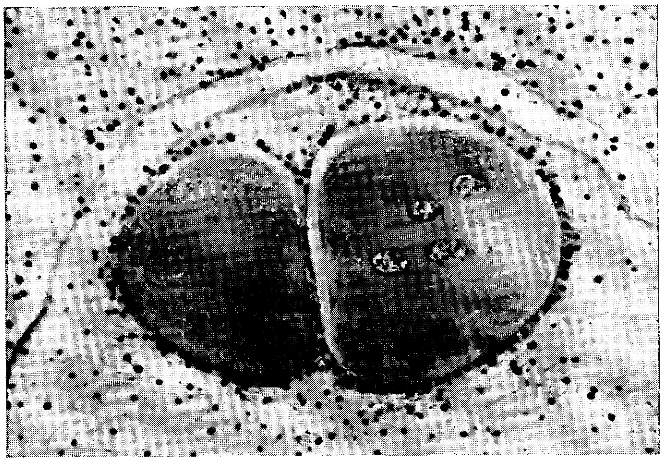

Fig. 8. — In the archegonium to the right the second mitosis of the hybrid embryo (father: *P. koraiensis*) has just passed. The four nuclei formed are still small. July 11. 1962. Magnification: × c. 71.

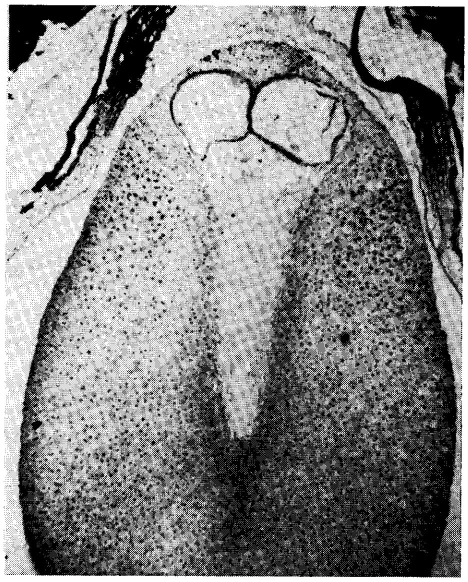

Fig. 9. — The endosperm of an ovule pollinated with pollen of *P. koraiensis* and the developing embryo cavity. The embryo have obviously degenerated at an early stage, because no sign of them can be observed in the ovulum. July 19. 1962. Magnification: × c. 26.

development seemed to continue. Their growth ceased rapidly, however. On the 20th of June they were about 3 cm long, when the pollinated cones on the same tree were 5 to 7 cm. At this date one of the unpollinated cones was fixed and all its ovules studied microscopically. They were already observed macroscopically to be flat and much smaller than the pollinated ovules. In none of the ovules were there any pollen grains. In every one the micropyle was closed, and the nucellus was completely destroyed. At the site of the female gametophyte was an empty cavity and on the edges of this cavity was destroyed tissue, as in *Fig. 10.*

Three weeks later (July 11th) there were only two cones left of those mentioned. One of these dropped when the twig was handled. A week later the last cone dropped. It was then 3.5 cm long.

Cones Treated with Growth Substances

Since it had been shown by many studies (see i. e. LUCKWILL, 1957 and JACOBS, 1962 for further references) that treatment with certain growth substances might bring about the parthenocarpic development of fruit and that the same effect in some cases in produced by pollen-extracts, some preliminary experiments were made with sprays on unpollinated cones. On tree No E 2062 26 isolated cones were sprayed 5 days after the date of pollination with a solution of gibberellic acid (10 p. p. m.). A group of 22 cones was sprayed with indolyl-3-acetic acid (100 p. p. m.) and 20 cones were sprayed with water in which pollen of *P. peuce*, from tree No E 1940, had been germinated for 3 days and from which the pollen and pollen tubes were filtered away.

On the 5th of June, 1962, all of the cones treated with IAA and all the cones treated with the "pollen germination water" had dropped. However, of the 26 cones sprayed with gibberellic acid, there were 4 cones left on two different twigs. They were firmly attached to the shoot and appeared to develop further. On 20th of June, one of these cones was fixed. It was about 4.5 cm long and was not noticeably behind the pollinated cones in development. All the ovules of this cone were examined microscopically. All of them were degenerated in the same way as the ovules of the control cones in tree No 1940 *(Fig. 10)*. Of the cones treated with gibberellic acid there was only one left on the 12th of July. It was 6 cm long and firmly attached to the shoot. It was taken down and its ovules inspected. All of them were small and flat. The decay of the cells had continued into the seed coats *(Fig. 11)*.

Fig. 10. — An ovulum from a strobilus left unpollinated but sprayed with gibberellin. On the site of the female gametyphyte is an empty cavity and the cells of the nucellus are dead. At the first half of the second growth season, June 20. 1962. Magnification: × c. 68.

Fig. 11. — An ovulum from a unpollinated, gibberelline treated cone on July 12. 1962. The degeneration has continued into the seed coat. Magnification: × c. 64.

Discussion

Self-Pollination

It seems obvious that no mechanism exists in *Pinus peuce* which prevents the germination of pollen on selfed strobili.

This observation also agrees well with the observations of Bingham and Squillace (1955) and Barnes *et al.* (1962) in *Pinus monticola* Dougl., and with the observation of Orr-Ewing (1957) in *Pseudotsuga menziesii* (Mirb.) Franco.

The second possible barrier to selfing would be that the pollen tube was not able to penetrate the nucellus in time for the receptive phase of the female gametophyte. In some of the ovules here studied all archegonia remained unfertilized, because the pollen tube had not grown (with sufficient speed through) the whole nucellus. This was, however, also the case in some of the cross-pollinated ovules. We do not have a sufficient quantity of material to make any significant calculations of a possible difference in pollen tube growth-rate between cross- and self-pollen tube growth. Therefore, the possibility still exists that this might be a means of blockage. Ferguson (1904) and Håkansson (1956) report the slight occurrence of cases where the pollen tube was insufficiently developed. Without a large body of material from controlled pollinations with pollen whose germination power has been throughly tested also in vitro, it is difficult to distinguish the cases of true incompatibility from the cases of reduced vigour in the male gametophyte itself, due to improper handling, its own constitution, or the constitution of its mother tree. That the latter condition might have some influence has recently been shown by Barnes *et al.* (1962). The difference in pollen tube growth might especially have importance in the cases of open pollination when cross- and self-pollen might compete in the same ovule. However, if a real incompatibility mechanism should exist, it seems to have to be so specific — as shown in the Angiosperms — that a mere growth rate difference could not suffice as the block. In almost all studied cases of self-incompatibility there seem to be involved specific biochemical reactions which lead to the blockage complete of the penetration of the pollen tube. If such systems exist in *Pinus,* it should be possible to detect cases in which all pollen ceased growth when selfed, although perfectly functional when used in cross polination. Such experiments have, so far the authors are aware, not yet been made (see also Sarvas, 1962, p. 127).

It is well known that the percent of empty seed after self-pollination is higher than after cross-pollination (Dengler, 1932; Plym Forshell, 1953; Ehrenberg *et al.* 1955; Ehrenberg and Simak, 1956; in *Pinus silvestris;* Bingham and Squillace, 1955 in *Pinus monticola;* Sarvas, 1962, in *Pinus nigra.*). Also the development of the embryos of filled seed after self-pollination is, in many cases, retarded. It seems therefore, that the cause must be a defect in the developing zygote rather than a barrier present before the fertilization takes place. Since the development of the pro-embryos in our material did not show any differences between self- and cross-pollination it seems that the degeneration will start at some later stage of development. This agrees with the hypothesis of Sarvas (1962) for *Pinus silvestris.* It must be left to a later and more extensive study to determine (at which stage the development is blocked or turned away from the normal course, and to what extent the different types and stages occur).

Interspecific Pollinations

Artificial hybridization within the genus *Pinus* has in many cases met with success, and some of the most promising hybrids are already used in commercial plantations. Recent descriptive lists have been published by Wright (1953, 1959) and Schütt (1959). However, many crosses have been reported as failures and there is very little information concerning at which stage the failure might have occurred. Wright (1959) reported that in the cross *P. peuce* × *P. koraiensis,* all the 6 strobili pollinated failed to develop into cones. He also reports the cross *P. peuce* × *P. cembra* as a failure. Buchholz (1944) reported cases in which after species crosses, the pollen failed to grow through the nucellus in coordination with the development of the female gametophyte. McWilliam (1959) in the cross *Pinus elliotii* Engelm. × *P. nigra* Arn. found the pollen to be unable to penetrate the nucellus. In the crosses *P. resinosa* Ait. × *P. rigida* Miller, *P. nigra* × *P. rigida,* and *P. resinosa* × *P. nigra* pollen tubes developed but the normal pollen tube growth was small. In about 70% of the ovules in the cross *P. nigra* × *P. resinosa,* the pollen either did not germinate or germinated with an ineffective tube.

In many cases the development of an empty seed is reported, which would indicate that the male gametophyte had survived at least to the second growth season of the conelets, since the failure of pollen germination and tube penetration seems to result in a collapse of the ovules during the first growing season. Exceptions do exist, however, as have been reported by Sarvas (1962) in *P. cembra,* where in some cases empty seeds develop even without pollination due to the exceptional development of the archegonia during the first growing season.

The comprehensive microscopical work which is necessary to detect the precise stage of the breakdown has obviously reduced the cases hitherto exactly described.

The pollen tubes of *P. koraiensis* grew very effectively through the nucellus of *P. peuce.* In most ovules pollinated with *P. cembra* pollen there was also at least one fertilization, although the growth of the pollen tubes in this combination was not so vigorous as in the cross with *P. koraiensis.* In the reciprocal cross *P. cembra* × *P. peuce* made on a small scale at this station in 1961 the pollen of *P. peuce* germinated but the penetration into the nucellus of *P. cembra* was very poor during the summer 1961.

It is possible, that the degeneration of the pro-embryos, at least in the cross *P. peuce* × *P. cembra,* started already at the four-nucleus stage.

The causes of the stop in the embryonic development are not easily understand. The behaviour of the chromosomes in the different phases of mitosis seemed to be quite normal.

It therefore seems that the hybrid nucleus was able to function as long as no organization of the new zygote was necessary. But at the first stage of differentiated development in the new individual, that is, at the stage when the 4 nuclei divide and the formation of cell walls starts at the base of the oöcyte, the nuclei were no longer able to communicate with the surrounding protoplasm. Hotta (1960) has shown that a sharp increase in protein concentration in the cell can be observed when two-dimensional growth starts in the at-the-beginning one-dimensional filamentous protonema of *Dryopteris erythrosa;* that is, at the moment when the direction of cell division of the apical cell of the protonema is converted from longitudinal to transverse. The RNA from the protallium in the two- dimensional growth possesses a nucleotide composition distinct from that in the one-dimensional growth. A biochemical differentiation of the internal pool of the cell's ribonucleic acid was also associated with the point of no return in morphological differentiation in the water fungus *Blastocladiella emersonii* (Cantino, 1962).

In animal eggs it has also been found (Brachet 1960, p. 88) that an increased cooperation between nucleus and cytoplasm starts at the stage of gastrulation. It seems therefore that the presence of an abnormal nucleus does not permit the synthesis of certain substances which are required for morphogenesis. Cytochemical methods have demonstrated an excess of RNA in the nuclei of the blocked hybrids (Brachet, op. cit. p. 89).

In view of recent research (see e. g. Ts'o, 1962) one hypothesis could be that the hybrid nucleus, formed from the chromosomes at two species, produces a "messenger-RNA" which cannot in all cases communicate with the ribosomes of the single species which are present in the plasma of the egg cell. Another possibility would be, that although the "hybrid message" is received, all of the amino acids which are necessary for the protein synthesis called for by this message are not available. McWilliam (1959) has shown that there are considerable differences in free amino acid concentrations between Austrian pine and red pine. The amino acids in ovules of *Pinus peuce, P. cembra,* and *P. koraiensis* have not yet been investigated.

It would therefore be interesting to attempt to determine whether a block to the development is also present at the free nuclei stage in other species hybrids in pine and if there could be detected a difference in the RNA content of the hybrid nuclei compared with normal nuclei at the stage of the beginning of the first differentiation.

There was no completely empty seed developed after the interspecific crosses here studied. The endosperm developed to almost its normal size, although the embryo cavity remained empty. It is therefore possible that the fertilization and first development of the zygote are sufficient for triggering the endosperm development mechanisms.

Unpollinated Controls

Strobili of *Pinus* in which none of the ovules has been pollinated usually drop, at the latest, at the beginning of the second growth season (Dengler 1932; Plym Forshell 1953; Wright 1953; Mergen 1954; Eklundh-Ehrenberg, and Simak (1956). There seems, however, to be at least some individuals of *P. peuce* in which a part of the control cones continue their development during a certain portion even of the second growth period. At the beginning of the second period all the 83 unpollinated conelets of tree No E 2061 had dropped. On the other hand, in the neighbour-tree No E 1940, 12 of the 18 control cones did continue their development. In this case only the cones developed further. The degeneration of the ovules had obviously started already during the later part of the first growing season.

McWilliam (1959) and Sarvas (1962) have shown that the ovules of unpollinated pine strobili start to degenerate in a few weeks after the time of flowering. It seems that the pollen either possesses, or at its germination produces, some substance necessary for the development of the ovule. This substance is however not absolutely species specific, as shown from many interspecific pollinations, even those in which no embryo develops. It might however be genus specific since, for example, pollen of *Picea abies* did not prevent the dropping of conelets of *Pinus silvestris* although the *Picea* pollen in many cases germinated on and penetrated into the nucellus of *Pinus silvestris* (Hagman and Mikkola, unpubl.). It might be that this substance is emitted also when pollen is germinated *in vitro,* in water. With such water we did not succeed in producing the development of unpollinated ovules. The treatment was done only once, and 5 days after normal pollination optimum. It might be, that with repeated treatment and/or another extraction method a positive effect could be obtained. McWilliam (1959) pollinated strobili of Austrian pine with heat-treated, dead pollen. This dead pollen retarded the degeneration of ovules for about one month.

In some few cases cones of pines have developed although they possessed only degenerated ovules (Dengler 1932; Wettstein 1940; Wright 1953; Plym Forshell 1953; Eklundh-Ehrenberg, and Simak 1956; Sarvas 1962). The last of the unpollinated and untreated control cones dropped in the midle of the second growth season, however.

The similarity between the dropping of conelets in pines and the dropping of fruits in several fruit tree species suggests that the mechanisms behind this phenomenon are of a similar nature. In fruits, fertilization and seed development are, with few exceptions, essential for fruit production. However it is not necessary for all ovules to develop and fruits may be produced with only a few seed. This agrees also with the observations made by Sarvas (1962) in *Pinus silvestris, P. cembra* and *P. peuce.* In *P. peuce* the aborted ovule percentage is reported to be (capable of becoming as high as) 70% before causing dropping.

Just as there are some fruit species and varieties which are completely parthenocarpic and other a which are parthenocarpic only under certain conditions, there may be variation in this quality among the pines as well.

In some plants parthenocarpic development of the fruit can be induced by sprays of growth substances, i. e. IAA, NAA, 2, 4, 5 T or gibberellin.

Of our 26 gibberellin-sprayed strobili 4 continued their development during the beginning of the second growth season, although all the 62 strobili treated with other substances or left completely untreated on the same tree had stopped their development. It is possible, although the amount of material is small, that the gibberellin treatment caused the further development of these conelets. It might be that the last of them, which was fixed on July 12th, could have continued its growth just as long as the pollinated cones. We tried also only one concentration, and since the system seems to be a very delicate as well as a complicated one and influenced by many factors (Jacobs 1962) more extensive studies are necessary before a conclusion can be reached. Such experiments are now in progress. Results similar to ours have recently been reported for apple (Dennis and Edgerton 1962). Gibberellic acid of a concentration of 500—1000 p. p. m. produced parthenocarpic fruits in the apple, but most of these fruitlets abscissed during the June drop.

Summary

1. The growth of the pollen tube, the fertilization, and the first embryo development are similar in self-pollinated and cross-pollinated ovules of *Pinus peuce.*

2. In all ovules studied which were pollinated with *Pinus koraiensis* pollen, and in most of the ovules pollinated with pollen of *Pinus cembra,* fertilization occurred. The embryos, however, degenerated already at the pro-embryo stage, inside the archegonia.

3. None of the unpollinated ovules developed. In two of the trees used, all the untreated control cones dropped at or before the beginning of the second growth season. In the third tree, a part of these cones continued their growth for a short time during the second growth season also. Four of the cones treated with gibberellin continued their growth during the second summer, although all the other unpollinated cones of the same tree had dropped. One of these was still firmly attached to the shoot on July 12.

Zusammenfassung

Titel der Arbeit: *Beobachtungen nach Kreuz-, Selbst- und Zwischenart-Bestäubungen bei Pinus peuce Griseb.*

1. Das Pollenschlauchwachstum, die Befruchtung und die erste Embryo-Entwicklung sind bei selbst- und bei kreuzbestäubten Samenanlagen von *Pinus peuce* ähnlich.

2. Bei allen untersuchten Samenanlagen, die mit Pollen von *Pinus koraiensis* bestäubt worden waren, und bei den meisten Samenanlagen, die mit Pollen von *Pinus cembra* bestäubt worden waren, trat Befruchtung ein. Die Embryonen jedoch degenerierten schon im Proembryo-Stadium im Archegonium.

3. Keine unbestäubte Samenanlage entwickelte sich. Bei 2 der benutzten Bäume waren alle unbehandelten Kontroll-Zapfen bei Beginn der zweiten Wachstumsperiode oder schon vorher abgefallen. Beim 3. Baum setzte ein Teil dieser Zapfen das Wachstum eine kurze Zeit lang auch in der zweiten Wachstumsperiode fort. 4 mit Gibberellin behandelten Zapfen wuchsen auch im zweiten Sommer weiter, obwohl sonst schon alle anderen unbestäubten Zapfen desselben Baumes abgefallen waren. Einer dieser Zapfen saß am 12. Juli noch fest am Zweig.

Résumé

Titre de l'article: *Observations sur les croisements intra et interspécifiques et des autofécondations chez Pinus peuce Griseb.*

1. La croissance du tube pollinique, la fécondation et le développement initial de l'embryon sont analogues dans les ovules autofécondés et dans les ovules fécondés avec un autre pollen.

2. Dans tous les ovules pollinisés avec du pollen de *Pinus koraiensis* et dans la plupart de ceux recevant du pollen de *Pinus cembra*, la fécondation s'est produite. Cependant, les embryos ont dégénéré dès le stade de pro-embryon dans les archégones.

3. Aucun des ovules non pollinisés ne s'est développé. Dans deux des arbres, tous les cônes témoins non pollinisés sont tombés au début de la seconde saison de végétation ou avant. Dans le troisième arbre, une partie de ces cônes a continué à croître pendant un certain temps au cours de la deuxième saison de végétation. 4 des cônes traités à la gibberelline ont poursuivi leur croissance au cours du second été, alors que les autres cônes non pollinisés du même arbre étaient tombés. L'un d'eux tenait encore solidement à la pousse le 12 Juillet.

Literature Cited

Barnes, B. V., Bingham, R. T., and Squillace, A. E.: Selective fertilization in *Pinus monticola* Dougl. II. Results of additional tests. Silvae Genetica **11**: 103—111 (1962). — Bingham, R. T., and Squillace, A. E.: Self-compatibility and effects of self-fertility in Western white pine. Forest Science **1**: 121—129 (1955). — Brachet, J.: The biological role of ribonucleic acids. Elsevier Publ. Co., Nijemegen, 1960, 144 p. — Buchholz, J. T.: The cause of sterility in cross-polinations between certain species of pines. Amer. J. Bot. **31**, Suppl.: 2 s. (1944). — Cantino, Edward C.: Transitional states of ribonucleic acid and morphogenesis in synchronous single generations of *Blastocladiella emersonii*. Phytochemistry, **1**: 107—124 (1962). — Dengler, A.: Künstliche Bestäubungsversuche an Kiefern. Zeitschr. Forst.- und Jagdw. **64**: 513—555 (1932). — Dengler, A.: Herkunfts- und Kreuzungsversuche im Versuchsgarten des Waldbauinstitutes Eberswalde. Mitt. Deutsch. Dendr. Ges. **55**: 157—169 (1942). — Dennis, F. G., Jr., and Edgerton, L. J.: Induction of parthenocarpy in the apple with gibberellin and the effects of supplementary auxin application. Proc. Amer. Soc. Hortic. Sci. **80**: 58—63 (1962). — Ehrenberg, C., Gustafsson, Å., Plym Forshell, C., and Simak, M.: Seed quality and the principles of forest genetics Hereditas **41**: 291—366 (1955). — Eklundh-Ehrenberg, C., and Simak, M.: Flowering and pollination in Scots Pine (*Pinus silvestris* L.). Medd. Stat. Skogsforskningsinst. **46** (12): 1—23 (1956). — Ferguson, M. C.: Contributions to the knowledge of the life history of *Pinus* with special reference to sporogenesis, the development of the gametophytes and fertilization. Proc. Wash. Acad. Sci. **6**: 1—202 (1904). — Håkansson, A.: Seed development of *Pinus abies* and *Pinus silvestris*. Medd. Stat. Skogsforskningsinst. **46** (2): 1—24 (1956). — Jacobs, W. P.: Longevity of plant organs: internal factors controlling abscission. Ann. Rev. Pl. Physiol. **13**: 403—436 (1962). — Luckwill, L. C.: Hormonal aspects of fruit development in higher plants. In the Biological action of growth substances. Symp. Soc. Exp. Biol. **11**: 63—85 (1957). — McWilliam, J. R.: Interspecific incompatibility in *Pinus*. Amer. J. Bot. **46**: 425—433 (1959). — McWilliam, J. R., and Mergen, F.: Cytology of fertilization in *Pinus*. Bot. Gaz. **119**: 246—249 (1958). — Mergen, F.: Self-fertilization in slash pine reduces height growth. U. S. Forest Serv. Southeastern Forest Exp. Sta. Res. Note 67, 1954. — Orr-Ewing, A. L.: A cytological study of the effects of self-pollination on *Pseudotsuga menziesii* (Mirb.) Franco. Silvae Genetica **6**: 179—185 (1957). — Plym Forshell, C.: Kottens och fröets utbildning efter själv- och korsbefruktning hos tall (*Pinus silvestris* L.). Summary: The development of cones and seed in the case of self- and cross-pollination in *Pinus silvestris* L. Medd. Stat. Skogsforskningsinst. **43** (10): 1—42 (1953). — Sarvas, R.: Investigations on the flowering and seed crop of *Pinus silvestris*. Comm. Inst. Forest. Fenn. **53** (4): 1—198 (1962). — Schütt, P.: Züchtung mit Kiefern. II. Kreuzungen, Resistenzzüchtung und Zytologie. Mitt. Bundesforschungsanst. Forst- u. Holzwirtsch. **42**: 1—40 (1959). — Squillace, A. E.: Variation in cone properties, seed yield and seed weight in Western white pine when pollination is controlled. Montana State Univ. School of Forestry Bul. **5**: 1—16 (1957). (Missoula.) — Squillace, A. E., and Bingham, R. T.: Selective fertilization in *Pinus monticola* Dougl. I. Preliminary results. Silvae Genetica **7**: 188—196 (1958). — Ts'o, P. O. P.: The ribosomes-ribonucleoprotein particles. Ann. Rev. Pl. Physiol. **13**: 45—80 (1962) — Wettstein, W. von: Zur Blütenbiologie von *Pinus silvestris*. Zeitschr. Forst- u. Jagdw. **72**: 404—409 (1940). — Wright, J. W.: Summary of tree breeding experiments by the Northeastern Forest Experiment Station 1947—1950. Northeast. Forest Exp. Sta., Sta. Paper **56**: 1—47 (1953). — Wright, J. W.: Species Hybridization in the white pines. Forest Science **5**: 210—222 (1959).