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Seed Sterility and Disturbances in Embryogeny in Conifers with particular Reference to Seed Testing and Tree Breeding in Pinaceae

Frösterilitet och störd embryoutveckling hos barrträden med speciell hänsyn till frökontroll och växtförädling hos Pinaceae

by

P.D. DOGRA

SKOGSHÖGSKOLAN
ROYAL COLLEGE OF FORESTRY
STOCKHOLM

In this work reference is made to several recently published and to some unpublished contributions by the author[1] (written singly or in joint authorship). These are listed under REFERENCES given at the end.

Studies on Swedish conifers were carried out and this manuscript prepared and completed at the Department of Forest Genetics, Royal College of Forestry, Stockholm 50, Sweden (1965—1967). Observations on Indian conifers were made at the Department of Botany, Punjab University, Chandigarh (1954—1961) and at the National Botanic Gardens, Lucknow (1962—1965), India.

[1] Visiting Scientist from National Botanic Gardens (Council of Scientific and Industrial Research), Lucknow, U,P., India.

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Introduction

The study of conifer embryogeny has followed two lines: one for seed testing in forestry, the other for academic interests in botany. SIMAK and GUSTAFSSON (1953 a, b, 1954) showed that conifer embryo and endosperm studies by X-ray technique could be successfully employed in tree breeding. In principle the X-ray technique is based on morphological methods of seed testing used by HEIKINHEIMO (1915, 1921), HAGEM (1917), WIBECK (1928 b, 1929 b), OLDERTZ (1921) and KUJALA (1927, 1928) and was developed by SIMAK and GUSTAFSSON and co-workers in numerous publications (SIMAK and GUSTAFSSON, 1953 a, b, 1954, 1957, 1959; PLYM FORSHELL, 1953; MÜLLER-OLSEN and SIMAK, 1954; EHRENBERG *et al.*, 1955; SIMAK, 1957, 1966; SIMAK *et al.*, 1957, 1961; SIMAK and KAMRA, 1963; GUSTAFSSON and SIMAK, 1958 a, b; MÜLLER-OLSEN *et al.*, 1956; KAMRA, 1963, 1964 a, b; KAMRA and SIMAK, 1965; ANDERSSON, 1965) and others (*e.g.* BARTELS, 1956; ROHMEDER, 1957; KLAEHN and WHEELER, 1961; NEKRASOV and SMIRNOVA, 1961; HANSEN and MUELDER, 1963; and KRIEBEL, 1966). In morphology conifer embryology has been studied exhaustively (see DOYLE, 1916—1963 *et seq.*; BUCHHOLZ, 1918—1950; SCHNARF, 1933, 1937; JOHANSEN, 1950; WARDLAW, 1955) and critically reviewed on the basis of later work by DOYLE (1957, 1963), DOGRA (1961, 1966 b) and CHOWDHURY (1962). The present discussion combines both practical and academic trends in embryological studies in order to obtain a better understanding of embryo and endosperm development which is shown to be important in evaluating seed quality and germination in conifers.

In conifers, classical techniques are as indispensible as X-ray methods, and are being profitably used in forest genetics in investigations on self and interspecific incompatibility, pollination mechanisms, seed setting and development, and seed sterility arising in experiments on self-pollination and in interspecific hybridization (DOYLE, 1916, 1918, 1945 b; DOYLE and

O'LEARY, 1935 a, b, c; DOYLE and KANE, 1943; HÅKANSSON, 1956, 1959, 1960, ORR-EWING, 1957 b; MCWILLIAM, 1958, 1959, 1960; BARNER and CHRISTIANSEN, 1960, 1962; ALLEN, 1963; SARVAS, 1962; HAGMAN and MIKKOLA, 1963; DOGRA, 1964, 1966 a; MERGEN *et al.*, 1965). MEHRA (1960) included embryology as one of the branches of study required for a joint approach to a systematic improvement of forest trees and GUSTAFSSON and MERGEN (1964) mentioned X-ray analysis of embryo and endosperm development as one of the many devices available for developing tree breeding programmes. A background of embryogeny may perhaps also be useful in such attempts; thus the normal pattern of embryo-endosperm development in Pinaceae, based on the present investigation, and on the analyses attempted by DOYLE (1954, 1957, 1963), DOGRA (1961, 1966 b) and MEHRA and DOGRA (unpublished), is presented here for use in tree breeding research.

SARVAS (1962) showed that determining embryo mortality in breeding experiments is difficult because embryo collapse in conifers is governed by complex factors. MERGEN *et al.* (1965) emphasized the need for correct understanding of embryology for accurate interpretation of stages of embryo failure. Embryology was successfully used by ORR-EWING (1954, 1957 b), HAGMAN and MIKKOLA (1963) and MERGEN *et al.* (1965) in studies of self-sterility in self-pollination and interspecific crosses in species of *Pseudotsuga*, *Pinus* and *Picea*. An understanding of the embryological disturbances responsible for embryo degeneration will no doubt be useful in such investigations on tree breeding, especially in analysis concerning the formation of empty seed.

A standard terminology based on clear morphological concepts is necessary for describing conifer embryo development. This was demonstrated by DOYLE (1954, 1957, 1963) who discussed the proembryo terms in detail and was also clearly recognized by SARVAS (1962). The modifications of terms (for proembryo and late embryogeny) used here were proposed by MEHRA and DOGRA (see DOGRA, 1961, 1966 b). Incorporated in these are new terms based on the work on Swedish conifers as well as terms given by DOYLE (1954, 1957, 1963) and others and three suggestions proposed by DOYLE (1963, p. 212) namely: "(a) the terms "rosette", "primary suspensor" and "prosuspensor" are dropped; (b) the basal nature of the normal proembryo type is clearly indicated; and (c) the derived advanced nature of the *Pinus* type and its relations to the normal type are suitably recognized". An effort is made here to keep the embryo terms simple, precise and easy to use.

This investigation deals with disturbances in embryogeny as a cause of inferior seed formation and seed sterility in some *Pinus* species, *Abies pindrow*, *Picea smithiana* and *Cedrus deodara* from the Northwestern Himalayas, India, and in *Pinus silvestris* and *Picea abies* from northern Sweden. The

work on Indian and Swedish conifers was undertaken on the suggestion of Professor P. N. MEHRA (Chandigarh) and Professor ÅKE GUSTAFSSON (Stockholm) respectively. The seeds of conifers from northern and southern Sweden were made available to me by Dr MILAN SIMAK whose generous help made this study possible. An attempt has been made to correlate embryo studies in seed testing by the use of X-ray techniques with principles of embryogeny. Aspects of embryo mortality and seed sterility are also discussed in relation to tree breeding.

Material and Methods

The ovules of *Pinus gerardiana*, *P. montezumae*, *P. nigra*, *P. patula*, *P. roxburghii*, *P. wallichiana*, *Cedrus deodara*, *Picea smithiana* and *Abies pindrow* from Himachal Pradesh in the North-western Himalayas in India were fixed in acetic acid and alcohol in ratio of 1:3 and preserved in 75 per cent alcohol during the years 1954—1964. Seeds of *Pinus silvestris* and *Picea abies* from northern Sweden were studied by X-ray radiographs (for technique see SIMAK and GUSTAFSSON, 1953 a, b, 1954 *et. seq.*) and by dissections of whole embryos by Buchholz's technique (BUCHHOLZ, 1918, 1929, 1938) from seeds presoaked in water. Safranin and fast green were used for staining microtome sections and acetocarmine for whole mounts of embryos.

Embryo Development and Terminology in Pinaceae

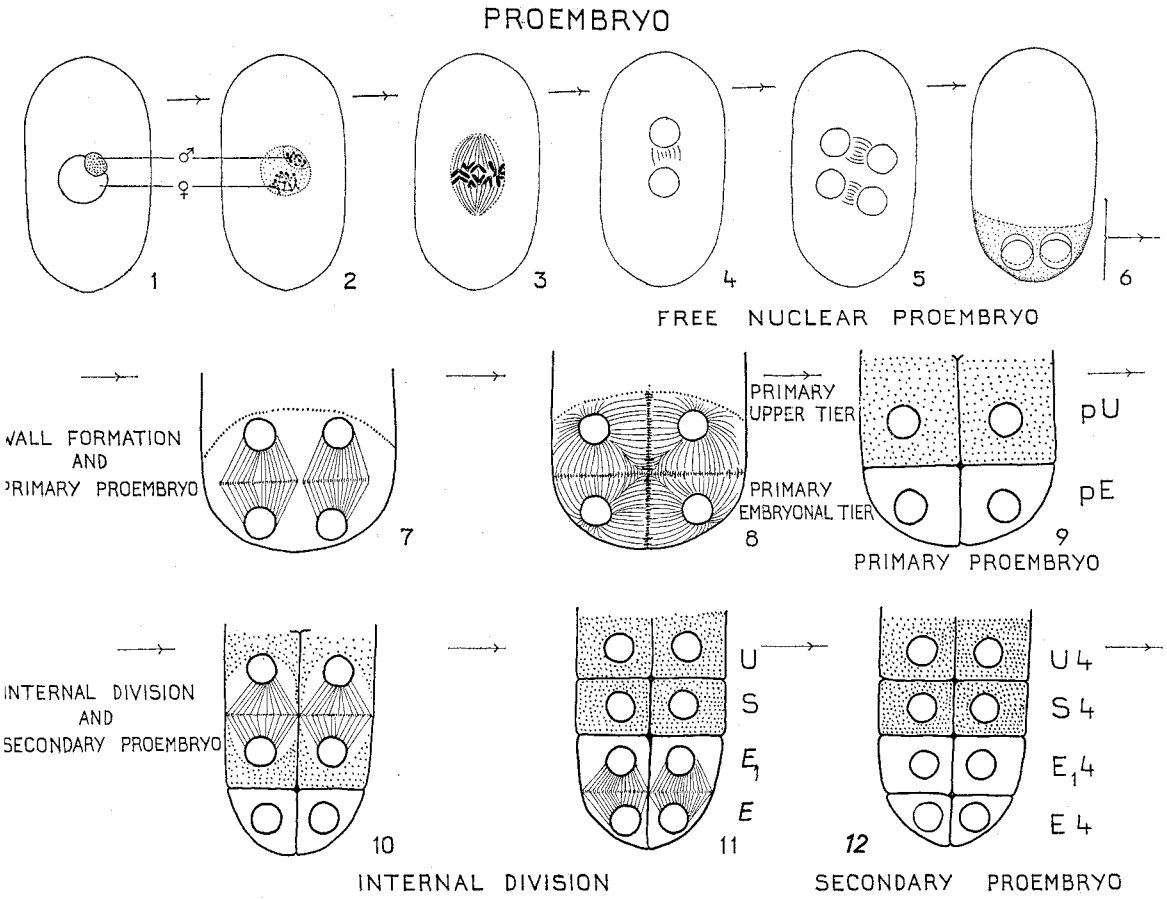
According to DOYLE (1957) the term embryology includes both pre-fertilization and post-fertilization stages while the term *embryogeny* is used only for post-fertilization stages. This account deals with embryogeny.

In gymnosperms embryogeny is studied in two separate phases. *Proembryo* (Fig. 3—12) includes all post-fertilization stages from gametic fusion to *suspensor* (for conifers in general) or substitute suspensor E_1 elongation (for Pinaceae). Further development is called *late embryogeny* (Figs. 14—23), which includes differentiation of tissues and organs in the embryo.

Proembryo

First proembryonal or zygote division: The male and female nuclei fuse (Fig. 1) and separate chromatin networks embedded in a common ground plasm are formed (Fig. 2). The egg after the fusion of male and female nuclei is called the *zygote*. In the first division a multipolar, later a bipolar spindle is formed (Fig. 3). The male and female chromatins form separate chromosome complements on a common spindle and a normal mitosis takes place (Fig. 3). The diploid chromosome number can be counted at metaphase. The *zygote* phase ends here (Figs. 1—3).

Free nuclear proembryo and wall formation: After two divisions *in situ* (Figs. 4, 5) four free nuclei move to the base by a morphogenetic process partly seen in the traction fibres (see JOHANSON, 1950, pp. 24—26) recorded in *Pinus* (SETHI, 1929; DOGRA, 1961, and see Figs. 35, 50). This is the last free nuclear stage (Fig. 6). Nuclei extruded from some free nuclear proembryos as seen in *Podocarpus andinus* are called *relict* nuclei by LOOBY and DOYLE (1944). During the third simultaneous mitoses of the proembryo, the vertically arranged spindles participate in wall formation, laying down horizontal walls (Fig. 7) which extend to the newly developed outer proembryonal wall formed by internal depositions in the lower part of the egg (Figs. 6, 50). Thus simultaneously occurring vertical spindles distribute four nuclei to the upper and four to the lower tier in a symmetrical arrangement which forms a characteristic feature of the proembryo in Pinaceae (Fig. 7). The vertical walls appear to arise on secondary horizontal fibres. Thus a multiple spindle system lays down, in two independent steps horizontal and vertical walls (Fig. 8) which extend to the outer proembryonal wall to form the *primary proembryo* (Fig. 9). *Primary type* of wall formation in the proembryo occurs



Figs. 1—12. Proembryo, development from fertilization to secondary proembryo. pU primary upper tier, pE, primary embryonal tier: U, secondary upper tier; S, suspensor tier; E_1, first embryonal segment; E, embryonal cells. Tiers are of 4 cells each. For further explanation see text.

on equatorial thickenings on the spindles of the previous division (Fig. 7), while in the *secondary type* the spindles do not *appear* to participate and walls originate on thickenings on *secondary* fibres (a term used by KILDAHL, 1907, see DOYLE, 1963, pp. 204—206; DOGRA, 1966 b). In gymnosperms a secondary type of wall formation is common but in Pinaceae wall formation in the proembryo occurs by both primary and secondary mechanisms.

Primary proembryo: *Primary proembryo* is the first cellular structure and consists of two primary tiers of four cells each which form two morphological parts (Fig. 9). *Tier* in an embryo is a term applied to cells lying in the horizontal plane, pU for *primary upper tier* (*Open tier* of BOYLE and DOYLE, 1954) and pE for lower *primary embryonal* cells (Fig. 9). pU cells are normally open from above; occasionally some, or in rare abnormalities all, may be partially and sometimes completely closed.

Internal division: *Internal division* (a term first used by BOYLE and DOYLE, 1954) occurs in the cells of the primary proembryo giving rise to the *secondary proembryo* (Figs. 10—12) described as U:S:E type by DOYLE (1957, 1963). It is the last proembryonal division taking place in the two primary parts. pU gives rise to the *secondary upper tier* U and the *suspensor tier* S; pE divides to produce two tiers of embryonal cells E_1 and E (Fig. 11). In Pinaceae, except for *Pseudotsuga* (ALLEN, 1946), internal division frequently takes place first in the cells of the pU, although pE are also known to divide first (KILDAHL, 1907; MEHRA and DOGRA, unpublished). The spindles of the internal division are arranged vertically and divisions in cells of a tier are synchronous, forming a *secondary proembryo* with four symmetrical tiers of four cells each (Fig. 12). In Pinaceae the egg is large but only a small portion is utilized in proembryo formation: this is a characteristic feature of conifer embryogeny.

Secondary proembryo: According to DOYLE (1963, pp. 181, 211) the secondary proembryo U:S:E has the construction U4:*v*S4:*e*S4:E4 ("*v*S" designating vestigeal suspensor and replacing the term "rosette tier"; and "*e*S" the *de facto* elongating cells, replacing the term "primary suspensor" and the embryo tier "E"). The general conifer plan however, shows the construction Ux: Sx: Ey arising from a variable number of cells in pU and pE (x, y designate the number of cells in each tier). For details the reader is referred to the original papers of DOYLE (1954, 1957, 1963). In conifers the *proembryo structure at this stage has a bearing on late embryogeny* and to avoid confusion I prefer to use a modification of an earlier suggestion by DOYLE ("U4: S4: E4+4" see DOYLE, 1957, p. 125), *viz.* U4:S4:$E_1$4:E4, where no distinction is made between vestigeal suspensor S4 and the first embryonal segment $E_1$4, (called substitute suspensor) of Pinaceae (Fig. 12) from functional suspensor Sx and Ey respectively of the general conifer plan. In Pinaceae U and S tiers have no function and they degenerate; the S tier may, however, show irregular divisions of the cells, here termed *proliferations* (p) in late embryogeny. Such proliferations of S cells have been called rosette embryos but there is not a single report on record where an embryo has been observed to develop from them and as proposed by DOYLE (1957, 1963), this term is discarded.

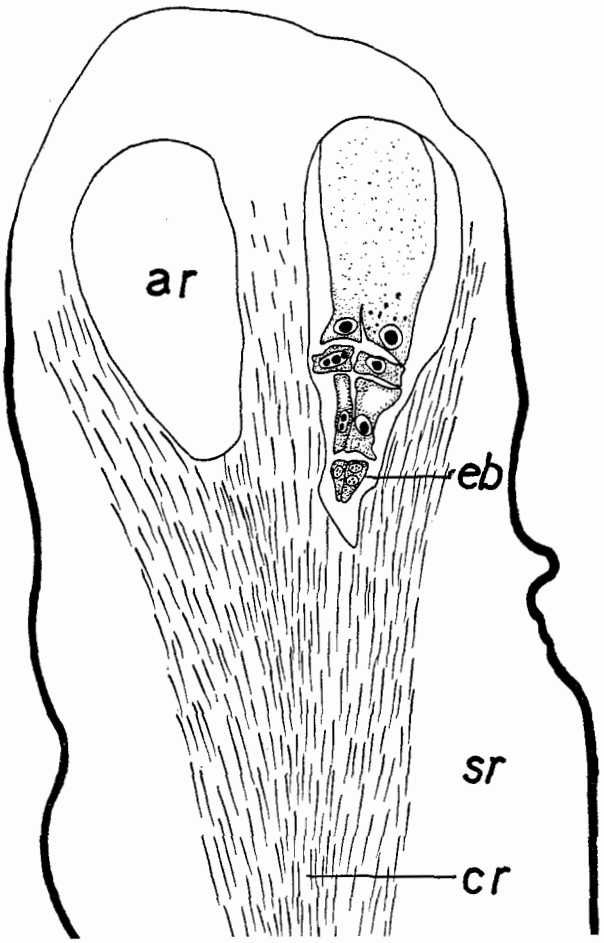


Fig. 13. *Picea smithiana* prothallus showing corrosion region (cr), storage region (sr), archegonia (ar) and embryo (eb). X 90.

Corrosion Region, Prothallus and Endosperm

During maturity a conical region of the prothallial tissue is differentiated below the archegonia (Buchholz, 1918; Doyle and Looby, 1939; Schopf, 1943; Orr-Ewing, 1957 b; Dogra, 1961). The cells of this region are dense, physiologically active and arranged in a linear pattern (Fig. 13). The embryos grow into this corrosion region, secrete enzymes into it and absorb the material. Disintegrated and liquified cells of the *corrosion region* are thus digested and absorbed by the embryos, while the outer tissue forms a storage

region utilized during germination of the seed (HÅKANSSON, 1956). The gametophyte in conifers is thus partly used for nutrition and formation of the embryo, but mainly it matures to form storage tissue (endosperm) which is emptied during germination of the seed. The female gametophyte in the maturing ovule and seed is termed *endosperm*, which is mainly the outer storage tissue; the corrosion region after being utilized by the young embryos (see DAHLGREN, 1931, Fig. 34) forms the *endosperm cavity* which fills with cotyledonous embryo in the seed. In gymnosperms the endosperm is haploid and forms a continuation of the female gametophyte, in contrast to angiosperms, where it is normally triploid in constitution if the two-polar nuclei and male cells which fuse to form it are haploid. The changes that take place in the *ovule* after fertilization result in the transformation of the ovule into a *seed*. The term *ovule* includes the ripening ovule containing post-fertilization stages to distinguish it from the *seed* collected from the ripened cones at the advent of winter.

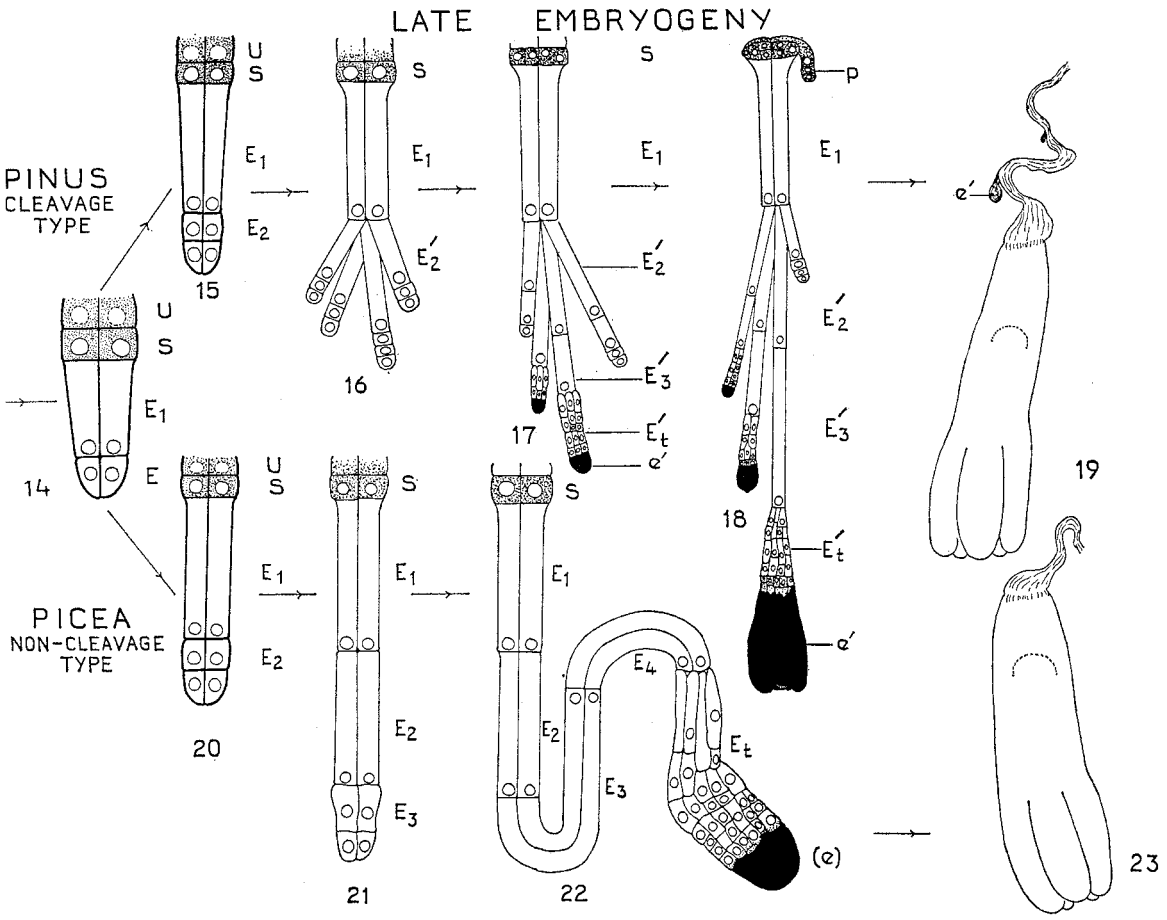
In species of Pinaceae which I studied in India the ovule contained a developed embryo in autumn (mid-October). A period of "seed maturation" as shown by BUCHHOLZ (1946, Fig. 7, p. 241) in *Pinus ponderosa* was seen in Indian species during autumn before the seeds were shed from the tree (see KONAR and RAMCHANDANI, 1958; KONAR, 1960, 1962).

In *Pinus silvestris* and *Picea abies* trees growing in northern Sweden, the embryos are seldom mature in most seeds at the time of seed collection before winter and variation in embryo stages is then a characteristic feature (Figs. 61—113).

Late Embryogeny

The proembryonal phase ends with the elongation of the substitute suspensor E_1 in Pinaceae (Fig. 14) or suspensor S in conifers in general (see DOYLE, 1957, 1963), and the embryo development which follows is called *late embryogeny* (Figs. 14—23).

Suspensor-system consists of suspensor S and all elongated suspensor-like E segments of the embryo. E_1, E_2, E_3 etc. denote tiers of simultaneously elongating cells attached to the suspensor (Figs. 17, 22, 59). In the formulae elongation is indicated by a sereis of dots thus, " . . . " (see p. 17). E_t is used for numerous proximal cells of an embryonal mass which elongate irregularly and do not form tiers (Figs. 17, 22). These may be formed directly after suspensor S elongation, as in Taxodiaceae (DOGRA, 1966 b) or after E_1, E_2, E_3 etc. as in Pinaceae. The production of E_1, E_2 etc. in the suspensor-system is evidently due to physiological processes similar to those which give rise to S with which they are continuous (WARDLAW, 1954).



Figs. 14—23. Late embryogeny in species showing cleavage (*Pinus*, Figs. 15—19) and non-cleavage (*Picea*, Figs. 20—23). U, secondary upper tier; S, suspensor tier; E_1, E_2 etc. embryonal segments; e, embryonal mass ofcells. For further explanation see text.

Polyembryony: Polyembryony means the occurrence of more than one embryo in an ovule. This feature of gymnosperms was first reported by Brown (1844). When this condition arises from fertilization of more than one archegonium it is termed as *archegonial polyembryony* (simple polyembryony of Buchholz, 1920; and polyzygotic polyembryony of Schnarf, 1933) when due to cleavage of terminal embryo units (Figs. 16—18) as *cleavage polyembryony* (Buchholz, 1918).

Embryo unit is used for a group of cells derived from one pE cell.

Non-cleavage is absence of cleavage (Fig. 22) and is denoted by "( )", (see p. 16—17).

An unstable non-cleavage condition may occur which is intermediate

between non-cleavage and cleavage condition. *Unitary lobing* (Fig. 49, re) results from different rates of growth of embryo units (4 in Pinaceae) in an embryonal mass (e). When it occurs temporarily it is *transitory;* when retained it is *persistent* (Fig. 77). Genera normally showing cleavage and non-cleavage sometimes show *partial cleavage* where pE units separate incompletely (some separate, other embryo units remain joined, Figs. 93 B, 98 B, 103) by differential elongation of E_t, E_1 and E_2 etc. of each lobe. If a persistent condition of unitary lobing or partial cleavage survives in the seed during germination it gives rise to joined isogenic twins. Seeds containing such embryos have been germinated under laboratory conditions in *Pinus gerardiana* by me and in *Pinus silvestris* by SIMAK. In *Pinus silvestris* such seedlings have survived.

Cleavage polyembryony is an organized and not accidental phenomenon occurring only between pE units and is termed *unitary cleavage* (Figs. 16—18). It is due to the growing independence of potential units of the primary proembryo and is expressed as")(". Unitary cleavage found in the conifer embryo is thus not comparable to the accidentally occurring cleavage seen in animals (*cf.* DOYLE and LOOBY, 1939; THOMSON, 1945; DOYLE, 1954). Abundant nutrition from the corrosion region no doubt makes polyembryony possible but it is mainly a gene-controlled phenomenon (WARDLAW, 1955). *Total cleavage* occurs when all pE units separate completely by differential elongation of E_t or E_1, E_2 etc. of each unit. It occurs early at E_2 (Fig. 16) in some species and late at E_3 or E_4 in others (Fig. 59) and is sometimes variable in the same species. A separate unit or part of a unit is designated by an accent, for example, E'_1, E'_t and e' (Figs. 17, 18). Sometimes the elongating E segment cells divide vertically to give rise to a more than one-celled E' (see figures in BUCHHOLZ, 1918, 1931; JOHANSON, 1950; WARDLAW, 1955; CHOWDHURY, 1962) which is indicated in the formulae by crossing E': thus "Ɇ' ". *Inhibitory cleavage* occurs from the failure of E_t, E_1, E_2 etc., elongations or by the inhibited growth of one or more units of an embryonal mass (Figs. 44, 46). This separation is sometimes expressed incompletely as *partial cleavage* and two to three units, rarely even four, may remain joined (Figs. 93 B, 98 B, 103).

In an ovule where polyembryony, both cleavage and archegonial, is present, the embryo with the best physiological constitution situated in the most suitable environment dominates over the others and survives (terminal embryo seen in late embryogeny, Fig. 18). Others degenerate: they encounter mechanical, nutritional and growth-inhibiting influences of the dominating embryo and of the environment. In the early stages the developing embryo grows into the stimulated gametophytic tissue along with the isogenic and heterogenic embryos from one or several archegonia

respectively. The ovule may be influenced variously at different stages of embryo-endosperm growth by changes in external environment such as those of sub-arctic climate in Sweden. A complex physiological relationship between embryo development and the nourishing gametophyte was shown by KONAR (1958 a, b) in *Pinus roxburghii* and the importance of such relationships was emphasized by WARDLAW (1955) and HÅKANSSON (1960). BUCHHOLZ (1918—1950) believed that selection in embryo competition was one of the major factors in evolution of the conifer embryo. The arguments and evidence presented in support of this belief (BUCHHOLZ, 1918, 1920 a, b, 1922, 1926, 1929, 1931, 1946, 1950) were criticized and corrected by DOYLE and LOOBY (1939), THOMSON (1945), DOYLE (1954, 1957, 1963) and HÅKANSSON (1956). STOCKWELL in 1939 pointed out the importance of preembryonic selection to tree breeding research. Future cytological and laboratory culture studies of embryos from archegonial polyembryony will probably show that BUCHHOLZ though not wholly correct in his main arguments, was not totally wrong either in some general observations (see DISCUSSION, pp. 83—84) though as shown by DOYLE, his concept of "simple polyembryony" (non-cleavage) versus cleavage polyembryony, his emphasis on the derived nature of non-cleavage and his application of embryological data to the solution of phylogenetic and taxonomical problems was wrongly based. WARDLAW (1955) states: "Until we have more knowledge of the factors involved in the inception and development of the various embryonic features, and some clue as to how these features change under the impact of genetical change, it will be difficult to determine finally the taxonomic relationships". DOYLE (1957) showed that embryological data in conifers can only be applied with great discretion, if at all, in such problems.

Polyembryony whether archegonial, cleavage, or both when it persists in the seed is called *persistent polyembryony* (Figs. 81—104, 106—113). Embryo mortality may occur at the proembryo stage or during late embryogeny in a maturing ovule or seed and the expression refers to the dominant, mostly terminal embryo. In the persistently polyembryonic condition the expression may refer to two to three dominant embryos if they are of nearly equal size. An embryo from a seed is considered to be dead only when it shows a clear-cut degenerated condition and is incapable of further development. Young embryos in the seed may be dead or living; the living embryos may show inhibited development and thus remain immature but growth and differentiation may be resumed as soon as conditions are favourable. Degeneration of the embryo is characterized by excessive vacuolation (Figs. 31, 40, 41), empty or shrunken cells (Figs. 64 B, 65 B, 74 B, 75 B), erratic staining behaviour (see ORR-EWING, 1947 b), loss of staining affinity (Fig. 75 b) or unusually deep staining of a part or the whole of the embryo (Figs. 44, 52, 58).

Another phenomenon *proliferation*, though less common in Pinaceae, is widespread in some conifer families, as in Taxodiaceae and Cupressaceae (DOGRA, 1961, 1966 b). *Proliferation* is unorganized prolific secondary lobing in an embryo unit or in the embryonal mass. Irregular cellular divisions of E_1, E_2 etc. E_t or of the so-called "rosette" or morphological S cells (Fig. 18) may also be termed proliferation, as suggested by DOYLE (1954) and within this category may be mentioned *secondary lobing*, which is of an embryonal unit and occurs either in an uncleaved or a cleaved unit. It may be *transitory* or *persistent*. *Secondary cleavage*, when it occurs, is false cleavage and the term is applied to separation of secondary lobes (DOGRA, 1961, 1966 b).

Pinaceae has ten genera of which the embryogeny of nine is well-known. No work on *Cathaya* is so far available. All members of the family, in general, show a stable pattern of proembryo development and there are no differences even at generic level (DOYLE, 1918, 1957, 1963; DOGRA, 1961; CHOWDHURY, 1962) except for *Pseudotsuga*, which according to ALLEN (1946) deviates from the standard pattern in non-division of the pU tier. An earlier account (ALLEN, 1943) of unusual proembryo development in *Pseudotsuga* reproduced by CHOWDHURY (1963) was contradicted by ALLEN (1946) and shown to be incorrect.

The general features of late embryogeny of nine genera are summarized by the formulae given below (for diagrams see BUCHHOLZ, 1918, 1920 a, 1931; SCHNARF, 1933; JOHANSON, 1950; WARDLAW, 1955; CHOWDHURY, 1962). The structure of the suspensor-system can sometimes vary in a species, as shown in *Pinus ponderosa* (BUCHHOLZ, 1918, p. 199, Fig. 2). The symbols used have already been explained in the above account (pp. 12—14). Intercalated linearly arranged cells (proliferations) of E_1, E_2, E_3 etc. seen in *Pseudolarix* (BUCHHOLZ, 1931), *Keteleeria* (SUGIHARA, 1943) and *Pinus roxburghii* (MEHRA and DOGRA, unpublished) and in some cases of *Cedrus deodara* (Fig. 45) are shown in the formulae by placing "p" on the segment concerned, for instance "$\overset{p}{E_1}$". Such proliferations were mistaken for embryos by BUCHHOLZ (1931) in *Pseudolarix*.

Genera showing non-cleavage:

Abies

USE_1 ..... E_t ..... (e) (BUCHHOLZ, 1920 a, 1926, 1931, 1942; SUGIHARA, 1947; MEHRA and DOGRA, unpublished).

Pseudotsuga

$pUE_1 \ldots\ldots\ldots E_2 \ldots\ldots\ldots ? \ldots\ldots\ldots E_t \ldots\ldots (e)$ (BUCHHOLZ, 1920 a, 1926, 1931; ALLEN, 1943, 1946).

Pseudolarix

$\overset{p}{U}SE_1 \ldots\ldots \overset{p}{E}_2 \ldots\ldots E_3 \ldots\ldots ? \ldots\ldots E_t \ldots\ldots (e)$ (BUCHHOLZ, 1931).

Picea

$USE_1 \ldots\ldots E_2 \ldots\ldots E_3 \ldots\ldots E_4 \ldots\ldots E_t \ldots\ldots (e)$ (BUCHHOLZ, 1920 a, 1942; MEHRA and DOGRA, unpublished).

Larix

$USE_1 \ldots\ldots E_2 \ldots\ldots E_3 \ldots\ldots E_4 \ldots\ldots E_5 ? \ldots\ldots E_t \ldots\ldots (e)$ (SCHOPF, 1943).

Genera showing cleavage:

Keteleeria

$\overset{p}{U}SE_1 \ldots \overset{p}{E}_2 \ldots E_3 \ldots E_4 \ldots E_5 \ldots E_6 \ldots ? \ldots) E'_t (\ldots\ldots e'.$ (SUGIHARA, 1943)

Cedrus

$\overset{p}{U}SE_1 \ldots) E'_2$(sometimes$\ldots$)$\not{E}'_3 (\ldots E'_4 \ldots E'_t \ldots\ldots e'.$ (BUCHHOLZ, 1920 a, 1926, 1931; CHOWDHURY, 1961; MEHRA and DOGRA, unpublished).

Tsuga

$\overset{p}{U}SE_1 \ldots\ldots) E'_2 (? \ldots\ldots) \not{E}'_3 (\ldots\ldots E'_t \ldots\ldots e'.$ (BUCHHOLZ, 1920 a, 1926, 1931).

Pinus

$\overset{p}{U}SE_1 \ldots\ldots) E'_2$(sometimes)$\not{E}'_{2-4} (\ldots\ldots E'_t \ldots\ldots e'.$ (BUCHHOLZ, 1918, 1920 a, 1926, 1931; KONAR and RAMCHANDANI, 1958; MEHRA and DOGRA, unpublished).

Observations on Some Indian Conifers

Proembryo

Free nuclear proembryo: Approximately 1.5 to five per cent of free nuclear proembryos (Figs. 4—7) studied in the species *Pinus nigra*, *Pinus montezumae*, *Pinus gerardiana*, *Picea smithiana* and *Abies pindrow* showed disturbances which lead to seed sterility. The frequency varied according to the year, locality, trees and species.

In *Pinus* after two divisions *in situ* the four proembryonal nuclei shift to the archegonial base by a mechanism indicated by fibre-like formations running from nuclei to the egg wall (Figs. 35, 50). This mechanism sometimes remains half effective or fails and the proembryo in such cases is formed near the middle or on a side-wall of the egg and degenerates (Figs 24, 25).

The four proembryonal nuclei sometimes do not divide simultaneously; some miss this division or a relict nucleus is extruded from the proembryo. In *Abies pindrow* and *Pinus nigra*, six and seven free nucleated proembryos were recorded (Figs. 33, 34). Some of these showed incomplete wall formation.

In *Abies pindrow* some of the free proembryonal nuclei were unequal, as shown in Fig. 37, which shows five unequal nuclei of which two are connected by a narrow isthmus. Such configurations may arise either because of abnormal proembryonal divisions as shown for *Cupressus arizonica* (Fig. 42) or because of fragmentation of embryo nuclei. Thus proembryos consisting of irregular cells and micronuclei are formed (Fig. 36).

Sixteen nuclei in the proembryos were observed in *Pinus gerardiana* and *Pinus nigra* (Fig. 43). Wall formation in primary proembryos with 14 nuclei with two extruded nuclei was observed in *Pinus gerardiana* (Fig. 38).

Primary proembryo: In *Pinus gerardiana* and *Pinus nigra* proembryos with six or seven nuclei in pE tier or one or two nuclei in pE tier were recorded (Fig. 39).

In *Pinus nigra* sometimes only one of the four pE cells of the primary proembryo divided; the three others degenerated (Fig. 54). In *Pinus* species and in *Cedrus deodara* it was sometimes observed that the four units of the primary proembryo did not develop together and that they separated at the proembryo stage. Degeneration of one to three embryo units took place, the remaining ones developed abnormally before degenerating.

In *Picea smithiana* in two cases eight-nucleated large proembryos formed on the side-wall of the egg. They had larger cells and nuclei about double those of the normal proembryo. More than half of the egg was used up in the proembryos, where normally only a small part is utilized (Figs. 27, 53).

About one to two per cent of the primary proembryos formed at the base or on the side-wall studied for *Pinus gerardiana, Pinus wallichiana, Pinus nigra* had smaller nuclei and cells, about half the size of those of the normal proembryo (Fig. 26). These small proembryos occupied a conspicuously small part of the egg. It is possible, although there is no direct evidence, that these proembryos result from a parthenogenetic division of the egg recorded in some cases in *Pinus wallichiana, Pinus nigra* (n=12, see DOGRA, 1966 a) and in *Pinus pinaster* (SAXTON, 1909).

In *Pinus gerardiana* and *Pinus nigra* several proembryos with irregular arrangement of cells without formation of pU and pE tiers were observed. They had large nuclei and micronuclei in variable numbers (Fig. 36).

Secondary proembryo: Abnormalities of the secondary proembryo were not as common as those of the primary proembryo. In *Cedrus deodara* in a few cases the E_1 tier degenerated and the secondary proembryo did not develop further (Fig. 52).

In *Pinus nigra* the pE units of the secondary proembryo were distorted into each other within the archegonium (Fig. 28).

In *Pinus wallichiana* five instead of the normal four tiers were observed in two proembryos.

Proembryo abnormalities varied from two to fifteen per cent of all proembryos studied in a species from different localities. This frequency varied in both trees and species in different years and localities. Numerical counts for particular trees were not made.

Late Embryogeny

Late embryogeny often shows more disturbances but the embryos survive more easily than those from the proembryonal disturbances. Cases leading to embryo degeneration are:

Failure of embryos to grow into the corrosion region: In 25 per cent of ovules of *Pinus nigra* fixed in 1958 at least one embryo in each failed to grow out of the archegonial wall. The secondary proembryos showed curved and distorted E_1 elongation. The four units mainly nourished by the egg were distorted and pushed to one side of the venter (Fig. 29). With E_1 elongation, the embryo coiled within the archegonium (Figs. 30, 51) and finally degenerated (Fig. 31). Such embryos were found developing together with normal ones from different archegonia in an ovule (Fig. 30).

Failure of elongation of segments of the suspensor-system: Failure of elongation or degeneration of any segment from E_1 to E_t occurred in five to ten per cent of the embryos studied in several species of *Pinus*, *Picea smithiana*, *Cedrus deodara* and *Abies pindrow*. This may be caused by failure of some hormonal, or enzymatic stimulus or of some other physiological process necessary for elongation of a segment (Fig. 60). This as well as embryo degeneration may possibly also be due to the inhibitory influence of a physiologically more active embryo from a separate archegonium (Figs. 32, 47, 56). Failure of elongation of E segments is sometimes accompanied by failure of cleavage; thus the following abnormal embryos, where E_t did not develop or elongate, were recorded in species of pines introduced in India.

Pinus montezumae

$USE_1 \ldots\ldots\ldots E_2 \ldots\ldots\ldots E_3 \ldots\ldots\ldots (e)$

Pinus nigra

$USE_1 \ldots\ldots\ldots E_2 \ldots\ldots\ldots (e)$

Pinus patula

$USE_1 \ldots\ldots\ldots\ldots\ldots\ldots (e)$

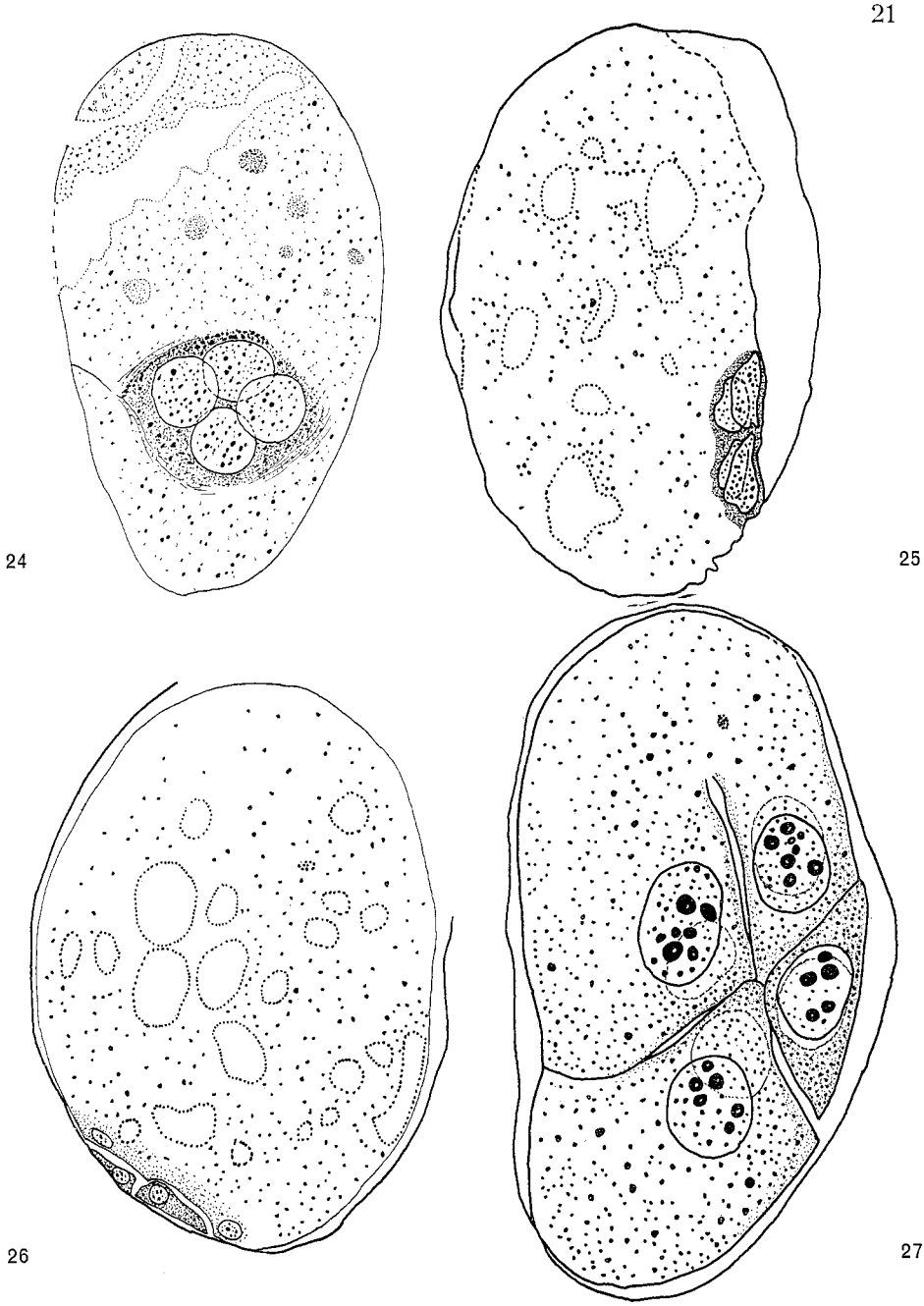
Such abnormalities are, however, rare in native pines. The abnormal embryos may or may not recover.

Young embryos dissected from ovules of *Picea smithiana* kept in fresh running water degenerated. The cells of the dead embryos were highly vacuolated and the four uncleaved units showed a loss of linear growth of cells and of E_1, E_2, E_3 etc. elongation. Irregular unit-lobes were formed directly after E_1 (Figs. 40, 41).

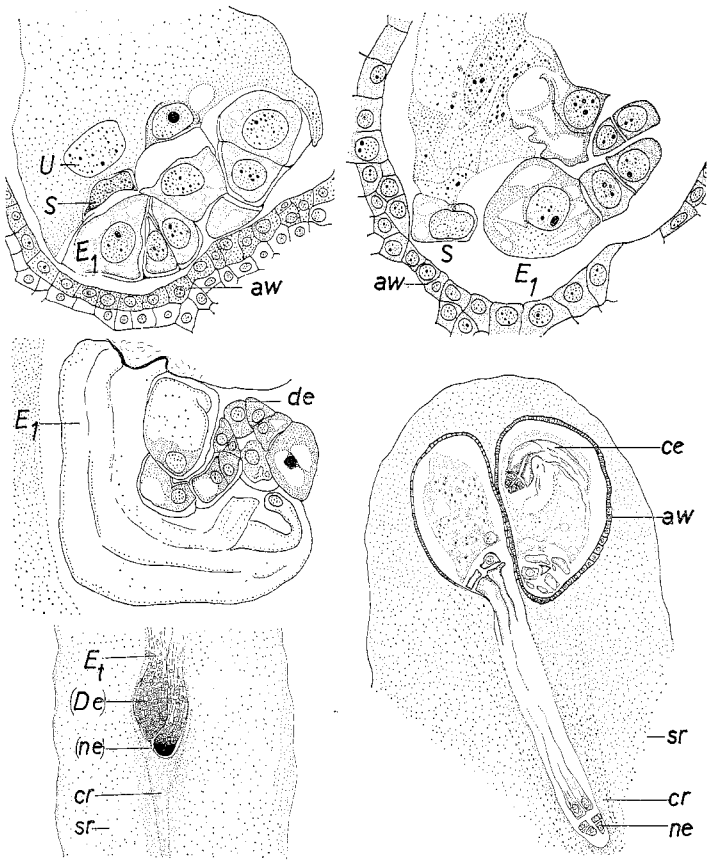
During E_1, E_2 etc. or E_t elongation the embryo sometimes turns and grows in the opposite direction towards the micropyle (Fig. 48) but a normal embryo from a different archegonium of the same prothallus can replace it to form the mature embryo (Fig. 49). An embryo reversed in this manner often degenerates (Dogra, 1961) as shown in figures 74 B, 78 B, but it may form a cotyledonous embryo in the seed as reported in *Picea abies* and in *Pinus silvestris* (Müller-Olsen et al., 1956; Håkansson, 1959).

Abnormal embryos with a variable number (less than four) of cells in early segments of the suspensor-system, or some with conspicuously dislocated, swollen or bulbous segments were also observed (Figs. 55, 57, 58).

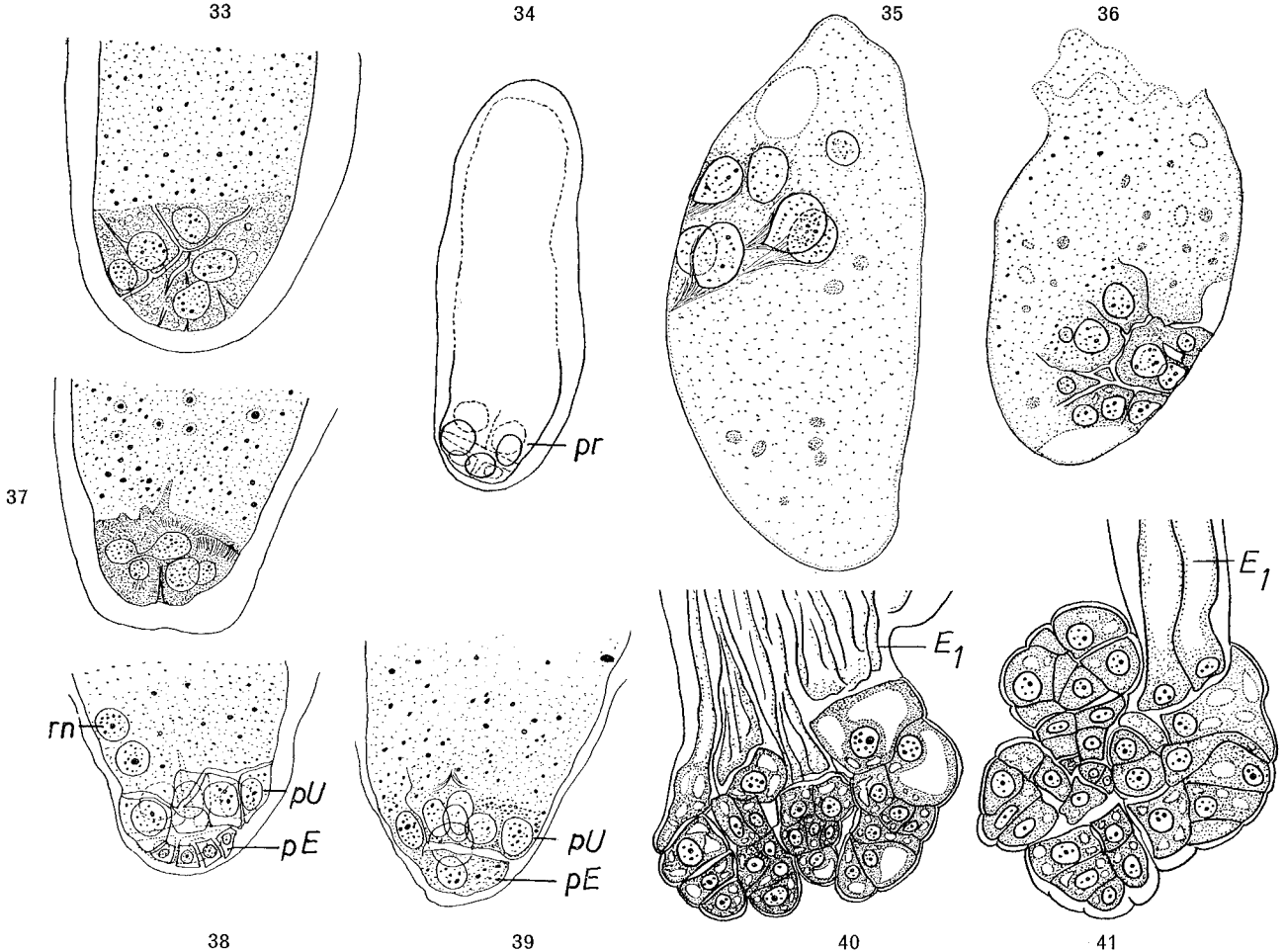
Inhibitory influences due to archegonial polyembryony: Embryos from different archegonia growing together in the same prothallus seem to inhibit each other (Fig. 56). Usually the terminal one is dominant and shows more organized growth, while embryos growing in close proximity but from different archegonia may show inhibited E_1, E_2 or E_t elongation. In such cases, cells of unsuccessful embryos degenerate or show a diffuse organization (Fig. 32).



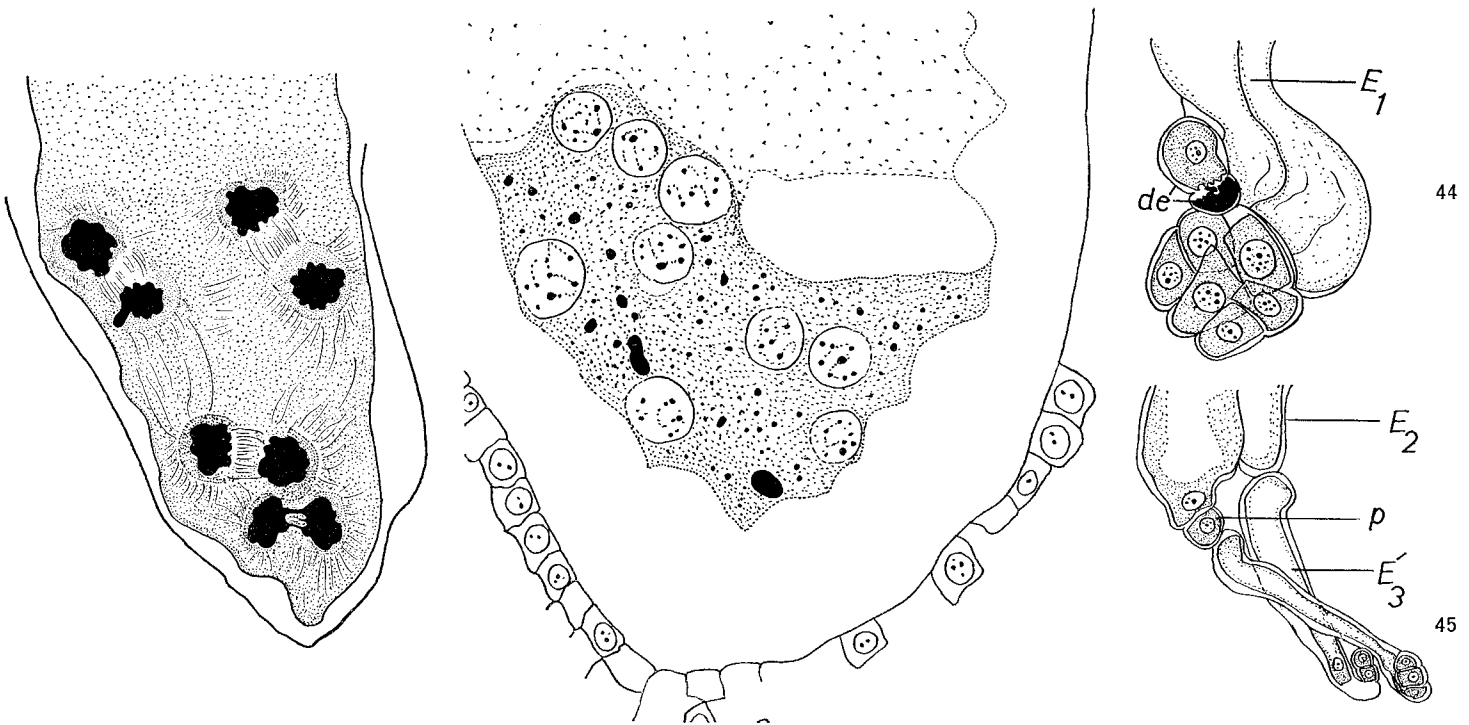
Figs. 24—27. Disturbances in proembryo development. 24—26 *Pinus gerardiana*. 24. Four free-nucleated proembryo formed near center of egg. 25. Same formed on side-wall of egg. 26. Primary proembryo smaller than half the normal proembryo. 27. *Picea smithiana*. Primary proembryo more than half the normal proembryo. 24—26, × 90, 27 × 105.



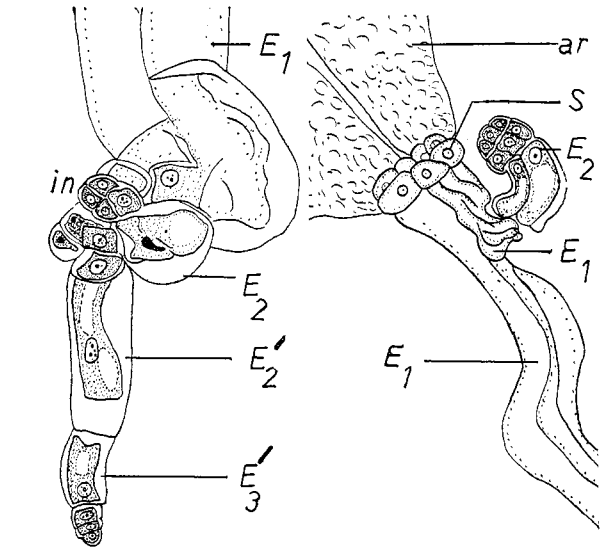
Figs. 28—32. 28—31. *Pinus nigra*. Different stages showing failure of embryo to grow out of archegonium. 28. Distorted secondary proembryo within archegonium. 29. Embryo pushed towards side-wall of archegonium by E_1 elongation. 30. Upper region of prothallus showing archegonial polyembryony. Archegonium on right side contains coiled embryo (ce) and the archegonium on left side bears normal embryo (ne) growing into corrosion region (cr). 31. Whole mount, degenerated coiled embryo from archegonium. 32. *Abies pindrow*. Whole mount, mid-region of prothallus showing archegonial polyembryony. Embryo (ne) on right side is normal, embryo (De) on left shows diffuse growth. U, secondary upper tier; S, suspensor tier; E_1, first embryonal segment tier; aw, archegonial wall; cr, corrosion region; sr, storage region; ne, normal embryo; ce, coiled embryo; de, degenerated embryo; De, diffused embryo. 28, 29, × 112; 30, × 90; 31, × 52; 32, × 40.



Figs. 33—41. 33. *Abies pindrow*. Six-nucleated proembryo showing incomplete wall formation. 34. *Pinus nigra*. Egg with seven-nucleated proembryo at base showing incomplete wall formation. 35. *Pinus gerardiana*. Egg showing four proembryonal nuclei associated with fibre-like formations and smaller nuclei not of proembryo origin. 36. *Pinus gerardiana*. Irregularly organized proembryo with micronuclei. 37. *Abies pindrow*. Proembryo after third division showing dumbbell-shaped nucleus, micronuclei and traces of wall formation. 38. *Pinus gerardiana*. Abnormal fourteen-nucleated primary proembryo showing two extruded relict nuclei (rn). 39. *Pinus gerardiana*. Eight-nucleated primary proembryo showing seven nuclei in pU and one in incompletely walled pE. 40, 41. *Picea smithiana*. Whole mounts of dead embryos, dissected from ovules kept in fresh water. Normal elongation of E_2, E_3 etc. is absent, four embryo units form a clump, dead cells become vacuolated, empty and take little or no stain. 33, 37, × 130; 34—36, 38, 39, × 90; 40, 41, × 105.



Figs. 42—45. 42. *Cupressus arizonica.* Proembryo showing third free nuclear division in telophase. Lowermost configuration shows sticky mitosis. 43. *Pinus nigra.* Abnormal sixteen free-nucleated proembryo. Nine nuclei are shown. 44. *Cedrus deodara.* Whole mount of embryo tip showing one inhibited degenerated embryo unit (dé). 45. *Cedrus deodara.* Whole mount, two embryo units after cleavage. One unit shows an intercalated cell (p) arising from proliferation of E_2. 42, × 900; 43, × 400; 44, 45, × 105.



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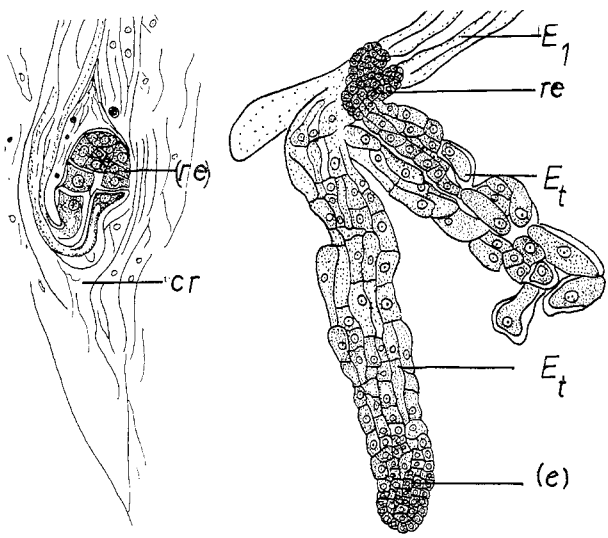

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Figs. 46—49. 46. *Pinus montezumae.* Whole embryo mount showing inhibitory cleavage in which three inhibited units (in) have degenerated. 47. *Cedrus deodara.* Whole embryo mount showing inhibited E_1 elongation in the embryo on right. The embryo on left (only E_1 shown) shows normal development. 48. *Abies pindrow.* Whole mount of part of corrosion region (cr) dissected to show the reversed embryo (re). 49. *Abies pindrow.* Whole mount of two archegonial embryos: (re) sharply bent at Et, shows unitary lobing, the other (e) on left replaces it and is normal. in, inhibited embryo units; ar, archegonium; S, suspensor; cr, corrosion region; re, reversed embryo; e, normal embryo. 46, 47, × 90; 48, 49, × 55.

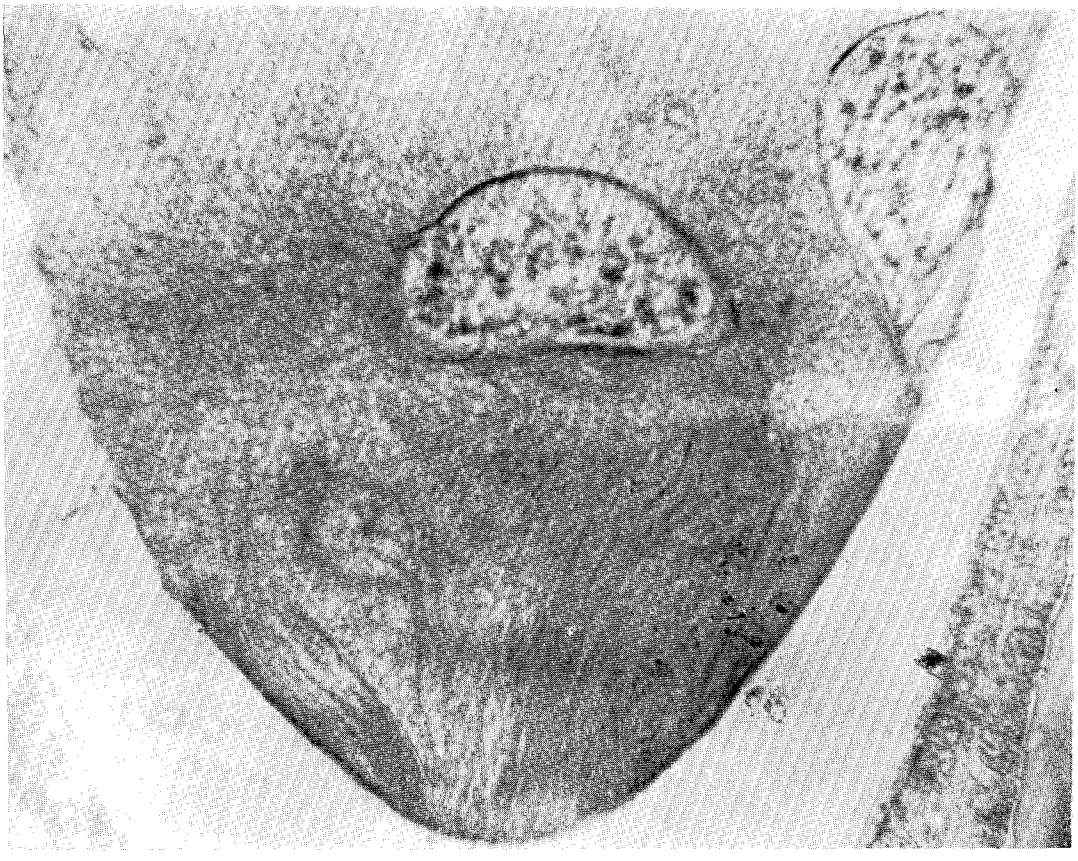

Fig. 50. *Pinus gerardiana.* Photomicrograph of base of egg showing four-nucleated proembryo, only two nuclei are shown with fibre-like "traction" mechanisms associated with downward migration of nuclei × 450.

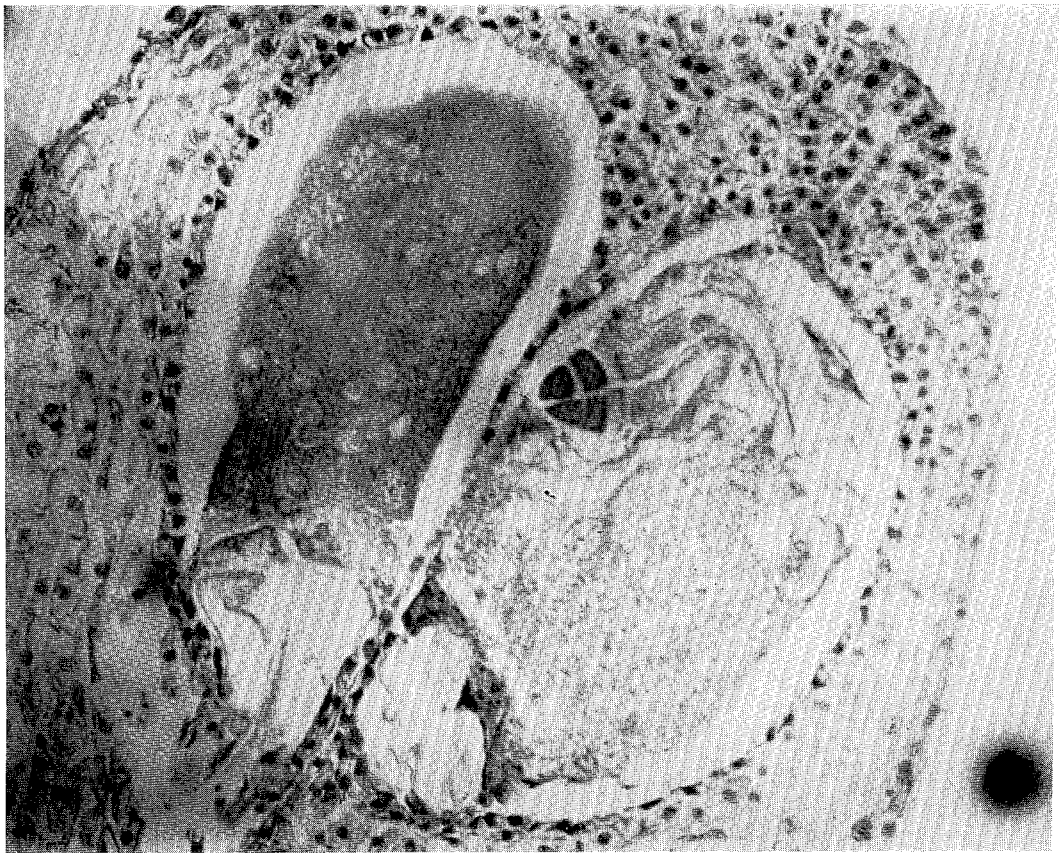

Fig. 51. *Pinus nigra.* Photomicrograph of micropylar region of prothallus. Archegonium on right shows embryo coiled within venter and archegonium on left shows normal embryo of which only upper part of E_1 is shown. $\times 160$.

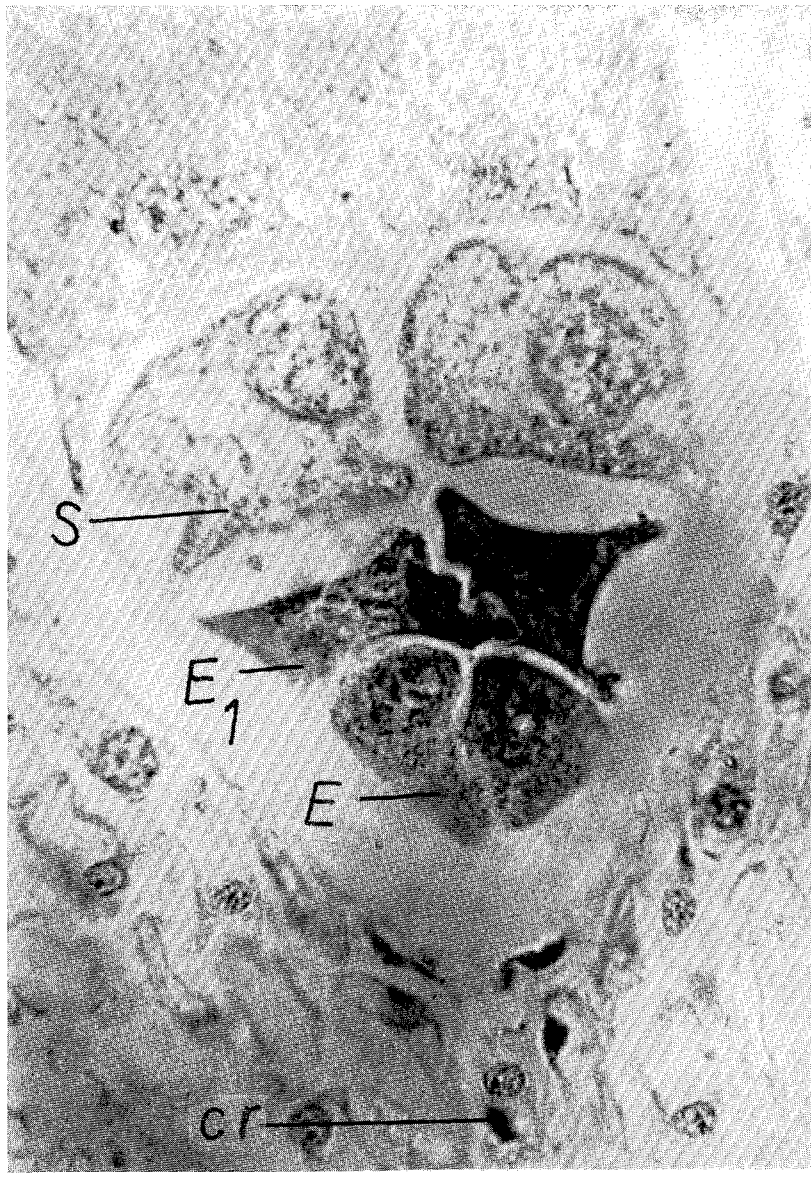

Fig. 52. *Cedrus deodara*. Photomicrograph of four-tiered secondary proembryo in which E_1 tier has degenerated. ×640.

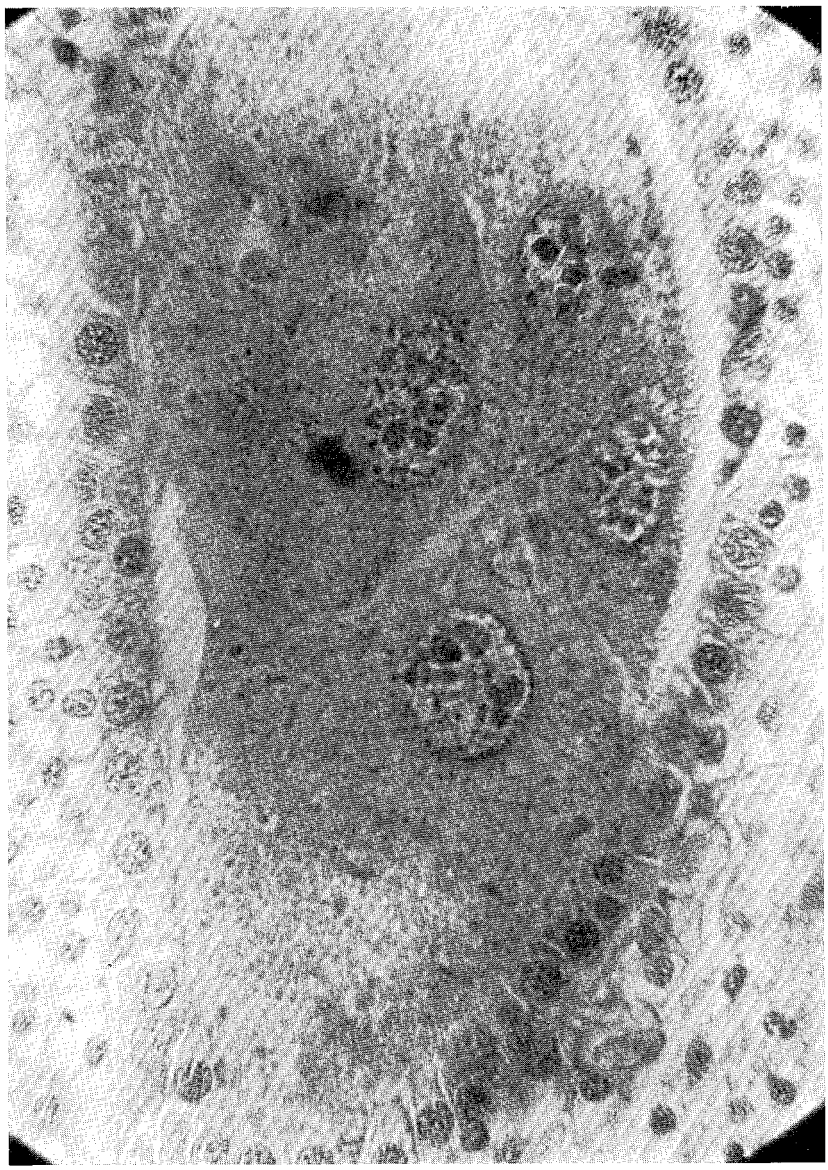

Fig. 53. *Picea smithiana.* Photomicrograph of abnormally large eight-nucleated proembryo (see Fig. 27) only four nuclei are shown. ×210.

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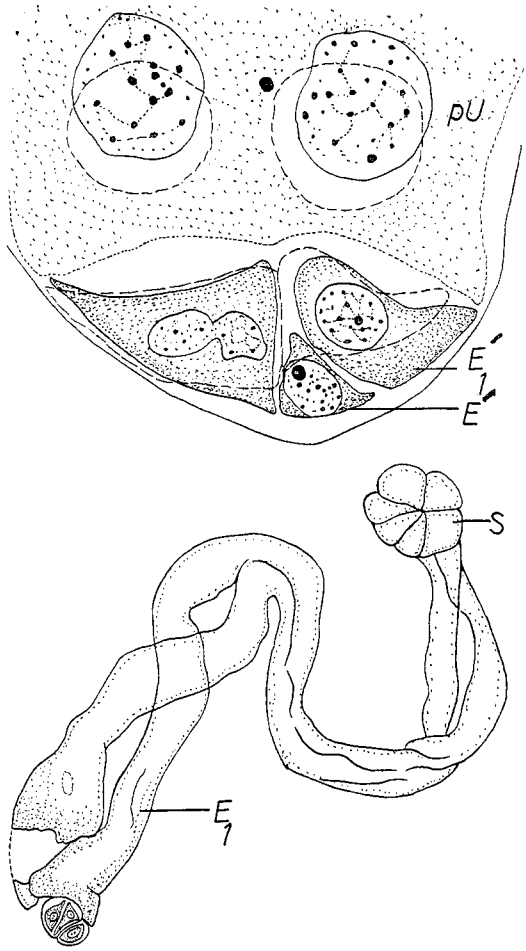

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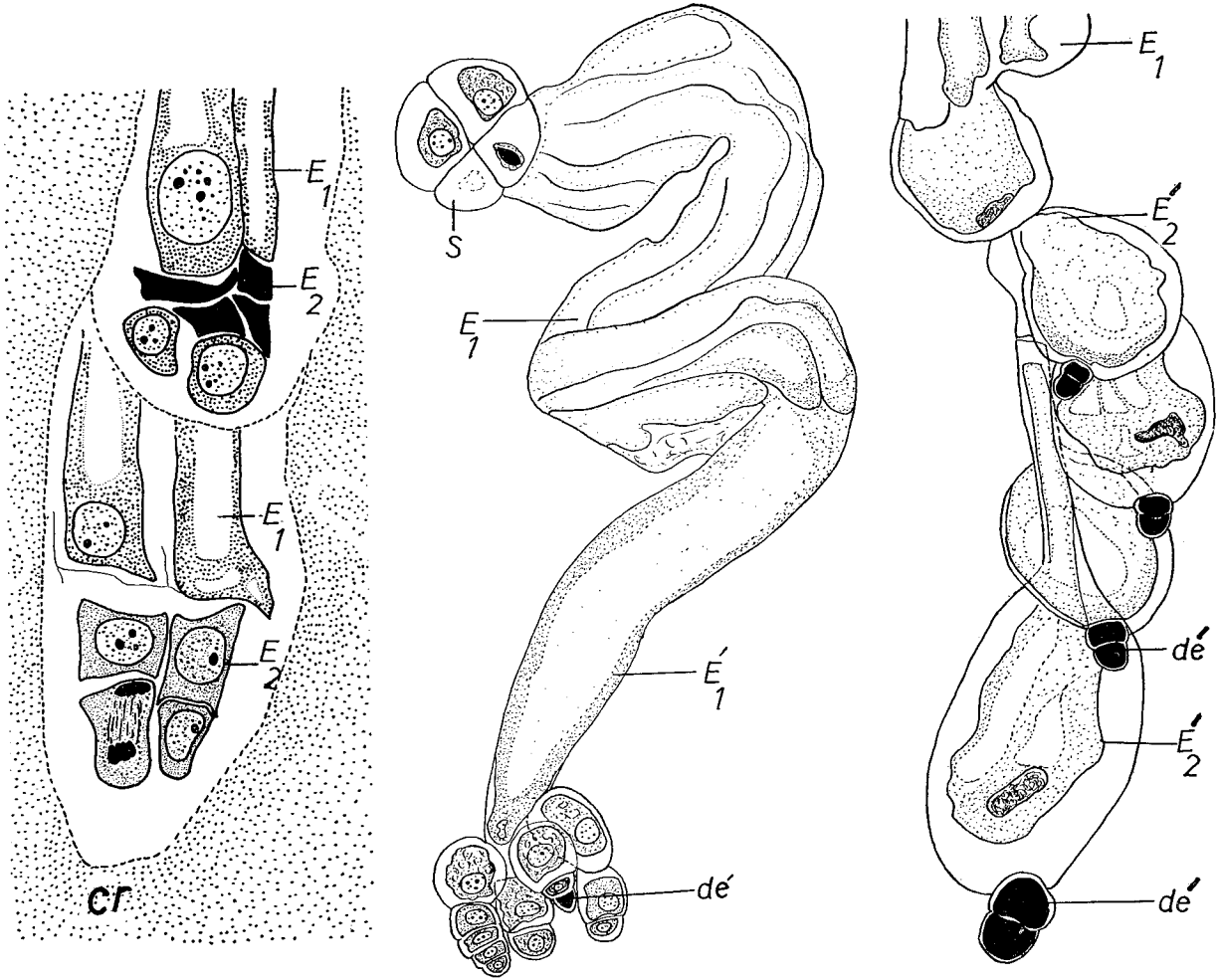
Figs. 54—55. 54. *Pinus nigra.* Primary proembryo showing undivided pU, three undivided pE cells and one two-celled pE unit. 55. *Pinus montezumae.* Whole mount of abnormal embryo showing two-celled E_1. 54, × 400; 55, × 70.



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Figs. 56—58. 56. *Pinus nigra*. Part of corrosion region showing two archegonial embryos growing alongside. Upper embryo shows degenerated E_2, E_3 segments, the terminal embryo is normal. 57. *Pinus montezumae*. Whole mount of abnormal embryo showing four degenerating units borne by one-celled E'_1. 58. *Cedrus deodara*. Whole embryo mount showing bulbous E'_2 cells and degenerated embryo units. cr, corrosion region; de′, degenerated embryo unit. S, suspensor. 56, × 225; 57, 58, × 90.

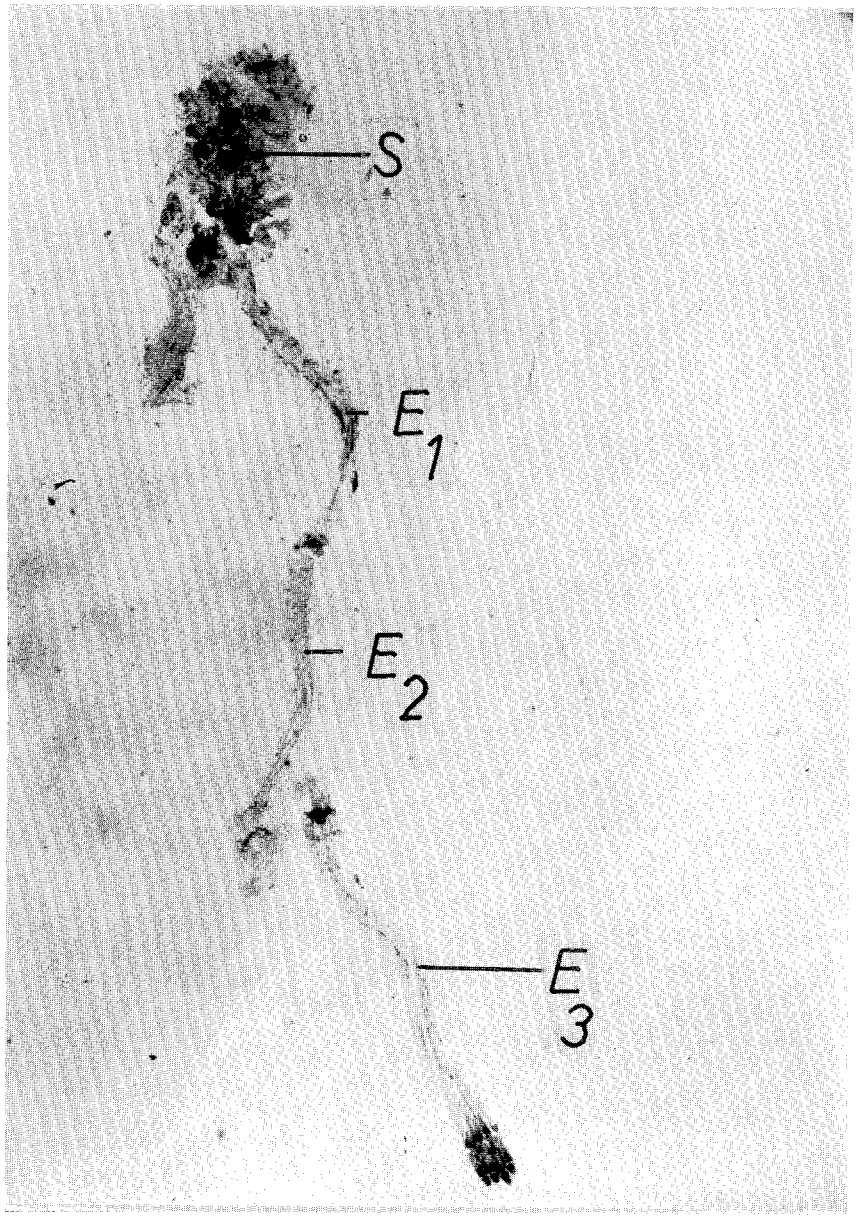


Fig. 59. *Pinus roxburghii*. Photomicrograph of whole embryo-mount showing typical segmented suspensor system of S, E_1, E_2 and E_3 with cleavage at E'_4. × 60.

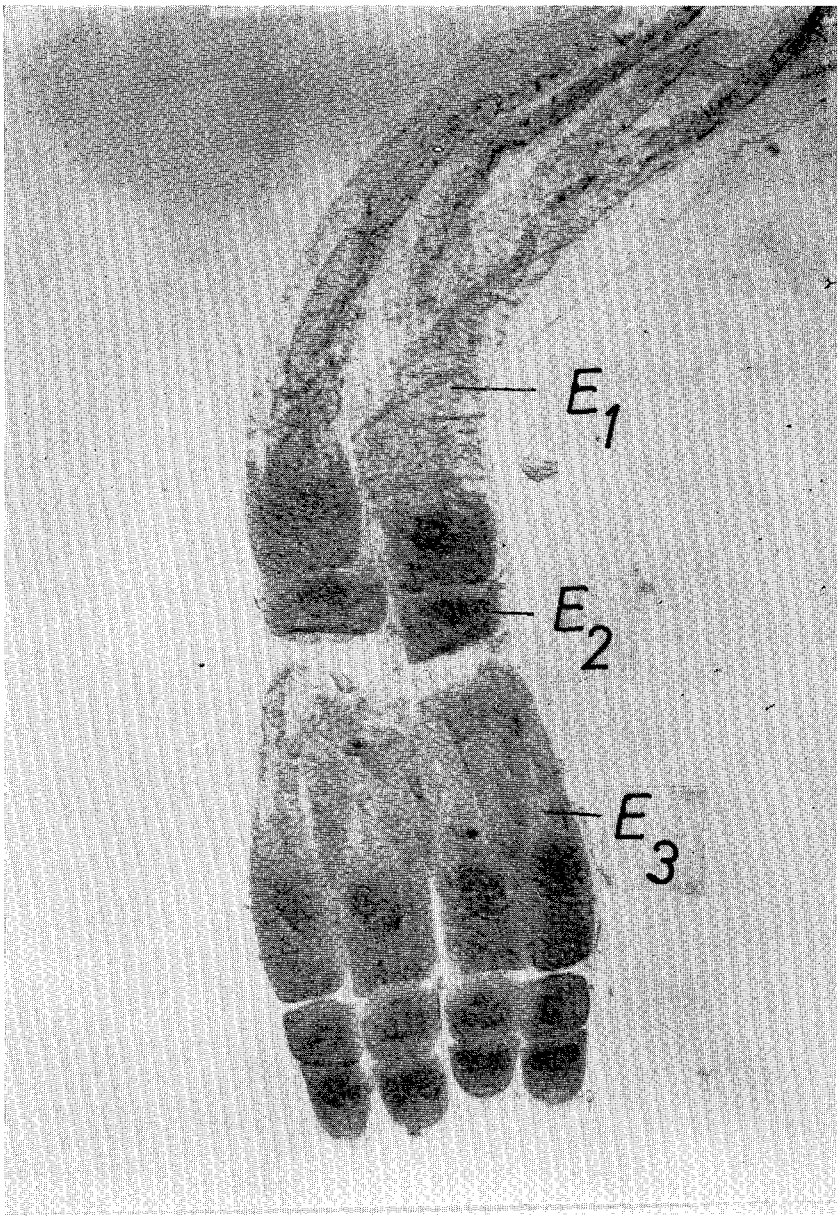


Fig. 60. *Pinus wallichiana*. Whole embryo-mount showing failure of E_2 elongation. ×160.

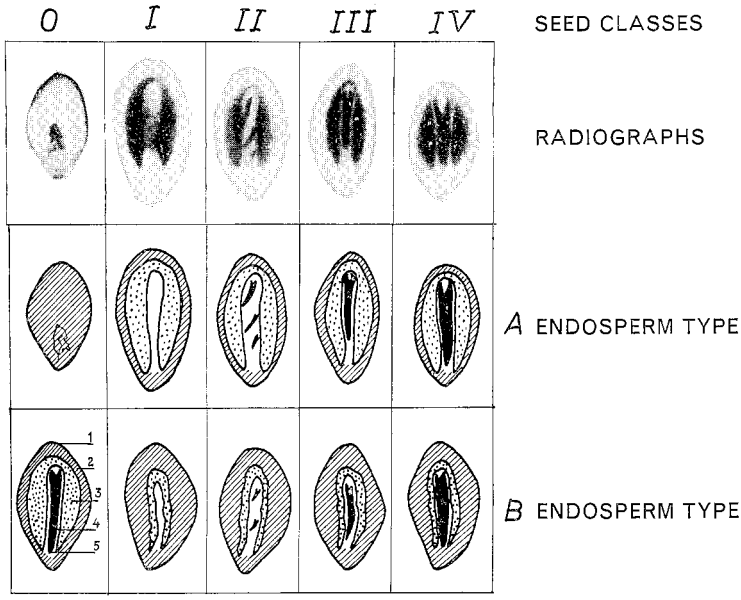
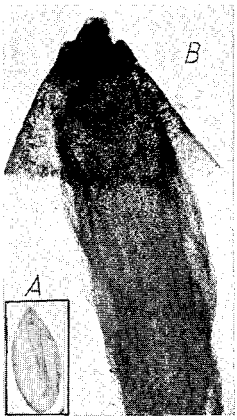


Fig. 61. *Pinus silvestris*. Radiographs (first row); corresponding drawings of 0—IV seed classes in endosperm types A and B (second and third row). 1, seed coat; 2, empty space; 3, endosperm; 4, endosperm cavity; 5, embryo. (Reproduced from Simak and Gustafsson, 1954, and Müller-Olsen and Simak, 1954.)



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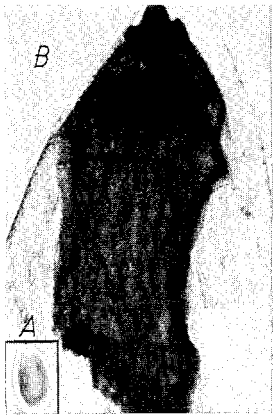

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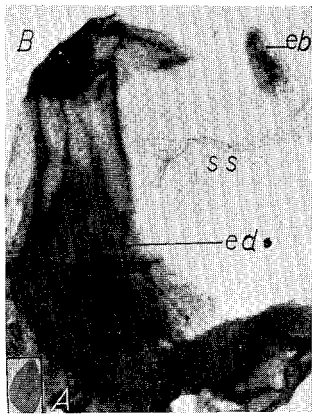

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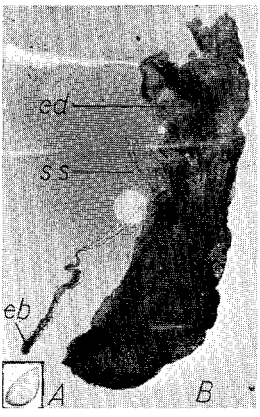

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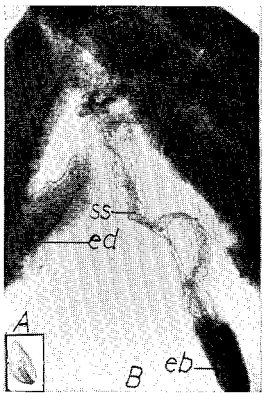

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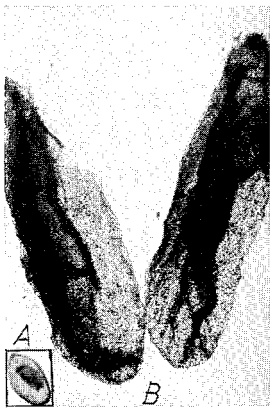

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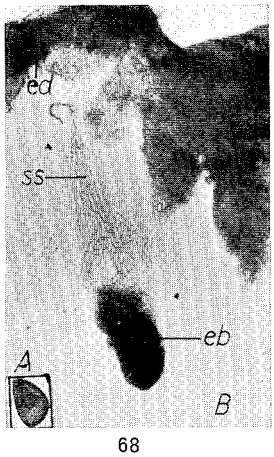

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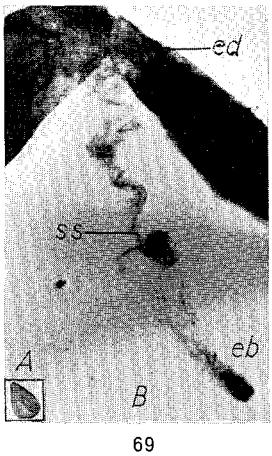

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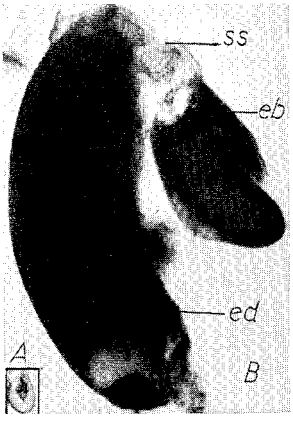

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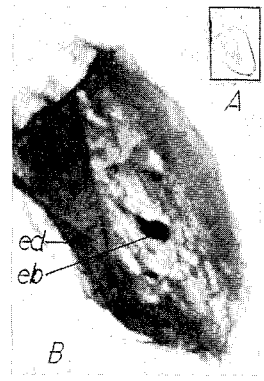
Figs. 62—70. Radiographs (A) of seed and photomicrographs (B) of whole mounts of embryos and endosperms, dissected from the corresponding seeds. 62, 63. *Pinus silvestris*. 62 A. Class 0. 62 B. Water-absorbent papery endosperm. 63 A. Class I. 63 B. Degenerated endosperm. 64 A—70 A. Class 0 seeds. 64 B—70 B. Water-soaked endosperms and embryos dissected from them. 64—66, *Picea abies*. 67. *Pinus silvestris*. Double endosperm in single seed. 68—70. *Picea abies*. ss, suspensor-system; ed, endosperm; eb, embryo. 62 A, × 4; 63 A—70 A, × 2; 62 B, 63 B, × 35; 64 B, 66 B × 50; 65 B, 67 B—70 B, × 45.



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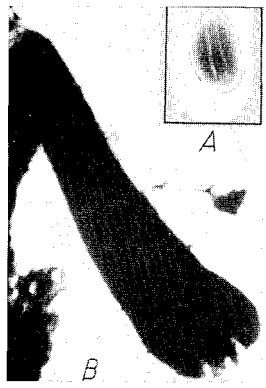

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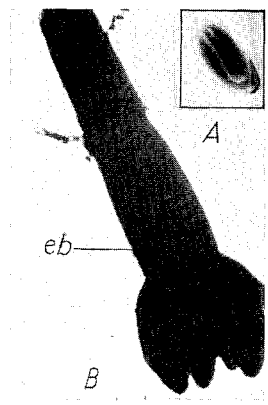

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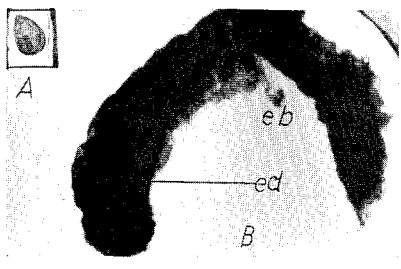

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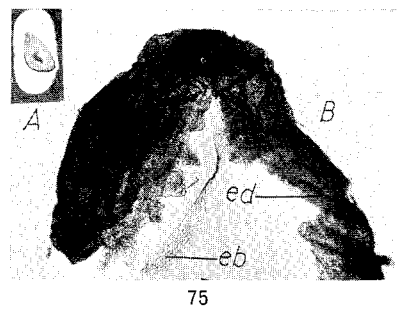

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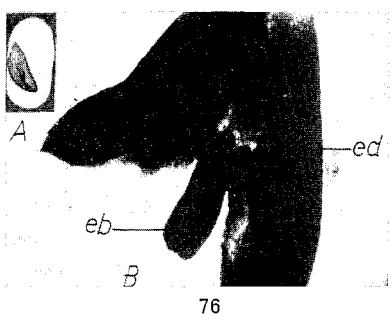

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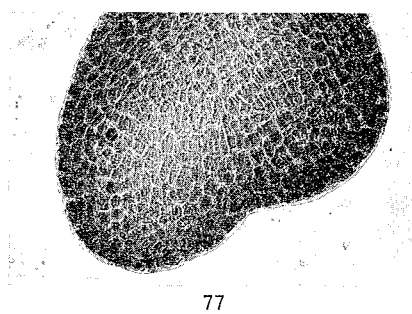

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Figs. 71—77. Radiographs (A) of seeds and photomicrographs (B) of whole mounts of embryos and endosperms dissected from the corresponding seeds. 71, 72. *Pinus silvestris*. 71 A. Class 0. 71 B. Degenerated endosperm containing a well formed deep-staining young embryo. 72 A. Class IV seed from southern Sweden. 72 B. Cotyledonous embryo dissected from same. 73—75. *Picea abies*. 73 A. Class IV seed from southern Sweden. 73 B. Cotyledonous embryo dissected from same. 74 A, 75 A. Class 0. 74 B, 75 B. Dead embryos and endosperms. 76, 77. *Pinus silvestris*. 76 A. Class 0. 76 B. Embryo, endosperm at advanced stage of development dissected from same. 77. Whole mount of embryo tip after partial cleavage showing unitary lobing because of failure of cleavage in two embryo units. ed, endosperm; eb, embryo; ss, suspensor-system. 71 A—73 A, × 3; 74 A—76 A, × 2; 71 B—73 B, × 45; 74 B—76 B, × 45; 77, × 205.

Observations on Some Swedish Conifers

Forests in Sweden, in the main, consist of two species, *Pinus silvestris* and *Picea abies*. Seed studies of conifers carried out with X-ray radiography follow the classification given by SIMAK and GUSTAFSSON (1953 a, b, 1954) and its later modifications. This investigation shows the embryological background of the five classes of seeds (Fig. 61) which are found in different frequencies in *Pinus silvestris* and *Picea abies* growing in northern Sweden. The pictures of seeds obtained by X-rays are called radiographs. Classification of seeds was confirmed by SIMAK from radiographs. The definitions of seed classifications are given with the descriptions of embryo and endosperm dissected from different seed samples. The seeds were soaked in water from six to ten hours and dissected under the microscope. About 200 seeds of a class from each sample of different localities and trees of a species were studied. The 0 class was particularly studied from numerous samples. Diseased seeds were excluded. Except where otherwise stated the description applies to both species studied, *viz. Pinus silvestris* and *Picea abies*.

Embryology of Seed Class 0

Definition: Neither embryo nor endosperm (= empty seeds), (Fig. 61, class 0).

The remains of endosperms of 0 class of seeds studied (50 to 90 per cent in *Pinus silvestris* and 80 to 95 per cent in *Picea abies*, varying in different samples) showed no embryos but a good percentage of 0 seeds in both species contained remains of different developmental stages of dead embryos.

Endosperms of 0 class without embryos: Dead endosperms without embryos had two types of tissue: 1) gelatinous or papery; translucent; and water-absorbent (Fig. 62 B); and 2) leathery, opaque; not water-absorbent. Double endosperms of these types in the same seed were also found (Fig. 67).

Endosperms of 0 class with embryos: The condition of endosperm with embryos, in general, depended mainly on the stage of embryo development. Embryos and endosperm were studied from youngest to oldest stages (Figs. 64—71, 74—76). The endosperms with young embryos were made either of dead empty cells (Fig. 64 B) or of leathery tissue (Fig. 65 B), but endosperms containing developed embryos were seen in different stages of food storage which were related to embryo stages in them. Such endosperms of 0 class of

seeds gave shrivelled shadows of different intensities on X-ray radiographs (Figs. 64 A—71 A, 74 A—76 A). It was, however, not possible to classify them in any clear sub-classes on the basis of morphology. Sometimes, badly developed endosperms showed well-formed young embryos with arrested development (Fig. 71 B).

Embryogeny: In the embryos dissected from 0 seeds, E_1 was well-developed and some embryos had a well-formed suspensor-system (Figs. 64—66, 68—70, 74, 75, 78, 80). Dominant embryonal tips in different seeds were of various sizes and differed in number of cells at the time of degeneration. The terminal embryonal tip varied from few empty cells (Fig. 80 B) to a multicellular mass (Figs. 64 B, 66 B, 68 B—70 B, 75 B) and in a few cases, even cotyledon primordia were differentiated (Fig. 76 B). The X-ray radiography showed all these as 0 class seeds.

Embryology of Seed Class I

Definition: Endosperm, embryo cavity developed but no embryo observed (Fig. 61, class I).

Endosperm: Dissected endosperms were of two types, with or without embryos (Figs. 63, 79, 81—83). Endosperm condition in the latter was related to the degree of embryo development. The embryos were mostly young with empty or dead cells and hence they did not absorb the X-radiation. X-ray radiographs record a well-formed endosperm and embryo cavity but not the embryos (Figs. 81, 82, 83).

Embryogeny: As noted for 0 class, the dissected embryos in class I were in different stages (Figs. 81, 88). Many embryos showed E_1 elongation, but further segments of the suspensor-system were not formed or they failed to elongate (Figs. 81, 82). In most cases there was no E_t elongation. Organized cleavage (as seen in Figs. 16, 17) due to different E_2, E_3 elongations in units was absent. Instead the four units of equal size (Figs. 81—83 *cf.* 17, 18) showed different degrees of irregular separation at E_1 level in many embryos (Figs. 81, 82). In class I seeds well-developed embryos were seen only rarely (Fig. 88). Some of these had E_1, E_2 and E_t in a developed suspensor-system but embryonal cells were empty and shrivelled. These embryos are not detected on X-ray radiographs as they are so shrivelled as to make differentiation by X-rays absorption difficult. In *Pinus silvestris* cleavage and archegonial polyembryony in one class I seed showed twelve embryos, but most embryos were in the same stage of development and size (Fig. 83). Some seeds showed reversed embryos sharply bent at different points on the suspensor-system (Fig. 78 B *cf.* Figs. 48, 49).

Embryology of Seed Class II

Definition: SIMAK and GUSTAFSSON (1959), SIMAK and KAMRA (1963) and SIMAK (1966) divide class II into two sub-classes IIP and II.

IIP: Endosperm containing one or more small embryos the length of which does not exceed their breadth (Figs. 84 A—87 A, 89 A—94 A, 98 A—100 A).

II: Endosperm, and one or several embryos, none of which longer than half the embryo cavity (Figs. 61, class II, 95 A—97 A, 104 A, 109 A, 111 A).

Sub-class IIP: Most endosperms containing IIP embryos were well-developed, milky-opaque and more rich in storage content than those of 0 and I.

Embryogeny: The suspensor-systems of embryos varied in length and development sometimes showing only E_1 or E_2 without E_t development. Dominant embryos in polyembryonic seeds were mostly undifferentiated embryos. Cleavage and archegonial polyembryony was common in *Pinus silvestris* (Fig. 99), but *Picea abies* showed only archegonial polyembryony (Fig. 96). Embryos from separate archegonia of the same seed differed in their ability to survive. Fig. 89 B shows eight embryos from two archegonia. Approximately all are at the same stage of development but four belonging to one archegonium have degenerated while the other four are normal.

Many embryos of *Pinus silvestris* showed loose separation of units at E_1. In Pinaceae cleavage at E_1 is absent. It normally occurs at E_2 (Fig. 16) or later *e.g.* at E_4 (Fig. 59). Unitary lobing and partial cleavage were frequent in *Pinus silvestris* due to failure of cleavage and sometimes in *Picea abies* due to failure of non-cleavage. Embryonal clumps of two or more overlapping embryo units were sometimes seen only as a single point on the X-ray radiographs (Fig. 101).

Normally the terminal embryo in unitary cleavage or in archegonial polyembryony is the largest and shows more differentiation than the others, which are progressively less differentiated according to their position in the endosperm cavity (Figs. 92, 99). Seeds from northern Sweden frequently do not show normal differences in development and differentiation between embryos from the same zygote or from different archegonia (Figs. 91 B, 104 B). Such a situation arises in ovules where the dominance factor of the terminal embryo and its effect over other embryos (a normal feature, see Fig. 18) does not function. There is thus no elimination or supression of supernumerary embryos. This is often accompanied by arrested seed maturity and persistent polyembryony results. In such seeds two, three or more embryos develop simultaneously to advanced stages (Figs. 101 B, 104 B) and some of these develop to more than one mature cotyledonous embryo in the seed (Fig. 102). Presence of intermediate stages show that

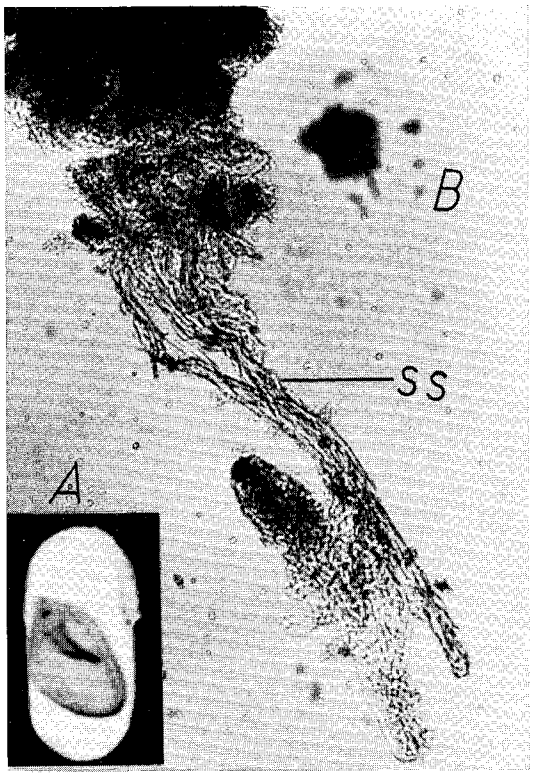
the effect of terminal embryo dominance may vary in degree (Fig. 111 B). Persistent polyembryony may also arise from arrested development of normal embryos in an ovule which forms an immature seed without the elimination of supernumerary embryos (Figs. 92, 99). The climatic factors which destroy the terminal embryo dominance in the northern conifer ovules are not known but seeds with normal dominance of the terminal embryo (Figs. 92, 99, 103), and sometimes randomly situated embryos (Fig. 95), found mixed with the affected seeds, perhaps result from seed immaturity caused by cold temperatures.

The point embryos show several pathological conditions not observable on X-ray radiographs, such as 1) failure or abnormal differentiation of internal tissues or embryo parts; (Figs. 90, 94, 101); 2) abnormal differentiation leading to distortions in shape (Fig. 87); suppression of cotyledons which thus vary in number from one to several; 3) increase in width but not in length (Fig. 85); and 4) dead embryo-tissue of opaque non-staining cells containing shrivelled contents (Fig. 94). Thus young embryos show deformation due to disturbed development (Figs. 85, 87, 90, 94). These, if they survive to maturity, are malformed and may have irregular cotyledon-number in embryos of II, III or IV classes (Figs. 95, 102, 104—113).

Sub-class II: Class II is embryologically not different from IIP except that the embryos are longer than they are broad and are not seen as point embryos on X-ray radiographs. However, it is necessary to point out that what may appear to be a class II embryo on the radiograph can actually be two or three not separated or closely appressed embryos often found in IIP seeds (Fig. 98). Sometimes well separated embryos overlap and give only one embryo configuration on an X-ray radiograph (Fig. 101, 102, 104, 108), but dissection and squashing reveal the correct situation. It must be remembered that X-ray radiographs give only a shadow of the contents of embryo cavity and not a picture of the embryos.

Endosperm: It is generally better developed than that of IIP as the embryo is more advanced.

Embryogeny: The embryos in this class show different shapes from elongated undifferentiated cell masses to small cotyledon-forming embryos at various stages of arrested differentiation and development. Some of the cotyledonous embryos show differentiation of parts but in size they remain small. Sometimes they form mis-shapen cotyledons or embryos. Persistent polyembryony is common but the X-ray radiographs do not always record the correct number of embryos present because of their overlapping and small size (Figs. 95, 97, 101, 104, 111).



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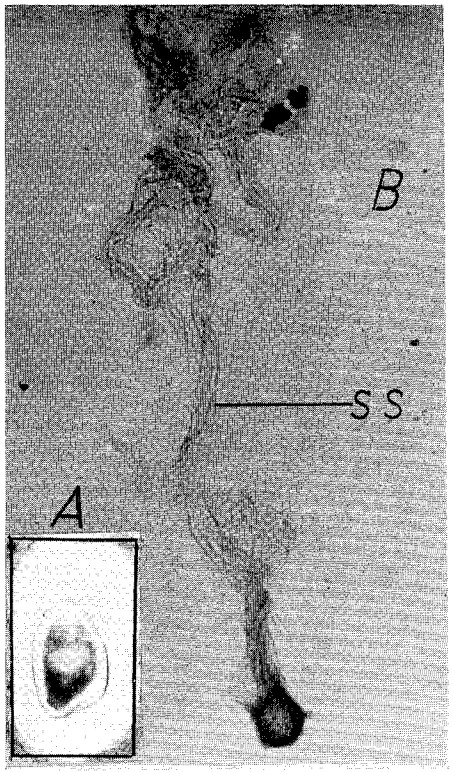

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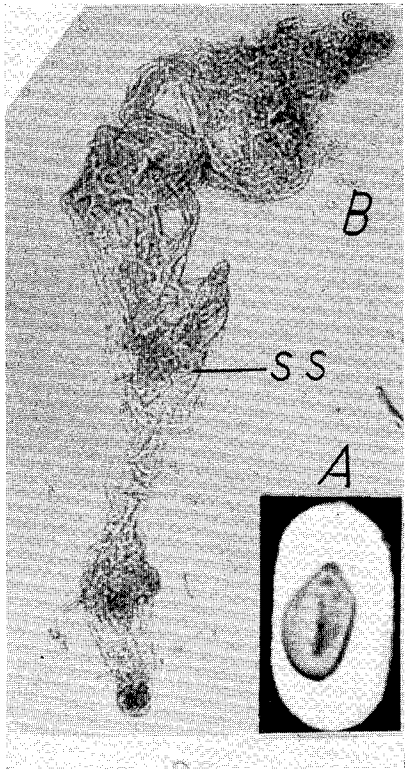

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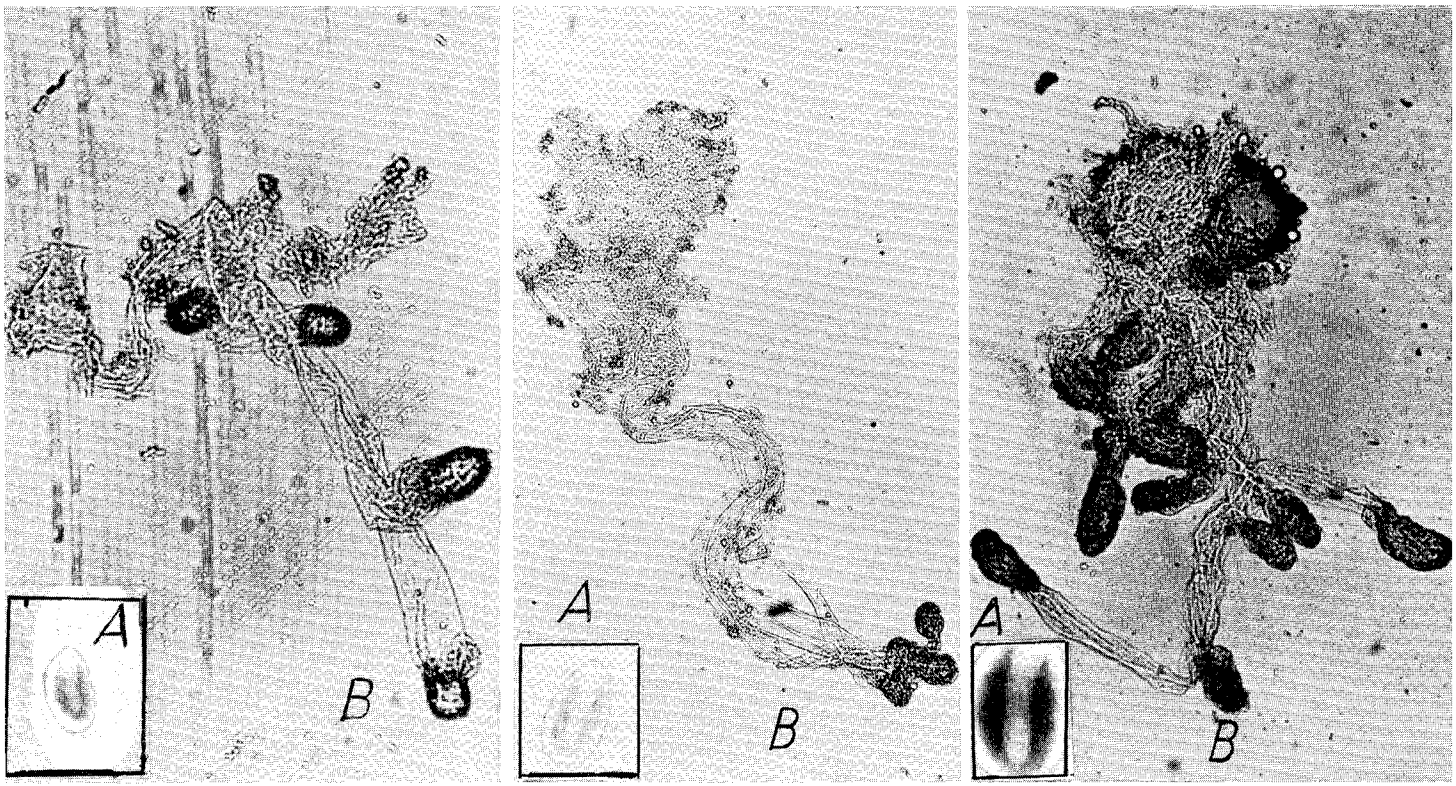
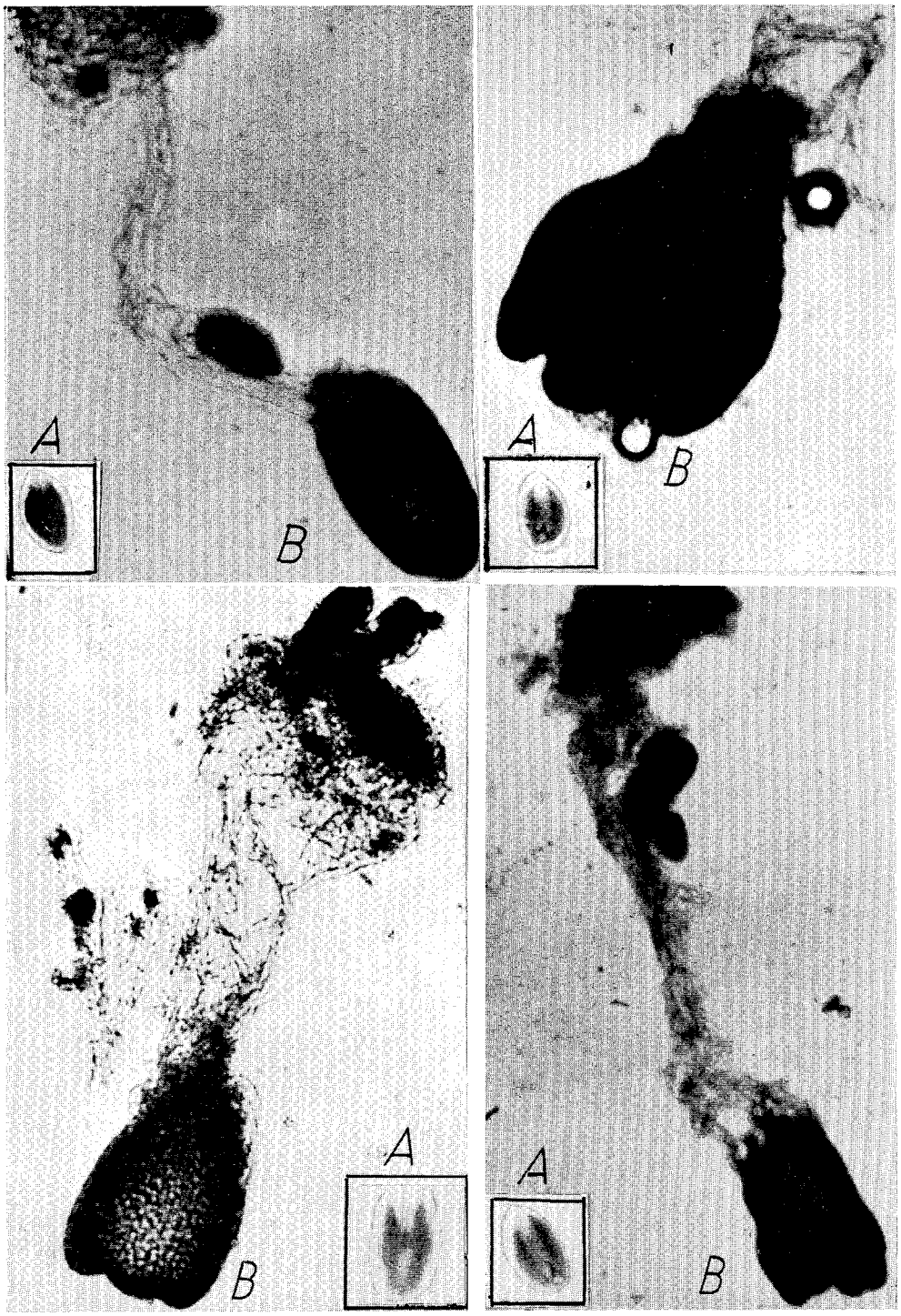
Figs. 78—80. Radiographs (A) of seeds and photomicrographs (B) of whole mounts of embryos dissected from the corresponding seeds. 78. *Pinus silvestris*. 78 A. Class 0. 78 B. Reversed terminal embryo, whole mount. 79. *Picea abies*. 79 A. Class I. 79 B. Whole embryo mount with developed suspensor system (ss). 80. *Pinus silvestris*. 80 A. Class 0. 80 B. Young terminal embryo with well developed suspensor-system (ss). 78 A, × 6; 79 A, 80 A, × 4; 78 B, × 112; 79 B, × 85; 80 B, × 115.

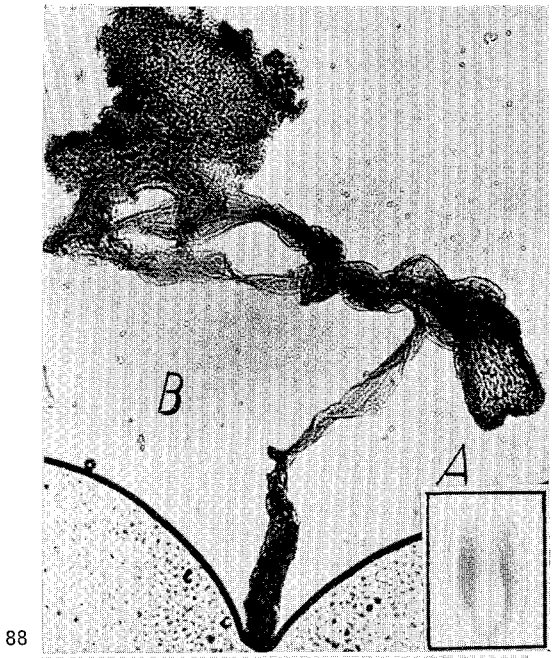


81 82 83

Figs. 81—83. *Pinus silvestris*. Radiographs (A) of class I seeds and photomicrographs (B) of whole mounts of embryos dissected from the corresponding seeds, which show irregular separation at E_1, embryo units of equal size and similar differentiation, and failure of Et elongation. Fig. 83 B shows embryos from three archegonia. 81 A, × 3; 82 A, × 4; 83 A, × 6; 81 ×, × 90; 82 B, × 100; 83 B, × 90.



Figs. 84—87. Radiographs (A) of class IIP seeds and photomicrographs (B) of whole mounts of embryos dissected from the corresponding seeds. 84. *Pinus silvestris*. Undifferentiated terminal embryos after cleavage. 85. *Picea abies*. Abnormally differentiated point embryo. 86 B. *Pinus silvestris*. Same. 87 B. *Picea abies*. Irregularly differentiated point embryo showing no increase in length. 84 A, 85 A, × 3; 86 A, ×5; 87 A, ×4; 84 B, ×100; 85 B, ×140; 86 B, ×100; 87 B, ×75.



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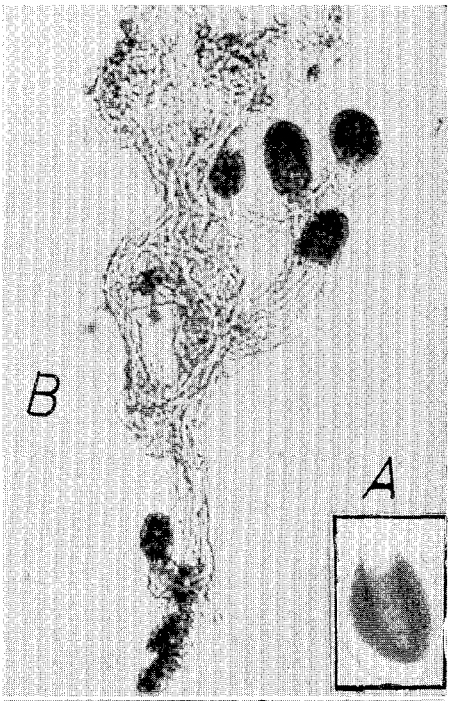

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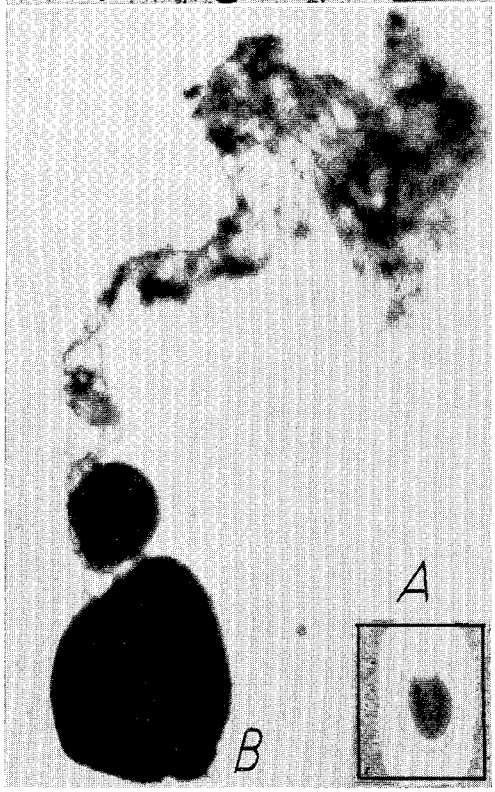

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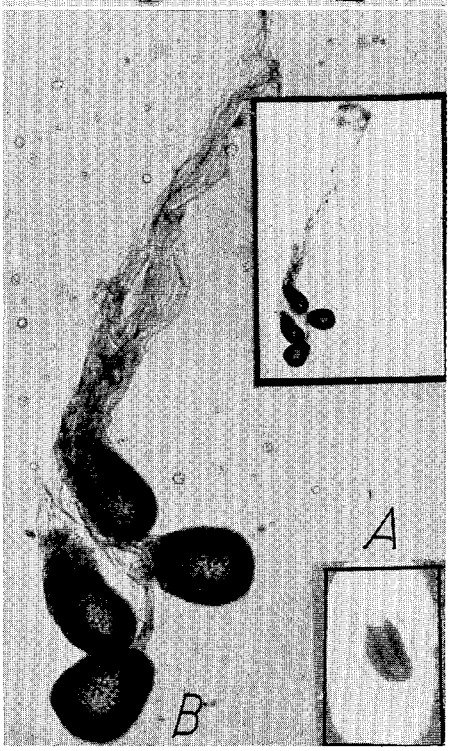

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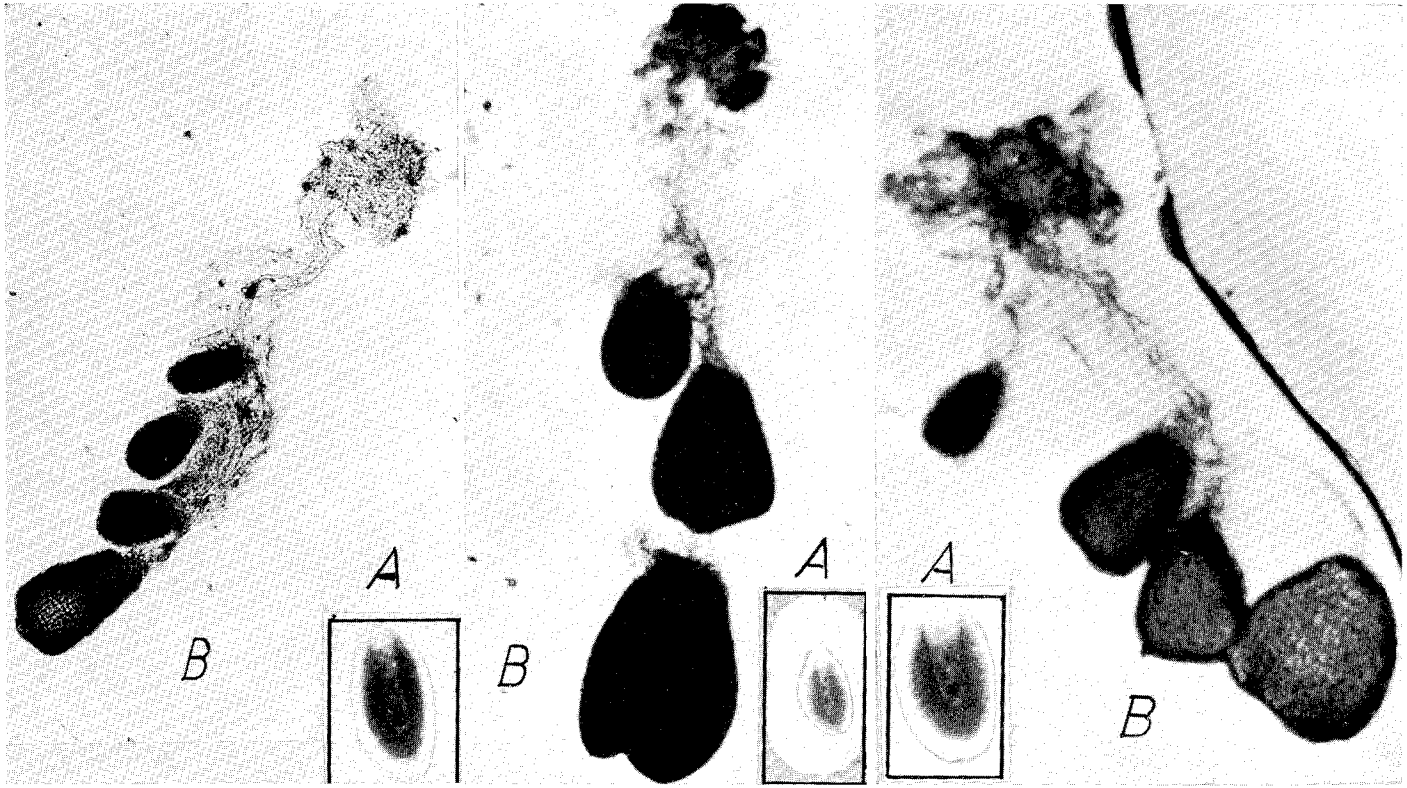
Figs. 88—91. *Pinus silvestris*. Radiographs (A) of seeds and photomicrographs (B) of whole mounts of embryos dissected from the corresponding seeds. 88 A. Class I. 88 B. Whole mount of embryos showing transparent tissue dissected from same. 89 A—91 A. Class IIP. 89 B. Embryos from two archegonia after cleavage, four embryo units from one archegonium have degenerated, the other four stain well and are not shrivelled. 90 B. Terminal embryo, rounded, irregularly differentiated, showing increase in width but not in length. 91 B. Embryo units separated irregularly after elongation of E_1 (see whole mount on right), E_2 absent, Et poorly developed or absent. 88 A, 89 A, $\times 6$; 90 A, 91 A, $\times 5$; 88 B, $\times 75$; 89 B, $\times 100$; 90 B, $\times 125$; 91 B, $\times 100$.



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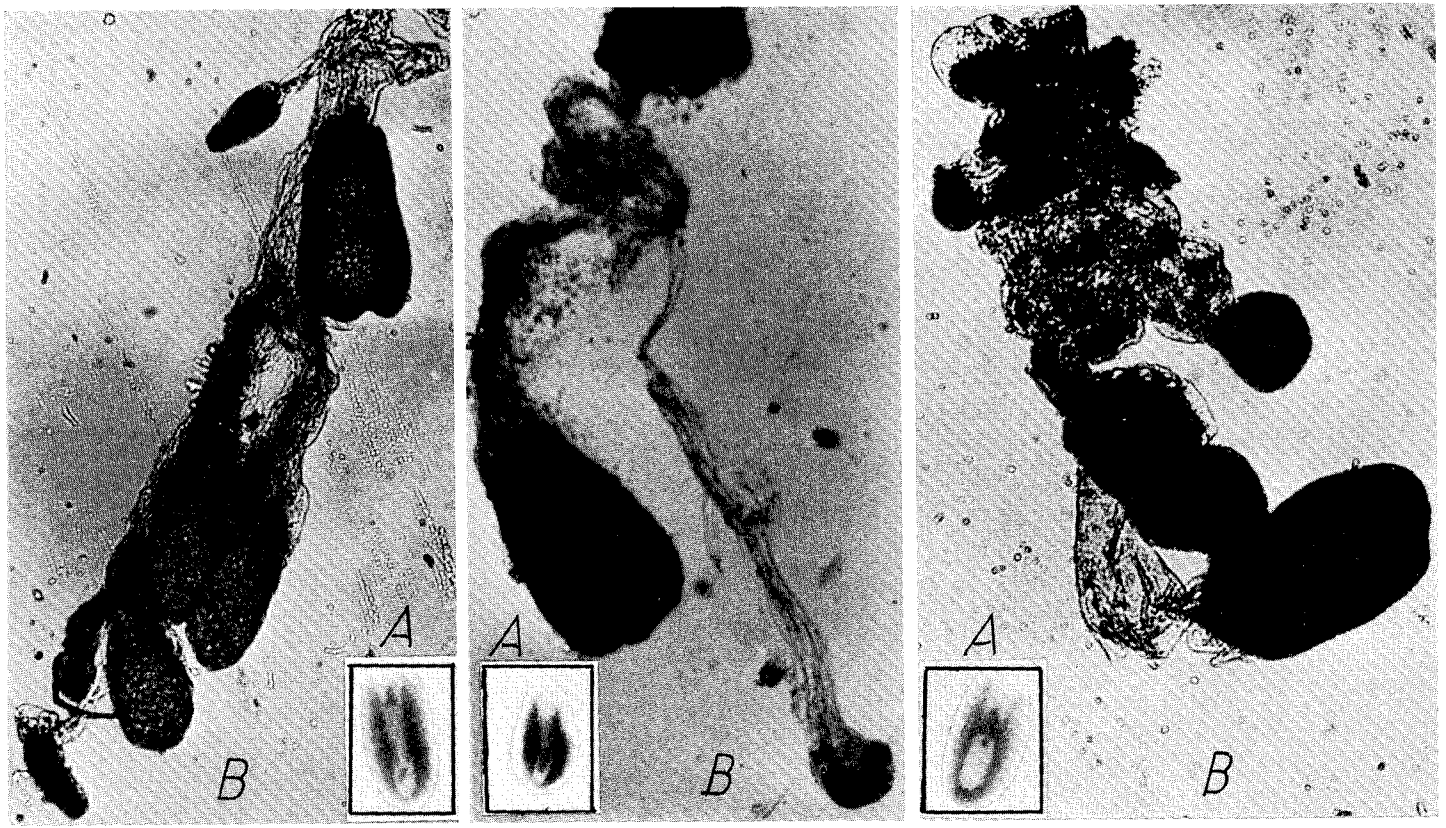
Figs. 92–94. *Pinus silvestris*. Radiographs (A) of class IIP seeds and photomicrographs (B) of whole mounts of embryos dissected from the corresponding seeds. 92 B. Normal terminal embryo dominance and differentiation of four embryo units after cleavage. 93 B. Partial cleavage of embryo units, terminal embryo consists of two units and shows unitary lobing. 94 B. Dead embryo units of compact tissue of shrivelled cells. 92 A, × 5; 93 A, × 3; 94 A, × 6; 92 B, × 75; 93 B, × 100; 94 B, × 112.



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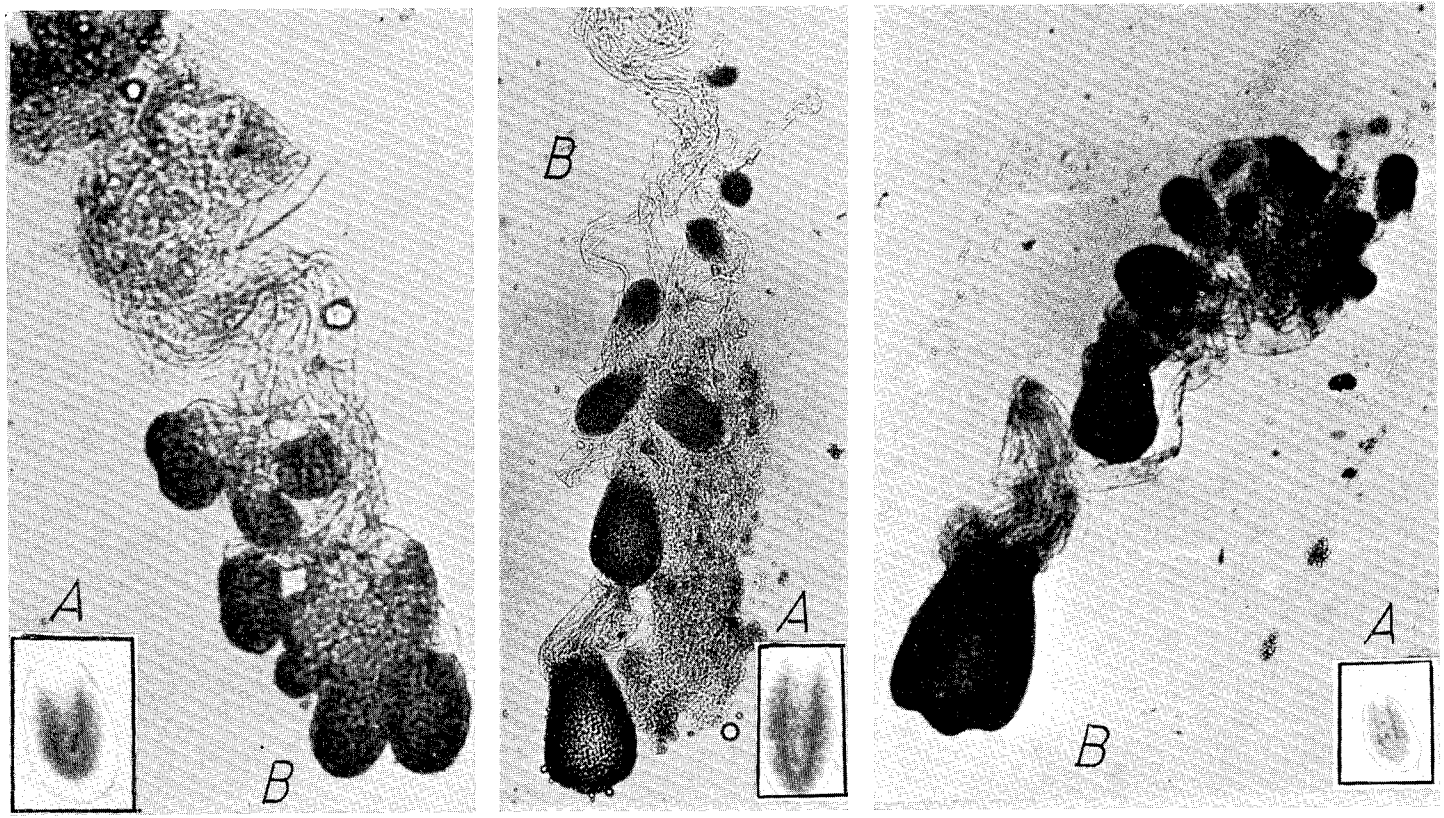
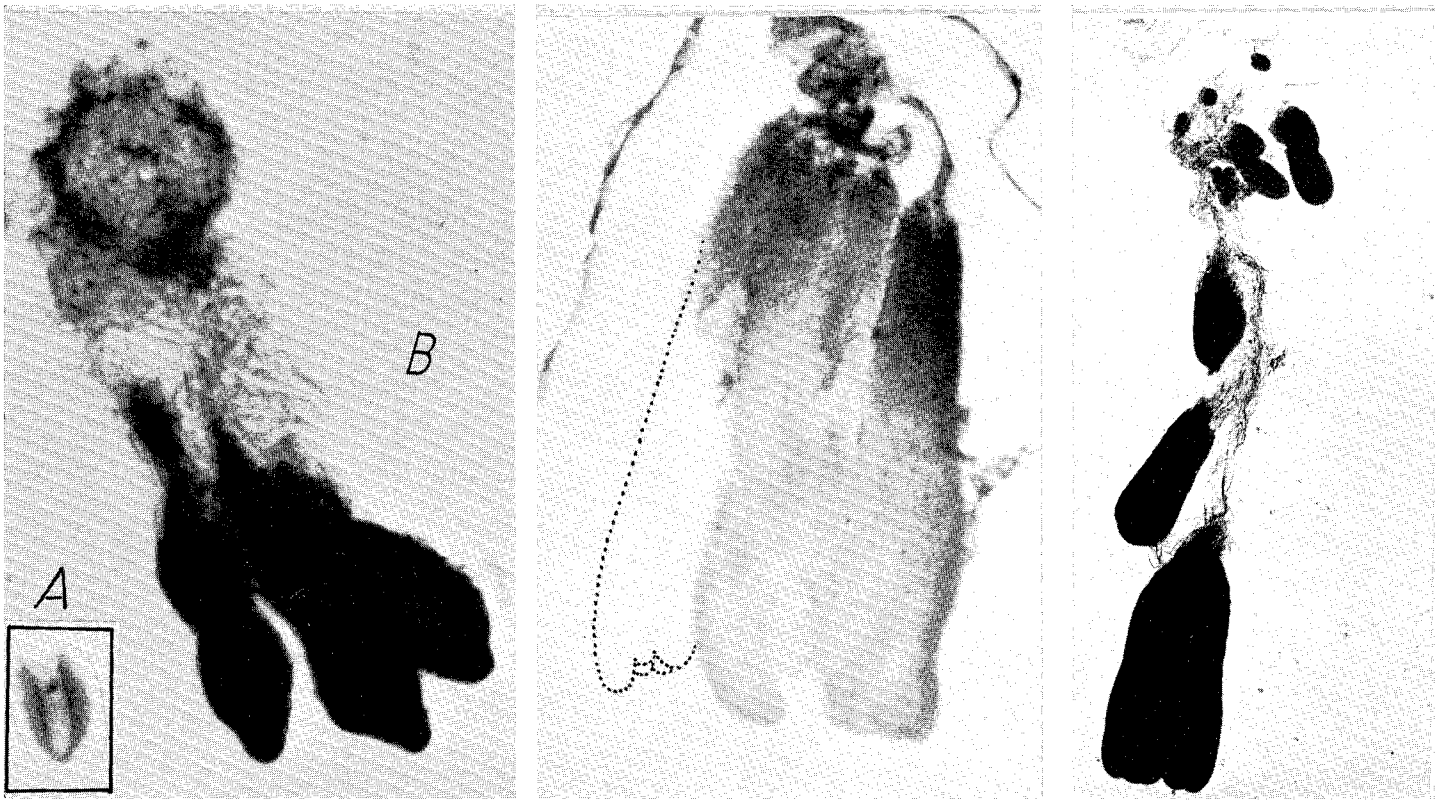
Figs. 95—97. Radiographs (A) of class II seeds and photomicrographs (B) of whole mounts of embryos dissected from the corresponding seeds. 95 B. *Pinus silvestris*. Seven embryos from a seed, showing same size and differentiation in only some embryos. 96 B. *Picea abies*. Two embryos from different archegonia in a seed. One with well-developed suspensor-system has degenerated. 97 B. Partial failure of cleavage of some units in embryos from different archegonia. 95 A, × 6; 96 A, × 5; 97 A, × 6; 95 B, 80; 96 B, × 75; 97 B, × 112,



98 99 100

Figs. 98—100. *Pinus silvestris*. Radiographs (A) of class IIP seeds and photomicrographs (B) of whole mounts of embryos dissected from the corresponding seeds. 98 B. Twelve embryo units in a seed from three archegonia showing unitary lobing and failure of cleavage. 99 B. Eight embryo units in a seed from two archegonia showing normal terminal embryo dominance, differentiation, and total cleavage. 100 B. Later stage of embryos seen in 99 B. 98 A, × 6; 99 A, × 6; 100, × 3; 98 B, × 112; 99 B, X 75; 100 B, X 70.



101 102 103

Figs. 101–103. *Pinus silvestris*. 101. Radiograph (A) of classIIP seed and photomicrograph (B) of whole mounts of embryos dissected from the corresponding seed. Three embryo units of almost identical size show similar differentiation. 102. Three mature cotyledonous embryos dissected from single class IV seed. 103. Embryos dissected from class II seed which belong to more than one archegonium. Partial cleavage and unitary lobing are seen due to failure of cleavage in two double units situated in upper half of seed. 101 A, $\times 6$; 101 B, $\times 125$; 102, 103, $\times 75$.

Embryology of Seed Class III

Definition: Endosperm and one or more embryos, the longest of which measures between half and three quarters of the embryo cavity (Fig. 61, class III).

Endosperm: The endosperm is rich in food material and though not so opaque and well-formed as that of class IV is nevertheless similar to it. The embryo cavity is clearly seen.

Embryogeny: The embryos are sufficiently long to give the proportion required for class III, but vary in width. The terminal one is generally largest and dominant (Fig. 112) but sometimes the dominant embryo occupies different positions (Fig. 95) due to disturbances in terminal embryo dominance, shrinkage of the suspensor-system or due to obstructions encountered in the endosperm cavity. Persistent polyembryony is common but the number of embryos present is difficult to interpret on X-ray radiographs.

The embryos filling from half to three quarters of the endosperm cavity show the following deformities: 1) They may be fully differentiated and sufficiently elongated to be classed in III but are often narrow. Embryo width varies in different seeds, is independent of length and does not fill the cavity breadthwise. 2) The normal terminal embryos of class III sometimes show little or no differentiation of cotyledons (Fig. 113). 3) The dominant embryo can have a distorted form with tumour-like lateral swellings (Fig. 107), and a variable number of suppressed or flattened cotyledons (Fig. 106, 108, 110). 4) Embryos consist of opaque non-staining partly or wholly dead tissue. 5) Two or more embryo units are sometimes loosely attached (Fig. 108) or joined because of initial failure of cleavage and presence of persistant unitary lobing which gives rise to embryo units joined along their whole length in the embryo. Sometimes embryo units are only partly and not completely joined. In these cases generally one embryo of the two is the better developed. Equal development in these is also common in seeds of northern conifers.

Embryology of Seed Class IV

Definition: Endosperm with one fully developed embryo completely or almost completely occupying the embryo cavity. Diminutive embryos rarely occur (Fig. 61, class IV).

Nearly all seeds of *Pinus silvestris* and *Picea abies* from southern Sweden were of class IV type (Figs. 72, 73). Class IV seeds occurred in a variable percentage in different samples from northern Sweden.

Endosperm: The endosperm was milky-white, rich in storage products and well-formed.

Embryogeny: Most class IV seeds from northern Sweden contained one well-formed cotyledonous embryo filling the endosperm cavity completely. The seeds recorded as normal class IV on X-ray radiographs sometimes show suppressed and deformed parts (Fig. 105), variable number of cotyledons, tumourlike swellings, and partly or wholly dead tissue in embryos. In addition, two to three mature embryos closely pressed and not discernible by X-ray radiographs because of overlapping were sometimes dissected from class IV (Fig. 102). Abnormal embryos were not common in class IV seed studied from southern Sweden.

Endosperm A and B

Definition:

A. The endosperm almost fills the seed coat to capacity and absorbs the X-rays well (Fig. 61, A).

B. The endosperm only fills the seed coat incompletely and is often shrunken or otherwise deformed. The X-ray absorption is inferior to that of class A (Fig. 61, B).

These two endosperm types although observable on X-ray radiographs were not clearly distinguishable from each other when dissected from seeds soaked in water, but differed only in visible food contents. It may, however, be added that endosperm A showed more vigorous embryos than those of B in classes IIP, II, III, IV.

Seed Abnormalities affecting Germination

SIMAK has recorded several seed abnormalities of endosperm, endosperm cavity, and embryo, from individual trees and provenances of *Pinus silvestris* with the help of X-ray radiographs which occur regularly and show inferior seed germination. These deserve a detailed embryological study. A close scrutiny of X-ray radiographs reveals the following: 1) endosperms showing bifurcated or trifurcated endosperm cavities of various shapes and sizes; 2) seeds with two endosperms, one degenerated and the other showing a normal endosperm cavity; 3) endosperm showing two separate endosperm cavities with embryos; 4) rimmed endosperm cavities of various shapes; 5) lateral endosperm cavities; 6) point embryos located near the micropyle; 7) embryos joined terminally; 8) embryos laterally joined or showing unitary lobing; and 9) inverted, luminous or diffused embryos.

Embryological Analysis of Seed Classes

Seed classification, on the basis of embryogeny, was first started by Scandinavian foresters who discovered that conifers growing in northern

latitudes bear unripened seeds with incompletely developed embryos (VESTERLUND, 1896; HOLMGREN, 1912; HEIKINHEIMO, 1915, 1921; HAGEM, 1914, 1917; VIKHAMER, 1919; OLDERTZ, 1921; KUJALA, 1927, 1928; and WIBECK, 1928 b, 1929 a, b). Incomplete ripening of ovules (due to latitude, altitude and climate) into seeds which contain different stages of not fully developed embryos governs seed quality in Scandinavia (HAGEM, 1917; KUJALA, 1927; WIBECK, 1928 b; SIMAK and GUSTAFSSON, 1953 a, b, 1954). HEIKINHEIMO (1915, 1921) and KUJALA (1927, 1928) classified seeds from Finland on an embryo-endosperm relationship in *Pinus silvestris*. KUJALA described six classes 0—V, two subclasses IA and IB and found polyembryony to be common in seeds collected from northern Finland. OLDERTZ (1921) and WIBECK (1928 b) confirmed the presence of a similar situation in seeds from northern Sweden. WIBECK (1928 b, 1929 b) showed a correlation between seed germinability and embryo length: seed length ratio. The ratio according to WIBECK was influenced by the distance of the embryo from the micropylar end of the seed. This was called the embryo ratio method in normal seed testing procedure (BALDWIN, 1942). This method required cutting of the seed, which was thus damaged.

SIMAK and GUSTAFSSON (1953 a, b) developed an X-ray technique which permitted a shadow study of embryo and endosperm to be made with radiographs without damage to the seeds. LUNDSTRÖM, in 1903, was the first to use X-ray studies to distinguish between full and empty seeds of forest trees (see SIMAK and GUSTAFSSON, 1953 b). The X-ray technique is sufficiently described in several papers and a detailed account is excluded to avoid repetition (see PLYM FORSHELL, 1953; SIMAK and GUSTAFSSON, 1953 a, b; FRÖLICH, 1954; MÜLLER-OLSEN and SIMAK, 1954; SIMAK, 1957, 1966; EHRENBERG *et al.*, 1955; BARTELS, 1956; GUSTAFSSON and SIMAK, 1958 a; EVRARD, 1957; ROHMEDER, 1957; SIMAK *et al.*, 1957; MÜLLER-OLSEN *et al.*, 1956; KAMRA, 1963, 1964 a, b; SIMAK and KAMRA, 1963). In this discussion embryological results, in the light of observations made in this study, are reviewed but studies on mechanical and insect damage are excluded.

SIMAK and GUSTAFSSON (1953 a, b) described with X-ray radiographs, the following types of *Pinus silvestris* seeds: 1) empty; 2) containing an endosperm without the embryo; 3) endosperm with embryos showing different stages of development; 4) abnormal turned, twisted or split embryos, polyembryony; and 5) embryos with different lengths. KUJALA (1927) classed empty seeds in 0; seeds containing endosperm without embryo in IA; and endosperm showing different stages of development of embryo in IB-V depending on stages at which embryo development stopped.

PLYM FORSHELL (1953, Fig. 11) studied seed formation in self and cross-pollination experiments in *Pinus silvestris* and used X-ray radiographs and

a classification in which 0, I, were similar to those described by KUJALA and SIMAK and GUSTAFSSON, but her II and III classes were based on polyembryony (1—4 embryos) and embryo size. Embryo size was designated as small in II, average in III and class IV was described as a normal seed with a full embryo. She concluded that self-pollination gives a smaller percentage of full seeds than cross-pollination.

SIMAK and GUSTAFSSON (1954) for the first time made seed classification more practical and precise. Class II was defined as seeds containing endosperm and one or several embryos none of which is larger than half the embryonic cavity; and III as endosperm and one not wholly developed embryo which fills between half to three quarter of the cavity. MÜLLER-OLSEN and SIMAK (1954) added two endosperm types: A, fills seed cavity and absorbs X-radiation well; B, fills seed cavity incompletely, is shrunken or deformed and its X-ray absorption is inferior to A. Classes 0, I and IV remained identical to the definitions given by PLYM FORSHELL (1953). Thus five seed classes 0—IV and two endosperm types A, B, which were shown to be correlated with germination in *Pinus silvestris* and *Picea abies* (SIMAK and GUSTAFSSON, 1954; MÜLLER-OLSEN and SIMAK, 1954) were standardized and successfully used with statistical methods for seed studies. SIMAK and GUSTAFSSON (1954) demonstrated that low germination was due to poor ripening conditions seen in a predominance of class II, sparse amounts of III and absence of IV classes in seeds of pine trees from northern Sweden during the year 1952. Vegetative material of six trees from northern Sweden when grown on grafts (grafted in 1947) at Bogesund (Stockholm) bore mostly class IV seeds (SIMAK and GUSTAFSSON, 1954, Fig. 18). This was the first experimental demonstration proving seed immaturity to be a modification arising from climatic conditions in northern Sweden.

That seed development can also be affected by genotypical differences in trees was shown by the presence of one tree (D: 6) in a *Pinus silvestris* population from middle Sweden which showed a high amount of empty seeds both on trees (30—35 per cent) in the North and on grafts (25—30 per cent) at Bogesund. Tree number 4 in another population of *Pinus silvestris* lying near the northern pine limit above the Arctic circle (Kiruna—Kaupinen) was discovered to be relatively superior and another tree (No. 5) to be poorest when compared in embryo quality with other trees of the same locality (EHRENBERG *et al.*, 1955; GUSTAFSSON and SIMAK, 1958 a). These two trees (Nos. 4, 5) growing under seemingly identical conditions and apparently of same age and 30 other trees from this population were grafted and laid out at Bogesund and Kiruna by SIMAK for future observation. Thus investigation of individual trees or provenances and their selection for superior seed with X-ray radiography was made possible (SIMAK and GUSTAFSSON, 1954;

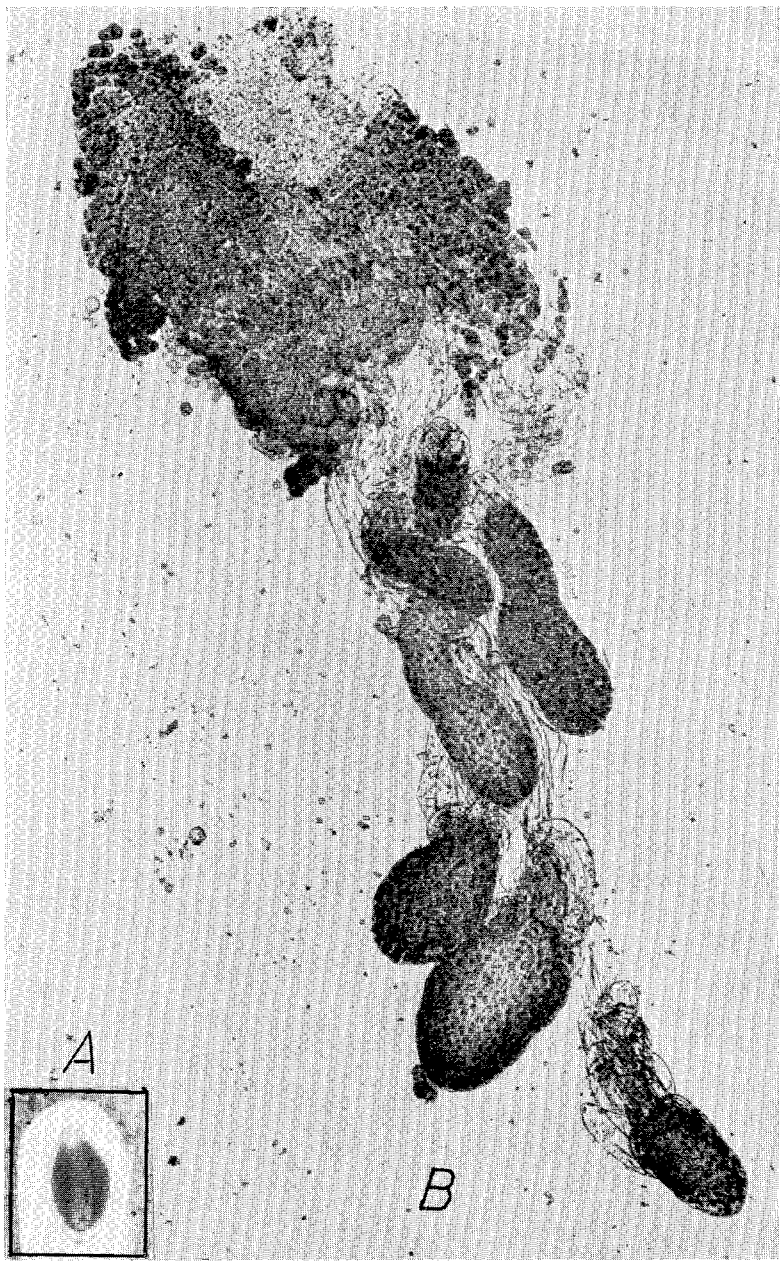
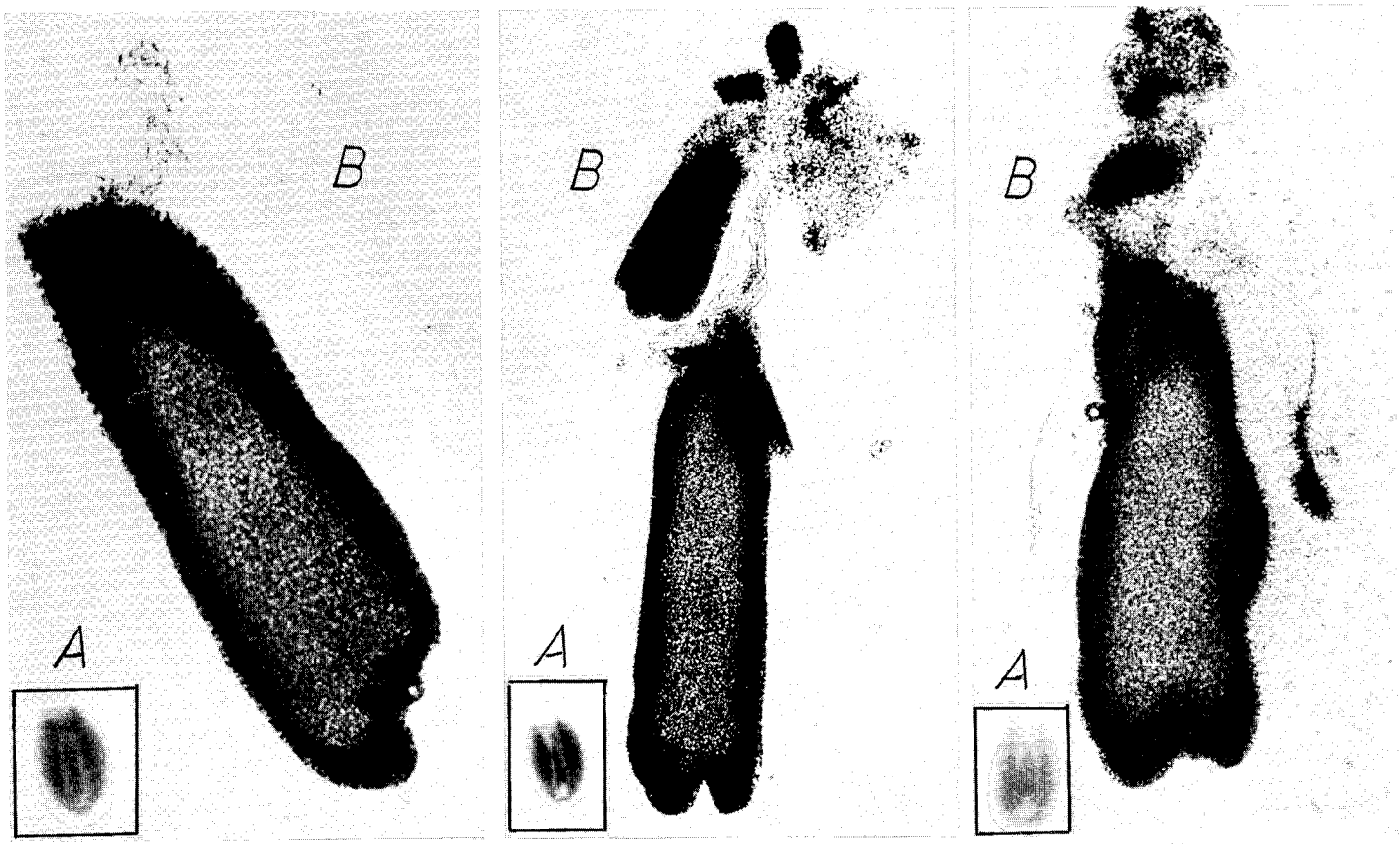
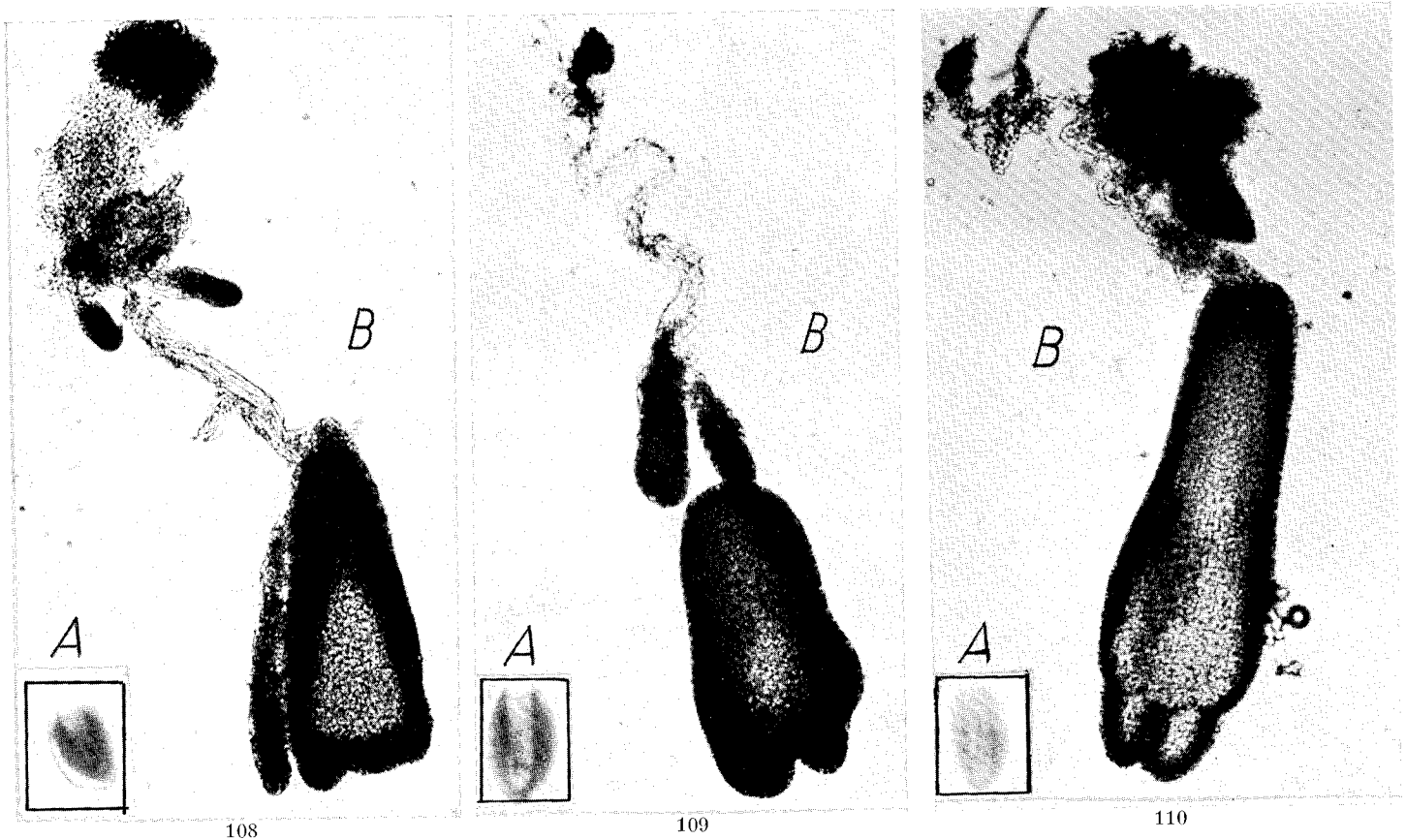


Fig. 104. *Pinus silvestris*. Radiograph (A) of a persistant polyembryonic seed shows only two embryos. Photomicrograph of whole mount of embryos dissected from the seed showing undifferentiated embryos arising from loss of terminal embryo dominance. 104 A, × 5; 104 B, × 125.



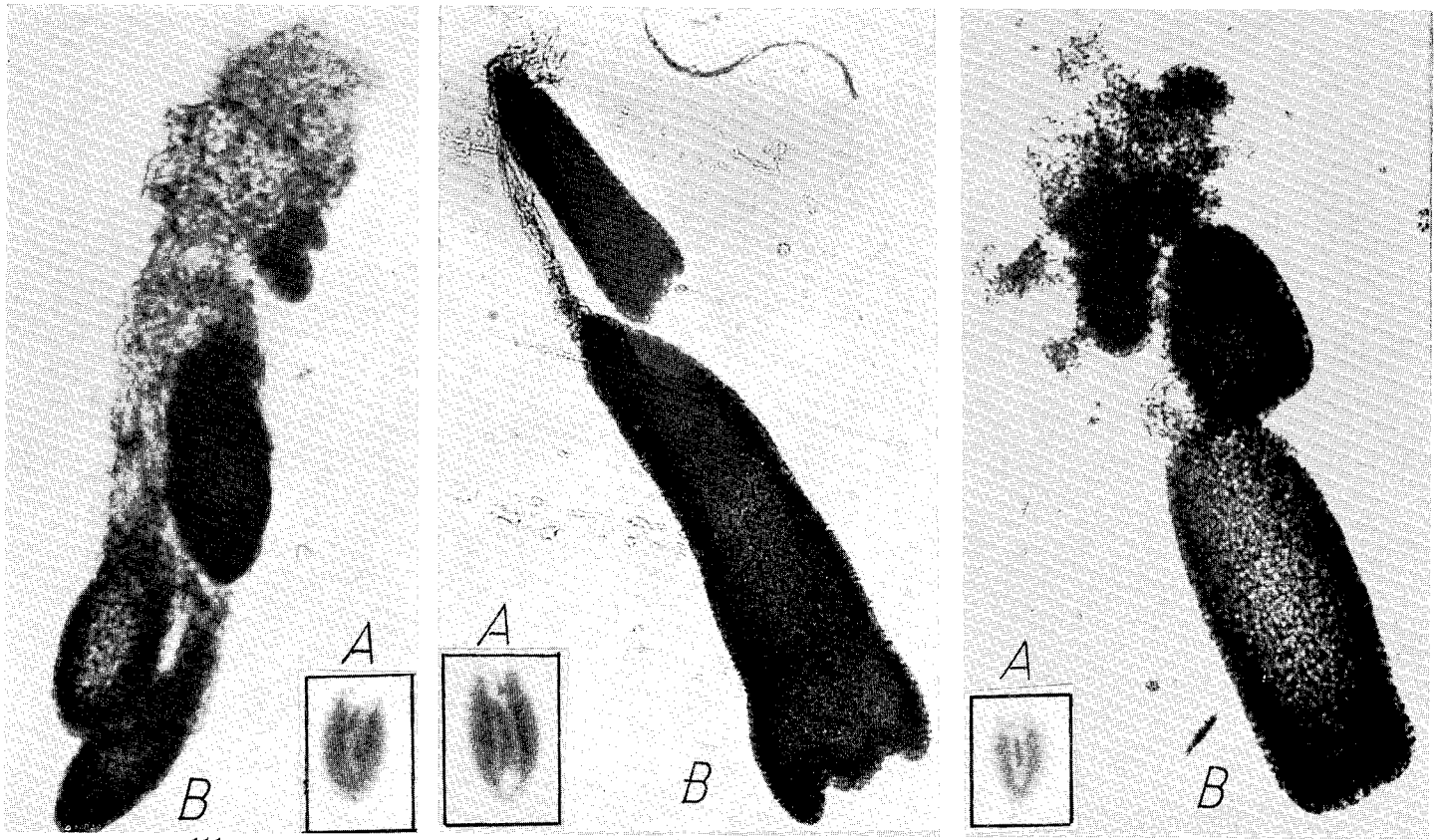
105 106 107

Figs. 105—107. *Pinus silvestris*. Radiographs (A) of class III, IV seeds and photomicrographs of whole mounts of embryos dissected from the corresponding seeds showing irregular differentiation 105 A, class IV; 106 A, 107 A, class III. Fig. 107 B shows a cotyledonous embryo with tumerous growths. 105 A, × 6; 106 A, × 5; 107 A, × 6; 105 B, × 100; 106 B, × 80; 107 B, × 100.



108 109 110

Figs. 108—110. *Pinus silvestris*. Radiographs (A) of seeds and photomicrographs (B) of whole mounts of embryos dissected from the corresponding seeds. 108 A. Class II. 108 B. Terminal embryo closely appressed to a degenerating embryo showing irregular differentiation. 109 A. Class IIP. 109 B. Embryo probably consisting of two uncleaved units borne terminally by a third unit. 110 A. Class III. 110 B. Mature embryo showing only two flattened cotyledons. 108 A, × 5; 109 A, × 6; 110 ×, × 6; 108 B × 75; 109 B, × 90; 110 B, × 80.



111 112 113

Figs. 111—113. *Pinus silvestris*. Radiographs (A) of seeds and photomicrographs (B) of whole mounts of embryos dissected from the corresponding seeds. 111 A. Class II showing two embryos. 111 B. Five embryos dissected from same, three of which show no differentiation but similar size. 112 A. Class III seed gives a correct picture of whole mount of embryos shown in fig. 112 B. 113 A. Class III. 113 B. Several embryos in which terminal embryo shows little external differentiation. 111 A, × 6; 112 A, × 6; 113 A, × 5; 111 B, ×90; 112 B, × 85; 113 B, × 125.

EHRENBERG *et al.*, 1955; GUSTAFSSON and SIMAK, 1958 a; ANDERSSON, 1965). SIMAK and GUSTAFSSON included in their investigation other studies such as seed morphology, cone weight and seed number, and seed germination in relation to seed classes. This paved the way for more investigations. A series of publications appeared dealing mainly, (1) with improvement of X-ray techniques in relation to seed quality and germination (MÜLLER-OLSEN and SIMAK, 1954; EHRENBERG *et al.*, 1955; SIMAK *et al.*, 1957; SIMAK, 1957, 1966; MÜLLER-OLSEN *et al.*, 1956; HAGNER and SIMAK, 1958; NEKRASOV and SMIRNOVA, 1961; HANSEN and MUELDER, 1963; KAMRA, 1963, 1964 a, b; SIMAK and KAMRA, 1963; KRIEBEL, 1966), (2) with effects of radiation on conifer seeds (SIMAK and GUSTAFSSON, 1953 b; GUSTAFSSON and SIMAK, 1958 b; SIMAK *et al.*, 1961; KAMRA and SIMAK, 1965), and (3) with studies of tree breeding (PLYM FORSHELL, 1953; EHRENBERG *et al.*, 1955; EHRENBERG and SIMAK, 1957; KLAEHN and WHEELER, 1961; KRIEBEL, 1966). For details the reader is referred to the original publications and further reference to these is made only with regard to data pertinent to the present theme.

Embryo development, a dynamic process, may sometimes stop at different stages in the ovules giving rise to what can be called *embryologically-static seed classes* (0—IV), containing different embryo stages in seeds. The amount of each class varies in trees depending on the climate, latitude and altitude in northern Sweden (MÜLLER-OLSEN and SIMAK, 1954; ANDERSSON, 1965). My observations show that this involves temporary inhibition of development in some or permanent embryo degeneration in other seeds in 0—IV classes. In general, temporary inhibition of embryogeny seen in II to IV classes may be said to indicate embryo-endosperm immaturity and seeds, mostly 0—I and sometimes II—IV, with dead embryos show seed sterility. Thus 0—IV seed-variation from the embryological point of view, is mostly the end result of a disturbed development.

HÅKANSSON (1959) analysed the seed classes given by SIMAK and GUSTAFSSON (1954). Class 0 according to HÅKANSSON arises due to failure of fertilization or ovule abnormality. Class I arises due to embryo degeneration during seed development and II—IV represent stages of normal development. This is broadly correct and as already hinted above, class 0—IV may show both inhibited living or dead embryos. A seed class is not related to any particular stage as different embryo stages are observable in each of 0—III classes. Class IV, though representing a mature cotyledonous embryo stage, is not always so. Observations on empty seed formation in relation to embryo mortality on Indian and northern Swedish conifers (DOGRA, 1961, present investigation) and breeding experiments in *Pinus* (SARVAS, 1962; HAGMAN and MIKKOLA, 1963), *Pseudotsuga* (ORR-EWING, 1957 b) and *Picea* (MERGEN *et al.*, 1965) show that embryo mortality is one

of the major causes of empty seed formation. It thus appears that the concept of empty seed (0 class) as pointed out by SARVAS (1962) or of the other classes I—IV is not as simple as it may at first seem.

0 class though defined as seeds containing "neither embryo nor endosperm" nevertheless contains shrivelled remains of endosperms. These endosperms which comprised 50 to 90 per cent in *Pinus silvestris* and 80 to 95 per cent in *Picea abies* contained no embryos at the seed stage. In the remaining endosperms embryos from late embryogeny were present. The condition of endosperm in these seeds was found to be related to stage of embryo development. The endosperms without embryos also showed differences which indicated that some of them were, perhaps, not initially embryo-less and that degeneration had taken place at the proembryo level. Data on proembryo degeneration in Swedish conifers are as yet not available, but those for Indian conifers (pp. 18, 19) demonstrate that proembryo mortality contributes to the formation of 0 class of seed. Empty seed can arise from other causes, such as lack of pollen as in *Picea abies* (ANDERSSON, 1965), *Abies pindrow* (DOGRA, 1966 a), *Pseudotsuga menziesii* (ALLEN, 1942; ORR-EWING, 1957 b), *Larix* and *Tsuga* (see ORR-EWING, 1957 a) and from endosperm and embryo degeneration between pollination and fertilization stages (SARVAS, 1962). Thus 0 class of seeds can result from several embryological conditions in the ovule.

The dry endosperm in 0 class of seeds gives shrivelled shadows of variable length, shape, and intensity on X-ray radiographs. On this basis 0 has been further sub-classified in *Picea*. KLAEHN and WHEELER (1961) divided 0 in three sub-classes 0_0, 0_1, and 0_2: 0_2 is completely empty seed; 0_1 gives an endosperm shadow, which is less than, and 0_2 more than, one third of the total length. ANDERSSON (1965) divided 0 class of *Picea abies* seeds from Kiruna (northern Sweden) into four sub-classes *viz.* 0_a, 0_b, 0_c, and 0_d. Seeds showing no visible contents were placed in 0_a; with small flake like remnants of collapsed prothalli in 0_b; with diffuse and often spongy endosperm masses without embryo cavities in 0_c; and with compact endosperm masses without embryo cavities in 0_d. 0_a and part of 0_b seed arise from unpollinated ovules and 0_c and 0_d, probably, after pollination (ANDERSSON, 1965, p. 29).

In an embryological study I could find no 0 type of seed in *Pinus silvestris* or *Picea abies* which was completely empty. KLAEHN and WHEELER (1961) pointed out that the highest proportion with 0_0 sub-class (completely empty seed) belongs to the unpollinated ovules. Unpollinated ovules are known to form empty seeds in several genera of Pinaceae. Such seeds are, however, not completely empty in the strict sense. In a study of unpollinated ovules of *Abies pindrow* (DOGRA, 1966 a) I found that membranous remains were always present in seeds formed from unpollinated ovules, which were

distinguishable from endosperms of pollinated ovules that degenerated after embryo collapse. Membranous remains of prothalli and their cavities in seeds formed from unpollinated ovules are not easy to detect on X-ray radiographs of dry seeds. That the fresh prothalli always showed the formation of an endosperm cavity in unpollinated ovules has been observed by ORR-EWING (1957 b) in *Pseudotsuga menziesii* and by me in *Abies pindrow*.

Endosperm condition in 0 class of undiseased seeds varies because of different degrees of stimulation provided to its tissue by pollen and by proembryo and embryo development, before degeneration takes place. That the embryo and endosperm development are closely related with regard to changes in chemical constitution and storage contents has been shown in *Pinus roxburghii* (KONAR, 1958 a, b). The present investigation indicates that the climatic effect, damage or injury to the endosperm influences the embryo which in return affects endosperm development. The endosperm contents of empty or immature seeds also show variable degrees of shrinkage, collapse or degeneration expressed differently in seeds, depending on the condition of their contents at the time of drying. It is, thus, difficult to state the real embryological origin of each sub-class of 0, even in a broad sense, on the basis of X-ray radiographs. In the undiseased 0 class of seeds X-ray configurations, condition and staining behaviour are a morphological expression of the degree of stimulation given to the endosperm by pollen and embryo development and of effects of degeneration, dehydration and shrinkage. It is concluded that the embryological background of the above sub-classes of 0 based on X-ray radiographs is difficult to establish and this type of classification will therefore be of little practical use.

Class I seeds contain a developed endosperm with clearly differentiated endosperm cavity but no embryo is observed on the X-ray radiographs. Fifty to seventy per cent of class I seeds had endosperm with no traces of embryo. They showed various stages of maturity and of storage of food products. The storage products develop because of stimulation provided to the endosperms by proembryos which, due to early degeneration, are not traceable in the seed. Embryo stages of late embryogeny were found in 30 to 50 per cent of class I seeds. The endosperms of these develop due to the presence of embryos, but degeneration of these embryos takes place in late embryogeny. Thus class I seeds which contain well-developed endosperms with clear cavities result, in general, from the presence of proembryos and late embryos, but the embryos do not survive and their remains are not recorded in the endosperm cavity seen on the X-ray radiographs.

In *Pinus silvestris* class II is common in northern Sweden (GUSTAFSSON and SIMAK, 1954) and in self-pollinated trees (EHRENBERG *et al.*, 1955). It shows poor germination when compared to III and IV (MÜLLER-OLSEN

and SIMAK, 1954). SIMAK and GUSTAFSSON (1959), SIMAK and KAMRA (1963) and SIMAK (1966) divided II into IIP and II. IIP seeds do not germinate and have small point embryos the length of which does not exceed the breadth. Embryogeny of class IIP shows a wide range of stages of late embryogeny (Figs. 84—87, 89—94, 98—100). The endosperm of IIP class is poorer in stored food than that of II, but persistent polyembryony is more common in the former. Increase of class II seeds results from the effect of cold climate on ovules and seed ripening during critical phases of embryogeny, for example, during, a) addition of the segments of suspensor-system and the subsequent elongation; b) establishment of the terminal embryo dominance in the polyembryonic ovule; c) elimination and degeneration of all supernumerary embryos except the dominant embryo, mostly in order of their positions from micropyle downwards, in the polyembryonic ovule or seed; d) growth and increase in size; and e) initial differentiation of tissues and parts of dominant embryo. Normal ovule and seed maturation is embryologically upset by shocks of cold climate which in some way disturb enzymatic, hormonal and other physiological processes of normal embryogeny. The physiological imbalance so created in the ovule causes, *inter alia*, loss of dominance of terminal embryo which leads to persistent polyembryony with several embryos of almost identical size and differentiation in the seed; to partial or complete failure or irregular mode of cleavage in the embryos; to no addition or no elongation of segments in suspensor-system; and to undeveloped size and no differentiation in the dominant embryo. These conditions manifested at IIP or earlier, when not fatal to the embryo find full expressions in II, III, and IV classes of seeds. Thus in II or III the dominant embryo sometimes shows: lack of differentiation of organs or tissues; failure of increase in size in length, breadth, or both; distorted embryos, suppression of cotyledon formation; death of any part or tissue of the embryo; and persistent polyembryony. Details of disturbances in late embryogeny due to climate, seen in the five classes, are given below.

Suspensor-system: Embryos with developed suspensor-systems (E_1, E_2 . . . E_t) were present in 0—IV classes but embryos with only E_1 formation were common in 0, I and IIP. In the latter E_2, E_3 etc. segments were not formed or they failed to elongate. Failure of E elongation in embryos was common in all five classes. The suspensor-system did not elongate remaining very short in some cases and the embryos remained undeveloped near the micropyle and subsequently degenerated. Thus the suspensor-systems of dominant embryos vary in length and in degree of segmentation but E_1 elongation is common in affected seeds of *Pinus silvestris* and *Picea abies* from northern Sweden. The structure of the suspensor-system cannot be clearly understood from a study of embryos from seeds only. For more accurate observa-

tions, investigations from fresh ovules, not carried out here, are recommended.

Failure of cleavage: In seeds of *Pinus silvestris* from northern Sweden failure of organized cleavage was common. The four embryo units sometimes separated irregularly and loosely at E_1. This is not a normal feature of genera showing cleavage in Pinaceae (MEHRA and DOGRA, unpublished); such separation occurs normally at E_2 or later (Fig. 16). In *Pinus silvestris*, which has cleavage, the embryos from seeds from northern Sweden sometimes showed no cleavage. In these, unitary lobing in uncleaved but clumped embryo units and different degree of partial cleavage were common and these conditions frequently persisted in the seed (Figs. 93, 98, 103). Joined seedlings which survived from partial cleavage were germinated in *Pinus silvestris* by SIMAK and in *Pinus gerardiana* by me (unpublished data). Non-cleavage condition of embryos in seeds of *Picea abies* from northern Sweden generally remained unaffected, with the exception of a few cases of unitary lobing and partial cleavage. Unitary cleavage of *Pinus silvestris*, though remaining predominant, frequently failed and seeds with embryos showing unitary lobing and partial cleavage were common. Primary unitary structure of four units in these embryos of *Picea abies* and *Pinus silvestris* was not affected.

Presence of cleavage or non-cleavage is a generic character in conifers (BUCHHOLZ, 1918—1950; SCHNARF, 1933; JOHANSON, 1950; DOYLE, 1954, 1957, 1963; WARDLAW, 1955; DOGRA, 1961, 1966 b; CHOWDHURY, 1962). In Pinaceae, *Abies*, *Pseudotsuga*, *Picea*, *Pseudolarix* and *Larix* show non-cleavage, while *Keteleeria*, *Cedrus*, *Tsuga*, and *Pinus* show cleavage (see pp. 16, 17). In northern Sweden the occurrence of cleavage and non-cleavage, in general, remains stable in embryos of *Pinus silvestris* and *Picea abies*. It is influenced by climate and is related to changes in the suspensor-system structure which may also be caused by climate. Instability of cleavage in *Pinus* and *Cedrus* and non-cleavage character of *Picea* and *Abies* (MEHRA and DOGRA, unpublished) and in other Indian conifer species (DOGRA, 1961) is observed in not uncommon cases.

Persistent polyembryony: Persistent polyembryony has been reported to be common in seeds of *Pinus silvestris* and *Picea abies* (KUJALA, 1927; SIMAK and GUSTAFSSON, 1953 a, b, 1954; PLYM FORSHELL, 1953; present investigation) from northern Scandinavia and in *Pinus aristata* trees growing near the timberline and at extreme northern limits of the species range (KRIEBEL, 1966). In *Pinus strobus* FOWLER (1959) noted that seeds from the fresh frozen cones collected from Quebec in 1956 were immature and had persistent polyembryony. As many as seven embryos were reported to be present in a single seed. Seed from fresh almost mature cones of the same species collected from southern Ontario in 1958 also had twin embryos but

in numbers not comparable to the 1956 Quebec seed. In northern Sweden embryo number is more conspicuous in *Pinus silvestris* seeds because polyembryony, both cleavage and archegonial, in this species gives rise to many embryos per ovule. *Picea abies* on the other hand has only archegonial polyembryony and thus shows fewer embryos in the persistently polyembryonic seeds. In both species persistent polyembryony is seen in all five classes but in IIP and II it is most common. It appears to arise in two ways. 1) In the ovule terminal embryo dominance is somehow destroyed or weakened at IIP stage by the sub-arctic climate. Size and differentiation of the embryos, normally regulated by the terminal embryo dominance, is thus weakened or is lost and growth, shape and size become identical for several embryos in a seed. 2) Embryonal development of these type of embryos or of normal embryos is sometimes arrested because of unusual low temperature effect on the cones and ovules. In both cases seeds form without the normal elimination of supernumerary embryos. Many embryos, thus, persist in the seed stage in IIP—IV classes, some of which survive at germination to give rise to seedlings. Cases of multiple seedlings from a germinating seed have been recorded as polyembryony by several workers in *Pinus* and *Picea* (*e.g.* TOUMEY, 1923; CLARE and JOHNSTONE, 1931; GRAVATT *et al.*, 1904; JOHNSTONE, 1940; NELSON, 1941; ILLIES, 1952, 1953, 1959, 1964; BLACK, 1960).

Undifferentiated dominant embryos are more common in IIP and less common in II—IV seeds but in severely affected ovules embryo differentiation fails to occur in II—IV seeds. There are differences, in different seeds and samples, in dominance effect of the terminal embryo. Thus persistent polyembryony results either from the partial or complete failure of dominance of the terminal embryo, or from arrested embryonal development or from both.

In a sampling analysis of seeds from free and controlled pollination of *Picea abies* (ILLIES, 1953) 135 out of 322 trees produced multiple seedlings. According to ILLIES the variation in frequency of production of multiple seedlings was due to differing inherent characteristics of the parent trees. In another investigation (ILLIES, 1959) on polyembryony of these trees she stated that the influence of the mother tree on the development of the polyembryony was strong but that of the father tree or of the environment could not be demonstrated. *Picea abies* is a non-cleavage species and the maternal influence on the frequency of production of persistent polyembryonic seeds depends on the number of archegonia fertilized and the environmental conditions in the ovule. Number of archegonia in a prothallus is variable in a species within a specific range (*e.g.* 1—6 in *Pinus*). The hereditary trend as to the number of archegonia in conifer species is as yet not fully investigated but an evolutionary trend from a primitive few to an advanced condition of many archegonia is shown at generic and sub-family

level in Podocarpaceae by DOYLE (1954). PLYM FORSHELL (1953), EHRENBERG *et al.* (1955), EHRENBERG and SIMAK (1957) and EHRENBERG (1963) state polyembryony to be one of the effects of self-pollination in *Pinus silvestris* and polyembryony occurs after "... self-pollination even in trees where it never is found after open pollination" (EHRENBERG and SIMAK, 1957, p. 21). A clear distinction, however, should be made between normal polyembryony (archegonial or cleavage) in an ovule and persistent polyembryony in the seed. Cleavage polyembryony is always present in ovules of pines but normally only one embryo survives in a seed. The elimination of supernumerary embryos is brought about by dominance of the terminal embryo during maturation from ovule to the seed. This elimination may not occur because of several factors. Occurrence of persistent polyembryony in seeds is thus variable within a species and it is not hereditary in the strict sense as cleavage polyembryony is in a species. Some trees, however, show conspicuous embryological disturbances which may occur regularly in annual seed crops as recorded in *Pinus silvestris* by SIMAK (see p. 50), and SARVAS (1962) and in *Picea abies* by ILLIES (1953, 1959). Persistent polyembryony, an end result of disturbed or arrested embryo development seen in the seed, results from the effect of environment on morphogenetic factors within the ovule where inherent qualities of the trees, provenances or species may or may not participate.

The multiarchegonial system in the prothallus plays a role in embryo survival in the seed. In *Picea abies* from northern Sweden, embryos from separate archegonia of a seed showed different behaviour in degeneration in which condition some embryos survived the effects of sub-arctic climate on the ovule and others did not. In *Pinus silvestris* after cleavage all four embryo units from one archegonium degenerated while four from the other in the same seed survived (Fig. 89). Similar observations on embryos of archegonial polyembryony have been made also in other species of *Pinus* and in *Picea smithiana*, *Cedrus deodara*, and *Abies pindrow* (DOGRA, 1961) and one such example from a naturally selfed *Pinus nigra* tree is given here (Fig. 30). In the ovules of these species the origin of embryos was traced to the archegonia from their suspensor-systems as shown by BUCHHOLZ (1918, 1920 a, 1926, 1929). Embryos of archegonial polyembryony can arise from fertilization of separate archegonia of an ovule by genetically different pollen and they can be inherently different.

The seed abnormalities recorded by MÜLLER-OLSEN *et al.* (1956) and SIMAK *et al.* (1961) may occur regularly in the annual seed crop of particular trees or provenances and affect seed germination. Some of these, as discussed, result from disturbed embryology. A scrutiny of X-ray radiographs placed at my disposal by SIMAK reveals the following:

X-ray radiographs showing two endosperm cavities with and without embryos in a seed can be explained by presence of two endosperms which on radiographs look like one. In normal development only one prothallus develops from a tetrad cell and after pollination the prothallus forms a single cavity for all embryos. Occasional development of more than one prothallus in an ovule or seed is observed commonly in *Pinus silvestris* (SARVAS, 1962), and in several other *Pinus* species, *Picea smithiana*, *Cedrus deodara* and *Abies pindrow* (personal observation). Such abnormalities occur more abundantly in some trees in a species as in *Pinus silvestris* (SARVAS, 1962). In an ovule only one or both of these gametophytes may survive to bear embryos if fertilized. Two asymmetrically arranged prothalli, each with a proembryo, are recorded in one ovule in *Podocarpus nivalis* (BOYLE and DOYLE, 1954).

Two endosperm cavities in an endosperm are seen in abnormal seeds of *Pinus silvestris* (EHRENBERG *et al.*, 1955).

Horizontally orientated endosperm cavities in some seeds are explained by the presence of lateral archegonia as shown in some prothalli of *Abies pindrow* (DOGRA, 1966 a) or by growth of embryos in a horizontal direction as shown in *Pinus silvestris* (HÅKANSSON, 1959).

Point embryos near the micropyle can arise due to inhibited elongation, undeveloped suspensor-system (Fig. 47) and due to shrinkage or mechanical causes resulting from the drying of seed contents.

Terminally joined embryos were observed in *Pinus silvestris* (Fig. 109) but real terminal fusion, if it occurs (see BLACK, 1960), can be shown only by an anatomical study.

Laterally joined embryos with joined tissues are sometimes found in non-cleavage genera, for example, in *Abies pindrow*, *Picea smithiana* and in cleavage forms like *Pinus* and *Cedrus* (MEHRA and DOGRA, unpublished). This condition arises from persistent unitary lobing or partial cleavage.

Inverted embryos are common and they develop due to their inability to grow by enzymatic dissolution of prothallial tissue of the corrosion region at some developmental stages. The suspensor-system in such cases becomes sharply bent and the embryo then grows in different directions, as in *Pinus silvestris* (HÅKANSSON, 1959) or in the opposite direction as shown in *Abies pindrow* (Figs. 48, 49) and *Pinus silvestris* (Fig. 78). This condition can also arise due to mechanical obstruction. Bifurcated and trifurcated endosperm cavities can arise by growth of from two to three young embryos in different directions in a prothallus. In several *Pinus* species, *Cedrus deodara*, *Picea smithiana*, and *Abies pindrow* inverted embryos were frequently observed to degenerate during development (DOGRA, 1961). In *Pinus silvestris*, *Picea abies* and in some other species they have been shown to survive and affect

seed quality (BUCHHOLZ, 1918; KUJALA, 1927; ILLIES, 1953, 1964; MÜLLER-OLSEN *et al.*, 1956; HÅKANSSON, 1959; BLACK, 1960).

Dead embryos of two types 1) with compact deeply staining shrivelled cell contents and 2) with diffuse tissues of almost non-staining empty cells are commonly observed. The luminous or diffused embryos recorded by MÜLLER-OLSEN *et al.* (1958) and SIMAK (unpublished) are perhaps related to a similar phenomenon of degeneration.

Seeds with rimmed or "distended" endosperm cavities reported in *Pinus silvestris* (SIMAK *et al.*, 1961) can only be explained by means of an embryological study which is necessary for understanding these abnormalities.

Embryology, Seed Classes, Storage and Germination

According to WIBECK (1928 b, 1929 b) germination of immature seeds is related to the distance of the embryo from the micropyle. Embryological observations on seeds containing young embryos show that this distance can also vary because of shrinkage of a developed suspensor-system or of the embryo, perhaps due to loss of water content. In II and III classes this value (of distance of embryo from micropyle) is useful in seeds with fairly well-proportioned embryos where mechanical displacement is negligible. The differences in the distance of the embryo from the micropyle in different seeds is actually due to variation in embryo length. This variation is better understood for practical purposes as: embryo length : endosperm cavity length ratio which forms the basis of the seed classification (for II—IV) adopted by SIMAK and GUSTAFSSON (1954). In *Pinus silvestris* and *Picea abies* from northern Sweden I observed that such embryos vary not only in length but also in width. Embryo length in relation to endosperm cavity is important for assessing seed quality and germination but differences in embryo width should also be included in such studies.

Pinus silvestris seeds from northern Sweden showed a germination of 0 per cent in 0, 4 per cent in I, 13 per cent in II, 63 per cent in III and 94 per cent in IV classes (SIMAK and GUSTAFSSON, 1954). Germination of class I seeds, also recorded by MÜLLER-OLSEN and SIMAK (1954) was reported to be due to misclassification. Relationship between germinability and seed classes was also observed in *Picea abies* (MÜLLER-OLSEN *et al.*, 1956).

MÜLLER-OLSEN and SIMAK (1954) included two endosperm types within seed classes showing superior and inferior germination. In *Pinus silvestris* and *Picea abies* II—IV class of seeds with endosperm A germinated better than those with B (MÜLLER-OLSEN and SIMAK, 1954; MÜLLER-OLSEN *et al.*, 1956). In dissections, these types were indistinguishable due to water absorption, but in all endosperms of II—IV seeds the A type was observed to be more vigorous and richer in stored food than B. Developmental studies

and chemical analysis of such seeds in relation to climate are necessary for a proper understanding of the two endosperm types in undiseased seeds. One explanation is that the B type of endosperm can result from a late climatic injury to an incompletely mature endosperm containing a developed embryo.

SIMAK and GUSTAFSSON (1954) demonstrated that the speed of germination is also related to seed classes. In *Pinus silvestris* a conspicuous delay in germination in II was noted, it was less in III and in class IV seeds the germination was fast. Grafted material of trees from northern Sweden at Bogesund (Stockholm) showed simultaneous germination of predominantly class IV seeds. In class II and III seeds with A and B endosperms of *Picea abies* (MÜLLER-OLSEN *et al.*, 1956) a stagnation period was noted between 13th to 25th day of germination, during which immature embryos completed a part of their development. This was shown by a comparison of X-ray radiographs before and during germination. During germination there was an increase in the number of 0 and IV seeds formed from II and III because of embryo degeneration or development. SIMAK and GUSTAFSSON (1959) demonstrated in another study an increase in seeds of 0, I and IV and decrease in II and III seeds of *Pinus silvestris* stored at 24° C while no such changes occurred in seeds stored at 4° C after storage for one month. The immature seeds showed improved germination after stratification. The stratification technique (SIMAK and GUSTAFSSON, 1957, 1959) is called "equilibration of immature seeds". It is clear that under storage conditions IIP embryos do not survive, as they are known not to germinate (SIMAK and KAMRA, 1963; SIMAK, 1966). In contrast, more developed and differentiated embryos which differ greatly in length (and which can be classed irrespective of degree of differentiation, not discernible on radiographs) can develop and survive under favourable storage and germination conditions. In germination the seed classes which survive are reported to show less vigour than IV (MÜLLER-OLSEN and SIMAK, 1954). This may be caused by inherent or physiological factors or by delayed effects of abnormal morphogenetic development of embryo and seedlings, *e.g.* in tissue differentiation seen in II—IV class of embryos. Studies of embryo development under different conditions of storage and germination with the use of X-ray radiography and conventional techniques can be useful for a better understanding of requirements for storage, stratification, and germination of immature and abnormal seeds.

Conclusion

Embryological investigations of seeds by X-ray radiographs are important for detecting and studying, without damage, general features like full or empty seeds with shrunken contents; polyembryony; variation in embryo

length or size in relation to endosperm cavity; embryo differentiation; embryo and endosperm abnormalities; for evaluating the frequency of their occurrence in a tree, or a species; and in tree breeding (SIMAK and GUSTAFSSON, 1953 a, b, 1954; PLYM FORSHELL, 1953; EHRENBERG *et al.*, 1955; EHRENBERG and SIMAK, 1957; KLAEHN and WHEELER, 1961; HANSEN and MUELDER, 1963; ANDERSSON, 1965; KRIEBEL, 1966; present investigation). The general embryology of the five seed classes is as follows: 0 and I result from degeneration of endosperm or embryo or both during pre-pollination and from pollination to early stages of late embryogeny (Figs. 1—16); II from early stages of late embryogeny to initiation in differentiation and cotyledon formation in the embryo (Figs. 17—19); III consists of older stages of late embryogeny (Figs. 18 and 19); and IV shows fully differentiated and developed embryo. Embryo length in relation to endosperm cavity varies in seeds of northern Sweden and it forms the main criterion of classification. Embryologically speaking, the classification is arbitrary, as embryo stages often overlap in 0—IV and same stages may be present in any seed class. Embryo width and differentiation (of tissues and cotyledons etc.) is independent of embryo length and these proportions should be incorporated in X-ray studies when a more critical germination analysis is needed. The general embryological background of 0—IV, in a broad sense, is sufficiently specific for a seed class to make X-ray radiographs valuable in bulk seed testing.

Analysis of seeds from inbreeding and experimental crosses of conifer trees, however, requires accurate interpretation in terms of embryology. Empty seed formation, effects of sub-arctic climate on fertilization and proembryogeny, mortality rate in proembryos and young embryonal stages, number of embryos and nature of polyembryony, archegonial or cleavage, failure of cleavage, initial embryo dominance, differentiation and other such characters can be roughly indicated by X-ray radiographs in the seed but not correctly interpreted. This is due to handicaps of shadow studies such as overlapping of parts or where living or dead cells in the seed give no impressions on radiographs. SIMAK (1957) points out that physiological changes in embryo and endosperm are seldom revealed on the radiograph.

Embryology is a dynamic process where X-ray study of seeds, an end result, is not sufficient to make clear the various embryological phenomena. For example from a study of *Pinus silvestris* seeds showing two endosperm cavities it is concluded that "The participation of two (or possibly more) archegons can often be decided; in such a case two different embryo cavities are formed" (EHRENBERG *et al.*, 1955, p. 292). It appears to be assumed here that separate cavities are formed by embryos from different archegonia, which is not the case in normal embryology of *Pinus silvestris* (HÅKANSSON, 1956, 1959) or of any other conifer (BUCHHOLZ, 1918—1950; DOYLE and

LOOBY, 1939; DOGRA, 1961). The only method to ascertain whether embryos belong to separate archegonia or not is to trace them from the origin of their suspensor-systems (BUCHHOLZ, 1918, 1920 a, 1926, 1929; SCHOPF, 1943). *Pinus silvestris* from northern Sweden shows frequent embryological abnormalities of various kinds (SIMAK and GUSTAFSSON, 1953 a, b; HÅKANSSON, 1956, 1959; SIMAK *et al.*, 1961, present investigation) and separate endosperm cavities in a seed is one of them. For the same reason my observations on embryogeny studied from *seeds* of Swedish conifers are also open to revision and confirmation with studies from developing *ovules*. Techniques of studying developing ovules with X-ray radiographs, if developed, can become useful for obtaining correct numerical data on embryology.

It is concluded that X-ray technique can become more useful and precise with parallel embryological studies which should precede bulk X-ray seed and germination analyses, wherever possible.

Embryology in Tree Breeding

Embryology of Self-pollinated Ovules in Trees of Some Species of Pinaceae

Self-pollination and inbreeding can be used to detect deleterious recessive genes, and to achieve the homozygosis required for improvement of trees by hybridization and heterosis. However, it is especially in the production of better seed from natural stands and seed orchards that the occurrence of natural selfing is recognized as a potential problem by forest geneticists (*e.g.* JENSEN, 1945; GUSTAFSSON, 1949; KLAEHN, 1953; LANGNER, 1953; LANGNER and STERN, 1955; SQUILLACE and BIHGHAM, 1958; STERN, 1959; BARNES *et al.*, 1962; KRAUSS and SQUILLACE 1963; ANDERSSON, 1963; FOWLER, 1965 a). Early experiments in self-pollination were made in *Picea abies* by SYLVÉN (1910), in *Pinus silvestris* by DENGLER (1932). KOLESNIKOFF in 1929 stressed the importance of inbreeding in trees. These were followed by many observations and experiments in species of *Pinus*, *Picea*, *Pseudotsuga* and *Larix* (PIATNITSKY, 1934; AUSTIN, 1927, 1937; LANGLET, 1940; WETTSTEIN, 1940; ALLEN, 1942; JENSEN, 1945; JOHANSON, 1945; ANDERSSON, 1947, 1965; DUFFIELD and STOCKWELL, 1949; DUFFIELD, 1950; TOYAMA, 1950; LANGNER 1951, 1957, 1959; MEYER, 1951; PLYM FORSHELL, 1953; WRIGHT, 1953, 1955; WRIGHT and GABRIEL, 1958; MERGEN, 1954; ORR-EWING, 1954, 1957 a, b, c, 1965; BINGHAM and SQUILLACE, 1955; EHRENBERG *et al.*, 1955; SYRACH LARSEN, 1956; MAGINI, 1956; EHRENBERG and SIMAK, 1957; RIGHTER, 1958; SQUILLACE and BINGHAM, 1958; PERRY, 1960; PETERS and GODDARD, 1961; DOGRA, 1961, 1964; BARNES *et al.*, 1962; SQUILLACE and KRAUS, 1962, 1963; DIECKERT, 1964 a, b; FOWLER, 1962, 1964 a, b, 1965 a, b, c, d; KLAEHN and WHEELER, 1961; SARVAS, 1962; HAGMAN and MIKKOLA, 1963; MERGEN *et al.*, 1965; and KRIEBEL, 1966). Some of these investigations show that embryo mortality is one of the major causes of empty seed formation. The embryology of empty seed formation is worked out only in a few species and some pertinent embryological conclusions have been drawn from studies of seeds with X-ray radiography. DENGLER (1932) considered empty seed formation in self-pollination to arise from a failure, either of fusion of gametes or of embryo development.

Specific mechanisms which prevent pollen germination on selfed ovules or strobuli have been shown to be absent in *Pinus monticola* (BINGHAM and SQUILLACE, 1955; BARNES *et al.*, 1962; FOWLER, 1962, 1965 a), *Pseudotsuga menziesii* (ORR-EWING, 1954, 1957 b), *Picea omorika* (LANGNER, 1957,

1959), *Pinus peuce* (HAGMAN and MIKKOLA, 1963) and *Picea glauca* (MERGEN *et al.*, 1965).

Pollen tubes were reported to fail to reach the archegonium in some cases in open pollinated ovules of species of *Pinus* (FERGUSON, 1904; SAXTON, 1909; HÅKANSSON, 1956). I observed the failure of pollen tube growth in rare cases in open pollinated ovules of *Pinus wallichiana*, *Cedrus deodara*, and *Picea smithiana*. In *Pinus peuce* (HAGMAN and MIKKOLA, 1963) the archegonia in both self and cross-pollinated ovules sometimes remained unfertilized because the pollen tube failed to grow with sufficient speed through the nucellus. In these and in the study of barriers in interspecific hybridization the role of environment in pollen germination (see MCWILLIAM, 1960) is as yet incompletely understood. FERGUSON (1904) noted that the pollen tube in *Pinus* grew normally even when sperm cells were not formed or when archegonia had degenerated and she concluded that pollen tube growth is not guided by any kind of attraction between sexual cells. In *Abies pindrow* (DOGRA, 1966 a, Figs. 18, 19) the physiological behaviour of the archegonial neck is interesting as it fills with an exudate and a neck passage is formed prior to the entrance of a conspicuously fast-growing pollen tube, a feature characteristic of *Abies* (HUTCHINSON, 1915; DOYLE and KANE, 1943; DOGRA, 1956; MEHRA and DOGRA, 1965). Although there is no evidence to show that this has any influence on the rate of pollen tube growth or that it inhibits self-fertilization to any extent, it is worth mentioning, since in some lower plants the entrance of sperms into the archegonial neck is known to be a chemotactic response (see SMITH, 1955).

PLYM FORSHELL (1953) studied empty seed formation from controlled self-pollinated trees of *Pinus silvestris* with X-ray radiographs. She concluded that inhibition of fusion of gametes was one of the barriers to selfing. On basis of an extensive embryological study ORR-EWING (1954, 1957 b) demonstrated that self-pollination in *Pseudotsuga menziesii* does not affect pollen germination or fertilization but that embryos degenerate during first stages of late embryogeny in many ovules. The inhibition of fusion of gametes proposed as an explanation for empty seed formation in selfing remains unsupported by embryological evidence. Moreover, embryological investigations in self-pollinated trees of *Pinus peuce* (HAGMAN and MIKKOLA, 1963) and *Picea glauca* (MERGEN *et al.*, 1965) confirmed that the defect after selfing arises in embryo development and not in fertilization as already demonstrated in *Pseudotsuga menziesii* by ORR-EWING. Genetical self-incompatibility is a physiological barrier arising between self-pollination and self-fertilization in certain angiosperms (see LEWIS, 1949, 1954; BATEMAN, 1952, 1954) and it differs from all other mechanisms which promote seed formation by cross-pollination. Gene controlled self-incompatibility, as demonstrated in some

angiosperms where specific biochemical reactions prevent germination of pollen or syngamy completely and block embryo inception, has so far not been demonstrated in any member of Pinaceae.

The embryological defect responsible for empty seed formation in selfed ovules when it occurs appears in embryo development (ORR-EWING, 1954, 1957 b; EHRENBERG *et al.*, 1955; EHRENBERG and SIMAK, 1957; SARVAS, 1962, 1963; HAGMAN and MIKKOLA, 1963; MERGEN *et al.*, 1965). Early proembryo development was usually normal in both self and cross-pollinated ovules in *Pseudotsuga menziesii*, *Pinus peuce*, and *Picea glauca* (ORR-EWING, 1957 b; HAGMAN and MIKKOLA, 1963; MERGEN *et al.*, 1965). Embryos in these species collapsed in selfed and not in cross-pollinated ovules at stages following the one shown in figure 20 (*cf.* ORR-EWING, 1957 b, Figs. 6—12, 15, pp. 181, 182; MERGEN *et al.*, 1965, Figs. 3 A—3 F, p. 193). The barrier occurred in some form of physiological incompatibility between early embryos and the female gametophyte tissue. The collapse of the terminal or the dominant embryos was general and ORR-EWING noted that no other embryo in the polyembryonic seed replaced the collapsed terminal one in *Pseudotsuga menziesii*. I have observed that the collapsed embryo of a conifer ovule in early stages can be replaced by an embryo from a different archegonium, but in advanced stages of late embryogeny such replacement may not take place. The cells of the gametophyte may remain normal regardless of the condition of the embryo. In *Pseudotsuga menziesii* the gametophytes showing early collapse of the embryo did not persist to maturity (ORR-EWING, 1957). Prothalli of *Pinus silvestris* and *Picea abies* in Sweden, however, sometimes continue to develop into endosperms even with degenerated embryos *e.g.* in class I (see Figs. 81—83).

Embryological defects of self-pollination have also been studied with X-ray radiography of seeds. Formation of 20—50 per cent in self in contrast to 0—3 per cent in cross-pollination of class I seeds (endosperm and cavity well-developed but embryo not seen) is recorded in *Pinus silvestris* (PLYM FORSHELL, 1953, Table 17, p. 42). EHRENBERG *et al.* (1955) discovered that in addition to abundant formation of 0 class, there were conspicuous amounts of class II seeds formed from arrested development in selfed trees of *Pinus silvestris* (on open or cross-pollinated trees class II seeds were absent). Embryos of class IV were less frequent and those of class I—III stages with persistent polyembryony were common. EHRENBERG and SIMAK (1957) concluded that self-pollination leads to an increase in inferior seeds of 0—III classes and decrease of class IV seeds in Swedish *Pinus silvestris*.

Observations on other species, however, do not show that self-pollination leads to immature embryos. KRIEBEL (1966) observed that the formation of defective or undeveloped seeds due to selfing was seldom found in *Pinus*

strobus. Sound seeds from selfing looked just the same on radiographs as those from outcrossing. An increase in 0 but not of II—III seeds was recorded in self-pollination experiments on *Picea abies* and *Picea glauca* (KLAEHN and WHEELER, 1961, Table III, p. 74; ANDERSSON, 1965, Appendix, Tables XL A and XL B). The endosperm tissue of different 0 class of seeds, however, showed different degrees of X-ray absorption and variation in length and configurations (KLAEHN and WHEELER, 1961; ANDERSSON, 1965) which result, *inter alia*, from embryo collapse at different stages of development. My observations on the embryology of 0 class confirms this.

A comparison of embryology of 0—IV classes (see pp. 66, 67) with that of self-pollinated *Pseudotsuga menziesii*, *Pinus peuce* and *Picea glauca* confirms that embryo degeneration due to selfing is mostly completed during stages seen in 0—IIP (Figs. 64—70, 78—94, 98—100, *cf.* ORR-EWING, 1957 b, Figs. 6—12, 15, pp. 181, 182; MERGEN *et al.*, 1965, Figs. 3 A—3 F, p. 193). This is supported by observations of abundance of class 0 (PLYM FORSHELL, 1953; EHRENBERG *et al.*, 1955; EHRENBERG and SIMAK, 1957; KLAEHN and WHEELER, 1961; ANDERSSON, 1965; KRIEBEL, 1966) and of class I (PLYM FORSHELL, 1953) in selfed trees of *Pinus* and *Picea* species. In *Pinus silvestris* EHRENBERG *et al.* (1955) and EHRENBERG and SIMAK (1957) observed that embryo development is inhibited because of self-pollination and that there is an increase of II and III seeds. This is probable because variations in embryological disturbances or in embryo mortality due to selfing can be considerable and its expression depends not only on trees or species but also on environment. However, confirmation that selfing can lead to inhibition of embryo development in *late* stages (of II—III) is desirable especially in trees growing in a more standard environment than that of northern Sweden. Effects of selfing in the form of embryo mortality, thus, occur in early stages of late embryogeny which are comparable to 0—IIP configurations of X-ray radiographs of seeds.

Embryo mortality does not, however, block selfing completely and this appears to vary in individual trees and species. ORR-EWING (1957 b) selected two trees of *Pseudotsuga menziesii* which showed marked effects of self-pollination in production of empty seeds. In tree number 1, one mature embryo was observed to develop and two viable seeds were obtained from 35 self-pollinated ovules. In *Picea glauca* two out of 20 self-pollinated trees produced no viable seeds (MERGEN *et al.*, 1965). Self-fertility of *Picea omorika* is characteristic of the species and it is as fertile in cross-pollination as in self-pollination (LANGNER, 1959). In controlled pollination experiments self-fertility, *i.e.* ability to produce germinable self-fertilized seeds, varies amongst individual trees of a species and in different species (*e.g.* PLYM FORSHELL, 1953; ORR-EWING, 1954, 1957 b; BINGHAM and SQUILLACE, 1955; SQUILLACE

and BINGHAM, 1958; LANGNER, 1957, 1959; BARNES *et al.*, 1962; SQUILLACE and KRAUS, 1963; FOWLER, 1962, 1964 b, 1965 a, b, c; KRAUS and SQUILLACE, 1964). I studied the embryogeny of a single isolated tree of *Pinus nigra* var. *austriaca* and one of *Pinus montezumae* var. *hartwegi* growing in the North-western Himalayas (DOGRA, 1961, 1964). Some of the disturbances in embryogeny in these species have already been discussed. The trees produced abundant pollen and were naturally self-pollinated. The conclusion that the number of ovules containing embryos ("fertile ovules") was higher in *Pinus nigra* (see DOGRA, 1964) was based on embryo inception and not on formation of full or empty seed. Embryo abnormalities and degeneration were frequent and 25 per cent of one type alone (Figs. 28—31, 51) where the embryo did not grow out of the archegonium were observed not to survive. Since the *Pinus nigra* tree from which the studies were made was introduced in Himachal Pradesh, the disturbances in embryogeny could be both environmental and inbreeding effects. SARVAS (1962) showed that selfing in *Pinus nigra* gives abundant and in *Pinus cembra* fewer empty seeds. In conifers the embryology of self-pollinated trees is not as yet fully investigated. However, embryological disturbances and embryo degeneration due to selfing can be said to vary in trees and species. "In this connection it must be remembered that the various individuals of the species may behave differently, and that the fertility does not need to be high for the progeny to be valuable" (SYRACH LARSEN, 1956).

FOWLER (1964) carried out controlled self-pollination in a *Pinus resinosa* tree located for a "genetic marker" and the progeny showed a number of mutant seedlings distinguishable from normal sibs by their pink hypocotyl and light yellow-green cotyledons. These mutants, which perished after few months, were the result of homozygosis for a certain recessive gene. The ratio between the mutant and normal seedlings in two selfed progenies raised from the tree during 1959, 1961, was one mutant to five normal instead of the expected 1 : 3. This significant divergence according to FOWLER could result from selection against embryos homozygous for this mutation. To support his explanation, FOWLER (1964) raised another progeny from the same tree during 1962, 1963 but this time decreased the number of viable pollen grains available to an ovule (by using self-pollen mixed with an equal quantity of dead *Pinus koraiensis* pollen) and reduced archegonial embryo competition. The departure from the expected 1 : 3 ratio in this case was not significant. The divergence from the expected 1 : 3 ratio of mutant to normal was thus interpreted to result from pre-germination selection against embryos homozygous for this mutation.

The climate in northern Scandinavia affects embryo development and inferior or empty seed formation is common in forests of this region. Some

degree of natural inbreeding may also be responsible for these disturbances in embryogeny. In general, the effect of selfing varies in trees and species and the degree of its expression in terms of abnormal development and embryo mortality (phenotype) will depend on the genetical constitution (genotype) and external and internal environment of the seed (cone) and embryo respectively.

Embryology of Some Interspecific Crosses in *Pinus*

In conifers interspecific hybridization has met success both in nature and in experimental trials (see SYRACH LARSEN, 1934, 1937, 1956; SMITH and NICHOLS, 1941; RICHENS, 1945; RIGHTER and DUFFIELD, 1951; DALLIMORE and JACKSON, 1961; WRIGHT, 1962) as predicted by SAX and SAX in 1933 on basis of a general uniformity of karyotype and chromosome number found in most conifer species except those of Podocarpaceae (MEHRA and KHOSHOO, 1956 a, b; HAIR and BEUZENBERG, 1958; KHOSHOO, 1959, 1961, 1962, 1963). Intra- and interspecific hybridization can be useful for combining desirable qualities of the parents and for introducing hybrid vigour and its merit from the evolutionary and tree breeding points of view is discussed by several authors (STEBBINS, 1950; SYRACH LARSEN, 1956; MEHRA, 1962; WRIGHT, 1962; GUSTAFSSON and MERGEN, 1964). Interspecific hybridization has been successfully carried out in some conifer species of forestry importance such as *Larix*, *Picea*, *Abies* and especially in *Pinus* (see RICHENS, 1945; SYRACH LARSEN, 1956; WRIGHT, 1955, 1962). *Pinus* is taxonomically divided into sub-genera, series, sections or sub-sections (SHAW, 1914; DUFFIELD, 1952). The crosses between species in the same series mostly succeed and those between species from different series fail. Failure in crosses between widely separated taxa can also be due to other causes, some of which are, as yet, incompletely investigated, *e.g.* different pollination mechanisms in conifers (DOYLE, 1945 b; BARNER and CHRISTIANSEN, 1960, 1962; DOGRA, 1964) but embryological failure is known to be one of the more important. Embryological failure of good seed setting occurs in both successful and unsuccessful crosses and can be used to determine incompatibility between two species.

BUCHHOLZ (1944) determined the embryological causes of seed-sterility after cross-pollination between certain species of pines. In full-grown cones he observed ovules: a) partially developed because of lack of fertilization; b) well-developed but empty because of failure of pollen tubes to reach the egg and effect fertilization; c) normally developed showing fertilization and early embryogeny but becoming sterile because of embryo degeneration at stages not determined. The seeds formed from the last two categories (*i.e.* b and c) were empty but contained shrivelled contents comparable to those of class 0.

McWILLIAM (1959) studied interspecific incompatibility between crosses of four species of *Pinus*, *viz. Pinus nigra* Arnold, *Pinus resinosa* Ait., *Pinus rigida* Mill. and *Pinus elliottii* Engelm. In *Pinus elliottii* × *Pinus nigra*, pollen germinated but pollen tubes failed to enter the nucellus. In other three crosses *Pinus resinosa* × *Pinus rigida*, *Pinus nigra* × *Pinus rigida* and in *Pinus resinosa* × *Pinus nigra*, pollen tubes penetrated the nucellus in some cases but the ovules showing a normal rate of pollen tube growth were few. UEDA *et al.* (1961) investigated the development of the female gametophyte after pollination in various interspecific crosses of pines in both crossable and non-crossable combinations and concluded that early nucellus and archegonial development proceeds normally in both (*vide* HYUN and YIM, 1963 a). Breakdown of the ovules after pollination was observed in *Pinus nigra* × *Pinus resinosa* (McWILLIAM, 1959) mostly starting during gynospore (megaspore) development before and after winter. Deterioration of the surrounding cells, collapse of the nucellus, and shrinkage of the ovules followed. No evidence of successful fertilization was observed in any of the sectioned ovules. The presence of fully developed seed coats observed on some aborted seeds, however, indicated that some ovules might have reached the fertilization stage. Complete inhibition of pollen tube growth, found only in one combination *Pinus elliottii* × *Pinus nigra*, showed an extreme type of incompatibility between widely divergent species. In crosses between intersectional and sectional species incompatibility was not complete as the pollen tube entered the nucellus in a small percentage of the ovules. Thus pollen development in the ovules of an incompatible pine species cross does not follow a set pattern. The pollen on an incompatible nucellus, as shown by *Pinus nigra* × *Pinus resinosa*, may show 1) no germination; 2) germination with slow ineffective pollen tube growth; and 3) normal germination with vigorous pollen tube growth. The two first mentioned conditions lead to early and the third to delayed degeneration of ovules.

HYUN and YIM (1963 a, b) studied fertilization in ovules cross-pollinated on a mass scale in: *Pinus rigida* × *Pinus taeda; Pinus rigida* × *Pinus radiata; Pinus rigida* × *Pinus elliottii* every year since 1955. These combinations gave 40 per cent, 32 per cent and 11 per cent fertile hybrid seeds respectively. HYUN and LEE (1964) added more information on fertilization in two of these combinations, *viz. Pinus rigida* × *Pinus taeda* and *Pinus rigida* × *Pinus radiata*.

In *Pinus rigida* × *Pinus taeda* pollen tubes completely penetrated and reached the archegonium and mature embryos were observed in the seeds. The growth rate of the nucellus was rapid. Although the early pollen tube growth was normal, in the upper part of the nucellus on the 3rd of June, the pollen tubes ceased to grow in some ovules after 10 to 16 days and only

50 per cent of the observed ovules were fertilized. The egg nuclei of the unfertilized ovules developed normally. The pollen tube growth fertilization and embryo development of this combination was slower than and inferior to that seen in open pollinated *Pinus rigida* ovules (HYUN and YIM, 1963 a; HYUN and LEE, 1964).

In *Pinus rigida* × *Pinus radiata* normal pollen tube growth was observed only in a few ovules (HYUN and LEE, 1964). The nucellus growth was inferior and the ovule shrivelled presumably due to failure of fertilization. Mature embryo formation was not detected by HYUN and YIM (1963) due to partial sterility. HYUN and LEE, however, observed fertilization in three out of 40 ovules, but the development of the zygote and embryo when formed was abnormal and slow.

In *Pinus rigida* × *Pinus elliottii* the pollen tube was observed to penetrate the nucellus; it reached the archegonium and a mature embryo was observed, but further details were not worked out.

From a chemical study of the nucellus and ovule extracts of *Pinus* species (MCWILLIAM, 1958, 1959) and from embryological studies discussed above, it is concluded that incompatibility results from differences in chemical constitution of the tissues of the ovules of different species which affect pollen tube growth during fertilization. These differences also influence the ability of the pollen tube to provide stimulus to the development of the female gametophyte and perhaps to the nucellus, as shown by different rates of growth of nucellus observed in different cross combinations of *Pinus* (HYUN and YIM, 1963 a). Physiological behaviour of the neck of the archegonium seen in *Abies pindrow* (DOGRA, 1966 a) or other embryological phenomena (see p. 70) may promote fertilization within a species only, by affecting pollen tube growth of alien species. The existence of such barriers has neither been demonstrated nor yet sufficiently investigated in any conifer species. During pollen germination the stage at which the breakdown of the prothallus and the nucellus occurs is different in different species combinations and this may indicate the degree of crossability of the different species. The failure of many crosses to produce seed could be attributed largely to genetic differences of a chemical nature (SAX, 1960) which inhibit gametophytic development, *i.e.* of pollen, pollen tube, prothallus and archegonia and as discussed below it can also occur due to embryo mortality.

WRIGHT (1959) reported failure of crosses of *Pinus peuce* with *Pinus koraiensis* and *Pinus cembra*. Proembryo mortality was determined to be the cause of this failure from studies of pollen germination fertilization and early development of the embryo in controlled pollination of *Pinus peuce* Griseb. with the pollen of *Pinus cembra* and *Pinus koraiensis* (HAGMAN and MIKKOLA, 1963).

Early stages of pollen tube growth of *Pinus cembra* in the nucellus of *Pinus peuce* were normal but later, growth slowed or stopped and the archegonia of a small number of the ovules in which this occurred, remained unfertilized. In most ovules at least one archegonium showed normal fertilization and early proembryo development. The proembryos, however, did not grow out of the archegonia and degeneration occurred at four to eight-nucleate stages.

In most fertilized archegonia four free nucleated proembryos were observed at the base where the development stopped or slackened. In subsequent stages degenerated remains of dense cytoplasm were recorded at the base of one archegonium, two abnormal eight free nucleated proembryos were observed in which wall formation did not occur. Sections of such ovules and prothalli after proembryo degeneration were similar in general appearance to radiographs of class I seeds, *i.e.* the endosperm cavity though formed remained empty (HAGMAN and MIKKOLA, 1963, Fig. 9, p. 76). In further development although the seed coat developed normally the prothallial development stopped early and the endosperm did not fill the seed cavity completely.

In *Pinus peuce* × *Pinus koraiensis* the pollen tube of *Pinus koraiensis* grew vigorously through the nucellus of *Pinus peuce* and fertilized at least one archegonium (in most three and in some four archegonia) per ovule. Normal proembryos developed at least up to the four-nucleate stage. One abnormal proembryo with six instead of four nuclei was observed. The exact stage of proembryo degeneration was not determined, but no case of an embryo growing out of the archegonium was recorded. All embryos were destroyed at an early stage and endosperms with embryo-less cavities developed as in the case of *Pinus peuce* × *Pinus cembra.*

Thus the pollen tubes of *Pinus koraiensis* grew effectively through the nucellus of *Pinus peuce* while those of *Pinus cembra* did not grow as effectively but were able to fertilize the archegonia of *Pinus peuce.* The hybrid zygote nucleus underwent normal mitotic divisions and the proembryonal nuclei developed but a cellular proembryo did not organize. Proembryo disturbances, whenever they occurred in these crosses, set in after the third mitotic division. They were similar to some of the disturbances observed by me (all of which are not recorded here) in small frequencies in natural populations of some species of *Pinus, Cedrus deodara, Abies pindrow, Picea smithiana* and in naturally self-pollinated pine trees growing in North-western Himalayas (see Figs. 24—31, 33—39). Embryo mortality did not, however, lead to empty seed formation; the endosperm developed almost to a normal size but the endosperm cavity remained empty as shown by radiographs of class I seeds.

Discussion on Disturbances in Conifer Embryogeny and Seed Sterility

The best seeds are secured by selection from nature or by growing them by controlled pollination and in seed orchards (see SYRACH LARSEN, 1937, 1956; GUSTAFSSON, 1949). That the knowledge of conifer embryology can be useful in these methods is shown by its role in studies of seed quality and sterility both in nature and in tree breeding.

The literature on gymnosperm embryology is voluminous; brief descriptions are, however, given by STRASBURGER (1872, 1879), COULTER and CHAMBERLAIN (1917), CHAMBERLAIN, 1935; JOHANSON (1950) and WARDLAW (1955). That these accounts, though fairly complete, need changes, is shown by recent studies carried out by DOYLE (1954, 1957, 1963). Conifer embryology is a complicated phenomenon and a clear picture of embryo development has been understood only recently (DOYLE, 1918—1963 *et seq.;* BUCHHOLZ, 1918—1950; DOGRA, 1961, 1966 b, present investigation; CHOWDHURY, 1962). Without entering into the details of the disputed issues raised and solved by various workers this discussion shows that in academic studies very little attention was paid to embryological details required in seed analysis and tree breeding (see SARVAS, 1962). Early attempts where embryological studies were used for bulk seed testing were made by several Scandinavian workers (*e.g.* KUJALA, 1927; WIBECK, 1928 b, 1929 b) and were followed up with X-ray radiography by SIMAK and GUSTAFSSON (1953 a, b, 1954, and co-workers) and with microtechnique by HÅKANSSON (1956, 1959, 1960) and SARVAS (1962).

The application of embryology with X-ray radiography in breeding and seed testing problems is wide and unexplored in both forestry (see EHRENBERG *et al.*, 1955; GUSTAFSSON and SIMAK, 1958 a) and agriculture (SWAMINATHAN and KAMRA, 1961; KAMRA, 1964 a, b). It must, however, be kept in mind that gymnosperm embryology differs fundamentally from that of angiosperms (see MAHESHWARI, 1950, 1963; *cf.* SCHNARF, 1933; JOHANSON, 1950; WARDLAW, 1955) and a separate understanding and treatment of both is necessary in application of embryological knowledge. X-ray radiography is the only method which can give adequate numerical data for embryological studies with a minimum of labour and time and without damage to the ovule or seed. X-ray radiography and embryological studies can thus be combined for analysis of valuable seed material in selection, inbreeding, and hybridization experiments, a possibility first mentioned by

SIMAK and GUSTAFSSON (1953 b). A scheme of embryo development is given for use in the breeding and seed analysis which in detail is open to further additions and modifications. The embryological disturbances found in normal embryo development which affect seed quality and sterility are discussed below.

Formation of abnormal prothalli in ovules influences embryo and seed development. Early development of the prothalli in *Abies pindrow* (DOGRA, 1966 a) was normal but fusions between free nuclei occurred during free nuclear or wall formation stages. Cell arrangement and development in these prothalli was abnormal due to formation of polyploid nuclei as reported in *Ephedra intermedia* (MEHRA, 1950). Such prothalli occurred in some species of *Pinus*, *Picea smithiana*, *Cedrus deodara* and in other conifer species in varying frequency in different trees and species. In *Abies pindrow* some prothalli showed polyploid archegonial initials; archegonia with polyploid or fused eggs; and archegonia laterally located or randomly scattered (DOGRA, 1966 a). Abnormalities of prothalli have been reported in several conifers in literature on gymnosperm embryology (*e.g.* FERGUSON, 1904; BUCHHOLZ, 1918; SCHNARF, 1933). More than one gametophyte in an ovule was sometimes observed in *Cedrus deodara*, *Picea smithiana*, *Picea abies*, *Abies pindrow*, *Pinus wallichiana*, *Pinus roxburghii*, *Pinus patula*, *Pinus nigra*, *Pinus silvestris* and in several species of Cupressaceae and Taxodiaceae (personal observation). In *Pinus silvestris* double prothalli in an ovule were reported in one per cent of ovules studied from some trees from northern Finland but such ovules were more abundant in two trees. Damage to the prothalli is sometimes caused by climate (SARVAS, 1962). Abnormal prothalli were usually observed to degenerate at different stages in some Indian conifers but in northern Sweden they frequently formed inferior seed.

Failure of fertilization due to insufficient pollen tube growth is sometimes observed in nature but it is more common in interspecific crosses of *Pinus* species (see pp. 75—77). In self-pollination, pollen germination or pollen tube growth does not stop self-fertilization but a high rate of embryo mortality causes seed sterility in *Pseudotsuga menziesii*, *Pinus peuce* and *Picea glauca* (ORR-EWING, 1954, 1957 b; HAGMAN and MIKKOLA, 1963; MERGEN *et al.*, 1965).

Considerable sterility seen in *Sequoia sempervirens* (LOOBY and DOYLE, 1937; BUCHHOLZ and KAISER, 1940; DOYLE, 1945) is probably due to the polyploid nature of this species (KHOSHOO, 1959). Ovular degeneration, also observed with X-ray radiography (HANSEN and MUELDER, 1963), was conspicuous in this species only at and after fertilization (LOOBY and DOYLE, 1937). In Pinaceae, once fertilization took place in a species, mitotic disturbances in the first two divisions of the proembryo were not common.

Disturbances in the proembryo development, when they occurred, started at the four-nuclear stage in nature (Figs. 5, 6, 24, 25) and in controlled interspecific crosses of some *Pinus* species (HAGMAN and MIKKOLA, 1963, Figs. 5, 8). The four nuclei sometimes failed to shift to the archegonial base or did not develop further. The proembryos in these cases formed or degenerated at the base, in the centre of the egg or on a lateral side of the archegonium (Figs. 24, 25). Relict nuclei were extruded from some of the four-nucleated proembryos in *Pinus* as already reported in *Cunninghamia lanceolata* (DOGRA, 1966 b) and some proembryos after the third division contained less than eight nuclei. Some of the four free proembryonal nuclei missed the third mitosis and proembryos with six or seven free nuclei were also formed in this way (Figs. 33, 34). These proembryos sometimes showed abnormal development before degeneration. Disturbances in some of the mitotic configurations were seen in some proembryos of Pinaceae as shown best for *Cupressus arizonica* (Cupressaceae, Fig. 42) and dumbbell-shaped proembryonal nuclei were thus formed either due to sticky mitosis or by nuclear fragmentation (*Abies pindrow*, Fig. 37). Proembryos showing fragmentation of the nuclei failed to develop further.

Proembryos with 16 free nuclei were seen in some cases (*Pinus nigra*, Fig. 43). Relict nuclei were sometimes extruded and pU and pE with variable number of nuclei in each were formed (*Pinus gerardiana*, Fig. 38). Wall formation, however, was not regular and these embryos degenerated at different stages, pU and pE in eight-nucleated proembryos sometimes had not four but a variable number of cells in each tier (*Pinus gerardiana*, Fig. 39), or formation of tiers did not take place. Wall formation and development in such proembryos were irregular. In some primary proembryos the pE cells developed or degenerated independently (*Pinus nigra*, Fig. 54).

Conspicuously large and small sized proembryos and nuclei (more than double or less than half the normal) may indicate the presence of polyploid proembryos (*Picea smithiana*, Figs. 27, 53) or haploid proembryos (*Pinus gerardiana*, Fig. 26). But differences in nuclear and proembryo sizes can arise due to other causes and confirmation of the presence of haploid and polyploid proembryos must come from chromosome counts of such proembryos. The haploid chromosome number (twelve) was counted by MEHRA and DOGRA in parthenogenetic divisions of the egg nucleus in naturally pollinated trees of *Pinus wallichiana* and *Pinus nigra* var. *austriaca* (DOGRA, 1966 a). ILLIES (1964) has also reported ten haploid (n = 12) seedlings obtained after open and controlled pollination of trees of *Picea abies*. Many of these trees, 41.9 per cent (ILLIES, 1953) were known to produce abnormal seedlings (ILLIES, 1952, 1953, 1959). Five of the ten haploids originated from seeds which had twins, and the others from inverted seedlings.

Secondary proembryo abnormalities were not common, but five-tiered instead of normal four-tiered proembryos or cases where E_1 degenerated were observed in *Pinus wallichiana* and *Cedrus deodara* (Fig. 52).

Variants of the typical proembryo which degenerate are recorded by LOOBY and DOYLE (1937, 1940) in *Sequoiadendron gigantea*, *Sequoia sempervirens*, *Callitris rhomboidea* and by me in some other members of Taxodiaceae and Cupressaceae (DOGRA, 1961, 1966 a).

Late embryogeny: The suspensor-system shows inhibition of E_1, E_2 ... etc. and E_t elongation probably due to effects of climate of northern Sweden (see p. 60) or of North-western Himalayas (see p. 20). Irregularities of the suspensor-system, in which E_2, E_3 etc. did not elongate, were induced in embryos of *Picea smithiana* (Figs. 40, 41). Variation in the suspensor-system is shown to occur within a species in *Pinus banksiana* (BUCHHOLZ, 1918, Fig. 2), in North-Swedish *Pinus silvestris* and *Picea abies*, and in *Pinus montezumae*, *Pinus nigra*, and *Pinus patula* introduced in Himachal Pradesh, India (see p. 20). Inhibition of elongation, degeneration of segments (E_1, E_2 etc.) or of an embryo can also occur due to physiological dominance of adjacent embryos from different archegonia (Figs. 47, 56). Formation of point embryos (IIP) near the micropyle of the seed could be due partly to these types of incomplete E_1, E_2 etc. elongation.

The suspensor-system is related to mode of unitary cleavage and any change in its structure can affect cleavage. This is *partly* responsible for the incomplete expression or abnormal mode of occurrence of cleavage seen in *Pinus silvestris*, *Pinus montezumae*, *Pinus nigra*, and *Pinus patula* (see pp. 20, 61).

The most common irregularities in late embryogeny concern the suspensor-system, cleavage, and non-cleavage in a species.

Young embryos showing reversed growth in ovules are sometimes observed in *Abies pindrow* (Figs. 48, 49), *Pinus silvestris* (Fig. 78), *Pinus banksiana*, *Picea smithiana*, *Cedrus deodara*, *Larix sibirica* and *Thuja orientalis* and in some other species of Cupressaceae and Taxodiaceae (LAND, 1909; BUCHHOLZ, 1918; HÅKANSSON, 1959, 1960, personal observation). In species growing in Himalayas these embryos mostly degenerated in young stages; they were rare in seeds as is stated by BUCHHOLZ (1918) for *Pinus banksiana*. Formation of *seeds* with reversed embryos (cotyledons orientated towards micropyle) has been more commonly observed in North-Swedish conifers. Several embryo-endosperm abnormalities discussed on pages 50, 63, 64 are embryological disturbances of these types.

Conspicuous proliferation of the embryo or its parts though not common in Pinaceae, is a dominant feature in some members of conifer families *e.g.* in Taxodiaceae, Cupressaceae (DOGRA, 1961, 1966 b) and Podocarpaceae

(DOYLE, 1954). Intensive proliferation of the dominant embryo, whenever it occurred in some members of Cupressaceae and Taxodiaceae, often led to embryo degeneration and seed sterility.

Failure of dominance or of enzymatic activity of the terminal embryo; growth inhibiting influences of adjacent embryos from different archegonia on each other in the polyembryonic ovule; mechanical hindrances, shape of the corrosion region and of surrounding storage tissue in the prothallus; changes in gene-controlled physiological processes; and modifications induced by external environment of the ovule; all contribute to disturbances in embryogeny. These disturbances can lead to embryo degeneration, but under favourable conditions the developed embryos but not proembryos (or young embryos) mostly overcome their deformations during further growth in the ovule or in seed-germination. Under general conditions the frequent occurrence of abnormal embryos or seedlings in the seed stage is not very common. In northern Scandinavia many embryological disturbances persist and give rise to inferior seed.

Disturbances of proembryo development were less common when compared with those of late embryogeny. In Indian conifers the occurrence of a particular abnormal proembryo stage in a species was by no means common but the frequency of all types of proembryo abnormalities, which differed according to trees, species and localities, varied approximately between two and fifteen per cent of proembryos studied for a species (studies on the species growing in the upper tree limit in the Himalayas were not included). Embryo disturbances and mortality were highest in North-western Himalayas in isolated, naturally self-pollinated and introduced trees of *Pinus nigra*, *Pinus montezumae*, and of some species belonging to Cupressaceae and Taxodiaceae (personal observation). Embryos in these, and in some cases in trees from natural populations, degenerated during stages similar to the ones described in controlled self-pollination and in interspecific hybridization experiments. Information available on embryo degeneration in controlled breeding experiments of different conifer species is as yet meagre. Embryo disturbances and mortality in conifers growing near tree limit altitudes, latitudes, and in unfavourable climates still remain to be investigated. LOOBY and DOYLE (1937) comment that "A percentage of abnormal young proembryos is probably more widespread in the conifers than can be judged from published accounts". My study confirms this statement. The same can, perhaps, be said for the occurrence of abnormal prothalli in the conifers (see p. 79). Further conclusions are, however, kept pending until more data are available. Attention is drawn to a need of numerical data in embryological studies, but such requirements, as pointed out by SARVAS (1962), can be fulfilled by extensive microscopic work. The

task would perhaps be made easier with X-ray radiography if it could be developed to study, perhaps with contrast methods, the internal structure of fresh ovules and seeds. For the present it is concluded that early embryo mortality (proembryo and young stages of late embryogeny 0—IIP) plays a more significant role in seed sterility in conifers than is known at present both in nature and in controlled breeding experiments.

Multiple archegonia and polyembryony in conifer ovules appear to lessen seed sterility caused by frequent embryo degeneration. Fertilization of two or more archegonia is common in gymnosperms. In members of Pinaceae in a single ovule I have counted from one to five pollen grains germinating on the nucellus and from one to three rarely four proembryos developing in different archegonia *e.g.* in several *Pinus* species, *Cedrus deodara*, *Picea smithiana* and *Abies pindrow*. As many as six pollen tubes in the nucellus, nine archegonia and a maximum number of four embryos developing from different archegonia are reported to occur in a prothallus of *Pinus* (Ferguson, 1904; Buchholz, 1918). The amount of pollen available to the ovule and to the archegonia from the nucellus also determines the number of archegonial embryos (Sarvas, 1962). Members of some other conifer families (*e.g.* Taxodiaceae and Cupressaceae) which show a higher number of archegonia have a higher number of archegonial embryos in an ovule (see Buchholz, 1929; Dogra, 1966 b) than shown in Pinaceae. The prothallus in Podocarpaceae, according to Doyle (1954), shows a tendency in increase in archegonial number, which in this family is most strikingly shown in *Eupodocarpus* and *Nageia* and also, though less markedly in *Michroachrys* and *Pherosphaera*. The increase in archegonial number ranges from one to three in the primitive and from six to many in the comparatively advanced taxa of the family. This tendency, though as yet not fully investigated, appears to be present in other conifer families too. The tendency towards an increase in archegonial number may be advantageous to a conifer species because, as is also stated by Sarvas (1962), the presence of several archegonia per ovule helps to reduce the empty seed percentage.

Archegonial embryos multiply considerably in species showing cleavage. According to Doyle (1957) cleavage polyembryony is another major trend in conifer embryogeny, a rare derivative phenomenon in angiosperms but a widespread one in conifers, characteristic only of more advanced and specialized forms in a family. It is seen that embryos or embryo units developing from different archegonia may differ from each other in physiological characteristics (Fig. 30) and in their ability to survive in an ovule subjected to unfavourable climate. During proembryogeny or in first stages of late embryogeny (Figs. 14, 15, 20, 21) degenerating embryos are sometimes replaced by embryos from different archegonia in a prothallus (*e.g.* Fig. 30).

Genetical differences between monozygotic embryos from cleavage are absent. Between archegonial embryos they can arise due to 1) fertilization of different archegonia of a prothallus by genetically different pollen; 2) presence or absence of heterozygosity for deleterious and other genes and 3) due to differences in chromosome number. Twins in polyembryonic seeds with different chromosome number: diploid and tetraploid in *Abies firma* (KANEWAZA, 1949), haploid, triploid, and heteroploid in *Picea abies* (ILLIES, 1952, 1964) and diploid and tetraploid in *Pinus thunbergii* (NISHIMURA, 1960), are probably cases of archegonial polyembryony. The triplets each having 2n = 24 chromosome number recorded from a seed of *Pinus thunbergii* (NISHIMURA, 1960) could be archegonial embryos or isogenic products of monozygotic cleavage. The occurrence of mutations, hidden in nature in a heterozygous state, which act as lethals and semilethals when homozygous in seedlings of conifer species is well-known (see GUSTAFSSON, 1962). They appear to occur frequently in some populations of *Pinus silvestris* (EICHE, 1955). Lethal and semilethal genes influencing embryo development can be as abundant as those shown for chlorophyll apparatus in *Pinus silvestris* by EICHE (GUSTAFSSON and SIMAK, 1958). That pre-germination selection against embryos homozygous for deleterious mutations may be possible is indicated by FOWLER (1955) in his experiments on self-pollination of a tree of *Pinus resinosa* containing a "genetic marker" (see p. 73). For the same reason in an earlier communication I remarked that in normal conditions the chances of survival of haploid embryos if formed are remote (DOGRA, 1966 a) except perhaps in monoarchegoniate prothalli. ILLIES (1964) has reported ten haploid (n = 12) abnormal seedlings. These were, however, obtained from trees known to produce seeds with persistent polyembryony and abnormal seedlings (ILLIES, 1952, 1953, 1959). Moreover, she found seedling abnormalities to occur in rather many trees, *i.e.* in 41.9 per cent (ILLIES, 1953). My investigations have shown that in persistent polyembryony, elimination of all the supernumerary embryos may not take place (see p. 61—63). The chances of survival of abnormal embryos (which include polyploids, haploids or embryos with recessive deleterious or other genes) if present along with normal diploid ones, may be greater in a persistently polyembryonic seed, than in a monoembryonic seed formed from a young normally polyembryonic ovule. Without touching upon the disputed issue of the possibility of pre-germination selection taking place in a polyembryonic ovule, it is concluded that seed sterility caused by embryo mortality (which may arise either from genetical or environmental influence or both), and classified as diplontic sterility by MÜNTZING (1930), is lessened in conifers by archegonial polyembryony.

The developing zygote and embryo in a plant is a complex *reaction system*

(WARDLAW, 1954, 1955, 1963). The pattern of development is controlled within certain limits by the genotype and cytoplasm of the egg. The egg cytoplasm and the nucleus in gymnosperms contains several organelles whose functions are as yet incompletely understood (FERGUSON, 1901, 1904; BRYAN and EVANS, 1956, MEHRA and DOGRA, unpublished). The prothallus structure and embryo development are morphogenetically interacting and their physiological relationships are as yet far from clear. In embryo development effects of genotype, egg cytoplasm, internal environment within the prothallus in which the embryo grows and the external environment of the ovule become apparent in the seed when it is formed.

The embryological disturbances of pine and spruce growing in northern Sweden show that climatic injury sometimes upsets the balance between different but interdependent tissues in the ovule (*e.g.* embryo and endosperm). This balance is necessary for normal growth, survival and maturity of the ovule. Damage to the prothalli, endosperms and embryos by sub-arctic climate is seen in *Pinus silvestris* and *Picea abies* growing in northern Scandinavia (SIMAK and GUSTAFSSON, 1954; SARVAS, 1962) and by drought conditions in *Cedrus deodara* and *Abies pindrow* in the North-western Himalayas, India, by me. Sharp changes in climate cause embryo death or abnormal endosperm-embryo development. Survival of abnormally developing embryos depends on the severity of the shock, stage of development at which it occurs (early or late) and genetical resistance of a species, a tree or of the provenance of the species to such shocks.

Climatic effects vary in degree and are shown by the number of ovules affected in different trees and species. In general they act less on the proembryo and more on late embryogeny. Climatic effects on proembryo and early embryo stages are fatal and give rise to abundant 0 class of seeds. Late embryogeny is conspicuously affected in pine and spruce trees of northern Sweden (see pp. 60—65). In a great number of ovules, however, the general plan of the embryo development remains either typical for that species or the embryos degenerate resulting in 0 class of seeds. In the surviving embryos the primary unitary structure (consisting of four embryo units) of the embryo, elongation of E_1, general expression of cleavage in pine and non-cleavage in spruce remain mostly unaffected. Embryological disturbances when they occur can be correctly interpreted in terms of broad phases of embryological development and not in numerical frequencies of particular type of abnormalities, which in number may not be significant. They may also differ in different ovules or collectively in ovules of particular species or in trees or provenances of a species. Such difficulties in seed analysis are also bound to arise in studies of seed-sterility (class 0, I, IIP) in tree breeding. It is for this reason that the general expression *embryological distur-*

bance is used for all embryological abnormalities throughout this discussion. In this way they can also be viewed from the broader perspective of an evolutionary phenomenon observed to be of widespread occurrence in living gymnosperms.

In natural populations embryological disturbances and embryo degeneration in conifers are mostly phenotypic expressions of different modifications, but they may also be due to different genotypes. Modifications, however, seem to be more common and they appear to conceal the disturbances of embryogeny caused by genotypes which are comparatively rare and sometimes apparently similar, especially in late embryogeny. On basis of an embryological study it is difficult to distinguish between the two. SIMAK and GUSTAFSSON have shown the only way to do so by studying the affected seeds by X-ray radiography of different trees and by comparing it with seeds borne by their grafts in a standard environment. Embryological study, vegetative methods of propagation, standard or preferably a phytotron controlled environment and X-ray radiography can thus be collectively used to throw more light on seed problems.

Summary

A scheme of embryo development in Pinaceae is given for the use in tree breeding research. The embryo terminology used is applicable to conifers in general. Disturbances in proembryo and in late embryogeny are described in Indian and Swedish conifer species.

The embryological analysis of the five (0—IV) seed classes shows that X-ray radiography is valuable in bulk seed testing but in analysis of seed formation microscopic studies are essential. However, X-ray radiography is the only method which can provide adequate numerical data with a minimum requirement of time and labour. The advantages of both techniques can be exploited by combining embryology and X-ray radiography in seed testing and tree breeding.

Conifer embryology can play an important role in determination of seed quality and sterility in natural populations and in tree breeding. In tree breeding it can also be used in determining incompatibility between species. The prothallus and embryo are physiologically interdependent and closely related, and abnormal prothalli can affect embryo development, and vice versa. Variants of the typical proembryo degenerate but those of late embryogeny may survive. Embryo mortality leads to an increase in empty seeds. Embryological disturbance appears to be a general phenomenon found in gymnosperms.

Disturbed embryology is often a climatic effect and varies considerably in

different localities, trees and species. As a rule embryo mortality is higher in early embryo stages. Development in late embryogeny is, however, more open to influences of climate but general characters of the embryogeny of the species remain unaffected in most surviving and developing embryos. Affected embryos either recover or degenerate during late development or during germination.

Abnormal embryogeny is common in self-pollinated and introduced isolated conifer species. It is also encountered in tree breeding experiments. In nature some embryonal disturbances appear to be similar to those occurring in tree breeding experiments and seem to be genotypically determined.

Embryological disturbances can thus be due to environmental causes (modifications) or may arise because of genotypical differences of trees in nature. It is difficult to distinguish between the two since modifications in embryogeny are common and often mask the comparatively rare genotypically induced disturbances. The only way to distinguish between the two is by studying them in controlled or standard environment with help of vegetative methods of propagation.

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Sammanfattning

Frösterilitet och störd embryoutveckling hos barrträden med speciell hänsyn till frökontroll och växtförädling hos Pinaceae

En generell översikt av embryoutvecklingen hos Pinaceae har utarbetats för användning inom den skogliga växtförädlingen. Den använda terminologien har i görligaste mån anpassats för samtliga barrträd. De störningar som uppträder i tidiga och sena embryostadier hos olika barrträdsarter från Indien och Sverige har underkastats en jämförande analys.

Med hjälp av radiografisk metodik (röntgenfotografering) urskiljes hos de svenska barrträden generellt fem fröklasser (0—IV). Den embryologiska detaljgranskningen visar att en dylik radiografisk analys otvivelaktigt är värdefull. Radiografin är den enda hittills utarbetade metodik som med ett minimum av tid och arbete och utan skador på materialet ger fullt tillförlitliga numeriska data rörande fröutvecklingen. Den bör emellertid kompletteras med mikroskopiska studier. Föreliggande avhandling vill visa att båda metoderna, dvs. såväl embryologiska undersökningar som avancerad radiografi, med fördel kan kombineras och ge resultat av värde i skogsträdens frökontroll och växtförädling.

Embryologisk analys ger viktiga upplysningar då det gäller att kartlägga fröets kvalitet och sterilitet i naturpopulationerna. Den klarlägger dessutom bakgrunden till korsningsförmåga och korsningssterilitet vid hybridisering inom och mellan arter. Mellan protallium och embryo sker en ömsesidig påverkan under hela fröutvecklingen. Bildningen av abnorma protallier influerar på embryonernas utveckling, och vice versa. Avvikelser i den tidiga embryoutvecklingen (proembryostadiet) leder ofta till degeneration. I senare embryostadier är de i betydligt mindre utsträckning letaliserande. Embryodödlighet leder till att tomfröhalten stiger.

Störningar i embryoutvecklingen är inte ovanliga hos gymnospermer. Ofta har de en klimatisk bakgrund och varierar därför starkt under olika klimatförhållanden. En viss genetiskt betingad inverkan föreligger likväl också, både hos olika biotyper inom en art och hos olika arter. Som nyss framhävts är embryodödligheten särskilt markerad vid tidiga embryostadier. Även sena stadier påverkas visserligen av ogynnsamma klimatbetingelser, men de karakteristiska embryologiska kännetecknen hos olika arter förblir i allmänhet oförändrade.

Självbefruktning, vare sig artificiell eller spontan, leder ofta till avvikelser i embryoutvecklingen. Detta gäller även vid förflyttning av främmande barrträdsarter (»exoter») till nya miljöer. Embryologiska störningar, som iakttas hos naturpopulationer, liknar ofta dem som uppträder vid artificiell hybridisering (inklusive artificiell självbefruktning).

Embryologiska störningar uppstår således både genom tillfälliga yttre miljöinflytelser (»modifikationer») och skillnader i den genetiska konstitutionen hos enskilda biotyper och arter (respektive hybrider). Det är ofta svårt att särskilja de två typerna av störningar, eftersom »modifikationer» är vanliga och ofta maskerar genetiskt betingade störningar i embryoutvecklingen. Den enda fullt säkra metoden att skilja på de två störningstyperna är att odla genetiskt enhetligt material av enskilda biotyper (t. ex. klonmaterial) i kontrollerad miljö.

ERRATA

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Page 6, par. 1, line 4: for MEHRA (1960) read MEHRA (1962)
Pages 8, 14, 16, 61, 78, 92: for JOHANSON (1950) read JOHANSEN (1950)
Page 12, par. 5, line 4: for sereis read series
Page 13, par. 1, line 5: for 1920 read 1920 a.
Page 15, par. 2, line 14: for 1947 b read 1957b
Page 16, par. 2, line 8: for 1963 read 1962
Page 18, par. 6, line 2, seventh word: for pE read pU
Page 43, figures on right: Upper, 85; lower, 87.
Page 49, par. 3, line 4: for (Fig. 95) read (*e. g.*, Class II, Fig. 95)
Page 49, par. 4, line 12: page 53, line 1: for persistant read persistent.
Page 58, par. 3, line 4, seventh word: for 0_2 read 0_0.
Page 59, par. 4, lines 1, 2: for GUSTAFSSON and SIMAK read SIMAK and GUSTAFSSON
Page 62, par. 1, line 19: for 1904 read 1940
Page 65, par. 2, line 4: for 1958 read 1956
Page 69, par. 1, line 7: for BIHGHAM read BINGHAM
Page 69, par. 1, line 8: for KRAUSS and SQUILLACE 1963 read KRAUS and SQUILLACE 1964
Page 69, par. 1, line 14: for JOHANSON read JOHNSON
Page 71, par. 2, line 21: for 1957 read 1957b
Page 79, par. 4, line 2: for 1945 read 1945b
Page 81, par. 6, line 4: for 1909 read 1902
Page 83, par. 2, line 18: for *Michroachrys* read *Microcachrys*
Page 84, par. 1, line 18: for 1958 read 1958 a
Page 89, line 13: for 1956 read 1965.
Page 91, line 3: for EKELUNDH read EKLUNDH
Page 92, line 23: for JENSON read JENSEN
Page 92, line 43: for G. M. Johri read B. M. Johri
Page 92, line 47: for KIHLDAHL read KILDAHL
Page 96, line 24: 1962 read 1963.