

Development of the ovulate strobilus in *Pinus kesiya* Royle ex Gordon (syn. *P. khasya* Royle) in relation to controlled pollination in Zambia¹⁾

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(Received for publication December 27, 1967)

Introduction

In Zambia the major exotic pine species used for commercial plantations is *Pinus kesiya* ROYLE ex GORDON (syn. *P. khasya* ROYLE) and an active tree breeding programme has been in progress for several years (COOLING, 1967). This programme has now reached the stage where many controlled pollinations will be required and the present study was undertaken to facilitate such work.

There have been several descriptions of the initiation and early development of pine strobili, e.g. *P. elliotii* (MERGEN and KOERTING, 1957), *P. ponderosa* (GIFFORD and MIROV, 1960), *P. densiflora* and *P. thunbergii* (Goo, 1961). Similarly development after pollination has been described (FERGUSON, 1904; McWILLIAM and MERGEN, 1958). However there have been few published details of the structure of the pine ovulate strobilus near the time of pollination. The classic account for several species given by FERGUSON (1904) has been confirmed and intensified in *P. silvestris* by SARVAS (1962), but there have been no other reports on pine comparable with those on *Larix* species (BARNER and CHRISTIANSEN, 1960) or *Pseudotsuga menziesii* (BARNER and CHRISTIANSEN, 1962).

Such information, together with the results of pollination carried out at various developmental stages, is essential for the determination of the optimum time for efficient, artificial pollination. Controlled pollination studies generally have been based on a recognition of various external, morphological stages (SNOW, DORMAN and SCHOPMEYER, 1943; CUMMING and RIGHTER, 1948; PERRY, 1954; WAKELEY and CAMPBELL, 1954; MERGEN, ROSSOLL and POMEROY, 1955; EHRENBURG and SIMAK, 1957; CAMPBELL and WAKELEY, 1961). In this laboratory a system has been adopted for classifying ovulate strobili into similar arbitrary classes based on macroscopic external features.

Five classes are defined and described specifically for field work. Examples of strobili in these classes are illustrated in Fig. 1 A–E and the corresponding details of expected isolation and pollination procedure are:—

Class 1.—No pollen can enter strobilus; surface should be sterilised and isolation bag attached.

Class 2.—Contamination by unwanted pollen can occur; probably too early for profitable controlled pollination; isolation bags must be in place already or strobilus should be rejected for controlled pollination work.

Class 3.—Artificial pollinations should be undertaken for maximum seed yield. (Illustration is before peak development of typical Class 3 strobili.)

Class 4.—Pollination is still possible but low seed yield would probably make controlled pollination uneconomical; isolation bags must remain in place. (Illustration is early Class 4.)

Class 5.—Pollination no longer possible; isolation bags should be removed.

The object of the research described here was to examine the morphology and anatomy of typical material in each class.

Methods

Ovulate strobili were collected during July and August, 1966, from four-year-old trees of *P. kesiya* (Luzon, Philippines provenance) growing on the Copperbelt of Zambia. Each strobilus was allocated to one of the five classes, fixed in FAA preservative and embedded in paraffin wax. Serial microtome sections were cut at 10–12 μ thickness from three or four in each class. They were stained with safranin and fast green prior to examination under a light microscope.

Results

Class 1.—The strobili were small (approximately 5 mm long) and the ovuliferous scales and bracts were completely concealed by the bud scales (Fig. 1 A, a). The sections showed that the bracts subtending the ovuliferous scales were closely overlapping each other (Fig. 1 a). The ovuliferous scales were merely bud-like protuberances in the axils of the bracts and showed little differentiation other than early vascularisation, although some of the upper scales showed darkly stained areas in the distal regions (Fig. 2 A). Occasionally a scale appeared to have a slight swelling in the region where an ovule would be expected to develop (Fig. 2 a).

Class 2.—Strobili in this class were larger and in side view the ovuliferous scales were still concealed by the subtending bracts (Fig. 1 B). However, the general appearance was less compact than that of strobili in Class 1. The sections confirmed this looser arrangement of the bracts which appeared to have been pushed apart by the enlarging ovuliferous scales (Fig. 1 b). On the upper ovuliferous scales distinct ovular swellings were to be seen and in some of these the integumental layers were distinct (Fig. 2 B). Some micropylar development could be seen but it was not complete (Fig. 2 b).

Class 3.—The strobili were further enlarged and the bracts no longer overlapped in the region with fully developed ovuliferous scales (Fig. 1 C). The sections showed the bracts tending even further towards the horizontal, many with their tips curled downwards (Fig. 1 c). The ovuliferous scales were further enlarged, yet the spaces between them were also larger. This appeared to have been caused by elongation of the strobilus axis (see FERGUSON, 1904) and also by the extension of the pedicels attaching individual scales to the main axis. Cell proliferation was still taking place within the ovular swellings as indicated by numerous mitotic figures.

¹⁾ Paper published with permission of Unesco and the Director, Agricultural Research Council of Central Africa.

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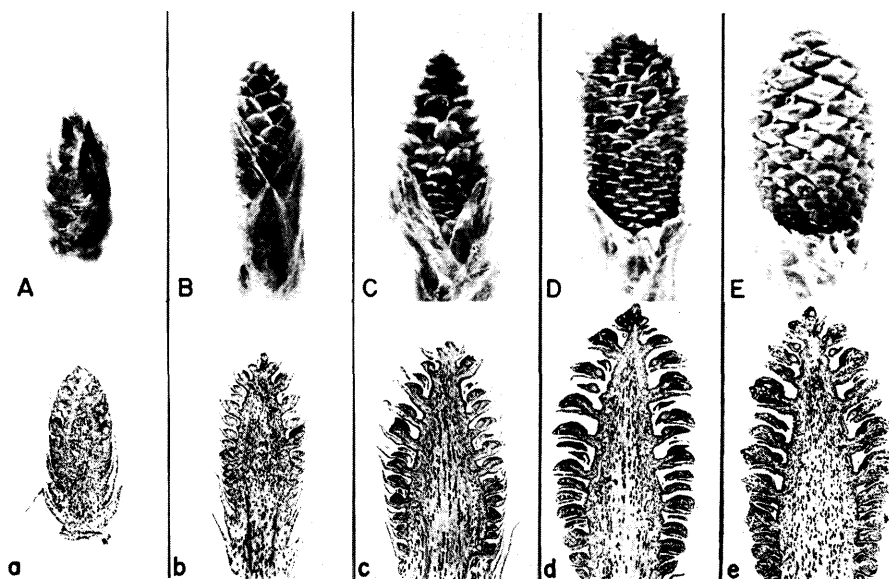


Fig. 1. — Development of *P. kesiya* ovulate strobili. — A—E: External morphology of typical strobili in Classes 1—5. — a—e: Median longitudinal sections of typical strobili in Classes 1—5. (These are not sections of the strobili shown in A—E.)

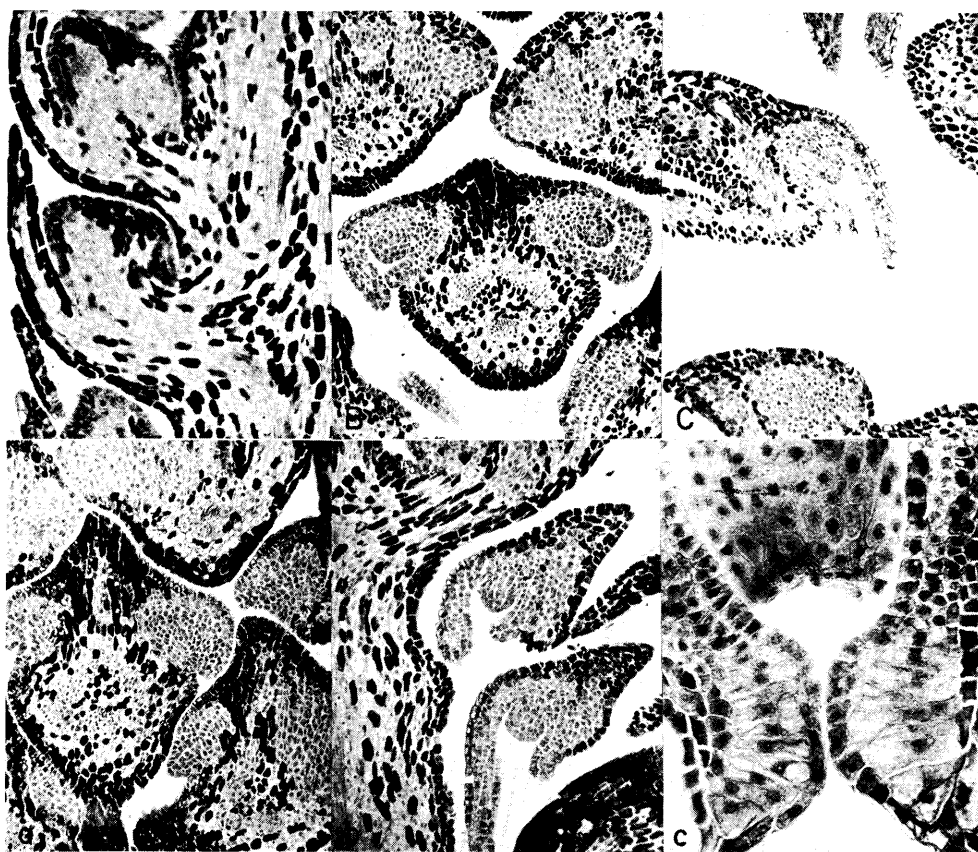


Fig. 2. — Development of ovuliferous scale and micropyle. — A, a: Longitudinal median (A) and tangential (a) sections through strobili in Class 1 showing early vascularisation, dark staining area and swelling at site of ovule (50 \times magnification). — B, b: Tangential (B) and longitudinal median (b) sections through ovuliferous scales of strobili in Class 2 showing early micropyle development (50 \times magnification). — C, c: Longitudinal median sections through micropyles of strobili in Class 3 (50 \times magnification) and Class 5 (300 \times magnification).

At the centre of the mass of cells forming the ovule there was frequently a large, hyaline cell, the megaspore mother cell (FERGUSON, 1904), with a large, unstained nucleus (Fig. 3 A). In the most advanced strobilus examined in this class, many of the megaspore mother cells contained small

vacuoles within the cytoplasm and chromatin material in the nuclei stained heavily (Fig. 3 B).

The structure of the open strobilus would allow pollen to reach the extensions of the ovular integument. As with other pine species, the integumental elongations which



Fig. 3. — Development of the macrospore mother cell (400× magnification). — A: Hyaline cell. — B: Chromatin appearance in nucleus. — C, D: Multinucleate cells.

formed the micropylar tubes projected downwards (Fig. 2 C). In the most advanced strobilus in Class 3 the distal ends of some of the micropylar tubes were seen to consist of three distinct layers of tabular cells. In many cases the micropylar tube broadened at the distal end to form a funnel shaped entrance to the micropylar canal.

Pollen grains were occasionally seen in the sections of the most advanced strobilus but none were seen in any sections of the other strobili examined in this class. Pollen is believed to pass through the micropylar canal by a nocturnal pollination drop mechanism (DOYLE and O'LEARY, 1935; McWILLIAM, 1958) but no evidence for or against this theory was obtained with this material.

Class 4. — These strobili were still larger than those in previous classes (Fig. 1 D). The sections showed the bracts to be almost horizontal and pushed back by the enlarging ovuliferous scales, some of which extended beyond the tips of the bracts (Fig. 1 d). In general there were still large gaps between the scales. Some ovuliferous scales had groups of large, thin walled cells in their distal regions. This enlargement probably leads to the complete closure of inter-scale spaces at the termination of the receptive period.

In the centre of these ovular swellings there were single, large, macrospore mother cells; some of these were still hyaline, while others had densely stained material in their nuclei, as in Class 3 (Fig. 3 A, B). Around each macrospore mother cell was a mass of small cells (the "spongy tissue" of FERGUSON, 1904), many of which were still in stages of mitosis. Towards the micropyle was an area of elongated, thin walled, vacuolate cells, which was frequently seen to

be capped by a mass of darkly stained, apparently disintegrating tissue (Fig. 2 c). This corresponds closely with the apical region of the nucellus at about the time of pollination, as described by SARVAS (1962).

The micropylar tubes were fully developed and still extended downwards at the proximal end of each scale. Many pollen grains were seen between the ovuliferous scales, within the micropylar tubes, and at the nucellar surface.

Class 5. — The inter-scale fissures were closed by swelling of the scales (Fig. 1 E, e), and the micropyle was closed as the middle row of integumental cells elongated radially and divided (Fig. 2 c), (see also FERGUSON, 1904; McWILLIAM, 1958). The arbitrary Class 5 appeared to cover several phases in the developmental process. In the least mature strobilus there was a single, large, central macrospore mother cell within each ovule. Some of these mother cells were hyaline, while others showed a darkly stained chromatin network in the nucleus (see Fig. 3 A and B). In the majority of such cells there were vacuoles in the cytoplasm and many of the small surrounding cells appeared to be dividing. At the micropylar end of the nucellus, elongated, vacuolate cells were capped by a mass of disintegrating cells as in Class 4 (Fig. 2 c). In another strobilus many of the macrospore mother cell nuclei contained distinct and darkly stained chromosomes, and were presumably about to undergo cell division. In many sections could be seen the distinctly concentric arrangement of the cells around the macrospore mother cell (SARVAS, 1962). In the most mature strobilus many of the macrospore mother cells were much enlarged, and possessed pale, vacuolate cytoplasm with several (at least four) large nuclei and no distinct cell walls (Fig. 3 C, D). FERGUSON (1904) reported that often 32 free nuclei were visible.

Discussion and Conclusions

These limited studies have provided some information on the pattern of development of ovulate strobili in *P. kesiya*, particularly on the relationship between external morphology and internal anatomy. The developmental sequence is similar to that described for other pine species.

The present work suggested that we were not accurately recognising in the field the optimum developmental stage for pollination. We now consider that this optimum stage extends from the developmental stage shown in Fig. 1 C to that shown in Fig. 1 D. The least mature strobili that would previously have been classified in Class 3 should now be included in Class 2. Similarly the least mature strobili in Class 4 should be reclassified with those in Class 3. Pollination of strobili more mature than that shown in Fig. 1 D may yield seed (in *P. silvestris* seed yields ranging from 60 to 80% of normal were obtained when the ovuliferous scales were nearly closed — NILSSON, 1964); however, because of the expense involved in controlled pollination, it is desirable to reduce the number of times that pollen is applied to a given strobilus. It is proposed therefore that pollen should be applied only to strobili which are now classified into Class 3. Isolation bags should be left on strobili at all stages of development in the present Class 4 but no controlled pollinations should be carried out after the stage shown in Fig. 1 D. These theoretical proposals must be tested by field trials of controlled pollinations at all developmental stages.

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Variation in Roots of Greenhouse Grown Seedlings of Different Scotch Pine Provenances

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(Received for publication February 10, 1968)

Scotch pine (*Pinus sylvestris* L.) is native over a wide geographic area in Europe and Asia. Its range reaches from above the Arctic Circle in northern Scandinavia to Spain and Turkey in the south and from Scotland in the west to northeastern Siberia near the Pacific Ocean in the east.

In southern and western Europe the range is discontinuous. In other areas it is generally continuous (Figure 1). Scotch pine has also been introduced extensively outside its native range. It is now the most commonly planted species for Christmas tree use in the United States. Many trees are

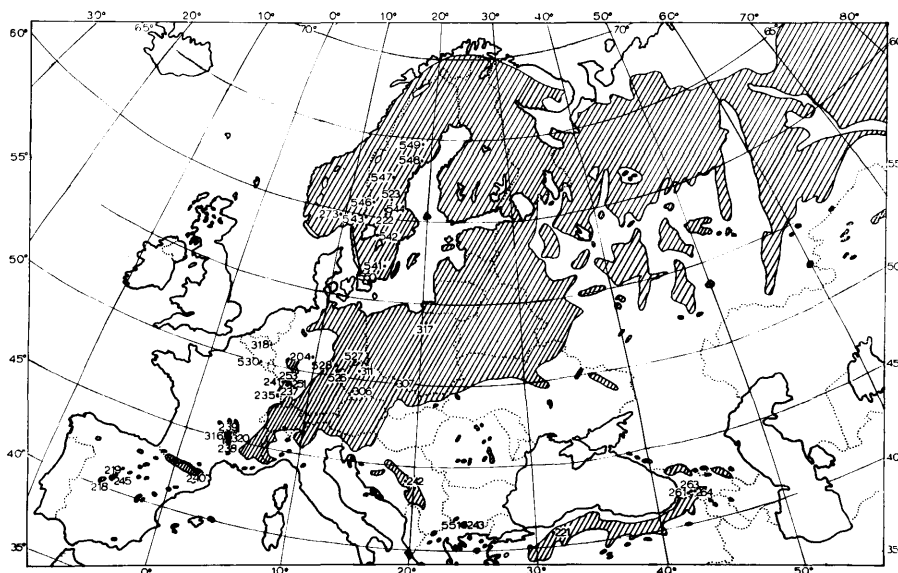


Figure 1. — Natural distribution of Scotch pine (shaded) and provenances included (numbered dots) in this experiment. In addition to those provenances shown, four additional sources from the eastern portion of the range (MSFG 234, 255, 256 and 254) were also used. (Range map from CRITCHFIELD and LITTLE, 1966)

¹⁾ The author is Associate Professor of Silviculture, Division of Forestry, West Virginia University, Morgantown, West Virginia. The paper is based on part of a Ph. D. thesis submitted to Michigan State University, East Lansing, Michigan. The work was supported in part by regional research funds from the U. S. Department of Agriculture under project NC-51, "Tree Improvement through Selection and Breeding" and in part by funds from Hatch 119, "Production of Plantation Grown Christmas Trees in West Virginia".

also planted for reclamation, stabilization and wood production.

Within its natural range Scotch pine shows wide variability. This variation has been the subject of a number of provenance studies (reviewed by WRIGHT and BULL, 1963 and RUBY, 1964). RUBY (1964) recognized 21 geographic varieties of the species.