INITIATION AND DEVELOPMENT OF FLOWER PRIMORDIA IN SLASH PINE

by

Francois Mergen, Yale Forestry Research Center
John A. Hartford Foundation, Forest Biology Program
(Illustrated with Kodachrome slides)

The pollination and fertilization pattern are fully described for several species of the genus Finns, but a systematic study of the time of flower primordia initiation, and a detailed morphological description of the rudimentary male and female flowers has not been made for pines. There has been a number of experiments which attempted to manipulate the flowering mechanism in pines by nutritional and other means. The timing of these treatments could only be guessed at for little precise information was available on the initiation of flower primordia. In applying the injury treatments and the fertilizer applications to the slash pine trees while I was at Lake City, I had little to rely on to decide on the time of application. It is of great importance that the flowering and fruiting cycles of our important forest tree species be known so that cultural methods can be developed to produce flowers in a more regular pattern in selected trees, and in trees growing in seed production areas and seed orchards.

The present paper presents the results of a study which was made to determine the time of male and female flower primordia initiation and the subsequent floral development of these structures on slash pine trees growing in the Lake City, Florida area. For the microsporangiate strobili, or the male catkings, the steps leading to the formation of the tetrad division of the microspore mother cells are described, and the description of the megasporangiate strobili or the female corselets, includes the various phases from flower primordia initiation to the formation of the ovule on the ovuliferous scale.

Materials and Methods

The buds for this study were collected at weekly intervals during the period from May 1953 to January 1954, from slash pine trees growing in an open old-field stand on the Olustee Experimental Forest in Baker County, Florida. The trees which were used had well-developed crowns and the collections were made from the distal branches in the upper part of the crown. Collections were restricted to trees which were flowering actively during the years prior to the study, and buds were collected only from branches which bore both male and female flowers in the previous two years. A weekly collection consisted of five buds each from five trees[1/]. Formalin-acetic acid-ethyl alcohol (FAA) was used

[1/] The collection of the buds was started while the author was stationed at the Lake City (Fla.) Research Center of the Southeastern Forest Experiment Station. The author thanks P. E. Hoeketra and E. E. Miles for carrying out of the field collections.

were larger in diameter, had a denser cytoplasm and accepted more stain than the surrounding cells.

The midrib or main axis was well marked by vascular bundles which connected the upper part of the rudimentary microsporophylls with the vascular system of the developing branch. Also, the outer layer of cells surrounding the microsporophylls, with the exception of those cells in the, area of the sporogenous cell activity, had become suberized and accepted the safranin stain. This mouccellular layer eventually forms the "epidermis" which surrounds the microsporophylls.

The sporogenous tissue enlarged considerably during the month of November. In some of the microsporophylls which had formed first, the microsporangia appeared to be fully developed by the early part of December. By December 14, the pollen sacs were filled with well-defined microspore mother cells which originated from the sporogenous tissue. They were surrounded by a tapetal layer consisting of three or four layers of cells. Also, at this stage a resin canal, complete with epithelial and sheath cells, had formed at right angles to the stalk close to the scalelike terminal appendage of the microsporphylls.

In the Lake City, Florida area the male strobili passed the "winter" in the microspore mother cell stage and the reduction division occurred during the middle of January. At the time the catkins were about 1.5 cm in length. Depending on weather conditions subsequent development from this stage on is very rapid. Catkins collected within three days from the same tree during the latter part of January, showed all stages from the microspore -mother-cell stage, through the tetrad stage, to the second vegetative division of the microspore.

B. Formation *of* the pistillate strobilus. The primordia for the female strobili are not laid down as early as those of the male catkins. The first evidence of pistillate flower primordia were observed during the latter part of August. They became first noticeable as slight protuberances in the axis of a developing cataphyll. This mass of cells was pointed, and vascular tissue in the central axis started to differentiate without any delay. Also, the protecting bud scales started to develop in a similar fashion us was described for the male flower. They continued to envelop the protuberance in a spiral manner until a tight hood had formed. By October 18, a hood of up to 12 layers of scales was observed around some of the primordia.

During this period of growth, there was pronounced differentiation into vascular tissue in the midrib area, and the apical part of the primordia assumed a more flattened appearance. The vegetative stalk also elongated considerably and formed xylem and phloem elements, and both longitudinal and horizontal resin canals were laid down.

The primordia of the bracts started to form during the latter part of October and were accompanied by active meristematic activity in the basal part of the rudimentary pistillate strobilus. They appeared as lateral outgrowths or papillae and progressed in an acropetal direction. They were

as a killing and fixing agent. The buds were dehydrated in an alcohol-chloroform series and embedded in Tissuemat (560C). Some of the longer bud scales were clipped off just before embedding to facilitate the sectioning. Serial sections were cut on a rotary microtome at thicknesses ranging from 10μ to 15μ Four types of staining techniques were used: haematoxylin and safranin, safranin and fast green; tannic acid, iron chloride, safranin, fast green; and haematoxylin and aniline glue. Smears of the latter develop, mental stages were stained with acetocarmine, The sections were mounted in Permount for future reference,

Results

A. Formation of staminate cone. In the buds collected during May and early June no evidence of staminate strobili primordia was noticed and the apical point of the bud was somewhat rounded, In some of the collections the apical point had elongated considerably and by June 11 had assumed a more pointed shape. Cell division was very rapid at this stage as evidenced by the number of mitotic figures in the apical area. In one of the buds collected on June 25, a distinct swelling could be observed in a axil of a nearly formed cataphyll before the cataphyll had reached its full size. The tissue in these mounds of cells was undifferentiated during the early stages but by July 12 a row of vascular tissue was evident in the lower vegetative areas of the primordia. By July 19 differentiation of vascular tissue had progressed toward the apical portion of the rudimentary catkin, During the latter part of July the lower meristematic area started to differentiate hood scales which began to envelop the rudimentary strobilus and by September 13, up to eight layers of scales had formed. The innermost layer pushed between the primordi and the previously formed scales, curving inward near the apex, and formed a protective arch. These scales have a thick epidermis, especially in the outer surface, which becomes suberized early during the development. Several of the scales which formed last, the involucral scales, elongated only slightly, At this stage the strobili primordia had formed a protective cuticle and this hood should prevent excessive evaporation from this succulent structure. During this period of growth, the only change recognizable within the primordi proper was a broadening, and a lengthening of the axis of the rudimentary catkin. Additional meristematic activity became pronounced during the latter part of September. It resulted in a lengthening of the axis of the strobilus and the rudimentary microsporophylls started to differentiate at the base. During the early stages they are similar in appearance to the primordia of the hood scales and their axis is at a right angle to the main axis of the strobilus.

During the remainder of the month of October differentiation of microsporophylls progressed towards the apex and the first formed microsporophylls started to turn upwards. This was the result of a symmetrical growth, namely cell division, both periclinal and anticlinal, was more rapid in the abaxial area than in adaxial surface. In several of the buds collected on October 4, sporogenous initials had been laid down in the abaxial pert of some of the early microsporophylls. In several of the sections, the sporogenous initials had already given rise to sporogenous tissue. These cells

formed by both periclinal and anticlinal cell divisions. This development period continued for a period of about three weeks. After this time the developing strobili were covered with slight protuberances which were arranged in a spiral manner. Also, vascular tissue had formed in the rudimentary bracts of the lower region. This tissue was connected to the vascular elements of the midrib area. During the later stages of growth, cell elongation and cell division was more rapid in the lower region, causing these bracts to curve slightly upwards towards the apex of the strobilus. The ovuliferous scale arcse in the axil of this bract and appeared at first as a conical mass of cells during the later part of December. Initiation of these primordia also progressed in an acropetal direction. Buds collected on January 6 showed well-defined mounds of cells which were subtended by the bracts. Vascularization in the midrib and as well as in the bracts had progressed rapidly. Also, the epidermal cells of the bract began to cutinize. There was distinct cell organization in the upper inward-part of the ovuliferous scale. Rapid cell division, as evidenced by a large number of mitotic divisions resulted in the formation of a protuberance. This was the first indication of the organization of an ovule. In this'ovular swelling were contained the sporogenous initials which will give rise to the sporogenous tissue. At this stage, which is about three weeks before pollination, the uncompleted strobilus proper was 4=4.5 mm in length, while the total length of the structure including the stalk and the hood was 12 mm. The strobilus is still completely enclosed by the interlocked hood scales and is whitish in color.

Discussion

The microsporangiate strobili were initiated during a period of about six weeks, while that of the female strobili was limited to about two weeks. In the samples studied there was only one distinct row of female strobili.

The period of initiation and development as given was applicable only for slash pine trees in the Lake City, Florida area for that particular year. The climatic and site conditions within the natural range of slash pine are varied and therefore affect their growth. As a result the period of initiation will fluctuate not only from year to year but will also vary over the range of the species. Therefore, the results of this study can only be applied with modification in another area.

The information obtained will be valuable not only in research work with this species but also should be helpful to the forest manager. The research forester can use these results to time the application of flower induction trials, to time the pruning of experimental trees in seed orchards so that he will have a large number of potential flower bearing branches, and to relate environmental and biotic conditions to the production of flowers. To the forest manager on the other hand these results will be of help during the timing of his cuts to convert a stand into a seed production area, or during intermediate fellings which are aimed at bringing about an increased seed crop.