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INTRODUCTION

A knowledge of differentiation of floral structures in trees is important in understanding the effects of stimulation treatments and environment on the initiation of these reproductive bodies. This study is an attempt to define the period of initiation of strobili in eastern white pine, <u>Pinus strobus L</u>.

Recent studies of initiation of strobili in <u>Pinus</u> have been on the 'hard" or "yellow" pines (Diploxylon). The results indicate that both male and female strobili are differentiated at the close of the growing season. However, marked differences between this group and the "soft" or "white" pines (Haploxylon) exist for many characteristics. Ferguson (1904) could find no primordia of male or female strobili in P. <u>strobus</u> as late as November, yet these structures always appeared the following spring. In southern New England <u>P. strobus</u> tends to flower later than other pines . These considerations suggested that initiation of strobili in P. strobus did not occur until spring.

METHODS AND MATERIALS

Terminal buds from the upper crown of four open-grown <u>P. strobus</u> trees in New Haven, Connecticut, were collected at intervals from March, 1959, until November, 1960. Two or more buds were selected from branches with and without oneyear-old cones at each sampling date (table 1).

The buds were fixed immediately after collection and stored in FAA fixing solution (70 percent ethyl alcohol: 37 percent formaldehyde: glacial acetic acid - 90:5:5, v:v:v). The buds were trimmed of needles and stems, dehydrated in a tertiary butyl alcohol series, and embedded in 56-58°C, Tissuemat or Histowax. Serial longitudinal sections were cut at 15 microns thickness on a rotary microtome. The sections were stained with safranin and fast green, dehydrated in an ethyl alcohol series, cleared in xylene, and mounted in Permount.

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RESULTS

The order of axial structures (sterile bracts, male strobili, brachyblasts or short shoots, female strobili) was the same as described by Doak (1935), except that no lateral bud primordia were found in the dormant bud. In 1959 the buds remained dormant until after April 3; mitosis was observed in material collected on April 12.

April 12 was the earliest date that an ovulate strobilus primordium could be identified with certainty (table 1). The next positive identifications were made on April 24. Development continued rapidly after that date, and ovulate strobili were emerging from between the bud scales by May 19. The exact period of differentiation of ovulate strobili could not be determined precisely, but it was apparent that development of the strobilus did not occur until bud activity had resumed in the spring.

Collection date	Branches with cones			Branches without cones		
	No. buds	Female	Male	No. buds	Female	Male
26 March 1959	10	(2)*		8	(1)	3
3 April	8	(3)		9	(5)	1
12 April	9	(4)		11	1 (3)	2
17 April	10	(2)		11	(3)	2
24 April	10	1 (4)		9	1 (2)	1
2 May	4	2 (1)		5	(1)	1
8 May	9	2 (2)		10	1	2
19 May	4	4		4	1	
10 August	8			8		
28 August	8			8		
17 November 196	0			18	(4)	7

Table 1. -- Presence of strobili in Pinus strobus at New Haven, Connecticut

*Numbers in parentheses indicate cases where positive identification could not be made.

The presence of completely differentiated male strobili in buds collected on March 26 indicated that differentiation occurred prior to bud dormancy. Material collected on August 10 and 28 contained differentiating basal bracts and brachyblast primordia, but no male strobili were detected. Material gathered on November 17, 1960, contained fully differentiated male strobili. The period of differentiation lies between these dates, possibly early September.

There was no relation between the presence of ovulate strobili primordia and the presence or absence of cones on the same branch. However, the presence of male strobili was significantly related to the absence of cones; no terminal buds from branches bearing developing cones contained any male strobili. No buds were observed to contain both male and female strobili.

DISCUSSION AND CONCLUSIONS

A period of slow, continuous development of the ovulate strobilus during the dormant season has been reported for three species of the Diploxylon group (Duff and Nolan, 1958; Gifford and Mirov, 1960; Mergen and Koerting, 1957). Bingham and Squillace (1957) reported that strobili of western white pine, <u>Pinus monticola</u> Dougl., are differentiated the season prior to flowering, but they cited no anatomical evidence.

However, there is no definite indication of differentiation prior to resumption of growth in the spring in this study. It is possible that whatever biochemical or cytological factor determines the differentiation of ovulate strobili has already had effect during the previous growing season although no recognizable differentiation occurs until resumption of growth. This idea is supported indirectly by the results of a flower stimulation experiment (Stephens, 1961). Stimulation treatments applied during late July increased the number of ovulate strobili the following season. However, the same treatments applied in early April had no effect on the differentiation of strobili during the same season.

The differentiation of male strobili of P. <u>strobus</u> occurs the season prior to flowering, probably early September in the vicinity of New Haven, Connecticut. The differentiation of ovulate strobili may possibly occur the season prior to flowering, but actual development of the strobili does not occur until resumption of growth by the bud in early spring.

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