NATURAL AND INDUCED FLOWERING IN YOUNG PINE TREES

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In forest trees we have numerous examples of distinct growth phases, and one of the more obvious one is the formation of the first sex organs. This stage, although easily recognizable in vivo, presents considerable difficulties when one attempts to explain it on biochemical grounds, or induce it at will. The literature abounds with reports of the failures to induce flowering at an early age in woody plants, and almost every person interested in general field of forest genetics has at one time or another attempted to induce flowering in forest tree seedlings.

Despite the many attempts, no reliable technique has evolved that induces flowering in very young seedlings, with the exception of the grafting work by Dr. Mirov (1951) . There have been, however, several successful attempts to considerably advance this "ripeness-to-flower stage". These treatments relied on either mutilation of the trees, fertilization of the trees, or on a combination of these two approaches. By this method, six-year-old slash pine seedlings, Pinus elIiottii Engelm., were brought into flowering by Hoekstra and Mergen (1957), and Wareing 1953) was able to induce male flowers on 12-year-old Scotch pine trees. It should be realized, however, that in both instances the trees had passed the juvenile stage in many other phases of their development, e.g. foliage, bark, and wood cells.

In this maturing process, the various tree characteristics appear to progress fairly independently of each other. Although a certain trend is present, the various steps do not seem to be dependent upon each other, e.g. mature conditions in one organ do not depend on the presence of mature traits in certain other ones. As an illustration might mention the observation where reproductive structures are produced before secondary needles are initiated. It seems to me that, although these various phases are interrelated and influenced by the environment, the physiological stages or phases of readiness for flowering proceed at their own pace and are only indirectly influenced by the maturation process in the other characteristics.

My observations on natural early flowering, as well as on induced early flowering, are based on experiments conducted over a period of six years. The observations are grouped together, as a matter of convenience, into six categories, namely: Report of early flowering; Relationship between first flowering and successive flowering; Effect of geographic seed source; Effect of hybridization; Attempts to stimulate flowers by altering the physical environment; Treatment with liquid fertilizer, high temperature and humidity; and Effect of physical and chemical treatment.

Observations

Report of early flowering: In 1939, Righter (1939) reported on the minimum ages for both staminate and ovulate flowers in 57 species and varieties of pines grown at Placerville, California. The average minimum age was 4.4 years for male flowers and 5.2 years for female flowers and one of the species bore male flowers when only one year old. During the spring of 1957, I observed male flowers with viable pollen on Mugo pine seedlings that were 10 months old (Mergers and Cutting, 1957) . Three plants from a total of about 330 seedlings, bore 12, 6, and 4 catkins respectively. The catkins were of normal size, and both the percentage of germination and pollen tube growth were normal when the pollen was cultured in distilled water. Of particular interest was the fact that these three seedlings produced reproductive structures prior to the initiation of secondary needles.
Relationship between first flowering and successive flowering: The Mugo pine seedlings that flowered while Tess than one year old were observed for four consecutive seasons, and in each year male flowers were produced. Whether the early manifestations of flowering in pine trees are of a transitory nature or whether they are an indication of sustained reproductive activity was discussed by Righter (1939), He felt that these early flowering were reliable indications that additional flowers would appear. A check on the relationship between the first flowering and flowering in the succeeding year was made on 14 different species and pine hybrids in my progeny testing plantations. A total of 89 trees were involved in the test. A regression analysis, using "Successive Flowering" as the dependent variable and "First Flowering" as the independent variable, indicated a highly significant relationship. The value for "b", the slope, in the regression equation was 0.468, meaning that approximately half of the trees flowered in the year succeeding their initial flowering. This would indicate that the early precocious flowerings need not be considered a freak of nature, but an indication that the so-called non-sexual cycle of these trees has been completed and that they are able to continue to produce functional reproductive structures.
Effect of geographic seed source: Jack pine seedlings not only flower at a very early age (2 years), but also produce numerous flowers of both sexes. From a statistical analysis of the number of male and female flowers on 4-year-old trees in a geographic seed source test, it became quite apparent that the seed source has a highly significant effect on the number of trees that flower, and on the number of flowers per tree. The trees under observation originated from seed collected in St. Louis, Pine, and Itasca Counties in Minnesota; and Wood and Oneida Counties in Wisconsin. The trees were obtained from the Lake States Forest

and Range Experiment Station of the United States Forest Service and were outplanted at Norfolk, Connecticut, The field design consisted of a randomized block layout with 6 replications, A X^2 test on the number of trees that flowered indicated that the origin of the seed significantly (1% level) affected the number of trees that flowered. Using the "New multiple range test'" by Duncan (1955) with the error term from an analysis of variance on the transformed data, the trees from Pine County, Minnesota showed both a significantly smaller percentage of trees that flowered, as well as a smaller number of flowers per tree. This seed source was less than 150 miles from its adjacent source, indicating that there are local ecotypes within the natural range of Jack pine that, when grown under Connecticut conditions, have a shorter interval from seed germination to flower production that is genetically controlled.

Effect of hybridization. From observations in our progeny testing plantations it is quite apparent that interspeciflc hybrids from both the Haploxylon and Diploxylon group flower occassionally at an earlier age than seedlings from either parents. It is possible that complementary genes present in the parental species shorten the juvenile non-reproductive stage. In addition, by interspeciific hybridization genes from early flowering species can be introduced into those flowering later.

Attempts to stimulate flowers by altering the physical environment: Experiment A) Two year-old seedlings o sand pine received three levels of chilling treatments during the summer and early fail. The treatments were ineffective in stimulating flowering, but the seedlings that were treated in the late fail grew most vigorously in the following growing season.

Experiment B) Seedlings of Scotch pine and Norway spruce were subjected to a photoperiod of 16 hours in a greenhouse during two growing seasons. The seed had been collected from four geographic locations, rind the seedlings were 8 months old when they were moved into the greenhouse. In this experiment combinations of photoperiod, temperature, and cold treatment were evaluated. At the conclusion of the study after two additional growing seasons, several of the pine seedlings that had received cold treatments and were subjected to a 16-hour-light period in the greenhouse produced male or female flowers. The flowering was too erratic, however, to make any type of statistical analysis, but the seedlings that flowered were the most vigorous ones. Experiment C) Using a randomized split plot design, one-year-old Scotch pine, seedlings from five geographic sources were placed under four light intensities in a greenhouse and in the field. The light conditions were: full light, 25% of normal light, 10% of normal light and 2% of normal light. The reduced light conditions were obtained by placing the potted seedlings in cages that were covered with layers of cloth. It should be pointed out that this treatment also affected the temperature and humidity conditions within these cages.

The results on flowering in this experiment were opposite to those of the study reported previously. The treatment that stunted the growth to the greatest extent, namely location under 10% of 2% of normal light intensity in the greenhouse without cold treatment, were the only treatments that stimulated male flowers. Using an exact statistical test on the data, the effect on flowering was significant at the 3% level . Of interest also was the fact that the male catkins matured during July, whereas older Pinus sylvestris trees in the vicinity of the greenhouse flowered in mid-May. The catkins on the seedlings were abnormal, were spherical in shape, and had the appearance of male flowers of Taxus. Possibly because of the stunted growth of the seedlings, this group had the highest nitrogen content (2.5%), as compared to the controls that had 1.9% O.D.W. basis. The most prolific flowering occurred on the most stunted seedlings and on seedlings from the northern sources (Inari, and Rovaniemi, Finland, and Tonset, Norway).

Experiment D) Mirov (1939) was able to stimulate flowering in five species of pine by in arching one-year-old seedlings into the crown of a mature, flower-bearing tree. Two years after grafting, male flowers were produced on all species but slash pine, Pinus elliottii Engelm. I repeated a similar study without success. Seedlings were marched into various positions in the crown of older slash pine trees, but during the three years that they were under observation, they maintained their juvenile appearance and none of them produced flowers.

Treatment with liquid fertilizer, high temperature and high humidity: From the evidence in the literature on time of initiation of flower primordia in members of the Diploxylon group, some of the primordia apparently are not laid down in the growing season prior to their anthesis, but during late winter or early spring before they appear. In a recent study by Stephens (1961) he was able to observe male strobili in white pine in the fall preceding flowering, but he was unable to locate female flowers until the spring preceding flowering. Therefore, members of this group are suited for experiments to obtain responses to treatments within a relatively short period.

Dormant 18 month-old potted seedlings of eastern white pine, Pinus strobus L., and Pinus griffithii McClelland x Pinus strobus hybrids were subjected to a sudden change in temperature and humidity by placing them into a 21°C. greenhouse during the beginning of January. As soon as the soil in the pots had thawed, the plants were watered with a liberal dose of a 1/2% aqueous solution of a 12-12-12 liquid fertilizer. After a short adjustment period the seedlings responded rapidly and normal growth started, and after seven-weeks in the greenhouse rudimentary male and female flowers appeared on the Pinus griffithii x Pinus strobus seedlings. Of a total of 156 seedlings in the greenhouse, 15 trees had flowers, while none of the controls produced flowers. Because none of the Pinus strobus nor Pinus griffithii seedlings produced flowers, this flowering was probably caused by a combination of the shock treatment, and the effect of interspecific hybridization. As in the other instances, the pollen of the male catkins was viable, and the female strobili appeared normal . Female strobili that were not pollinated or that were selfed, withered and fell off during the early part of the summer; those that were cross-pollinated continued normal development.

Needle samples for N determination were collected from the flowering seedlings as close to the locus of flowering as possible; needles from the new growth were not taken. For the controls, needles were selected from similar locations on non-flowering seedlings. The C/N ratios of the needles were the same for both the flowering and non-flowering seedlings (72.186 vs. 72.814).

Effect of Physical and chemical treatment. In a randomized 2 x 2 x 2 factorial experiment the effects of root-pruning, stem injury and fertilization were evaluated on 3-year-old Pinus clause seedlings that were growing in a greenhouse. The treatments were started during June and continued over a period of one year. For the root-pruning, one half of the roots were removed and the trees were placed back in the original pot. Aluminum foil was placed along the edge of the cut surface to prevent the roots from growing into the other part of the flower pot. For the stem injury a 2 cm wide girdle (bark and cambium) were removed, leaving about one quarter of the stem intect. The fertilized trees received at weekly intervals 100 cc of an aqueous solution of a 12-12-12 soluble fertilizer.

Observations on flowering were made during a period of two years, and although a considerable number of trees flowered (43/96), there was no relationship between treatment and the production of reproductive structures. Flowering was distributed randomly among the seedlings and both male and female flowers were produced in abundance.

At monthly intervals during the first year after treatment, needle samples were collected from each tree to determine the O.D.W. , and the content of nitrogen. The purpose of these analyses was to see whether there was a relationship between flowering and a specific C/N ratio, or whether flowering was associated with a change in the ratio during the period while flower initiation took place. For the analysis the individual tree data were plotted, along with indicator lines of the approximate C/N ratioes. In this experiment flowers were produced in trees with ratios varying between 1 .0 and 1 .7, and in absolute amounts of N varying by a factor of 2.

Various graphs were prepared of the changes in C/N that took place during the spring and summer when flowers were initiated. The differences in C/N ratio between July 15 and August 14, or during the time when flower primordia initiation took place were plotted and it became quite obvious from this graph that flower production and a change in C/N ratio, (either positive or negative), were not associated.

General

In our greenhouse I have a fairly large number of slash pine, loblolly pine, and longleaf pine seedlings. These seedlings are kept under a high level of N fertilization. As a result of this treatment flowering is quite common amongst three-year-old slash and loblolly pine, and this past spring we had 8 longleaf pines out of a total of that produced female flowers while they were still in the grass-stage. In addition, some seedlings flowered during the first year after they started height growth.

There is an interesting phenomenon associated with high N fertilization. Already when Hoekstra and myself worked on the stimulation of flowers in slash pine we noticed an increase in the number of hermaphroditic male flowers that were produced as a result of fertilization (Hoekstra and Mergen, 1957). In a recent study on the induction of flowers in young white pine hybrids, I observed a similar phenomenon. A large number of the male flowers were hermaphroditic, and the upper part of the strobili had changed into female structures. Germination of pollen collected from these hermaphroditic flowers germinated normally in vitro, and the sex inversion was complete in some instances so that viable seed was obtained after pollination.

DISCUSSION

From the results presented one can conclude that, 1) the ability to flower at an early age is genetically controlled, and 2) in flowering trees the mechanism that controls the change from a vegetative shoot to a sexual one can be modified to some extent by the environment. As in any other growth phenomenon, the resultant is the product of the interaction of a particular genotype with its environment. It appears that in certain species the degree of genetic control of the juvenile period is more plastic and therefore these species lend themselves better to manipulative flower induction techniques.

The results of studies by previous investigators, as well as those reported by me, are of little help in explaining the biochemical and physiological changes that bring about early flowering in trees. Neither do they throw any light on the various theories that try to explain flowering. The question of whether early flowering is induced by an excess of auxin or by a lack of auxin is still unanswered. The majority of the successful techniques rely on changing the C/N ratio, with the concentration of nitrogenous compounds being affected to the greatest extent. What effect this has on shortening the juvenile stage is not known, nor can a specific C/N ratio, or even a range of ratios, be assigned as an index of when the change from vegetative growth to reproductive growth will take place. A particular ratio will not give the absolute amounts of its two variables, and a wide range of conditions can give he same ratio.

To check on some of the theories, and to analyze chemically the experimental material, we need to find manipulative techniques that are reliable, and that can be repeated over a period of years by investigators in various parts of the world. Environmental changes, although quite effective and important, need to be backed up by studies that check the correlated modifications. To find reliable techniques, a great deal of empirical work needs to be done, such as testing the effects of certain treatments under very uniform conditions. With the current popularity of controlled light and temperature rooms, it should be much 'easier to carry out these studies. It is also important that information on failures and successes be exchanged even if the results are not too striking.

The entire field of cell differentiation, of which juvenility is a part, is a fascinating one that has challenged the ingenuity of researchers for quite some time. It is one of the most basic areas in biology, and needs to be explored cooperatively by the biophysicists, the enzyme chemists, and the physiologists, and the botanists.

Summary

In summarizing, I would like to mention that: 1) Mugo pine seedlings have flowered when less than one year old and before they had produced secondary needles, 2) once a tree has begun to flower, it can continue to do so, 3) there is evidence that within Pinus banksiana some geographic ecotypes are more precocious than others, 4) interspecific hybrids flower occassionally at an earlier age than the parent species, 5) flower induction may be stimulated in two-year-old Pinus sylvestris seedlings by either severe retardation or stimulation of growth, and 6) treatment with a 12-12-12 fertilizer along with a temperature and humidity shock, induced male and female flowers on Pinus griffithii x Pinus strobus hybrids that were less than two years old.

Literature Cited

Duncan, D. B. 1955: Multiple range and multiple F tests. Biometrics 2: 1-42.

Hoekstra, P. E., and F. Mergen. 1957. Experimental induction of female flowers on young slash pine. Jour. For. 55: 827-831.

Mergen, F., and S. G. Cutting. 1957. Male flowers on one-year-old Mugo pine seedlings. For. Science 3: 355-356.

Mirov, N. T. 1951. Inducing early production of pine pollen. U.S.D.A., Cal. For. and Range Exp. Station, For. Res. Note 80: 3 pp.

Stephens, G. R., Jr. 1961. Flower induction in woody plants. Ph. D. dissertation. Yale University. 75 pp.

Righter, F. I. 1939. Early flower production among the pines. Jour. For. 37: 935 - 938.

Wareing, P. F. 1953. Experimental induction of male cones in Pinus sylvestris. Nature 171: page 147.