STIMULATION OF EARLIER FLOWERING AND SEED PRODUCTION IN JACK PINE SEEDLINGS THROUGH GREENHOUSE AND NURSERY CULTURE

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Attempts are continually being made to reduce the time between generations in forest trees through flower induction and stimulation studies and by selection for precocious flowering.

The pines have been of primary interest in research on early flowering. Righter (1939) surveyed the minimum ages of flower production among the pines grown at Placerville, Calif. The average minimum age for 39 species was 4.4 years for staminate strobili production; and for 55 species it was 5.2 years for ovulate strobili production. Jack pine (*Pinus banksiana* Lamb.) was reported as producing both male and female strobili at 3 years of age. More recently precocious flowering in pines has been described by Mergen and Cutting (1957), Reines and Greene (1958), Greene and Porterfield (1962, 1963), and others. Wright (1964) has summarized the information on flowering age in forest trees. In the pines he found earliest flowering in the *Lariciones* and *Insignes* series. Jack pine is a member of the *Insignes* group. Wright (1964) notes that jack pine seedlings grown on a wide spacing in a well-watered nursery can be expected to produce flowers by 3 years of age. However, the proportion of trees normally flowering at this age is extremely small. An examination of 100,000 three-year-old jack pine seedlings in standard nursery beds in Lower Michigan, but growing at a closer spacing than that described by Wright, showed that only approximately 0.3 percent of the seedlings bore first-year cones. Staminate strobili occurred with about equal frequency.[2]

The available evidence in the literature suggests, therefore, that early flowering or flowering before the age of 3 or 4 years in the pines is rare. However, as Wright (1964) points out, experiments indicate a possibility of reducing the flowering age of seedlings considerably by appropriate fertilizer and cultivation treatments.

Results given in this paper indicate that the flowering age in jack pine can be reduced and the proportion of flowering seedlings increased through cultural treatments.

Materials and Methods

In June and November 1961, jack pine seed from three sources was collected: (1) 90 individual trees grown from X-rayed seed on the Argonne Experimental Forest in northern Wisconsin, (2) a bulked lot from a control plot of trees grown from nonirradiated seed, and (3) a stand of mixed plantation and natural origin on the Argonne Experimental Forest. The origin of (1) and (2) was the Chippewa National Forest in Minnesota. The seed was extracted from the cones immediately after each collection. Although the seed collections were made for another study (Rudolph 1966),[3] the observations reported here were made on the same plant material.

The first sowing was made on June 29, 1961, in fine sand on a greenhouse bench. Each of the individual tree collections was represented by a single 16-inch row containing 100 seeds. The control populations were represented by 4,500 seeds similarly arranged in 100-seed rows. The sand in the greenhouse benches was treated with a Tersan fungicide solution as required to check damping-off in the young seedlings. Commercial 10-10-10 fertilizer was applied as necessary to maintain the plants in a vigorous condition. A 20-hour photoperiod was maintained throughout the greenhouse phase of the study. Temperatures in the greenhouse were normally 75° F. during the day and 60° F. at night. However, day temperatures occassionally exceeded 75° F., especially in the summer and early autumn, and reached 90 to 95° F. on hot, clear days. During the summer and until about October 1, a 50-percent-shade saran cloth covered the greenhouse.

When the seedlings were from 2 to 3 months old, the following groups were transplanted into 2½ inch clay pots: (1) a random sample of 6 plants each from 53 of the 90 individual tree progenies, (2) a random sample of 120 plants from the nonirradiated control, (3) 160 plants from the natural stand collection, and (4) 50 plants in each of the cotyledon-number classes from 3 to 6 from the natural stand collection. Also, 17 seven-cotyledon plants were potted.

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[2] *Rudolph, T. D. 1962 Unpublished report on file at the Institute of Forest Genetics, Rhinelander, Wis.*

[3] *Also Rudolph, T. D. The effects of X-irradiation of seed on X_1 and X_2 generation progeny in Pinus banksiana. Ms. in preparation.*

A second sowing was made on December 15, 1961, following the same arrangement as that in June. Greenhouse conditions were the same, except that day temperatures did not reach the high levels experienced occasionally with the first sowing. When the seedlings were about 3 months old, a random sample of 6 seedlings from each of the 37 progenies not previously sampled was potted into 4-inch clay pots; this completed the potting of all 90 progenies being studied. All potted seedlings from this and the earlier sowing were subsequently grown under similar greenhouse temperature and photoperiod conditions. A commercial 10-10-10 fertilizer was applied to the pots as needed and the pots were watered daily.

Late in June 1962 the seedlings were transplanted into the nursery at a spacing of 10 x 10 inches. The seedlings with known cotyledon numbers were planted into 5 replications of 10 trees; all other seedlings were planted in identified row plots. A 50-percent-shade saran cloth covered the seedlings in the nursery bed for the first 6 weeks. The seedlings were fertilized in the nursery as required to maintain foliage color and vigor.

In late May and early June 1963, flowering was scored for each tree. At this time the older seedlings were 23 months and the younger group 17 months old. All seedlings in the individual tree progenies bearing female strobili were bagged for isolation and subsequent backcrossing to the female parent. A small number of flowering plants in the control and cotyledon-number groups were also bagged for subsequent controlled pollination. All other flowering seedlings were open to wind-carried pollen. Cones from the controlled pollinations and from the cotyledon-number population were collected in September 1964. The seed was extracted by hand and counted. Germination tests were made during the 1964-65 winter in the greenhouse.

Results and Discussion

The results in table 1 show that female strobili production was stimulated in jack pine seedlings grown under the conditions described above. The large number of seedlings with female strobili (62

Table 1.--Female flowering and seed production on 17- and 23-month-old[1/] jack pine seedlings

Date sown and seedling type	Trees observed: Number	Trees observed: Percent with female strobili	Avg. no. female strobili per tree	Percent of cones maturing	Avg. seeds per cone	Percent seed germination
June 29, 1961	Section A. In relation to seedling cotyledon number:					
3 cotyledon	50	40.0*	1.75	50.0	25.1	87.2
4 cotyledon	49	65.3	2.19	54.3	26.1	76.2
5 cotyledon	50	72.0	2.14	48.1	24.9	70.7
6 cotyledon	47	66.0	2.16	47.8	30.3	79.7
7 cotyledon	16	75.0	2.08	56.0	23.4	-- 2/
Total or mean	212	61.8	2.08	50.5	26.4	77.0
Dec. 15, 1961	Section B. In X-irradiated individual tree progenies and control populations:					
37-X_2 generation progenies 3/	216	23.1	1.34	10.4	25.9	35.6
June 29, 1961						
53-X_2 generation progenies 3/	309	41.4	1.80	29.0	26.6	53.3
Non-irradiated control	120	47.5	1.60	--	--	--
Mixed planted and natural stand	151	56.5	1.32	--	--	--
Total or mean	796	40.3 4/	1.56	24.8	26.4	51.4

* Percent of trees is significantly (5-percent level) lower than in other seedling types.

1/ Female strobili counted on June 1, 1963, when pollination was practically completed.

2/ Seed damaged during extraction; no germination data available.

3/ Seed yield and quality from these seedlings are based on results of controlled backcrosses to the female parent. Seed from all other seedling types was from open-pollinations.

4/ The mean for the seedlings sown on June 29, 1961, in Section B is 46.7 percent.

and 47 percent, respectively, in two lots of 23-month-old seedlings) contrasts sharply with 0.3 percent flowering on seedlings 1 year older grown under normal commercial nursery conditions. Noteworthy but unexplainable from the results of this study is the significantly smaller proportion of trees bearing female strobili in the 3-cotyledon seedlings. The decreased flowering in this group is apparently not related to height differences since there was no significant difference in height development between seedlings of the various cotyledon classes.

More than one-half of the female strobili produced developed into mature cones under wind-pollination conditions. The nearest pollen source was in a windbreak planting more than 50 yards from the seedlings in the transplant bed. In the controlled pollinations the proportion of female strobili developing into mature cones was reduced to slightly over 10 percent in the 17-month-old seedlings and 29 percent in the 23-month-old plants. Several factors may in part account for this. First, some of the female strobili may have been beyond the stage of receptivity before pollen from the female parents was available and applied. Secondly, some heat injury to the seedlings was noted due to temperature buildup within the cellulose casing-kraft paper double isolation bags. Further desiccation and injury to the developing strobili appeared to occur immediately following bag removal as a result of hot, dry weather conditions. Thirdly, the possibility cannot be discounted that some of the backcrosses attempted were incompatible. Finally, the lower proportion of female strobili maturing in the younger (and smaller) of the two seedling lots may be explained by more severe heat injury in this group due to two things: a larger proportion of the seedling was enclosed in the pollination bags, and the isolation bags were closer to the ground where higher temperatures prevail.

Seed yields per cone were comparable in the open- and control-pollinated groups; both fell within the range of average seed yields per cone reported by Rudolf (1958). Seed viability, however, was much higher in the open-pollinated seed than in that from the backcrosses. The 77-percent germination for the open-pollinated seed is markedly higher than the 68-percent average for jack pine (U. S. Forest Service 1948). The relatively high germination is even more impressive, as it is based on the actual number of seeds collected including empty seeds. The lower viability in seed obtained from the backcrosses may be accounted for to some degree by incompatibility that can occur at various stages of seed development in crosses between closely related genotypes. The potential increase in homozygous lethals at this level of inbreeding no doubt also tends to decrease embryo and seed viability. Furthermore, heat injury within the isolation bags as described above also may have influenced female gametophyte development and thereby subsequent embryo and seed development.

Strikingly, male strobili production was almost totally absent on the seedlings observed in this study. Less than 1 percent of the seedlings produced male strobili. The lack of male strobili and the stimulation of female strobili production may have been influenced by the environmental manipulations that the seedlings were subjected to during the period of study. Although the factors responsible for this response cannot be identified from the results, nor was the experiment designed for this purpose, several possible influencing factors are suggested.

In this study the environmental factor most drastically changed from what would occur in nature was the photoperiod. Although flowering in the pines has been considered to be day-neutral, Larson (1961) presents evidence suggesting that in jack pine photoperiod cannot be ruled out as a factor influencing flower formation. We have noted that in jack pine a 20-hour photoperiod applied continuously from the time of seed germination results in practically continuous vegetative growth for a year or more with only short intermittent hesitation periods for primordia formation and bud differentiation. The seedlings in this study were in a stage of active vegetative growth when transplanted into the nursery and into the decreasing photoperiod occurring after the summer solstice. Since, during a normal growing season, male strobili initiation in the pines occurs several weeks or even months before the female flower bud (Mergen and Koerting 1957; Larson 1961), it is most possible that the plants when moved to the nursery were subjected to a photoperiod not suitable for male strobili initiation. Further experimentation would be necessary to test this hypothesis.

In addition to consideration of the possible influence of photoperiod on flower initiation, it is reasonable to speculate that the increased vegetative growth induced in the seedlings by the extended photoperiod, resulting in abnormally large seedlings relative to their chronological age, may be related to the increased female flowering. Nienstaedt (1961) has suggested that age in the chronological sense can hardly be a determining factor in early flowering. He further suggests that size of a seedling as it is related to the balance of assimilating tissue to total amount of meristematic area may have an imporant influence on early flowering. A significant increase in the relative amount of assimilating tissue in the jack pine seedlings grown under long photoperiods in this study was evident. Needles, for example, often developed to a length of 10 inches, whereas under natural environmental conditions the needles rarely exceed 2 inches in length. Perhaps the long photoperiod regime influenced the formation of

increased numbers of female strobili in this indirect manner, as well as more directly through its effect during a particular plant growth phase on flower primordia initiation.

Further progress in stimulating earlier flowering could be expected if we could determine accurately the exact stage in vegetative development and the specific time during the annual growth period during which both female and male strobili are initiated. The necessary environmental manipulations could be applied more effectively during such critical periods of flower primordia initiation.

Conclusions

The data presented in this paper suggest that flowering in jack pine, a species normally flowering at a young age, can be obtained even earlier and in increased magnitude through simple environmental manipulation during the first year of seedling development. The results further show that the breeding of such young trees, at least on a population or family basis, is feasible.

Female strobili formation on as many as two-thirds of the seedlings in a 23-month-old population and on up to one-fourth of 17-month-old seedlings, resulting from merely providing good growing conditions in the greenhouse and nursery, offers improved possibilities for the forest geneticist and tree breeder to accelerate their programs. With the ability to reduce the time between sexual generations to less than three years (including the period required for seed maturation), the forest geneticist has in jack pine an "experimental tree" species that will permit genetics research progress approaching that now enjoyed by nonwoody annual plant geneticists. And the tree breeder can more quickly test the feasibility and demonstrate the potential gain from a tree improvement program by choosing jack pine as the best species. Analysis of advance-generation progeny tests can be accomplished in this species through stimulation of early flowering in a fraction of the time normally required for most forest tree species.

Undoubtedly, stimulation of female flowering in jack pine on the majority of plants prior to 2 years of age, as described in this paper, is not the ultimate in what can be accomplished in stimulation of early flowering. Through more critical environmental control along with genetic selection for early flowering it is not unreasonable to expect that eventually the age for consistent formation of both male and female flowers can be reduced to 1 year in jack pine.

Although flowering age may be further reduced in the future, the present results strongly suggest that jack pine may be a wise choice as an experimental species for fundamental research on the genetic behavior of the pines. Furthermore, jack pine warrants careful consideration as a "pilot" species in a full-scale tree improvement program.

Literature Cited

Greene, J. T. and H. D. Porterfield. 1962. Early cone production in loblolly pine through selection and control-pollination. Georgia Acad. Sci. Bull. 20: 6.

——— and ———. 1963. Selection and progeny testing for early cone production in loblolly and slash pines. Forest Genet. Workshop Proc. 1962: 9-10.

Larson, P. R. 1961. Influence of date of flushing on flowering in *Pinus banksiana*. Nature 192: 82-83.

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

——— and L. E. Koerting. 1957. Initiation and development of flower primordia in slash pine. Forest Sci. 3: 145-155.

Nienstaedt, H. 1961. Induction of early flowering: a critical review of recent literature. *In* Recent Advances Bot., sect. 14: 1658-1662.

Reines, M. and J. T. Greene. 1958. Early cone production in loblolly pine. J. Forest. 56: 855.

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

Rudolf, P. O. 1958. Silvical characteristics of jack pine. U. S. Forest Serv., Lake States Forest Exp. Sta., Sta. Paper 61, 31 pp.

Rudolph, T. D. 1966. Segregation for chlorophyll deficiencies and other phenodeviants in X_1 and X_2 generation progeny of irradiated jack pine. Forest Genet. Workshop Proc. 1965 (this issue).

U. S. Forest Service. 1948. Woody-plant seed manual, U S. Dep. Agr. Misc. Publication 654, 416 pp., illus.

Wright, J. W. 1964. Flowering age of clonal and seedling trees as a factor in choice of breeding system. Silvae Genet. 13: 21-27.