Flowering Induced on Young Loblolly Pine Grafts by Out-of-Phase Dormancy

Abstract. *Loblolly pine trees 3 to 7 years old grown in a heated greenhouse under a 20-hour photoperiod had quiescent buds induced by lowering the temperature and shortening the photoperiod. This treatment resulted in the formation of both male and female strobili. Overall, 38 percent of the ramets produced females, and 68 percent produced males. There was no significant difference in the frequency of flowering among 3-, 5-, and 7-year-old scions.*

Shortening the generation turnover time in most forest tree breeding programs depends on being able to induce functional reproductive organs on trees that are considered juvenile. The definition of juvenility in most woody plants includes lack of ability to flower regularly (*1*). The cause of reproductive incompetence during the juvenile period in woody plants is not well understood, although precocious flowering can be induced in some otherwise juvenile conifers by gibberellin (*2*). Loblolly pine, one of the fastest growing and most widely distributed pines in the southeastern United States, has a juvenile phase that appears to last 10 years or more (*3*), and gibberellins alone are not particularly successful in terminating it (*4*). In addition, other members of the Pinaceae do not flower well in response to gibberellin (*2*).

I now present evidence that loblolly pine is reproductively competent even when very young, but fails to flower because the normal growth behavior of young trees does not allow enough time for differentiation of strobilus buds to occur. Young loblolly pine trees grow vegetatively for a much longer period than mature trees and set a quiescent bud relatively late in the growing season (*5*). However, flower bud initiation and differentiation is a time-consuming process, which apparently requires earlier formation of a quiescent bud (*4*).

Scions from 2-, 4-, and 6-year-old loblolly pine trees (grown from seed) representing six clones and three half-sibling families from North Carolina were grafted in April 1976 onto 1-year-old root stock potted in a sand, vermiculite, and peat moss mixture in a ratio of 2:1:1 and topdressed with a timed-release fertilizer. All grafted ramets were kept in a greenhouse under natural day length until early October 1976. At that time the ramets from each family and clone were divided into two equal blocks. One block was moved outdoors while the other was kept in the greenhouse, where the day temperature was kept at 20° to 25°C and night temperature at 8° to 15°C. Day length inside was extended to 20 hours with incandescent lighting at 80 μW/cm^2. On 14 February 1977, the indoor block, which was still actively growing, was transferred to an unheated greenhouse with no supplemental lighting.

Although day temperatures ranged from 19° to 25°C, night temperatures were at or near freezing in the unheated greenhouse. In addition, the natural photoperiod was cut nearly in half, to about 10 hours, which reduces height growth of loblolly pine seedlings by about sevenfold (*6*). Under these conditions the ramets set quiescent buds shortly after their transfer to the unheated greenhouse, and practically no elongation was observed until mid-May, more than 2 months later. In contrast, ramets moved outdoors in October 1976 began to elongate vigorous vegetative flushes in mid-March 1977.

In mid-May 1977, receptive female conelets were observed on ramets of the indoor block; they continued to emerge until mid-August. Male strobilus buds began to appear beginning in mid-August and continued through mid-September (Table 1). Both male and female strobili occurred on all ages of scion material, and there was no significant difference in the frequency of male and female flowering between 3-, 5-, and 7-year-old scions ($\chi^2 = 3.28$ for females and $\chi^2 = 0.45$ for males, both with 2 degrees of freedom). Ramets from all six clones and three half-sibling families produced male strobili, and ramets from all six clones and one of three half-sibling families produced female strobili. Sixty-eight percent of the ramets produced male strobili, and 38 percent produced female strobili. Two trees with male strobili were found on trees of the outdoor block, and a single female cone was observed in March 1977 (Table 1). These data support the results of a similar pilot study conducted the previous year.

The developmental pattern for male and female strobili on the trees receiving lowered temperature and photoperiod differed from that on trees grown in the open. The female buds were located below the male buds on the shoot, and therefore must have initiated before them. In addition, they differentiated faster and formed receptive conelets in the same season that they were formed. In contrast, male development was slower and appeared to be following a more normal time sequence, with most of them passing the winter in a quiescent condition.

By prolonging vegetative growth through the winter, and then forcing bud set in the early spring with lowered temperature and photoperiod, we have been able to induce both male and female strobili on scion material 3, 5, and 7 years old, grown from seed. To my knowledge, these results represent the highest frequency of both male and female flowering ever reported on loblolly pine of this age. Both male and female strobili have also been induced on top-worked scions of loblolly pine as young as 3 years, grown from seed, but at a lower frequency (*4*).

The reproductive competence of 3-year-old scions, both topworked into large trees or grafted onto seedling root stock, appears to be related to the induction of a quiescent bud that persists for several months during temperature and photoperiod conditions favorable for vegetative growth. In 19-year-old loblolly pine seed orchard trees producing heavy crops of both male and female strobili, a quiescent bud is usually set in early July (*4*). Male strobilus primordia initiate in this bud during late July, and females initiate in August. They do not differentiate into recognizable reproductive structures until late September (*4*). Therefore the normal time course of strobilus bud formation spans well over 2

Table 1. Number of ramets that produced female and male strobili.

Scion age (years)	Grown indoors			Grown outdoors		
	Total ramets	Producing		Total ramets	Producing	
		Females	Males		Females	Males
3	15	2	8	15	0	1
5	12	5	8	14	0	0
7	46	21	32	47	1	1
Totals	73	28	48	76	1	2

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months. During that time the bud remains enclosed in bud scales and develops short shoots and lateral primordia, but no internode elongation occurs on either the terminal or lateral buds until the following spring. In contrast, young trees produce lateral primordia and short shoots, which quickly begin to elongate internodes and complete their development.

Three-year-old loblolly pine may sequentially produce in one growing season as many as four temporary buds that begin to elongate almost immediately. The final quiescent bud that will overwinter is not formed until September (*4*). Therefore, lateral primordia which might be potentially reproductive may form too late in the growing season to allow the lengthy process of strobilus differentiation to be completed. The material that flowered in this study was induced to form a quiescent bud that persisted for longer than 2 months during favorable spring conditions. Material of similar age that flowered after topworking also set buds early in the growing season, just like the large adult material to which they were grafted (*4*).

The transition to the adult reproductive phase in loblolly pine thus appears to be at least in part a function of loss of ability to elongate several buds during the growing season. Reproductive competence in young material would therefore be masked by bud set too late to permit the differentiation of reproductive structures.

Flowering in woody plants is a long, drawn-out process easily reversible in its early stages; a decline in vegetative growth usually precedes it (*1*). I therefore propose that a critical event in the transition to the adult reproductive phase in loblolly pine and possibly other temperate-zone woody plants is the change in vegetative growth behavior that results in the formation of quiescent buds earlier in the growing season. This change then allows sufficient time for the slow process of initiation and differentiation of reproductive structures to occur during good growing conditions. Whatever causes phase change would therefore act directly on vegetative growth and would only indirectly affect reproductive development.

This hypothesis is further supported by the observation that stimulating vegetative growth by frequent watering and fertilizing resulted in no female flowering and greatly reduced male flowering on 2-year-old potted grafts of 50- to 60-year-old mature scion material. These grafts were vegetatively more vigorous than similar grafts of the same clones which were simultaneously water-stressed or given photoperiod and temperature shock as previously described. The ramets receiving the last two treatments have formed a heavy crop of both male and female strobili.

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Light-Stimulated Morphogenesis in the Fruiting Myxobacterium *Stigmatella aurantiaca*

Abstract. *When the fruiting myxobacterium* Stigmatella aurantiaca, *a gliding prokaryote, is starved on an agar surface, the cells form multicellular aggregates resulting from morphogenetic movements. In the presence of incandescent light, each aggregate develops into a structurally complex fruiting body, possessing a stalk and several sporangia. In contrast, this pattern of development is not seen when cultures are incubated in the dark. The cells form irregular interconnecting aggregates, which rarely develop into fruits. However, aggregates formed in the light will develop into fruits even if placed in the dark, suggesting that the light produced a relatively stable alteration in the phenotype of the cells.*

The fruiting myxobacteria are typical of other Gram-negative bacteria in structure and physiology, but differ in that multicellular interactions are a prominent and important part of their life cycle (*1*). These organisms exist as swarms of individual cells embedded in a secreted extracellular matrix material commonly referred to as slime. When the cells are starved on an agar surface, cell movements result in the construction of multicellular aggregates and fruiting bodies of genetically determined shape. Their life cycle makes the myxobacteria invaluable for the study of cell interactions and morphogenetic movements. We have isolated a dispersed-growing strain of *Stigmatella aurantiaca* that retains its fruiting ability even when repeatedly subcultured in liquid. The new strain, designated DW-4, was isolated as a natural variant from a nondispersed growing strain (CCf). We have devised conditions to produce rapid and synchronous fruiting of strain DW-4 (*2*). A typical fruit of *S. aurantiaca*, strain DW-4, is approximately 50 μm tall and possesses a stalk supporting several sporangia (Fig. 1).

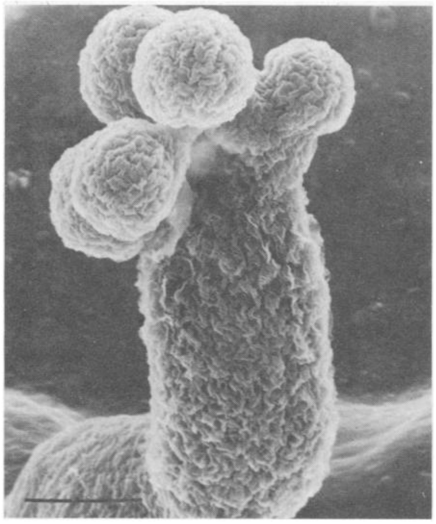

Fig. 1. Fruiting body of *S. aurantiaca* strain DW-4. Small blocks of agar containing fruiting swarms were mounted on specimen stubs, air-dried, and coated with metal in a vacuum evaporator. The first coat was carbon, followed by a mixture of gold and palladium (60:40). Specimens were examined with an Autoscan (ETEC Corp.) scanning electron microscope. The bar represents 20 μm.

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