

# POLLEN DEVELOPMENT CLASSIFICATION SYSTEM FOR LOBLOLLY

D. L. Bramlett and F. E. Bridgwater

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Abstract.--A pollen development classification system (PDCS) for loblolly pine is presented that is parallel to the six-stage female development system currently used in tree breeding of southern pines. Ninety-four individual ramets at two sites in the Weyerhaeuser Company's loblolly pine seed orchard at Lyons, Georgia, were observed at 2-day intervals, and catkin development was scored from 3.0 to 6.0 during the period of March 7-30, 1988. In PDCS, Stage 3 covers development of individual sporophylls, Stage 4 is the start of pollen release, Stage 5 is maximum pollen release, and Stage 6 is the end of pollen release. Stages 3 and 5 are further subdivided.

With the system, duration of pollen release and percentage of pollen shed can be calculated for individual ramets in the orchard. Averages of the pollen development classes are then used to separate clones into early, middle and late pollen release in loblolly pine clones.

Keywords: Pinus taeda L., seed orchard, pollen distribution, forest genetics.

## INTRODUCTION

As improvement of southern pines progresses with continuing cycles of breeding, progeny testing, selection, and establishment of advanced generation orchards, the genetic and economic value of the seeds increases. One of the basic assumptions of the program is that panmixia of the orchard breeding population occurs (Zobel and Talbert 1984). However, it is known that contamination from background pollen dilutes genetic gain from seed orchards and variability in clonal phenology prevents panmixia.

In addition, variability in numbers of male and female strobili lead to unequal parentage in the seed mix from a given orchard. In research studies and pollen distribution models, it is important to define the phenological development of pollen parents and to quantify the gametic production of each male ramet (Askew 1988). This paper reports a pollen classification procedure that relates catkin development to parallel stages of female flower development in loblolly pine (*Pinus taeda* L.).

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1/ Research Plant Physiologist and Research Geneticist, USDA Forest Service, Southeastern Forest Experiment Station, Macon, Georgia and Raleigh, North Carolina.

## METHODS

Two areas of loblolly pine were selected in the Weyerhaeuser seed orchard at Lyons, Georgia. At each site, pollen catkin development was observed on all trees within a 90 foot radius of three target female parents. Pollen catkin phenology was classified and the proportions of pollen grains shed by each ramet were estimated for the 1988 pollen season. Fifty-seven ramets were observed at site 1 and 37 ramets observed at site 2.

Ten branch tips were tagged on each ramet, and a strip of flagging was attached to the branch for easy identification. Each branch tip included 1-6 clusters of pollen catkins (microsporangiate strobili). Three branch tips were located in the upper crown, four in the middle crown, and three in the lower crown. The branches were typically located in the aspect of the crown that was most accessible from the hydraulic lift truck. Since the sampled aspect varied from tree to tree, all aspects of the study trees were sampled. One to eight ramets of each clone appeared in the sample as a function of their frequency in the orchard at the sites selected for the study.

Catkin clusters were scored with a 6-stage pollen development classification system (PDCS) at the time of tagging and then at 1-2 day intervals for the remainder of the pollen season:

- Stage 1.0 Catkins can be distinguished in the fall as individual buds on the vegetative shoot but are enclosed by bud scales.
- Stage 2.0 Individual catkins emerge from their protective bud scales, and slowly elongate from November to February.
- Stage 3.0 Individual microsporophylls develop as catkins increase in length over an extended period. Pollen catkins exude a clear fluid when pressed between the fingers.
  - Substage 3.3 Pollen catkins exude a yellow fluid when pressed between the fingers. This stage occurs approximately 7-10 days before pollen release.
  - 3.6 Catkins increase further in length and exude a clear fluid when pressed between the fingers. This stage occurs approximately 3 to 5 days before pollen release.
  - 3.9 Very little if any fluid can be pressed from the catkin. Microsporophylls are separating. Catkins bend easily and spaces are visible between sporophylls. Catkins feel light and "rubbery" when bent. This stage occurs approximately 1 to 2 days before pollen release.
- Stage 4.0 Microsporophylls begin to release pollen. Pollen release begins at the proximal end of the catkin and progresses acropetally. Pollen released from cluster is less than 10 percent of the total.
- Stage 5.0 Maximum pollen release for the catkin cluster. The majority of individual catkins within a cluster are releasing pollen. Pollen release can be divided into substages based on a visual estimate of the percent of pollen released from the catkin cluster.
  - Substage 5.2 Twenty percent of the pollen released from the catkin cluster.
  - 5.4 Forty percent released from catkin cluster.
  - 5.6 Sixty percent of pollen released from catkin cluster.
  - 5.8 Eighty percent of pollen released from catkin cluster.

;tage 6.0 Pollen release completed. All microsporophylls have opened and released pollen. Catkins are light to dark brown in color and are dry.

The 94 trees were scored with the PDCS from March 7 to March 30, 1988. When a cluster reached Stage 6.0, the individual catkins were counted and removed from the vegetative shoot. In all 27,072 individual catkins were observed on 2,108 clusters. The phenology of female flowers was also scored on the six target female sample trees using the standard 6-stage development procedure (Bramlett and O'Gwynn, 1980). The phenological scores were averaged for each ramet and clone and plotted over dates of observations.

## RESULTS AND DISCUSSION

As the spring season progressed, the observation of pollen development on individual ramets increased from Stage 3.0 through Stage 6.0. The pattern of this development cycle followed a sigmoid curve with clones showing different starting dates (Stage 4.0), midpoint release dates (Stage 5.5) and completion dates (Stage 6.0). Figure 1 illustrates the range in development among clones.

These distinct pollen development patterns can have a large effect on the availability of pollen to the female flowers of specific clones in the orchard. For cross pollination to occur among specific seed orchard clones, it is obvious that the receptive stage of the female parent must coincide with pollen release of the male parent.

The primary advantage of the pollen development classification system is that it permits an estimation of proportions of pollen released over time rather than simply a record of whether pollen is being shed or not.

For example, female strobili on clone 081069 progressed through Stages 4.5-5.6, during which most pollinations are expected to occur (Bramlett and O'Gwynn 1980), from March 22 through March 25 (Figure 2). Males 071053 and 071059 had released essentially all pollen before March 22, thus would not have pollinated female strobili on clone 081069. The other three males contributed to the pollen pool in different proportions during the period of female receptivity. Males 061025 and 081077 released 80% and 40%, respectively, during the 4 days of interest; while male 081005 released no more than 10% during the last 2 days of the same period. Thus, the PDCS scores permit a refinement of estimates of the proportionate parentage among males that qualitative scores would not.

Synchronization of male and female development on the same tree was compared on the six designated female target trees (Table 1). From these observations it appears that designated pollen development class when described as a number, may be either ahead or behind female flower development class on the same ramet depending on the individual clone. For example, in clone 71022, female flower development class was consistently ahead of the pollen class, whereas in clone 81069, the female development class was consistently behind the pollen class number.

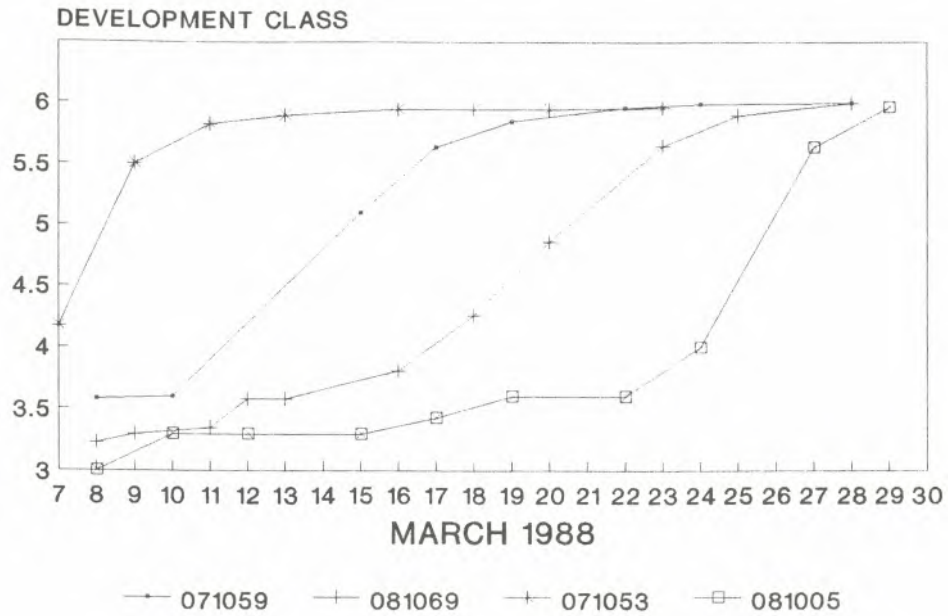


Figure 1. Average pollen development stage during March 1988 for four loblolly pine clones in the Loblolly Pine Seed Orchard, Weyerhaeuser Company, Lyons, Georgia.

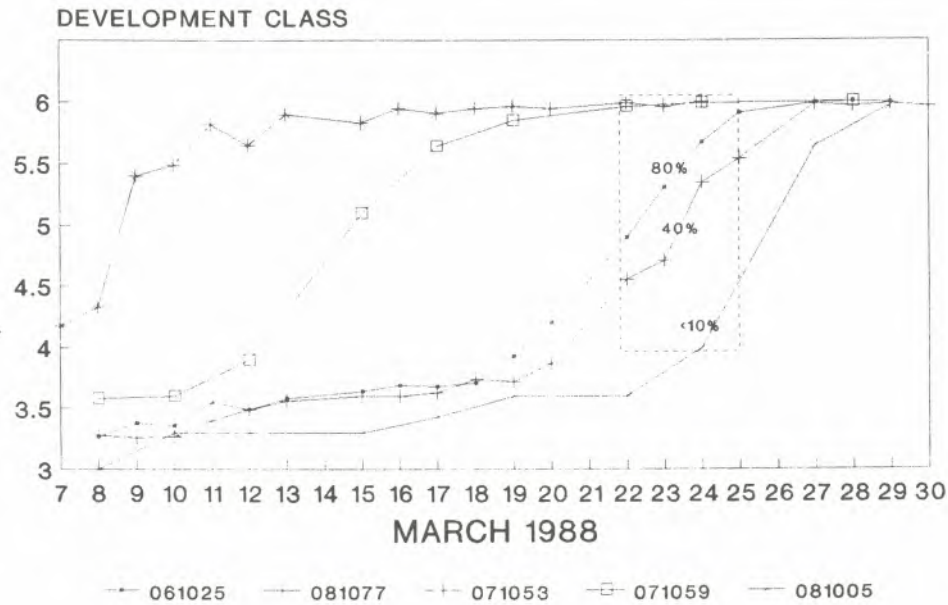


Figure 2.--Female strobilus receptivity for clone 081069 compared to catkin development and pollen release of five neighboring males. The period of maximum receptivity for the female clone (March 22-25, 1988) is enclosed in the rectangle.

Table 1.--Average scores of male and female development stages for six loblolly pine clones in the Weyerhaeuser Seed Orchard, Lyons, Georgia.

CLONE	MALE	FEMALE	DEVELOPMENT STAGE			
			MALE	FEMALE	MALE	FEMALE
021021	4.0	4.5	5.0	5.3	5.5	5.9
061031	4.0	3.7	5.0	4.5	5.5	4.8
071022	4.0	4.6	5.0	5.6	5.5	5.8
081069	4.0	3.6	5.0	4.3	5.5	4.6
081086	4.0	3.3	5.0	3.9	5.5	4.2
AVERAGE	4.0	3.9	5.0	4.7	5.5	5.1

These differences may be partially explained by the fact that the classes themselves are artificial values that are assigned to the class for identification only. Nonetheless, assigning a number to the class does allow us to average the value for a specific date and consequently to make comparisons between clones. One problem with the PDCS is that in actual practice Stage 5.0 the onset of rapid pollen release, cannot be exactly identified. Pollen release begins from an individual cluster in Stage 4.0. Designation of that stage implies that only a very small fraction of the pollen contained in the entire cluster is being released. The next identifiable class would then be 5.2, when 20 percent of the pollen has been released from the entire cluster. It is conceivable to use 5.1 or 5.05 to indicate smaller amounts of pollen release, but in practice it would be difficult to give an estimate of the amount of pollen dispersed in percentages less than 20 percent.

In observing pollen release, the normal pattern is that the more proximal catkins on the cluster begin pollen release and progressively release pollen over time acropetally. Individual catkins also begin pollen release at the proximal part of the catkin and progress acropetally. On an individual tree, a rather wide diversity of pollen development stages were observed. For example, once a tree began shedding pollen (at least one cluster classified as 4.0), shedding continued for an average of 10 days until all clusters and reached stage 6.0.

Once individual clusters reach Stage 4.0, they moved rapidly through Stages 5.2 - 5.6. The time required for a ramet to move from an average of Stage 4.0 to 5.0 was about 3 days. Another 2 days were required for the average to reach 5.5 and then in 2 more days the average was 5.8. To reach a time when all clusters were 6.0 was somewhat longer because of the delay of

some individual catkins. For practical purposes, however, when the average reached 5.8-5.9, pollen dispersal was essentially completed for a tree.

These numeric classes are not quantitative variables. Nevertheless, when they are averaged they appear to fairly accurately reflect loblolly pine pollen dispersal. Variation among ramets of the same clone was remarkably low. Even from different sites in the orchard, pollen release values agreed very closely for ramets of the same clone. Thus, the conditions that influence pollen catkin development must have been fairly constant within the orchard, and the PDCS is repeatable.

#### CONCLUSIONS

The PDCS appears to reflect the development pattern of loblolly pine pollen dispersal, and to be highly repeatable. Clones can be ranked on the basis of their pollen release dates. When key pollen development stages are averaged for a ramet, Stage 4.0 equals the beginning of pollen release, Stage 5.5 equals mid-point of pollen release, and Stage 6.0 equals cessation of pollen release.

Using PDCS, pollen release dates can be compared to female receptivity periods and proportionate paternity can be predicted for ramets or for an orchard as a whole.

Since PDCS scores were consistent among ramets in the seed orchard, it may be possible to use a small number of trees to reflect annual variation in pollen dispersal for a clone. However, the minimum numbers of observations to describe the pollen dispersal patterns for ramets, clones, and orchards have not been determined. If the dispersal pattern can be related to weather variables, extensive observation of catkins may not be needed to predict the pollen release patterns for ramets, clones, or a seed orchard.

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