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# SUPPLEMENTAL STUDY PLAN

## LONGLEAF PINE SEED PRODUCTION

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# LONGLEAF PINE SEED PRODUCTION

## INTRODUCTION

The time of peak pollen shed by longleaf pine (Pinus palustris Mill) is closely correlated with total heat accumulation after December 31, according to 10 years of record on the Escambia Experimental Forest in southwest Alabama. Heat sums were expressed either as cumulative degree-days or degree-hours above a specific threshold temperature. A threshold value of 50°F. was the best among six screened (30°, 40°, 45°, 50°, 55°, 60° F.). Total degree-days for each day was the number of degrees that maximum temperature exceeded the threshold. Degree-hours above the threshold were derived from daily maximum-minimum temperatures using the Lindsey-Newman formulas (8) 1/. Heat sums, by formula, closely approximate actual sums from thermograph traces for periods in excess of 10 days, according to comparisons made for winter-early spring temperatures near Brewton, Alabama, and Durham, North Carolina. Correlation coefficients were $r = 0.988$ for 93 days in South Alabama with a ratio (regression coefficient) of 1.063 to 1, and $r = 0.948$ for 61 days in North Carolina with a ratio of 1.068 to 1. The temperature data used for computation of degree-hours were those recorded at the local Weather Bureau Station about 3 miles NNE of the Experimental Forest.

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1/ Numerals in parentheses refer to Literature Cited.

Degree-days from January 1 to peak longleaf pine pollen shed, for the 10 years from 1957 through 1966, ranged from 1118 to 1274 and averaged 1193, with a standard error of 48.27, and a coefficient of variation of 4.05 percent. There was no relationship between degree-days and time, in days, from January 1 to peak flowering. The January 1 starting date was the best among 12 between November 1 and February 19.

Degree-hour heat sums to peak flowering declined significantly with increasing time. The relationship between degree-hours and days to peak pollen shed was best expressed by the regression:

$Y = 19059.04 - 90.193 X$, in which Y is estimated degree-hours to peak pollen shed, and X is number of days beginning with January 1. This highly significant regression accounted for 94 percent of the observed variation in degree-hours to peak flowering.

These results from 10 years of observation of longleaf pine pollen shed strongly suggest a close relationship, probably linear, between heat accumulation and development of longleaf pine flowers. The heat sums used here were all derived from air temperatures in a weather instrument shelter some 3 miles from the forest, and do not represent the temperatures actually prevailing near the trees, and flowers, that contributed to the pollen shed record. It is safe to conclude only that heat sums from shelter air temperatures were closely correlated with an environmental factor, or factors, that exerted a dominant influence on the flower development process. Presumably, this is also temperature, but is it air temperature surrounding the tree or adjacent to the flower buds? Or is it the internal temperature of the buds or shoots or other parts of the tree? Or is it some combination of all of these?

The important effect of temperature, or temperature-related factors, on the springtime growth and development of forest trees has been observed frequently, particularly with flowering phenology (1, 2, 4, 5, 7). This has been amply illustrated by the latitudinal and altitudinal sequence of flowering within a species. The action of higher air temperatures in speeding flower development is utilized, particularly in tree breeding, when shoots bearing flower buds are cut and "forced" in a greenhouse to provide a supply of pollen before female flowers on the trees are receptive (2, 6, 9, 11, 12). Normally, cut shoots are kept in water or a weak nutrient solution (11, 12). Flower-bearing shoots of longleaf pine have been forced by grafting them onto rootstocks in the greenhouse (9) since cut shoots do not otherwise produce viable pollen. Forcing in the greenhouse will result in pollen shedding as much as 2 weeks to a month (2, 11) ahead of normal, depending on the date shoots were cut, and temperatures to which they are exposed. Snyder (11) observed a significant increase in strobilus growth on cut shoots kept at 25°C. air temperature over those at 20°C.. He also reported that he, and others, had forced pollen shedding as much as 2 weeks prematurely by covering the strobili on the trees with sausage casing bags. The environment within various types of pollination bags has been investigated (10). Inside the bags, there is a pronounced increase in air temperature--as much as 18°C.--under conditions of positive radiation, and a fall below outside temperatures under conditions of negative radiation. Branches wrapped in a homogeneous material, such as polyethylene, are exposed to a saturated hot-house atmosphere that is apparently deficient in $CO_2$.

A short-term study, from December 1967 through March 1968, is proposed here to explore experimentally the relationship between temperature and the maturation of staminate strobili of longleaf pine. The variety of natural environmental conditions experienced over the 10 years of record cannot be duplicated in 1 year. In this respect, each year yields only one sample in a specific region. However, based on the observations cited above, it appears that flower development can be accelerated by artificial application of heat to flower-bearing shoots alone, whether cut and placed in the greenhouse, or left on the tree and enclosed within pollination bags. The latter procedure should more closely simulate reality. In this way it will be possible to artificially create a range of environmental conditions by exposing selected shoots to higher temperatures for varying periods of time.

## SCOPE

This short-term study supplements an existing long-term study[2/] concerned with factors behind year-to-year variations in longleaf pine seed production. It will amplify, through acquisition of experimental data, the temperature-flowering relationships hypothesized from the 10-year record of longleaf pine pollen shed. The major drawback is that temperature variations in this study will be artificially induced. The unavoidable departure from natural conditions may influence the relationships under study, but this is a risk inherent in the method. Further pertinent information will be provided by an additional two years (1967-68) of records of longleaf pine pollen shed on the Escambia Experimental Forest, and also at two locations (Sanford and Southern Pines) in North Carolina.

## OBJECTIVES

The primary objective of this supplemental study is to obtain experimental data on temperature and flowering of longleaf pine, to support or modify the following hypotheses derived largely from observations of longleaf flowering from 1957 to the present.

(1) Air temperatures, in the vicinity of flower-bearing shoots, is an environmental factor closely correlated with the development of staminate strobili on longleaf pine.

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2/ Boyer, W. D. Longleaf pine seed production. 1964. (Unpublished study plan, FS-SO-1103-1.32, U. S. Forest Service, South. Forest Expt. Sta.)

(2) The best expression of the temperature effect on flower development is cumulative heat sums, in degree-days or degree-hours, above a threshold value of 50°F.. The cumulative heat effect is linear throughout the range of temperatures normally encountered, and begins during the first weeks of the winter season (late December, early January). An upper limit to flower development rate exists, however, and heat exceeding that necessary for the maximum rate will have no effect.

(3) Degree-days to peak flowering are not influenced by variations in the time, intensity, or duration of heat exposure.

(4) Degree-hours to peak flowering decline as length of time to flowering increases. The relationship is linear.

(5) Temperatures prevailing near the flower buds have the predominant effect on development rate, rather than temperatures affecting other parts of the tree, or the tree as a whole.

## METHODS

### STUDY AREA

This study will be established on the Escambia Experimental Forest, Escambia County, Alabama. A stand of longleaf pine of flowering age will be selected, preferably consisting of younger trees with lower branches within reach of the ground. The stand should be of light density, or else form a wall of timber next to a clearing, so that all trees in the study will have the same southern exposure.

### TREATMENTS

Treatments will be a deliberate variation of the rate of heat accumulation by staminate flower buds of longleaf pine. This will be done by enclosing shoots within polyethylene bags for varying lengths of time. Air temperatures within the bags can be expected to range up to as much as $35^{\circ}$F. higher than those prevailing outside, thus increasing rate of heat accumulation and presumably speeding development of enclosed buds. Bags will be vented on the underside to prevent excessive moisture accumulation and a $CO_2$ deficiency.

Ten trees will be selected for study, and each of the following eight treatments applied, at random, to two shoots among the 16 selected on the south side of each tree. A total of 160 shoots will be represented in this phase of the study.

Treatments are:

(1) Shoots bagged for 2 weeks, beginning January 2, 1968 (off January 16)
(2) Shoots bagged for 2 weeks, beginning January 16, 1968 (off January 30)

(3) Shoots bagged for 2 weeks, beginning January 30, 1968 (off February 13)

(4) Shoots bagged for 4 weeks, beginning January 2, 1968 (off January 30)

(5) Shoots bagged for 4 weeks, beginning January 9, 1968 (off February 6)

(6) Shoots bagged for 4 weeks, beginning January 16, 1968 (off February 13)

(7) Shoots bagged for 6 weeks, beginning January 2, 1968 (off February 13)

(8) Unbagged controls

In addition to the above, two additional shoots on the north side of each tree will be marked for observation, to compare rate of natural development between flowers primarily in the shade and those exposed to direct sunlight.

A problem will arise if the coming season is one of those years with a small longleaf pine flower crop. Should the number of shoots with staminate strobili be too few to meet requirements, the number of treatments will be reduced, as necessary, by successively dropping treatments 6, 4, 1, and 2. This will reduce requirements for the exposed side of each tree from 16 to 8 shoots with staminate flowers. This will be the lower limit for conduct of the study.

## MEASUREMENTS

Flowering.--The following phenological observations will be recorded:

(1) Date first staminate strobilus on each sample shoot begins to shed pollen

(2) Date approximately 50 percent of flowers on each sample shoot are shedding pollen.

(3) Date all flowers on each sample shoot that have shed or are shedding pollen.

Temperature.--Air temperatures near flower buds, within and outside the bags, will be monitored by thermocouples connected to a portable recorder. Thermocouples will be shielded from the direct rays of the sun. At least two separate records of each condition (inside-outside) will be maintained. The recorder should be housed in a shelter, preferably a standard weather instrument shelter, in which a hygrothermograph and another thermocouple are also located. The hygrothermograph will provide a continuous standard against which the thermocouple temperature records can be checked, and adjusted as necessary. All should be calibrated with a standard mercury-in-glass thermometer before field installation. Thermocouples monitoring within-bag temperatures will be located in bags of treatment 7. Thermocouples recording open air temperatures will be kept near shoots of treatment 8. For the remaining treatments, it will be assumed that prevailing temperatures within and without the bags will average the same as those being recorded concurrently by the thermocouples. Sample data sheets to be used for recording and summarizing temperature data are appended.

Gaps in the temperature records for flowering shoots are likely to occur, on occasion, during the 3 months of study. However, the diurnal march of temperature recorded by the shelter hygrothermograph will undoubtedly be closely correlated with temperatures recorded at flowering shoots.

These correlations will be used to estimate missing temperature data by computing regressions of recorded shoot data (open and enclosed separately) over equivalent values from the hygrothermograph. Regressions for the following temperature variables will be obtained:

(1) Daily maximum temperature

(2) Daily minimum temperature

(3) Daily degree-hours

In event of recorder failure, an alternative method of estimating degree-hour totals at the shoots will be required. If so, daily maximum minimum temperatures at the shoots will be obtained from maximum-minimum thermometer, supplemented, as necessary, by estimates from the regressions. Maximum-minimum termperatures at the shoots will be used to compute daily degree-hour values by the Lindsey-Newman formulas. Degree-hour values, both measured and by formula, will also be acquired from the hygrothermograph record for each day, and the ratio between the two values obtained. Then the formula value for the shoots will be multiplied by the hygrothermograph ratio for that day to obtain an estimate of the actual degree-hour value for the shoots.

## ANALYSES

The results of this study will be evaluated in terms of (1) total days to peak flowering, starting with January 1, and (2) total heat sums from January 1 to peak flowering, in both degree-days and degree-hours above a threshold temperature of 50°F.

The existence of a temperature effect induced by the treatments will be tested by an analysis of variance of total days from January 1 to peak flowering. A significant treatment effect will indicate that shoot temperatures do influence the rate of flower development. The analysis will have the following form:

| Source | df |
|---|---|
| Treatments | 7 |
| Trees | 9 |
| Treatment x trees | 63 |
| Error | 80 |
| Total | 159 |

Single df comparisons will be made of each treatment against the check.

Next, the same analysis will be applied to total heat sums to peak flowering, for both degree-days and degree-hours. Assuming the relationships suggested by the 10-year data hold up, then treatments will not have a significant effect on total degree-days to peak flowering. This will indicate that the degree-day sums required for flowering are similar for all treatments, confirming the temperature effect. Treatments should have a significant effect on total degree-hours to peak flowering, because of the linear relationship between degree-hour requirements and flower development time. If so, the regression of degree-hours to peak flowering over development time will be computed, based on the mean values of the two variables derived from each of the eight treatments.

Should results show consistently higher heat sums for flowering of treated than untreated shoots, or a large coefficient of variation in heat sums to flowering, then the following possibilities must be considered.

(1) Temperatures within the bags exceeded optimum levels part of the time. This can be checked by subtracting from the degree-hour total for each treatment the number of degree-hours above a temperature threshold of, say, 90°F.. If such an effect is suspected, the factor may be included as a second independent variable in the regression analysis.

(2) Exposure of the shoots alone may meet only part of the flowering requirements, particularly if the whole-tree environment also enters into the picture. The regression of degree-hours over time obtained from the 10-year data suggests that some physiological requirements of the tree must be met before flowering, and hence a decrease in days to flowering must be compensated by an increase in total heat sums. Even higher shoot temperatures may be required to compensate for exclusion of the tree from the high temperature environment. If, after checking possibility (1) above, the slope of the regression of degree-hours over time derived from the experimental data, still differs significantly from that of the 10-year data, or if residual variance of the experimental regression is considerably greater, then it may be assumed that the whole-tree environment does indeed play a part. The difference between the two regressions should provide an indication of the relative importance of shoot versus whole-tree environment in the flower development process. A difference in the level of the two regressions can be expected, because of the comparatively distant source of the temperatures used to compute degree-hours for the 10-year regression.

## PRESENTATION OF RESULTS

The final results of this study will be included in a progress report covering the flowering phase of the parent study. This progress report will also be submitted, as a PhD dissertation, to the Department of Forestry of the Graduate School of Arts and Sciences, Duke University, Durham, North Carolina.

## ADMINISTRATIVE DETAILS

### ASSIGNMENT

| | |
|---|---|
| Professional | W. D. Boyer |
| Technician | A. A. Thomas |

### DURATION OF STUDY

December 1967 through March 1968.

### MAN-POWER REQUIREMENTS

| | Man-days |
|---|---|
| Professional | 10 |
| Technician | 15 |
| Clerical | 2 |

### VEHICLE REQUIREMENTS

Sedan or pick-up truck (Brewton to Escambia Experimental Forest)
500 miles

### SAFETY

No special hazards are involved in this study. Safety precautions appropriate for normal field work will be observed.

## ESTIMATED EXTRA COSTS

Travel.--(Boyer) Durham, North Carolina to Brewton, Alabama.

| | |
|---|---|
| December 1967 | |
| Per diem ($5\frac{1}{2}$ days @ $15) | $ 82.50 |
| Mileage (1400 miles @ $ .09) | 126.00 |
| March 1968 | |
| Per diem ($3\frac{1}{2}$ days @ $15) | 52.50 |
| Mileage (1400 miles @ $ .09) | 126.00 |
| Total travel | $ 387.00 |
| Analyses:--Automatic data processing of study results results | 60.00 |
| Total estimated extra costs | $ 447.00 |

## CHRONOLOGICAL LIST OF JOBS

| Job | Assignment | Date |
|---|---|---|
| Locate and mark trees and sample shoots, set up equipment | Boyer & Thomas | December 18-19 1967 |
| Place bags of treatments 1, 4, 7. | Thomas | January 2, 1968 |
| Place bags of treatment 5 | Thomas | January 9, 1968 |
| Place bags of treatments 2 & 6; remove bags of treatment 1 | Thomas | January 16, 1968 |
| Place bags of treatment 3; remove bags of treatments 2 & 4 | Thomas | January 30, 1968 |
| Remove bags of treatment 5 | Thomas | February 6, 1968 |
| Remove bags of treatments 3, 6, 7. | Thomas | February 13, 1968 |
| Change recorder charts | Thomas | Weekly |
| Check equipment & flower development | Thomas | Week-days, as necessary |
| Terminate and dismantle study | Boyer & Thomas | March 25, 1968 |

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Supplemental Study Plan Summary
W. D. Boyer - Brewton, Ala.

Aim of this study is to test the following hypotheses:

1. Air temperature is correlated with the development of longleaf pine staminate strobili.

2. The best expression of the temperature effect on flower development is cumulative heat sums.

3. Degree-days to peak flowering are not influenced by variations in the time, intensity, or duration of heat exposure.

4. Degree-hours to peak flowering decline as length of time to flowering increases. The relationship is linear.

5. Temperatures prevailing near the flower buds have the predominant effect on development rate rather than temperatures affecting other parts of the tree.

This supplemental study will be installed on longleaf pine seed trees on the Escambia Experimental Forest in South Alabama beginning in January 1968. Temperatures will be recorded near 160 flower shoots with thermocouple equipment. Differences in temperature for study purposes will be induced by inclosing flower shoots in plastic bags on predetermined schedules. Data will be analyzed by both analysis of variance and regression procedures. Results from this supplemental study and the parent study will provide data to be used in writing a Duke University thesis.