chromosomal connections were sufficiently removed to allow the chromosomes to untangle.

From a cytological point of view, it is important to have good methods to untangle meiotic prophase chromosomes, so that processes like pairing and genetic recombination can be studied at the chromosome level. A technique often used to untangle prophase chromosomes is to treat specimens with water or hypotonic salt solutions (KLASTERSKA, *et al.* 1976, BEÇAK, *et al.* 1977). These pretreatments were tried in conjunction with the GWDMC technique but were not effective (BONGA, 1978). However, the GWDS-GWS technique is an effective alternative method to untangle prophase chromosomes, at least for those of *Pinus resinosa.*

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Use of the Weibull function to quantify sweetgum germination data

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Summary

The three-parameter Weibull function was used in germination analyses of seeds from four stands of sweetgum *(Liquidambar styraciflua* L.) in central Mississippi. Stratification periods of 4 to 32 days gave equal stimulation of germination; 64 days provided much greater stimulation. There were no significant differences between stands, but there was a significant interactioa between stands and stratification period in the "b" parameter which describes the time-scale of germination. Estimates of germination response based on the Weibull parameters were very close to actual germination, and they facilitated interpretation of other components of variance.

Key words: programming (Computer), variance analysis, percentiles

Zusammenfassung

Samen von *Liquidambar styraciflua* L. von vier Herkünften aus Zentral-Mississippi wurden unterschiedlich lang stratifiziert. Hierbei zeigten sich erst nach einer Behandlung von 64 Tagen Unterschiede im Keimprozent. Zwischen den Hexkünften konnten keine signifikant unterschiedlichen Keimproeente beobachtet werden, jedoch significante Unterschiede im Keimungsverlauf.

Introduction

Germination tests are commonly used to measure seed viability and to describe the relative quality of the seed lot. The pattern of germination frequency in these tests is interpreted as an indicator of seed quality-its vigor. Quantification of differences in the pattern of germination frequency has been a traditional problem in seed research. Recently BONNER and DELL (1976) used the Weibull function for quantifying the trend in the cumulative proportion of seed germinated. The objective of this study was to evaluate the use of the Weibull function to quantify differeaces in germination between sweetgum *(Liquidambar styracif lua* L.) seed lots from different stands and trees within stands when subjected to various periods of stratification.

Procedure

Four natural stands in eastern Oktibbeha County, Mississippi, were selected for their proximity to Mississippi State University. The stands were separated from each other by a distance of at least 2 km. Within each stand five selected parent trees were an average of 20 meters apart, although this spacing varied between 10 and 25 meters. From a biological standpoint stands and trees within stands were considered as random selections.

Mature fruits were collected from the middle portion of the crown of parent trees (generally at a height of 6 to 9 meters). After air drying seeds were extracted by hand from the fruits, and empty seeds were removed with compressed air. The cleaned seeds were stored at 5.6° C until germination or pretreatment.

Samples of 200 seeds from each parent tree were subjected to each of six stratification treatments. These treatments consisted of chilling the seeds at 4.4° C for periods of 0, 4, 8, 16, 32 or 64 days after soaking for 24 hours at room temperature.

The seeds were germinated in a Stults germinator, on standard blotters at a 20°—30° C regime with a light period of 11 hourc during the higher temperature period.[1]) For a period of 15 days germination was scored daily; seeds were scored as germinated when radicles exhibited positive geotropism. At the end of the germination period all ungerminated seeds were cut open to determine the number of seeds that had the capacity to germinate.

DELL and BONNER[2]) provide comprehensive consideration

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[1]) Mention of trade names is solely to identify material used and does not imply endorsement by the U.S. Department of Agriculture.

[2]) DELL, T. R., and F. T. BONNER. Quantifying germination trends over time using the Weibull function. Unpublished manuscript.

of the Weibull function for quantification of germination patterns. In their notation any specific point in time after initiation of the germination test, is denoted by x. The proportion of germination that is witnessed at time x is F(x), the cumulative distribution function. The derivative of F(x) or probability density function is f(x), and it defines the instantaneous change in the cumulative proportion germinated occurring at time x. F(x), then, depicts the generally S-shaped curve of cumulative proportion germinated, and f(x) depicts the associated mound-shaped curve of change. By adopting the Weibull model these functions are defined as:

$$F(x) = 1 - \exp(-[(x-a)b^{-1}]^c) \quad (1)$$
$$0 \leqslant a \leqslant x, b > 0, c > 0$$

and

$$f(x) = cb^{-1}[(x-a)b^{-1}]^{c-1} \exp(-[(x-a)b^{-1}]^c) \quad (2)$$

where $\exp(-[(x-a)b^{-1}]^c)$ specifies that e, the base of the natural logarithm, is raised to the power $-[(x-a)b^{-1}]^c$. The three Weibull parameters "a", "b", and "c", are estimated from the data of a given germination test; and their interpretation will be covered in the results section of this paper.

With these estimates of the parameters it is possible to compute model-based estimates of the cumulative proportion germinated for specific times. And considering the inverse of F(x), one can also compute estimates of the time required to witness specific proportions of the cumulative germination. Other descriptive statistics such as the mean, mode and variance can also be computed with the parameter estimates.

For this study the Weibull parameters were estimated with the Fortran routine FITTER (Bailey 1974) using data from each tree and stratification period. Presently, a comprehensive program to summarize germination data, including the estimation of Weibull parameters with complete or censored data, is available from the Southern Forest Experiment Station. The Weibull parameters and various functions of them were used as response variables. Univariate analyses of variance were employed recognizing stratification treatment, stands and trees within stand as factors. Implications related to assumptions underlying these analyses were considered.

Results

Varying the stratification period resulted in a significantly ($\alpha < .05$) altered pattern of germination as illustrated by the Weibull parameters and other expressions which can be derived from them (*Tables 1* and *2*). The parameter "a" is the earliest point in time beyond which the predicted cumulative proportion germinated is greater than zero. Average values of "a" decreased from 3.10 in the control treatment to 1.95 after 64 days stratification.

Table 1. — Analysis of variance of Weibull parameters.

Source	df	"a" MS	"a" F	"b" MS	"b" F	"c" MS	"c" F
Stand	3	1.47	3.13 NS	7.71	1.67 NS	1.74	2.52 NS
Trees/Stand	16	.47	1.34 NS	4.61	8.23SIG	.69	2.09SIG
Stratification	5	4.09	9.09SIG	17.54	10.89SIG	4.32	8.82SIG
Stand x Stratification	15	.45	1.29 NS	1.61	2.88SIG	.49	1.48 NS
Trees/Stand x Stratification	80	.35		.56		.33	
	119						

Expected values of mean squares

Source	df	EMS
Stand	s - 1	$\sigma_\varepsilon^2 + r\sigma_{\mathcal{E}}^2 + tr\sigma_\alpha^2$
Trees/Stand	s(t - 1)	$\sigma_\varepsilon^2 + r\sigma_{\mathcal{E}}^2$
Stratification	r - 1	$\sigma_\varepsilon^2 + t\frac{r}{r-1}\sigma_{\alpha\beta}^2 + ts\frac{\Sigma\beta^2}{r-1}$
Stand x Stratification	(s - 1)(r - 1)	$\sigma_\varepsilon^2 + t\frac{r}{r-1}\sigma_{\alpha\beta}^2$
Trees/Stand x Stratification	s(t - 1)(r - 1)	σ_ε^2

Table 2. — Average Weibull parameters and the associated mean, mode and variance values of the probability density function for each stratification period.[1])

Stratification Period	"a"	"b"	"a + b"	"c"	Mean Germination Time	Mode	Variance
0 days	3.10	5.26	8.36	2.13	7.76	7.01	5.30
4 days	2.48	3.90	6.38	2.10	5.93	5.34	2.96
8 days	3.05	3.21	6.26	1.89	5.89	5.21	2.46
16 days	2.95	3.37	6.32	2.34	5.94	5.61	1.83
32 days	2.42	3.51	5.93	2.99	5.55	5.48	1.29
64 days	1.95	2.44	4.39	2.96	4.13	4.08	0.65

[1]) Average Weibull parameters are based on parameters fitted to germination frequency distributions of seed from twenty trees.

Another description of the changing germination period is given by the sum of parameters "a" and "b". This value defines the total time (in days) at which approximately the first 63 percent of the germination is anticipated. By increasing the stratification treatment from zero to 64 days, this time period was cut almost in half (8.36 to 4.39) (*Table 2*).

Since "a" establishes the time that germination begins and "a + b" gives the time at which approximately 63 percent of the germination is accomplished, we can think of "b" as the period of time beyond "a" during which the first 63 percent of the germination occurs. Additional insight into the scaling or multiplicative role of the "b" parameter can be obtained by reviewing equations for various statistics given in Dell and Bonner[2]).

The curve depicting the change in proportion germinated is usually assumed to be a single mound. With the Weibull model the shape of this curve is described by the "c" parameter. In the vicinity of "c" = 3.6 the shape of this curve is nearly symmetrical and approximates a normal distribution. As "c" decreases below this vicinity, the shape becomes progressively more positively skewed and exhibits a more pronounced tail to the right. When "c" is increased to values beyond 3.6, negative skewness increases. In this study increasing the stratification treatment resulted in a more symmetric germination frequency pattern (*Table 2*).

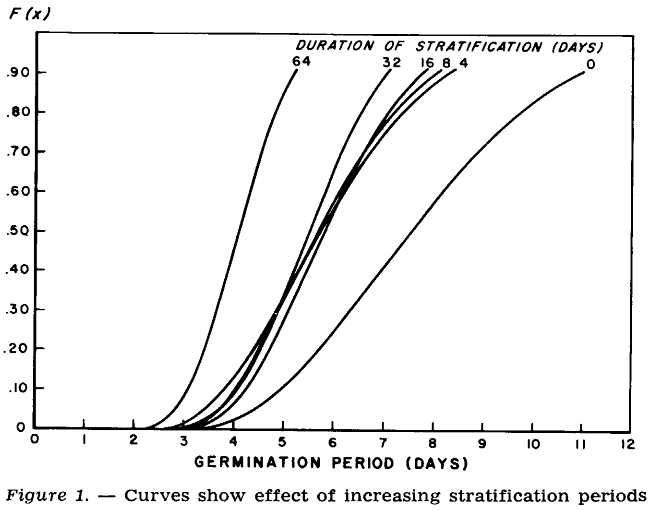

Figure 1. — Curves show effect of increasing stratification periods on cumulative proportion of germination using average Weibull parameters (*Table 2*).

Table 3. — Average "b" parameter corresponding to each stand and stratification period.

Stratification Period	STAND 1	2	3	4
0	7.12	4.64	4.34	4.93
4	4.86	3.50	3.03	4.20
8	3.54	2.32	3.36	3.59
16	3.71	3.22	3.13	3.45
32	3.76	2.68	3.80	3.80
64	2.30	1.90	2.73	2.84

There was also a trend toward a reduction in the mean, mode and variance with increased stratification periods.

The Weibull parameters were also used to compute the time it took to reach given proportions of germination; these proportions are plotted against time using the cumulative Weibull distribution function *(Figure 1)*. The total time required to attain 90 percent of the total germination was decreased by one-half by increasing the stratification period from zero to 64 days. With the latter treatment, however, 90 percent of the germination occurred within a three-day period (number of days from initial germination until 90th percentage attained) as compared with almost an eight-day period for non-stratified controls *(Figure 1)*. Time required to obtain 50 percent germination, a level commonly employed by seed analysts, was reduced almost by half. These cumulative curves in *Figure 1* suggest that there is little difference among the effects on germination of stratification periods of 4 to 32 days, but the 64 day stratification is far superior. The Weibull parameter values of *Table 2* also show this effect.

When each of the Weibull parameters was viewed as a response variable in an analysis of variance, the component of variance among stands was estimated to be zero *(Table 1)*. There was, however, a signficant interaction of stratification period and stand for the "b" parameter *(Table 1)*. Seed from some stands responded to stratification treatment differently than others. *Table 3* shows that with no stratification the "b" parameter mean for Stand 1 was much greater than the means for the other stands. The decrease in the "b" parameter mean from none to 64 days of stratification was 4.82 for Stand 1 and 2.74, 1.61 and 2.09 for Stands 2, 3 and 4 respectively. Since all stands had similar times of initial germination, these results suggest that seed lots from Stand 1 contained a considerable proportion of slowly germinating seeds which required chilling for quick germination.

The component of variance for trees within stands was significant for the "b" and "c" parameters but not for the "a" parameter *(Table 1)*. This implies that the time to reach a given percent germination is a function of the individual female parent tree and it becomes more evident when comparisons are made among time requirements for seeds of different trees to reach 90 percent germination *(Table 4)*. Trees 1—1, 2—5, 4—2, and 4—5 yield seed which may be called slow germinators, while seed of trees 2—1 and 3—3 may be called fast germinators. However, the terms fast or slow germinators are primarily applicable to seeds exposed to no stratification or to only the shortest stratification periods; the differences among seeds exposed to 64 day stratification treatments seem to be minimal. These data show that the beneficial effect of stratification is not only to hasten germination, but also to increase its uniformity (note decreasing variance values in *Table 2*). The significant component of variance for trees within stands and the decreasing variance with increased stratification treatments suggest genetic control over germination.

The application of univariate analysis of variance to the Weibull parameters and functions of them, such as a given percentile, necessitates consideration of the underlying assumptions. However, it should be clear that there is no firm basis either pro or con for a general decision regarding acceptable compliance with the assumptions for these particular quantitative expressions of the germination trend. If, for example, we had simply used the witnessed proportion germinated by the end of five days as a response variable, there would be no reason to assume a greater degree of compliance with the analysis of variance assumptions than if we had fitted the Weibull parameters for each group of seeds and used them to compute estimates of F(5). The fact is judgement of acceptable compliance with the assumptions must be rendered for each separate response expression and experiment regardless of whether the Weibull function is employed. We can at least be assured that the Weibull parameters do not express variates that are on a nominal or ordinal scale.

In this study we were willing to say that the independence assumption was justified by the experimental design and procedure employed. Additivity has been already addressed in the interpretation of interactions and so we need consider only homogeneity of variance and normality.

For each of the eight response variables ("a", "b", "c", 25th, 50th, 63rd, 75th and 90th percentiles) ther were indications from BARTLETT's tests of heterogeneous variance associated with the overall analysis *(Table 1)*. Separate analyses for each level of the stratification treatment disclosed a trend of reduced variance among trees of the same stand as the duration of stratification increased. The trend was very well defined for the scale parameter and each of the five percentiles. This is just additional evidence that stratification causes more uniform as well as rapid germination. Nevertheless, the circumstance does cast doubt on the validity of the analyses of variance. Since the larger means were associated with larger variances the logarithmic transformation was adopted and all analyses were repeated. The transformation served very well to eliminate the pattern in the variances. However, the conclusions re-

Table 4. — Effect of length of stratification treatment on time (in days) to reach 90 percent germination of seed of twenty trees.

Stratification Period	Tree No. 1-1	1-2	1-3	1-4	1-5
0	19.3	11.6	10.4	10.7	11.1
4	12.1	9.3	7.7	9.0	7.4
8	11.0	8.4	7.9	8.4	7.2
16	10.0	8.4	7.3	7.5	7.6
32	9.5	7.1	6.8	6.7	7.4
64	5.6	4.7	4.9	4.9	4.5
	2-1	2-2	2-3	2-4	2-5
0	7.7	9.4	10.4	8.9	12.3
4	5.7	7.0	7.1	6.2	10.4
8	5.4	7.2	6.5	6.1	10.0
16	6.0	7.1	6.8	6.7	10.8
32	6.0	6.0	6.5	6.2	8.2
64	4.5	3.9	4.6	4.4	6.1
	3-1	3-2	3-3	3-4	3-5
0	12.0	9.1	7.8	10.7	11.5
4	8.6	8.3	6.2	8.3	8.6
8	9.2	7.7	6.5	7.9	9.3
16	9.1	7.0	7.0	7.7	9.1
32	7.6	6.9	6.9	7.5	7.3
64	5.1	6.0	5.1	5.9	5.6
	4-1	4-2	4-3	4-4	4-5
0	11.7	12.6	8.1	9.0	13.2
4	9.2	11.3	6.2	6.3	11.8
8	9.2	10.2	6.6	7.1	9.5
16	8.3	10.0	6.3	6.0	8.5
32	7.1	9.2	6.2	6.6	7.2
64	6.3	6.8	5.1	4.6	5.8

garding significance were the same as those given in *Table 1.*

The question of normality was pursued through the use of a procedure by SHAPIRO and WILK (1965). For each of the eight response variables, data from the sample of five trees of a given stand — stratification level were utilized in computing the test of normality. Such tests were made with both original and transformed values. A total of 384 W values were thus computed. In the overview these tests provided strong assurance that the assumption of normality was acceptable with each of the response variables. For example, with the significance level set at the 1 in 20 level and after having conducted 24 tests related to the "b" parameter only one test declared a significant departure from normality. To some extent the tests for normality on the location parameter were hampered by the coarseness that was accepted in fitting this parameter. The previously mentioned program for summarizing germination data does provide more refinement in the estimation of the location parameter. Another conclusion regarding these tests was that with Stand 1 and no stratification there was a disproportionate occurrence of normality rejections for the collection of response variables considered. The conclusion as has already been presented is that one of five trees in Stand 1 produced seed which were very slow to germinate without stratification.

Discussion

It is already known of course, that sweetgum exhibits shallow seed dormancy, yet responds well to cold, moist stratification (BONNER, 1974). However, the extent of variation in seed response to stratification among separate stands and trees within individual stands was not known. Fitting Weibull parameters to the data from this study clarified the stratification effect and provided a way to aptly examine the variation in the populations sampled.

The overall stimulation of germination by stratification is clearly seen by the plots cumulative proportion germinated over time *(Figure 1)*. Since initial germination is hastened by only about 2 days, one can infer that stratification had its greatest effect on the slow-germinating seeds of the population. This effect is also illustrated by the increasing distance among plot curves. There is a wider spread in time required to reach 90 percent germination than is required at the lower percentiles *(Figure 1)*. Stratification also alters the germination frequency curves toward more symmetrical distributions.

Stratification periods of 64 days clearly provided the greatest stimulation to germination, but 64 days may be longer than most users want to leave their seed in stratification. Thirty days is a more common stratification period although these results indicate very little difference in germination between a 4 day and 32 day stratification period. On the basis of parameters "a", "b" and "a + b" *(Table 2)* and the time required to attain 90 percent germination (*Figure 1*), effectiveness of stratification may be classified into 3 categories: rapid uniform germination (64 day treatment), intermediate germination (4 to 32 day treatment), and slow heterogeneous germination (control). The "c" parameter does not provide such a clearcut classification; the overall trend is for values to increase with longer stratification periods.

One point that was not examined, which could explain some of the interaction of stratification period and stands and trees within stands, was the relative phenology of the trees and stands. Differences in maturity date among trees at the time of seed collection can certainly influence the response of seed to stratification. Immature seeds should benefit more form stratification than fully mature seeds; thus, seeds from Stand 3 may have been more mature at collection than seeds from Stand 1.

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