Genetic and Maternal Influences on Virginia Pine Seed Germination

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Summary

In Virginia pine seed lots from a complete diallel mating design (excluding self pollination), the germination time trend was under strong genetic influence. General combining ability (GCA), specific combining ability (SCA), and reciprocal and maternal effects were evaluated in an analysis of variance of seed germination parameters related to the Weibull function. The GCA, maternal, and reciprocal effects were consistently significant for parameters of the time trend in the proportion germinated; SCA effects were not significant and explained only a small portion of the total variance. Tree breeders and nurserymen could utilize these genetic and maternal effects on germination performance to produce uniform seedlings for outplanting.

Key words: Combining ability, Weibull distribution, dormancy.

Zusammenfassung

Bei *Pinus virginiana* Mill. Sämlings-Nachkommenschaften eines vollständigen Diallels (das Selbstung ausschließt), war der Trend in der Keimzeit unter starkem genetischen Einfluß. Die allgemeine Kombinationseignung (GCA), die spezifische Kombinationseignung (SCA) sowie reziproke und mütterliche Effekte wurden in einer Varianzanalyse mittels Keimparametern geschätzt, die zur Weibull-Funktion in Beziehung gesetzt wurden. Die allgemeine Kombinationseignung, mütterliche und reziproke Effekte waren für die Parameter des Zeittrends im Keimverhalten durchweg signifikant. Die spezifische Kombinationseignung war nicht signifikant und erklärte nur einen kleinen Teil der Gesamtvarianz. Züchter und Baumschuler könnten diese genetischen und mütterlichen Effekte des Keim-Ablaufs benutzen, um Sämlinge zu erzeugen, die zum gleichen Zeitpunkt ausgepflanzt werden können.

Introduction

Speed and uniformity of germination are important for the production of quality seedlings in forest tree nurseries. Rapid germination permits seedlings to establish themselves before the seedbed deteriorates, and when all the seeds in a bed germinate at about the same time, culling may not be needed. A seedling that germinates long after its neighbors is at a competitive disadvantage, and often must be culled. Considerable progress has been made on modifying environmental conditions to obtain rapid, uniform germination, but little information is available on genetic influences. The study described here evaluated the germination time trend of full-sib seed lots from a complete diallel cross in Virginia pine (*Pinus virginiana* Mill.).

Materials and Methods

Five Virginia pines were selected on the basis of accessibility in a young open-grown stand on the Lee Experimental Forest, Buckingham County, Virginia. The five trees were pollinated in a complete diallel mating design in April 1971, and cones were enclosed in screen wire cages in April 1972. All mature cones from each full-sib family in the diallel cross and additional open-pollinated cones from the same female trees were collected in October 1972. The seeds were extracted and kept separate by individual cones in each pollination treatment. Empty seeds were separated from filled seeds by flotation in 95 percent ethanol. The filled seeds from each of four randomly selected cones per cross were placed on moistened Kimpak pads in separate 13 × 17 × 7 cm plastic trays. The number of filled seeds tested for each family varied from 106 to 363 for the cross pollinations, from 1 to 64 for self pollinations, and from 164 to 305 for wind pollinations. A total of 5608 seeds were observed.

Stratification is not normally prescribed for Virginia pine seeds and was not performed in this study. The 114 trays were arranged on a laboratory bench in 4 randomized blocks under continuous fluorescent lighting. Ambient temperatures for the study were 22—26° C. Germination was recorded at 4, 6, 7, 10, 15, 21, and 30 days. The test was terminated at 30 days, and x-radiographs showed that all ungerminated seeds were filled and assumed capable of germination.

The response variables used for analysis were the parameters and related statistics from the Weibull function. This function has been used by Bonner and Dell (1976) and Rink, *et al.* (1979) to quantifiy the time trend in the germination response of forest tree seeds. Two additional manuscripts contain background on the method (Dell and Bonner[1])) and on the specifics of the computer program (Polmer and Dell[2])) that is used to facilitate applications including the ones reported here. A critical assumption is that the distribution of germination times for the seed that will ultimately germinate is unimodal.

The following provides notation and definitions related to the Weibull function used in this paper. Extensive background is given in Bonner and Dell (1976). The cumulative and probability density functions are also specified in Rink *et al.* (1979).

Let x represent a speciefied period of time, in days, since the germination test started. Three parameters (a = location, b = scale, and c = shape) define for the Weibull function the trend in the cumulative proportion germinated, for seed that have the capacity to germinate. The a parameter can be interpreted as the earliest time at which the proportion germinated is greater than zero. The b parameter has a multiplicative or scaling role. For the Weibull, approximately 63 percent of the germination takes place by time a + b. Thus, b can be viewed as the period of time beyond time a required to accomplish the first 63 percent of the germination. The c parameter defines the shape of the distribution, which is essentially symmetric for c = 3.6. The cumulative distribution function defines for a given time (x) the proportion germinated. The inverse of this operation for the Weibull distribution is given as,

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[1]) Dell, T. R., and F. T. Bonner. Quantifying germination trends over time using the Weibull function. (Unpublished manuscript, Southern Forest Experiment Station, U.S. Forest Service.)

[2]) Polmer, B. H., and T. R. Dell. GERM — A computer program to summarize data of seed germination tests with an option for fitting the Weibull function. (Unpublished manuscript, Southern Forest Experiment Station, U. S. Forest Service.)

x_p = (time to reach a specified percent (p) of germination) $= a + b\,[-\ln(1\text{-}p)]^{1/c}$ where ln indicates the natural logarithm. Thus, $x_{50} = 5.5$ specifies 50 percent germination by 5½ days.

For each tray of seeds we estimated values for the parameters a, b, and c by maximum likelihood methods (POLMER and DELL[2])).

Based on these parameters, we then computed the time to reach 25, 50, 75, 90, and 95 percent germination. The genetic components of variance for observations of the germination test were analyzed with a general least squares analysis for a diallel mating design (SCHAFFER and USANIS 1969). The germination observations of self-pollinated seeds were excluded from the analyses because of the relatively low number of filled seeds from self pollinations.

Results and Discussion

Germination of individual families began after 3 to 4 days, and the cumulative germination increased rapidly during the next 10 days for most families. Differences in germination percentages for a given observation day were consistently related to the female parent. The average cumulative germination graphs were plotted for each family *(Fig. 1).* The differences in the family germination curves are very pronounced and demonstrate a strong genetic influence on germination behavior. For example, families with tree 2 as a female parent have a cumulative germination curve that is considered ideal for nursery production *(Fig. 1,* female 2). These families have a rapid initial germination time and achieve almost total germination by 10 days. This germination performance would conceivably produce optimum growing space conditions in the seedbed as all trees would be approximately the same age and a high percentage of seeded spots would have a seedling.

In contrast, families with female tree 5 as a parent *(Fig. 1,* female 5) germinated slowly, taking about 15 days to reach 50 percent cumulative germination. After 30 days, the cumulative germination was only around 70 percent for families with female tree 5. This type of germination is obviously undesirable for nursery production. Between these two extremes, families 1, 3, and 4 showed intermediate cumulative germination curves with the male parent 2 generally showing the most favorable germination response and male parents 3 and 5 showing the least favorable germination response and male parents 3 and 5 showing the least favorable germination response *(Fig. 1).*

Although only a few seeds were present in some of the self-pollinated families, germination was poor for families 5 × 5, 4 × 4, and 3 × 3. Family 1 × 1 performed relatively well, but only a small number of seeds were tested.

The germination trends for wind-pollinated families for each of the female parents were generally intermediate among the specific single crosses. It would appear from these data, that the background mix of pollen has an average effect on the germination trends that is approximately equal to the average of the single crosses in this study.

Effects of general combining ability, specific combining ability, female parent, and reciprocals on the germination

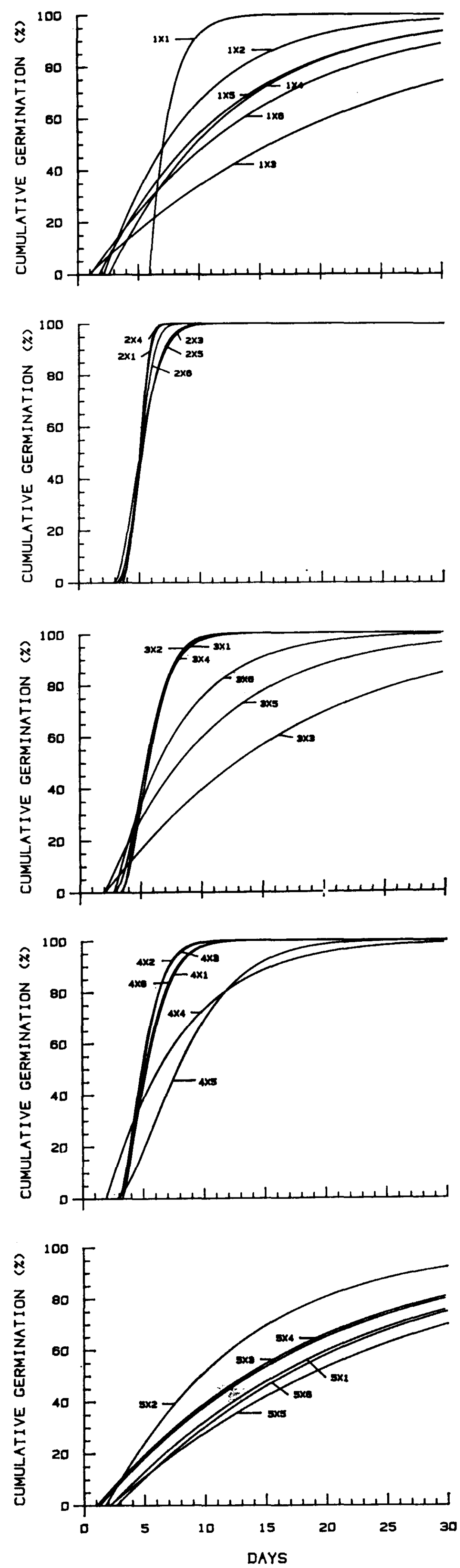

Figure 1. — Average cumulative germination of Virginia pine seed lots plotted from the Weibull distribution function parameters over a 30-day observation period. Each sagment represents a different female tree, and the female parent is listed first. Thus, 1—5 is the cross between female 1 and male 5. Male 6 = wind.

Table 1. — Estimates of variance components for Virginia pine response variables of Weibull function.

Component of variance	Variable							
	a	b	c	x_{25}	x_{50}	x_{75}	x_{90}	x_{95}
	----------------percent of total variance----------------							
General combining ability	9	32*	17*	14*	29*	33**	34**	34**
Specific combining ability	1	5	3	0[1/]	2	3	4	4
Maternal	10*	15*	8	4	11*	13*	14*	14*
Reciprocal	0	9*	11*	6	9*	9*	9*	9*
Error	80	39	61	76	49	42	39	39

1/ Negative estimates were replace by zeros.

* SATTERTHWAITE's (1946) approximate F-test was significant at the 5 percent level.

** SATTERTHWAIT's (1946) approximate F-test was significant at the 1 percent level.

observations were evaluated in the analysis of variance of the diallel mating design *(Table 1)*. Bartlett's test for heterogeneous variance was performed for the eight response variables (a, b, c, 25th, 50th, 75th, 90th, and 95th percentiles). Data from each cross--diallel and wind-pollinated--were included in Bartlett's tests where a sufficient number of replications were available for computing a sample variance. Heterogeneity was indicated for each of the eight response variables in a pattern similar to that reported in previous work with germination data (RINK, *et al.* 1979). Rapid germination tended to be associated with small variation among replications. For each response variable, except the shape parameter, families with larger means also have larger variances.

The standard remedial procedure for this pattern of heterogeneity is the logarithmic transformation. However, Bartlett's test indicates that the logarithmic transformation failed to stabilize the variances of any variable except the scale parameter. This result contrasts with that of RINK *et al.* (1979).

Analyses on the untransformed data are reported here with the caution that the homogeneity assumptions for analysis of variance were not met by our data. This failure distorts the significance levels and reduces the sensitivity of the F-tests to an unknown degree.

In the analysis, general combining ability (GCA) was a significant source of variation for the b and c parameters from the Weibull function, the 25th, 50th, 75th, 90th, and 95th percentiles *(Table 1)*. The significance level increased with time, and the percent of total variance explained by GCA increased as the percentile increased from 25 to 95. At number of days to reach 95 percent germination (x_{95}), GCA was the major source of genetic variation in the analysis and accounted for 34 percent of the total variation.

The significant effects of general combining ability indicate an additive genetic variance among the parent trees. They indicate that a positive response would be expected from a recurrent selection program for the germination characteristics observed in this study. This conclusion is also supported by the similarity in germination between wind-pollinated seeds from a female parent and the average performance of that in female parent in specific crosses.

The specific combining ability (SCA) had no significant influence on the observed germination variables and consistently explained only a small percentage of the total variation (0—5 percent). The lack of a significant SCA response for any germination variable indicates that specific crosses in the diallel mating design are not deviating from the general performance of the parent tree.

The maternal influence was statistically significant for the percent of normal germination (PNG), Weibull parameters a and b, and for days to reach 50, 75, 90, and 95 percent germination *(Table 1)*. The proportion of variation explained by the maternal influence ranged from 10 to 15 percent for the significant responses.

The maternal effects were consistently important in the analysis of variance of the diallel cross for each germination time with the exception of x_{25}. Greathouse (1966) has observed a similar effect for full-sib Douglas-fir *(Pseudotsuga menziesii* (MIRB.) FRANCO) families. Maternal influences in this mating design are usually interpreted as effects due to the special environment of the female parent (FALCONER 1960). This effect could be expected in the five separate female Virginia pine parents where nutrition, microsite, or other edaphic features could influence seed development. Pines also have a rather unique seed development sequence. The "endosperm" of the pine seed is actually female gametophyte tissue and is consequently haploid. There is no triploid tissue or union with the pollen parent nuclei in the "endosperm." During germination, the only male influence is in the embryo itself, and any influences of the female gametophyte tissue on seed germination would appear in the analysis as maternal influence. The female gametophyte and the female egg nuclei are also genetically identical since they originate from a single cell of the meiotic division of the megaspore mother cell. Thus, in pines, the female effect in a diallel analysis includes not only the special environment of the female tree and a possible effect of the seedcoat but also the gametophyte effect of the "endosperm" on seed germination. These two effects could be separated in clonal seed orchards if the diallel crosses were replicated on individual ramets of a given clone.

Reciprocal crosses of the best germinators, trees 2 and 4, produced the highest germination percentages. With tree 2 as a female parent and 4 as the male, germination was 100 percent after 7 days. The reciprocal cross 4 × 2 had germination of 97 percent at 7 days and increased to 99 percent by 10 days.

In contrast, reciprocal crosses of trees 5 × 1 and 1 × 5 had much poorer average germination--only 69 and 82 percent at 30 days. This difference reflects a reciprocal effect since it cannot be explained on the basis of maternal effects alone. Reciprocal effects were significant for Weibull parameters b and c, and for days to reach 50, 75, 90, and 95 percent germination. The percent of total variance explained by the reciprocal effects, however, was small--9 to 11 percent.

Conclusions

The germination of the full-sib Virginia families from a diallel mating design indicates strong genetic influence on the germination process. Since seed dormancy is an interaction of the environmental conditions with the genotype of the seed, these results should be interpreted as reflecting genetic influences under the specific environment of the study. It is possible that other environmental conditions could mediate the genetic influence on germination. For example, stratification could increase the speed of germination for the families with slower rates of germination, and families such as tree 1 should be routinely stratified. However, tree 2 would not benefit from stratification. In a mix of such families, stratification would increase overall uniformity.

The greatest differences between germination of these seed lots occurred during the first 4 to 10 days of germination. As the study progressed, cumulative germination increased and differences between seed lots gradually decreased. The results indicate, however, that both the germinative energy and the germinative capacity are related to the genotype of the parents.

The Weibull function appears to be useful in quantifying tree seed germination. Furthermore, the parameters associated with the function are valid response variables for consideration in an analysis of variance (RINK *et al.* 1979). The advantage of the Weibull function in describing the germination process of pines is that the function smoothes out the cumulative germination curve for visual or statistical comparison of particular sources of seed germination. Furthermore, the parameters a, b, and c can also be used as response variables.

Nurserymen and tree breeders could improve speed and uniformity of germination through recurrent selection but would have to weigh potential benefits against possible gains or losses in other genetic characteristics of the seed supply. The general lack of specific combining ability of seed lots in this study suggests that little could be gained by specific crosses for seed germination. The strong maternal influence could be a very valuable source of control of seed germination. Individual families of wind-pollinated seedlings would have more uniform germination than mixed seed lots and would certainly be expected to produce greater uniformity in nursery beds. The strong maternal influence could also be useful in the selection of female parent trees for controlled pollinations. Females that consistently produced highly viable seed with favorable germination records would be most desirable as progeny test female parents.

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Sensitivity of Seed Germination and Seedling Root Growth to Moisture Stress in four Provenances of Pinus halepensis Mill.

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Summary

The effects of water potential of the substrate on germination and on the first stage of root growth in seeds from four sources (Israel, Marocco, Greece and Italy) of *Pinus halepensis* MILL. have been studied.

They were kept for thirty days in light (16 hours photoperiod at 10.000 lux) and at the temperature of 20 ± 0.5° C. The water potentials between 0 and −8 bars are obtained by the use of polyethylenglycol solution (PEG 6.000).

Germination energy, germination rate and root growth appear to be substantially different in the four sources.

Significant reduction of germination percentage and of germination value (GV) are already observed at −2 bars, while root growth, which appeared to be less susceptible to water stress, is affected only at −4 bars.

The behaviour of the four provenances under stress was very differentiated: the water potential which induces significant reductions of germination percentage and of root growth varies from −2 bars to −6 bars in the more or less susceptible provenance, respectively.

The survival ability of the seedlings under situations of water stress, also estimated in respect to root growth, showed to be significantly different between the provenances.

The results are also discussed in relation to the climatic conditions of the zones of seed origin.

Key words: Pinus halepensis, provenances, moisture stress, germination, root growth.

Zusammenfassung

Es wurde der Einfluß des Wasserpotentials des Substrats auf die Keimung und die erste Wurzelentwicklung bei 4 Saatgutherkünften von *Pinus halepensis* MILL. aus Israel, Marokko, Griechenland und Italien untersucht. Die Keimung erfolgte bei 20 ±0.5° C unter 16stündiger Tagesbelichtung mit 10000 lux über 30 Tage. Die Wasserpotentiale zwischen 0 und −8 Bar wurden durch Polyäthylenglycol-Lösungen (PEG 6000) erreicht. Keimkraft, Keimprozent und erstes Wurzelwachstum waren bei den 4 Herkünften unterschiedlich. Das Keimprozent sank schon bei −2 Bar, während das Wurzelwachstum, welches vom Wasserstreß weniger beeinflußt wurde, erst bei −4 Bar reagierte. Die 4 Provenienzen verhielten sich unter Streßbedingungen sehr unterschiedlich, d. h. dasjenige Wasserpotential, welches das Keimprozent und das Wurzelwachstum signifikant negativ beeinflußte, variierte bei den Provenienzen von −2 bis −6 Bar. Die Überlebensfähigkeit der Keimlinge zeigte unter Wasserstreß ebenfalls signifikante Provenienzunterschiede. Die Ergebnisse werden auch im Zusammenhang mit den klimatischen Bedingungen in den Saatgutursprungsgebieten diskutiert.

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