VARIATION IN SEEDLING GROWTH RATES: THEIR GENETIC AND PHYSIOLOGICAL BASES

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Abstract.--Studies of 45 progenies of loblolly pine (Pinus taeda L.) reveal that seed coat thickness, seed weight, condition of the embryo, seed treatment, and rate of germination can account for some 20% of the variation in initial seedling size and growth rates. These and other seed properties are affected by the genotype and environment of the mother tree and can affect progeny test results as well as having a strong influence on the results of nursery management practices.

Additional keywords: Seed properties, genetics, Pinus taeda L., growth rate.

INTRODUCTION

There has been a great deal of effort expended on attempts to reduce the generation time for tree improvement programs--particularly through the use of infrared gas analyzers and seedling selection on the basis of greenhouse and nursery performance. These attempts were largely unsuccessful. This lack of success, we believe, was due in part to the confounding effects that the genotype of the mother tree, year-to-year variation in the environment, and other factors have on early germination and growth of tree seedlings.

The studies reported here are part of an effort to quantify and separate these confounding factors from the effects of the genetic characteristics of the zygote on the growth and yield potential of the seedlings under field conditions. These efforts have been only partly successful. Nonetheless, the results have important implications for tree planters, managers of forest nurseries and workers in the field of Tree Improvement.

Previous findings

Simple correlations between seedling size in the nursery and size at increasing ages decrease systematically and are particularly poor when the seedlings used for the correlation are less than 3 years of age (Lambeth 1980), This concept is well developed in discussions of changes in heritability with time (Franklin 1979, Namkoong and Conkle 1976, Namkoong et al. 1972).

Seed weight, seed coat thickness, polyembryony, seed stratification requirements, number of days required to germinate, and a number of other seed and seedling characteristics affect initial growth rates and seedling size (Righter 1965, Nanson 1965, 1967, 1969, Perry 1976). These characteristics

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are often determined by the genotype of the mother tree and the nursery environment. They have little to do with the genotype of the zygote and can be considerably modified from year to year (Silen and Osterhaus 1979, Perry 1976). Often the differences in seedling size generated by these seed and early germination phenomena persist for 15 or more years but the intrinsic growth rate of the seedlings is not altered (see for example Overton and Ching 1978, Sluder 1979, and Wakeley 1963).

Large seedlings can suppress small seedlings growing in the nursery bed and exaggerate initial differences in seedling size. Hence arrangement of plants in the nursery and arrangement of plants after they are out planted can considerably bias progeny test results (unpublished data of Adams et al. 1973, Dierauf).

Accurate prediction of future growth on the basis of early progeny performance requires that the various factors, genetic and environmental, that regulate early germination and growth be taken into account. Two recent attempts to do this with progenies of loblolly pine (P. taeda L.) have been relatively successful (Cannell et al. 1978, Robinson and van Buijtenen 1979).

Cannell et al. (1978) have made correlations between seedling growth rates and progeny performance under field conditions. They achieved their results by delaying the measurements of the seedlings of the 16 loblolly pine progenies they studied until they were 140 mm tall ("when the effects of seed size no longer affected daily growth rates") and by growing the progenies under varying conditions of water stress (the major variable of the field environment). There was an interaction between progeny and water stress such that those progenies that grew best under conditions of low water availability had daily growth rates that were correlated with the results of 8-year-old tests of the same progenies on upland sites and those progenies that grew most rapidly in well watered nursery conditions had growth rates that correlated positively with the results of 8-year-old tests on dry sites. There were appropriate differences in the root-shoot ratios of the progenies that corresponded well with the interaction with water stress and with differences in performance under field conditions. The literature review on early progeny testing for this excellent paper is probably one of the most comprehensive in the forestry literature.

Robinson and van Buijtenen (1979), in a study of seed weight, percent of seedlings of a progeny with distinct terminal buds, and percent of seedlings of a progeny without basal branches showed a positive correlation with 5, 10, and 15-year volumes for a loblolly pine progeny test in Texas.

Studies of diallel crosses with white pine (P. strobus L.), Sitka Spruce (Picae sitchensis (Bong.) Carr.), and Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco var. menziesii) indicate that a large proportion of the variation in size among progenies is attributable to differences among mother trees (Campbell 1971, Kriebel et al. 1972, Samuel et al. 1972). Reciprocal crosses with slash pine (P. elliottii Engelm. var. elliottii) showed 30% differences in volume and gum yield at age 11. There were significant differences in growth rates of 4 and 7 **pairs** of reciprocal crosses with slash pine at age 3 (Susan Kossuth, personal communication, U. S. Forest Service).

Progeny tests with loblolly pine conducted by the North Carolina Tree Improvement Cooperative indicated that maternal determination of seed size may be one of the variables related to early progeny performance (Table 1 and Figure 1, Perry 1976). The correlation between seed weight and early progeny performance is often poor and other seed variables affect early seedling size and growth rates. These variables include polyembryony, seed coat thickness and ability to shed seed coats promptly.

Table 1.--Relative weights of open-pollinated seed taken from selected and unselected trees of loblolly pine (Federal Paper Board Co., North Carolina). Commercial check = seed from trees selected at random in natural stands. Rogued clones = seed from mediocre clones which have been rejected in the tree improvement program. Select clones = seed from outstanding trees. Some of the difference between commercial check and seed orchard trees is attributable to the superior cultural circumstances of the trees in the orchards and some is a reflection of genetic differences in ability to produce seed among the different categories of trees.

Classification	Seed Weight (mg seed-1)	% of Commercial Check	% of Rogued Check
Commercial check	23.2		90
Rogued clones	26.8	116	
Select clones	36.5	157	137

MATERIALS AND METHODS

Open-pollinated seed from 40 loblolly pine clones of the North Carolina Forest Tree Improvement Program (North and South Coastal clones of the Weyerhaeuser Company) were used in the studies reported here.

One hundred seed of each progeny were weighed individually to the nearest .1 milligram and then mounted so that X-ray images of their internal structure could be prepared. The seed were classified into 5 categories on the basis of their X-ray images:

1. Seed apparently sound.
2. Embryo defective: image fuzzy, not opaque, not fully developed, distorted, cracked, other.
3. Gametophyte defective: criteria as above.
4. Both embryo and gametophyte defective: same criteria as above plus: seed empty and seed with indistinct residue of embryo and gametophyte (a ghost image).
5. Seed polyembryonic.

The relative thickness of the seed coats of a given progeny was estimated in thousandths of an inch by using a graduated air-photo-dot scale and placing it

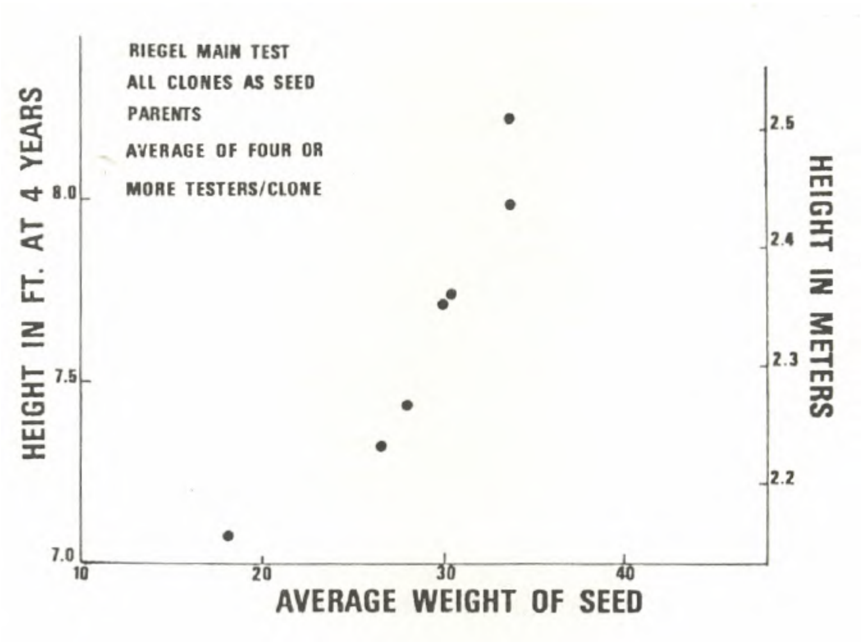

Figure 1.--Effects of seed weight of maternal parent on progeny performance when pollen from a single parent is used (loblolly pine data age 4, Federal Paper Board Company). Differences among female parents accounted for 88 percent of the variation in seed weight while differences among males only accounted for 12 percent of the variation (Perry 1976).

over the X-ray image of the seed. The averaged value of measurements from 10 seeds was used in subsequent statistical analyses.

Each seed was then planted in an individual container and daily records of its early germination and growth were kept. The nominal dimensions of the containers used to grow each seedling were 3" x 2" x 9" deep. "Pro Root", a calcined clay equivalent to "Sorbolite" or "Kitty Litter" screened to an average particle size of 2 mm was used as the growth medium. The containers were then used in two separate experiments. For the first experiment reported, they were placed in the 26° day, 22 night of the Phytotron at IN.C. State University. The watering schedules and nutrient solutions and general care of the plants represented the standard practices of the N. C. facility (Downs and Bonaminio 1976). For the second experiment, they were placed in a typical greenhouse with the thermostat set for 23 during the day and 17 at night. Incandescent lights were used to interrupt the dark period in both the phytotron and the greenhouse.

For the first experiment, 20 seeds of progeny (still kept individually), were placed in a pipette washer with 10_{100} to 13 water flooding over them and changing every 5 minutes for 96 hours before they were planted in the phytotron.

For the second experiment, 10 seeds of each progeny were planted without treatment in the greenhouse in the same type of containers and soil medium. They were watered by a mist system for 5 minutes twice a day. They were fertilized twice a month with fertilizer solution in accord with the recommendations of the manufacturer.

Records of germination and growth were taken daily in the phytotron for 86 days (i.e., when germination was complete). The plants were then moved to a test plot at our School Nursery where they have been measured once a week during the active growing season and at least once a month during January and February. There were 1350 seed at the beginning of the phytotron study. Some 1050± seedlings from these studies are still being measured.

Records of germination and growth were taken daily in the greenhouse for 201 days (in order to allow time for complete germination). The seedlings from this study have been discarded.

The data from these studies were stored on magnetic disc drives of the IBM 370 at the Research Triangle Computing Center and the Statistical Analysis System (SAS) was used for the subsequent analyses.

RESULTS

The results are illustrated in Figures 2-11. Statistical analyses reveal that there were significant differences among progenies in all characteristics measured: seed coat thickness, seed weight, number of days required to germinate, number of days required to shed seed coats and the average size of progenies on all dates measured.

Multiple regressions of seed weight, seed coat thickness, number of days required to germinate and number of days between germination and seed coat shed reveal that variation in the number of days required to shed seed coats is the best single predictor of initial seedling size and accounts for 13% of the variation in seedling size at day 86. The best multiple regression equation for predicting initial seedling size only accounted for 20% of the variation in initial seedling size and included the variables of number of days required for seed coat shed, seed weight and seed coat thickness. Appropriately, seed coat thickness was negatively correlated with initial seedling size.

Surprisingly, the frequency distribution of seed sizes for several of the progenies was discontinuous and at least bimodal. This plus the observed relationship between seed coat thickness and seed weight may explain in part why average seed weight can sometimes be such an inadequate predictor of seedling size and subsequent growth.

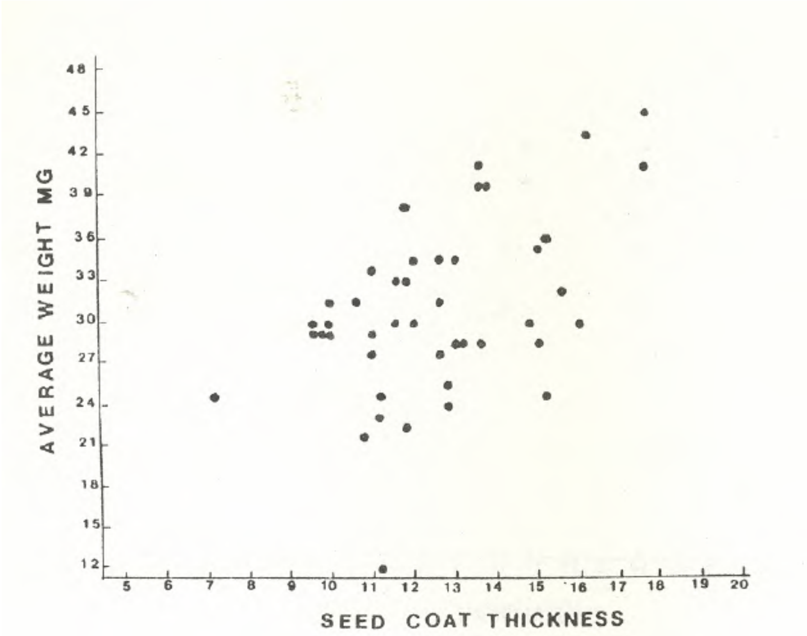

Figure 2.--Average weight vs. seed coat thickness. The seed coat is maternal tissue and its thickness and weight affect early growth and progeny performance. Seed weight = 13 + 1.36 (seed coat thickness), r^2 = .26. Weight is in milligrams. Seed coat thickness and other variables confound the direct relationship between seed weight and initial seedling size and growth rate.

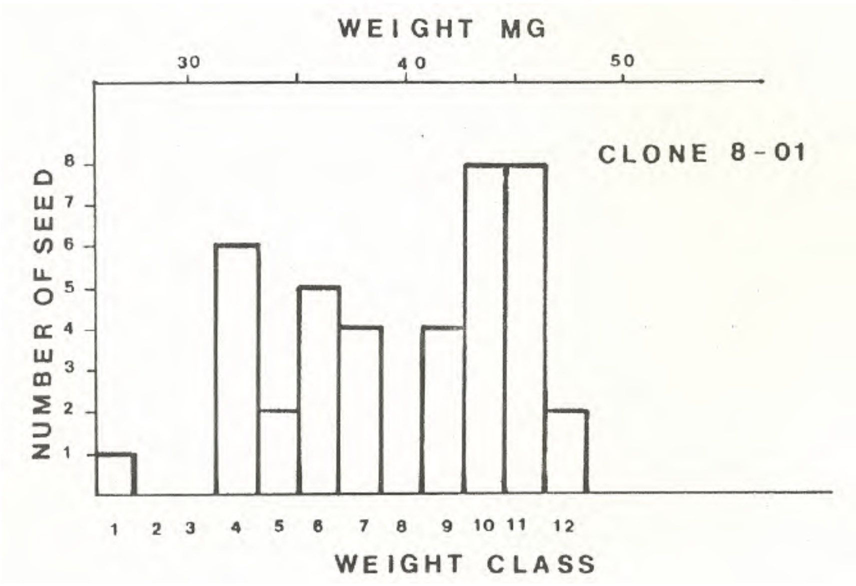

Figure 3.--Frequency distribution of seed weights for clone 8-10. Several progenies had seed weights which yielded discontinuous frequency distributions of the type observed here. Could the seed have come from different ramets in the orchard? Peculiar frequency distributions of the type observed here could he one of the reasons why the relationship between initial size and seed weight is so poor.

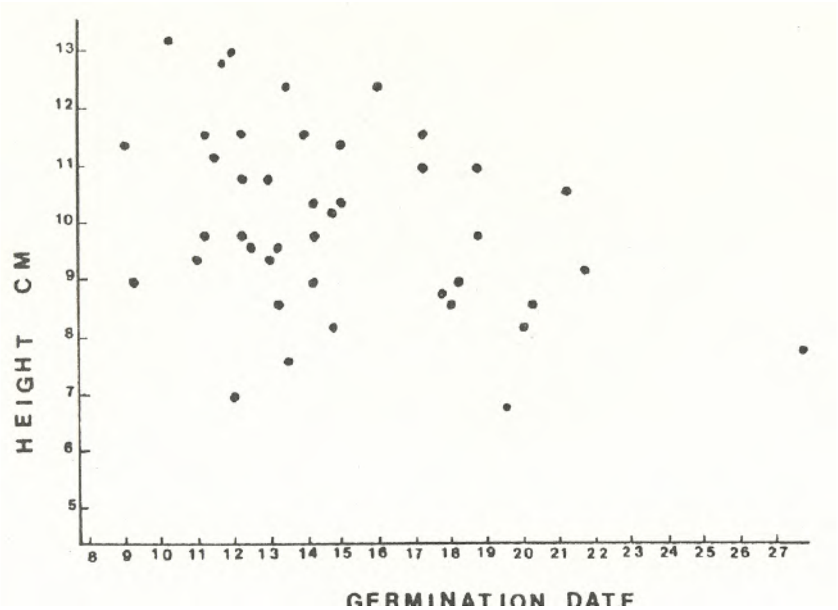

Figure 4.--Height of seedlings in the phytotron at day 86 vs. germination date. These seed were cold soaked in well oxygenated running water for 96 hours prior to planting. The slowest family to germinate required an average of 28 days. This contrasts markedly with the studies of the same seedlot germinated without treatment in a greenhouse. See Figure 8.

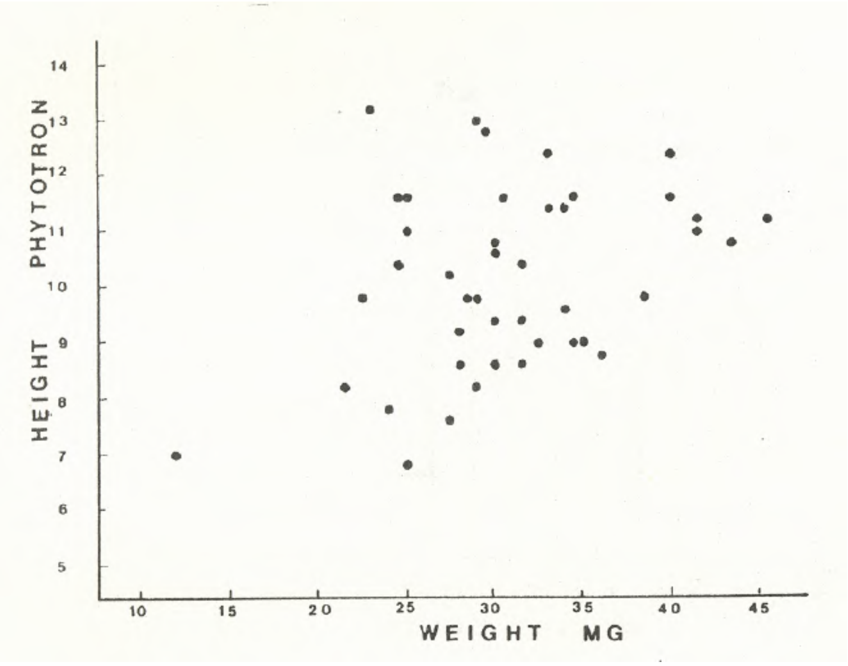

Figure 5.--Height in the phytotron (day 86) vs. seed weight in milligrams. With the exception of 5 families, there is a fair trend. Contrast these results with those obtained in the greenhouse (Figure 9).

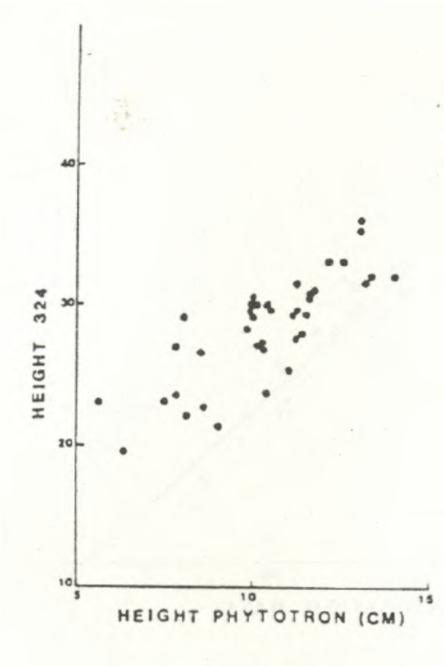

Figure 6.--Height on day 324 in the field vs. height on day 86 in the phytotron. At the end of the first year in the field the relative sizes of the progenies did not change materially.

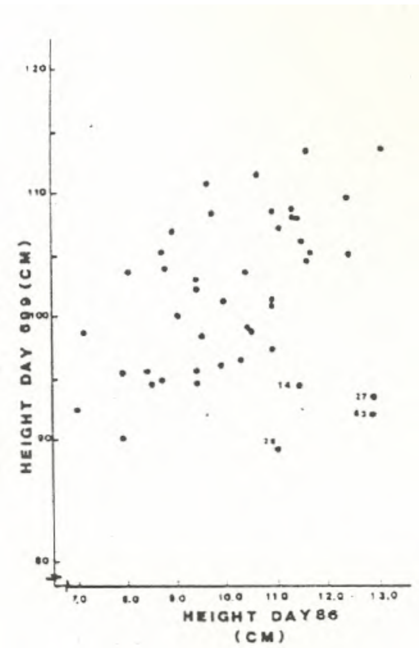

Figure 7.--Height day 699 in the field vs. height day 86 in the phytotron. Only four families have made major changes in rank relative to their sizes at day 86 in the phytotron. The effects of initial plant size are still apparent.

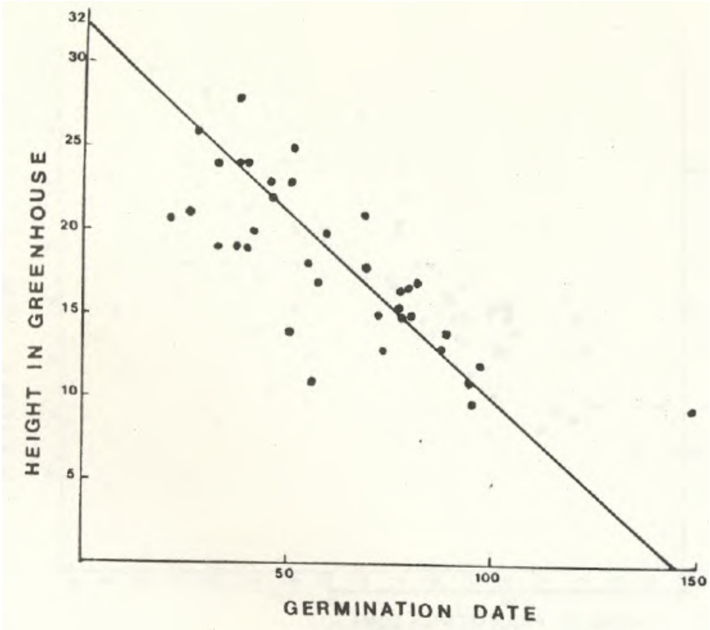

Figure 8.--Height in the greenhouse, day 201 from planting vs. germination date. In this experiment, the seeds were planted with no pretreatment. Germination date had a major effect on the initial height of the seedlings. The results contrast markedly with those observed with partially stratified seed in the phytotron. (r = 0.61. Other variables not significantly correlated with initial height: seed weight, seed coat thickness, weight x thickness.) Compare with Figure 4.

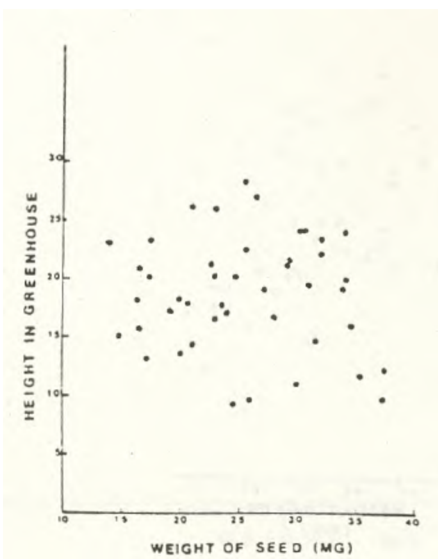

Figure 9.--Weight of seed in the greenhouse vs. height in the greenhouse at day 201. There is no detectable correlation. This contrasts with the phytotron results. See Figure 8.

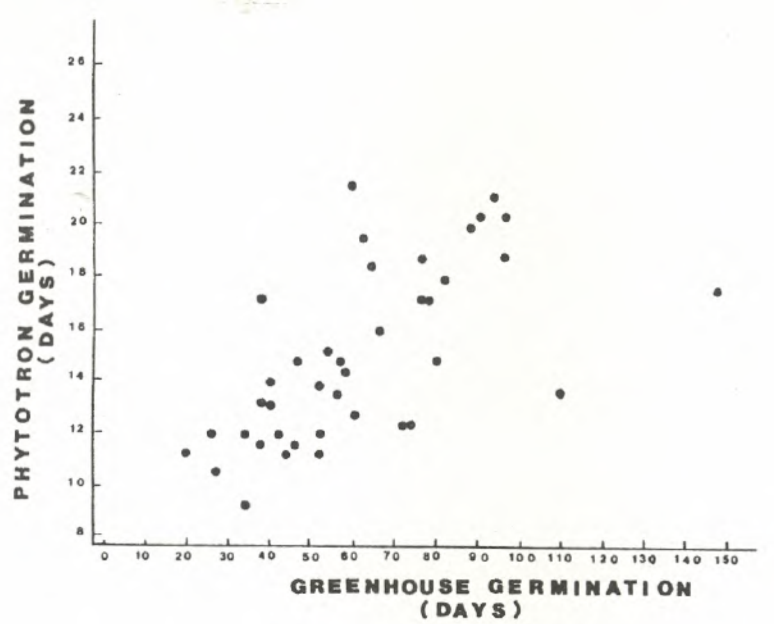

Figure 10.--Correlation between the number of days required to germinate in the phytotron and the number of days required to germinate in the greenhouse. There was a definite correlation in spite of the differences in seed treatment (r^2 = .35).

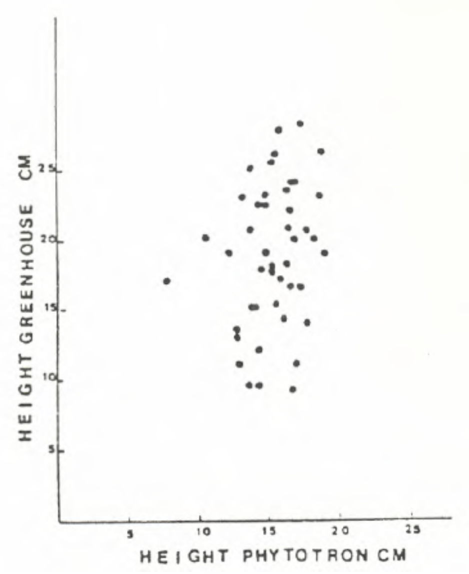

Figure 11.--Height in the greenhouse vs. height in the phytotron. There may be some weak correlation. The greater spread of the greenhouse heights is a reflection of the lack of pretreatment of the greenhouse seeds. Seed handling, treatment, and nursery environments can alter the variability of progeny test results.

Examination of individual seedlings and progenies during the entire study reveals factors that affect initial size and growth rates. One of these is the characteristic of some progenies to have their cotyledons trapped for long periods. Over 80% of the seedlings of one progeny had their cotyledons trapped by the seed coat for 77 days after planting. Initial growth of these seedlings was stunted and crooked.

The results of attempting to relate initial size and growth performance with condition of the seed and gametophyte as estimated from examining their X-ray images were not consistent. Sometimes seed classified as sound did not perfo/m any better than seed with embryos or gametophytes which were classified as fuzzy or defective. For some progenies there were distinct differences in the growth performance of seeds that were classified as sound or defective; while for others, the defective seeds of a progeny were as likely to give a superior performance as were the sound ones. Seeds with obvious defects (category 5) either did not germinate or produced weak plants that did not survive in the phytotron.

There was no correlation between seed weight and number of days required to germinate. Nor was there between seed coat thickness and number of days required to germinate or to shed seed coats.

Comparison of the results in the phytotron and in the greenhouse (Figures 4, 5, 8, 9, 10, 11) reveal that seed handling and rearing conditions can considerably alter the relative importance of seed variables in determining initial size and growth rates. Seed weight and seed coat thickness were significant variables in determining initial size and growth rates when seed were prewashed in well oxygenated cold water for 96 hours and grown in the phytotron. The number of days required to germinate masked the effects of other seed variables in determining initial seedling size and growth rates when seeds were not pretreated (the greenhouse study).

SUMMARY AND DISCUSSION

Non-destructive measurements of seed properties (seed coat thickness and seed weight) plus variation in the number of days required to shed seed coats accounted for only 20 percent of the variation in the size of seedlings 86 days after planting. Number of days required to shed seed coats was a better predictor than number of days required to germinate (show any sign of emerging radicle) in accounting for variation in size at day 86. These variables could only account for 7 percent of the variation in size after two years of growth in a test planting.

Attempts to account for early differences in seedling size on the basis of examination of X-ray images of the seeds prior to planting gave inconsistent results. Identification of partially developed seeds with obviously defective embryos and gametophytes was relatively easy. However, it was not possible to consistently rate other seed on the basis of X-ray images that were distinct or indistinct, as being sound or defective, or to correlate these classifications with early seedling size and growth.

The trapping of cotyledons by seed coats and various unknown seed characteristics may account for part of the residual 80% of the variation in initial seedling size. Some of the variation may be due to differences in the properties of the zygote.

Changes in seed stratification and other nursery management practices, such as root and top pruning can radically alter the importance of seed and seed germination characteristics on initial seedling size.

Frequent measurements during two years since outplanting reveal that progenies with seedlings that were initially large or small tend to remain relatively large or small. There were no significant differences in the duration of seasonal growth, or in K, the specific rate of growth ($(dH/H)/dT = k$, H = height, T = time) that could account for the variation in progeny sizes after two years of measurements. The correlation between initial size and current size decreased with time. However, only 4 of the 45 original progenies have made height growth radically different than would have been predicted on the basis of their initial size.

In many of our progeny tests, we are not separating the effects of genetic variation of the zygote from genetic and environmentally determined attributes of the seed and the seedling environment. The seed coats and gametophytic tissue of a conifer seed commonly amount to 80% or more of the weight of the seed and are determined by the genotype of the mother tree.

Seed size and seed quality increase as the ramets of a seed orchard increase in age and vigor. Seeds from orchards which are fertilized, irrigated, and protected from insects are going to be larger and of better quality than "commercial check" seed which was gathered from random trees in the forest.

Changes in the environment during a particular year of seed maturation and changes in nursery practice can further alter the size and relative performance of a progeny.

These non-genetic effects can account for a large portion of the variation in the size of progenies at age 4. These non-genetic effects decrease with time but continue to confound interpretation of progeny test results for 20 years or more. They seriously alter estimates of additive and non-additive genetic variance and can lead to exaggerated estimates of the amount of gain in vigor or growth that can be attributed to altering the genotype.

The array of genetic and environmental factors that combine to effect the size and condition of seeds is complex. No single measurement, such as seed weight, seed coat thickness, or condition of the embryo is sufficient to account for all of the variation observed. Even with careful stratification, and sorting of seed by size, there are unpredictable variations in the average size of seedlings produced from a given ramet in a given year.

In the nursery, large seedlings of one progeny interact to suppress the smaller seedlings of another progeny. Hence potential differences in individual seedling size and average size of a progeny can be greatly exaggerated by random or mixed plantings in the nursery.

The literature reviewed in this paper (Campbell 1971, Kriebel et al. 1972, Perry 1976, Samuel et al. 1972) indicate that the factors shown to be of influence in these studies of initial size and growth rates are affected by the genotype and environment of the mother tree and may have a strong influence on the early years of seedling development. Seed from reciprocal crosses, from different ramets of the same clone, and from different year classes may vary markedly in their potential for germination and growth. Mixture of seed of these different categories could confound experimental and practical results of tree improvement programs when decisions are made at early ages.

We recommend that both research and commerical collections of seed from a given mother tree be kept separate, receive custom treatment, and be planted separately in the nursery.

Seeds of progenies with a long stratification requirement or a low germinative energy or germinative capacity should receive special seed stratification treatments and be planted earlier in the nursery and at different seed bed densities than seedlings with contrasting characteristics.

The simple act of managing the seeds of different progenies and different year-classes separately will increase the yields of plantable seedling per hectare of nursery, reduce the magnitude of variability among seedlings, and yield a more uniform and vigorous plantation in the field.

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