in the early and the late part of the growing season was generally similar. The average density was slightly reduced as a result of the most intense thinning and fertilizer treatment.

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Statistical Validity of Single-Tree Plot in Forest Genetics Research

By E. C. Franklin[1])

Single-tree plots have received much attention in recent literature, primarily because some researchers in forestry are proving to themselves what agricultural researchers were reporting over 40 years ago: that, because of the large amount of environmental variability on the average planting site, large block sizes result in poor statistical precision (Wood and Stratton 1910, Batchelor and Reed 1918).

The single-tree plot permits minimum block sizes for a given number of entries in a complete block design. However, several arguments have been advanced against the use of single-tree plots. This discussion will center on several questions about the use of single-tree plots raised by Shiue and Pauley (1961) and Evans et al. (1961). My purpose is to show that the theoretical arguments and experimental results presented by these authors can be interpreted in a way which suggests that the obstacles to the use of small-plot designs, single-tree plots in particular, are not as serious as is generally assumed. I am not arguing against or in favor of the use of single-tree plots, because the choice of plot size in forest genetics research usually involves many considerations other than those which are purely statistical (Wright and Freeland 1960).

Normality and Homogeneity of *Error* Variation

The major points made by Shiue and Pauley concerning plot size can be briefly summarized. In provenance and progeny tests, error variation consists of two parts: environmental and genetic. "Such genetic [error] variation, especially in the case of controlled pollination, is not necessarily distributed normally in the offspring" (Shiue

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and **Pauley** 1961, p. 117). Environmental error variation will satisfy the normality assumption in most cases where conventional experimental designs are properly used. Thus, if genetic error variance constitutes a large proportion of the total error variance, the normality assumption may not be satisfied. When single-tree plots are used, a large amount of genetic diversity in a species could lead to nonnormal distributions of the genetic portion of error variation. Under similar circumstances, error variation in single-tree plots will probably fail to satisfy the homogeneity assumption for the analysis of variance, in which case single-tree plots cannot be used. The normality and homogeneity assumptions should always be tested statistically before analysis of variance is attempted on data from provenance and progeny tests using single-tree plots.

Evans *et al.* (1961, p. 76) also argue against the use of single-tree plots. They, too, feel that "... perhaps the most formidable argument against the single-tree plot is the likelihood that the genetic portion of variation from plot to plot is non-normal." These authors do not support their argument with references or data, but their discussion is in general agreement with **Shiue** and **Pauley** (1961).

According to the central limit theorem (**Freund** 1962), the distribution of plot means will approach the standardized normal distribution of errors as plot size is increased. Therefore, if there were nonnormality with data from single-tree plots, the means of larger plot sizes would be expected to approximate more closely the standardized normal distribution. But the probability of finding nonnormality or heterogeneity in single-tree plot data, the utility of testing for failure of assumptions for the analysis of variance, and the handling of such data when certain assumptions do fail are questions which deserve re-examination on the basis of the discussion by **Shiue** and **Pauley**.

Influence of Genetic Diversity on Normality and Heterogeneity Assumptions

Presence of a particular degree of genetic diversity in a population is not sufficient evidence for predicting the form of the distribution of genetic sampling variation which might obtain under a given experimental design. Presence of genetic diversity means only that there is genetic variance. That the distribution of random genetic error variation is not necessarily normal is a rather unformative statement in the absence of any evidence against the generally accepted normality assumption (**Cochran** and **Cox** 1957, p. 91). In fact, there are several good arguments in favor of the normality assumption for this type of data. Genetic segregation in quantitative traits creates a continuous range of variation because of the actions and interactions of many alleles at many loci. Stabilizing natural selection causes frequencies of intermediate genotypes to be highest (Lerner 1958), thus tending to mold the array of genotypic values into something approaching a normal distribution. This tendency is also found in most cases of artificial selection (**Haldane** 1954). Even when the number of loci for a trait and the number of alleles at those loci are small, it is only at extreme allelic frequencies that arrays of genotypic values might deviate substantially from normality.

Ratio of Genetic to Environmental Variation

The main points for discussion concerning the relative amounts of genetic and environmental variation in provenance and progeny tests have been clearly outlined by **Shiue** and **Pauley**: "Although the environmental variation can be safely assumed to be normally distributed, the total variation due to genetic and environmental factors may or may not deviate from normality depending on the type of genetic variation and the relative magnitude of genetic and environmental variation. In cases where the genetic variation is abnormally distributed and has a similar or greater magnitude than the environmental variation, data from a single-tree-plot test cannot be validly analyzed with the ordinary methods. When the source of variance is predominantly from the environmental effects such as is the case in most agricultural crops, the assumption of homogeneity of variance may be valid, but provenance or progeny test samples of forest trees will frequently not satisfy this assumption, and single-tree-plots are therefore not usable" (1961, p. 117—118).

Experience has shown that provenance or progeny tests of tree species established on representative ranges of sites will frequently have higher proportions of environmental variation than agricultural crops, because they are tested on sites of much greater diversity. In any case, "Metrical traits almost inevitably show, upon analysis, a large environmental component of variation" (**Brewbaker** 1964, p. 53). With provenance and progeny tests, the most commonly encountered situation is that the environmental component is greater than the genetic component. Therefore, no matter what distribution genetic values take, the distribution of phenotypic values will usually approximate the normal. Furthermore, if nonnormality or heterogeneity is present, corrective procedures are available which are independent of plot size (**Box** 1953); therefore, single-tree plots as well as multiple-tree plots are useful in these cases.

Experimental Evidence

Nonnormally distributed error variation in data from single-tree plots of red pine, reported by **Blake** (1959), was discussed by **Shiue** and **Pauley**. They argued that, since red pine had considerably less genetic diversity than jack pine, data from jack pine progeny and provenance tests using single-tree plots would probably not satisfy either the normality or heterogeneity assumptions. But these authors failed to clarify at least two important facts:

(1) In testing for kurtosis and skewness, **Blake** found significant kurtosis for height and diameter distributions based on data from single-tree plots. But **Blake** also reported significant skewness for 16-tree plots in diameter distributions and significant kurtosis in 9-tree and 25-tree plots for diameter distributions. These multiple-tree plots were grouped from the same data which were analyzed as single-tree plots.

(2) **Blake** did not have any way of specifying what proportion of the total variation was genetic; therefore, the critical question of the nonnormality of the genetic portion of the total variation cannot be approached on the basis of this data. Thus, **Blake**'s results do not in any way support the position of **Shiue** and **Pauley**.

Efficacy of Statistical Tests of Nonnormality and Heterogeneity

Throughout the discussion of plot size, **Shiue** and **Pauley** emphasize the importance of the normality and homogeneity assumptions for the analysis of variance. They recommend that statistical tests be routinely done to validate these assumptions prior to using the analysis of variance. From the practical point of view, how much deviation from normality is permissible without loss of effectiveness for a given decision rule? Nonnormality affects statistical efficiency, the confidence levels of tests of significance, and confidence ranges for estimates of variables (Cochran 1947). When the F statistic or the two-tail t is used to test differences among means, the confidence level is affected very little by deviations of the magnitude usually encountered in experimental data (Box 1953, Cochran 1947). True significance levels usually range between 4 and 7 percent for the 5-percent level and between ½ and 2 percent for the 1-percent level (Cochran 1947). The one-tail t test can be more strongly affected by deviations from normality. Tests among variances and variance components are sensitive to deviations from normality, and special caution is warranted for these. But for the most commonly used tests, i. e., F and two-tail t tests, the normality assumption is usually satisfied by biological data.

According to Cochran (1947), there are two principal effects of heterogeneity of error variation:

(1) Loss of statistical efficiency, and
(2) Loss of sensitivity of tests of significance.

The magnitudes of these effects are roughly proportional to the size of the differences between error variances. The most important fact again is that the F test is so insensitive to heterogeneity of error variance that Box (1953) says it is neither necessary nor useful to test statistically for it. He explains that, when plot sizes are about equal, preliminary testing for heterogeneity may lead to more wrong conclusions than if homogeneity is assumed. The most serious distortions of significance levels due to heterogeneity come about throughout use of pooled error terms such as those often used for F tests (Cochran 1947). Use of pooled error terms requires extra caution.

In summary, Shiue and Pauley (1961) have overemphasized the efficacy of statistical tests for determining the validity of assumptions for the analysis of variance, and, while recognizing the dangers of heterogeneity, they have neglected to emphasize the general robustness of the frequently used F and two-tail t tests.

Summary

Presence of genetic diversity in a population is not sufficient basis for predicting, *a priori*, nonnormality or heterogeneity in data from progeny and provenance tests with single-tree or multiple-tree plots. Rarely will the environmental component of error variation be so small that the genetic component could cause failure of the assumption of normality in the analysis of variance of such data. Data previously presented and discussed in the literature cannot be unambiguously interpreted to mean that single-tree plots are more likely to yield nonnormal distributions of error variation than are multiple-tree plots.

Confidence levels for the most frequently used tests of significance, F and two- tail t tests of means, are affected very little by deviations from normality and homogeneity of the magnitude usually encountered in experimental data. Therefore, it is neither necessary nor useful to routinely apply statistical procedures for testing normality and homogeneity.

When corrective procedures for nonnormality and heterogeneity of error variation are needed, they can be applied to data from single-tree plots as well as multiple-tree plots. Therefore, no special restrictions are encountered through the use of single-tree plots on this account.

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The Morphology and Behavior of Meiotic Chromosomes of Douglas-Fir

(Pseudotsuga menziesii)

By Gordon K. Livingston[1])

Introduction

Two recognized species of *Pseudotsuga* occur in North America (Munz and Keck, 1959). *Pseudotsuga menziesii* (Mirb.) Franco is the most widespread, and ranges 3000 miles from Canada to Mexico and extends eastward 1000 miles from the Pacific coast to the Rocky Mountains. *Pseudotsuga macrocarpa* Mayr., on the other hand, is restricted to southern California. Four other species occur in the eastern part of Asia and on the islands of Formosa and Japan. There are seven published accounts of the chromosomes of the American species, one of which (Thomas and Ching, 1968) deals in part with an Asiatic species. With the exception of Thomas and Ching's study on the somatic chromosomes of Douglas-fir in central Oregon, no studies have dealt with the chromosomal cytology of *Pseudotsuga* growing in its natural range. The author knows of no published report describing the meiotic chromosomes of Douglas-fir in its native habitat and of only one study in Europe, where this species was introduced in the 19th century.

Sax and Sax (1933) first reported the chromosome number of *Pseudotsuga menziesii* as $n = 13$. Their observations were based on haploid, female gametophyte tissue from trees planted at the Arnold Arboretum. However, both

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