REPEATABILITY OF TRACHEID CHARACTERISTICS IN DOUGLAS-FIR SEEDLINGS GROWN UNDER TWO PHOTOPERIODS

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INTRODUCTION

When it is possible to make multiple measurements of the same character on an individual the phenotypic variance can be partitioned into the variance between individual s and the variance within individuals. From this subdivision it is possible to obtain a measure of the constancy of the repeated measurements within individuals by calculating the intra-class correlation coefficient. This coefficient is called the repeatability (Falconer, 1960) and is calculated thus :

$$r = \frac{\sigma B^2}{\sigma W^2 + \sigma B^2}$$

where: r = repeatability; σB^2 = between-individual variance component; σW^2 = within-individual variance component.

The genetic interpretation of the two components is as follows: The within-seedling variance component is due entirely to differences in environment operating within the individual. For measurements repeated in time the variation is due to temporary changes in environment between successive measurements. For measurements repeated in space, the within-individual variance is due to localized circumstances within the individual operating during development. This temporary or localized variation is termed special environmental variance. The between-individual variance is caused by variations in the genotypes of different individuals and by environmental variation which affects each individual permanently, in the case of temporal repetition; or as a whole, in the case of spatial repetition. This is termed general environmental variance. Therefore, the genetic meaning or significance of repeatability is thus:

$$r = \frac{V_G + V_{Eg}}{V_G + V_{Eg} + V_{Es}}$$

where: V_G = genetic variance; V_{Eg} = general environmental variance; V_{Es} = special environmental variance.

One use to which this ratio can be put is as an estimate of the upper limit of broad-sense heritability. If general environmental variance were nonexistent, which is extremely unlikely, the formula would be:

$$r = \frac{V_G}{V_G + V_{Es}} = \frac{V_G}{V_P}$$; where, V_P = phenotypic variance.

This ratio is therefore equal to broad-sense heritability.

When making suppositions about heritability from repeatability, one must be certain that the repeated measurements were made on what is in fact, genetically the same trait. That is, they were the result of identical physiological and developmental processes,

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A second use of repeatability and where it finds its greatest use in animal breeding is in determining how many measurements are necessary to obtain a sufficiently accurate estimate of an individual's real productive ability. If there is only slight variation between repeated measurements, the repeatability is high and the first measurement would be almost as reliable as the average of four (Lush, 1945).

There are few instances where repeatability was calculated in forestry experiments' One study was concerned with crown characteristics: interwhorl length, number of branches per whorl and a knottiness index in 15-to 20-year-old Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco) (Campbell, 1961). The only repeatabilities significantly greater than zero were in the order of 30 to 40 percent, for trees growing on an area with very uniform site conditions. The author concluded that selection of superior phenotypes with respect to crown characteristics would be successful only on the most' uniform sites.

Another study was on yearly shoot elongation in red pine [*Pinus resinosa* Ait.) plantations (Lester and Barr, 1966). Repeatabilities were calculated for ages 4 to 6 and 9 to 11. Between 4 and 6 years the repeatabilities averaged approximately 50 percent while between 9 and 11 years they fell to around 30 to 35 percent. The drop was thought to be related to the onset of competition between trees at about age The authors therefore recommended that progeny tests be planted at wide enough spacings so that growth up to an age of approximately 10 years would be limited only by the potential of individual trees and not by factors of competition.

MATERIAL AND METHODS

The material studied in this experiment was Douglas-fir seedlings grown for 132 days under a short (10 hour) and a long (15 hour) photoperiod. Fifteen seedlings from the short day and 12 from the long day were examined. Measurements of radial cross-sectional tracheid characteristics were obtained from six radial rows of tracheids equally spaced around the cross-section but omitting compression wood. Earlywood and latewood were characterized by taking the mean value of five cells per radial row in the respective zones. The whole ring value was calculated as the mean of all cells in the radial row. This varied between 14 and 90 in different seedlings. Tangential cell diameter was obtained from a sample of 10 cells from four locations around the ring located at points one-third of the width of the ring in from the outer circumference. An index of specific gravity was calculated by dividing tangential double wall thickness by radial cell diameter.

Variance components were obtained from an analysis of variance (table 1) by equating the expected mean square with the calculated one.

Table 1.--Analysis of variance for calculation of within-seedling and between-seedling variance components.

Source of variation	Expected mean square
Between seedlings	$\sigma_W^2 + n\sigma_B^2$
Within seedlings	σ_W^2

Note: n = number of estimates within a seedling.

The repeatabilities of these spatially repeated measurements could therefore be calculated.

RESULTS AND DISCUSSION

The variance components are graphed and the repeatability values calculated from them are noted in figures 1 to 5. Explanations for some of the more outstanding variation patterns might be speculated upon.

The first graph (fig. 1) is concerned with radial growth as measured by number of cells in the radial direction. The repeatabilities are quite high because of the comparatively large between-seedling variance components in each case. In the long day there is a very large between-seedling variance component in comparison with the short day. The reason may be that in the short day a single environmental factor (photoperiod) severely limits growth in all seedlings while in the long day this specific factor is not (or is less) limiting and therefore the seedlings could grow to their fuller individual potentials limited only by a variety of other factors (environmental, genetic and interactions) which might be different for different seedlings.

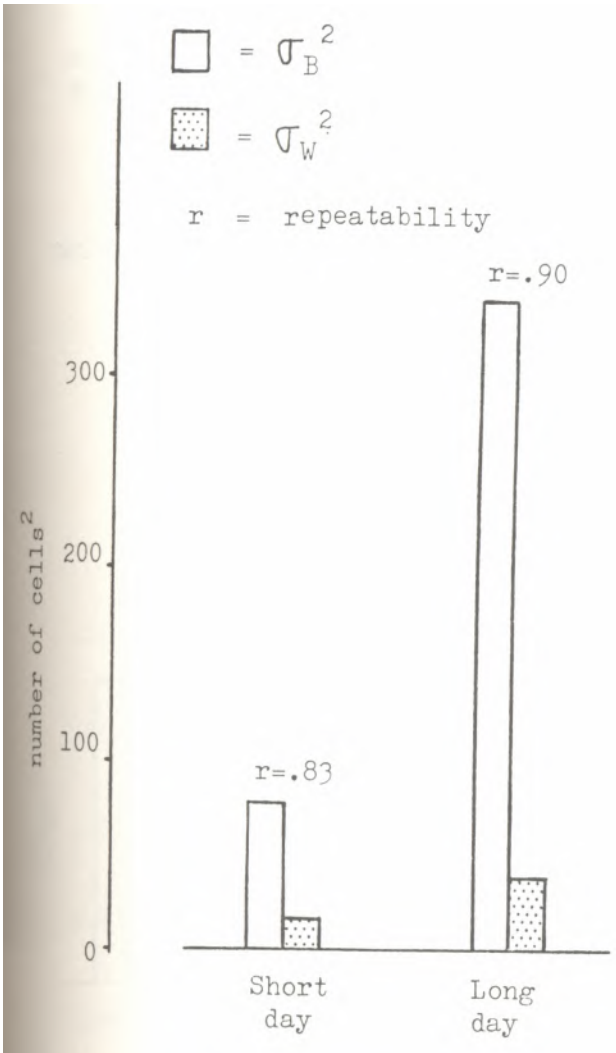

Figure 1.--Variance components and repeatabilities for radial growth.

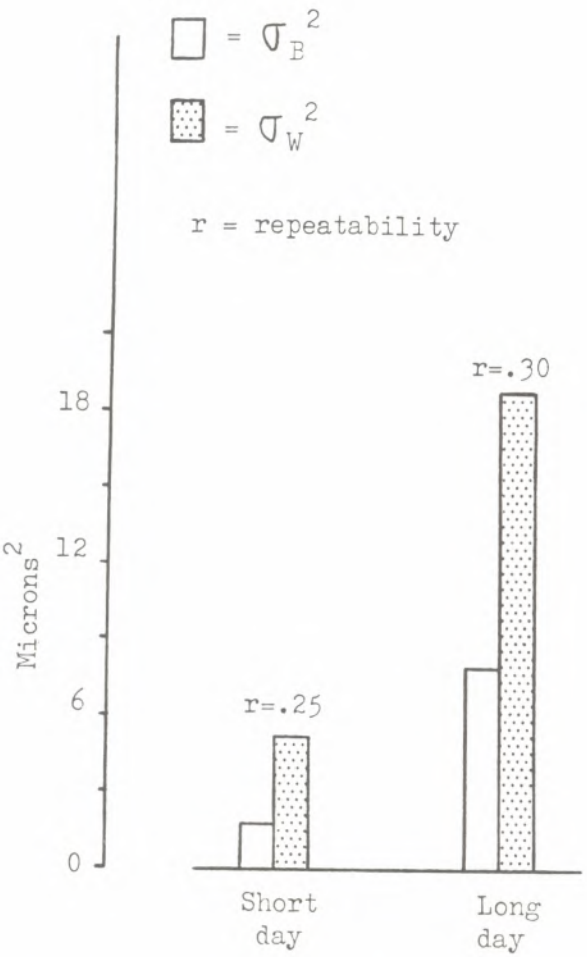

Figure 2.--Variance components and repeatabilities for tangential cell diameter.

With tangential cell diameter (fig. 2) the repeatabilities are low because of comparatively large within-seedling variation. The total variance is less in the short day. In older wood the repeatability might be much higher due to less within-seedling variation. At this early age the rate of expansion in stem circumference in relation to rate of expansion in diameter is greater than at any later age and therefore there might be less competition between the derivatives of anticlinal divisions in the cambium (Bannan and Bayley, 1956) than at a later age. Therefore more of the smaller cells might survive causing greater within-seedling variance. This theory is based only on geometrical considerations. Differences in rate of cell division at different ages should be considered.

The variance components and repeatabilities for radial cell diameter in the earlywood zone, the latewood zone and the average of the whole ring are graphed in figure 3. Much of the within-seedling variance is cancelled out when the whole-ring average is taken. The within-seedling variance components differ quite little between earlywood and latewood zones but there are substantial differences in between-seedling variance components. In the earlywood this component is much smaller in the long photoperiod because possibly this environment allowed all seedlings to carry out the necessary processes for cell enlargement. On the other hand, the short day environment might have allowed only a few, perhaps the more vigorous, to carry out these processes while others could not and therefore a large between-seedling variation arose.

In the latewood a different set of processes hold sway. At a time when seedlings are on the verge of dormancy after a long period of growth, the short photoperiod may be more optimum for all, and therefore a smaller between-seedling variation might arise.

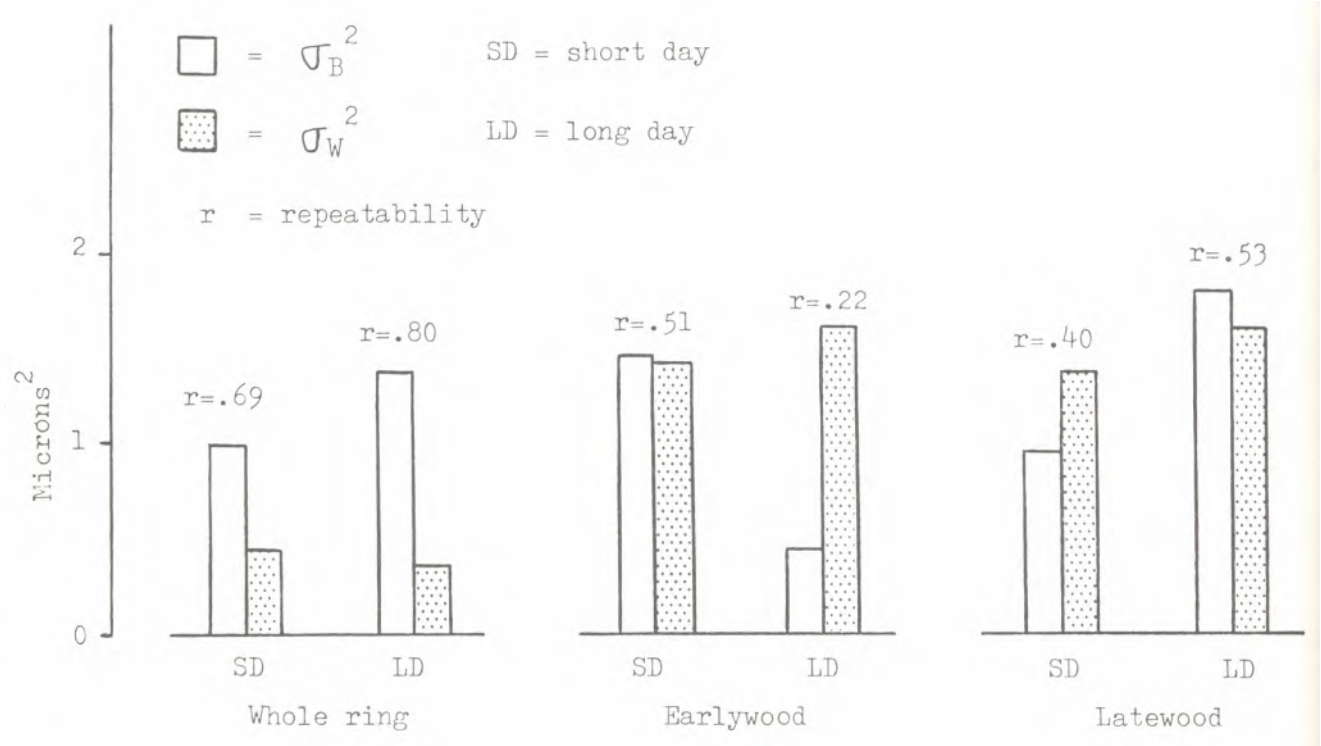

Figure 3.--Variance components and repeatabilities for radial cell diameter.

For tangential double wall thickness (fig. 4) the repeatabilities are in the more or less moderate range. The between-seedling variation pattern is quite similar to the one for radial cell diameter. The reasons might also be similar since it was found that cell diameter and wall thickness tend to be positively correlated except in the longday earlywood.

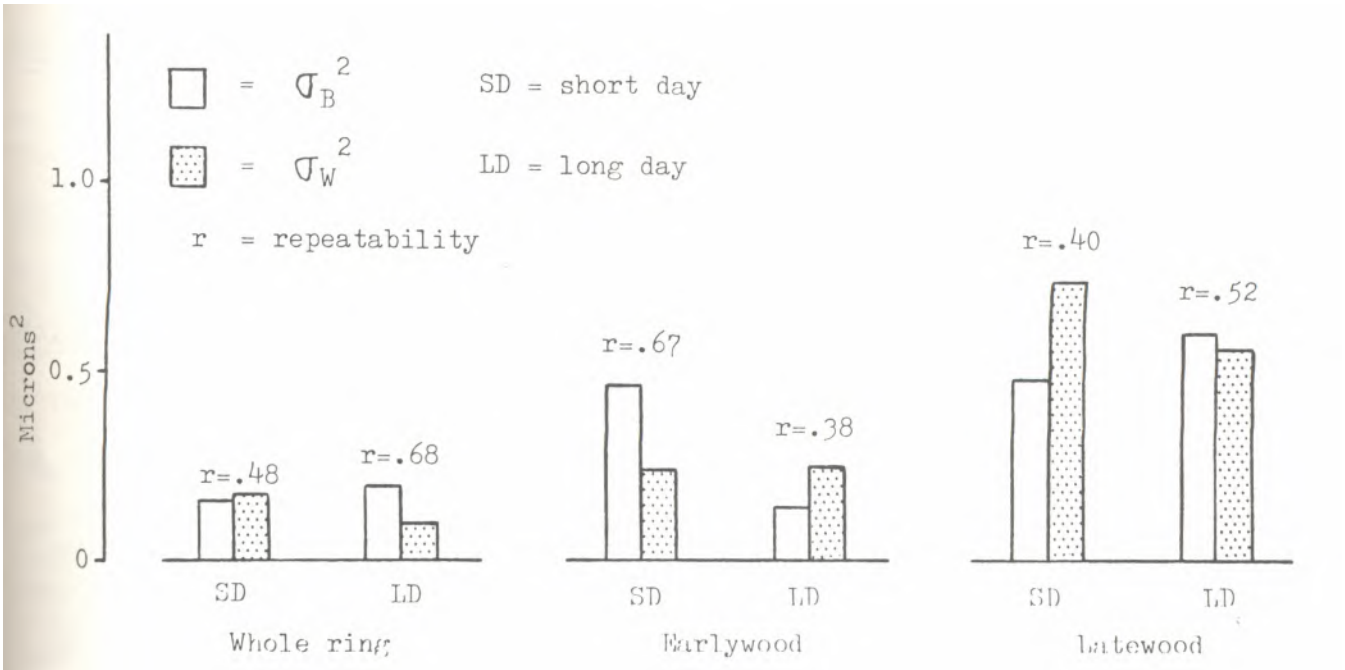

Figure 4.--Variance components and repeatabilities for tangential double wall thickness.

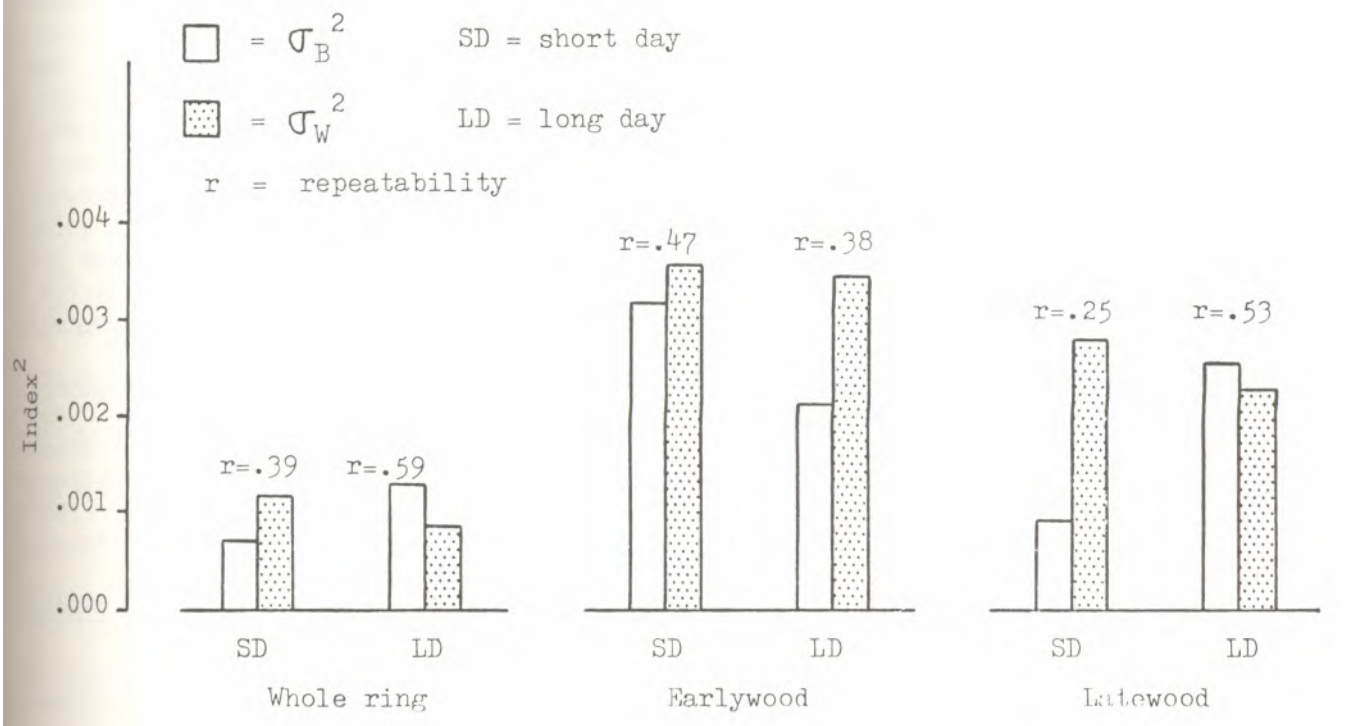

Figure 5.--Variance components and repeatabilities for specific gravity index.

With specific gravity index (fig. 5) the variance is greater if the two values making up the ratio are poorly correlated. Within-seedling variance was quite similar for different treatments in the same zone. There was less total variation in the latewood than the earlywood for both treatments indicating that the two measurements are better correlated in that zone as indeed a correlation analysis verified.

CONCLUSION

This study has shown that the variances of tracheid characteristics are greatly influenced by photoperiod and associated environmental factors just as other investigators have shown the mean values to be. For radial cell diameter and tangential double wall thickness there was less between-seedling variation when environmental factors were favorable for a particular stage of growth. That is, they were less for the earlywood in the long day and for the latewood in the short day. Within-seedling variance components showed no pronounced pattern.

Repeatability does not seem to have much utility as an estimator of broad-sense heritability because too many assumptions must be made about the relative magnitudes of genetic and general environmental variance. With tracheid characteristics, the assumption that the repeated values are dependent on the same genes is probably not valid because the distribution of growth promoting and growth inhibiting substances would likely not be uniform around the whole ring, and therefore, developmental and physiological processes would be different in quality or rate at different locations.

Repeatability seems to be most useful as an easily calculated and understood indicator of firstly, the extent of sampling necessary within an individual or secondly, the number of successive records one should have when evaluating an individual for the purposes of selection. It may also be used as an indicator of when new environmental or genetic factors become important in a tree's development.

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DISCUSSION

STAIRS - I'd like to ask Miss Sweaney what the moisture content of the seeds was when irradiated.

SWEANEY - I do not have the list of moisture contents with me. The seeds were stored in the refrigerator at all times except during irradiation, so we assume that the moisture content has remained at the level at which the seeds were delivered from the seed plant.

STAIRS - For all species?

SWEANEY - No it isn't. It varies from about 2 to 7 percent.

STAIRS - Then it appears that moisture content is not a reason for the difference between species. Do you have a hypothesis as to why jack pine should be that much more resistant?

SWEANEY - I think it's going to be characteristic of the species rather than the moisture content. The six species have different traits, such as growth rates and enzyme systems, which are determined by the genome. The particular genome will determine the radiosensitivity. Dr. G. H. Newcombe, at Chalk River, has been raising trout from irradiated sperm and eggs. He had expected to find abnormal embryos developing from these sperms and eggs but few were observed. His tentative explanation for this was a scrubbing of the genome following irradiation which results in the elimination of damaged cells from the population.

RUDOLPH - I was very much interested in your paper and the results, Miss Sweaney, but I'd like to differ with your conclusion regarding "scrubbing of the genome." I think that what you are dealing with here are primarily physiological effects and really you have not had any information on what you've done as far as genetic damage is concerned. My reason for saying this is that in jack pine, at least, we have now some second generation results following seed irradiation and I think you'd be very much surprised at the results. The trees in the X1 generation, the ones that survive to sexual maturity, do not differ much but there is a tremendous amount of hidden genetic damage that selfing and various crossing patterns will show. I have no argument with your conclusion that you're eliminating some of the weaker seedlings; this is probably true. But as far as scrub bing the genome is concerned, using this phraseology is, I think, rather misleading. Ithink you're really inducing a great amount of genetic damage that in this first generation you have no way of evaluating.

SWEANEY - Dr. Newcombe was only half serious when he suggested this explanation. In my work no visibly abnormal trees were found. In other studies conducted by our laboratory the seedlings survived to higher dose levels. The phrase appealed to me as expressing the removal of the abnormal trees I had expected to find. The trees that did survive would probably show genetic damage if they were permitted to reproduce.

YEATMAN - Question for Dr. Farrar; what light intensity did you use? I'm interested in this because the question arose when I was growing jack pine seedlings ingrowth cabinets where I was using a fairly high light intensity. Under these conditions, nutritional imbalance seemed to show up quite quickly. I had some seedlings that were shaded and the marked deficiencies were not evident in these seedlings. It was not a fair comparison because I had only a few seedlings in the shade but I have a strong impression that light intensity and nutrition interact strongly under these controlled environment conditions.

FARRAR - I agree with you that under high light intensity nutritional disorders tend to show up more noticeably, and if you want to grow healthier looking seedlings, it's better to cut the light intensity down to something under 2,000 foot-candles.

NICHOLSON - In this experiment the light intensity was 1,500 to 2,000 foot-candles; it varied between locations in the growth chamber.

FARRAR - When you consider that full daylight is around 10,000 foot-candles in mid-day in mid-summer, I think that anything under 2,000 is low.

HOLST - I would like to ask Jack Farrar a question. You applied the standard solution plus calcium chloride -- now the effect that you had could be a chloride poisoning, couldn't it? Why should the chloride poison the provenances from the granitic sites more than those from the limey sites?

FARRAR - I'd be surprised if it were chloride, but it is a possibility as far as this experiment is concerned.

HOLST - You had the same variation in chloride as in calcium, but I don't see why provenances from limey sites should not have as much chloride toxicity as those from the granitic sites. As Jack pointed out there are many factors that might have an effect on ecotypes, but in this particular experiment you had it on the chart as Ca++, but no mention of chloride. Perhaps you should change it a little bit and say reaction to calcium chloride in solution.

HOWE - Dr. Farrar, what do you feel might have been the effect of the pH alone; you mentioned the differences in availability of ions, but might there have been other differences resulting from pH?

NICHOLSON - Perhaps I should answer since I took most of the readings. The pH was between 4.2 and 5 for all treatments. I expect that if we had varied the range of pH between treatments we might have obtained better differentiation of provenances or that we might have gotten better results at some other pH. Have you any suggestions yourself?

HOWE - No.

NICHOLSON - We could not test the effect of pH at that time.

HUNT - I would like to comment on the chloride ion concentration. It would seem to me that one could plot chloride parts per million as just double the number of calcium cations. If you suspected an excessive concentration of chlorides, would you repeat the study using another anion or a mixture of calcium salts?

FARRAR - Yes, we could have used sulphate, for example. That would be a good thing to try.

HOWE - Miss Sweaney, would you care to speculate on this interesting phenomenon of stimulation at rather low dosages?

SWEANEY - The stimulatory effect has been in controversy now for some 30 or 40 years. It was reported soon after the discovery of X-ray, and has been discussed in various seminars, but nobody can get any definite data that they can say 50 or 100 Roetgens with certain radiation will do certain things, In our pine work we have found, in nearly all species, stimulation at low doses. It's more pronounced in my work than in simulated fallout work, and in the acute work. I have found a stimulation of numbers up to 200 percent control. This increase is a function of the

severity of the damage on the control, and in the experiment I'm reporting on, there was not sufficient replication to really test the amount of population variation. The stimulation on size is significant in some cases, but it can be reproduced with fertilizer and other things. As far as agricultural crops are concerned, there are authors that report very large stimulatory effects and somebody else who tries to do the work says there's none at all.

HOWE - Have you run any cytological or chemical analyses of your irradiated material?

SWEANEY - We are starting now to check these characteristics, the cell size and nuclear volume. Sparrow at Brookhaven has brought out a hypothesis that the severity of damage is the function of the chromosome volume, which is obtained by measuring the nuclear volume of the tissue and dividing by the chromosome number, He finds a direct correlation of sensitivity with the increasing chromosome volume. We are checking on this as a possibility to explain the differences in response in our own seed. We're trying to explain why the difference between white pine and jack pine is so large.