THE PROBLEM OF SITE VARIATION WITHIN RED PINE PROVENANCE EXPERIMENTS

by Mark J. Holst1

In spite of care taken in the selection of site and experimental design of provenance experiments, site heterogeneity within the experimental area may be more complex than was anticipated when the experiment was established. The present paper describes a problem of this nature encountered in a red pine (*Pinus resinosa* Ait.) provenance experiment at the Petawawa Forest Experiment Station. An appropriate design for minimizing this problem is proposed.

Background

Red pine grows best on fertile sands with good aeration and moisture supply. On these soils, it is a fine straight tree of high economic value. It is not surprising that it has been widely planted both in Canada and the United States. Failure owing to planting on heavy soil has been reported (Stone et al. 1954, Richards and Stone 1964), but there is little information concerning red pine's reaction to fertility, drought, and frost (Wilde 1964).

Red pine shows a remarkable stability in taxonomic characteristics, and the stem form is uniformly good. One could, therefore, easily get the false impression that there are few important differences in the species associated with provenance. However, recent studies show clearly that physiological races do exist (Hoist 1964b) although they are hard to distinguish morphologically, and the range in rate of growth is relatively limited.

The older red pine provenance experiments established in the Lake States (Lester 1964, Nienstaedt 1964, Rudolf 1964) and Pennsylvania (Hough 1952) were planted on moderately fertile and uniform sands in congenial climates. In these circumstances, survival was good, the trees appeared healthy, and reports about them mention no excessive variation because of soil heterogeneity. Differences among provenances were small, and none of the provenances was damaged by frost or the effects of drought.

In Canada where red pine provenance experiments have been planted near the northern limit of the species or on marginal soil, differences among provenances have been more evident (Hoist 1964a). On the marginal soils red pine has reacted strongly to variations in microsite.

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Effects of Macro-Site Differences

Provenance experiments conducted on uniform, fertile, and weed-free nursery soil are better than tests planted on less uniform forest soils for providing estimates of differences among provenances. This was demonstrated in one of our oldest red pine provenance experiments.

Ten Ontario provenances were sown in 1951. A nursery provenance experiment was planted in 1952 with 25-tree plots and three replications. A field experiment was planted in 1955 on a somewhat frosty and variable site? with 100-tree (10x10) plots and three replications. Single division rows of Petawawa red pine were planted between the plots to aid the assessment of any site differences that might appear later. Thus 44 division row plants were associated with each plot.

A comparison of the 1954 heights in the nursery test with the 1957 heights in the field test is shown in figure 1. One particular provenance is clearly

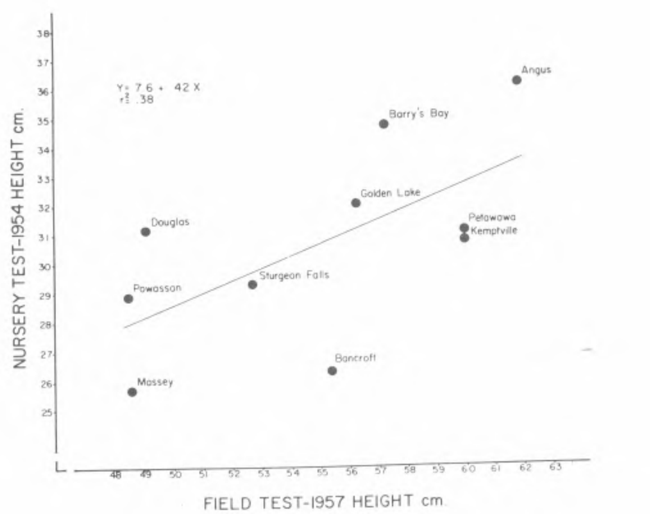

FIGURE 1. — Comparison of heights of red pine provenances in a nursery test on uniform soil and in a field test on variable soil.

2The test site was an old beach line with sand of variable texture and shallow frosty depressions. The mounds had better soil than the depressions. Thus there is a confounding effect of soil fertility and frost that could be mapped, but not removed by the experimental design. Mounds accounted for roughly 15 percent, depressions 60 percent, and intermediate 25 percent of the experimental area.

best in both instances, but in general there is a great lack of consistency between the two plantings. The relationship between height and length of growing season for the areas of seed origin is significant in the nursery test (r = 0.78 **), but is not significant in the field test (r = 0.62). Duncan's Multiple Range Test at the 5 percent level of probability distinguished four groups in the nursery test, but only three groups in the field test. The F ratios for provenances were about the same in both experiments.

The 1959-60 winter was severe and was followed by a late spring frost that caused considerable injury to the red pine in these experiments. The opportunity was taken to study spring frost damage in relation to provenance. In the early summer of 1960, all trees in plots and surround rows were scored as follows:

Score	*Degree of frost injury*
1	No damage.
2	No damage on upper part of the crown, but up to five branches nearer ground level browned by frost.
3	Less than 75 percent of the crown browned by frost.
4	More than 75 percent of the crown browned by frost.

In viewing the experiment as a whole, it was evident that the intensity of frost injury was related to minor variations in elevation over the area of the experiment. The area was therefore stratified according to three general levels of frost intensity: heavy (low elevation), medium (intermediate elevation), and light (high elevation). Individual trees and provenances exhibited varying degrees of frost injury within each intensity level. Average scores of frost injury were calculated for each plot, and for subplots formed within plots by the boundaries of the general zones of frost injury. In view of the confounding effect of site variation due to elevation, the problem was to determine the component of variation in frost injury associated with provenances.

The analyses of variance among plot means, plot means adjusted for surround rows by covariance (deviation of particular surround rows from the overall surround row mean), and plot means in percent of surround rows, are listed in table 1. Differences among provenances based on observed plot means were not significant, but reached significance (5 and 1 percent) after the adjustments for surround rows were made. The error variances for the observed plot means and plot means adjusted for surround rows by covariance were directly comparable and indicated a threefold gain in precision.

These are standard procedures but they may give a false impression of the real pattern of injury. The relatively few plots occurring in the medium and light frost areas made it difficult to run separate regressions for each injury zone. Instead I plotted the frost damage relative to surround rows in the heavy frost area only against the plot means adjusted for surround rows by covariance (fig. 2). The scatter is considerable. Since it is considered that a truer relationship between frost damage and provenance is to be found in the area with heavy frost, the conclusion must be that the standard covariance methods for adjustment of plot means were inadequate, even when based on a grid of surround rows.

In this particular layout, mapping of the three levels of frost intensity gave a better rating of provenance differences than plot means adjusted for surround rows, which again were three and a half times better than comparisons of the unadjusted plot means. The ratio between plot plants and surround plants was nearly 2:1 (100:44). A more accurate estimate could be made by omitting the surround rows and instead establishing plot plants and check plants in a ratio of 1:1. I shall return to this problem later.

Table 1.--Analyses of variance of frost damage index

Source	Observed plot means (C.V.= 18)[1]			Plot means adjusted for surround rows (C.V.= 10)[1]			Plot means in percent of surround rows (C.V.= 11)[1]		
	d.f.	M.S.	F	d.f.	M.S.	F	d.f.	M.S.	F
Total	29			28			29		
Blocks	2			2			2		
Provenances	9	0.28	1.0	9	0.29	3.6*	9	584	4.2**
Error	18	0.28		17	0.08		18	138	

1/ C.V. = coefficient of variation = $100\frac{S}{\bar{x}}$ percent.
Significance levels: **, 1 percent; *, 5 percent.

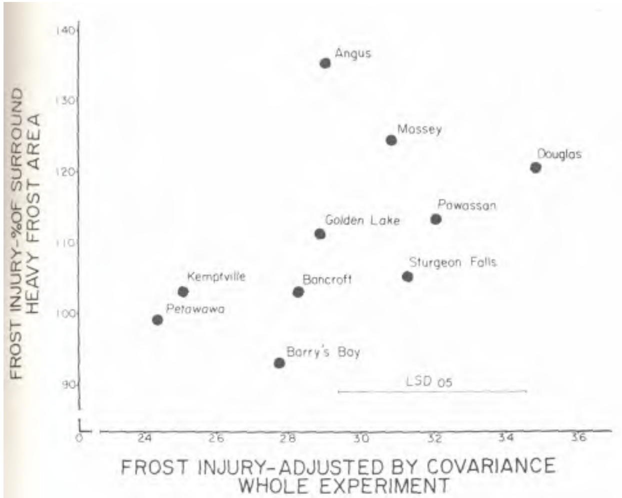

FIGURE 2. — Spring frost injury on red pine provenances. Comparison of spring frost injury relative to surround row (Petawawa provenance) in area of heavy frost, and index of spring frost injury adjusted for surround row performance by covariance.

In establishing experiments of standard design where there are no surround rows or check plants to serve as indicators of soil heterogeneity, the best way to deal with the excessive variability induced by important macro-site differences, is to prepare a map of the site differences. These differences are best judged on a basis that is independent of the experimental plants, such as soil characteristics or elevation. Even a crude separation can be helpful in applying meaningful adjustments that improve the interpretative value of the experiment. Where the macro-site differences are less pronounced we have found that it is possible to get acceptable results by measuring the dominant trees only.

Effects of Micro-Site Differences

The other point I should like to demonstrate is the effect of micro-site on tree height and survival. In this red pine provenance experiment striking trends in tree height were observed within the area of the experiment. The trees were spaced at 4x4 feet. Within the space of four or five trees (i.e. 15 to 20 feet) one could see a gradient from tall trees to short or even missing (dead) trees. As this is an area of outwash sand and gravel one would suspect differences in moisture-holding capacity that would influence the survival and growth of the red pine. This has been demonstrated in red pine plantations on similar sites at the Petawawa Forest Experiment Station. [3] However, there also may be some soil factor which is poisonous to red pine roots.

During the summer of 1965, the soil was sampled along 16 tree height gradients. As the seedlings were planted in plowed furrows, samples were taken from the undisturbed soil at the bottom of the furrows. Tree heights were recorded along each gradient. The soil samples were set out in the greenhouse, watered with distilled water, and sown with red pine seed. Table 2 shows the relation between tree heights in the plantation and germination. It demonstrates delayed germination in the soil taken under trees with intermediate growth, and both delayed and low germination in the soil taken under the trees of stunted growth.

Trees grown under adverse conditions of moisture, nutrition, or gas exchange are more susceptible to injury by frost than trees grown under optimal soil conditions. In addition to the variability induced by these causes, there appears to be some factor which inhibits germination and

[3] Several research workers of the Petawawa Forest *Experiment Station have studied fail areas in red pine plantations. Some are of the opinion that certain fail areas are caused by antagonistic effects of sweetfern (Comptonia peregrina (L) Colt), sometimes associated with an attack by Saratoga spittlebug (Aphrophora saratogensis Fitch). ,4 recent investigation by Dr. P. J. Rennie, head of the Soils Section, indicated that some fail areas are associated with a high silt content which creates a compact, poorly aerated rooting medium for red pine. It has also been noted that the poorly developed red nines in those areas were damaged by frost.*

Table 2.-- Germination of red pine seed in soil taken along a deficiency gradient as indicated by tree height

Tree height (cm)	Germination percent 1/							
	Date in August							
	12	14	16	18	20	22	26	28
345	2	10	19	22	28	58	73	79
240	5	15	25	31	42	62	77	79
110	0	2	2	4	19	37	72	78
80	0	2	4	4	10	16	47	56

1/ Mean of two samples of 100 seed.

may also limit root development. In milder cases growth is retarded. In severe cases the plant is killed.

In an experiment with different seed-covering material we have noticed that washed gravel from our local gravel pit also resulted in delayed germination of red pine seed. The cause has not yet been identified.

Difficulties in experimentation arising from micro-site differences can be reduced by increasing the number of plants per plot and then measuring only the taller trees in each plot.

Soil Heterogeneity and Experimental Error

Provenance experiments are designed in part to estimate population site interactions in yield. The purpose may relate directly to problems in plantation management; or it may be to provide estimations of genetic parameters essential for a program of selection and breeding. Experimental plots should be large enough to minimize error in yield estimates that may be induced by variations among plots in stand density, competition, site, or soil.

To keep the experimental error at a minimum the site chosen for experimental tests should be as homogeneous as possible. It is difficult to distinguish a homogeneous site because under natural conditions stand stratification may result from differences in age, site, competition, genotype, and their interactions. A mature stand of widely spaced trees may give a false impression of site uniformity which is dispelled when plantations are established at close spacing.

It has been shown that standard statistical designs are inadequate to account for site heterogeneity found in some forest experiments (Wright and Freeland 1959, 1960). This is a common situation. I have seen many provenance experiments in both Europe and North America in which, in spite of careful attention to experimental design, the level of precision has been low owing to marked soil heterogeneity. The precision of these experiments could have been increased by the inclusion of suitable checks on soil heterogeneity.

In the red pine experiment, the error variance was reduced by accounting for some of the site effect with surround row plants. Here the ratio of plot:check plants was approximately 2:1. The error would be further reduced and the sensitivity of the covariate increased if check plants were established in a 1:1 ratio with plot plants, as in the black and white squares on a checker board. Each plot plant could then be rated relative to four adjacent check plants.

The primary advantage of this intensive system of check and plot plants is that it permits the estimation of intra-plot as well as intra-block variation in levels of site productivity.

From this it follows that:

1. Plot size would not be critical in relation to soil heterogeneity.
2. Estimates of plot and block variation could be made independently of the plot plants.
3. Analysis would yield values for check x provenance x block interactions that estimate the stability of individual provenances within the experiment.
4. Extremely poor parts of the experiment could be objectively identified and rejected if necessary.
5. After the check trees have provided early estimates of site heterogeneity, they may be thinned or retained according to need.

If the main objective of the experiment is to compare exotic provenances with the local provenance, then the check trees should be from a selected population of local origin. In this case the most important comparison is made with a very high degree of precision.

The check plants should normally be of the same species as the plot plants. Seed for the check plants should be collected from natural stands rather than from plantations. The population of which the check plants are a sample should be chosen according to the objective of the experiment, e.g. a slow-growing type may be desirable. The check plants should be treated with the same care and attention in the nursery as the plot plants, and should be heavily graded for uniformity before being planted out. It may be desirable to include four different populations as sources of check plants to guard against specific population x environment interactions[4] (see fig. 3).

This method of accounting for environmental heterogeneity is proposed as a means of dealing with the common problem of controlling experimental error. It is my hope that the inclusion of check plants in suitable experiments will be tested by a number of investigators in the field of forest genetics.

Summary

To demonstrate the effect of macro- and micro-site differences, a red pine provenance experiment planted on a variable site at the Petawawa Forest Experiment Station was analysed. By subdividing the area into three classes of frost intensity, and by expressing plot means relative to their surround rows, the error term was reduced by one-fourth of the error term obtained when the plot means were compared directly.

Variation in micro-site was also pronounced in this experiment. Soil seed germination antagonism was demonstrated from soil sampled from fail areas. Delayed germination of red pine seed occurred in the soil taken under trees with intermediate

[4] *Suggestion made by Dr. W. J. Libby at the Forest Genetics Workshop.*

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ONE PROVENANCE USED AS CHECK PLANTS      FOUR PROVENANCES USED AS CHECK PLANTS

x x x x x x x x x x x x                  x x x x x x x x x x x x

x x x x x x x x x x x x                  x x x x x x x x x x x x

x x x x x x x x x x x x                  x x x 2 x 4 x 2 x 4 x 2

x x x 0 x 0 x 0 x                        x x 3 0 1 0 3 0 1

x x x x 0 x 0 x 0                        x x x 4 0 2 0 4 0 2

x x x 0 x 0 x 0 x                        x x 1 0 3 0 1 0 3

x x x x 0 x 0 x 0                        x x x 2 0 4 0 2 0 4

x x x 0 x 0 x 0 x                        x x 3 0 1 0 3 0 1

x x x x 0 x 0 x 0                        x x x 4 0 2 0 4 0 2

x x x                                    x x 1   3   1   3

x x x                                    x x x

x x x                                    x x 3

0 = plot plants;  x = check plants and outer surround rows.
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FIGURE 3. — Sample layouts of alternate planting of plot plants and check plants.

growth, and both delayed and low germination in the soil taken under trees with stunted growth. The cause of delayed or low germination was not identified.

It is suggested that a better estimation of the influence of soil heterogeneity could have been obtained by alternate planting of plot plants and check plants so any plot plant could be rated relative to the four adjacent check plants. The advantages of such a system are: plot size is not critical; size of experimental area is not a limiting factor (which is important when many provenances are tested); the estimation of population differences will be more precise; extremely poor parts of the experiment can be identified (and rejected if necessary); after the check plants have served their purpose as indicators of soil heterogeneity they can be removed if desired.

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