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A THREE-DIMENSIONAL LATTICE DESIGN FOR STUDIES IN FOREST GENETICS

BY

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(Contribution from Forest Service)

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A THREE-DIMENSIONAL LATTICE DESIGN FOR STUDIES IN FOREST GENETICS[1]

By BESSE B. DAY, *associate statistician*, and LLOYD AUSTIN, *in charge, Institute of Forest Genetics, Forest Service, United States Department of Agriculture*[2]

INTRODUCTION

The logical procedure for the improvement of all wild stock of any kind, whether plant or animal, is practically the same: segregation of varieties, races, and strains of the wild population; the evaluation of the characteristics of each group; the selection of the best individuals from each of the best strains; utilization of these in breeding and selection; and finally the production of new types (*5*).[3]

In forest genetics, both in breeding and in mass reforestation, the initial step involves seed selection. It is evident that there are many valuable inherent characteristics in forest trees. These need to be discovered, isolated, and defined. The measure of heredity is to be found in the offspring. Seeds from individual seed trees must be collected and sown and the behavior of the progeny therefrom studied. This necessitates the making of fairly extensive progeny tests.

The same difficulty arises in individual seed-tree progeny studies as in other plant research in varietal testing—the lack of homogeneity of the medium, soil, in which such tests are to be made. It is a well-established fact that there is much variation even in soil which seemingly has the most constant texture and quality and that the variation is reflected in the growth of the plants to such an extent that variety difference may be so completely obscured as to be lost entirely. It is only when the area is exceedingly small that soil effects may be ignored. As the number of varieties to be tested increases, the area necessary for a complete set becomes increasingly larger and the variation of soil and other growing factors are likewise magnified, resulting in what may be a considerable lack of precision. All attemps to solve this problem proved unsatisfactory for one reason or another until Yates (*7*, *8*, *10*) conceived the idea of arranging the varieties in a series of small blocks, instead of the previous arrangement in one block, distributing them in such a way that a variety variance could be calculated which would be freed of block effects. This would yield an error variance appropriate for making significance tests of differences found in the measurements of the varieties or progenies. Designs on this principle, termed "quasi-factorial" and "incomplete randomized blocks," are readily adaptable to all phases of agronomy where varietal tests are made.

The theoretical aspects of this type of design have been treated in earlier writings (*3*, *7*, *10*). It is believed, however, that the application of this design to a particular field problem will be of interest in

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[1] Received for publication December 5, 1938.

[2] Grateful acknowledgment is due Prof. R. A. Fisher, Galton Laboratory, University College, London, for proposing this type of design for the experiment; to F. Yates, chief statistician, Rothamsted Experimental Station, for invaluable counsel and interest in the development of this experiment; and to Prof. P. M. Barr, Division of Forestry, University of California, who made numerous suggestions leading to a more precise presentation.

[3] Italic numbers in parentheses refer to Literature Cited, p. 118.

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that it affords an exact test of the effectiveness of the design in ironing out heterogeneity. For this purpose the description is here given of such a design as applied to the progeny test nursery planted in April 1937 at the Institute of Forest Genetics, Placerville, Calif.,[4] and the procedure that was followed for correcting the resulting data and making tests of significance of differences. Actual data are employed and the appropriate statistical procedure has been carried through on germination counts. In these data factors known to produce variability of conditions were actually introduced, as in the watering of sections of the experiment at unequal intervals. Their influence was reflected in the results. With the application of the correction procedure such effects were eliminated.

The objectives of the 1937 tests were as follows: (1) To determine the hereditary nature of the numerous local strains of ponderosa pine occurring in the various localities through El Dorado County, Calif., including probable casual relationships with elevation and other factors of the seed-trees' environment; (2) to discover which individual seed trees in each strain, or in each elevational belt, have the inherent ability to produce the most rapidly growing offspring, as determined by measurement of the height, diameter, and branching of the progenies.

The experiment was limited to 729 seed selections or strains, 696 ponderosa pine (*Pinus ponderosa*) and 33 Jeffrey pine (*P. jeffreyi*), gathered from 17 consecutive 500-foot altitudinal zones in what was known as the El Dorado transect, an area in El Dorado County, extending less than 100 miles across the main range of the Sierra Nevada and about 50 miles parallel to the range. Some of the seeds were obtained from the same trees but in different years. In many cases two or more of the seed trees were growing in the same field plot. It was desired to design a nursery in such a way that measurements on progenies would be available free from the influence of all heterogeneity in growing conditions and with an estimate of error adequate for testing the significance of differences between particular progenies.

THEORETICAL BASIS AND DESCRIPTION OF THE DESIGN

The design best adapted to the testing of an unusually large number of varieties is termed the "three-dimensional quasi-factorial" with three groups of sets ("pseudo-factorial" in earlier publications), or more simply, the cubic lattice design (6). The initial requirement is that the number of varieties tested be a perfect cube. In this experiment 729, or 9^3, individual seed selections were made. Nine plots or varieties were grouped together to make up a block. It follows, then, that 81 blocks are needed for one complete replication. It was believed that this block size was sufficiently small to eliminate unavoidable heterogeneity therein. Fundamentally this process of arranging fewer than the total number of treatments (individual seed selections) in a block, in other words more than one block to a replication, results in confounding (*10*). Some of the information on treatment or interaction effects is sacrificed by being entangled with fertility differences between blocks in order that the precision may be increased through a reduction of the standard error due to elimination of block

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[4] A part of the California forest and Range Experiment Station.

division be so arranged that they cut across those of all the other divisions. Cutting the cube by two sets of parallel planes through the intersections—the first set parallel to the right-front face and the second parallel to the upper face—yields 81 blocks, each nine plots (small cubes) long. The numbers 111, 211, 311 ------ 911, form a set, another is 112, 212, 312 ------ 912, and so until 81 such blocks are designated. It will be noted that in each of these sets or blocks

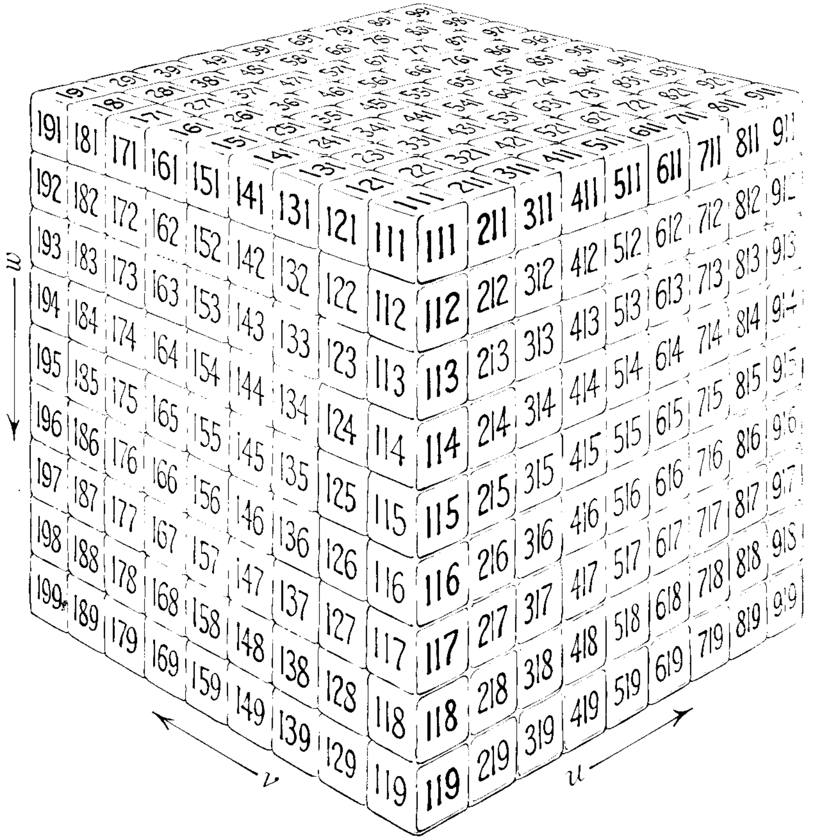

FIGURE 1.—Cube to illustrate the method for determining the sets in the X, Y, and Z groups for the three-dimensional lattice progeny test nursery.

vw numbers are constant and u varies from 1 to 9. This group of 81 sets or blocks was designated by the letter X, and each block number is formed by the combination of its constant vw and small x, e. g., 11x, 12x, etc. Group X is given in detail in the first vertical section of table 1.

Passing planes through the cube parallel to the left front face and then horizontally cuts across the sets in the first group making certain that no two plots which appeared together in the X group are now together. The new sets form the Y group. In these u and w are constant and v varies from 1 to 9. One such block is 111, 121, 131 ------ 191; another 911, 921, 931 ------ 991. Consistent with the first group these would be blocks 11*y* and 91*y*. The second vertical section of table 1 shows the sets of the *Y* group.

TABLE 1.—*Numbering system for progenies in the 1937 progeny test nursery, showing block assignments of plots for groups X, Y, and Z*

Block No.	Group *X* (·*vw*)					Block No.	Group *Y* (*u*·*w*)					Block No.	Group *Z* (*uv*·)				
11*x*	111	211	311	---	911	11*y*	111	121	131	---	191	11*z*	111	112	113	---	119
12*x*	112	212	312	---	912	12*y*	112	122	132	---	192	12*z*	121	122	123	---	129
13*x*	113	213	313	---	913	13*y*	113	123	133	---	193	13*z*	131	132	133	---	139
.	.	.	.		.	.	.	.	.		.	.	.	.	.		.
.	.	.	.		.	.	.	.	.		.	.	.	.	.		.
19*x*	119	219	319	---	919	19*y*	119	129	139	---	199	19*z*	191	192	193	---	199
21*x*	121	221	321	---	921	21*y*	211	221	231	---	291	21*z*	211	212	213	---	219
22*x*	122	222	322	---	922	22*y*	212	222	232	---	292	22*z*	221	222	223	---	229
23*x*	123	223	323	---	923	23*y*	213	223	233	---	293	23*z*	231	232	233	---	239
.	.	.	.		.	.	.	.	.		.	.	.	.	.		.
.	.	.	.		.	.	.	.	.		.	.	.	.	.		.
29*x*	129	229	329	---	929	29*y*	219	229	239	---	299	29*z*	291	292	293	---	299
31*x*	131	231	331	---	931	31*y*	311	321	331	---	391	31*z*	311	312	313	---	319
32*x*	132	232	332	---	932	32*y*	312	322	332	---	392	32*z*	321	322	323	---	329
33*x*	133	233	333	---	933	33*y*	313	323	333	---	393	33*z*	331	332	333	---	339
.	.	.	.		.	.	.	.	.		.	.	.	.	.		.
.	.	.	.		.	.	.	.	.		.	.	.	.	.		.
39*x*	139	239	339	---	939	39*y*	319	329	339	---	399	39*z*	391	392	393	---	399
.	.	.	.		.	.	.	.	.		.	.	.	.	.		.
.	.	.	.		.	.	.	.	.		.	.	.	.	.		.
91*x*	191	291	391	---	991	91*y*	911	921	931	---	991	91*z*	911	912	913	---	919
92*x*	192	292	392	---	992	92*y*	912	922	932	---	992	92*z*	921	922	923	---	929
93*x*	193	293	393	---	993	93*y*	913	923	933	---	993	93*z*	931	932	933	---	939
.	.	.	.		.	.	.	.	.		.	.	.	.	.		.
.	.	.	.		.	.	.	.	.		.	.	.	.	.		.
99*x*	199	299	399		999	99*y*	919	929	939	---	999	99*z*	991	992	993	---	999

The third and last or *Z* group of sets, which cuts across each of the other two groups, is formed from the cube by passing sets of planes in both vertical directions. The blocks will stand vertically in the figure. Block 11*z* will be 111, 112, 113 ------ 119 while block 91*z* is made up of plots 911, 912, 913 ------ 919, as in the last vertical section of table 1.

Groups *X*, *Y*, and *Z* were each replicated three times in the nursery, making nine complete replications for each progeny.

DESCRIPTION OF NURSERY BEDS

The most desirable set-up, both from the standpoint of the technical phases and of the field work, was to use beds 4.5 feet by 48 feet, running east and west. Each bed was divided into 288 plots 1½ feet long and 6 inches wide running across the bed, making 3 plots to the width (north, center, and south) and 96 to the length. Nine plots at each end were kept as guard plots, leaving 270 test plots in each bed, which would yield 10 blocks of 9 plots each in each of the north, center, and south positions (figs. 2 and 3A). Each test plot held a row of 6 spots spaced 3 inches apart and planted to 6 seeds each; later these were to be thinned to one seedling per spot. Thus a replication of the 729 seed selections, each represented by 1 plot, required 2.7 seedbeds; and the entire set-up of 9 replications, 24.3 beds.

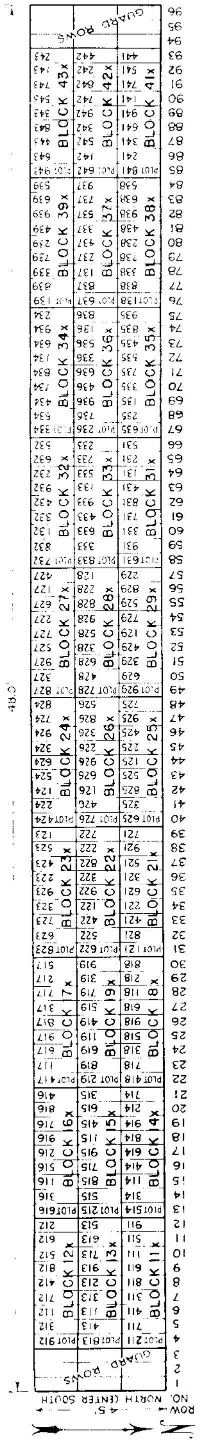

FIGURE 2.—Illustration of arrangement of plots and blocks in a typical nursery bed (bed No. 1).

For the first replication the strains were listed so that certain ones (usually in order of elevation) would appear together in the same block and particular blocks would come in consecutive order, but every precaution was taken to insure that the plot positions within these blocks, as well as those in all the other replications, be determined in a random manner. To equalize any effect due to crosswise position in the seedbeds each particular block, of which there were three replications, appeared once in each of the three positions, north, center, and south. It was not essential that every plot in the nursery be subject to exactly the same treatment simultaneously—that is, that they be sown, watered, etc., on the same day, or receive the same amount of water—but only that precaution be taken that those within the same block be treated closely alike. To insure a completely random experiment it was decided to randomize the blocks within each of the longitudinal positions with the exception of replication X, in which the blocks occurred in consecutive order. Tippett's Random Sampling Numbers (*4*) was used for this work.

For recording the field data with efficiency and rapidity in a design as complicated as this, forms must be devised to fit particular measurement, the total for the plot, and the total for the block. In the example here described, the germination count data, days from time of planting until germination, were recorded on the original sowing charts of which figure 2 is a sample.

OUTLINE OF PROCEDURE FOR CORRECTING MEASUREMENTS[5]

As previously stated, the chief purposes of this design were to eliminate differences in progenies which might be due to soil or other treatment heterogeneity under which the individual progenies have been growing and to arrive at a valid estimate of error for making individual

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[5] Very recently Yates has done considerable additional work on the development of methods for the recovery of the interblock information. Since this paper was presented for publication, he has very kindly made available to the authors his results (in manuscript form) on the three-dimensioned lattice. By a somewhat different computational procedure from that outlined here, it is possible to determine an estimate of the interblock variance, freed from varietal effects. Adjustments to the varietal means where the interblock and intrablock comparisons are correctly weighted may then be made. While the amount of computation required for this analysis is a little more than that described here, it yields a larger efficiency factor which is also always greater than that for the ordinary randomized blocks, except for the limiting case when there is no reduction of variance due to the use of smaller blocks. When interblock information is ignored, the efficiency factor is not always greater.

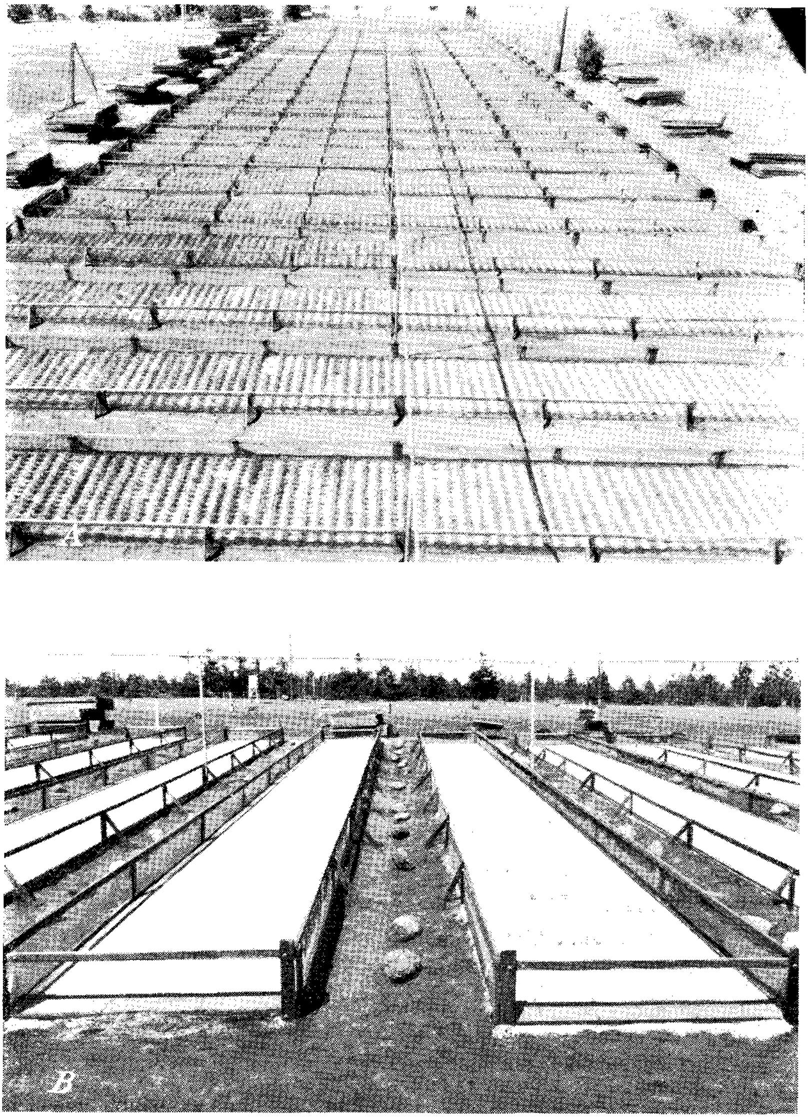

FIGURE 3.—*A*, General view of the entire progeny test experiment. *B*, Bed 16, right, was watered immediately after sowing; bed 17, left, was watered several days later—an example of an unavoidable difference that may occur in treatment and illustrating the need for a design that will eliminate variations due to such causes.

comparisons. The analysis of variance takes the following general form:

Degrees of freedom	Sum of squares	Mean square	F
Incomplete blocks $(3np^2-1)=728$			
Progenies $(p^3-1)=728$			
Error $(3np^3-3np^2-p^3+1)=5{,}104$			
Total $(3np^3-1)=6{,}560$			

Where $p=9$, the number of plots to the block
$p^2=81$, the number of blocks in each replication
$p^3=729$, the total number of progenies or strains
$n=3$, the number of replications of each of the three groups
$3n=r=9$, the total number of replications
$3np^2=729$, the total number of blocks in the nursery
$3np^3=N=6561$, the total number of plots in the nursery

The usual procedure may be followed in computing the sum of squares corresponding to the 728 degrees of freedom for the blocks and the 6,560 making up the total. As has been pointed out earlier, the block differences have been confounded with progenies (varieties) and hence there will be no valid mean square for blocks.

The first step in determining the sum of squares due to progeny differences is to apply a correction factor to the gross average for each variety which will eliminate the differences due to soil and treatment heterogeneity, leaving a value which is truly representative of the particular strain insofar as its characteristics were embodied in the seeds sown. A cubic lattice with dimensions p may be likened to a factorial experiment (*7, p. 432*) involving three factors each with p values. The main effects of each of these factors would be confounded in two of the three groups or replications and the p^3-1 degrees of freedom (here strains) for treatment would be as follows:

First factor (A)	$p-1$
Second factor (B)	$p-1$
Third factor (C)	$p-1$
Interactions:	
First order (AB, AC, BC)	$3\,(p-1)\,(p-1)$
Second order (ABC)	$(p-1)\,(p-1)\,(p-1)$
Total	p^3-1

The precision of the estimate of the main effects would be just one-third that of an unconfounded experiment with the same error variance per plot; that for the first order interactions would be two-thirds, while the second order would be entirely free of confounding.

In this progeny test the interest lies in the differences between single progenies rather than main effects and interactions. Estimates of the yield for each variety or strain may be expressed in terms of the gross mean yield, the main effects, and the interactions, the latter two being equivalent to removing differences due to soil or treatment heterogeneity. For factorial experiments involving two or more factors (*9, pp. 12–13*) the yield of any treatment combination is equal to the mean yield and the sum of plus or minus *one-half* of all the main

TABLE 2.—*Computation of corrected progeny means; First of series of nine; w=1*

SECTION 1: X_{uvw}

v \ u	By groups 1	2	3	4	5	6	7	8	9	
1	82	62	72	89	65	76	92	70	66	674 $X_{.vw}$
2	63	99	64	83	98	59	89	70	86	711
3	64	60	74	95	82	104	68	88	67	702
4	80	73	101	71	79	90	75	67	103	739
5	85	74	83	66	93	86	73	72	73	705
6	67	71	88	80	66	79	98	70	74	693
7	77	96	111	70	98	116	83	97	69	817
8	72	91	66	93	72	80	99	76	70	719
9	89	92	94	95	77	67	89	84	102	789
$X_{u.w}$	679	718	753	742	730	757	766	694	710	6549 $=X_{..w}$

SECTION 2: Y_{vuw}

v \ u	1	2	3	4	5	6	7	8	9	
1	77	64	66	86	62	75	104	77	74	685 $Y_{.vw}$
2	56	89	63	80	89	64	88	65	94	688
3	51	59	69	81	74	77	63	79	73	626
4	76	78	88	66	70	81	74	72	111	716
5	77	75	81	60	92	77	66	67	75	670
6	59	65	87	76	62	82	107	72	96	706
7	67	78	91	63	88	100	73	83	66	709
8	84	99	64	90	73	85	102	74	84	755
9	67	82	80	83	63	62	86	73	90	686
$Y_{u.w}$	614	689	689	685	673	703	763	662	763	6241 $=Y_{..w}$

SECTION 3. Z_{uvw}

v \ u	1	2	3	4	5	6	7	8	9	
1	79	67	72	80	66	68	86	80	63	661 $Z_{.vw}$
2	69	99	61	79	80	59	90	68	67	672
3	57	49	73	67	82	87	65	86	67	633
4	76	80	92	58	92	87	72	81	102	740
5	79	74	84	66	86	79	62	65	68	663
6	64	67	97	83	61	91	108	66	89	726
7	63	83	107	58	95	86	69	80	67	708
8	79	92	71	98	79	84	84	86	66	739
9	71	83	79	86	62	60	78	67	87	673
$Z_{u.w}$	627	694	736	675	703	701	714	679	676	6215 $=Z_{..w}$

$\Sigma(t_{uvw}\ T_{uvw})=508,939.949.$

SECTION 4: T_{uvw}

v \ u	For all groups 1	2	3	4	5	6	7	8	9	
1	238	193	210	255	193	219	282	227	203	2,020 $T_{.vw}$
2	188	287	188	242	267	182	267	203	247	2,071
3	172	168	216	243	238	268	196	253	207	1,961
4	232	231	281	195	241	258	221	220	316	2,195
5	241	223	248	192	271	242	201	204	216	2,038
6	190	203	272	239	189	252	313	208	259	2,125
7	207	257	309	191	281	302	225	260	202	2,234
8	235	282	201	281	224	249	285	236	220	2,213
9	227	257	253	264	202	189	253	224	279	2,148
$T_{u.w}$	1,930	2,101	2,178	2,102	2,106	2,161	2,243	2,035	2,149	19,005 $=T_{..w}$

SECTION 5: $T'_{uvw/3n}$

v \ u	1	2	3	4	5	6	7	8	9	
1	26.444	21.444	23.333	28.333	21.444	24.333	31.333	25.222	22.556	−0.124 $C_{.vw}$
2	20.889	31.889	20.889	26.889	29.667	20.222	29.667	22.556	27.444	−.333
3	19.111	18.667	24.000	27.000	26.444	29.778	21.778	28.111	23.000	−1.237
4	25.778	25.667	31.222	21.667	26.778	28.667	24.556	24.444	35.111	−.527
5	26.778	24.778	27.556	21.333	30.111	26.889	22.333	22.667	24.000	−.847
6	21.111	22.556	30.222	26.556	21.000	28.000	34.778	23.111	28.778	−.053
7	23.000	28.556	34.333	21.222	31.222	33.556	25.000	28.889	22.444	−1.664
8	26.111	31.333	22.333	31.222	24.889	27.667	31.667	26.222	24.444	.156
9	25.222	28.556	28.111	29.333	22.444	21.000	28.111	24.889	31.000	−1.680
$C_{u.w}$	.296	−.037	.438	.043	.290	.074	−.531	.056	−1.111	

SECTION 6: CALCULATED CORRECTED PROGENY MEANS, t_{vuw}

v \ u	1	2	3	4	5	6	7	8	9	
1	27.440	21.760	23,734	30.771	21.982	25.094	31.020	25.555	22.521	25.542 $t_{.vw}$
2	20.577	32.324	21.606	27.797	31.484	20.904	29.058	22.915	28.644	26.145
3	19.265	19.648	23.924	27.331	25.944	29.371	20.870	27.152	21.376	23.876
4	26.531	25.883	32.109	23.566	26.518	29.569	25.037	24.868	35.166	27.694
5	27.390	24.149	26.913	21.178	30.235	27.069	22.630	22.913	23.587	25.118
6	22.073	23.783	31.700	27.355	21.961	28.085	33.795	24.589	28.505	26.872
7	23.159	26.931	32.923	20.836	30.727	32.882	23.319	29.028	19.572	26.597
8	26.998	32.892	22.403	32.483	26.109	28.424	33.689	27.372	24.880	28.361
9	25.180	28.100	27.468	28.425	21.766	19.662	26.791	24.858	29.143	25.710
$t_{u.w}$	24.290	26.163	26.976	26.638	26.303	26.784	27.357	25.472	25.933	

$\Sigma(X_{.vw}\ t_{.vw})=171,768.205.$

$\Sigma(Y_{u.w}\ t_{u.w})=163,827.666.$

effects and interactions. Analogous to this, the formula (7, *p. 436*) for the estimate of an individual strain, t_{uvw}, is

$$t_{uvw}=m_{uvw}+\frac{1}{2}(\bar{m}_{.vw}+\bar{m}_{u.w}+\bar{m}_{uv.})-\frac{1}{2}(\bar{m}_{u..}+\bar{m}_{.v.}+\bar{m}_{..w})$$

$$+\frac{1}{2}(\bar{X}_{u..}+\bar{Y}_{.v.}+Z_{..w})-\frac{1}{2}(\bar{X}_{.vw}+\bar{Y}_{u.w}+\bar{Z}_{uv.})\text{ ------------- }(1)$$

Where m_{uvw} is the mean yield of the nine replications of a strain, the first and the third quantities within parentheses are considered main effects and the other two interactions. This formula has been developed in a detailed manner, but the procedure has not been included here since it involves considerable algebra inappropriate to the purposes of this article.

For a better understanding of the terms making up the above formula a brief explanation of the mathematical notation is needed. Subtotals of all the plots (3 of these) in each of the three groups which are numbered exactly alike are designated X_{uvw}, Y_{uvw}, and Z_{uvw}, respectively, while the total of the three groups (9 of these) is called T_{uvw}. In table 2 the first item in section 1, 82, is the sum of the three plots in the X group with the number 111; in section 2, 77 is the sum of the three plots in the Y group with the number 111, and finally in section 3, 79 is the sum of the three 111's in the Z group; and the total of all these is 238 in section 4. A dot appearing in place of the u, v, or w in a subscript indicates a summation of all plots whose numbers in the same position as the dot range from 1 to 9; i. e., $X_{.vw}$ is the summation of all plots with the same vw but with u ranging from 1 to 9, and $X_{..w}$ that of all plots with w constant but all values of u and v. Thus 674 is the sum of the 27 progeny measurements in group X whose numbers end in 11; and 6,549 is the sum of the 243 progeny values in group X with $w=1$, u and v taking on all values from 1 to 9. This notation is extended for all terms with uvw subscripts and is quite adequate for all summations. The corrected progeny mean is denoted t_{uvw} while $t_{.vw}$, $t_{u.w}$, and $t_{uv.}$ are the average progeny means for the nine progenies having constant vw, uw, and uv designations. For ease of computation it has been found desirable to combine certain of the correction terms into three symmetrical parts, designated $C_{.uw}$, $C_{u.w}$, and $C_{uv\cdot}$. Their composition and consistency will appear from the discussion to follow.

It is now possible to show the application of formula (1) to the data at hand, as follows:

$m_{uvw}=\frac{T_{uvw}}{3n}$ (section 5 of the first nine computational tables of which table 2 is a sample);

$\bar{m}_{\cdot vw}$, $\bar{m}_{u\cdot v}$, and $\bar{m}_{uv\cdot}$=the same average of $3np$ (or 81) plots making up $T_{\cdot vw}$, $T_{u\cdot w}$, and $T_{uv\cdot}$, respectively (section 4 of the series of 10 tables);

$\bar{m}_{u\cdot\cdot}$, $\bar{m}_{\cdot v\cdot}$, and $\bar{m}_{\cdot\cdot w}$=the average of $3np^2$ (or 729) plots in $T_{u\cdot\cdot}$, $T_{\cdot v\cdot}$, and $T_{\cdot\cdot w}$, respectively (section 4 of the series of 10 tables);

$\bar{X}_{u\cdot\cdot}$, $\bar{Y}_{\cdot v\cdot}$, and $\bar{Z}_{\cdot\cdot w}$=the average of np^2 (or 243) plots making up $X_{u\cdot\cdot}$, $Y_{\cdot v\cdot}$, and $Z_{\cdot\cdot w}$, respectively (sections 1 and 2 of the last table of series [table 3], and section 3 of the first 9 [table 2];

TABLE 3.—*Computation of corrected progeny means: Summary for all values of w*

SECTION 1: X_{uv}.

v \ u	By groups 1	2	3	4	5	6	7	8	9		
1	727	672	719	785	662	712	724	752	712	6,465	$X_{.v}$
2	719	784	761	666	773	728	768	706	792	6,697	
3	816	776	674	662	723	824	758	726	743	6,702	
4	801	822	875	710	780	826	781	751	808	7,154	
5	800	832	789	807	849	743	863	733	782	7,098	
6	776	807	839	763	784	799	725	805	796	7,094	
7	817	752	766	748	782	841	749	757	800	7,012	
8	759	766	718	817	790	788	793	793	764	6,988	
9	759	795	748	758	693	766	824	730	804	6,877	
X_{u}	6,974	7,006	6,889	6,716	6,836	7,027	6,885	6,753	7,001	$62,087 = X_{..}$	

SECTION 2: Y_{uv}.

v \ u	1	2	3	4	5	6	7	8	9		
1	671	660	676	750	671	712	715	697	706	6,258	$Y_{.v}$
2	737	753	715	675	735	759	745	677	762	6,558	
3	752	702	650	602	706	733	687	686	668	6,186	
4	745	725	748	679	699	740	708	714	726	6,484	
5	737	806	724	715	780	684	712	697	746	6,601	
6	705	795	753	703	714	778	702	760	776	6,686	
7	737	697	747	679	749	790	744	740	738	6,621	
8	730	708	692	762	722	792	747	741	735	6,629	
9	723	734	714	679	657	716	748	660	759	6,390	
Y_{u}	6,537	6,580	6,419	6,244	6,433	6,704	6,508	6,372	6,616	$58,413 = Y_{..}$	

SECTION 3: Z_{uv}.

v \ u	1	2	3	4	5	6	7	8	9		
1	680	677	726	626	678	684	737	740	666	6,214	$Z_{.v}$
2	798	753	724	636	645	705	781	688	617	6,347	
3	743	606	639	571	720	755	698	736	701	6,169	
4	741	760	768	564	783	711	665	708	684	6,384	
5	722	848	811	771	801	674	647	679	693	6,646	
6	730	744	743	730	732	821	791	711	768	6,770	
7	701	768	807	676	736	779	750	655	831	6,703	
8	757	670	783	766	735	785	621	739	691	6,547	
9	680	712	718	722	659	757	759	614	760	6,381	
Z_{u}	6,552	6,538	6,719	6,062	6,489	6,671	6,449	6,270	6,411	$58,161 = Z_{..}$	

$\Sigma(Z_{uv} t_{uv}) = 1,587,292.030$.

SECTION 4: T_{uv}.

v \ u	For all groups 1	2	3	4	5	6	7	8	9		
1	2,078	2,009	2,121	2,161	2,011	2,108	2,176	2,189	2,084	18,937	$T_{.v}$
2	2,254	2,290	2,200	1,977	2,153	2,192	2,294	2,071	2,171	19,602	
3	2,311	2,084	1,963	1,835	2,149	2,312	2,143	2,148	2,112	19,057	
4	2,287	2,307	2,391	1,953	2,262	2,277	2,154	2,173	2,218	20,022	
5	2,259	2,486	2,324	2,293	2,430	2,101	2,122	2,109	2,221	20,345	
6	2,211	2,346	2,335	2,196	2,230	2,398	2,218	2,276	2,340	20,550	
7	2,255	2,217	2,320	2,103	2,267	2,410	2,243	2,152	2,369	20,336	
8	2,246	2,144	2,193	2,345	2,247	2,365	2,161	2,273	2,190	20,164	
9	2,162	2,241	2,180	2,159	2,009	2,239	2,331	2,004	2,323	19,648	
T_{u}	20,063	20,124	20,027	119,022	19,758	20,402	19,842	19,395	20,028	$=178,661\ T_{...}$	

SECTION 5: CALCULATION OF CORRECTION FACTOR, C_{uv}.

v \ u	1	2	3	4	5	6	7	8	9
1	.824	.477	.087	2.519	.372	.811	.342	.401	1.200
2	−.275	.805	.612	1.198	1.860	.941	.255	.636	2.644
3	1.095	2.255	.723	1.525	.447	.756	.860	.222	.724
4	.984	.780	.976	2.383	−.023	1.355	1.539	.895	1.693
5	1.163	.255	−.234	.649	.681	.953	1.675	1.037	1.545
6	.719	1.317	1.093	.809	.724	.064	−.399	1.475	.891
7	1.527	.076	−.184	1.235	.879	.916	.514	1.747	−.097
8	.435	1.440	−.524	1.062	.774	.527	2.397	.938	1.391
9	1.342	1.261	.599	.729	.712	.268	.891	1.593	.934

SECTION 6: CALCULATED CORRECTED PROGENY MEANS, t_{uv}.

v \ u	1	2	3	4	5	6	7	8	9
1	26.044	24.799	26.056	28.653	24.770	26.290	26.680	26.873	26.308
2	27.258	28.736	27.697	25.200	28.151	27.597	28.190	25.791	28.965
3	28.556	26.867	24.105	22.998	25.918	28.118	26.155	25.552	25.541
4	27.832	27.828	29.326	24.996	26.521	27.968	26.653	26.216	27.502
5	28.021	29.868	27.644	27.815	29.655	25.749	26.750	25.924	27.747
6	27.168	29.386	29.281	26.962	27.412	28.710	26.044	28.607	28.746
7	28.554	26.587	27.863	26.274	28.059	29.745	27.301	27.384	28.151
8	27.416	27.115	26.021	29.155	27.773	28.866	28.237	28.134	27.494
9	27.023	27.870	26.720	26.262	24.509	26.788	28.566	25.205	28.416

And, finally,

$\bar{X}_{\cdot vw}$, $\bar{Y}_{u\cdot w}$, and $\bar{Z}_{uv\cdot}$ = the average of np (or 27) plots in $X_{\cdot vw}$, $Y_{u\cdot w}$, and $Z_{uv\cdot}$, respectively, sections 1 and 2 of the first 9 tables [table 2] and, section 3 of the last of series [table 3].

For computational work a slightly different form has been found most suitable. Substituting the foregoing in formula (1) gives—

$$t_{uvw}=\frac{T_{uvw}}{3n}+\frac{1}{2}\left[\frac{T_{\cdot vw}}{3np}+\frac{T_{uv\cdot}}{3np}+\frac{T_{u\cdot w}}{3np}\right]-\frac{1}{2}\left[\frac{T_{u\cdot\cdot}}{3np^2}+\frac{T_{\cdot v\cdot}}{3np^2}+\frac{T_{\cdot\cdot w}}{3np^2}\right]$$
$$+\frac{1}{2}\left[\frac{X_{u\cdot\cdot}}{np^2}+\frac{Y_{\cdot v\cdot}}{np^2}+\frac{Z_{\cdot\cdot w}}{np^2}\right]-\frac{1}{2}\left[\frac{X_{\cdot vw}}{np}+\frac{Y_{u\cdot w}}{np}+\frac{Z_{uv\cdot}}{np}\right]$$

which may be reduced to

$$t_{uvw}=\frac{T_{uvw}}{3n}+\frac{1}{6np^2}\Big[pT_{\cdot vw}-3pX_{\cdot vw}-T_{\cdot v\cdot}+3Y_{\cdot v\cdot}\Big]+\frac{1}{6np^2}\Big(pT_{u\cdot w}$$
$$-3pY_{u\cdot w}-T_{\cdot\cdot w}-3Z_{\cdot\cdot w}\Big]+\frac{1}{6np^2}[pT_{uv\cdot}-3pZ_{uv\cdot}-T_{u\cdot\cdot}+3X_{u\cdot\cdot}]$$

With the terms containing the brackets represented by $C_{\cdot vw}$, $C_{u\cdot w}$, and $C_{uv\cdot}$ the formula for correcting an individual progeny becomes—

$$t_{uvw}=\frac{T_{uvw}}{3n}+C_{\cdot vw}+C_{u\cdot w}+C_{uv\cdot} \tag{2}$$

It will be seen later that the computation of the C's is a very simple matter. $C_{v\cdot w}$ and $C_{u\cdot w}$ appear in the margins of section 5, table 2, $C_{uv\cdot}$ in section 5, table 3. Table 2, section 6, gives the corrected mean germination days of the 81 progenies so numbered that $w=1$. There will be similar sections for w equal to 2, 3, ------ 9.

Using the corrected progeny means it would be possible to find the sum of squares for progenies by the usual procedure. It can be proved, however, that the same results may be arrived at with the formula—
SS (corrected progeny total)

$$=\Sigma(t_{uvw}T_{uvw})-[\Sigma(X_{\cdot vw}t_{\cdot vw})+\Sigma(Y_{u\cdot w}t_{u\cdot w})+\Sigma(Z_{uv\cdot}t_{uv\cdot})] \tag{3}$$

and with very much less work.

Had there been no confounding in this experiment, the variance of every comparison between pairs of progencies would have been $\frac{2s^2}{r}$, s being the error value determined from the analysis of variance table and r the number of replications. Because of the confounding, pairs of progenies will be classified in three ways on the basis of their relative block locations, for comparison by means of the variance of the mean difference. The variance for each of these is expressed in the following three formulas—

$$V(t_{211}-t_{111})=\frac{2s^2}{rp^2}(p^2+p+1)\text{ ---------------- } \tag{4}$$

$$V(t_{122}-t_{111})=\frac{s^2}{rp^2}(2p^2+3p+4)\text{ ---------------- } \tag{5}$$

$$V(t_{222}-t_{111})=\frac{s^2}{rp^2}(2p^2+3p+6)\text{ ---------------- } \tag{6}$$

depending on whether the progenies differ in one, two, or three of the letters *uvw*.

The mean variance of all comparisons is—

$$V_m=\frac{s^2}{r}\frac{(2p^2+5p+11)}{p^2+p+1} \qquad (7)$$

Formulas 4, 5, and 6 for variances of mean difference were derived in the same manner as were the formulas for correcting progeny means, in terms of main effect and interactions (*7, pp. 433–437*).

The resulting standard errors from extracting the square roots of each of the four variances are the error factors to be used in making individual comparisons of progenies by "Student's" *t* test.

The factor by which each of the above variances differ from $\frac{2s^2}{r}$ is a measure of the increase in variance that results from the division of the varieties into sets when the error variance per plot is unaltered by the resultant reduction in block size. The reciprocal of such factor, called efficiency factor of the arrangement (*9, p. 86*), is a measure of the inherent strength of the arrangement.

Thus the increases in variance are—

$$\frac{2s^2}{rp^2}(p^2+p+1)\div\frac{2s^2}{r}=\frac{p^2+p+1}{p^2}$$

$$\frac{s^2}{rp^2}(2p^2+3p+4)\div\frac{2s^2}{r}=\frac{2p^2+3p+4}{2p^2}$$

$$\frac{s^2}{rp^2}(2p^2+3p+6)\div\frac{2s^2}{r}=\frac{2p^2+3p+6}{2p^2}$$

and for all comparisons—

$$\frac{s^2}{r}\frac{2p^2+5p+11}{p^2+p+1}\div\frac{2s^2}{r}=\frac{2p^2+5p+11}{2(p^2+p+1)}$$

With $p=9$, the efficiency factor is in each case then—

$$\frac{p^2}{p^2+p+1}=.890,$$

$$\frac{2p^2}{2p^2+3p+4}=.839,$$

$$\frac{2p^2}{2p^2+3p+6}=.831,$$

and for the mean variance of all comparisons—

$$\frac{2(p^2+p+1)}{2p^2+5p+11}=.835$$

APPLICATION OF PROCEDURE TO GERMINATION DATA

With the preceding notation and formulas as a basis, the actual computations involved in correcting the progeny means for heterogeneity are very simple. The process can best be carried through in tabular form using a series of 10 computational tables of six sections each, of which table 2 illustrates the first ($w=1$) and table 3 the last—a summary of the other nine tables. For illustrative material, the time of germination was recorded for each of the 6,561 plots. This time was defined as the number of days from planting until the first day when three or more spots (six spots to the plot) had one or more seedlings (six seeds planted to the spot) visible above the ground regardless of whether or not they had been injured by damping-off or otherwise.

The procedure may be demonstrated as follows:

(1) Using a convenient index for finding the location in the nursery of the replications of individual progenies, the first step is to find the sum of the three plot measurements having the same *uvw* number in each of the three groups and record them in tabular form. For example, in table 2, for totals in the X group, the readings for No. 111 were $31+27+24=82$; in the Y group, the readings for the same progeny were $28+27+22=77$; and in the Z group they were $28+26+25=79$. This process is repeated for each of the other 728 progenies.

(2) The sum of the three totals above, $82+77+79=238$, or T_{111}, the total for the 9 replications of progeny No. 111. This and similar totals for the progenies whose numbers end in 1 are recorded in the fourth section of table 2.

(3) Marginal totals are found for the first four sections yielding $X_{u\cdot w}$, $X_{\cdot vw}$, $X_{\cdot\cdot w}$, and these are repeated for Y, Z, and T, as indicated in table 2.

(4) The average $\frac{T_{uvw}}{9}$ is computed for each value of T in the nine tables—a very simple matter. These form the fifth section of each. In table 3, for No. 111, $\frac{238}{9}=26.444$.

(5) For $X_{uv\cdot}$ the items occupying the same position in each of the nine tables are added. The first summation is $82+93+84+70+68+74+97+90+69=727$. Of these, the first value only, 82, may be found in table 2. This result, 727, is the first item in the tenth table, here represented by table 3. This process is repeated for each of the other progenies.

(6) Marginal totals here yield $X_{u\cdot\cdot}$ and $X_{\cdot v\cdot}$. The same procedure is followed for Y, Z, and T.

(7) The next important step is the calculation of the correction factors $C_{\cdot vw}$, $C_{u\cdot w}$, and $C_{uv\cdot}$. Substituting in the formulas given above for these:

$$C_{\cdot vw}=\frac{1}{6\times 3\times 9^2}\,[9\times 2020\text{ (table 2)}-27\times 674\text{ (table 2)}-18{,}937\text{ (table 3)}+3\times 6258\text{ (table 3)}]=-0.124\text{ (vertical margin of fifth section, table 2).}$$

$C_{uw} = \frac{1}{6 \times 3 \times 9^2}$[9 × 1930 (table 2)−27 × 614 (table 2)−19,005 (table 2)+3 × 6215 (table 2)]=0.296 (horizontal margin of fifth section, table 2).

$C_{uv} = \frac{1}{6 \times 3 \times 9^2}$[9 × 2078 (table 3)−27 × 680 (table 3)−20,063 (table 3)+3 × 6974 (table 2)]=0.824 (fifth section, table 3).

Attention is called to the fact that the coefficients of these three formulas are such as to give equal weighting to all items. The sum of all the C's should equal zero, which is a check on the accuracy of the computations.

(8) The final operation in correcting the individual progenies for heterogeneity is to apply these correction values to the original average in section 5, table 2, using formula 1—

$$26.444 - 0.124 + 0.296 + 0.824 = 27.440$$

which is the corrected number of days from planting until germination for progeny No. 111.

With each of the 729 progeny means corrected, it is a very simple matter to carry through the computations necessary for obtaining the items in the analysis of variance table form above. If d_{111}. d_{211} ------ d_{999} be the original germination time, then summing over all replications—

$\Sigma d_{uvw} = 178{,}661$ and the correction factor becomes $\frac{(178{,}661)^2}{6561} = 4{,}865{,}074.37$

$\Sigma d^2_{uvw} = 5{,}122{,}921$

Total $SS = 5{,}122{,}921 - 4{,}865{,}074.37 = 257{,}846.63$.

For the variation due to blocks, b,

$\Sigma b^2 = 44{,}601{,}697$

SS due to blocks $= \frac{44{,}601{,}697}{9} - 4{,}865{,}074.37 = 90{,}669.74$

The usual procedure for finding the sums of squares for the corrected progeny measurements could have been used, but formula (3) simplifies and shortens the labor to a considerable degree. Substituting therein—

SS due to progenies $= 5{,}011{,}580.822 - [1{,}689{,}976.220 + 1{,}592{,}598.640 + 1{,}587{,}292.030] = 141{,}713.932$.

These known items may now be tabulated for the analysis of variance and the error term, 25,462.96, obtained by subtraction, table 4.

TABLE 4.—*Analysis of variance of the germination period in days*

Variation due to	Degrees of freedom	Sum of squares	Mean square	*F*
Blocks	728	90, 669. 74	----------	----------
Progenies	728	141, 713. 93	194. 66	[1] 39. 02
Error	5, 104	25, 462. 96	4. 988824	----------
Total	6, 560	257, 846. 63	----------	----------

[1] Highly significant.

The error mean square in table 4 is the squared standard error, that is, $s^2=4.988824$. The comparison of individual progeny means falls into three groups, which may be determined from the progeny number, with distinct standard errors of the difference. These are expressed in formulas (4) to (7). Substituting in these

$$V(t_{211}-t_{111})=\frac{2\times 4.988824}{729}\times 91=1.245495 \quad SE=1.116$$

$$V(t_{122}-t_{111})=\frac{4.988824}{729}\times 193=1.320772 \quad SE=1.149$$

$$V(t_{222}-t_{111})=\frac{4.988824}{729}\times 195=1.334459 \quad SE=1.155$$

Mean variance of all comparisons

$$V_m=\frac{4.988824}{9}\times\frac{218}{91}=1.327917 \qquad SE_m=1.152$$

With these standard errors it is now possible to make any individual comparisons of means desired using the well-known *t* test. $t=\frac{\text{Mean}_1-\text{Mean}_2}{SE}$ and referring to the *t* table (*1, p. 166*) for the probability that the difference might be due to random sampling. This *t* is, of course, the one first established by "Student" in 1908. An adequate treatment of it is given by Fisher (*1*). To compare progenies No. 111 and No. 211.

$$t=\frac{21.760-27.440}{1.116}=-5.090$$

Entering the *t* table (*1, p. 166*) at $n=16$ ($n=n_1+n_2$ where n_1+1 and n_2+1 are each equal to 9), the computed value of *t*, 5.090, is found to be far beyond the range of the table, showing that the propability, *P*, is extremely small. This justifies the conclusion that the seeds from the tree whose progeny is No. 111 germinate at a slower rate than those from the seed tree of progeny No. 211.

In an earlier paragraph the efficiency factors for this design were computed. For the mean variance of all comparisons, this efficiency factor was found to be 0.835. In other words, 16.5 percent was lost because the ordinary randomized block per replication was not used. The reduction in error variance due to the design will, however, more than compensate for this loss. It is possible to take into account the information accruing from the block comparisons, since this experiment has a sufficient number of replications (nine in all with three for each of the groups *X*, *Y*, and Z) to give an adequate estimate of error for interblock as well as the intrablock comparisons. This adds greatly to the attractiveness of the design.

To use the information from the interblock comparisons most accurately, all the blocks forming a complete replication *X*, *Y*, or *Z* should be arranged in a compact unit on the ground, with these three groups randomized for positions with reference to each other in addition to the randomization of blocks within groups and plots within blocks (*9, pp. 30, 31, 86*). Although utilization of interblock comparisons has been introduced since the experiment was installed and the pattern outlined was not exactly followed, still it is possible to recover much of the lost information. With the present design, the 728 degrees of freedom ascribed to blocks (these confounded with main effects) may be broken down as follows:

	D/f
Groups	2
X grouping:	
Blocks	80
Error	162
Y grouping:	
Blocks	80
Error	162
Z grouping:	
Blocks	80
Error	162
	728

Combining the three terms for error gives 486 degrees of freedom. $\frac{\Sigma(X_{.vw})+\Sigma(Y_{u.w})+\Sigma(Z_{uv.})}{27}$ minus the correction factor will give the part of the sum of squares for blocks other than error. Subtracting this from the total sum of squares due to blocks leaves the error term attributed to the 486 degrees of freedom.

This computation becomes

$$\frac{48{,}003{,}111+42{,}409{,}381+42{,}040{,}325}{27}-4{,}865{,}074.37=40{,}585.52.$$

$$90{,}669.74\ (SS\text{ due to blocks})-40{,}585.52=50{,}084.22.$$

This may be set up in the following form:

Item	*D/f*	*SS*	*MS*
Blocks	486	50,084.22	103.05
Within	6,074		4.988824
Total	6,560		12.25

The mean square for the 6,560 degrees of freedom, 12.25, is found thus:

$$\frac{50{,}084.22+(6074\times 4.988824)}{6560}=12.25$$

Following Fisher (*2, pp. 255–258*), the expression for precision is $\frac{n+1}{(n+3)s^2}$, where n is the degrees of freedom and s^2 the sampling variance. Hence the ratio of the two sampling variances above, $\frac{12.25}{4.99}=$ 2.45, or 245 percent, will measure the recovery of information due to reduction in error variance by the use of this design. This means that the experiment is about 2½ times as precise as it would have been if the ordinary randomized block design had been used.

Earlier, account was taken of the loss of information due to the confounding of main effects with blocks, with the result that the efficiency of the experiment was assessed at 0.835. The net efficiency from these two sources is then

$$0.835 \times 2.45 = 2.05$$

This net gain of 105 percent makes it evident that the use of the cubic lattice design for this experiment was most worth while.

EXPLANATION AND SUMMARY

An examination of the body of germination data after the correction factors have been applied gives most conclusive evidence of the effectiveness of this type of design for the purpose for which it was evolved, namely, to eliminate differences in yields or measurements due to soil or treatment heterogeneity. Besides the expected soil variations, a known variable factor was introduced in the watering time of the nursery. It is granted that watering will tend to hasten germination. The entire nursery was watered once, beds 1–16 on April 27 and beds 17–25 on May 7. The sowing started on April 20 with bed 1 and continued consecutively until finished on May 5. This means that the greatest number of days between planting and watering for the first set would have been for bed 1, and the least for bed 16; in the second set the greatest number of days for bed 17, the lowest number of days for bed 25 (fig. 3 *B*).

The number of days from watering to germination were also recorded for each of the 6,561 plots. Using the original average values, time to germinate from planting date minus the time to germinate from watering varied from plot to plot, a range of 3.3 to 5.9.

When the average number of days from watering time to germination for each progeny was corrected, as was done for the planting time, the differences between corrected planting and corrected watering time became practically constant at 4.6 days. This is evidence that this design does eliminate effects of plot differences upon the average values, whether they be initial or some later happening, as in the incident of watering the nursery.

The correction of the 729 progeny means for heterogeneity due to location and the setting up of the procedure for making tests of significance of the differences of these individual progeny means completes the objectives of this article. From the standpoint of the purposes for which this experiment was designed, however, it marks only the initial step in the selection of seed trees for the improvement of the strains of timber trees; it is necessary to learn what conditions affect the growing characteristic of the seedlings. Hence, the next step, which is beyond the scope of this article, would be to subject the corrected data to such standard statistical procedures as seem most applicable.

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