Experimental Design

Sampling versus experiments

- similar to sampling and inventory design in that information about forest variables is gathered and analyzed
- experiments presuppose intervention through applying a *treatment* (an action or absence of an action) to a unit, called the *experimental unit*. The experimental unit is an item on which the treatment is applied.
- The goal is to obtain results that indicate cause and effect.

Definitions of terms and examples

- For each experimental unit, measures of the *variables of interest* (i.e., *response* or *dependent variables*) are used to indicate treatment impacts.
- Treatments are randomly assigned to the experimental units.
- *Replication* is the observation of two or more experimental units under identical experimental conditions.
- A *factor* is a grouping of related treatments.

Examples:

1. 1,000 seedlings in a field. Half of the seedlings get a "tea bag" of nutrients, others do not, *randomly* assigned.

   Experimental unit: the seedling.

   Treatments are: no tea bag, and tea bag.

   Factor: only one – fertilizer (none, tea bag)

   Replications: 500 seedlings get each treatment

2. 300 plant pots in a greenhouse: Each pot gets either 1) standard genetic stock; 2) genetic stock from another location; 3) improved genetic stock.

   Treatments: the three types of genetic stock

   Experimental Unit: The pot

   Factor(s): Genetic Stock (one factor only)

   Replications: 300 pots /3 treatments = 100 pots /treatment

3. The number of tailed frogs in different forest types is of interest. There are six areas. Three are cut and the other three are not cut.

   Treatments: cut, uncut

   Experimental Unit: each of the six areas

   Factor(s): only one, cutting with two levels

   Replications: six areas/ two cutting levels = 3 replicates per treatment.

4. Two forest types are identified, Coastal western hemlock and interior Douglas fir. For each, a number of samples are located, and the growth of each tree in each sample is measured.

   Treatments: NOT AN EXPERIMENT!!

   Experimental Unit:

   Factor(s):

   Replications:

What does it mean that *treatments are randomly assigned to experimental units*?

- Haphazard vs. random allocation
- Practical problems and implications

Other terms:

- The *null hypothesis* is that there are no differences among the treatment means. For more than one factor, there is more than one hypothesis
- The sum of squared differences (termed, *sum of squares*) between the average for the response variable by treatment versus the average over all experimental units represents the variation attributed to a factor.
- The *degrees of freedom*, associated with a factor, are the number of treatment levels within the factor minus one.

Example of hypotheses:

Factor A, fertilizer: none, medium, heavy (3 levels)

Factor B, species: spruce, pine (2 levels)

Number of possible treatments: 6 e..g, spruce, none is one treatment.

Experimental Unit: 0.001 ha plots

Replicates planned: 2 per treatment (cost constraint). How many experimental units do we need?

Variable of interest: Average 5-year height growth for trees in the plot

Null hypotheses:

There is no different between the 6 treatments. This can be broken into:

1) There is no interaction between species and fertilizer.
2) There is no difference between species.
3) There is no difference between fertilizers.

- *Experimental error* is the measure of variance due to chance causes, among experimental units that received the same treatment.
- The degrees of freedom for the experimental error relate to the number of experimental units and the number of treatment levels.
- The impacts of treatments on the response variables will be detectable only if the impacts are measurably larger than the variance due to chance causes.
- To reduce the variability due to causes other than those manipulated by the experimenter, relatively homogenous experimental units are carefully selected.

- Random allocation of a treatment to an experimental unit helps insure that the measured results are due to the treatment, and not to another cause.

Example: if we have applied the no fertilizer treatment to experimental units on north facing sites, whereas moderate and heavy fertilizer treatments are applied only to south facing sites, we would not know if differences in average height growth were due to the application of fertilization, the orientation of the sites, or both. The results would be *confounded* and very difficult to interpret.

Variations in experimental design

Introduction of More Than One Factor:

- Interested in the interaction among factors, and the effect of each factor.
- A treatment represents a particular combination of levels from each of the factors.
- When all factor levels of one factor are given for all levels of each of the other factors, this is a *crossed experiment*.

  Example: two species and three fertilization levels = six treatments using a crossed experiment.

Fixed, Random, or Mixed Effects:

- *Fixed factors*: the experimenter would like to know the change that is due to the particular treatments applied; only interested in the treatment levels that are in the experiment (e.g., difference in growth between two particular genetic stocks) [*fixed effects*]
- *Random factors*: the variance due to the factor is of interest, not particular levels (e.g., variance due to different genetic stocks—randomly select different stock to use as the treatment) [*random effects*]
- Mixture of factor types: Commonly, experiments in forestry include a mixture of factors, some random and some fixed [*mixed effect*].

Restricted Randomization Through Blocking: Randomized Block (RCB), Latin Square, and Incomplete Blocks Designs:

- Randomize treatments with blocks of experimental units
- Reduces the variance by taking away variance due to the item used in blocking (e.g., high, medium and low site productivity
- Results in more homogeneous experimental units within each block.

Restricted Randomization Through Splitting Experimental Units:

- Called "split plot"
- An experimental unit is split. Another factor is randomly applied to the split.

Example: The factor fertilizer is applied to 0.001 ha plots. Each of the 0.001 ha plot is then split into two, and two different species are planted in each. Fertilizer is applied to the whole plot, and species is applied to the split plot. Species is therefore randomly assigned to the split plot, not to the whole experimental unit.

Nesting of Factors

- Treatment levels for one factor may be particular to the level of another factor, resulting in nesting of treatments.

Example, for the first level of fertilizer, we might use medium and heavy thinning, whereas, for the second level of fertilizer, we might use no thinning and light thinning.

Hierarchical Designs and Sub-Sampling:

- Commonly in forestry experiments, the experimental unit represents a group of items that we measure. E.g. several pots in a greenhouse, each with several plants germinating from seeds.
- Treatments are randomly assigned to the larger unit (e.g, to each plot not to each seedling). The experimental unit is the larger sized unit.
- May want variance due to the experimental unit (pots in the example) and to units within (plants in the example). These are 1) nested in the treatment; 2) random effects; and 3) hierarchical
- A common variation on hierarchical designs is measuring a sample of items, instead of measuring all items in an experimental unit.

Introduction of Covariates

- The initial conditions for an experiment may not be the same for all experimental units, even if blocking is used to group the units.
- Site measures such as soil moisture and temperature, and starting conditions for individuals such as starting height, are then measured (called covariates) along with the response variable
- These covariates are used to reduce the experimental error.
- Covariates are usually interval or ratio scale (continuous).

Designs in use

- The most simple design is one fixed-effects factor, with random allocation of treatments to each experimental unit, with no 1) blocking; 2) sub-sampling; 4) splits; or 5) covariates
- Most designs use combinations of the different variations. For example, one fixed-effects factor, one mixed-effects factor, blocked into three sites, with trees measured within plots within experimental units (sub-sampling/hierarchical), and measures taken at the beginning of the experiment are used as covariates (e.g., initial heights of trees.

Why?

- Want to look at interactions among factors and/or is cheaper to use more than one factor in one experiment than do two experiments.
- Experiments and measurements are expensive – use sampling within experimental units to reduce costs
- Finding homogeneous units is quite difficult: blocking is needed

BUT can end up with problems:

- some elements are not measured,
- random allocation is not possible, or
- measures are correlated in time and/or space.

In this course, start with the simple designs and add complexity.

Main questions in experiments

Do the treatments affect the variable of interest?

For fixed effects: Is there a different between the treatment means of the variable of interest? Which means differ? What are the means by treatment and confidence intervals on these means?

For random effects: Do the treatments account for some of the variance of the variables of interest? How much?

Completely Randomized Design (CRD)

- Homogeneous experimental units are located
- Treatments are randomly assigned to experimental units
- No blocking is used
- We measure a variable of interest for each experimental unit

CRD: One Factor Experiment, Fixed Effects

Main questions of interest

Are the treatment means different?

Which means are different?

What are the estimated means and confidence intervals for these estimates?

Notation:

Population: $y_{ij} = \mu + \tau_j + \varepsilon_{ij}$ OR $y_{ij} = \mu_j + \varepsilon_{ij}$

y_{ij} = response variable measured on experimental unit *i* and treatment *j*

j=1 to *J* treatments

μ = the grand or overall mean regardless of treatment

μ_j = the mean of all measures possible for treatment *j*

τ_j = the difference between the overall mean of all measures possible from all treatments and the mean of all possible measures for treatment *j*, called the *treatment effect*

ε_{ij} = the difference between a particular measure for an experimental unit *i*, and the mean for the treatment *j* that was applied to it

$$\varepsilon_{ij} = y_{ij} - \mu_j$$

For the experiment:

$y_{ij} = \bar{y}_{\bullet\bullet} + \hat{\tau}_j + e_{ij}$ OR $y_{ij} = \bar{y}_{\bullet j} + e_{ij}$

$\bar{y}_{\bullet\bullet}$ = the grand or overall mean of all measures from the experiment regardless of treatment; under the assumptions for the error terms, this will be an unbiased estimate of μ

$\bar{y}_{\bullet j}$ = the mean of all measures for treatment *j*; under the assumptions for the error terms, this will be an unbiased estimate of μ_j

$\hat{\tau}_j$ = the difference between the mean of experiment measures for treatment *j* and the overall mean of measures from all treatments; under the error term assumptions, will be an unbiased estimate of τ_j

e_{ij} = the difference between a particular measure for an experimental unit *i*, and the mean for the treatment *j* that was applied to it

$$e_{ij} = y_{ij} - \bar{y}_{\bullet j}$$

n_j = the number of experimental units measured in treatment *j*

n_T = the number of experimental units measured over all treatments = $\sum_{j=1}^{J} n_j$

Example: Fertilization Trial

A forester would like to test whether different site preparation methods result in difference in heights. Twenty five areas each 0.02 ha in size are laid our over a fairly homogeneous area. Five site preparation treatments are randomly applied to 25 plots. One hundred trees are planted (same genetic stock and same age) in each area. At the end of 5 years, the heights of seedlings in each plot were measured, and averaged for the plot.

i = a particular 0.02 ha area in treatment *j*, from 1 to 5.

Response variable y_{ij} : 5-year height growth (one average for each experimental unit)

Number of treatments: *J*=5 site preparation methods

n_T = the number of experimental units measured over all treatments = $\sum_{j=1}^{5} n_j$ =25

$n_1 = n_2 = n_3 = n_4 = n_5$ =5 experimental units measured each treatment

Schematic of Layout:

3	4	4	5	1
1	2	3	5	2
2	1	2	4	2
5	4	3	1	5
4	3	1	5	3

Data Organization and Preliminary Calculations

For easy calculations by hand, the data could be organized in a spreadsheet as:

Obs:	Treatment, j=1 to J					
i=1 to n_j	1	2	3	…	J	
1	y_{11}	y_{12}	y_{13}	…	y_{1J}	
2	y_{21}	y_{22}	y_{23}	…	y_{2J}	
3	y_{31}	y_{32}	y_{33}	…	y_{3J}	
…	…	…	…	…	…	
n	y_{n1}	y_{n2}	y_{n3}	…	y_{nJ}	
Sum	$y_{\cdot 1}$	$y_{\cdot 2}$	$y_{\cdot 3}$	…	$y_{\cdot J}$	y..
Averages	$\bar{y}_{\bullet 1}$	$\bar{y}_{\bullet 2}$	$\bar{y}_{\bullet 3}$		$\bar{y}_{\bullet J}$	$\bar{y}_{\bullet\bullet}$

$$y_{\bullet j} = \sum_{i=1}^{n_j} y_{ij} \quad \bar{y}_{\bullet j} = \frac{y_{\bullet j}}{n_j} \quad y_{\bullet\bullet} = \sum_{i=1}^{J}\sum_{i=1}^{n_j} y_{ij} \quad \bar{y}_{\bullet\bullet} = \frac{y_{\bullet\bullet}}{n_T}$$ NO

TE: may not be the same number of observations for each treatment.

Example:

J= 5 site preparation treatments randomly applied to n=25 plots.

Response Variable: Plot average seedling height after 5 years

Plot Average Heights (m)

	Treatments					Overall
Observation	**1**	**2**	**3**	**4**	**5**	
1	4.6	4.9	4.0	3.4	4.3	
2	4.3	4.3	3.7	4.0	3.7	
3	3.7	4.0	3.4	3.0	3.7	
4	4.0	4.6	3.7	3.7	3.0	
5	4.0	4.3	3.0	3.4	3.4	
SUMS	20.600	22.100	17.800	17.500	18.100	96.100
Means	4.120	4.420	3.560	3.500	3.620	3.844
n_j	5	5	5	5	5	25

Example Calculations:

$$\bar{y}_{\bullet 1} = \sum_{i=1}^{5} y_{i1} = (4.6 + 4.3 + 3.7 + 4.0 + 4.3)/5 = 4.12$$

$$\bar{y}_{\bullet\bullet} = \frac{\sum_{j=1}^{5}\sum_{i=1}^{5} y_{ij}}{\sum_{j=1}^{5} n_j} = (20.6 + 22.1 + 17.8 + 17.5 + 18.1)/5 = 96.1/25 = 3.844$$

We then calculate:

1) Sum of squared differences between the observed values and the overall mean (*SSy*):

$$SSy = \sum_{j=1}^{J}\sum_{i=1}^{n_j}\left(y_{ij} - \bar{y}_{\bullet\bullet}\right)^2 \quad df = \sum_{j=1}^{J} n_j - 1$$

Also called, sum of squares total (same as in regression)

2) Sum of squared differences between the treatment means, and the grand mean, weighted by the number of experimental units in each treatment (SS_{TR})

$$SS_{TR} = \sum_{j=1}^{J}\sum_{i=1}^{n_j}\left(\bar{y}_{\bullet j} - \bar{y}_{\bullet\bullet}\right)^2 = \sum_{j=1}^{J} n_j\left(\bar{y}_{\bullet j} - \bar{y}_{\bullet\bullet}\right)^2 \quad df = J - 1$$

3) Sum of squared differences between the observed values for each experimental unit and the treatment means (*SSE*)

$$SSE = \sum_{j=1}^{J}\sum_{i=1}^{n_j}\left(y_{ij} - \bar{y}_{\bullet j}\right)^2 \qquad df = n_T - J$$

$$SSy = SS_{TR} + SSE$$

Alternative formulae for the sums of squares that may be easier to calculate are:

$$SSy = \sum_{j=1}^{J}\sum_{i=1}^{n_j} y_{ij}^2 - \frac{y_{\bullet\bullet}^{\ 2}}{n_T}$$

$$SS_{TR} = \sum_{j=1}^{J} n_j \bar{y}_{\bullet j}^2 - \frac{y_{\bullet\bullet}^{\ 2}}{n_T}$$

$$SSE = SSy - SS_{TR}$$

For the example, differences from treatment means (m):

	Treatments					Overall
Obs.	**1**	**2**	**3**	**4**	**5**	
1	0.480	0.480	0.440	-0.100	0.680	
2	0.180	-0.120	0.140	0.500	0.080	
3	-0.420	-0.420	-0.160	-0.500	0.080	
4	-0.120	0.180	0.140	0.200	-0.620	
5	-0.120	-0.120	-0.560	-0.100	-0.220	
SUMS	0.000	0.000	0.000	0.000	0.000	0.000
Sum of Squares						
Error	0.468	0.468	0.572	0.560	0.908	2.976
n_j	5	5	5	5	5	25
s^2_j	0.117	0.117	0.143	0.140	0.227	

Example Calculations:

$$SSE \text{ for treatment } 1 = \sum_{i=1}^{5} (y_{i1} - \bar{y}_{\bullet 1})^2$$

$$= (4.6-4.12)^2 + (4.3-4.12)^2 + (3.7-4.12)^2 + (4.0-4.12)^2 + (4.0-4.12)^2 = 0.468$$

$$s^2_1 = \frac{SSE \text{ for } treatment\ 1}{n_1 - 1} = \frac{0.468}{5-1} = 0.117$$

$$SSE = \sum_{j=1}^{J} \sum_{i=1}^{n_j} (y_{ij} - \bar{y}_{\bullet j})^2$$

$$= SSE \text{ for } treatment\ 1 + \text{SSE for } treatment\ 2 + \ldots + \text{SSE for } treatment\ 5$$

$$= 0.468 + 0.468 + 0.572 + 0.560 + 0.908 = 2.976$$

Differences from grand mean (m)

	Treatments					Overall
Obs.	**1**	**2**	**3**	**4**	**5**	
1	0.756	1.056	0.156	-0.444	0.456	
2	0.456	0.456	-0.144	0.156	-0.144	
3	-0.144	0.156	-0.444	-0.844	-0.144	
4	0.156	0.756	-0.144	-0.144	-0.844	
5	0.156	0.456	-0.844	-0.444	-0.444	
SUMS	1.380	2.880	-1.420	-1.720	-1.120	0.000
Sum of Squares						
Total	0.849	2.127	0.975	1.152	1.159	6.262
n_j	5	5	5	5	5	25

$$SSy = \sum_{j=1}^{J} \sum_{i=1}^{n_j} (y_{ij} - \bar{y}_{\bullet\bullet})^2$$

$$= SSy \text{ for } treatment\ 1 + \text{SSy for } treatment\ 2 + \ldots + \text{SSy for } treatment\ 5$$

$$= 0.849 + 02.127 + 0.975 + 1.152 + 1.159 = 6.262$$

Difference between treatment means and grand mean (m)

	Treatments					Overall
	1	**2**	**3**	**4**	**5**	
Mean	4.120	4.420	3.560	3.500	3.620	
Difference	0.276	0.576	-0.284	-0.344	-0.224	0.000
Sum of Squares						
Treatment	0.076	0.332	0.081	0.118	0.050	3.286
n_j	5	5	5	5	5	25

Example Calculations:

$$SS_{TR} = \sum_{j=1}^{J} n_j \left(\bar{y}_{\bullet j} - \bar{y}_{\bullet\bullet}\right)^2 = \left(5 \times (4.120 - 3.844)^2\right) + \left(5 \times (4.420 - 3.844)^2\right)$$
$$+ \left(5 \times (3.560 - 3.844)^2\right) + \left(5 \times (3.500 - 3.844)^2\right) + \left(5 \times (3.620 - 3.844)^2\right)$$
$$= 3.286$$

Test for differences among treatment means

The first main question is: Are the treatment means different?

H_0: $\mu_1 = \mu_2 = \ldots = \mu_J$

H_1: not all the same

OR:

H_0: $\tau_1 = \tau_2 = \cdots = \tau_J = 0$

H_1: not all equal to 0

OR:

H_0: $(\phi_{TR+}\sigma^2_\varepsilon) / \sigma^2_\varepsilon = 1$

H_1: $(\phi_{TR+}\sigma^2_\varepsilon) / \sigma^2_\varepsilon > 1$

Where σ^2_ε is the variance of the error terms;

ϕ_{TR} is the effect of the fixed treatments (see page 234 for more details on what this is).

If the treatment does not account for any of the variance in the response variable, then treatment effects are likely all = 0, and all the treatment means are likely all the same.

Using an analysis of variance table:

Source	df	SS	MS	F	p-value
Treatment	J-1	SS_{TR}	$MS_{TR}=SS_{TR}/(J-1)$	$F=MS_{TR}/MSE$	Prob F> $F_{(J-1),(nT-J),(1-\alpha)}$
Error	n_T-J	SSE	$MSE=SSE/(n_T-J)$		
Total	n_T-1	SSy			

$$F=\frac{SS_{TR}/(J-1)}{SSE/\sum_{j=1}^{J}(n_j-1)}=\frac{SS_{TR}/(J-1)}{SSE/(n_T-J)}=\frac{MS_{TR}}{MSE}$$

Under H_0, and the assumptions of analysis of variance, this follows an F-distribution. If

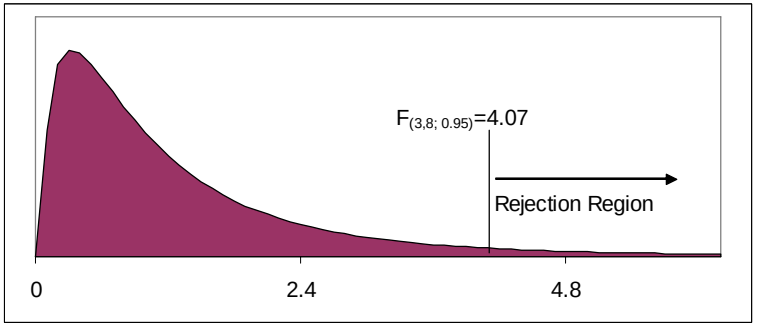
$$F > F_{(J-1,n_T-J,1-\alpha)}$$

We reject H_0 and conclude that there is a difference between the treatment means.

Notice that this is a one-sided test, using 1-α

This is because we are testing if the ratio of variances is > 1.

For example, if we have 4 treatments, and 12 experimental units, and we want α=0.05:



If the calculated F is larger than 4.07, we reject H_0: The treatments means are likely different, unless a 5% error has occurred.

OR: We take our calculated F value from our experiment and plot it on this F curve. Then, find the area to the right of this value (p-value). We reject a hypothesis if the probability value (p-value) for the test is less than the specified significance level.

For the example:

If assumptions of ANOVA are met then interpret the F-value.

H_0: $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5$

H_1: not all equal

Analysis of Variance (ANOVA) Table:

Source	df	SS	MS	F	p-value
Treatment	5-1=4	3.286	0.821	5.51	0.004
Error	25-5=20	2.976	0.149		
Total	25-1=24	6.262			

If assumptions of ANOVA are met then interpret the F-value.
NOTE: Fcritical for alpha=0.05, df treatment=4 and df error=20 is 2.87.

Since the p-value is very smaller (smaller than alpha=0.05), we reject H0 and conclude that there is a difference in the treatment means. <u>BUT</u> this is only a good test if the assumptions of analysis of variance have been met. Need to check these first (as with regression analysis).

<u>Assumptions regarding the error term</u>

For the estimated means for this experiment to be unbiased estimates of the means in the population, and the MSE to be an unbiased estimate of the variance within each experimental unit, the following assumptions must be met:

1. Observations are independent – not related in time nor in space [independent data]
2. There is normal distribution of the y-values [or the error terms] around each treatment mean [normally distributed]
3. The variances of the y's around each treatment mean [or the error terms] are the same (homogeneous) for all treatment means [equal variance]

Similar to regression:

- a normal probability plot for the error terms can be used to check the assumption of normality, and
- a residual plot can be used to visually check the assumption of equal variance.

OR, these can be tested using (1) normality tests (as with regression); (2) Bartlett's test for equal variances (for more than one factor or for other designs with blocking, etc. this becomes difficult).

Transformations to meet assumptions

Similar to regression:

- logarithmic transformations can be used to equalize variances
- arcsine transformation can be used to transform proportions into normally distributed variables
- rank transformation can be used when data are not normally distributed and other transformations do not "work" [nonparametric analysis of variance using ranks]

Unlike regression you must transform the y-variable

Process:

- do your analysis with the measured response variable
- if assumptions of the error term are not met, transform the y-variable
- do the analysis again and check the assumptions; if not me, try another transformation
- may have to switch to another method: generalized linear models, etc.

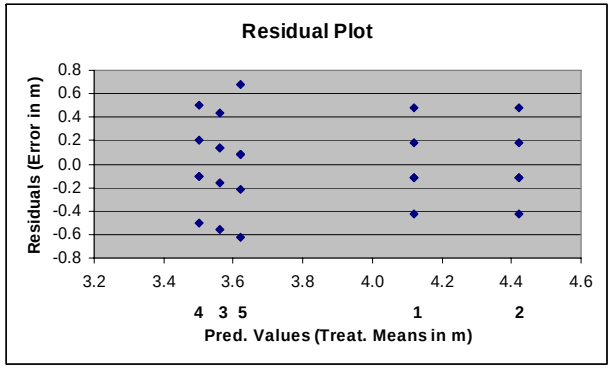
Expected values:

Under the assumptions of analysis of variance, MSE is an unbiased estimate of σ^2_ε and MS_{TR} is an unbiased estimate of $\phi_{TR+}\sigma^2_\varepsilon$. Therefore, this F-test will give the correct probabilities under the assumptions.

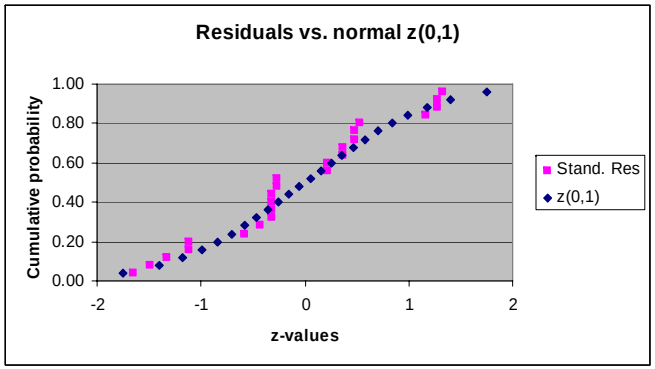
This is the same as saying that the expected value of MSE is σ^2_ε, and the expected value of MS_{TR} is $\phi_{TR+}\sigma^2_\varepsilon$. The F-test is then a measure of how much larger the value is when the treatment means are accounted for.

For the example, before interpreting the ANOVA table, we must check assumptions of ANOVA:

Is there equal variance across treatments? (estimated by MSE as 0.149 on our ANOVA table). Using a residual plot and EXCEL:



Are residuals normally distributed? Again using EXCEL:



Where standardized residuals are calculated by:

$$e_i\,(\text{standardized}) = \frac{e_i - 0}{\sqrt{MSE}}$$

Compare these to z-values for a standard normal distribution with a mean of zero and a variance of 1 (z(0,1))

Differences among particular treatment means

If there are differences among means detected, which means differ?

Can use:

- Orthogonal contrasts – see textbook
- Multiple comparisons

Multiple comparisons (or contrasts):

- Many different types, e.g.
  - T-test for every pair of means; must adjust the alpha level used by dividing by the number of pairs.
  - Scheffe's multiple comparisons
  - Bonferonni's adjustments

- Try to "preserve" the alpha level used to test all the means together (the F-test)

For the example, given that there is a difference among treatment means, which pairs of means differ?

t-test for pairs of means:

- determine the number of pairs possible

$$\binom{5}{2} = \frac{5!}{3!2!} = 10 \quad \text{possible} \quad \text{pairs} \quad \text{of} \quad \text{means}$$

Comparing Treatments 2 (largest estimated mean) versus 4 (smallest estimated mean):

$H_0 : \mu_2 - \mu_4 = 0$ OR $H_0 : \mu_2 = \mu_4$

$H_1 : \mu_2 - \mu_4 \neq 0$

$$t = \frac{(\bar{y}_{\bullet 2} - \bar{y}_{\bullet 4}) - 0}{\sqrt{MSE\left(\frac{1}{n_2} + \frac{1}{n_4}\right)}}$$

$$t = \frac{(4.4 - 3.5)}{\sqrt{0.149 \times \left(\frac{1}{5} + \frac{1}{5}\right)}} = 3.686$$

Under H_0: This follows:

$$t_{1-\alpha/2, n_T - J}$$

Using alpha=0.005 (0.05/10=0.005), for 5 treatments and 25 observations, the t-value is 3.153. Result?

Another way to assess this is to obtain the p-value for t=3.686, with 20 degrees of freedom (25-5).

This is 0.001464. Since this is less than 0.005, we reject H_0 and conclude that these two means differ.

<u>Confidence limits for treatment means</u>

Under the assumptions, confidence intervals for each treatment mean can be obtained by:

$$\bar{y}_{\bullet j} \pm t_{(n_T - J), 1-\alpha/2} \sqrt{\frac{MSE}{n_j}}$$

Since MSE estimates the variance that is assumed to be equal,

and the observations are normally distribution and independent.

For the example:

$$\bar{y}_{\bullet j} \pm t_{(n_T - 1), 1-\alpha/2} \sqrt{\frac{MSE}{n_j}}$$

$\bar{y}_{\bullet 1} = 4.1 \quad \bar{y}_{\bullet 2} = 4.4 \quad \bar{y}_{\bullet 3} = 3.6 \quad \bar{y}_{\bullet 4} = 3.5 \quad \bar{y}_{\bullet 5} = 3.6$

All $\sqrt{\frac{MSE}{n_j}}$ are all the same since n_j are all equal

$$\sqrt{\frac{0.149}{5}} = 0.173 \qquad t_{20,0.975} = 2.09$$

For treatment 1:

$4.1 \pm 2.09 \times 0.173$ $\qquad$ 4.1 ± 0.36

$(3.74, 4.46)$

Using SAS and R:

For entry into statistical programs like SAS and R, the data should be organized as:

Treatment j=1 to J	Obs: i=1 to n_j	Response
1	1	y_{11}
1	2	y_{21}
1	3	y_{31}
…	…	...
1	n_1	$y_{(n1)\,1}$
2	1	y_{12}
2	2	y_{22}
2	3	y_{32}
…	…	...
2	n_2	$Y_{(n2)\,2}$
…	...	...
J	1	y_{1J}
J	2	y_{2J}
J	3	y_{3J}
…	…	...
J	n_J	$y_{(nJ)\,3}$

For the example, we can put the data into an EXCEL file:

Treatment	Observation	AveHt
1	1	4.6
1	2	4.3
1	3	3.7
1	4	4.0
1	5	4.0
2	1	4.9
2	2	4.3
2	3	4.0
2	4	4.6
2	5	4.3
3	1	4.0
3	2	3.7
3	3	3.4
3	4	3.7
3	5	3.0
4	1	3.4
4	2	4.0
4	3	3.0
4	4	3.7
4	5	3.4
5	1	4.3
5	2	3.7
5	3	3.7
5	4	3.0
5	5	3.4

Power of the Test:

A Type I error rate (α, significance level), the chance of rejecting a null hypothesis when it is true (you reject when the means are actually the same) must be selected. Given:

- a particular number of experimental units
- sizes of the differences between true population means, and
- variation within the experimental units

this will set the Type II error rate (β), the chance of accepting a null hypothesis when it is false (you fail to reject when the means are actually different)

The power of the test is 1- β, the probability you will reject the null hypothesis and conclude that there is a difference in means, when there IS a difference between population means.

If the difference between population means (real treatment means) is very large, than a small number of experimental units will result in rejection of the null hypothesis.

If the number of experimental units is very large, then even a small difference between population means will be detected.

If the variation within experimental units is very small, then the difference will be detected, even with a small difference between population means, and even with only a few treatment units.

Statistical Significance is not the same as differences of Practical importance! UNLESS you:

- have some idea of within experimental unit variation from a previous study with the same conditions (e.g., MSE from a previous study)
- know the size of the difference that you wish to detect
- have selected the α level

Then:

You can calculate the number of experimental units per treatment that will result in rejection of H_0: when the differences are that large or greater.

Alternatively:

You can calculate the power of the test for an experiment you have already completed.

[see examples in www.forestry.ubc.ca/biometrics course materials for FRST 430/533]

Methods based on maximum likelihood rather than least squares

ML methods can be used when:

- Treatments are random rather than fixed (more on this later)
- Transformations do not result in assumptions being met
- Your dependent variable is a count, or it is a binary variable (e.g., yes or no; dead or alive; present or absent)

CRD: Two Factor Factorial Experiment, Fixed Effects

Introduction

- Treatments can be combinations of more than one factor
- For 2-factor experiment, have several levels of Factor A and of Factor B
- All levels of Factor A occur for Factor B and vice versa (called a *Factorial Experiment*, or *crossed* treatments)

Example:

- Factor A, (three levels of fertilization: A1, A2, and A3)
- Factor B (four species: B1, B2, B3 and B4)
- Crossed: 12 treatments
- Four replications per treatment for a total of 48 experimental units
- Measured Responses: height growth in mm

Schematic and Measured Response for the Example:

A1B1=10	A3B2=25	A3B4=35	A2B2=23	A1B2=14	A2B3=24
A1B4=24	A2B2=22	A1B2=15	A2B4=28	A3B3=32	A3B2=25
A3B2=27	A1B4=23	A3B3=29	A3B2=26	A1B3=17	A1B1=11
A3B4=35	A1B2=13	A1B4=22	A1B1=11	A2B3=24	A3B3=30
A1B3=19	A2B1=18	A2B4=30	A3B3=31	A2B3=23	A1B4=22
A3B1=22	A2B4=29	A3B1=23	A2B1=18	A1B2=15	A3B1=23
A2B2=25	A3B4=37	A1B1=9	A3B1=24	A3B4=36	A2B4=28
A1B3=17	A2B1=18	A2B2=20	A2B1=18	A2B3=26	A1B3=18

A1B1=10 indicates that the response variable was 10 for this experimental unit that received Factor A, level 1 and Factor B, level 1. Treatments randomly assigned to the 48 experimental units.

Organization of data for analysis using a statistics package:

A	B	result
1	1	10
1	1	11
1	1	9
1	1	11
1	2	15
1	2	15
1	2	13
1	2	14
1	3	17
1	3	18
1	3	17
1	3	19
1	4	22
1	4	23
1	4	24
1	4	22
2	1	18
2	1	18
2	1	18
2	1	18
2	2	20
. . .		
3	3	32
3	4	35
3	4	36
3	4	37
3	4	35

Main questions

1. Is there an interaction between Factor A and Factor B (fertilizer and species in the example)? Or do the means by Factor A remain the same regardless of Factor B and vice versa?
2. If there is no interaction, is there a difference
    a. Between Factor A means?
    b. Between Factor B means?
3. If there are differences:
    a. If there is an interactions, which treatment means differ?
    b. If there is no interaction, then which levels of Factor A means differ? Factor B means?

Notation, Assumptions, and Transformations

Models

Population: $y_{ijk} = \mu + \tau_{Aj} + \tau_{Bk} + \tau_{ABjk} + \varepsilon_{ijk}$

y_{ijk} = response variable measured on experimental unit *i* and factor A level *j*, factor B level *k*

j=1 to *J* levels for Factor A; *k*=1 to *K* levels for Factor B

μ = the grand or overall mean regardless of treatment

τ_{Aj} = the *treatment effect* for Factor A, level *j*

τ_{Bk} = the *treatment effect* for Factor B, level *k*

τ_{ABjk} = the *interaction* for Factor A, level *j* and Factor B, level *k*

ε_{ijk} = the difference between a particular measure for an experimental unit *i*, and the mean for a treatment:

$$\varepsilon_{ijk} = y_{ijk} - (\mu + \tau_{Aj} + \tau_{Bk} + \tau_{ABij})$$

For the experiment:

$$y_{ijk} = \bar{y}_{\bullet\bullet\bullet} + \hat{\tau}_{Aj} + \hat{\tau}_{Bk} + \hat{\tau}_{ABjk} + e_{ijk}$$

$\bar{y}_{\bullet\bullet\bullet}$ = the grand or overall mean of all measures from the experiment regardless of treatment; under the assumptions for the error terms, this will be an unbiased estimate of μ

$\bar{y}_{\bullet jk}$ = the mean of all measures from the experiment for a particular treatment *jk*

$\bar{y}_{\bullet j\bullet}$ = the mean of all measures from the experiment for a particular level *j* of Factor A (includes all data for all levels of Factor B)

$\bar{y}_{\bullet\bullet k}$ = the mean of all measures from the experiment for a particular level *k* of Factor B (includes all data for all levels of Factor A)

$\hat{\tau}_{Aj}, \hat{\tau}_{Bk}, \hat{\tau}_{ABjk}$ = under the error term assumptions, will be unbiased estimates of corresponding treatment effects for the population

e_{ijk} = the difference between a particular measure for an experimental unit *i*, and the mean for the treatment *jk* that was applied to it

$$e_{ijk} = y_{ijk} - \bar{y}_{\bullet jk}$$

n_{jk} = the number of experimental units measured in treatment *jk*

n_T = the number of experimental units measured over all treatments = $\sum_{k-1}^{K}\sum_{j=1}^{J} n_{jk}$

Means for the example:

Factor A: 16 observations per level

A1=16.25, A2=23.38, A3=28.75

Factor B: 12 observations per level

B1=17.08, B2=20.83, B3=24.17, B4=29.08

Treatments (A X B): 4 observations per treatment

Sums of Squares:

$SSy = SS_{TR} + SSE$ as with CRD: One Factor. BUT

SS_{TR} is now divided into:

$SS_{TR} = \quad SSA \quad + \quad SSB \quad + \quad SSAB$

SSy: The sum of squared differences between the observations and the grand mean:

$$SSy = \sum_{k=1}^{K}\sum_{j=1}^{J}\sum_{i=1}^{n_{jk}}\left(y_{ijk} - \bar{y}_{\bullet\bullet\bullet}\right)^2 \quad df = n_T - 1$$

SSA: Sum of squared differences between the level means for factor A and the grand mean, weighted by the number of experimental units for each treatment:

$$SSA = \sum_{k=1}^{K}\sum_{j=1}^{J} n_{jk}\left(\bar{y}_{\bullet j\bullet} - \bar{y}_{\bullet\bullet\bullet}\right)^2 \quad df = J - 1$$

SSB: Sum of squared differences between the level means for factor B and the grand mean, weighted by the number of experimental units for each treatment:

$$SSB = \sum_{k=1}^{K}\sum_{j=1}^{J} n_{jk}\left(\bar{y}_{\bullet\bullet k} - \bar{y}_{\bullet\bullet\bullet}\right)^2 \quad df = K-1$$

SSAB: Sum of squared differences between treatment means for *jk* and the grand mean, minus the factor level differences, all weighted by the number of experimental units for each treatment:

$$SSAB = \sum_{k=1}^{K}\sum_{j=1}^{J} n_{jk}\left((\bar{y}_{\bullet jk} - \bar{y}_{\bullet\bullet\bullet}) - (\bar{y}_{\bullet\bullet k} - \bar{y}_{\bullet\bullet\bullet}) - (\bar{y}_{\bullet j\bullet} - \bar{y}_{\bullet\bullet\bullet})\right)^2$$

Since some of the estimated grand means cancel out we obtain:

$$SSAB = \sum_{k=1}^{K}\sum_{j=1}^{J} n_{jk}\left(\bar{y}_{\bullet jk} - \bar{y}_{\bullet\bullet k} - \bar{y}_{\bullet j\bullet} + \bar{y}_{\bullet\bullet\bullet}\right)^2$$

$$df = (J-1)(K-1)$$

SSE: Sum of squared differences between the observed values for each experimental unit and the treatment means:

$$SSE = \sum_{k=1}^{K}\sum_{j=1}^{J}\sum_{i=1}^{n_{jk}}\left(y_{ijk} - \bar{y}_{\bullet jk}\right)^2 \qquad df = n_T - JK$$

Alternative computational formulae:

$$SSy = \sum_{k=1}^{K}\sum_{j=1}^{J}\sum_{i=1}^{n_{jk}} y_{ijk}^2 - \frac{\bar{y}_{\bullet\bullet\bullet}^{\,2}}{n_T} \qquad SSA = \sum_{k=1}^{K}\sum_{j=1}^{J} n_{jk}\bar{y}_{\bullet j\bullet}^2 - \frac{\bar{y}_{\bullet\bullet\bullet}^{\,2}}{n_T}$$

$$SS_{TR} = \sum_{k=1}^{K}\sum_{j=1}^{J} n_{jk}\bar{y}_{\bullet jk}^2 - \frac{\bar{y}_{\bullet\bullet\bullet}^{\,2}}{n_T} \qquad SSB = \sum_{k=1}^{K}\sum_{j=1}^{J} n_{jk}\bar{y}_{\bullet\bullet k}^2 - \frac{\bar{y}_{\bullet\bullet\bullet}^{\,2}}{n_T}$$

$$SSAB = SS_{TR} - SSA - SSB \qquad SSE = SSy - SS_{TR}$$

[See Excel Spreadsheet for the Example]

Assumptions and Transformations:

Assumptions regarding the error term

- Must meet assumptions to obtain unbiased estimates of population means, and an unbiased estimate of the variance of the error term (same as CRD: One Factor)
  - independent observations (not time or space related)
  - normality of the errors,
  - equal variance for each treatment.
- Use residual plot and a plot of the standardized errors against the expected errors for a normal distribution to check these assumptions.

Transformations:

As with CRD: One Factor, you must transform the y-variable

Process:

- do your analysis with the measured response variable
- if assumptions of the error term are not met, transform the y-variable
- do the analysis again and check the assumptions; if not me, try another transformation
- may have to switch to another method: generalized linear models, etc.

Test for Interactions and Main Effects

The first main question is: Is there an interaction between the two factors?

H_0: No interaction
H_1: Interaction
OR:

$$H_0: (\phi_{AB+}\sigma^2_{\varepsilon)} / \sigma^2_{\varepsilon} = 1$$
$$H_1: (\phi_{AB+}\sigma^2_{\varepsilon}) / \sigma^2_{\varepsilon} > 1$$

Where σ^2_{ε} is the variance of the error terms;

ϕ_{AB} is the interaction effect of the fixed treatments.

Using an analysis of variance table:

Source	df	SS	MS	F	p-value
A	J-1	SSA	$MSA = SSA/(J\text{-}1)$	$F = MSA/MSE$	Prob F> $F_{(J\text{-}1),(dfE),\ 1-\alpha}$
B	K-1	SSB	$MSB = SSB/(K\text{-}1)$	$F = MSB/MSE$	Prob F> $F_{(K\text{-}1),(dfE),1-\alpha}$
A X B	(J-1)(K-1)	$SSAB$	$MSAB = SSAB/(J\text{-}1)(K\text{-}1)$	$F = MSAB/MSE$	Prob F> $F_{dfAB,dfE,,1-\alpha}$
Error	n_T-JK	SSE	$MSE = SSE/(n_T\text{-}J)$		
Total	n_T-1	SSy			

Source	df	MS	E[MS]
A	J-1	MSA	$\sigma_\varepsilon^2 + \phi_A$
B	K-1	MSB	$\sigma_\varepsilon^2 + \phi_B$
A X B	(J-1)(K-1)	$MSAB$	$\sigma_\varepsilon^2 + \phi_{AB}$
Error	n_T-JK	MSE	σ_ε^2
Total	n_T-1		

See Neter et al., page 826, Table 19.8 for details on expected mean squares; ϕ is used here to represent fixed effects.

For the interactions:

$$F = \frac{SSAB/(J-1)(K-1)}{SSE/(n_T - JK)} = \frac{MSAB}{MSE}$$

- Under H_0, this follows $F_{df1,df2,\ 1-\alpha}$ where df1 is from the numerator (J-1)(K-1), and df2 is from the denominator (n_T-JK)
- If the F calculated is greater than the tabular F, or if the p-value for F calculated is less than α, reject H_0.
    - The means of Factor A are influenced by the levels of Factor B and the two factors cannot be interpreted separately.
    - Graph the means of all treatments
    - Conduct multiple comparisons all treatments (rather then on means of each Factor, separately
    - Not as much power (reject H_0 when it is false), if this occurs.

If there are no interactions between the factors, we can look at each factor separately – fewer means, less complicated.

Factor A:

$$H_0: \mu_1 = \mu_2 = \ldots = \mu_J$$

OR:

$$H_0: (\phi_{A+}\sigma^2_\varepsilon)/\sigma^2_\varepsilon = 1$$
$$H_1: (\phi_{A+}\sigma^2_\varepsilon)/\sigma^2_\varepsilon > 1$$

Where σ^2_ε is the variance of the error terms;

ϕ_A is fixed effect for Factor A.

From the ANOVA table:

$$F = \frac{SSA/(J-1)}{SSE/(n_T - JK)} = \frac{MSA}{MSE}$$

- Under H_0, this follows $F_{df1,df2,\ 1-\alpha}$ where df1 is from the numerator (J-1) and df2 is from the denominator (n_T-JK)
- If the F calculated is greater than the tabular F, or if the p-value for F calculated is less than α, reject H_0.
    - The means of Factor A in the population are likely not all the same
    - Graph the means of Factor A levels
    - Conduct multiple comparisons between means for the J levels of Factor A, separately

The analysis and conclusions would follow the same pattern for Factor B.

Analysis of Variance Table Results for the Example

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F	p
A	2	1258.17	629.08	514.70	<0.0001
B	3	934.75	311.58	254.93	<0.0001
A X B	6	17.00	2.836	2.32	0.0539
Error	36	44.00	1.22		
Total	47	2253.92			

If assumptions met, (residuals are independent, are normally distributed, and have equal variances among treatments), we can interpret the results.

Interpretation using α =0.05:

- No significant interaction (p=0.0539); we can examine species and fertilizer effects separately.
- Are significant differences between the three fertilizer levels of Factor A (p<0.0001), and between the four species of Factor B (p<0.0001).
- The mean values based on these data are:

  A1=16.25, A2=23.38, A3=28.75

  B1=17.08, B2=20.83, B3=24.17, B4=29.08

Did not have to calculate these for each of the 12 treatments since there is no interaction.

Further analyses, for each Factor separately:

- Scheffé's test for multiple comparisons, could then be used to compare and contrast Factor level means.
  - The number of observations in each factor level are: 16 for Factor A, and 12 for Factor B
  - Use the MSE for both Factor A and for Factor B (denominator of their F-tests)
- t-tests for each pair of means could be used instead.
  - Again, use MSE, and 16 observations for Factor A versus 12 for Factor B
  - Must split alpha level used in the F-tests by the number of pairs

<u>Factor A: t-tests for pairs of means</u>

Determine the number of pairs possible

$$\binom{3}{2} = \frac{3!}{1!2!} = 3 \text{ possible pairs of means}$$

Use a significance level of 0.05/3 pairs=0.017 for each t-test

Comparing Factor Levels 1 and 2: A1 vs. A2

$$H0 : \mu_{1\bullet} - \mu_{2\bullet} = 0 \qquad H1 : \mu_{1\bullet} - \mu_{2\bullet} \neq 0$$

$$t = \frac{(\bar{y}_{\bullet 1} - \bar{y}_{\bullet 2}) - 0}{\sqrt{MSE\left(\frac{1}{\sum_{k=1}^{K} n_{1k}} + \frac{1}{\sum_{k=1}^{K} n_{4k}}\right)}}$$

$$t = \frac{(16.25 - 23.38)}{\sqrt{1.22 \times \left(\frac{1}{16} + \frac{1}{16}\right)}} = -18.258$$

Critical t value from a probability table for:

- df(error) = 36 based on ($n_T - JK$), and 0.017 significance level (For α =0.05 use 0.05/3 pairs for each t-test), 2-sided test
- Using an EXCEL function: =tinv(0.017,36), returns the value of 2.50 (this assumes a 2-sided test).
- Since the absolute value of the calculated t is greater than 2.50 we reject H0.

OR

- enter your t-value, df (error), and 2 (for 2-sided) into the EXCEL function =tdist(18.258,36,2)
- Returns a p-value of <0.000. (NOTE that you must enter the positive value, and the p-value is for the two "ends" (area greater than 18.258 plus area less than -18.258)
- Since p<0.017, we reject H0

The mean of treatment A1 differs from the mean of A2.

<u>For Factor B</u>

- Recalculate the number of possible pairs for 4 factor levels

  (will be 6 pairs; divide alpha by this for each test)

- The observations per factor level is 12, rather than 16

- Df(error) and MSE are the same as for Factor A.

A Different Interpretation using α =0.10:

- There is a significant interaction (p=0.0539) using α =0.10;

  cannot interpret main effects (A and B) separately.

- The mean values based on these data are: [Excel]

  A1B1=10.25 A1B2=14.25 A1B3= 17.75 A1B4= 22.75
  A2B1=18.00 A2B2=22.50 A2B3= 24.25 A2B4=28.75
  A3B1= 23.00 A3B2=25.75 A3B3=30.50 A3B4=35.75

12 mean values as there is a significant interaction

Further analyses:

- Scheffé's test for multiple comparisons (or others) could then be used to compare and contrast treatment means (pairs or other groupings of means). The number of observations in each treatment are 4 [lower power than if there was no interaction], and use the MSE.

- Using t-tests for pairs of means, the number of observations are 4 for each *jk* treatment, use the MSE, and recalculate the number of possible pairs out of 12 treatments (will be 66 pairs! Retaining $\alpha = 0.10$, we would use $0.10/66 = 0.0015$ for each t-test)

<u>Confidence limits for factor level and treatment means</u>

Treatment means:

$$\bar{y}_{\bullet jk} \pm t_{(n-JK),1-\alpha/2}\sqrt{\frac{MSE}{n_{jk}}}$$

Factor A means:

$$\bar{y}_{\bullet j\bullet} \pm t_{(n-JK),1-\alpha/2}\sqrt{\frac{MSE}{\sum_{k=1}^{K} n_{jk}}}$$

Factor B means:

$$\bar{y}_{\bullet\bullet k} \pm t_{(n-JK),1-\alpha/2}\sqrt{\frac{MSE}{\sum_{j=1}^{J} n_{jk}}}$$

[see www.forestry.ubc.ca/biometrics course materials for FRST 430/533 for other designs]