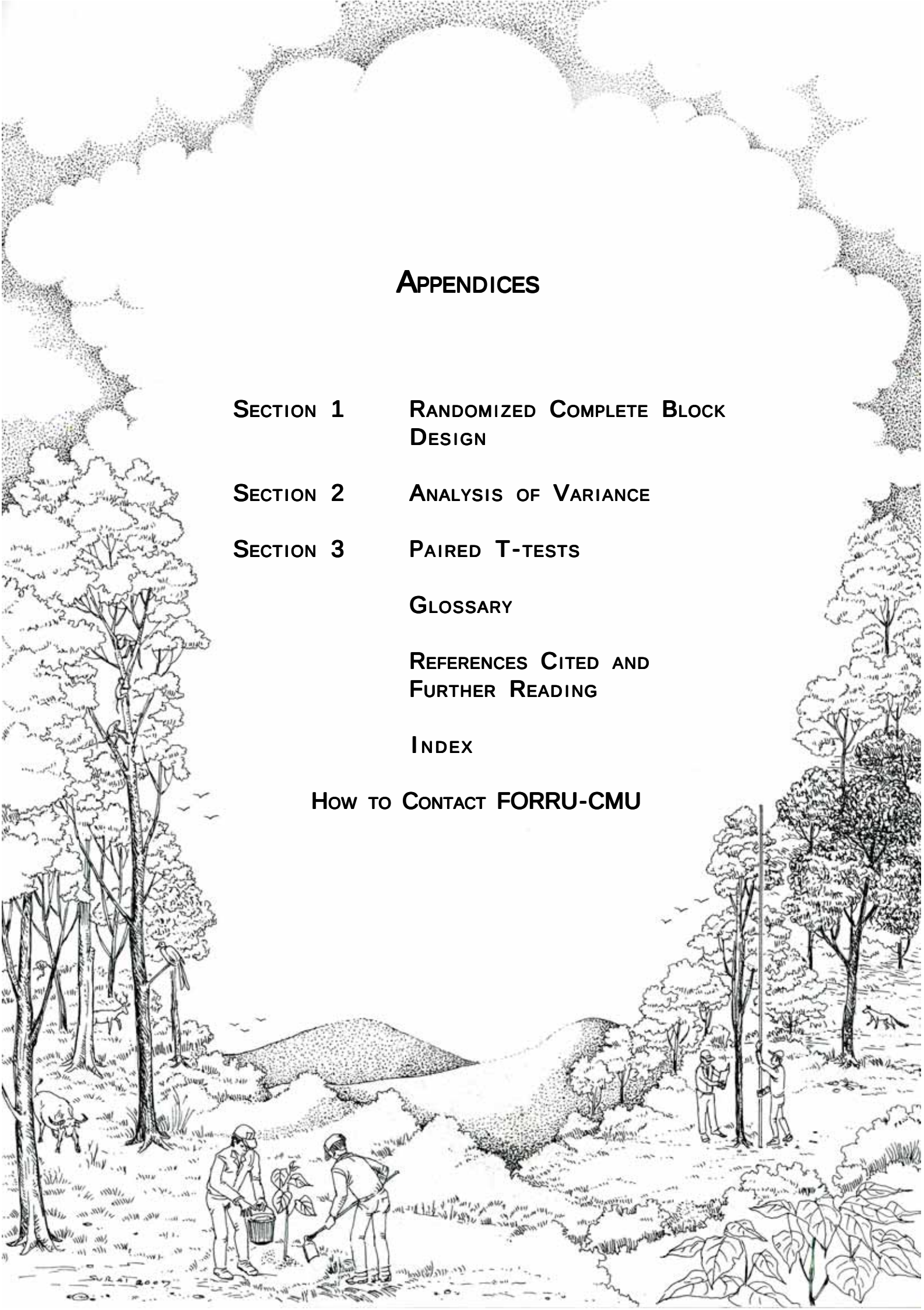
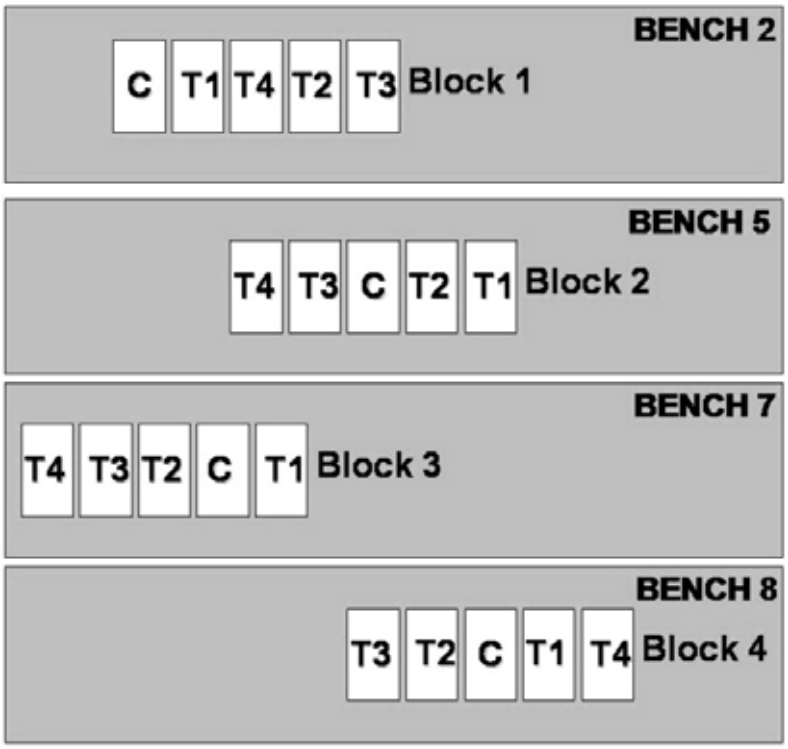
# Appendices

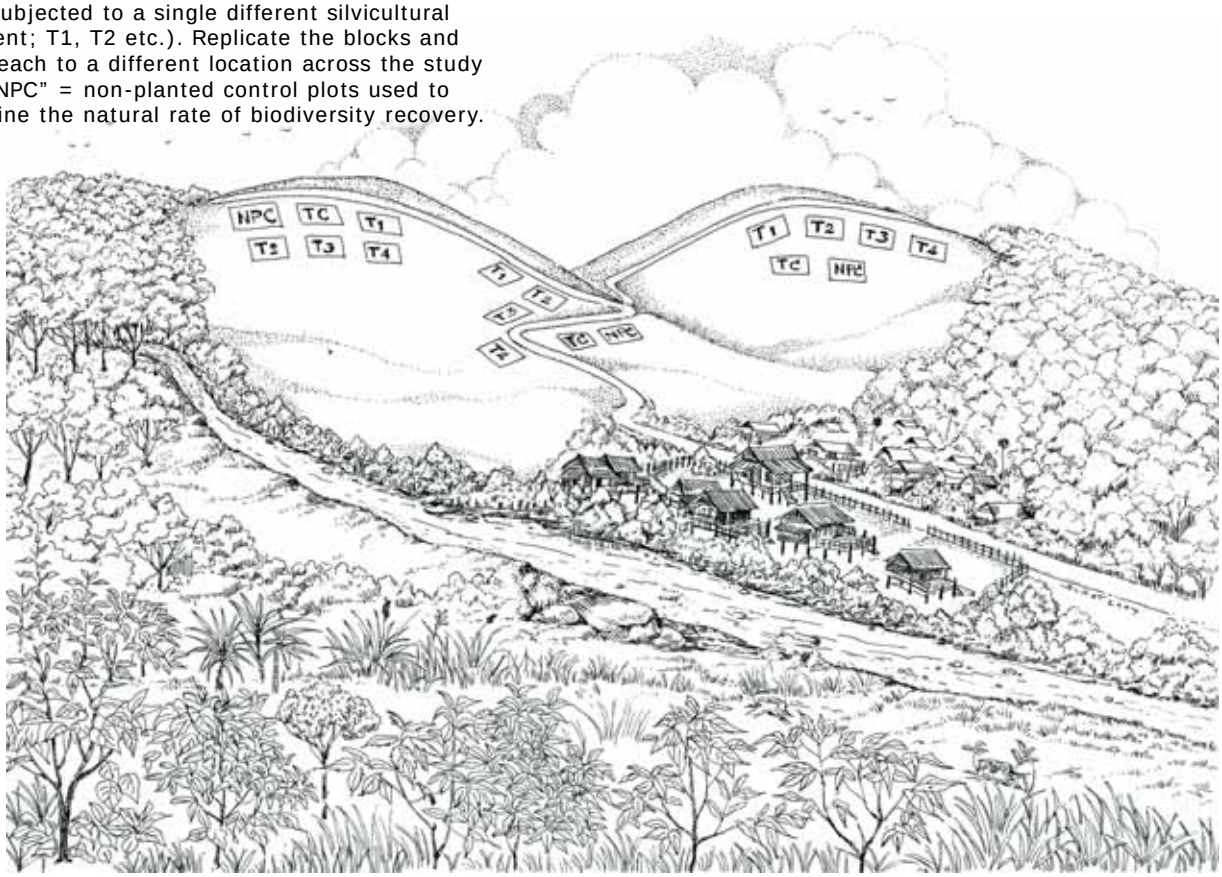




For testing pre-sowing seed treatments, place a control germination tray (C) and several treatment trays (each one containing the same number of seeds subjected to a different pre-sowing treatment; T1, T2..etc.) adjacent to each other on a nursery bench as a "block". Sow seeds that have been prepared in the standard way in the control tray. In each of the treatment trays, sow seeds that have also been prepared in the standard way, but with a single additional treatment applied. Position the trays randomly within each block. Randomly assign each block to a different bench in the nursery.




To compare performance of planted trees in field trials, group together a treatment control plot "TC" (trees planted in the standard way) with treatment plots (each subjected to a single different silvicultural treatment; T1, T2 etc.). Replicate the blocks and assign each to a different location across the study area. "NPC" = non-planted control plots used to determine the natural rate of biodiversity recovery.



# SECTION 1 - RANDOMIZED COMPLETE BLOCK DESIGN EXPERIMENTS

As with all biological experiments, those described in this manual will generate highly variable results. Therefore, experiments must be repeated or "replicated" several times and the results must be presented as mean values, followed by a measure of variation among replicates of the same treatment (e.g. variance, standard deviation etc.). Luckily, most of the experiments required for forest restoration research (germination tests, seedling growth experiments and field trials) can all be set up using the same basic experimental design and the same method of statistical analysis: i.e. a "randomized complete block design" or RCBD, with results analyzed by a two-way analysis of variance (ANOVA), followed by pair-wise comparisons.

### What is a RCBD?

A RCBD experiment consists of replicated "blocks", each one consisting of 1 replicate of the control, plus 1 replicate of each of the treatments being tested. Each treatment and the control are represented equally in every block (i.e. by the same number of seeds, plants etc.). In each block, the positions of the control and the treatments are allocated randomly. The replicate blocks are placed randomly across the study area (or nursery).

### Why use RCBD?

The purpose of using this experimental design is to separate the effects due to environmental variability from the effects of the treatments being tested. Each block may be exposed to slightly different environmental conditions (light, temperature, moisture etc.). This creates variability in the data, which can obscure the effects of the treatments applied. However, since a control replicate and treatment replicates are grouped together in each block, all germination trays or plots within a block are exposed to similar conditions. Consequently, the effects of variable external conditions can be accounted for and the effects of the treatments applied (or absence of effects) revealed by a two-way ANOVA (see Section 2).

### How many blocks and treatments?

Ideally, the combined number of blocks and treatments used should result in at least 12 "residual degrees of freedom" (rdf) according to the equation below…

$$rdf = (t-1) \times (b-1)$$

…where t = number of treatments (including the control) and b = number of blocks. In reality, it is often very difficult to achieve a rdf of >12 in nursery or field experiments, due to shortages in availability of seeds, trees, land or labour. If so, a rdf of <12 may still yield robust results, if you ensure as much uniformity among the blocks as possible. Otherwise, you may use a simpler experimental design (paired experiments, which compare a single treatment with a control) and simpler analytical methods (e.g. Chi Square for germination tests).

# ANOVA - Worked Example

Type data into an Excel spreadsheet - rows are blocks and columns are treatments. In this example, the data are the number of seeds germinating out of 50 seeds sown in each replicate tray in each block. Different treatments were applied to the seeds to try to increase germination. T1 = soaking in hot water for 1 hour; T2 = scarification with sand paper; T3 = soaking in acid for 1 minute; T4 = soaking in cold water overnight.

| | Control | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|
| Block 1 | 24 | 26 | 30 | 23 | 25 |
| Block 2 | 22 | 26 | 31 | 21 | 26 |
| Block 3 | 26 | 26 | 35 | 22 | 27 |
| Block 4 | 29 | 32 | 30 | 23 | 35 |

Running an "Anova: Two Factor Without Replication" in Excel produces two output tables. The first one presents a summary of mean values. In this example, it looks like treatments 1, 2 and 4 increase germination, whereas treatment 3 reduces it slightly; but are these difference larger than what could be expected due to random variation?

Anova: Two-Factor Without Replication

| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Block 1 | 5 | 128 | 25.6 | 7.3 |
| Block 2 | 5 | 126 | 25.2 | 15.7 |
| Block 3 | 5 | 136 | 27.2 | 22.7 |
| Block 4 | 5 | 149 | 29.8 | 19.7 |
| | | | | |
| Control | 4 | 101 | 25.25 | 8.9167 |
| T1 | 4 | 110 | 27.50 | 9.0000 |
| T2 | 4 | 126 | 31.50 | 5.6667 |
| T3 | 4 | 89 | 22.25 | 0.9167 |
| T4 | 4 | 113 | 28.25 | 20.917 |

The answer is - yes. Look at the P-values. In this case the probability that there are ***no*** differences among the treatments ((“columns”) is 0.21%, which means it is highly probable that at least some of the treatments have significant effects. Likewise, there were significant differences among the blocks (“rows”) - with only a 4.3% chance of ***no*** differences. This suggests that there were environmental differences, which affected germination among the benches within the nursery. The use of the randomized complete block design was, therefore, justified.

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | ***P-value*** | *F crit* |
|---|---|---|---|---|---|---|
| Rows | 65.35 | 3 | 21.783 | 3.6869 | **0.0433** | 3.490295 |
| Columns | 190.70 | 4 | 47.675 | 8.0691 | **0.0021** | 3.259167 |
| Error | 70.90 | 12 | 5.9083 | | | |
| | | | | | | |
| Total | 326.9 | 19 | | | | |

## SECTION 2 - ANALYSIS OF VARIANCE

Data from RCBD experiments can be analyzed by a rigorous standard statistical test called an analysis of variance (ANOVA). There are several forms of this test. The one used to analyse RCBD experiments is called a "two-way ANOVA (without replication)". The "without replication" part is confusing, since treatments *are* replicated across the blocks but in statistical jargon, it means that there is only one value for each treatment in each block e.g. for germination experiments, one value for the number of seeds germinating in each replicate germination tray.

The simplest way to perform an ANOVA is to use the Analysis ToolPak, which comes bundled with Microsoft Excel, so first make sure that you have the Analysis ToolPak installed on your computer.

If you are using Windows XP, open Excel and click on "Tools" in the toolbar and then click on "Add-Ins...". Make sure that the box next to "Anlaysis ToolPak" has a tick in it. If the tick box does not appear, you must re-run Excel set-up and install the Analysis ToolPak add-in.

With Vista, click on the Microsoft Office button (top left), then on the Excel Options button (bottom right of the menu), then on "Add Ins" and finally on the "Go" button next to "Manage Excel Add Ins". Tick the box labeled "Analysis ToolPak".

Open a new spreadsheet and type in your data with blocks as rows and treatments as columns, as shown opposite. In the example, we are using number of seeds germinated (out of 50) subjected to different pre-sowing treatments. But the same analysis could equally well be applied to the mean height of seedlings subjected to different fertilizer treatments during growing on or the number of seedlings surviving 1 year after planting out, subjected to different mulching treatments etc.

Next, if using Windows XP, click on "Tools" and then on "Data Analysis...". With Vista click on the "Data" tab at the top of the screen and then on "Data Analysis" (top right). A dialogue box, containing a list of various statistical tests, will appear. Click on "ANOVA: Two-Factor Without Replication" and then click "OK".

Another dialogue box will appear. Click on the square button to the right of the "Input Data" box. Then, using the mouse, drag the cursor across the data table to select the entire data set, including column and row headings. Back in the dialogue box, make sure there is a tick in the "Labels" box and that the value in the "Alpha" box is 0.05. Click on the circular radio button, "Output Range:" and then on the square button to the right of the output range box. In the spreadsheet, move the cursor to a cell immediately below your data table and click. Then go back to the dialogue box and click "OK".

Two tables of output results will appear below your data table. The upper one summarizes mean values for each treatment and for each block, along with a measure of variability (i.e. variance). The lower one will tell you if there are significant differences among the treatments.

In the example opposite, variances within blocks (among treatments) are generally higher than variances within treatments (among blocks), suggesting that the effects of the treatments are stronger than random variations due to differences in conditions among the blocks. It looks like treatments 1,2 and 4 increase

germination compared with the control, whereas treatment 3 reduces it. But are these results significant? The lower table answers this question.

In the table, "Rows" refers to blocks and "Columns" refers to treatments. ANOVA tests the 'null hypothesis' that there are *no* real differences among the control and the treatments tested and that any variation among the mean values is just due to chance. Consequently, if large differences among the mean values for treatments and blocks are found, then the assumption will be false, and at least one of the treatments has had a significant effect.

The important values to look at are the P-values, which quantify the probability that the null hypothesis (i.e. no differences) is valid. The previous table, therefore, shows that there is only a 0.21% probability that differences among treatments do not exist (and hence a 99.79% probability that they do). Similarly, real differences among the blocks are highly probable (95.7% likely). The significant differences among blocks, show that an RCBD was necessary in order to remove a substantial amount of variation due to differences in the micro-environment affecting each block.

Although this ANOVA shows significant differences among treatments, it does not say which of the differences are significant. In order to determine that, it is necessary to perform a pair-wise comparison.

For further information about ANOVA and for a wider choice of analytical techniques, please refer to Dytham (1999) and Bailey (1995).

## Paired t-Tests - Worked Example

| | ***Control*** | ***T1*** | ***T2*** | ***T3*** | ***T4*** |
|---|---|---|---|---|---|
| Mean | 25.25 | 27.5 | 31.5 | 22.25 | 28.25 |
| Variance | 8.91666667 | 9 | 5.66667 | 0.91667 | 20.9167 |
| Observations | 4 | 4 | 4 | 4 | 4 |
| Pearson Correlation | | 0.837218 | 0.02345 | 0.67041 | 0.87258 |
| df | | 3 | 3 | 3 | 3 |
| t Stat | | -2.63493 | -3.3113 | 2.44949 | -2.4495 |
| P(T<=t) one-tail | | 0.038997 | 0.02267 | 0.04586 | 0.04586 |
| t Critical one-tail | | 2.353363 | 2.35336 | 2.35336 | 2.35336 |
| P(T<=t) two-tail | | 0.077994 | **0.04535** | 0.09172 | 0.09172 |
| t Critical two-tail | | 3.182446 | 3.18245 | 3.18245 | 3.18245 |

## SECTION 3 – PAIRED T-TESTS

Once ANOVA has confirmed the presence of significant differences among the mean values, pair-wise comparisons are needed to confirm which differences are significant. It seems logical to compare each treatment with every other treatment, but this "shot gun" approached is frowned upon by statisticians. The more tests you perform, the more likely it is that you will find significant differences. So, it is best to adopt the so-called "*a priori*" approach, i.e. decide on the questions you want to answer beforehand and only carry out the tests necessary to answer those questions. In this case, the main question is "do treatments significantly increase or reduce performance compared with the control". To do this, use the paired t-Test in the Analysis ToolPak in Microsoft Excel. First, install the ToolPak if necessary and follow the instructions in Section 2. In "Data Analysis", click on "t-Test: Paired Two Sample for Means" and then click "OK".

In the dialogue box, click on the square button to the right of the "Variable 1 Range" box. Then, using the mouse, drag the cursor down the table to select the data set for "Control", including the column heading. Repeat for "Variable 2 Range" by selecting the data set for "T1". Back in the dialogue box, select a "Hypothesized Mean Difference" of "0" (the null hypothesis being that there is no significant difference between the treatment data). Make sure there is a tick in the "Labels" box and that the value in the "Alpha" box is 0.05. Click on the circular radio button, "Output Range:" and then on the square button to the right of the output range box. In the spreadsheet, move the cursor to a cell immediately adjacent to your data table and click. Then go back to the dialogue box and click "OK". A table of output results will appear adjacent to your data table. Repeat the process for all pair wise comparisons that you decided were useful.

The results tables opposite show higher mean values for treatments 1,2 and 4 and a lower mean value for treatment 3. For these differences to be significant, the value of "t Stat" must be higher than a critical value determined from the number of degrees of freedom and the acceptable value of P (usually 5%). The significance of the differences is therefore determined by looking at the value for "P(T<=t) two-tail". If that value is less than 0.05, the difference is significant. It means that there is a 5% probability that the null hypothesis (i.e. the difference between the means is zero) is correct. In the example opposite only one treatment, T2, satisfies this condition. So the result is that scarification most probably increased germination compared with the control from around 27/50 seeds to around 31/50. The other treatments most probably had no effect.

# GLOSSARY

**Agro-forestry:** a landuse that combines agriculture with forestry involving growing agricultural crops or raising livestock with trees.

**Analogue Forestry (AF):** analogue forestry retains the overall structure of mature tropical forest; substituting economic species for each of the plant life forms that contribute to forest structure.

**Accelerated (Assisted) Natural Regeneration (ANR):** management actions to enhance the natural processes of forest restoration, focussing on encouraging the natural establishment and subsequent growth of indigenous forest trees, whilst preventing any factors that might harm them.

**Accelerated Pioneer-Climax Series (APCS)**: a plantation design that follows the principles of natural succession by planting rows of a small number of pioneer species, followed later by inter-row planting with climax species.

**Biodiversity:** the variety of life encompassing genes, species and ecosystems.

**Candidate Framework Species:** local tree species currently undergoing nursery and field performance testing against framework species criteria to determine their suitability as a framework species.

**Climax Forest:** undisturbed, stable, forest at maximum development in terms of structure and species composition, determined by soil and climatic conditions.

**Climax Tree Species:** the tree species that comprise climax forest, with shade tolerant seedlings.

**Community Forest:** a forest that is managed collectively by local people, usually with timber and non-timber forest product extraction.

**Conservation:** the preservation, management, and care of natural and cultural resources.

**Deciduous**: shedding leaves annually or periodically; not evergreen.

**Deforestation**: conversion of forest into other land uses with less than 10% tree cover e.g. arable land, pasture, urban use, logged area, or wasteland.

**Degradation**: disturbance leading to decrease forest quality and impeded ecological functioning of the forest ecosystem.

**Direct Seeding:** the establishment of trees on deforested sites by sowing seeds rather than by planting nursery-raised saplings.

**Dormancy:** a period during which viable seeds delay germination, despite having conditions (moisture, light, temperature etc.) that are normally favourable for the later stages of germination and seedling establishment.

**Ecto-mycorrhiza:** association between vascular plant roots and fungi, resulting in a fungal sheath on root surfaces and between root cortical cells.

**Endemic:** indigenous to and confined to a particular area.

**Enrichment Planting:** planting trees to i) increase the population density of existing tree species or ii) increase tree species richness by adding tree species to degraded forest.

**Evergreen:** a plant that retains green foliage throughout the year.

**Exotic:** of species – introduced, not native.

**Extinction:** the complete loss of a species globally; when no more individuals of a species exist.

**Extirpation:** the disappearance of a species from a certain area (but it survives elsewhere).

**Extractive Reserve:** designated conservation areas, in which natural resource extraction is carried out complementary to the objective of conserving biological diversity and the natural resource base.

**Field Trial Plot System (FTPS):** a set of small plots, each one planted with a different mixture of candidate framework tree species for testing and subjected to a different silvicultural treatment.

**Forest Landscape Restoration (FLR):** integrated management of all landscape functions in deforested or degraded areas to regain ecological integrity and enhance human well-being; usually including some forest restoration.

**Forest Restoration:** any activity aimed at re-establishing the forest ecosystem originally present on a deforested site before deforestation occurred; a specialized form of reforestation.

**FORRU:** Forest Restoration Research Unit – established to develop methods to harness and accelerate the natural processes of forest regeneration, so that biodiversity-rich forest ecosystems, similar to the original forest, can be re-established.

**Foster Ecosystem:** using tree plantations of not necessarily indigenous species to facilitate the natural regeneration of native species in their understoreys.

**Framework Species Method (or Framework Forestry):** planting the minimum number of indigenous tree species required to re-instate the natural processes of forest regeneration and recover biodiversity. It combines the planting of 20-30 key tree species with various ANR techniques to enhance natural regeneration, creating a self-sustained forest ecosystem from a single planting event.

**Framework Tree Species:** indigenous, non-domesticated, forest tree species, which, when planted on deforested sites, rapidly re-establish forest structure and ecological functioning, whilst attracting seed-dispersing wildlife.

**Frugivorous:** fruit-eating.

**Genetic Diversity:** diversity within a species.

**Geographic Positioning System (GPS):** a handheld or vehicle-mounted system that uses satellite communications to determine the geographical position and other navigational information.

**Growing On:** the time that young trees are grown in the nursery between potting and transportation to the planting site. Includes both seedlings and wildlings.

**Germination:** the growth of seeds or spores after a period of dormancy; emergence of an embryonic root through the seed coverings.

**Herbarium:** a repository for easy accessible collections of dried, preserved and well-labeled specimens of plants and fungi.

**Hyphae:** a long, branching filamentous cell of a fungus; the main mode of vegetative growth; collectively called “mycelium”.

**Indigenous:** native to an area, not introduced: the opposite of exotic.

**Intermediate Seeds:** seeds which can be dried to low moisture contents approaching those of orthodox seed, but they are sensitive to chilling when dried.

**Keystone Tree Species:** tree species vital to support animal populations, usually by flowering or fruiting at times when other food resources are in short supply.

**Maximum Diversity and Miyawaki Methods of Forest Restoration:** restoring as much of the tree species richness of the original forest as possible, without relying on natural seed dispersal.

**Mycorrhiza:** symbiotic (occasionally weakly pathogenic) association between a fungus and the roots of a plant.

**Mycorrhizal Inoculums:** artificial preparations that contain mixtures of common mycorrhizal fungi spores, that can be added to plants.

**Natural Regeneration:** the recovery of forest following disturbance, in the absence of human intervention. Resulting in increasing ecosystem functionality, vegetation species diversity and structural complexity, habitat availability etc.

**Non-Governmental Organization (NGO):** a legally constituted organization created by private persons or organizations with no participation or representation of any government.

**Non Timber Forest Products (NTFP’s):** broadly includes all non-timber vegetation in forests and agro-forestry environments that have commercial value; classified into four major product categories: culinary, floral and decorative, wood-based, and medicinal and dietary supplements.

**Nurse Tree Species:** extremely hardy, usually fast-growing pioneer tree species planted specifically to develop the soil on a site, or improve its fertility.

**Orthodox Seeds:** seeds that are easy to store for many months or even years.

**Permaculture:** a word originally coined by Bill Mollison and David Holmgren in the mid 1970's to describe an "integrated, evolving system of perennial or self-perpetuating plant and animal species useful to man".

**Phenology:** the study of the responses of living organisms to seasonal cycles in environmental conditions e.g. the periodic flowering and fruiting of trees.

**Pioneer Forest:** Forest in the early stages of recovery following a large disturbance event, with higher solar radiation, wind exposure and depleted soils than for climax forest.

**Pioneer Tree Species:** Early-successional species that germinate only in full sun or the largest gaps. They exhibit high photo-synthetic and growth rates, have simple branching patterns, and require high temperature and/or high light intensity for germination. Usually short-lived, and are characteristic of pioneer forest.

**Primary Forest:** undisturbed forest at maximum development in terms of structure and species composition (=climax forest).

**Production schedule:** a concise description of the procedures for producing planting stock of optimum size and quality from seed (or wildlings) by the optimum planting out time. It combines all availabel knowledge about the reproductive ecology and cultivation of a species.

**Protected Area:** an area of land and/or sea conserved for protection and maintenance of biological diversity, and of natural and associated cultural resources, and managed through legal or other effective means.

**Recalcitrant Seeds:** seeds sensitive to drying and chilling.

**Recruit Species:** additional (non-planted) tree species that establish naturally in forest restoration sites.

**Reforestation:** planting trees to re-establish tree cover of any kind; includes plantation forestry, agro-forestry, community forestry and forest restoration.

**Remnant Forest:** small areas of forest that survive in a landscape following large-scale deforestation.

**Root Collar:** where the above-ground parts of a plant meet the tap-root.

**Secondary Forest:** a forest or woodland area which has re-grown after a major disturbance but is not yet at the end point of succession (climax forest), usually distinguished by differences in ecosystem functionality, vegetation species diversity, structural complexity, etc.

**Seed Bank:** seeds, often dormant, stored within the soil of terrestrial ecosystems. A seed bank can also refer to storage of seeds as a source for forest restoration activities.

**Seed Rain:** the movement of seed into an area through natural processes. This can occur via various mechanisms of dispersal, for example wind and animal dispersal.

**Senescent Leaves:** leaves losing their chlorophyll (green colour) just before leaf fall.

**Silviculture:** controlling the establishment, growth, composition, health, and quality of forests to meet diverse needs and values of landowners.

**Site Capture:** elimination of herbaceous vegetation by the shading effects of planted trees or by ANR.

**Standing Down:** the time that containerised seedlings are kept in the nursery, from potting until transportation to the planting site.

**Target Forest:** a forest ecosystem which defines the goals of a forest restoration program in terms of tree species composition, structure, and biodiversity levels etc.; usually the nearest surviving patch of primary forest, remaining in the landscape, at a similar elevation, slope, aspect etc. to those of the restoration site.

**Vesicular Arbuscular Mycorrhizae (VAM):** mycorrhizal fungi that grow into the root cortex of the host plant and penetrate root cells to form two kinds of specialized structures, arbuscules and vesicles. Also known as arbuscular mycorrhizae.

**Voucher Specimens:** dried specimens of tree leaves, flowers and fruits etc. kept for confirmation of species names (of phenology study trees, seed collection trees etc.).

**Wildings:** seedlings or saplings growing naturally in native forest.

**Wildlife:** all non-domesticated, plant and animal species living in natural habitats.

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