CHECK-LISTS, SOURCES AND RECORDS

Check-lists, sources and records C 60
- check-list for nursery trees not growing well

Feature	Possible reasons for poor growth	See sheet number
LEAVES		
twisted	Aphids attacking plant?	C 45
	Leaf damaged earlier by drought or insects?	C 13, 41, 45
pale colour	Soil lacks nutrient(s)?	C 14, 33
	Nutrients unbalanced?	C 14, 33
	Natural feature of new leaves - colour develops later?	C 12
very small	A feature of the particular genetic origin?	C 5
	Young tree has branched a lot?	C 5, 12, 55
	Not enough shade?	C 41
	Previous water stress?	C 13, 25, 41, 46
	Shortage of nutrients?	C 14, 33
prone to wilt	Shade reduced too quickly?	C 41, 47
	Soil too rich and leaves too big?	C 6, 23, 33, 34
	Roots damaged in potting or transplanting?	C 4, 40, 42
	Roots have not had time to grow into new soil?	C 42, 47
	Roots attacked by pest or disease?	C 45
fall off early	Sudden change in environment?	C 4, 25, 40, 46
	Too rapid hardening?	C 47
	Pots too small?	C 6, 63
	Watering problems?	C 43, 52
	Plants attacked by pest or disease?	C 45
have holes	Caterpillars or leaf-miners?	C 45
	Leaf-cutting ants?	C 45
torn	Large, soft leaves damaged by wind?	C 25, 45
	Careless handling of plants?	C 40, 50, 52
	Birds taking insects?	C 45
STEMS		
spindly	Plants too close to each other?	C 42
	Shade too heavy?	C 41
	Pots too small?	C 6
	Poor potting mix?	C 6
	Competition from weeds?	C 44
growth stopped	The species grows in height by periodic flushing?	C 12, 55
	Soil unsuitable?	C 6, 20, 23
	Plants short of nutrients?	C 14, 33
	Watering problems?	C 43
	Environment too shady?	C 41
bent	Genetic characteristic of the species?	C 5
	Temporary feature of young shoots?	C 12
	Edge plant with one-sided foliage?	C 7, 48
	Not enough shelter from wind?	C 25, 46

STEMS (continued)

die-back of tip	Soil waterlogged?	C 6, 20, 23, 67
	Previous severe water stress?	C 13, 41, 55
	Insect attack?	C 45
broken tip	Careless handling?	C 40, 50
	Severe storm?	C 3, 25
	Stem-boring insects?	C 45
	Large animal in nursery?	C 3, 25, 46
forking	Natural feature of the species or clone?	C 5
	Birds attacking buds?	C 45
	Response to previous die-back or breakage?	C 55

ROOTS

pot-bound	Containers too small?	C 6
	Tree too long in same pot?	C 6, 42
few seen	Damaged in potting up?	C 42
	Unsuitable pH of potting mix or bed?	C 6, 23
	Most roots are in the ground beneath the pot?	C 4, 41, 42
many dead	Soil poorly aerated or waterlogged?	C 6, 23
	Root disease?	C 45
	Nematodes or other pest damaging them?	C 45
small clusters along roots	Beneficial nitrogen-fixing nodules?	C 32
	Root aphis or similar pest?	C 45
fine threads near roots	Beneficial mycorrhizal fungus?	C 31
	Harmful fungus?	C 45

WHOLE PLANT

stunted	Seed-lot with inbreeding depression?	C 5
	Shoot development is naturally slower than root growth?	C 11, 12
	Plants need to be repotted into larger containers?	C 6, 42
	Different potting mix or nurery soil required?	C 6
	Shortage of a nutrient?	C 14, 33
	Inoculation for mycorrhizas or nodules needed?	C 30, 31, 32
	Altered shading required?	C 41
trees dying	Unsuitable species or provenance?	C 5
	Poor clone?	C 5
	Unfavourable nursery environment?	C 3, 4
	Insufficient care of young trees?	C 40, 50, 52
	Virus disease?	C 45, 53
	Serious pest?	C 45, 53

GENERAL APPROACH

Unless the batch is specially valuable, break up the soil on a few sample plants and examine the roots, root-collar, stems and leaves in detail, to find out what may be wrong with them.

For unsuitable genetic origins: C 5

(A) Try several different provenances, local seed sources, or clones;
(B) Avoid collecting seed from single trees or very small groups.

Difficult nursery soils: C 23

Work into the topsoil of seed and transplant beds, *if the nursery soil is:*
(a) *too acid,* either ground limestone, lime or a fertiliser that increases the pH;
(b) *alkaline,* add flowers of sulphur or a fertiliser that lowers the pH;
(c) *too heavy,* work in some sharp sand;
(d) *too sandy,* work in some silt and extra organic matter;
(e) *too hard,* try cultivation and extra organic matter, sand or forest topsoil;
(f) *too wet,* try digging drains and using raised nursery beds;
(g) *too dry,* try sunken beds and mulching.

Container problems: C 6

(1) Try out pots of different size, shape or type;
(2) Consider using 'root-trainers';
(3) Make up a different potting mix;
(4) Stand the containers on a different surface.

Unsuitable potting mixtures: C 6, 30, 42

(1) *If drainage is poor,* add more sharp sand or grit, check the holes in the containers, and also the standing ground;
(2) *If pots drain too freely,* add more organic matter and finer components, and consider whether the young trees should be potted up more firmly;
(3) *If the pH is unsuitable,* treat the potting mixture as for nursery soils above, but avoiding too much of any nutrient;
(4) *If a crust of algae forms on the surface,* use a mixture less rich in nutrients, and break up the crust with a sharpened stick;
(5) *If a micro-nutrient is lacking,* add some good topsoil or sieved compost or a small amount of a suitable fertiliser;
(6) *If the species has a close association with a micro-organism,* mix in chopped roots and topsoil from under a thriving stand of the same species, nursery soil from a bed where it has grown well, or a special inoculum if this is available.

Problems with watering: C 24, 40, 43, 66

(A) Check water purity and availability, and install a reserve supply;
(B) Check the type of container, potting mix and potting up techniques;
(C) Increase the spacing of plants to allow water to reach each pot;
(D) Consider changing the way the water is put on;
(E) Explain the desired watering method more clearly, and check that it is adopted.

Difficulties over light and shade: C 41, 47, 48

(a) Put the young trees under temporarily heavier shade when their root systems have been disturbed, but avoid keeping them in deep shade too long;
(b) Reduce shading gradually, rather than suddenly, allowing for changing seasons;
(c) Consider planting suitable shade trees.

Shelter problems: C 25, 46, 48

(A) Plant hedges to check the force of the wind and reduce water loss by the young trees;
(B) Raise small seedlings and delicate species under a roof to protect them from heavy raindrops;
(C)Consider building a shadehouse or simple greenhouse.

Dealing with insect pests: C 45, 61-C

(1) Look out for early signs of a pest attack, including a check on the roots of the trees;
(2) Take off or squash the insects, and check again every day or two;
(3) Remove any nearby plants or weeds that may be acting as a centre for spread;
(4) Spray affected trees with water containing a little detergent, or if necessary with an insecticide.

Other pest problems: C 45, 61-C

(a) Try to find out what kind of pest is present, and how it can be controlled;
(b) Follow (1-4) above, where appropriate;
(c) Avoid spreading the resting stages of the pest to other plants, for instance through topsoil, compost or mulch.

Difficulties due to disease: C 45

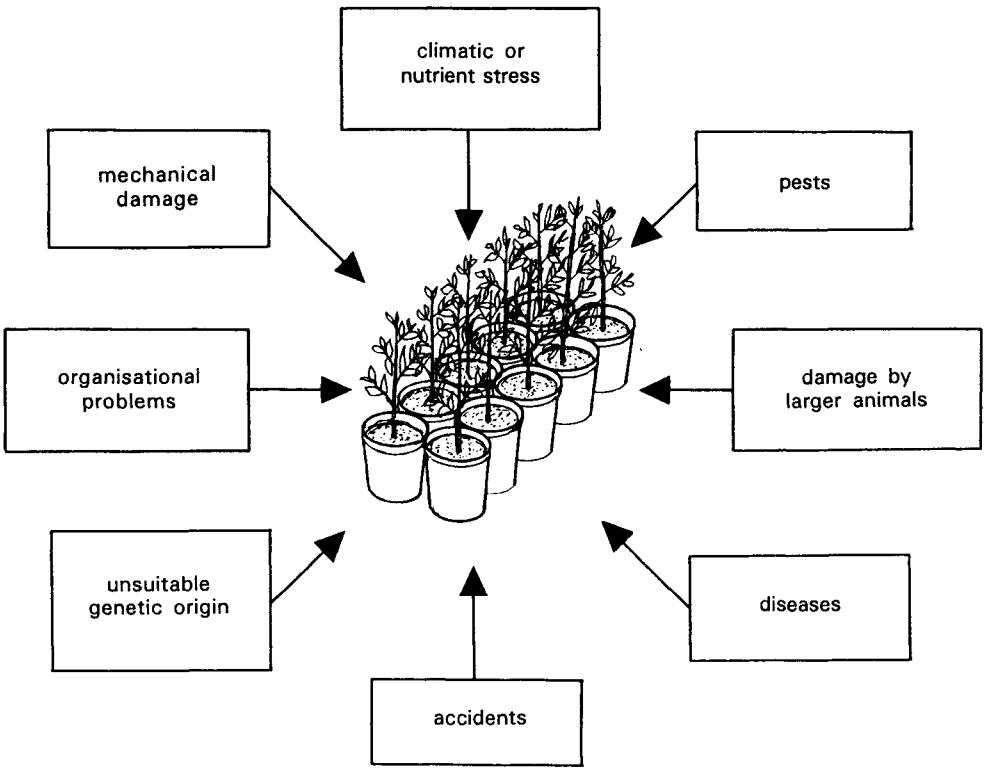
(A) Try not to have very damp, cool and shady environments, except for poly-propagators;
(B) Remove weeds and rotting leaves regularly;
(C) Look out for signs of problems, such as moulds on young leaves, cankers on stems or roots that are dying;
(D) Increase ventilation and consider decreasing shade and watering;
(E) Search for a book or someone who can identify what kind of micro-organism is responsible;
(F) If necessary, spray with a fungicide, or add one to the potting mix.

Careless handling of plants: C 40, 50, 52

(1) Explain the reasons for handling young trees carefully;
(2) Correct anyone who is treating them roughly;
(3) If work is paid by the amount done, consider changing to a system with a bonus for the number of good plants raised.

Other problems:

(a) For poorly rooted cuttings, see sheets A 2, A 50 and A 61 in Manual 1;
(b) For poor seed germination, see Manual 2.



Check-lists, sources and records C 61
- *some information about nurseries*

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Notes:

(1) See sheet A 62 in Manual 1 for more information on vegetative propagation, and sheet A 63 for some sources of chemicals and materials;
(2) More information on seed collection, storage, dormancy and germination will be given in Manual 2.

Check-lists, sources and records C 62
- *some information on tree growth*

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Notes:

(1) See *Forest Genetic Resources,* published by FAO, Rome, Italy; and sheet D 70 in Manual 4 for more sources on genetic conservation of tropical trees;
(2) See sheet D 71 for more sources on agroforestry, mycorrhizas, nitrogen-fixing species, and organisations providing information;
(3) See sheet D 72 for more sources on formal and informal experiments.

Check-lists, sources and records C 63
- *estimating quantities*

Introduction:

Unless your tree nursery is very small, setting it up and running it are likely to involve estimating:
(A) how many young trees will be needed (Manual 5);
(B) the nursery space needed for the different types of growing environments;
(C) how big the nursery should be; ***and***
(D) the amounts of various items that will be needed.

If you under-estimate, the work is likely to be held up, and insufficient numbers of young trees produced;
If you over-estimate, time and materials may be wasted, and too many plants produced.

This sheet gives some hints for making reasonably accurate predictions of what is likely to be needed.

(A) Estimating the number of trees to be grown:

(1) List the different kinds of trees and shrubs that are of interest.
(2) Estimate how many plants of each are likely to be needed, and when they are to be planted.
(3) Then increase the numbers to allow for losses, because:
(a) the percentage of seeds germinating and cuttings rooting will be less than 100%;
(b) some young trees may die during propagation, or be culled as unsuitable for planting; ***and perhaps***
(c) it may be necessary to replace some planted trees that fail to establish.
(4) Work out the approximate totals to be grown in the nursery.

(B) Calculating the space needed:

(1) **For seed beds,** a rough estimate can be made by assuming that:
(a) *larger* seeds should be sown at around *twice their diameter apart;* ***and***
(b) *smaller* seeds should be spaced on average *no closer than 5 mm to each other.*
Then each square metre should produce approximately the following number of seedlings if they are well looked after (Manual 2); and the space needed for 1000 plants would be:

Seed diameter (mm)	Number of plants per m^2 of bed	Amount of space (in m^2) needed for 1000 plants assuming a germination percentage of 75 %	50 %	25 %
1	26,500	0.05	0.07	0.15
3	12,000	0.08	0.16	0.33
5	4,200	0.32	0.48	0.95
8	1,700	0.78	1.2	2.4
10	1,175	1.1	1.7	3.4
15	600	2.2	3.3	6.7

Seed trays have the advantage of being movable, but need around 25% more space.

When larger seeds are sown directly into a pot, the space may be calculated as in (B 3).

(2) **For cuttings** (Manual 1), assume that:

(a) *leafy cuttings* will need to be spaced in the polypropagator so that the leaves are not touching each other; ***and***

(b) *leafless cuttings* (if rooted in the nursery) should be placed in the propagation bed with spaces between them that are at least twice the diameter of the stems. Then:

Spacing of cuttings (cm x cm)	Number of cuttings per m^2	Amount of space needed for 1000 cuttings (m^2)
2 x 2	2500	0.40
3 x 3	1090	0.92
4 x 4	625	1.6
5 x 5	400	2.5
7.5 x 7.5	170	5.9
10 x 10	100	10.0
12.5 x 12.5	64	15.6
15 x 15	44	22.7

(3) **For standing ground for young trees in pots:**

Widest diameter of pot when filled (cm)	Number of pots across an 80 cm wide standing area	Running length of bed to hold 100 pots (m)
5	16	0.65
10	8	1.3
15	5	3.0
20	4	5.0

More space may be needed to allow enough room for trees with bushy shoots (C 42), or if the nursery has to be put on a steep slope (C 20).

(4) For **transplanting into beds** instead of using containers, the table in (B 2) can be used to estimate the space needed. Choose:

(a) *wider* spacings for trees that are to be planted as soil blocks (to leave enough room for the roots to be pruned), and for striplings and bare-rooted planting stock; ***but***

(b) *narrower* spacings for stumps (since both roots and shoots will be heavily pruned).

(C) Considering the total area needed for the tree nursery:

Add together the estimates for each of the growing areas. Multiply by a safety factor of 1.25. Then assume that you will need as much space again for paths, roads, buildings and so on, so double the figure to give a rough idea of the total size of the nursery.

For example, if you needed:

(1) 25 m^2 for seedbeds, 5 m^2 for seed trays, and 50 m^2 for large seeds sown into pots;

(2) 20 m^2 for polypropagators, and 15 m^2 for propagating leafless cuttings;

(3) 900 m^2 for standing ground for pots, and 335 m^2 for transplant beds;

then this gives a total of 1350 m^2. Twenty-five percent of that is 338 m^2, making a total of nearly 1700 m^2 for all the growing areas. The whole nursery might then occupy about 3375 m^2 (0.34 ha), not including room to expand. On the other hand, less space will be needed if different species can occupy the same growing areas at different times of year.

(D) Estimating how much potting mix will be needed:

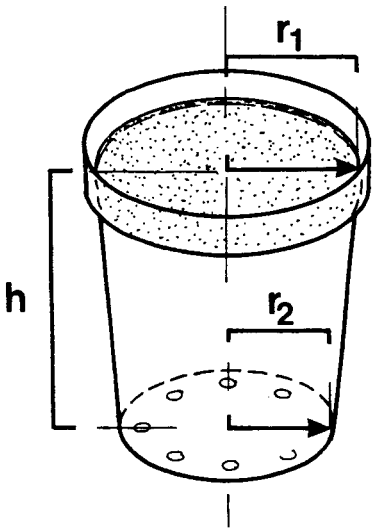
(1) **In each container:** the volume of soil that different containers hold can be estimated in two ways:

(a) by closing the holes in the bottom of a pot of each size (C 6), and filling them up with water to the proper level for the top of the soil (C 42). Pour the water from each pot into a *measuring cylinder* to find out how many millilitres (ml) of water each contains. This is a rough estimate of the number of cubic centimetres (cm^3) of firmed down soil they will hold;
or
(b) by measuring the diameter and the height up to the soil level, and then calculating the volume:

For roughly cylindrical pots: divide the diameter by two to get the *radius.* The soil volume equals the radius squared, multiplied by 3.14, and multiplied by the height.
Volume = $\pi r^2 h$.
Example (a): if the diameter is 6 cm and the height 7 cm, then the soil volume is about 250 cm^3. *(Smaller than this, pots are only suitable for very small trees.)*
Example (b): if the diameter is 14 cm and the height 16.5 cm, the soil volume will be about 2500 cm^3. *(Larger than this, cylindrical pots are very heavy to use.)*

For tapered pots: divide the diameters at the top (soil level) and bottom by two to get the top radius and the bottom radius. Then the soil volume equals the top radius squared, plus the top radius multiplied by the bottom radius, plus the bottom radius squared; then multiplied by 3.14, then multiplied by the height and divided by 3.
Volume = $\pi^h/_3(r_1^2 + r_1r_2 + r_2^2)$.

Example (c): if the two diameters are 13 cm and 10 cm, and the height 11 cm, then the soil volume is about 1150 cm^3.



$$V = \pi \frac{h}{3}\left(r_1^2 + r_1 r_2 + r_2^2\right)$$

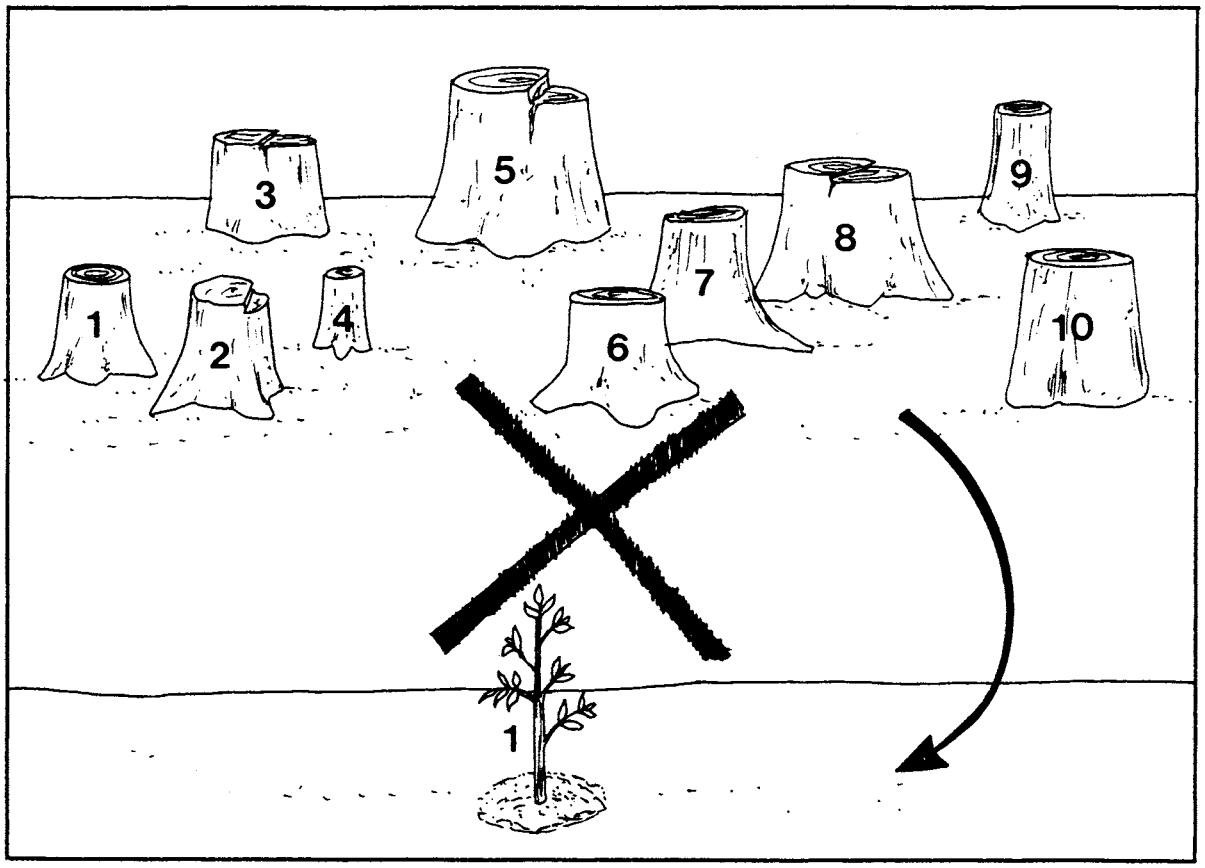
(2) To pot up 100 young trees: In (D 1) above, the volume of potting soil required will be about 0.025 m^3 for Example (a); 0.25 m^3 for Example (b); and 0.115 m^3 for Example (c).

(3) To pot up 10,000 trees a year: then the amount of the components needed for potting mix B1 in sheet C 6 for the 14 x 16.5 cm pots in example (b) above would be about 5 m^3 of coarse sand, 10 m^3 of loamy topsoil, 7.5 m^3 of weathered sawdust and 2.5 m^3 of compost each year.

(E) What other points need to be taken into account:

(1) You will also need reliable sources of good seeds (C 5 and Manual 2) and enough stock-plants to supply the shoots to make into cuttings (Manual 1).
(2) You might consider the potential for sale of unwanted planting stock.

See sheet C 61 for further information about nurseries.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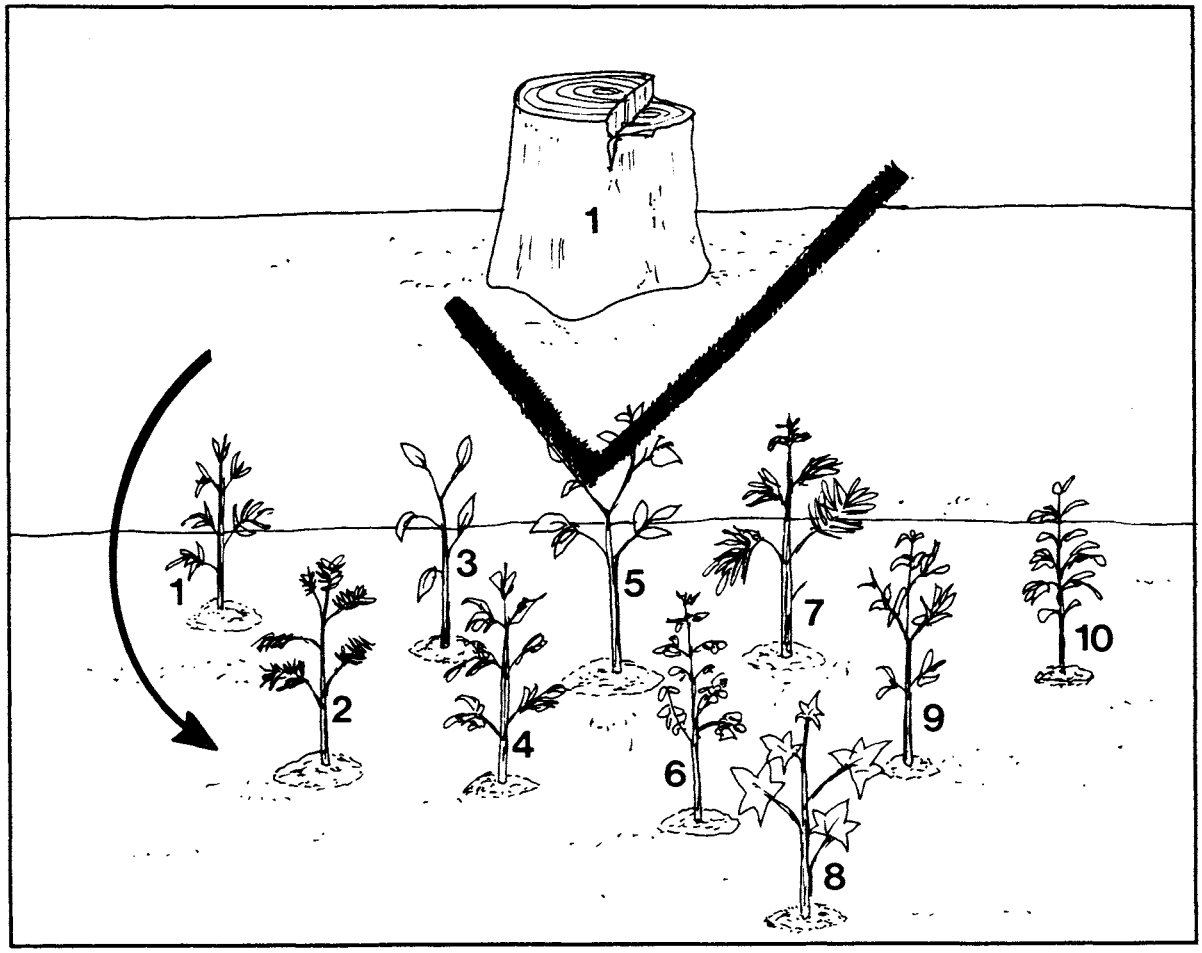
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Check-lists, sources and records C 64

- record sheet for seeds, cuttings and plants collected or received

Identity number	Species	Type of material	Quantity	Date collected	Date received	Origin	Notes
Examples:							
98/1	Leucaena leucocephala	Seeds	250g	12/97	4/1/98	Ibadan, Nigeria	scarify (hard seeds)
98/2	Triplochiton scleroxylon	Cuttings	120	5/1/98	6/1/98	Mbalmayo, Cameroun	20 x 6 clones
98/3	Lovoa trichilioides	Plants	85	(1997 fruiting)	9/1/98	local forest	wildings

Check-lists, sources and records C 65
- record sheet for batches of plants grown

SPECIES: **IDENTITY NUMBER:**

PLACE OF ORIGIN OF SEEDS OR CUTTINGS:

Date of collection - / / Collected by -

Country - Provenance/Land race -

Exact locality - Approximate altitude -

SEEDS SOWN:

Date - / / Approximate amount sown - Where propagated -

Seed beds or seed trays? Germination medium -

Germination: very good/good/moderate/poor/nil, after weeks (% germinated)

Approximate number potted/transplanted - after weeks (% survived)

CUTTINGS SET:

Date - / / Approximate numbers of each clone -

Location of stockplants - Height at which cuttings taken -

Approximate length of cuttings - cm. +/- Auxin?

Where propagated - Rooting medium -

Number rooted - on / / (% rooted)

Number potted - on / / (% potted)

INTENDED USE OF THIS BATCH OF PLANTS:

For planting out - at site -

For potted plant experiments - For other research use -

For stockplants - To grow larger -

Other purposes -

Check-lists, sources and records C 66

- record sheet for checks made during nursery propagation

WEEK STARTING:

DAILY CHECKS:

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Appearance of young trees:							
Wilting seen?							
Soil too wet?							
Leaves discoloured?							
Several leaves falling?							
Insect damage?							
Other animal damage?							
Growing conditions:							
Water supply in order?							
Shading intact?							
Weeding needed?							
Breaks in hedges or fences?							
Animal droppings?							

WEEKLY CHECKS:

Is each batch of young trees surviving and thriving?

Are any plants damaged, dead or missing?

Is it time to reduce any of the shading?

Are some batches ready for potting/transplanting?

Are weeds, insects and disease being kept in check?

What problems were found during the week?

Were they successfully dealt with?

Check-lists, sources and records C 67

- record sheets for measurements and analyses of variance

Note:

Page 210 is a blank assessment sheet for measuring trees.

Page 211 is the same sheet, with a simple worked example.

Page 212 is a blank analysis of variance sheet.

Page 213 is the same sheet, with a worked analysis of the figures on page 211.

ASSESSMENT OF ______________________________

SPECIES ;

EXPERIMENT NUMBER:

IDENTITY NUMBER:

DATE OF TREATMENT:

DATES OF ASSESSMENT:

DETAILS OF MEASUREMENTS MADE:

DATES →												OBSERVATIONS AND NOTES
TREATMENT ↓ TREE NUMBER ↓												
TOTAL												
NUMBER												
MEAN												

ASSESSMENT OF Gain in height growth

SPECIES: Ceiba pentandra

EXPERIMENT NUMBER: 3/97

IDENTITY NUMBER: 96/12

DATE OF TREATMENT: 15/12/97

DATES OF ASSESSMENT: 15/12/97, 5/1/98

DETAILS OF MEASUREMENTS MADE:
Height in cm measured from the top of a peg in the soil to the estimated position of the main shoot tip

DATES →		15/12/97	5/1/98		OBSERVATIONS AND NOTES
TREATMENT ↓	TREE NUMBER ↓	Initial height	Height	Gain	
1 (small pots)	1	20	24	4	
	2	18	26	8	
	3	21	23	2	
	4	15	25	10	
	Total	74	98	24	
	Number	4	4	4	
	Mean			6.0	
2 (medium sized pots)	5	19	25	6	
	6	17	26	9	
	7	20	28	8	
	8	(22)	—	—	attacked by aphids
	Total	56	79	23	
	Number	3	3	3	
	Mean			7.7	
3 (large pots)	9	18	32	14	
	10	20	31	11	
	11	(17)	—	—	broken off
	12	(21)	—	—	died back
	Total	38	63	25	
	Number	2	2	2	
	Mean			12.5	
	TOTAL	168	240	72	
	NUMBER	9	9	9	
	MEAN			8.0	

Species: Experiment number:

ANALYSIS OF VARIANCE

Effect of:

Assessment of: Units:

Date of treatment: Date of assessment: Assessment number:

Treatment number → / Treatment →							OVERALL TOTALS
Total (Σx)							TOTAL (ΣX)
Number (n)							NUMBER (N)
Mean ($\bar{x}$)							MEAN ($\bar{X}$)
Differences: size: significance:							C.F. = $\frac{(\Sigma X)^2}{N}$
$\Sigma(x^2)$							(A)
$\frac{(\Sigma x)^2}{n}$							(B)

Source of Variation	Sums of Squares	Df	Variance Estimate (Mean square)	Variance Ratio (sign.)	F (tables) (to exceed) (at level)
•					
•					
•					
•					
•					
•					
Treatment	(B − CF)				
Error	(total − treatment)				
Total	(A − CF)				

LSD = $t_{(\text{error Df})} \sqrt{\text{error variance} \times \left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$

Coefficient of variation: %

Standard error of the mean:

at the
5%
1%
0.1%
levels

CONCLUSIONS:

Species: Ceiba pentandra

Experiment number: 3/97

ANALYSIS OF VARIANCE

Effect of: pot size

Assessment of: gain in height

Units: cm

Date of treatment: 15/12/97 Date of assessment: 5/1/98 Assessment number: 1

Treatment number → / Treatment →	1 small	2 medium	3 large				OVERALL TOTALS
Total (Σx)	24	23	25				TOTAL (ΣX) 72
Number (n)	4	3	2				NUMBER (N) 9
Mean ($\bar{x}$)	6.0	7.7	12.5				MEAN ($\bar{X}$) 8.0
Differences: size: significance:	1.7	4.8	Treatment 3 = 2.1 × Treatment 1				C.F. = $\frac{(\Sigma X)^2}{N}$ 576
	6.5*						
$\Sigma(x^2)$	184	181	317				(A) 682
$\frac{(\Sigma x)^2}{n}$	144.00	176.33	312.50				(B) 632.83

Source of Variation	Sums of Squares		Df	Variance Estimate (Mean square)	Variance Ratio (sign.)	F (tables) (to exceed) (at level)
.						
.						
.						
.						
.						
.						
Treatment	(B − CF)	56.83	2	28.42	3.47 n.s.	5.14 (5%)
Error	(total − treatment)	49.17	6	8.194		
Total	(A − CF)	106.00	8			

LSD = $t_{(\text{error Df})} \sqrt{\text{error variance} \times \left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$

Coefficient of variation: 35.8 %

Standard error of the mean:
for 4 replicates = ± 1.4
for 3 replicates = ± 1.7
for 2 replicates = ± 2.0

at the
5% — 6.1
1% — 9.2
0.1% levels

CONCLUSIONS: Overall treatment effect not significant.
Growth in large pots more than twice that in small pots.
This probably significant difference needs further study with many more trees in each treatment.

Check-lists, sources and records C 68
- *assessment by scoring*

(A) Need for scoring methods:

Scoring is a valuable way of getting a rapid, general view of a situation in biology. It can take one further than the recording of an observation (C 55), without embarking on long and detailed measurements that may or may not be appropriate and productive.

Scoring is especially useful when the features to be assessed are difficult or impossible to record by measurement or counting; for example when differences are:

(1) primarily **qualitative**, such as the rooting of cuttings, the germination of seeds, or the stopping or re-starting of shoot elongation (C 12, C 55);
(2) rather **subjective,** like the branching habit of a young tree, or the colour of its leaves; ***or***
(3) needing to be estimated **without sacrificing** sample leaves (for example to measure leaf areas) or whole plants (to obtain dry weights).

Scoring can also be helpful later on, when the trees are **too big** for easy measurement, or there are **too many** items to count.

(B) How to score:

(1) Decide on the **feature** of the trees which you want to assess;
(2) Choose a set of recognisable **categories** or **stages** that cover at least the range of variation shown, and which can be seen without disturbing their growth;
(3) Label an example of each category or stage, and number them in sequence;
(4) Try giving a score to a few plants, and modify the categories if needed; ***and then***
(5) Score all the trees in the experiment (see the blank record sheet in C 67).

Suggestions on scoring leaf colour are given in sheet C 55.

(C) Some weaknesses of scoring methods:

(1) It can be difficult to standardise the categories, and the intervals between them are not necessarily equal;
(2) Bias (C 15) is harder to avoid than when measuring;
(3) After some time, one's brain may refuse to carry on scoring without a rest;
(4) Not all statistical tests (C 67, C 69-B2) can be done on the results, and extra care is needed not to mislead oneself.

(D) Hints on scoring:

The main aims when choosing a scoring method are to minimise these weaknesses, and to achieve a valid, useful assessment simply and promptly. Some hints are:

(1) Look through the young trees first in order to discover whether they have yet reached a suitable stage of development for scoring, and to gauge the range of variation to be expected in the feature(s) to be scored;
(2) Choose between 5 and 10 convenient categories or stages. For example, when assessing:
  (a) **categories** of *branching habits,* 1 might be used for young trees with unbranched main stems, and 5 for very bushy trees; ***and***
  (b) **stages** in *outgrowth of new shoots,* 1 might stand for "buds still unopened", and 10 for "first new leaves fully expanded";
(3) Do the scoring with at least one other person - difficult features may need three or four observers. Discuss the categories or stages together, but then score independently;
(4) Aiming for consistency of scoring is more important than whether you tend to score higher or lower than other people;
(5) Don't try and score too many different features at the same time; ***and***
(6) To reduce bias in experiments, arrange to do the scoring without knowing the treatment or genetic origin of the trees. Expectations can influence results!

(E) Analysis of scored data:

(1) Chi-square (χ^2) tests are especially appropriate for comparing categories.
The 2×2 χ^2 test is quick to calculate, and gives a simple estimate of the *significance* (see C 69-E, G, H, I) of qualitative differences such as the presence or absence of something. For example:

Comparing the number of cuttings that rooted in two different rooting media:

Treatment	Number rooted	Number not rooted	Total number	Percentage rooted
sand	19 (a)	46 (b)	65 (g)	29 %
sawdust/sand	12 (c)	3 (d)	15 (h)	80 %
both	31 (e)	49 (f)	80 (N)	

This is the sum for calculating the chi-square:

$$\chi^2 = (bc-ad)^2N/efgh$$

Note: when the numbers are small (some totals less than 30), three points apply:

(1) the test is only valid when the 'Expected Frequency' in each of the 4 main boxes a-d is at least 5 *(for example the expected frequency in box d = fh/N = 9.2 and so is valid);*

(2) 'Yates's Correction' should be applied before doing the test *(take ½ from higher value and add it to smaller);* ***and***

(3) the chi-square will only be significant if the difference between the percentages is quite large *(for example, a difference of 30 percentage points will not be significant until the total in each sample exceeds 25).*

Result of the chi-square analysis (using Yates's Correction): $\chi^2 = 11.18$ ***.

With one *degree of freedom* (see C 69-I) between the two treatments, the values of chi-square to be exceeded are 3.84 (5% level); 6.64 (1%); and 10.83 (0.1%). In this example, the difference in rooting percent is highly significant (C 69-H), indicated by ***.

When features have been scored into several categories, larger tables can be constructed, and the combined chi-square calculated. If such a test would be invalid because of low 'expected frequencies' in some boxes in the table, then categories can be amalgamated and a simpler table prepared. *(For instance, categories 1+2 and 3-5 might be put together and the larger groups compared in a 2×2 chi-square test.)*

(2) **Analysis of variance** can also be applied to scored data, and the variation between independent observers included in the analysis (see C 69-F), provided that:

a) the intervals between categories are reasonably even;

b) an appropriate *transformation* (see C 69-O) is used because the numbers are expressed in a small number of discrete categories, and may not be 'normally distributed' (C 69-B,2,g). Transformations may also be relevant when the categories are non-linear (for example, with numbers of branches in categories of 0, 1, 2-5, 6-14, 15+).

If there are many zero values, you could compare the presence or absence of the feature by a chi-square test, and confine the analysis of variance to the cases where the feature is present.

(F) Summary:

Used with judgement, scoring methods can provide a rapid and useful complement to more precise and fully quantitative measurements. They are especially valuable when time is short and the features do not lend themselves to easy measurement.

Although the data obtained are only semi-quantitative, it may be possible to carry out valid statistical tests of significance.

Check-lists, sources and records C 69
- *analysing the results of experiments*

(A) Why experimental results generally need analysing:

Looking carefully at what happened in your experiment can clarify:

(1) whether there were differences between any treatments or genetic origins;
(2) when they started to occur, and how big they became; ***and***
(3) possible linkages between observations and measurements, or between different assessments (C 55).

Statistical analyses are particularly important, helping one to **avoid being misled** when drawing conclusions about the results. They indicate how likely it is that any differences between the growth of various groups of young trees in your experiment are due just to chance, rather than to the conditions being studied (C 62-F).

(B) Two questions before starting a statistical analysis:

(1) Is it unnecessary? A formal analysis may not be needed for example when:

(a) numerous treated plants are thriving, while all the controls remain stunted;
(b) the trees of one genetic origin are growing, and those of the other are all dead; ***or***
(c) you were just doing a preliminary 'look-see' trial with a few plants, to try something out before a full experiment.

(2) Would the analysis be valid? It may not be, if, for instance:

(a) there were no controls or other standards to compare with the treated trees;
(b) the treatments were not applied randomly, or otherwise without bias;
(c) one treatment influenced another (for example if fertiliser in treated containers could have washed out and been taken up by control trees);
(d) a suitable layout of the young trees wasn't used during the experimental period;
(e) some of the experimental trees were subjected to severe stress (C 41) before the time of the assessment;
(f) the figures were arithmetically invalid *(for instance, ratios of percentages);* ***or***
(g) the pattern of variation between the individual plants didn't approximate to a normal distribution *(but see section O)*.

(C) Steps in analysing the results:

(1) Calculating the **average values** for all treatments, Blocks *(D 55 in Manual 4)*, genetic origins, and other factors; from the measurements you have done, at each assessment;
(2) Finding out what **patterns of variation** occur - from selected samples of the individual values - and looking at how one set of figures might be linked to another;
(3) Preparing the main results as a **graph,** histogram, pie-chart, diagram or table, so that differences between sets of trees can be more easily seen and appreciated;
(4) Doing some **tests of significance** on the most relevant figures; ***and***
(5) Drawing **conclusions** about what the experiment has shown.

(D) Which figures to analyse?

This depends on the circumstances, but it may often be best to start with:

(1) assessments made at the end of the experimental period;
(2) stages when sizeable differences had recently appeared between various sets of experimental trees; ***or***
(3) differences that deal with the main hypotheses of the experiment.

A decision can then be taken about which other sets of figures might be worth analysing.

(E) Tests of significance for 'Yes/No' situations:

If the difference between two sets of experimental trees is **qualitative** - that is, a simple choice between damaged/undamaged; alive/dead; leafy/leafless; terminal bud sprouting/not sprouting - then the **Chi-square test** (χ^2) is a straightforward one to use.
(see worked example of a 2×2 χ^2 test in sheet C 68-E.)
Chi-square tests can also be performed with more than two samples.

(F) Tests of significance for 'More/Less' situations:

Where the difference between various sets of experimental trees is **quantitative**, several kinds of tests of significance are available. One of the most adaptable and widely used is the **Analysis of Variance** (ANOVA) - see blank sheet and worked example in C 67). What this does is to estimate:

(1) how much variation exists between all the individual values being analysed;
(2) how much of this variation can be assigned to the treatments applied, to overall differences between Blocks, to various genetic origins, or to other factors; ***and***
(3) how much then remains unassigned (the 'Error' or 'Residual' estimate).

A simpler version is the **t-test,** but this can only handle a single comparison at a time, so is generally less informative and useful. *(See also J - standard error of the mean.)*

(G) Significant and non-significant effects.

The starting assumption *('null hypothesis')* on which tests of significance are based is that there are <u>not</u> any real differences between the various groups of plants in the experiment - they just show chance variation around the overall mean. However, if it turns out that considerably more of the variation is assigned to treatment than to error, the assumption is found to be false and the treatment is said to have had a *significant* effect. If, on the other hand, roughly similar variation is assigned to treatment and to error, the original assumption stands, and the overall treatment difference is said to be *'not significant'* (n.s.). The same applies to Blocks, genetic origins, and other factors.

(H) Levels of significance.

If a test of significance gives a number that is **larger** than the value given in the relevant table for the 5% level of probability (p = 0.05), this means that variation like this might happen anyway by chance in one out of more than 20 such trials. We say that such a difference is *"probably significant"* (and it is usually given one *). If the test gives a number that is bigger than the value in the table for the 1% level (p = 0.01), such a difference would only be likely to happen by chance once in more than 100 trials, and it is called *"significant"* (**). If the value for the 0.1% level (p = 0.001) is exceeded, the difference would probably only occur by chance once in more than 1000 trials, and is called *"highly significant"* (***).

(I) Degrees of freedom and significance in statistical tables.

Between two treatments, there is only one comparison to be made; between ten seed-lots only nine independent comparisons. The number of degrees of freedom (d.f.) is the number of trees, replicates, Blocks, treatments, seed lots, clones, and so on that are involved; minus one. So when looking up tables for:

(1) *Chi-square tests:* With one d.f. between 'yes' or 'no', the values of chi-square to be exceeded are 3.84 (5% level); 6.64 (1%); and 10.83 (0.1%).

(2) *ANOVA* (See worked example in C 67):

(a) Divide each of the *sums of squares* by the appropriate d.f. to get the *mean squares* (estimates of variation);
(b) Divide the mean square for treatment by the error mean square, to get the calculated value of F *(variance ratio);*
(c) Look up a Table of F values, using the d.f. for treatment along the top, and the error d.f. down the side. If your calculated F is larger than the one in the Table, this means that there is a significant **overall effect** of treatment *(see J and L for individual pairs of treatments);*
(d) Similarly, the mean squares for Blocks, clones, 'triplets' (C 15) or other groupings are divided by the error mean square to find out whether any of them show significance.

(J) Calculating the Standard Errors of the Means:

The standard error of the mean (S.E.) is the simplest estimate of how reliable an average is. It can be calculated for any set of figures by dividing the standard deviation by the square root of the number of trees:

$$\text{S.E.} = \frac{\text{standard deviation}}{\sqrt{n}}$$

After an ANOVA, a more accurate S.E. is calculated by dividing the error mean square (residual variance estimate - see I-2) by the number of values that have been averaged in a particular treatment, and then taking the square root.

$$\left(\text{S.E.} = \sqrt{\frac{\text{error mean square}}{n}}\right)$$

The average (mean) is then written for example as 5.6 ± 1.2, and on a graph or histogram the S.E. is usually shown to scale, as a vertical bar above and below the average value.

If their 'error bars' do not overlap, this is commonly taken as an indication that two means are probably significantly different from each other. If they do overlap, any differences may just be due to chance.

(K) Interactions.

Consider a 2 x 2 trial with a control, mulch only, fertiliser only, and mulch plus fertiliser (D 6 and D 55 in Manual 4). If, for example the effects of fertiliser were dependent on whether the plants were mulched or not, then an *interaction* is occurring (Manual 5). The two factors, mulch and fertiliser, are not acting independently from each other. Interactions are important in understanding more about growth, because they suggest that the two factors are acting upon the same process. On the other hand, if there is no interaction, the separate effects of the two factors will just be added together, or the one subtracted from the other. Interactions:

(1) can only be detected in experiments examining more than one factor. These might for instance be two different types of treatment, or one kind of treatment and a difference of genetic origin;
(2) need to be considered before looking at the effects of the main factors on their own;
(3) may, if significant, mean that a 'breakdown analysis' (usually, re-analysing the experiment in two parts) is needed to determine whether the individual effects are also significant.

(L) Examining the significance of differences between individual pairs:

(1) Find **the difference** between the average values in the pair to be compared *(for example between treatments 1 and 3 in the worked example in C 67);*
(2) Calculate a value called the ***least significant difference*** (L.S.D.):

$$\text{L.S.D} = t \times \text{the standard error of the difference.}$$

$$\text{L.S.D.} = t\sqrt{\text{error mean square} \times \left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$$

The value 't' is taken from tables at the 5%, 1% and 0.1% levels of probability, using the **error** degrees of freedom. n_1 and n_2 are the numbers of plants in treatment 1 and control;
(3) If the difference between the two averages is larger than the L.S.D., the difference is significant at the appropriate level.

This is the simplest method. Although various authors suggest alternatives (C 62-F), these are more complicated to calculate. The L.S.D. can be a useful guide, provided the following points are remembered:

(a) if 20 genetic origins were tested in an experiment, you would expect the LSD at the 5% level to be exceeded once, just by chance, not because there was a real difference;
(b) similarly, if the treatments in an experiment involved 3 factors at 3 different levels, at least one of the 26 possible comparisons would be expected to be significant at the 5% level, without meaning that a real difference had been found.

(M) Various reasons for lack of significance:

If a test does not show significance, this merely means that the null hypothesis stands (see G). It **does not** prove that the treatment is ineffective.

Significant effects might not have been found because:

(1) there was too much variation in the experiment for them to show up;
(2) there weren't enough replicates, as in the worked example in C 67;
(3) the experimental plants were growing slowly, and errors in measurement were too large for an effect of treatment to be found;
(4) the effect of another factor was masking that of the treatment in question; ***or***
(5) the particular treatment would only show significant effects in a different environment.

(N) Reducing variability:

(1) *Re-analysing data from the same experiment:*

(a) You could recalculate the data as the gain since the start of the experiment (C 55). This removes the variation due to the trees starting off at different sizes, and is in any case desirable when studying the periodicity or rates at which shoots are growing;
(b) For *relative* growth rates, you could do an analsis of *covariance,* based on the values for individual trees at the beginning of the experiment, and after a given period, or a regression (see Q);
(c) Transformations (see O) may have the effect of reducing variation, because they often give less weight to occasional very high values;
(d) Some computer programmes (see R-2) can be set to ignore data points that are further from the mean than a set distance. This is risky when dealing with variable species and environments, as these points may well be true values.

(2) *Repeating the experiment.* For instance, you might do this using:

(a) more replicates;
(b) young trees that had been grown beforehand under more uniform conditions (C 7);
(c) a different experimental area that provided similar light levels to all the plants. If necessary, you could have 3-5 Blocks running from the sunnier to the shadier parts;
(d) a 'surround' of similar trees that were not part of the experiment, to reduce 'edge' effects;
(e) treatments that were more contrasting than before; ***and***
(f) more careful handling and watering (C 42, C 48)

Only after several experiments would you conclude that the treatment probably has little or no effect on those aspects of growth of that tree species.

(O) Transformations.

These are sometimes needed in order to put the figures into a form where a valid analysis can be done (see B-2). Here are some examples:

(1) Chi-square tests with small numbers - apply Yates's Correction (see C 68-E).

(2) ANOVA with a non-normal distribution - if the mean value lies well towards the low end of the distribution, transforming all the original data ('x') may make the distribution reasonably normal. If so, do the ANOVA on the transformed figures ('z'). Some common transformations include:

(a) square root transformation: $z = \sqrt{(x + 0.375)}$
(b) log transformation: $z = \log^{10} (x + 0.375)$; or $z = \ln (x + 0.375)$

(0.375 is added to each number to avoid problems with values of zero and one.)

(3) ANOVA of percentages - use the arcsin transformation:

$$z = \sin^{-1}\sqrt{\frac{x\%}{100}}$$

Note: if you want to de-transform the results before presenting them, the standard error of the mean (see J) and the least significant difference (see L) require care. Because transformations (2) and (3) above are not linear ones, the S.E. bars will be of unequal length above and below a mean.

(P) Missing plants or readings.

It is still possible to do ANOVAs when there are different numbers of readings in the various treatments or genetic origins, for example because:

(1) there was a shortage in some groups of plants;
(2) only a few trees could be treated, but more controls were available;
(3) some trees were accidentally damaged during the experiment;
(4) some dieback of shoot tips occurred, or death of plants (C 55).

If the ANOVA has only one factor (see K), then calculate as in C 67.
If it has more than one factor, you could analyse them separately, though without being able to look at any interactions. Alternatively see statistical textbooks (C 62-F) for how to estimate missing values, noting that for each of them one d.f is deducted before calculating the error mean square.

(Q) Correlation and regression.

These are ways of examining how closely two sets of readings may be connected. For example, height and diameter growth in a set of young trees might often (though not always) be closely linked, with the shorter trees thinner, and the taller ones thicker.
Moreover, you might expect that the growth of the trees could be linked with soil depth, moisture or fertility, or with an aspect of the weather.

When correlations or regressions show a close relationship, significance values are often given to them. But here it is particularly important not to be misled, because:

(1) the significance has not come from the experimental testing of a hypothesis;
(2) the apparent link may simply depend on connections to a third factor; ***and***
(3) one in twenty comparisons amongst the many factors affecting the young trees may be expected to show a 'probably significant' relationship just by chance.

(R) Aids to calculation.

(1) **Calculators:** These have the advantages of being small, portable, robust and relatively cheap, and of working reliably from long-lasting batteries or solar energy. They are invaluable for transformations (see O), to obtain and check totals and averages, and for other simple calculations (C 63).

Some types contain programmes that automatically calculate the standard deviation and standard error of the mean when a set of figures is totalled. Others will perform more detailed analyses, or allow you to write a programme yourself.

(2) **Computers:** These offer opportunities for storing large amounts of data, doing complex calculations and analyses, and almost limitless possibilities for displaying the results. They can also be programmed to accept electronically recorded information about the environment. However, computers are relatively expensive, require a steady and reliable electricity supply, and need to be kept free of dust and high humidity. Some types can operate from rechargeable batteries, but they are too delicate to be really portable.

(S) A final hint:

Check ***at each stage*** for errors in recording numbers, and in calculations. If not, sooner or later you will find yourself having to start back at the beginning, re-analysing and re-drawing graphs (and perhaps even changing slides and the proofs of publications). Just because a computer has done the analysis does not mean that there cannot be errors.