The DOOR Manual for Plant Nurseries

Reprint – information current in 1996

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This publication has been reprinted as a digital book without any changes to the content published in 1996. We advise readers to take particular note of the areas most likely to be out-of-date and so requiring further research:

- Contacts—many of the contact details may have changed and there could be several new contacts available. The industry organisation may be able to assist you to find the information or services you require.
- Organisation names—most government agencies referred to in this publication have had name changes. Contact the Business Information Centre on 13 25 23 or the industry organisation to find out the current name and contact details for these agencies.
- Additional information—many other sources of information are now available. Contact an agronomist, Business Information Centre on 13 25 23 or the industry organisation for other suggested reading.

Even with these limitations we believe this information kit provides important and valuable information for intending and existing growers.

This publication was last revised in 1996. The information is not current and the accuracy of the information cannot be guaranteed by the State of Queensland.

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Queensland Government

THE EXPERIMENTAL APPROACH IN FINDING SOLUTIONS

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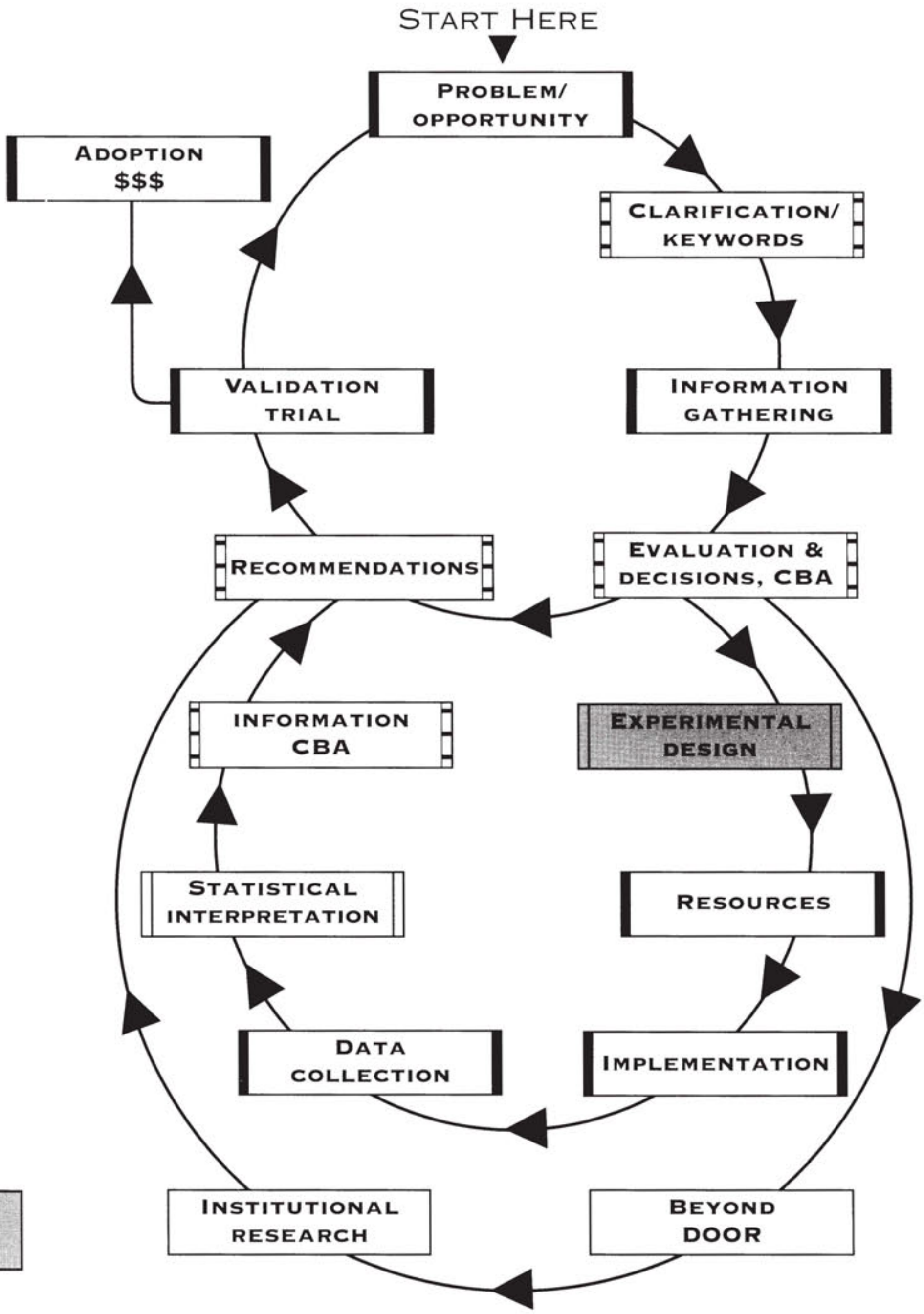
DOOR
Implementation Cycle



Legend

Chapter Topic

Action Key

Consultant only

Joint

Operator only

CBA = cost–benefit analysis

5.1 INTRODUCTION

Experimentation aims to solve a problem, fine tune an existing practice or develop a way of exploiting an opportunity. It challenges the old way of doing things. In the nursery industry many processes have been adopted on an intuitive "best bet" basis, without any subsequent verification.

Conclusions in the DOOR context are based on clearly defined data or information rather than on intuition.

Analysis of these data allows you to conclude whether the gains obtained by the new way of doing things (or treatment), are real or significant and not just a chance occurrence. DOOR experimentation involves designing appropriate experiments, selecting treatments and collecting data from objective measurements, followed by appropriate statistical analysis. Not only can you be confident of your conclusions, you can also be sure that small but economically important effects will be detected in an efficient and cost-effective way.

5.1 INTRODUCTION

- Experiments aim to solve a problem, fine tune a practice or exploit an opportunity.
- Intuition is not enough on which to base changes—statistically sound information is.
- Statistical analysis determines whether a result can be repeated or whether it is due to chance.

5.2 EXPERIMENTAL DESIGN

During the experimental design phase, treatments are defined, the conditions of testing are specified, decisions are made on the number of replications (how often treatments are repeated), the method of allocating units (usually pots, punnets, or trays in nursery research) to treatments and the variables to be measured are determined (e.g. plant height, number of leaves, rating of disease, etc.).

5.2 EXPERIMENTAL DESIGN

- In the design phase, tactics and strategies are decided.

5.2.1 TREATMENT SELECTION

Treatments are the different conditions being tested in the experiment e.g. four different potting mixes, or six nitrogen-phosphorus fertiliser combinations or three sources of supply of seedling material, etc.

There can be complicating factors. Consider an experiment comparing the effects of different rates of superphosphate on growth. In this case it is impossible to conclude whether any beneficial effect of increased fertiliser application is due to phosphorus, calcium or sulphur present in superphosphate, or perhaps even some combination of these. The effects are said to be confounded (mixed up). When testing the response to phosphorus alone, the other elements need to be added so that all treatments have the same level of calcium and sulphur.

Control treatments

Each experiment should include a control treatment: a standard treatment, a nil treatment or an ideal treatment.

For instance, when comparing a number of potting mixes, the standard potting mix used by the nursery would be included. This control treatment gives a base reading against which the new mixes can be compared.

5.2.1 TREATMENT SELECTION

- Experiments test different conditions called treatments, e.g. different cultivars, fertiliser levels, media, etc.
- Control treatments provide a base line for comparisons.
- Factorial treatments test two (or more) treatments at the same time.
- When conducting experiments that have factorial treatments, you can determine if the treatments are interacting.
- Confounding occurs when the treatment effects cannot be separated from one another.

In testing water retention agents, control treatments could be both the wetting agent usually used by the nursery (if any) and also no wetting agent. Including this latter treatment allows you to discover the benefit obtained by using a water retention agent. Of course, if this was already well established, it may not need to be included.

In a weed-control experiment, treatments could be a number of herbicides and three control treatments. The first control could be the standard herbicide (if there is one), the next control would be left untreated (to assess growth of the plant in the presence of weeds) and the third control would be the ideal state brought about by hand weeding (to measure growth of the plant unimpeded by weeds or reduced by the negative effects of herbicides).

Generally, inclusion of control treatments helps you interpret treatment effects.

Factorial treatments

Sometimes two types of treatments are tested at the same time, e.g. the effect of different levels of nitrogen (N) and the effect of different levels of phosphorus (P). If there are three levels of nitrogen (low, medium and high) and two levels of phosphorus (low and high) the experiment would include six treatments in total (listed below).

1. low N, low P
2. low N, high P
3. medium N, low P
4. medium N, high P
5. high N, low P
6. high N, high P

Including all six treatments allows you to estimate the average effect of adding three different amounts of nitrogen, the average effect of adding two levels of phosphorus and also whether the effect of adding additional phosphorus varies depending on the level of nitrogen applied. This last effect is called the interaction between nitrogen and phosphorus levels.

Other examples of factorial experiments include the 12 combinations of four media and three fertiliser treatments, or the 18 treatments formed from three potting mixes, three levels of application of fertiliser and the addition, or not, of ferrous sulphate.

Conditions for testing

Experiment results will be affected by the conditions under which the treatments are to be tested. Define the type of plant (seedlings, size classification, cultivar, source of supply, etc.), and management factors such as water and fertiliser applications, environmental conditions (if controlled), etc. The best combination of treatments for growth may well depend on the conditions under which the plants are grown.

Consider a comparison of different amounts of fertiliser on the growth of a bedding plant where water is supplied by overhead irrigation. Plants that show big responses to high levels of fertiliser will consume more water than the smaller, less-fertilised plants. Increasing the irrigation to meet the requirements of the larger plants probably means that the smaller plants will be over-irrigated, with the excess water leaching out fertiliser and thereby exacerbating the difference between the two treatments. Conversely, applying water to suit the smaller plants will prevent the larger ones from reaching their potential. This is another case of confounding where treatment differences are caused by two factors: different amounts of fertiliser and water. The contribution of the two cannot be separated.The solution to this would be to irrigate using capillary flow or subirrigation, where each treatment could be assessed under its own optimum watering conditions.

Because different conditions affect treatments differently, keep records of conditions under which treatments are compared. Include information on the conditions of plants before treatments were applied, any cultural treatments (fertiliser, water, etc.), weather conditions, any incidence of pests or diseases, etc. Often experiments are repeated in another season or another year to allow comparisons to be made under different conditions.

5.3 VARIABILITY

In all biological experimentation one common feature is the variability of the experimental material.

Twenty plants from the same source, same cultivar, history, etc., treated similarly for three months and then measured (e.g. height, weight, canopy size) may show quite large differences. These differences are caused by slight differences in watering, fertilisation, pot media composition and handling, as well as variations in original biomass (cutting, seedling or seed size) and inherent differences between outcrossed plants (some are just destined to be bigger and healthier in the same way that humans vary). Also, some will have been in a slightly more favourable position in the glasshouse, perhaps receiving slightly more or less sun or breeze. There may also be small differences in measuring technique.

This variability, always present in biological research, has a big influence on the design of our experiments.

5.3 VARIABILITY

- Variation in biological material is natural.

5.3.1 REPLICATION

Variability means that experiments have to be carried out more than once (replicated). Instead of having one pot of each treatment, include several to reduce the likelihood of a treatment performing well just because it had the luck to be allocated to the plant with the best potential. Replication results in a better estimate of the effect of each treatment. Also, the higher the replication, the better is our estimate of the variability between plants that is not caused by treatments. This is important when doing statistical analysis.

5.3.1 REPLICATION

- Use replication to accurately estimate treatment effects.
- Use replication to estimate variability. This is necessary for statistically sound analysis.

5.3.2 BLOCKING

Variability must be reduced or controlled as much as possible so that it does not interfere too much in any estimation of the treatment effects. Reduce this variability by selecting uniform plants, containers, etc. for experiments and by carefully controlling all experiments so that no plants receive an advantage over others.

Blocking is another technique for reducing the effects of variability. If you expect that bigger plants will perform better in the experiment, divide the plants into size groups and ensure that each treatment is applied to one plant in each group. In this way all treatments are applied to plants of all sizes, so no treatment receives an advantage and you can also calculate the effects of different size groups on subsequent growth of the plants.

5.3.2 BLOCKING

- Blocking reduces the effects of variability.
- Blocking separates variability from known causes.
- Blocking accounts for differences that arise during the experiment.

If the experiment involves 60 plants and six treatments, divide the plants into 10 groups, with the six biggest plants in block 1, the next biggest six in block 2, etc., with the six smallest plants in block 10. Each treatment would be allocated to one plant from each size group (block). When the statistical analysis is done, as well as comparing the effects of the six treatments on plant growth, you can compare the performance of the 10 size groups.

You can block according to the condition of the plants before the experiment, for example vigour or source of supply.

You can block according to differences which might develop during the experiment. Some glasshouse benches may receive more sun or shade than others so put block 1 plants in the sunniest position, block 2 plants in the next sunniest position, etc. Similarly, positions closer together will probably be more alike so blocking might be on geographical position.

Make use of blocking during the experiment. Complete procedures like fertilising and measuring by blocks so that despite interruptions, at least all of a block can be completed at one time. If two operators are assessing the experiment, then one might assess the first five blocks and the second the last five.

Thus, in an experiment, block 1 might consist of the plants which were most vigorous before treatments were applied, are positioned on the northern edge of the experiment, are fertilised first and are assessed first by Fred (Tom assesses some of the other blocks).

The analysis might tell us that block 1 was the best performing block. It cannot tell us whether this was because it started with the most vigorous plants, or because it had the sunniest position or because Fred's technique gives larger measurements than Tom's. This is another example of confounding but, because it is blocking factors that are confounded, it does not affect our comparison of treatments.

5.3.3

Randomisation

- Randomisation gives each plant an equal chance of being allocated to any treatment.
- Randomisation prevents bias. This is necessary for statistically sound analysis.
- Randomise treatments by using a random number generator or picking numbers out of a hat.

5.3.3

Randomisation

Randomisation means that each plant has an equal chance of being allocated to each treatment. Randomisation prevents bias — vital if the results are to be analysed statistically.

Randomisation avoids any conscious or unconscious bias when selecting plants. It works this way: the experiment calls for eight treatments on 12 pots. Number the pots from 1 to 96 and then randomly choose 12 pot numbers which then become treatment 1. One way of doing this is to place all the numbers in a hat. Allocate the first 12 numbers drawn to treatment 1, the next 12 to treatment 2, etc. You can also use random number tables or a computer randomisation.

Randomisation protects experiments from problems that may or may not arise.

The experiment described above (eight treatments, each with 12 pots) did not include blocking but experiments would usually include that technique as well.

Randomisation and blocking together work as follows. Suppose you had an experiment with six treatments and 10 replicates, where the replicates corresponded to 10 blocks. The blocks might be based on plant vigour, for example. The pots would be numbered from 1 to 6 within each block. Within each block you could randomly decide which treatment was applied to which pot so that, for example, pot 1 becomes treatment 4, pot 2 treatment 6, etc. Repeat the procedure for the other nine blocks. A possible allocation of treatments for pots 1 to 6 in each of 10 blocks is given below.

Table 5.1

	Pot 1	Pot 2	Pot 3	Pot 4	Pot 5	Pot 6
Block 1 (most vigorous plants)	4	6	1	5	2	3
Block 2	3	2	6	4	1	5
Block 3	3	4	6	2	1	5
Block 4	6	1	4	5	2	3
Block 5	1	4	3	5	2	6
Block 6	3	2	1	4	5	6
Block 7	1	4	5	2	6	3
Block 8	6	4	2	1	3	5
Block 9	6	3	4	5	2	1
Block 10 (least vigorous plants)	1	5	3	2	6	4

Suppose pots have been blocked according to distance from the northern wall of the glasshouse. There will still be some differences between positions within a block in regard to light, sun exposure, etc. Also, those plants next to rapidly growing larger plants may suffer from shading. Overcome this problem by regularly rerandomising the placement of pots within blocks (e.g. once a week). Only do this if the pots can be moved without damage to the plants.

5.4 LAYOUT OF EXPERIMENT

Draw up a physical layout of the experiment, indicating dimensions and the random distribution of pots indicated.The consultant must check that this layout is appropriate before the experiment commences.

5.5 VARIABLES

Things that can be measured on experimental plants are called variables: height, vigour, disease rating, plant girth, number of leaves. Obviously it is only practical to measure a few variables.

Choose those traits that can be measured relatively easily and quickly and are functionally least related, for example, plant height and shoot number. The value of collecting different sorts of data lies in their different responses to the treatments being investigated. More information is gained from the responses that occur in a diverse set of variables rather than from a group of variables that are closely related.

5.4 LAYOUT OF EXPERIMENT

- Draw up a plan of your experiment.

5.5 VARIABLES

- Measure only a few variables at a time.
- Select variables that are easily measured.

5.5.1

OBJECTIVE VARIABLES

- Objective variables are more reproducible and scientific than subjective measurements.

5.5.2

SUBJECTIVE VARIABLES

- Subjective variables are scored intuitively by individuals.
- Subjective scoring is less helpful in detecting changes over time.

5.5.1

OBJECTIVE VARIABLES

Objective variables are measured as numbers of units (e.g. counts of flowers, shoots, etc.) or with some measuring device (a ruler, a measuring cylinder or scales or other specialised equipment). Useful variables may include plant height, plant width, leaf number, the number of dead leaves, the number of shoots, the number of flower buds, the number of open flowers, the length of the flower stem, the width of the flower and the width of the topmost fully expanded leaf.

If plants can be sacrificed at the end of the experiment, cut them off at ground level (include bulbs and rhizomatous material, free of roots) and weigh then immediately before they lose water. Alternatively, place all material from each pot in individual, pre-weighed plastic bags, seal, and weigh them when convenient (within 6 hours unless stored in a cool room). Material can also be dried at about 70°C in paper bags for two days or so (hotter temperatures may cause dry matter losses). The difference between fresh weight and dry weight (amount of water) is also a useful variable. Percentage moisture content can also be derived from these data.

Only a limited amount of data can be collected on root growth during the course of the experiment without plant damage. One variable could be the number of roots that cross an imaginary line marked down the pot wall, measured after carefully removing the intact root ball from the pot. This may prove difficult until the pot volume is occupied by enough roots to keep the whole root system intact. Roots may be recovered for weighing at harvest by washing out the medium, but some nursery media such as peat may not separate satisfactorily. Partial drying of the media may help.

5.5.2

SUBJECTIVE VARIABLES

Subjective variables are scored intuitively rather than by objective measure. These variables are often rated on a scale 1–10, with 1 being equivalent to nil, and 10 being the maximum. Alternatively, an average plant is scored as 5 and other scores increase or reduce depending on growth relative to the average. Subjective variables may include plant colour, overall growth or vigour and a plant's water stress. However, because scores are rarely well related to time, it is difficult to establish time-based changes in responses.

Rating levels must be equally spaced, for example, if the difference between 3 and 4 is equivalent to 30 per cent then this should also apply to the difference between 7 and 8.

5.5.3

OBJECTIVE VERSUS SUBJECTIVE ASSESSMENTS

Objective measurements are better than subjective ones simply because the ability to detect subjective differences varies between people, with time and according to personal bias. However, aesthetic appeal may be a very important variable. Try to establish whether there are any objective variables involved. Measuring these could greatly enhance the value of the aesthetic appeal variable. Ask a number of people to conduct their own aesthetic-appeal rating in order to minimise individual bias.

Subjective scoring is quicker than objective measurements and can greatly complement objective assessments. Unfortunately, such scores tend to be more variable than objective measurements.This may not be an issue where treatment responses are large, but they do limit interpretation where real responses are small (less than 10 per cent).

5.5.4
Estimates of vigour

The plant with the greatest proportion of young flush is likely to be the most attractive. Such characteristics indicate more rapid growth and thus better growing conditions, an important factor to the discerning buyer. Growth rate may thus constitute an important objective variable, perhaps in terms of leaf-area development or height increase over time.

5.5.4

Estimates of vigour

- Growth rates are an important objective variable to be measured over time.

5.5.5
Chemical assays

Chemical assays of whole plants or particular leaves for particular nutrients greatly aid interpretation. Usually this process involves sampling a designated plant part that is collected at a particular time in the growth cycle of the plant. This information is particularly useful in sorting out plant responses in nutritional experiments.

5.5.5

Chemical assays

- Chemical analysis of plant tissues and media can be very informative.

5.5.6
Non-plant variables

Variables of the media that could be expected to respond to the various treatments could include pH, electrical conductivity, air-filled porosity, water-holding capacity, nutrient concentrations, and nitrogen and phosphorus draw-down. With careful sampling, these data may be collected during the course of the experiment without doing too much damage, but information on the whole pot may mean sacrificing the whole plant. These independent variables can be used to explain plant responses and explain why treatments were effective.

5.5.6

Non-plant variables

- A number of variables can be measured during an experiment that are not plant-related but are still caused by treatment effects.

5.6
Measurements

All measuring techniques need to be critically described. For example, in measuring height, identify a fixed reference point such as the surface of the medium or the lowest visible and permanent mark on the plant. Measure from this reference point to the highest growing point each time you measure.Avoid measuring to a position on the leaf blade since this is not particularly well fixed, being responsive to the time of the day and soil water status. Measure maximum distance from leaf tip to leaf tip on the same plane as an estimator of width. Measure diameter or circumference of stem at a constant height, for example, 15 cm above the soil.

5.6

Measurements

- Critically describe all measuring techniques.