On woody plant experiments

Essay for the Experimental Plant Science course

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Scientific inquiry

Scientific inquiry often begins with asking a question and proposing a hypothetical answer. This part is normally based on observation and experience with the system under study, and perhaps derived from testing of a previous hypothesis. Under the assumption that a hypothesis is true, we can make predictions about the system under study. We can now test whether these predictions hold. Traditionally we will do so by attempting to reject them.

Two basic approaches exist to testing of hypotheses, the descriptive approach which involves analytical exploration and comparison, and the experimental approach in which we attempt to influence the system under study in a controlled manner before analysis. Both approaches have advantages and shortcomings. The virtue of the descriptive approach is that it attempts to leave the system untouched until it is analyzed, and thus avoids the necessary compromises and artifacts of an experimental setup; on the other hand, the descriptive approach can only demonstrate statistical correlations, while the experiment is closer to an actual demonstration of cause-and-effect. Both approaches may involve meticulous repetition of analyses, large data sets, and sophisticated analytical equipment, but can also be minor studies and involve simple measurements or grading.

What is an experiment?

To sum up, an *experiment* is a methodical procedure carried out with the goal of falsifying or supporting a hypothesis. Since one can only *support,* but not prove a hypothesis, it is customary to formulate a 0-hypothesis and attempt to falsify that instead.

As a simple example, supposing that the hypothesis is:

"A higher temperature leads to a higher tissue concentration of substance Z",

the 0-hypothesis could be

"A higher temperature does not increase the concentration of substance Z", Experiments always rely on repeatable treatments that represent *an interference* with the system or process under study. This usually requires a control group that is not manipulated.

For practical reasons, an experiment is limited in time and space. It follows that many branches of research do not lend themselves easily to experimentation. How to make experiments with processes that happens extremely slowly or at a very large scale, such as geological or astronomical processes? Most biological processes happen within a time frame comparable to the duration of human patience, but even large animals and slow-growing trees can be difficult, costly and time-consuming to experiment with. Even so, most plant material is easily produced and handled, and destructive experimental procedures usually do not provoke ethical concerns. Furthermore, plant science is in a favourable position that similar questions may be addressed with both descriptive and experimental approaches.

Example: Supposing we want to learn about climatic effects on annual growth ring patterns in trees, and ask whether a warm July influences the cambium to switch to latewood production at an earlier date than it would in cool summers? If so, the prediction is that earlywood/latewood ratio is lower in years that we define as "warm". A descriptive approach to test this hypothesis would be to analyse the growth rings produced in the past and compare the observations with climate records in corresponding years. A statistical significant negative correlation of earlywood/latewood ratio and July temperatures would support the hypothesis, and a lack of correlation would reject it. An experimental approach to the same question would be to expose groups of similar trees to different regimes of July temperatures, other climatic factors being kept equal, and a subsequent analysis of the growth rings produced. A statistical significant negative effect of the treatments on the earlywood/latewood ratio would support the hypothesis. This approach will probably require that the experimental scale is limited to young trees in pot culture.

Considerations before an experiment

Generally, it is useful to consider if the questions under investigation is sufficiently interesting to justify the labour and costs involved in the study. Also, it might be a good idea to consider what can go wrong, what the most trivial outcome of the study could be, and whether that in itself would be adequate for a report? On the whole, an experimental approach is usually risky: the plant material may succumb to disease unrelated to the treatments, the climate regulation can break down, the treatments may turn out to be ineffective, and so on. The longer time the experiment is to run, the greater the risk that unplanned events interfere.

Before taking on an experiment, a lot of essential decisions and planning is required:

- Practical application of the treatments
- Number of replicates and repeated treatments
- Appropriate level of treatments to yield a significant reaction
- Relevant and appropriate control groups
- Relevant (and accessible) analytical methods
- Timing and sampling plan
- Structure of resulting data, and a plan for the statistical analysis, based on the experimental design.

Different experimental environments

Plant experiments are carried out in different environments: *in vitro*, in growth chambers, in green houses, in the field or in the natural habitat. It may involve whole plants or plant parts in tissue or cell culture. The choice of location depends on the research questions asked, including the plant material we are interested in. Usually, the experiment is about varying the factor or factors of interest while keeping other factors as stable as possible. Generally, the smaller units, the easier it is to apply treatments and regulate external factors, but this regulation requires a series of choices that are complicated to make unless there are already highly developed growth protocols for the plant material in question. Choosing experimental settings that are in any way unrealistic may also produce results that only apply under very special conditions.

Field and nature experiments are subject to the outside factors that cannot be controlled, such as seasonal temperatures. There is a challenge in containing the treatments within the plots actually being treated while avoiding side effects on the control plots. Careful monitoring of uncontrolled factors and recording of accidental interfering events are essential during experiments, so that the results can be related to the ranging conditions. Outdoor experiments are often more difficult to interpret , but they are also closer to real life.

Sources of variation

The ambition of any experiment is that the treatments should generate a change in the features analyzed (such as, number of flowers produced, stem width increment, chlorophyll a/b ratio), which can be demonstrated statistically over and above the natural variation in these features. The natural variation is genotypic as well as phenotypic. Cloned plants, for instance derived from cuttings, are genotypically alike, but nevertheless vary in accordance with the size of the original cutting and its place on the mother plant. Working with pure lines and other material with restricted genetic variation may reduce phenotypic variation, but does not eliminate it. Variation is also produced by the environment: a greenhouse or a growth chamber with sophisticated climate controls cannot prevent edge effects and gradients from side to side from occurring.

One solution to natural variation involves using many replicate plants and several plots with the same treatment scattered over the growing site. With a better statistical assessment of the control group, it becomes easier to identify minor changes introduced in the treated groups. One may attempt to narrow down the variation by selecting a subset of the available plant material, and subsequently assign them in a standardized way to treatments. The main thing is that, before experiment, the groups should be as similar as possible. With variable material and in small experiments one may keep track of individual units so that starting values can be used as covariates in a final analysis of variance, and errors and outliers be easily detected.

Furthermore, there is variation arising from the measurements. When the same instrument is used throughout the recording of an experiment, its *accuracy* needs not be a concern, as it affects all groups equally. However, if the instrument is repaired or replaced during the recording, it is essential to test if its accuracy has changed. The *precision* with which the meter is read needs to be analyzed by repeated measurement of the same sample (in case of destructive methods, a subdivided sample). Ideally, the same experienced person should read the meter consistently throughout the recording.

When drawing conclusions from statistical analyses, it should be borne in mind that critical P-values (usually 0.05, 0.01 or 0.001) are chosen by convention, and express the chance that the result is incidental (and thus in support of the 0-hypothesis). If P is smaller than 0.05 , there is low chance (5%) for the outcome at hand to be incidental, which allows us to formally reject the 0-hypothesis and support the hypothesis. However, a small chance remains that this judgement is wrong. The same applies if the data indicate that we should accept the 0-hypothesis. P-values close to the chosen threshold clearly cause some uncertainty as well. For instance, a 7% likelihood ($P=0.07$) requires that we accept the 0-hypothesis, but we might suspect that repeated or more extensive experiments could actually support the hypothesis.

Different kinds of plant material

Major parts of modern plant physiology are based on studies of model species, in particular the annual *Arabidopsis thaliana* (Brassicaceae). While the same environmental stimuli and basic physiological processes govern plant life generally, each species bases its particular life strategy on differential expression of these processes and differential reaction to their environment. For instance, even if the synthetic pathway for a certain secondary metabolite is the same, different species may differ in the amount produced under similar external stimuli.

In connection with growing plants for a particular usage, e.g., food crops, ornamentals and timber trees, the interest often lies in the reaction of particular species or varieties to the growing conditions. Experiments will thus often deal with species not ideally suited for experimentation.

Experiments involving trees

The study of tree physiology is topical not only in connection with ornamental trees, fruit and timber production, but also with respect to production of biological fuels. Environmentally, the trees define most of the world's vegetation, and their carbon storage is a primary climate ameliorator in the ecosystems.

In tree experiments, time limits are a concern, since most experiments needs to be carried out at least within the duration of a scientific career (although some studies have been known to be passed on from generation to generation of scientists). The physiology of trees changes during their life span, and thus experiments with very young trees (cf example on page 2) are not always a relevant solution to the time (and size!) limits of a practical experiment. In some cases, excised tree parts, such as branches or roots are used experimentally for a limited time.

In experiments with larger trees, the existing plant body represents a buffer that conceals experimental effects for a time. For instance, fertilization experiments in Christmas tree production with 8-10 years old conifers may require 2-3 growth seasons for a statistically valid result. When applying experimental treatments to trees that have grown for a long time on a particular site, adequate control individuals or control plots must be carefully selected at the outset, to match the ones that are given treatments.

Low control of environmental conditions reduces reproducibility, and experiments with trees are typically large to very large in terms of number of replicates. For the same reason, the features traditionally analyzed are often only easily recorded growth or yield measures. When considerable costs and time have been invested in setting up a long-term experiment, it is often used for several purposes, and new uses introduced during time. Long lasting experiments may thus constitute a stable resource, actually a part of the scientific infrastructure in tree research.

Concluding remarks

During this course you will be presented with a number of simple plant experiments. Several compromises have been taken for fitting them into the limited time of the course, especially the whole-plant experiments. Most of the planning mentioned on page 3 will have been done in advance. In order to reduce tideous repetitious data collection, the number of treatments is low, and the replicates and repeats are few. For learning purposes, you are encouraged to take turns at the instruments. In a realistic experiment, however, the number of similar analyses is usually great and carried out with great consistency to yield statistically valid results. It is also an ambition of the course to introduce a nice range of current and useful analytical methods of which quite a few require some previous skill to carry out consistently.

With this in mind, it is not surprising if the numerical results of the course experiments in some cases turn out to be difficult to interpret. Hopefully this should not discourage you from using the methods and skills learned in this course to set up solid future experiments of your own.

Literature

This essay is intensionally held in non-technical language, and based entirely on experience with own experiments through time and personal reflections to the Experimental Plant Science course. It should be regarded as an introduction only, and participants should obtain more information in a regular textbook on experimental design and statistical methods. There is a rich literature, and since many of the books are good, almost any one of them will be helpful. Among many offered at the university library, I have come up with a few titles that sound particularly interesting and relevant, listed below as a suggestion. The last mentioned is a classic favourite.

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