GLOBAL TREES CAMPAIGN

How to solve germination problems

Credit: Sociedade Chauá

> " Information on germination contributes to a better understanding of plant reproduction "

Baskin and Baskin (1998).

This brief was written by Santiago Velazco, Pablo Hoffmann and the Chauá team[1]

1. Sociedade Chauá is a NGO working for the conservation of natural ecosystems and biodiversity in Paraná, Brazil: http://www.chaua.org.br/

Introduction

Different species of tree require different conditions to support germination and growth. So what should you do if you have tried and failed (or don't know how) to grow seedlings from your species of concern? One option is to carry out your own informal experiments to test whether application of certain factors can affect and improve germination. The purpose of this brief is to provide guidance on how to carry out these experiments and then analyse and use the data you collect.

Who is this guidance for?

This brief is for non-specialists tasked with the germination, growth and restoration of rare or threatened tree species. Because the techniques described here are more advanced than earlier briefs in this series, we recommend that your team has some training and experience in horticulture before completing experimental trials.

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Before you start

Germination trials are experiments that allow you to test: (a) **which** conditions lead to highest rates of germination and (b) **how many days, weeks or months** it takes for seeds to germinate. In the long-term, this information can save a huge amount of time and effort in your nursery and ultimately allow you to grow a better quality and higher quantity of seedlings.

However in the short-term, germination trials can be time-intensive, involving regular data collection. To make sure that your team is as prepared as possible we recommend that you familiarise yourself with general procedures for growing trees from seed (see GTC brief 7). Then, before you start: (1) research the biology of your target species; (2) ensure your team has the right skills, (3) set up an appropriate facility with the necessary equipment and (4) obtain a source of seed.

STEP 1: Know your target species in advance

A) Check if existing protocols have been developed by other people

Other people may have already developed germination protocols for your target species (or closely related species). You may be able to access these by (i) searching Kew's Seed Information Database: http://data.kew.org/sid/ or other online resources, (ii) reviewing published literature, (iii) visiting botanic gardens or seed banks or (iv) consulting anybody known to grow or use the species.

Even if protocols already exist for your target species, you may still want to carry out your own experiments. It is important to work out which conditions work best for the seeds collected in your locality.

B) Understand the ecology of your species

General information on the ecology of your target species can help you to develop relevant research questions relating to its germination. Below we list a few factors to consider when you are searching the literature.

What climate does the species typically tolerate?
This may indicate optimal temperature or moisture levels for breaking dormancy or promoting germination.

What type of habitat and soil type is it typically found in?
This may indicate optimal growing medium for the species. For example, many tree species require certain fungi or bacteria to be present in the soil.

What ecological community does it belong to (i.e. is it a pioneer, secondary or climax species)?
This may indicate optimal shade levels (e.g. pioneer species are more likely to require or tolerate sunlight whereas climax species may require or tolerate shade).

Do the seeds from your target species exhibit dormancy, and if so, how is it broken?
This may indicate whether seeds need to be treated or stratified before germination.

Are seeds recalcitrant (i.e. they have low storage capacity) or orthodox (i.e they have a higher storage capacity)?
This may inform the timing and potential duration of your experiment.

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STEP 2: Make sure your team has the right skills

The procedures explained in this brief are more complex than those described in GTC Brief 7 (explaining basic germination). As a result make sure that at least one person in your team has well developed **horticultural skills** and that at least one member is comfortable with basic **data analysis and statistics.**

The whole process requires regular data collection and data entry so make sure that your team is well briefed in advance and that each member is highly organised and has good time management skills.

STEP 3: Establish facilities and acquire equipment

Facility

Very basic germination trials can be completed in a greenhouse or nursery, providing that your team has access to a sheltered workspace and running water.

For more complex trials - involving more accurate control of temperature, humidity and photoperiod - you may need to use a small building to act as a laboratory. Access to electricity will be important if you plan to use germination chambers.

Basic equipment and resources useful for all germination trials

- Modular seed trays (e.g. plastic trays with small holes for each seedling)
- Growing medium
- Mesh covers
- Shade cover (e.g. netting, palm leaves, bamboo etc.) to test light vs. shade
- Watering cans
- Labels, pens, correction fluid pens, pencils
- Notebooks, datasheets
- Access to a laptop or desktop computer for data entry

Additional / optional equipment and resources for testing particular conditions (depending on what you want to test)

- Mortar and pestle, knives or a cement mixer to test the effects of scarifying seeds
- Acids and chemicals **(to be handled with extreme care)** to test different pre-treatments
- A kettle or pan to test boiling water as a pre-treatment
- Different growing mediums (e.g. sand, vermiculite, paper)
- Simple germination chambers to test different temperatures.
- Sophisticated germinators chambers to test different temperatures, light levels, moisture levels or photoperiod

STEP 4: Obtain a source of seed to carry out trials

Finally, make sure you have a reliable source of seed (see GTC Brief 5 for advice on seed collection). For your experiments make sure you use seed from the same provenance (i.e. the location of the seed producing trees) to make sure seed source does not influence the results.

Seeds will also need to be cleaned and processed in advance of your trials (see GTC Brief 6 for advice on how to do this)

Designing your experiment

Various factors may influence germination of your target species including:

(1) Physical characteristics of the seed themselves
- **Degrees of maturity of seeds or fruits**
- **Internal moisture levels of seeds**
- **Size and shapes of seeds**

(2) Applications to help seeds overcome dormancy
- **Scarification (removing part of the seed coat)**
- **Soaking in cold water or in hot water**
- **Chemical treatment**
- **Freezing or heat treatment**

(3) Environmental factors
- **Light conditions (natural, artificial, shade or absence of light)**
- **Photoperiod (relationship between hours of light and dark)**
- **Temperature**
- **Environmental moisture levels (adjusted within germination chambers)**
- **Substrates (sand, vermiculite, paper, soil and presence of fungi or bacteria)**
- **Substrate depth (some species only germinate on top of the substrate)**

For your experiment, **select one or two these factors (known as treatments)** to test on your target species. You may select treatments based on the research completed under Step 1 (i.e. how the species grows and reproduces in the wild may provide some clues on its germination in the nursery) or by making small adjustments to previous attempts you have made to germinate the species.

You may want to compare how different **types** of treatment influence germination. For example: testing the effects of different methods used to break dormancy.

Treatment 1 (T1)	Treatment 2 (T2)	Treatment 3 (T3)
Soaking seeds in cold water	Soaking seeds in hot water	Scarifying seeds

In other cases you may want to compare different **levels** of a treatment. For example: testing the effects of different ambient temperatures on germination.

Treatment 1	Treatment 2	Treatment 3
18°C	22°C	26°C

In more complex experiments you may test several different factors (and how they interact with each other) at the same time. For example: testing the effects of different light levels and the effects of different substrates on germination. In this case you would end up with six different treatments.

In any case, we recommend keeping your experiment as simple as possible. The more treatments you use, the greater the resources you will have to expend and the more complex data collection and analysis will be.

Substrate	Light	
	Natural	**Without**
Paper	Treatment 1 (T1)	Treatment 2 (T2)
Vermiculite	Treatment 3 (T3)	Treatment 4 (T4)
Sand	Treatment 5 (T5)	Treatment 6 (T6)

Standardised methods, replicates and controls

Individual trees produce seeds that vary in size, structure and viability. Within a random batch of seeds, some may germinate and some may not, and this may have absolutely nothing to with the treatments you apply in an experiment.

This variation can cause problems for your experiment. For example, when comparing germination success, **how can you know for sure** that differences in germination between two different treatments (e.g. different temperatures) are really due to temperature, and not just down to chance alone (i.e. the seeds used for Treatment 2 just happened to contain a larger number of damaged seeds)?

Standardized methods, **replication** and use of **controls** are three methods you can use to solve this problem.

If, for example, you are comparing the effects of different temperatures on seed germination then, as much as possible, try to make sure that **all other factors that may influence the results are standardized** (i.e. the same for all treatments). This involves using the same seed types for all treatments (e.g. from the same provenance and avoiding use of damaged seeds) and ensuring that all other factors (in this case: light, water and substrate) are exactly the same for all treatments.

Replication of treatments reduces the chance that your results will be influenced by some unknown factor (e.g. low natural germination success for a proportion of seeds in the batch). Try to replicate each treatment at least four times (or more if you have enough seed and available resources). The final result will be averaged across all replicates (reducing the chance that atypical results from one replicate will have an influence on the overall results for the treatment).

Finally include **controls** within your experiment as a means to verify whether the effects you observe are really down to differences between treatments. A control batch of seed would have no treatment applied to it all – allowing you to compare germination rates with what would have happened anyway.
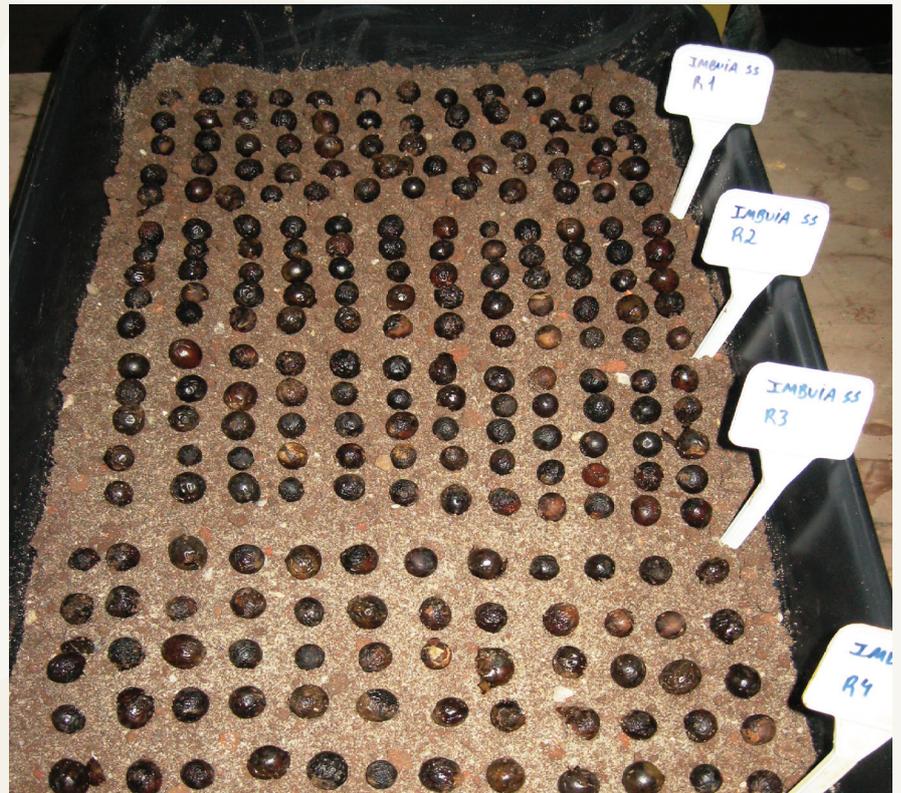
In this experiment a treatment applied to seeds from the Imbuia tree *(Ocotea porosa)* is replicated four times to improve reliability of the results. Credit: Sociedade Chauá.

Pre-treatment	Light	Treatment	Replicates	Seeds per replicates	Total seed for Treatment
Soaking seeds Cold water	Natural	T1	4	50	200
	Without	T2	4	50	200
Soaking seeds hot water	Natural	T3	4	50	200
	Without	T4	4	50	200
Nothing (Control)	Natural	T5	4	50	200
	Without	T6	4	50	200
Total		**6**	**24**	**300**	**1200**

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Implementing your experiment

After you have selected an experimental design (describing number of treatments, replicates, and seeds per replicate you aim to use), you should be ready to set-up your first experiment.

Prepare one modular germination tray for each treatment by sowing a single seed into each module. If you are sowing seeds in soil, don't sow seeds too deeply – you will need to observe each seed as it germinates. If experiments are carried out in your nursery you may want to cover seed trays with wire mesh to protect them from animals.

Next, group the tray for each different treatment together to form one block in your experiment. Then replicate each block the desired number of times (remembering to label each tray with a Treatment and Block number).

Replicate	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Block 1	50 seeds	50 seeds	50 seeds	50 seeds
Block 2	50 seeds	50 seeds	50 seeds	50 seeds
Block 3	50 seeds	50 seeds	50 seeds	50 seeds
Block 4	50 seeds	50 seeds	50 seeds	50 seeds

A germination tray for Treatment 1 – Block 1
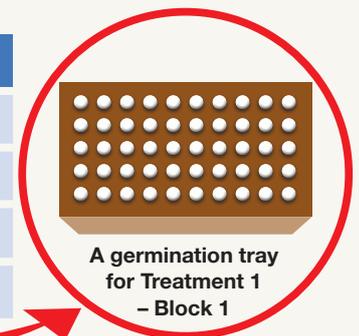
Data collection – what are you measuring?

Typical data collection involves inspecting all germination trays at least once a week (or more often if seeds germinate quickly). This will allow you to measure: **1)** the **total number of seeds** that germinate, failed to germinate or were attacked by pathogens, fungi or insects by the end of the trial and **2)** the **speed of germination.**

Prepare a datasheet to be updated after each inspection. On each inspection day, use a correction fluid pen, or permanent marker, to make a dot against each module with a newly germinated seed. Then on your datasheet write down the total number of dots that have germinated.

	Block 1			
Date	Treatment 1	Treatment 2	Treatment 3	Treatment 4
15/09/2014	0	0	0	0
18/09/2014	1	0	0	0
21/09/2014	4	1	0	0
24/09/2014	7	2	0	1

You may also want to measure the number of seeds that germinated but subsequently died. To do this, at the end of the experiment, count the number of dots which have dead seedlings in them.

Analyzing and writing up results

After completing your experiments, your next step may be to analyse data and present results to your project team and other interested stakeholders. The simplest way to present results for each treatment is to show the percentage of seeds that have germinated at the end of your experiment:

Percentage germinated seeds = Number germinated seeds/Total seeds sown x 100

However, you may also want to **compare the different treatments** with some basic statistical analysis. This will help you to demonstrate how reliable your results really are.

To do this, upload your data to Microsoft Office Excel and calculate the mean (average) numbers of seeds germinated under each treatment. Then, using statistical software, compare differences between all of the means by carrying out an 'analysis of variance' (ANOVA) test. Differences between means are statistically significant when test results have a P value <0.05. If you observe significant differences, confirm your result by carrying out 'pair-wise comparisons' between the mean of each individual treatment and the mean of the control.

Germination curves and indices

Another way to represent the results from germination trials is to create germination curves. These are graphs that show how the percentage of germinated seeds for different treatments changes over time (from the original sowing date to the end of the experiment).
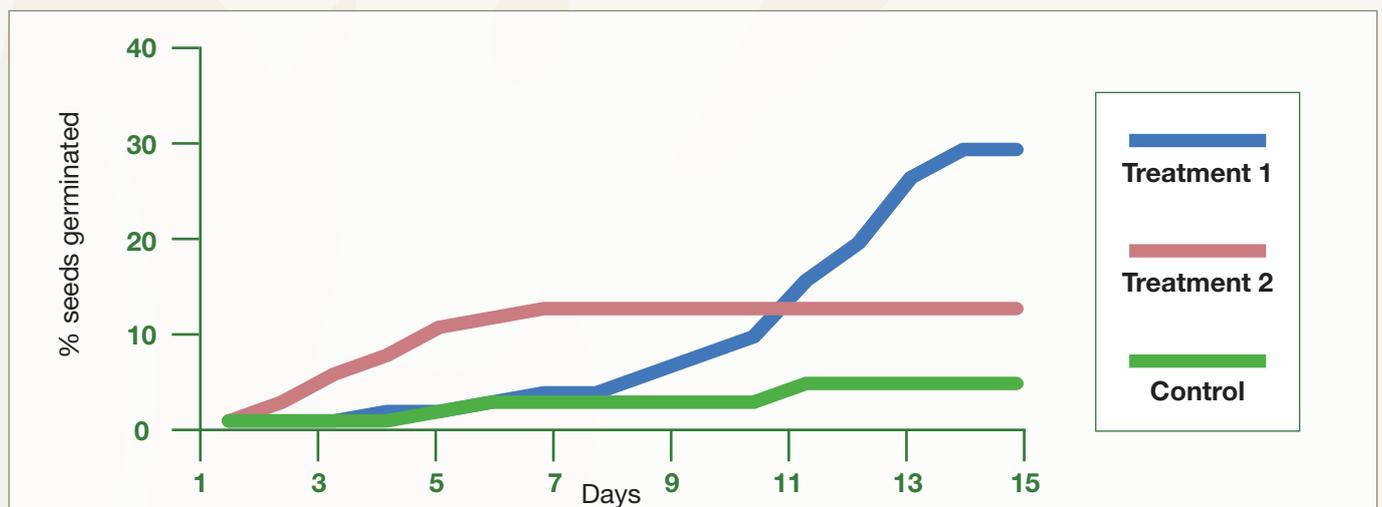
Time and speed of germination can also be represented by indices. These include the Germination Speed Index, Average Germination Time and the Average Speed of Germination (see references on Page 8 for guidance on how to calculate these).

Using information from germination trials

By comparing the results of different germination treatments you should be able to optimise germination conditions for your target species. Make sure you write up your methods and results to create a germination protocol. This can be followed and adapted by your team or other groups in the future.

Information on the speed and timing of germination can also be incredibly useful. If you aim to plant seedlings from your target species into their natural habitat then you most likely have to do this at a particular time of year (e.g. in the seasonal tropics, this is often at the beginning of wet season). If you know how long it will take to (a) break the dormancy of your species, (b) achieve high rates of germination and (c) how long seedlings take to reach suitable size and vigour for planting, then you can start to plan suitable sowing dates for your species.

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Selected references and further guidance

References and further guidance on some of the methods described in this brief are provided below

Guidance on germination experiments

Baskin, C. C. and Baskin, J. M. (2001). Seeds: ecology, biogeography, and evolution of dormancy and germination. Elsevier. Available to order at: **http://bit.ly/gtc_ref_8c**

Elliot, S., Blakesley, D. and Hardwick, K. (2013). Restoring Tropical Forests: a practical guide, Royal Botanic Gardens Kew; 344pp. Available at: **http://bit.ly/gtc_ref_3i**

Guidance on measuring germination rates

Ranal, M.A. and Garcia de Santana, D. (2006). How and why to measure the germination process? Revista Brasil. Bot., 29(1), 1-11. Available at: **http://bit.ly/gtc_ref_8a**

Guidance on seed behaviour and overcoming seed dormancy

Millennium Seed Bank Resources: **http://bit.ly/gtc_ref_8b**

Willan R.L. (1987). A Guide to Forest Seed Handling, with Special Reference to the Tropics. Rome, Food and Agriculture Organization of the United Nations. Available at: **http://bit.ly/gtc_ref_4d**

Examples of germination experiments

Daws, M.I., Burslem, D.F.R.P., Crabtree, L.M, Kirkman. P., Mullins, C.E. and J.W. Dalling (2002). Differences in seed germination responses may promote coexistence of four sympatric Piper species, Functional Ecology, 16(2): 258-267. Available at: **http://bit.ly/gtc_ref_8d**

Examples of germination protocols

Kew's Seed Information Database: **http://bit.ly/gtc_ref_6e**

Román, F., De Liones, R., Sautu, A., Deago, J. and Hall, J.S. (2012). Guía para la Propagación de 120 Especies de Árboles Nativos de Panamá y el Neotropico. Environmental Leadership and Training Initiative – ELTI, Yale School of Forestry & Environmental Studies. Available at: **http://bit.ly/gtc_ref_7a1**

For more information, or to download the other briefs in this series, visit **www.globaltrees.org/resources/practical-guidance**

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