_Improving the germination of mediterranean forest species seeds

Laboratory studies to develop a methodology for direct forest seeding

Semillistas a.c.

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1. Introduction

1.1. Presentation and content

This 2025 study presents research by the Semillistas Association on improving the germination of seeds from 10 forest species commonly used in Mediterranean region restoration. It presents methods and data from laboratory studies in language that is accessible to people working in forest restoration, even if they do not have specialised knowledge. Although we have tried to make the information in this document accessible, some scientific knowledge is needed to understand what is meant; for example, knowing how to use simple formulae, read an X-Y graph, calculate percentages, etc.

The species studied are:

- *Pinus halepensis* (Aleppo pine)
- *Pinus nigra salzmannii* (black pine)
- *Ceratonia siliqua* (carob)
- *Tetraclinis articulata* (Cartagena cypress)
- *Rhamnus alaternus* (evergreen buckthorn)
- *Rhamnus lycioides* (licorice buckthorn)
- *Pistacia lentiscus* (mastic tree)
- *Retama sphaerocarpa* (retama)
- *Genista umbellata* (Spanish broom)
- *Quercus ilex* (holm oak)

All these species are native to the southeastern Iberian Peninsula and are characteristic of the Mediterranean climate.

For each species the study covers:

- Understanding classical germination, i.e., the germination type traditionally used in tree nurseries and as described in books and tree seed manuals.
- Batch improvement, i.e., techniques to eliminate non-germinating seeds that reduce the batch germination percentage.
- Optimal priming treatments for improving germination.
- Comparison of seed germination with and without priming treatments at suboptimal temperatures, i.e, temperatures expected in the field when direct seeding is carried out.
- Information on how different pre-germination methods affect seeds before sowing under suboptimal temperature conditions.

1.2. Justification

Semillistas carried out this research to develop a working methodology that makes it possible to direct-seed forest areas successfully in Mediterranean environments. Direct seeding is rarely practiced anywhere in the world because the results are unpredictable. It has not been possible until recently to solve two major drawbacks: uncertainty about both field germination and seed predation rates. For this reason, the most widely used method

worldwide is the transplanting of young trees (i.e., saplings) previously grown in a nursery. However, the need to reforest billions of hectares worldwide is driving the search for cheaper and more scalable solutions. In our case, Mediterranean environments with long, dry, hot summers mean that tree transplanting requires enormous effort, involving summer irrigation. We therefore searched for alternatives to transplanting and have developed a focus on direct seeding.

Semillistas also wanted to explore solutions to reforestation by imitating natural regeneration processes, so that tree roots could develop naturally and w are simply the 'little birds' that disperse seeds, without any later requirement to care for any trees that emerged from these seeds. This path towards ensuring reliable direct seeding has been possible thanks to two developments:

1. Academic development of seed biotechnology, also known as 'seed enhancement technologies'. This knowledge provides a deeper understanding of forest tree germination processes, as well as identifying some possible improvements.
2. Our own development of a connection with our own local woodlands, which has increased our understanding of natural regeneration processes through a combination of observation and academic knowledge. This has enabled us to design a sowing methodology that uses seed technology adapted to our local ecosystem.

The first development mentioned above was developed by the agricultural industry to meet market needs in the context of climate change. Only a handful of academics around the world have subsequently dedicated themselves to transferring this knowledge to the forestry field because much of it remains commercially confidential within large agrifood corporations.

The second development above is based on work by Masanobu Fukuoka and his philosophy that observes the limitations of the human mind and seeks to guide action from love and respect for the way nature works.

This document addresses only the first of these (seed enhancement), because without this knowledge it is difficult to train the eye to look for regeneration potential within complex ecosystems.

1.3. Approach

The studies in this document were carried out at the Semillistas SeedLab, located in La Tahá, in the Alpujarra region of Granada. This is an open-source, replicable laboratory designed both for scientific development and to meet the needs of a practical forest restoration project based on direct seeding. Our equipment currently includes 8 germination chambers, a precision oven, a pelletiser based on a concrete mixer, and balances with 0.1 mg and 0.01 g precision.

The goal of Semillistas is to carry out applied science studies focused on solutions for forest direct seeding. This has led us to design simple experiments that generate sufficient data for us to carry out field sowing with a high likelihood of success, in contrast to extensive, expensive, and time consuming preliminary experimental work in replicated plots. This

approach allows us to adapt seed technology knowledge to our ecosystem both efficiently and effectively.

1.4. Knowledge transfer

The Semillistas project responsible for transferring any generated knowledge is called *SeedToSeed* (S2S). Our approach is “open science” with total transparency in order to support the current momentum for forest regeneration. The target organisations for this knowledge are those working worldwide to regenerate their local woodlands and forests. These organisations, rooted in their locality and with a love for their native woodlands, are the ones that can best carry out reliable, coherent, and lasting regeneration work.

S2S provides training to organisations that want to explore the paradigm shift from transplanting to direct seeding. This paradigm shift is profound because it is not easy to change something that is extensively practiced worldwide. As Masanobu Fukuoka said, it is not possible to change one process within a complex system without affecting the whole system, since all processes are related. Therefore, to change seedling establishment methods in a restoration project, perspectives must change along with our actions in relation to other system elements. That is why such knowledge transference to other organisations takes time.

Two types of training can be distinguished:

- *Laboratory projects:* The organisation receiving the training intends to establish its own laboratory and therefore receives knowledge necessary to study relevant species, carry out seed treatments, and adapt the methodology to its own ecosystem. Such projects may involve several separate seeding projects in different areas.
- *Seeder projects:* The organisation receiving the training learns how to use seeds that have been prepared by a laboratory project and receives support in adapting the methodology to its local ecosystem. The amount of information transferred in a seeder project is much less than for laboratory projects.

This guidance manual is intended to facilitate these training programs by providing basic information on seeds plus specific information on several species relevant to the Mediterranean ecosystem. It therefore serves as a reference for seeding projects, especially in the Mediterranean, and supports decision-making for the design of sowing plans.

2. Abbreviations

%mc	% moisture content
%mcfw	% moisture content (fresh weight basis)
%mcdw	% moisture content (dry weight basis)
SMP	Solid Matrix Priming.
CMC	Control Moisture Content
TMC	Target Moisture Content
HP	Hydropriming
AS	Aerated Soaking
LDPE	Low-Density Polyethylene
%HR	% relative air humidity
T0	time at which first germination occurs
T90	time at which 90% of maximum germination in germination test occurs
FW	fresh weight
DW	dry weight

3. Foundational concepts

3.1. What is germination?

Germination occurs when the root becomes visible as it breaks through the seed coat. The germination process includes events that occur from the moment seed hydration begins, until its root is visible. *Germination* itself occurs at the end of the *germination process*.

There are several stages after a root becomes visible:

- *G0:* The root begins to elongate but is only a tiny protrusion, less than half a millimetre long. Later, this manual identifies this as the start of FIII of the germination process.
- *G1:* The root elongates and separates from the seed coat, reaching a length of about 1 mm.
- *G2:* The root turns downward due to *geotropism* (a plant's growth response to gravitational pull), forming a right angle if the seed was sown horizontally. Normally a root makes this angle when it is about 2 mm long (i.e., after G1).
- *GX:* The root continues elongating downward.

Many factors affect the germination process and each species is sensitive in different ways to each of these factors, the most important of which are temperature, soil moisture, oxygen, light, nitrogen, and other nutrients.

In addition, seeds emit odours (volatile organic compounds) during the germination process, and later from their roots. These volatiles are linked to internal chemical processes during germination, but secondarily, and no less importantly, they can attract microorganisms and predatory fauna. For example, saprophytic fungi and mice use this smell to feed on the seed, and mycorrhizal fungi and bacteria are attracted by the smell to form symbioses or other kinds of beneficial exchanges.

3.2. What is emergence?

Emergence is the moment when the downward-heading root 'grips' the soil and the *cotyledons* (embryonic leaves) begin to emerge upward from the seed. When the cotyledons rise above the soil surface they open and begin to photosynthesise.

There are some seed types with rudimentary cotyledons inside the seed coat. When the root 'grips' the soil these cotyledons move toward the surface and the seed disappears, transformed into a downward root and upward cotyledon. This process is called *epigeal germination*. Examples of this type of germination are found in tomatoes, lentils, pine, mastic tree, and genistas (e.g., broom).

In other species, the cotyledons inside the seed remain in the soil. When the root has penetrated deeply enough a stem emerges upward from the seed itself, from the same point at which the root also emerged. This stem has buds that become the first leaves of the plant when they rise above the soil surface and receive light. This process is called *hypogeal*

germination. Examples of this type of germination are found in acorns and peas. In these species the seed remains attached to the plant (i.e., to both the root and stem) for a period before eventually detaching and decomposing. This is important in the case of acorns because the acorn remains active underground even though the plant can be seen to grow. It therefore remains exposed to predators such as mice and wild boar.

Germination is what is usually observed in the laboratory, with only *emergence* seen when seeds are sown in the field, where not all seeds that germinate will manage to emerge. An indicative reference value would be that if a maximum germination percentage of ~90% is observed in the laboratory, emergence of ~80% might be seen in the field.

3.3. What is *survival*?

A plant begins to grow above ground once its cotyledons have emerged. Under ideal conditions of soil moisture, temperature, and the absence of predators the plant can continue growing. However, reality is complex and each young plant encounters various events that can compromise its life. That is why measuring survival is so important.

If a seed germinates after limited rainfall in soil that is not moist at depth then it is very likely that the young plant will die from lack of soil moisture. Also, if the seed germinates in late spring it will not be sufficiently developed to withstand summer conditions.

Semillistas uses the following definitions when performing and monitoring field sowings:

- *Emergence percentage* as the number of plants that have emerged compared with the number of seeds that were sown.
- *Survival percentage before the first summer* as the number of plants that have survived until the end of spring compared with the number of seeds that were sown.
- *Survival percentage after the first summer* as the number of plants that have survived by the beginning of autumn. This can be measured either based on the number of seeds sown or the number of live plants before the first summer.

3.4. What is *direct seeding*?

Direct seeding methods distribute seeds directly in the field, in contrast to the usual forestry technique of sapling transplants. One of the reasons why sapling transplant practices are used around the world is the unpredictability of direct seeding. The lack of control over field germination and high rates of predation can make sapling transplants preferable, despite the much higher cost of this when compared with direct sowing.

Semillistas has overcome these direct seeding obstacles to ensure predictable results in the semi-arid Mediterranean environment. We had to change our perspectives to do so, move into a different paradigm, and build methodologies from almost nothing.

Direct seeding in agriculture is carried out under semi-controlled conditions. However, in forest seeding the conditions under which seeds are planted are largely beyond our control. With the Semillistas methodology we control those factors that can be controlled and leave the rest to nature. What *can* be controlled is the field emergence percentage (germination

and predation control) and the sowing date (soil moisture control and survival control after the first summer).

Direct seeding involves several possible techniques which may be classified as:

- Buried seeding in which seeds are buried at an appropriate depth.
- Surface seeding in which seeds are spread over the soil surface (with or without pelleting).

The unpredictability of surface seeding (as far as current techniques allow) has led us to explore buried seeding.

Two types of buried seeding can be distinguished, selected according to ecosystem type:

- Dry seed sowing before it rains.
- Pre-germinated seed sowing immediately after it rains.

Rainfall is unpredictable in our Andalusian Mediterranean climate. Rain may fall in early autumn and continue through winter and spring, or it may rain only once in autumn or winter, or follow another pattern. It is also impossible to know for certain how much rain may fall on each date. Semillistas realised how important this rainfall unpredictability is as a determining factor in our sowing methodology when one year we sowed thousands of seeds in early October, expecting them to be available for subsequent rain. That year it did not rain until early March (five months later), by which time mice and insects had eaten all the seeds. On another occasion we sowed dry seeds before it rained. The first rain was sufficient for seed germination but was then insufficient to keep the plants alive until the next rain.

How then can germination and survival be guaranteed if rainfall is unpredictable? The answer can be found in dryland cereal sowing techniques in which farmers sow wheat *after* sufficient rain. At Semillistas seeds are prepared so that they are on the brink of germination (via laboratory pre-germination) at the moment they are sown in the field after rainfall. That is why this manual stresses the importance of both priming and pre-germination processes.

3.5. What is a *batch*?

A batch (or *mother batch*) can be defined as a set of seeds of the same species, harvested from the same place (its *origin*), in the same year. For a mother batch to have sufficient genetic diversity for forest regeneration it must meet certain requirements. The most important is that seeds should be harvested from at least 50 parent plants separated from each other by at least 50 metres. Unfortunately, commercial seed companies do not usually provide this information.

It is impossible to obtain this high level of genetic diversity in a single year if a project is quite small. However, since sowing will usually be carried out in the same area over a period of several years, seeds can be harvested each year from different places with the same provenance, and genetic diversity can therefore be increased over time.

Germination tests, batch-improvement processes, priming treatments, dormancy-breaking treatments, etc. are performed on small samples of the mother batch directly after harvest (but after extraction, cleaning, and storage). If a batch-improvement process is required,

followed by a priming process, and finally a germination test, the number of seeds needed for the entire process should be calculated and removed from the mother batch so that the latter subsequently remains intact and untouched until it is required.

A batch contains live seeds with viability characteristics that vary from year to year depending on the storage method, which affects each species differently.

3.6. What are *germination chambers*?

Chambers are required in which temperature control and stability are guaranteed in order to carry out any kind of controlled temperature process (e.g., germination tests, priming, pre-germination, etc.). These *germination chambers* must allow a constant temperature (within an acceptable margin of error), or allow programmed alternating temperatures (i.e., different temperatures through the day) to simulate temperature in the field..

This type of chamber is one of the indispensable elements in a seed laboratory, but commercial models are expensive (about €6,000 each). Semillistas has developed an open-source prototype of a germination chamber based on a household refrigerator. In addition to reducing cost, we can adapt the chamber to the laboratory's specific needs. The assembly manual for such chambers will be published by the end of 2026.

3.7. What is *moisture content*?

The moisture content of a seed or a soil sample is the percentage of water it contains. For example, 10% moisture means that in 100 g of seeds there is 10 g of water and 90 g of dry matter (this is defined more carefully below). In our studies moisture percentage is defined based on the *weight* of water and dry matter, not on their volume.

For seeds, the definition of moisture percentage is based on fresh weight (FW), while for soil the convention is to base it on dry weight (DW). Both moisture percentages express the same value of water content:

$$\%mcfw = \frac{FW-DW}{FW} \times 100$$

(Moisture content fresh weight basis)

$$\%mcdw = \frac{FW-DW}{DW} \times 100$$

(Moisture content dry weight basis)

FW is the weight of the seeds whose %mc needs to be calculated. DW is the dry weight (without water) of those seeds, which is calculated by removing all the water from the seeds in an oven before weighing them again. FW-DW is therefore the amount of water in the seeds whose %mc we wish to calculate. FW or DW is chosen as the reference to obtain the percentage of water in the seed.

Figure 1 shows the hydration curve of *Pinus nigra* expressed in both ways, as fresh weight and dry weight. The convention used subsequently throughout this manual is to refer to seed moisture as %mcfw and soil moisture as %mcdw.

Some further general comments on %mc are:

- In seeds stored without plastic or glass protection the %mc will fluctuate with changes in air humidity.
- If seeds are stored hermetically they will have a constant moisture level.
- Not all seeds in a batch will have the same %mc, although each individual seed %mc will usually be close to the mean of a set of seeds. For most seeds we therefore calculate the mean %mc, but acorns are such large seeds that with a cheap balance we can obtain the %mc of individual acorns.

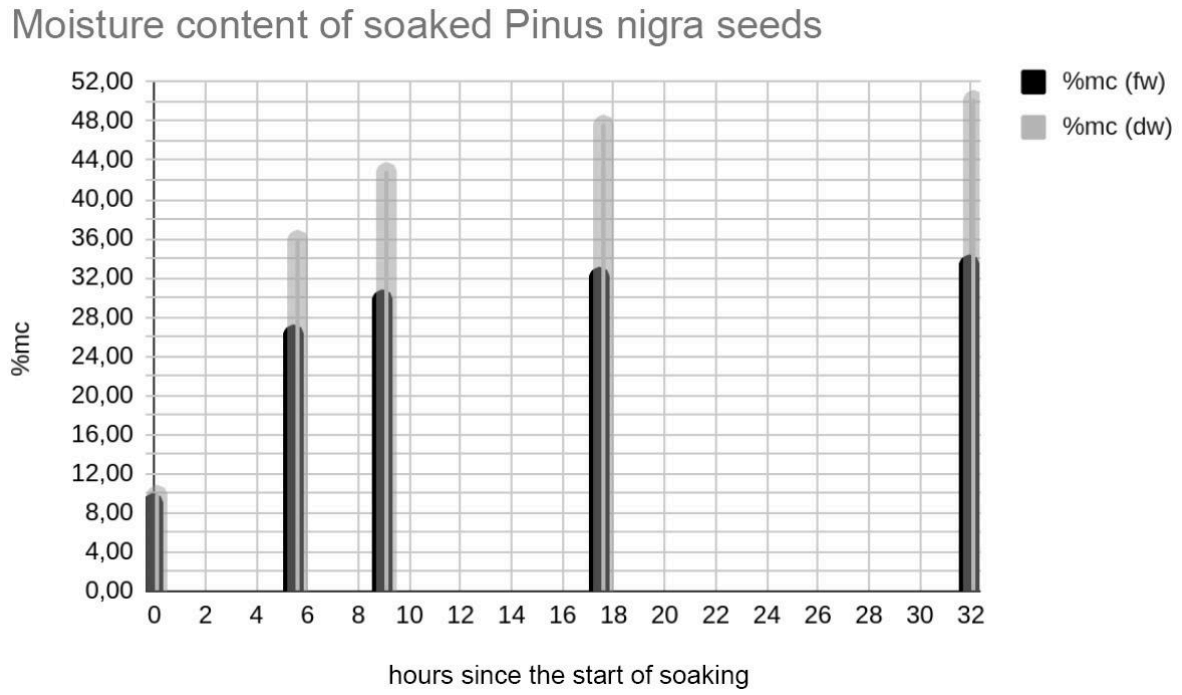

Figure 1: Hydration curve of Pinus nigra

3.8. What is *seed storage*?

Seeds can be classified as *recalcitrant* (e.g. *Quercus*), *orthodox* (most species), or *intermediate* in behaviour (we will not discuss the last of these). These descriptions refer to the seed's response to desiccation, which is directly linked to storage possibilities.

Orthodox seeds can be stored dry, whereas recalcitrant seeds cannot. Orthodox seeds include the vast majority of trees, shrubs, and grasses. For example, lentil seeds in a kitchen jar are almost dry, but they respire and remain alive, showing desiccation tolerance. As they mature on the plant, lentils are full of moisture, but once maturation is complete they dry to a low moisture content (becoming almost completely dry) and can then be stored for months, years, or decades. By contrast, recalcitrant seeds (e.g., acorns and chestnuts) die as they dry.

See the Semillistas *Best practice manual for Quercus restoration projects in Mediterranean environments*[1] for advice on the storage of recalcitrant seeds.

[1] https://www.semillistas.es/wp-content/uploads/2025/07/202506_Manual_buenas_practicas_EN.pdf

Storing orthodox seeds is relatively simple, but if we want to extend seed viability then two factors should be considered:

- Seed moisture must be as low as possible. At relative air humidity of 50%, seed moisture remains at around 9-10% mcfw. Seed life doubles with every percentage point of moisture reduction, so ideal storage is between 3% and 6% mcfw, depending on the species.
- The temperature must also be low. This is not as important as seed moisture, but it helps preservation. The simplest method is to keep seeds in the refrigerator at 4°C. They can also be stored at -18°C, but this requires different protocols.

Orthodox seed life can therefore be extended by drying them as much as possible and then storing them in airtight containers (plastic or glass) at low temperature.

Complex laboratory systems are unnecessary for pre-storage drying. Instead, we can take advantage of warm, dry summer days as a simple method to extend seed life. On such days (30-35°C, 20% RH) seeds can be spread on a sieve and placed outdoors in the shade. Seed moisture will usually have declined from 9% to 5% mcfw within 8 hours.

3.9. What are *hydration curves* and *phases of germination*?

Only orthodox seeds are considered in this section. For recalcitrant seeds please see Semillistas' *Best practice manual for Quercus restoration projects in Mediterranean environments* (see footnote 1 above).

When a pine seed is stored dry it still contains a small percentage of moisture (the seed is 'dry' but not completely so). If it is well stored the seed will contain between 5% and 10% mc. If this 'dry' pine seed is sown in moist soil it begins to absorb water and its moisture content increases (FI, Phase I of imbibition). After about 18 hours of imbibition (Figures 2 and 6), the pine seed can no longer absorb much more water and its internal moisture content (%mc) stabilises. During this second phase of stable moisture content (FII, Phase II), seed metabolism increases (it respires more) and it carries out DNA repair, nutrient mobilisation, RNA processes, breakdown of starches into sugars and proteins into amino acids, and many other processes. When the pine seed has done everything it is genetically programmed to do to prepare for root emergence, it breaks down fats, the embryo elongates, the seed coat breaks, and the root tip emerges (FIII, Phase III). When FIII begins, the seed once again absorbs moisture rapidly. The length of FI and FII, and the %mc of FII, are species-specific.

Figure 2 shows the hydration curve of a set of seeds. The X-axis is time and the Y-axis is %mc. This type of curve is valid for all orthodox seeds. In some seeds FII lasts a few hours and in others months. In some seeds the %mc during FII is 25% mcfw and in others 65% mcfw. The duration of FI is usually short - between a few hours and 1 day.

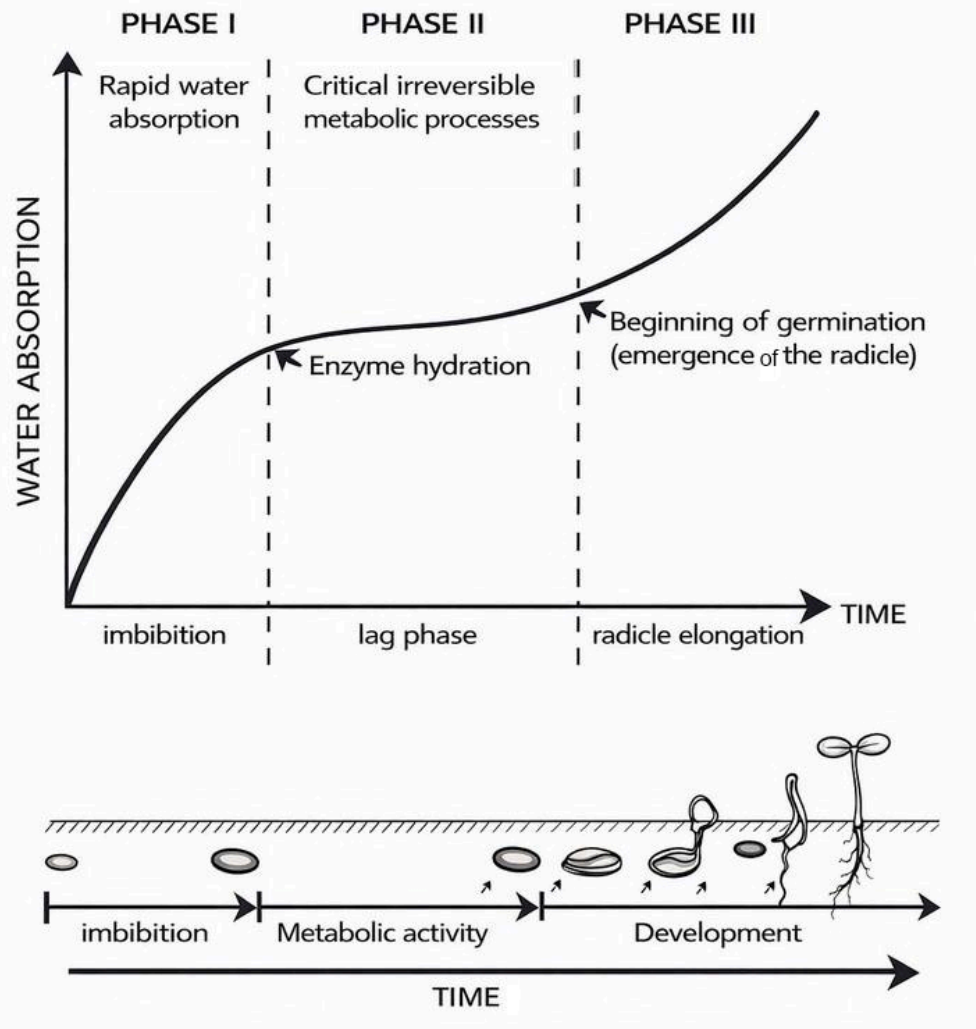

Figure 2: Phases of germination

A hydration curve is used to:

- Find out how long it takes a seed to reach FII. This period is important because a batch of seeds should not be soaked beyond that time, since seeds cannot continue their FII processes without oxygen.
- Gain an initial estimate of the %mcfw during FII. This value is used in the SMP process.
- The %mcfw value during FII is also used to discover how much water the seeds need in order to hydrate from their dry state to FII. This is important because it shows how much water inside a Petri dish the seeds will absorb and allows adjustment of the moisture content of the substrate.

3.10. What is *classical germination*?

Classical germination is the 'all-at-once' germination at optimal temperature, which is the temperature producing the best germination results for the species (i.e., maximum germination percentage, germination speed, and uniformity).

'All-at-once' germination is the type that humanity has practiced since the beginning of agriculture. It is based on sowing, watering, and maintaining soil moisture until the seed germinates or emerges. However, that is not usually how germination happens in nature. Instead, a seed lying in the soil is dry, becomes wet when it rains, and then begins its germination process. If it does not have time to germinate and the soil dries out, the seed returns to its dry state. When it rains again the seed becomes wet once more and continues its germination process, except that this time it will now germinate faster, having already completed part of the germination process after the previous rain. This is the basis of priming treatments (i.e., hydration-dehydration cycles) and is why we distinguish between the 'all-at-once' germination of a batch and the germination of a batch that has previously been subjected to priming treatments. In nature, classical germination can occur, but usually the germination process involves several hydration-dehydration cycles. Priming treatments are discussed later.

Classical germination includes the treatments required to remove any dormancies that 'prevent' germination. Acid, boiling water, or sanding treatments can be used for species with physical dormancy before a germination test is performed. However, in the case of morphophysiological dormancy, warm and cold stratification are part of 'all-at-once' germination. We therefore hydrate the seeds and keep them cold for X months before moving them to spring temperatures, without breaking the continuity of seed moisture, so they can germinate.

In summary, classical germination is the type carried out by farmers and in plant nurseries and the one found in the manuals and rules of the International Seed Testing Association (ISTA).

3.11. What is batch improvement?

When fruits are harvested from trees and shrubs and their seeds are extracted, cleaned, and dried, a seed batch remains that may sometimes yield a very low germination percentage. This is because many of the seeds are empty, damaged, or infested by insects.

Batch improvement is the suite of processes carried out to increase the germination percentage of a batch (as measured in a classical germination test). These processes can be simple or complex. Some are integrated into post-harvest cleaning processes, while others are performed after storage. In some species batch improvement is carried out during priming treatments. For example, after acorns are harvested they are cleaned and disinfected before bagging. Floating acorns are removed during this process because they have been eaten by weevils. In another example, floating mastic fruits (mostly the red ones) are removed before their seeds are extracted because these fruits contain only empty seeds.

Soaking and then separation of floating seeds after a specific time period is the usual process for batch improvement after storage. Seeds that sink are dried again for storage. This new batch significantly improves the germination percentage because most floating seeds are empty.

A process that we have not yet studied in depth is called IDS (incubation, drying, separation). It consists of hydrating a batch to the maximum extent and then letting it dry. Seeds that will not germinate dry faster than viable ones, so at a certain point during this drying process they can be separated into floaters and sinkers because of density differences.

It is true that during these processes some seeds that could have germinated are removed, so whether batch improvement is worthwhile should be assessed on a case-by-case basis.

3.12. What are *priming treatments*?

A priming treatment consists of drying the seeds at some point after hydration has begun. If a farmer is asked what happens when seeds are sown and watered, thereby initiating germination, but the soil then dries out before germination is complete, they will usually tell you that the seeds will die and sowing should be repeated. It was not until the 1970s that knowledge developed to include the possibility of carrying out hydration-dehydration cycles during seed germination. Since then this knowledge has developed further in agriculture and has recently attracted interest in forest ecological restoration circles.

The benefits of priming treatments in agriculture are:

- Acceleration of germination.
- Uniformity of emergence.
- Resistance to different types of stress (e.g., salinity, drought, or extreme temperatures) both in the seed and in the growing seedling.
- Increased seedling vigour.
- Increased yield (typically 10% more).
- Breaking of dormancy (e.g., thermodormancy and photodormancy),

Priming treatments are first discussed as a type of germination process, and then defined as a treatment.

Germination can occur in two ways:

- Through a classical germination process.
- Through a germination process with one or more hydration-dehydration cycles.

A seed has entered the germination process from the moment it leaves its mother tree and dries in or on the soil. When it rains the seed will hydrate, undertake some metabolic processes necessary for germination and then, at this point, three things can occur:

1. Soil moisture remains high enough for long enough for the root to emerge and penetrate the soil. In other words, the seed completes germination and the seedling begins to develop.
2. The soil dries superficially (where the seed is located) just *after* the root emerges and before it has time to penetrate the soil, so the seed and its tiny root dry out. The seed

germinated (completed the germination process), but the seedling rapidly died before the cotyledons emerged.

3. The soil dries superficially *before* the root emerges, so the seed dries again but remains alive (with a very low metabolism) awaiting the next rain, with the advantage of having already carried out some of the metabolic processes necessary for germination. In the future it will rain again and any of these three options may occur once more.

Semillistas understands the germination process in this way, as a continuum that begins the moment the seed falls from the tree. If understood in this way:

- Each seed in the woodland soil seed bank is at a different point in its germination process, depending on the hydration-dehydration cycles it has undergone.
- Dormancy-breaking is not an absolute process, but a condition that must occur along a germination process continuum so that the seed can sprout.
- Seed storage is not a condition prior to 'all-at-once' germination, but is part of the germination process in which the seed is at different stages of dehydration.

When working with seeds in our laboratory priming is understood as a treatment. Semillistas looks for a priming treatment (a hydration-dehydration cycle), based on situations that can occur in nature, that produces germination characteristics meeting these current goals:

- Maintaining or increasing the maximum germination percentage.
- Speeding up germination (i.e., making the day germination begins (T0) occur earlier than in classical germination).
- Making germination more uniform (i.e, reducing the duration between T0 and T90).
- Improving germination at the temperatures expected when field sowing.

The priming treatments used at Semillistas are of two types:

- Hydropriming (HP).
- Solid Matrix Priming (SMP).

The HP process consists of carrying out classical germination that is not allowed to complete, at an optimal temperature. The seeds are dried and returned to storage %mcfw before any roots begin to emerge (the start of FIII),. This treatment can be carried out in any way that allows seed moisture to follow a classical germination pattern. It can be done by placing the seeds in moist sand (or any other substrate), on moistened paper, or by aerated soaking. Figure 3 shows the moisture of mastic seeds during classical germination in blue and during the hydropriming treatment in red. The germination curve for classical germination is also shown in green. FIII begins between days 5 and 6, so seeds must be dried before then in the HP process .

The SMP process consists of bringing the seeds to a moisture level below that of classical germination FII, at an optimal temperature. This moisture (TMC: the Target Moisture Content) must lie within a range that allows development of metabolic processes occurring during FII, but does not allow root emergence. The TMC is obtained empirically for each species. Figure 3 shows the moisture of mastic seeds during SMP treatment in yellow. Note that the duration of the SMP treatment is around the time it takes for classical germination to complete. This means that since each seed during standard germination enters FIII between days 5 and 19, all the seeds during the SMP process will have completed all FII metabolic

processes without germinating and before they dry. Drying seeds on day 4 during the HP process achieves 'good priming' (i.e., drying the seeds once they have completed FII), but only for those seeds that classically germinate in the first few days. Seeds that take 15-19 days to germinate classically cannot complete FII during the HP process if dried on day 4. SMP treatments are more expensive than HP, but only species that complete classical germination rapidly are candidates for an optimal HP.

SMP treatment is one way to carry out a type of priming called Control Moisture Content (CMC). There are also other ways to perform CMC, such as osmopriming using polyethylene glycol (PEG), or drum priming (in a rotating drum with seeds and no medium).

In all these priming treatments only temperature, seed moisture, and treatment duration are manipulated. These factors are the basis of priming treatments. Other types of treatments can be developed from this that add further improvements, such as halopriming (priming with salts) or biopriming (priming with microorganisms).

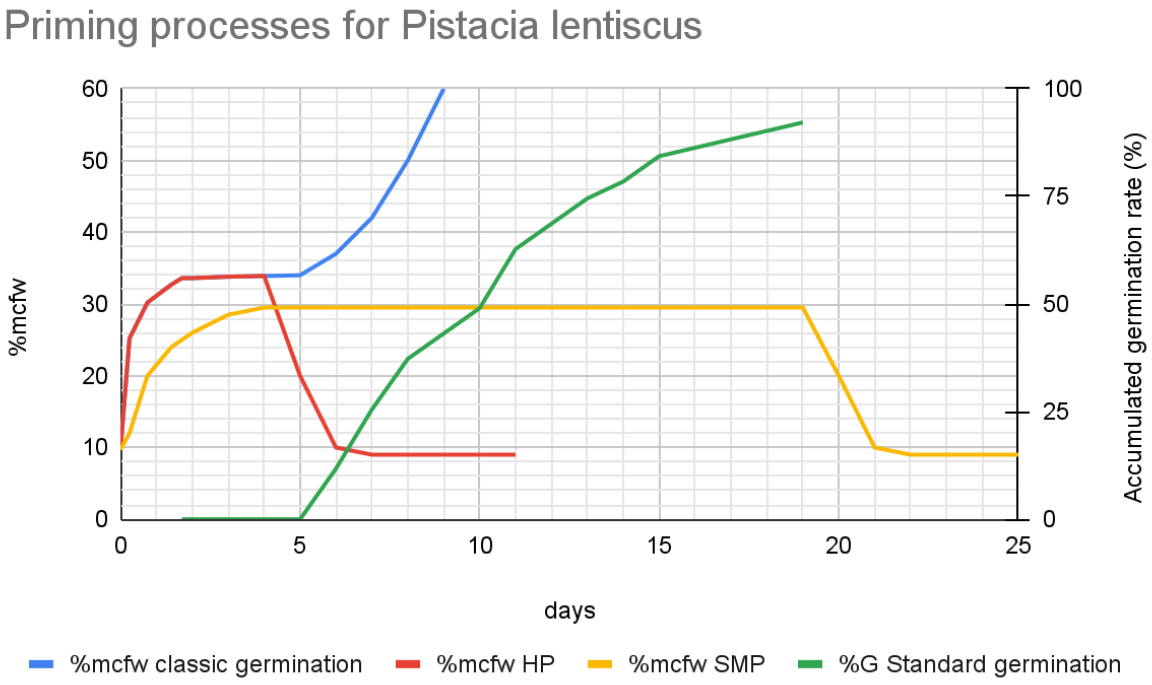

Figure 3: Priming in Pistacia lentiscus

3.13. What is *seed dormancy*?

Dormancy indicates that a seed will not germinate in the laboratory at the temperature and other conditions at which it emerges in the field. For example, a hackberry seed emerges in the field in early spring at around 5/15°C (night/day). However, failure will occur if it is germinated in the laboratory at this temperature. In another example, retama (broom) may emerge in autumn and spring, but this species will also fail if sown in the laboratory at any temperature.

Dormancy is a mechanism that makes it possible for seeds to germinate spatially far from their mother tree and temporally away from the moment at which dispersal began. Dispersed in time and space, they move away from their mother tree and can survive for a long time in the soil seed bank. In addition, dormancy increases the probability that seeds will germinate at the time of year most favorable for seedling development.

The term dormancy is often confusing in its definition and can have a complex academic meaning (which is still unresolved). Semillistas takes a practical position and uses the term to define information on the necessary conditions during germination for a seed to germinate.

Several types of dormancy can be distinguished:

- *Physical:* Seeds cannot hydrate because they have an impermeable coat. In nature, microorganisms (or other abiotic factors) create tiny fissures that eventually allow water to enter the seed.
- *Physiological:* Seeds require a period of cold stratification, or a period of dry after-ripening, or slight scarification.
- *Morphological:* The embryo is underdeveloped when seeds are dispersed from their mother tree and they need a subsequent period of warm stratification to complete embryo development.
- *Morphophysiological:* In addition to an underdeveloped embryo, seeds need cold to complete FII. Certain periods of warm stratification followed by cold stratification are required for each species.
- *Physical plus Physiological:* Seeds have an impermeable coat and require a period of cold stratification once this condition is overcome.

Physical dormancies are discussed below because these are the types present in this study's seeds.

Different types of scarification can be used to overcome physical dormancy in the laboratory using:

- Sulphuric acid
- Hot water
- Dry heat
- Enzymatic action
- Mechanical action (e.g., sandpaper or scarification machines)

The most appropriate treatment is chosen depending on the species and treatment volume.

The complexity of breaking physical dormancy lies in the fact that different batches of the same species may require different treatment times. Seed coat thickness may change year on year even in seeds with the same provenance. Therefore each year a small trial must be carried out to adjust the intensity of the scarification method. Few seeds will hydrate if the intensity is too low; if it is too high then the interior (embryo) of the seed will be damaged. In addition to this complexity, the coat thickness also varies within the same batch. This means that when the chosen treatment is applied, a percentage of seeds will achieve optimal scarification, another percentage will not be able to hydrate, and a further percentage will be damaged. The choice of scarification intensity comes after comparing these percentages.

When legume seeds are hydrated after scarification they can be observed to see whether they swell in order to evaluate the effectiveness of the chosen method without a germination test.

Once physical dormancy has been broken a classical germination test or priming treatment can be carried out.

3.14. What are *pre-germination treatments*?

Pre-germination treatment is a controlled germination process applied to a batch of seeds before they are sown in the field. While the term could encompass all classical germination processes, such as dormancy breaking and soaking, Semillistas uses it specifically to describe the treatments applied to seeds (regardless of whether they have undergone priming) immediately before field sowing. To avoid ambiguity, we distinguish between:

- Classical germination treatments
- Priming treatments, and
- Pre-germination treatments for field sowing.

Pre-germination treatments are used for field-sown seeds because surface soil dries rapidly, and at typical sowing temperatures (e.g., in winter), seeds (even those primed) require time to germinate. Pre-germination ensures a predictable germination rate under a wide range of field conditions. For example, consider seeds that have been primed and stored while awaiting rainfall for field sowing. In the laboratory, germination is initiated at 20°C. Once rain has fallen and soil conditions are suitable, the seeds are sown in the field just as they reach the brink of germination (end of Phase II). Depending on the species, this may occur after a few hours or days. Sown in moist soil at temperatures of approximately 5–15°C or 10–20°C, the seeds germinate quickly before the soil dries out.

Semillistas sows pre-germinated seeds after rainfall, rather than sowing dry seeds before rainfall. In our climate, predicting the "right" rain is impossible. Sowing dry seeds while waiting for rain presents two risks:

- The seeds may remain in the field for weeks or even months before rain arrives, leaving them vulnerable to predation by rodents or insects.
- The initial rainfall may trigger germination but prove insufficient to sustain the seedlings if subsequent rain is delayed.

Sowing dry seeds after rainfall is also an option, but surface soil dries quickly. To guarantee germination and achieve a predictable germination rate, it is preferable to sow pre-germinated seeds that are on the verge of germination at the time of sowing, even if this complicates pre-sowing logistics.

4. Methodology used

4.1. Classical germination and germination tests

We studied the classical germination patterns of each species, focusing on the "all-at-once" germination. To achieve this, we obtained germination curves at an optimal and constant temperature for each species, typically 20°C, based on tests conducted in previous years.

For species with physical dormancy, we performed germination tests using seeds that had been pre-treated with 96% sulphuric acid for varying immersion times. During immersion, the seeds were stirred every 10 minutes, then washed thoroughly with water and left to dry before starting the germination test in Petri dishes.

All germination tests were performed in Petri dishes, except for *Quercus ilex*. The characteristics of the setup for these tests were:

- Petri dishes filled with fine sand (less than 300 microns) with a moisture content of 11.5% mcdw;
- Seeds placed on top of the sand and pressed into it, with the Petri dish closed with parafilm and placed in a germination chamber at the appropriate temperature;

Disinfection (we prefer the term disinfection rather than sterilisation because an autoclave was not used):

- Water used to moisten the sand was disinfected by boiling for 15 minutes and then stored in vacuum-sealed glass jars;
- Sand was disinfected with dry heat at 200°C for 3 hours and then stored in vacuum-sealed glass jars;
- All instruments needed to set up the germination tests were boiled for 15 minutes;
- Seed coats were disinfected with different proportions and times of bleach under agitation (depending on the species) followed by washing with disinfected water;
- The work area was disinfected by spraying a solution of alcohol, hydrogen peroxide and water;
- Work was carried out near a Bunsen burner.

The bleach percentages used refer to the total volume of the solution. Thus, a 30% bleach solution contains 30% bleach and 70% water. Commercial bleach for drinking-water disinfection was used, with a chlorine concentration of 3.5%.

Quercus germination tests were performed in 50-micron LDPE zip bags filled with sand between 500 and 1000 microns (three times the volume of the seeds), with a moisture content of 11.5% mcdw. The disinfection and counting details were the same as for the Petri dishes, except that the LDPE bags were not disinfected.

All germination tests were conducted in darkness, except when counting germination success.

Each germination test consisted of 1 or 2 replicates of between 30 and 60 seeds, depending on the species. The data shown in each species sheet are the mean of both replicates.

Counts were made every 3–4 days at the start of germination and every 7 days after 2 weeks of germination. Germination was considered to have occurred when the root measured 2 mm. In *Quercus ilex*, germination was considered to have occurred when the root turned downward. During the counts, the number of germinations was recorded for each root length range (less than 2 mm, 2 to 5 mm, 5 to 10 mm, etc.), in order to estimate the number of daily germinations. The germination figures show the data from these estimates.

In most cases, the germination test was performed on the batch then subsequently studied and used for field sowing. However, in some species there were different batches with different storage methods and storage periods; the results of classical germination of these batches are also shown.

4.2. Batch improvement

Three methods were used to improve the batches of some of the species studied.

The improvement process during seed extraction and cleaning was discussed earlier in Section 3.11 on batch improvement.

The improvement process based on the soaking time of stored seeds involves soaking the seeds for a specific period, then removing the sinking ones. Germination tests were then performed on both the sinking seeds from different time intervals and the floating seeds to determine the optimal soaking time. This allowed us to remove the sinkers and increase the batch germination percentage.

When the parameters for improving a species batch are clear, the process can be applied to the quantity of seeds required for use in the short term. During this process, any floating seeds are discarded after separation and sinking seeds are dried again for storage. In some species, carrying out this improvement process accelerates the deterioration of the newly stored seeds. For this reason, this improvement process is used only on the seeds that will be used within the next six months, thereby preserving the original batch, which responds better to storage.

The batch-improvement process is integrated into the priming treatments for species with physical dormancy. The process is as follows:

1. Treat with sulphuric acid for the optimal time, then store again.
2. Start the hydropriming treatment by aerated soaking.
3. After 24 hours of aerated soaking, some of the seeds will swell and others will not. Separate the swollen from the non-swollen seeds using an appropriate sieve.
4. Keep the swollen seeds in aerated soaking for the optimal priming time, then dry them to finish the treatment.
5. Keep the non-swollen seeds in aerated soaking in another container.
6. After another 24 hours of aerated soaking, some of the seeds that had not swollen will swell and some will not. Separate them.

7. Keep the swollen seeds in aerated soaking for the optimal priming time, then dry them to finish the treatment. Note that if they had not been separated at step 3, these seeds would not have experienced the optimal priming duration.
8. Step 5 can continue to be repeated, depending on the number of swollen seeds or their economic value.

With this method, the seeds that will not germinate are eliminated (those that do not swell after several cycles), and an optimal-duration priming for all seeds is achieved.

4.3. Obtaining the optimal priming treatments

The choice of priming treatment type for each species is based on observations of classical germination. Specifically, the number of days it takes for germination to begin (T0) and how long germination lasts (T90, the time it takes to reach 90% of the possible germinations in that batch) were noted. Physiological or morphophysiological dormancy were also taken into account. Among our study species, we only found physical dormancy, so we have species whose classical germination is T0 between 1 and 5 days, and T90 between 1 and 10 days. For these cases, hydropriming techniques are used because these are species without dormancy whose seeds germinate quickly and uniformly (this includes species whose physical dormancy has been removed with sulphuric acid). For species whose seeds have slow, non-uniform, erratic germination, CMC techniques are used, with SMP being one of the possible ways of carrying out CMC in our study.

All species can receive CMC-type priming treatments, but not all can receive hydropriming (at least not with optimal results). The T0 and T90 ranges shown are indicative and depend largely on how the species responds to hydropriming treatments. In other words, it may seem that a species within those ranges will work with hydropriming, but this is empirically not the case and CMC is required. Hydropriming techniques are easier and cheaper to carry out, so if classical germination falls within those ranges, it is the first technique to try.

Hydropriming

The form of hydropriming used in this study is aerated soaking at the optimal temperature for classical germination. In addition to temperature, the other factor defining hydropriming is treatment duration. This duration cannot exceed T0, the time in which the process goes from Phase II to Phase III. Seeds that germinate during aerated soaking will die during the subsequent drying. Thus, for the different species to which this priming technique is applied, 2 or 3 treatment durations shorter than T0 are chosen and compared with results obtained via germination tests at the optimal temperature.

The study process is as follows:

1. The priming process temperature is the same as the optimal temperature for classical germination.
2. Two or three process durations shorter than T0 are used.
3. Aerated soaking for each duration is performed.
4. The seeds are 'slow dried' in an environment of about 15–20°C and 40–60% RH.
5. The seeds are stored.

6. Germination tests are performed at the optimal temperature for the different priming treatments performed and then compared with each other and with the untreated seeds.

Solid Matrix Priming

This technique requires more control and precision during the process than hydropriming. The parameters that define this priming treatment are treatment duration, the %mcfw of the seeds during the priming process (TMC, target moisture content), and temperature.

In general, the temperature of the SMP process is the same as the optimum for classical germination. If the temperature is very high (more than 20°C) or the seeds are prone to fungal development, it requires a very careful sterilisation process. For this reason, we carried out the SMP process for *Pinus halepensis* at 15°C. This species germinates well at 15°C, and fungal infestations are significantly reduced.

The TMC must have a value that does not allow germination for the duration of the SMP treatment. To achieve this, preliminary tests must be conducted to obtain the %mcfw value at which no germination occurs. This value is lower than the %mcfw value during Phase II in the classical germination process. Once the %mcfw value at which germination occurs has been obtained, two or three hypotheses for the TMC value are developed. For example, if in Phase II the %mcfw is 30% and the value at which no germination occurs is 27%, then 26% and 24% are used as TMC hypotheses. Values of 26% and 25% are not selected because of the precision with which %mcfw can be achieved through SMP, which is around ±1% mcfw.

SMP is put into practice by placing a specific amount of seeds in a 50-micron LDPE zip bag with a specific amount of 500–1000 micron silica sand and water. The amount of sand for all the SMPs in the study is 30 times the weight of the seeds (in previous years preliminary tests were conducted to determine this value for seeds of about 2–5 mm diameter). The amount of water to add depends on the TMC value, the species, and the storage %mcfw of the seeds, and is calculated empirically. With those empirical data, the %mcdw of the sand to is related to the %mcfw reached by the seeds during the SMP process using a regression line.

The study process is as follows:

1. The priming process temperature is the same as the optimal temperature for classical germination.
2. The %mcfw of the batch in storage is calculated.
3. The %mcfw of the seeds in Phase II of the classical germination process is calculated.
4. Several %mcfw values as TMC are proposed.
5. The %mcdw of the sand needed to reach the TMC values is estimated.
6. An SMP using the %mcdw estimates is conducted to obtain a regression line relating sand %mcdw to the %mcfw reached by the seeds during the SMP process.
7. An SMP is conducted based on the regression with different TMC values and maintained for an unlimited time to observe at what %mcfw the seeds germinate during the process.

8. Once the temperature, the regression (sand %mcdw vs. seed %mcfw), and the %mcfw at which the seeds germinate during the SMP process is known, the optimal duration of the process can be determined.
9. Two to three possible TMC values are chosen below the %mcfw at which the seeds germinate. For each TMC, the SMP is conducted with different process durations. We then choose the time around which the process durations are based on the T90 time of classical germination, the time it takes the seeds to complete classical germination.
10. At the end of the SMP process, the seeds are 'slow dried' at 15–20°C and 40–60% RH.
11. The seeds are stored.
12. Germination tests are conducted at the optimal temperature for the different priming treatments performed and compared with each other and with untreated seeds.

4.4. Pre-germination treatments

Our first hypothesis for preparing seeds (primed and stored) using pre-germination techniques was that they should be maintained at the optimal germination temperature until they were on the brink of germination and then immediately transported to the field for sowing. To initiate the pre-germination process, 50-micron LDPE bags were prepared containing 500–1000 micron sand at 11.5% mcdw mixed with the seeds. These bags were kept at 20°C in the laboratory for the maximum possible time without exceeding T0, the time at which germination begins. The pre-germination process began when rainfall ensured sufficient water in the soil to guarantee seedling development, so that when seeds were sown in the field, two conditions were met:

- The seeds must not have already germinated;
- Not so much time must have passed since the rains that the soil was dry on the surface.

Each species at each temperature, with its priming treatment, has a different T0. Therefore, depending on the sowing schedule, the pre-germination process for each species must start on different days.

Normally, the process begins by disinfecting the seeds (primed and stored) in a bleach solution, and then placing them in a bag with moist sand in a chamber at 20°C. When it is time to sow, the sand is separated from the seeds under running water, seeds are lightly surface-dried, and then coated with micronised diatom film coating to make them manageable (so they do not stick together). The seeds with diatomaceous earth are placed in a plastic jar which the field sowers subsequently use.

The study carried out in the laboratory aimed to determine how different species would respond to these pre-germination treatments by following this procedure for each species:

1. Germination tests were set up in Petri dishes (using the procedure described in *Methodology/Classical germination*) with both primed seeds and unprimed seeds (control).
2. Some Petri dishes were placed directly at 5/15°C and 10/20°C for germination tests without pre-germination.

3. Other Petri dishes were placed at 20°C for *X* days, then moved to 5/15°C and 10/20°C.
4. The procedure described in *Methodology/Classical germination* for the germination counts was followed, with the caveat that time t=0 of the germination graphs in the case of pre-germination is when the Petri dish was moved to the suboptimal temperature.

The *X* days of pre-germination depend on each species (and the treatment it receives) and are determined by a maximum time (T0 at 20°C).

We realised that some species do not respond well to the change from 20°C to suboptimal temperatures and that pre-germination treatments can slow germination rather than speed it up. For this reason, a second study was conducted (only with *Pinus nigra*) to observe the response to pre-germination treatments at different temperatures.

4.5. Scarification

The method used to scarify the seeds of Spanish broom, retama, and carob was 96% sulphuric acid. Different immersion times (with stirring every 10 minutes) were evaluated for each species. After immersion, the seeds were sieved and cleaned under running water for about 5 minutes. They were then spread out to dry and stored.

4.6. Hydration curve

The hydration curves obtained during the study aimed to identify when the transition between Phase I and Phase II occurs and the %mcfw of Phase II. The hydration curve for the moment of entry into Phase III was not recorded because it is not useful in practice. Besides, it is already known that the transition from Phase II to Phase III occurs through the onset of germination observed in the germination tests.

The procedure for obtaining the curve is as follows:

1. Measure the batch storage %mcfw.
2. Weigh the seed sample. We use only one 1 g replicate (for large seeds, we increase the sample weight).
3. Place the sample in soaking water at the temperature at which the priming treatments are performed.
4. At time intervals defined for each species, remove the sample from the soak in a strainer, surface-dry with paper towels, weigh, and return to soak.
5. Repeat the process until the weight stabilises.
6. After the final weighing, calculate the %mcfw of the seeds (in the oven), which corresponds to the moisture during Phase II.

4.7. Moisture content

An oven at 103±1°C for 17±1 hours is used to determine the moisture content of the seeds.

When seed moisture needs to be calculated, the seeds (FW) are weighed and placed in numbered containers. We use glass jars with twist-off lids and an inner rubber gasket. The containers are left until there are enough to fill the oven. After 17 hours in the oven, lids are placed on the glass jars and the seeds left to cool until they reach room temperature (about 1 hour). If the process had gone well, the jars form a vacuum as they cool, ensuring that the humidity of the outside air has not entered the jar and altered the seed moisture. After 1 hour, the jars are opened, the seeds poured into a container placed on a 0.0001 g precision balance, and the weight (DW) is recorded.

The moisture content based on fresh weight is calculated using the following formula:

$$\%mcfw = \frac{FW-DW}{FW} \times 100$$

The balance we used for weighing had a precision of 0.1 mg. The error it introduced to the weight measurement was ±0.2 mg. Using these data and the error propagation method based on partial derivatives, the error in %mcfw for a 0.8 g sample was less than 0.1% mcfw.

4.8. Germination tests at suboptimal temperatures

Germination tests were conducted following the process described in Section 4.1, *Classical germination and germination tests*.

To obtain germination data adapted to field conditions, the temperatures of the germination chambers were adjusted to 10/20°C and 5/15°C, following the hourly pattern corresponding approximately to the months of February and January, respectively (Figure 4). The error in the temperature oscillations inside the chambers was at most ±1°C.

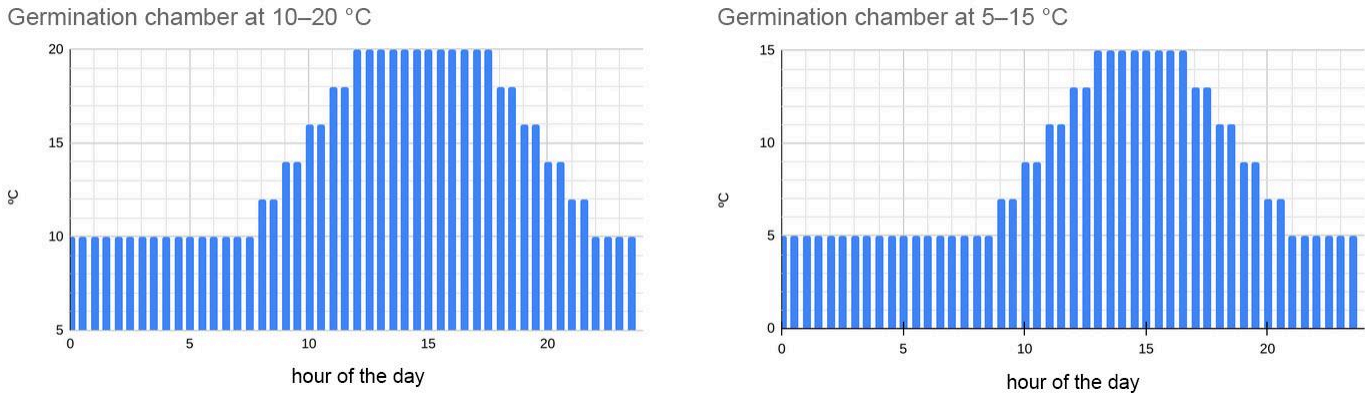

Figure 4: Germination chamber temperatures

5. Research sheets for each species

Clarifying Information

Information for each species is structured as follows:

- Batch
- Classical germination
- Batch improvement
- Hydration curve
- Priming treatments
- Germination tests at suboptimal temperatures
- Pre-germination treatments

All germination curves show results from either one replicate or the mean of two replicates.

When the text states that a seed germinates between 2 and 6 days, this is calculated as:

- **T0**: the time at which the first 2 mm germinated seeds appear.
- **T90**: the time at which 90% of the germinations in that germination test are completed.

Thus, when the text states *"it germinates between 2 and 6 days, up to 85%,"* this means:

- T0 = 2 days
- T90 = 6 days
- Maximum germination percentage of the germination test = 85%
  Note that 85% is not the germination percentage at T90, but at T100.

The legends of each figure provide a summary of the characteristics of the treatments applied:

- **SMP treatments**: treatment name + %mcfw at the end of the treatment + treatment duration + treatment temperature + drying.
- **Pre-germination treatments**: information about the SMP treatment + pre-germination (sand or soaking) + pre-germination temperature + pre-germination duration + germination-test temperature after pre-germination.
- **Scarification treatments**: the type of scarification (96% acid) and the immersion duration.

In germination curves from pre-germination treatments, the process is as follows:

1. The seeds are placed to pre-germinate in moist sand at 20°C for a set time.
2. Without drying the seeds, they are transferred after pre-germination to 10/20°C or 5/15°C.
3. Time 0 of curves from pre-germinated seeds corresponds to the moment of transfer to the suboptimal temperatures, as this simulates the moment of field sowing.

The data shown in the suboptimal-temperature and pre-germination figures refer to the main batch with which classical germination and the optimal priming treatment were conducted. However, the SMP or HP treatment used to obtain these suboptimal-temperature and pre-germination graphs may be slightly different from the optimal one. The figure legends show the relevant data on the priming parameters for the germination curves they display.

SPECIES PROFILES

PINUS HALEPENSIS

BATCH

The seed lot used was purchased from the El Serranillo Forestry Centre and originated from the Subbética Mountains (2014 harvest).

During the study the seeds were stored in airtight containers at 4°C. The moisture content of the seeds from each sublot used throughout the year was: 6.8%, 6.6%, and 6.7% mcfw.

The parent lot corresponds to a harvest from a wide variety of trees, resulting in a fairly heterogeneous seed size.

BATCH IMPROVEMENT

It was not deemed necessary to improve the seed lot, as germination rates were around 90%.

CLASSIC GERMINATION (Figure 5)

	Optimal T^a (ºC)	96% acid time (min)	Batch improvement	Bleach disinfection	T0 (days)	T90 (days)	%maxG
P. halepensis	20ºC	--	--	30% 15'	7	15	88%

1 or 2 replicates of 40 to 50 seeds were used for all germination tests with this species.

20ºC HYDRATION CURVE (Figure 6)

	FI-FII hours	%mcfw FII
P. halepensis	15-20	30%

PRIMING TREATMENTS (Figure 5)

P. halepensis	Priming Tª (ºC)	Priming duration (days)	%mcfw FII	%mcfw SMP (no germinations)	%mcfw final SMP	GT Tª (ºC)	GT T0 (days)	GT T90 (days)	%maxG. GT
Control	--	--	--	--	--	20ºC	7	15	88%
Optimal SMP	15ºC	32	30%	29%	24%	20ºC	3	7	94%

SMP treatments instead of HP.were used because the completion time for conventional germination is 15 days.

The temperature for the SMP process was changed from 20°C to 15°C to reduce the risk of fungal infestation during SMP treatment. This was possible because this species germinates in a similar way at both 15°C and 20°C, except slightly more slowly at 15°C.

The %mcfw during FII was 30%.Germination occurred with an SMP treatment at 15°C for 30 days with seeds at 29.3% mcfw, but at values below 29% mcfw germination did not occur.

We conducted a large number of SMP tests with various moisture contents (Figure 5) and treatment durations. Bringing the seed moisture content during SMP treatment close to 29% mcfw would normally yield the best results for most species, but this was not the case for this species.

Figure 5 shows germination curves for the different treatments. All germination tests were conducted at a stable 20°C. All SMP treatments improved germination characteristics, although some treatments were more effective than others. Listing the treatments from most to least optimal, the curves in Figure 5 show:

- The most optimal treatment, shown by the red line, germinates at 20°C within 3 to 7 days compared to control seeds, which germinate within 7 to 15 days. The optimal SMP treatment for *Pinus halepensis* is therefore TMC = 24% mcfw and Duration = 32 days.
- The solid-line curves in the figure represent near-optimal treatments. They correspond to treatments with TMC ≈ 24% and durations of less than 30 days; plus durations of 30 days and 26.7% mcfw.
- The dotted-line curves show a decrease in germination rate, although they still provide a significant improvement in germination. They correspond to the %mcfw of the seeds at:
    - 21% and 22%, regardless of the duration of the process.
    - 23.6% mcfw with 18 days.
    - 28.3% mcfw with 30 days.

In other words, if we deviate from TMC = 24%mcfw and Duration = 32 days (either above or below these values) we lose some but not all of the improvements achieved with optimal priming.

SMP tests were conducted at 20°C during the priming process (Figure 5, dashed line). Susceptibility to fungal growth, both during the SMP process and in subsequent germination tests, was higher than when the SMP process was performed at 15°C. The results point to the same conclusions as when SMP was performed at 15°C: approaching the TMC of 24%mcfw and 30 days improves treatment quality.

GERMINATION TESTS AT SUBOPTIMAL TEMPERATURES (Figures 7 & 8)

	GT T^a (ºC)	T0 (days)	T90 (days)	%maxG.
Control	10/20ºC	12	26	70%
SMP	10/20ºC	6	13	90%
Control	5/15ºC	26	61	70%
SMP	5/15ºC	14	32	88%

Seeds treated with SMP significantly increase germination rates compared to the control group, while also increasing the overall germination rate, at both optimal and suboptimal temperatures.

PRE-GERMINATION TREATMENTS (Figures 7 & 8)

Pre-germination treatments were performed at 20°C on the control and SMP seeds for a specified pre-germination period, after which the seeds were transferred to 10–20°C and 5–15°C.

- Because control seeds germinate in 7 days at 20°C, pre-germination lasted 6 days.
- Becaus SMP seeds germinate in 3 to 4 days at 20°C, pre-germination lasted 3.5 days.
- At 10/20°C (Figure 7), pre-germination of the SMP seeds (green line) managed to advance the germination curve by 2 days, reaching germination percentages similar to those of seeds without pre-germination (red line). In contrast, pre-germination of control seeds (yellow line) greatly increased their germination rate, but the process took longer, and achieved only 50% germination.
- At 5/15°C (Figure 8), pre-germination of the SMP seeds (green line) advanced the germination curve by 7 days, but reduced the germination percentage to 60% compared to SMP seeds without pre-germination (red line). In contrast, pre-germination of the control seeds (yellow line) achieved only 20% germination, albeit rapidly.

The pre-germination results were not as expected. The shift from stable 20°C to suboptimal temperatures results in a decline in germination characteristics (rate and percentage of germination). This decline is evident when moving from 20°C to 5–15°C. When moving to 10/20°C, SMP seeds do manage to improve germination characteristics. Observing this decline in several species we have studied led us to investigate more thoroughly the consequences of moving from a pre-germination temperature to one that mimics field conditions (e.g., the study on *Pinus nigra*).

SEED MOISTURE CONTENT VARIABILITY AT THE END OF SMP TREATMENT (Figure 9)

To verify the reproducibility of the SMP method, several treatments with identical characteristics were conducted to determine the %mcfw reached by seeds at the end of the treatment. Monitoring the %mcfw reached by seeds is critical to the quality of the seeds obtained. Additionally, the aim was to observe the range of %mcfw values, i.e., the variability in %mcfw among seeds at the end of the SMP process.

Fourteen SMP treatments were conducted using two different sand moisture levels and various treatment durations. The moisture content was calculated for six replicates of approximately 0.8 g of seeds for each treatment (Figure 9).

	Average initial %mcfw (seeds)	Initial %mcdw (sand)	Average final %mcfw (seeds)	Std. deviation final %mcfw (seeds)
P. halepensis	6.6%	1.05%	24.3%	0.6%
P. halepensis	6.6%	0.9%	21.6%	0.5%

Based on our analysis of numerous germination curves for different species treated with SMP, we conclude that the maximum standard deviation for an effective SMP treatment should not exceed 1%; in other words, seeds should have a %mcfw value of TMC ± 1% at the end of the process. If this variability increases then the improvement process will not be uniform across all seeds in the treatment.

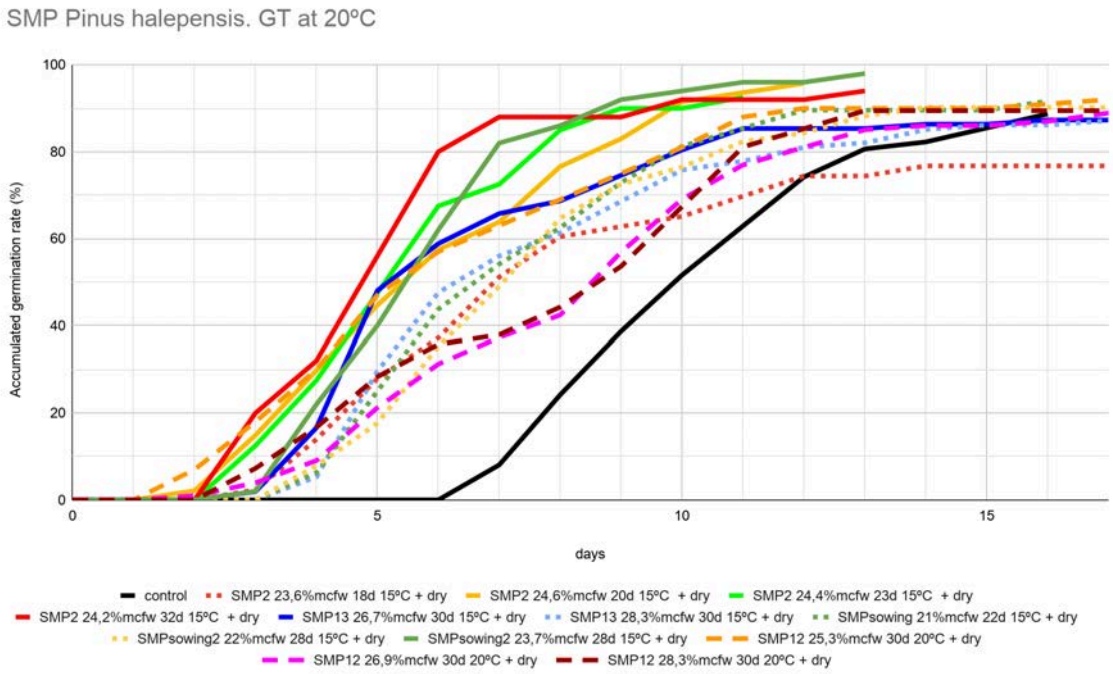

Figure 5: Germination curves for Pinus halepensis

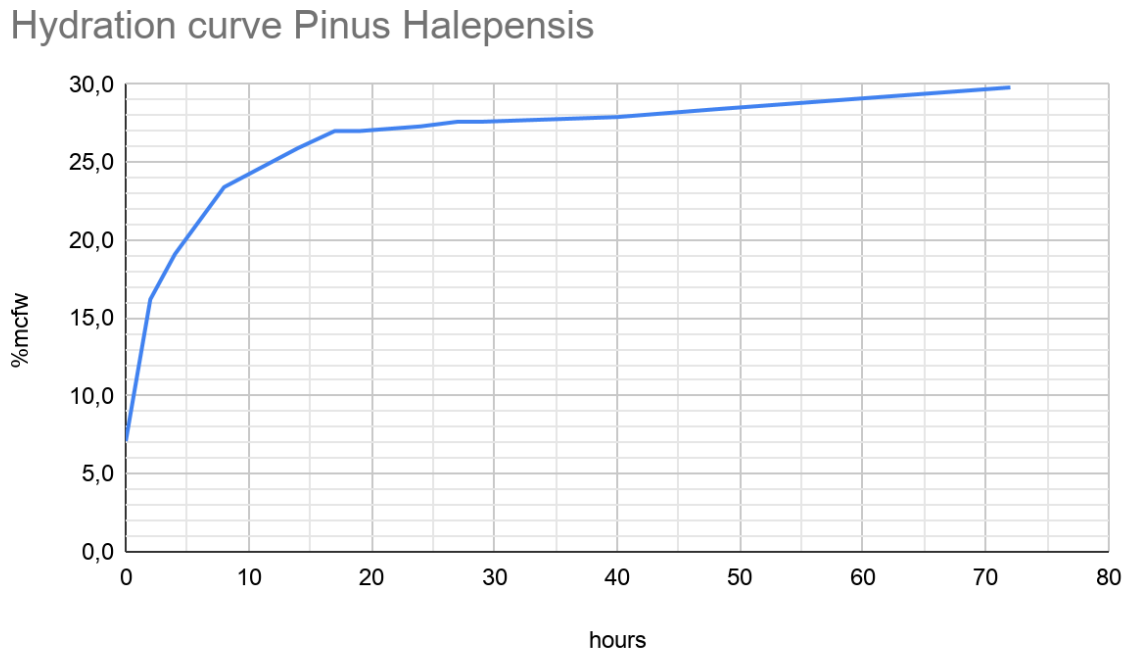

Figure 6: Hydration curve for Pinus halepensis

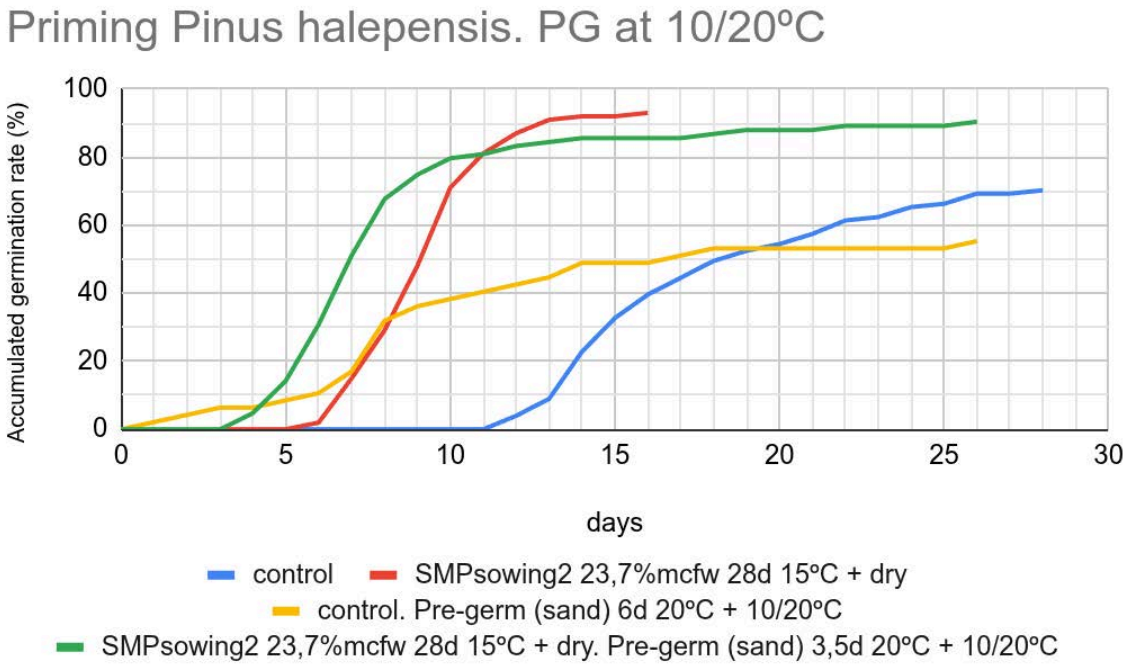

Figure 7: Germination curves for Pinus halepensis

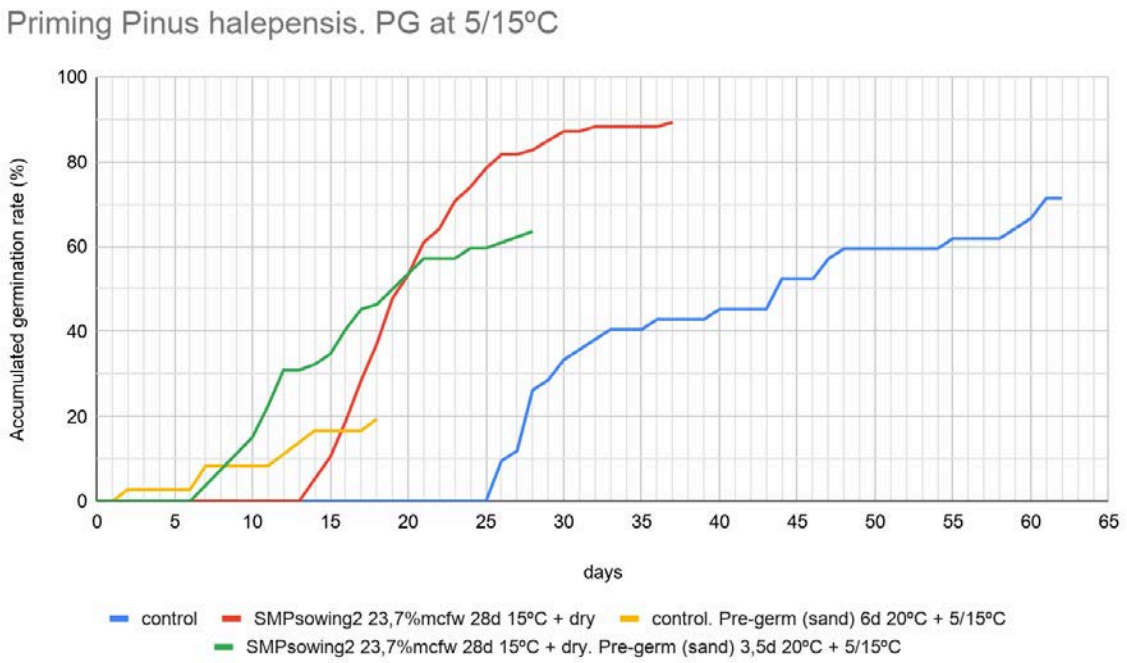

Figure 8: Germination curves for Pinus halepensis

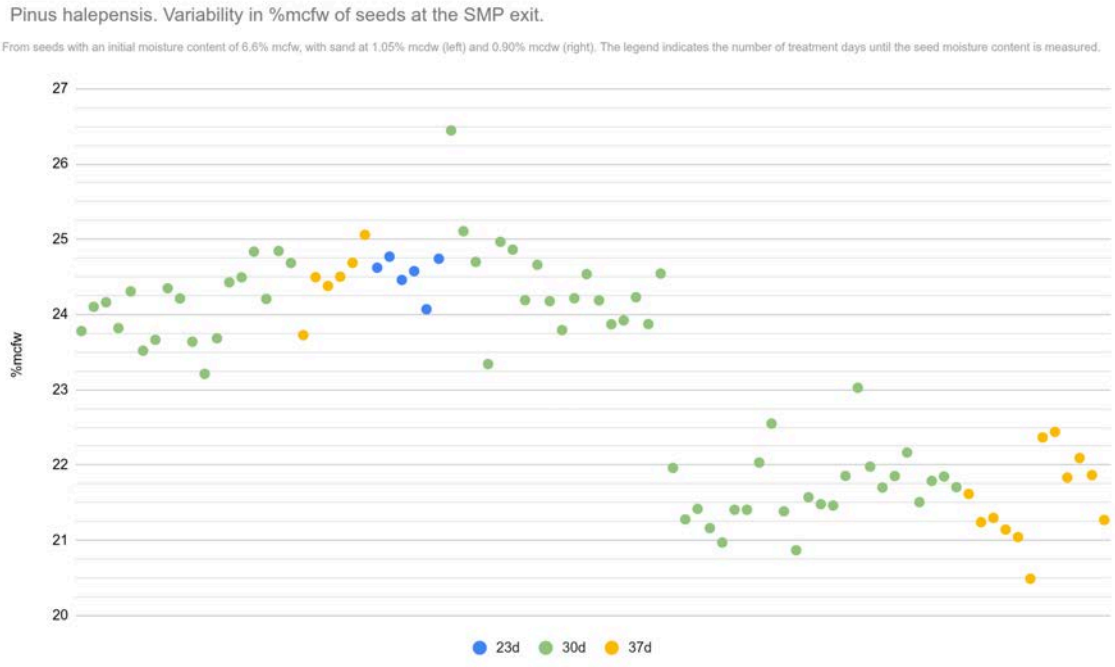

Figure 9: Variability in moisture content for Pinus halepensis *during SMP*

PINUS NIGRA SALZMANNII

BATCH

The seed lots used were purchased from the El Serranillo Forestry Centre and originated from the Cordilleras Béticas (2014 harvest) and Sierra Mágina (2014 harvest). With the exception of the special pre-germination section (which we conducted only with this species on the Mágina lot), all other tests were performed using the Béticas lot.

During the study the seeds were stored in airtight containers at 4°C. The moisture content of the seeds in each sublot used was: 7.1% mcfw for Béticas and 6.3% mcfw for Mágina.

The parent lot corresponds to a harvest from a wide variety of trees, resulting in a fairly heterogeneous seed size.

BATCH IMPROVEMENT

It was not deemed necessary to improve the seed lot, as germination rates were around 90%.

CLASSIC GERMINATION (Figure 10)

	Optimal T^{a} (ºC)	96% acid time (min)	Batch improvement	Bleach disinfection	T0 (days)	T90 (days)	%maxG
P. nigra salzmannii	20ºC	--	--	10% 15’	3	10	85%

For all germination tests with this species 1 or 2 replicates of 30 to 50 seeds were used.

20ºC HYDRATION CURVE (Figure 11)

	FI-FII hours	%mcfw FII
P. nigra salzmannii	10-15	32%

PRIMING TREATMENTS (Figure 10)

P. nigra salzmannii	Priming Tª (ºC)	Priming duration (days)	%mcfw FII	%mcfw SMP (no germinations)	%mcfw final SMP	GT Tª (ºC)	GT T0 (days)	GT T90 (days)	%maxG. GT
Control	--	--	--	--	--	20ºC	3	10	85%
Optimal SMP	20ºC	9	32	29.5	29.5	20ºC	1	3	90%
Optimal HP	20ºC	45 hours	--	--	--	20ºC	1	3	90%

SMP and HP treatments were studied because the typical germination period for this species is 10 days.

The %mcfw during FII was 32%. Germination began with an SMP treatment at 20°C for 10 days with seeds at 30.3% mcfw, but did not occur at values below 29.5% mcfw, regardless of treatment duration.

Since the onset of FIII at 20°C occurs between 2 and 3 days, HP treatments were limited to a maximum duration of 2.5 days. The selected durations were 35 h, 45 h, and 55 h.

Figure 10 shows the germination curves for the different treatments. All germination tests were conducted at a stable 20°C, and both SMP and HP treatments were performed at 20°C. All treatments improved germination characteristics similarly. The curves in Figure 10, listed from most to least optimal, are as follows:

- 55-hour HP in AS + drying: Germination reached 90% within 1 to 3 days, compared to control seeds, which reached 85% within 3 to 10 days.
- 35-hour and 45-hour HP treatments: Both performed similarly, with the 35-hour treatment germinating slightly slower.
- SMP with 30.1% mcfw for 9 days: Yielded germination characteristics similar to the HP treatments.
- SMP with similar %mcfw for 6 and 13 days: Slightly slowed germination.

The optimal priming treatments for *Pinus nigra salzmannii* are:

- 55-hour HP in AS at 20°C + drying.
- 9-day SMP with TMC = 29.5% mcfw + drying.

At the end of the 55-hour HP treatment, the seeds were observed beginning to open, with a small white bulge (the start of root elongation) which we call G0 (0 mm germination). Drying the seeds in this state does not kill the *Pinus nigra* seeds. However, we consider this too risky. Since the shorter HP durations studied show a similar improvement, we recommend performing HP for between 35 and 45 hours, rather than 55 hours.

GERMINATION TESTS AT SUBOPTIMAL TEMPERATURES (Figures 12 & 13)

	GT T^a (ºC)	T0 (days)	T90 (days)	%maxG.
control	10/20ºC	6	12	88%
SMP	10/20ºC	3	7	98%
control	5/15ºC	9	26	86%
SMP	5/15ºC	5	12	95%

Seeds treated with SMP significantly increase germination rates compared to the control group, while also increasing the overall germination rate, both at optimal and suboptimal temperatures.

PRE-GERMINATION TREATMENTS (Figure 14)

Pre-germination treatments were conducted at 20°C on control and SMP seeds for a specified duration, after which seeds were transferred to 10–20°C or 5–15°C.

For control seeds, which germinate in 3 days at 20°C, the pre-germination period was 2.5 days. For SMP seeds, which germinate in 1 day at 20°C, pre-germination lasted 15 hours.

At 10/20°C (Figure 12):

- Pre-germination of SMP seeds (green line) did not improve germination and was much slower compared to SMP without pre-germination (red line).
- Pre-germination of control seeds (yellow line) increased the germination rate compared to the control without pre-germination (blue line), but only achieved 67% germination.

At 5–15°C (Figure 13):

- Pre-germination of SMP seeds (green line) advanced the germination curve by 1 day compared to SMP without pre-germination (red line).
- Pre-germination of control seeds (yellow line) significantly advanced germination compared to the control without pre-germination (blue line), but germination dropped to 75%.

Due to the varied germination responses when initiating germination at one temperature and then changing it, a preliminary study was conducted in preparation for 2026 research on the effects of mid-process temperature changes on germination characteristics.

An SMP treatment was performed on the Sierra Mágina batch with TMC = 29.7% mcfw for 8 days at 20°C, followed by drying. Various pre-germination treatments were then carried out at different temperatures and durations to initiate pre-germination, after which the Petri dishes were transferred to 5/15°C.

The duration of each pre-germination treatment was determined by the maximum time seeds could remain at that temperature before germination began (as indicated in the legend of Figure 14). The

control seeds (SMP without pre-germination) are represented by the purple line. All pre-germination treatments improved the germination rate, though some were more effective than others.

SEED MOISTURE CONTENT VARIABILITY AT THE END OF SMP TREATMENT (Figure 15)

To verify the reproducibility of the SMP method, multiple treatments with identical characteristics were conducted to determine the %mcfw of the seeds at the end of the treatment. Monitoring the final %mcfw is critical for ensuring seed quality, as it also allows assessment of the range of %mcfw values; i.e., the variability among seeds after SMP.

For the Sierra Mágina batch, 10 SMP treatments were performed using sand with a moisture content of 1.4% mcdw for 7 days. For each treatment, the moisture content was calculated for 6 replicates of approximately 0.8 g of seeds (Figure 15).

	Average initial %mcfw (seeds)	Initial %mcdw (sand)	Average final %mcfw (seeds)	Std. deviation final %mcfw (seeds)
P. nigra salzmannii	6.3%	1.4%	29.9%	0.5%

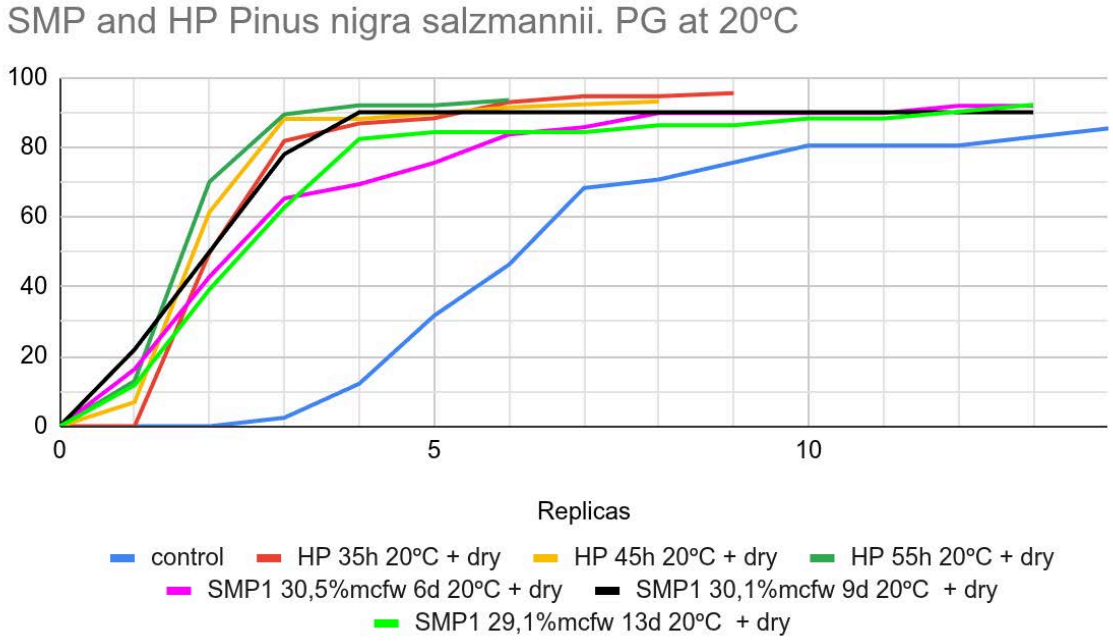

Figure 10: Germination curves for Pinus nigra salzmannii

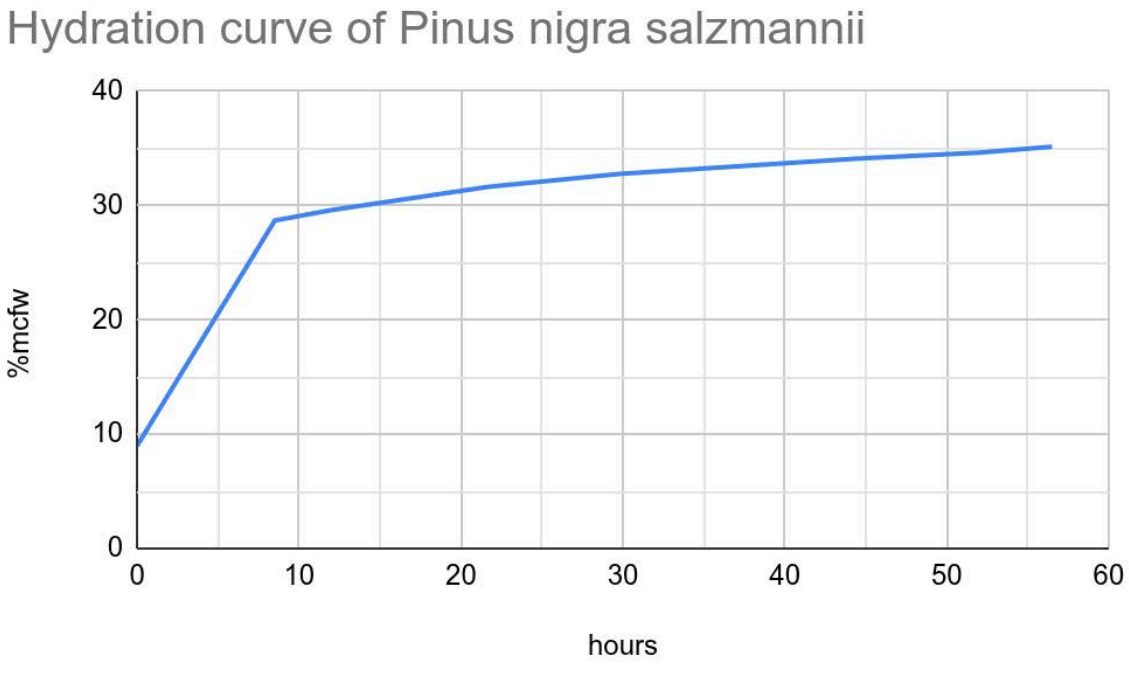

Figure 11: Hydration curve for Pinus nigra salzmannii

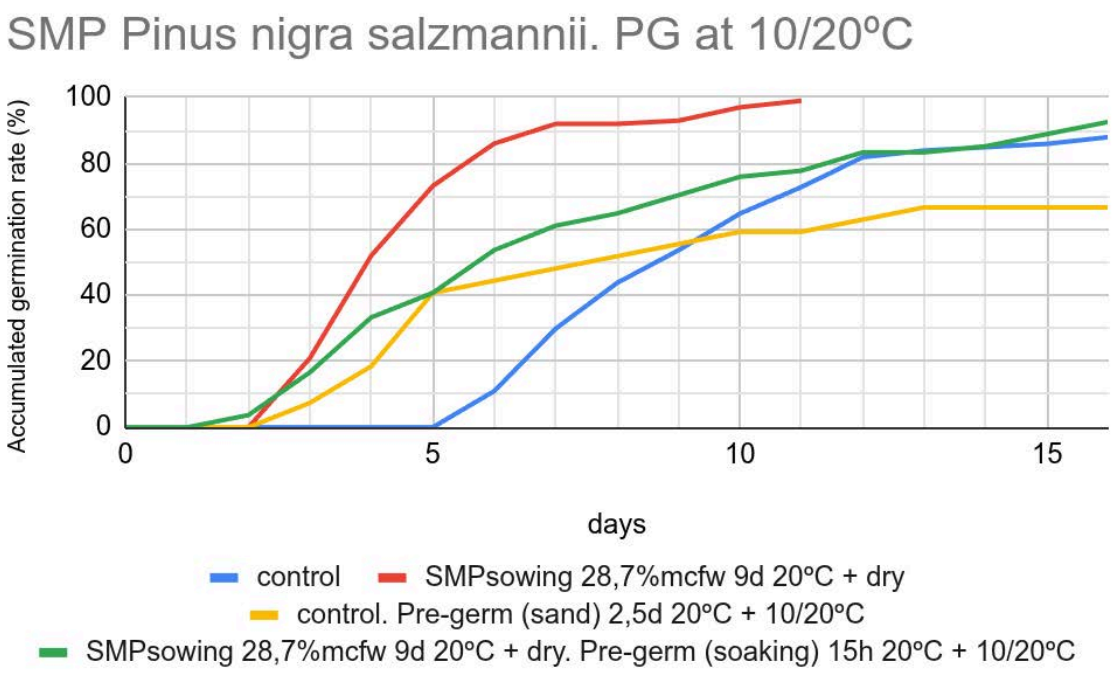

Figure 12: Germination curves for Pinus nigra salzmannii

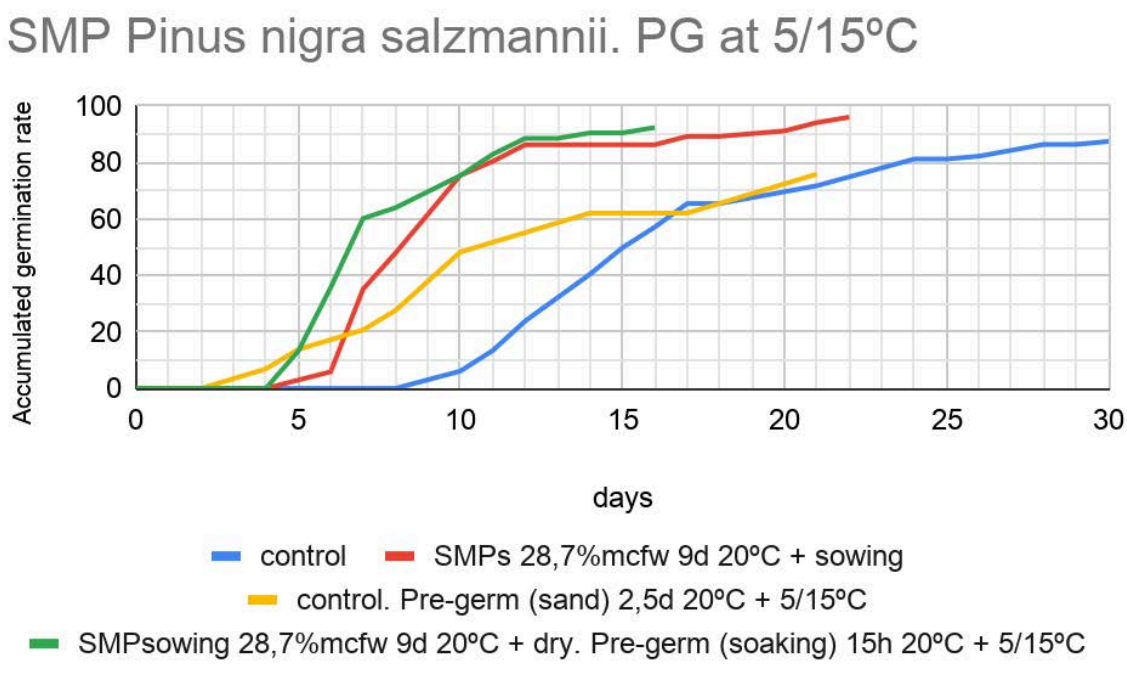

Figure 13: Germination curves for Pinus nigra salzmannii

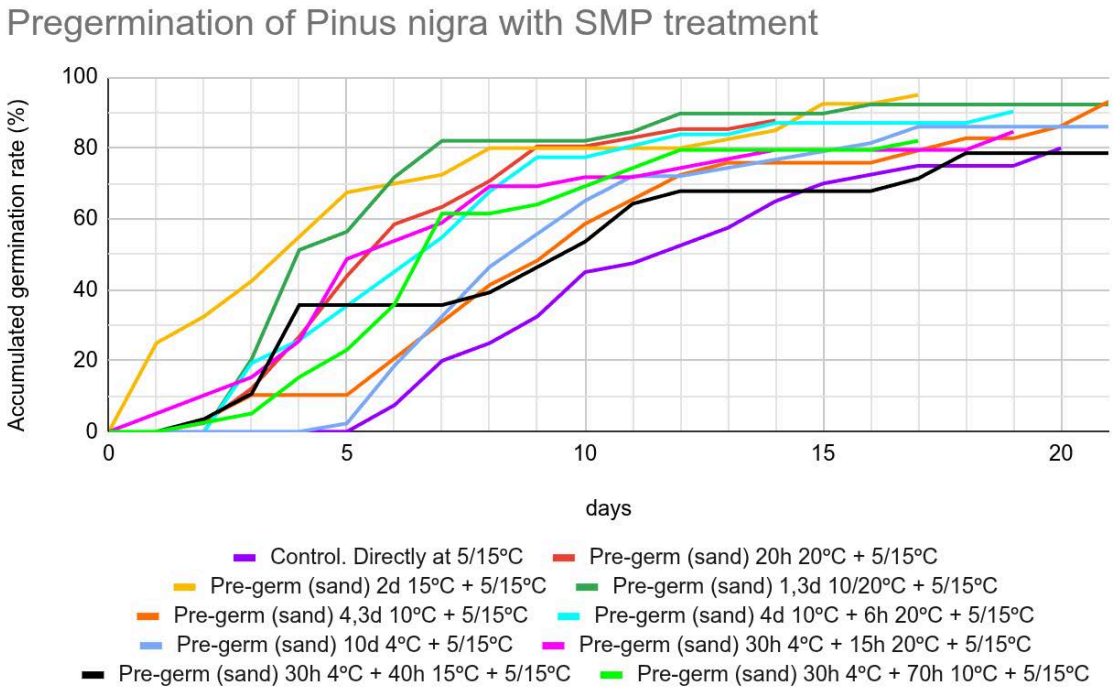

Figure 14: : Germination curves for Pinus nigra salzmannii

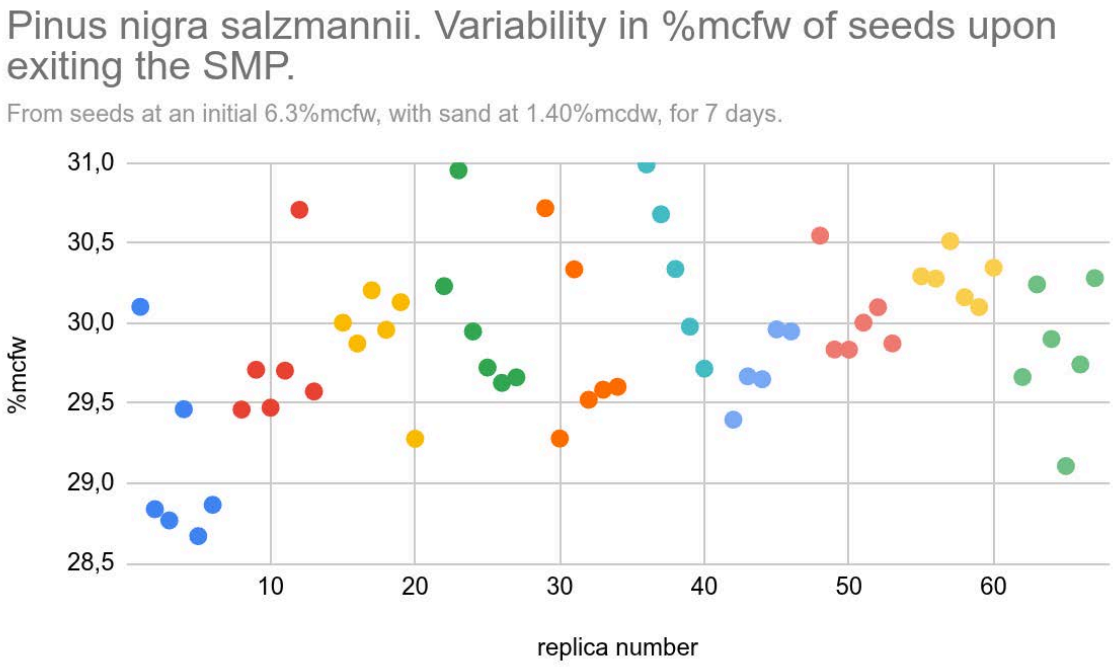

Figure 15: Variability in moisture content for Pinus nigra salzmannii *during SMP*

CERATONIA SILIQUA

BATCH

The batch used was harvested in the Alpujarra region (Soportujar) in 2022. It came from a single tree.

The seeds were stored in airtight containers at 4°C after harvest and extraction. For the purposes of this study it was not necessary to measure the moisture content of the seeds.

BATCH IMPROVEMENT

It was not deemed necessary to improve the seed lot, as germination rates were around 100%.

CLASSIC GERMINATION (Figure 16)

The germination temperature used for all tests was 20°C. Although germination occurs more rapidly at higher temperatures the seeds are also more susceptible to fungal contamination.

An acid scarification time of 90 minutes was chosen for all subsequent tests. 1 or 2 replicates of 30 seeds were used in all germination tests with this species.

	Optimal T^{a} (ºC)	96% acid time (min)	Batch improvement	Bleach disinfection	T0 (days)	T90 (days)	%maxG
C. siliqua	20ºC	60	--	10% 10’	6	8	100%
C. siliqua	20ºC	75	--	10% 10’	5	8	100%
C. siliqua	20ºC	90	--	10% 10’	4	6	100%

20ºC HYDRATION CURVE (Figure 17)

	FI-FII hours	%mcfw FII
C.siliqua (acid 90' + HP2d)	20-25	63%

The hydration curve was generated using seeds subjected to a 90-minute acid treatment followed by a 2-day HP treatment.

The test was conducted using both soaked and moist sand to confirm that hydration dynamics remained consistent. For the moist sand method, the initial sand moisture content had to be adjusted to accommodate the high water absorption of the large carob seeds. While sand at 11.5% mcdw is typically used for seeds like pine or buckthorn, carob seeds require an initial sand moisture content of 14% mcdw (3:1 vol sand:seed). This ensured that by the end of hydration the sand moisture content would be around 9.5% mcdw. Using 11.5% mcdw would have caused the sand moisture to drop too quickly, slowing the seed hydration rate.

PRIMING TREATMENTS (Figure 18)

C. siliqua (acid 90')	Priming Tª (ºC)	Priming duration (days)	%mcfw FII	%mcfw SMP (no germinations)	%mcfw final SMP	GT Tª (ºC)	GT T0 (days)	GT T90 (days)	%maxG. GT
Control	--	--	--	--	--	20ºC	4	6	100%
Optimal HP	20ºC	2	--	--	--	20ºC	1	3	100%

HP was selected as the priming treatment due to the typical 6-day germination period.

Since acid-treated seeds begin germinating after 90 minutes and reach FIII in 3 to 4 days, HP with AS was performed for 2 to 3 days at 20°C.

The 3-day HP treatment failed to germinate because, by the end of the RA, the seeds had open testa in a state referred to as G0 (0 mm germination). Drying these seeds after RA resulted in death, as they had already reached FIII.

GERMINATION TESTS AT SUBOPTIMAL TEMPERATURES (Figures 19 & 20)

	GT T[a] (ºC)	T0 (days)	T90 (days)	%maxG.
Control	10/20ºC	6	8	100%
HP 2d	10/20ºC	4	6	95%
Control	5/15ºC	15	19	86%
HP 2d	5/15ºC	11	16	95%

HP-treated seeds germinate rapidly at 20°C—significantly faster than the control—but at suboptimal temperatures an HP treatment does not significantly improve germination rates.

To verify these results, the HP treatment will be repeated, and germination will be observed at suboptimal temperatures. The data may be incorrect, and inconsistencies in the pre-germination treatment section support this possibility. This task will be conducted in 2026.

PRE-GERMINATION TREATMENTS (Figures 19 & 20)

Pre-germination treatments were conducted at 20°C on control and HP seeds for a specified duration, after which seeds were transferred to 10/20°C or 5/15°C.

For control seeds, which germinate in 4 days at 20°C, the pre-germination period was 3 days. For HP seeds, which germinate in 1 day at 20°C, pre-germination lasted 20 hours.

At 10/20°C (Figure 19):

- Pre-germination of HP seeds (green line) improved germination timing from 1 to 4 days, compared to 4 to 6 days for HP seeds without pre-germination (red line).
- Pre-germination of control seeds (yellow line) increased germination time significantly, from 6–8 days to 1–3.5 days, compared to the control without pre-germination (blue line).

At 5–15°C (Figure 20):

- Pre-germination of HP seeds (green line) advanced the germination curve from 11–16 days to 6–8.5 days, compared to HP seeds without pre-germination (red line).
- Pre-germination of control seeds (yellow line) significantly advanced germination from 15–19 days to 3–5 days, compared to the control without pre-germination (blue line).

Although pre-germination of HP seeds substantially improves germination compared to untreated HP seeds, this improvement is far more pronounced in non-HP seeds. As noted previously, potential errors in the process may skew the data, and the study must be repeated.

The comparison between pre-germination by soaking and moist sand placement is notable. Soaking delays germination by nearly 1 day, a pattern observed in other species. The soaking duration is critical to avoid germination delays. The hypothesis is that soaking should not exceed the duration of hydration FI.

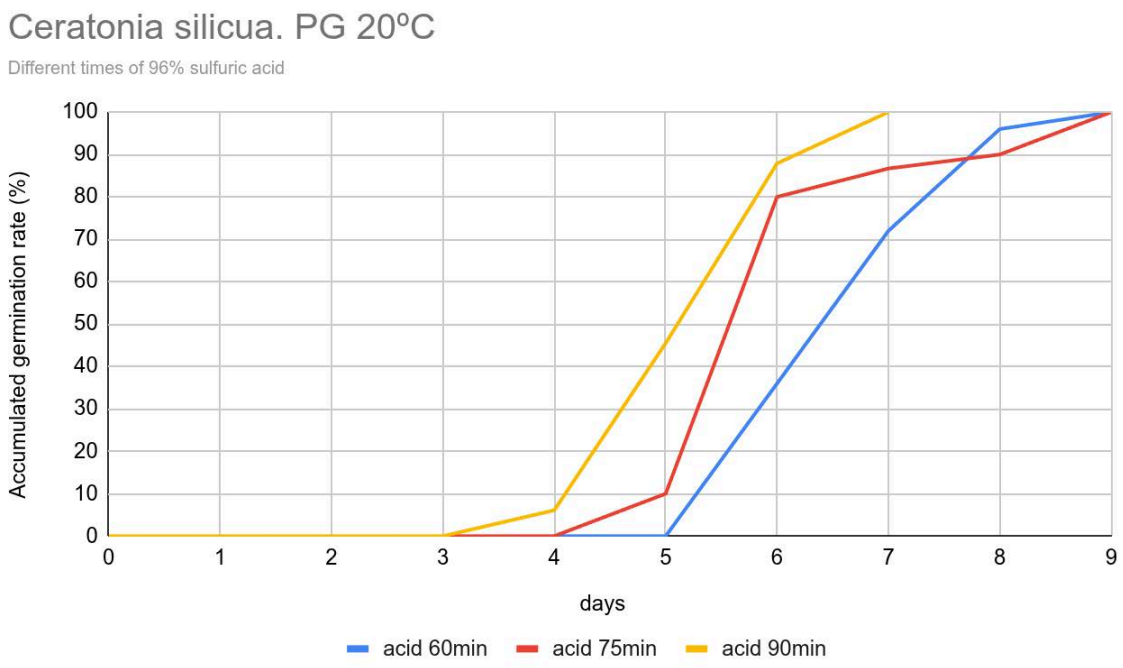

Figure 16: Germination curves for Ceratonia siliqua

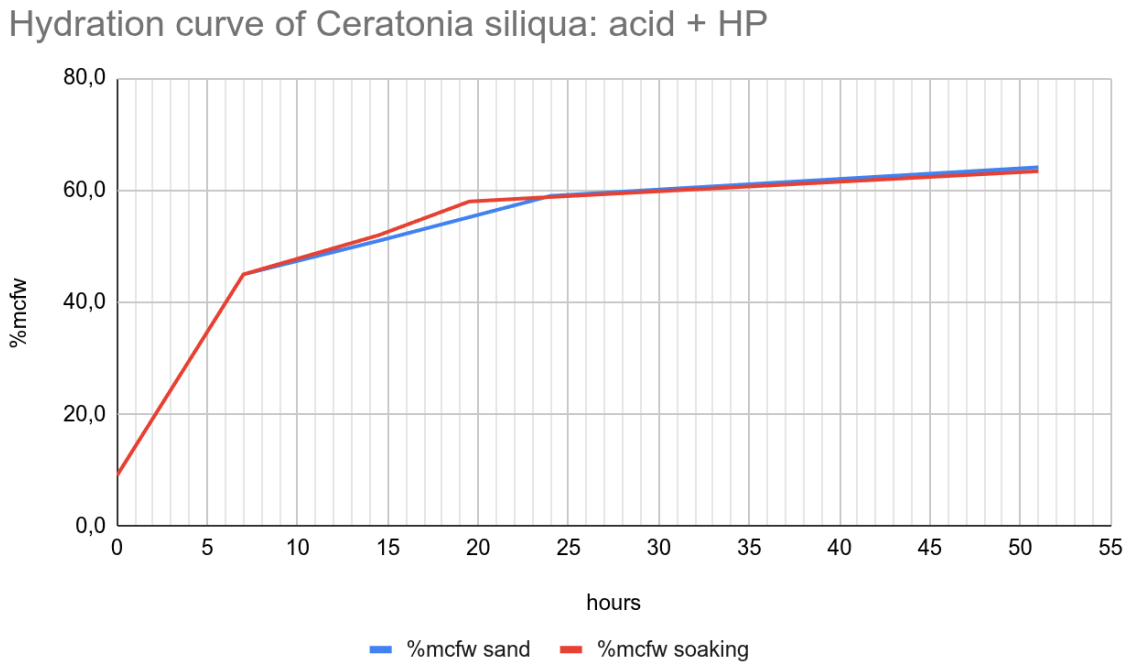

Figure 17: Hydration curve for Ceratonia siliqua

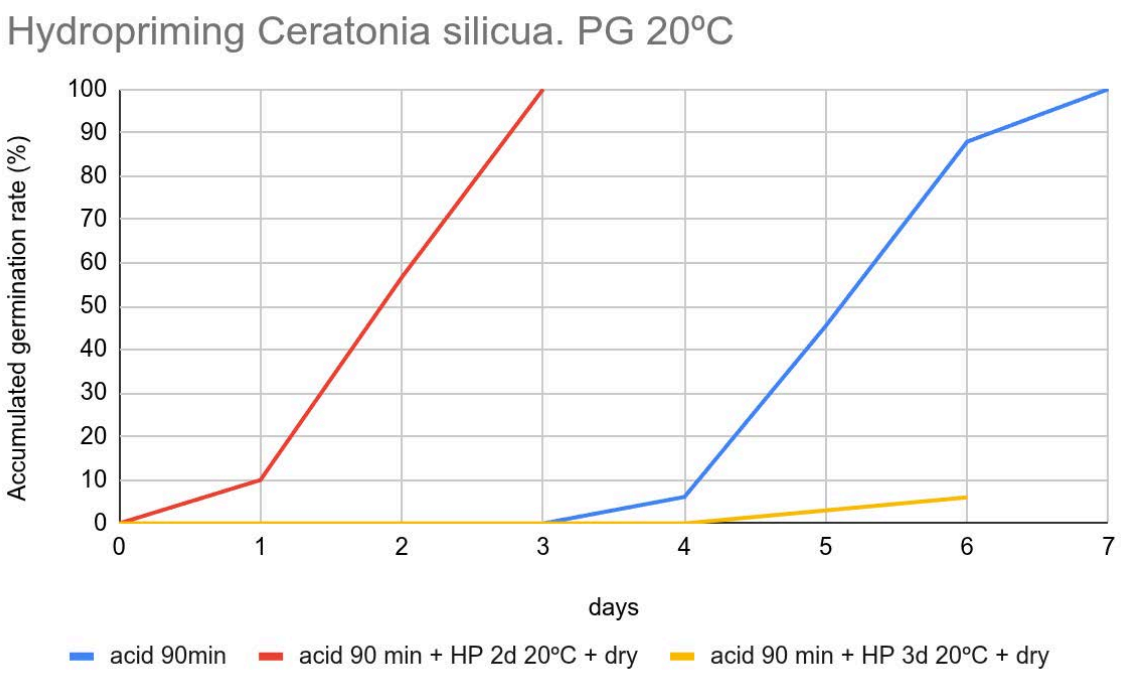

Figure 18: Germination curves for Ceratonia siliqua

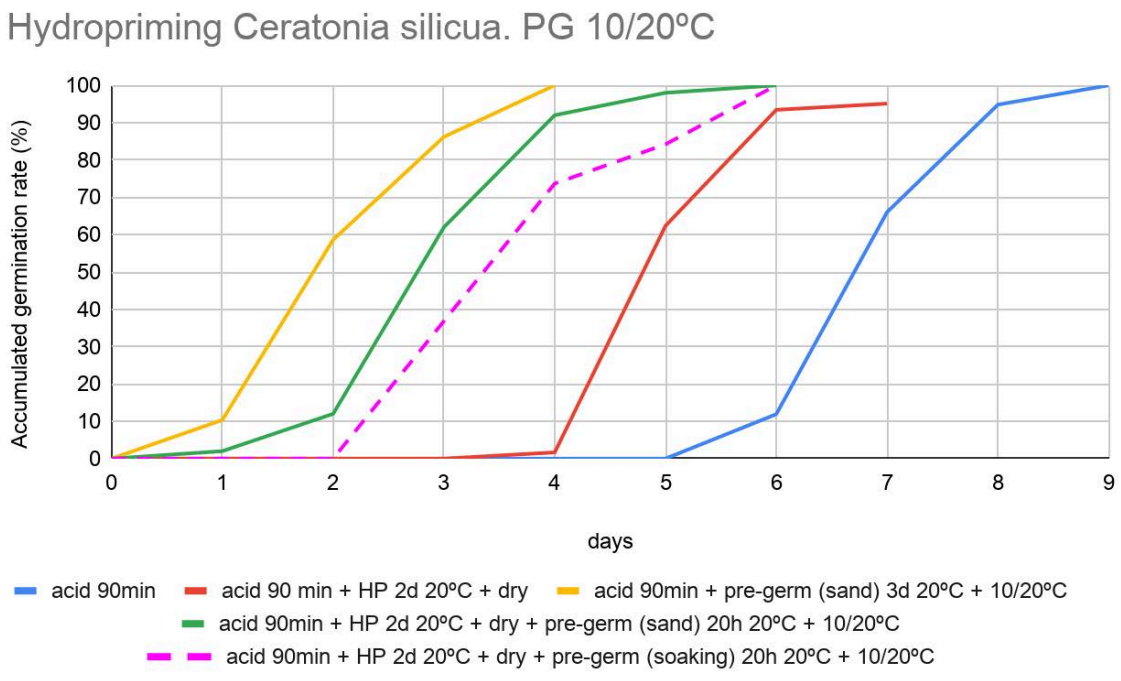

Figure 19: Germination curves for Ceratonia siliqua

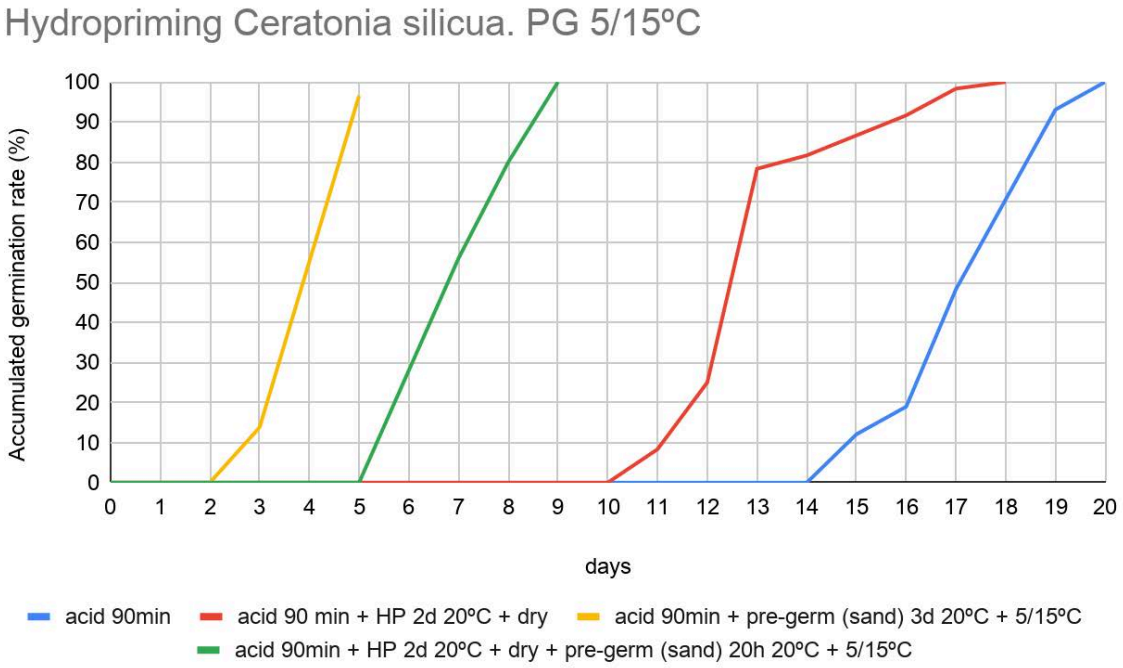

Figure 20: Germination curves for Ceratonia siliqua

TETRACLINIS ARTICULATA

BATCH

Different seed batches were used in the study. For HP tests, a batch from Semillas Cantueso—originating from Sierra Morena cultivation and harvested in 2024—was used, with tests conducted within the first year of storage. Viability tests were also performed on the following batches:

- A batch from Cartagena, stored for 3 years.
- A batch from Semillas Cantueso (Sierra Morena, harvested in 2023), stored for 1.3 years by the company.
- A batch from Semillas Cantueso (Sierra Morena, harvested in 2024), stored for 1 year by the company, and the same batch stored for 1 year by Semillistas.
- A batch from Elx, stored for 2 months.

Prior to storage at Semillistas at 4°C, the %mcfw of each batch was reduced by exposing the seeds to air in the shade for 8 hours on a warm day (approximately 25°C and 30% RH). The storage %mcfw was not measured. The storage method used by Semillas Cantueso is unknown. Given the critical impact of storage conditions on viability, this aspect will be further investigated in 2026.

BATCH IMPROVEMENT

We used a batch from Semillas Cantueso, originating from cultivation in the Sierra Morena, harvested in 2024 and stored for 3 months.

The improvement applied to this batch consisted of soaking the seeds at 20°C for 24 hours and then separating sunk seeds from floating ones. During this stage it is necessary to agitate the soaking solution to remove any micro air bubbles that are attached to the seeds so that they sink.

	Sunk at 24 hours	Floating at 24h
Number of seeds	55%	45%
Germination	92%	17%
Discarded viable seeds	8%	--

This batch improvement is applied directly within the HP process, as shown later. Therefore, it was not necessary to dry the seeds again after separating the sunk and floating fractions, allowing the original (mother) batch to be kept intact until priming treatment.

CLASSIC GERMINATION (Figure 21)

	Optimal Tª (ºC)	96% acid time (min)	Batch improvement	Bleach disinfection	T0 (days)	T90 (days)	%maxG
T. articulata (Cartagena 3 years of storage)	20ºC	--	--	10% 10'	8	10	22%
T. articulata (Harvest 2024, 3 months of storage)	20ºC	--	sunk 24h	10% 10'	3	10	92%
T. articulata (Harvest 2024, 3 months of storage)	20ºC	--	floating 24h	10% 10'	5	6	17%
T. articulata (Harvest 2024, 1 year of storage in Semillistas)	20ºC	--	sunk 24h	10% 10'	4	11	90%
T. articulata (Harvest 2024, 1 year of storage in Cantueso)	20ºC	--	--	10% 10'	7	13	43%
T. articulata (Elche, 2 months of storage)	20ºC	--	sunk 24h	10% 10'	3	17	62%

Data for different batches and storage durations are presented because this species rapidly loses viability (Figure 21).

The Cartagena batch, stored for 3 years, showed almost no germination. The batch stored for 1 year by Semillas Cantueso also exhibited very low germination. However, the same batch (harvested in 2024) was acquired by Semillistas at the end of 2024 and stored at low %mcfw. Storage at low %mcfw likely maintained its viability, as its germination curve after 1 year at Semillistas matched its post-harvest germination.

The Elx batch, despite being recently harvested, showed lower viability than other batches, with a maximum germination of 60% compared to 90% for the 2024 Semillas Cantueso batch. This may be due to differences between batches of different origins.

All germination tests for this species used 1 or 2 replicates of 25 to 50 seeds.

20ºC HYDRATION CURVE (Figure 22)

	FI-FII hours	%mcfw FII
T. articulata HP 2d	20-25	50%

We conducted the test under soaking conditions and in moist sand at 11.5% mcdw to ensure that there were no differences in hydration dynamics.

PRIMING TREATMENTS (Figure 23)

T. articulata (sunk at 24 hours)	priming Tª (ºC)	priming duration (days)	%mcfw FII	%mcfw SMP (no germinations)	%mcfw final SMP	GT Tª (ºC)	GT T0 (days)	GT T90 (days)	%maxG. GT
control	--	--	--	--	--	20ºC	3	10	92%
optimal HP	20ºC	3	--	--	--	20ºC	2	4	83%

The Semillas Cantueso batch—originating from Sierra Morena cultivation, harvested in 2024, and stored for 3 months—was used for this study.

Since classical germination requires approximately 10 days to complete, HP was selected as the priming treatment. Given that classical germination begins (entry into FIII) in about 3 days, HP in AS was performed for 3 days at 20°C. The drying step in the HP process occurred just before entry into FIII, which had minor consequences, discussed later.

The HP treatment began in AS at 20°C. After 24 hours, seeds were removed from AS and separated into sunken and floating fractions. Floating seeds were discarded, while sunken seeds remained in AS for 2 additional days before drying.

The 3-day HP treatment (Figure 23) increased the germination rate but reduced the maximum germination percentage. This reduction likely resulted from drying too close to the onset of FIII, as seeds at 20°C germinated at a rate of ~6% in 3 days (2 mm germination), corresponding to the time spent in RA. This caused the death of all seeds that entered FIII during RA.

For this batch, HP should have been applied for 2.5 days in AS at 20°C to achieve a similar germination rate without reducing the maximum germination percentage.

GERMINATION TESTS AT SUBOPTIMAL TEMPERATURES (Figures 24 & 25)

C.siliqua (acid. 90')	GT T^a (ºC)	T0 (days)	T90 (days)	%maxG.
Control	10/20ºC	12	26	72%
HP 2d	10/20ºC	6	13	92%
Control	5/15ºC	26	37	77%
HP 2d	5/15ºC	14	16	90%

The Semillas Cantueso batch—originating from Sierra Morena cultivation, harvested in 2024, and stored for 1 year—was used. With this storage duration, germination begins in 4 days, slightly slower than the same batch stored for 3 months, which began in 3 days.

In this case, performing HP for 3 days does not reduce the maximum germination percentage because the seeds had not yet entered FIII when dried.

The proposed priming treatment significantly increases the germination rate at suboptimal temperatures while maintaining the maximum germination percentage at the same level as germination at 20°C.

PRE-GERMINATION TREATMENTS (Figures 24 & 25)

Pre-germination treatments were conducted at 20°C on both control and HP seeds for a specified duration, after which seeds were transferred to 10/20°C or 5/15°C.

For control seeds, which germinate at 20°C in 3 days, pre-germination lasted 2 days. For HP seeds, which germinate at 20°C in 2 days, pre-germination lasted 1 day.

Control seeds underwent 1 day of soaking to remove floating seeds, after which sunken seeds were placed in Petri dishes. For non-pre-germinated controls, soaking occurred at 10/20°C or 5/15°C, with time 0 in the figure marking the start of soaking. For pre-germinated controls, seeds were soaked for 1 day at 20°C, followed by 1 day in sand, before transfer to 10/20°C or 5/15°C. The transfer to suboptimal temperatures is considered time 0 in the figure, simulating field sowing.

At 10/20°C (Figure 24):

- Pre-germination of HP seeds (green line) did not improve germination speed compared to HP seeds without pre-germination (red line) and reduced the maximum germination percentage from 90% to 80%.
- Pre-germination of control seeds (yellow line) slightly increased germination speed compared to non-pre-germinated controls (blue line), from 8–13 days to 5–13 days, with a marginally higher germination percentage.

At 5/15°C (Figure 25):

- Pre-germination of HP seeds (green line) advanced the germination curve compared to HP seeds without pre-germination (red line), from 7–16 days to 2–14 days, while maintaining the same maximum germination percentage.

- Pre-germination of control seeds (yellow line) slightly advanced germination compared to non-pre-germinated controls (blue line), from 16–38 days to 12–35 days.

Pre-germination at 20°C does not improve results when Petri dishes are transferred to 10/20°C and offers only minor improvements at 5/15°C. Exploring other pre-germination temperatures may be necessary to optimise the process.

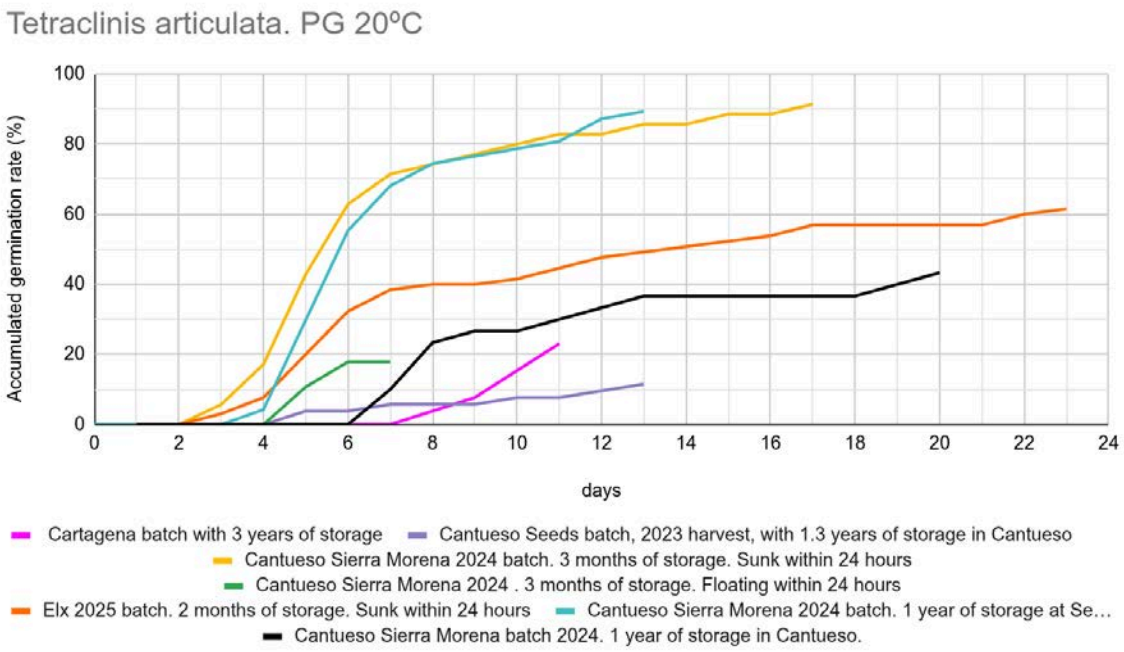

Figure 21: Germination curves for Tetraclinis articulata

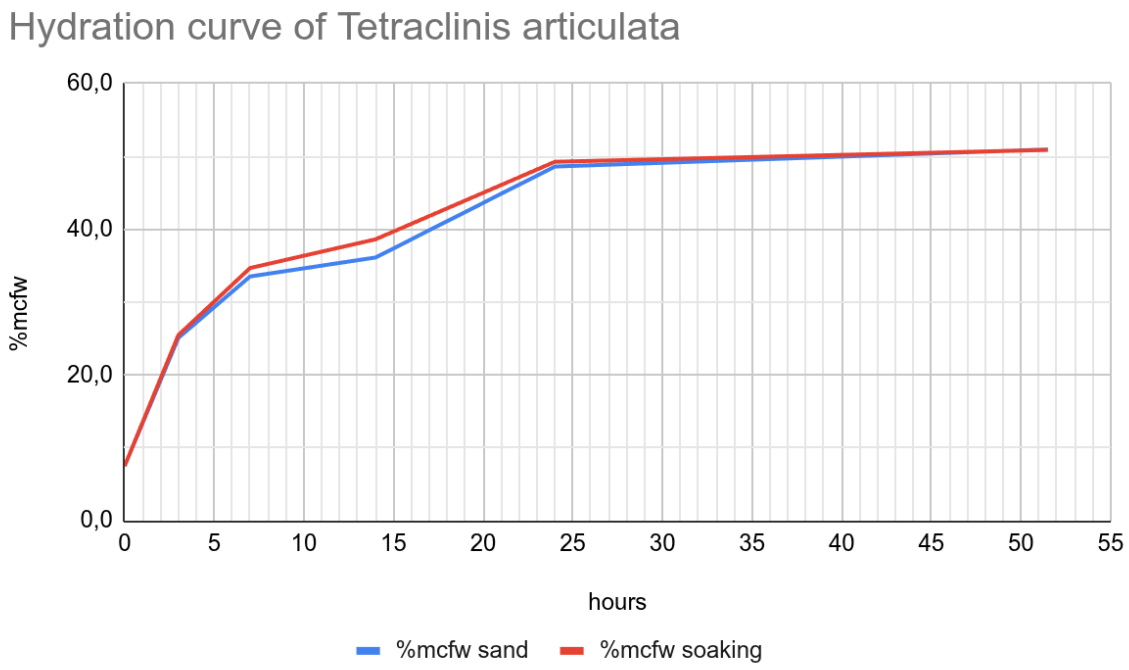

Figure 22: Hydration curve for Tetraclinis articulata

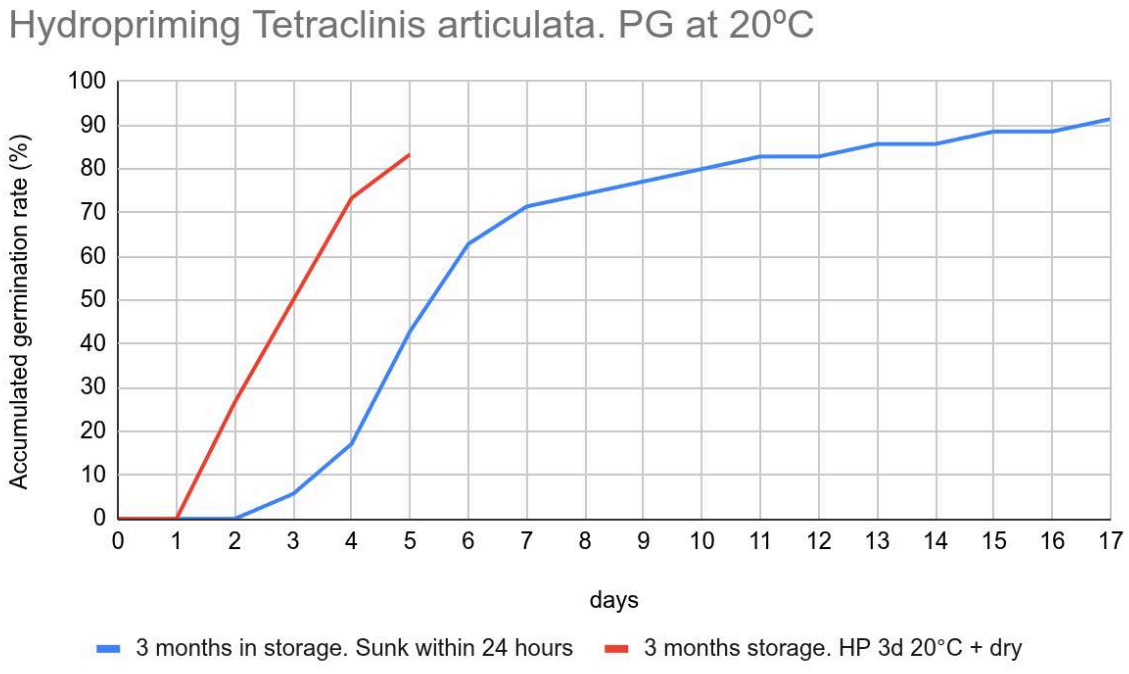

Figure 23: Germination curves for Tetraclinis articulata

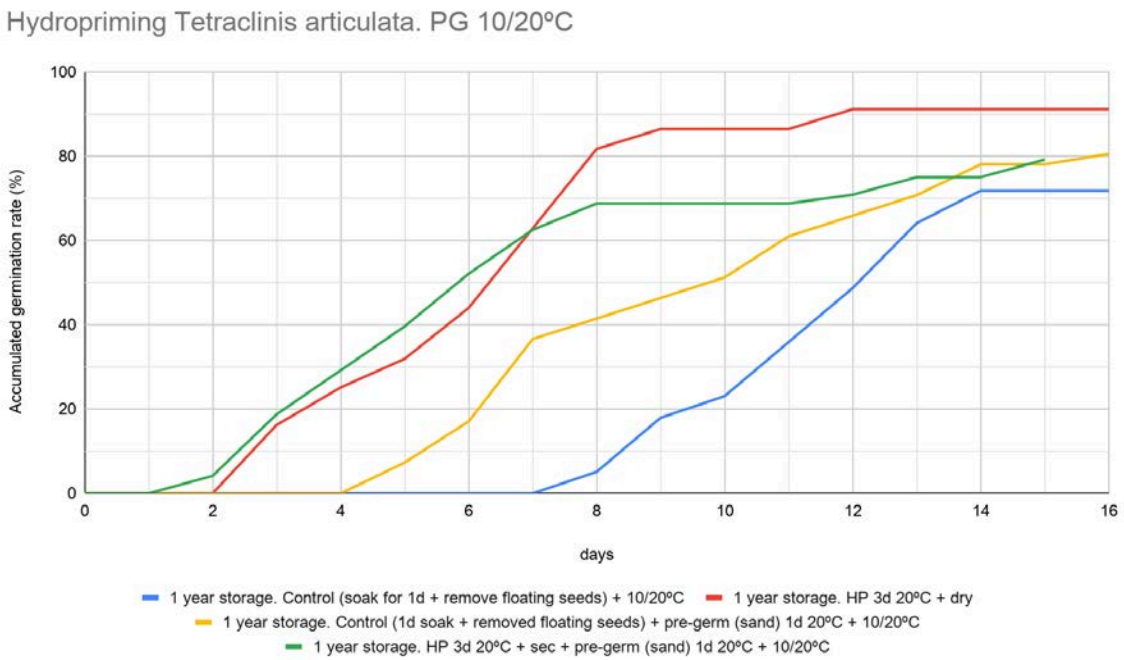

Figure 24: Germination curves for Tetraclinis articulata

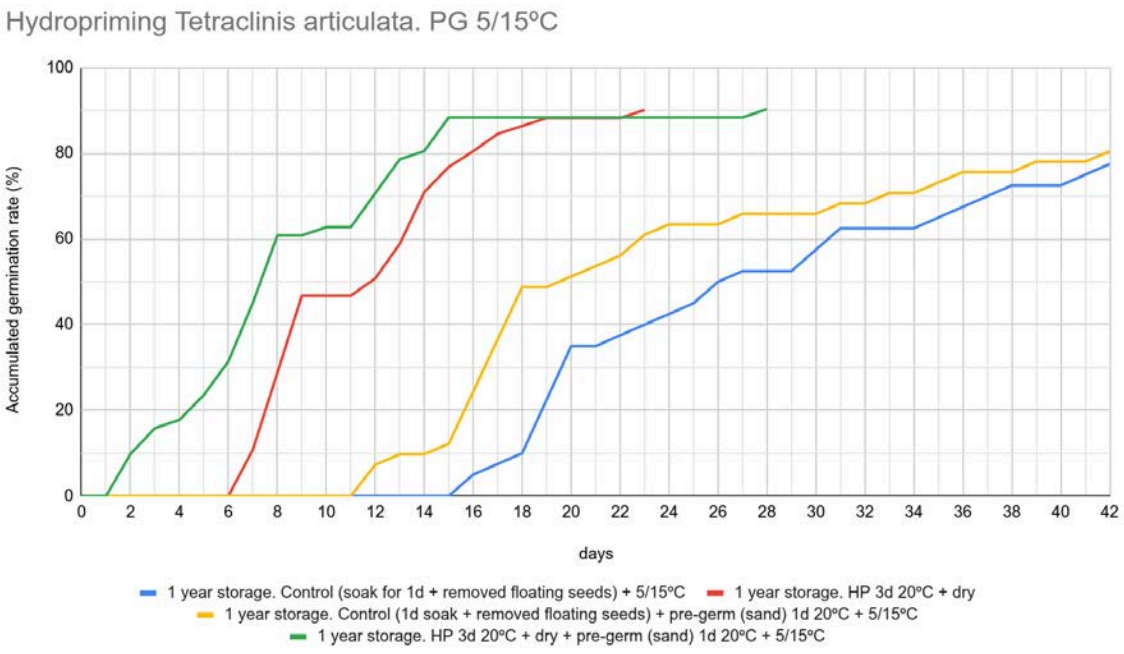

Figure 25: Germination curves for Tetraclinis articulata

RHAMNUS ALATERNUS

BATCH

The batch used was harvested in 2023 from the Alpujarra region (between Órgiva and Pitres) from approximately 30 plants. During the study, seeds were stored in airtight containers at 4°C, with a moisture content of 4.9% mcfw in the mother batch.

For batch improvement tests, a batch from the same origin but harvested in 2022 was used.

Additionally, the optimal priming treatment was studied on a batch from Sierra Mijas, harvested in 2025 by colleagues from WWF Málaga, to whom training was provided.

BATCH IMPROVEMENT

These tests were carried out using a batch harvested in the Alpujarra in 2022.

The technique of discarding floating seeds after a set period of soaking at 20°C was used.

	All seeds	Sunk at 11 hours	Sunk between 11h and 20h	Floating at 20h
Number of seeds	100%	53,6%	21.8%	24.6%
Germination	42%	72%	19%	0%
Discarded viable seeds	--	4%	0%	--

The batch improvement applied to the 2023 batch consisted of selecting the sunk seeds after 10 hours of soaking at 20°C.The resulting batch achieved up to 92% germination. After soaking the seeds were dried again to a low %mcfw (5%) for storage.

CLASSIC GERMINATION (Figure 27)

	Optimal T^{a} (ºC)	96% acid time (min)	Batch improvement	Bleach disinfection	T0 (days)	T90 (days)	%maxG
Rhamnus alaternus	20ºC	--	Soaked for 10h	60% 15'	9	23	92%

The disinfection intensity was insufficient to prevent fungal contamination during germination tests, with 10–15% of seeds in each Petri dish developing fungi. These seeds were likely those with little or no viability, as they mobilise endogenous microorganisms that the seed's defenses cannot control. Increasing disinfection intensity was avoided as it would complicate the removal of bleach residues from the seed surface, potentially harming germination.

All germination tests for this species used 1 or 2 replicates of 45–55 seeds.

20ºC HYDRATION CURVE (Figure 26)

	FI-FII hours	%mcfw FII
Rhamnus alaternus	3-4	32%

PRIMING TREATMENTS (figures 27 y 28)

Rhamnus alaternus	Priming Tª (ºC)	Priming duration (days)	%mcfw FII	%mcfw SMP (no germinations)	%mcfw final SMP	GT Tª (ºC)	GT T0 (days)	GT T90 (days)	%maxG. GT
Control- Alpujarra	--	--	--	--	--	20ºC	9	23	92%
Optimal SMP - Alp.	20ºC	36	32%	27%	26%	20ºC	4	15	85%
Control- Mijas	--	--	--	--	--	20ºC	9	41	70%
Optimal SMP - Mij.	20ºC	36	?	?	26%	20ºC	5	25	78%

SMP treatments were proposed instead of HP because classical germination requires 23 days to complete.

The SMP process was conducted at 20°C. The %mcfw during FII was 32%. Germination began with an SMP treatment at 20°C for 20 days with seeds at 28% mcfw, but did not occur at values below 27% mcfw.

Figure 27 shows the germination curves for the different treatments. All germination tests were conducted at a stable 20°C. All SMP treatments improved germination characteristics, though some were more effective than others. The most optimal treatment (black line) germinated at 20°C between 4 and 15 days, compared to control seeds, which germinated between 9 and 23 days. The optimal SMP treatment was therefore defined as TMC = 26% mcfw and Duration = 36 days. The treatment with 26% mcfw and a duration of 29 days achieved nearly the same improvement. All other SMP treatments showed germination speeds between the optimal treatment and the control, as seen in the pink, red, yellow, and dark green lines, with %mcfw around 24–25% regardless of duration.

This species is highly prone to fungal development during laboratory germination tests. For this reason, disinfection with a high bleach concentration is necessary. The variation in maximum germination percentage among the tests in Figure 27 is due to differing levels of fungal contamination in each Petri dish.

Comparison of Priming Treatments in Two Batches of Different Origins

The 2023 Alpujarra batch was compared with the 2025 Sierra Mijas batch (Figure 28). The Mijas batch underwent the same batch improvement as the Alpujarra batch (removal of floating seeds after 10 hours and drying).

Standard germination of the Mijas batch was much slower than that of the Alpujarra seeds (Mijas: 9–41 days; Alpujarra: 9–23 days), with a lower maximum germination percentage (70% vs. 92%).

For the SMP treatment of the Mijas batch, similar parameters were applied: 24% and 26% mcfw, with durations of 36 and 43 days. The three treatments produced similar improvements in seed performance, although, as with the Alpujarra batch, the treatment with 26% mcfw was the most effective.

GERMINATION TESTS AT SUBOPTIMAL TEMPERATURES (Figures 29 & 30)

	GT T^a (ºC)	T0 (days)	T90 (days)	%maxG.
Control	10/20ºC	16	30	92%
SMP	10/20ºC	7	24	86%
Control	5/15ºC	38	61	73%
SMP	5/15ºC	14	36	83%

Seeds subjected to SMP treatments show a considerable increase in germination speed compared to the control at suboptimal temperatures.

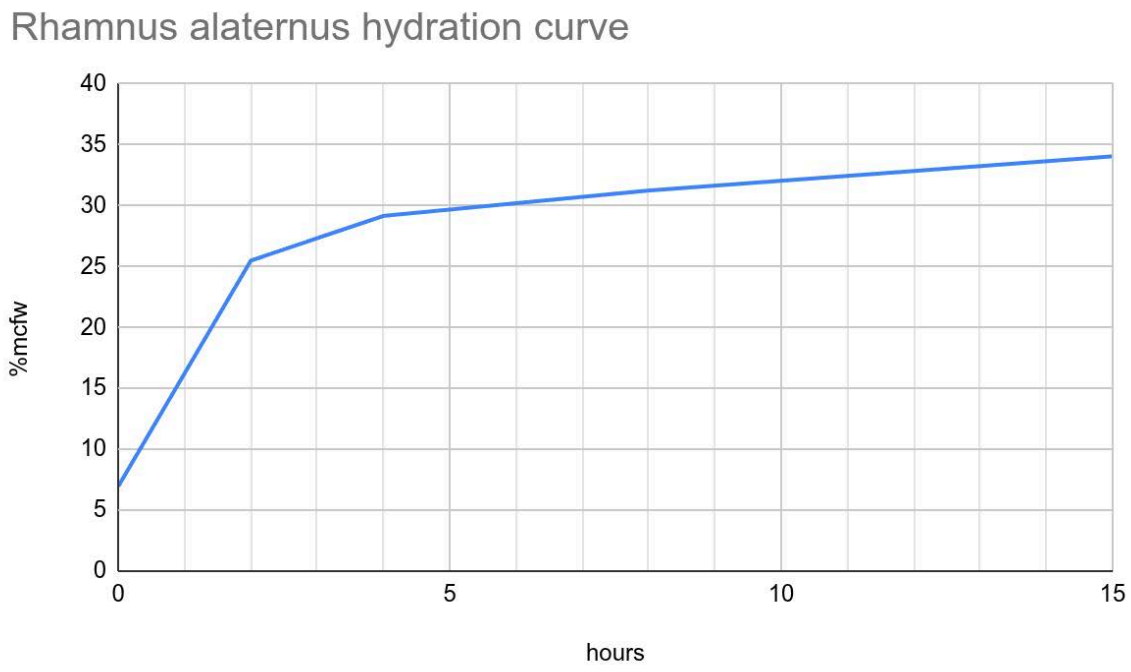

Figure 26: Hydration curve for Rhamnus alaternus

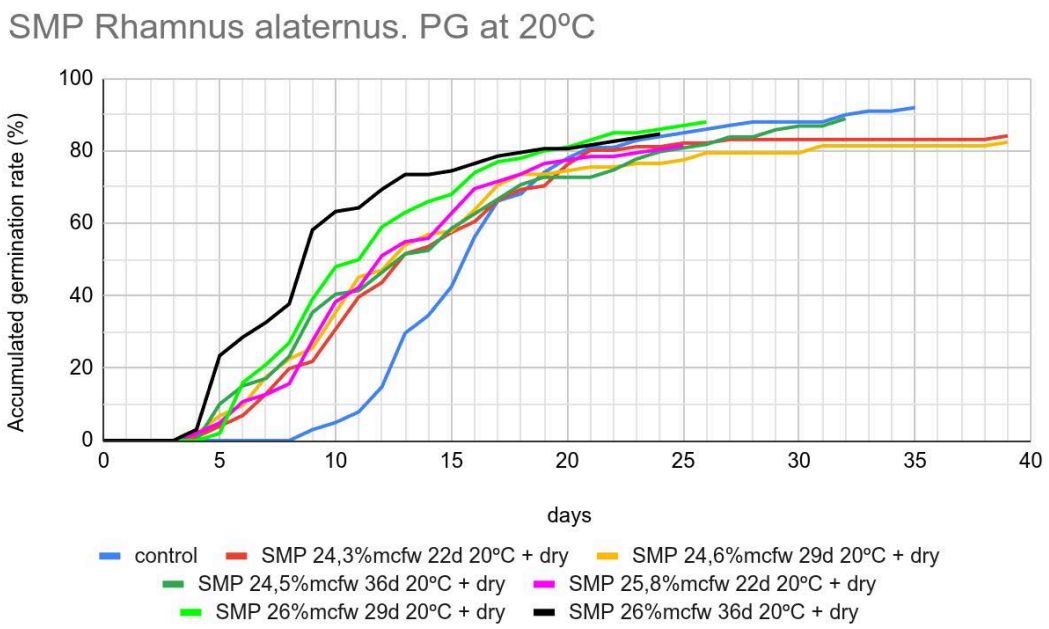

Figure 27: Germination curves for Rhamnus alaternus

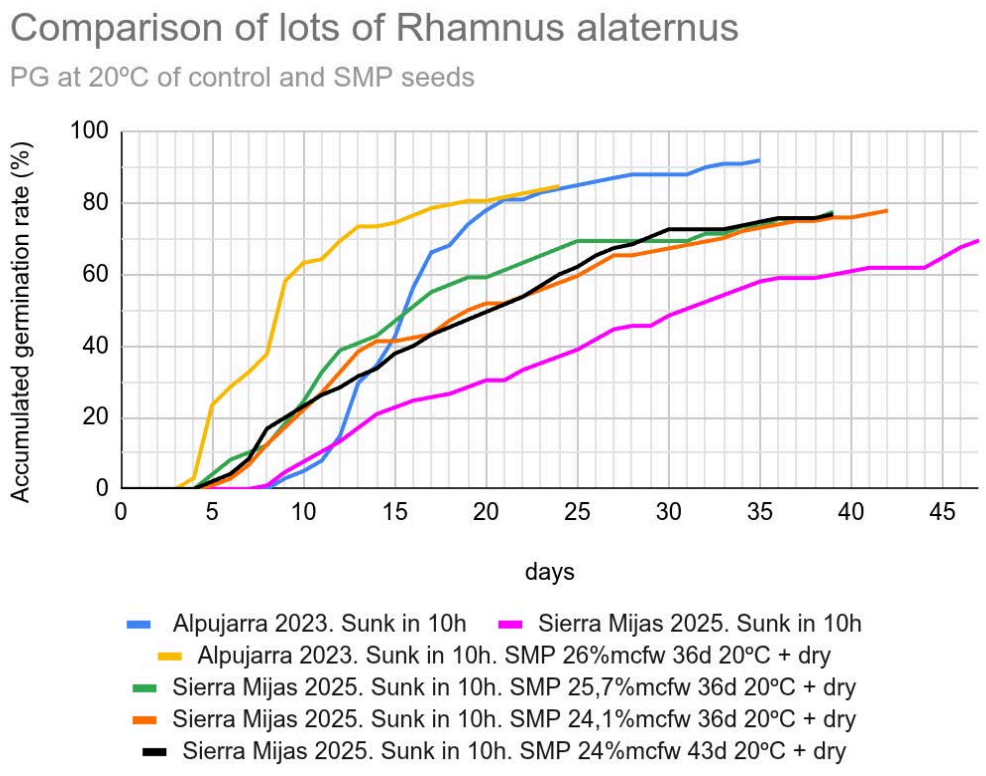

Figure 28: Germination curves for Rhamnus alaternus

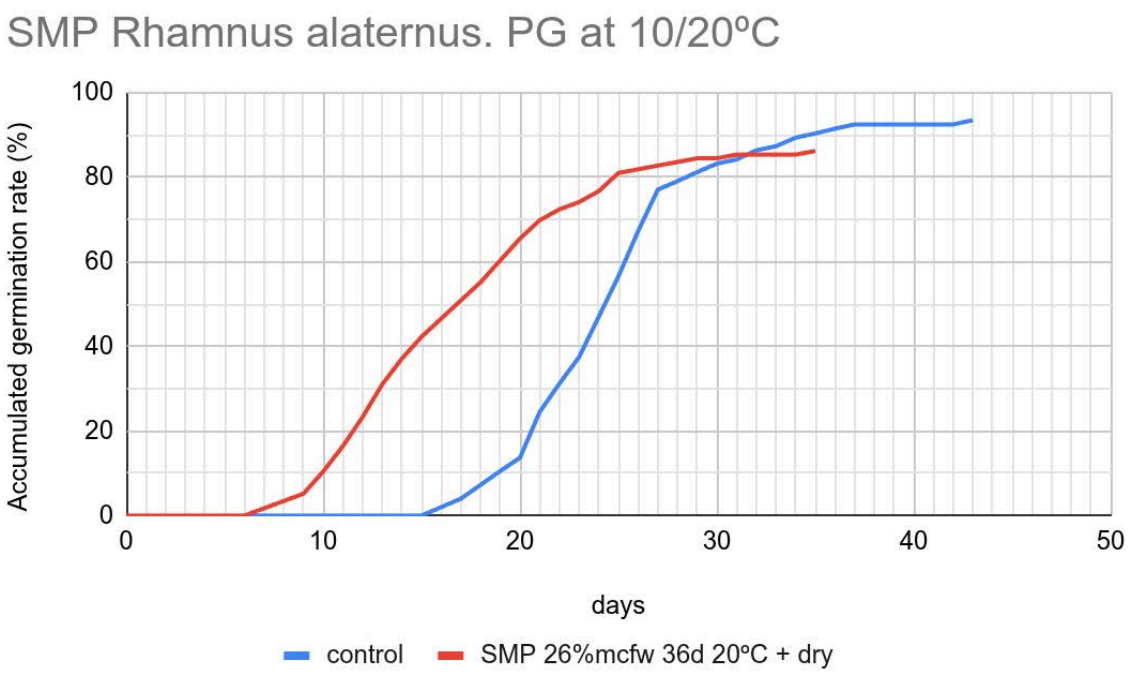

Figure 29: Germination curves for Rhamnus alaternus

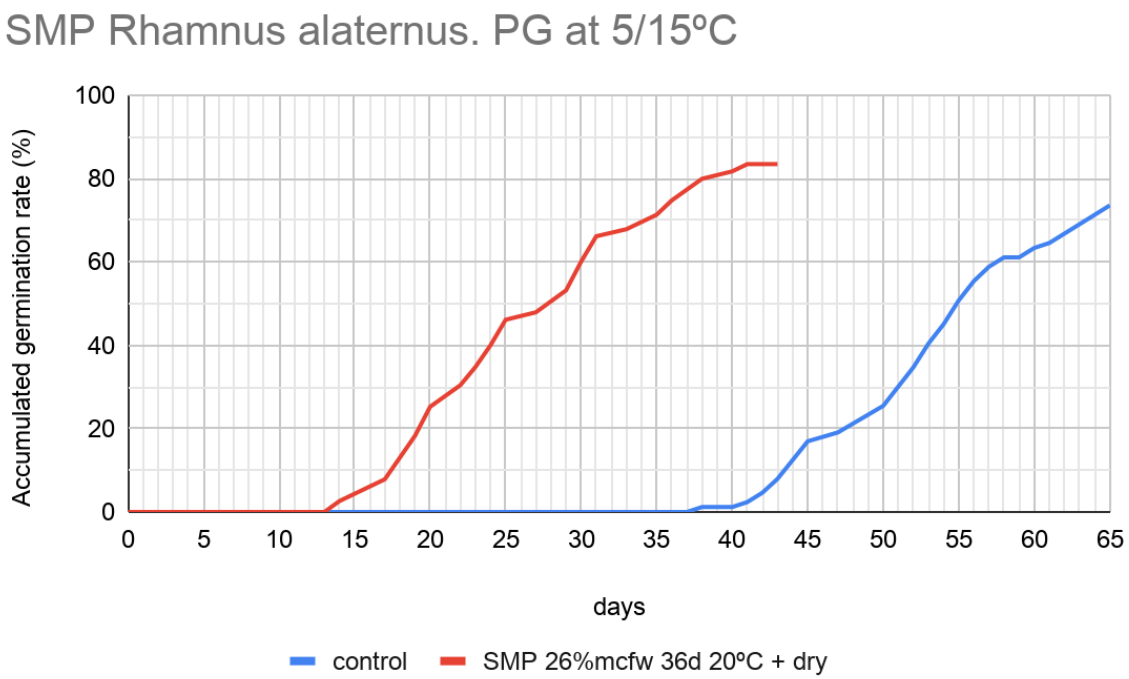

Figure 30: Germination curves for Rhamnus alaternus

RHAMNUS LYCIOIDES

BATCH

The batch used was obtained from Semillas Cantueso in 2023, with seeds originating from the Sierra de Baza. During the study, seeds were stored in airtight containers at 4°C.

In 2024, batch improvement was performed (selection of sunk seeds after 10 hours), after which the seeds were dried and stored at 6% mcfw until the studies were conducted in 2025.

BATCH IMPROVEMENT

The technique of discarding floating seeds after a set period of soaking at 20°C was used.

	sunk at 10 hours	sunk between 10h and 20h	floating at 20h
Germination	78%	25%	20%

Due to a procedural error, the weight of each treatment was not recorded, and data on the percentage of seeds in each treatment are unavailable.

Batch improvement involved selecting sunken seeds after 10 hours of soaking at 20°C, achieving up to 78% germination. After soaking, the seeds were dried to 6% mcfw for storage. The improved batch retained its viability characteristics for at least 1.5 years after the batch improvement treatment.

CLASSIC GERMINATION (Figure 32)

	Optimal T[a] (ºC)	96% acid time (min)	Batch improve ment	Bleach disinfection	T0 (days)	T90 (days)	%maxG
Rhamnus lycioides	20ºC	--	sunk at 10 hours	40% 15'	4	19	78%

Approximately 10–15% of seeds developed fungi during germination tests. After SMP treatments, some seeds darkened and later developed fungi, likely due to low or no viability that could not be eliminated during batch improvement.

All germination tests for this species used 1 or 2 replicates of 40–50 seeds.

20ºC HYDRATION CURVE (Figure 31)

	FI-FII hours	%mcfw FII
Rhamnus lycioides	3-5	35%

PRIMING TREATMENTS (Figure 32)

Rhamnus lycioides	Priming T[a] (ºC)	Priming duration (days)	%mcfw FII	%mcfw SMP (no germinations)	%mcfw final SMP	GT T[a] (ºC)	GT T0 (days)	GT T90 (days)	%maxG. GT
Control	--	--	--	--	--	20ºC	4	19	78%
Optimal SMP	20ºC	14	35%	29.5%	28.5%	20ºC	2	6	85%

SMP treatments were proposed instead of HP because classical germination requires 19 days to complete.

The SMP process was conducted at 20°C. The %mcfw during FII was 36%. Germination began with an SMP treatment at 20°C for 21 days with seeds at 29.5% mcfw, but did not occur at values below 29% mcfw.

Figure 32 shows the germination curves for the different treatments. All germination tests were conducted at a stable 20°C. All SMP treatments improved germination characteristics, although some were more effective than others.

The most optimal treatment (black line) germinated at 20°C between 2 and 6 days, compared to control seeds, which germinated between 4 and 19 days. The optimal SMP treatment was therefore defined as TMC = 28.5% mcfw and Duration = 14 days.

The treatment with TMC = 29.3% mcfw and Duration = 17 days showed slightly faster germination than the optimal treatment, but the maximum germination percentage was lower. This may be because it was too close to the upper limit of %mcfw at which seeds can germinate (29.5% mcfw and 21 days), causing some seeds to die during drying after SMP as they had already entered FIII.

All other SMP treatments had lower germination speeds than the optimal treatment, although they still improved upon the control. This occurred when using a TMC lower than 28.5% and/or a longer treatment duration.

GERMINATION TESTS AT SUBOPTIMAL TEMPERATURES (Figures 33 & 34)

	GT T^a (ºC)	T0 (days)	T90 (days)	%maxG.
Control	10/20ºC	9	25	78%
SMP	10/20ºC	5	12	73%
Control	5/15ºC	20	49	74%
SMP	5/15ºC	9	21	80%

The SMP treatment used for these tests was not optimal. A treatment with TMC = 27.8% mcfw and Duration = 21 days was applied. As shown in Figure 32, this treatment did not achieve the improvements of the optimal treatment at 20°C, though it still improved germination compared to the control seeds. The reason for using this non-optimal treatment was the limited seed availability: sufficient seeds from this treatment were available to conduct germination tests at suboptimal temperatures and for pre-germination.

Seeds subjected to SMP treatments showed a considerable increase in germination speed compared to the control at suboptimal temperatures. Notably, these improvements occurred even when the SMP treatment was not optimal, providing a substantial margin for error when applying SMP treatments for field sowing.

PRE-GERMINATION TREATMENTS (Figures 33 & 34)

Pre-germination treatments were conducted at 20°C on both control and SMP seeds for a specified duration, after which seeds were transferred to 10/20°C or 5/15°C.

For these tests, the pre-germination time was adjusted as closely as possible to T0 at 20°C (when the first seeds reached 2 mm). Control seeds, which germinate at 20°C in 4 days, underwent pre-germination for 4 days. SMP seeds, which germinate at 20°C in 2–3 days, underwent pre-germination for 2.5 days.

At 10/20°C (Figure 33):

- Pre-germination of SMP seeds (green line) advanced the germination curve by just over 1 day, achieving similar germination percentages to SMP seeds without pre-germination (red line).
- Pre-germination of control seeds (yellow line) began germinating earlier than non-pre-germinated controls (blue line) but germinated slowly, with a reduced maximum germination percentage of 53%.

At 5/15°C (Figure 34):

- Pre-germination of SMP seeds (green line) had no effect compared to SMP seeds without pre-germination (red line).
- Pre-germination of control seeds (yellow line) increased germination speed by 4–5 days compared to non-pre-germinated controls (blue line), but reached only 57% germination.

Pre-germination at 20°C, followed by transfer to suboptimal temperatures, does not benefit *Rhamnus lycioides*. Alternative pre-germination strategies will need to be studied for this species.

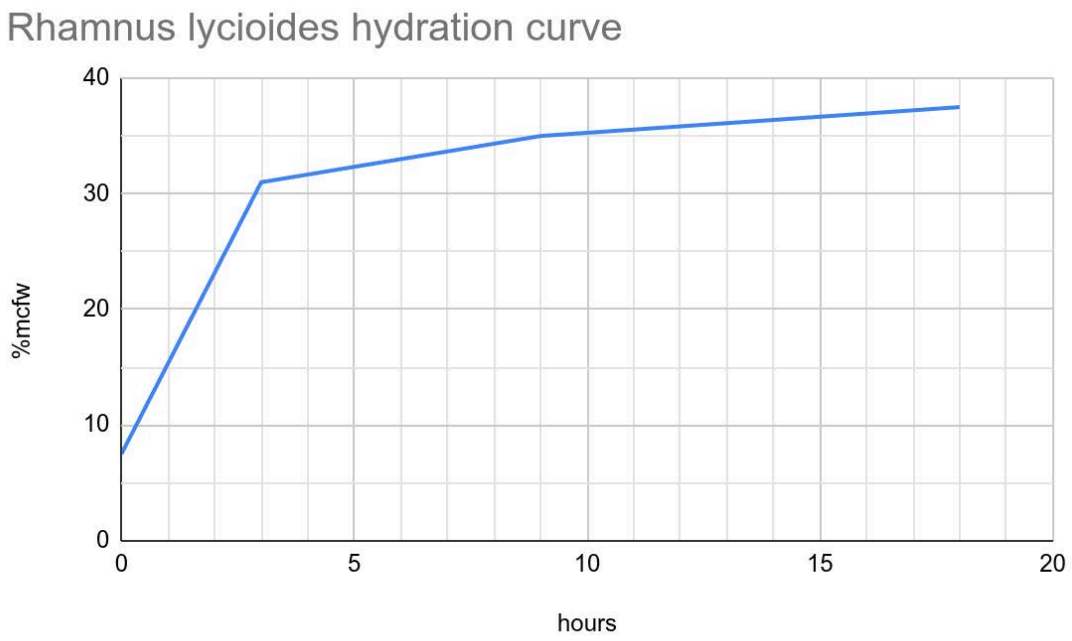

Figure 31: Hydration curve for Rhamnus lycioides

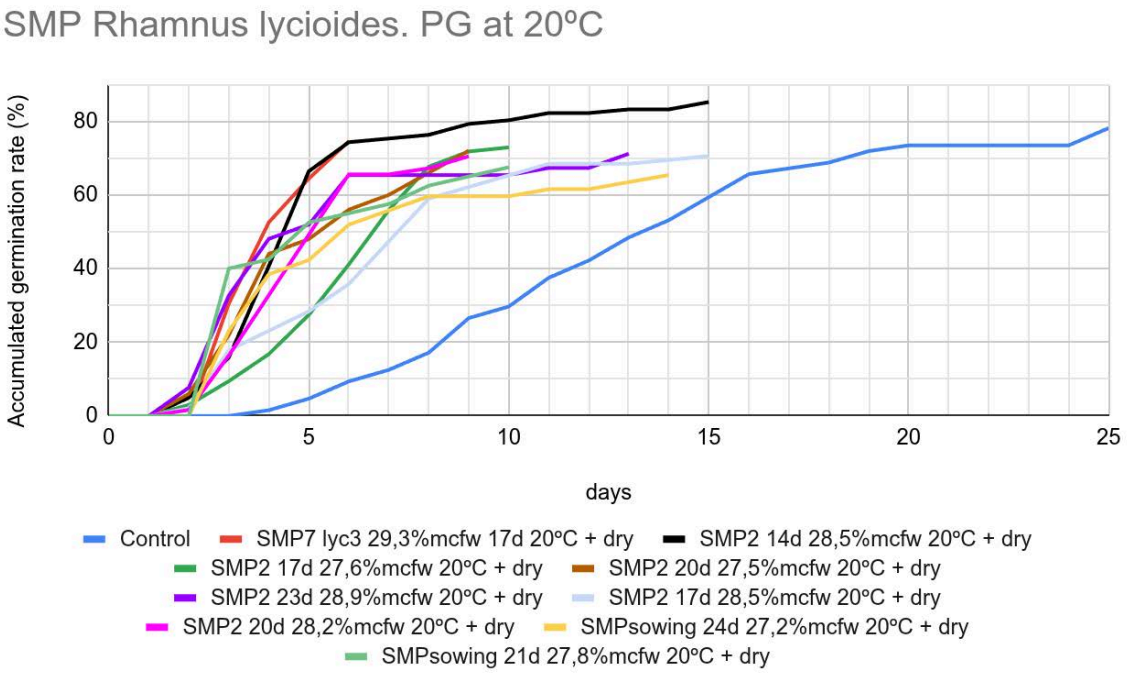

Figure 32: Germination curves for Rhamnus lycioides

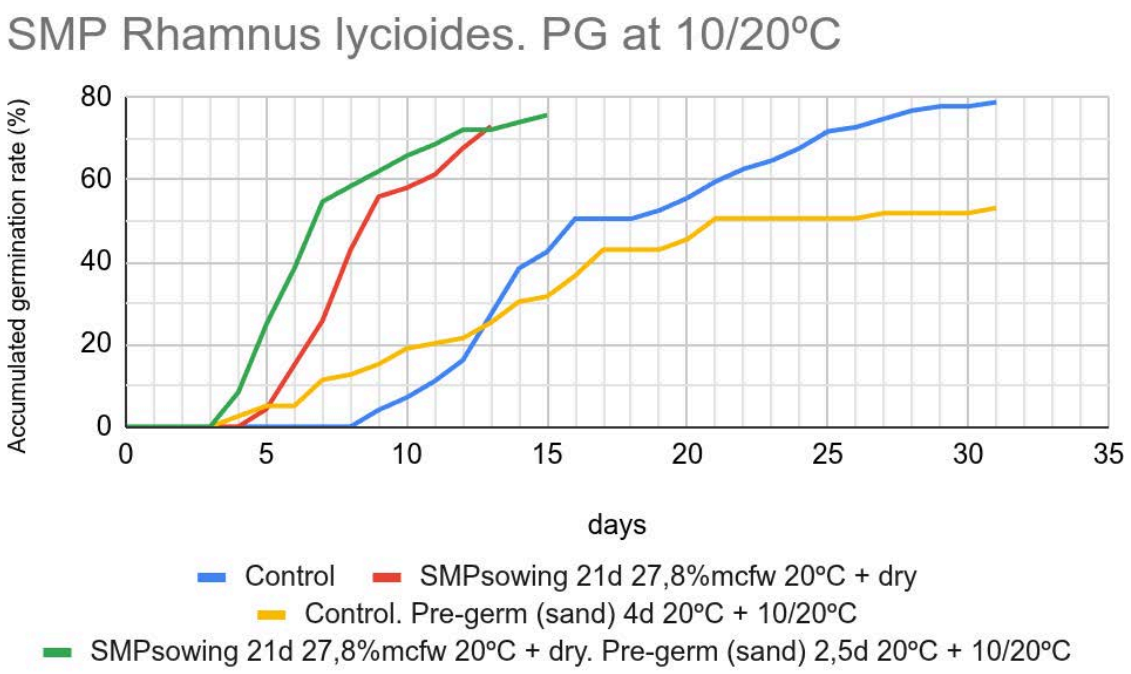

Figure 33: Germination curves for Rhamnus lycioides

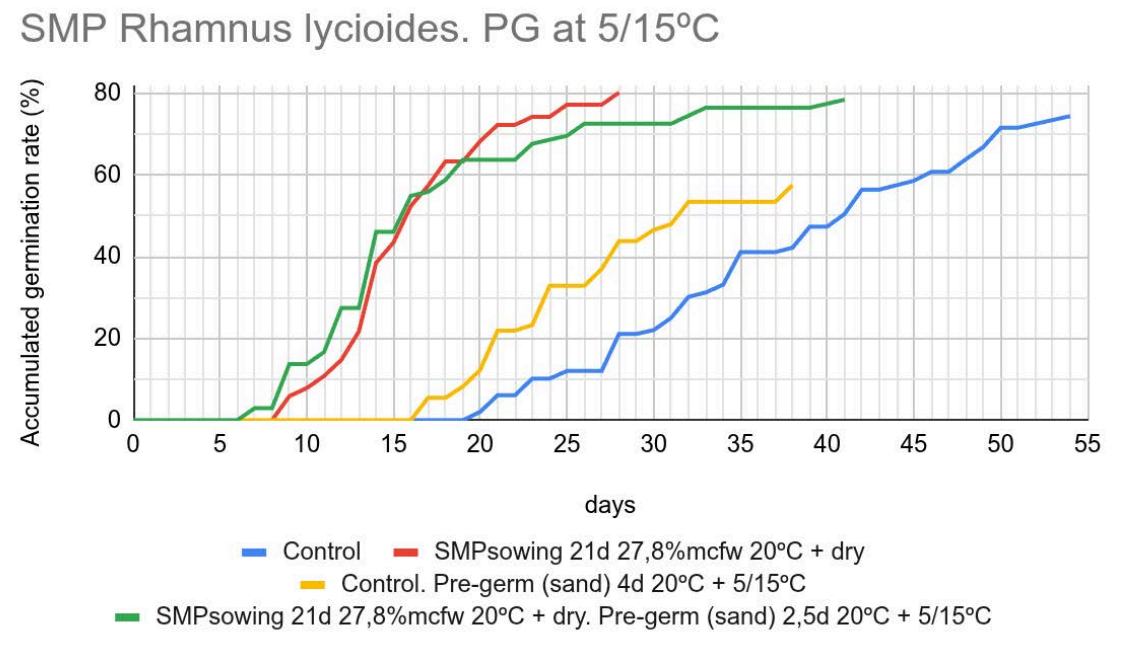

Figure 34: Germination curves for Rhamnus lycioides

PISTACIA LENTISCUS

BATCH

The batch used was harvested in Sierra Lújar in 2023.

A batch from the same harvest location but from 2021 was used for the hydration curve..

During the study, seeds were initially stored in airtight containers at 4°C. Before conducting the study in 2025, batch improvement was performed (selection of sunk seeds at 0 hours), after which the seeds were dried and stored at approximately 7% mcfw.

During extraction after harvest, floating fruits (red in colour) were removed, as they mostly contained empty seeds.

BATCH IMPROVEMENT

The technique of discarding floating seeds after a set period of soaking at 20°C was used.

	Control	Sunk at 0h	Sunk between 0h and 2.5h	Floating at 2.5h
Number of seeds	100%	34%	40%	26%
Germination	72%	94%	87%	22%
Discarded viable seeds	--	35%	6%	-

For logistical reasons the priming study commenced with seeds that sank at 0 hours. Storage of these seeds (7% mcfw) leads to lost viability after 1 year, so the mother batch (harvest) must be stored and batch improvements performed gradually.

CLASSIC GERMINATION (Figure 36)

	Optimal T^{a} (ºC)	96% acid time (min)	Batch improvement	Bleach disinfection	T0 (days)	T90 (days)	%maxG
Pistacia lentiscus	20ºC	--	Sunk at 0h	30% 15’	5	14	94%

1 or 2 replicates of 50–60 seeds were used for all germination tests with this species.

20ºC HYDRATION CURVE (Figure 35)

	FI-FII hours	%mcfw FII
Pistacia lentiscus	15-20	32%

PRIMING TREATMENTS (Figure 36)

Pistacia lentiscus	Priming T^{a} (ºC)	Priming duration (days)	%mcfw FII	%mcfw SMP (no germinations)	%mcfw final SMP	GT T^{a} (ºC)	GT T0 (days)	GT T90 (days)	%maxG. GT
Control	--	--	--	--	--	20ºC	5	14	94%
Optimal SMP	20ºC	19	32%	30,1%	29%	20ºC	2	8	96%
HP	20ºC	4	--	--	--	20ºC	4	11	90%

SMP treatments were proposed for this species, as classical germination requires 14 days to complete, although some HP treatments were also conducted for comparison.

The SMP process was conducted at 20°C. The %mcfw during FII was 32%. Germination began with an SMP treatment at 20°C for 21 days with seeds at 30.1% mcfw, but did not occur at values below 30% mcfw.

At the most optimal treatment (red line) seeds germinated at 20°C between 2 and 8 days, reaching 96%, compared to control seeds, which germinated between 5 and 14 days, reaching 92%. The optimal SMP treatment was therefore defined as TMC = 29% mcfw and Duration = 19 days.

The treatment with 31.4% mcfw and 16 days, although exceeding the maximum %mcfw for germination during the treatment, had not germinated at 16 days. This treatment and the treatment with 29.2% mcfw and 16 days performed similarly to the optimal treatment.

All other SMP treatments, with durations longer than 19 days, provided little benefit compared to the control. HP treatments slightly improved germination characteristics compared to the control.

The margin for SMP parameters to improve mastic tree seeds is narrower than in other species, likely requiring parameter adjustments for each batch.

GERMINATION TESTS AT SUBOPTIMAL TEMPERATURES (Figures 37 & 38)

	GT T^a (ºC)	T0 (days)	T90 (days)	%maxG.
Control	10/20ºC	7	23	90%
SMP	10/20ºC	6	12	80%
Control	5/15ºC	14	43	90%
SMP	5/15ºC	9	22	83%

The differences in maximum germination percentages are due to fungal proliferation during the germination tests, so they are not an effect of priming.

Although seeds treated with SMP show improved germination speed at suboptimal temperatures, this improvement is not as pronounced as in other species.

PRE-GERMINATION TREATMENTS (Figures 37 & 38)

Pre-germination treatments were conducted at 20°C on both control and SMP seeds for a specified duration, after which seeds were transferred to 10/20°C or 5/15°C.

For control seeds, which germinate at 20°C in 5 days, pre-germination lasted 3 days. For SMP seeds, which germinate at 20°C in 2 days, pre-germination lasted 1.5 days.

At 10/20°C (Figure 37), pre-germination of both SMP seeds (green line) and control seeds (yellow line) reduced germination characteristics compared to non-pre-germinated seeds.

At 5/15°C (Figure 38), the same occurred for pre-germinated SMP seeds (green line). In contrast, pre-germination of control seeds (yellow line) did not reduce germination but provided no significant benefit.

Pre-germination at 20°C for a set period, followed by transfer to suboptimal temperatures, does not benefit *Pistacia lentiscus*. Alternative pre-germination strategies will need to be studied for this species.

SEED MOISTURE CONTENT VARIABILITY AT THE END OF SMP TREATMENT (Figure 39)

To verify the reproducibility of the SMP method, multiple treatments with identical characteristics were conducted to observe the %mcfw reached by the seeds at the end of the treatment. Controlling the final %mcfw is critical for ensuring seed quality, as it also allows assessment of the range of %mcfw values; i.e., the variability among seeds after SMP.

For the 2023 Sierra Lújar batch (sunk at 0 hours), eight SMP treatments were performed using sand with varying moisture contents (1.4%, 1.25%, and 1.10% mcdw) and different durations (21, 28, and 35 days). For each treatment, moisture content was measured in six replicates of approximately 0.8 g of seeds. The data for these treatments are shown in the following table and in Figure 39.

	Average initial %mcfw (seeds)	Initial %mcdw (sand)	Average final %mcfw (seeds)	Std. deviation final %mcfw (seeds)
Pistacia lentiscus	7.6%	1.4%	30.7%	0.5%
Pistacia lentiscus	7.6%	1.25%	29%	0.6%
Pistacia lentiscus	7.6%	1.1%	27.4%	0.3%

With an initial sand moisture content of 1.4% mcdw, seeds germinated during the treatment as they reached %mcfw values above 30%. The %mcfw measured at the end of this treatment was calculated only for non-germinated seeds.

These data demonstrate the level of control achievable with SMP treatments using a sand medium. With the employed methodology, seeds can be brought to the desired moisture content with an error of ±1% mcfw. This precision ensures that all seeds in a treatment receive consistent benefits and guarantees that repeating the treatment with a new batch will yield the expected results.

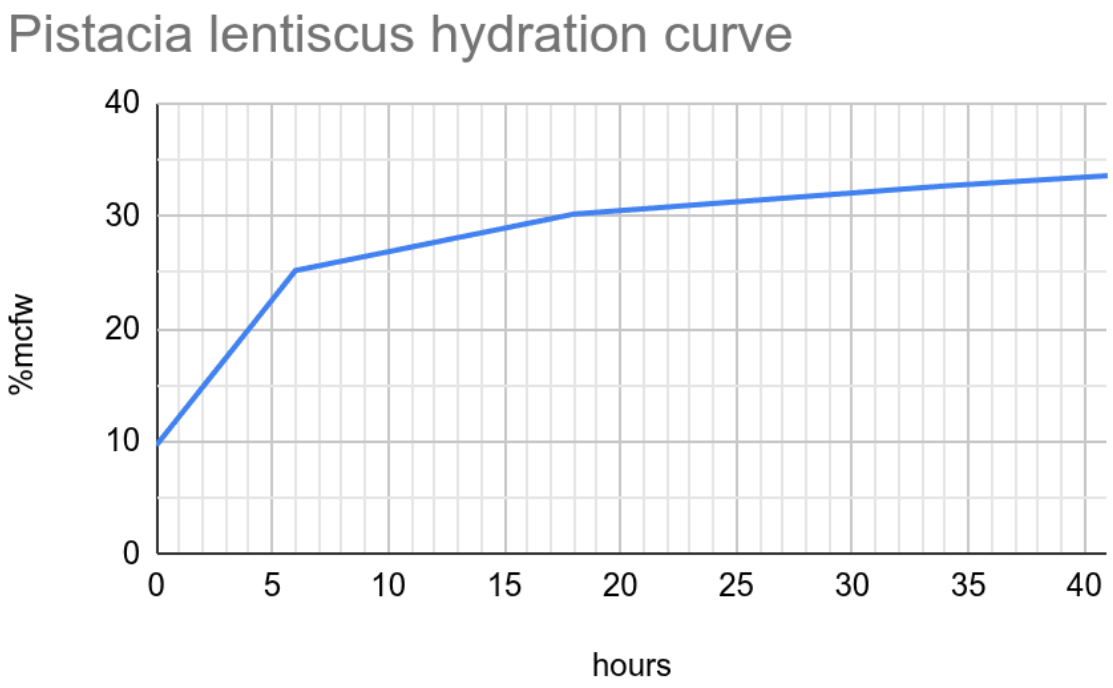

Figure 35: Hydration curve for Pistacia lentiscus

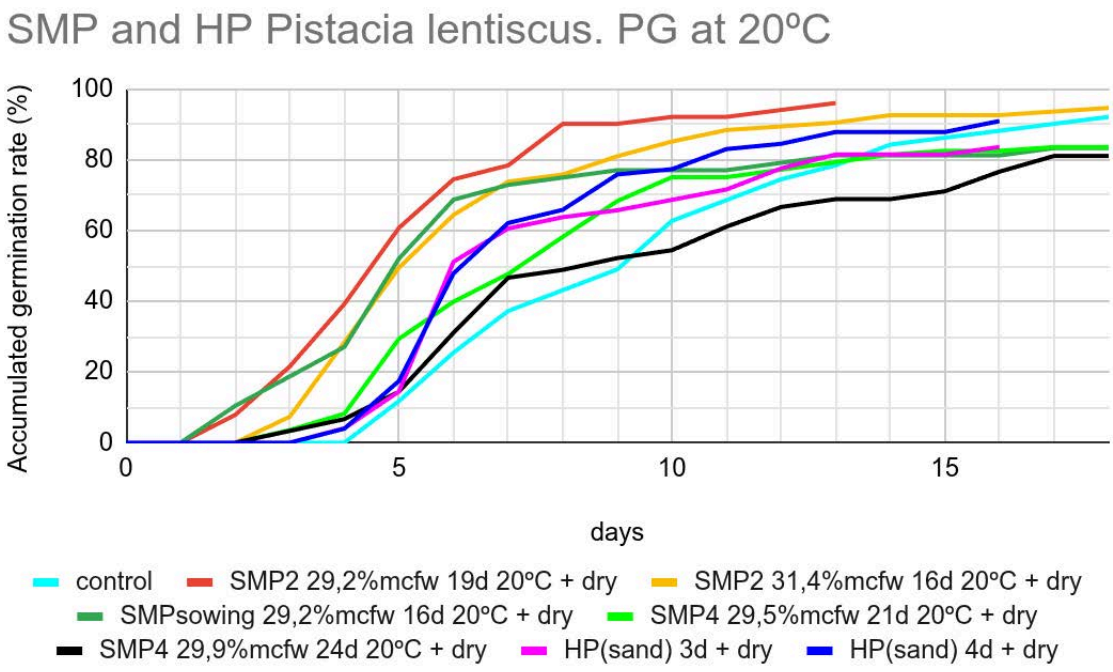

Figure 36: Germination curves for Pistacia lentiscus

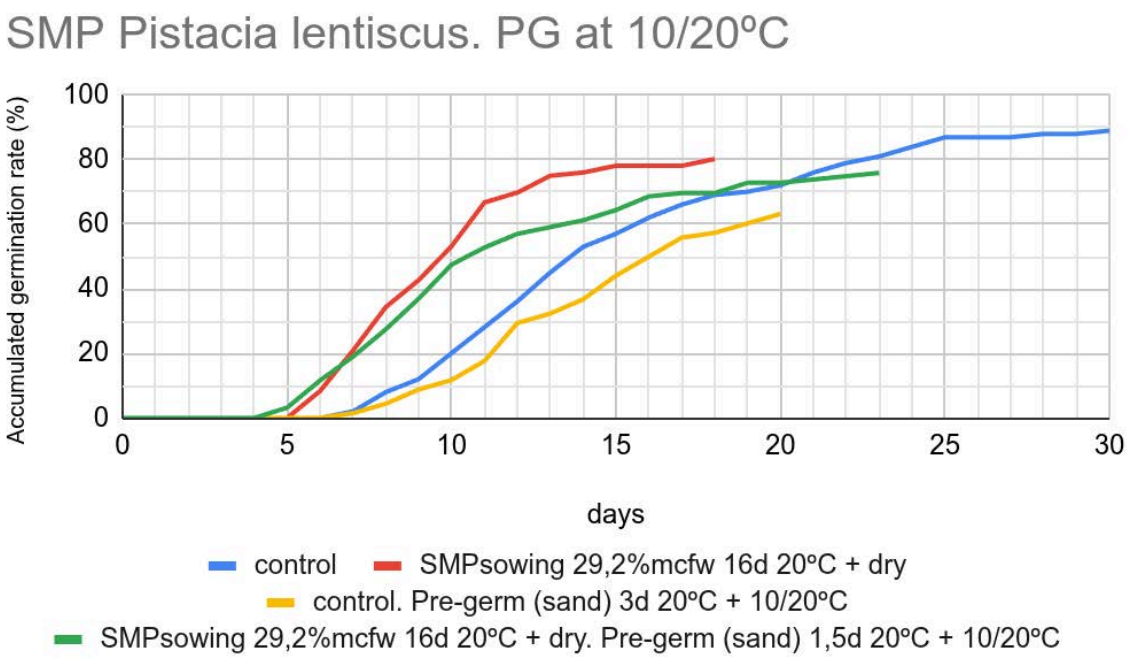

Figure 37: Germination curves for Pistacia lentiscus

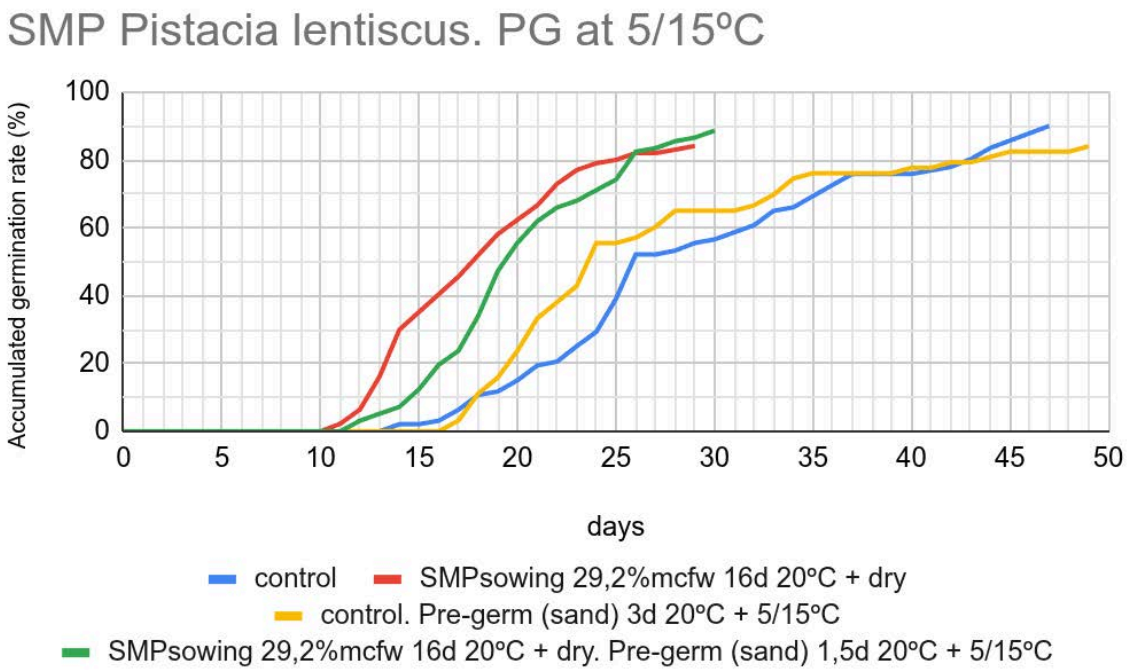

Figure 38: Germination curves for Pistacia lentiscus

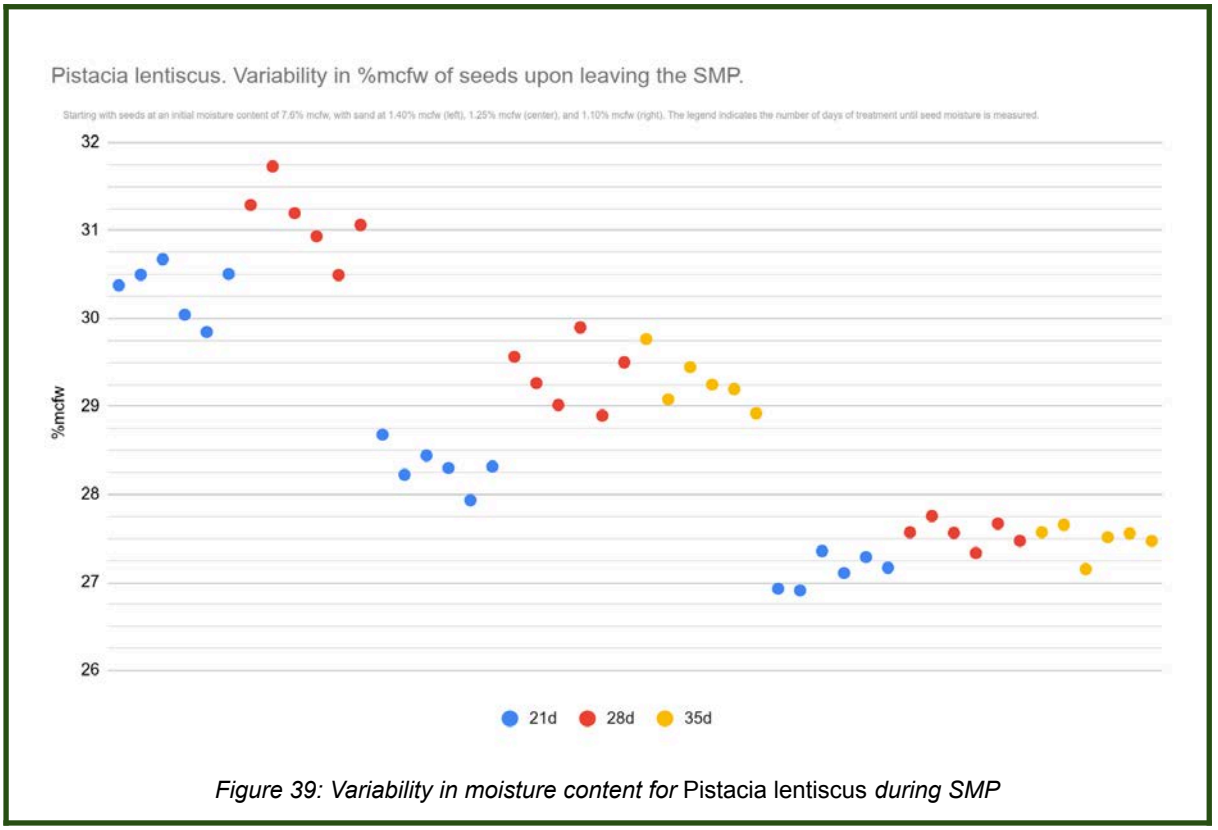

Figure 39: Variability in moisture content for Pistacia lentiscus *during SMP*

RETAMA SPHAEROCARPA

BATCH

The batch used was harvested in the Alpujarra (Pitres) in 2023.

The seeds were stored in paper bags in a warehouse after harvest. Seed extraction from the dry pods was carried out in 2025. The seeds were then dried on a warm day and stored in airtight containers at 4°C. Measuring the seed moisture content was not necessary for this study,

BATCH IMPROVEMENT

Retama seeds, once scarified and soaked, can swell within the first 24 hours, 2 days, or longer. Swelling indicates entry into Phase II (FII), while non-swelling seeds remain in Phase I (FI). Separating seeds based on swelling time ensures grouping by hydration profile, forming the basis for batch improvement in this species. The specific procedure will be detailed in the priming section.

All germination tests of retama seeds with HP implicitly use seeds that swell in less than 24 hours. For comparison with control seeds (without HP), germination tests without HP use seeds that swell after 24 hours of hydration.

CLASSIC GERMINATION (Figure 40)

	Optimal Tª (ºC)	96% acid time (min)	Batch improvement	Bleach disinfection	T0 (days)	T90 (days)	%maxG
Retama sphaerocarpa	20ºC	90	Swollen in 24h	30% 15'	5	29	67%
Retama sphaerocarpa	20ºC	135	Swollen in 24h	30% 15'	5	17	79%
Retama sphaerocarpa	20ºC	150	Swollen in 24h	30% 15'	4	16	65%
Retama sphaerocarpa	20ºC	190	Swollen in 24h	30% 15'	5	12	38%

This species is challenging to manage in terms of fungal control under controlled conditions (without fungicides). Using standard laboratory techniques, 10–20% of seeds in germination tests develop fungi.

This species exhibits physical dormancy, preventing water penetration into the seed. Scarification is required to promote germination. Dormancy was broken using 96% sulphuric acid. After immersion for varying durations (with agitation every 10 minutes), seeds were drained, thoroughly washed with water, dried, and stored under cold conditions. Germination tests, HP treatments, and hydration curves were then conducted.

Four acid immersion times were tested: 95, 135, 150, and 190 minutes. The germination curves are shown in Figure 40, representing seeds that swelled after 24 hours of soaking. Seeds treated for 95 minutes germinated more slowly. Exposure beyond 150 minutes reduced the maximum germination percentage achieved at 135 minutes, as the inner embryo is damaged by acid exposure longer than this. A 135-minute acid treatment was selected for all subsequent tests.

All germination tests for this species used 1 or 2 replicates of approximately 40 seeds.

20ºC HYDRATION CURVE (Figure 41)

	FI-FII hours	%mcfw FII
Retama sphaerocarpa	15	64%

The hydration curve was developed using seeds treated with 135 minutes of acid followed by a 2-day HP treatment.

While the hydration curve for Retama seeds treated with acid but without HP has not been calculated, it is expected that hydration would occur more slowly. In HP-treated seeds, the swelling and drying process weakens the testa, facilitating faster subsequent hydration.

PRIMING TREATMENTS (Figure 42)

Retama sphaerocarpa	Priming Tª (ºC)	Priming duration (days)	%mcfw FII	%mcfw SMP (no germinations)	%mcfw final SMP	GT Tª (ºC)	GT T0 (days)	GT T90 (days)	%maxG. GT
Control	--	--	--	--	--	20ºC	5	17	79%
Optimal HP	20ºC	2	--	--	--	20ºC	1	8	85%

Although classical germination requires 16 days to complete, HP was selected as the priming treatment due to positive results from previous experiments.

Since seeds treated with 135 minutes of acid begin germinating between 4 and 5 days (entry into FIII), HP with AS was performed for 2 and 3 days at 20°C.

During the HP treatment, the batch improvement process was carried out to ensure seeds were at the same germination stage (beginning of FII). Combined with HP, this process homogenises the resulting batch. The procedure is as follows:

1. Start with dry seeds treated with 135 minutes of acid.
2. Place the seeds in AS to initiate HP.
3. After 24 hours in AS, remove the seeds and separate swollen from non-swollen seeds using a sieve.
4. Swollen seeds continue HP in AS for 1 more day, completing 2 full days in AS, after which they are dried.
5. Non-swollen seeds remain in AS in another container.
6. After 24 hours in the new AS, remove these seeds and again separate swollen from non-swollen seeds.
7. Newly swollen seeds require 1 more day to complete 2 full days in AS, after which they are dried.
8. The process can be repeated for seeds that have still not swollen.

This ensures all seeds completing HP start at the same point (the initiation of hydration) within the last 24 hours. For Retama seeds, the duration under water does not matter until swelling begins, as the germination process does not start until then.

Both the 2-day and 3-day HP treatments produced similar results. The 2-day HP treatment germinated at 20°C between 1 and 8 days, reaching 85% (Figure 42), significantly faster than seeds without HP, which germinated between 5 and 17 days.

GERMINATION TESTS AT SUBOPTIMAL TEMPERATURES (Figures 43 & 44)

	GT T^a (ºC)	T0 (days)	T90 (days)	%maxG.
Control	10/20ºC	6	22	85%
HP	10/20ºC	3	7	85%
Control	5/15ºC	13	28	79%
HP	5/15ºC	4	12	82%

Seeds treated with SMP show a considerable increase in germination speed compared to the control at suboptimal temperatures.

PRE-GERMINATION TREATMENTS (Figures 43 & 44)

Pre-germinative treatments were conducted at 20°C on control and HP seeds for a specified duration, after which seeds were transferred to 10/20°C or 5/15°C.

Control seeds, which germinate at 20°C in 4–5 days, underwent pre-germination for 3.6 days. HP seeds, which germinate at 20°C in 1 day, underwent pre-germination for 24 hours.

At 10/20°C (Figure 43):

- Pre-germination of HP seeds (green line) slightly improved germination, from 3–7 days to 1–5 days, compared to HP seeds without pre-germination (red line).
- Pre-germination of control seeds (yellow line) considerably increased germination speed, advancing from 6–22 days to 3–14 days, compared to control seeds without pre-germination (blue line).

At 5/15°C (Figure 44):

- Pre-germination of HP seeds (green line) shifted the germination curve forward, from 4–12 days to 3–7 days, compared to HP seeds without pre-germination (red line).
- Pre-germination of control seeds (yellow line) substantially advanced germination, from 13–28 days to 3–20 days, compared to control seeds without pre-germination (blue line).

Pre-germination provides only a slight improvement for HP seeds compared to HP seeds without pre-germination, but the improvement is much more pronounced in seeds without HP. Although further optimisation of the pre-germination process for HP seeds may be possible, germination is already very fast (3–7 days), which meets current field requirements (soil drying time vs. germination time at low temperatures).

Additionally, pre-germination via soaking was compared to placing seeds in moist sand. Although the germination curves were very similar, soaking delayed the onset of germination by more than 1 day compared to using sand. It is hypothesised that in any species, when soaking time approaches or exceeds the duration of germination FI, germination performance decreases in either percentage or speed. This is likely due to a higher oxygen requirement during FII that is not met when seeds are submerged in water.

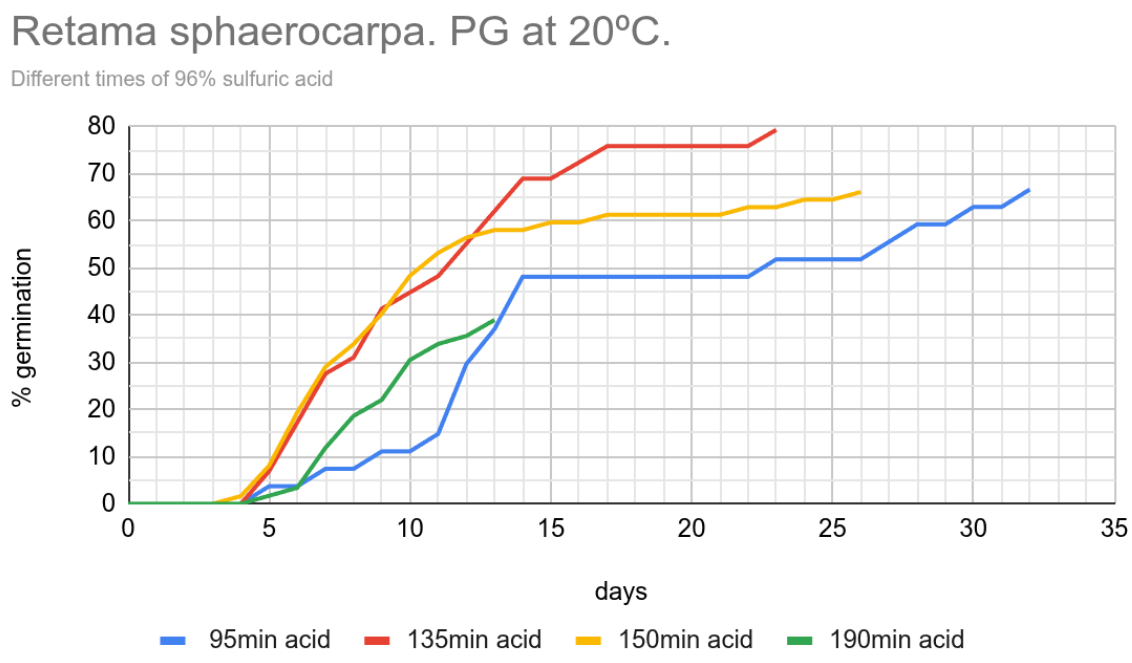

Figure 40: Germination curves for Retama sphaerocarpa

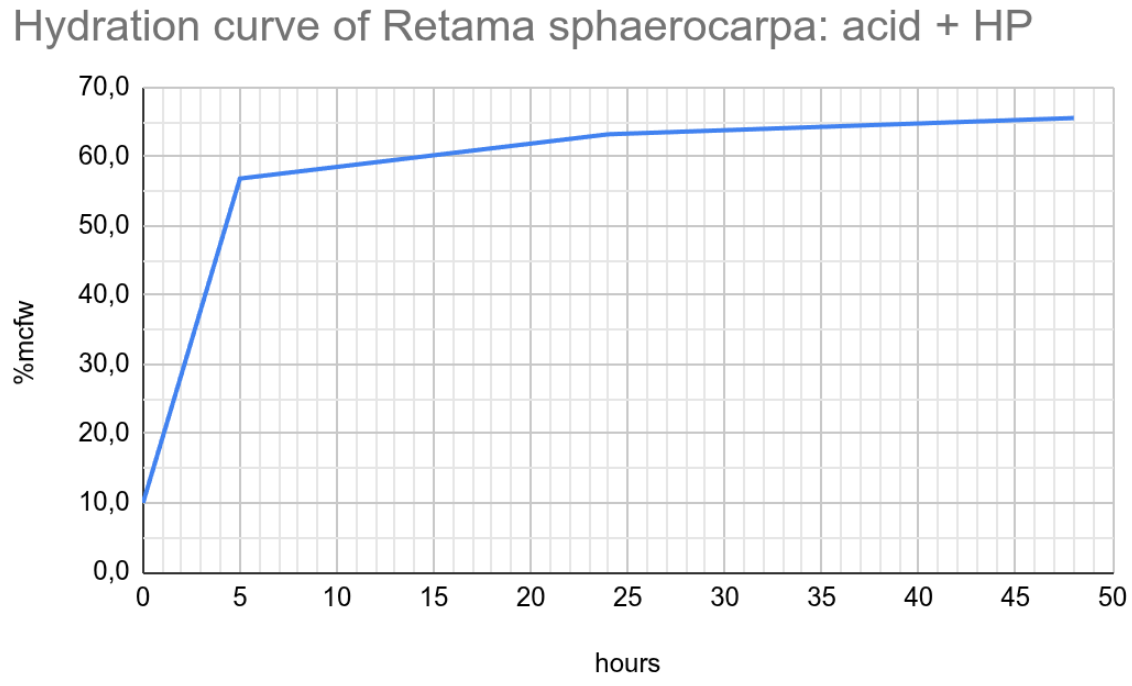

Figure 41*: Hydration curve for* Retama sphaerocarpa

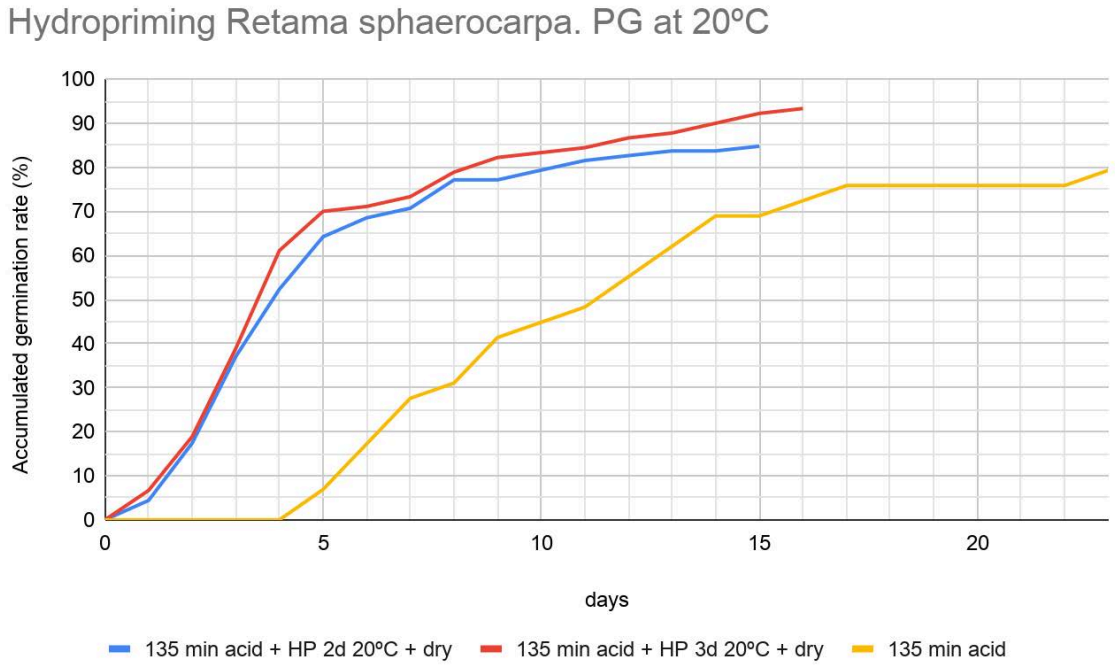

Figure 42: Germination curves for Retama sphaerocarpa

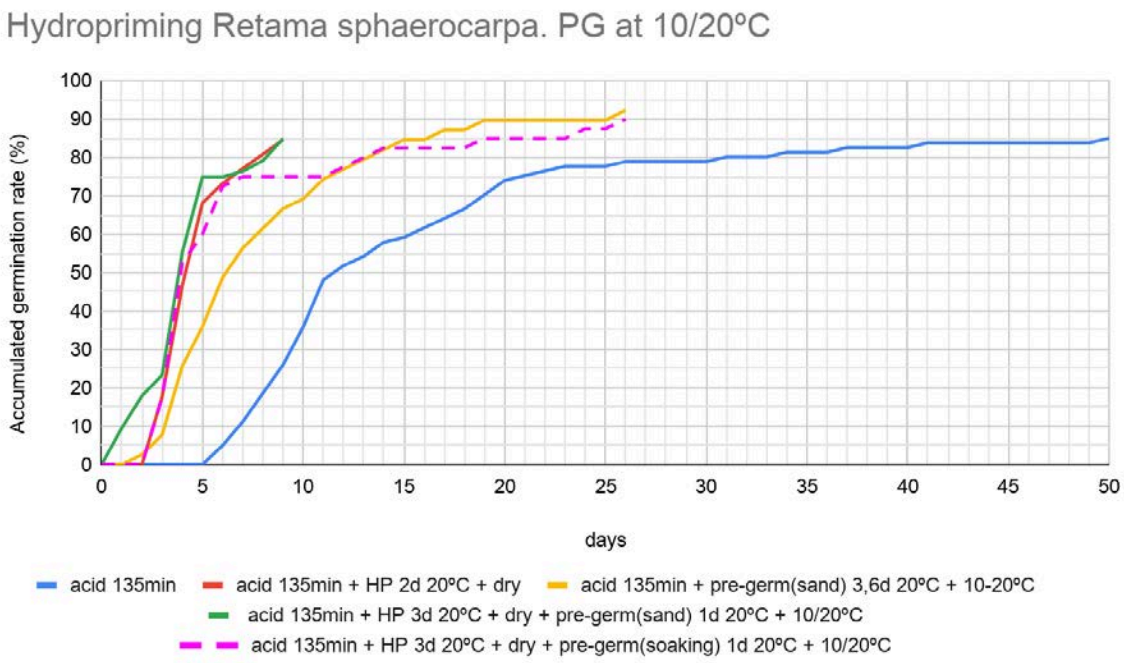

Figure 43: Germination curves for Retama sphaerocarpa

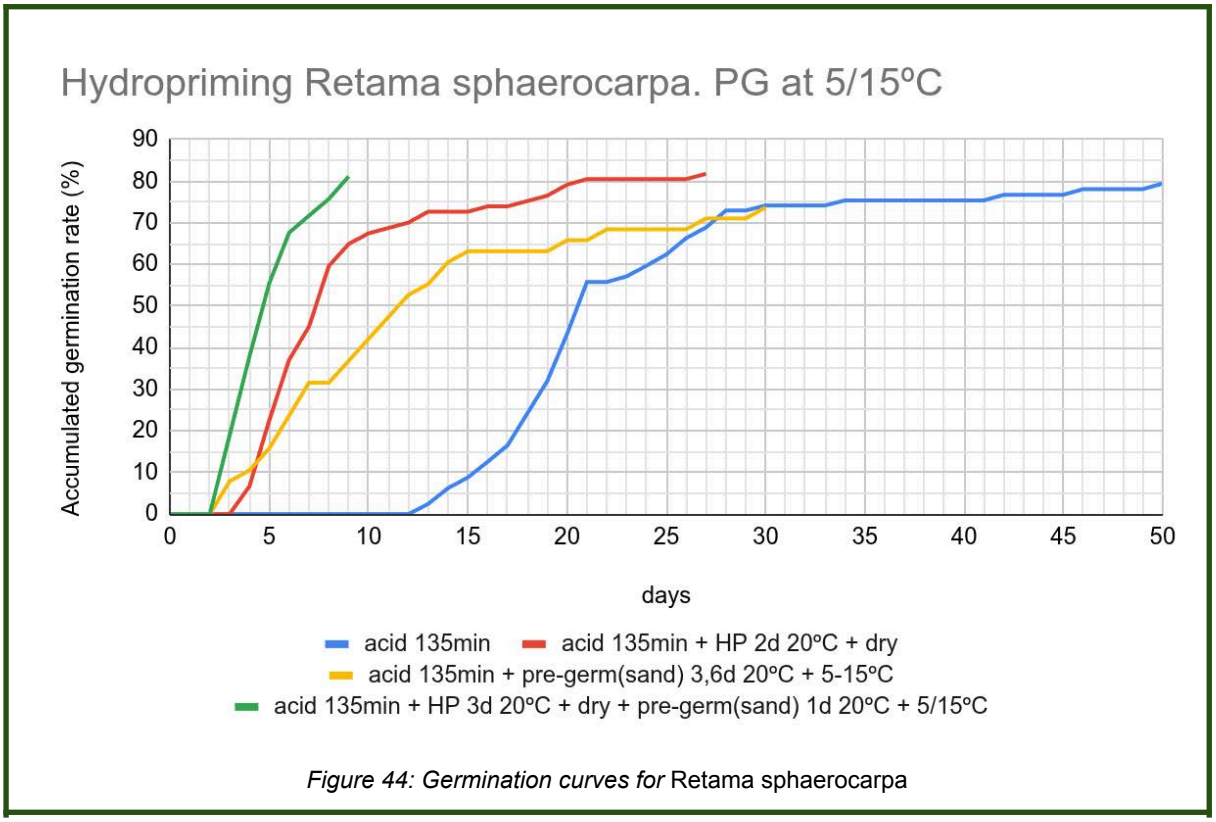

Figure 44: Germination curves for Retama sphaerocarpa

GENISTA UMBELLATA

BATCH

The batch used was harvested in the Sierra Lújar (Órgiva) in 2023. Germination tests to break dormancy are also shown for the batch harvested in La Alpujarra (Pitres) in 2020.

The seeds were stored in paper bags in a warehouse after harvest. Seed extraction from the dry pods was carried out 3 months after harvest. Moisture content was minimised, and the seeds were stored in airtight containers at 4°C. Determining the seed moisture content was not necessary for this study.

BATCH IMPROVEMENT

As with *Retama*, the batch improvement procedure for *Genista* (Spanish = Bolina) seeds is also linked to the hydropriming (HP) process, which will be detailed in the priming section. The procedure involves selecting seeds that have swollen after a soaking period.

All germination tests with Bolina seeds and HP assume the seeds swell within less than 30 hours. All germination tests of Bolina seeds without HP assume the seeds swell within less than 13 hours.

The 13-hour swelling period was not extended to 30 hours (for seeds without HP) because excessive soaking time negatively affects germination characteristics. Additionally, separating swollen seeds during the AS phase of the HP process at 13 hours was avoided for logistical reasons.

CLASSIC GERMINATION (Figure 45)

	Optimal T^{a} (ºC)	96% acid time (min)	Batch improvement	Bleach disinfection	T0 (days)	T90 (days)	%maxG
Genista umbellata	20ºC	65	swollen at 13h	10% 10’	2	4.5	90%

This species exhibits physical dormancy, preventing water penetration into the seed. Scarification is required to promote germination.

For the La Alpujarra 2020 batch, reused 96% sulphuric acid was used to break dormancy. After immersion for 20, 35, 50, and 65 minutes, germination rates were 20%, 16%, 44%, and 56%, respectively, with swelling observed in 24%, 23%, 49%, and 60% of seeds during the germination test. When fresh acid was used for 65 minutes, 79% of seeds germinated, and 86% swelled.

For the Sierra Lújar 2023 batch, seeds treated with fresh acid for 65 minutes and soaked for 13 hours were manually or mechanically separated into swollen and non-swollen seeds. The germination tests below include only seeds that swelled during the first 13 hours of soaking. This ensures comparability between seeds with and without priming, as the priming process for *Genista umbellata* (like *Retama sphaerocarpa*) involves separating swollen from non-swollen seeds at a specific stage.

With 65 minutes of acid treatment and selection of seeds swollen at 13 hours from the start of soaking, *Genista umbellata* germinates at 20°C in 2–4.5 days with 90% germination (Figure 45).

All germination tests for this species used 1 or 2 replicates of 25–30 seeds.

PRIMING TREATMENTS (Figure 45)

Genista umbellata	Priming T^a (ºC)	Priming duration	%mcfw FII	%mcfw SMP (no germinations)	%mcfw final SMP	GT T^a (ºC)	GT T0 (days)	GT T90 (days)	%maxG. GT
Control	--	--	--	--	--	20ºC	2	4.5	90%
Optimal HP	20ºC	30 hours	--	--	--	20ºC	1	3	90%

Since classical germination requires 4.5 days to complete, HP was selected as the priming treatment.

Seeds treated with 65 minutes of acid begin germinating between 1 and 2 days (entry into FIII), so HP with AS was performed for 30 hours at 20°C. No other HP durations were tested, as the optimal treatment time was already established from previous experiments.

During the HP treatment, the batch improvement process was carried out to homogenise the resulting batch. The procedure is as follows:

1. Start with dry seeds treated with 65 minutes of acid.
2. Place the seeds in AS to initiate HP.
3. After 30 hours in AS, remove the seeds and separate swollen from non-swollen seeds using a sieve.
4. Dry the swollen seeds.
5. Discard the non-swollen seeds.

Unlike *Retama*, non-swollen seeds are not returned to AS because only a small percentage (10–15%) fail to swell.

The 30-hour HP treatment germinates at 20°C in 1–3 days, reaching 90% (Figure 45), which is faster than seeds without HP, which germinate in 2–4.5 days, also reaching 90%.

GERMINATION TESTS AT SUBOPTIMAL TEMPERATURES (Figures 46 & 47)

	GT T^a (ºC)	T0 (days)	T90 (days)	%maxG.
Control	10/20ºC	3	6.5	78%
HP	10/20ºC	3	5	85%
Control	5/15ºC	4	8	69%
HP	5/15ºC	2	6	65%

This species germinates relatively quickly at low temperatures, and HP treatments provide only minimal improvements in germination characteristics. Nevertheless, for field sowing this species is used with HP due to the other advantages conferred by priming treatments, particularly increased resistance to extreme temperature and drought stress acquired by plants derived from primed seeds.

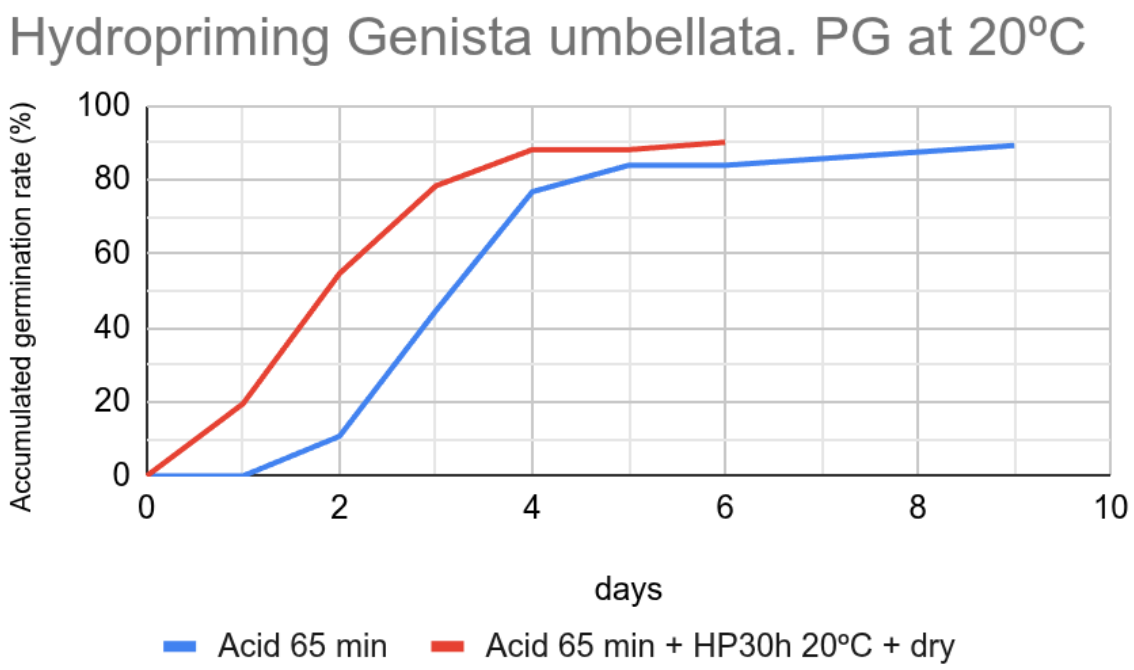

Figure 45: Germination curves for Genista umbellata

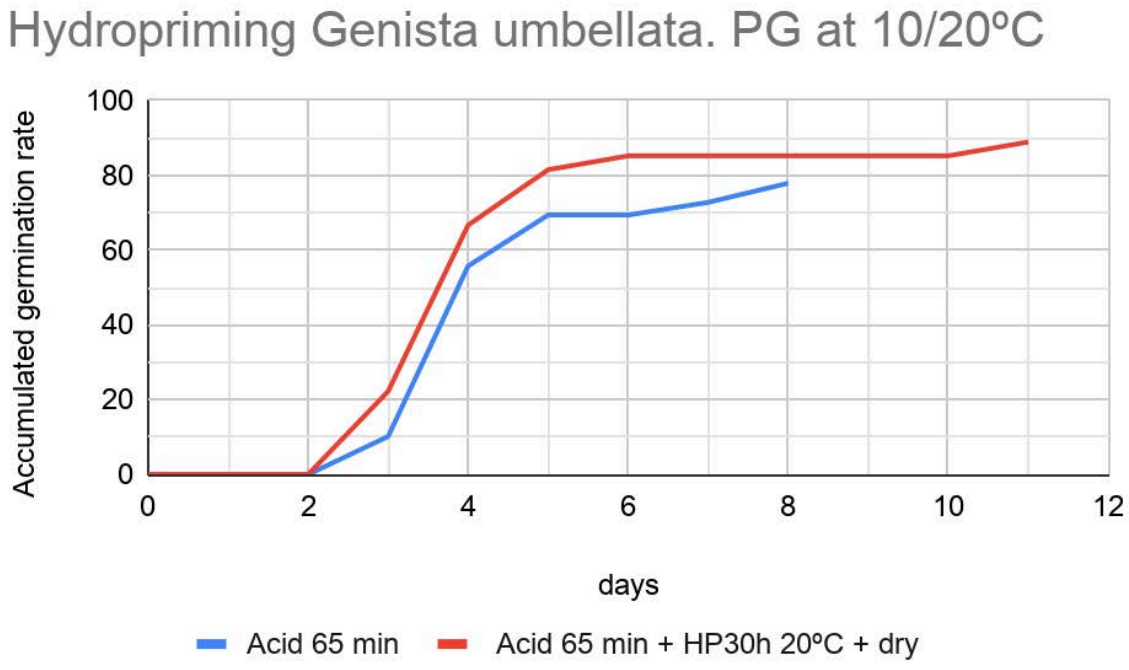

Figure 46: Germination curves for Genista umbellata

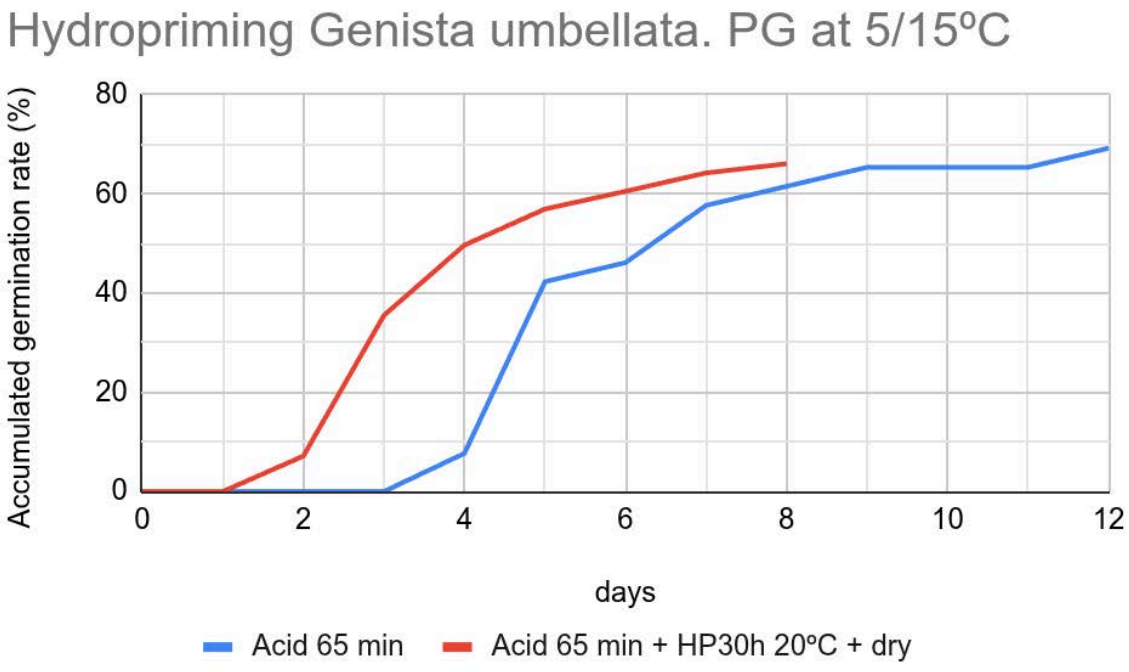

Figure 47: Germination curves for Genista umbellata

QUERCUS ILEX

BATCH

The batches used were harvested in La Alpujarra (Pitres) in November 2024. Each batch consisted of acorns (harvested directly from the tree) from 3–6 trees with similar colouration characteristics. After cleaning and disinfection, acorns were dried to achieve a %mcfw between 39% and 40%. One batch, which was very brown at harvest, was stored without drying, as its moisture content after disinfection was 37.4% mcfw.

Storage was carried out in LDPE bags (50 microns) at 4°C. The %mcfw shown for each batch is the mean moisture content of 10 individual acorns, with a maximum variation of ±2% within each batch.

NOTE: This species is described in detail in the *"Best Practice Manual for Quercus Restoration Projects in Mediterranean Environments."*

The datasheet below provides information on the viability of the batches used for field sowing, as well as a small experiment observing the effects of different pre-germinative treatments on the acorns.

CLASSIC GERMINATION (Figure 48)

	Batch	Tª (ºC)	%mcfw storage	Bleach disinfection	T0 (days)	T90 (days)	%maxG
Quercus ilex	1	15	40	10% 15’	10	37	82%
Quercus ilex	2	15	37.2	10% 15’	12	36	90%
Quercus ilex	3	15	39.5	10% 15’	12	34	91%
Quercus ilex	4	15	40.3	10% 15’	9	25	93%

Quercus ilex acorns germinate effectively at temperatures ranging from 4°C (or even as low as 1°C) up to at least 20°C. At low temperatures, the onset of germination is delayed, and root growth is slow. At high temperatures, the opposite occurs.

The viability of four batches, stored for 1 month and intended for field sowing, was assessed through germination tests at 15°C in a sand medium within an LDPE bag. Maximum germination percentages for each batch were similar, ranging from 83% to 93%.

PRE-GERMINATION TREATMENTS (Figure 49)

In our experience, the germination phases of this recalcitrant species can be understood similarly to orthodox species. Starting from an initial %mcfw of, for example, 37%, acorns gradually gain moisture during water imbibition (FI) until reaching around 44% mcfw. At this point, they enter FII, and within a few hours the root emerges (FIII). Thus, during germination and from the moment acorns are placed in a moist substrate, they remain primarily in FI. This has implications for designing pre-germinative treatments, as oxygen requirements are low during FI, supporting the hypothesis that soaking treatments (as pre-germinative methods) could exceed 48 hours, which is a common practice for acorns.

Pre-germinative treatments were studied at two temperatures (4°C and 16°C) with two durations (1.5 and 4.5 days) and two methods (soaking and aerated soaking) (Figure 48). The aim was to determine whether treatments longer than 48 hours, with or without aeration, affected germination.

The treatments were applied to a fifth batch, stored for 6 months. This batch was stored post-harvest at 40.5% mcfw, and at the time of the study, moisture was measured at 42% mcfw. The reason for the moisture increase during storage remains unclear, although the most plausible explanation is that LDPE bags allowed entry of high ambient humidity from the storage chamber (belonging to a fruit and vegetable company in Órgiva). Despite the increase in %mcfw during storage, batch 5 did not germinate during this period.

Figure 48 shows germination for each pre-germinative treatment. Note that time 0 of each germination curve corresponds to the end of the pre-germinative treatment. Key observations include:

- Maximum germination percentage for the batch ranged from 90% to 100%, confirming the viability of 6-month storage.
- Pre-germination for 4.5 days triggered faster germination than 1.5 days, as expected.
- For both 4.5-day and 1.5-day soaking treatments, aeration during soaking had no effect.
- For 1.5-day soaking, temperature (16°C vs. 4°C) had no effect.

We conclude that acorn soaking times can be extended beyond 48 hours, at least up to 4.5 days. This facilitates logistics for preparing pre-germinated acorns for field sowing. Future work will explore the temporal limits of soaking duration.

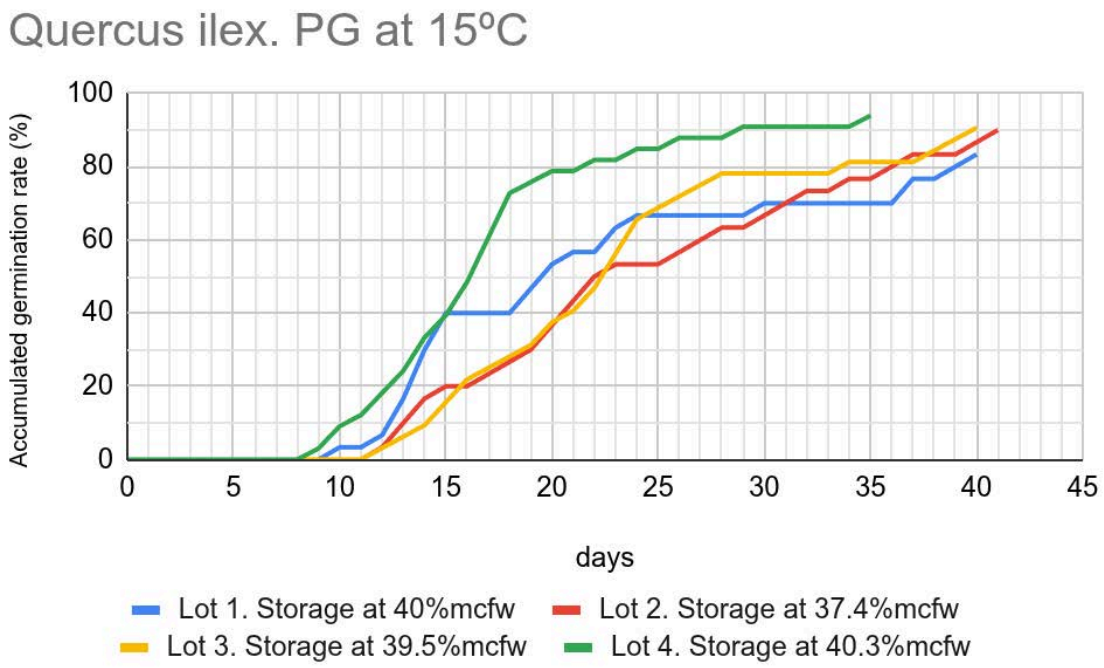

Figure 48: Germination curves for Quercus ilex

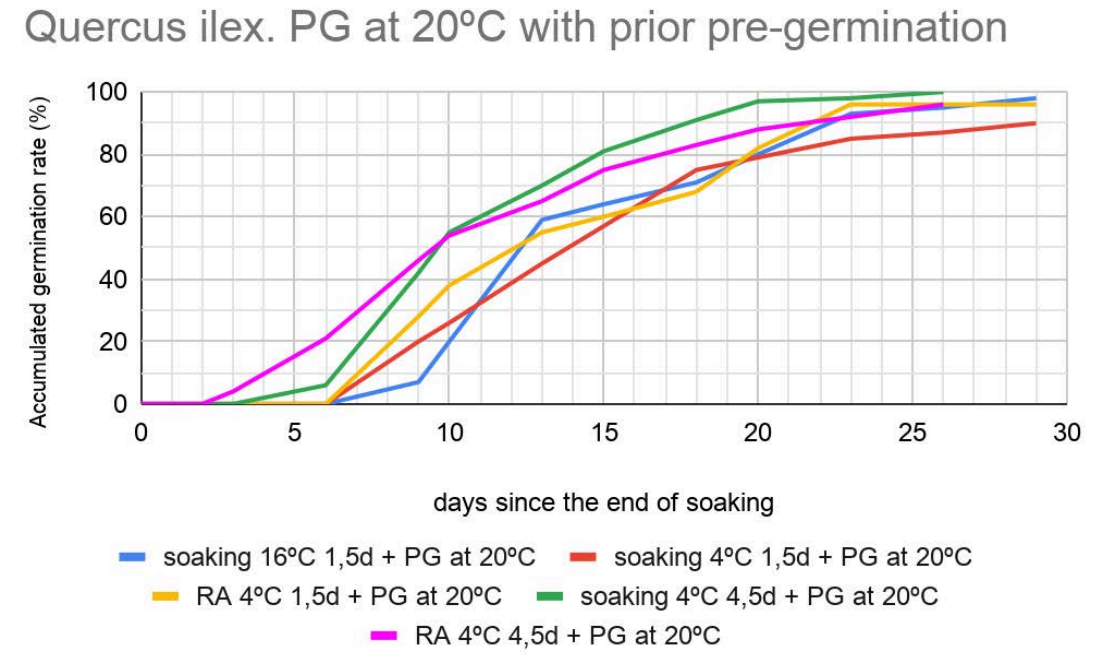

Figure 49*: Germination curves for* Quercus ilex

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