_Best Practice Manual

for Quercus Restoration Projects in Mediterranean Environments

Semillistas a.c.

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Introduction

Semillistas is a non-profit association, located in the Alpujarra (Granada). Since 2018 we have been dedicated to forest restoration using seed planting methods instead of traditional tree planting. In our work, caring for human health and the rural community in which we live go hand in hand. Social participation through volunteering and awareness, with talks and workshops are also part of our regenerative action.

Our organisation's objectives, as well as its internal functioning, are based on four pillars that define our way of understanding society, nature and our relationship with it.

- We take care of our land: We halt desertification and climate change, with reforestation and regeneration of land eroded by fire and human action. We promote biodiversity.
- We take care of our health: We promote the physical and mental health of the local population, as this is inseparable from environmental health. We implement internal mechanisms that promote health at work and in labour relations.
- We care for our rural community: We create new economic opportunities in the enrichment and care of our natural heritage, in a sustainable and respectful way. We promote a participatory and resilient society. We educate for the valuation and conservation of natural heritage, promoting land stewardship and its reappropriation.
- We take care of our knowledge: We publish the results of our research and development in a free and non-profit way. No patents or copyrights.

For more information on the work carried out by Semillistas please consult the activity reports available in the Downloads section of our web site[1].

The need for this manual

The creation of this manual is motivated by the need to take action against the state of degradation of our forests and our land, and all the consequences this has on life. There are several issues that lead us to act to support the natural regeneration of the land and to increase biodiversity. Among them are increased fire occurrence and intensity, increased desertification, degradation by human activities, and loss of biodiversity.

For more than a century forest restoration has been carried out around the world based on nursery-raised plant transplanting techniques. At Semillistas we have been dedicated for several years to the development of a methodology that allows restoration using seeds directly. The reasons we to want to change the way we approach forest restoration are due to the problems associated with transplanting practices in semi-arid climates. These include the need for summer irrigation, the cost of these projects, their impact on the restored ecosystem, and the impossibility of large-scale restoration by transplantation. In addition to these reasons, we also have faith in the principle that the best action in restoration is that which imitates natural regeneration processes[2]. The first step to imitate nature is to let the root of a plant develop naturally, without the constraints of a container, which is an essential approach in a climate as dry as the Mediterranean.

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[1] https://www.semillistas.es/descargas/

[2] Fukuoaka M. 1978. *The One-Straw Revolution*. New York Review of Books, New York.

On the other hand, the small associations that in each village do their restoration work are very limited by the transplanting technique, as the number of trees they are able to manage (in reference to the effort of summer watering) is very small. However, with a successful direct seeding system, small associations could multiply the scope of their actions. We think that there is a gap, a niche market if you prefer, in which ecological restoration can be professionalized and, in rural areas, people who do love their forests, can live economically from agroforestry restoration. This manual, and the SeedToSeed (S2S) project of Semillistas, are directed towards the recovery of the sovereignty of our land, which historically has been delegated to the administrations and/or large companies. It seems essential to us that, in every village around the world, there is a 100% local entity that knows how to harvest and sow its own seeds for the restoration/conservation of forests and pastures.

The path of Semillistas to make forest planting possible has been based on seeking solutions to the bottlenecks that in the past made the planting method the technique of choice in reforestation. These bottlenecks are: low and/or uncontrollable germination rates and high seed predation. The solution was found by exploring the knowledge in seed biotechnology. This has involved sorting out dispersed or even inaccessible (trade secrets) knowledge on the use of seeds.

This manual presents a methodology to help Mediterranean restoration projects find solutions to enable direct seeding of acorns with guaranteed success. Improving orthodox seeds[3] requires more work and we are therefore building another type of proposal (the S2S project[4]) so that small organisations can access this technology.

Finally, we provide our experience of different aspects that we consider essential for the implementation of an ecosocial restoration project by small and medium-sized organisations.

Structure of the manual

This manual is Split into two independent chapters and a series of introductory and concluding points.

In CHAPTER 1 - THE PROJECT, we list and define several factors that we consider important when initiating a collective project successfully and, more specifically, an integrated ecological restoration project.

In CHAPTER 2 - THE *QUERCUS*, we deepen our understanding of the seeds of the *Quercus* genus for use in a direct seeding forest restoration project.

Given the changing and incomplete nature of the knowledge we are trying to present here, we have opted to reduce the amount of text that makes up each point, and to expand the information by means of hyperlinks that provide information that can be updated over time.

Target audience

This manual is intended for individuals and organisations that have some experience with acorn planting. There are several manuals relevant to Spain that explore various aspects of direct seeding very well. The guides that we consider essential are the Guía Bellotera de Reforest-Acción[5], the Guía Práctica del IFAPA[6],

[3] Seeds which will survive drying and/or freezing during *ex situ* conservation, as opposed to recalcitrant seeds, which will not.
[4] https://www.semillistas.es/en/projects/seed2seed/
[5] [2] https://lagranbellotadaiberica.org/
[6] https://www.juntadeandalucia.es/agriculturaypesca/ifapa/servifapa/registro-servifapa/7c258946-6e55-4f46-b92f-0d482607a9b0/download

the ARBA manuals[7], the Guía Rápida de J. Castro (UGR)[8], and the Técnicas de Reforestación de Montoya[9]. All these guides are available for download on the Internet. What we offer in this manual is also complemented by a recent WWF publication[10].

CHAPTER 1 offers a guide for those who wish to undertake a reforestation activity, but do not know which steps to take. This includes guidance on the order of activities and important factors to take into account so that a project can be developed successfully. It is common to find groups with good ideas and great motivation but who do not know what steps to take (administratively or operationally), who lack the knowledge to plan a project, or who ignore other important factors that compromise project success.

This guidance emerges from our own knowledge and experience. It is not intended to be a rigid model to be copied but is a guide to a pathway which should be adapted to the particular conditions of each group and project. We are also aware that any biological project exists in circumstances subject to continuous change and development, so we consider the information we present as valid now, but potentially subject to future modification.

This chapter was born out of a desire to facilitate the path for other people and organisations, so that they do not need to “reinvent the wheel”, and to accompany and advise them along a common path to regenerate the earth, while also taking care of the people involved. We want to share what we consider good practices for the positive development of projects and activities, in which everyone involved feels fulfilled and in which they gain and grow together with their regeneration projects.

CHAPTER 2 of this manual goes deeper into some aspects that the guides mentioned above do not cover, so it will only make sense to those who have experience with acorns and who feel that something is missing in our understanding of the transition between "the acorn in the tree" and "the established oak".

The foundation of CHAPTER 2 is understanding the influence of acorn moisture content on storage and germination physiology. In the light of this concept, we are better able to understand past mistakes and how to adapt our way of working with acorns in specific ecosystems subject to different annual dynamics. Some basic knowledge of mathematics is required (e.g. understanding an x-y graph, use of formulas, solving simple equations of degree, calculation of percentages, etc.). It also requires basic knowledge of ecological restoration and its associated concepts, such as genetic diversity, germination, establishment, habitats, soil types, etc. That is why we recommend this manual for those already have a background in acorn planting and that this knowledge can help them to refine the work they already do. If a person has a good conceptual capacity, but not much experience in acorn planting, it is recommended to first soak well in the existing manuals, and then put the finishing touches with this manual.

We do not pretend that in Semillistas we have answered every question, but we have resolved them sufficiently to provide a methodology that guarantees the success of our sowings. Every year when the acorns begin to mature we become aware of further aspects that were unknown to us, in the never-ending search for regularities in nature that we can use in a practical way for regeneration.

Although this manual suggests a series of optimal "recipes" that can be followed for use with acorns, it is more important to understand the physiology of acorn germination so that we can adapt the decisions we make each year from the time we start harvesting to the time we finish planting. We must not forget that nature is a complex system and that every year is different. Only understanding, observation, and continuous learning can guarantee successful and permanent regeneration activity.

[7] https://arba-s.org/para-leer/#manuales
[8] https://ecologia.ugr.es/sites/dpto/ecologia/public/inline-files/Guia_rapida_para_bellotadas.pdf
[9] https://www.mapa.gob.es/ministerio/pags/biblioteca/hojas/hd_1995_07-08.pdf
[10] https://www.google.com/url?q=https://www.wwf.es/?59941/Estandares-para-la-certificacion-de-proyectos-de-restauracion-de-ecosistemas-forestales&sa=D&source=docs&ust=1728032783424784&usg=AOvVaw1nzC2-3Zx1mDciUAeIgH1a

CHAPTER 1 - THE PROJECT

Patricia Cañas Rios. Director of the SiembraBosques project

1.1 Project design and planning

There are many methodologies for project design and planning. In this guide we present the two we work with and outline the basic points they have in common, plus others that we consider important. These two methodologies are:

- **Logical Framework Approach (LFA)**[11] – which is an analytical tool, developed in 1969, for objective-oriented project planning. It is frequently used by international cooperation agencies and is very useful when writing proposals for grants for public or private entities.
- **Dragon Dreaming Collective Project Design**[12] – which is a systematic design process, philosophy, and methodological framework for the realisation of collaborative and regenerative projects, organisations, and platforms.

In LFA the following points are developed for each project:

- A general objective;
- The **specific objectives**;
- **Expected results**;
- **Activities** necessary to achieve these results;
- **Resources needed** to develop the activities;
- **External constraints** of the program or project;
- Measurable and objective **indicators** to evaluate the program or project; and
- The **procedure** for determining the indicators.

Dragon Dreaming has a more holistic perspective and uses 12 steps to develop a project, divided into four phases: Dream, Plan, Do, and Celebrate (Figure 1). In each of these phases we work on several important aspects, which are listed below:

- Creation of a team
- Define general and specific SMART[13] Objectives
- Define and assign activities and tasks
- Budget
- Management and administration
- Timing

[11] https://www.usaid.gov/logical-framework
[12] https://dragondreaming.org/
[13] Objectives that are Specific, Measurable, Achievable, Realistic, and Time-limited.

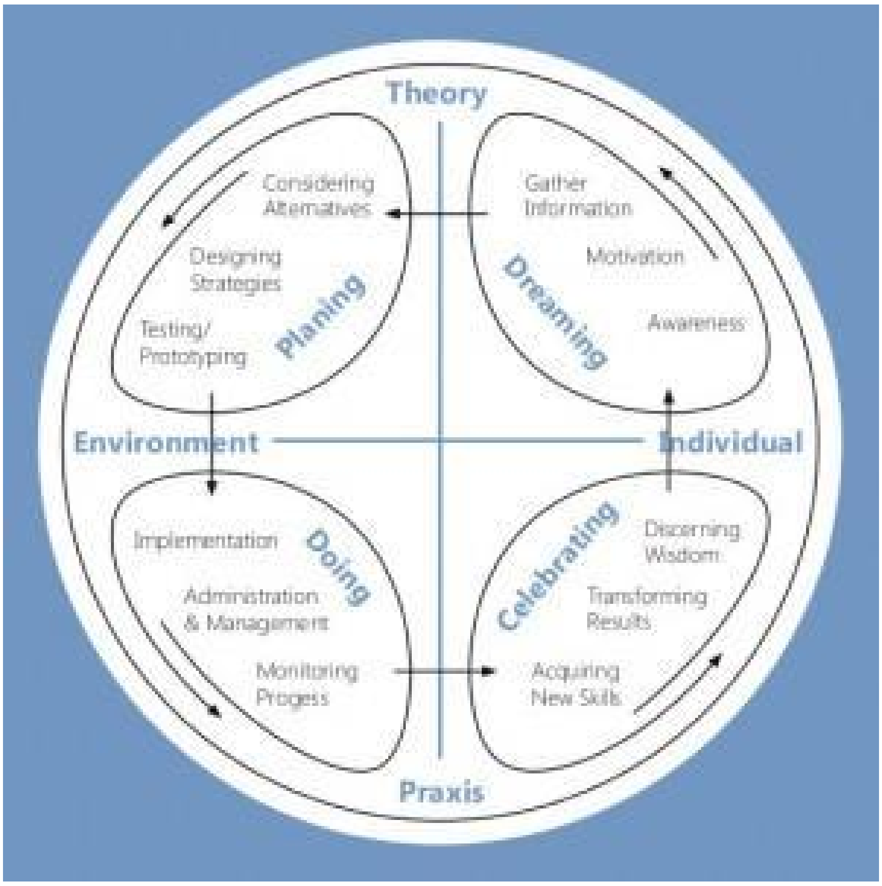

Figure 1. The Dragon Dreaming Project Wheel

1.2 Land and property search

Finding suitable land for an ecological restoration project requires thorough knowledge of an area. First, it is essential to define the objectives of the project clearly, such as the type of ecosystem to be restored and the goal of this restoration. Extensive research must then be carried out to identify potential areas, using maps, environmental databases and consultations with local conservation experts. Collaboration with conservation organisations and government authorities can provide valuable information on available land and applicable environmental regulations.

There are several factors that will have a significant impact on the costs associated with a project, such as the accessibility of the land, its topography, travel distance to project activities, etc.

Land ownership is also an important factor. Regeneration activities require time and it is therefore necessary to have some assurance that a project will be preserved and that future activities will not reverse regeneration. The options available in relation to land ownership are purchase of land by the organisation, or agreements with private landowners or the administrators of public land (e.g. town councils, autonomous communities, the state, publicly owned companies, etc.).

Semillistas has not pursued the purchase of land due both to lack of funds and also because there is plenty of available public and private land in our area of interest. Semillistas started by regenerating the land of local interested people in small stages and with simple verbal agreements. After a few years we then contacted a sympathetic municipality that administered most of the Sierra de Lújar[14] and signed an agreement with them, renewed annually.

In recent years, when Semillistas has become better known, we have received numerous contacts from people who want to offer their private land for reforestation activities. We have also contacted other municipalities, in collaboration with local organisations, individuals, and agents, to sign collaborative agreements for land owned by them.

1.3 Legislation

Initiating an ecological restoration project in Spain requires a solid understanding of the environmental legislation in force. In Spain, the legislation surrounding environmental restoration and conservation is extensive and detailed. One of the fundamental aspects to consider is the Forestry Law, which establishes the basis for the sustainable management of forest resources and regulates the planting and reforestation of land. This law also establishes the obligation to restore degraded areas and encourages reforestation as an essential measure to combat erosion and maintain biodiversity.

European Union regulations are also important and have a significant impact on Spanish environmental legislation. Spain must comply with European Directives related to nature conservation and the management of protected areas, such as the Habitats Directive[15] and the Birds Directive[16]. These Directives establish an obligation to protect and restore natural ecosystems, including the reforestation of degraded areas.

In addition, it is essential to consider local and regional regulations that may vary depending on the part of Spain where the reforestation project is to be carried out. These regulations may influence aspects such as the necessary permits, the plant species to be used, and the execution deadlines. Therefore, before starting a reforestation project in Spain, it is crucial to consult with the competent authorities, including the local town hall and relevant environmental agencies, and obtain the necessary legal advice to ensure compliance with all applicable environmental regulations.

In our experience, communication with the Delegación Territorial de Sostenibilidad, Medio Ambiente[17] in Granada has been very positive. Although sometimes it has taken us time to connect with the correct person due to the volume of work they handle, their collaboration and guidance has been of great help to the project.

For this reason, we encourage you to establish collaborative relationships, as far as possible, with councillors, town council staff, personnel working for territorial environmental delegations, and regional ministries.

1.4 Permits: landowners and environment

As mentioned in point 1.2, once suitable land has been located and an agreement has been reached with the person or entity that owns it, this agreement must be formalised as a permit, agreement, or contract.

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[14] https://es.wikipedia.org/wiki/Sierra_de_L%C3%BAjar
[15] https://environment.ec.europa.eu/topics/nature-and-biodiversity/habitats-directive_en
[16] https://environment.ec.europa.eu/topics/nature-and-biodiversity/birds-directive_en
[17]https://www.juntadeandalucia.es/organismos/sostenibilidadymedioambiente/consejeria/delegaciones/granada.html

After this, and before starting the reforestation project, a permit must be requested from the competent environmental authority. In our case, this is currently the Territorial Delegation of Environment of Granada, since we act in this province.

1.4.1 Landowners' permits.

When we want to agree a permit for public land, such as with a town council, we must follow certain administrative steps which are a little more complex and time-consuming than with private ones. Once the land/plots have been identified and agreed with the municipality, an agreement is prepared for these plots, based on a model agreement[18]. These permits, renewable either annually or every two years, are:

- For access to and use of the land only, and not for transfer of ownership.
- Specify the protection of reforested areas for a minimum of 15 years, which can be extended, avoiding, for example, earthworks, construction, or the entry of livestock.
- Sometimes the town council or other public body requires us to prepare the agreement in a draft that they can modify.
- Once drafted, this agreement must be presented at a meeting of the town council for approval.
- All this takes time, from six months to a year, so our recommendation is to start this process as soon as possible.
- A permit with a person with private land is as easy as drawing up a document of agreement (you can take as a model the agreement with town council) and for this to be signed by both parties. In this case there is no maximum time of the agreement, and it can be renewable after five years, instead of the one or two years that is standard for a public body.

1.4.2 Environmental Permit

Once we have the owner's permission, we must apply for a permit from the competent environmental authority. At Semillistas we wrote an Action Plan and submitted it to the Territorial Delegation of Sustainability, Environment and the Blue Economy in Granada for approval.

In every case it is necessary to know which authority has jurisdiction over the land to be reforested. If you do not know this, you can ask the Consejería de Medio Ambiente or the corresponding provincial delegation.

This permit application must be accompanied by an action report proposal. In this we present what we want to do, with the necessary data and information provided in the following sections:

0. General information about the project and the organisation
1. Detailed description of the area(s) of activity.
    a. Name of each area
    b. Description of the area
    c. Surface area of the project
    d. Location coordinates
    e. Polygon/plot
    f. Type of soil
    g. Slope
    h. Altitude

[18] https://www.semillistas.es/wp-content/uploads/2024/05/Modelo-convenio-aytos.pdf

i. Dominant woody species
j. Under-represented woody species
k. Description of the work to be performed:Method to be used
l. Species to be planted: number of plants, location (if there are several zones), hectares
m. Planting density and design
n. Origin of plant material (from seeds)
o. Material and human resources (to carry out the activity)

2. Schedule of work
3. Access to work areas
4. Safety measures (fieldwork, fire prevention, occupational risk plan, etc.).
5. Digital mapping of the action areas

1.5 Team (paid and volunteer staff)

The people who dedicate their time to a project are the driving force behind it. Without them there is no project. This team can be made up of people acting on a voluntary basis and/or hired staff.

It is essential to be clear about the objectives of the project and what is required to achieve these objectives, including the human resource requirements. It is then possible to assess whether the project goals can be achieved with only the time offered by volunteers or whether it needs paid staff to dedicate more time and perhaps specific knowledge or skills.

At Semillistas we realised that to achieve our initial objectives we would need the full-time dedication of several people. At the same time, at the start of the project we still had no funds, so all the work had to be done on a voluntary and unpaid basis, but with professional dedication. This was an initial investment by the volunteer project team as they worked to raise funds to pay subsequently for staff. This situation is common to many projects and associations.

If the objectives to be achieved are small then perhaps volunteer time and energy will suffice, but if they are large, we believe they cannot be left to the volunteer time of individuals.

At Semillistas we choose to combine paid and volunteer staff. We have a team of contracted personnel to ensure that important tasks or tasks that require specific knowledge and skills are carried out. We also have the invaluable help of volunteers in different areas:

- Volunteers from the local community for reforestation activities (combined with community awareness and cohesion activities);
- The voluntary collaboration of experts in this area of research; and
- Specific formal volunteer programs such as the European Solidarity Corps[19].

At this point we would like to emphasise the importance to Semillistas of quality and care in teamwork relationships. Some of the actions taken to achieve this are:

- Periodic meetings between the management team, starting with time to share how each person is doing and what their needs are;
- Regular meetings with the wider team to evaluate and share information transparently, celebrate, and create our relational fabric;

[19] https://youth.europa.eu/solidarity/mission_en

- An annual convivial meeting;
- Mutual support in work tasks;
- Measures for the care of personal wellbeing in the fulfilment of objectives, for example: decent salaries above the minimum, adaptation of the working day to personal needs (family, health, etc.).
- Encouragement of personal commitment to health care (physical and mental).
- Use of tools to facilitate group processes, meetings, decision making, as well as to avoid and resolve conflicts: sociocracy, non-violent communication, decision making by consensus, etc.

1.6 Community involvement

We believe that the involvement of the local community is essential for the success of a project. This starts with the founders of the project or closely associated members. It is important that at least one member of this group belongs to the local community. This helps to build trust in the community, provides knowledge about the area where the project will operate, and opens many doors to resources, contacts and possibilities.

At Semillistas we work to promote the involvement of the local community in the care and custody of their locality in different ways:

- For those who wish to be involved with greater commitment, being an active part of the reforestation project and involved in its design, planning, decision making, action;
- For those with less commitment, being part of the project but only for some of its aspects, such as performance of tasks and search for resources;
- For those who wish to be involved on an occasional basis, an opportunity for engagement in specific activities.

To this end, we have carried out various types of activities such as public presentations of the project with the support of the town council, collaborations with volunteer platforms, volunteer activities, and workshops and free talks on topics of interest to the local population.

Through these activities we want to bring the project closer to the local population, with the aim of integrating and opening it up to their participation and involvement.

We have found this work really challenging. Only those people who were already sensitive to the issue of the regeneration and care of their local environment have become more involved in the project. On specific volunteer days, the response is variable, with days of high participation and others with very low participation. Out of every 100 people who have contact with the project, four will volunteer repeatedly and one or two others become more involved. We are aware that this is a job that can take many years: cultivating and achieving community involvement growth can take as long as planting a forest and watching it grow.

Another relevant issue in the local community is to identify which people or groups may be affected by our actions (i.e., "interest groups" or "stakeholders"). In our case in the Sierra de Lujar these are shepherds and hunters (we originally thought that there might also be mining activity in the area, but this was not the case). There could be other stakeholders depending on each location, so every project will need to identify its own. It is of vital importance to communicate and create a good relationship with stakeholders, as far as possible, otherwise an entire project may fail. It is also important to reach clear agreements and rely on any agreements signed with municipalities to be respected. In Semillistas, when the planting season starts we agree with the hunting community the areas where we will plant and where there will be volunteer activities on weekends (which is also when hunting occurs). We agree on the days and areas where we will carry out these activities to avoid risks to volunteers and our team. This is a complex relationship that requires determination and patience. We do not have much experience with the shepherding community because there are only two shepherds in the whole of the Sierra de Lújar. The relationship is cordial with both of them and they understand and respect the work being done, which does not interfere with their shepherding.

1.7 Associated awareness-raising activities

We believe that public awareness activities are very important in order for our work to be known and understood, including any problems we are dealing with and their importance.

The response to awareness-raising activities varies greatly depending on a number of factors, including the target audience, the date and time, the venue, the context, and the type of activity, to name a few.

With these factors in mind, Semillistas have carried out various types of activities, examples of which are listed below: serve as inspiration:

- Presentations oo the project with the support of members of the town council;
- Information stands at other events and festivals, e.g., Sulayr and Fest Reforesta;
- Talks within other events, e.g., Fest Sulayr, Jornadas de lucha campesina, Curso ODS Universidad de Granada, and Ecoencuentro de Almócita;
- Children and family workshops within other events, e.g., Ambientfest and Sulayr; and
- Talks and workshops promoted by the organisation itself.

In our experience we have had greater participation when the activity has been part of the program of another event - at the request of, or in collaboration with, another organisation - than when the activity has been a unilateral initiative of Semillistas.

1.8 Human health and Earth health

At Semillistas we believe that human health and the health of the earth are deeply interrelated. This is something that ancient cultures already knew and that modern science reinforces with living systems theory among others. Therefore, although Semillistas is a project focused on the regeneration of the earth, we do not want to lose sight of care for human health, but to integrate it, as much as possible, in all of our activities. This is one of the distinctive features of our project.

Some of the ways in which we implement this in our project are:

- Before volunteer planting activities we dedicate 30 minutes to guided stretching, warm-up, and body awareness activities. This is a brief activity to help us connect with the natural environment. Instructions are given on how to take care of the body and avoid strain and injury during the reforestation activity, and rest breaks are taken as many times as necessary.
- Local volunteers who have participated in three or more days, and European volunteers from the ESC program are offered a free body care treatment (chiromassage, shiatsu, reflexology, or osteopathy) to be chosen from a pool of local professionals with whom Semillistas collaborates.
- Members of the Semillistas team are also offered these treatments. As mentioned above, a personal commitment to health care (physical and mental) by Semillistas team members is something desirable that is promoted alongside ecological values.
- Free lectures and workshops are held on a regular basis for the general public on aspects of human health care such as shiatsu, breathing for health, etc.

We would like to be able to expand our activities in this area, both for the team and for the local population in general, so that this is not something that is occasional, but becomes an important activity that is well integrated and recognisable within the project.

1.9 Broadcast

Communication about the project, what it does, its achievements, and its needs is an important process in any activity and project that is linked to the community.

Dissemination, whether locally or online, plays an essential role. At Semillistas we use it to:

- invite people to our awareness events: lectures and workshops;
- attract people for volunteer reforestation days;
- publicise the results of regenerative activities;
- publish and share technical developments;
- create support networks with other organisations, groups, and individuals;
- ask for support when we have a very specific need, such as voting for an award, occasional help for a very specific activity, etc.; and
- create an involved community for future fundraising campaigns.

1.10 Collaborations

An ecological restoration project is something exciting that awakens sympathy and a desire to collaborate in many of the people who live in and value their local area. Knowing how to seek, channel, and formalise these relationships is important, and can solve many bottlenecks that cannot be addressed with money.

In Semillistas we count on the support of and collaboration with several people and organisations, in different areas and at different levels, ranging from the local to the international. Some of these collaborators were sought by us, and others have sought out Semillistas. As the project progresses, obtains results, and becomes better known, it has become easier to attract the attention of collaborators or to ask for their collaboration. We identify some of them below, to illustrate their variety:

- At the level of restoration and awareness-raising activities:
  - Town councils (either the Environment Department or Mayor's Office);
  - Municipal environmental technicians;
  - Various local or provincial reforestation or ecology associations;
  - Neighbourhood associations;
  - National and international reforestation organisations and foundations; and
  - Festivals, conferences, events organised by municipalities, universities, organisations or related persons.
- At the research and academic level:
  - Department of Ecology, University of Granada;
  - Companies and organisations for reforestation, seeds, and soils;
  - Programming and electronics professionals;
  - Professionals in specific subjects such as holohomeopathy of ecosystems, forestry scientists, etc.; and
  - Forest restoration academics.
- At the organisational, administrative, and economic level:
  - Web design company;
  - Local businesses, companies related or close to team members; and
  - International Permaculture CoLab.

1.11 Search for funds

Starting an ecological restoration project requires human effort and resources. It is important to define a fundraising strategy to raise the resources needed for the project, whether it is supported by volunteers or is intended to be a project that generates employment to support the lives of the people who make it possible. A minimum stable income is required to maintain the commitment of people involved in the project and keep it active and growing.

Nowadays the search for funds has become an almost professional job, and for projects such as Semillistas that are born from volunteering, it is a considerable challenge to find the necessary resources and funds. To date we have not found a stable source of funding and we continue to search for this with the help of professionals.

We have found some sources of funding that have helped us to grow and develop over the first five years, and others that we have considered but have not developed for various reasons. We share these with you below:

Grants and awards

There are many of these at different levels, including public and private at local, provincial, national, European, and international levels. There are simpler and more complicated ones. There are subsidy search pages, such as: Soluciones ONG[20], Infoayudas[21], Plataforma del Voluntariado de Granada[22] (each province has a Platform), Grant Finder[23], and Funds for NGOs[24].

Indirect donations through digital platforms

To access these platforms, an organisation must pass a selection process, create a profile on the platform and comply with the monitoring and verification commitments. These platforms include Tree-nation[25], Plant for the Planet[26], and One Tree Planted[27].

Direct donations from individuals or companies.

Donation certificates can be issued if the donor needs it and regular donations can be formalised through tools such as Patreon[28], Paypal[29], Teaming[30], etc.

Other lines of financing

In addition to these, there are **other lines of financing**, but we have not opted for them for the time being. These include:

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20 https://www.solucionesong.org/
21 https://www.infoayudas.com/
22 https://enmarcacion.com/plataforma/?page_id=335
23 https://www.grantfinder.co.uk/
24 https://www2.fundsforngos.org/
25 https://tree-nation.com/
26 https://www.plant-for-the-planet.org/
27 https://onetreeplanted.org/
28 https://www.patreon.com/
29 https://www.paypal.com/es/home
30 https://www.teaming.net/

- Crowdfunding Campaigns;
- Donations from major philanthropists;
- Membership dues;
- Voluntary carbon markets;
- Proprietary cryptocurrency
- Financing rounds; and
- Payment for services offered, such as training courses, reforestation services, seed treatments, etc.

CHAPTER 2 - THE ACORN

Daniel Calatayud Cabrera. SeedLab Project Manager

2.1 Germination of orthodox seeds

In general, seeds can be classified into orthodox (they can be stored dry), recalcitrant (they cannot be dried for storage) and intermediate behaviour. Orthodox seeds are those in usually found in our fruit and nut orchards and in most trees, shrubs, and grasses. The lentil seeds we have in a jar in the kitchen are almost dry, but they respire: they are alive and desiccation tolerant. As they mature on the plant, lentils are loaded with moisture and, after maturation is complete, they dry to a low (almost dry) moisture content. In recalcitrant seeds (e.g., acorns and chestnuts), the end of seed maturation on the tree also coincides with the maximum moisture value in the seed, but the acorn dies as it dries out. Before we start talking about acorns, let's understand orthodox seed germination and the concept of seed moisture content.

When a lentil seed is stored and dried it contains a small amount of moisture (we call it "dry" but it is not completely dry). If it is well stored it will contain between 5% and 10% moisture content (%mc). This means that in every 100 g of seeds there is 5-10 g of water. If that "dry" lentil is sown in wet soil begins to absorb water and its moisture content will increase (Figure 1. Stage I, imbibition).

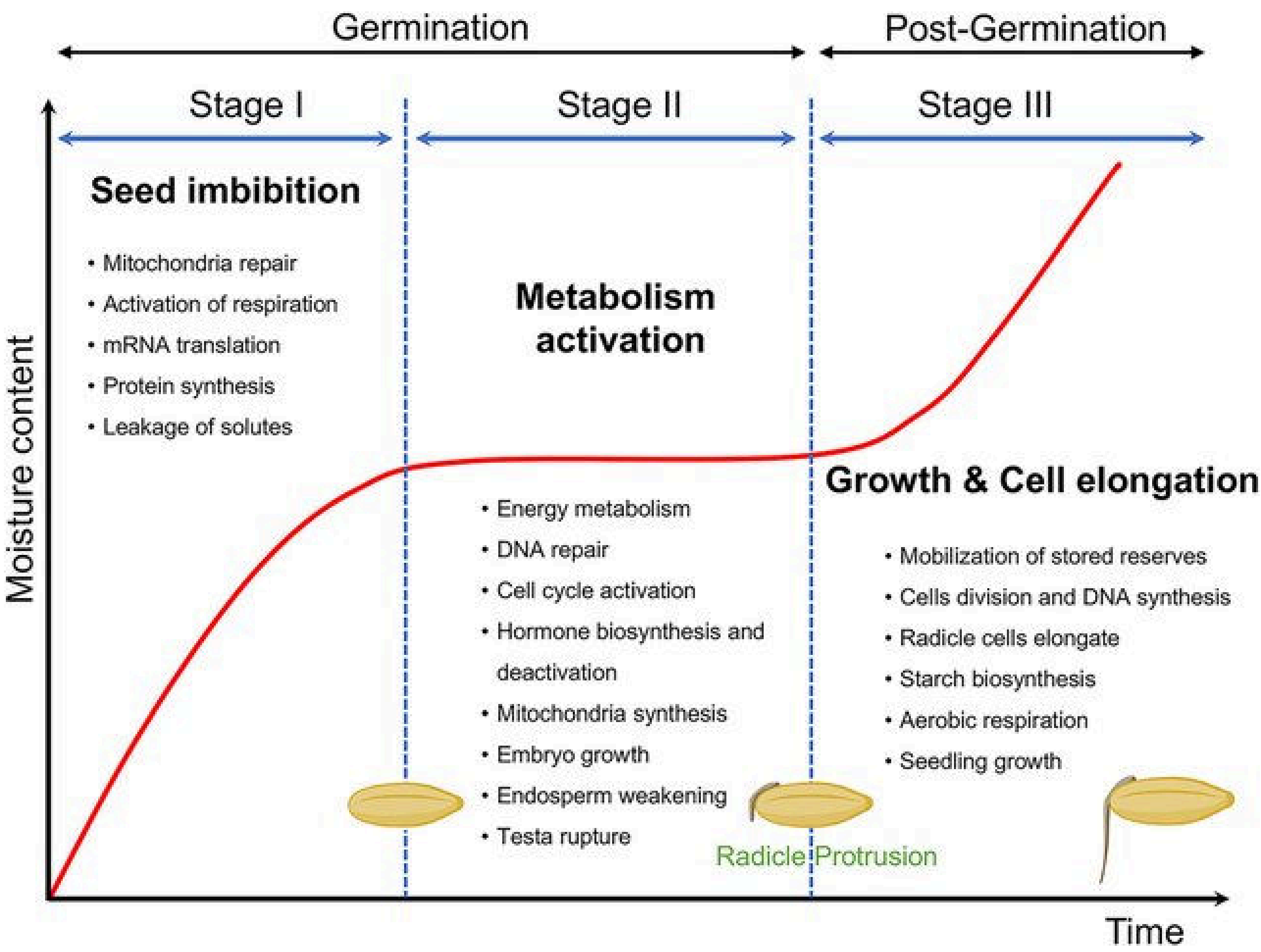


Figure 1. Phases of germination

After about 10 hours of imbibition, the lentil will not be able to absorb any more water and its internal moisture content will stabilise. During this stable moisture content phase (Stage II, metabolism activation) seed metabolism increases its metabolism (it respiures more) and DNA repair, nutrient mobilisation, the breakdown of starches into sugars and proteins into amino acids, and a host of other processes occur. When the lentil seed has done everything it is genetically programmed to do to prepare for germination it then breaks down fats, the embryo elongates, the seed coat breaks, and the root tip emerges (Stage III, growth and cell elongation). At the onset of Stage III the seed quickly absorbs moisture again. The duration of

Stages I and II, and the %mc of Stage II are species-specific.

What we have just described is the standard germination of an orthodox seed, i.e., from an almost dry state the seed absorbs moisture until germination. Unnoticed during most of the history of agriculture, but known since the 1970s, is the possibility of re-drying seeds during the germination process while they are in Stage II. If a seed does not produce a radicle (Stage III) it can re-dry repeatedly without dying. However, if the root begins to elongate and the soil dries out, the seed will die (although there are exceptions). Therefore, if we sow lentils and water them, but they dry out before they germinate (e.g. because of too much sun), these newly dry lentils remain alive until the next watering. When they are watered again they will germinate faster because in the previous hydration-dehydration cycle they already went through most of the necessary steps to germinate. This effect is called priming and is a generally unknown fact with great benefits for agriculture.

If we consider the seeds in a forest seed bank, each one will have a history of hydration-dehydration cycles. Depending on this history each seed will be more or less likely to germinate after the next rain. Thus a diversity of physiological states is generated in seeds of the same species within the soil after each rain. Some seeds on the soil surface will dry quickly after each rain (these will need many cycles of rain to germinate). Others will be slightly buried and with each rain will advance considerably through Stage II before drying out again. Others will be buried more deeply but because of low oxygen levels will not be able to respire adequately to advance through Stage II despite adequate moisture. This diversity of states, a product of the microsite where seeds from the same parent tree fall, produces forest resilience. The seeds on the forest floor, including those that are dormant, are not in a state of rest awaiting perfect conditions to germinate, they are continually moving towards germination with each rainfall.

When dehydration of seeds that are in Stage II occurs, the plant's stress-related DNA is activated in addition to obtaining new dry seeds that are closer to germination. This means that in years of low rainfall, plants from primed seeds produce more than untreated ones. Priming is used in agriculture for the following benefits:

- To increase and homogenise germination;
- To improve germination at non-optimal temperatures;
- To improve plant responses to water and salt stress; and
- To increase in production

It is not the purpose of this manual to delve into the intricacies of orthodox seed priming, but we hope it will whet your appetite to learn more.

Before moving on to acorns, let's explore %mc a little further.

The FW is the undried weight of the seeds for which we want to calculate the %mc. The DW is the dry weight (without water) of those seeds and is calculated by removing all water from the seeds in an oven and reweighing. Thus, (FW-DW) is the amount of water in the seeds for which we wish to calculate %mc. To obtain the percentage of water in the seed we need a baseline, which can be either the dry weight of the seeds or their fresh weight, depending on the purpose (e.g. %mcdw for food, transport, or substrates and %mcfw in seed biotechnology). The %mcdw is calculated by dividing the amount of water by the baseline we choose. In our case we will work with %mcfw.

For the %mcfw, the formulas relating the three concepts are:

- %mcfw= (FW - DW) / FW * 100
- DW = FW * (1 - (%mcfw / 100))
- FW = DW / (1 - (%mcfw / 100))

There are two ways of expressing the moisture content of a seed, depending on whether it is based on dry weight (%mcdw) or fresh weight (%mcfw):

- based on seed dry weight: %mcdw= (FW-DW)/DW*100
- based on seed fresh weight: %mcfw= (FW-DW)/FW*100

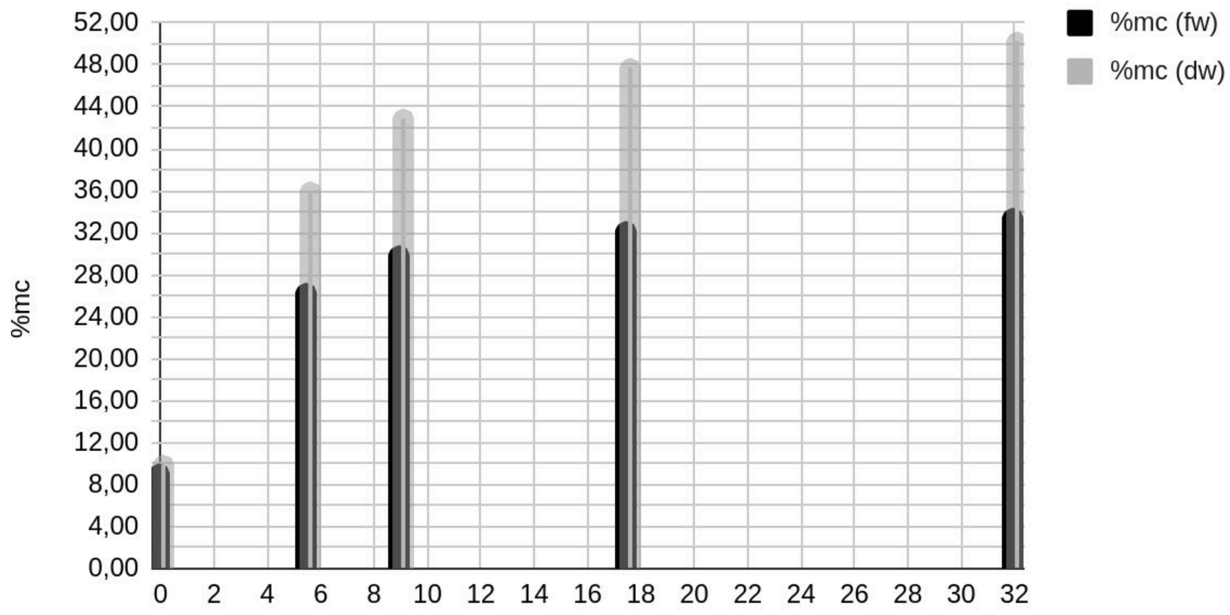


Figure 2. Moisture content of Pinus nigra salzmannii (stored seed, Phase I and entry into Phase II) over a 32 hour period (Semillistas data)

Some more points about %mc:

- In seeds stored in the open (i.e. not in plastic or glass containers) the %mc fluctuates with differences in air humidity;
- If seeds are stored hermetically they will experience a constant humidity level;
- Not all seeds in a batch will have the same %mc. There is variability which means each seed has a different %mc albeit close to an average for that batch. We usually calculate the average of a sample of seeds, but acorns have such a large seed that with a cheap balance we are able to measure the %mc of individual acorns.
- Orthodox seeds can usually be stored for many years between 5 and 6%mcfw at 4^{o}C. As a rule of thumb, each 1%mc decrease during storage doubles the shelf life of the seeds. At normal room temperature and relative air humidity (20^{o}C and 50%) seeds have between approximately 8% and 11%mc.

2.2 Moisture content of acorns

Acorns have a recalcitrant behaviour in relation to dehydration. This means that if we treat them like orthodox seeds they will die when left to dry. Some other seeds, such as chestnuts, are also recalcitrant, although not all recalcitrant seeds behave in the same way as described here. This manual is applicable to almost all *Quercus* (except *Quercus* of intermediate behaviour), and in particular ***Quercus ilex*** will be the main species considered throughout the manual.

Since seeds are so sensitive to desiccation, measuring their moisture content is essential to increase control. In this manual we will explain how to quantify acorn moisture to gain a good idea of the state of viability of acorns and what to do with them before sowing.

We begin this quantification by looking at the moisture content of the acorns during harvest:

- At the time of harvest (November), acorns should be around 42-46%mcfw (%mcfw). Acorns reach maturity when their moisture content reaches a maximum while still on the tree (%mcfw greater than 44%). They then turn brown as they dry out (below 44%mcfw). As time passes, brown acorns in a tree (if no wind blows them away) lose moisture, and brown acorns can be found on trees at a moisture content of less than 37%mcfw.
- A shiny brownish-brown colour indicates high %mcfw (~42%mcfw); as this shine decreases so does the %mc (to ~36%mcfw); if acorns are not fully mature (i.e., green and brown on the same acorn) the %mc will be high (43-50%mcfw). Acorns collected from the ground can be from 20 to 40%mcfw (depending on the number of days after they fell, soil moisture, sunshine, wind, etc). If the brown colouration of acorns on the ground starts to become light and dull it indicates very low %mc (~30%mcfw).

Once harvested, the viability of the acorns can be assessed by studying their germination percentage. Figure 3 shows that if the moisture content of English acorns is reduced to 40%cfm then 100% of them germinate; at 38%cfm germination is reduced to 80%; at 36%cfm 60% germinate; and at 34%cfm only 20% germinate. This last result can be reached after only one week of drying so if we do not control the moisture dynamics of an acorn it is relatively easy to ruin the viability of our crop.

Explained in another way, during maturation and after falling each acorn dries out. On dry ground after a few days in the sun the acorns will have a moisture content of, ~34%mcfw. If left in the sun for longer they will continue to lose moisture and if after those few days in the sun, when they are at 34%mcfw, we rehydrate them, only 20% of them will germinate; 80% will have died.

In Figure 3 we can also observe variation in the desiccation tolerance of *Q. ilex* from different locations in Europe. The %mcfw at which viability is reduced to 50% germination is 35%mcfw in acorns from England, 28%mcfw in acorns from France, 33%mcfw in acorns from Teruel and 32% in acorns from Zamora. Those from the south of France tolerate drying better. It is important to us to repeat this study in Andalusia, but while we do not have results yet, we can extrapolate based on the four studies in Figure 3 and another one from Tunisia (Amimi, 2023). Viability starts to decrease when *Q. ilex* acorns are dried below 40%mcfw. In the face of uncertainty, for the purpose of this manual we use the data from the Teruel study, which is close to the few data we have obtained on acorns from Sierra Nevada (Granada).

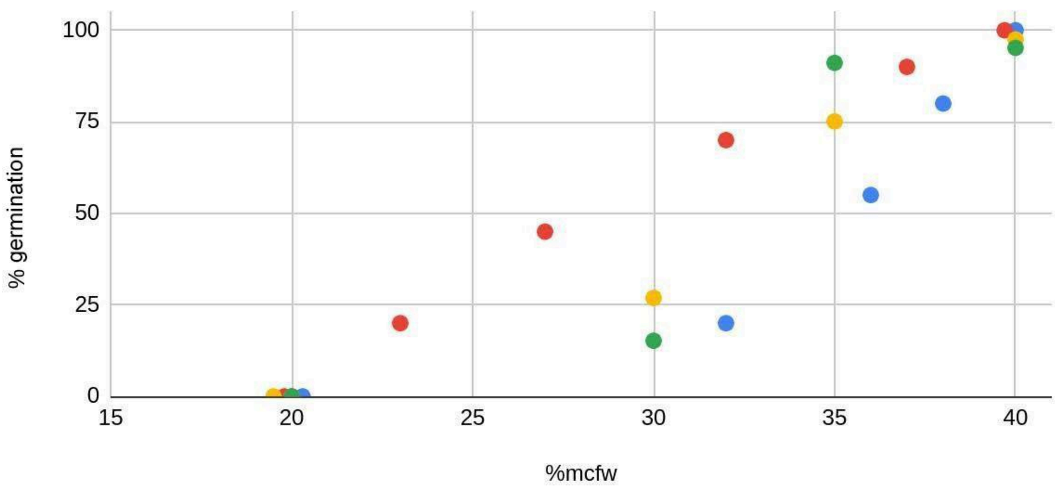


Figure 3. Tolerance to desiccation of Q. ilex acorns from different sources.
Data taken from Pedro León-Lobos, 2018 (England; blue); Joët, 2013 (South of France; red);
Salomón, 2012 (Teruel – yellow and Zamora – green).

To summarise, drying of *Q. ilex* acorns should never go below 40%mcfw if we do not want to lose viability. It does not matter how long an acorn remains at a low humidity level because viability is reduced just by reaching that level of dehydration. However, it is not always possible to obtain acorns at an optimum humidity level. Throughout the manual we will see what it means in practice to go below this level of humidity and how we can deal with the problems it induces (although the loss of viability – and % germination - due to dehydration cannot be counteracted).

Some other details:

- A graph of sensitivity to drying can be constructed for each species with recalcitrant seeds, which is essential for working with that species. Graphs for different *Quercus* species can be found in the academic literature and depend on the particular species and its origin (e.g., Figure 4).
- The acorn casing is designed to prevent rapid desiccation (orthodox seeds dry from 40%mc to 10%mc in 1-3 days, while acorns that are not exposed to the air or sun lose only 1.5%mc per day). The casing allows slow desiccation, but also slow rehydration and respiration. Almost all an acorn's water and air exchange with the external environment is through the hilum (the base of the acorn). The brown testa of acorns is quite impermeable.
- After harvest, if brown acorns (which already have less than 44%mcfw) are rehydrated (i.e., sown in moist soil) germination will occur when the acorn reaches 44%mcfw.
- If an acorn does not undergo the maturation process (drying to below 44%mcfw) because they are harvested when quite green (i.e., green and brown at the same time), their moisture content will be between 44 and 50%mcfw. These green, immature acorns (≥44%mcfw) cannot germinate even if planted in moist soil (Figure 5). The harvesting of green acorns above 44%mcfw is not well studied and is discussed in section 2.19.

In the next section we discuss acorn storage and examine the dynamics of germination as a function of initial %mc. In the light of these data we can understand where the common errors in acorn sowing occur.

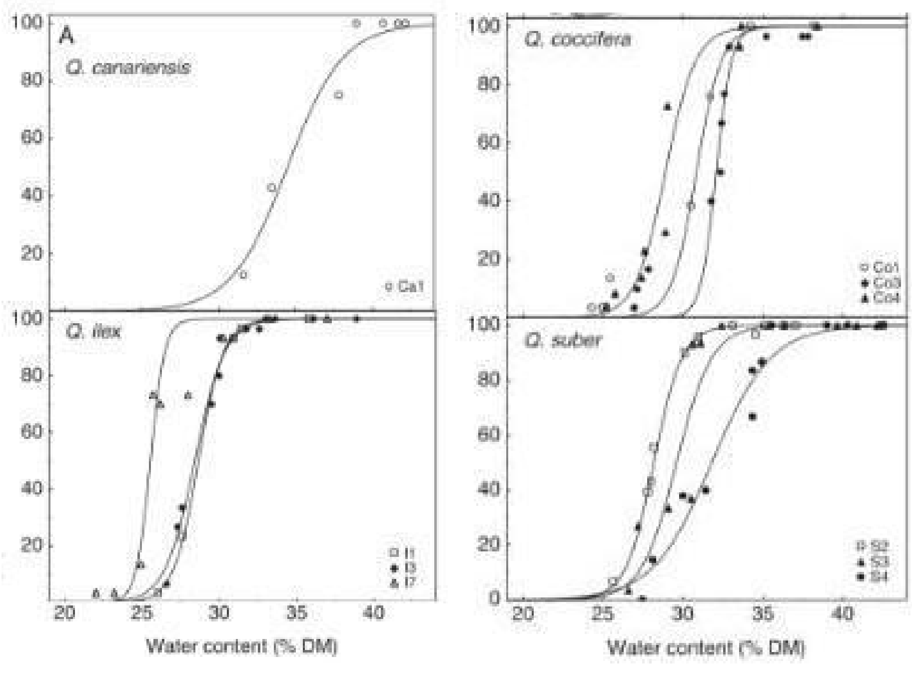


Figure 4. Sensitivity to drying of Quercus species from Tunisia (Amimi, 2020)

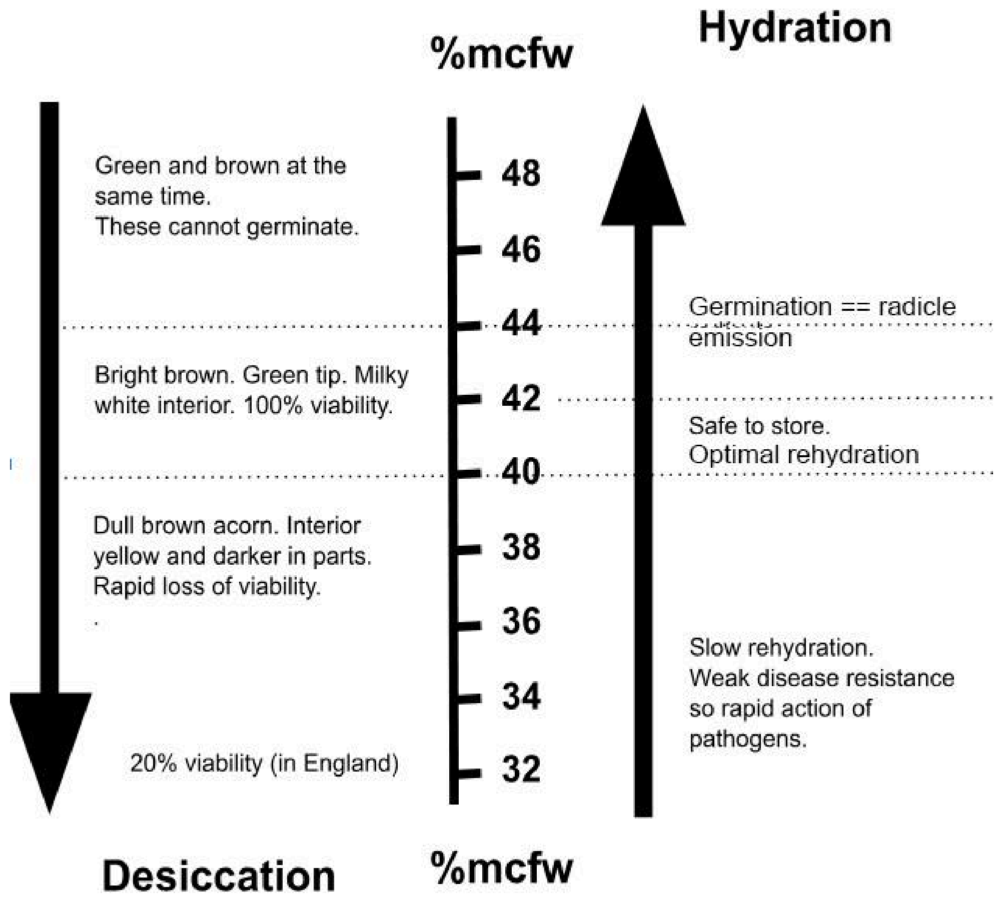


Figure 5 Acorn status at different values of %mcfw

2.3 Optimal storage of acorns

The reasons we need to store acorns properly are:

- Progressive drying rapidly leads to loss of viability; and
- We cannot always sow after harvest but need to wait for sufficient rain.

These reasons have a special weight in Andalusia, or in those areas with irregular rainfall. We must store the acorns until the soil has moisture at sufficient depth. However, if acorns cannot be dried then how do we store them? Traditionally acorns have been stored in sacks or boxes in a cool place without air movement, but if thorough control of the moisture content is not carried out then the results will be variable. We have measured this for acorns in large paper sacks stored at 15-17°C and found that 0.2-0.3%mcfw is lost per day. If conditions are improved by providing cooler air in a cellar with reasonable humidity then the drying process could be slowed, but in the conditions available to most small projects in Spain the acorns will progressively dry out.

Let us examine what an acorn needs to promote its medium-term storage:

- Moisture must be stabilised so that the acorn does not continue to dry out. We must try to keep the humidity of the acorns above 40%mcfw.
- We need to avoid germination. For this the %mcfw must be lower than 44% and ideally no higher than 42%.
- Low temperature: When we lower the temperature to 4°C, the respiration and metabolism of

acorns is very slow and the action of any saprophytic microorganisms is also slower (do not forget that we have sacks of humid and living organic matter which might rot). The closer we get to 1°C, the more effective the storage will be (below 0°C requires special care). The higher the storage temperature (≥8°C), the more acorn respiration and action of microorganisms occurs and more acorn viability is lost during storage.

- The solution to this is to store acorns in zip bags, made of low density polyethylene (LDPE) 30-50 microns thick (the zip bags sold by dollar store), at 40-42%mcfw and between 1 and 4°C. This type of plastic allows gas exchange and can sustain a slow rate of respiration at low temperatures. LDPE limits water exchange (although not totally) and keeps acorn moisture stable throughout several months. Low temperature also limits the action of microorganisms. This storage even improves germination over two to five months from the beginning of storage, although germination is reduced to 60% after one year. Between 3-4 kg of acorns can be stored in each zip bag in a domestic refrigerator.
- Prior to bagging floating acorns are discarded and the remaining acorns are disinfected in 10% bleach for 15 min, surface dried and their storage moisture is adjusted by drying them to the desired %mc. We dedicate a special section later to the issues of harvesting and storage moisture adjustment. However, it is not always possible to store acorns between 40% and 42%mcfw because different degrees of maturity harvest logistics mean that some acorns will be harvested with less than 40%mc.

The process described above is cold storage and not cold stratification, which is a term used to refer to the breakdown of tough seed coats via cold treatment. Cold stratification does not apply to acorns, but a cold *priming* method can be used which involves mixing the acorns with a humid substrate (in Tupperware or a plastic bag) and storing this at 4°C. This allows the acorns to rehydrate, i.e., progress towards germination. During this slow rehydration of the acorns, when they reach 44% mcfw they begin slow germination (slow due to the cold). The tip of the acorn opens and within days the root elongates, albeit very slowly (mm per day, versus cm per day during germination at 20°C). Cold priming is used to progress the acorns towards germination, but by slowing down the emergence process in such a way that when, after two to four weeks of cold, we sow the acorns at a higher temperature (10-20°C) they all germinate very quickly. Cold priming is therefore a pre-germination treatment used to initiate germination in the laboratory, so that when we sow acorns in the field germination is faster and more uniform.

Pasquini et al. (2012), compared two storage methods for Q. ilex acorns with an ~42%mcfw: in bags without substrate and in containers with wet peat, with both treatments stored at 3°C and 60% relative humidity. Germination tests were performed after every two months of storage (Figure 6). In both cases there is an increase in germination after 3-6 months storage, but in the peat treatment the acorns germinated during storage. After eight months of storage the viability of the acorns began to decrease, especially in the peat treatment.

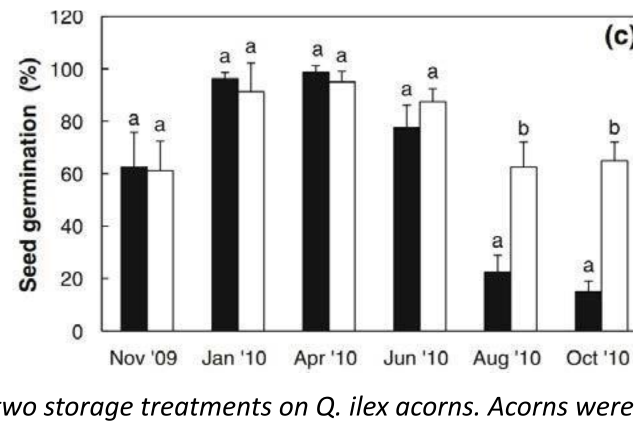


Figure 6. Effect of two storage treatments on Q. ilex acorns. Acorns were stored in a plastic bin with peat (closed bars) or in polyethylene bags (open bars). Values represent the mean (±SD) of at least four independent replicates. Means with different letters are significantly different at P

<0.05, as determined by a Fisher LSD test (Paquini et al. 2012)*

Summary of optimal storage:

- Separate the acorns from their cup and any leaves (the same day or the day after harvest);
- Remove floating acorns, disinfect the remaining ones (10% bleach for 15 min) and dry at 40-42%mcfw (if they already have <42%mcfw only dry superficially);
- Place acorns in In 50 micron LDPE bags (250x350mm bags with 3-4 kg of acorns) and store at 1-4°C;
- Ideally, each bag needs space for gas exchange (do not pile up the bags);
- If stored between 42% and 44%mcfw more microorganisms will develop and the acorns will germinate.

Disinfection is with 10-20% bleach (100-200ml in 900-800ml of water) for 10-20 min (depending on the condition of the acorns) with subsequent rinsing with plenty of water.

If during storage (after 4-6 months) fungus is observed growing on the base of the acorns, they can be removed, disinfected again, superficially dried, and stored in new LDPE bags. Acorns stored below 40%mcfw do not store optimally and tend to develop more fungus than those stored at 40-42%mc. The bleach we use is sodium hypochlorite at 3.5% active chlorine, without additives (i.e. the one used for water purification).

Other details:

- Acorns containing grubs in which the grub has not eaten the embryo can germinate (the endosperm has been eaten, but not the part of the tip of the acorn where the embryo is located). Although these acorns tend to develop fungus if stored, they can be used for planting immediately after harvest. For the storage protocol, these acorns must be removed, using the same technique as removal of floating acorns.
- Rehydration of acorns prior to storage is not recommended. If acorns are at 36%mcfw they should be stored at this humidity, rather than rehydrated to 40-42%mcfw and stored at that level. We have not tested this, nor have we seen any relevant studies for *Q. ilex*, but it is recommended not to do so. We know that there are other *Quercus* species that may benefit from rehydration as a means to readjust the %mcfw before storage.
- Semillistas harvests approximately 30000 acorns/year, which we need to store for 4-5 months (to wait for rain or for late sowings at altitudes higher than 1400 m). To store this amount of acorns we rely on the cold storage of Braojos de Orgiva, a local company dedicated to the distribution of fruits and vegetables.
- It is not possible to store acorns in LDPE bags at high temperatures. The recommendation is 1-4°C. At higher temperatures there may be a progressive loss of viability. However, once at Semillistas we had to store acorns at 6-7°C and we did not observe a loss of viability after four months of storage. Care must be taken to ensure that there is not too much metabolic activity in such a closed container.

2.4 Rehydrating stored acorns

In a study conducted by Semillistas in 2022 (Figure 7) we wished to understand the germination process of acorns that were stored at different moisture contents. We knew that the maximum germination percentage decreases as the %mc of storage decreases, but we did not know how the germination process was affected at different %mc.

Figure 7 shows the moisture content of 6 groups of 25 acorns each over time. In this factorial study we looked at:

(acorns stored at 40 and 34%mcfw) X (at each of these moisture levels we tested the germination of whole acorns at 20°C, whole acorns at 4°C, and scarified acorns at 20°C). Scarified acorns had a piece (1 cm) of the testa removed from the tip of the acorn. All acorns had been stored for eight months at 4°C.

The study found that acorns with low %mc (34%mcfw) took 22 days to begin germinating at 20°C, compared to five days for acorns stored at 40%mcfw. In addition, a very low percentage of the acorns stored at 34%mcfw germinated. Figure 7 shows:

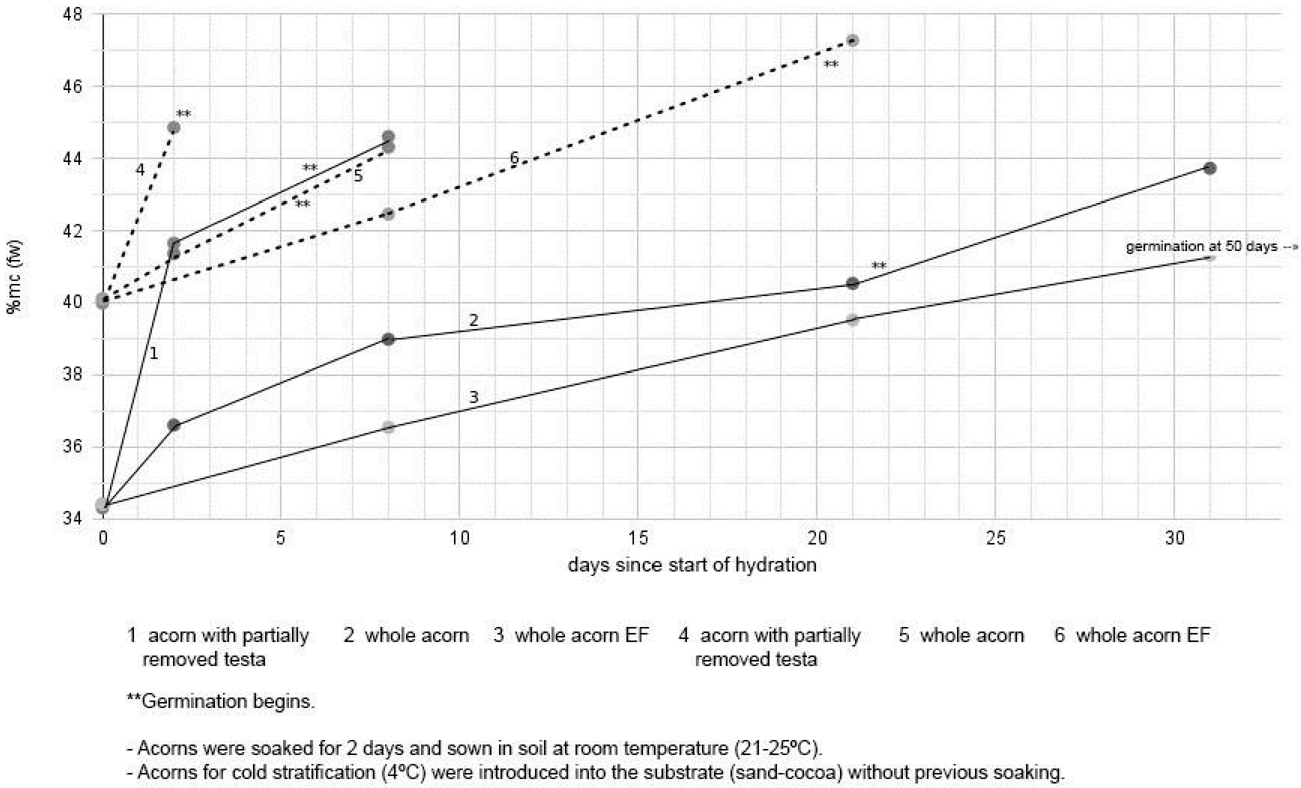


Figure 7 Rehydration dynamics of Q. ilex acorns in 6 cases.

- Acorns stored at low humidity (34%) took 30 days at 20°C and 50 days at 4°C to attain about 44% mcfw. However, if part of the testa is removed then they hydrate very quickly. These acorns with low moisture are very prone to fungal infection during storage, take a long time to rehydrate, and germinate at a very low percentage. The cold priming approach is a safe method (increased fungus control) to rehydrate these acorns.
- Acorns with 40%mcfw, rehydrate to 44%mc in 6 days at 20°C. Rehydration is a little slower at 4°C than at 20°C. If the testa tip is removed then it takes less than two days to reach 44%mc.

Partial removal of the testa is not practical in a project (due to the time required to perform the operation); it would be more practical to cut 0.5 cm from the base of the acorn, which also results in rapid rehydration. However, other problems are introduced by leaving the endosperm unprotected because microorganisms will feed on the acorn. Cutting the base of an acorn to accelerate hydration (in the USA a machine is under development to cut acorns automatically) requires fungicide to control pathogens. Semillistas has not pursued these techniques because phytochemicals are necessary.

This study shows that:
- The acorn testa is the first limitation for rehydration;
- Root elongation begins once the acorn has rehydrated to about 44%mcfw;
- The drier the acorn, the longer it will take to reach 44%mcfw;
- The rate of acorn rehydration is similar in acorns stored at low and optimum humidity (approx. 0.5%mc/day);
- Rehydrating at 4°C is a little slower than at 20°C.

The study provides information on how we should pre-germinate acorns before sowing them in the field. That is, how much pre-germination time they need to start germinating, depending on the moisture content of the acorns during storage (more on this later). For now it suffices to say that the field sowing we consider ideal is one in which the acorns are sown very close to 44%mcfw and, regardless of the state of the acorns during storage, we should achieve this, otherwise we will have the problem we describe in the following section.

Figure 3 (Section 2.2) shows that acorns from England and southern France had different tolerances to desiccation. Those from France could be stored at 32%mc while retaining 70% germination, while the equivalent figure for acorns from England was only 15%. Although acorns from France retain viability at low %mc, it is costly for germination rates to rehydrate them to 44%mcfw.

Q. ilex acorns have a three-phase germination (Romero, 2018): Stage I (hydration) and Stages II and III (root emergence). We can imply from Figure 7 that acorns, after soaking, remain in Stage I from the %mc of storage until they are at around 44%mcfw. They remain about 24h in Stage II (Romero, 2018) and quickly enter Stage III. This way of looking at acorn germination helps to relate the priming processes of orthodox seeds to the storage processes of recalcitrant seeds.Germination of acorns in the field.

2.5 Acorn germination in field

Sown acorns may not quickly reach the 44%mc needed to germinate, depending on rainfall, soil exposure, nurse plants, and initial acorn moisture. Acorns sown at more than 40%mc will quickly reach 44%mc. They could also be sown at 44% if a germination treatment is performed before sowing. In Figure 8, acorns sown at 37%mcfw fail to germinate with the first rain. Before reaching 44%mcfw they begin to dry out again (due to the drying of the surface soil). When it rains again in February, these seeds rehydrate and reach 44%mcfw in March.

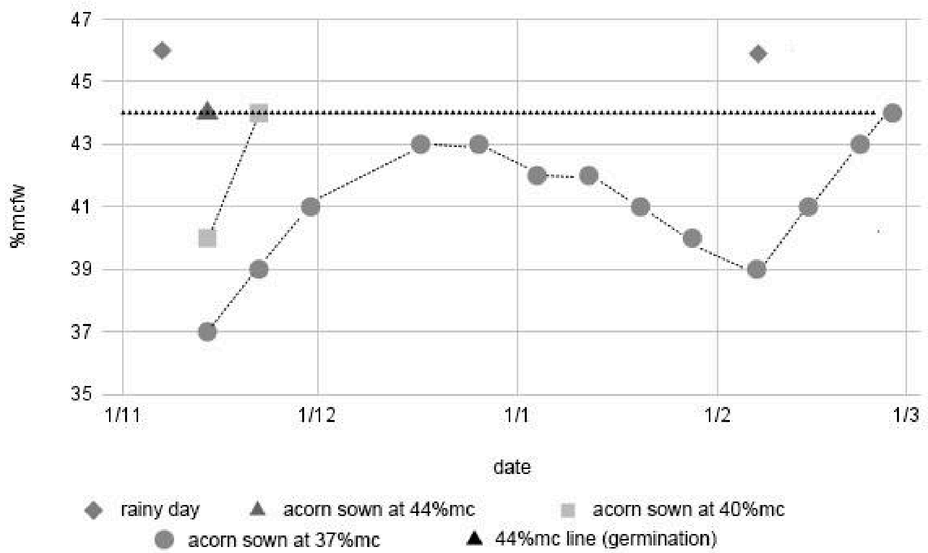


Figure 8 Hypothetical hydration dynamics of acorns planted in the field at different %mcfw, with two rainfall events, in November and February.

If we have acorns stored with low %mc and they are planted in that state we cannot ensure immediate germination. These acorns will be exposed to the drying rate of the soil, so it is likely that the acorns will not germinate and will return to the drying process. This is a typical problem in small reforestation projects. They do not store the acorns optimally and the acorns are at a very low %mcfw at the time of planting. Even if they are soaked for 2 days, they will still have a low %mcfw level and will take a long time to germinate.

It is important to ensure immediate germination after planting so that the acorn root begins to penetrate deeply as early as possible before summer. In the hypothetical case shown, acorns with a low moisture content could have started to develop roots in November, but because they did not reach 44%mcfw they must wait for February rains to do so.

Acorns sown at more than 40%mc can be sown at a shallow depth (3 cm), as they will quickly germinate and if the soil dries out superficially it will not be a problem because the root will already have reached a sufficient depth). Those sown at 37%mc should be sown 6 cm deep to try to maintain soil moisture around the acorn until it can rehydrate completely and germinate. However, the deeper the acorns are planted the less surrounding air they will be exposed to and germination will be slower. Usually the hole in which acorns are planted is stepped on and compressed so that the soil dries out less, although acorn respiration will also decrease.

In summary, storing acorns with low %mc not only reduces the viability of the acorns but also delays their germination in the field unless a pre-germination treatment is carried out before they are sown.

2.6 Emergence of acorns in the field

Germination occurs when the root breaks the acorn cover and begins to elongate. Emergence occurs when the stem peeks above the soil. Acorns have hypogean germination, i.e. the inside of the acorn does not develop into the first cotyledons, but the root emerges downwards and the stem upwards from the acorn, which remains underground and attached to the plant for 1-2 years.

When do germination and emergence occur? The Table below shows the germination and emergence of *Q. ilex* in seedbed trials with pre-germinated acorns at about 44% mcfw.

	Weeks of the 1st month after planting								
Sowing conditions	**1st**	**2nd**	**3rd**	**4th**	**2nd month**	**3rd month**	**4th month**	**5th month**	**6th month**
At 10-20°C	Germination		Emergence						
At the end of February at 1100 m	Germination				Emergence				
In December at 1600 m	Germination								Emergence

Although emergence is late the root develops immediately after sowing, although at different rates depending on the temperature. The colder it gets, the longer it takes to complete germination, the root growth rate decreases, and emergence is delayed.

The timing of emergence does not seem to be relevant for survival after the first summer. Acorns sown in December and emerging in June had 88% survival after the summer. What is important in *Quercus* is root development, which is favoured by such a large seed as the acorn, which provides nutrients.

Further details:

- Why do acorns sown at 10-20°C take two weeks to complete germination if they are sown close to 44%mcfw? The answer is that acorns from the same batch do not have the same %mcfw: some will have a lower and some a higher than average value. Thus, sowing close to 44%mcfw ensures that a large proportion of the acorns will germinate within a few days after sowing. However, another part of the same batch with an %mcfw lower than the batch average will take longer to reach 44%mcfw.
- In very cold areas (such as planting in December at 1600 m) sowing can be delayed until

temperatures rise or when the soil thaws. In the case above acorns were sown before the first frost and the ground froze from January to March. Germination of these acorns was lower (45%) than acorns sown at 1100 m in February (65%), possibly due to frost damage.

2.7 Laboratory germination of acorns

In Figure 9 the different germination lines correspond to different geographical origins. Germination at a nighttime and daytime temperatures of 10ºC and 20ºC is a little slower (2-3 days) than at a constant temperature of 20ºC. Below 10ºC acorns germinate more slowly but, more importantly, the root grows very slowly once they have germinated.

If acorns are near 44%mcfw when sown in a moist substrate at 20°C, germination is rapid and complete (for all acorns) in less than two weeks. In the Caliskan study, there is no information on the acorn %mcfw at the time the germination test was performed.

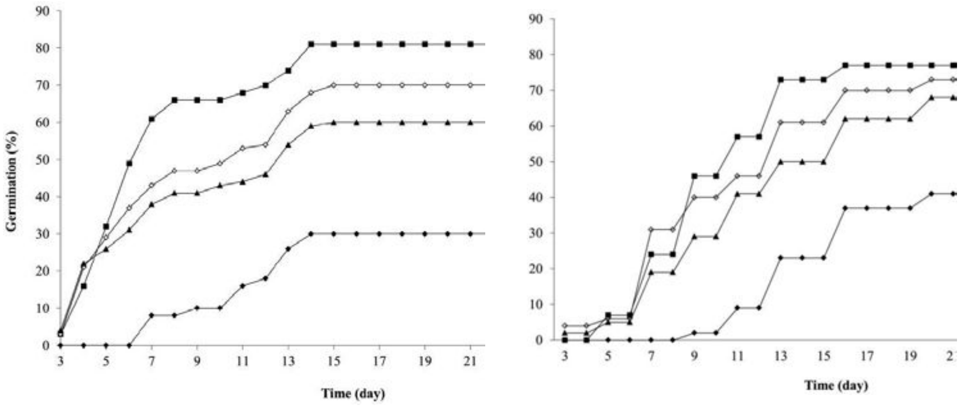


Figure 9. Germination at 20°C (left) and alternating at 10-20°C of Q. ilex from four different locations in Turkey (Caliskan, 2014)

2.8 Root growth

Corchero et al. (2002; Figure 10) found that that until March there was hardly any root growth (measured as the sum of the length of short roots from each seedling) in *Q. ilex* seedlings transplanted on the dates indicated.

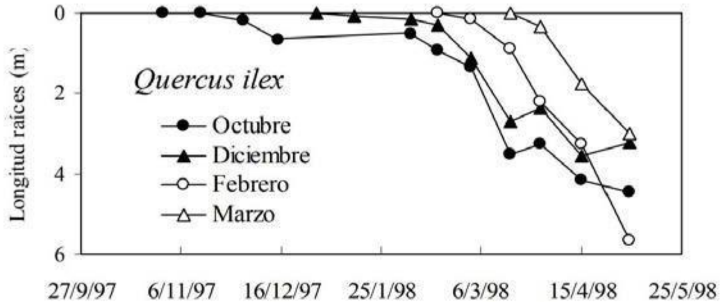


Figure 10. Growth of transplanted seeding roots (Corchero et al., 2002).

Pemán et al. (2006) sowed acorns of ~44%mcfw into long containers (temperature not reported). In a 1 m deep container the root exceeded 30 cm after one month, 60 cm after two months and was more than 1 m long before summer arrived. Root growth does not stop during the summer, although it is very slow.

We do not have conclusive academic data on the effect of planting date on the root development of a direct seeded acorn. Our recommendation is to plant in the field even if the weather is cold (≤5ºC). The root will then develop slowly until the spring.

2.9 Pre-germinative treatments

Depending on the condition of the acorns during storage, it may be necessary to perform one of the following pre-germination treatments, with the objective of sowing acorns as close as possible to 44%mcfw in the field, thus ensuring that germination will occur quickly and successfully.

The sequence of work will be:
- Acorns are stored at a known %mcfw;
- Depending on when we want to sow, a quantity of acorns is taken out of storage and a pre-germination treatment is performed; and
- When the pre-germinative treatment is finished, the acorns are sown in the field.

A Table of recommended pre-germinative treatments according to %mcfw during storage is shown below:

Stored acorns %mcfw	**Recommended treatment**
42	2-3 days of soaking at 4 or 20ºC.
40	7 days in humid substrate at 15-20ºC; or 4ºC for 10-15 days; or 5-6 days soaking at 4ºC
38	Cold priming at 4ºC for 20-30 days; or Long aerated soaking at 4ºC
36	Cold priming at 4ºC for 30-40 days
34	Cold priming at 4ºC for 50 days (this is uneconomical due to loss of viability)

More than two days of soaking is counterproductive for the acorn because it cannot respire and anaerobic fermentation is promoted. If you wish to continue hydration of acorns after two days they should be placed in a humid substrate (at 20ºC or 4ºC) or the soaking solution should be aerated (using an aquarium air pump). Our experience at the time of writing this manual is that it is possible to rehydrate by soaking for at least five days at 4ºC with comparable results to cold priming. With an aerated soak we can certainly extend the rehydration period further. Our preference for soaking rather than cold priming is that it requires much less space to pre-germinate the same number of acorns.

Some acorns during storage will have been home to grubs. This occurs even if we have removed any floaters before storing them, because at the time of storage the grub had barely eaten any of the acorn or was still in the larval stage; that is why the acorn did not float. Therefore, it is necessary to place acorns in water once again after storage to remove any new floaters. These floaters might still germinate and it will depend on each project's aims whether to use them (although not all of these acorns will germinate because some will have had their embryo eaten).

When the acorns are removed from storage they will most likely need a bleach disinfection (10% bleach for 15 min) as the base of some acorns will have developed a fungal infection. Some of these acorns can be dissected to see whether the fungi have reached the endosperm, or they have only remained in the testa. It is possible that fungi may have broken through the casing in acorns with a low storage %mc. When dissecting these acorns we can also observe whether they maintain a white colour (≥40%mcfw) or they have yellow and darkened parts (≤40%mcfw; see images in 2.20).

If there is a long pre-germination treatment (and fungal growth is observed) another disinfection can be done before sowing. In the undesirable case that the roots have already emerged (because of their delicacy when transporting and planting), disinfection can also be done (reduced to 5% for 10 min and rinse well). It is recommended to wash the acorns by rubbing and disinfect at a higher concentration (up to 20% bleach for 30 min) if substantial development of fungi is observed

Cold priming at 4°C is the preferred method to promote germination in low %mc acorns (i.e., to rehydrate the acorns to 44%mcfw). If acorns are placed in a humid substrate at 20°C to hydrate them there is a risk of microorganism proliferation if the time spent at 20°C is prolonged, or if details of disinfection of substrate, container, acorns and water have not been taken care of. At 4ºC it is safer.

Cold priming requires use of a 3:1 volume of substrate:acorns. The substrate moisture should be at field capacity (~50% of the substrate saturation or use a feel test[31]). If the %mc of the acorns is low, after several weeks check that the substrate still contains enough moisture because it is possible that during rehydration the acorns have absorbed all the moisture of the substrate.

The logistics of an organisation's planting scheme should take into account the time required for cold priming. If there is sufficient rainfall and sowing is possible, then the first two weeks of sowing can begin with acorns that have close to optimal %mcfw in storage (plus a soak). Acorns stored with low %mcfw can begin cold priming when it first rains and then sown 3-4 weeks later.

In summary, it is important to know the moisture content of acorns in order to choose the most suitable pre-germination treatment. For this treatment, soaking in water at 20°C for 2 days or soaking in water at 4°C for 5-6 days, or cold priming at 4°C in moist substrate are the preferred options.

2.10 Planting date

Q. ilex acorns are usually harvested in November (or also in October or December, depending on altitude, temperature, etc.).

Sowing can be performed from the harvest date until a date that depends on the altitude (i.e. the mean temperature): the earlier the start of summer, the earlier an acorn should be sown, since summer arrives earlier at lower altitudes. Sowing is usually best done:

- <300 m above sea level: between November and December;
- >300 - <800 m above sea level: between November and January;
- >800 - <1200 m above sea level: between November and February; and
- >1200 - 1600 m above sea level: between February and March (after the ground thaws).

The earlier an acorn is sown, the better developed the root will be before the summer. If the mean temperature is less than 5°C the root grows very slowly, and planting can be delayed.

[31] https://www.cdpr.ca.gov/docs/county/training/inspprcd/handouts/soil_moist_feel_test.pdf

However, the sowing date depends on rainfall and it is always necessary to sow when there is moist soil on both the surface and at depth. We therefore need to store acorns correctly until there is sufficient rain. This is the key in Mediterranean ecosystems with sporadic rainfall. Until the soil contains enough moisture for the acorn to grow deep roots it is very risky to plant (since we do not know when it will rain again). The acorn would still germinate but if there has not been enough rain then its roots will not be able to penetrate deep into the soil. Depending on the type of soil, altitude, date, etc. this will require at least 100 L of non-intensive rainfall (i.e. 100 mm).

An example of this is that if there is 50 L (i.e., 50 mm) of rain in November and we sow, then the acorns will germinate but will not have access to moisture deep in the soil for the root to penetrate. In this case, if it rains again in January then the planting will be successful because before the root reaches the dry soil it will be soaked by the new rain. However, if it does not rain again until May the root will not be able to grow to depth until May and, until then, it will spiral in the wet soil. During summer survival will be poor even if an acorn has germinated well. Therefore, even if there is 50 L of rain in November, we should wait to see how the soil moisture continues to recharge. In our example, if it rained in January we would decide to sow then. If it rained in May, we would leave sowing for the following autumn/winter, even if it means a year without sowing and spoiling of the acorn crop (but note that with good storage we could save 50-60% of the acorns).

If the soil is very exposed (i.e., there is a lack of vegetation), south facing, at low altitude, or very sandy, the surface will dry out quickly. In such cases it is necessary to sow as soon as possible after rain. Once the acorn has germinated the root will grow to depth even if the soil is dry on the surface.

2.11 Rapid dry weight determination of a sample of acorns with microwave oven

The Backer method (Backer & Walz, 1987) is described below:

1. Dry a paper towel (although the amount of moisture on a paper towel is very low and will not make a large difference if this step is not followed).
2. Weigh the paper towel with a balance and record its weight (P_{paper}). See Section 2.13 for choice of balance.
3. With the towel on the balance, place the acorn(s) on the towel and record the weight (P_{fresh})
4. Cut the acorn into 2-3mm slices, taking care that no small pieces are lost. This step is only necessary for large seeds such as acorns.
5. Place the towel and acorn pieces in the microwave oven.
6. Heat in the microwave for four-minute intervals. Every four minutes remove the sample and weigh it. When the difference in weight is small start weighing at two-minute intervals until there is no change in weight. Lack of weight indicates that the moisture has been removed (P_{final}).
7. If several measurements are made in the microwave over a short period check whether the glass tray of the microwave is heating up. If it is, allow the tray to cool down before performing another measurement. If a sample starts to smoke or appears charred then start another measurement (with the microwave at lower power) after the microwave and tray cool down.
8. Calculate:
    a. Dry weight: $P_{dry} = P_{final} - P_{paper}$
    b. $\%mcfw = (P_{fresh} - P_{dry})/P_{fresh} * 100$

When the sample is removed from the oven and placed on the balance you will notice that the weight gradually increases. This is because the sample is very hot and it quickly absorbs moisture from the air. Each person should establish their own protocol for dealing with this. For example, remove the sample from the oven quickly and weigh it, recording the minimum weight before it begins to increase.

The preferred method for calculating %mcfw is by using a precision oven to hold pieces of acorn at 103°C ± 2°C for 17h. At Semillistas we have developed the hardware and software necessary to transform a domestic kitchen oven into a precision laboratory oven[32]. The help of a computer scientist is required to do this.

2.12 Calculation of seed moisture content

We will first explain the calculation of %mc in any seed and in the next section we will discuss acorns. We recommend practice with these procedures before formally using them, for example with lentils that you may at home.

We should aim to make accurate measurements. If a seed is lost or a small piece of acorn is misplaced then measurements will be incorrect. This requires order and attention and use of a spreadsheet to save time.

A practice example with %mcfw calculations for a set of seeds might be to calculate the moisture increase of a sample of soaked seeds:

1. We have a set of seeds in a plastic bag. We calculate its storage %mcfw with a small sample of seeds (e.g. %mcfw = 9%). We do this by using the microwave method described above or by using an oven set at 103°C for 17 hours.
2. We take another sample of seeds from the bag and see how its %mc increases if we soak them or leave them in the air.
3. When that seed sample was removed from the bag its %mcfw is 9%. We measure its fresh weight at that point (out of the bag, and before soaking) P_{fresh1} = 2.5 g. Knowing this, we can calculate the dry weight of that seed sample (P_{dry} = 2.275 g), using the formulae above. The dry weight remains constant while the seeds hydrate or take up moisture from the air.
4. We add the seeds to water.
5. At intervals we weigh the seed sample and calculate the new %mcfw. For example, 2h after soaking, and after drying the seeds superficially with a paper towel (to eliminate any water on the seed surface), they are weighed (P_{fresh2} = 2.6 g) and a new %mcfw is calculated, since we know the P_{dry} (%mcfw=12.5%).

Summary:

1. Measure the %mcfw of the bag in storage: 9%mcfw
2. Weigh a sample: P_{fresh1} = 2.5 g
3. Calculate the dry weight of the sample: P_{dry} = 2.5*(1-9/100) = 2.275
4. Weight after hydrating the sample for 2 hours, and drying superficially: P_{fresh2} = 2.6 g
5. Calculate the new %mcfw of the sample: (2.6-2.275)/2.6*100 = 12.5%.

Further details:

- It is prudent to measure several replicates when calculating %mcfw in a microwave or oven, as well as for monitoring of the %mcfw of soaked seeds. This prevents errors and establishes the degree of deviation from the mean.
- If we want to track the %mcfw of a seed during germination and it enters phase II, respiration (sugar consumption) will cause the dry weight of the seed to decrease over time and we cannot use this method after several days in phase II to find the value of the new %mcfw. The effect of this depends on the precision we want and the respiration rate of each species. In a small seed, such as pine, the error that we introduce in the calculation of the %mcfw after 5 days in phase II is of the order of 0.5%mcfw if we use the initial dry weight of the seeds as a constant,

[32] All documentation can be found at https://gitlab.com/semillistas/seedlab

2.13 Measurement of harvested acorn %mcfw

Several strategies can be followed at different times to measure the %mcfw of harvested acorns. We can also use a microwave either to calculate the %mcfw of a batch of acorns or for each acorn separately (so we have an idea of the variability of %mc within a batch of harvested acorns and can calculate the mean and standard deviation of the batch). For good sample accuracy the %mcfw calculation must be performed with at least 25 acorns.

Strategy 1

1. After harvesting keep the acorns in batches in bags (either from each tree separately or together);
2. Remove leaf and cup debris from each batch;
3. Disinfect the acorns with bleach;
4. Spread out the acorns for drying out of direct sun and wait for them to surface-dry (use a fan and remove dry acorns);
5. Remove two samples of 10 acorns (G1 and G2) and surface-dry them further if water remains;
6. Weigh G1 (WFG1) and leave them to dry together with the other acorns (but do not mix);
7. Calculate the %mcfw of the other 10 acorns (G2) in a microwave or oven (collectively or separately) and calculate the mean %mcfw;
8. Assume that the initial mean %mcfw of G1 and G2 was the same, so we can now calculate the dry weight of G1 (of the set of acorns) and thus calculate the %mcfw as the batch dries:
    - The %mc of G1 will be the same as G2: %mcfwG1 = %mcfwG2
    - Calculate the dry weight of G1: WDG1 = WDG1 * (1 - %mcfwG1/100)
    - At intervals weigh the acorns in G1: $WFG1_{new}$
    - Calculate the new %mcfw: %mcfw = ($WFG1_{new}$ - WSG1)/$WFG1_{new}$ * 100
9. When G1 has an appropriate %mcfw, store the batch in LDPE bags at 4^{o}C (when storing the acorns they should be surface dry, without water residues).

Microwave measurement is rapid and can be done while the acorns are spread out and surface-drying

Strategy 2

Surprises are avoided if the %mcfw is calculated as soon as the acorns are harvested so that on the next day, when cleaning of batches is complete, we already have data on which to base decisions. These situations can occur:

- The %mcfw is higher than 43%: proceed as explained in strategy 1.
- The %mcfw is between 40% and 42%: proceed as before, but it is not necessary to calculate %mcfw again. The acorns are bagged after disinfection and complete surface drying. Since exposed acorns lose 1.5%mc per day, provide just a few hours of drying to make sure that they are well surface-dried.
- The %mcfw is between 36% and 40%: the acorns cannot be stored optimally. We therefore proceed as in the case of those between 40 and 42%. When we receive these acorns from the harvest we can store them covered in plastic sheet to prevent them from drying out more. If there are not many of them it is better to bag them and place in a refrigerator.
- The %mcfw is less than 36%: proceed as in the case of those between 36 and 40%, although there is a risk that the % germination after storage will be very low.

Having monitored the %mcfw we will know at what %mcfw the acorns are stored. When harvesting large quantities of acorns we can perform a confirmatory measurement by re-measuring the %mcfw about two weeks after storage begins.

With this information we can choose how to pre-germinate the acorns for sowing in the field.

2.14 Choice of balance

Balances can be purchased with different degrees of accuracy, cost, and reliability, from 0.1 mg with manual calibration at 1000€, to 1 mg without calibration at 20€. We can opt for a cheap balance for use in a small reforestation project with acorns, but we need to take into account the error they introduce, which depends, in addition to their accuracy, on the weight of the sample.

In the Table below show some examples of errors that depend on the amount of seed weighed and the accuracy of the balance.

For example, if we measure a %mcfw of 8.3% with a 0.001 g accuracy balance on a 1 g sample, our measurement will be %mcfw = 8.3 ± 0.195%.

The Table shows in brown the error that can be assumed in our calculations (less than 0,1%). Depending on the need of the calculation to be performed, it would be possible to assume an error of 0.2% or higher.

An acorn weighs between 2 and 10 g. If we weigh acorns individually we will need a 0.001 g accuracy balance. If we weigh a group of acorns, a balance with accuracy of 0.01 g will be sufficient.

Error in %mcfw	**Sample weight (g)**									
Balance accuracy (g)	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.7**	**1**	**2**	**5**	**10**
0.0001	0.1950	0.0975	0.0650	0,0488	0.0390	0.0279	0.0195	0.0098	0.0039	0.0020
0.001	1.950	0.975	0.650	0.488	0.390	0.279	0.195	0.098	0.039	0.020
0.01	19.50	9.75	6.50	4.88	3.90	2.79	1.95	0.98	0.39	0.20

Calculated with error propagation method of partial derivatives, assuming the worst case.

There are Chinese manufactured balances with 0.001 g accuracy that measure up to 50 g maximum, and others with 0.01 g accuracy that measure up to 300 g. Both cost around 20-30€. Although these balances do not have manual calibration we do not need more than this for use in adjusting acorn moisture. In any case, to gain accuracy, before going to the more expensive scales, we can buy test weights to identify the error that a cheap balance introduces.

2.15 Choice of planting microsite

In semi-arid areas of the Mediterranean countryside, survival is greatly improved by planting acorns under the shelter of shrubs or nurse plants. A nurse plant is any herbaceous plant or shrub that has a facilitating effect on the survival of another plant that grows under its protection. What occurs as facilitation in the Mediterranean becomes competition in environments with an Atlantic or Northern European climate.

Facilitation occurs at several levels when compared to planting on bare soil:

- Reduction of soil moisture loss due to the effect of the nurse plant's shade;
- Protection from direct sunlight during the summer;
- Improvement of microsite climatic characteristics;
- Protection of young seedling from herbivores;

- Rapid access to the nurse plant's endomycorrhizae; and
- More favourable microbiota in the soil under the nurse plant.

Each species may have a nurse plant that suit it better than others, depending on its needs. The best way to choose the "perfect nurse plant" for a species we want to plant is to observe any natural regeneration in the border zone of our reference ecosystem. At high altitudes (over 1000 m), holm oaks grow in the interior of broom (i.e., Cytisus oromediterraneus, Vella spinosa, etc). At lower altitudes they often grow inside broom. We will not always be able to plant within these nurse plant, but they will give us an idea of the conditions preferred by the plants we want to introduce. In the case of holm oaks these conditions are surrounding plants with spikes or low branches to protect the acorn from wild boars, which let ~60% of sunlight pass through (e.g., broom), and as hidden as possible from herbivores during the first years of growth.

The following text and figure are from a study conducted in the Sierra Nevada (Castro et al. 2002, 2004) that demonstrates the facilitating effect for survival of a reforestation carried out with nursery-raised plant transplants:

"Seedling survival also depended on the nurse plant. The effect ranged from a high increase in survival under legumes and shrubs (d+=1.74 and d+=0.98, respectively), to a negative effect of cistaceae (d+=-1.52), the only group that did not favour seedling survival. As for environmental gradients, facilitation by shrubs was greater at low altitude (d+=1.90) than at high altitudes (d+=0.80), greater in the solana (d+=1.52) than in the umbria (d+=0.90), and greater in dry years than in the rainy year."

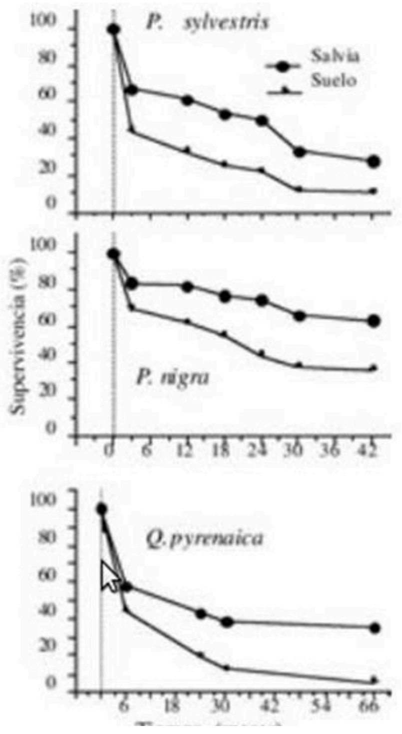


Figure 11. Pinus sylvestris, Pinus nigra, and Quercus pyrenaica percent seedling survival under nurse plants (Salvia) versus the traditional planting technique (in bare soil) throughout the sampling period (four years for P. sylvestris and P. nigra; six years for Q. pyrenaica) (Castro et al. 2004)

Nurse plants can grow very thickly in the case of holm oak. If there is low light (less than 60%) the oaks will take longer to grow but will have a better chance of surviving the first summer. If a nurse plant is tall (e.g. a gorse 1 m high), the holm oak will be well protected and will grow slowly until it exceeds this height and receives more light. This is also the case with the planting of acorns in reforested pine plantations where there is little species diversity. In this case, the oaks will grow slowly under the shade of the pines but their root system will become extensive. When the pines fall (e.g. from snowfall, wind, or logging) these small oaks will grow rapidly. It is said that a holm oak can live for 200 years waiting for the pine tree that shades it to fall. This work of acclimatizing repopulation pine groves is an almost infinite task on the Iberian Peninsula.

Semillistas uses the north side of each nursery for sowing because these sites are better at conserving soil moisture during the months after sowing.

In addition to individual nurse plant there is also the concept of “islands”. The regenerating Mediterranean scrub can be viewed as a collection of shrub islands. Each island comprises several species growing together and intermingled. Between these islands there are corridors through which larger forest animals (e.g., foxes, wild boars, and goats) travel. If we sow in these corridors that plantings will be quickly trampled and destroyed (graph 9).

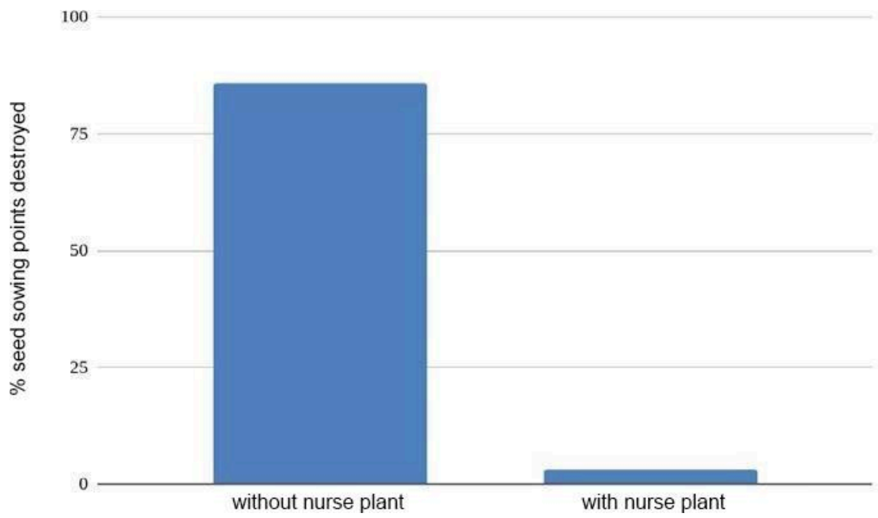


Figure 12. Percent of bolina blows destroyed, 6 months after sowing (Semillistas, 2021).

In nurse plant, whether individual plants or islands, there are special areas that we call "untouched areas". For example, 1 m² of dry, half-decomposed gorse where it is clear that no animal has passed through for years. Or there may be decaying material under a rosemary plant that has not been moved by wild boar for years. Use of these places for planting ensures that the new plant will remain undisturbed.

Finally, once the microsite has been chosen, we can simply plant or make a small pit. The high possibility of torrential rains in the Mediterranean means that sloping areas, clay soils, and areas with sparse vegetation will retain more water if we make a small pit or infiltration trench. We are not talking about large ditches or keyline systems, but rather something similar to the sub-Saharan Zai system[33], but in a soil full of nurse plants. Semillistas dig these trenches on the side of the nurse plant so that the ridge of the trench connects with its trunk or branches. We then sow the acorn on the north side of the nurse plant, outside the trench. The choice of whether to make a trench, and how much effort to expend on this, will depend on each plot and the experience of the planter or previous trials in the area. These small trenches make sense if they improve survival in the first two years and will not persist much beyond this. That is why we call them small trenches and they should be both easy and quick to construct.

2.16 Predation by mice and wild boars

[33] https://farmingafrica.net/2014/09/zai-system-overcomes-desertifacation/?lang=en

Ecology of dispersion

Before we discuss planting by humans we will consider the relationship of acorns with rodents and wild boar. In dry areas, when the acorn falls from the oak tree it dies after a short period. The dispersion of acorns depends on the action of jays and rodents, which take the acorns and bury them in their home territory for later consumption. Jays can carry acorns away from the mother oak while mice extend the forest boundary little by little. More information on the ecology of acorn dispersal by mice can be found in Muñoz & Bonal (2011), Sunyer et al. (2015 & 2016), (Villalobos et al. (2020), and Gomez et al. (2003).

An interesting curiosity is the interaction between weevils and mice (Perea et al., 2012). The acorn weevil (*Curculio elephas*) lays its eggs in acorns, which then hatch, feed on the acorn and fatten into the typical maggot. This maggot emerges from the acorn, making a visible hole, and then buries itself in the soil to continue its metamorphic cycle into following year when it becomes a flying weevil that lays eggs in another acorn. Perea et al. (2012) observed that when mice eat acorns under an oak tree they prefer those with maggots. When they have eaten enough they then preferentially select acorns containing a maggot to bury nearby for future consumption. Many of these hidden acorns are eaten by either the same mouse or other mice during the winter, but some remain uneaten and manage to germinate. This suggests that historically it is more probable that more oaks originated from acorns with maggots than from those without. The opposite is now the case with mainly anthropic regeneration.

Mice and wild boar detect acorns by their scent (Engman, 2020). It seems that the ecological reason for acorns to produce volatiles with an odour is to attract microorganisms, because the survival of an oak sapling depends on rapid symbiosis with endomycorrhizae. Without odours the microbial spores are not prompted to germinate. An acorn can remain attached to its sapling for up to two years after germination, emitting volatiles and supplying nutrients. At Semillistas we consider it essential to understand the period of volatiles emissions as this determines how long we must maintain physical barriers to wild boar.

The smell of acorns when they are germinating and rehydrating is quite noticeable to humans. But when they are drying we cannot detect their smell. In the USA a squirrel species was studied that only found acorns when they were in the process of rehydration. There are no studies on this in any Mediterranean country. Once, in Semillistas we planted acorns in dry soil to check for rodents and the acorns were not predated until after a rain. It is therefore possible that acorns do not emit volatiles if they are not germinating.

Before closing this section on dispersal ecology we would like to suggest one way in which wild boar may have a valuable ecosystem role in relation to acorns. At first sight, wild boar are acorn-eaters and seem to hinder their natural regeneration. However, by eating acorns under an oak tree and disturbing the soil they also ingest large quantities of endo- and ectomycorrhizal spores. They then deposit their faeces throughout the ecosystem. If they did not do this work there would be no endomycorrhizal spores in areas without holm oak, since these spores are not dispersed aerially like ectomycorrhizae. We might therefore hypothesise that wild boar are the major dispersers of endomycorrhizal spores.

Restoration

When we consider direct seeding, in addition to the proper management of acorns to ensure their germination, a big problem is their appeal as food to numerous animals. We refer mainly here to mice and wild boar, which are the ones that we experience.

There are usually many mice in the countryside: on average 1000 per ha. There are areas where there are temporarily no mice and others where they always live. Before sowing it is prudent to survey the area to be planted to determine mouse predation pressure. Wherever we sow In the Sierra de Lujar mice consume <u>all</u> the acorns in less than three days. Sometimes wild boar consume the acorns even faster than the mice. Surveying for this consists of sowing a few acorns and observing them over the following days and should be done during the sowing period. The Mediterranean mouse population is low in the summer (in contrast to northern Europe) and remains at a low population level until it rains in autumn and food resources increase. We should therefore not carry out surveys in summer but should perform them at sowing time (if there is rain in the autumn, winter or spring). Surveying should be extended to areas bordering our area of activity since it is likely that we do not have mice in our area, but they move in from nearby areas after sowing.

If it has not rained much there will not be many mice (and we will not be able to plant either because we need to wait for sufficient rain). When it rains we may initially be able to sow without many mice, but the wild boar will be hungry and can smell acorns from far away. In the Sierra de Lujar in years of low rainfall and low production of acorns and other wild boar food, no planting lasted more than a single night. Boar were even able to bite through the wire mesh used to protect acorns. When there is sufficient rainfall and food in the countryside this does not happen.

Before we examine the available options to stop predators, let us look at what does not work:

- Repellents. No effective solution has yet been found. Even predator odour (e.g., mink) has been tested on mice, but without clear results. Cayenne (capsaicin) causes some repellent effect, but reduces acorn germination to 50% and also delays it (Villalobos et al., 2019; Birkedal 2010). RTA50 (empyreumatic oil) is also marketed to deter wild boar. This has a persistent odour but needs continuous applications and is not 100% effective.
- Odour barriers. No effective solution has yet been found. Biochar is known to act as an odour barrier but no conclusive results have yet been obtained.
- Toxic substances. The toxic ingredients in oleander can kill mice and wild boar and are a risk to humans. Their use is prohibited. Rodenticides were used to kill rodent populations before planting in the past.

The only way to stop mice and wild boar currently is to use physical barriers to obstruct mice, wild boar, or both:

- Reque Protector (Reque & Martin, 2015) (Photo 2 in Section 2.20). Construction of this protector is slow and very expensive, but it does not require subsequent removal.
    - The acorns are enclosed in a metal mesh to protect them from mice. The lower wing prevents a mouse from gaining access by burrowing and could lead to the design of other simple devices to prevent mouse access, e.g., a prism (half seed shelter) with a lower wing. Mice have no memory; the protector will be attacked successively over time by many mice.
    - Protection against wild boar, based on their memory. A boar may dig up the guard but it cannot access the acorns and, while trying to do so, it punctures the roof of its mouth on the guard's wires. When the boar digs up a few more guards it learns that this is useless and stops digging; however, it is necessary for all the wild boar in a sowing area to learn this. Semillistas has tried this in the Alpujarra (a region of abundant food) and it worked. We assembled 300 units and planted them in the Sierra de Lujar in a year when the wild boars were hungry due to a lack of rain and therefore cut the guard wires with their teeth.
- Protection solution for mice (see point 2.21). Inexpensive materials (0.3€/unit), but slow installation (5-7 min), and removal of the protector is required afterwards. The protector is based on a mouse not persisting when it encounters an obstacle. We have discarded this solution because of the high installation cost.
- Seed shelter (Photos 3 and 4 in Section 2.21). University of Granada patent (Castro et al., 2015). This uses a non-biodegradable material (polypropylene) and a single company (Sylvestris Group) has the marketing rights (0.43€/unit). Prior preparation is required to assemble the shelter and fill it with substrate and acorns prior to planting. Installation in the field is simple and subsequent removal of the protector is not required. This protector works perfectly with mice and has the great advantage of being easy to install in a way that is very similar to planting an acorn. A biodegradable material for this design that resists mice has not yet been found, but at Semillistas we are testing a design with pressed cardboard in 2024.

- Broom plants (Photo 6 in Section 2.21). We are experimenting with this to stop wild boar at Semillistas. The approach requires a seed shelter so that it can be easily installed inside the broom plant. This method should be useful for preventing mouse and boar predation in areas where these bushy structures exist. It is relatively easy to sow acorns in this way, although hand and arm protection are necessary to avoid pricks from the broom (e.g., Cordura® fabric). We are fine-tuning the installation method in which we install the acorn without breaking the broom, so that the boar decides not to damage its snout on the broom's spikes. The results are satisfactory so far.
- Electric fence for wild boar. Addition of a decoy is 100% effective for an electric fence to work for wild boar (Photo 1 in Section 2.20). Without a decoy the boar will go through the fence without any problem, as its thick skin and hair act as an "armour". The decoy causes the boar to approach slowly and fearfully to investigate the foreign element. When it approaches the decoy it does so with its snout, which is its vulnerable part, and that is where it is shocked. An approved electric fence does not leave any after-effects, just a sharp pain which is stored in the boar’s memory. The disadvantage of this method is its installation and maintenance cost. We are studying how long it is necessary to leave a fence energizer active before the boars lose interest in the protected acorns. We use an internet-connected fence voltage sensor to help us respond quickly and reduce maintenance costs (a fence alarm from Luda.Farm[34]); we have also started to design an open source device to better meet our monitoring needs).

Below you can see the costs of some of the systems described above. These are the operating costs plus organisational costs. The percent survival indicates survival with respect to the number of seeds sown (which we have defined as Type 2 survival in Section 2.17). The cost is calculated for a plot of 3 ha, near a track or road, at a distance of 45 min from the meeting point with workers, and includes salaries, social security costs, and the indirect costs of the organisation. It is calculated based on two projects implemented in 2023 in the Sierra de Lujar, at 1000 and 1600 metres above sea level.

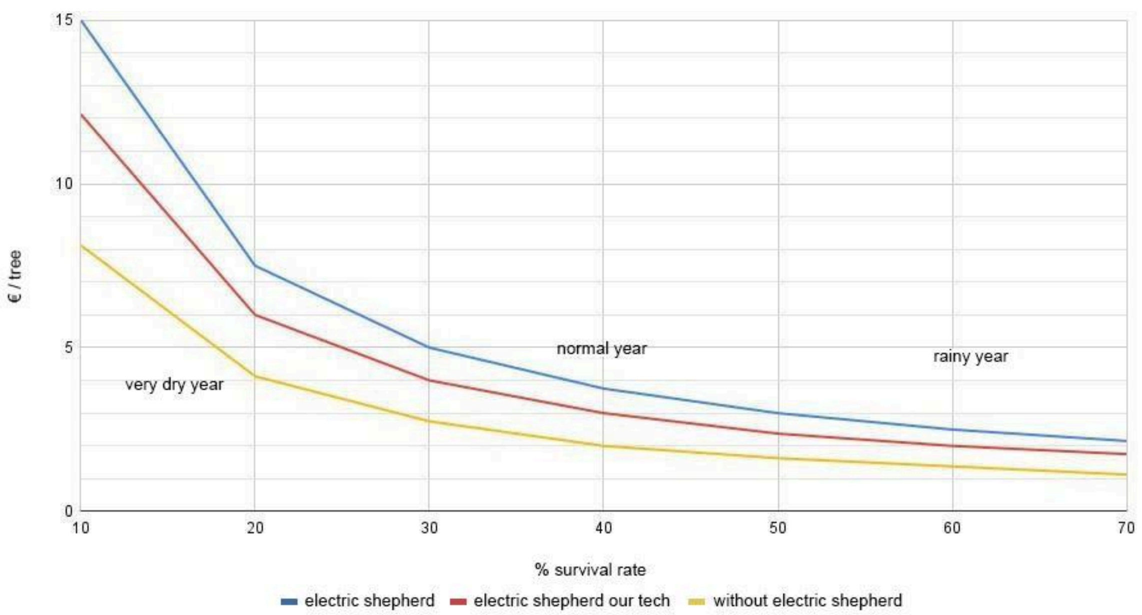


Figure 13 Cost of planting acorns on 3 ha (Semillistas). Blue line - electric fence, seed shelter and commercial remote monitoring; red line - electric fence (EC standard), our rodent protector and our remote monitoring. This is an estimate, as we have not yet finalized the development of these solutions; yellow line - without electric fence: sowing within broom plants, with seed shelter.

Other possible options or lines of research to prevent rodent or boar predation are:

- Observation of wild boar population movements and tracking of areas without mice. We have

[34] https://www.luda.farm/products/luda-fencealarm

observed that wild boar use human tracks and trails as highways for their movements. Planting far (more than 100-200 m) from these increases the chance of success. But this is very risky as boars should not approach the area for 1-2 years.

- Steel wool to obstruct mice. This inexpensive and biodegradable material is used in construction to stop mice. The fine steel wires cut the mouse's lips when it tries to pass through and discourages further progress. At Semillistas we have enclosed acorns with moist substrate inside a steel wool ball with good results against predation, but the air left inside the wool prevents the root from growing through the steel wire and it is left spiralling in the small amount of substrate inside the wool ball. We think the approach is promising but needs further research. For example, a cellulose material could be designed with steel wool fibres.
- Can acorn odour be eliminated for two years? This idea needs more research before there is a viable solution. Biochar could be used as a barrier to the movement of volatiles. Our experience trying to mask acorn odour has not been satisfactory (using RTA50 or essential oils).
- Can we take advantage of the fact that a mouse can eat an acorn after germination (for example, after the first summer of seedling growth) so that a wild boar will no longer act? To reduce the costs of the electric fence we need to reduce the time it is installed and maintained. If, after the first summer, the acorn is removed from its seedling the wild boar would not be a problem. However, this is very expensive to do manually. If, on the other hand, the mouse guard allows acorn protection for a while and then degrades, mice could remove the acorns and we would no longer need to maintain the electric fence to deter wild boars.
- Seed shelter with biodegradable material. Introducing polypropylene into an ecosystem is not a sustainable solution for us, although we temporarily plan to use shelters made in this way. We are searching for solutions with geotextiles, pressed cardboard, and other materials.

2.17 Brief introduction to the use of microorganisms in forest restoration

We cannot deal extensively with microorganisms because the subject is too complex and we have little experience in this area. We therefore consider two aspect of this subject and list in the bibliography some information that seem to us to provide some basic information, as well as some interesting academic studies:

- Brundrett, 1996. It is the bible of symbiont microorganisms.
- Restrepo (2007), Facio (2017), and Simon (2014). Bioreactors, mountain microorganisms, and applications in agriculture.
- Biotechnology for the extraction of microbial communities from healthy soil (by freeze-drying) for introduction into disturbed soils: post-fire in the Guajares (Niza Costa et al., 2023) and post-mining in Australia (Pedrinni S. et al).
- Tichavsky, 2018. Holohomeopathy.
- Ectomycorrhizal independence (Oria 1991; Garcia de Jalón et al., 2020)

Microorganism tourism

With the rise of microbial knowledge and the benefits microbes bring, large agricultural multinationals are breeding soil microorganisms in Canada, which are of New Zealand origin, and selling them to farmers in India. Globalization has eaten into the ethics and care of aspects that we still do not know the consequences of.

We believe that just as we take care of the provenance of the genetics of our trees and shrubs, we should do the same with the genetics of fungi, bacteria, and other microscopic life.

Extraction of endomycorrhizae

Plant species form mycorrhizal associations with specific fungal species. Ectomycorrhizae spread their spores through the air, but endomycorrhizae sporulate inside the roots of the host plant. If the ecosystem is degraded or at an early stage of plant succession the endomycorrhizae necessary for the trees and shrubs we want to introduce will not be present. At Semillistas we wrap seeds with these endospores (specific to each plant species and of local provenance) before sowing to increase the chances of an optimal symbiosis as soon as possible.

The endomycorrhizal spores are harvested from the fine, superficial roots of different plants of the species we want to introduce. We choose an unimpacted place in the local forest, make a small hole near the trunk of the tree we wish to sample from and collect fine roots and soil (the old fine roots are part of the organic matter of the surface soil) in very small quantities. We do this for pine, mastic, buckthorn, and other species to obtain plant-specific symbionts. We crush the roots and sieve the soil and the resulting powder is used to wrap the seeds.

2.18 *Quercus* direct seeding and planting studies

Two studies in Andalusia, in addition to data from Semillistas' sowings in 2023, are discussed in this section to show the effectiveness of direct sowing of acorns compared to planting of saplings

We must distinguish between different ways of calculating survival (Figure 14):

- Survival rate (type 1) of live plants after summer compared to the number of live plants before summer;
- Percent survival rate (type 2) of live plants after summer with respect to the number of seeds sown; and
- Germination percent (live plants before summer in relation to the number of seeds sown).

At Semillistas we use the germination percent (which reflects the success of our decisions regarding the storage process, sowing date, and sowing microsite) and Type 1 survival percent, which gives a clear idea of survival with respect to live plants before the summer and depends on both our decisions and that year's weather. We do not use Type 2 survival percent because it mixes two almost independent processes: germination and surviving the first summer.

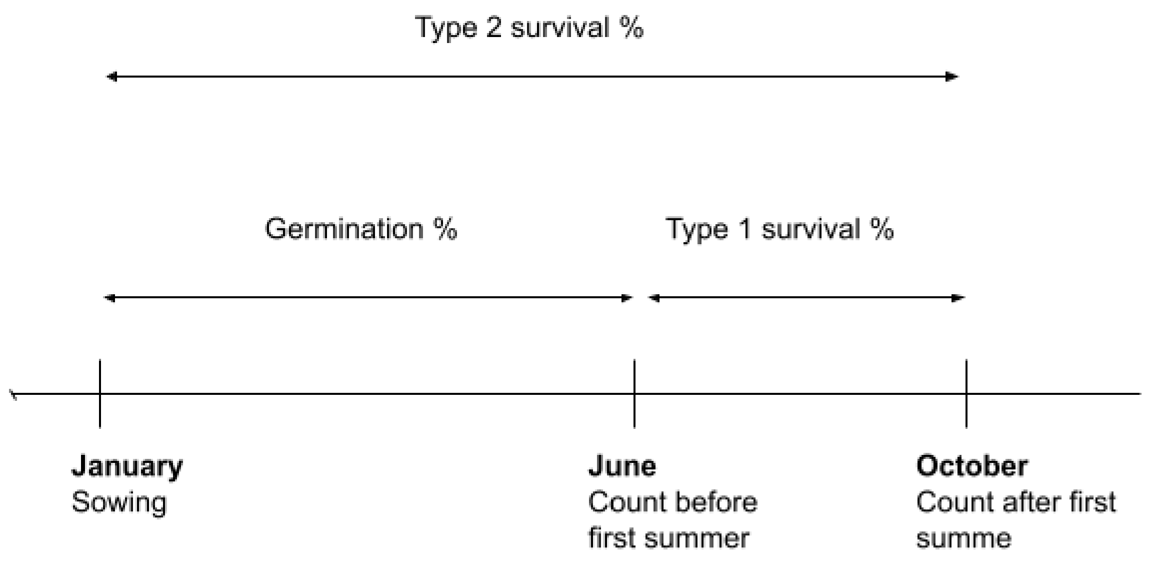


Figure 14 Methods for calculating plant survival

Experience of oak regeneration in the northern mountains of Seville (Tejeiro et al., 2004).

Type 2 survival of 288 seeds or seedlings several months after sowing or planting was studied. The month 0 in the Table below is November and planting was by hand in bare soil in the dehesa near Seville at 300 m above sea level with no summer irrigation. The data in the Table require explanation. Acorns alive at month 8 correspond to those that actually germinated before the first summer and therefore survival at 41 months is 29.9% with respect to the number of acorns sown, but 78.8% with respect to those that actually germinated. On the other hand, in the case of planting at 1 sap, only 34% survived at month 8, or 18% at month 41. In other words, if we compare Type 1 survival, we have survival of 78.8% when sown and 18.3% when oak saplings were planted.

	Mes 0	Mes 8	Mes 16	Mes 41
Bellotas	100	41,07	38,39	29,91
1 savia	100	34,82	27,68	18,30
2 savias	100	28,57	14,73	8,48

Deep sowing with the aid of protective tubes. Results of comparative sowing and planting trials under arid conditions in Vélez-Rubio (Carreras Egaña et al., 1997).

Planting was near Vélez Rubio at 1000 m above sea level on a 20% west-facing slope. The site was subsoiled to 50 cm and there was no irrigation, with planting in January in bare soil. The Table below shows Type 2 survival one year after planting. During the study year only 100 L of rain fell in October.

Deep sowing (first column) was at 9 cm below the soil surface (a 9 cm hole was made and acorns sown at 6 cm below the new soil level).

As in the previous example, not all the seeds germinated and the percent survival rate shown is in relation to the total number of acorns sown, not to the acorns that germinated. Data for Type 1 survival cannot be calculated from the data presented. However, the low survival of the shallow sowings (0-13%) is probably due to non-germination, rather than Type 1 non-survival, as the sowing was 3 months after rainfall and the soil at 6 cm depth may have been too dry.

Similarly, the high survival of the deep seedings may be due to high germination (at that depth there was moisture to complete germination), rather than high survival of the plants that did germinate.

This shows how important it is to know what percent survival refers to for good data analysis.

	6 + 9 cm + tube	**6 cm + tube**	**6 cm without tube**
Acorns sown	56%	13%	0%
Planted trees	51%	14%	4%

Planting in the Sierra de Lujar (Semillistas, 2023)

Rainfall for this hydrological cycle (2022/23) was 15 L in November 2022, 235 L in December 2022, and 106 L at the end of May/beginning of June 2023 (SAIH Hidrosur data for the summit of Sierra Lujar).

Olías Characteristics

- The less rocky zone is about 30-40 cm deep before reaching a fractured bedrock. The more rocky zone has this fractured bedrock emerging at the surface.
- Soil with presence of clay and a NE orientation. Dense cover of mainly esparto grass, rosemary, rock roses and gorse, with some scattered oaks.
- Electric fences and cone and mesh mouse guards were installed.
- Acorns were stored at 39%mcfw on average. Prior to seeding they had 2 to 6 weeks of cold priming.
- Planting was carried out between the end of February and the beginning of March 2023, in moist soil at a depth of about 4 cm.
- With the exception of a few sowings, the chosen microsite was in the interior of shrub islands, preferably on their northern side.

Summit Features

- Zone of fractured rock emerging at the surface.
- Soil with presence of clay and an N orientation. Dense cover of broom and sage.
- No protection from predation. Of all the areas planted at the summit, this was the only one where there was neither mice nor boar activity.
- Acorns were stored at 39%mcfw on average. Before sowing they were soaked for two days.
- Sowing was carried out on December 22, 2022, in moist soil at a depth of about 6 cm inside nurse plant.

Germination was better at the Olías site than at the summit, probably due to:

- Cold priming carried out for Olías acorns; and
- Heavy frosts in January and February at the summit, which froze the soil to a depth of 3 cm. This perhaps reduced the viability of germinating acorns.

Type 1 survival at the summit was somewhat higher due to the altitude, which implies a shorter summer. Type 1 survival at Olías depended on soil depth.

	Olías (1000 m above sea level)			**Summit (1500 m above sea level)**
	Very rocky area	**Less rocky area**	**Entire plot**	**Entire plot**
Germination %	61	63.4	66.7	41.1
Type 1 survival %	29.4	77.9	59.6	86.1
Type 2 survival %	17.8	49.4	39.7	35.4
sampled blows	14	82	578	123

2.19 %mcfw of acorns at harvest

To conclude this presentation of Semillistas' experience over the last few years, we are going to discuss the moisture content of acorns at harvest time. This is something that we have not yet been able to explore in depth, but which we present as a tentative understanding of optimal harvesting. We are sure that in the future we will have more a comprehensive idea.

The objective of this section is to understand the condition of acorns at the time of harvest so that we may decide when to harvest and how to store them. The genetic variability of each holm oak leads to great variability in acorn maturation. In this section we analyse some cases to help develop understanding of this variability so that each project can make its own decisions.

Acorns should be picked from the tree (not from the ground, as they will probably be too dry) and all should be ripe (with a little green and a lot of brown colouring, so we are sure that they are already below 44%mcfw; if green acorns are harvested at the same time as green and brown ones they should be allowed to dry below 44%mcfw). The time of harvest is critical because when acorns are ripe they fall from the tree. Each tree has a different acorn maturation time and it is important to collect acorns from trees with different maturation dates (so that there is genetic variability in flowering time). This ensures subsequent acorn production in the area we are regenerating because flower set can be affected by late frosts or high soil moisture. Ensuring a wide flowering period provides resilience to the ecosystem that we are helping to regenerate. At Semillistas we collect mature acorns at least at the beginning and end of November.

We analyse moisture content data from different batches, each with acorns harvested from several trees that we place together in the same batch if the external colour characteristics are similar. Figures 15 and 16 show the mean %mcfw and the standard deviation for different batches. The measurements were made on individual acorns in order to provide information on the %mcfw range of a batch.

Our earlier recommendation for the measurement of %mcfw of a batch to decide the pre-drying time before storage in LDPE bags, is to perform the calculation with a sample of acorns, in order to obtain the average of the batch. The analysis we present (with measurement of individual acorns) is intended to understand better the condition of the acorns at the time of harvest, rather than to help us make storage decisions.

The first set of batches we are going to look at was harvested at the end of November 2023 (Figure 15). The acorns were kept in paper sacks at 15-18ºC until cleaning (during the whole process from tree to %mcfw measurement we estimate that no more than 0.5%mc was lost). During harvesting acorns from different trees with similar maturity characteristics (external colour) were mixed.

Figure 15 shows different types of individual acorns:

- "m": 100% brown.
- "pv": brown with green tip (less than 10% of green acorn cover).
- "25% v": brownish green with 20 to 30% green acorn cover.

Characteristics of each lot:

- A1 50-70% were brownish green, rest "m" and "pv".
- A2 95% were "m", of which 20-30% were "pv". The remaining 5% were brownish green.
- A3 99% were "m", and 1% were "pv".
- A4 15% were brownish green, the rest (85%): 50% "m" and 50% "pv".

(Note: brownish green means that the lot has acorns with a testa from 100% green to 15% green. An acorn with less than 10% green is called green tip).

The %mcfw of individual acorns (just after cleaning) was measured for 10 to 15 acorns from each batch, except A2pv and A4m, for which only 5 acorns were measured. A 0.1 mg accuracy scale was used. We know that this analysis should use more acorns but the economic resources of Semillistas are very limited.

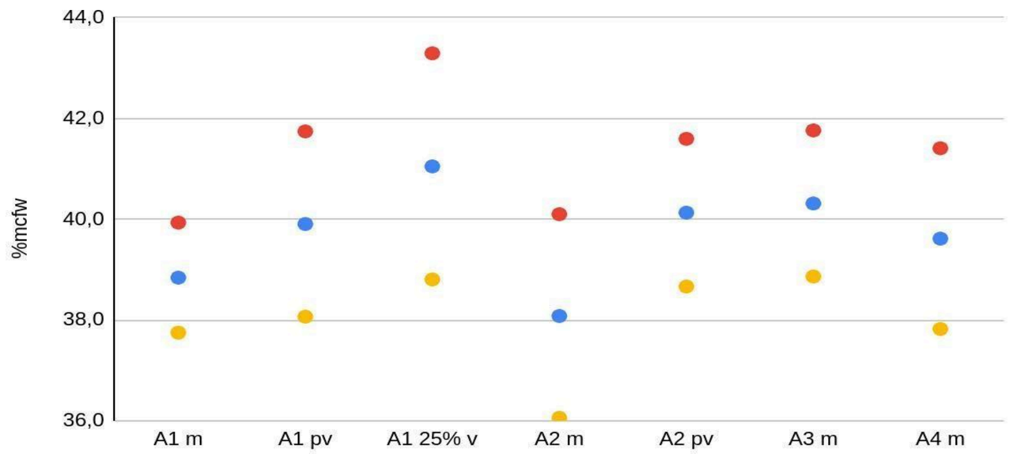


Figure 15. Mean (blue) and standard deviation (red-yellow) of acorn %mcfw one day after harvesting (Semillistas data).

Batch A1 has acorns in quite different stages of maturation. Acorns that are already brown are around 39%mcfw, those with green tips are ~40%mcfw, and those with 25% green are ~41%mcfw. The batch had brownish green acorns with a higher percentage of green, for which the %mcfw was not measured, but which we know to be higher (43-48%mcfw). This case is the most complex in deciding how much to let the acorns dry before storage as there is considerable disparity among the acorns in the batch. Due to the high percentage of green acorns we decided to let them dry for two days and then to store them (on average they lost 3%mcfw). We thus ensured that most of the brownish green acorns had dropped below 44%mcfw, although the brown acorns stayed at a low %mcfw.

Batch A2 is more homogeneous. The brown ones have 38%mcfw on average, while the green-tipped ones have 40%. These acorns should be stored immediately.

Batch A3 is even more homogeneous. They are all brown and between 39% and 42%mcfw, but have a greater %mcfw than the brown acorns in batch A2, because the latter were on the tree and drying for longer without falling off. These acorns should be stored immediately.

Batch A4 is less homogeneous. The brown acorns have between 38% and 41.5%mcfw. Those with green tips should be 1% higher (not measured). This is a similar batch to A2, but A4 would have been on the tree and maturing for a shorter period (there is a greater percentage of brownish green acorns) and its brown acorns still have almost 40%mcfw on average. Since there are some brownish green acorns in this batch it is best to lower the %mcfw. Allow one day of surface drying before bagging these acorns.

Conclusions:

- Each tree has acorns within a different moisture content range, depending on the their stage of maturity.
- If most of the acorns are already brown we will not know how long they have been on the tree and they may have a %mcfw of 36% to 42%. If the dry climate of Andalusia is not relevant to a project then it is possible that drying on the tree, when the acorns are already brown, will not be as pronounced.
- If the acorns are brown but there is a high percentage of green tipped acorns we know that the

brown ones are at around 39-40%mcfw and the green tipped ones at around 40-42%mcfw.

- If there is a high percentage of brownish green acorns at harvest, the moisture content of these acorns will be above 42-44%mcfw, although acorns that are already brown will have dropped to 39-40%mcfw.

Recommendations:

- As soon as possible after harvesting, clean and calculate the %mcfw to decide whether to dry the acorns a little more or to store them.
- If many trees are harvested it is possible to reduce the number of batches kept separate for the %mcfw calculation by separating acorns that are at similar maturity times. For example, batch acorns that are:
    - 100% brown.
    - Brown and green tip, with very little brownish green.
    - Brown and green tip, with ~30% brownish green.
    - Brown and green tip, with ~60% brownish green.
    - Greater than 75% brownish green.
- If fewer trees are harvested they can be managed separately as each one will have a %mcfw within a different range.
- It is not possible to keep separate batches based on how long they have been brown on the tree, since we do not know their maturity (unless we have monitored their maturation rate). If we want to be very cautious we would not mix brown acorns from different trees as the acorns from each tree will have a different drying time. However, this is not practical because of the workload involved, unless we have a very abundant harvest from each tree.

We are now going to analyse another harvest, this time from early November (Figure 16). The nomenclature we use differs a little from the previous example. We are still looking for the best way to express these data and this example was our first. Apologies for the inconvenience.

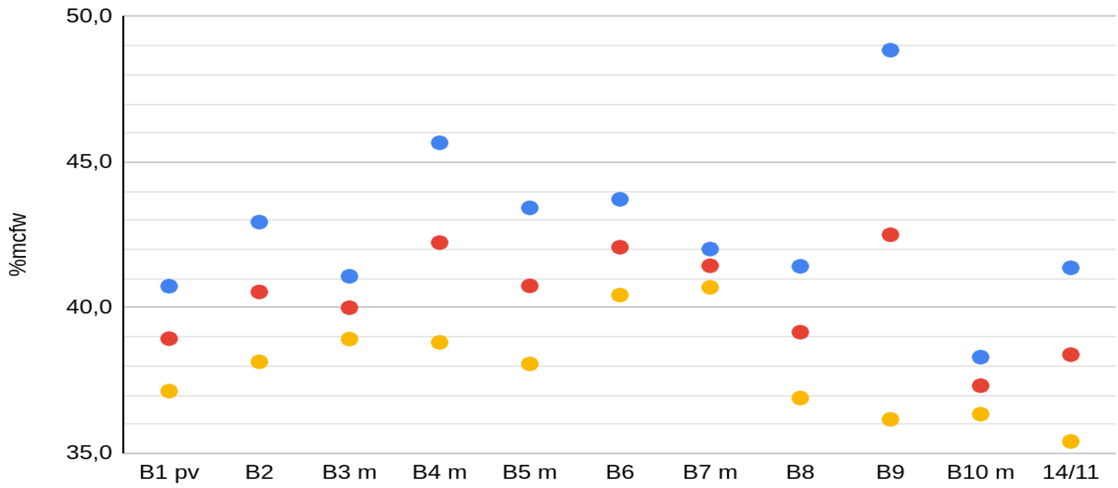


Figure 16. Mean (red) and standard deviation (blue-yellow) of acorn %mcfw after harvesting (1 day), cleaning (1 day), drying (1 day) and storage at 4°C in LDPE bags (1 week). (Semillistas data).

In batches B1, B2, B6, B8, and 14/10 10% of the acorns were brownish green, and 90% were brown or green tip. Where "m" is shown the batch was almost 100% brown acorns. Where "pv" is shown the batch was almost all with green tips. Batch 14/11 had two days of drying (by mistake).

In this harvest, acorns from each tree were kept separate, without pooling acorns that were at similar stages of maturation. Although each tree had several trunks we assume that they derive from the same original acorn. The %mcfw of individual acorns was measured for between 5 and 10 acorns from each batch on a 0.1 mg accuracy scale. The subsequent cleaning process was slow and the acorns should not have been left to dry for a day after cleaning, so the initial %mcfw would have been around 1.5%mc higher in each of batches B1, B3, B8, B10, and 14/11. Therefore, in order to compare Figures 15 and 16 we assume an additional 1.5%mc for Figure 16 because the acorns in Figure 16 did not have a day of drying.

We could have successfully combined the 100% brown acorns in batches B3, B4, B5, and B7 (excluding B10 because its acorns would have been on the tree for some time: they were brown but did not fall). Excluding B10 we would have acorns of between 38% and 45%mcfw, with an average of approximately 41.5%mcfw. Excluding the one-day drying, they would average 43%mcfw. Therefore, the one-day drying was beneficial in adjusting the moisture content to the optimum for storage.

Of the 100% brown acorns in Figure 15, A3 was 40%mcfw on average. This percentage is lower than in the 100% brown acorns in Figure 16 (41.5%mcfw on average), probably due to drying on the tree without falling.

Acorns B1, B2, B8, and 14/11 with 10% brownish green colouration have on average 39%mcfw (if batch 14/11 had only one day of drying). The same batch B6, with the same characteristics, was 42%mcfw on average. With the exception of B6, the other batches would have averaged 40.5%mcfw if they had not dried for one day. We could have combined these batches (B1, B2, B6, B8, and 14/11) of similar visual characteristics during harvest. Batches may be combined if it seems quite likely that their %mcfw is similar, but in the two cases shown above there is one batch that is an exception.

Choice of acorn drying time without %mcfw calculation

After harvesting, if acorns are stored in closed, ventilated bags (e.g., paper, raffia, etc.) and are not exposed to sunlight or heat, but are kept in a cool place overnight (10-15°C), they will lose only 0.2-0.4%mcfw;

The day after harvest if these acorns are cleaned, disinfected, and spread out for surface drying, they will have the following %mcfw:

- On uniformly maturing trees:
    - If the acorns are brown they will be between 38% and 42%mcfw, so should be stored quickly.
    - If the acorns are brownish green they will be between 43% and 48%mcfw and will need 2-3 days of drying (spread out indoors at 17°C, with 1.5%mcfw loss per day). It is necessary to calculate the %mcfw of the acorns to know the required drying time.
- On non-uniformly maturing trees:
    - Separate the acorns from each tree according to the percent of acorns that are brownish green.
    - If the brownish green acorns are more than 70% of the total they will need 2-3 days of drying. If they are more than 40% of the total they will need 1-2 days of drying. In any case it is advisable to calculate the %mc to know the required drying time.
    - If there are fewer than 15% green acorns we can store them immediately after ½ day of drying.

Final details

We have observed the appearance of water condensation inside LDPE bags used to store acorns. Some condensation is normal due to the metabolic activity of the acorns. However, when it is abundant it coincides with acorn germination during storage. We are currently uncertain as to why this is, but it could be due to:

- High relative air humidity when bagging the acorns, so that when the bags are placed in the refrigerator the humidity increases further and condensation occurs. A possible solution is to leave the bag slightly open when placing in the fridge and close the bag when the temperature equilibrates.

- When transferring bags from one refrigerator to another during a journey of more than 1h without maintaining the cold chain. The rise in temperature would cause an increase in respiration and, consequently, an increase in relative humidity inside the bag. The solution would be to maintain the cold chain.
- The acorns stored in the bag having a high %mc (>44%mcfw) because brownish green acorns have been stored with brown acorns. In an inhomogeneous batch if we do not want the brown acorns to be stored at a low %mc then the brownish green acorns will not fall below 44%mcfw. With storage time in the refrigerator acorns will tend to balance their %mc and it is possible that after several months the acorns will begin to germinate inside the bag without substrate. A possible solution is that all brownish green acorns should have a %mcfw less than 43-44%mcfw, even if brown acorns from the same batch remain with a low %mc.

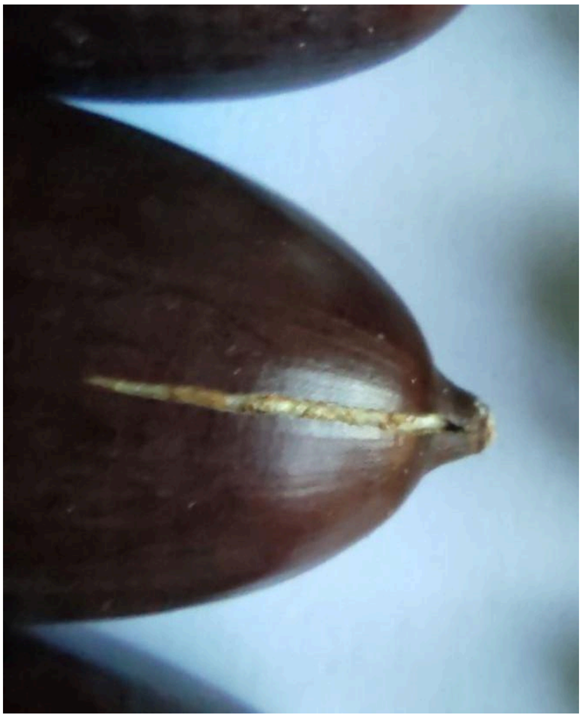
2.20. Images of the interior of holm oak acorns

1. Acorn embryo in good condition (the one on the right is starting to elongate). Endosperm with good bright white and creamy colour. Storage was at %mcfw ~ 41%.

2. Rotting embryo (perhaps fungus), although the endosperm is white. All acorns in a bag stored at 41%mcfw experienced this for unknown reasons.

3. The white grubs are *Curculio elephas* and will develop into weevils (beetles).

4. Acorn after a long time in substrate at 20ºC, initially at 35% mcfw. The testa is starting to open for germination. The marks on the testa are from bacteria that are degrading it.



5. Acorns initially at 41% germinate quickly at 20ºC. When the testa starts to crack it is a sign of immediate root emergence.

6. Acorn with yellow colour in endosperm with %mcfw ~ 34%. Fungi in the acorn's base passed through the testa into the endosperm during storage.

7. Fungi on the base of the acorn beginning to pass through the testa. At the time of cutting, the endosperm was not yet contaminated.

8. The acorns on the right, stored at 40%, maintain the white colour of the endosperm without the appearance of dark spots. The acorns on the left, stored at 36%, turn yellow, dark spots appear, and fungus develops more easily. Each acorn may or may not germinate depending on the degree of fungal infestation. The leftmost acorn has an area near the base that has been eaten and has worm faeces on it.

9. Acorns stored at 36%. The endosperm yellows and dark spots appear. Despite this the embryo remains viable and can be seen to elongate.

10. Acorn from storage at 34%mcfw. This acorn is visibly drier than 34%. It has turned from a yellowish colour to light brown with a glassy texture (which splits). This acorn will not germinate. Air voids are visible between the endosperm and the testa (which are noticeable to the touch from the unbroken acorn).

11. Acorn from storage at 34%mcfw. It has not yet reached the vitreous stage and retains the yellow colour. The embryo does not appear to be in good condition and the dark spot occupies almost the entire endosperm. It is difficult for a seed like this to germinate.

12. Acorn with the testa removed in the embryo area so that it can hydrate faster. The beginning of germination can be observed with the endosperm beginning to split and the skin around the endosperm cracked.

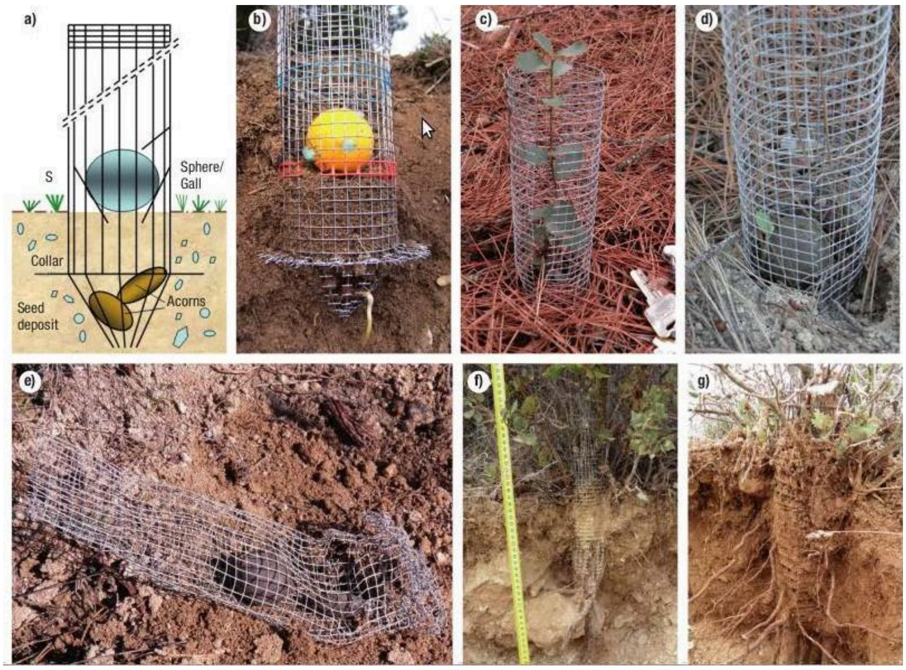
13. Acorn with strong root and stem emerging. A good white colour of the endosperm is visible, a sign of good health of the acorn-plant.

14. Acorns at different stages of maturation. The brown colour always advances from the base to the tip. From green it changes to ochre and then brown. Brownish green acorns can germinate, but they must first fall below 44% mcfw. They may be 43% to 50% mcfw and should be stored between 40-42%.

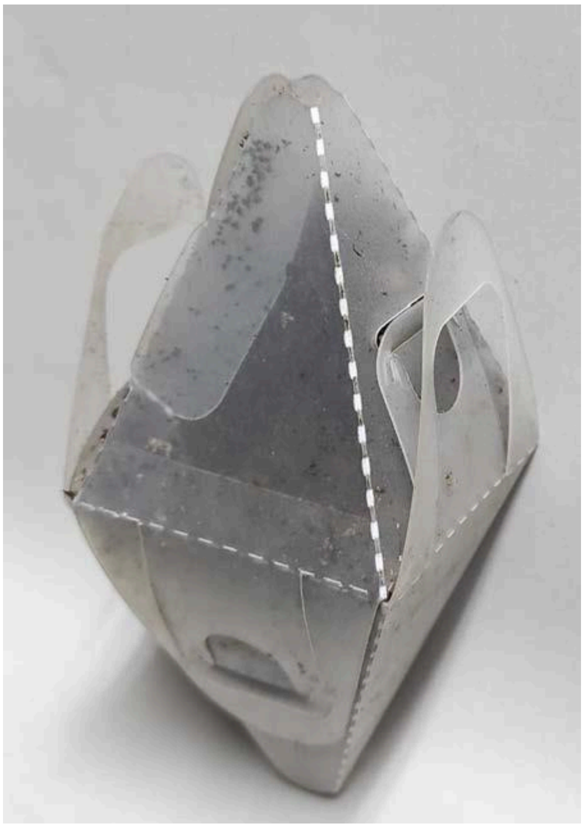
15. Acorns with the base cut off. The testa is cracking for root emergence.

2.21. Images of predation

1. Decoy for wild boars. A tight knot that does not touch the thread below. For installation especially at places where there are wild boar trails. Three wires at 20 cm from the ground and 15 cm apart. The centre wire is connected to ground. In 2024 we tested with only 2 wires which worked just as well. The top wire is connected to a tensioner and the bottom wire to the ground. Two wires provide more leeway to remove growing plants that touch the bottom wire. We are currently using compostable plastic lures made from starch.



2. Reque Protector.



3. Seed shelter (Sylvestris Group), made of polypropylene (PP).

4. Seed Shelter after several years.

5. Different protectors tested in 2024, to avoid the use of polypropylene. The protectors we are testing for mice:
   - PLA glasses have worked (right photo), but we believe that hungry mice could break through them.
   - Pressboard and Supertex seed shelters have good moisture resistance. They are under experimentation.
   - Stapled parallel plates made of pressboard and Supertex are the cheapest option. We are focussing on the smaller ones. They are also under experimentation.
   - Paraffin-treated cellulose seed shelters are too flimsy for seeding logistics.

6. Sowing seed shelters inside broom on the summit of Sierra de Lujar. By placing the shelter in the interior, and not on the edge, acorns can be sown without danger of being preyed upon by wild boar. The hole that is made in the shrub to introduce the seed shelter must be small so that the wild boar cannot fit its snout into it.

7. Detail of the necessary protection for planting in broom. Dense Cordura® type fabric, gloves, and duct tape.

8. Protection of acorns from mice. No matter how much we cover the surface, the mouse passes underneath. 6.

9. Semillistas' solution to mouse predation: a textile cone with two stones to stabilize it which allows the mouse to reach the cone and start to make a vertical hole to reach the acorn.

10. The cone has mesh underneath. The mouse reaches this and withdraws. The mesh could be much smaller. Alternatively, if the cone had wings on its base the mesh would not be required.

11.The mesh has a few small cuts made in it so that the cone can pass through.

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