Management and long term storage of cork-oak acorns

FAIR5-CT97-3480

Introduction

The natural regeneration of cork-oak is impeded by abiotic stresses, pests and diseases, as well as by grazing and management practices of agro-forestry, where they exist.

Cork-oak trees can have unpredictable reproduction patterns, with some trees producing seeds only one every 2 to 5 years. The problem of the irregularity of mast-years is complicated by dormancy-related heterogeneity in germination of freshly harvested acorns. The long time taking by acorn to germinate increases the risk of their viability loss caused by rodents and drought.
Due to these problems, **the long-term storage of acorns** may be an important alternative for reforestation and rejuvenation of cork-oak stands.

Until now, the nurserymen encountered difficulties to store cork-oak acorn for long period due to the inadequate processing, poor seed handling and inappropriate equipment. The long-term storage of recalcitrant seeds is very complex, but possible if old practices are overcome.
Storing acorns successfully requires attention to the entire seed storage process starting from seed harvest. Steps like seed maturity, time of harvesting (physiological status), method of drying, water content, treatment with thermotherapy, storage temperature, type of storage bags need to be carefully considered and tested.

The **objective** of this handbook is to provide the knowledge obtained during 3 years experiments with cork-oak acorn storage (**European Project FAIR5 CT97-3480**), describing the practical aspects to ensure the success of the long-term storage of cork-oak acorns.

SEED COLLECTION:
Where, How and When

It is known that plant survival and production are affected by seed quality. In fact, to ensure high quality of acorn, we should collect acorns:

In the pure stands, and from healthy trees

Avoid collecting from isolated trees due to inbreeding

To predict the period of complete acorn maturity easily, visual observations and moisture content (MC) determination are needed. At full maturity the whole acorn MC ranges between 43 to 50% corresponding to about 42% in cotyledons. This MC seems a good indicator of acorn maturity.

The stands and the trees are then selected and identified.

Complete maturation is affected by weather patterns and geography. It occurs, in Portugal, between 10 and 25 November.

How to predict acorn maturity

Successful acorn storage starts with harvest. If acorns are not mature, their germination rate will be poor and their storage life will be short.

During maturation, the weight of acorn increases to reach a full size, varying with parental tree and localities, their pericarp turning progressively brown.

Usually when acorns start dropping (first week of November), most of the acorns remaining in the tree are ripe. **At this moment efforts should be made to organise the acorn collection**. It has been shown that the harvest duration and acorn state (fresh or dehydrated) affect the acorn long-term storage.

1- Install nets under the canopy of all trees already selected, or clean the ground to eliminate the fallen acorns.
Or simply move the net among selected trees.

2 - Put the rope around the fine branch and shake slightly to induce drop. The acorns are easily detached from caps without damage to the acorns.

3 - Assemble collectors in number to reduce harvest time as much as possible.
Collect the acorns fallen on the nets and put them into mesh bags.

Traditional harvest may be a slow process. It consists of collecting acorns from the ground during a long period, increasing the risk of seed viability loss and pre-germination and therefore reducing the possibility of storage. The loss of viability may be due also to insect and fungi attacks as well as acorn dehydration.

The advantage of the controlled harvest is the reduction in harvest time, providing fresh acorns of high quality. Acorns may also be gathered from the ground, if they have fallen recently.

Choose the acorns that are green-brown indicating their recent downfall

Avoid to mix acorns collected from the ground with those harvested from the trees

Light brown color usually indicates that acorns have been on the ground for a long time and have probably became dehydrated.

Dark brown color indicates that acorns have been fully infested by fungi. Sometimes acorns appear to be good, but they are probably infested inside.

! Do not collect !

Acorns with holes: especially *Curculio elephas* attacks acorns on the tree, reducing acorns weight and affecting negatively plant survival and development.

Irregular shape: acorns with a shape alteration are usually affected by insects.

Seedling resulting from damaged acorns exhibited a slower growth rate and lower biomass production

Inadequate handling between collection and processing can greatly shorten acorn storage life.

Separate acorns collected from trees to those from ground

Avoid leaving acorns under the sun during the day

Due to their rapid metabolism (recalcitrant seeds), put each daily acorns collection in mesh bags (70-80 Kg each) or in vented boxes (15-20 Kg) to allow air circulation.

Avoid using plastic bags

During transport, acorns need to be well-ventilated and should be not exposed to high temperatures. So:

Use covered or cooled vehicles

Avoid leaving acorns under the sun or inside closed vehicles

The mesh bags should not be packed so tightly to allow heat and gas removing and to avoid anaerobic conditions

Successful acorn storage depends greatly on a fast storage processing. Therefore the seedlot collected daily (if it is large enough) **or at maximum during 3 days should be immediately prepared for storage.**

If the harvest is performed over 3 days, the seedlot should be put daily under cool conditions (4-5º C) to minimize water losses

SEED STORAGE:

Cleaning

At reception the seedlot usually shows impurities such as debris of branches, leaves, empty acorns...etc

Clean the seedlot to make acorn storage easier and to ensure potentially better acorn quality

How to clean ?

Submerge the acorns in water and stir gently

The debris of leaves, branch and the unhealthy acorns (empty) will float,

and the healthy ones will sink to the bottom.

This phase is easier and quicker if the acorn collection was performed with the nets or from the ground after cleaning

Discard all floating acorns

However, some acorns with holes may also sink to the bottom. So, perform

Hand cleaning

Remove all remaining debris (branch...). Discard all acorns with holes, or with pericarp of dark-brown and black color (fully infested by fungi)

Thermotherapy

The thermal treatment is a step indispensable to ensure a successful long-term storage of acorns. This treatment (45 ±1ºC for up to 2 hours) proved to be effective against larvae of *Curculio elephas, Lepidoptera Tortricidae* and inside fungi proliferation.

How to treat ?

Immediately after cleaning submerge the acorns in the water previously heated at 45 ºC

Wait until water temperature reachs again 45ºC and record 2 hours

discard again the floating acorns

stir occasionally for temperature uniformity

After treatment remove the acorns from the container and drain them for some time

We have proved that the thermotherapy do not affect the acorns and the seedling quality and storability.

After 1 to 2 hours draining,
the acorns should be checked
for quality before drying and storing

The most practical ways to check the seed quality to confirm that they are viable and to give a reference for future drying degree will be described below.

Moisture content

Germination

Embryo Electrolyte Leakage

A successful storage and the viability of acorns greatly depend on the moisture content (MC) of acorn or embryo

The drying phase is necessary to avoid premature germination inside the bags during storage. This is likely to occur if acorns are stored at high moisture content (42-47%)

	MC (%)	Storage duration (months) 1	3	6	13
Stored at fresh MC	47	0	0	21.1	65.4
	42	0	0	10.7	42.9
Stored after a moderate drying	41	0	0	0	0
	40	0	0	0	3.5
Stored after an excessive drying	31	0	0	0	0

% of pre-germination within bags during storage

However, drying should not be excessive since it leads to acorn death.

How to dry ?

Drying should be speeded in controlled chamber

Adjust a temperature and relative humidity (RH) of a controlled chamber to 30°C and 30% RH

Spread the acorns in a thin layer (<10 cm) in opened containers and place them in the controlled chamber

If the air flow is not available
use over saturated solutions of mineral salts: (calcium chloride $CaCl_2$, lithium chloride $LiCl_2$, silica gel..)
$CaCl_2$ maintains a RH of 30% at 25°C and is inexpensive

Because it is difficult to control the exact RH in the chamber due the evaporation of water present inside the acorn, it is recommended to use **the air flow chamber to avoid moisture accumulation**

Stir the acorns occasionally

Under these conditions, 24 to 36 hours should be sufficient to reach the **desired acorn moisture content**

Optimal acorn MC to store should be 40 - 42%

***Traditionally*,**

acorns are stored in mesh bags without any treatment, except fungicide application.
This type of container allows seed desiccation and therefore induces the loss of acorn viability.
Storing in mesh bags lead to death of 60% acorns after 4 months storage due essentially to their dehydration.

How to store

When desired MC is attained (40%), acorns should be taken out of chamber and **immediately** stored to prevent undesirable physiological changes and water condensation on the inner wall of the bags.

Weight and split the acorn in Polyethylene bags 30 - 50 ?m thick

These types of bags reduce the water loss and allow the CO_2 to reach a concentration of 1-4% inside the bag

Add fungicide (e.g. thiram) to prevent fungi development

Seal the bags tightly but leave inside an air volume similar to that of the seeds

The quantity of acorns should not be large since the bag walls are fragile:

- Manipulate the bags gently
- No more than 15-20 kg to facilitate handling
- Avoid stockpiling the acorns bags

Put the bags in open containers to allow air circulation between containers

Store the containers at 0 ? 1°C in controlled chamber

Sometimes due to the stockpiling containers, moisture condensation appears on the bag walls

Move the container occasionally to avoid condensation

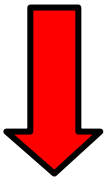
Quality certification

The establishment of forest plantations throughout the world, and particularly in European regions, requires a high seedling quality. It is known that seedling quality is affected by the seed quality. Seed certification, which standardizes the quality of seed including the specific identity, origin, genetic characters and the purity of lots, is now adopted by many countries. Seed quality depends on many factors, among which purity, weight, moisture content, seed viability and health assessment.

Viability

Traditionally germination and Tetrazolium tests, using ISTA (International Seed Testing Association) methods, were used to test the seed viability. However, the disadvantage of these tests is that the first requires a long time to respond (at least 28 days) and, the second is subjective. Therefore, some countries (Canada, USA) do not recognize the Tetrazolium test officially and encourage following-up with a germination test.

Seed viability is the first test requested by the purchaser to buy seeds. In case of the cork-oak acorns which are very sensible (**recalcitrant**), **the long period to obtain a response could be a disadvantage.**



Therefore, the interest for the two parts (seller and buyer) is to develop rapid and reliable methods as a replacement for germination test.

For this intention the Institute of Agronomy of the Technical University of Lisbon (Portugal), partner in the European Project FAIR5 CT5-3480, has developed an innovative and rapid method.
Embryo Electrolyte Leakage and Embryo Moisture Content seem reliable techniques to evaluate rapidly the germination capacity of the acorns.

The main objective in the nursery is to obtain high percentage and rapid and uniform seedling emergence. This is very difficult with fresh acorns since they are dormant. Therefore, storage could be an alternative.

Fresh cork-oak seeds have a low level of dormancy, expressed by slow and not uniform germination. During storage at low temperature this dormancy is released and stored acorns will germinate faster and more uniformly. However, prolonged storage period may lead to deterioration, which declines and retards germination again.

Germination test

1 Soak acorns for 48 hours in water preferentially at 20ºC (or in greenhouse)

2 Sterilize the imbibed acorns in 80% sodium chloride for 10-15 min

3 Compress acorns within a layer of sterilized moist substratum (peat, sand). The substratum should be saturated by adding water. Wetting occasionally by Thiram (1.5 g/l)

4 Germination tests should be carried out at 20°C

Acorns are considered germinated when radicle protrudes at least 0.5-1 cm

Emergency test

Not all germinated seeds are able to develop in a normal and healthy plant. Emergency test evaluates the number of seedlings that can be obtained by a seedlot. Seeds are sown in phytocells containing sand and peat (1:1). The phytocells are put in a greenhouse and regularly irrigated. Weekly, the seedling emergency is scored. The emergency test requires more time than the germination test, but after 6 weeks it is already possible to get data, namely the diameter at the collar, the stem height, the root length, the number of leaves, the dry weight of the stem and of the roots, the produced biomass.

Innovative and rapid tests

The embryo is the active part of the acorn, responsible for the root and stem development.
Therefore, the cell moisture status and its membrane integrity should be in the optimal conditions.

Moisture content

Usually, only the moisture content (MC) of the whole acorn is examined by the analysts. Sometimes, the MC of acorns collected in the same locality is different. This difference is due to pericarp thickness, which can vary between trees. To the contrary, cotyledon and embryo MC is much more uniform among acorns from different trees.

The determination of cotyledons and embryo MC is recommended for consistency of results.

The embryo MC of fresh acorn varies with the harvest locality. It ranges usually between 56 to 62%. When the embryo MC decreases below 50% the germination is affected.

How ?

- Determine the MC in at least 3 repetitions of 15 pooled acorns or in at least 25 individual seeds
- Quickly cut the seeds into two pieces and weigh them (FW)
- Oven dry the samples for 17 hours at 103 ±2ºC
- After that, place the samples in a desiccator for 40 min
- Weigh them again (DW)
- To determine MC of embryos adopt the same procedure but use only embryos

Calculate the moisture content

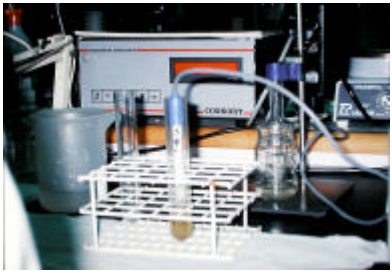
%MC = (FW - DW) x 100 / FW

Embryo Electrolyte Leakage EEL

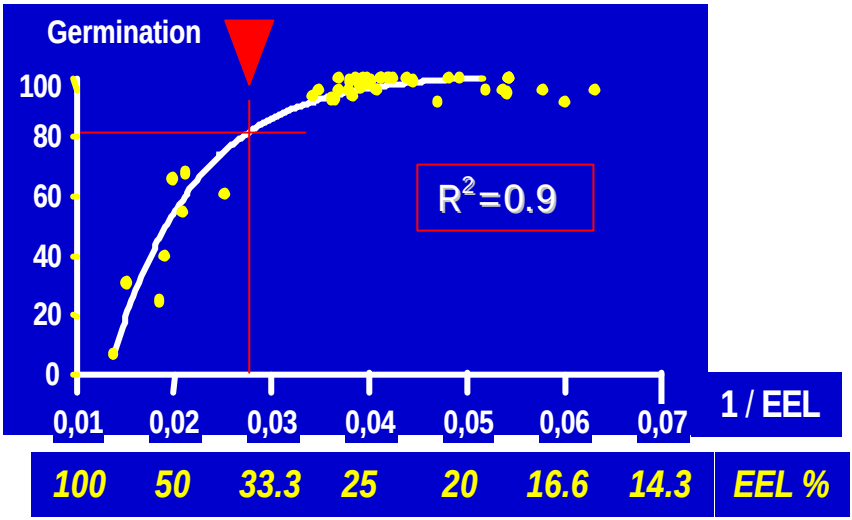
Initially, Electrolyte Leakage was used to evaluate leaves and roots damages caused by different constraints (cold, heat, drought, containers...).
The hypothesis of this technique is that membranes of damaged tissues leak more electrolyte out the cells than those of healthy cells.

Determination of electrolyte leakage should be done on at least 30 individual axes for each seedlot of 100 kg.

- More than 60 experiments of 30 excised embryos each were performed during 4 years.
- Different physiological status of acorns (fresh, dried at different moisture contents, stored, attacked or not...) were used to evaluate embryo membrane integrity and were followed by germination tests and moisture content determination.



The method shows a good correlation (R^2 = 0.93) between a germination test and the 1/EEL as illustrated in the Figure.



This rapid and reliable technique shows that acorn viability is greatly affected when the % of EEL is higher than 30%.