

PRACTICE NOTE

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SEPTEMBER 2002

SUMMARY

The fruits of oaks, chestnuts and sycamore are extremely perishable. They are shed at a high moisture content and, unlike most fruits and seeds, are killed by very little drying. They are also frequently infected with fungi which thrive in the moist conditions necessary to avoid dehydration damage. The best advice is to nursery sow (or direct seed) these fruits in autumn as soon after collection as possible.

If storage is unavoidable, it is preferable to begin with seeds which show a combination of a high moisture content ($\geq 35\%$), high germination percentage ($\geq 85\%$) and little or no fungal infection. Sealed containers must be avoided as sufficient gaseous exchange to permit respiration is essential. Temperatures below -3°C will freeze these seeds to death. Optimum storage conditions combine low temperatures (-3 to $+5^{\circ}\text{C}$) to slow fungal growth and acorn deterioration, with high humidity to retard drying. It may be beneficial to spray or soak fruits during storage; this appears to have a rejuvenating effect on acorns that have suffered a non-lethal water loss. Even under ideal conditions, slow deterioration will take place in the 18–24 weeks between acorn collection (October/ November) and spring sowing (March/April). Typically, germination values will decline from 90% to 50% in this time interval.



Acorn collection in the New Forest.

INTRODUCTION

Large fruits such as those of oak, sycamore, sweet- and horse-chestnut all have a characteristic that you would least associate with seeds: they are highly perishable. In nature, they either germinate soon after shedding, are eaten, or die. Even the best current methods of artificial storage merely retard deterioration, they cannot prevent it. More detailed consideration of the difficulties involved is contained in Gordon (1992), Gosling and Aldhous (1994) and Suszka *et al.* (1996).

This Note is aimed principally at commercial seed collectors, foresters and nursery managers who want to collect, handle (and if unavoidable, store) large quantities of seeds of these species as a prelude to raising commercial quantities of trees. But the same principles apply to small quantities of seeds. Therefore anyone with an interest in raising even a small number of trees from these quirky seeded species can apply similar techniques. As a word of warning, the correct botanical term for oak, sycamore, sweet- and horse-chestnut propagules is 'fruit', but in this Note, 'seed' is often used to avoid the text becoming over-'fruity'!

'ORTHODOX' VERSUS 'RECALCITRANT' SEED

The ease with which the seeds of most plants can be handled and stored is ultimately dependent upon whether or not they are killed by drying. Seed moisture content is therefore an important characteristic (see Box 1).

Most seeds can be dried without harm, and because they are in the majority they are frequently referred to as 'orthodox'. After drying, orthodox seeds are relatively easy to store.

But the seeds of a significant minority of plants are killed by drying. Their cells are like the cells of most other animal and vegetable tissues – when they are dried, they die. This makes handling exceptionally difficult and

anything other than relatively short-term storage virtually impossible. In 1973, Roberts coined the word ‘recalcitrant’ as an apt description of such seeds. Oak, sycamore, sweet and horse-chestnut fruits all fall into this category. To maintain their viability the fruits must be kept at as high a moisture content as possible, in a breathable atmosphere. (See Box 2 for further details.)

SEED SUPPLY AND THE LAW

Two legal documents directly affect commercial tree seed trade in the UK, they are the *Plant Varieties and Seeds Act* (1964) and the *Forest Reproductive Material (FRM) Regulations* (1977), Statutory Instrument No. 891. Their aim is to ensure that the seeds and planting stock of the most important forest tree species are of ‘high quality’ and ‘genetically suited’ to their planting site. At the time of publication the only recalcitrant fruits which are affected by this legislation belong to the oak genus. However, sweet chestnut and sycamore may be included soon in response to the recent publication of a new European Community Directive (No. 1999/105/EC) on the ‘Marketing of forest reproductive material’. Horse chestnut is not likely to be included.

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Other legislation that might have a bearing on tree seed trading includes the *Plant Health (Great Britain) Order* (1987); the *Plant Health (Forestry) (Great Britain) Order* (1989). In addition there is legislation on: Health and Safety; Control of Pesticides; Control of Substances Hazardous to Health; Trade Descriptions; and Consumer Protection. Finally, any person proposing to collect seeds should obtain permission from the landowner.

DIFFERENTIATION BETWEEN SESSILE & PEDUNCULATE OAKS

Sessile oak (*Quercus petraea*) and pedunculate oak (*Quercus robur*) are frequently referred to as separate species. However, it is widely acknowledged that the two species are very closely related and liable to hybridise. Species authentication is tricky, even when trees are alongside each other! And when the only material

available is an acorn, differentiation is often a practical impossibility. Figure 1 highlights acorn characteristics which are believed to typify each species – but always be prepared to be confronted with acorns exhibiting overlapping features. For this reason the rest of this Note will not differentiate between fruits of the two species, but merely refer to them collectively as ‘acorns’.

ACORN COLLECTION

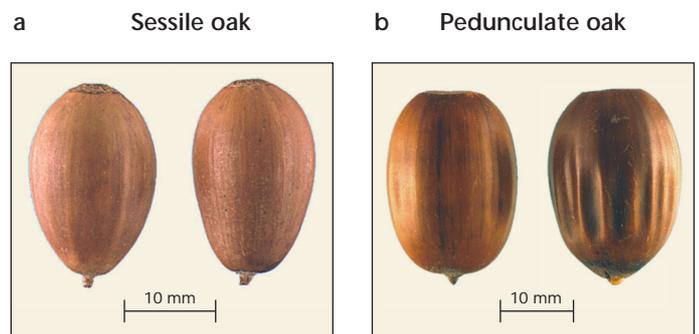
Developing acorns are green and turn brown when fully ripe. They begin to fall from the trees in October, particularly during strong winds and /or after the first frosts. Depending upon the weather conditions, in some years many acorns will fall while they are still largely green. But in most years they are retained on the tree for longer and do not fall until completely brown.

Small collections of acorns (a few hundred or so) can be made by either picking the green / browning fruits from lower branches or waiting until they drop and selecting individual, intact and apparently healthy fruits. Large quantities of fruits are normally collected from the ground after they have fallen from the trees. In these instances, earlier site preparation, for example clearing ground and maybe covering it with tarpaulins or suitably sized netting, can pay dividends.

Figure 1

Typical shapes of (a) sessile (*Quercus petraea*) and (b) pedunculate oak (*Quercus robur*) acorns.

(Aide mémoire – robur acorns: more robust, ridged and wrinkled!)



	a Sessile oak	b Pedunculate oak
Size:	Usually smaller (330* pure seed kg ⁻¹)	Usually larger (248* pure seed kg ⁻¹)
Shape:	Long egg/tear shape	Squat cigar shape, blunt ends
Colour:	Darker brown	Lighter brown (usually)
Surface:	Rarely ridged/wrinkled	More prone to ridges/wrinkling
Other:	More prone to sprouting	Less prone to sprouting

*Based on c. 100 seedlots of each species.

HANDLING ACORNS

As soon as any collection of highly perishable, recalcitrant seed has been completed, it is vital to obtain the earliest possible indication of quality – this will allow deductions to be made on likely storability and potential performance, and enable decisions to be taken on how best to proceed with handling. ‘Moisture content’ and ‘viability’ are two important properties to measure.

Seedlot moisture content (mc) should be determined within 24 hours of collection (see Box 1). The test itself will generally take another 24 hours and in the meantime seed should be treated according to the summary on page 5. If the moisture content is <35% moisture content on a fresh weight basis (mcfw) the seedlot is probably not worth storing. If it is $\geq 35\%$ it should be maintained by spraying or soaking.

Box 3 summarises how to assess ‘viability’ within a few hours using a ‘cut-test’.

Continental seed merchants frequently apply fungicidal treatments to combat fruit decay, the spread of mould growth and the concomitant germination losses but UK pesticide legislation means that there are no fungicides covered for use on tree seeds (see Box 4).

Depending upon the cut-test results, it may be worth using flotation to remove impurities or mouldy, insect damaged and empty seeds (see Box 5). Seedlots with an initial viability of less than 85%, or with significant percentages of mouldy, insect damaged and empty seeds are rarely worth attempting to store.

Immediately after a moisture content or viability test, or flotation, the best advice is to minimise any storage interval and sow recalcitrant fruits as soon as possible. Recently collected green fruits will have a moisture content of about 50% (mcfw), and the moisture content of brown fruits is probably only marginally lower at about 40–45%. Both types of fruits will be respiring vigorously and highly susceptible to damage from any further drying. All handling, temporary storage, transport and longer term storage techniques should make every effort to avoid drying, slow down metabolism and minimise fungal growth. For longer term storage, it is preferable to spread acorns on a cool, dry surface, no deeper than 150 mm (see Figure 2). A less preferred option is to place them in sacks (see Figure 3). A summary of practical advice at each stage for achieving these aims is given on page 5.

Figure 2

Approximately 2000 kg of acorns spread on a cool, dry, concrete floor.



Figure 3

Loosely woven 25 kg polypropylene sacks. Note spaces between alternate rows to allow ventilation, permit access and dissipate heat.



N.B. Sacks must be made of a material with a loose enough weave to allow gaseous exchange (for acorn respiration), tight enough to retard moisture loss (and impede drying) but not too tight as to allow condensation to collect. Hessian and cotton sacks are good, but once used can retain moisture and harbour fungal spores. Loosely woven polypropylene sacks do not absorb moisture and are therefore probably better.

Penultimately, ‘sprouting’ is another phenomenon which often causes concern during the handling of acorns (see Figure 4). However, the problem is more perceived than real. Sprouted acorns are more awkward to handle and sow, but the large food reserves available to the growing embryo mean that even if the radicles are damaged or broken during handling or sowing, there will usually be enough storage material for the living embryo to resume growth. Every attempt should be made to avoid sprouting (by using the combination of techniques described above) but it is more likely to be a nuisance than fatal.

Figure 4
Acorns at a high enough moisture content to remain viable are very prone to germinating prematurely (sprouting).



Finally, details of nursery production can be found in Mason (1994) and direct seeding in Willoughby *et al.* (1996).

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Summary of practical advice for large-scale acorn handling and storage

At collection site

1. Try to avoid putting newly collected acorns into sacks until it is time to transport them. It is best to spread them to ≤ 150 mm deep on any surface that is cool, dry, and sheltered from wind, rain or sun (see Figure 2). An unheated, well-ventilated shed, with a clean, dry, wooden or concrete floor is adequate. **PROTECT FROM RODENTS and use traps!**
2. If the acorns are wet from rain, allow them to slowly surface dry but never dry with warm or hot air. They will need to be turned and mixed twice daily to allow uniform drying.
3. Inspect daily, looking for signs of drying out, shrivelling or fungal growth and remove obviously damaged or dying fruits. Carefully turn at least weekly. In the event of drying, sprinkle or spray with water.

Transport

- Place acorns in loosely woven sacks.
- If an open lorry is used, it is essential to cover the acorns with a waterproof tarpaulin – this will prevent excessive moisture loss if conditions are dry and waterlogging if the weather is wet.
- When the sacks are piled high on the lorry, under a tarpaulin, there will be a considerable build-up of heat due to the acorns' respiration. When the tarpaulin is removed after the journey, it is not uncommon to observe 'steam' billowing from the load. Therefore unload the sacks as soon as possible to minimise the potential damage.

Further storage

- Storage for ≤ 2 weeks
 - Try to avoid keeping or putting acorns into sacks. It is best to spread them to ≤ 150 mm deep on any damp-proof surface. An ideal environment is in a humi-store or direct-cooled cold store at -2 to $+4^{\circ}\text{C}$. Alternatively use a cool, well-ventilated room at 2 to 10°C sheltered from wind, rain or sun. **PROTECT FROM RODENTS!**
 - If storage in sacks is unavoidable, then arrange the sacks in double rows with a gap in between. This arrangement will prevent too much heating up, allow some ventilation and permit access and inspection (see Figure 3).
- Storage for > 2 weeks
 - It is best to spread the acorns to ≤ 150 mm deep on any damp-proof surface. An ideal environment is in a humi-store or direct-cooled cold store at -2 to $+4^{\circ}\text{C}$. Alternatively use a cool, well-ventilated room at 2 to 10°C sheltered from wind, rain or sun. **PROTECT FROM RODENTS!**
 - Inspect daily, looking for signs of drying out, shrivelling or fungal growth. Carefully turn at least weekly. In the event of drying, sprinkle or spray with water.

SUPPORTING INFORMATION

Box 1: Seed moisture content

Seed moisture content is generally reported as the weight of water, expressed as a percentage of the fresh weight of the seeds. This is called the moisture content on a fresh weight basis (mcfw). (Beware the difference between this and moisture content calculated on a dry weight basis.)

A reasonably accurate measurement of seed moisture content can be carried out on acorns, chestnuts and sycamore fruits in the following way:

- Take, say 30 clean, firm, healthy and apparently live fruits.
- Cut the fruits into approximately 3 x 3 mm cubes and mix the sample as quickly as possible to minimise drying.
- Accurately weigh a 10 g sub-sample of the cut fruits. (This is the 'fresh weight'.)
- Dry the sub-sample (preferably at *c.* +105°C for between 18 and 24 hours or until two consecutive weighings give a constant weight).
- Take a final 'dry weight'. Assuming that any reduction in weight reflects moisture loss then:

$$\text{Moisture content (as \% fresh weight)} = \frac{(\text{fresh weight [g]} - \text{dry weight [g]})}{\text{fresh weight [g]}} \times 100$$

N.B. For fruits containing significant amounts of volatile substances such as resins, fats and oils, this is slightly erroneous because these substances are also driven off by heating. However, even for these species the error is usually relatively small.

Box 2: Storage characteristics of orthodox and recalcitrant fruits

Tree seeds with orthodox storage characteristics can be safely dried to moisture contents of 4–8 % (mcfw) without harm. At these moisture levels seed metabolism is virtually undetectable, oxygen is unnecessary for respiration, and hence ageing and deterioration are dramatically reduced – even at ambient temperatures. An additional benefit of low seed moisture content is that the cells can tolerate sub-zero temperatures – this is because the small amount of water present is chemically bound to the biological molecules and

does not expand upon freezing to cause tissue damage. Orthodox seeds can therefore be stored at low moisture contents plus sub-zero temperatures and this combination extends their lifespan still further. Commercial facilities routinely store orthodox tree seeds for up to 20 years at –5 to +5°C and *c.* 7% mcfw. Genebanks reduce storage temperatures and seed moisture contents still further (e.g. –20 to –80°C and *c.* 4% mcfw). Under these conditions potential longevity can be extended to centuries.

Recalcitrant fruits (such as acorns) are killed by drying. Moisture contents should therefore be kept as high as possible – at least 35–40% mcfw. However, at such high moisture levels active metabolism takes place and sufficient gaseous exchange must be provided to allow respiration to keep the seeds alive. Unfortunately, these conditions lead to unwanted ageing and deterioration! The only way to slow down the metabolism of tissues at high moisture content is to reduce storage temperature. Carefully controlled temperatures of between –3 to +5°C are best for the storage of temperate, recalcitrant tree seeds, but of course sub-zero temperatures below –3°C must be avoided as they will bring about freezing damage. A further complication to the storage of recalcitrant seeds is that even at low temperatures, high seed moisture contents provide ideal conditions for fungal growth! (See Box 4.)

If storage is unavoidable:

- it is preferable to begin with a seedlot of oak, chestnut or sycamore exhibiting a combination of a high moisture content ($\geq 35\%$), high germination percentage ($\geq 85\%$) and little or no fungal infection (Kehr and Schroeder, 1997).
- even under ideal conditions, slow deterioration will take place in the 18–24 weeks between acorn collection (October/November) and spring sowing (March/April) (Gosling, 1989).
- there is some evidence that acorns which have been subjected to a non-lethal loss of moisture may be rejuvenated by a 48 h soak at +2°C (Gosling, 1989), but this soak period should not be exceeded, or repeated more than monthly.

N.B. For best viability maintenance, the seedlot should be kept at as high a moisture content as possible ($\geq 35\%$ mcfw), at between –3 to +5°C and adequately aerated.

Box 3: Seed germination/viability tests

A germination test, as the name implies, measures the percentage of seeds within a sample that are actually capable of germinating under a particular set of conditions. Unfortunately, intact acorns and most other recalcitrant seeds are so slow to germinate (5–15 weeks) that the parent seedlot may have significantly deteriorated by the time a sub-sample has completed a germination test. It is possible to speed up the germination process (4–6 weeks) by partially dissecting recalcitrant fruits, but this is an exceedingly labour intensive and time-consuming process which is frequently confined to ‘Official Seed Testing Stations’ for statutory control purposes.

It is much quicker for seed collectors to measure seed ‘viability’. However, it is vital to appreciate that although a viability test permits seeds to be classified as either alive (= viable) or dead, it is not a measure of the percentage of seeds capable of germinating. ‘Viable’ and ‘germinable’ are not synonymous.

The ‘cut-test’ is probably the oldest, crudest, simplest and most appropriate viability test for recalcitrant fruits. As the name implies it relies on using a sharp knife or scalpel to cut open the fruits prior to classifying them. A reasonably accurate measurement of seed viability can be made by taking a random sample of say 100 fruits and identifying the following categories and sub-categories:

% not viable

- empty more than 50% of tissues missing
- mouldy more than 50% of tissues missing or dead from fungal attack
- insect damaged more than 50% of tissues missing or dead from insect attack
- dead fruit containing all tissues considered essential for germination, but where embryonic axis is at all unhealthy or more than 60% of the cotyledon tissues are clearly dead

% viable

- filled fruit containing all tissues considered essential for germination, where embryonic axis is firm, fresh, healthy and apparently alive, and no more than one-third of the cotyledon tissues are unhealthy

- filled and sprouted as above, but with protruding, live radicle

N.B. It is also useful to note the levels of impurities such as leaves, twigs, cupules, soil, sand and gravel.

Box 4: Fungal infection

Acorns, especially from continental sources, are liable to carry the fungus *Ciboria batschiana* (Zopf) Buchwald (= *Sclerotinia pseudotuberosa* = *Stromatinia pseudotuberosa*) living on the inside and outside of the fruit case.

Unfortunately, acorns are shed at a high moisture content and must stay moist to remain alive and these are exactly the conditions which are highly conducive to fungal growth. *Ciboria batschiana* can therefore be a significant cause of germination losses and fruit decay.

Treatments to combat this fungal disease have been widely studied in France, Poland, Czech Republic and Germany for over 20 years. Three treatments are common: fungicidal application, a hot water treatment (called ‘thermotherapy’) and a combination of the two. However, although each is successful in small scale research trials, it has not proved possible to scale them up to reliably treat commercial quantities of acorns. For example, while many chemical treatments are effective at killing fungi, they have also been phytotoxic and can significantly reduce germination percentage and seedling survival. Thermotherapy requires that fruits are incubated for 2.5 hours at precisely 41°C. To be effective, this temperature must be achieved quickly and maintained extremely accurately, and not surprisingly this is rather difficult on more than a few kg of acorns. A slightly lower temperature or shorter exposure does not kill the fungus while a slightly higher temperature or longer exposure kills both fungus and fruits. The combination of thermotherapy plus fungicides unfortunately has the potential to be even more harmful. It is therefore rare for any of these techniques to be used as a prophylactic treatment; they tend to be applied only after a fungal outbreak for curative purposes. Even then they are unable to eliminate the fungal contamination, they only reduce it. One final drawback is that UK pesticide legislation actually means that there are no fungicides cleared for use on tree seeds anyway!

Box 5: Flotation

It is frequently suggested that poor quality acorns can be very simply separated from good quality acorns by flotation in a suitable volume of water. This is based on the idea that dead, mouldy, decaying and empty acorns plus most other unwanted debris will be less dense than water and therefore float, so that they can be skimmed off and discarded. Conversely, filled, live acorns will be more dense than water and therefore sink. The theory is extremely attractive, and most descriptions of the technique imply that acorns can be quickly thrown into a tub of water, and those that float immediately removed. However, in practice it is rarely this simple, and the degree of success may be quite variable. It is therefore always advisable to try flotation on a representative sample of any seedlot first, followed by a cut-test (see Box 4) on the floaters and sinkers to verify that the expected separation is taking place. The most common problem is that a significant proportion of live seeds are often not dense enough to sink. The floating fraction therefore often contains a significant proportion of live seeds. Sometimes this can be remedied by leaving the acorns soaking, during which time filled, live seeds may absorb more water, increase in density and sink. But it should always be remembered that any immersion in water will facilitate the spread of fungal contamination. The use of flotation should therefore always take these potential difficulties into account.

RECALCITRANT SEED: DOs and DON'Ts

-  Do **protect from rodents**
-  Do **allow respiration**
 - Don't **seal acorns in a polythene bag**
-  Do **keep moisture content above 35% (soak or spray intermittently)**
 - Don't **do any more than surface-dry fruits**
-  Do **handle with extreme care**
 - Don't **bruise, crush or drop**
-  Do **keep cool – to retard fungal decay, slow respiration and minimise heat build-up**

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