Project title: Application of post-harvest treatments to extend storability

of pedunculate acorns (Quercus robur L.) without loss of

viability or germinability.

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The results and conclusions in this report are based on an investigation conducted over a two-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

Report authorised by:

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headlines

- The project has identified methods that extend the shelf-life acorns for at least 60 weeks so that these acorns can be used between mast years.
- Acorns entirely coated with bees- or microcrystalline waxes retained high moisture contents during storage. However, germination was hampered due to mechanical resistance of the coatings, which often prevented radicle emergence.
- Acorns stored in polyester bags retained moisture content above the critical threshold and still had 49% germination after 60-weeks storage. Fungal infection caused 16-18% losses from 24-weeks onwards.

Background

Pedunculate oak (*Quercus robur* L.) produces good acorn crops during mast years that occur every few years. Acorns are classed as difficult to manage or 'recalcitrant' because fruits are shed with high moisture content and lose viability rapidly below a species-specific threshold. In addition, the acorns remain metabolically active, producing carbon dioxide, water and heat as by-products of respiration. Under traditional 'wet storage' conditions, the recalcitrant nature of acorns can promote premature sprouting and deterioration due to mould (Figure 1h).

So far, research has been unable to determine a single solution for successful storage of acorns. This results in supply and demand problems for seed traders and nurseries during inter-mast years. As a result, acorns are often imported from Europe to meet these requirements. However, this is not without risks, which include the suitability of provenance for the local climate and the accidental release of pests and pathogens into Britain. Thus, there is a need to extend the shelf-life acorns for at least 60 weeks so that acorns could be ready for use during inter-mast years.

The aim of this proof-of-concept project was to use two different approaches to extend the shelf-life of acorns without significant loss of seed quality. Throughout the experiments, seed quality was tracked using a range of tests to:

- 1. determine water loss by measuring moisture content.
- 2. detect changes in seed occupancy (shrinkage) by taking x-rays.
- 3. detect membrane damage by measuring electrolyte conductivity/solute leakage.
- 4. determine loss of viability/germinability of acorns.

In addition, control and treated acorns were sown in a nursery trial and the resulting seedlings were graded according to industry standards.

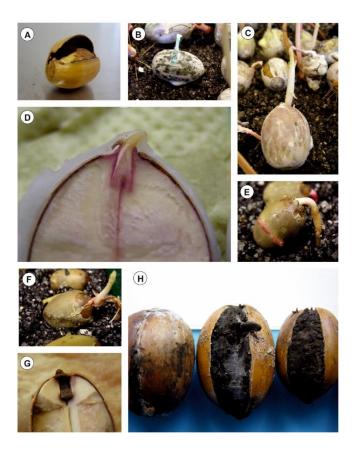


Figure 1: A - Control acorn that has split, exposing seed, B - Acorn coated entirely with soya wax that is covered with superficial mould, C - Seedling from acorn entirely coated with soya wax, D - Acorn entirely coated with microcrystalline wax with trapped radicle, E - de-capped acorn coated entirely with bees wax germinating; and F - producing shoots, G - Acorn with necrotic embryo due to fermentation, H - acorns succumbed to *Ciboria batschiana*.

Part 1 - Coatings

The key to successful storage is to maintain the moisture content of acorns above a critical threshold. In pedunculate oak, this critical threshold is about 38% (fresh weight [fw]) below which acorn viability decreases rapidly. Therefore, the first approach aimed to reduce water loss by coating acorns with either a commercial anti-transpirant (Wiltpruf®) or different types of waxes viz. bees, soya and microcrystalline wax. These coatings were applied in two ways, resulting in either partially or entirely coated acorns (Figures 2a-i).

In general, control acorns, which were stored loosely in trays, lost water rapidly during storage (Figures 2a and 3). This resulted in corresponding losses in germination as moisture content decreased below the critical threshold. The partially coated acorns followed similar trends to the control acorns although moisture loss was slightly slower. Water loss, therefore, occurs over the entire pericarp (acorn shell) not just through the cup scar of the acorns. The entirely coated acorns responded differently depending on wax type. This reflected the chemical and physical properties of the waxes, which in turn influenced the pliability and thickness of the coatings.

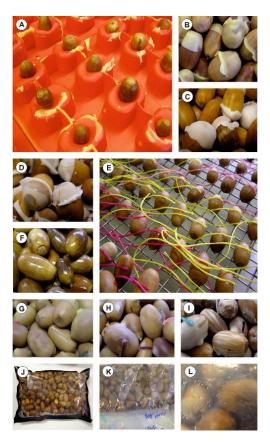


Figure 2. A - Ice-cube jig used to partially coat cup-scar end of acorns with wax; Acorns partially coated with B - bees-, C - microcrystalline-, or D - soya wax, E - Threads were glued to acorns, which were then dipped entirely into molten wax; Acorns coated entirely with F - Wiltpruf ®, G - bees-, H - microcrystalline-, or I - soya wax, J – Acorns stored in polyester bags, K – peforated biopolymer bags with L- 25 microperforations per square inch.

The acorns entirely coated with soya wax germinated as successfully as the control acorns at the beginning of the experiment (Figure 1c). However, the thin, brittle coatings subsequently flaked-off during storage. Thus these acorns lost moisture rapidly with very few acorns germinating after 24-weeks storage. Soya wax was also susceptible to superficial fungal infection but this did not appear to have a negative impact on acorn viability (Figure 1b).

The acorns entirely coated with bees- or microcrystalline waxes had moisture content close to the critical threshold at 60-weeks (Figure 3). In both cases, waxes hampered germination by trapping the radicles even at the beginning of the trial (Figure 1d). This was largely due to mechanical resistance as some 'decapped' acorns subsequently germinated but very slowly after 36-weeks storage (Figure 1e & 1f). At longer storage durations, acorns lost viability due to fermentation with cut-tests revealing only 25-35% fresh acorns at 60-weeks. Thus after 60-weeks storage, germination was 8.3% and 6.7% for acorns coated entirely with bees- or microcrystalline waxes respectively.

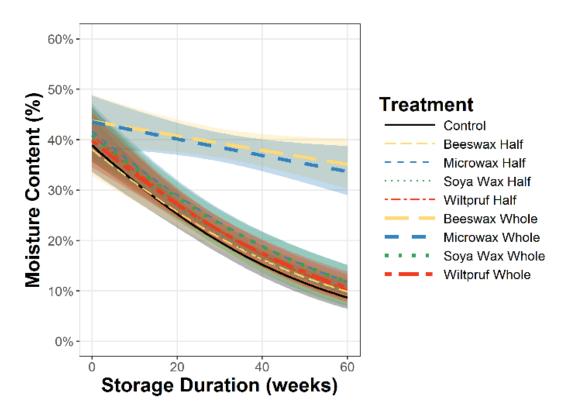


Figure 3. Wax data best fit model for moisture content, predicted by storage duration and treatment. Coloured ribbons indicate 95% confidence intervals. Microwax = microcrystalline wax

Part 2 - Bags

In common with other recalcitrant seeds, acorns remain metabolically active, producing carbon dioxide, water and heat as by-products of respiration during storage. Thus, the second approach aimed to reduce respiration by storing acorns in different types of bag viz. biopolymer, polyethylene or polyester (Figures 2j-I). These bags have different properties, which interact with the respiring fruits to passively modify the storage atmosphere.

The acorns stored in polyethylene bags had high moisture content even after 60 weeks storage (Figure 4). Nonetheless, acorns lost viability rapidly, which was largely due to fermentation as oxygen became depleted in these bags. These 'fermented' acorns were characterized by necrotic embryos (Figure 1g). Thus, acorns had 39% germination after 12-weeks storage.

The acorns stored in biopolymer bags (perforated and non-perforated) followed a similar trend to the control acorns. Thus acorns lost viability largely due to moisture loss, which was slower in the non-perforated bags (Figure 4). In the non-perforated bags, however, there was some fermentation particularly at the longer storage durations, which highlights the trade-off between better ventilation and the increased risk of moisture loss.

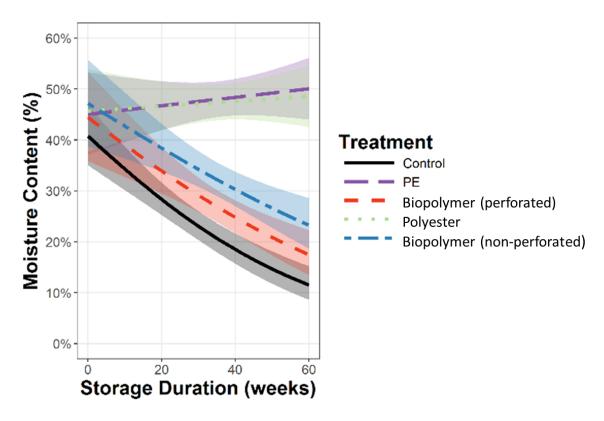


Figure 4. Bag data best fit model for moisture content, predicted by storage duration and treatment. Coloured ribbons indicate 95% confidence intervals. PE = polyethylene

The acorns stored in polyester bags had high moisture content during storage. After 12-weeks storage, the acorns germinated rapidly and uniformly, reaching similar levels to the control acorns at the start of the experiment. However, the acorns were prone to fungal infection (Figure 1h), which caused large losses (16-18%) from 24-weeks storage onwards. Nonetheless, germination remained high (47-52%) even after 60-weeks storage.

Part 3 - Nursery trials

Nursery managers usually select germinating acorns for sowing so as to maximise crop production. It is expected that about 50-65% of an acorn seed lot produces saleable seedlings depending on seed source and quality. As the initial seed lot had 88% viable acorns, it would not be unreasonable for it to produce 44-57% saleable seedlings. The best coating treatment was acorns entirely coated with microcrystalline wax, which produced 30% saleable seedlings, while the best bag treatment was the perforated biopolymer bags, which produced 35% saleable seedlings. However, these figures could be improved by: (1) improving the methods of storage described above, and (2) allowing unsaleable seedlings (<20 cm) to grow on for a further season.

Conclusions

The two approaches used in this study provided useful new information, indicating potential areas for further research and development. Firstly, coating acorns entirely with bees- or microcrystalline wax reduces moisture loss during storage and maintains viability for up to 36-weeks. Results suggest that shelf-life could be extended further by modifying the wax formulations to reduce problems with mechanical resistance and fermentation. Secondly, storing acorns in polyester bags maintained high moisture content for 60-weeks. Critically, germination remained constant between 47-52% from 24- to 60 weeks storage. Results suggest that germination could be close to the target 70% if the fungal infection that caused 16-18% losses in this treatment was controlled more effectively during storage, perhaps by monitoring the gas atmosphere within bags.

Financial Benefits

From the seed trader's perspective, the cost-benefit depends on several factors including:

- 1. probability of an acorn crop in the following year;
- 2. value of the fruits, which is influenced by provenance;
- 3. availability/suitability of alternative provenances;
- 4. storage duration;
- 5. germination percent after storage.

Thus, the cost-benefit is hard to judge as these factors are unknown when the fruits enter storage. The main benefit is that seed traders have fruits for sale when none would be available otherwise for customers.

Action Points

For successful storage, it is critical to:

- Use high-quality seed lots and remove any damaged or germinating acorns where possible before storage.
- Check acorn seedlots periodically for fungal infection caused by the storage pathogen, Ciboria batschiana, and if possible, remove infected acorns.
- Ensure that moisture content of acorns remains above 38% (fw) to prevent loss of viability; also maintain temperature between -2 and +2°C to reduce respiration, and maintain RH as high as possible to reduce water loss. Current industry practice is to store between -2 and 0°C.
- Prevent condensation on acorns as this promotes cross-infection between clean and infected acorns particularly if storing acorns in polyethylene or polyester bags.
- Ensure that there is adequate gas exchange to prevent fermentation particularly if acorns are stored in bags, which have different properties and influence water loss and gas exchange.