Increasing acorn moisture content followed by freezing-storage enhances germination in pedunculate oak

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Summary

The effect of acorn moisture content (MC) during storage at –3°C on the germination response after storage of pedunculate oak (*Quercus robur* L.) acorns was investigated. Acorns from two lots (42–43 per cent MC at time of arrival in laboratory) were soaked in tap water for 5 days at 4°C to achieve the highest MC level (46 per cent). Some of these acorns were then dried back over 1–3 days at ~20°C to the other MC levels (37, 40 and 43 per cent). The MC of other acorns was not adjusted (controls). The acorns were stored at –3°C in perforated plastic bags for 2, 4 or 6 months. Germination (radicle emergence) after 6 weeks at 15°C was evaluated. Germination of the control (non-soaked) acorns was only 18 per cent at the time of storage. Both percentage germination and speed of germination after storage increased as acorn MC before storage was increased. Acorns adjusted to 46 per cent MC and stored for 6 months had the highest germination (76 per cent), while the controls and those adjusted to 37 per cent MC has the lowest values (17 per cent for both treatments). The germination of the control acorns (42–43 per cent MC) was significantly lower than seeds that were soaked and then dried back to similar levels. The exact reason for the improvement in germination following storage at high seed MC levels is not known, but the treatment may have influenced acorn dormancy (through an effect on the pericarp) or other metabolic factors.

Introduction

The successful storage of oak (*Quercus* spp.) seeds [For convenience, the term seed is used interchangeably with acorn throughout the text, although in reality an acorn is a one-seeded fruit with a woody pericarp.] largely depends on the moisture content (MC) of the seeds and storage temperature (Connor and Sowa, 2002), assuming that harvesting and handling operations have not adversely affected storability (Tanaka, 1984). Most tree seeds have 'orthodox' characteristics and can be readily dried down to a moisture content of <12 per cent for long-term storage. However, oak seeds are 'recalcitrant', which means that they do not tolerate moisture loss without adversely affecting viability, making it difficult to

store them for a useful period (Gordon, 1992b). In particular, seeds of the white oak (subgenus *Quercus*) group are generally considered difficult to store successfully over one winter (Schroeder and Walker, 1987; Gosling, 1989; Connor and Sowa, 2002, 2003). Seed MC appears to be the most critical factor for the successful storage of oak acorns and seeds of other tree species that have recalcitrant characteristics (Schroeder and Walker, 1987; Gosling, 1989; Tompsett and Pritchard, 1998; Berjak and Pammenter, 1999; Connor and Bonner, 2001; Connor and Sowa, 2002, 2003; Sowa and Connor, 2003). The results of several studies have shown that the critical MC for storage of pedunculate oak (*Quercus robur* L.) acorns is about 40 per cent, below which viability declines rapidly (Suszka and Tylkowski, 1980; Gosling, 1989; Poulsen, 1992). Finch-Savage (1992) suggested that this critical MC stage in oak acorns occurred at about the point where all 'free' (unbound) cellular water was lost. Furthermore, he suggested that viability is primarily related to the MC of the cotyledons, which subsequently causes a decline in viability in the embryonic axis.

The effect of storage temperature on the viability of acorns of several oak species, including members of the white oak group, is inconclusive (Schroeder and Walker, 1987; Connor and Bonner, 1999, 2001; Connor and Sowa, 2002, 2003). Although other factors may also have been important, it is likely that differences in seed MC and the amount of desiccation damage caused during storage probably explains the variable results. The acorns of some species stored best at mild freezing temperatures (about –2 or –3°C), whereas others stored best at normal refrigerator temperatures (about 2–4°C) (Schroeder and Walker, 1987; Connor and Bonner, 1999, 2001; Connor and Sowa, 2002, 2003). Pedunculate oak acorns appear to store well over the full range of these temperatures (about –3°C to 4°C).

The classical method of short-term storage (over one winter) of pedunculate oak acorns is to mix the seeds in a medium (such as dry peat or sawdust) and place them in containers or sacks in cold (~2–4°C) storage (Gordon, 1992b). Acorns can also be kept in cold storage in containers (e.g. plastic bags) without a medium (Gosling, 1989). However, Suszka (1996) reported that pedunculate oak acorns (at 42–45 per cent MC content) could be stored for up to 3 years with only a modest decline in viability if they are maintained in a modified environment (high CO_2 and low O_2 concentrations) at –3°C. Furthermore, acorns should be treated (usually using hot water) prior to storage to reduce the risk of fungal damage (Suszka and Tylkowski, 1980; Suszka *et al.*, 1996). However, there is much less risk of fungal damage if the acorns are stored at freezing temperatures since the spread of the most important pathogen, *Ciboria batschiana* (Zopf) Buchwald, is greatly slowed (Schröder, 2002).

Several hypotheses have been advanced to explain the reasons for decline in acorn viability during storage, but the exact causes are not known. The three main hypotheses, largely based on the results of research on seeds of other plant species, are (1) deleterious change in lipid composition (Flood and Sinclair, 1981), (2) damage to cell membranes (Seewaldt *et al.*, 1981) or (3) abnormal metabolic functioning during hydrated storage (Pammenter *et al.*, 1994), including the potential effect of desiccation damage (Berjak and Pammenter, 1997). Desiccation damage is more likely than rapid ageing to cause a loss in viability of oak acorns during storage (Finch-Savage, 1992). Furthermore, Finch-Savage *et al.* (1996) suggested that damage to pedunculate oak acorns during drying was caused by an inadequate protection from high respiration coupled with damage from free radicals. The concentrations of jasmonic acid and its methyl ester and ABA increase following desiccation stress in acorns, which would likely delay germination after subsequent imbibition (Finch-Savage *et al.*, 1992).

Seed dormancy intensity in oak is weak, although there is evidence of some epicotyl dormancy (Bonner and Vozzo, 1987). Epicotyl dormancy is expressed most strongly at low germination temperatures (<15°C) (Corbineau *et al.*, 2003). However, it has been reported that chilling at cold or mild freezing temperatures sometimes improves the germination of pedunculate oak seeds (Suszka and Tylkowski, 1980; Poulsen, 1992; Suszka *et al.*, 1996), but the effect does not appear to be consistent. The dormancy mechanism appears to function through the pericarp, either through mechanical resistance, delayed water uptake, chemical inhibition, light or other factors (Peterson, 1983; Pritchard and Manger, 1990). Finch-Savage and Clay (1994) provided evidence

that light, ethylene promoters and ABA mediated the germination response in pedunculate oak.

Because of storage difficulties, acorns are often sown in the autumn and allowed to break dormancy naturally in the seedbed (Gordon, 1992a). Nevertheless, significant losses can occur over the winter due to predation, waterlogging, frost, etc. (Aldous and Mason, 1994). In Ireland, oak acorns (about 38–43 per cent MC) are sown in the autumn or stored under operational conditions in Hessian bags at –3°C over one winter. Phytosanitary measures are not normally necessary for seeds stored at –3°C for one winter. Acorns are no longer stored at cold temperatures (1–4°C) in Ireland, mainly because the risk of fungal damage is high. However, seed germination after sowing in the nursery in the spring is not highly reliable. New germinants also tend to emerge over a long period, perhaps contributing to size variability and a low yield of target-size seedlings (P. Doody, Coillte, pers. comm.).

There is little information on the effect of acorn MC during freezing storage on the post-storage germination of pedunculate oak. The objective of this study was to determine the effect of different storage moisture content levels (37–46 per cent) and storage duration (2, 4 and 6 months) at –3°C on the germination response of pedunculate oak acorns at 15°C. The germination test temperature was expected to provide more meaningful results than a constant 20°C recommended by the International Seed Testing Association (ISTA, 1996). For example, soil temperatures near the surface ranged from 7 to 15°C during the period of germination at Ballintemple Nursery in Ireland (52° 44' N, 6° 42' W; 100 m) (data on file).

Materials and methods

Seed material

The experiments were carried out using two lots of pedunculate oak acorns obtained from the Coillte National Seed Centre (Ballintemple, Co. Carlow) in early November 2002, about 2–3 weeks after harvesting in October. The seeds were collected in 2002 in the same seed zone in the Netherlands (02(492)2-C09; 02(492)2-C10). The acorns were stored at 2–4°C from the time of arrival in Ballintemple until the time of treatment at University College Dublin (UCD).

The mean moisture content of the acorns at delivery time was 42–43 per cent (fresh weight basis). Moisture content was determined after drying four replicates of five acorns each per seedlot at 105°C for 24 h. The official seed test results, carried out in early November by the Department of Agriculture according to ISTA rules, showed that the acorns had a germination of 86 per cent (seed lot C09) and 89 per cent (C10). The acorns were soaked for 48 h and approximately one-third of the 'scar end' was removed before testing.

Seed moisture content and storage treatments

Seeds of each lot were adjusted to approximately 37, 40, 43 and 46 per cent MC. The acorns were soaked in water (approximately four volumes of water to one of seeds) in plastic boxes at 2–4°C for 5 days. The acorns had absorbed very little moisture after 2 days (see Gosling, 1989), so a lengthy soak period was required. The water was changed several times during this period. The MC of the acorns was ~46 per cent after soaking. Some seeds were then dried back (weight loss basis) at about 20°C in a well-ventilated greenhouse. The acorns reached 43, 40 and 37 per cent after 1, 2 and 3 days drying, respectively. The seeds were spread evenly on a table and mixed regularly to encourage uniform drying. The MC of other seeds was not adjusted (controls).

After adjusting their MC, the seeds representing each of the five treatments (control, 37, 40, 43 and 46 per cent) were placed in polyethylene bags at –3°C for 2, 4 or 6 months. The bags (20 × 30 cm) were manually perforated to allow gas exchange. No water was supplied during storage. The MC of the seeds changed little during storage, as determined by weighing the bags containing the acorns at regular intervals during storage. The temperatures in the room and inside the bags were recorded over a 1-week period during storage. The mean temperatures at both locations were almost identical, but the fluctuations in temperature inside the bag were much smaller than in the room (data not shown).

In a separate study, some of the acorns of seedlot C10 were soaked for 2 days (see Gosling, 1989) just after arrival at UCD and again after 4 and 6 months' storage to determine the effect of

post-storage soaking on germination. Unfortunately, no data are available for 2 months' storage due to an inadvertent error during processing.

Premature germination

The number of prematurely germinated seeds was estimated based upon a random sample of 100 acorns of each seed lot per treatment combination, using a separate sample of acorns than were used in the germination tests. Germination was considered to have occurred if the radicle had started to emerge, regardless of the length of the protrusion.

Germination tests

The seeds were germinated in transparent rectangular plastic boxes (17 × 10.5 × 6 cm) (Hoffstätter & Ebbesen A/S, Espergaerde, Denmark) containing a layer of cotton wool moistened with distilled water. Four replications (boxes), each containing 30 seeds, represented each of the 15 treatment combinations (5 MC levels × 3 storage durations). Similarly, the (non-soaked) control acorns were tested immediately after arrival at UCD (0 months' storage), but the soaked acorns were not evaluated at this time. The boxes were placed in a germination cabinet (CMC Germination cabinet, 400l, D/L, Glesborg, Denmark) set at 15°C with 8 h lighting. An acorn was considered to have germinated when the radicle protruded >1 cm. The number of seeds that germinated was recorded every 3 or 4 days for 45 days. Percentage germination and mean germination time (MGT) was calculated from these data. MGT was calculated as the mean number of days for the seeds to germinate over the 45 days. However, no attempt was made to determine the reason why some seeds did not germinate, using other methods such as a viability (e.g. tetrazolium) test.

Data analyses

The data were analysed according to a full factorial ANOVA design to test for the effects of seedlot, MC, storage temperature, and storage duration on percentage germination (after square root arc sine transformation to normalize the data) and MGT. Similarly, the effect of soaking for 2 days after storage was evaluated using an ANOVA to test for the effect of soaking, storage duration and their interactions (seedlot C10 only). Means were compared further using the least significant means test.

Results

Acorns that had their MC adjusted to levels <43 per cent and the non-soaked controls did not germinate prematurely. However, nearly 3 per cent of those adjusted to 43 per cent MC germinated after 6 months' storage, while 10–12 per cent of those adjusted to 46 per cent MC had germinated prematurely after 4–6 months' storage. Radicle emergence was <0.5 cm in all cases.

Moisture content and storage duration had the largest effect on percentage germination, although most treatment interactions were also significant (Table 1 and Figure 1). Means were pooled across seedlots because treatment effects were generally consistent within each lot.

Germination potential of the (non-soaked) control acorns was only 18 per cent at the time of storage (Figure 1). The control group had significantly lower germination than the acorns that were soaked and then re-dried to similar levels (the 40 per cent and 43 per cent MC treatments). Germination potential increased during storage as MC prior to storage was increased. Germination was better after 4 months' storage than after 2 months' storage, although differences were not significant in all cases (Figure 1). The pattern for the effect of storage duration on the (non-soaked) controls was similar. After 4 months' storage, seeds stored at 46 per cent MC had 76 per cent germination compared with only 32 per cent for the controls (42–43 per cent MC at time of storage) and 17 per cent for acorns adjusted to 37 per cent MC.

Soaking of the control seeds for 2 days after storage had no significant effect on germination (Table 2). However, storage duration had a highly significant effect ($P = 0.0034$) on this response, similar to the pattern observed in Figure 1. There was no significant interaction between soaking and storage duration.

Treatment effects on MGT showed a similar pattern to that of percentage germination (Table 1 and Figure 2). The speed of germination increased

Table 1: ANOVA of the effects of seed lot, seed moisture content, and storage duration on percentage germination (after arc sine square root transformation) and mean germination time (MGT) in pedunculate oak

		Germination			MGT		
Source of variation	d.f.	MS	*F*	*P*	MS	*F*	*P*
Seed lot (SL)	1	0.0326	3.5	0.0666	70.4260	6.5	0.0125*
Moisture content (MC)	4	1.5915	168.1	0.0001*	1391.4100	128.4	0.0001*
Storage duration (SD)	2	0.2040	21.6	0.0001*	68.5017	6.3	0.0027*
SL × MC	4	0.0495	5.2	0.0008*	24.1180	2.2	0.0725
SL × SD	2	0.0481	5.1	0.0081*	25.2279	2.3	0.1034
MC × SD	8	0.0280	3.0	0.0055*	30.6961	2.8	0.0075*
SL × MC × SD	8	0.0382	4.0	0.0004*	7.3796	0.7	0.7072
Error	90	0.0095			10.8375		

*Significant values ($P \leq 0.05$)

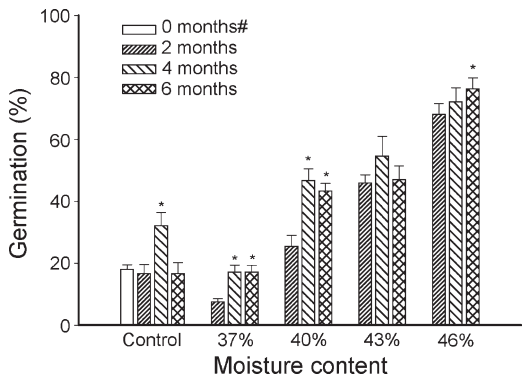

Figure 1. Effect of freezing storage for up to 6 months on the germination of oak acorns adjusted to different moisture levels prior to storage. Acorns (40–42 per cent moisture content) were soaked and then re-dried to the various moisture levels, except for the controls which were not soaked. Means within each seed moisture level that are significantly different ($P \leq 0.05$) from the 2-month value are shown (*). #, Available for control acorns only.

(or MGT declined) as MC increased, but the effect of storage duration on this response was relatively small.

Discussion

The most significant finding of this study was that increasing the moisture content of oak acorns followed by freezing at –3°C greatly increased percentage germination and germination speed compared with that achieved by the standard (non-soaked) control. The effect of soaking was largest for seeds that had been adjusted to the higher MC levels. Others have also revealed that pendunculate oak acorns are sensitive to MC during storage (Gosling, 1989; Finch-Savage, 1992; Suszka *et al.*, 1996), but to the authors' knowledge there is no information on the effect of acorn MC level during freezing storage on germination potential. Jensen (2002) found that acorns stored at 48 per cent MC for up to 16 weeks at 4°C germinated better than those adjusted to 52, 40 or 42 per cent MC. However, a high proportion of the seeds germinated prematurely in that study, probably because they were stored at a higher temperature than that used in this study. Suszka *et al.* (1996) also showed that seeds of pedunculate oak germinated better after 100 days prechilling (temperature not given) at 44 per cent MC that when prechilled between 23 and 37 per cent MC. Similar results have been reported for *Quercus macrocarpa* Michx. (Schroeder and Walker, 1987). Although some

Table 2: Effect of soaking acorns for 2 days after storage on the percentage germination of pedunculate oak from seedlot C10

Storage duration (months)	Non-soaked	Soaked
0	17.5^{a}	23.3^{ab}
4	41.7^{c}	31.7^{bc}
6	25^{ab}	25^{ab}

Means followed by the same superscript letter are not significantly different ($P \leq 0.05$).

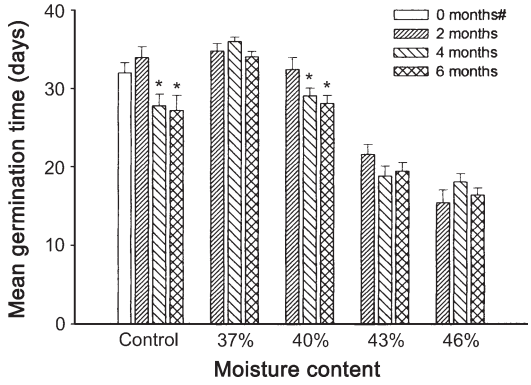

Figure 2. Effect of freezing storage for up to 6 months on mean germination time of oak acorns adjusted to different moisture levels prior to storage. Acorns (40–42 per cent moisture content) were soaked and then re-dried to the various moisture levels, except for the controls which were not soaked. Means within each seed moisture level that are significantly different ($P \leq 0.05$) from the 2-month value are shown (*). #, Available for control acorns only.

acorns germinated prematurely at –3C after 4–6 months' storage at high seed MC levels in this study, the proportion was low (<13 per cent) and no radicle exceeded 0.5 cm. It is unlikely that acorns with short protruding radicles would be damaged during handling and sowing operations (Jensen, 2002).

The exact reason for the beneficial effect of freezing storage following soaking on the germination response (Figures 1 and 2) is unclear, but several theories might be advanced. The initial drying of the acorns to 42–43 per cent MC just after harvesting (prior to time of arrival for treatment) may have caused some desiccation damage or metabolic adjustments. However, most desiccation damage is caused at MC <40 per cent (Suszka and Tylkowski, 1980; Gosling, 1989; Poulsen, 1992; Finch-Savage *et al.*, 1996). The additional water supplied during soaking prior to storage might have facilitated the functioning of repair mechanisms (see Villiers, 1974). Others have also shown the benefits of re-hydration in improving the germination response in oak (Bonner and Vozzo, 1987), including pedunculate oak (Gosling, 1989). There is evidence that the ability of the membrane lipid structure to adjust between gel and liquid crystalline phases in response to drying and rehydration declines as germination potential decreases (Priestley and Leopald, 1983; Connor and Sowa, 2003), and similar changes might be expected as seeds age during storage. This might help explain the decline in seed germination following 6 months' (except the 46 per cent MC treatment) compared with values following 4 months' storage (Figure 1). The critical MC for acorn survival appears to be related to the level of matrix-bound water (Finch-Savage, 1992; Grange and Finch-Savage, 1992) and the MC of the cotyledons may be the most critical factor (Finch-Savage, 1992). Membrane damage and the loss of viability during drying may be caused by high respiration rates and inadequate protection by free-radical scavengers (Finch-Savage *et al.*, 1996).

Although pedunculate oak has an epicotyl form of dormancy (Bonner and Vozzo, 1987), which is expressed mainly at temperatures <15°C (Corbineau *et al.*, 2003), radicle dormancy is considered weak (Suszka *et al.*, 1996). However, mild freezing temperatures are known to be highly beneficial in releasing dormancy in Norway maple (*Acer platanoides* L.) (Suszka *et al.*, 1996), and a similar response might have occurred in pedunculate oak in this study. Furthermore, Suszka *et al.* (1996) reported that germination potential of pedunculate oak seeds sometimes increased and they responded better to low germination temperatures after freezing storage, further supporting the view that mild freezing temperatures affected the dormancy response. Poulsen (1992) also provided evidence that cold (non-freezing) temperatures improved germination in pedunculate oak seeds.

The dormancy mechanism in oak seeds appears to be mediated by the pericarp (Suszka *et al.*, 1996). The pericarp of red oak (*Quercus rubra* L.) acorns exerts dormancy through chemical constraint, mechanical inhibition or other means (Peterson, 1983), and a similar mechanism might operate in pedunculate oak. Acorns tested before storage had a germination potential of only 18 per cent (Figure 1), compared with 89 per cent after the 'scar end' of pericarp was removed for the official seed tests. If the mechanism of germination control was exerted through the pericarp, it appears that 2–4 months' storage at –3°C was adequate to release dormancy. Freezing moist

storage might have helped to 'soften' the pericarp. Gosling (1989) also reported that soaking improved the germination of acorns that had been stored for up to 24 weeks after first being slowly dried in a cold store for 28 weeks. He suggested that soaking could be used to retard acorn deterioration for all except the freshly harvested seeds (45 per cent at time of harvest in that study). However, it is difficult to compare the results of that study with those of this study because the temperature and the duration of drying and storage differed between studies.

The results of this study also bring into question the usefulness of the data obtained by the ISTA-approved method of excising the scar end of the acorns for the germination tests. While the method may allow the rapid assessment of acorn viability, the data provided may be less reliable in nursery operations.

A 2-day soak period was used to treat the control acorns after storage because Gosling (1989) showed that this treatment was adequate to improve germination after storage. However, in this study soaking the control acorns for 2 days after storage did not improve germination. The highly beneficial effect of the 5-day soak pre-storage soak period on germination was an unexpected outcome (Figures 1 and 2). Further studies are underway to determine if post-storage soaking for 5 days improves acorn germination.

Many seeds did not germinate during the test period, especially those that had low MC levels during storage. In some cases the seeds succumbed to disease during the test. However, since the viability of acorns was not assessed at the end of the test, the exact reason why the seeds did not germinate could not be ascertained.

Some seedlot differences were significant, including some interactions with other factors, but the response of the seeds to treatment was almost identical in each seedlot. All interactions were minor compared with the main effects. For example, the increase in germination potential following 4 months' storage was slightly greater in one lot than in the other. Seedlot differences are not unexpected since the physiological condition of seeds is influenced by genetic factors (e.g. provenance), environmental conditions during maturation (Baskin and Baskin, 1988), and by procurement and processing methods used (Tanaka, 1984). Furthermore, preliminary results from an on-going study in 2003/04, using two additional seedlots, showed an identical pattern to that described in this study.

In conclusion, acorns which have dried (usually to about 40–42 per cent MC) during normal handling after harvesting and temporary storage should be soaked to increase their MC to ~46 per cent and then stored at –3°C for up to 6 months. This treatment should increase the germination potential of the acorns, probably largely through its effect on the functioning of the pericarp, but the exact mechanism is not known.

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