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## Research Note

# Comparison of seed desiccation sensitivity amongst *Castanea sativa*, *Quercus ilex* and *Q. cerris*

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## Abstract

The distribution and propagation by seed of species with recalcitrant seed storage behaviour requires knowledge of the lowest safe moisture content (LSMC) before desiccation damages seed survival. The LSMCs were comparatively high and varied amongst the forest tree species *Castanea sativa* (58%), *Quercus cerris* (40-47.5%) and *Q. ilex* (44%) (Fagaceae) collected at one site in one year. Relations between lower seed moisture contents and viability (radicle emergence) were sigmoidal and quantified by logistic curves, with *Q. ilex* showing the smallest seed-to-seed variation.

**Keywords:** *Castanea sativa*, desiccation tolerance, Fagaceae, germination, *Quercus cerris*, *Quercus ilex*, recalcitrant

## Experimental and discussion

Seeds of most plant species are orthodox (Roberts, 1973); they survive desiccation and their longevity is increased in a predictable manner by reducing the moisture content and temperature of storage. The term 'recalcitrant' (Roberts, 1973) describes the smaller number of plant species whose seeds do not survive (considerable) desiccation. Recalcitrant seeds are difficult to store long-term.

Seed survival for at least short-term periods is required for seed collection and distribution in order to maintain, distribute and propagate a species, but it is difficult to know how much moisture loss is acceptable before viability is reduced when handling recalcitrant seeds. Considerable variation in the lowest safe moisture content (LSMC,

below which, further desiccation reduces viability) has been reported amongst species with recalcitrant seeds; for example, from as low as 15% (wet basis, w.b.) in *Quercus ithaburensis* Decne. ssp. *Ithaburensis* (Ganatsas *et al.*, 2017) up to 61-62% in *Avicennia marina* (Forsk.) Vierh. (Farrant *et al.*, 1985; Le Tam *et al.*, 2004).

The forest trees *Castanea sativa* Mill. (sweet chestnut), *Quercus cerris* L. (Turkey oak) and *Q. ilex* L. (holm oak) (Fagaceae) are native to Europe (de Rigo *et al.*, 2016). They produce large seeds shed at high moisture content with seed storage behaviour classified as recalcitrant (*C. sativa*, *Q. cerris*) or probably recalcitrant (*Q. ilex*) (Hong *et al.*, 1996); *Q. ilex* is now confirmed as desiccation intolerant (Joët *et al.*, 2013). Reports of loss in viability following desiccation in recalcitrant species tend, inevitably, to be carried out at different sites in different years. We compare here the effect of desiccation on ability to germinate on seeds of three Fagaceae species harvested at a single site in one season in order to identify and compare the LSMC amongst these three forest tree species.

Fresh, mature seeds of *C. sativa* were collected from trees and recently-shed seeds of *Q. ilex* and *Q. cerris* from the ground in October 1997, at Whiteknights Park, University of Reading, UK (51° 26' N latitude, 0° 57' W longitude). Seeds were cleaned by hand immediately after collection, and initial seed viability and moisture content of controls (not dried) determined. Germination tests were conducted on samples of 100 seeds in four replicates of 25 between moist paper towels (Kimberley Clark Professional 6803 HOSTESS, Natural, 240 × 350 mm, Greenham Sales, UK) in plastic boxes at alternating 20/30°C (16 hours/8 hours) with light for 8 hours day<sup>-1</sup> (during the warm phase). Seeds were monitored for germination regularly; those that had begun to germinate (radicle > 2 mm) were counted and removed. Germination tests ended after 45 days; no firm seeds remained at this time. Seed moisture content was determined by the low-constant temperature oven method and is expressed on the fresh weight basis (ISTA, 1999) to the nearest 0.5%.

The remaining seeds of each species were divided into sub-samples at random and dried in a forced-air cabinet at 15-17% relative humidity with 15-17°C. Samples were removed after different periods of drying. Each sub-sample was then stored at 5°C for five days, to allow moisture to equilibrate within and amongst the seeds. Germination and habitat requirements are linked in *Quercus* (Xia *et al.*, 2015): 5°C is within the site's minimum to average October - November ambient range and hence this temperature is not extreme. Samples were then divided and one subjected to moisture content determination and the other tested for ability to germinate. Seeds expected to be at 10% moisture content or below (based on loss in sample weight) were humidified above water at 20°C for 24-48 hours before the germination test to avoid imbibition damage (Hong and Ellis, 1996).

Seeds of *C. sativa* had the highest moisture content at collection (58%), then *Q. cerris* (47.5%) and *Q. ilex* the lowest (44%) (figure 1). *Quercus ilex* and *Q. cerris* showed greater ability to germinate at collection than *C. sativa*. In all three species, ability to germinate of each seed population showed a sigmoidal response to moisture content during desiccation, described here by the logistic regression

$$\text{Germination (\%)} = 100 \times (\alpha / (1 + \beta \cdot e^{(-\gamma \cdot m)})) \quad (1)$$

where  $\alpha$ ,  $\beta$  and  $\gamma$  are fitted constants, and  $m$  is seed moisture content (% w.b.).

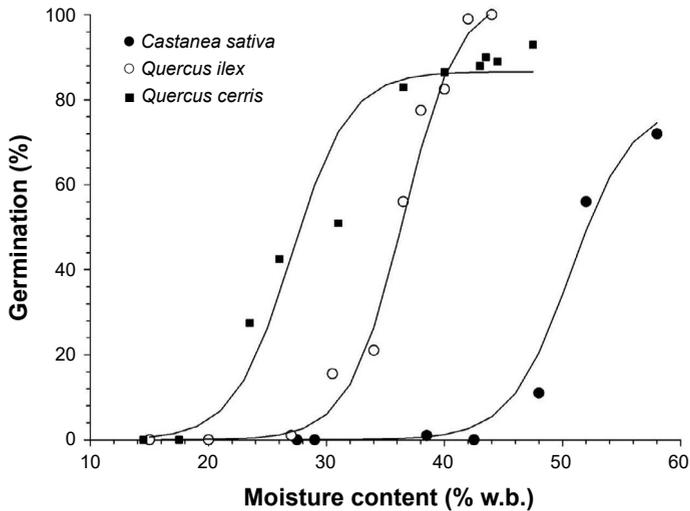


Figure 1. Relationship between seed moisture content (% w.b.) and ability to germinate in *Castanea sativa* (●), *Quercus ilex* (○) and *Q. cerris* (■). Fitted lines are logistic regressions (equation 1).

Seeds of *C. sativa* were the most desiccation intolerant (figure 1). They showed a marked decline with the initial desiccation treatment and almost no germination was detected following desiccation to 42.5% moisture content or below. The response provided a LSMC of at least 58% in *C. sativa*. This could be below the true value. Given the low ability to germinate initially (figure 1), desiccation may have occurred in tree before collection. The value of 58% is greater than earlier estimates for this species (Hong *et al.*, 1996), and particularly so in comparison with the report that 60% of excised embryonic axes survived desiccation to 25% moisture content (Pence, 1992). Embryos of recalcitrant species are known to be considerably more tolerant of desiccation, however, than whole seeds (Hong *et al.*, 1996).

*Quercus ilex* and *Q. cerris* seeds also showed desiccation sensitivity, but this response was displaced to lower moisture contents than *Castanea sativa* (figure 1). Seeds of *Q. ilex* were more sensitive to desiccation on average than *Q. cerris*, and possibly showed a little less seed-to-seed variation in the minimum moisture content for survival. Whereas desiccation of *Q. cerris* seeds from 47.5 to 31% moisture content reduced viability from 93 to 50%, similar desiccation in *Q. ilex* (44 - 30%) reduced viability from 100 to 17%. The estimated LSMC was the seed collection moisture content for *Q. cerris* (47.5%) and *Q. ilex* (44%) from the observations. In *Q. cerris*, however, the logistic curve fitted suggested desiccation from 47.5 to 40% resulted in little damage (figure 1), suggesting the LSMC could be closer to 40%.

Little or no germination was detected in *Q. cerris* (0%) and *Q. ilex* (1%) once dried to 17.5 or 27% moisture content, respectively. The population response of seed survival to desiccation determined here for seeds of *Q. ilex* collected from one site in southern England

was similar to that reported by Joët *et al.* (2013) for seeds collected from 42 woodlands in southern France. Their LSMC was only around 37%, however. Our greater LSMC (figure 1) might result from the cooler, wetter climate at the higher latitude. More widely across the literature for *Quercus*, our LSMC estimates are slightly greater than previously reported upper extremes of 43% for (temperate) *Q. fabri* Hance (Tian and Tang, 2010) and 44% for (temperate to sub-arctic) *Q. macrocarpa* Michx. (Schroeder and Walker, 1987); and considerably above the lowest reports of 15 and 25% for (Mediterranean-adapted) *Q. ithaburensis* ssp. *ithaburensis* and ssp. *macrolepis*, respectively (Ganatsas *et al.*, 2017).

We hypothesise, from our results and the summary above, a trend of greater LSMC with adaptation to cooler and wetter climates within *Quercus*. This is compatible with Daws *et al.* (2004), where seeds of *Aesculus hippocastanum* L. from trees further north across Europe were less developed when shed due to cooler temperatures. They concluded that variation in recalcitrant seed traits was explained by environment within *A. hippocastanum*, and hypothesised that this might also apply to other species.

All three species showed the seed desiccation sensitivity expected for recalcitrant seeds. Certain recalcitrant seeds can survive limited desiccation after collection. The moisture content of seeds of *Acer pseudoplatanus* L., for example, can be reduced by around 5 to 10% and sometimes more after collection without loss in viability (Hong and Ellis, 1990). In contrast, seeds of *C. sativa* and *Q. ilex*, and possibly *Q. cerris*, survived no post-collection desiccation before some seeds lost viability (figure 1). Hence in all these three species it is imperative to avoid, or minimise, loss in moisture from seed samples collected to propagate these trees. Moreover, as rainfall patterns alter with climate change (IPCC, 2013) the natural regeneration of these trees will benefit from natural environments which conserve moisture between shedding and germination with, for example, the incorporation of shed seeds deep into leaf litter reinforcing the latter's importance in ecosystem dynamics.

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