

A comparative study of desiccation responses of seeds of Asian Evergreen Oaks, *Quercus* subgenus *Cyclobalanopsis* and *Quercus* subgenus *Quercus*

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Received 24 June 2010; received in revised form 5 March 2011; accepted 3 May 2011

Abstract

The fruit biology of *Quercus* subgenus *Quercus* has received considerable attention. However, considerably less is known about the fruit (hereafter referred to as seed) biology of subgenus *Cyclobalanopsis* which is distributed mainly in tropical and sub-tropical Asia. Consequently, we investigated the responses to desiccation of seeds of 11 species from subgenus *Cyclobalanopsis* and compared these with responses of 11 species from subgenus *Quercus* from both China and Europe. Similar to species in the subgenus *Quercus*, we found that all 11 subgenus *Cyclobalanopsis* species had desiccation-sensitive (recalcitrant) seeds. For the 22 species, which had seed dry masses spanning 0.57 to 6.41 g, there were wide differences in drying rates, but drying rate was not related to either seed dry mass or oil content. Drying rates also varied across the individual seeds within a species. These differential drying rates within a seed resulted in some individual seeds remaining significantly moister than the mean water content which may explain the apparent ability of some seeds of several species e.g. *Quercus schottkyana* to tolerate desiccation.

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Keywords: Acorn; Desiccation; Drying rate; Recalcitrant; Seed mass

1. Introduction

Based on their response to desiccation, seeds are divided into three broad categories; orthodox, intermediate or recalcitrant. Orthodox seeds can tolerate desiccation to low moisture contents (<7%) with little negative effect on viability (Roberts, 1973). In contrast, recalcitrant seeds are killed by drying to moisture contents as high as 20–30% (Pritchard, 2004). Intermediate seeds tolerate desiccation to moisture contents of ca. 10–12%, but further desiccation reduces viability, and/or there is a more rapid loss in viability during storage at cooler as opposed to warmer temperatures (Hong et al., 1998).

The genus *Quercus* (Fagaceae) contains two subgenera, *Quercus* and *Cyclobalanopsis*. Based on current knowledge of

seed storage behaviour for subgenus *Quercus*, most species appear to have recalcitrant seeds (Hong et al., 1998). However, Nyandiga and McPherson (1992) reported that *Quercus emoryi* may have intermediate seed storage behaviour. Because of this prevalence of recalcitrant seeded species within this subgenus, this group has attracted much interest as a model system for understanding recalcitrant seed storage behaviour (e.g. Bonner, 1996; Finch-Savage, 1992; Grange and Finch-Savage, 1992; Pritchard, 1990), facilitated by the wide distribution of this subgenus throughout temperate and subtropical montane areas of the Northern Hemisphere (Frodin and Govaerts, 1998; Huang et al., 1999; Nixon, 1993).

However, subgenus *Cyclobalanopsis* is restricted in distribution to tropical and subtropical Asia (Huang et al., 1999; Luo and Zhou, 2001; Nixon, 1993). In a few Chinese surveys (National Service Center for State-Owned Forest Farms and Forestry Seed Seeding Affairs of the Forestry Administration,

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Table 1

Collection date and location for the study species. Species names in bold are subgenus *Cyclobalanopsis*, the remaining species are subgenus *Quercus*.

Species	Collection date	Collection location	Altitude (m)
Chinese species			
<i>Q. annulata</i> Sm. [†]	11/10/06	GIB, Guilin, Guangxi: N 25°01', E 110°17'	230
<i>Q. austroglauca</i> (Y.T. Chang) Y.T. Chang [†]	08/11/06	Xichou, Yunnan: N 23°26', E 104°40'	1495
<i>Q. camusiae</i> Trel. ex Hickel & A. Camus [†]	27/09/06	Dawei Mountain, Pinbian, Yunnan: N 22°54', E 103°41'	2010
<i>Q. fleuryi</i> Hickel & A. Camus [†]	03/11/07	GIB, Guilin, Guangxi: N 25°01', E 110°17'	230
<i>Q. glauca</i> Thunb. [†]	05/10/06	Hengshan Mountain, Hunan: N 30°01', E 110°08'	1090
<i>Q. lamellosa</i> Sm. [#]	23/10/06	Wuliang Mountain, Jingdong, Yunnan: N 24°43', E 100°30'	2398
<i>Q. multinervis</i> (W. C. Cheng & T. Hong) Govaerts [†]	04/10/06	Hengshan Mountain, Hunan: N 27°25', E 112°86'	1090
<i>Q. myrsinifolia</i> Blume [†]	23/10/06	Lushan Mountain, Jiujiang, Jiangxi: N 29°33', E 115°59'	1032
<i>Q. schottkyana</i> Rehder & E. H. Wilson [#]	02/11/06	KIB, Kunming, Yunnan: N25°01', E102°41'	1900
<i>Q. sichourensis</i> (Hu) C. C. Huang & Y. T. Chang [†]	20/11/07	Funing, Yunnan: N23°44', E 104°53'	980
<i>Q. stewardiana</i> A. Camus [†]	23/10/06	Lushan Mountain, Jiujiang, Jiangxi: N 29°33', E 115°59'	1032
<i>Q. fabri</i> Hance [#]	17/10/07	Xishan Mountain, Kunming, Yunnan: N 24°97', E 102°62'	2050
<i>Q. franchetii</i> Skan [#]	30/10/07	KIB, Kunming, Yunnan: N25°01', E102°41'	1900
<i>Q. variabilis</i> Blume [#]	23/10/07	KIB, Kunming, Yunnan: N25°01', E102°41'	1900
European species			
<i>Q. cerris</i> L. [#]	11/06	Rolasco, Alessandria, Italy: N 44°55', E 08°37'	95
<i>Q. ilex</i> L. [#]	11/06	Bosco della Tesolo, Ferrara, Italy: N 44°50', E 11°37'	9
<i>Q. macranthera</i> Fisch. & C. A. Mey. ex Hohen. [#]	12/06	Gardabani, Georgia: N 41°27', E 45°5'	311
<i>Q. petraea</i> (Matt.) Liebl. [#]	12/06	Kirstinebjergvej, Årslev, Denmark: N 55°19', E 11°28'	3
<i>Q. pubescens</i> Willd. [#]	11/06	de Fasse-Pissoiotto, Verona, Italy: N 45°26', E 10°59'	59
<i>Q. robur</i> L. [#]	11/06	Bosco della Partecipiano, Novara, Vercelli, Italy: N 45°27', E 08°37'	162
<i>Q. rubra</i> L. [#]	11/06	R.N.O. Boscofontana, Milano, Italy: N 45°27', E 09°11'	120
<i>Q. suber</i> L. [#]	11/06	Ducua Fecuglio, Grosseto, Italy: N 42°46', E 11°06'	10

[†]stored at 15 °C prior to experimentation; [#]stored at 5 °C prior to experimentation.

1999), the seeds of this subgenus have been described as short-lived requiring moist storage. However, the actual responses of the seeds to desiccation have been little studied. The scarcity of seed biology knowledge of subgenus *Cyclobalanopsis* may also result from confusion regarding the taxonomy of the subgenus. In some works, this subgenus is treated as a genus (e.g. Huang et al., 1999) while Hong et al. (1998) reported some species from this subgenus as genus *Cyclobalanopsis* and others as genus *Quercus*. Hong et al. (1998) presented data for five species in subgenus *Cyclobalanopsis*: *Q. acuta*, *Q. lamellosa*, *Q. lineata*, *Q. myrsinifolia* (all listed as genus *Quercus*), and *Q. glauca* (listed as genus *Cyclobalanopsis*), out of a total of 150 known species (Huang et al., 1999).

The subgenus *Cyclobalanopsis* is important in Asia both as a dominant component in evergreen broad-leaved forests and for timber. Because of the ecological importance of this group, coupled with limited knowledge of the seed biology of subgenus *Cyclobalanopsis*, we investigated the desiccation tolerance of these seeds. In addition, some of the species in this subgenus have been reduced to only a small number of individuals; conservation of these species is therefore of high importance (Xia et al., 2008). Understanding their seed storage behaviour will facilitate the planning of appropriate conservation strategies. In this study, the response to desiccation of fruits (referred to as 'seeds') from 11 species in subgenus *Cyclobalanopsis* from S and SW China was characterised. For comparison, seeds of 11 species from subgenus *Quercus*, eight from Europe and three from SW China were also investigated. The differences in desiccation response across species are reported and discussed.

2. Materials and methods

2.1. Fresh material

Fruits (acorns; referred to as seeds throughout) of 11 species from subgenus *Cyclobalanopsis* and three species from subgenus *Quercus* were collected from S and SW China in late autumn 2006 and 2007, at the time of natural dispersal (Table 1). Seeds of eight species of *Quercus* subgenus *Quercus* were obtained from Italy, Denmark and Georgia late autumn–winter 2006 (Table 1). Upon receipt, seeds were stored at either 15 °C (for subtropical subgenus *Cyclobalanopsis* species) or 5 °C (for temperate subgenus *Quercus* species and *Q. lamellosa* and *Q. schottkyana* from subgenus *Cyclobalanopsis*, whose seeds were collected in a temperate climate region), based on ambient temperatures at the time of dispersal (Table 1). Germination and desiccation experiments commenced within two weeks of receipt. Prior to experiments, seeds were visually checked and any infested by fungi or weevils were discarded.

2.2. Initial moisture content and germination

Upon receipt, 15–25 seeds of each species were dissected into their component parts (embryonic axis, cotyledons, and seed coat [botanically the pericarp]) for gravimetric determination of moisture content by drying in an oven at 103 °C for 17 h (ISTA, 2007). Moisture contents are expressed on a fresh weight basis (% fr.wt). A further sample of seeds was placed into the sample chamber of a HygroPalm water activity monitor with AWVC-DIO sensor (Rotronic Ltd., UK) for non-

destructive measurement of equilibrium relative humidity (eRH).

For each species, samples of 25 seeds (as sub-samples of 12 and 13) were sown to test for germination at 20 °C. Only 25 seeds were used due to the difficulty of obtaining large numbers; similarly low numbers of seeds have been used elsewhere for large seeded tree species (e.g. Daws et al., 2004a). Where possible, seeds were also sown to test for germination at 15 and 25 °C. Seeds were incubated in sandwich boxes (174 × 115 × 60 mm, L × W × D) on top of an approximately 10 mm deep layer of 1% agar-water. Germination was defined as radicle emergence by at least 2 mm. Germination was recorded until no further germination was observed for at least 1 month.

2.3. Seed desiccation responses

To determine seed responses to desiccation, a modified version of the 100-seed test was used (Pritchard et al., 2004). For each species, 30 or 37 seeds were dried using freshly regenerated silica gel in a 1:1 ratio by weight. Seeds and silica gel were mixed and sealed in press-seal polythene bags (140 × 140 mm, 230 × 150 mm, or 255 × 355 mm depending on seed-size) and placed at 15 °C. The fresh weight of each batch of seeds was determined before desiccation and recorded on alternate days during desiccation. When the seed weight had declined by ca. 50%, the eRH of the desiccating seeds was measured weekly using the HygroPalm water activity monitor with AWVC-DIO sensor. Desiccation was ended when the eRH

of the seeds was approximately 15%. Concurrent with the desiccation trials, duplicate samples of seeds were stored in press-seal polythene bags with an equal volume of moistened vermiculite at 15 °C, as a moist (non-desiccated) control.

Once drying was complete, 5 or 12 seeds of both the desiccated and moist-stored seeds were used for moisture content determination (individual, intact seeds). The remaining 25 seeds in each batch were sown (as subsamples of 13 and 12 seeds) on 1% agar water at either 20 or 25 °C (Table 2). The moisture contents (MC, % fresh weight basis) of the seeds during desiccation were estimated using the following equation:

$$\text{EstimatedMC} = 100 \times [W_f - (W_d - (W_d \times MC_d))] / W_f$$

Where W_f = the seed fresh weight during drying, W_d = final (after desiccation) seed fresh weight and MC_d = final seed moisture content.

2.4. Full assessment of desiccation tolerance (*Q. schottkyana* and *Q. lamellosa*)

A full assessment of desiccation tolerance was carried out for *Q. schottkyana* and *Q. lamellosa* following the approach in Hong and Ellis (1996). For desiccation, six (*Q. schottkyana*) and eight (*Q. lamellosa*) samples of 100 seeds each were placed in press seal polythene bags with an equal weight of freshly regenerated silica gel. The bags were held at 15 °C. During drying, seeds were periodically re-weighed to estimate their final moisture content (based on their initial moisture content

Table 2

Seed characteristics of the 22 *Quercus* species investigated. All data are means ± 1SE. Germination data are the maximum germination percentages at the optimal temperature (where 15, 20, and 25 °C were tested). Dry mass and initial moisture contents on 25 individuals, germination tests were carried out on a total of 25 seeds (as sub-samples of lots of 12 and 13 seeds) and oil contents were (dry mass basis) obtained from Xia et al. (2010).

Species	Dry mass (g)	Oil content (%) [†]	Initial moisture content (%)				Maximum germination (%)	Temp. (°C) [‡]
			Whole seed	Axis	Cotyledons	Coat		
<i>Q. annulata</i>	1.63 ± 0.08	0.9 ± 0.3	37.2 ± 1.0	55.0 ± 1.1	43.1 ± 1.6	21.8 ± 0.3	90.0	15
<i>Q. austroglauca</i>	2.43 ± 0.14	–	35.6 ± 1.3	49.3 ± 0.8	40.5 ± 1.7	19.8 ± 0.3	40.1	20
<i>Q. camusiae</i>	1.79 ± 0.12	–	42.2 ± 1.4	50.9 ± 1.8	50.9 ± 1.8	22.0 ± 0.5	72.1	20
<i>Q. cerris</i>	4.63 ± 0.24	7.2 ± 0.2	34.5 ± 0.4	50.1 ± 0.5	35.6 ± 0.4	27.5 ± 0.4	100	25
<i>Q. fabri</i>	0.74 ± 0.03	2.5 ± 0.6	42.6 ± 0.9	54.2 ± 0.4	47.4 ± 1.1	23.7 ± 0.5	96.2	20
<i>Q. fleuryi</i>	6.41 ± 0.19	1.5 ± 0.2	33.6 ± 0.6	51.6 ± 0.6	36.0 ± 0.9	28.5 ± 0.6	96.3	15
<i>Q. franchetii</i>	0.59 ± 0.02	7.1 ± 0.8	39.9 ± 0.5	50.8 ± 3.2	45.3 ± 0.6	20.0 ± 0.2	100	15, 25
<i>Q. glauca</i>	0.57 ± 0.01	0.9 ± 0.3	44.0 ± 0.6	58.0 ± 0.8	49.2 ± 0.8	19.8 ± 0.4	87.8	20
<i>Q. ilex</i>	2.00 ± 0.12	3.8 ± 1.3	37.7 ± 0.6	51.2 ± 0.7	39.2 ± 0.7	29.0 ± 0.3	72.4	20
<i>Q. lamellosa</i>	4.55 ± 0.30	0.7 ± 0.3	47.4 ± 1.1	61.7 ± 0.7	47.6 ± 1.5	46.5 ± 0.7	32.7	20
<i>Q. macranthera</i>	2.74 ± 0.19	–	32.3 ± 0.8	43.8 ± 0.9	34.3 ± 0.9	24.6 ± 0.8	8.3	20
<i>Q. multinervis</i>	0.72 ± 0.03	1.1 ± 0.6	48.4 ± 0.9	58.7 ± 0.9	53.4 ± 1.1	20.5 ± 0.5	70.0	25
<i>Q. myrsinifolia</i>	0.71 ± 0.03	–	31.4 ± 0.5	47.5 ± 0.8	35.0 ± 0.5	14.6 ± 0.3	32.4	20
<i>Q. petraea</i>	1.36 ± 0.08	5.7 ± 0.3	49.5 ± 0.9	54.0 ± 0.6	41.9 ± 0.7	29.2 ± 0.6	96.2	15
<i>Q. pubescens</i>	1.98 ± 0.10	6.5 ± 0.5	35.4 ± 0.3	46.6 ± 1.8	36.9 ± 0.4	25.3 ± 0.2	42.0	25
<i>Q. robur</i>	2.74 ± 0.09	3.5 ± 0.9	34.1 ± 0.3	50.6 ± 0.9	36.0 ± 0.3	24.9 ± 0.1	82.6	15
<i>Q. rubra</i>	2.94 ± 0.11	18.0 ± 1.2	36.1 ± 0.5	39.8 ± 0.7	28.6 ± 0.5	23.9 ± 0.3	87.8	15
<i>Q. schottkyana</i>	1.02 ± 0.04	3.8 ± 0.5	36.6 ± 0.4	55.5 ± 1.1	39.8 ± 0.4	21.0 ± 0.6	92.0	25
<i>Q. sichourensis</i>	6.25 ± 0.40	1.2 ± 0.1	43.0 ± 1.2	55.2 ± 1.8	49.1 ± 2.1	35.4 ± 1.1	28.0	25
<i>Q. stewardiana</i>	0.70 ± 0.05	3.2 ± 0.7	36.5 ± 0.6	50.3 ± 1.0	40.4 ± 0.8	16.0 ± 0.3	44.2	20
<i>Q. suber</i>	2.62 ± 0.15	1.5 ± 0.5	48.4 ± 3.1	58.7 ± 1.6	52.3 ± 1.0	32.9 ± 1.2	80.0	25
<i>Q. variabilis</i>	1.33 ± 0.06	2.1 ± 0.6	38.0 ± 0.70	54.5 ± 1.5	42.4 ± 1.2	27.5 ± 0.7	77.6	20

[†]Data from Xia, et al. (2010); [‡]Germination temperature.

and starting weight). This meant that it was possible to stop the drying process at a range of target moisture contents for moisture content determination and germination testing. Concurrent with the desiccation experiments, duplicate samples of hydrated seeds were held at 15 °C, as a control, in polythene bags containing an equal volume of moistened vermiculite. These seeds were also used for moisture content determinations and germination tests. For all the germination tests, seeds (four replicates of 20) were sown on the surface of 1% agar in water in sandwich boxes at 25 °C and scored for germination every 2–3 days. The remaining 20 seeds were used for individual determination of seed moisture content, as before.

2.5. Statistical analysis

Drying curves of estimated MC vs. desiccation period were analysed using the FITNONLINEAR directive in GenStat 13 (VSNi Ltd., UK) to fit the equation,

$$\text{Estimated MC} = 100 \times [\text{MC}_{\text{eq}} + ((\text{MC}_i - \text{MC}_{\text{eq}}) \exp(-kp^n))]$$

Where MC_i is the initial, measured moisture content, p is the desiccation period in days, and the parameters to be estimated are the equilibrium moisture content, MC_{eq} (since for many species, seeds had not equilibrated with the silica desiccant); k and n (Page, 1949 cited in Bruce, 1985; Stoyanova et al., 2007). An index of seed drying rate was then calculated as the decline in moisture content over the first 28 d. Linear regression was used to test for a relationship between seed oil content or mean dry mass and drying rates using Minitab 13 (Minitab Inc., Pennsylvania, USA). Oil content data for these species were obtained from Xia et al. (2010).

3. Results

3.1. Initial seed characteristics

Initial characteristics of the seeds are shown in Table 2. The largest seeds were those of *Q. fleuryi* and *Q. sichouensis* with a mean seed dry mass of ca. 6 g. The smallest seeds, those of *Q. franchetii* and *Q. glauca*, had a mean seed dry mass of 0.6 g. Upon receipt, seeds of all species had a high eRH (82.9 to 97.9%, data not shown) and whole seed moisture content (31.4 to 49.5%). Across the different seed components, the axis had the highest moisture content followed by the cotyledons and the seed coat. With the exceptions of *Q. austroglauca*, *Q. lamellosa*, *Q. macranthera*, *Q. myrsinifolia*, *Q. pubescens*, *Q. sichouensis*, and *Q. stewardiana* germination was $\geq 70\%$ for at least one of the germination temperatures indicating the good quality of the seeds.

3.2. Drying rates and post-drying germination

After drying with silica gel, the whole seed moisture content of most species had decreased to less than 12% (Table 3). However, *Q. franchetii* and *Q. schottkyana* still had a mean whole seed moisture content of ca. 20%. All species had a

similarly low moisture content for seed coat (<9%, data not shown) and the axis and cotyledons of most species had fallen to less than 16 and 14%, respectively (Table 3). However, there was some variation in final moisture content between individual seeds (Fig. 1), in particular for *Q. franchetii* and *Q. schottkyana* which meant that the mean moisture content after drying for the axis and cotyledons of these species was relatively high (>29 and >22% for axis and cotyledons, respectively; Table 3). For example, after desiccation, 80% of *Q. franchetii* seeds had a whole seed moisture content >20% and an axis moisture content $\geq 34\%$; other *Q. franchetii* seeds had a whole seed and axis moisture content <10%.

Between species, the drying rates (defined as the decline in moisture content over the first 28 d) of the seeds varied from 4.1% to 36.0% for *Q. franchetii* and *Q. suber*, respectively (Table 3; Figs. A.1 and A.2). The variation in drying rates between species was neither related to seed dry mass nor the seed oil content (linear regression analysis, $R^2=0.06$, d.f.=21, $p>0.05$ for dry mass and $R^2=0.001$, d.f.=17, $p>0.05$, for oil content). At the point when drying was stopped, only some species (e.g. *Q. sichouensis*, *Q. ilex*, *Q. petraea*, *Q. rubra*) showed evidence of seeds reaching equilibrium (Figs. A.1 and A.2).

Except for *Q. franchetii*, *Q. robur*, and *Q. schottkyana*, no germination was observed after desiccation (Table 3). For all species, germination was still observed after hydrated storage, ranging from 3.3 (*Q. macranthera*) to 100% (*Q. fabri*).

3.3. Full assessment of desiccation tolerance (*Q. lamellosa* and *Q. schottkyana*)

Fig. 2 shows the moisture loss of the seeds of *Q. lamellosa* and *Q. schottkyana*. The seeds of *Q. schottkyana*, while having a lower initial mean moisture content, dried more slowly than those of *Q. lamellosa* (Fig. 2A). The seed coat of both species lost moisture very rapidly (Fig. 2B). During the initial rapid loss of water from the seed coat, the cotyledons and the axis of *Q. schottkyana* lost moisture slowly such that while the seed coat moisture content had been reduced to 10.3% after 52 days, the cotyledons and axis still had moisture contents of 37.6 and 51.7%, respectively. Subsequently, the cotyledons and axis lost moisture more quickly. After an initial rapid loss of moisture over the first 16 d, the moisture content of the seed coats of *Q. lamellosa* continued to decline steadily up to 143 d (Fig. 2B). The moisture content of the cotyledons and axis also declined steadily over this period (Fig. 2C and D). During desiccation, the variation (represented by standard deviation) in individual seed moisture contents of both the species increased (from 5.0 to 7.4 for *Q. lamellosa* and from 3.8 to 9.2 for *Q. schottkyana*) and then decreased (from 7.4 to 1.2 for *Q. lamellosa* and from 9.2 to 5.9 for *Q. schottkyana*) as seeds reached their final moisture content.

Germination of *Q. lamellosa* and *Q. schottkyana* seeds declined as moisture content was reduced during drying (Fig. 3). After 192 d drying with silica, the whole seed moisture content of *Q. schottkyana* had fallen to 9.9% and there was still 5.0% germination (Fig. 3B). *Q. lamellosa* seeds did not

Table 3

Desiccation and moist storage results for *Quercus* seeds of 22 species. Data are means \pm 1SE. Drying rates are presented as the decline in moisture content over the first 28 d.

Species	Desiccation						Moist storage		
	Drying rate (%)	Drying period (d)	eRH (%)	Moisture content (%)			Germination (%)	Germination (%)	Temperature (°C)
				Whole seed	Axis	Cotyledons			
<i>Q. annulata</i>	15.7	67	15.9	8.7 \pm 0.4	10.5 \pm 0.7	9.6 \pm 0.5	0	27.9	20
<i>Q. austroglauca</i>	13.6	72	12.5	6.7 \pm 0.5	8.8 \pm 0.5	9.3 \pm 0.8	0	23.7	20
<i>Q. camusiae</i>	19.1	66	15.1	11.7 \pm 2.1	14.1 \pm 2.3	13.3 \pm 2.4	0	7.7	20
<i>Q. cerris</i>	22.6	41	14.0	10.3 \pm 0.3	12.5 \pm 0.6	10.7 \pm 0.3	0	54.2	20
<i>Q. fabri</i>	16.4	90	9.1	6.6 \pm 0.1	7.0 \pm 0.3	7.3 \pm 0.2	0	100	20
<i>Q. fleuryi</i>	7.3	132	14.1	6.4 \pm 0.3	7.8 \pm 0.5	6.8 \pm 0.2	0	88.0	25
<i>Q. franchetii</i>	4.1	164	4.6	22.8 \pm 4.6	34.2 \pm 7.1	27.8 \pm 5.7	59.9	96.4	25
<i>Q. glauca</i>	14.8	79	14.0	10.2 \pm 1.2	15.8 \pm 3.1	11.4 \pm 1.5	0	32.4	20
<i>Q. ilex</i>	25.1	60	13.7	7.8 \pm 0.1	7.0 \pm 0.2	7.9 \pm 0.1	0	50.0	20
<i>Q. lamellosa</i>	27.1	52	14.4	11.7 \pm 0.5	11.8 \pm 1.1	13.6 \pm 0.7	0	28.2	20
<i>Q. macranthera</i>	17.9	35	15.0	10.5 \pm 0.4	9.6 \pm 0.2	11.3 \pm 0.5	0	3.3	20
<i>Q. multinervis</i>	21.6	69	10.2	7.9 \pm 0.3	11.0 \pm 0.4	8.6 \pm 0.3	0	11.9	20
<i>Q. myrsinifolia</i>	19.0	38	11.1	8.4 \pm 0.4	8.9 \pm 0.8	9.2 \pm 0.5	0	25.0	20
<i>Q. petraea</i>	20.1	69	11.9	7.6 \pm 0.3	7.2 \pm 0.3	7.9 \pm 0.4	0	87.8	20
<i>Q. pubescens</i>	17.5	56	14.6	8.1 \pm 0.2	9.2 \pm 0.4	8.6 \pm 0.2	0	7.7	20
<i>Q. robur</i>	18.8	44	14.5	10.9 \pm 1.1	9.7 \pm 0.9	11.9 \pm 1.4	24.7	51.6	20
<i>Q. rubra</i>	20.0	43	15.3	6.1 \pm 0.1	6.2 \pm 0.2	6.0 \pm 0.1	0	67.6	20
<i>Q. schottkyana</i>	8.0	78	15.9	19.5 \pm 2.7	29.3 \pm 4.0	22.3 \pm 3.2	27.6	87.8	25
<i>Q. sichourensis</i>	25.1	85	9.2	6.4 \pm 0.2	7.0 \pm 0.2	8.0 \pm 0.4	0	7.7	25
<i>Q. stewardiana</i>	14.2	73	15.7	8.1 \pm 0.2	7.6 \pm 0.7	8.7 \pm 0.3	0	10.0	20
<i>Q. suber</i>	36.0	41	11.4	8.7 \pm 0.5	9.6 \pm 1.0	9.0 \pm 0.6	0	71.4	20
<i>Q. variabilis</i>	17.8	90	10.1	10.5 \pm 3.7	13.5 \pm 6.1	12.8 \pm 5.5	0	54.2	20

germinate after the whole seed moisture content had been reduced to 29.1% (Fig. 3A). After hydrated storage for the same period of time (192 d and 143 d for *Q. schottkyana* and *Q. lamellosa* respectively), *Q. schottkyana* and *Q. lamellosa* still germinated to 81% and 44% of the initial germination level. The relationship between loss of seed viability and extent of desiccation for *Q. lamellosa* and *Q. schottkyana* was appeared to be linear for the majority of the regression lines ($p < 0.05$; Fig. 3).

4. Discussion

In this study, seeds of ten of the 11 species in the subgenus *Cyclobalanopsis* and nine of the 11 species investigated in subgenus *Quercus* were shown to display recalcitrant seed storage behaviour; desiccation to a whole-seed moisture content of $c. 10\%$ (eRH $< 16\%$) resulted in a complete loss of viability. As viability in the moist-stored controls was maintained, viability loss was a consequence of desiccation *per se* rather than storage duration. In contrast, there was some germination of seeds of *Q. franchetii* (60%), *Q. robur* (25%), and *Q. schottkyana* (28%) after drying until an eRH $< 16\%$ was recorded (Table 3). However, this result does not necessarily mean that these species cannot be classified as having recalcitrant seed storage behaviour; moisture content determinations on component parts of individual seeds of these species showed that there was wide variation in the rate at which individual seeds dried. Thus, although seed coat moisture content had been reduced to $< 9\%$ after 164 or 78 days of drying for *Q. franchetii* or *Q. schottkyana*, respectively, the mean

whole seed moisture content was $c. 19\text{--}23\%$, but ranged from 5 to 32% for *Q. franchetii* and from 8 to 34% for *Q. schottkyana* (Fig. 1). The mean moisture content of the axis and cotyledons was 29–34% and 22–28%, respectively, but ranged between 7–11 to $\geq 47\%$ and between 6–7 to $\geq 32\%$ for individual axes or cotyledons (both species), respectively. Similarly, the moisture content of the internal seed tissues of one of the *Q. robur* seeds remained relatively high (18 and 25% for the axis and cotyledons, respectively) after 44 days drying.

A slow as well as variable rate of drying of *Q. schottkyana* seeds was confirmed by the fuller study carried out for this species and for *Q. lamellosa*, where moisture content was actually determined during drying (rather than making estimations based on declining fresh weight). The moisture content-germination co-plot shows that seeds of *Q. schottkyana* can germinate at a mean seed moisture content of $c. 10\%$. However, at this stage, some seeds still had moisture contents in the range 15 to 27%: it is likely those seeds that germinated were those that were still at high moisture contents.

However, since desiccation substantially decreased the seed viability, it is likely that all the species studied here are desiccation sensitive. The viable seeds of some species when drying was seemingly complete ($\sim 15\%$ eRH) were not desiccation tolerant but had a moisture content higher than both the mean moisture content and the critical moisture content. Such apparent desiccation tolerance for a proportion of the population has been observed for other recalcitrant seeds such as *Zizania palustris* (Probert and Longley, 1989), *Acer pseudoplatanus* (Dickie et al., 1991), *Q. robur* (Finch-Savage, 1992), and *Vitellaria paradoxa* (Daws et al., 2004b) and

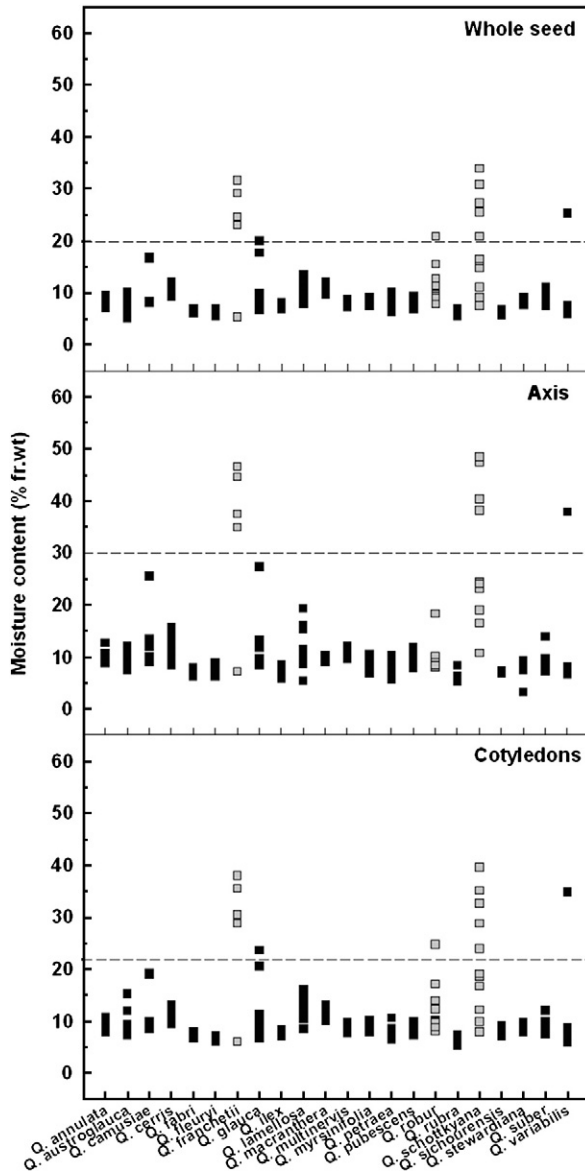


Fig. 1. Moisture contents of individual seeds, axes, and cotyledons at the conclusion of the desiccation tests for 22 *Quercus* species. The species (*Q. franchetii*, *Q. robur*, and *Q. schottkyana*) which had some post-desiccation germination are marked with grey squares.

attributed to seed-to-seed variation in post-drying moisture content. Interestingly, Finch-Savage (1992) demonstrated that viability was lost for individual seeds of *Q. robur* at a moisture content of ca. 19%. While our data demonstrated limited variation in moisture content between the *Q. robur* seeds studied, the wettest seeds (after desiccation) had a moisture content greater than 19%. Hence, this result is not incompatible with that of Finch-Savage (1992).

Even within the recalcitrant or ‘desiccation intolerant’ category, there are marked differences in the responses of seeds of different species, which led to the suggestion of highly-, moderately-, and minimally recalcitrant behaviour (Berjak et al., 1989; Farrant et al., 1988). This study demonstrated that seeds of some *Quercus* species (i.e. *Quercus robur*) could tolerate drying to a lower seed moisture content

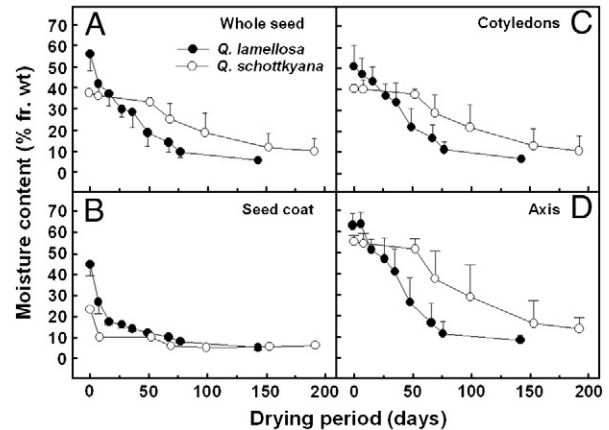


Fig. 2. Time-course of moisture loss during drying at 15 °C (using silica gel) and 15% RH of whole *Q. lamellosa* (closed cycles) and *Q. schottkyana* (open cycles) seeds. Results are presented for the whole seed (A), the seed coat (B), the cotyledons (C), and the axis (D). Each sample point represents the mean \pm SD of 20 seeds.

(ca. 20%, which was the highest moisture content amongst individual seeds) and might be described as minimally recalcitrant. Other *Quercus* species (i.e. *Q. glauca* and *Q. variabilis*) did not germinate after drying, even though some of the seeds placed to germinate are likely to have been above this same moisture content when drying was stopped; these species might be described as highly recalcitrant (Fig. 1). However, since detailed desiccation tolerance tests were only carried out for *Q. lamellosa* and *Q. schottkyana*, it is difficult to be sure where the other *Quercus* species fall within the recalcitrant category. These conclusions represent new records of desiccation sensitivity for eight species in subgenus *Cyclobalanopsis* (including *Q. schottkyana*) and two species in subgenus *Quercus* (including *Q. franchetii*) (Liu et al., 2008; Tian and Thang, 2010). The desiccation sensitivity for these species is consistent with both their relatively high seed masses (Daws et al., 2006) and high moisture content at shedding (Hong and Ellis, 1998).

Variation in seed drying rates between species was not related to either the oil content or size (mass) of the seeds. This is perhaps surprising since the surface area for moisture loss is directly related to seed volume (approximately equivalent to seed mass) and suggests that internal properties of the seed (such as coat structure) may regulate water loss. This is also suggested by the fact that even when the measured seed eRH was around or less than 15%, the axis and cotyledons within the seeds of some species such as *Q. franchetii* could still have a high moisture content (and presumably high eRH, had this been measured).

Pammenter et al. (1998, 2000) described two types of damage that could occur in desiccation sensitive seeds on drying: strict desiccation damage due to the loss of macromolecular structures caused by the removal of water; and aqueous-based oxidative damage that occurs at intermediate water contents and which is a consequence of unregulated metabolism. Rapidly dried seed/tissue might survive drying to lower water contents than those dried slowly because the seed/tissue

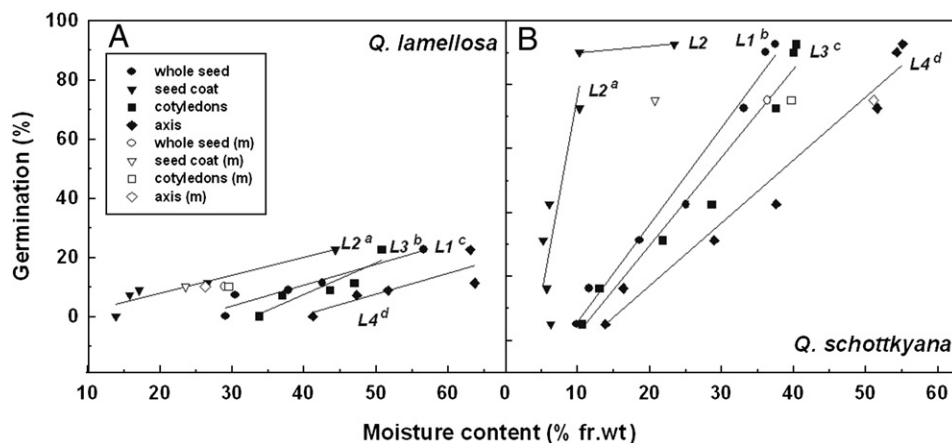


Fig. 3. The effect of moisture content on *Q. lamellosa* and *Q. schottkyana* seed germination. Whole seeds were desiccated with silica gel (at 15 °C and 15% RH). Moisture contents are the mean of determinations carried out using 20 individuals. Germination was carried out on a total of 80 seeds. Open and closed symbols represent the desiccated samples and the moist stored samples (m), respectively. For *Q. lamellosa*, slopes of lines are: L₁, $k=0.706$ ($R^2=0.924$, $p=0.009$); L₂, $k=0.614$ ($R^2=0.896$, $p=0.015$); L₃, $k=1.074$ ($R^2=0.846$, $p=0.027$); and L₄, $k=0.709$ ($R^2=0.729$, $p=0.066$) corresponding to % viability loss per 1% reduction in moisture content for whole seed, seed coat, cotyledons, and axis, respectively. For *Q. schottkyana* slopes of lines are: L₁, $k=3.042$ ($R^2=0.979$, $p<0.0001$); L₂, $k=12.520$ ($R^2=0.620$, $p=0.114$) and 0.194; L₃, $k=2.760$ ($R^2=0.964$, $p<0.0001$); and L₄, $k=1.969$ ($R^2=0.966$, $p<0.0001$). The regression lines with the different letters within the same species were significantly different at $p<0.0001$ (assessed using ANCOVA).

does not spend sufficient time at intermediate water contents for damage caused by aqueous-based reactions to accumulate (Pammenter et al., 1998, 2000). In this study, the period of time required to dry *Quercus* seeds to <16% eRH ranged from 35 to 164 d (Table 3). In fact, none of the seeds were dried as rapidly as Pammenter et al. (1998, 2000) would prescribe and they may have been damaged by deleterious aqueous-based reactions at intermediate water contents.

There were different patterns of moisture loss between seeds of *Q. lamellosa* and seeds of *Q. schottkyana* (Fig. 2). For *Q. lamellosa*, the pattern of moisture loss from the seed coat followed a typical drying curve with an initial faster rate of moisture loss over the first 16 d, followed by more gradual decline in moisture up until 143 d. For the cotyledons and axis there was a near-linear decline in % moisture content until 77 d, followed by further slow moisture loss until the conclusion of drying at 143 d. In contrast, for *Q. schottkyana*, the cotyledons and axis did not start to lose much moisture, until after 52 d, once the seed coat had dried. The different patterns of moisture loss imply differences in the moisture exchanging properties of the seed coat for these species. A study on the seed coat micromorphology of *Q. suber* (subgenus *Quercus*) showed that in that species, the seed coat functions to maintain water within the seed (Sobrinho-Vesperinas and Viviani, 2000). The ability to maintain seed moisture content by slow drying may be an adaptation to facilitate seed survival post dispersal — a potentially valuable strategy for desiccation sensitive seeds, especially since many *Quercus* species occur in arid and semiarid regions (Trehane, 2007).

To conclude, seed desiccation sensitivity is likely a widespread trait not just in subgenus *Quercus* but also subgenus *Cyclobalanopsis*. The outer coat of the seeds may play a protective role against possible desiccation, by preventing moisture loss from the seeds; since seeds of different species dried at different rates, this function of the seed coat appears to vary between species. Since a

number of these species are rare and threatened, effective *ex situ* measures such as cryo-preservation or living collections, may be required to ensure their continued existence.

Supplementary materials related to this article can be found online at doi:10.1016/j.sajb.2011.05.001.

Acknowledgements

The authors wish to thank Dr. M. Cao, Dr. Y.-H. Ji, Dr. M.-Q. Liu and T. Su for collecting the seeds. The authors also thank H. Everett and Z. Cook for their assistance in the laboratory work. This work was supported by the Millennium Seed Bank Project of the Royal Botanic Gardens, Kew, UK, the Fund for Applied Basic Research of Yunnan Province, China (2008CD165), the National Basic Research Program of China (973 Program; No. 2007CB411601), Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany (KIB), Chinese Academy of Sciences (CAS), China (2006DP173034) and the Running Cost Funding of CAS Large-scale Scientific Facility for Germplasm Bank of Wild Species, KIB, CAS, China.

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