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Influence of acorn size and storage duration on moisture content, germination and survival of *Quercus petraea* (Mattuschka)

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Abstract: *This study was conducted to evaluate how acorn size (small, medium and large) and acorn storage duration (0, 5 and 17 months) influenced Quercus petraea (Mattuschka) moisture content and germination. Acorn size and storage duration did not significantly affect acorn moisture content, but they significantly affected acorn germination performance. When averaged for three acorn sizes, loss of germination performance occurred after 17 months of storage even when the moisture content did not reduce significantly and remain at the initial level (32.6%). Maximum germination percentage was observed in large and medium size classes before storage (93 and 95%, respectively) and after 5-month storage (94 and 93%, respectively), but after 17-month storage medium acorn size class exhibited the highest germination (68%). Small seed size class exhibited the lowest germination percentage and rate in each acorn storage duration. Acorn size also significantly affected seedling emergence and survival in the nursery, and seedling emergence and survival was the lowest in small seed size class (85 and 80%, respectively). Although seedling survival of one-year seedlings in the nursery increased up to large seed size class, maximum survival in nursery conditions was observed in large and medium size classes (89 and 91%, respectively). Thus, acorn size grading in Q. petraea may result in higher germination performance within in a seedbed.*

Key words: *Acorn weight, Germination performance, Seedling emergence, Seedling survival*
PDF of full length paper is available online

Introduction

Quercus petraea (Mattuschka) Liebl. (sessile oak) is a species of oak native to most of Europe, and into Anatolia. It is a large deciduous tree up to 20-40 m tall in the white oak section. The fruit is an acorn 2-3 cm long and 1-2 cm broad, which matures in about 6 months (Yaltirik and Efe, 1994; Rushforth, 1999). Productive oak stands cover about 2.0 million ha and among the oak species sessile oak are the most important species which grow in pure stands and are also found growing with other deciduous and coniferous species in Turkey (Yaltirik and Efe, 1994; Anonymous, 2006). The problems associated with oak regeneration start with the acorn (Cecich, 1993). Dormancy is the inability of a seed to germinate, even under conditions that are normally considered favorable for germination. Stratification, scarification and gibberellins have a promotive effect on the germination of many species of angiosperms and gymnosperms (Bradbeer, 1988; Bewley and Black, 1994; Esen *et al.*, 2007; Tilki, 2008). With a few exceptions, the members of the white oak group produce acorns that do not have complete dormancy, and with only a few exceptions, acorns of the white oak group cannot be successfully stored more than 4 to 6 months. These non-dormant seeds sprout very readily in storage and die rapidly. If white oaks are held over winter for spring planting, the best conditions are almost the same as those recommended for red oaks: temperatures just above freezing (2-3°C), maximum acorn moisture content (45-50%), and containers that allow gas exchange (Bonner and Vozzo, 1987).

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Environmental influences during the development of seeds combined with genetic variability can result in variations in seed dimensions (Willan, 1985). The higher seed dimensions could be attributed to better differential seed filling based on locality or site factors. The performance of the seeds immediately after germination is related to seed size (Willan, 1985). Seedlings resulting from large seeds rich in food reserves confer competitive advantages on seedlings (Fenner, 1985). Large seeds can withstand unfavourable environmental conditions over a long period of time, while smaller seeds under the same conditions deplete their reserves in the process of respiration and physiological rearrangements. When conditions improve, the seedlings emerging from smaller seeds are either unable to changeover to autotrophic feeding and die, or their growth and development are strongly retarded (Ovcharov, 1977; Khera *et al.*, 2004).

A large variability in seed size is common in oak species and could affect seedling quality. Large seeds have traditionally been viewed as advantageous in closed communities, such as forests, whereas small seeds would be more suitable for open successional communities (Gross, 1984). Although large seeds increased germination and seedling growth of some species (Dirik, 1993; Kormanik *et al.*, 1998; Ke and Werger, 1999; Khera *et al.*, 2004; Navarro *et al.*, 2006), occasionally the large seeds performed less well than medium or small seeds (Indira *et al.*, 2000; Alptekin and Tilki, 2002; Khera *et al.*, 2004). Thus, the aim of this study was to investigate the effect of acorn size and acorn storage duration on germination and survival of *Q. petraea*.

Materials and Methods

Acorn collection: Acorns were collected from natural sessile oak stands (10-15 trees) in Ardanuc, Turkey (lat. 41°08' N, long. 42°10' E, alt. 650 m a.s.l.) in mid-October 2005. After collection, acorns obviously defective were first discarded by visual inspection and then the acorns were floated in water to remove acorn cups and to eliminate insect-damage or dead acorns (any that floated) (Pichon and Guibert, 2001). Acorn size classes were determined by mixing and spreading acorns on a flat surface, and the seeds were visually separated into three seed sizes, small, medium and large. Average seed weight of each group was determined in four replicates of 100 acorns. Length and width of 100 seeds with four replications were also determined with high precision calipers (Table 1).

Acorn storage: To test the effect of acorn storage on germination, acorns were stored in black polyethylene bags for 0, 5 and 17 months at 4 °C. After each acorn storage duration, acorn moisture content, germination percentage and germination rate were determined in laboratory.

Moisture content determination: Moisture content of whole acorns was determined for five replicates of three acorns each after 0, 5 and 17 months of storage by the procedures recommended for large seeds with high moisture contents (Bonner, 1981; ISTA, 1993). Randomly selected acorns were cut into quarters and dried in aluminum cans at 103°C for 24 hr in an oven (Connor and Sowa, 2003).

Germination test: Seed germination tests were performed in the plastic trays for each experiment, and acorns were soaked overnight in tapwater prior to germination testing. The trays were filled with sterilized sand, which was watered throughout the entire incubation period and the acorns were laid out on the surface of the media. Experiments were carried out in a germination chamber at 20±1°C under 12 hr photoperiod (Pichon and Guibert, 2001).

For each experiment, there were five replicates with twenty seeds each per treatment arranged in a completely randomized design, and the germinated seeds were counted every day for 30 days following first signs of germination (ISTA, 1993). Acorns were placed in moist sand so that the distal end, containing the axis, protruded from the sand and was visible for germination (radicles>5 mm) monitoring.

The germination data were calculated each day and expressed as germination percentage of seeds (GP%) that had germinated after 30 days. Germination rate was calculated and expressed as peak value (PV), an index of germination speed which is the highest number obtained when percentage germination is divided by the number of elapsed days (Czabator, 1962).

Seedling emergence and survival: Seeds stored for 5 months were sown in nursery beds to look at the effect of seedling size on seedling emergence and survival at Ardanuc Forest Nursery (altitude 700 m), Artvin, Turkey. The nursery soil was clay loam with a soil pH of 7.1. Four replicates of 100 seeds for each seed size were sown by hand in a randomized block design. The seeds were sown in nursery bed at 60 seed m^{-2} density in March 2006 and one seed was sown at each spot in 3-5 cm depth. Seedbeds were standard 1.2 m wide with five rows. Before sowing, the seeds were soaked overnight in water. Acorns were also fumigated with Pomarsol Forte before sown in nursery bed. During the vegetation period, following the sowing date, the only treatment given was irrigation and weed control-hoeing.

Seedling emergence in nursery was determined at the end of the April 2006 (after four weeks following first sign of germination) (Pichon and Guibert, 2001), and seedling survival percentage was determined in December 2006.

Statistical analyses: An analysis of variance was used to compare the germination performance and seedling survival to the seed sizes and storage durations. Percent data (germination percentage and survival) were arcsine transformed to stabilize any heterogeneous variance (Zar, 1996). When significant differences were found, Duncan' new multiple range test was performed for comparison of the means. Statistical analyses were performed with the help of the computer software package SPSS.

Results and Discussion

The moisture content of the acorns as a percentage of fresh weight before storage was found to be 35% when averaged for three acorn size classes (Table 2). Storage for 5 months increased moisture contents although not significantly when averaged over three seed sizes but 17 months seed storage reduced moisture content in each seed size but not significantly ($p<0.05$), and acorn size and storage duration did not significantly affect acorn moisture content.

The duration of acorn storage significantly affected acorn germination percentages and germination rates (Table 3). Acorns stored at 4°C for 5 months retained their viability in each seed size as the average moisture content was increased to 35.5 %. When averaged over three acorn size classes, more than 90% viability of acorns before storage was reduced to 62% after 17 months storage during which the moisture content was reduced to 32.5% (Table 2,3). Stored acorns are sown in nursey during spring, thus, they have to be stored for 4-6 months or 16-18 months. Acorn germination percentage decreased with long seed storage in the present study. After 5 months of acorn storage, germination and moisture content did not fall significantly in each acorn size. Average germination of acorns stored for 17 months was close to 60%. This low germination was not associated with acorn moisture content. Loss of germination percentage occurred after 17 months of storage even when the moisture content did not reduce significantly and remain at the initial level. In some *Quercus* species germination percentage decreased significantly in stored acorns although moisture content increased during storage (Clatterbuck and Bonner, 1985; Finch-Savage *et al.*, 1996).

Table - 1: Biometric characteristics of the acorns used in the study

Acorn size	Diameter (mm)	SE	Length (mm)	SE	Weight SE	SE
Large	19.65 [a]	0.30	36.83 [a]	0.40	8.38 [a]	0.28
Medium	16.76 [b]	0.22	32.59 [b]	0.35	5.96 [b]	0.21
Small	13.14 [c]	0.17	24.82 [c]	0.28	3.37 [c]	0.16

Means in the same column followed by the same letter are not significantly different at p<0.05

Table - 2: Moisture content (MC %) of the stored acorns

Acorn size	MC (%)		
	0-month storage	5-month storage	17-month storage
Large	34.8	36.1	32.9
Medium	36.0	35.8	33.3
Small	34.3	34.5	31.8
Mean	35.0	35.5	32.6

Table - 3: Germination percentage (GP %) and germination rate (PV) of *Q. petraea* acorns after storage

Acorn size	GP (%)			PV		
	0-month storage	5-month storage	17-month storage	0-month storage	5-month storage	17-month storage
Large	93 [Aa]	94 [Aa]	64 [Bb]	4.7 [Aa]	4.9 [Aa]	4.1 [Ab]
Medium	95 [Aa]	93 [Aa]	68 [Ab]	4.4 [Aa]	4.8 [Aa]	3.7 [Ab]
Small	87 [Ba]	85 [Ba]	54C [b]	3.5 [Bab]	3.9 [Ba]	3.0 [Bb]
Mean	91.7 [a]	90.7 [a]	62.0 [b]	4.2 [a]	4.5 [a]	3.6 [b]

Means in the same column followed by the same capital letter are not significantly different at p<0.05, Means in the same row followed by the same lowercase letter are not significantly different at p<0.05

Table - 4: Effects of acorn size on seedling emergence and survival in the nursery

Acorn size	Seedling emergence (%)	Seedling survival (%)
Large	91 [a]	89 [a]
Medium	92 [a]	91 [a]
Small	85 [b]	80 [b]

Means in the same column followed by the same letter are not significantly different at p<0.05

Seed storage may be a way to palliate the irregular acorn production and to maintain a regular supply of acorns to nurseries (Merouani *et al.*, 2001). Temperate recalcitrant seeds such as *Quercus* spp. are desiccation-sensitive and cannot be dried below a relatively high moisture content but can be stored at near freezing temperatures (1-3°C). Thus, seeds of *Quercus* will be injured by prolonged sealed storage, and genetic and environmental factors (initial seed quality, seed moisture content, storage temperature, oxygen, *etc.*) affect seed longevity in storage (Bonner and Vozzo, 1987; Wang *et al.*, 1993). Storage of oak in moist media is only adequate for short-term storage of a few months and is unlikely to be effective for long term. The most successful method of storing recalcitrant seeds has been sealed storage in polyethylene bags. The results of this study indicated that *Q. petraea* acorns could be stored for 5-month in sealed storage of polyethylene bags at 3-5°C without losing their germination performance, but acorn germination percentage decreased with long seed storage (17-month) even when the moisture content did not reduce significantly in the present study.

Studies on *Q. rugosa* and *Q. laurina* showed that seedling size was significantly affected by the amount of reserves originially available in the cotyledons (Blake and Sutton, 1987). Therefore, the much decrease in germination percentage from small seeds at 17 months of acorn storage could be explained by the initial amount reserves and their depletion during storage in the present study. It is known that soluble carbonhydrates generally decline with seed ageing (Petruzelli and Taranto, 1989).

Acorn size significantly affected acorn germination percentages and germination rates (Table 3). In each storage duration germination was much poor in small seed size class. Maximum germination percentage was observed in large and medium size classes before storage (93 and 95%, respectively) and after 5-month storage (94 and 93%, respectively), but after 17-month storage medium acorn size class exhibited the highest germination percentage (68%). Small seed size class also exhibited low germination rate in each acorn storage duration.

Acorn size also significantly affected seedling emergence and survival in the nursery. Seedling emergence was the lowest in small seed size class (85%) and survival was also much poor in small seed size class (80%) and although survival increased up to large seed size class, maximum survival in nursery conditions were observed in large and medium size classes (89 and 91%, respectively).

The better performance of larger seeds may simply be a reflection of the greater amount of nutrients available to the embryo (Abideen *et al.*, 1993). The higher seed dimensions could be attributed to better seed size indicates better quality and germination of seeds and genetic potential (Davidson *et al.*, 1996). Large size in *Q. ilex* increased germination rate and seedling survival (Gomez, 2004). The effect of seed size was significant for *Q. rugosa* and *Q. laurina*, with a positive correlation between seed mass and survival (Bonfil, 1998). The smallest acorns of *Quercus rubra* also produced low germination and seedling survival (Kormanik *et al.*, 1998). In the present study small sized acorns had also the poorest seed germination and seedling survival. Although large seeds increased germination and survival of some other tree species (Ke and Werger, 1999; Khera *et al.*, 2004; Cicek and Tilki, 2006; Pizo *et al.*, 2006) as found in the present study, large seed did not produce the highest germination and seedling survival in some tree species (Shepard *et al.*, 1989; Khera *et al.*, 2004; Tilki and Alptekin, 2005; Delgado *et al.*, 2009).

The present study pointed out that 5 months of acorn storage did not reduce germination performance and moisture content significantly but acorn germination percentage and germination rate decreased significantly with long seed storage (17-month storage) in each acorn size. Acorn size affected germination performance in laboratory and seedling survival of one-year seedlings in the nursery, and small acorns performed poorly. The results suggest that nursery manager can collect *Q. petraea* acorns in autumn and store them for 5 months at low temperature until sow in early spring without losing their viability and that seed sizing of *Q. petraea* may result in higher germination within in a seedbed.

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