Acorn size influence on the quality of pedunculate oak (*Quercus robur* L.) one-year old seedlings

**Jovana Devetaković¹☆, Marina Nonić¹, Bojan Prokić¹, Vladan Popović², Mirjana Šijačić-Nikolić¹**

¹Faculty of Forestry – University of Belgrade, Belgrade, Serbia  
²Institute of Forestry, Belgrade, Serbia

☆jovana.devetakovic@sfb.bg.ac.rs

**Abstract**

Pedunculate oak (*Quercus robur* L.) is one of the most important deciduous tree species in the Serbian and European forests. Different negative factors limit natural regeneration of pedunculate oak forests, so producing of high quality seedlings becomes imperative to ensure successful forest restoration. This research was conducted with to aim to determine whether acorn size is related to seedlings quality. Acorns were collected from the natural pedunculate oak forest and divided into two groups by their size. Acorns were sown in the spring into plastic containers after five months cold storage. Size of acorns was in the standard species range, though germination was low (< 20 %). Germination was more then double in group of large acorns in comparison to group of smaller acorns (19.2 %, respectively 8.6 %). Height and root collar diameter of produced seedlings were lower than seedlings produced in seedbeds in commercial nurseries in Serbia, but it was in range of container produced seedlings in some similar researches. Height to root collar diameter ratio and shoot to root ratio were considered as satisfactory for containerized seedlings. There was not strong correlation between acorn size and seedlings quality attributes.

**Keywords**

Acorn size; Pedunculate oak; Seedlings quality

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**1 Introduction**

Pedunculate or English oak (*Quercus robur* L.) is one of the most important deciduous tree species in Europe from the ecological and economic aspect (Ducousso and Bordacs 2004). Pedunculate oak forests represent one of the most valued forests in the forest fund of Serbia, though they only cover 1.4 % of total forest area (Banković et al. 2009). Restoration and planting of pedunculate oak forests on the larger scale is
required because of the high value of pedunculate oak wood has caused harvesting pressure on this forest and adverse site conditions have limited natural regeneration (Dubravac and Dekanić 2009; Medarević et al. 2009; Tikvić et al. 2011; Bauer et al. 2013). From the genetic aspect an advantage is given to natural regeneration of oak forests (Ducousso and Bordacs 2004), but the regular yield of quality seeds is necessary to ensure natural regeneration. Irregular yield and problematic natural regeneration of forests increases the need for growing and planting seedlings to ensure restoration success. To ensure high outplanting success, high quality seedlings are required. If quality of seedlings are defined with morphological parameters (Tompson 1985; Mexal and Landis 1990), we want to be able to define which parameters best define seeding development. The main aim of this research was to relate acorn size to quality parameters in the first year seedling development.

Oaks can be considered as typical of a large seeded tree species. Seed is characterized with two large cotyledons which provide large amount of metabolic reserves for growing young plants, especially during first growing season (Andersson and Frost 1996, Perez-Ramos et al. 2010). We hypothesize that larger acorns indicate a greater nutritional reserve which will result in initially better growth of young seedlings. Other studies looking a seed size to young seedling development have been conducted within the genus Quercus, with some studies confirming (Navarro et al. 2006; Roth et al. 2009; Tilki et al. 2009; Roth et al. 2011: Popović et al. 2015), while other work not confirming (Ivanković et al. 2011) this hypotesis.

2 Materials and methods

The acorns of pedunculate oak were collected from its natural habitat on the area FE “Sremska Mitrovica “ (Vojvodina, North Serbia) in mid-October 2014. We chose 10 maternal trees with different morphological characteristics of acorns for seed collection (5 trees with large acorns and 5 trees with small acorns). Acorns were expose to standard seed treatment in FSC “Morović“ (Ivetić 2013). Total of 1000 acorns (10 trees X 100 acorns) were transferred to the Faculty of Forestry – University of Belgrade where the trial was conducted. Acorns were divided into two groups: Group L (larger acorns) and Group S (smaller acorns). Seeds were cold stored at +4 °C until the start of the experiment.

Thousand seed mass (TSM) was calculated for both seed size populations (Ivetić 2013). Acorns were numbered and measurement of diameter (mm), length (mm) and weight (g) were conducted. Diameter and length of acorns were measure using digital calliper with an accuracy 0.01 mm. Weight was measured by electronic scale with an accuracy of 0.01 g. After measurement, it was determining length to diameter ratio (L/D) and volume of acorn as volume of roller ($V = \pi r^2 H$).

In March 2015, acorns were sown into plastic containers (Plantagrah I: 120cm$^3$). A total of 1000 acorns were sown into 20 containers, 10 containers per group. The containers were filled with the substrate consisting from humus, peat and sand (1:1:1). Containers were placed in the Nursery of the Faculty of Forestry - University of Belgrade.

The germination dynamics were monitored and every day watering during germination, later watering was reduced on the period of two to three days. Weeding was performed optionally. Fungicidal protection was provided because Microsphaera alphitoides attach was recorded. At the end of the growing season seedlings were
measured for height, root collar diameter and seedling dry weight. Root collar diameter and height were measured using digital calliper with an accuracy of 0.01 mm, respectively 0.1 cm. Height to diameter ratio (H/RCD) was also determined. Seedling dry weight was determined as dry weight of root (m_r) and dry weight of shoot (m_s) after drying at 68 °C ± 2°C during 48h, and relation between dry weight of shoot and root (m_s/m_r) was calculated also (Ivetić 2013).

Relationship between measured parameters of acorn size and seedling morphological development were determined by simple linear correlations (Pearson R). Statistical analysis was performed using Statistica 7.0 software.

3 Results and discussion

There were differences between the populations of large and small pedunculate oak acorns (Table 1). The thousand seed mass (TSM) was almost double for the large acorns compared to the small group of acorns. This parameter was used as the main descriptor for the groups, because it is useful in operational practice due to ease of determination. Length, diameter, weight and volume of acorns as mean values of large and small groups show clearly different values for different groups (Table 1), with a large range from minimum to maximum values for all observed parameters (Figure 1). Never the less, all measured parameters were significantly different between large and small groups of acorns (T-test, p<0.05, Table 2). Both groups of acorns have size characteristics comparable to previous research on pedunculate oak (Ivanković et al. 2011; Popović et al. 2016).

Table 1. Mean length, diameter, L/D ratio, weight, volume, thousand seed mass (TSM) and germination of pedunculate oak acorns (standard deviation of mean values).

| Acorn Groups | Length (mm) | Diameter (mm) | L/D | Weight (g) | Volume (mm$^3$) | TSM (g) | Germination (%) |
|--------------|-------------|---------------|-----|------------|-----------------|--------|----------------|
| Large        | 34.15 (2.19)| 17.28 (1.08) | 1.98 (1.11) | 6.01 (1.13) | 8071.91 (1341.40) | 6005.82 | 19.2           |
| Small        | 25.41 (1.58)| 15.05 (0.93) | 1.70 (0.09) | 3.28 (0.65) | 4600.51 (791.27)  | 3284.18 | 8.6            |
| Average      | 29.78 (4.9) | 16.17 (1.71) | 1.84 (0.21) | 4.62 (1.69) | 6332.21 (2135.55) | 4645.00 | 13.9           |

Acorn weight and volume were strongly related to the acorn diameter ($R^2=0.93$; $R^2=0.95$) (Figure 2), then acorn length ($R^2=0.88$; $R^2=0.92$), (Figure 3). These findings are comparable to other work on pedunculate oak (Roth et al. 2011) and red oak (Popović et al. 2015).

Germination was very low for both groups (<20%), with germination of acorns from the large acorn size group more than double the small acorn size group (Table1). Large seed size at Quercus petraea also increases germination percent (Tilki 2008). Low germination percent can be caused by many factors (seed moisture, temperature, storage, moisture of substrate etc.) and it is hard to explain without extensive research. It is assumed that some negative effects related to spring sowing and five months of acorn cold storage in paper bags affected seed germination.
Figure 1. Distribution of length (a), diameter (b), L/D (c), weight (d) and volume (e) of pedunculate oak acorns.

Table 2. T-test (p<0.05) for L and S group of pedunculate oak acorns for all measured parameters.

| Parameter | t-value | df  | p   | F-ratio | P var. |
|-----------|---------|-----|-----|---------|--------|
| Length    | 61.68299| 998 | 0.00| 1.722153| 0.0000 |
| Diameter  | 27.19615| 998 | 0.00| 1.219918| 0.0266 |
| Weight    | 41.48197| 998 | 0.00| 2.072638| 0.0000 |
| L/D       | 30.77268| 998 | 0.00| 2.138991| 0.0000 |
| Volume    | 44.13723| 998 | 0.00| 1.636886| 0.0000 |
From 1000 sown seeds, at the end of growing season only 139 seedlings were produced (96 seedlings from the L group of acorns and 43 seedlings from the S group of acorns). There was a significant difference in the dry weight of root and shoot to root ratio of seedlings from the different acorn size groups (t-test, p<0.05), (Table 3 and Table 4); with the L group of acorns having greater root mass. Height and RCD of pedunculate oak seedlings produced in this research were lower than seedlings produced in seedbeds in commercial nurseries in Serbia (Ivetić et al. 2017), though seedling size was in a comparable range in comparison to container grown seedlings (Ivanković et al. 2011; Roth at al. 2011). Orešković et al. (2006) were conducted research about container type influence on seedlings quality and marked container similar as used in this research as inappropriate for pedunculate oak seedlings production. On the other hand, dry weight of shoot and root had good ratio (1:2, respectively 1:1, Table 3) for containerized seedlings (Ivetić 2013) and similar results were reported for barren root seedlings of deciduous species in Serbia (Stjepanović and Ivetić 2013; Ivetić et al. 2013; Popović et al. 2015). Height to RCD ratio can be also considered as satisfactory for containerized seedlings (Ivetić 2013).
Table 3. Mean values of height (H), root collar diameter (RCD), height to root collar diameter ratio (H/RCD), dry weight of shoot (m_r), dry weight of root (m_r) and shoot to root ratio (m_r/m_r) for seedlings from large and small seed size groups (standard deviation) of pedunculate oak seedlings.

| Group  | H (cm)       | RCD (mm) | H/RCD     | m_r (g)    | m_r (g)    | m_r/m_r    |
|--------|--------------|----------|-----------|------------|------------|------------|
| Large  | 13.30 (4.99) | 2.09 (0.53) | 6.48 (1.56) | 0.49 (0.36) | 1.34 (1.08) | 0.52 (0.37) |
| Small  | 14.27 (3.46) | 2.05 (0.31) | 7.01 (1.77) | 0.49 (0.19) | 0.72 (0.38) | 0.97 (0.76) |
| average| 14.02 (4.53) | 2.07 (0.48) | 6.64 (1.64) | 0.49 (0.36) | 1.19 (0.92) | 0.66 (0.56) |

There were significant differences between seedlings of different groups in root dry weight and shoot to root ratio of seedlings (Table 4, Table 5). Seedlings weight is related to a larger seedlings biomass (Gonzales-Rodriguez et al. 2011) and specially proved for total aboveground biomass (Perez-Ramos et al. 2010; Roth et al. 2011). In this research shoot dry mass did not dependent from seed size and contradictory to Roth et al. (2011) shoot to root ratio is higher at seedlings from the group of small acorns.

Table 4. T-test (p<0.05) for seedlings from large and small groups of acorns for all measured morphological parameters (m_r – shoot dry weight, m_r – root dry, m_r/m_r – shoot to root ratio, RCD – root collar diameter, H – height, H/RCD – height to root collar diameter ratio) of pedunculate oak seedlings.

| Morphological Parameters | t-value | df  | p     | F-ratio | P var. |
|--------------------------|---------|-----|-------|---------|--------|
| m_r                      | -0.068207 | 137 | 0.9457| 3.049244| 0.0001 |
| m_r                      | 4.357244  | 137 | 0.0000| 7.862345| 0.0000 |
| m_r/m_r                 | -4.68893  | 137 | 0.0000| 4.363571| 0.0000 |
| RCD                      | 1.015964  | 137 | 0.3114| 2.779030| 0.0004 |
| H                        | -0.303269 | 137 | 0.7621| 1.744857| 0.0456 |
| H/RCD                    | -1.78111  | 137 | 0.0771| 1.286042| 0.3150 |

Acorn size did influence seedling height, H/RCD ratio and shoot dry weight (Table 5). There was a significant correlation between acorn size and dry root weight and shoot to root ratio. Measured seedling morphological parameters showed similar relationships with other one-year old deciduous but bareroot seedlings grown in Serbian nurseries (Stjepanović and Ivetić 2013; Ivetić et al. 2013; Popović et al. 2015).

Table 5. Correlation coefficients (R) between the observed parameters of pedunculate oak acorns (L-length, D - diameter, m - weight, L/D, V - volume) and seedlings (m_r – shoot dry weight, m_r – root dry weight, RCD – root collar diameter, H – height, m_r/m_r – shoot to root ratio, H/RCD – height to root collar diameter ratio).

| N=139 | P<0.05 | ACORNS | SEEDLINGS |
|-------|--------|--------|-----------|
|       |        | L      | D         | m         | L/D      | V         | m_r      | m_r      | RCD      | H        | m_r/m_r  | H/RCD |
|       |        |        |           |           |          |           |          |          |          |          |          |        |
| ACORNS|        | 1.00   | 0.70*    | 0.84*     | 0.73*    | 0.88*    | 0.10     | 0.34*    | 0.14     | 0.06     | -0.25*   | -0.08  |
|       |        |        |           |           |          |           |          |          |          |          |          |        |
|       |        | 1.00   | 0.93*    | 0.28*     | 0.95*    | 0.97*    | 0.15     | 0.41*    | 0.25*    | 0.09     | -0.21*   | -0.09  |
|       |        |        |           |           |          |           |          |          |          |          |          |        |
|       |        | 1.00   |           | 1.00      |          | 1.00     | 0.17*    | 0.48*    | 0.25*    | 0.11     | -0.29*   | -0.03  |
|       |        |        |           |           |          | 1.00     | -0.01    | 0.09     | -0.00    | 0.00     | -0.27*   | -0.09  |
|       |        |        |           |           | 1.00     |          | 0.13     | 0.42*    | 0.19*    | 0.00     | 0.24*    | 0.08   |
|       |        |        |           |           |          | 0.00     | 1.00     | 0.18*    | 0.25*    | 0.04     | 0.66*    | 0.01   |
|       |        |        |           |           |          | 0.00     | 1.00     | 0.02     | 0.21*    | 0.21*    | 1.00     |        |
|       |        |        |           |           |          | 0.00     | 1.00     | 0.02     | 0.27*    | 0.27*    | 1.00     |        |
4 Conclusion

In this study, two groups of pedunculate oak acorns divided into large and small groupings by thousand seed mass (TSM) showed statistically significant differences between measured acorn parameters (length, diameter, weight and volume). The large differences in TSM and seed size can be used as a method to partition acorns by size. Germination percent was more than double for the group of larger acorns. Large acorns produced seedlings with greater dry weight of roots and lower shoot to root ratios. However, low overall germination, possibly due to seed storage practices, limited the ability to conclusively describe young seedling development in relation to acorn size. Thus one cannot conclusively confirm there was a relationship between acorn size and subsequent seedling development. Further work is required to determine whether acorn mass of pedunculate oak provides a good measure of subsequent seedling development in the nursery.

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