

Tree testing and estimation of heritability using the pedunculate oak *Quercus robur* L. seed orchard in the Krotoszyn Forest District

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Abstract. In this paper, we present the results and analyse of tree height measurements, phenological observations (bud flushing), oak powdery mildew symptoms and multiple shoots of pedunculate oaks *Quercus robur* L. at the age of 12 years. The examined oaks belonged to 29 open pollination families. Family as well as individual heritability scored very highly for their respective indices. The calculated indices were 0.83 for tree height, 0.98 for flushing of buds, 0.53 for oak powdery mildew symptoms and 0.58 for the multi-stem index. Family seed orchards established using a completely randomized design are not a reliable substitute for progeny testing, because of the commonly small number of collected families.

Keywords: tree breeding, heritability, seedling seed orchards

1. Introduction

The pedunculate oak *Quercus robur* L. is one of the species included in the ‘Program to protect the genetic resources of the forest and selective tree breeding in Poland in 2011–2035’ (Chałupka et al. 2011). The selection of oaks is important because the progressive naturalisation of tree stand species composition is leading to a decrease in the share of coniferous species, which produce significant quantities of valuable raw material, to the benefit of less productive deciduous species. As a consequence, the reconstruction of species composition may reduce the production potential of forests. The selection of oaks having greater productivity or higher quality raw material should reduce the negative effects of this reconstruction. Individual selection requires testing the parents of future generations of trees. Inferring the breeding value of parents is based on comparing the offspring constituting a group of related individuals. Such a related group is known as half-sibs. In the case of open-pollinated trees, half-sibs are the generative progeny of one tree. Half-sibs are therefore the offspring of the best (plus) trees in seedling seed orchards. The principles of designing and establishing seedling seed orchards in the State Forests, referred to as seed orchards, are consistent with the methodology of establishing vegetation field experiments. The principle of

repeating (many trees belong to a family) and the principle of randomisation (random placement of trees in the orchard) is respected, which is why these orchards can be used to test the progeny of plus trees and to estimate heritability.

The aim of this study is to verify the thesis about the usefulness of family seed orchards for testing the plus trees of the pedunculate oak and to determine several genetic parameters of the Krotoszyn population of this species.

2. Materials and Methods

The orchard is located in the Smoszew Forest Range in division 70 b, which has an area of 4.8 ha. The habitat was defined as a fresh forest (Lśw). The soil formed from sandy boulder clays was classified as surface-water gleysol. The vegetative period lasts 215 days. The average July temperature is 18 °C and the average January temperature is –2.1 °C. The sum of annual rainfall is 579 mm.

The orchard was established in the spring of 2003 from the generative progeny of 30 plus trees selected from the Krotoszyn Forest District. Each family represented 2-year-old seedlings (40–42), except for the 7448 line, from which only four seedlings were obtained. Seedlings belonging to various families were randomly placed in three quarters, tre-

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ated in this study as blocks while maintaining certain limitations as to the minimum distances between trees from the same families. The soil was prepared at the point of planting (spots of 60×60 cm made by hand or with a soil cutter). The planting distance was 6×6 m.

In the spring of 2012, bud flushing was noted using an eight-point scale:

- 0 – buds in resting phase, dark brown, tightly closed bud scales,
- 1 – buds swollen, lighter at the tip, bud scales still not opened,
- 2 – buds elongated with green-yellow tips, scales clearly parted,
- 3 – buds strongly elongated, broken at the tip, with greenish tops, wide breaks between the scales, leaves still not visible,
- 4 – the buds are broken, beginning leaf development, visible leaf edges, but the whole leaf is invisible,
- 5 – fully unfurled leaves are visible, but they are still very small and facing upwards,
- 6 – fully unfurled leaves, mainly directed downwards, but not yet completely developed, shoot growth beginning,
- 7 – fully developed leaves, shoot growth continues.

In September, after growth had completed, the height of all trees was measured with a telescopic levelling stave. The degree of leaf infestation by powdery mildew (*Erysiphe al-phitoides*) (Griffon & Maubl. by U. Braun and S. Takam) was measured on a four-point scale: 1 – no infestation, 2 – weak infestation (one-third of the leaf surface area affected), 3 – medium infestation (half of the leaf surface area infested), 4 – strong infestation (over two-thirds of the leaves covered with powdery mildew).

The form of the tree was also estimated according to a scale: 1 – trees with a distinct main stem and single shoot apex, 2 – trees with two crowns and 3 – multi-stem trees (Krahl-Urban, 1959). For the purpose of presenting the values of the families and analysing multiple features, the qualitative scale was reversed so that its desirable values were the highest, for example, a tree without mildew damage received a score of 4 and a tree with strong infestation was scored 1. The quality scale (ranking) was treated in the analyses as a quotient scale (continuous) with equal intervals between the levels, which can be done for the convenience of the calculations if the distribution of the features among the intervals is close to the normal distribution.

Determining the results began with an analysis of the correlation by calculating the Pearson linear correlation coefficient of every feature with each of the remaining ones.

Then, a one-way analysis of variance according to a random classification model was used. When a significant difference among the studied items was obtained, the general variance was divided into the following components: objects and residuals. The size of the components was estimated by

comparing the obtained mean squares (deviations from the arithmetic means) with the expected values of the mean squares. The components were used to calculate the heritability coefficients according to the formulas:

- family heritability:

$$h_r^2 = \frac{\sigma_r^2}{\frac{\sigma_e^2}{n_0} + \sigma_r^2}$$

- individual heritability:

$$h_i^2 = \frac{\sigma_r^2}{0.25 (\sigma_r^2 + \sigma_e^2)}$$

where:

σ_r^2 – is the family component,

σ_e^2 – is the residual component of the total variance and

n_0 – is the estimated average number of families according to the formula:

$$n_0 = \frac{1}{a-1} (N - \frac{1}{N} \sum n_i^2)$$

where:

$a-1$ – denotes the number of degrees of freedom for the families,

N – is the total number of trees and

n_i^2 – square of the number of the i family.

Based on the heritability coefficients, the expected genetic gain was estimated at different intensities of selection, expressed in standardised units.

The summary assessment of families was somewhat problematic when taking into aggregate assessment of all the features together. Usually, selection indices are used when simultaneously selecting many traits (Smith 1936; Hill et al. 1998). This index is the sum of the products of the breeding value of each feature and its economic weight (Žuk 1989). In case of features such as the bud flushing index or the extent of powdery mildew symptoms, it is difficult to choose an objective economic weight. Therefore, a weight of 1 was assumed for each feature.

3. Results

Table 1 presents average tree height values, average bud flushing indices, mean powdery mildew symptoms indices and mean multi-stem indices, together with standard deviations. All features significantly differentiated the half-sib groups. The phenological phase was found to be the most variable (with a coefficient of variation of 72.40%). Half-sibs were least distinguished by height (the coefficient of variation was 26.15%).

Table 2 presents the components of variance and the indices of family and individual heritability calculated from them. For three features (height, powdery mildew symptoms

Table 1. The mean values of features of pedunculate oak plus trees half-sibs in the seedling seed orchard in Krotoszyn Forest District in 2013: \bar{x} – mean value, σ – standard deviation, V [%] – coefficient of variation, α – significance level

Family	No of trees	Height [m]		Mean index of bud flushing		Mean index of powdery mildew symptoms		Mean multi-stem index	
	n	\bar{x}	σ	\bar{x}	σ	\bar{x}	σ	\bar{x}	σ
7443	41	3.68	0.91	1.41	1.09	1.73	0.81	1.51	0.68
7444	32	2.97	0.94	3.72	1.75	1.81	0.78	1.94	0.67
7445	41	3.69	0.78	1.22	0.94	1.51	0.68	1.49	0.60
7446	38	3.33	0.79	1.66	1.28	1.58	0.68	1.97	0.59
7447	42	3.78	0.88	1.57	1.48	1.69	0.68	1.52	0.59
7449	39	3.49	0.93	0.95	0.97	1.41	0.85	1.56	0.68
7450	40	3.54	0.82	2.30	1.59	1.65	0.74	1.45	0.64
7451	36	3.48	0.73	1.81	1.43	1.72	0.70	1.28	0.57
7452	24	3.70	0.94	2.54	1.28	1.67	0.92	1.25	0.44
7453	39	3.82	0.85	2.13	1.36	1.77	0.63	1.64	0.71
7455	42	3.69	0.86	5.62	1.48	1.29	0.71	1.48	0.59
7456	38	3.71	0.82	2.76	1.40	1.68	0.81	1.66	0.58
7457	40	4.36	0.92	5.83	1.26	1.45	0.60	1.38	0.59
7458	41	3.76	0.74	0.98	0.91	1.41	0.77	1.63	0.80
7459	42	3.64	0.87	4.95	1.43	1.67	0.69	1.26	0.45
7460	41	4.03	0.73	5.88	1.03	1.39	0.86	1.44	0.59
7461	41	3.99	1.02	5.76	1.34	1.34	0.66	1.41	0.67
7462	38	4.09	0.69	1.97	1.13	1.74	0.60	1.50	0.60
7571	37	3.41	0.99	2.49	1.35	1.68	0.71	1.51	0.61
7572	37	3.33	1.11	1.24	1.14	1.57	0.77	1.68	0.67
7573	38	3.77	0.69	1.87	1.55	1.89	0.56	1.45	0.65
7574	42	3.33	0.87	4.95	1.17	1.50	0.59	1.45	0.59
7575	40	3.32	0.94	4.78	0.83	1.43	0.55	1.58	0.68
7578	41	3.00	0.87	1.46	1.16	1.90	0.80	1.51	0.71
7579	41	3.18	1.01	4.68	1.68	1.80	0.75	1.46	0.64
7581	28	3.43	1.13	5.04	1.57	1.82	0.77	1.57	0.79
7583	40	4.12	1.00	2.15	1.33	1.43	0.75	1.48	0.64
7584	40	3.19	0.75	1.45	1.57	1.63	0.87	1.55	0.71
7585	14	2.76	1.17	3.43	1.99	1.43	0.94	1.86	0.66
Razem	1093	3.60	0.94	2.98	2.16	1.60	0.74	1.53	0.65
σ	-	0.9401	-	2.1602	-	0.7397	-	0.6494	-
V [%]	-	26.15	-	72.40	-	46.12	-	42.58	-
α	-	0.0000	-	0.0000	-	0.0005	-	0.0000	-

Table 2. Components of variance and heritability's estimation – among half-sibs – family heritability, within half-sibs – individual heritability

Feature	Source of variation	Variance components	Heritability h^2
Height	among half-sibs	0.1025	0.8309
	within half-sibs	0.7844	0.4621
	total	0.8869	-
Spring phenology (bud flushing)	among half-sibs	3.0035	0.9846
	within half-sibs	1.7671	>1
	total	4.7706	-
Powdery mildew symptoms	among half-sibs	0.0161	0.5327
	within half-sibs	0.5319	0.1176
	total	0.5480	-
Multi-stem index	among half-sibs	0.0150	0.5803
	within half-sibs	0.4075	0.1418
	total	0.4225	-

index and multi-stem index), relatively high heritability values were found, which were higher than the value of individual heritability. The lowest heritability was found for the powdery mildew symptoms index (family $h^2=0.53$, individual $h^2=0.12$). In the case of the phenological index, family heritability was close to 1, and the value of individual heritability reached 2.52. By definition, heritability cannot exceed 1. If this is the case, it means that the maternal component was higher than assumed by the method of estimating heritability, that is, the female influences the appearance of the phenotype more strongly than the male.

The correlation between the observed features may be interesting. A significant linear correlation coefficient was obtained only between height and the multi-stem index (Table 3). Trees with many stems are generally lower than trees with one dominant crown stem. The examined features can therefore be considered as mostly independent.

At an early age, an important feature of a tree's adaptation is height growth. The heritability of this feature at the family level was $h^2=0.83$ and at the individual level $h^2=0.46$, which indicates the possibility of effectively selecting trees at the age of 12 by their height. The expected genetic gain at a different selection threshold is shown in Table 4. When selecting families equal to or above the average, the expected genetic gain will be 23 cm, or 6.6% of the average. At a higher selection threshold, for example, when selecting families with a height higher than the average increased by the standard deviation, the genetic gain will be 45 cm, or 12.71% of the average.

The assessment of the total value of the half-sibs is shown in Table 5. In the last column, the rank of each half-sib is noted after linear ordering. The best of the 29 analysed families were the seedlings of plus trees numbered 7583, 7458, 7445 and 7449. The worse were the progenies of 7444, 7585, 7581 and 7579.

4. Discussion

Pedunculate oaks originating from a relatively small geographical area and selected as the best trees (plus trees) show relatively high phenotypic and genetic variability in terms of economically important features. This may be due to the artificial origin of the Krotoszyn oak forests and the eventual introduction of propagation material, but this hypothesis cannot be verified without undertaking molecular or biochemical genetic studies. Genetic diversity is evidenced by the high rates of heritability, which at the same time indicates the possibility of effectively selecting all the considered features. The results for the family heritability of trees for the start of spring growth are not surprising. In this experiment, this heritability was $h^2=0.98$, so it is close to the theoretical maximum. This feature appears to be under full genetic control. This result only confirms a known regularity (Stojković 1991; Jensen 1993; Baliuckas et al. 2001; Gailink et al. 2005; Czubkowski 2013). A value exceeding 1 was obtained for the individual heritability.

Table 3. The values of Pearson's coefficient of correlation among the features of oaks families (half-sibs). Only between the height and multi-stem index is the correlation significant (at $\alpha=0.05$ level)

Feature	Height	Bud flushing	Powdery mildew symptoms
Bud flushing	-0.1428	-	
Powdery mildew symptoms	0.2737	-0.3202	-
Multi-stem index	0.4928*	-0.2236	0.0103

*) $\alpha=0.05$ **Table 4.** The estimation of genetic gain in height of trees at the age of 12 years, obtained due to the selection of the half-sibs with the average or higher height (selection threshold= $+\sigma$) and the half-sibs with the height no less than the mean value increased by standard deviation (selection threshold= $+\sigma$).

Parametr	\bar{x}	$\bar{x}+\sigma$
Selection threshold [m]	3.5718	3.9389
Selection difference [m]	0.2826	0.5461
Intensity of selection $i=\sigma/S$	0.7697	1.4873
Family heritability h^2	0.8309	0.8309
Genetic gain [m]	0.2348	0.4538

lity of the bud flushing index. This is possible only when the genetic component associated with the female is higher than the component of the male, and thus when the assumptions of the method of estimating heritability by comparing half-sibs are not met. Baliuckas et al. (2001) obtained similar results in an experiment conducted in Lithuania and linked this to the possibility of cytoplasmic inheritance.

The results for height heritability were $h^2=0.83$ (family) and $h^2=0.46$ (individual). In various experiments, different values were obtained, depending on the age, the environmental variance of the study site and the genetic variance of the material being tested. In Denmark, Jensen (1993) received a heritability result (sensu lato) of $h^2=0.871$ for height. For 17-year-old Dutch oaks in Denmark, Jensen et al. (1997) obtained a height heritability result of 0.76 (family) and 0.34 (individual). Vidaković et al. (2000) obtained height heritability results in Croatia varying from 0.74 to 0.90, depending on the provenance. In Poland, in provenance-half-sib experiments with pedunculate oaks, heritability findings differ slightly. In experiments in the Choczewo Forest Inspectorate for 5-year-old trees, Fober (1999) established family heritability at 0.66–0.68 and individual heritability at 0.35–0.38. In the experiment in the Milicz Forest Inspectorate, family heritability was determined to be 0.45 and individual 0.10 at the age of 11 (Barzdajn, 2008). In the experiment in the Bolesławiec Forest District for oaks aged 9–11 years, individual heritability of 0.22–0.34 was

obtained, with significant differences between provenances: for the Zalesie population: heritability 0.02–0.12, for the Zaporowo population: 0.13–0.27 and for the Tronçais provenance: 0.58–0.61 (Barzdajn 2004). In the Namysłów Forest District, the family and provenance heritability of the height of 11-year-old oaks was 0.98, while the individual heritability was 0.21 (Czubkowski 2013). The quoted values, except for a few exceptions, are very high and indicate the large selection potential of pedunculate oaks, even when they originate from a small area.

At the Krotoszyn Forest District orchard, the height of oaks correlated negatively with the multi-stem index, which means a preference for single-stem forms. The amount of heritability of this feature is moderate (family – 0.58 and individual – 0.14); however, it is far from 0 and this feature should be taken into account during selection.

In the described study, evidence was obtained that the degree of powdery mildew infestation is largely genetically controlled, which means that immune selection is possible.

The purpose of selection is to obtain improved values of a given feature in the next generation. In biology, this improvement is called selection response and in breeding, genetic gain. Estimating the amount of the expected genetic gain for height showed that negative selection, based on rejection half-sibs that do not reach average values, will result in a genetic gain that has no practical significance. For genetic gain to be significant, the breeder should decide on very

Table 5. Relative breeding value of half-sibs (standardized effect \times family heritability)

Nominal number of family	Height	Bud flushing index	Index of powdery mildew symptoms	Multi-stem index	Sum of relatives breeding values	Rank of family
7443	0.24	0.91	-0.39	0.07	0.84	13
7444	-1.36	-0.42	-0.63	-1.36	-3.78	29
7445	0.27	1.02	0.29	0.15	1.74	4
7446	-0.54	0.77	0.08	-1.49	-1.18	25
7447	0.46	0.82	-0.26	0.03	1.05	11
7449	-0.19	1.18	0.60	-0.10	1.49	5
7450	-0.07	0.40	-0.13	0.28	0.48	14
7451	-0.21	0.68	-0.36	0.86	0.98	10
7452	0.28	0.26	-0.19	0.96	1.31	6
7453	0.56	0.50	-0.50	-0.36	0.19	19
7455	0.26	-1.52	0.99	0.19	-0.08	12
7456	0.31	0.13	-0.24	-0.42	-0.22	21
7457	1.79	-1.64	0.48	0.53	1.16	2
7458	0.42	1.16	0.59	-0.34	1.84	3
7459	0.16	-1.14	-0.19	0.92	-0.25	15
7460	1.03	-1.67	0.67	0.32	0.34	9
7461	0.94	-1.60	0.82	0.40	0.55	7
7462	1.18	0.59	-0.40	0.11	1.47	8
7571	-0.36	0.29	-0.21	0.07	-0.22	20
7572	-0.54	1.01	0.12	-0.48	0.11	17
7573	0.44	0.65	-0.89	0.29	0.49	16
7574	-0.55	-1.14	0.33	0.27	-1.08	22
7575	-0.56	-1.04	0.56	-0.14	-1.18	23
7578	-1.31	0.88	-0.91	0.07	-1.26	24
7579	-0.89	-0.98	-0.61	0.24	-2.25	26
7581	-0.33	-1.19	-0.66	-0.13	-2.31	27
7583	1.25	0.48	0.56	0.20	2.49	1
7584	-0.86	0.89	-0.06	-0.06	-0.09	18
7585	-1.83	-0.26	0.55	-1.09	-2.63	28

intense selection. The selection threshold should not be set below the average value increased by the standard deviation. This also means that the material to be selected should be numerous so that the trees of the next generation will not

be the offspring of only a few parents. Seedling seed orchards usually have 30–40 families, from which several can be selected. In the described example, above average height increased by the standard deviation was exceeded by only

five half-sibs, so five plus trees were verified. This number is far too small to create a seed orchard of 1.5 generations. However, it is possible to select forward, that is, selecting, for example, 50 trees belonging to five verified half-sibs for the next selection cycle. The total breeding value totalling more than the average increased by the standard deviation was exceeded by only three families: 7445, 7449 and 7583. Apparently, there were only three families with a total breeding value lower than the average minus the standard deviation, numbered 7579, 7581 and 7585. Thus, seedling seed orchards can be useful only for a preliminary understanding of the selection possibilities (estimating the heritability of important economic features) and will not replace offspring tests that include over 100 half-sibs.

5. Conclusions

1. The most strongly genetically controlled feature was the spring phenological (bud flushing) index, but all of the studied features exhibited high heritability and are useful in selection.

2. The height of oaks is positively correlated with the multi-stem index, which means that selection for height will simultaneously limit the occurrence of multi-stem forms.

3. Seedling seed orchards have value as research areas, but for the purposes of practical selection, they are unable to replace progeny tests using hundreds of tested objects.

Conflict of interest

The authors declare no conflicts of interest.

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Authors' contribution

W.B. – contributed to the concept, statistical analysis and writing the text; M.B. – did the fieldwork, prepared the research documentation, data sets and performed literature search

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