#### ORIGINAL PAPER

# Effect of the changing seedlings density of *Quercus robur* L. grown in nursery containers on their morphological traits and planting suitability

Jacek Banach<sup>(1)</sup>, Mariusz Kormanek<sup>(2)</sup>, Stanisław Małek<sup>(1)</sup>, Grzegorz Durło<sup>(1,3)</sup>, Kinga Skrzyszewska<sup>(1)</sup>

<sup>(1)</sup> Department of Ecology and Silviculture, Faculty of Forestry, University of Agriculture in Krakow, Al. 29 Listopada 46, 31-425 Kraków, Poland

<sup>(2)</sup> Department of Forest Utilization, Engineering and Forest Techniques, Faculty of Forestry, University of Agriculture in Krakow, Al. 29 Listopada 46, 31-425 Kraków, Poland

<sup>(3)</sup> NaviGate LLC, Wadowicka 8a, 30-415 Kraków, Poland

#### ABSTRACT

The density of seedlings in a nursery affects their growth parameters and subsequently their adaptation to the growth conditions of their planting sites. Density is especially important in the case of deciduous seedling species, such as pedunculate oak, as it significantly influences their size and leaf size during the production season. The research was conducted in 2015 and 2016 at the container nursery in Nedza (Rudy Raciborskie Forest District). The aim of the study was to investigate the effect of reduced density on the growth of pedunculate oak seedlings in Hiko V265 containers. Approximately three months after sowing in each container the seedlings were sorted into tall (TS) and short seedlings (SS). Subsequently, the TS seedlings were transferred to a new container until reaching 50% capacity. This provided double the growing space for the transplanted seedlings in the second half of the production season. Each year seedlings were analyzed at 14 dates in total in two-week intervals. During the first five dates one container was selected, and after sorting, two containers (one with TS and one with SS) were selected during the remaining nine dates. On each date seedling height, root collar diameter, the dry weight of the roots, shoots, leaves, and total seedlings were measured. Each seedling was assessed to determine three indices of its breeding suitability including: sturdiness quotient (SQ), shoot-to-root ratio (S/R) and Dickson quality index (DOI). The study found that the SS seedlings that were retained in the container after sorting were on average characterized by a lower height and root collar diameter than the TS seedlings. However, by the end of the production season the SS seedlings reached the required quality class. Both the sorted seedling groups exhibited a comparable sturdiness quotient and shoot-to-root ratio during the growth season, whereas only the TS seedlings had a higher Dickson quality index. Overall, the study results support the idea of reducing seedling density in a container during the production season by transferring the tallest seedlings to new containers. This procedure significantly increased the number of good-quality seedlings (by more than 100%) compared to the method without this process.

Received: 21 December 2022; Revised: 22 February 2023; Accepted: 3 March 2023; Available online: 2 June 2023

<sup>🖂</sup> e-mail: jacek.banach@urk.edu.pl

#### **KEY WORDS**

growth, Hiko container, quality indicators, seedling efficiency

#### Introduction

Seedlings with a covered root system are produced in nursery containers growing uniformly spaced and usually at a high density. The density of the substrate is important for the proper growth of seedlings in nursery containers (Alameda and Villar, 2009; Jourgholami et al., 2017; Kormanek et al., 2021; Pajak et al., 2022) however, the growth density of the seedlings is much more important. Many studies have described the effect of density on seedling characteristics of coniferous species including morphological traits (Jinks and Mason, 1998; Radoglou et al., 2007; Kostopoulou et al., 2010), crop adaptation (Simpson, 1991; Salonius et al., 2000; Dominguez-Lerena et al., 2006; Aghai et al., 2014), and water stress and frost resistance (Timmis and Tanaka, 1976). However, there are fewer similar studies for deciduous species, such as Betula pendula Roth, for which seedlings were grown at different numbers of seeds sown into a nursery container (Aphalo and Rikala, 2003).In Poland, the effect of seedling density was studied by Wrzesiński (2015) who showed its influence on the morphological traits of Fagus sylvatica L. mainly on the development of the root system. Studies investigating the breeding of planting stock at different seedling densities in a nursery container have revealed that seedling density is a factor that significantly affects their growth at the nursery stage and their subsequent adaptation after planting on the forest crop. Simpson (1991) highlighted the importance of determining the optimum density of seedlings in a container for different forest tree species, as it can affect the efficiency of production and the quality of the nursery stock.

Unlike coniferous species, deciduous species require more space for growth, so larger containers with smaller cell numbers per unit area are used. In Poland, two types of containers are used for growing oak seedlings: Hiko V265 (28 cells, length 35.2 cm, width 21.6 cm, height 15.0 cm) which is made of high-density polyethylene and Marbet V300 (53 cells, length 65.0 cm, width 31.2 cm, height 18.0 cm) which is made of polystyrene. The containers differ in terms of the growth area which is 27 and 38 cm<sup>2</sup>, respectively (a difference of about 40%). The efficiency of oak seedling production is also influenced by variation in the onset of acorn germination, mainly when seed scarification is not performed. Uneven germination may result in the death of seedlings growing from the later germinated acorns due to shading by the leaves of already growing seedlings (Skrzyszewska *et al.*, 2019).

This study aimed to evaluate the effect of changing the seedling density by reducing the number of pedunculate oak seedlings grown in a Hiko V265 polyethylene nursery container. For purpose of this study, the tallest seedlings were transferred to a new container in the middle of the production period. In addition, the study investigated whether sorting the seedlings into 'tall' (TS) and 'short' (SS) seedlings while reducing their number in the container influences their later growth and quality indicators which characterize the seedlings in the groups. Furthermore, using results from another experiment set up in 2015 the yield per container and parameters of pedunculate oak seedlings grew with reducing seedling density (VS) and without this treatment (VNS) were compared.

### Materials and methods

The research was conducted at the Nursery Farm in Nędza (50°10'08" N, 18°19'04" E) over two consecutive production seasons (2015 and 2016). The seedlings were grown in Hiko V265 nursery containers (28 cells, 265 cm<sup>3</sup> each) in a mixed substrate composed of peat (90%) and perlite

(10%). The same substrate was used to grow the seedlings in both study years. In both years, the measurements were conducted on approximately the same dates (Table 1). Acorns were scarified before sowing by cutting off about one-third of the cotyledon part of the seed. Only viable seeds were sown in nursery containers, while those with necrosis were discarded.

Seedlings were analyzed on 14 dates in total at two-week intervals. Immediately after sowing the acorns, 14 nursery containers were randomly selected from five pallets with a total of 135 containers and assigned numbers from 1 to 14, which denoted the order in which they were taken for analysis. During the first five dates, one container with a maximum of 28 seedlings was selected. After sorting, two further containers (one with TS and one with SS) were selected during the remaining nine dates. Sorting and reducing the number of seedlings in the container involves transferring the tallest seedlings from the original container to a new one (TS). This is the standard procedure used at the container nursery in Nedza for producing oak seedlings. This usually involves the selection of 11 to 13 of the tallest seedlings out of a maximum of 28 growing in the container. The TS container was half planted (14 cells), and the seedlings were placed in a chequerboard pattern. This procedure doubled the growing area for the transplanted seedlings. If the number of TS seedlings transferred to a new container was less than 14, it was planted with the tallest seedlings from the nearest container. After the sorting was done and the density of the seedlings was changed, the containers with tall (TS) seedlings were marked. After sorting, the container with the remaining SS seedlings (13-16) had ad hoc adjustments made to the seedling positions so that they did not grow next to each other. A total of 341 seedlings were analyzed in 2015 and 346 seedlings in 2016. During the production season, foliar fertilization was applied with Floralesad fertilizer at a rate of 0.258 dm<sup>3</sup>·m<sup>-2</sup> (2015) and 0.114 dm<sup>3</sup>·m<sup>-2</sup> (2016). The applied fertilizer consisted of 1.2% NO3-N, 8% NH2-N, 3.5% P2O5, 5.0% K2O, and 0.5% MgO as well as microelements such as S, Fe, B, Cu, Mn, Mo, and Zn. Each year, irrigation in the production field was carried out according to the procedure used in the container nursery which consisted of a subjective assessment of the substrate moisture by the staff member supervising the seedling's growth and deciding whether to switch on the irrigation ramp.

On each date, seedling height (SH, cm) and root collar diameter (RCD, mm) as well as the dry weight of the roots (RDW, g), shoots (SDW, g), leaves (LDW, g), and total seedlings (STDW, g) were measured. The plant material was dried in a Memmert UF 110 laboratory dryer at +65°C for 72 hours, and after cooling, it was weighed on a laboratory balance with an accuracy of 1 mg. Each seedling was assessed to determine three indices of its breeding suitability including sturdiness quotient (SQ) [eq. 1], shoot-to-root ratio (S/R) [eq. 2], and Dickson quality index (DQI) [eq. 3] (Dickson *et al.*, 1960; Iverson, 1984; Thompson, 1985; Banach *et al.*, 2020a) based on the following equations:

$$SQ = \frac{SH}{RCD}$$
[1]

$$S/R = \frac{SDW}{RDW}$$
[2]

Table 1.	
Dates of sowing	and analysis

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Year	Sowing date	Start of the analysis	Sorting of seedlings	End of the analysis	
2015	March 31	May 5	July 5	November 2	
2016	March 24	April 25	June 28	October 24	

$$DQI = \frac{STDW}{SQ + S/R}$$
[3]

These indices are an indicator of a seedling's resistance to abiotic factors and predict its adaptation in the first years after planting (Dickson *et al.*, 1960; Thompson, 1985; Olivo and Buduba, 2006; Haase, 2007; Grossnickle, 2012; Ivetić *et al.*, 2016b; Skrzyszewska *et al.*, 2019; Banach *et al.*, 2020a). Further, the mean values of the analyzed traits of the pedunculate oak seedlings were calculated for both research years. Regression plots were generated to determine the course of changes over the entire production cycle. The minimum values for seedling height and root collar diameter for class I and class II in PN-R-67025 (1999) were also used to assess the quality of the seedlings.

The results of another experiment set up in 2015 (18 containers in total) were used to compare the yield per Hiko V265 container and the growth parameters of seedlings that grew with the application of sorting to TS and SS and density reduction (VS variant) and without application of this treatment (VNS variant). The number of germinated acorns and seedlings grown were also determined in this study. At the end of October 163 seedlings in the VNS variant were analyzed. The same seedling growth parameters were measured as in the main experiment, *i.e.* VS variant.

# Results

SEEDLING YIELD. In both experimental variants, with sorting and density reduction (VS) and without (VNS), the proportion of germinated seeds after 90 days (one week before sorting) was similar at an average of about 88%. At the end of the production period (October), the total yield per container differed by 42.5% in favor of the VS variant. A similar disproportion (40.9%) was found in the productivity of good-quality seedlings which was assessed based on the PN-R-67025 (1999) standard. Both methods of oak production clearly differed in favor of the VS variant regarding the average height of seedlings. Slightly better S/R and DQI values were obtained for seedlings from the VNS variant. No significant differences in RCD, dry seedling weight and SQ were observed between the seedlings grown in either variant (Table 2).

GROWTH PERFORMANCE. In both measurement years, reduced density positively affected the two growth traits: SH and RCD. The rate of change in the average values in both groups was

		Experimenta	ıl variant
Trait		Without reducing	Reducing seedling
		seedling densityVNS	densityVS
Number of acorns sown in the container [pcs.]		28	28
Share of germinated seeds [%]		90.9 ±2.1 a	86.2 ±1.7 a
Seedling yield [%]	Total	32.1 ±1.8 b	74.6 ±2.0 a
Securing yield [70]	Good quality*	22.2 ±2.3 b	63.1 ±2.1 a
Seedling mean height (SH, cm)		24.3 ±1.1 b	32.4 ±0.6 a
Root collar diameter (RCD, mm)		5.9 ±0.3 a	6.1 ±0.1 a
Total seedling dry weight (STDW, g)		7.73 ±0.63 a	7.49 ±0.15 a
Sturdiness quotient (SQ)		4.36 ±1.90 a	4.74 ±1.00 a
Stem-to-root ratio (S/R)		0.39 ±0.01 a	0.51 ±0.04 b
Dickson quality index (DQI)		1.63 ±0.09 a	1.43 ±0.03 b

Table 2.

Comparison of mean yield and mean parameters ( $\pm$ SE) of the pedunculate oak seedlings grown in both variants in 2015 (a, b – homogeneous groups determined by Tukey's test, p=0.05)

\*Rated according to the PN-R-67025 standard

best described by quadratic and power equations with a high coefficient of determination values in the range of 0.70-0.96. The height and RCD of TS exceeded the quality class I limit according to the PN-R-67025 (1999) standard. The SS also had large diameters in both measurement years, while those grown in 2015 reached quality class II. For SH and RCD, a decrease in the disproportion between TS and SS was observed in both measurement years at the end of the production period (Fig. 1).

DRY WEIGHT. During the production season the STDW of the TS increased to above 10 g while the root system increased to 6-7 g. The course of changes observed in 2016 in the dry seedling weight of TS was best described by quadratic equations and SS by power equations with a very high coefficient of determination value of about 0.95 (Fig. 2).

QUALITATIVE INDICATORS. In contrast to the growth and dry matter traits, no differences were observed in the quality indices values between the two seedling groups analyzed after sorting. The SQ index did not change significantly throughout the production period in each measurement year. The value of SQ remained within the acceptable level (SQ=6.5) and reached the lowest value at the end of the seedling culture. The S/R in both measurement years decreased with time to about 0.6 with a slightly better value of 0.4 for SS in 2015. The DQI increased with time to about 1.8 for TS and to about 1.2 for SS. The rate of change in the coefficients over the production season was best described by quadratic and power regression equations with a very high coefficient of determination value (ca. 0.9) for the DQI index and lower, more varied values ranging from 0.2 to 0.8 for the other indices (Fig. 3).

#### Discussion

The study showed that producing pedunculate oak in a Hiko V265 container with seedling density reduction about three months after sowing (97 days) by transferring TS to another container



Fig. 1.

Comparison of height (SH) and root collar diameter (RCD) of pedunculate oak seedlings grown in 2015 and 2016, separated during the production season (DAS=97) into tall (TS) and short seedlings (SS); horizontal lines indicate the first (I) and second (II) quality class according to the PN-R-67025 standard; DAS – days after sowing



Fig. 2.

Change in the dry weight of the roots (RDW) and total seedlings (STDW) grown in 2015 and 2016, DAS – days after sowing



Fig. 3.

Comparison of the quality indicators of pedunculate oak seedlings grown in 2015 and 2016; DAS – days after sowing, SQ – sturdiness quotient, S/R – shoot-to-root ratio, DQI – Dickson quality index

resulted in significantly higher productivity. The number of grown and good-quality seedlings in one container was higher by about 40% compared to the variant without density correction. In contrast, the number of germinated seeds was comparable (ca. 87-90%) in both variants. The results show the effectiveness of thinning seedlings to reduce their density in the container. For example, sowing 1 million cells in nursery containers with scarified acorns results in the germination of 880,000 from which an average of 641,000 good-quality seedlings will be obtained combined through sorting and a reduction in seedlings in the container. In contrast, only about 222,000 seedlings will be obtained if these procedures are not applied. The profit resulting from the increased number of good-quality seedlings obtained with density reduction during the growing season is significantly higher than the costs of this nursery treatment. A similar result was obtained in a study on *Fraxinus angustifolia* Vahl, wherein rearing seedlings at lower densities resulted in a better yield than those with higher densities (Fidan, 2017).

The results obtained for different coniferous and deciduous species indicate that seedling density during nursery growing affects the growth parameters obtained (Jinks and Mason, 1998; Aphalo and Rikala, 2003; Dominguez-Lerena et al., 2006; Cicek et al., 2007; Dini-Papanastasi et al., 2012; Aghai et al., 2014; Wrzesiński, 2015) which later determine the adaptation of seedlings as forest crops (Barnett, 1980; Dey and Parker, 1997; Jackson et al., 2012; Banach et al., 2020b). Higher densities increase seedling height, while lower densities influence RCD and dry weight and cause a reduction in SQ values as observed for *Pinus taeda* L. (Carneiro et al., 2007). In contrast, this study on pedunculate oak did not confirm such a relationship. Seedlings growing under higher density conditions were 33% lower at the end of the production period. However, the DQI obtained for these seedlings that indicated their adaptability (Olivo and Buduba, 2006; Ivetic et al., 2016a, b) was lower by 37% with similar average values of dry seedling weight and RCD in both variants. Comparable results were observed in similar studies carried out using V265 (390 cells·m<sup>-2</sup>) and V250 (243 cells·m<sup>-2</sup>) containers, where the use of the latter led to higher values of the growth parameters for Q. robur seedlings (Wesoly et al., 2017). The significant influence of the nursery container type on the growth of oak seedlings was also shown by Mariotti et al. (2015) who reported that the Tubex container was ideal for growing Q. robur and that it improved the growth of shoot and root systems of the seedlings. Further, better performance of seedlings grown in containers with fewer cells was shown in a study on *Chamaecyparis obtusa* (Siebold & Zucc.) Endl. (Cho et al., 2014). Similar results were also obtained in the field nursery where the density of Quercus rubra L. seedlings the size of the root system. Increasing the seedling density from 18 to 148 pcs.·m<sup>-2</sup> significantly reduced RDW (Tomlinson *et al.*, 1997). Similarly, the spacing of seedlings within and between rows significantly influenced the seedling parameters including the size of the root system which increased with increasing seedling spacing (Cicek et al., 2007).

In this study, seedling sorting and density reduction during the second half of the production period decreased the disproportion in SH between the TS and SS groups after sorting from 75% to 25% at the end of October 2015 and from 38% to 22% in 2016. Similarly, the disproportion in the dry weight decreased but not in the RDW of pedunculate oak seedlings grown in 2015. These results are consistent with the observation of Simpson (1991) who found that the optimal density of seedlings is critical for forming an even biomass of seedlings. The findings also confirmed the results of Wesoły *et al.* (2017) who achieved a similar breeding result using the reducing the density of oak seedlings in a container in late June/early July.

The increase in RCD in both height variants depended on the weather conditions and the energy and water balance in the production fields. Although 2015 was an average year in terms of temperature with a mean temperature in the growing season of  $15.1^{\circ}C$  (±6.2°C), it was low in

precipitation during that time (225 mm). The precipitation irregularity index W (Radzka and Rymuza, 2020) was 35%. However, in 2016 the precipitation pattern was close to the climatic norm. The total precipitation during the growing season was 340 mm, and the W index value was 43%. The average temperature of the growing season was similar to that of 2015 which was 15.2°C (±5.9°C). The value of the W index indicated slightly irregular precipitation in both years. However, the average index values do not support the fact that the energy and water balance in the production fields were fundamentally different in the two variants. In addition, the irrigation system consistently compensated for the water deficit during the summer months of 2015. These assumptions are confirmed because the leaf area index (LAI) of the pedunculate oak in 2015 reached its maximum value only in the middle of August. In contrast, in 2016 the LAI peaked at the beginning of July. These differences significantly affected the short-circuiting rate of the assimilative apparatus within containers, pallets and the entire production field (Durlo *et al.*, 2016). LAI is an important determining factor for sorting oak seedlings in container nurseries.

A more uniform RCD was obtained in 2016. However, in both years the average values for seedlings, regardless of whether they belonged to the TS or SS group, clearly exceeded the minimum value for quality class I determined by the PN-R-67025 (1999) standard. A high RCD value is essential for the subsequent survival from seedling to crop. This was shown by Aphalo and Rikala (2003) in a five-year study on *B. pendula* Roth and by Jinks and Mason (1998) on *Pinus nigra* var. *maritima* Melv., *Pinus sylvestris* L. and *Pseudotsuga menziesii* (Mirb.) Franco. Further, Simpson (1991) also pointed out the need to determine the correct seedling density which significantly affects the efficiency and quality of nursery production, and thus the adaptation of seedlings to crop. The importance of seedling density is confirmed by a study on *Pinus pinea* L. in which a clear difference was observed in the adaptation of seedlings grown using different nursery procedures (Dominguez-Lerena *et al.*, 2006). According to the authors of the study, the morphological characteristics of seedlings and their density are also influenced by the ratio of the container depth to diameter.

Of greater importance for good adaptation of seedling to crop are not so much the single seedling parameters obtained at the nursery stage, but synthetic indices calculated using a more significant number of traits (Olivo and Buduba, 2006; Haase, 2007; Grossnickle, 2012; Ivetić *et al.*, 2016a, b). For example, Wrzesiński (2015) showed that density significantly affects the SQ and S/R indices in beech seedlings, especially the latter. This is due to an increased root system weight relative to decreasing seedling density. In the presented study comparable values for both indices were obtained for the two height groups and increased with the production period. The SQ coefficient value did not exceed the maximum level for either height group. This confirms the results of changing seedling density by sorting them and reducing their number to about half in a nursery container. However, more favorable higher DQI values were obtained for the TS seedlings. This may give a slight advantage to such seedlings after planting in a forestry crop in terms of improved adaptation.

#### Conclusions

- The study confirmed the expediency of sorting seedlings into tall (TS) and short (SS) and by reducing seedling density in the container by transferring TS seedlings to new containers in the middle of the production season. This treatment results in significantly higher quality seedlings than growing them without.
- Throughout the growing season the value of the SQ and S/R indices did not depend on the seedling's height, which positively affected only the value of the DQI index.

The seedlings' breed suitability indices (SQ, S/R, and DQI) changed during the production season. The best values were obtained at the end of the production period (the second half of October).

# Authors' contributions

All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by J.B., M.K., S.M., G.D. and K.S. The first draft of the manuscript was written by J.B. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

#### Conflicts of interest

The authors declare that they have no conflict of interest.

### Funding

The research was carried out as part of the project ER-2717-4/14, 'Optimizing the production of seedlings with covered root system in the selected container nurseries,' funded by the Directorate General of State Forests Warsaw (Poland).

#### Acknowledgments

The authors would like to thank the staff of the container nursery in Nędza for their assistance with the 2015-2016 research and Grzegorz Bownik, M.Sc., for his help with the measurements.

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11

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#### **STRESZCZENIE**

# Wpływ zmiany zagęszczenia sadzonek *Quercus robur* L. rosnących w pojemnikach szkółkarskich na ich cechy morfologiczne i przydatność do zakładania upraw

Zagęszczenie sadzonek w trakcie ich hodowli w szkółce wpływa na uzyskane parametry wzrostowe, które później determinują adaptację sadzonek po posadzeniu na uprawie leśnej. Przestrzeń wzrostowa w kwaterze szkółkarskiej lub w kontenerze jest szczególnie ważna dla sadzonek gatunków liściastych, takich jak dąb szypułkowy, które w trakcie sezonu produkcyjnego znacząco zwiększają swój rozmiar, w tym wielkość aparatu asymilacyjnego. Badania dotyczące przebiegu wzrostu sadzonek debu szypułkowego przeprowadzono w latach 2015-2016 w szkółce kontenerowej Gospodarstwa Nasienno-Szkółkarskiego w Nędzy (Nadleśnictwo Rudy Raciborskie). Przedmiotem badań był wpływ redukcji zagęszczenia sadzonek dębu hodowanych w kontenerach Hiko V265 na przebieg ich wzrostu do końca okresu produkcyjnego. Po około 3 miesiącach od momentu wysiewu żołędzi w każdym kontenerze podzielono sadzonki na wysokie (HS) i niskie (LS), przenosząc następnie wysokie sadzonki do nowego kontenera i wypełniając go tylko w 50% (w systemie szachownicy). Zastosowany zabieg pozwolił na dwukrotne zwiększenie przestrzeni wzrostowej dla hodowanych sadzonek w drugiej połowie sezonu produkcyjnego. Każdego roku sadzonki analizowano w odstępach dwutygodniowych, łącznie w 14 terminach (tab. 1). W pierwszych pięciu terminach pobierano losowo jeden pojemnik z sadzonkami, a po przesortowaniu na sadzonki wysokie i niskie pobierano w dziewięciu terminach dwa pojemniki (jeden z HS i jeden z LS). W każdym terminie mierzono wysokość, średnicę w szyi korzeniowej oraz suchą masę korzeni, pędów, liści i całej sadzonki. Dla każdej sadzonki obliczono trzy wskaźniki przydatności hodowlanej, tj. współczynnik wytrzymałości (SO), współczynnik pedowo-korzeniowy (S/R) oraz indeks jakości Dicksona (DQI). Stwierdzono, że sadzonki niskie (pozostające w kontenerze po przesortowaniu) charakteryzowały się przeciętnie mniejszą wysokością i grubością w szyjce korzeniowej w porównaniu do sadzonek wysokich. Jednak na koniec okresu produkcyjnego także i te sadzonki osiągały wymaganą klasę jakości (ryc. 1). Sadzonki wyższe w momencie sortowania (HS) cechowały się przeciętnie wyższą suchą masą do końca okresu hodowli, jednak

różnica w każdym terminie pomiarowym względem sadzonek LS utrzymywała się na zbliżonym poziomie (ryc. 2). Przez cały sezon produkcyjny sadzonki w obydwu grupach wysokościowych (HS i LS) charakteryzowały się porównywalnymi wartościami współczynnika wytrzymałości SQ i współczynnika pędowo-korzeniowego S/R. Z kolei wyższe wartości indeksu jakości Dicksona DQI uzyskano dla sadzonek z grupy wysokich (ryc. 3). Dodatkowo w 2015 r. przeprowadzono ocenę wpływu redukcji zagęszczenia sadzonek w kontenerze w porównaniu do jej niezastosowania. Produkcja w wariancie ze zmniejszeniem zagęszczenia charakteryzowała się istotnie wyższą wydajnością, a wyhodowane sadzonki były wyższe i cechowały się lepszym indeksem DQI (tab. 2). Wyniki badań dla dębu szypułkowego hodowanego w szkółce kontenerze w trakcie sezonu produkcyjnego przez przeniesienie najwyższych sadzonek do nowych kontenerów. Taka procedura pozwoliła na znaczące zwiększenie liczby sadzonek dobrej jakości (o ponad 100%) w stosunku do wariantu porównawczego.