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# The influence of seedling density in containers on morphological characteristics of European beech

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**Abstract.** This study examines the influence on growth parameters, in particular the morphological features of the root system, of 1-year-old European beech seedlings cultivated in containers with two different densities. The experiment was conducted in the container nursery in Skierdy (Forest District of Jabłonna) in spring 2011. After 10 months of cultivation in Hiko polyethylene containers, above- and below-ground parts of the seedlings were measured. The measurements of the root system were conducted with a scanner and the WinRHIZO software. No influence due to the seedling density on either shoot height or thickness was observed, but instead the research showed that different seedling densities affected the development of root systems. The mean root thickness and dry mass of the European beech seedlings were significantly higher at the lower density. The influence of seedling density on the development of root mass deserves special attention as it is the most important factor affecting future growth of the seedlings during cultivation. This tendency also suggests that the amount of nutrients allocated to shoot development may be higher in order to improve the efficiency of photosynthesis. At both densities, differences in biomass accumulation affected the root-toshoot ratio. In seedlings cultivated at the lower density, the increased dry root matter of the seedlings resulted in a significant increase in the root-to-shoot ratio. This may cause a potential growth advantage of these seedlings after they are planted and may thus result in a more productive cultivation.

**Keywords:** seedling density, seedling size, shoot height, seedling root system, container type, container nursery

## 1. Introduction

The purpose of nursery production is to breed planting material of adequate quality to ensure high adroitness of the established forest plantations. In this aspect, nurseries play an important role in the process of shaping future forest stands. The influence of quality and immunity of seedlings on stress factors after planting assumes importance in this context.

The thrust of forest nursery in Poland is breeding of seedlings with open root system in traditional field nurseries. Production of container seedlings in container nurseries increases its share in general breeding, despite the fact that it causes many new technical and organisational problems for forest practices. Proper selection of parameters and shapes of containers assumes significance because of their essential influence on morphology and physiology of seedlings, arising from their root system (Gilman 2001), their quality, growth potential (Davis, Jacobs 2005) and their mass (Haase 2011). These parameters often decide the adroitness of the seedlings. The number and volume of cells in containers are two relevant determinants shaping the quality of seedlings (Kinghorn 1974). Those determinants are strongly connected with each other and dependent on physiological needs of bred species, breeding cycle, growth conditions and mechanisation degree and automation (Szabla, Pabian 2009). They are also one of most important factors influencing production costs (Chirino et al. 2008).

Many scientists have studied which variables of container (i.e. diameter, depth, volume and number of cells) have the biggest influence on the growth and quality of planting material in nursery and after planting. Which seedlings show the highest survivability in defined habitat conditions (tro-

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phic, moisture) (Rose et al. 1997; Dominguez-Lerena et al. 2006; Pinto et al. 2011; Aghai et al. 2013), competition (Newton et al. 1993; Hunt 2002) and other limiting factors occurring in cultivation (Lamhamedi et al. 1997; South et al. 2005) were also observed. Therefore, studying the influence of those variables is important in order to optimise the usable space in container production while maximising the quality of seedlings. The influence of seedlings density in containers on their morphological features in reference to chosen species and types of containers (Barnett, Brissette 1986; Simpson 1991) was also analysed.

The aim of the present research was to compare the influence of two different densities of European beech seedlings in Hiko-type container on their growth parameters, especially on morphological features of root system.

## 2. Methodology and research material

The experiment was conducted in economic conditions of Skierdy container nursery in Jabłonna Forest Inspectorate. One-year-old European beech seedlings with covered root system were chosen for research. The breed of seedlings used were Hiko type polyethylene containers, produced by Swedish company BCC AB, in two variants of cell density determining seedlings spacing. The first variant, Hiko V-250, sized 352 × 216 × 160 mm, consists of 18-cell in spacing 72 × 59 mm, each of volume 250 $cm^3$. The second variant, Hiko V-265, sized 352 × 216 × 150 mm, consists of 28 cells in a spacing of 54 × 50 mm, each of volume 265 $cm^3$. The single cells in container had square upper section, tapering downwardly with fishnet bottom and four ribs running vertically on the inner walls. Both types of containers used in research were selected according to breeding needs of the given species. Seedlings from both variants were subjected to controlled mycorrhisation with fungi *Hebeloma crustuliniforme* (Bull.) Quél.

The containers were filled with substratum. On 11 May 2011, in each cell, one sprouted beech seed was sown. For three weeks, containers were kept in greenhouse block, which provides optimal microclimate conditions for seedling development. Then the seedlings were transferred to open production fields where they stayed for 10 months until the end of the experiment. Containers with 18 and 28 cells were placed on separate pallets. After 10 months, 30 seedlings were randomly collected from each variant.

Each seedling was treated as an experimental unit (repetition), i.e. representing a single container. Seedlings chosen for research were a part of the planting material produced for Forest Inspectorate's needs. The seedlings were transported to laboratory where they were removed from containers and substratum was removed from the root system. Then, the seedlings were scanned and analysed comprehensively with the use of computer program WinRHIZO from Regent Instruments company (2008). The analysis allowed for defining following morphological features of examined root systems: joint length of all roots in root system [cm], average root thickness [mm] and volume of root system [$cm^3$]. The dry mass of root system [g] was also determined. Deformations of root systems were evaluated visually. Two classes of deformations were distinguished: (1) properly shaped and (2) with changed direction of growth. The characteristics of above-ground part was conducted on the basis of measurements of the following features: height [cm] and thickness in shoots' neck root [mm] and total dry mass of shoot [g]. Above-ground parts and roots of seedlings were dried in temperature 104°C for 24 hours, and then they were weighed on laboratory weight with accuracy up to 0,001 g. In the experiment, the relation of shoots height to thickness (slenderness H/D) and dry mass of roots to dry mass of shoots were calculated. These are one of the parameters used for evaluation of seedlings quality, allowing for predicting their survivability and growth in cultivation.

Statistical analysis was performed with the use of computer program Statistica 10 (StatSoft 2011). In the analysis the normal distribution of observations, in particular variants of experiment with the use of W Shapiro-Wilk test and the homogeneity of variance with the use Brown-Forsyth test, was checked. For the evaluation of influence of cell arrangement in the container on morphological features of the examined seedlings univariate analysis of variance (ANOVA) was used. The significance of differences at level $\alpha = 0,05$ was defined with the use of Student's t-test. A linear correlation analysis was also performed. It defined the influence of height and thickness in seedlings' neck root on other features after 10 months of growth of the seedlings. The strength of relation was evaluated by significance of linear correlation at level $\alpha = 0.01$ and $\alpha = 0.05$. Besides features used in variance analysis, the number of epicormic shoots and the number of tops and root branching were additionally included in the correlation.

## 3. Results

The average height of beech seedlings from 18-cell containers was 36.6 cm, whereas those from 28-cell containers was 39.7 cm. Seedlings growing in bigger density had therefore higher shoot than seedlings from second variant of the experiment. The difference was, however, not significant statistically. The average thickness in neck root of bred seedlings was 6.11 mm in 18-cell containers while in 28-cell containers it was 5.73 mm. In terms of analysed features, no significant statistical differences between density variants was observed. Total dry mass of one seedling's shoot ranged from 0.41 g in 18-cell container variant to 5.56 g. The average mass of European beech shoot bred in containers with 28 seedlings was 2.78 g, while in containers with 18 seedlings, it was insignificantly smaller by 0.07 g. The Calculated slenderness of seedlings was for variant with smaller density 1:6, while in variant with higher density it was 1:7. Seedlings' slenderness (H/D) varied statistically significantly between variants (Table 1).

At smaller density, seedlings developed the biggest root systems of total length 11,410.60 cm. In the same variant of the container, the average length of roots reached 5702.3 cm, while in containers with bigger density they measured 5581.2 cm. The shortest total length of roots among analysed seedlings measured 716.2 cm. The variance analysis showed that this feature between variants did not differ significantly (Table 1).

The average thickness of roots of seedlings bred in 18-cell containers was 0.30 mm, whereas in 28-cell containers it measured 0.28 mm. Differences between those values are relevant statistically. One-year old seedlings of European beech bred in container nursery in containers with smaller density had average volume of root system at 4.1 $cm^3$, while seedling from second density variant had slightly smaller average volume of root system at 3.4 $cm^3$. This difference was not significant statistically (Table 1).

Seedlings from containers with smaller density were characterised by significantly higher values of total dry mass of roots in comparison to seedlings from second variant of the experiment. On the basis of our analysis of ratio of dry mass of roots to dry mass of shoots, the influence of density of seedlings in container on this ratio was significant. The ratio of root system mass to over-ground part mass was on average was 1:1.4 for seedlings from 18-cell containers and 1:1.0 for seedlings from 28-cell containers (Table 1).

The important part of evaluation of seedlings from container is the construction of the root system, especially its deformation. In following study, among all examined European beech seedlings, as much as 65% had deformed root system. It was observed that increased deformation occurs when seedlings are being bred at higher density.

In order to define the relationship between height and thickness in seedlings' neck root and remaining morphological features, factors of linear correlation were calculated. The significance of relations was examined at level $\alpha = 0.01$ and $\alpha = 0.05$. From our analysis, it was found that height of seedlings, regardless of density variant, has a positive influence on thickness of the neck root, dry mass of shoot, dry mass of roots and total length of roots. Only in 18-cell containers, this positive correlation between height and volume of root system and number of root tops was observed.

Height had no influence on the average thickness of roots and number of branching in both variants of the experiment. The number of epicormic shoots was correlated with height of seedlings only in the higher density variant. The analysis of linear correlation dependence showed that, apart from root thickness, there is a significant relation between thickness in neck root and remaining values of seedlings' features. The strongest correlation from all of the analysed features applied to total dry mass of roots regardless of the density variant. A slightly weaker correlation between thickness of shoot and its dry mass was observed (Table 2).

**Table 1.** Effect of density of seedlings on the analysed parameters of European beech
(P – significance level)

| Variable | Average calculated for density: | | Coefficient of variation calculated for density: | | Significance level (P) |
|---|---|---|---|---|---|
| | 18 seedlings | 28 seedlings | 18 seedlings | 28 seedlings | |
| Height of the seedlings (H) | 36.6 | 39.7 | 19.1 | 16.8 | 0.084 |
| Thickness of the neck root (D) | 6.11 | 5.73 | 16.3 | 15.1 | 0.124 |
| Dry mass of the shoot (SMP) | 2.71 | 2.78 | 36.0 | 38.3 | 0.797 |
| Root dry mass (SMK) | 3.75 | 2.93 | 40.4 | 42.8 | 0.027 |
| Total root length | 5702.3 | 5581.2 | 41.9 | 39.5 | 0.839 |
| Thickness of the roots | 0.30 | 0.28 | 12.3 | 16.6 | 0.021 |
| Root system of volume | 4.10 | 3.40 | 38.6 | 47.0 | 0.092 |
| The ratio of height to thickness of the neck root | 1:6 | 1:7 | 17.5 | 12.3 | 0.001 |
| The ratio of dry mass of the shoot to root dry mass | 1:1.4 | 1:1.0 | 8.6 | 23.4 | 0.001 |
| Percentage of root deformation | 68.0 | 62.0 | 24.9 | 24.0 | 0.599 |

## 4. Discussion

The density of seedling distribution may have influence on their development, both in open nurseries and in controlled conditions. Barnett and Brissette (1986) concluded that the main factor influencing the phenotype of container seedlings was the density of cells in the container and not their shape or volume. Timmis and Tanaka (1976) and Simpson (1991) stated that breeding of seedlings in containers with higher density results in high and thin seedlings of coniferous species, while breeding of seedlings in container with smaller density results in smaller and thicker seedlings. The results of this research with European beech confirm those findings. It should be noted, however, that the lack of statistically significant differences in height and thickness of seedlings' shoot between the examined density variants. Similar regularity was observed with *Pinus nigra* and *Pinus sylvestris* seedlings (Jinks, Mason 1997), *Picea mariana* (Salonius et al. 2000). But a different result was obtained by Peterson (1996). He observed reduction of *Picea mariana* seedlings' height with an increase of their density in the container.

The size of shoot is very important because in places with access to water and nutrients in soil, resources connected with space, the competition for light between seedlings and vegetation of forest litter seems to be the main factor limiting their growth and development. According to numerous studies, in case of strong competition, higher seedlings are characterised by better survivability and growth. Those seedlings grew in higher densities (Mason et al. 1996; Mohammed et al. 1998; South, Mitchell 1999; Puértolas et al. 2003). The ratio of height to thickness of seedlings' shoot (H/D) is also a good indicator of their quality. A lower H/D ratio indicates that seedlings are more resistant to wind and drought but it also has an influence on their increased growth and survivability in dry places (Johnson, Cline 1991). On the basis of conducted research a statistically significant influence of seedlings' density in container on ratio of height to shoot's thickness was observed. Seedlings growing in smaller density were characterised by lower H/D ratio, which may suggest their better breeding quality. It is important to emphasise that the thickness of shoot shows bigger degree of correlation with weight of the roots than with the mass of over-ground part. A bigger size of root system occu-

**Table 2.** Coefficients of correlation (r) between height of the seedlings and thickness of the neck root and other characteristics morphologic

| Variables | | r coefficient calculated for density: | |
|---|---|---|---|
| *X* | *Y* | 18 seedlings | 28 seedlings |
| Height of the seedlings | Thickness of the neck root | 0.609** | 0.688** |
| | Number of the offshoots | 0.259 | 0.624** |
| | Dry mass of the shoot | 0.712** | 0.856** |
| | Root dry mass | 0.578** | 0.702** |
| | Total root length | 0.492** | 0.356** |
| | Thickness of the roots | 0.277 | 0.062 |
| | Root system of volume | 0.681** | 0.272 |
| | The number of roots' tops | 0.578** | 0.270 |
| | Number of roots' branching | 0.239 | 0.316 |
| Thickness of the neck root | Number of the offshoots | 0.492** | 0.652** |
| | Dry mass of the shoot | 0.893** | 0.863** |
| | Root dry mass | 0.931** | 0.926** |
| | Total root length | 0.599** | 0.521** |
| | Thickness of the roots | 0.263 | 0.141 |
| | Root system of volume | 0.771** | 0.562** |
| | The number of roots' tops | 0.576** | 0.412* |
| | Number of roots' branching | 0.385* | 0.391* |

** – relationship significant at P = 0.01
* – relationship significant at P = 0.05

rs as the thickness increases in neck root of seedlings with an open root system (Janson 1969; Ritchie 1984) and container seedlings (Grossnickle 2000). This dependence was also noted with European beech seedlings in present experiment.

The values of average thickness and dry mass of root were significantly lower for higher-density European beech seedlings in the container than for smaller-density seedlings. It is confirmed by numerous research results, for example *Picea glauca* (Scarratt 1972), *Pseudotsuga menziesii* and *Pinus contorta* (Simpson 1994), *Betula pendula* (Aphalo, Rikala 2002), *Pinus palustris* (South et al. 2005) and *Pinus pinea* (Dominguez-Lerena et al. 2006). It was also observed that values of the volume of root system were constant between two levels of density, despite significant differences in root dry mass. A similar finding was observed by Aghai and others (2013) while breeding *Larix occidentalis*.

One of the features best characterising quality of seedlings was parameters of the root system. The best feature for parametric characterisation is the mass of the root system. Previous research showed that seedlings with bigger mass of roots while planting had bigger growth and adroitness in comparison to those with smaller mass, even if they had a smaller diameter of the shoot (Jacobs et al. 2005; Grossnickle 2012). Bigger mass of roots is the indicator of their absorption capacity and it increases the possibility of overcoming the planting stress. The effects of initial size of root system may have a lasting impact on further growth of the seedlings. Sundström and Keane (1999) noticed that initial size of root system was correlated with the increase of diameter breast height and root biomass in 10-year-old Douglas firs, which they dug up.

The morphology of seedlings is visibly dependent on their density in the container, which significantly influences the availability and quality of light. It is well known that density of foliage influences the development of the whole seedling by shading. More specifically, the change of photosynthetically active radiation and quality of light has an influence on shoot phenotype and pace of root increase (Aphalo, Ballare 1995). Previous research (Vance, Running 1985), in terms of shading, indicate that lower levels of lighting at higher density should strengthen the increase in growth, not limiting at the same time the diameter of the root and dry mass of the root. Mitchell and Arnott (1995) stated that shading of coniferous seedlings causes the increase of height and decrease in branches and root growth. The seedlings' tendency to compensate light limit by allocating bigger part of photosynthesis products in the growth of shoot at the expense of root growth (Drew 1983) may explain why European beech seedlings in this research show smaller accumulation of mass in root with an increase of breeding density. The differences in accumulation of biomass considerably changed the ratio of root to shoot between examined levels of density.

An appropriate balance of roots and shoots is an important morphological attribute as it is the measure of water loss and the capacity of water capture by the plants during the planting time (Burdett 1990; Grossnickle 2000). The proportion of over-ground to underground part of the seedling is also a predictor of seedling's tolerance to transplanting stress and resistance to drought (Grossnickle 2005). Drying is the most stress-causing factor influencing the young seedlings. This stress lowers seedlings' usefulness for renewals and afforestation. That is why earlier information characterising the quality of planting material is needed and also it is important to know where the planting has been planned. It is believed that the functional value of seedlings is higher, higher the ratio of root system mass to over-ground part mass (Gunia, Sobczak 1981). Therefore, higher values of root system among examined European beech seedlings may be connected with higher adroitness of cultivation. At smaller density of cultivation, bigger dry mass of seedlings' root meant the increase of root to shoot ratio. This implies potential advantage for those seedlings after transplanting in relation to seedlings bred at high density. It was confirmed by Aghai and others (2013) in simulated field trials, in which *Larix occidentalis* seedlings from scheme with smaller density after transplanting had significantly bigger increase of root mass and efficiency. Aphalo and Rikala (2002) also stated that a reduction in density of *Betula pendula* nursery had an influence on seedlings' morphology after planting, but had little impact on survivability. However, according to Dominguez -Leren and others (2006), the density of seedlings influenced the morphology, nutrition and efficiency only in nursery.

A majority of the cited research and the results obtained in the present study suggest that higher density leads seedlings to allocate disproportionately large quantity of photosynthesis products into shoot growth, resulting in less developed roots. The increase of seedling's height at the cost of root system may be a disadvantageous change in terms of quality of production. On the other hand, the increase in dry mass of root, root diameter and ratio of root to shoot obtained by breeding in smaller density may turn out to be a profitable investment, especially if the result obtained is more durable seedling, which may show higher growth potential. Higher growth potential presumably means higher survivability after planting, which results in smaller number of corrections. Generally, benefits from better developed root system as a result of breeding at smaller density seem to surpass benefits resulting from increase of photosynthetic tissue. Breeding of seedlings in containers at smaller cell density will be justified only if planting material produced in such a method will result in high adroitness of cultivations. This issue creates new challenges for forest nursery in Poland and requires conducting further research evaluating the impact of methods used in seedling production on adroitness and development in different habitat conditions.

## 5. Conclusions

On the basis of research results, it is possible to formulate following conclusions:

1. Use of different density of European beech seedlings in containers did not have significant influence on their height and thickness of the shoot.
2. A significant influence of density on seedlings' slenderness (H/D) was observed.
3. Our research showed that change of seedlings' density in the container has a large impact on development of root system. It should be emphasized that the influence of this feature on development of root mass is the most important element for their further development in cultivation.
4. Density of seedling in container is an important factor influencing the growth and quality of the bred planting material. At smaller density of examined seedlings, bigger dry mass of roots of seedlings led to a significant increase in root mass to shoot ratio. That creates potential advantage in the growth of those seedlings after planting and may result in greater adroitness of plantations.

## Conflict of interest

The authors declare that there were no potential conflicts of interest.

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