Effects of fertilisation on development and nutrient uptake of black locust saplings grown in pots

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Abstract: Currently, black locust is the most important tree species in Hungary with significant economic value. Intensification of its cultivation and the improvement of the timber quality should include the use of highly productive clones and reasonable fertilization. Nutrition management should be based on reliable data from exact experiments. In our trial, nutrition intake of Turbo Obelisk OBE01 clone saplings was examined during a four-month period. Osmocote Pro (18:9:10 + 2Mg) was used as fertilizer at a dose of 2.5 and 5 kg m⁻³ mixed to a peat-based substrate. At the end of the growing period, saplings reached a height of 260-280 cm and a stem diameter of 16-18 mm. Nutrient intake order was found to be the following: Ca (3.3-4.2 g) > N (3.1-3.6 g) > K (2.1-2.9 g) > Mg (0.35-0.5 g) = P (0.3-0.5 g). Based on our results, a lower N:P and N:K rate fertilizer is recommended, especially if a non-peat based substrate and longer growing period is planned with a higher rate of nitrogen fixation. Considering nitrogen resource, a dose of 5 kg m⁻³ was proved to be less effective than a concentration of 2.5 kg m⁻³. However, the higher concentration of phosphorous, potassium and magnesium were well-utilized by the plants.

Keywords: nutrient concentration, nutrient distribution, dry matter, Turbo Obelisk clone Received 23 May 2020, Revised 6 October 2020, Accepted 7 October 2020

Introduction

Black locust (*Robinia pseudoacacia* L.) originated from North America, is the second most important cultivated deciduous tree species in the World after the eucalyptus (Bartha et al. 2006). It has global economic importance and widely cultivated in temperate North America, Europe, and Asia. It was introduced to Hungary between 1710 and 1720 and spread widely in the 19th century (Rédei et al. 2008). In 2018, the surface of 454 000 hectares of black locust forest meant a quarter of Hungarian forest surface (KSH). Considered as an important and recognized tree species, black locust was declared as Hungaricum in 2014 (http1).

The main aim of black locust plantation is to exploit its fast and intensive growing. Although it is widely planted on medium quality or on poor soils where it is only able to produce firewood quality, the species can provide high-quality timber on suitable sites with nutrient-rich, well-aerated soil. Under favourable conditions, height growth of trees can have a peak in five years while it can reach the final diameter after 10 years, producing 8-12 tonnes of dry timber for a hectare. These characteristics make black locust one of the most important tree species of timber plantations in Hungary. At the same time, technology applied for less intensive species cannot be adopted for black locust (Rédei et al. 2008; Rédei 2015).

Due to the huge surface of Robinia forests and plantations, a great amount of black locust timber is available in the market but still at low quality, useless for furniture or construction industry. Improvement of timber quality can be reached by the use of selected varieties and intensive cultivation technology (Keresztesi 1988; Rédei 2015). Besides poplar and willow, black locust is also used in energy forests to provide the

highest aboveground biomass in the shortest time (Orlovic and Klasnja 2004). Wood and his colleagues (1977) called the attention to the problem that intensive forest management practices and short rotation cycles can severely deplete the nutrient pool of the soils. Short rotation forestry, therefore, requires reasonable nutrient management but Hungarian literature on the topic is scarce, especially under nursery conditions.

Black locust fixes 75-150 kg/ha atmospheric nitrogen in a year (Boring et al. 1981), being one of the most effective tree species (Olesniewicz and Thomas 1999). Consequently, black locust can enhance the growing of poplars (*Populus* sp.) or oriental arborvitae (*Platycladus orientalis*) when planted in mixed forests (Shen et al. 1998; Chen et al. 2018). However, careful nutrient management should be carried out until the associated nitrogen-fixing Rhizobium bacteria develop on the roots.

Pope and Andersen found (1982) that phosphorous and potassium fertilization significantly improved dry biomass in the first and second years of planting, especially on poor sites. Based on the results of Keresztesi (1988), optimal available phosphorous and potassium content in the black locust nursery beds should reach 150-200 mg kg⁻¹ and $100-150 \text{ mg kg}^{-1}$, respectively. The findings of Tsiontsis and his colleagues (2001) revealed that available calcium and magnesium determines the improvement of Robinia. In Hungary, 2,5 kg m⁻³ Osmocote (18:9:10 + 2Mg) is generally applied in nurseries while the manufacturer, although for ornamental purposes, recommends 5 kg m^{-3} .

Nutrient demand of Robinia is not considered high: Wen and his colleagues (1998) calculated that 11.7 kg nitrogen, 0.7 kg phosphorous, 3.66 kg potassium, 15.1 kg calcium, 2.3 kg magnesium and 0.3 kg sulphur is necessary for 1 ton of dry biomass. Pope and Andersen (1982) revealed that nutrient con-

tent and distribution between leaf and stem of Robinia is stable. Site quality, spacing and fertilization had no effect on the distribution of dry matter, nitrogen and phosphorous between the foliage and the stem. Seed provenance had no considerable effect on nutrient status either when comparing seeds from Hungary and Iran (Moshki et al. 2012). Even site quality proved to have a minor effect on the order of nutrients quantities in thirty-year-old plantations, resulting in a sequence of Ca > N > K > Mg > P (Moshki and Lamersdorf 2011).

'Turbo' Robinia is a variety bred for intensive early-stage growing with faster timber mass development than common black locust by 30-50%. The variety is both suitable for energy plantation and forest establishment. 'Turbo Obelisk' is an asexually propagated clone group selected from 'Turbo' variety for its very fast early-stage growth. It can produce higher timber mass by 100% compared to traditional varieties, being able to reach maturity with a straight stem at the age of 15 on good sites (Pataki et al. 2016; Silvanus Forestry homepage).

Our study aimed to investigate the effect of two fertilizer doses (2.5 and 5 kg m⁻³) on growth, dry matter and nutrient uptake and distribution of 'Turbo Obelisk' clones during a four-month pot trial.

Materials and Methods

The experiment was carried out in the research unit of Szent István University in Gödöllő-Szárítópuszta (N 47°58' E 19°37'). Micro-propagated saplings of Turbo Obelisk OBE01 provisional variety was planted on the 15th May 2019 to seedling trays of 66 cells with a cell size of $3.5 \times 3.5 \times 4.5$ cm filled with perlite. Saplings were placed in an unheated, plastic sheet covered greenhouse and were fertigated on demand. After 22 days of growing, properly rooted saplings were planted to polypropylene pots of 12cm

diameter filled with a potting mixture of 90 v/v% peat and 10% v/v% perlite. Peat mix of Klassman TS3 Medium Basic contained 140 mg/L nitrogen, 41 mg L^{-1} phosphorous, 149 mg L^{-1} potassium and 100 mg L^{-1} magnesium based on manufacturer's information. Three treatments were carried out: control did not receive additional fertilizer, the standard treatment was supplied by 2.5 $kg m^{-3}$ of Osmocote Pro (18:9:10 + 2Mg) 8-9 months, while elevated fertilizer treatment included the adding of 5 kg m^{-3} of the above fertilizer. 28 saplings per treatment were grown until 28th June 2018 when height and stem diameter were measured. Then 16 saplings with average values were planted into 12-litre polypropylene plant bags filling them with 10 litres of the potting mix of the same composition used before. Plant bags were placed outdoor on plastic pallets covered by geotextile to separate from the soil surface. Treatment units of 4 bags were randomly placed. Bamboo sticks and a high wire trellis system were used. Shading net was used for the first two weeks. Further fertilization was not made during the growing period. Daily irrigation was made by drip irrigation sticks. During the growing period from 28th June to 2nd October, each plant bag received a total of 282 L water from irrigation and a total of 135 mm rainfall. The average air temperature was measured at 20.7 °C.

Development of saplings was monitored by measuring height and stem diameter of each plant four times during the growing period (28th June, 28th July, 27th August and 27th September). Plant height from potting mix surface to the apex was measured by a metric gauge and was recorded in centimetres. Stem diameter was described by a digital vernier calliper measuring at the height of 5 cm from potting mix surface with the accuracy of 0.1 mm.

During the growing period, lateral shoots were removed three times (23rd July, 11th

August and 12th September). Cut lateral shoots were grouped in the same block of four pots and were dried at 65 °C. Dry weight was measured with a scale at an accuracy of 0.01 g. Based on the results measured on the 27th September, from every four-pot unit two plants with middle values were selected and cut on the 2nd of October. Then leaves and stem were separated, milled and were dried. Dry weight of both stem and leaves was measured. Lateral shoots were mixed with previous cuttings to give one sample/treatment. Nitrogen, phosphorous, potassium, magnesium and calcium content of lateral shoot and stem samples were measured in an accredited laboratory based on MSZ-08-1783 standard. Nutrient uptake of aboveground biomass was calculated based on nutrient content and dry matter weight. Nutrient uptake values of fertilized plants were reduced by nutrient values of control plants, resulting in the amount of nutrients taken up from the fertilizer. This value was compared to the total nutrient quantity of the fertilizer added to each plant bag.

Statistical analysis was made with Microsoft Excel Analysis Tool Pack. In the case of height and stem diameter, all the plants were measured therefore 16 data from each treatment was available. In the case of dry weight and nutrient uptake, 4 data were calculated from the 4-unit blocks for each treatment. After the normality test and the verification of homoscedasticity of the data, one-way ANOVA was used to analyse the data. Fischer test was used as post-hoc test at 95% likelihood.

Results and Discussion

Plant size

No significant differences in size were measured among control and fertilized plants of the small-pot experiment (Figure 1), which means that nutrient included in the peat mix was sufficient for the development of the

plants in the first month. Therefore, the second trial period was started with saplings of the same size in case of all the three treatments.

After one month (28th July) the size of the control plants was significantly smaller than those of the two fertilized treatments. This trend remained true for the subsequent two measuring dates. A higher concentration of fertilization resulted in slightly higher and thicker plants compared to standard dosage $(2.5 \text{ kg m}^{-3} \text{ Osmocote})$ on the 28^{th} July, but the difference did not prove to be significant either in the case of height or stem diameter (Figure 1.). At the last two measuring dates (27th August and 27th September) height data was still not significantly different between the two fertilized treatments. However, the stem diameter of elevated fertilizer dose showed a significantly higher number than standard dosage at both dates. Therefore, stem diameter proved to be a more efficient parameter of plant development than height. It can be concluded that elevated fertilizer dose resulted in bigger, more developed plants than standard dose. At the same time, differences between control and standard dose were more pronounced than those between standard and elevated dose of fertilizer. Hence, the 'first' dose of 2.5 kg fertilizer eventuated a higher rate of production growth than the 'second' dose of 2.5 kg fertilizer.

Heights of 260-280 cm and stem diameter of 16-18 mm of fertilized plants mean an outstanding result during the growing period of 4.5 months, compared to international literature. Pope and Andersen (1982) measured a height of 57-139 cm and a stem diameter of 7.1-17.3 mm for a one-year breeding of bare-root black locust seedlings, strongly depending on site quality. Moshki and his colleagues (2012) planted out 3-week-old saplings to plastic pots. After breeding them for four months, height was measured 150 cm and stem diameter was 5 mm. This value

changed to 200 cm and 6 mm, respectively, at the end of the 6-month-experiment. The exceptional values of the current study can be explained by the genetic potential of Turbo Obelisk OBE01 clone and the effect of irrigation.

Dry matter production

Similarly to the tendencies occurred in plant sizes, dry matter weight showed significant differences among treatments. Control plants had significantly the lowest aboveground biomass and elevated fertilizer treatment showed significantly the highest values (Table 1). The standard dose of fertilizer doubled the biomass compared to control one, while elevated fertilizer dose improved a further 30% on it. When analysing stem, leaf and lateral shoot biomass values separately, similar tendencies can be observed. Treatments had no significant effect on the rate of the stem of the whole biomass. Notwithstanding the tendency that enhanced fertilizer level improved the rate of stem biomass, although still not at a significant level (Table 1).

The above dry biomass data confirm again the exceptional growth capacity of Turbo Obelisk clone. Eigel and his colleagues (1980) measured a dry weight of 164 g as an average of seven different plantations. Plants were in situ sown, they were fertilized during the two-year trial period and plant density was 28,700 plant ha⁻¹. Pope and Andersen (1982) measured a dry weight between 50 and 600 g after a three-year trial. Weight was dependent on fertilization and site quality. Leaf rate of 46-49% in our experiment considered high but fits the age of the plants while it will decrease with the time. Eigel and his colleagues (1980) measured 38% leaf-rate at a five-year-old stand whilst it was 11% at the age of 13 years.

Nutrient content and nutrient uptake

The tendency of different nutrients was variable in the stem. Nitrogen content was sig-

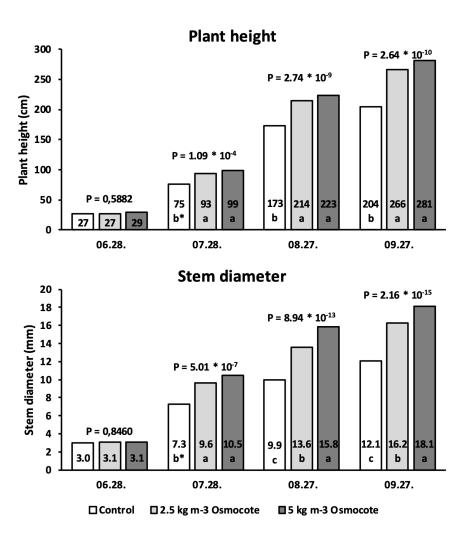


Figure 1. Effect of different fertilization doses (kg m⁻³) on the height and stem diameter of pot-grown Black locust saplings. (*Average values of the same date and letter mean no significant differences based on Fischer's least significant difference test.)

nificantly the lowest in the stem in the elevated fertilizer treatment while it was nonsignificant between the standard treatment and the control, although control values were the highest (Table 2). The reason for this phenomenon could be the low dry biomass of control plants (Table 1). The high nitrogen content of the stem of control plants was observed even though plants definitely showed nitrogen deficiency. The absolute nitrogen values of 6-9 mg kg⁻¹, which is considerably higher than the values measured by Moshki and Lamersdorf (2011) on elder plants (2-

3 mg kg⁻¹) is one of the signs of juvenile stage.

Phosphorous content of the plants was significantly different from each other at all the treatments and it increased with the elevation of the doses (Table 2). The measured values can be considered low compared to the results (2-3 mg kg⁻¹) of a previous study on pot-grown Robinia plants (Moshki et al. 2012). In the case of calcium, magnesium and potassium, no significant effect (P > 0.05) of the treatments was detected. The reason for these results can be

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	Aboveground	Stem	Leaves and lateral	Ratio of stem
	parts	$(g plant^{-1})$	shoots	weight
	$(g plant^{-1})$	(g plant)	$(g plant^{-1})$	(%)
Control	78.8 c*	39.9 c	38.9 c	50.6%
$2.5 \text{ kg m}^{-3} \text{ Osmocote}$	178.1 b	93.5 b	84.6 b	52.7%
$5 \text{ kg m}^{-3} \text{ Osmocote}$	229.7 a	125.3 a	104.4 a	54.5%
P-value	2.59×10^{-7}	4.10×10^{-7}	5.37×10^{-6}	0.1930
LSD 5%	21.8	12.9	13.6	=

Table 1. Effect of fertilizers on aboveground biomass of pot-grown black locust saplings

(*Average values of the same parameter and letter mean no significant differences based on Fischer's least significant difference test.)

that the potting mix contained a relatively high amount of magnesium, while irrigation water, given equally to all the plants, contained a considerable amount of calcium. Among the above mentioned mineral elements, fertilization had the greatest impact on potassium, resulting in considerable, but not significant differences among treatments. Nitrogen, potassium and calcium content of the stem varied between 8 and 9 mg kg⁻¹. These results are in harmony with the findings of Moshki and his colleagues (2012) except calcium which was measured almost twice more in our experiment.

Considering the nutrient uptake of the stem, the same tendency occurred for every examined nutrient except the nitrogen. With the elevation of fertilizer doses, nutrient uptake increased significantly (Table 2). However, similarly to the dry matter values, the difference between control and standard dose was greater than that of between standard and elevated dose. Nitrogen uptake of the stem did not differ between standard and elevated level of fertilizer treatments. Thus, the significantly bigger stem dry matter compensated the significantly lower nutrient concentration. Probably, higher carbohydrate accumulation can explain the lower rate of nitrogenbased compounds and therefore lower nitrogen concentration for the elevated level treatment.

Nitrogen concentration of leaves showed different tendencies than that of the stem. Despite the smaller dry weight, control had lower values (Table 2), so data reflected the well visible nitrogen deficiency. The values of the two fertilized treatments were nearly the same, so leaf-nitrogen was not affected by the adding of extra 2.5 kg m^{-3} fertilizer to the plants. Potassium showed the same tendency, although elevated dose treatment resulted in a slightly higher concentration of potassium than standard-dose treatment. The tendency of phosphorous and magnesium values was equal: with the elevation of fertilizer doses, nutrient content of the leaves increased significantly. The values of calcium showed a totally different trend. The highest value was detected in the control treatment while it was equally lower in the two fertilized treatments. It is important to emphasize that all the treatments received an equal quantity of calcium through the irrigation water. It is worth to note that the concentration of calcium in the leaves was higher than those of potassium and, even the level of nitrogen. All the concentration of the measured nutrients, except potassium, showed equality to values published by Moshki and Lamersdorf (2011) and Moshki and his colleagues (2012). However, potassium levels

Table 2. Effect of fertilization doses on the nutrient content and uptake of aboveground biomass of pot-grown black locust saplings

	Nutrient concentration in the stem (mg g ⁻¹ DW)							
	Nitrogen	Phosphorus	Potassium	Magenesium	Calcium			
Control	9.45 a*	0.43 c	7.40	0.90	8.75			
$2.5 \text{ kg m}^{-3} \text{ Osmocote}$	8.15 a	1.08 b	8.53	0.90	7.88			
5 kg m ⁻³ Osmocote	6.60 b	1.28 a	8.73	0.95	8.68			
P-value	0.0138	3.40×10^{-6}	0.0653	0.9087	0.3628			
LSD 5%	1.71	0.17						
	Nutrient uptake of the stem (g plant ⁻¹)							
Control	0.374 b	0.017 c	0.295 с	0.036 c	0.349 с			
$2.5 \text{ kg m}^{-3} \text{ Osmocote}$	0.765 a	0.100 b	0.796 b	0.085 b	0.736 b			
$5 \text{ kg m}^{-3} \text{ Osmocote}$	0.817 a	0.160 a	1.093 a	0.119 a	1.093 a			
P-value	0.0002	1.55×10^{-7}	0.0059	0.0003	0.0166			
LSD 5%	0.155	0.019	0.122	0.029	0.206			
Nutrient concentration in the leaves and lateral hoots (mg g ⁻¹ DW)								
Control	18.05 b	1.15 c	11.88 b	2.38 c	35.50 a			
$2.5 \text{ kg m}^{-3} \text{ Osmocote}$	27.80 a	2.65 b	15.98 a	3.28 b	29.70 b			
$5 \text{ kg m}^{-3} \text{ Osmocote}$	26.88 a	3.35 a	17.15 a	3.70 a	29.75 b			
P-value	0.0067	1.78×10^{-7}	0.0002	1.81×10^{-5}	0.0075			
LSD 5%	5.96	0.31	1.71	0.32	3.59			
	Nutrient uptake of the leaves and lateral hoots (g plant ⁻¹)							
Control	0.703 b	0.045 c	0.464 c	0.092 c	1.379 b			
$2.5 \text{ kg m}^{-3} \text{ Osmocote}$	2.353 a	0.223 b	1.343 b	0.276 b	2.538 a			
5 kg m ⁻³ Osmocote	2.789 a	0.349 a	1.785 a	0.386 a	3.099 a			
P-value	8.05×10^{-5}	1.29×10^{-8}	1.11×10^{-7}	1.09×10^{-6}	$3.03 \times 10-4$			
LSD 5%	0.622	0.031	0.174	0.050	0.589			
Total nutrient uptake of above-ground parts (g plant ⁻¹)								
Control	1.077 b	0.062 c	0.758 c	0.128 c	1.727 c			
$2.5 \text{ kg m}^{-3} \text{ Osmocote}$	3.118 a	0.323 b	2.139 b	0.361 b	3.274 b			
5 kg m ⁻³ Osmocote	3.606 a	0.509 a	2.878 a	0.505 a	4.191 a			
P-value	2.51×10^{-5}	5.48×10^{-9}	8.32×10^{-9}	2.77×10^{-6}	5.09×10^{-5}			
LSD 5%	0.656	0.041	0.207	0.071	0.664			
Total amounts of nutrient provided by the fertilizer (g container ⁻¹)								
2.5 kg m ⁻³ Osmocote	4.05	0.88	1.87	0.27	-			
$5 \text{ kg m}^{-3} \text{ Osmocote}$	8.10	1.76	3.74	0.54	-			
Utilisation of nutrients provided by the fertilizer (%)								
2.5 kg m ⁻³ Osmocote	50%	30%	74%	86%	-			
$5 \text{ kg m}^{-3} \text{ Osmocote}$	31%	25%	57%	70%	-			

(*Average values of the same parameter and letter mean no significant differences based on Fischer's least significant difference test.)

in our experiment were only half of those almost identical to the results of stem analyreported by their trial made of young, potgrown plantlets.

Nutrient uptake tendencies of the leaves were

sis except that in the case of calcium no significant difference was detected between the two fertilizer treatments. The same trend was described in the case of nitrogen content, no significant difference was measured between the two fertilizer dozes. Significant differences were observed in the case of phosphorous, potassium and magnesium as higher fertilizer dose produced higher nutrient uptake.

Consequently, the total nutrient uptake consisting of the stem and leaf nutrient uptake followed the earlier described tendencies (Table 2). Only nitrogen uptake remained nonsignificant between the two fertilizer dose treatments. The nutrient uptake of the five elements was obviously significantly the lowest in the case of control plants. Compared to the standard treatment, calcium uptake of control plants was less than half of standard-dose treatment, in the case of phosphorous, this number was around 20%. Results of the elevated fertilizer treatment revealed that during the four-month-trial, potential nutrient uptake of the plants was nearly 4 g per plant in the case of nitrogen, more than 4 g per plant in the case of calcium and more than 3 g per plant in the case of potassium. Phosphorous and magnesium uptake ended around 0.5 g per plant.

These results can serve a base for a fertilization system which allows exploiting the maximal growing potential of the sapling. Our results support the high growing capacity of Turbo Obelisk clone as Eigel and his colleagues (1980) reported similar nutrient uptake (N 3.5, P 0.2, K 1.7, Ca 1.1, Mg 0.3 g per plant) in the case of two-year-old, in-situ sown, fertilized Robinias. Interestingly, the nutrient uptake order by quantity was proved to be identical what Moshki and Lamersdorf (2011) found in plantations.

The nutrient uptake tendencies underpin the previous statement that the 'second' dose of 1.3.1-VKE-2017-00022 project.

 2.5 kg m^{-3} fertilizer was not as effective, as the first one. The highest decrease in efficiency was observed in the case of the nitrogen (Table 2), which means that the dose of 8 g per container seemed to be a luxury quantity, based on our results. An extra dose of phosphorous and potassium proved to be more beneficial, 1.75 g of phosphorous and 3.75 g of potassium per container did not seem as excessive quantities. The highest rate of nutrient consumption was described in the case of magnesium. The reason for this result could be that - similarly to calcium uptake - plants partly covered their nutrient need not only from the fertilizer but also from the irrigation water.

Conclusions

Based on our results, Turbo Obelisk clone could produce very fast growing and high nutrient uptake under optimal fertilization conditions. Nutrient uptake order by quantity was Ca > N > K > Mg = P. Based on our results, a lower rate of N: P and N: K should be used than the fertilizer applied in the experiment (18:9:10). Between the two doses of fertilizer (standard: 2.5 kg m⁻³ and elevated: 5 kg m⁻³) elevated amount proved to be less effective than the standard one, especially in the case of the nitrogen. Moreover, nitrogen fixation was probably still at low efficiency due to peat-based potting mix and a relatively short, four months growing period.

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