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Elements of growth and structure of narrow-leaved ash (*Fraxinus angustifolia* Vahl) annual seedlings in the nursery on fluvisol

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Abstract

Background and Purpose: *For the process of optimisation of annual seedling production of narrow-leaved ash (*Fraxinus angustifolia *Vahl) planned for the reforestation of marshland sites morphology, height and diameter growth and structure of narrow-leaved ash were researched in the nursery on fluvisol in different microsite conditions (microsite A – sandy-loamy fluvisol and microsite B – loamy fluvisol).*

Material and Methods: *The measurements included the length of the axis above the cotyledons* (h)*, and hypocotyl diameter* (d_0)*. The length of internodes and two intersecting diameters in the middle of each internode were measured on three highest seedlings in the seedbed (h=208.7–223.7 cm).*

Results and Conclusion: *The elements of seedling growth were affected by microsite conditions and growing space. The analysis of covariance showed that growing space did not have a significant effect on mean seedling height* (h_a, h_L, $h_{g20\%}$)*, and on mean square diameter of 20% largest-diameter seedlings, which indicates that these growth elements were more affected by site conditions, i.e., indirectly, by silvicultural treatments. This shows that the conditions for narrow-leaved ash were more favourable on microsite B than on microsite A. The analysis of covariance showed that growing space had a significant effect on arithmetic mean diameter* (d_a)*. The results pointed out that fluvisol was a very suitable natural environment for the production of narrow-leaved ash bare-root seedlings.*

INTRODUCTION

Narrow-leaved ash (*Fraxinus angustifolia* Vahl) is a tree species occurring in the Mediterranean, Caspian, Black Sea, Atlantic and Pannonian regions *(1)*. According to *(2)*, the species *F. angustifolia* is subdivided into two subspecies: *F. angustifolia subsp. angustifolia* and *F. angustifolia subsp. pannonica*. According to *(3, 4, 5)*, *F. angustifolia subsp. pannonica* belongs to the Pannonian part of the range.

In the Pannonian region, in Serbia and Croatia, narrow-leaved ash is a widely distributed tree species of monodominant and mixed forests in the alluvial areas of large rivers *(6, 7)*. Monodominant forests grow on gley soils where narrow-leaved ash is a pioneer species and has the coenological optimum. Mixed forests, with black and white poplar, common oak, common elm, European white elm, hornbeam and field maple, grow on developed alluviums and semigley soils. In mixed forests, narrow-leaved ash has the ecological and productivity optimum and its stands are distinguished by high productivity *(8, 9)*. In Serbia, the most significant complex of lowland forests is in the area of the river Sava lower course (Srem), where monodominant narrow-leaved ash forests cover 1,401 ha, and forests of common oak and narrow-leaved ash (*Fraxino angustifoliae-Quercetum roboris* Jovanović et Tomić 1979) and forests of common oak and narrow-leaved ash and hornbeam (*Carpino-Fraxino-Quercetum roboris* Miš. et Broz 1962), which are syndynamically closely related, cover the area of 22,181 ha *(10)*. In lowland forests in Croatia, which occupy the area of 278,362 ha, narrow-leaved ash covers 27,296 ha *(9)*. In mixed forests with common oak, narrow-leaved ash is a valuable species for veneer industry and its rotations are 80 to 140 years. Mixed forests of common oak and narrow-leaved ash in Serbia and Croatia are traditionally naturally regenerated by regeneration cutting *(11, 12)*. On alluviums, in the Danube Basin and Drava Basin, narrow-leaved ash is fragmentary, predominantly in black and white poplar forests (*Populetum nigro-albae* Slav. 1952), and forests of narrow-leaved ash and European white elm (*Fraxineto-Ulmetum effusae* Slav. 1952). In the past period on alluvial soils, narrow-leaved ash growing was significantly neglected, predominantly because of Euramerican poplars. On the narrow-leaved ash sites, for already more than a hundred years, the introduced white ash (*F. americana* L.) and green ash (*F. pennsylvanica* Marshall) have been well-adapted; however, these species are less suitable for growing in long rotations compared to narrow-leaved ash, and their stands are today predominantly in need of reconstruction *(13)*.

Narrow-leaved ash is a very productive tree species on developed alluviums, where it attains the height of 40–45 m at the stand age of 70–100 years and the volume above 800 $m^3 \cdot ha^{-1}$ *(14, 15)*. A small number of insect species make significant economic damage to the narrow-leaved ash, its leaves are not used by gypsy moth (*Lymantria dispar* L.); according to *(16)* endophagy insects (leafminers) are not found in narrow-leaved ash. Today, because of changes in hydrological conditions and vegetation succession *(17, 18)*, it is a significant species which can be applied in the process of rehabilitation and reforestation of alluvial forest ecosystems. Reforestation is performed with bare-root or containerized narrow-leaved ash seedlings produced in nurseries. Bare-root seedlings are more often produced. Usually, 100–150 seedlings are produced per m^2 *(19)* by classical method with dense sowing in nursery beds, or 150–250 seedlings per m^2 *(20)*, but the latter are predominantly more poorly developed. Taking into account that vigorous weed vegetation appears in marshland ecosystems already in the first year after planting, it is necessary to adapt seedling characteristics to such conditions in the field to reduce costs in the future period.

According to literature data on seedling growth elements in the nursery, narrow-leaved ash shows highly variable growth in height (and diameter) at the juvenile stage of development in different growing conditions. Mean height of container seedlings, using different substrates and different mineral fertilization, in the first year is 3.37–29.93 cm, and in the second year 5.10–37.10 cm *(21)*. Container seedlings in different nursery conditions, in the open and in plastic-covered greenhouses, using different substrates and different mineral fertilization, in the first year reach the height of 33.3–87.9 cm *(22)*. The height of 1+0 bare-root seedlings is 60–80 cm *(23, 24, 25)* report the height up to 110 cm. According to *(26)*, the height of 2+0 bare-root seedlings is 60–80 cm, and according to *(27)*, mean height of 2+1 seedlings in the field, in three test plantations with 75 half-sib families from 9 natural Sava Basin narrow-leaved ash populations, ranged from 36.4 to 53.9 cm. *(20)* pointed out the significance of growth space for the production of narrow-leaved ash annual seedlings of specified characteristics and reported that the mean height of seedlings in the first year at different spacings ranged between 36.7 and 94.9 cm.

The aim of this paper was to study the morphology and growth and structure elements of narrow-leaved ash seedlings aged 1+0 in the nursery, in the conditions in which narrow-leaved ash showed high variability in height and diameter growth and reached significantly larger dimensions than those reported in the cited literature. This paper points out the fast growth of narrow-leaved ash seedlings recorded in the traditional procedure of nursery production. The study results could serve as the starting point for the process of optimization of annual seedling production of narrow-leaved ash intended for the reforestation of marshland sites.

STUDY AREA

The research was performed in the area of the Special Nature Reserve »Gornje Podunavlje« (Upper Danube Basin), which is a unique and compact marshland complex in the area of Upper Danube Basin in the Republic of Serbia, between 45° 31' 47" and 45° 51' 3" north latitude and 18° 49' 8" and 19° 5' 43" east longitude. Total protected area of »Gornje Podunavlje« is 19,648 ha. Dominant plantations are Euramerican poplars, and narrow-leaved ash stands are fragmentary on about 190 ha.

The climate of the study area is semiarid, with mean annual air temperature 11.1° C and mean annual precipitation 569 mm. Forest vegetation is primarily conditioned by floodwater or groundwater influences related to the Danube water level.

The nursery in which the narrow-leaved ash seedlings were produced is in a flood-protected area on a plateau of mildly rolling relief, elevation between 82.8 m and 84.7 m. Based on hydrographical position before the erection of an embankment in 1965, the location of the nursery was the site of narrow-leaved ash and European white elm (*Fraxineto-Ulmetum effusae* Slav. 1952), and the site of black and white poplars (*Populetum nigro-albae* Slav. 1952). The lowest part was the site of white willow (*Salicetum albae* Issl. 1936). After the erection of the embankment in 1965, Euramerican poplars were planted on the site of narrow-leaved ash and European white elm and on the site of black and white poplars. The experimental nursery production of narrow-leaved ash bare-root seedlings was organized on anthropogenized soil of fluvisol type. Annual seedlings for analysis were sampled at two microsites (Table 1) selected on the basis of on micro-relief forms and textural class stratigraphy of soil profiles. Microsite A was at the highest elevation of 84.7 m (ridge) on the sandy-loamy type of fluvisol, and Microsite B was on a wide plateau, at the elevation of 84.0 m on the loamy type of fluvisol.

Main differences in soil characteristics on study microsites were soil depth and particle-size distribution. At microsite A, groundwater was at the depth of 250 cm, and at microsite B, groundwater was at the depth of 180 cm. Particle-size distribution of microsite A was heterogeneous (sandy loam), especially the top soil and deeper layers, as opposed to microsite B where particle-size distribution was rather homogeneous (silty loam) to the depth of 140 cm. Particle-size distribution at microsite B enabled the capillary rise of ground water to the root zone (0–50 cm) and a greater supply of readily available water in the capillary pores. On microsite A, the greater percentage of sand fraction in horizons below 45 cm (>79%) stoped the capillary rise of ground water, which compared to microsite B, means the lower amount of water in the rhizosphere zone. On microsite A, the content of humus in the humus-accumulation horizon was 1.99%, and it was 4.49% on microsite B. The above soil characteristics indicate more favorable conditions for the development of narrow-leaved ash seedlings on microsite B, compared to microsite A.

MATERIAL AND METHODS

Seedlings originated from seeds from free pollination which were collected in the seed year, early in the autumn of 2006. The seed was collected from several trees in a medium-aged stand, in a local micro-population of narrow-leaved ash in the area of the Special Nature Reserve »Gornje Podunavlje«. On some sites in that area narrow-leaved ash has superior growth and, as a highly productive tree species, at stand age 70–100 years, it attains the height of 40–45 m and the volume above 800 $m^3 \cdot ha^{-1}$ *(14, 15)*. Immediately after collection, the seeds were sown into seedbeds of standard width – 120 cm. The seedbeds were laid in the direction north-south, and they occupied a narrow ridge (microsite A) and a wide plateau, which was the area between the ridge and the lower land (microsite B). Each seedbed consisted of four rows, spaced 40 cm. According to instructions of *(28)*, about 10 $g \cdot m^{-1}$ of seed was sown manually into rows, at the depth of about 3–5, cm and was covered with soil. In view of the fact that the location of the nursery was in the area of low precipitation, during the vegetation period in 2007, the seedbeds were watered abundantly from the channel Danube-Tisza-Danube, which is in the vicinity of the nursery. In addition to classical measures of the site preparations for seed sowing, the mechanical removal of weeds was the only tending measure during vegetation period. The appearance of narrow-leaved ash seedlings in different microsite conditions in the nursery is presented in Figure 1.

TABLE 1

Physical-chemical characteristics of the soil and textural classes of the microsites A and B.

Horizon	Depth	*Particle-size distribution [%]*						Textural class	Chemical characteristics					
		Coarse sand	Fine sand	Silt	Colloid clay	Sand	Clay		pH	Humus	C_aCO_3	N	P_2O_5	K_2O
		>0.2	0.2–0.02	0.02–0.002	<0.002	>0.02	<0.002							
	[cm]	*[mm]*	*[mm]*	*[mm]*	*[mm]*	*[mm]*	*[mm]*		*in* H_2O	*[%]*	*[%]*	*[%]*	$[mg \cdot 100g^{-1}]$	$[mg \cdot 100g^{-1}]$
								Microsite A						
A_{mo}	0–16	1.4	43.5	36.6	18.5	44.9	55.1	Loam	7.61	1.99	23.85	0.210	11.8	9.6
I	16–45	5.6	26.4	48.6	19.4	32.0	68.0	Loam	7.70	0.19	25.14	0.0188	14.0	11.4
IIG_{so}	45–150	1.3	77.9	12.6	8.2	79.2	20.8	Loamy sand	7.98	1.10	13.35	0.0188	5.6	4.4
$IIIG_{so}$	150–250	2.6	95.4	1.1	0.9	98.0	2.0	Sand	7.83	0.02	17.02	0.001	2.2	1.6
								Microsite B						
A_{mo}	0–18	5.7	25.5	50.7	18.1	31.2	68.8	Silty loam	7.53	4.49	20.65	0.215	14.2	11.6
IG_{so}	18–85	1.3	27.8	52.9	18.0	29.1	70.9	Silty loam	7.68	0.66	22.71	0.182	14.6	12.0
IIG_{so}	85–140	1.8	28.6	51.7	17.9	30.4	69.6	Silty loam	7.69	0.89	29.34	0.185	14.4	11.4
$IIIG_{so}$	140–180	4.8	74.8	12.1	8.3	79.6	20.4	Sandy loam	7.68	0.28	16.19	0.161	5.6	4.4

Figure 1. *Narrow-leaved ash seedlings in the seedbeds in different microsite nursery conditions (2nd June 2007).*

In late autumn 2007, from the seedbed where soil profiles had previously been opened within microsites A and B, the seedlings were sampled from internal rows along 2.0 m, in 4 repetitions. The analysis included a total of 431 seedlings.

On leafless seedlings, visible quantitative biological characters were measured: length of the axis above cotyledons (height – h) and hypocotyl diameter (diameter – d_0). The heights were measured to the nearest 1 cm, and the two cross diameters in the hypocotyl were measured by digital micrometer to the nearest 0.1 mm. The positions of opposite cotyledon buds were previously defined on each seedling, and the length of the axis above cotyledons was measured from their points to the top of the terminal bud. To get total seedling height (h_t), another

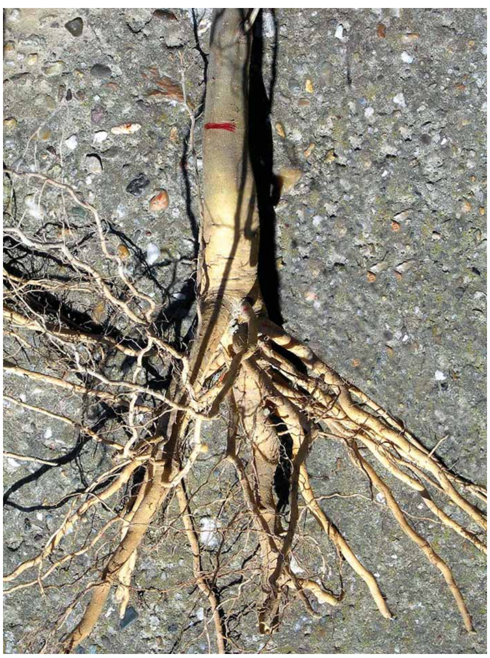

Figure 2. *Hypocotyl and root of the dominant narrow-leaved ash annual seedling.*

about 3–5 (8) cm of the hypocotyl part of a seedling was added to the measured length of the axis above cotyledons (h), The hypocotyl part, depending on the sowing depth and tending measures, is partly above soil surface, and partly in the soil. The hypocotyl diameter (d_0) was measured immediately below the point of cotyledon bud, because of easier ocular determination and more correct cross section, compared to the lower parts of the hypocotyl (Figure 2). In this way, the biological trait usually called the root collar, was more precisely defined.

To emphasize the fast growth of narrow-leaved ash in the nursery over the first vegetation period, three highest seedlings in a seedbed were morphometrically analyzed. The morphometric analysis included the digital micrometer measurement of internode length and two cross diameters in the middle of each internode, to the nearest 0.1 mm. The internodes were on each seedling defined from the insertion point of the cotyledon leaf, i.e. the first node, which on the seedless axis consists of two opposite cotyledon buds.

The data were processed by standard statistical methods, using the basic parameters of diameter and height structure. The analysis of covariance was applied to determine the significance of the effect of seedling number, i.e. growth space, on seedling growth elements in different microsite conditions:

$$Y_{ij} = \mu + \tau_i + \beta(X_{ij} - \overline{X}) + \varepsilon_{ij}$$

where: Y_{ij} – measured value, μ – mean; τ_i –effect of factor i; β – coefficient of covariance; X_{ij} – covariance, ε_{ij} – random error.

The relationship h=f(d_0) was computed by correlation and regression analysis. Modeling of seedling diameter and height distribution per diameter, i.e. height classes were computed by Weibull function, and they were compared by Kolmogorov-Smirnov non-parametric test. Parameters of height distribution per Weibull function were calculated by *(29)* methods. Statistical analysis was performed by EXCEL and STATISTICA packages. Photographs supplement the manuscript. The list of symbols with explanations is presented in Table 2.

Figure 3. *Narrow-leaved ash annual seedlings in the nursery (31st Sept 2007).*

TABLE 2

List of used symbols.

Symbol	Dimension	Description	Symbol	Dimension	Description
d_a	[mm]	Arithmetic mean diameter	h_a	[cm]	Arithmetic mean height
d_g	[mm]	Mean square diameter	h_L	[cm]	Lorey's mean height
$d_{g20\%}$	[mm]	Mean square diameter of 20% largest-diameter trees	$h_{g20\%}$	[cm]	Height of the tree with diameter $d_{g20\%}$
s_d	[mm]	Standard deviation – diameter	s_h	[cm]	Standard deviation – height
c_d	[%]	Coefficient of variation – diameter	c_h	[%]	Coefficient of variation – height
d_{min}	[mm]	Minimal diameter	h_{min}	[cm]	Minimal height
d_{max}	[mm]	Maximal diameter	h_{max}	[cm]	Maximal height
v_{w_d}	[mm]	Variation width – diameter	v_{w_h}	[cm]	Variation width – height
α_3		Coefficient of skewness	α_4		Coefficient of kurtosis

RESULTS

Morphology of dominant seedlings

In study conditions, seedling development over a vegetation period was unobstructed (monopodial growth), and the length of the axis above cotyledons of dominant seedlings was more than 2 m.

In analyzed dominant seedlings (h=208.7–223.7 cm), 29–32 internodes were formed along the length of the axis above cotyledons (Figure 4).

Nodal increment of analyzed dominant seedlings was characterized by a short internode-epicotyl (1.5–2.2 cm), and subsequent internodes were gradually elongated, with minor oscillations, up to 18th–26th nodes, and then more or less similar growth continued till the end of the vegetation period. Taking into account that leaf arrangement in the genus *Fraxinus* is decussated, 56–62 compound leaves were formed on dominant seedlings. An axillary bud was formed in each leaf axil, and in leaf axils on dominant seedlings, there were often also secondary buds in addition to primary buds (Figure 5). Some thinner branches were formed from the secondary buds only on individual seedlings, but seedling growth was always characterized by a pronounced apical tendency.

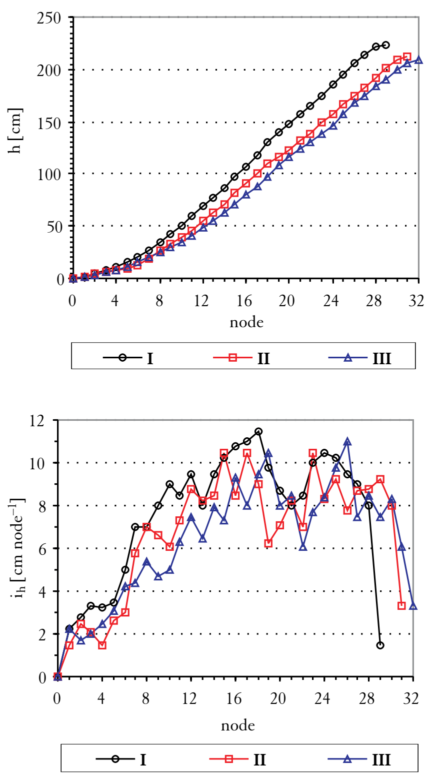

Figure 4. *Total and nodal increment of dominant seedlings in height.*

Dependence between height and diameter in narrow-leaved ash annual seedlings at both microsites was described by an linear equation (y=a·x+b). A high coefficient of correlation (R>0.90) between seedling height and diameter (Table 3, Figure 6) was calculated based on correlation and regression analyses.

Elements of seedling growth and structure

In the applied procedure of nursery production of narrow-leaved ash seedlings, seed germination was not uniform, which resulted in a nonuniform number of sampled seedlings (coefficient of variation: 27.4% on microsite B and 43.8% on microsite A).

TABLE 3

Parameters and evaluation of the model of seedling height dependence on diameter.

	Microsite A	Microsite B
a	10.74	8.833
b	–8.83	9.709
R^2	0.854	0.821
R	0.924	0.906
s_e	12.06	20.07
s_a	0.261	0.352
t	41.098***	25.119***

Figure 5. *Primary and secondary buds (serial arrangement) on annual dominant narrow-leaved ash seedlings in the nursery (31st March, 2008).*

Variability in mean heights (h_a) between individual samples (c_h=11.9% on microsite B and c_h=15.2% on microsite A), was higher than variability in mean heights of dominant seedlings ($h_{g20\%}$) (c_h=2.2% on microsite B, and c_h=10.2% on microsite A). Relative variability in seedling heights in individual samples was also high, c_h=29.3–49.8% on microsite A, and c_h=33.7–46.7% on microsite B. On microsite B, absolute variability in heights in individual samples (s_h=44.1–50.0 cm) was higher than that on microsite A (s_h=24.9–32.2 cm). On both microsites, minimal seedling heights were 11 cm. Maximal heights were greater on microsite B (178–211 cm) compared to microsite A (132–141 cm). On both microsites, left skewness in seedling height structure was predominant, as well as platykurtic distribution (Table 4).

Variability in mean diameters (d_0) of individual samples was higher (c_d=12.6% on microsite B, and c_d=17.2% on microsite A) compared to variability in dominant di-

TABLE 4

Numerical parameters of height structure of narrow-leaved ash annual seedlings.

Parameter of structure	Microsite – A				Microsite – B			
	I rep.	II rep.	III rep.	IV rep.	I rep.	II rep.	III rep.	IV rep.
n [pcs.]	72	71	113	35	26	28	46	40
h_a [cm]	66.8	80.7	61.5	85.1	111.7	130.8	110.3	98.3
h_L [cm]	82.3	98.3	82.5	93.9	132.9	150.5	143.1	133.1
$h_{g20\%}$ [cm]	96.1	117.5	101.9	117.8	153.7	161.5	160.5	160.1
s_h [cm]	30.5	32.2	30.7	24.9	42.4	44.1	50.0	45.9
c_h [%]	45.6	39.9	49.8	29.3	38.0	33.7	45.4	46.7
h_{min} [cm]	11	13	11.5	15	13	30	14	15
h_{max} [cm]	132	141	132	133	190	178	211	187
v_{w_h} [cm]	121	128	121	118	177	148	197	172
α_3	–0.273	–0.251	0.238	–0.504	–0.509	–1.042	–0.296	–0.057
α_4	–0.927	–0.395	–0.891	0.644	–0.228	–0.185	–0.741	–0.681

TABLE 5

Numerical parameters of diameter structure of narrow-leaved ash annual seedlings.

Parameter of structure	Microsite – A				Microsite – B			
	I rep.	II rep.	III rep.	IV rep.	I rep.	II rep.	III rep.	IV rep.
n [pcs.]	72	71	113	35	26	28	46	40
d_a [mm]	7.3	8.2	6.4	9.5	12.5	13.5	10.6	10.5
d_g [mm]	7.7	8.6	6.8	9.7	13.3	14.3	11.5	11.6
$d_{g20\%}$ [mm]	10.9	12.2	10.2	12.0	18.6	19.4	17.1	18.0
s_d [mm]	2.51	2.72	2.51	2.06	4.45	4.84	4.57	4.99
c_d [%]	34.5	33.3	39.4	21.6	35.5	35.9	43.2	47.7
d_{min} [mm]	2.0	2.6	1.9	3.2	2.7	3.5	2.0	2.8
d_{max} [mm]	11.7	17.6	13.6	13.9	20.1	24.5	21.6	20.1
v_{w_d} [mm]	9.7	15.0	11.7	10.7	17.4	21.0	19.6	17.3
α_3	0.038	0.507	0.516	–0.723	–0.337	–0.186	0.190	0.309
α_4	–0.778	0.935	–0.243	1.225	–0.32	–0.255	–0.473	–0.811

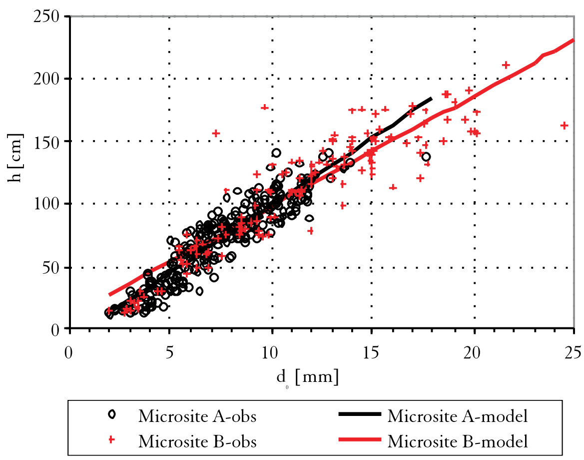

Figure 6. *Dependence of seedling height and diameter in microsite conditions A and B.*

ameter (c_d=5.4% on microsite B, up to c_d=8.2% on microsite A). Also, there was a high relative variability of seedling diameters in individual samples c_d= 21.6–39.4% on microsite A, and c_d=35.5 – 47.7 % on microsite B. On microsite B, absolute variability in seedling diameter (s_d=4.5–50.0 mm) was higher than that on microsite A (s_d=2.1–2.7 mm). On both microsites, minimal seedling diameter was 2.0 mm, and maximal diameter was larger on microsite B (20.1–24.5 mm) than on microsite A (11.7–17.6 mm) (Table 5).

Statistical test of the analysis of covariance showed that a different number of seedlings, i.e. seedling growing space, did not have a significant effect on mean height (h_a, h_L, $h_{g20\%}$), and on mean square diameters of 20% largest-diameter seedlings. However, different growing space had a significant effect on arithmetic mean diameter (d_a) and square diameter (d_g) (Table 6). Mean heights (h_a, h_L, $h_{g20\%}$) were significantly greater on microsite B than on microsite A, which indicated that the conditions for narrow-leaved ash were more favorable on microsite B than on microsite A. Also, mean square diameter of 20% largest-diameter seedlings ($d_{g20\%}$) and mean square diameter (d_g) were significantly larger on microsite B than on microsite A. The difference in arithmetic mean diameter (d_a) between microsites A and B was not significant at 5% risk level. With emphasized variability among seedling samples, it shows that the arithmetic mean diameter (d_a) was under the effect of growth space.

TABLE 6

Results of the analysis of covariance test.

Growth element	*Mean values*		*Test results of analysis of covariance*			
	Microsite A	Microsite B	$F_{treatment}$	$p_{treatment}$	$F_{regression}$	$p_{regression}$
h_a [cm]	73.5 *b*	112.8 *a*	7.08785^{*}	0.0448	3.75943^{ns}	0.1102
h_L [cm]	89.2 *b*	139.9 *a*	31.8271^{**}	0.0024	0.93724^{ns}	0.3775
$h_{g20\%}$ [cm]	108.3 *b*	159.0 *a*	34.3879^{**}	0.002	1.59974^{ns}	0.2617
d_a [mm]	7.8 (8.7 *a*)[1]	11.8 (10.9 *a*)[1]	5.67771^{ns}	0.0629	9.28227^{*}	0.0285
d_g [mm]	8.2 (9.1 *b*)[1]	12.7 (11.8 *a*)[1]	11.5072^{*}	0.0194	9.19311^{*}	0.0290
$d_{g20\%}$ [mm]	11.3 *b*	18.3 *a*	68.3239^{***}	0.0004	5.41284^{ns}	0.0675

[1] Corrected mean values of the analysis of covariance and LSD test at 0.05 risk level

TABLE 7

Results of Kolmogorov-Smirnov nonparametric test for the diameter and height structure.

			Microsite – A				Microsite – B			
			I rep.	II rep.	III rep.	IV rep.	I rep.	II rep.	III rep.	IV rep.
			Height structure – |D| statistics							
Microsite A	I rep.	Diameter structure -|D| statistics	–	0.218^{ns}	0.125^{ns}	0.329^{*}	0.549^{***}	0.758^{***}	0.559^{***}	0.422^{***}
	II rep.		0.160^{ns}	–	0.285^{**}	0.112^{ns}	0.394^{**}	0.623^{***}	0.417^{***}	0.267^{ns}
	III rep.		0.210^{*}	0.300^{***}	–	0.395^{***}	0.506^{***}	0.723^{***}	0.517^{***}	0.392^{***}
	IV rep.		0.481^{***}	0.322^{*}	0.569^{***}	–	0.434^{**}	0.65^{***}	0.444^{***}	0.307^{ns}
Microsite B	I rep.		0.615^{***}	0.551^{***}	0.657^{***}	0.058^{***}	–	0.299^{ns}	0.145^{ns}	0.173^{ns}
	II rep.		0.679^{***}	0.609^{***}	0.715^{***}	0.621^{***}	0.195^{ns}	–	0.325^{ns}	0.429^{**}
	III rep.		0.435^{***}	0.350^{**}	0.475^{***}	0.378^{**}	0.229^{ns}	0.290^{ns}	–	0.193^{ns}
	IV rep.		0.353^{**}	0.309^{*}	0.423^{***}	0.275^{ns}	0.340^{ns}	0.404^{**}	0.160^{ns}	–

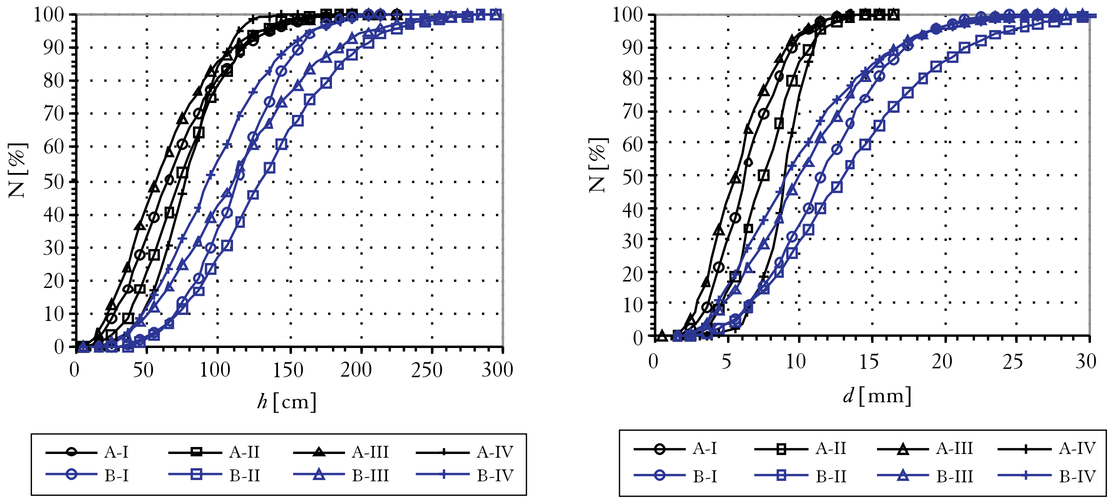

Figure 7. *Summary curves of height structure (interval width 10 cm) and diameter structure (interval width 1 mm) of narrow-leaved ash annual seedlings per repetitions.*

The Kolmogorov-Smirnov non-parametric test (Table 7) shows that, on microsite B, height and diameter structures were mainly homogeneous and, on microsite A, the homogeneity of height structure, and especially of diameter structure, was not confirmed. |D| statistics of the Kolmogorov-Smirnov non-parametric test showed differences between microsite A and B in almost all diameter and height structures.

The Weibull function model of height and diameter structure showed good agreement with empirical data (Tables 8 and 9). The parameters of the model of height structure varied between individual repetitions, especially on microsite B. Great variation of parameter »a« (c_h= 45.7%), which was in direct relation with minimal heights, could be taken as random on microsite B. However, the variation of parameter »c« (c_h=25.3% on microsite B, and c_h=34.9% on microsite A), which defined the form of distribution, was in direct relation with the conditions in which the seedlings were developing. The analysis of covariance showed the significant effect of growth space on the form of height distribution (parameter »c«), whereas microsite effect was not significant. Microsite effect was significant in parameter »b«, which defined the proportion of the distribution of seedling height structure (Table 10).

TABLE 8

Parameters of height distribution – Weibull function.

Parameter	*Microsite A*				Microsite B			
	I rep.	*II rep.*	*III rep.*	*IV rep.*	*I rep.*	*II rep.*	*III rep.*	*IV rep.*
a	10.999	12.968	11.481	11.049	10.979	28.615	13.967	14.994
b	71.501	76.032	62.519	78.951	119.02	124.39	119.03	97.506
c	1.8334	2.323	1.6473	3.4464	3.61	2.3983	2.1659	2.3183
|*D*|	0.1389^{ns}	0.1268^{ns}	0.0796^{ns}	0.1429^{ns}	0.1538^{ns}	0.1786^{ns}	0.1087^{ns}	0.125^{ns}
n	72	71	113	35	26	28	46	40

TABLE 9

Parameters of diameter distribution – Weibull function.

Parameter	*Microsite A*				*Microsite B*			
	I rep.	*II rep.*	*III rep.*	*IV rep.*	*I rep.*	*II rep.*	*III rep.*	*IV rep.*
a	1.9576	2.5993	1.8991	2.2237	2.4652	3.1774	1.9006	2.7976
b	5.6424	6.2007	5.0009	7.7763	11.135	12.423	10.299	8.6024
c	2.3089	2.6263	1.928	5.3002	2.5363	1.9895	2.0434	1.5826
|*D*|	-0.0972^{ns}	0.0423^{ns}	0.0354^{ns}	0.1714^{ns}	0.1154^{ns}	0.1071^{ns}	0.0652^{ns}	0.075^{ns}
n	72	71	113	35	26	28	46	40

TABLE 10

Results of the analysis of covariance test.

Growth element	Para-meter	Mean values		Results of the analysis of covariance test			
		Microsite A	Microsite B	$F_{treatment}$	$p_{treatment}$	$F_{regression}$	$p_{regression}$
h	*a*	11.624 *a*	17.139 *a*	0.68808^{ns}	0.4446	0.02576^{ns}	0.87876
	b	72.251 *b*	114.99 *a*	15.6564^{*}	0.01078	2.4377^{ns}	0.1792
	c	2.3125 (2.7843 *a*)[1]	2.6231 (2.1514 *a*)[1]	1.78657^{ns}	0.23894	8.60186^{*}	0.03252
d_0	*a*	2.17 *a*	2.5852 *a*	0.12375^{ns}	0.73934	0.78021^{ns}	0.41752
	b	6.155 *b*	10.615 *a*	7.29387^{*}	0.04275	4.65861^{ns}	0.08336
	c	3.0409 (3.8082 *b*)[1]	2.0379 (1.2705 *a*)[1]	17.2224^{**}	0.00891	13.6481^{*}	0.01408

[1] Corrected mean values of the analysis of covariance and LSD test at 0.05 risk level

The variation of parameters of diameter structure was also high, especially of parameter »c« on microsite A (c_v=50.4%). The analysis of covariance showed a significant effect of growth space on the form of diameter structure distribution (Table 10). The effect of microsite on diameter structure was significant, both on the distribution form (parameter »c«), and on its proportion (parameter »b«).

Summary curves of seedling height and diameter structure differed both by position and by form, which points to high variability in seedling height and diameter in seedling samples from particular microsite conditions (Figure 7). On both microsites, there were seedlings with heights from 10 to 15 cm and with diameters from 2 to 3 mm, but seedling heights and diameters on microsite B were greater. However, the summary curves of seedling height and diameter structure indicate microsite differences more clearly.

DISCUSSION AND CONCLUSION

Based on the above investigations, it can be concluded that growth elements of narrow-leaved ash annual seedlings in the nursery were the consequence of interaction between genetic potential (genotype) of the micro-population from which seeds were collected and microsite conditions in which seedlings developed. Seedlings in the nursery originated from seed mixture collected from only a few trees from autochthonous narrow-leaved ash population with abundant fructification on the site of narrow-leaved ash and European white elm (*Fraxineto--Ulmetum effusae* Slav. 1952). In the nursery, also on autochthonous site of narrow-leaved ash, the seedlings showed significant variability in height and diameter growth in different microsite conditions on anthropogenized soils of the fluvisol type. The observed variability could be assigned to a significant degree to phenotype plasticity of narrow-leaved ash characters observed in the experiment.

Long ago, foresters reported fast juvenile growth of narrow-leaved ash *(30, 31)*. Based on the comparison of growth elements of narrow-leaved ash seedlings produced in nursery, it can be concluded that the height and diameter of bare-root annual seedlings *(20, 23, 24, 25)* are superior to the height and diameter of container grown seedlings *(21, 22)*. In the production of bare-root seedlings, in addition to other factors, growing space was decisive for the production of narrow-leaved ash annual seedlings with superior heights and diameters *(20)*. Different reactions of seedlings to different growth conditions point to the wider adaptation potentials of nursery production to specific afforestation demands of different sites. In this context, the study of biological characters of narrow-leaved ash in the juvenile phase is the basis of the process. Also, the study of superior sizes of annual seedlings, such as those observed in our nursery experiment by traditional silvicultural procedure, can contribute to the process.

Narrow-leaved ash has an intermediary type of seedling. The aboveground part consists of: hypocotyl, opposite cotyledons, epicotyl above cotyledons, two opposite primary leaves which alternate with cotyledons, and terminal and axial buds *(32)*. In unfavorable conditions, seedling growth in the first vegetation period can end with the formation of epicotyl with primary leaves and one or a few internodes with compound leaves. In the study nursery conditions, 29–32 internodes were formed on the axis above cotyledons of dominant seedlings (h=208.7–223.7 cm), which indicated that narrow-leaved ash in favorable environmental conditions was characterized by continued growth in height over the vegetation period. *(33)* reported that from the beginning of growing season, narrow-leaved ash continually grew in height for 81 days and that only 1.8% of the total increment was attained after summer pause. *(34)* also emphasized the continuity of the growth in height in the genus *Fraxinus* (*Fraxinus alba* and *Fraxinus excelsior*) and reported the heights of annual seedlings up to 2 m under special nursery measures.

In our experimental procedure with narrow-leaved ash seedlings, full germinability of seed was not ensured. After germination, the spacing between seedlings in rows was not uniform. Based on the number of annual seedlings in samples it can be concluded that, after sowing of about 10 g·m^{-1}, the average number of seedlings was 17.5–56.5 m^{-1} on microsite A and 13.0–23.0 seedlings m^{-1} on microsite B. Average spacing between seedlings in rows was 5.7–1.8 cm on microsite A and 7.7–4.3 cm on microsite B. Taking into account the uniform spacing between rows (40 cm), we produced 44–141 seedlings per m^2 on microsite A and 33–58 seedlings on microsite B. Non-uniform spacing between seedlings in rows affected seedling growth elements and caused their variability. Phenotype plasticity of narrow-leaved ash in study conditions was more expressed because of the biological differentiation of seedlings. Seedling growth elements in nursery were affected by microsite conditions and by seedling growth space. On microsite B (where 40.7%–74.3% seedlings m^{-1} were produced), mean seedling heights (h_a, $h_{20\%}$, h_L) were greater by 49–57%, and mean seedling diameters (d_a, d_g, $d_{g20\%}$) were larger by 58–62% compared to microsite A. Statistical test of the analysis of covariance showed that growing space did not have a significant effect on seedling mean height (h_a, h_L, $h_{g20\%}$), and on mean square diameter of 20% of largest-diameter seedlings, which indicates that these growth elements were affected directly by site conditions. This shows that conditions for narrow-leaved ash were more favorable on microsite B than on microsite A. Statistical test of the analysis of covariance showed that growing space had a significant effect on arithmetic mean diameter (d_a).

The study results showed that anthropogenized soil of fluvisol type was a very suitable natural environment for the cultivation of narrow-leaved ash bare-root seedlings. The described traditional procedure of narrow-leaved ash annual seedling production under dense sowing in rows resulted in the processes of biological differentiation of seedlings and their natural selection in the early period. In the procedure, growing space had different effects on seedling heights and diameters. Consequently, study results can be used as pragmatic basis for optimization of production of narrow-leaved ash annual seedlings planned for the reforestation of alluvial sites. The production procedure should be optimized in further investigations.

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