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The growth of one-year-old narrow-leaved ash seedlings is strongly related to the leaf area parameters

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Abstract

The leaf area of plant leave scan be considered as an indicator of the plant growth conditions, and its variability is usually associated with adaptation and response to the changing environment. The aim of this study is to investigate the relation of the leaf area parameters on growth of the narrow-leaved ash 1+0 seedlings from the nursery trial and determining variability between the 18 half-sib lines. The seedlings were obtained from seeds collected from 18 maternal trees found in the area of the special nature reserve Upper Danube Region. Ten randomly selected 1+0 seedlings per half-sib line were selected for further study, and all leaves per seedling were collected, herbarized and scanned. The leaf area of each leaf (LACL) was measured using an open-source image processing program – ImageJ, and the total leaf area per seedling (TLA) was calculated. Also, the number of leaves per plant (NCL) as well as the number of leaflets within each imparipinnate compound leaf (NLCL) were counted. The results showed that differences between studied attributes were statistically significant among 18 selected half-sib lines ($p < 0.05$). The most variable parameter was the total leaf area per seedling (TLA; CV=68.98%), which is strongly correlated to the leaf area per each leaf (LACL; r=0.95) and the number of leaflets (NLCL; r=0.94). In this study, we confirmed that leaf parameters were highly correlated to the growth of one-year-old *Fraxinus angustifolia* seedlings.

Keywords

Leaf area; Morphometric traits; Narrow-leaved ash; Half-sib lines

Contents

1 Introduction

Fraxinus angustifolia Vahl (narrow-leaved ash) is a broadleaved forest tree species found throughout southern and eastern Europe in mixed broadleaved woodlands (Fraxigen 2005). In Serbia, it commonly occurs in monodominant and mixed forests in the alluvial area of large rivers (Tomić 2004). This pioneer species commonly grows on gley soils where it builds monodominant forests (Bobinac et al. 2010). Seedlings of narrow-leaved ash, as a hygrophytic tree species, do not tolerate dry conditions and no watering (Drvodelić et al. 2016), which indicates the high vulnerability of this species in the context of climate change. Climate change extremes, such as extreme temperatures and long dry periods, uneven distribution of precipitation directly affect the dynamics of groundwater levels and the dynamics of precipitation and floodwater. The causes of the tree declining comprise also a drop in groundwater levels, changes in floodwater dynamics, water logging and drying of habitats, the pollution of flood and precipitation water, etc. (Drvodelić et al. 2016).

Since the leaves are the principal photosynthetic plant organs (Wright et al. 2004), a variety of biological processes (plant growth, survival, reproduction, and ecosystem function) are affected by the size of leaves (Koch et al. 2004; Tozer et al. 2015). Also, leaf size (i.e. leaf area) profoundly affects a variety of biological carbon, water and energy processes (Wang et al. 2019). Leaf size is linearly negatively correlated with leafing intensity (Kleiman and Aarssen, 2007). The fitness benefits of higher leafing intensity (namely small leaves) are primarily associated with the fitness benefits of a larger pool of axillary buds, which in turn provide the greater facility for wide phenotypic plasticity in the allocation of these meristems to vegetative versus reproductive functions (Kleiman and Aarssen, 2007). Leaf size evolution is one of the fundamental adaptation strategies of plants to environmental changes (Yang et al., 2008) and it can be understood by the implications of leaf size-number trade-off (Wang et al. 2019). In adult trees, leaf area can be variable depending on their position within the crown and light influence (Šrámek and Čermák 2012). However, if the ecological conditions are the same (as in a nursery, for example), the leaf area can be considered as a parameter under the influence of genetic control. Nursery influence can be great on the leaf morphology, so some studies show the greater influence of water regime on the leaf traits than the origin (Black-Samuelsson et al. 2003). A strong relationship between leaf morphological characteristics and seedling morphological parameters has been proved (Ivetić et al. 2014).

In the context of climate change, with temperatures rising, leaf area studies and measurements will become even more important. If too much water is lost through transpiration, the plant needs to absorb more water from the ground, but if there is not enough water, plants would be stressed. In plant production, excessive transpiration means more irrigation and fertilizer use. A larger leaf area indicates greater photosynthetic and transpiration potential, but also it means that seedlings would be more stressed if there is not enough water in the soil (Wang et al. 2019).

It is very demanding to use leaf parameters as morphometric quality parameters of seedlings since this is a time-costing and invasive method, but it can be used to show the relation of these parameters with the growth of seedlings. Production and planting of the best quality narrow-leaved ash seedlings are crucial for regeneration since this species lack natural regeneration on the floodplains (Andrić 2018).

This paper aims to study the relation of leaf area parameters to the growth of one-year-old narrow-leaved ash seedlings in the nursery trial and determining variability among the 18 half-sib lines. The study results contribute to the knowledge base of narrow-leaved ash seedlings production.

2 Material and method

Maternal trees of the narrow-leaved ash, characterized by the superior phenotypic characteristics, were selected among those found in mixed forests in the area of the special nature reserve Upper Danube Region ("Gornje Podunavlje"). Eight trees (labeled from FA_2 to FA_9) were selected from the Management Unit (MU) "Karapandža", and 10 trees from the MU "Monoštorske šume" (labeled from FA_10 to FA_14 and, from FA_16 to FA_20). The diameter at the breast height (DBH) and the total height (H) were measured, and the age and GPS coordinates (Gauss Kruger coordinates) of all trees were determined (Table 1).

Table 1. Characteristics of the maternal trees: MU – management unit; K – MU „Karapandža"; M – MU „Monoštorske šume"; DBH – diameter at the breast height; H – total height.

Maternal tree	Coordinates	MU	DBH [cm]	H [m]	Age [years]
FA_2	7334249 5079633	K	41	27.2	39
FA_3	7334567 5079405	K	29	28.5	40
FA_4	7334604 5079421	K	33	32.2	40
FA_5	7334543 5079441	K	50	30.8	40
FA_6	7334565 5079429	K	28	32.4	40
FA_7	7334269 5077755	K	69	32.5	32
FA_8	7334286 5077692	K	33	32.6	32
FA_9	7334347 5076811	K	53	27.7	56
FA_10	7336637 5077102	M	36	21.8	42
FA_11	7336850 5077090	M	68	21.8	42
FA_12	7336895 5076948	M	47	28.7	42
FA_13	7337156 5076819	M	39	17.3	24
FA_14	7337107 5076885	M	54	24.4	21
FA_16	7337692 5071692	M	41	20.5	23
FA_17	7337654 5071725	M	69	25.4	23
FA_18	7339959 5068150	M	39	28.2	32
FA_19	7339614 5067982	M	60	33.9	32
FA_20	7339925 5068561	M	96	32.7	32

The seeds collected from maternal trees in October 2016 were used for the establishment of the progeny test in the nursery of the Faculty of Forestry, University of Belgrade. The seeds were sown immediately in a seedbed with natural soil, and during the growing season, cultural practices were performed once a week. The seedbeds were irrigated when needed, based on soil moisture.

All leaves per seedling were collected in August 2017 from 10 randomly selected 1+0 seedlings representing each of the 18 half-sib lines. Collected leaves were herbarized and scanned. The number of leaves per plant (NCL) was counted, as well as the number of leaflets within each imparipinnate compound leaf (NLCL). Also, the leaf area of each leaf (LACL) was measured using an open-source image processing program designed for scientific multidimensional images – ImageJ, and then, the total leaf area per seedling (TLA) was calculated. The root collar diameter (DIA) of seedlings was measured as well.

Descriptive statistics parameters (mean values, standard deviation, and minimal and maximal values) for all measured parameters, as well as One-Way ANOVA test, Tukey post-hoc test for each half-sib line, and the correlation coefficient between all morphological parameters were performed using software Statistica 7.0. The coefficient of variation was calculated in STATGRAPHICS Centurion XVI.I.

3 Results

The maximal mean number of leaves per plant (NCL) ranged from 9 to 16, and was observed in the half-sib line FA_7 (12) (Table 2). The minimal mean number of leaves per plant ranged from 5 to 9 and was recorded in the half-sib line FA_8. The lowest mean number of leaflets (NLCL)of 4.68 was recorded in half-sib line FA_19, while the highest NLCL of 8.06 was recorded in the half-sib line FA_4. Mean values of leaf area per leaf (LACL) ranged from 8.48 cm^2 (half-sib line FA_19) to 27.82 cm^2 (half-sib line FA_4). The mean total leaf area per seedling (TLA) ranged from 58.38 cm^2 (half-sib line FA_19) to 346.37 cm^2 (half-sib line FA_4).

OneWay ANOVA revealed that that the differences among 18 selected half-sib lines with respect to the studied attributes were significant ($p < 0.05$) (Table 3).

The variability of measured morphological attributes was large (Table 4). The most variable parameter was the total leaf area per seedling (TLA; CV=68.98%), which was strongly correlated to the leaf area per each leaf (LACL; r=0.95) and the number of leaflets (NLCL; r=0.94). The least variable parameter was the number of leaflets within each imparipinnate compound leaf (NLCL; CV=21.83%). All correlation coefficients were positive (Table 4).

The mean values of height (HT) and root collar diameter (DIA), (Kerkez et al. 2018) were used to calculate the coefficient of correlation (Table 5). Height (HT) was strongly correlated to all leaf morphological parameters (NLCL, LACL, TLA), with the exception of the number of leaves per plant (NCL), which was moderately to strongly correlated to the height of seedlings. Root collar diameter (DIA) was strongly correlated to the leaf area per each leaf (LACL) and the total leaf area per seedling (TAL), moderately to strongly correlated to the number of leaflets (NLCL), and moderately correlated to the number of leaves per plant (NCL).

Table 2. Mean values and standard deviation (sd) with post-hoc test groups, and extreme values of measured morphological parameters of leaves: the number of leaves per plant (NCL), the number of leaflets (NLCL), leaf area per each leaf (LACL) expressed in cm^2 and the total leaf area per seedling (TAL) expressed in cm^2 according to half-sib line.

	NCL		NLCL		LACL		TAL	
Half-sib line	mean value (sd)	min-max	mean value (sd)	min-max	mean value (sd)	min-max	mean value (sd)	min-max
FA_2	8.10^{abc} (2.47)	5-13	6.03^{abcd} (0.79)	4.67-7.27	26.24^{e} (10.21)	14.41-47.36	210.72^{bcd} (93.78)	90.77-372.04
FA_3	11.00^{bcd} (2.31)	8-15	7.26^{de} (1.18)	5.54-9.10	26.02^{de} (11.95)	11.04-51.47	272.92^{de} (98.14)	121.42-424.75
FA_4	12.30^{d} (2.45)	9-16	8.06^{e} (0.98)	6.67-9.94	27.82^{e} (5.75)	19.90-37.22	346.37^{e} (114.90)	195.87-523.33
FA_5	11.10^{bcd} (1.97)	9-15	7.23^{de} (0.83)	6.00-8.47	21.88^{bcde} (4.79)	14.58-30.28	245.49^{cde} (74.89)	131.22-349.62
FA_6	9.60^{abcd} (1.51)	8-13	6.69^{cde} (0.63)	5.80-8.11	28.33^{e} (6.31)	19.79-42.84	273.55^{de} (81.45)	178.15-428.38
FA_7	12.00^{cd} (2.40)	9-16	7.82^{e} (1.51)	5.30-10.07	24.20^{cde} (8.11)	15.85-42.25	294.50^{de} (144.52)	179.00-675.95
FA_8	7.20^{ab} (1.32)	5-9	5.73^{abc} (0.63)	5.14-7.00	14.09^{ab} (4.67)	8.78-26.20	104.38^{ab} (46.03)	43.89-209.60
FA_9	7.70^{ab} (3.16)	4-14	5.63^{abc} (0.75)	4.33-6.80	14.63^{ab} (4.88)	6.53-24.99	104.35^{ab} (33.39)	61.77-149.95
FA_10	8.70^{abcd} (2.54)	6-14	5.50^{abc} (0.83)	4.07-7.00	15.81^{abc} (3.21)	9.69-19.57	134.95^{abc} (40.88)	80.90-215.27
FA_11	8.50^{abcd} (3.14)	5-16	5.52^{abc} (0.77)	4.00-7.06	16.95^{abcd} (4.26)	10.60-23.76	139.88^{abc} (48.01)	63.58-245.12
FA_12	8.00^{ab} (3.16)	4-14	5.34^{abc} (1.02)	3.75-6.86	12.11^{a} (7.12)	5.79-28.77	107.77^{ab} (110.46)	32.74-402.82
FA_13	10.40^{abcd} (3.37)	7-18	5.25^{abc} (0.57)	4.55-6.57	8.03^{a} (2.09)	5.66-11.97	81.94^{a} (27.93)	51.36-130.12
FA_14	7.90^{ab} (1.73)	6-11	5.81^{abcd} (1.46)	4.17-9.13	13.53^{ab} (7.51)	6.07-32.10	103.89^{ab} (49.33)	48.59-192.62
FA_16	7.40^{ab} (2.67)	4-12	5.00^{ab} (0.62)	3.71-5.92	12.90^{ab} (2.10)	8.61-14.65	96.21^{a} (41.68)	51.64-175.79
FA_17	8.40^{abcd} (2.46)	6-13	5.28^{abc} (0.55)	4.50-6.00	11.65^{a} (3.19)	7.05-16.12	100.59^{ab} (45.65)	42.28-190.50
FA_18	9.10^{abcd} (3.11)	6-16	5.23^{ab} (0.46)	4.75-5.91	9.87^{a} (1.57)	7.63-12.06	89.84^{a} (32.40)	48.96-148.95
FA_19	7.00^{a} (2.58)	4-13	4.68^{a} (1.01)	3.00-6.56	8.48^{a} (1.89)	4.90-11.47	58.38^{a} (22.68)	38.25-99.37
FA_20	8.90^{abcd} (1.60)	6-11	6.14^{bcd} (1.31)	5.10-8.83	12.49^{a} (3.57)	8.50-18.84	108.74^{ab} (28.01)	67.98-160.26

[*]Tukey post-hoc test, $p<0.05$

Table 3. OneWay ANOVA based on measured leaf parameters: the number of leaves per plant (NCL), the number of leaflets (NLCL), leaf area per each leaf (LACL) and the total leaf area per seedling (TAL).

	Between groups			Within groups				
	SS	df	MS	SS	df	MS	F	p
NCL	458	17	26.92	1024.5	162	6.32	4.2560	0.0000
NLCL	167	17	9.82	141.2	162	0.87	11.2673	0.0000
LACL	8088	17	475.76	5649.9	162	34.88	13.6414	0.0000
TLA	1330151	17	78244.16	841920.6	162	5197.04	15.0555	0.0000

Table 4. Coefficient of variation for all morphological parameters of leafs within and between half-sib lines, and coefficient of correlation between all morphological parameters: the number of leaves per plant (NCL), the number of leaflets (NLCL), leaf area per each leaf (LACL) and the total leaf area per seedling (TAL).

	Coefficient of variation [CV%]			
Half-sib line	NCL	NLCL	LACL	TLA
FA_2	30.49	13.16	38.91	44.50
FA_3	20.99	16.26	45.95	35.96
FA_4	19.93	12.14	20.68	33.17
FA_5	17.74	11.49	21.90	30.51
FA_6	15.68	9.42	22.28	29.77
FA_7	20.03	19.30	33.53	49.07
FA_8	18.29	11.02	33.17	44.10
FA_9	41.09	13.25	33.33	31.99
FA_10	29.20	15.05	20.32	30.29
FA_11	36.89	13.89	25.14	34.32
FA_12	39.53	19.00	58.81	102.50
FA_13	32.43	10.82	26.05	34.09
FA_14	21.88	25.10	55.47	47.48
FA_16	36.15	12.33	16.26	43.32
FA_17	29.27	10.34	27.39	45.38
FA_18	34.15	8.81	15.88	36.06
FA_19	36.89	21.66	22.29	38.85
FA_20	17.92	21.36	28.59	25.76
Total	31.72	21.83	51.70	68.98
	Coefficient of correlation [r](p < 0.05, n=18)			
Parameter	NCL	NLCL	LACL	TLA
NCL	1,00	0,85	0,60	0,80
NLCL	0,85	1,00	0,84	0,94
LACL	0,60	0,84	1,00	0,95
TLA	0,80	0,94	0,95	1,00

Table 5. Coefficient of correlation (n=18) between height (HT) and root collar diameter (DIA) and leaf morphological parameters: number of leaves per plant (NCL), number of leaflets (NLCL), leaf area per each leaf (LACL) and total leaf area per seedling (TAL).

	NLCL	LACL	TLA	NCL
DIA	0,85	0,90	0,90	0,67
HT	0,95	0,92	0,97	0,78

4 Discussion and conclusion

Indication of frequent drought episodes due to global warming predicted by the climate models (Salinger et al. 2005), emphasizes the urgent need to study the morphological and physiological adaptation strategies of plants to environmental changes including future climate change (Wang et al. 2019). Also, variability in individual leaf size and its trade-off with total leaf number in a plant have particularly important implications for understanding the adaption strategy of plants to environmental changes (Wang et al. 2019). Plants growing in the same habitat with a great variety of leaf sizes are expected to have distinct abilities of thermal regulation influencing leaf water loss and shedding heat (Wang et al. 2019). In hot and dry environments and at high intensities of solar radiation smaller leaves are advantageous, while large leaves with less efficient energy exchange capacity are advantageous in cooler, moister and lower irradiance environments (Niinemets et al. 2006; Meier and Leuschner 2008; Tozer et al. 2015). *Fraxinus angustifolia* is species native to the moist sites, which according to Long and Jones (1996) may have the greatest capacity for high rates of leaf area production. Also, high genetic variability in *Fraxinus angustifolia* natural population (Jarni 2009; Jarni et al. 2011; Papi et al. 2012; Jeandroz et al. 1996; Kremer et al. 2010; Čortan et. al. 2017; Temunović et al. 2012; Abbate et al. 2020) indicates high adaption strategy of this species.

Leaf parameters can be considered as a valuable morphological attribute of seedlings quality, strongly correlated to other morphological attributes (Ivetić et al. 2014), especially with growth elements height and root collar diameter. In this study total leaf area per seedling (TLA) can be considered as the most variable parameter, and it is strongly correlated with growth elements (HT and DIA). Also, the correlation coefficient in this study is higher compared to correlations reported by Ivetić et al. (2014) between TLA and DIA/HT, but lower between NCL and DIA/HT. Strong correlations between leaf traits and shoot size are logical (Ivetić et al. 2014). Thus, we confirmed high variability and differences in all investigated leaf parameters. One of the main tasks, according to FRAXIGEN (2005) is to identify superior reproductive material for production and genetic improvement programmes for narrow-leaved ash, which at first mean evaluation of seed sources. High genetic variability means that narrow-leaved ash has a great genetic potential for conservation and directed utilization (Kerkez et al. 2018). The number of half-sib lines in reforestation determines the degree of genetic diversity and adaptability and depends on the seed source (seed stands or orchards), seed processing and nursery production (Ivetić et al. 2016).

According to Ivetić et. al. (2014) total leaf area and average leaf area are suitable for testing the seedling's quality (SQ). As additional knowledge and contribution, results from this study confirmed high variability of leaf parameters and their high correlation to the growth of one-year-old *Fraxinus angustifolia* seedlings.

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