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Relationships between leaf traits and morphological attributes in one-year bareroot *Fraxinus angustifolia* Vahl. seedlings

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Abstract. Leaf traits were investigated as morphological attributes for quality estimation of one-year-old bareroot seedlings of *Fraxinus angustifolia* Vahl. Leaf traits (number of simple – NSL and compound leaves – NCL, leaf average – ALA and total area – TLA and leaf area ratio – LAR) show similar relations to morphological attributes, the strongest to shoot growth (height – HT, root collar diameter – DIA and shoot dry weight – SDW). Increases of seedling morphological attributes are strongly related to increases of ALA. Given to strong positive correlations with other morphological attributes, ALA (R = 0.68-0.88) and TLA (R = 0.46-0.92) are suitable for seedlings quality testing. TLA explained a larger proportion of the variation of SDW (R^2 = 0.85) and ALA explained a large proportion of the variation of DIA (R^2 = 0.81). Leaf traits can be considered as valuable morphological attributes of seedling quality, in addition to strong correlation with other morphological attributes.
Keywords total leaf area, average leaf area, seedlings quality, Narrow-leaved ash.

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Introduction

Fraxinus angustifolia Vahl. (Narrow-leaved ash) is a fast growing tree species with quality timber, distributed from Spain and Portugal in the west, Slovakia and southern Moravia to the north, and to Iran and southern Russia to the east (Fraxigen 2005). In Serbia it covers 25.200 ha (Banković et al. 2009) in riparian and floodplain forests (Jovanović 2007). Beside timber production as primary management goal, this species can be used in silvopastoral systems (Klossas et al. 2013). Despite its relative importance, little is known about its plantation establishment (Cicek et al. 2010).

Leaf area is a plant-biometric index, important for leaf energy and water balance (Cornelissen et al. 2003). Beside leaf area, operational environmental factors that influence the rate and duration of photosynthesis are water, carbon dioxide, light (intensity, duration, quality), heat, macro- and micronutrients and air pollutants (Hobbs 1992). Large leaf area as seedling morphological attribute indicates higher potential for photosynthesis and transpiration, from which newly planted seedlings can benefit in favorable environmental conditions. However, larger transpirational area can have a negative impact on seedling development or even survival after planting, in cases when the root system cannot supply enough water to maintain a proper water balance (Grossnickle 2005).

Leaf area ratio (LAR) is a measure of photosynthetic potential per unit of plant biomass. Radford (1967) suggested the term, for the ratio of the total leaf area to the total plant dry biomass. LAR, together with net assimilation rate (NAR), is one of two components of relative growth rate (RGR). The RGR is the product of NAR (largely the net result of carbon gain and losses, expressed per unit leaf area) and LAR (indicating the fraction of total plant weight allocated to the leaves) (Poorter and Remkes 1990). LAR is the product of a morphological component: specific leaf area – SLA (amount of leaf area per unit of leaf mass), the ratio of leaf area and leaf weight, and the leaf mass ratio – LMR (the fraction of the total plant biomass allocated to leaves) (Lambers et al. 2008).

Leaf traits as morphological attributes of seedling quality are more often studied for conifers, mainly as needle surface area – a direct measure of potential photosynthetic or transpirational surface area (Grossnickle et al. 1991). For broadleaved species there is a limited number of studies. Relation between leaf traits and morphological attributes was studied on seedlings of *Quercus rubra* L. (Larson 1977) and *Castanea sativa* Mill. (Ozturk and Serdar 2011), and for eight Mediterranean woody species (Sanchez-Gomez et al. 2007). Jijeesh and Sudhakara (2013) reported that positive correlation between drupe size and growth of teak seedlings is mainly due to the larger leaf area of seedlings with a larger drupe size.

The objective of this study was to investigate the value of leaf traits (number, area and LAR) as morphological attributes in quality estimation of *Fraxinus angustifolia* seedlings.

Material and methods

Seedlings production

Seedlings were produced from the same seedlot: autochthonous, selected seed sources (RS-2-2-fan-81-315), region of provenances 81 – Sava-Danube-Morava basin, from the Danube river bank, southeast Serbia, at an altitude of 70 m. Seeds were collected in October 2011, and stratified from December 2011 to April 2012, in mixture of sand and peat (1:1 v:v), at temperatures between 3-5° C. Seeds were sown in the first week of April 2012, in seedbed previously prepared by mixing of 1,500 L of sphagnum peat with 10 m^3 of upper 10 cm of natural soil. Distance between rows was 20 cm, and distance between seed spots in rows was 5 cm, two seeds were sown at each seed spot. After sowing, a pre emergence herbicide Prometrin was used for weed control (2 $kg \cdot ha^{-1}$). After germination, a (2%) microbiological fertilizer with the commercial name Slavol (Agrounik Ltd., Serbia) was used for foliar fertilization. This irrigation was performed by a sprinkler system on demand, based on substrate moisture.

Seedlings measurements

One-year bareroot seedlings were sampled in the first week of November 2012. A total length of seedbed (60 m) was divided into four segments, and from each quarter, 25 seedlings were randomly sampled for morphological attributes' measurements. A total sample of 100 seedlings was measured for: height (HT), root collar diameter (DIA), shoot dry weight (SDW), root dry weight (RDW), root volume (RV), total root length (RL), number of simple leaves (NSL), number of compound leaves (NCL), average leaf area (ALA) and total leaf area (TLA). Leaf area ratio (LAR) was calculated from total area and total dry weight.

The HT was measured as distance between the root collar and the base of terminal bud of dormant seedlings, with an accuracy of 0.1 cm. DIA was measured at or near the root collar, with an accuracy of 0.1 mm. Shoots were separated from roots at the root collar and separately oven dried in open paper bags for 48 hours, at 80° C. SDW and RDW were measured on an electronic scale with an accuracy of 0.001 g. RV was measured by water displacement method (Burdett 1979), with an accuracy of 1 mL. RL was measured using the intersection method (Tennant 1975). NSL and NCL were counted as number of simple and compound leaves per seedling. TLN represent a total number of single leaves and leaflets in compound leaves. ALA and TLA (Figure 1) were measured using LAMINA software tool (Bylesjö et al. 2008). LAR ($cm^2 \cdot g^{-1}$) was calculated by dividing of TLA with (SDW+RDW).

Statistical analysis

Mean value, standard deviation (SD) and minimum and maximum values of measured morphological attributes were calculated.

Relation between leaf traits and other morphological attributes was examined by correlation coefficient (R) for bivariate data with scatter diagrams showing linear association. Infrequent observations in terms of outliers were removed to calculate correlations. R was used to calculate coefficient of determination (R^2) in order to examine how much variability is shared by two variables. Equations of regression lines were calculated for these bivariate data with $R > 0.50$ and where residual plots did not show any trend or linear relation.

Measurement data was statistically analyzed using Statistica 7 software (StatSoft, Inc. 2004).

Results

One-year bareroot seedlings of *Fraxinus angustifolia* produced oversized minimum requests for the first class seedlings by the Serbian standard for broadleaved species (SRPS D.Z2.112, 1968). However, morphological attributes showed large variability (Table 1). Distribution of NCL, RV, SDW and RDW was not normal, resulting in larger standard deviations than the mean values. However, scatter plots with leaf traits and other morphological attributes showed linear tendency. A slightly larger part of biomass was located in the root system (S:R = 0.85). Some seedlings had only four single leaves, without compound leaves developed.

Leaf traits showed the weakest correlation with RV and RL (Table 2). NSL had weak and negative correlations with morphological attributes. Other leaf traits showed similar correlations, the strongest with shoot absolute growth (HT, DIA and SDW). The strongest

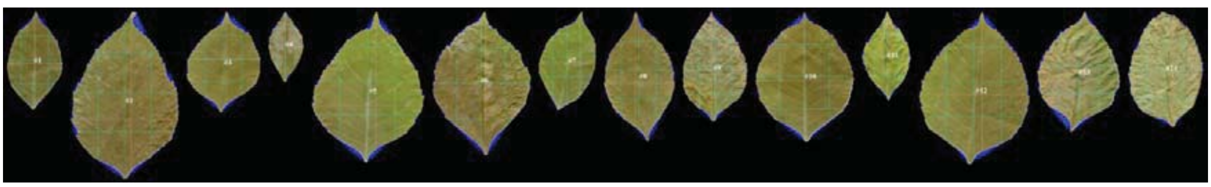

Figure 1 Leaf area measured by Lamina software tool

Table 1 Mean, minimum and maximum values and standard deviation of *Fraxinus angustifolia* one-year bareroot seedlings' morphological attributes

	Mean	Minimum	Maximum	SD
HT	31.65	14.10	73.10	10.44
DIA	4.23	1.60	11.16	2.01
RV	2.88	0.50	32.00	4.24
RL	175.01	47.00	365.00	83.52
SDW	1.68	0.16	10.98	1.94
RDW	1.97	0.19	14.05	2.35
NSL	11.12	2.00	22.00	4.09
NCL	3.56	0.00	23.00	4.99
TNL	22.12	4.00	81.00	16.07
ALA	8.99	6.25	14.53	1.44
TLA	225.84	40.08	1089.69	184.65
LAR	91.25	16.04	267.23	51.30

Note. Abbreviations: height (HT), root collar diameter (DIA), shoot dry weight (SDW), root dry weight (RDW), root volume (RV), total root length (RL), number of simple leaves (NSL), number of compound leaves (NCL), average leaf area (ALA), total leaf area (TLA) and leaf area ratio (LAR).

Table 2 Coefficients of correlation (*R*) between leaf and morphological attributes.

	HT	DIA	RV	RL	SDW	RDW
NSL	-0.26*	-0.17	-0.11	-0.07	-0.22	-0.21
NCL	0.88**	0.81**	0.59**	0.49**	0.86**	0.80**
TNL	0.88**	0.83**	0.57**	0.47**	0.89**	0.81**
ALA	0.80**	0.87**	0.74**	0.68**	0.88**	0.88**
TLA	0.84**	0.81**	0.63**	0.46**	0.92**	0.84**
LAR	-0.59**	-0.66**	-0.42**	-0.47**	-0.52**	-0.54**

Note. Marked correlations are significant at $p < 0.05$ (*) and $p < 0.01$ (**) N = 97.

correlation was found between TLA and SDW, followed by correlations between ALA and dry weights. LAR showed negative, moderate and significant correlations with seedlings morphological attributes (Figure 2).

Seedling morphological attributes showed the largest increase with an increase of ALA, leading to the largest slopes of regression lines (Table 3). TLA explained a larger proportion of the variation of SDW (85%). ALA explained a large proportion of the variation of DIA (81%) and NL a large proportion of variation of SDW (79%).

Discussion

One-year bareroot seedlings of *Fraxinus anfustifolia* examined in this study satisfied minimum requests for first class one-year bareroot seedlings by the Serbian standard, but they are much smaller compared to bareroot seedlings of the same age reported by Cicek et al. (2007), especially in terms of SDW and RDW. This is mainly because our seedlings were not fertilized. Variation of morphological variables was large and can be explained by genetics and by stretched germination time (two weeks).

Recorded correlations in this study are much higher compared to correlations reported by Ozturk and Serdar (2011) between DIA and NL, ALA, TLA; and between HT and NL, but not between HT and ALA and TLA for *Castanea sativa* Mill. However, this comparison needs to be taken with caution, considering interspecies variation and different operational environment.

Strong correlations between leaf traits and shoot size are logical. Leaf weight contributes to total SDW and larger shoot HT is followed with larger number of leaves. Strong correlation between leaf area and DIA is consistent

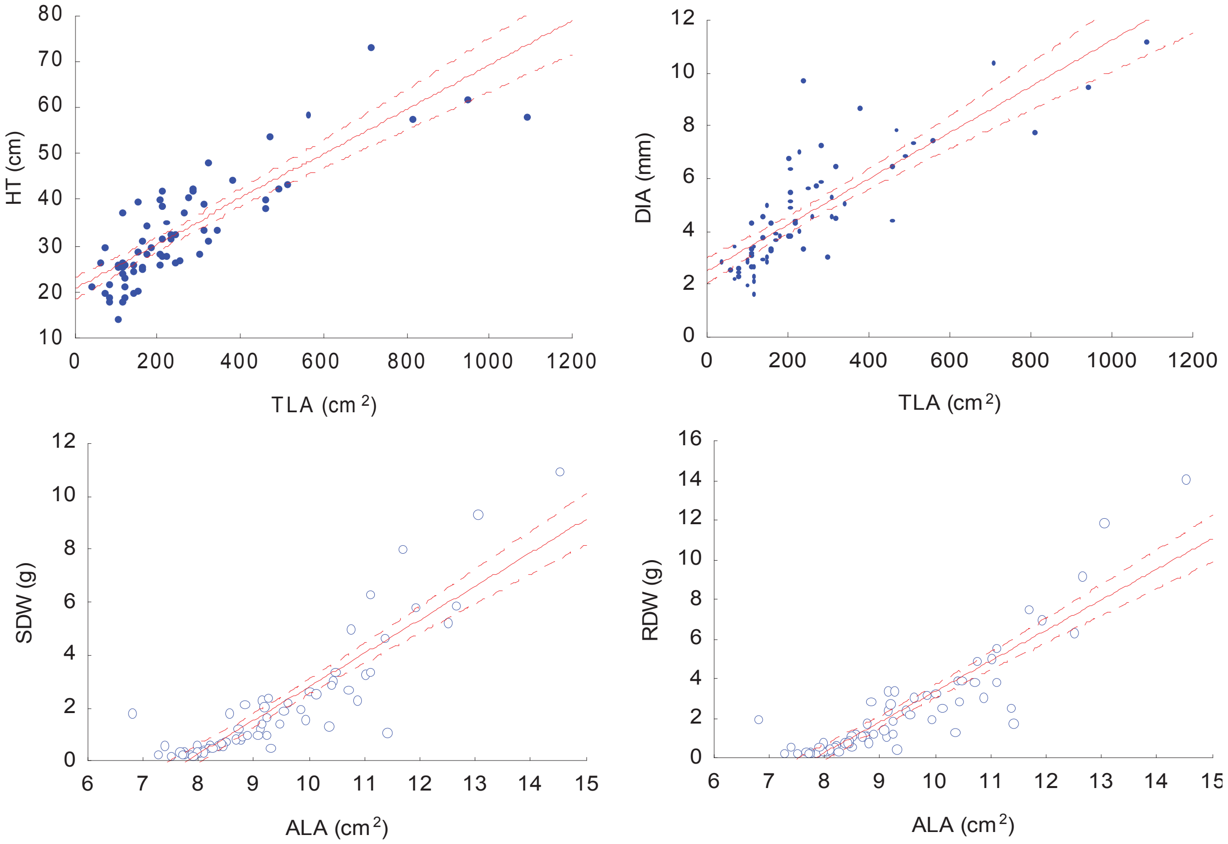

Figure 2 Relationship between different size characteristics and total average leaf area in *Fraxinus angustifolia* one-year bareroot seedlings

with the pipe model theory (Shinozaki et al. 1964a, 1964b, Mencuccini and Bonosi 2001).

Negative correlation between LAR and other morphological attributes can be explained by the calculation method based on the absolute growth of seedlings. Looking on relative growth, a high LAR enables plants to grow fast (Lambers et al. 2008). Larger investment in leaf area per unit of total plant weight enhances the growth rate (Reich et al. 1992). RGR of plants is much more correlated with LAR of plants than with net photosynthetic capacity of their leaf area (Raghavendra 2000) and the faster growth of the deciduous trees could be explained by their higher LAR relative to evergreens (Antúnez et al. 2001). However, Sanchez-Gomez et al. (2007) reported the importance of low LAR and low phenotypic plasticity as potential determinants of enhanced performance under shade during the very early seedling stages of Mediterranean woody species. Species native to moist sites, which do not need a large root system for optimum growth (like *Fraxinus angustifolia*), may have the greatest capacity for high rates of leaf area production (Long and Jones 1996).

In research of leaf structure of tree species, other indexes are more investigated, like leaf area index or specific leaf area (e.g. Reich et al. 1998). However, we believe that non-destructive measurement of ALA and TLA can improve the seedling quality estimation; as well as LAR in seedling testing programs which are involved as a measure of dry weights. In terms of the strength of correlations with other morphological attributes, ALA and TLA are suitable for testing the seedlings quality.

Table 3 Linear regressions and coefficients of determination (R^2) of leaf and other morphological attributes of *Fraxinus angustifolia*

y	a_0	$a_1 \cdot x$	R^2
HT	24.968	1.877 · NCL	0.77
DIA	3.297	0.324 · NCL	0.66
RV	1.095	0.538 · NCL	0.35
SDW	0.474	0.351 · NCL	0.74
RDW	0.668	0.396 · NCL	0.64
HT	18.439	0.600 · NL	0.77
DIA	2.117	0.106 · NL	0.69
RV	-0.680	0.168 · NL	0.32
SDW	-0.830	0.116 · NL	0.79
RDW	-0.752	0.128 · NL	0.66
HT	-23.530	6.046 · ALA	0.64
DIA	-7.463	1.299 · ALA	0.75
RV	-19.010	2.397 · ALA	0.55
RL	-174.300	37.468 · ALA	0.46
SDW	-9.870	1.268 · ALA	0.77
RDW	-12.040	1.541 · ALA	0.77
HT	20.867	0.048 · TLA	0.70
DIA	2.498	0.009 · TLA	0.66
RV	-0.502	0.156 · TLA	0.40
SDW	-0.487	0.010 · TLA	0.85
RDW	-0.418	0.011 · TLA	0.70
HT	44.050	-0.122 · LAR	0.35
DIA	6.975	-0.025 · LAR	0.43
SDW	3.822	-0.020 · LAR	0.27
RDW	4.696	-0.026 · LAR	0.29

Note. Abbreviations: height (HT), root collar diameter (DIA), shoot dry weight (SDW), root volume (RV), root dry weight, (RDW), total root length (RL), number of compound leaves (NCL), number of leaves (NL), average leaf area (ALA), total leaf area (TLA) and leaf area ratio (LAR).

Conclusions

To find methods of testing the seedling quality are important for reforestation projects since they indicate seedling mortality after planting. The imperative is to define the most simple and fastest methods for SQ estimates. However, additional information are beneficial in some cases. Leaf traits can be considered as valuable morphological attributes of seedling quality, due to their strong correlation with other morphological attributes.

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