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MORPHOLOGICAL DIFFERENTIATION BETWEEN ROMANIAN LIME SPECIES (TILIA SPP.): A CASE STUDY

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Abstract: *Three lime species are known to occur in Romania: Tilia cordata, T. platyphyllos and T. tomentosa. The aim of this study iss to highlight the morphological traits which differentiate the three species by using Discriminant Analysis. One hundred fifty lime individuals were sampled and eleven leaf and twig descriptors were assessed. The results of this multivariate statistical analysis confirmed the high discriminating power of certain traits. Abaxial laminal pubescence, lamina length and bud pubescence were the variables that separate the three species and were used to develop three discriminant functions.*

Key words: *lime, Tilia, morphological descriptors, Discriminant Analysis*

1. Introduction

In general, woody plants can be easily identified by certain morphological traits that are species-specific. But in many cases, for example in species-rich genus like *Quercus* L., morphological differentiation between closely related tree species is difficult to assess [4], [5], [10].

Another example is genus *Tilia* L., which includes, according to different authors, between 25 and 50 tree species native throughout the temperate northern hemisphere, in Asia, Europe and North America [6], [17], [21], [23].

Recently, several studies [7], [19], [22], [32], [33] were carried out for lime species (*Tilia* spp.) in order to assess their morphological variability by using different descriptors, especially leaf traits. By contrast, no detailed leaf and/or twig morphological assessment was done in Romania.

Genus *Tilia* L. belongs to the family *Tiliaceae* Juss. (Order *Malvales* Juss.). It is represented by economic and ecological important tree species.

In Europe, only four lime species occur naturally, namely *Tilia cordata* Mill. (small-leaved lime), *T. platyphyllos* Scop. (large-leaved lime), *T. tomentosa* Moench. (silver lime) and *T. dasystyla* Stev. (Caucasian lime) [21]. The first three of them are also present in Romania, where besides their multiple ecological and silvicultural roles they are also very appreciated for their ornamental value [16]. Among them, in our country, the most widespread is small-leaved lime [24], while the less common in large-leaved lime [27].

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Small-leaved lime can be found on plains of approximately 900 m elevation [25]. Its longevity is around 200 years [25], but according to recent determinations, *T. cordata* can reach around 300 years old [28].

According to several studies, many lime species can hybridize [14], [15], [22], [31] and in these cases species differentiation is difficult to assess [33]. For example, in Romania seven lime hybrids are known to occur [23].

A right identification of a species *sensu lato* can be done by taking into consideration relevant morphological descriptors recommended by the literature and by using several multivariate analyses.

One of the most used multivariate statistical techniques for species differentiation which provided good results in similar morphological studies dealing with closely related species is Discriminant Analysis [8], [11], [12], [13].

The objective of this study is to evaluate the leaf, bud and twig morphology of the autochthonous lime species, namely *T. cordata*, *T. platyphyllos* and *T. tomentosa*. Secondly, three easy to use discriminant functions for lime species identification were constructed.

2. Materials and Methods

Plant material (undamaged and fully developed twigs, buds and leaves) was collected from two mixed lime stands in the autumn of 2013.

From the first stand, located in Cocani Forest, Dâmbovița County (N44,62182; E25,88087), 180 individuals were sampled, while from the second one, located in Babadag Forest, Tulcea County (N44,87982; E28,72131), only 90 lime trees were sampled. All three species were represented in both sampled stands, but in different proportions. In total, there were sampled 270 individuals.

The minimum distance between the trees was about thirty meters.

Species identification was done according to morphological descriptions from specialized manuals [6], [18], [20], [27]. Almost half of the sampled trees (108 individuals) presented intermediate morphology (putative hybrids?).

Therefore, in order not to include the above mentioned individuals in the analysis and to construct three equal groups corresponding to the three species, only fifty lime individuals corresponding to each species were chosen for performing the morphological analysis. Thus, three to five leaves, three or four buds and one to three twigs were sampled from every tree.

In order to assess the morphological variability of the three lime species, eleven descriptors were used.

For each leaf, four dimensional characters, namely lamina length (LL), maximum lamina width (LW), length of lamina from base to widest point (WP) and petiole length (PL), were measured (Figure 1).

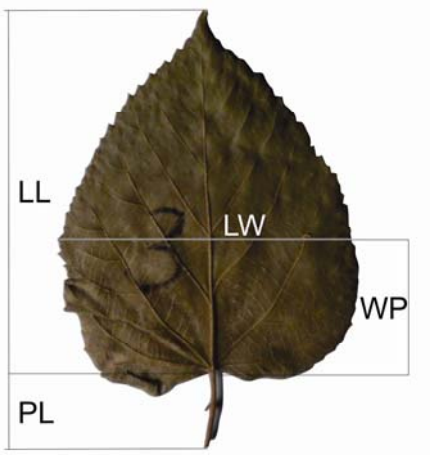

Fig. 1. *Description of the dimensional leaf traits*

In addition, two observed variables, *i.e.* abaxial laminal pubescence (LP) and basal shape of the lamina (BS), were assessed.

Abaxial laminal pubescence (LP) was assessed at the leaf vein axils level, especially at the bottom of the leaf as shown in Figure 2 by the red arrows. It was scored as an index as follows: 1 – no tufts of hairs in the leaf vein axils, 2 – big tufts of reddish-brown hairs in the leaf vein axils and 3 – white or yellow hairs in the leaf vein axils.

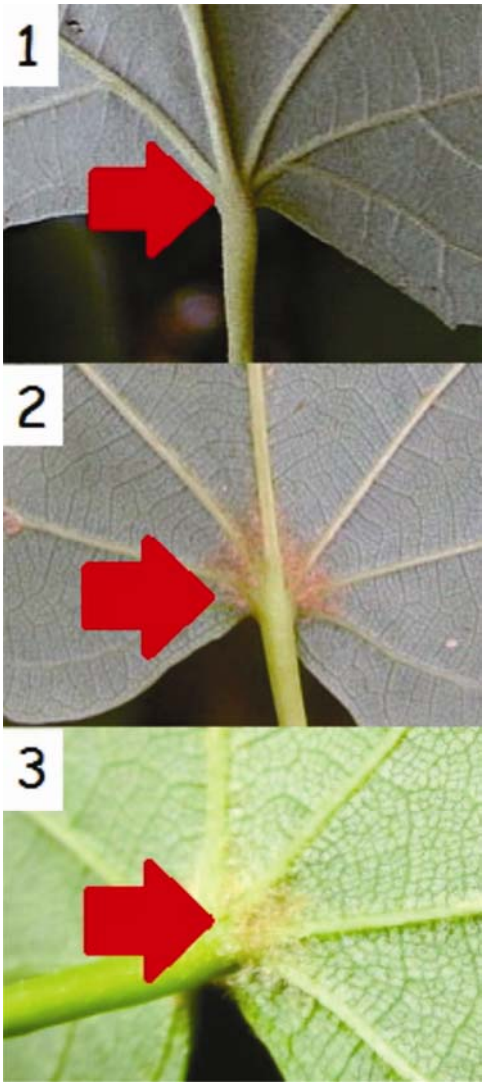

Fig. 2. *Description of the abaxial laminar pubescence*

Basal shape of the lamina (BS) was scored as an index varying from 1 to 9, according to the angle of the basal part of lamina. So, if the value of the angle was less than 20 then BS was 1, if the value was between 21 and 40, BS was 2, a.s.o.

For twig morphological assessment two variables were taken into consideration, namely twig color (TC) and twig pubescence (TP).

Twig color was scored as follows: 1: green, 2: brown-reddish and 3: grey (Fig. 3).

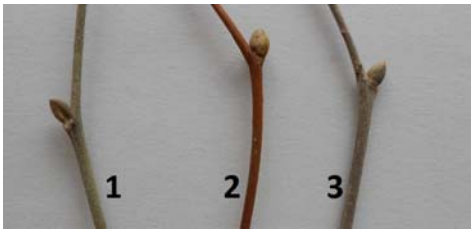

Fig. 3. *Twig color assessment*

In the case of the second twig trait, only two values were used, namely 1 (glabrous twigs) and 2 (pubescent twigs).

Regarding the buds, three traits were assessed: number of scales (NS), bud length (BL) and bud pubescence (BP). For bud pubescence, the same scale as for twig pubescence was used, namely 1 (glabrous buds) and 2 (pubescent buds).

In the case of pubescence assessment, naked-eye evaluation was used.

In the case of the five dimensional leaf and bud traits (LL, LW, WP, PL and BL) the measurements were made with a digital slide caliper with accuracy of 0.01 mm, model HW0812-4311.

Afterwards, morphological data were processed with STATISTICA software v.8.0 and mean values, standard deviations and coefficients of variation for every morphological descriptor were calculated.

Discriminant Analysis was carried out with the same software and Forward stepwise method with tolerance 0.01 was used.

3. Results and Discussions

Mean and standard deviation values of the eleven assessed morphological traits are given in Table 1 (150 trees, 50 individuals for each lime species).

Except the value for BS in the case of *T. tomentosa*, the values for the coefficient of variation were below 30%, which means that the mean is representative for the data set.

Regarding leaf dimensional traits, it can be seen that *T. platyphyllos* had the biggest leaves, followed by *T. tomentosa* and T. *cordata*. This is in accordance with the data from Romanian [2], [3], [6], [9], [27], [30] and Italian [1] literature.

Lamina length ranged from 65.1 to 89.8 mm in the case of small-leaved lime, from 67.2 to 149.7 mm for large-leaved lime and from 70.1 to 127.4 mm for silver lime, respectively.

In the case of *T. cordata*, according to our results, bigger leaves were identified compared with the ones measured in a study made in Timişoara city [26]. This could be explained by the fact that the trees located inside the cities, in polluted areas, have smaller leaves that the ones situated outside the cities. This was demonstrated in a study carried out recently on limes [29].

Leaf shape resulted as the ratio between lamina length (LL) and lamina width (LW) combined with the position of LW along the LL. In our study, this ratio was close to 1 for all three species, so that the overall shape of the leaf was more or less round.

The petiole length was on average approximately 1 mm longer in *T. platyphyllos* and *T. tomentosa*, compared with *T. cordata*.

Basal shape of lamina was cordate in all cases, the mean values for BS descriptor ranged from 4.6 to 5.3.

As a consequence of selecting only 50 trees per species with the typical morphology, according to Romanian Dendrological literature [3], [6], [9], [20], [25], [27], the standard deviations for abaxial laminal pubescence (LP) were almost 0.0. The same remark is also valid for morphological descriptor number of scales (NS).

Table 1

Mean and standard deviation (SD) values of morphological descriptors

Descriptor	Statistics	Lime species		
		T. cordata	*T. platyphyllos*	*T. tomentosa*
LL [mm]	Mean	78.1	104.0	91.7
	SD	6.9	17.1	13.3
LW [mm]	Mean	74.3	97.2	90.6
	SD	8.7	19.0	14.6
WP [mm]	Mean	25.9	33.8	30.6
	SD	3.5	6.6	5.5
PL [mm]	Mean	34.8	44.9	42.5
	SD	8.1	7.3	9.5
LP	Mean	2.0	2.9	1.0
	SD	0.1	0.2	0.0
BS	Mean	4.6	5.3	4.7
	SD	1.3	1.4	1.5
TC	Mean	1.8	1.7	3.0
	SD	0.5	0.5	0.0
TP	Mean	1.1	1.1	2.0
	SD	0.3	0.2	0.2
NS	Mean	2.0	3.0	2.0
	SD	0.0	0.0	0.0
BL [mm]	Mean	4.5	5.8	4.8
	SD	0.7	1.2	1.0
BP	Mean	1.1	1.1	2.0
	SD	0.2	0.2	0.0

Regarding the twig color (TC), in the case of *T. cordata*, most of the twigs were brown-reddish, but a few green or grey twigs were identified. The same results were also found for *T. platyphyllos*, while in the case of *T. tomentosa* all the twigs were grey.

It is worth to mention that sometimes it was very difficult to decide whether the most predominant color of the twig is green or grey, especially when the twigs were pubescent and most of the hairs were grey.

With regard to twig pubescence (TP), for *T. cordata* and *T. platyphyllos* most of the twigs were glabrous (*i.e.* no hairs) (92% and 94%, respectively), while for *T. tomentosa* almost all the twigs were pubescent (96%).

On average, the buds of large-leaved lime were bigger with 1 to 1.3 mm than the buds of the other two lime species.

Regarding the bud pubescence, in the case of silver lime all buds were pubescent, while for *T. cordata* and *T. platyphyllos* most of the buds (96% in both cases) were glabrous.

The Discriminant Analysis was firstly performed by using the three groups of individuals corresponding to the three lime species.

The results weren't relevant, so three separated pairs were constructed: i) *T. cordata – T. platyphyllos*; ii) *T. cordata – T. tomentosa* and iii) *T. platyphyllos – T. tomentosa*.

In all three cases, all the variables except number of scales (NS) were used. NS wasn't included in the analysis because the variance for this variable was zero.

For the first pair (*T. cordata – T. platyphyllos*), the variables with the lowest Partial Wilks' Lambda values (variables that discriminate best between the two species) were: abaxial laminar pubescence (LP), lamina length (LL) and bud length (BL) (Table 2).

These three variables were retained for the construction of the following discriminant function: $\mathbf{DF_1}$=1652.3 - (501.6×**LP**) + (3.9×**LL**) - (19×**BL**).

According to the software, this function returns positive values for *T. cordata* and negative values for *T. platyphyllos*, respectively.

Table 2

Values of Partial Wilks' Lambada for the Variables included in the model for the pair T. cordata – T. platyphyllos

	Partial Wilks' Lambda	**p-level**
LP	0.228404	0.000000
LL	0.809095	0.000000
BS	0.956706	0.041921

Regarding the second pair (*T. cordata – T. tomentosa*), the morphological descriptors with the higher discriminating power between the two lime species were: abaxial laminar pubescence (LP), bud pubescence (BP), twig color (TC) and petiole length (PL) (Table 3).

Table 3

Values of Partial Wilks' Lambda for the variables included in the model for the pair T. cordata – T. tomentosa

	Partial Wilks' Lambda	**p-level**
LP	0.276525	0.000000
BP	0.810881	0.000010
TC	0.849799	0.000096
PL	0.874552	0.000400

These four variables were retained for the construction of the following discriminant function: $\mathbf{DF_2}$=520.7 - (917.7×**LP**) + (268.7×**BP**) + (118.8×**TC**) + (3.9×**PL**).

This function gave positive values for *T. tomentosa* and negative values for *T. cordata*, respectively.

For the last pair (*T. platyphyllos* – *T. tomentosa*), six out of the ten morphological traits were found in the analysis ('variables in the model'): abaxial laminar pubescence (LP), bud pubescence (BP), twig color (TC), bud length (BL), lamina length (LL) and maximum lamina width (LW) (Table 4).

Table 4

Values of Partial Wilks' Lambda for the variables included in the model for the pair T. platyphyllos – T. tomentosa

	Partial Wilks' Lambda	**p-level**
LP	0.245616	0.000000
BP	0.793215	0.000004
TC	0.908278	0.003007
BL	0.952625	0.035085
LL	0.918506	0.005287
LW	0.957946	0.047395

These six variables were retained for the construction of the following discriminant function: $\mathbf{DF_3}$=728.4 - (574.9×**LP**) + (287.1×**BP**) + (92.3×**TC**) + (26.1×**BL**) – (3.6×**LL**) + (2.5×**LW**).

This function returns positive values for *T. tomentosa* and negative values for *T. platyphyllos*, respectively.

As seen from the results of the Discriminant Analysis, among the ten analyzed variables, abaxial laminal pubescence (LP) is the variable with the highest discriminating power between the three lime species (it had the lowest Partial Wilks' Lambda value in all cases). On the other hand, the variables: length of lamina from base to the widest point (WP), basal shape of lamina (BS) and twig pubescence (TP) were included in any analysis. This means that they don't have any discriminating power between the three studied lime species and their assessment (in order to obtain similar discriminating functions) is useless.

4. Conclusions

The results of this brief morphological survey are in the accordance with the data from the literature, the values for the morphological descriptors being identical with those from similar studies or Dendrological manuals.

Nevertheless, it is important to underline that the three discriminant functions have no value (failed to discriminate between species) when individuals with intermediate morphology (putative hybrids) are sampled.

Last but not least, we suggest that in order to obtain functions with a higher discriminating power more lime stands should be sampled across species' distribution ranges. Additionally, only individuals for which no doubt regarding their hybrid status exists, which are very hard to find especially in mixed stands, should be sampled. At the same time, more macro-morphological descriptors should be assessed.

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