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SMALL-SCALE MORPHOLOGICAL DESCRIPTORS ANALYSIS IN FOUR ROMANIAN OAK STANDS REPORTED TO SERIES LANUGINOSAE SIMK.

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Abstract: *Two oak taxa from series Lanuginosae are known to occur in Romania: Quercus pubescens and Q. virgiliana. The taxonomic status of the latter taxon is still debated: distinct species or intraspecific unit of Q. pubescens. The aim of this research was to evaluate the existence of these taxa in the studied area on the basis of leaf and fruit descriptors. Four stands situated in different geographic regions were sampled. In three of them the occurrence the two taxa was reported in Romanian literature. Our results indicate that most of the oak individuals can be classified as Q. pubescens due to sessile or nearly sessile fruits, in combination with specific leaf descriptors. No typical Q. virgiliana specimens were detected.*

Key words: *Quercus pubescens, Q. virgiliana, taxonomical assessment, morphological descriptors.*

1. Introduction

Oaks from series *Lanuginosae* - downy oak (*Q. pubescens* Willd.) and Italian oak (*Q. virgiliana* Ten.) - are very important for the Romanian forest ecosystems situated in the wood steppe as a consequence of their adaptive thermophilic and xerophilic traits. In fact, in the context of the climate change due to global warming revealed by increasing mean of annual temperature [25], which can determine essential changes in forest ecosystems [22] the importance of these taxa will increase.

In relation to different taxonomical classifications, *Q. virgiliana* is sometimes nominated as a distinct species [2], [8], [9], [14], [24], [26], sometimes as a intra-specific unit of downy oak or even it is not mentioned as a taxon or infrataxon [6], [7], [20]. Other botanists [13] consider *Q. pubescens* as a species, analysed in *sensu lato*, subdivided in other *sensu stricto* species, among which is also *Q. virgiliana*. Actually, these different perspectives regarding the taxonomical differentiation between the two taxa are derived from their morphological similarities, with interferences between particular leaf descriptors (lamina length, petiole length) and fruit descriptors (peduncle length, scales shape), as reflected in various morphological descriptions [8], [9], [14].

2. Objectives

The objective of this study was to determine the existence of *Q. virgiliana*, in

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complex with downy oak, in phytocoenoses nominated in Romania in this respect by using statistic morphological data of leaf and fruit and multifactorial analysis which were useful in similar studies [3], [13], [16], [19].

3. Materials and Methods

Plant material was sampled from three phytocoenoses in which, according to the Romanian literature [2], [23] the two taxa coexists: two in Dobrogea, in southeastern Romania (Măcin and Ciucurova) and another one in Banat, in southwestern Romania (Clisura Dunării). There were also analyzed samples from Petiş, in Transilvania - central Romania, a NATURA 2000 sit for downy oak. A total of 119 oak trees were mapped with a GPS (36 trees from Măcin, 19 trees from Ciucurova, 14 trees from Clisura Dunării and 50 trees from Petiş). Branches from the upper part of the crown with fully developed leaves were sampled. Thus, five representative leaves were chosen from every tree to form the herbarium collection. Cup collections were also done.

Leaves were scanned with WinFolia software and 14 variables were assessed, according with the methodology described in a similar study done in Europe [19]: five dimensional characters - *lamina length* (LL), *petiole length* (PL), *lobe width* (LW), *sinus width* (SW), *length of lamina at largest width* (WP), two counted variables - *number of lobes* (NL) and *number of intercalary veins* (NV), two observed variables - *abaxial laminar pubescence* (PU), evaluated according to the grading system from 1 (no pubescence) to 6 (dense hairness) [17] with an microscope (x30) and *basal shape of the lamina* (BS), scored as an index varying from 1 to 9 and five transformed variables - *lamina shape* or *obversity* (OB), *petiole ratio* (PR), *lobe depth ratio* (LDR), *percentage venation* (PV) and *lobe width ratio* (LWR).

As a consequence of the importance of peduncle length**,** which is considered to be relevant for differentiation between typical specimens of *Q. virgiliana* and *Q. pubescens,* the following two descriptors were assessed: *peduncle length up to the first fruit* - l_{ped} (this situation is similar with the case when there is only one fruit on the peduncle) and *peduncle length up to the last fruit of the inflorescence* - L_{ped}. Peduncle length was assessed with a digital slide caliper with accuracy of 0.01 mm.

Morphological data were processed with STATISTICA software v.8.0 and the mean values and standard deviations for every morphological descriptor were calculated. Correlation coefficients between traits were calculated.

ANOVA test was applied to evaluate among and within phytocoenoses differences. Also, for each phytocoenose and for all a PCA (Principal Component Analysis) was done.

4. Results and Discussions

The mean values, standard deviations and the results of ANOVA test of the leaves and fruit descriptors are shown in Table 1.

It can be seen that the two Dobrogean phytocoenoses (Măcin and Ciucurova) have more pronounced morphological similarities. In general, the oaks from these two locations have leaves with a longer petiole (PL) and fewer intercalary veins (NV) and number of lobes (NL). But, knowing that the two oaks coexist in Măcin, Ciucurova and Clisura Dunării areas we expected that this will be reflected in the inter- and intraphytocoenotic morphological analysis. Indeed, the mean value of lamina length falls within the specific interval for the two taxa.

However, oaks with typical characters for *Q. virgiliana* were not present and the coefficients of variation indicate only a moderate variability in all four phytocoenoses.

Means (x), standard deviations (SD) and ANOVA for all descriptors Table 1

Variable	Phytocoenose				All stands	ANOVA	
	Ciucurova	Măcin	Petiş	Clisura Dunarii		Fisher test	
	x ± SD	*x* ± SD	*x* ± SD	*x* ± SD	*x* ± SD	Among	Within
PU	4.2±0.87	4.3±0.81	4.7±0.72	4.1±0.74	4.4±0.79	0.71 ns	2.56 ***
BS	3.9±0.80	4.0±0.88	4.1±0.93	4.9±1.06	4.1±0.92	0.89 ns	2.48 ***
NL	8.9±1.11	8.4±1.21	10.3±1.23	11.1±2.16	9.6±1.43	2.24 ns	1.92 **
NV	2.0±1.9	1.9±1.23	2.6±1.40	2.3±1.25	2.2±1.22	1.23 ns	2.99 ***
LL [mm]	85.2±15.00	79.8±11.97	76.9±11.81	89.0±17.08	80.5±13.69	1.96 ns	1.75 *
PL [mm]	15.5±2.36	13.8±2.87	12.5±3.09	11.4±4.01	13.3±3.12	1.48 ns	1.86 **
LW [mm]	31.5±6.03	26.1±4.36	27.3±5.19	29.2±4.71	27.8±4.80	1.23 ns	2.36 ***
SW [mm]	14.7±5.15	11.8±3.57	11.1±3.72	10.8±3.87	11.8±3.53	1.31 ns	3.48 ***
WP [mm]	45.6±9.23	44.8±7.35	41.6±7.34	49.1±9.79	43.1±8.06	1.16 ns	2.04 **
OB [%]	53.5±4.09	56.2±4.26	54.1±5.37	55.3±4.81	54.8±4.55	0.84 ns	2.37 ***
PR [%]	15.6±2.59	14.8±3.01	14.1±2.98	11.5±3.57	14.2±3.09	0.24 ns	1.89 ***
LDR [%]	53.3±13.91	54.9±11.05	59.2±11.04	63.3±11.36	57.4±11.55	0.19 ns	2.01 **
PV [%]	22.3±11.77	22.0±13.95	26.3±15.85	21.6±13.24	23.8±13.59	1.11 ns	3.14 ***
LWR [%]	36.9±2.75	32.9±4.47	35.6±4.59	33.2±4.75	34.7±4.17	1.46 ns	4.32 ***
lped [mm]	3.4±1.48	4.1±2.05	2.4±1.72	nv	2.6±	**2.43** ns	**5.02** ***
Lped [mm]	4.7±2.92	5.6±2.83	2.8±2.21	nv	3.5±	**1.36** ns	**4.74** ***

Legend: ns - non significant; * - significant; ** - highly significant; *** - extremely significant; nv - not evaluated; **in bold** - results for the 95 oaks for which the length of peduncle was evaluated.

The lower intraphytocoenotic variation of lamina length was found in Măcin (CV = 15%). In the context of the coexistence of the two taxa and knowing that lamina length is an essential criterion of discriminating between them, this value seems to be very low. However, from all sampled oaks there were only two trees from Măcin and one from Petiş whose maximum value for petiole length was more than 20 mm. It is known that the specific interval for downy oak for this descriptor is 0 to 20 mm. Therefore, the sampled oaks were classified by this criterion as downy oak.

The maximum abaxial laminar pubescence was found in Petiş, while the coefficient of variation for this descriptor in this phytocoenose had the lower value (CV = 15.3%). For the two Dobrogean phytocoenoses the values for this trait were very close and the interphytocoenotic coefficient of variation ranged from 18.8% to 20.7%.

The maximum value for NV was found also in Petiş (2.6), which is higher than the average value for all four stands. In the case of Petiş and Măcin stands, which had very close values for LL, NV clearly separated them, having a 36.8% higher value in the first case.

However, the interphytocoenotic variation is very high (CV = 55.4% for all analysed material), which seems to be a consequence of the polygenic control by the action of neutral genes.

Total length of the fruit peduncle (L_{ped}) and the length up to the first fruit of the peduncle (l_{ped}) had slightly higher values in Ciucurova, followed by Măcin. However, for all of the three locations where these descriptors were assessed the values were not significantly different, falling within the specific intervals for *Q. pubescens.* Only one tree, from Petiş phytocoenose, had the length of the fruit peduncle of 21 mm, but the mean value of the whole phytocoenose was only 2.8 mm.

ANOVA revealed nonsignificant interphytocoenotic values for all analysed descriptors. This demonstrated the existence of a spatial uniformity of the analysed material, which could be the consequence of an advanced genetic cohesion. The fact that most of the variation was found at intraphytocoenotic level could not be interpreted as an effect of the existance of two different taxa (*Q. pubescens* and *Q. virgiliana*), because such situation is typical for forest trees, including also downy oak [13].

Table 2 shows the correlations between morphological descriptors for all four phytocoenoses.

When **LL** was considered **as a reference descriptor,** our data indicate that it does correlate negatively with the laminar

Correlations between traits Table 2

Variable	Phytocoenose			
	Ciucurova	Măcin	Petiș	Clisura Dunării
A. LL as a reference variable				
PU	-0.08	-0.25	-0.47	-0.25
BS	-0.06	-0.16	-0.19	0
NL	0.57	-0.01	0.21	0.71
NV	0.16	-0.04	0.21	-0.36
PL	0.23	0.11	0.31	0.1
LW	0.92	0.67	0.72	0.66
SW	0.45	0.7	0.53	0.51
WP	0.92	0.87	0.82	0.87
OB	0.11	-0.11	-0.03	-0.13
PR	-0.69	-0.49	-0.31	-0.47
LDR	0.08	-0.37	-0.04	-0.28
PV	-0.01	-0.01	0.13	-0.58
LWR	0.05	-0.32	-0.16	-0.49
L_{ped}			0.09	
B. PL as a reference variable				
PU	-0.08	-0.17	-0.1	0.02
BS	-0.06	-0.07	-0.4	-0.81
NL	-0.12	-0.2	-0.3	-0.37
NV	0.41	0.26	0.02	-0.49
LL	0.23	0.11	0.31	0.1
LW	0.28	0.15	0.37	0.42
SW	0.06	0.01	0.29	0.28
WP	0.07	0.18	0.31	-0.27
OB	-0.14	0.2	0.07	-0.6
PR	0.59	0.8	0.8	0.85
LDR	0.12	0.1	-0.1	-0.14
PV	-0.42	0.27	0.11	-0.29
LWR	0.33	0.06	0.17	0.39
L_{ped}			0.35	

pubescence intensity. This is in accordance with the field observation regarding the intensely pubescence of the trees with smaller leaves. They can be considered as belonging to a biotype with more pronounced adaptability to arid conditions that could be recommended for ecological restoration in the present and future climate conditions.

In all cases, between lamina length and petiole length there is a positive correlation, but low in intensity, so, in the determination of Italian oak it can capture imperfections due to the existence of some *Q. pubescens* specimens with relatively longer leaves than usual. In all four phytocoenoses, the correlation between LL, firstly, and LW, SW and WP, secondly, are positive and intense like the cases revealed in other studies [11], [19].

The correlation between LL and $L_{ped,}$ although positive, has a weak intensity (r = 0.09), which means that there is the possibility of misinterpretation of the two taxa only or mainly based on these descriptors. However, taxonomic determinations which use a combination of characters LL-L_{ped} (l_{ped}) proves to be a better option, but other relevant descriptors (PL, NL, PU) should be considered.

Regarding **PL as a reference descriptor** it can be seen that it does correlate negatively, but with relatively low intensity, with NL, which suggests that the specimens analyzed belong to *Q. pubescens*. This was because if there were *Q. virgiliana* individuals, these two descriptors would have to be positively correlated, because leaves of this taxon have a longer petiole and more pairs of lobes than *Q. pubescens* [1], [21], [27]. The positive correlation and with a sufficient intensity between PL and L_{ped} (r = 0.35) indicates the possibility to use these descriptors in the morphological discrimination between these two oak taxa.

In all of the examined material, the first two components of **PCA** explain 45.4% of total variation, from which 24.7% on the first axis, with great influence caused by LL and WP.

In Măcin PCA diagram there is a small trend to form a bimodal distribution. The main morphological group belongs to typical specimens of *Q. pubescens,* while the second group contains specimens which showed transitional characters between *Q. pubescens* and *Q. virgiliana.* The existence of small morphological differences in these four phytocoenoses, as shown above can be explained by the environmental particularities. In fact, there was reported for the European oaks the existence of adaptive reactions as a reply to environmental conditions [18], although studies done in some species do not always reveal such situations [5].

Compared with average values for the same descriptors determined in an Italian study [4], the average values for our four phytocoenoses are higher for LL (with 16%) and PL (with 13.7%), respectively, and lower for NL (with 14.4%), SW (with 19.2%), PR (with 1.4%). In Martinsky stand from Slovakia [15] there were reported higher values than ours with 27.9% for LL, 30.8% for PL, 20.8% for NL and 28.8% for SW. To some degree, these differences may be genetically determined, but dimensional characters are more influenced by environmental conditions, as a consequence of the mainly polygenic control.

Compared with the average value of number of intercalary veins (NV) found in a similar study, which is considered capable to discriminate between pedunculate oak, sessile oak and downy oak [10], our values for this descriptor is two times higher (2.2).

ANOVA test for leaf descriptors in 10 populations of *Q. pubescens* from Croatia [13] revealed that the intrapopulational variability has higher values than interpopulational. This pattern for distribution of variability was confirmed also in our study, including the three phytocoenoses in which the two taxa have been nominated in the past.

In the morphological differentiation between *Q. pubescens* and *Q. virgiliana* there are often used the specific values for descriptors LL, PL, L_{ped} and NL [1], [2], [9], [14]. Generally, for trees analysed in our study these descriptors are typically for *Q. pubescens:* leaf length up to 12 cm [1], petiole length up to 20 mm [2] - but usually up to 10 mm, with 3-6 pairs of lobes, although for some trees the values of these descriptors match the reference ones for *Q. virgiliana* (LL is greater than 8 cm, PL is greater than 15-20 mm, respectively NL is greater than 12). However, in our opinion, the desciptor Lp, with extremely rare values up to 10 (12) mm, discriminates best between the two taxa. For *Q. virgiliana*, the minimum value of this descriptor is 20 mm [8] or 30 mm [1], [21]. Under these values taxonomic classification corresponds to *Q. pubescens.* A single tree out of the 119 analyzed, located in Petiş, has the length of peduncle of 21 mm.

The general idea resulted from the PCA is that the principal components generate a single morphological group, specific to *Q. pubescens*. In fact, the repartition and grouping of the analysed trees in PCA graphics indicates the existence of unimodal distributions, corresponding to a single morphological group. By contrast, in the study done on sessile oak and pedunculate oak, PCA analysis showed a bimodal distribution, corresponding to the two taxa [19].

Instead, in Măcin phytocoenose, although the leaf and cupule descriptors do not differentiate clearly the specimens with characters for *Q. virgiliana*, there is a discrete tendency to form two morphological groups. In fact, according to morphological descriptions of the aforementioned literature, in the second group there were present specimens that were labeled in the field as being ambiguous, with possibly intermediate characters between *Q. pubescens* and *Q. virgiliana.* However, if we consider the morphological description proposed by Doniță [9], namely fruit peduncle length, these intermediate specimens should be reported to *Q. virgiliana,* according to PCA results.

In Slovenia, comparative morphological studies could not make possible their morphological differentiation from *Q. pubescens* [16]. Still, in Turkey, using PCA there resulted a quite obvious separation of two morphological groups determined by leaf descriptors used in characterization of the trees belonging to these two species [3]. Recent data [12], based on micromorphological studies of leaves revealed better possibilities to differentiate *Q. pubescens* from *Q. virgiliana* than those given by the macro-morphological traits.

5. Conclusions

From the classical phenotypical analysis, except some notes [9], nearly all trees from the mentioned phytocoenoses sampled from the *Q. virgiliana* distribution range (in complex with *Q. pubescens*) are typical forms of *Q. pubescens,* because LL, PL and NL, respectively, fall within the interval of variation for this taxon. Moreover, the acorns are sessile or nearly sessile, which also corresponds to morphoplogy of *Q. pubescens*. Only a few individuals showed intermediate characters between the two taxa regarding these descriptors. Correlations between PL and L_{ped} confirmed the discriminating power of these descriptors for taxonomic classification of downy oak and Italian oak. The mean values of descriptors from analysed phytocoenoses are situated within those reported in abroad populations, suggesting a dependence of quantitative traits to habitat conditions.

ANOVA indicated nonsignificant differences among the four phytocoenoses, but highly to extremely significant within them. By using PCA, in three out of four phytocoenoses only a single morphological group was revealed, that corresponded to *Q. pubescens*. Instead, in Măcin, according to fruit descriptors (peduncle length), there is a small trend to form a bimodal distribution. The main morphological group belongs to typical specimens of *Q. pubescens,* while the second group contains specimens which showed transitional characters between *Q. pubescens* and *Q. virgiliana.* In this context, we appreciate that future research is needed by using methods with higher resolution (i.e. *genetic markers*), which will detect possible differences existing at molecular level between trees reported to the two taxa.

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