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Anatomical and Morphological Analysis of the Dalmatian black pine [*Pinus nigra* ARNOLD subsp. *dalmatica* (Vis.) Franco] needles

ZINKA PAVLETIĆ & ZLATKO LIBER

Summary

The anatomical structure of needles in the Dalmatian black pine (*Pinus nigra* Arnold subsp. *dalmatica* (Vis.) Franco) has already been the scope of a detailed analysis, but on the individual level. In this paper, an attempt to perform the anatomical parameter analysis on the population level has been made. The Biokovo, Brač, Pelješac and Hvar populations have been analysed at the same time. The number of resin ducts and the number of stomata on the cross cut in the one-, two- and three-year needles appeared to show a distinctly clinal distribution. In the direction Biokovo-Brač-Pelješac-Hvar the number of resin ducts (7,6-11,10-11,36-12,65) and the number of stomata (15-20-22-24) increase in a regular way. As this direction coincides with the change of ecological conditions, the climate in the first place, the determined clinal distribution can be indicated as the ecocline. We can assume that under the influence of the local microclimate the adequate ecotypes have been developed.

Zusammenfassung

Die anatomische Struktur der Nadeln der dalmatinischen Schwarzföhre (*Pinus nigra* Arnold subsp. *dalmatica* (Vis.) Franco) war bereits das Objekt einer Detail-Analyse, aber auf der Individualebene. In dieser Arbeit versuchte man, die anatomischen Parameter auf der Populationsebene zu analysieren. Parallel wurden die Populationen Biokovo, Brač, Pelješac, Hvar analysiert. Es wurde festgestellt, daß die Anzahl der Harzkanäle und der Spaltöffnungen auf dem Querschnitt der ein-, zwei- oder dreijährigen Nadeln eine ausgesprochen clinale Reihenfolge zeigt. In Richtung Biokovo-Brač-Pelješac-Hvar steigt die Anzahl der Harzkanäle (7,6-11,10-11,36-12,65) und die Anzahl der Spaltöffnungen (15-20-22-24) gleichmäßig. Da sich in dieser Richtung auch die ökologischen Bedingungen, in erster Linie das Klima, ändern, kann die festgestellte clinale Reihenfolge als Öko-Cline bezeichnet werden. Es wurde festgestellt, daß sich unter dem Einfluß des lokalen Mikroklimas die entsprechenden Ökotypen entwickelt haben.

Keywords: Croatian flora, endems, *Pinus nigra* subsp. *dalmatica*, anatomical analysis, population investigations

Introduction

Both the morphological and the anatomical structure of the Dalmatian black pine (*Pinus nigra* Arnold subsp. *dalmatica* (Vis.) Franco) needles were the scope of a detailed analysis conducted by VIDAKOVIĆ (1957) as a part of the needle-anatomy-based analysis of the black pine species in former Yugoslavia. As can be seen from the said, extensive work, in compliance with the possibilities of that time, the analysis was made on the individual level from a relatively large number of populations. As a matter of fact, a variable number of trees from individual populations, sometimes only one tree, was used and a detailed analysis of a series of individual anatomical structure parameters was made.

Certain parameters proved to be highly significant for individual subspecific black pine taxons, while others (such as the epidermis structure, the number of hypodermic layers) in some populations are distributed in the way to give impression of transitional forms between some intraspecific taxons.

It is evident that such very complicated state of the black pine - *P. nigra* s.l. needle anatomical structure is also conditioned, at least partly, by artificial raising of black pine cultures using seeds of presently unknown provenience, followed by their mutual hybridization, partly with local populations as well if they were located in the proximity. This is supported by VIDAKOVIĆ's observations (1957) on the island of Brač, for instance, where he could distinguish well the stand in the locality Fantovi dolci from other Dalmatian black pine stands on the same island among other things by the habitus of trees, and the specimens from the Fantovi dolci population differ from those significant for the Dalmatian black pine by the anatomy of their needles, too.

As the Dalmatian black pine is included in the population research program on the molecular and biological level by the DNA analysis, a detailed anatomical structure research of needles is carried out simultaneously for the same specimens from the same populations for which the DNA analysis is made. Some of the analyzed anatomical structures show significant interrelations, which will be discussed here.

MATERIAL AND METHODS

Plant material

Branches of individual Dalmatian black pine trees containing three generations of needles and several mature cones were collected in natural localities during winter, namely: on Biokovo 1994, Brač, Pelješac and Hvar 1996.

Nine samples from the island of Brač were collected on various altitudes, the upmost point being the highest peak on the island of Brač - Vidova gora (773 m above sea level).

Nine samples from the island of Hvar were collected on various altitudes, the

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upmost point being the highest peak of Sv. Nikola (626 m above sea level).

Nine samples from the Biokovo mountain were collected below the Šibenik peak (1200-1450 m above sea level).

Nine samples from the Pelješac peninsula were collected in the stands near the highest peak on the peninsula Sveti Ilija (961 m above sea level).

Methods

Upon return from field work, the material collected in natural localities was placed in the refrigerator on -20°C . During treatment, the material was taken out from the temperature of minus 20°C in smaller quantities, and needle unfreezing was done gradually to avoid tissue damage. This research included the analysis of needle length and thickness, number of stomata and number of resin ducts.

To obtain the said anatomical data, needles were cross cut in their central part, and each tree was represented by nine needles in all three generations (thirty needles in total).

Results

On the Dalmatian black pine material originating from four locations of its habitat (Biokovo, Brač, Pelješac, Hvar), the analysis of needle length and thickness, the number of stomata and the number of resin ducts was made. Each of these parameters was analyzed on one-, two- and three-year needles taken from 9 trees in each population. In total 1080 needles were analyzed.

First, the deviations appeared in the results obtained between the one-, two- and three-year needles for each individual tree inside the population (the individual variability), as well as between individual populations (the population variability).

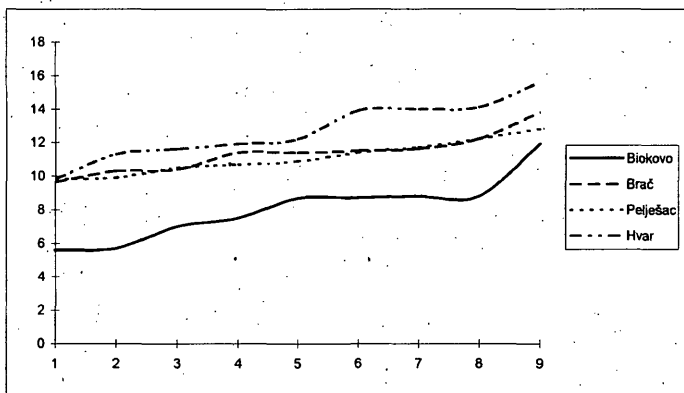
The needle length and thickness show no regularity in their variability either between the trees inside the population or between the populations. The existence of variability between the one-, two- and three-year needles of the same tree is quite unusual, so the problem of needle length and thickness as well as the regularity of their variability should be solved by a separate research with previous testing of sample quality (needle sampling for analysis purpose).

On the other hand, the number of resin ducts and the number of stomata on the cross cut vary in an absolutely identical way showing a certain regularity in their variability.

As can be seen in the attached graphs (Fig. 1), the lowest number of resin ducts and the lowest number of stomata were noted for the Biokovo population. The number of resin ducts ranges from 5,6 to 11,9 and that of stomata from 12,2 to 20,1. Their variability curves behave very similarly in the one-, two- and three-year needles.

The highest number of resin ducts and the highest number of stomata have been noted in the Hvar population, ranging between 9,8 to 15,6 and between 16,5 to 29,1 and respectively, with similar variability for all analyzed needle ages.

a)	1	2	3	4	5	6	7	8	9
Biokovo	5,6	5,7	7	7,5	8,7	8,7	8,8	8,8	11,9
Brač	9,6	10,3	10,4	11,4	11,4	11,5	11,6	12,2	13,8
Pelješac	9,8	9,9	10,5	10,7	10,9	11,4	11,7	12,2	12,8
Hvar	9,8	11,3	11,6	11,9	12,2	13,9	14	14,1	15,6



b)	1	2	3	4	5	6	7	8	9
Biokovo	12,2	12,8	13	13,1	14,3	16,2	16,7	18,5	20,1
Brač	16,1	17,9	18	19,1	20,1	21	21,1	23,3	23,3
Pelješac	18,5	19,1	19,7	19,8	20,8	21,8	23,2	24,5	28,3
Hvar	16,5	21,8	21,9	24,3	25,2	25,5	25,6	26,2	29,1

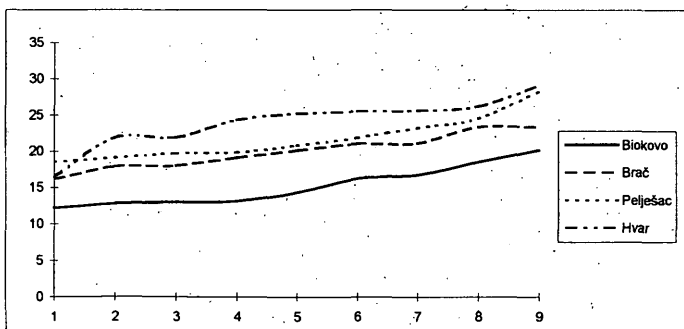


Fig. 1: Average numbers of resin ducts (a) and stomata (b) in one-year old needles of four populations of Dalmatian black pine (each population is represented by nine individuals)

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The number of resin ducts and the number of stomata vary also on the level of the one-, two- and three-year needles (Fig. 2), but these differences are negligible.

With regard to the number of resin ducts and the number of stomata, the Brač and Pelješac populations are intermediary with a tendency to approach the Hvar population.

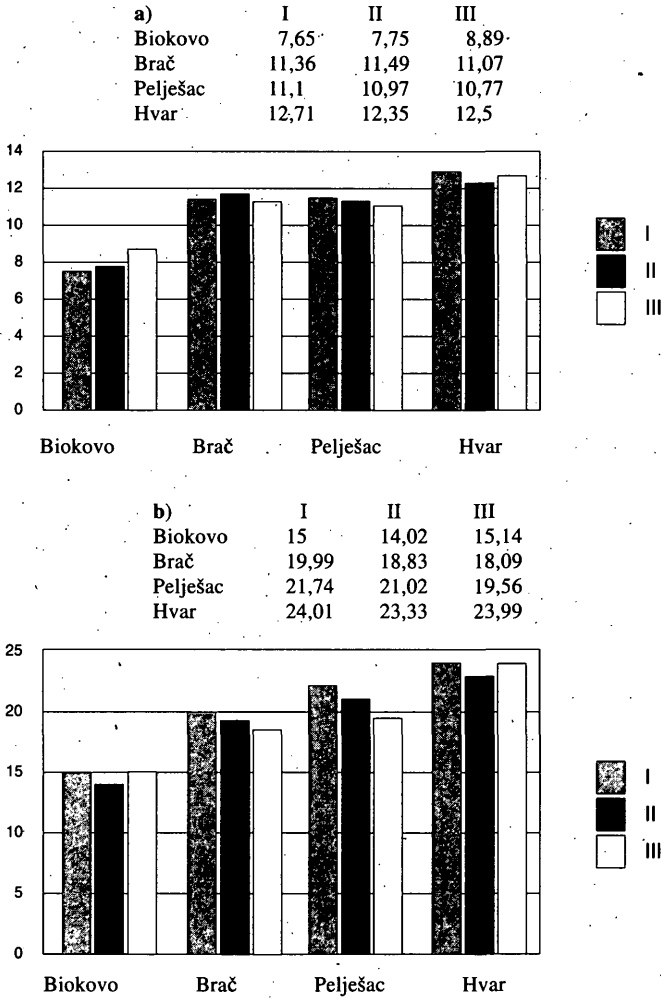


Fig. 2: Average numbers of resin ducts (a) and stomata (b) in one, two and three-year old needles (I, II, II) of four populations of Dalmatian black pine

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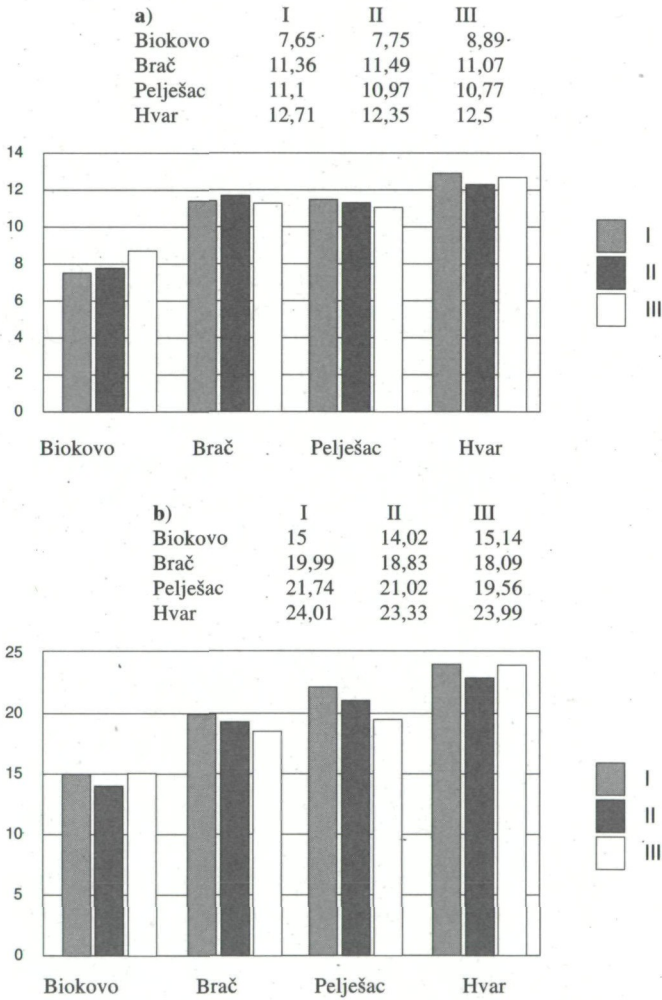


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Discussion

If we start from the assumption that the anatomical needle structure in the pine (the genus *Pinus*) is determined genetically and that during the speciation and specialization process it has been affected by certain ecological factors, it can be concluded that each of the analyzed Dalmatian black pine populations has been differentiated in dependence on the environmental conditions under which its development took place.

With respect to the altitude, the Biokovo population has developed on 1100 m, the Brač population on 700 m, the Pelješac population on approx. 800 m and the Hvar population on +/-600 m. As far as the general climatic conditions are concerned, the Biokovo population has developed under conditions of a special Mediterranean and montane climate characterized by cold winters with regular snow cover and warm and dry summers, that caused the development of a special climazonal premontane beech forest of the ass. *Doronico columnae-Fagetum* (comp. TRINAJSTIĆ 1990). Other populations have developed under the Mediterranean climatic conditions, within the Mediterranean and montane vegetation belt of the ass. *Ostryo-Quercetum ilicis* (comp. TRINAJSTIĆ, 1982). The Brač and Pelješac populations have developed under conditions of a somewhat more humid and cooler climate, sometimes with snow cover in winter. The Hvar population is markedly xerothermic.

From the above said it is clear that the general climate in the direction of Biokovo-Brač, Pelješac-Hvar becomes warmer and dryer. This coincides exactly with the direction of change in the number of resin ducts and the number of stomata, so we could speak about the ecotypical variability, which in space shows a clinal variability depending upon environmental conditions. From this it can be concluded that there is a connection between the climate and the number of stomata and the number of resin ducts, and, therefore, such relation of the distribution of the number of resin ducts and the number of stomata to the climate could be indicated as the ecoclina.

The clinal variability of individual morphometric parameters relating to the height above sea level has already been noticed, for instance, for the Common oak - *Quercus robur* (comp. TRINAJSTIĆ and KRSTINIĆ, 1993), and the clinal variability relating to the general climatic conditions (ecocline) for the species *Q. robur* in the lowlands of Croatia in the east-west direction (comp. FRANJIĆ, 1994). Differences in the number of stomata depending upon the geographic area (DUBRAVEC and TRINAJSTIĆ, 1984) have been noticed and analyzed also in the populations of the species *Phillyrea angustifolia* in the West-Mediterranean part of its area (narrow lanceolate shape) and in the Adriatic basin (wide lanceolate shape), even in the case when both shapes grow in the same stand (comp. BRITVEC and TRINAJSTIĆ, 1996).

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Adress of the authors:

Prof. Dr. Zinka PAVLETIĆ
Mr. Zlatko LIBER

Botanički zavod PMF-a
Maruličev trg 20/II
10000 Zagreb
Hrvatska

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